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Analysis of amino acids levels of freeze-dried termite queen *Macrotermes gilvus* Hagen

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Abstract

Objective: Termites (*Macrotermes gilvus* Hagen) are the social insects with colonies divided into 'castes' that include workers, soldiers, winged adults, a queen and a king. Queen of termites were analyzed for amino acid content.

Methods: Amino acid analyses were carried out on the part reproductive termites to determine the nutrient composition. The queen termites were collected from palm plantation, Taluak Kuantan, Riau, Indonesia. They were opened from nest, freeze-dried to fine powder. Amino acids were determined using ultra pressure liquid chromatography (UPLC).

Results: Freeze-dried termites queen showed that crude protein contain the essential amino acid. The highest content of amino acid was lysine, while the least content of amino acid was tryptophan.

Conclusion: The queen of termites was the nutrient-rich insects that can supply adequate quantities of the various amino acids for pharmaceutical nutrient.

Keywords: Termites, UPLC, Lysine, Tryptophan, *Macrotermes gilvus*

Introduction

Termites are most widely used as food in Africa. They are social insects with colonies divided into "castes" that include workers, soldiers, winged adults and a queen. The queen becomes very large and she lays thousands of eggs. Colonies of some species build huge earthen mounds, called termitaria, which may be up to 20 feet high. Periodically, the winged adults emerge in huge swarms, mate while in flight, and then start new colonies. They are highly attracted to lights, even candlelight, and that is one way they are captured for use as food. The wings are broken off, and, fried, termites are delicious. The queens are considered a special treat and are often reserved for children or grandparents [1].

Information on insects as food in Nigeria was collected by reviewing the literature of what scientists have done locally in the country. Lists of 22 edible insect species from six orders were compiled. Of these, 77.3% were Lepidoptera (27.3%), Coleopteran (27.3%), Orthopnea (22.7%) and 22.7% Isopteran, Hemisphere and Hymenoptera. These insects are rich in protein, vitamins and minerals. Some antinutritional factors are detected from some insects but the contents are below toxic level to man. Edible insects have a wide range of host plants from forest trees to agricultural crops and their consumption stages are available at different periods of the year. They are collected by hand picking, digging of soil and luring into water traps at night. Processing of collected insects could be carried out by boiling, sun drying, frying and roasting methods. To manage insects in the interest of food security, more attention should be given to environmentally sustainable collecting methods. These insects could be made better available throughout the year by developing improved conservation methods or by raising them as animal livestock. Considering the economic, nutritional and ecological advantages of this traditional food source, its promotion deserves more attention both from national governments and assistance programmers [2].

In Indonesia, *Macrotermes gilvus* Hagen (order Isoptera, family Termitidae, and sub family 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. *Macrotermes gilvus* Hagen have been investigated as antihyperlipidemic activity [3], acute and sub acutetoxicity [4] and immunomodulatory activity [5]. This study was therefore designed to determine amino acids composition to get information about nutritional potential of the termite.

Materials and methods

Sample collection

The queen termite *Macrotermes gilvus* Hagen were collected in the early morning hours 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 store d at 4 ºC until analysis.

Method of amino acid analysis

Two to three mg of each dried termite sample was transferred into a tarred glass ampoule. Norleucine, an amino acid not commonly found in proteins, was the internal standard used in all determinations. After 1.0 mL of 6 N HCl was added, the samples were flushed with nitrogen, evacuated, sealed and placed in an oven at 110 °C for 24 h. Following hydrolysis, a 10 mL aliquot was withdrawn and subjected to derivatization. Samples to be used for the determination of cysteine were first oxidized with performic acid [80% formic acid: 30% hydrogen peroxide, 9:1, (v/v)] for 18 h at room temperature [6]. Performic acid was removed in an evaporative centrifuge and the samples were hydrolyzed with HCl as described above.

The tryptophan content was determined separately. With regard to the tryptophan analysis, 450 μL of 4.67 M KOH containing 1 % (w/v) thiodiglycol was added to each sample [7]. Hydrolysis was performed in plastic tubes within an evacuated ampoule at 110 °C for 24 hrs. After allowing the hydro lysate to cool, 0.5 mL of 4.2 M perchloric acid and 50 μL of acetic acid were added to neutralize the solution. The samples were mixed thoroughly using a Thermolyne Maxi mixer, chilled on ice, and centrifuged. Fifteen microliters of the supernatant were transferred to 6 x 50 mm glass tubes and dried in a speedvac in preparation for derivatization. Duplicate lysozyme controls were analyzed for quality control purposes.

The samples were dried using 20 ml of ethanol: triethylamine: water (2:1:2, v/v) and derivative with 20 ml phenylisothiocyanate reagent [ethanol: triethylamine: water: phenylisothiocyanate (7:1:1:1 v/v)] for 20 min at room temperature. Excess reagent was removed in a speedvac. Derivative and dried samples were dissolved in 100 μL of equilibration buffer.

Analysis of the amino acids was performed with a Waters C18 column (3.9 x 150 mm). The gradient solution was the same as that described by Bidlingmeyer *et al.* (1984) [8]. The solvents utilized were the sodium acetate buffer and acetonitrile (300 ml ACN, 200 ml water, 0.2 ml CaEDTA). Twenty microliter aliquots were injected onto the column. Tryptophan analysis was performed according to Hariharan *et al*. (1993) [9]. Elution of the amino acids was achieved by increasing the acetonitrile concentration in the eluent, causing individual amino acid to be eluted at predetermined times. Quantitation was achieved by monitoring the absorption of the column at 254 nm and comparing the absorbance of individual peaks with that of the corresponding amino acid standard.

Results and discussion

Result of this study revealed that amino acid profile of freeze-dried queen termite *Macrotermes gilvus* Hagen was showed in Table 1.

Table 1: Amino acids profile of freeze dried termite queen *Macrotermes gilvus* Hagen

S.No	Amino acids	Unit	Result	Limit of detection	Method
1	L-Phenylalanine	ppm	30561.01	-	18-5-17/MU/SMM-SIG, UPLC
2	L-Valine	ppm	24128.61	-	18-5-17/MU/SMM-SIG, UPLC
3	L-Threonine	ppm	25837.02	-	18-5-17/MU/SMM-SIG, UPLC
4	L-Tryptophan	ppm	3560.64	-	18-5-63/MU/SMM-SIG, UPLC
5	L-Isoleucine	ppm	20296.23	-	18-5-17/MU/SMM-SIG, UPLC
6	L-Methionine	ppm	11051.67	-	18-5-17/MU/SMM-SIG, UPLC
7	L-Leusine	ppm	38341.64	-	18-5-17/MU/SMM-SIG, UPLC
8	L-Lysine HCl	ppm	39013.74	-	18-5-17/MU/SMM-SIG, UPLC

Protein content and amino acid composition

The protein content of the queen termite (which is the anatomical part consumed by Human) was rather high, accounting for 43.44 g/100 g of the dry weight (43.44% dry weight). This value is equal to the sum of all the amino acids (Table 1). However, the essential amino acid composition of the samples, queen proteins was of higher quality. In fact, the chemical index, calculated by comparing the essential amino acid content of the sample protein with that of a WHO standard protein, was rather high, with methionine, the sulphuric amino acids, being the limiting amino acids. Interestingly, tryptophan, which is generally limiting in the food insects [10], was present in adequate amount (Figure 1 and 2).

The termites that were the focus of this study, particularly queen termite, contain large amounts of protein. Overall the amino acid composition of the queen is good compared with that of the WHO standard protein, except for the somewhat low values for the sulfur amino acids (methionine) that are thus limiting. The chemical index of these proteins (85%) is nevertheless high and comparable with that of conventional meats.

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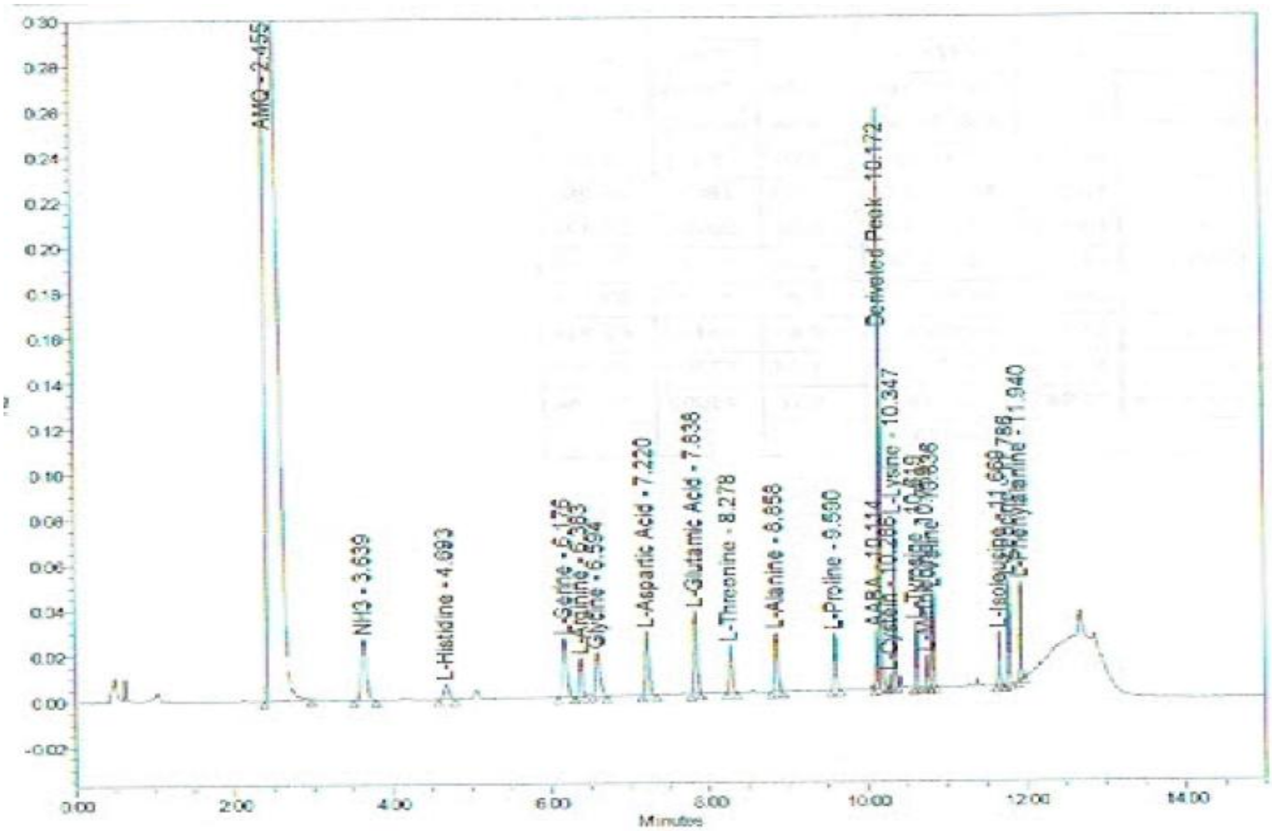

Figure 1: Chromatogram of amino acids of queen termite

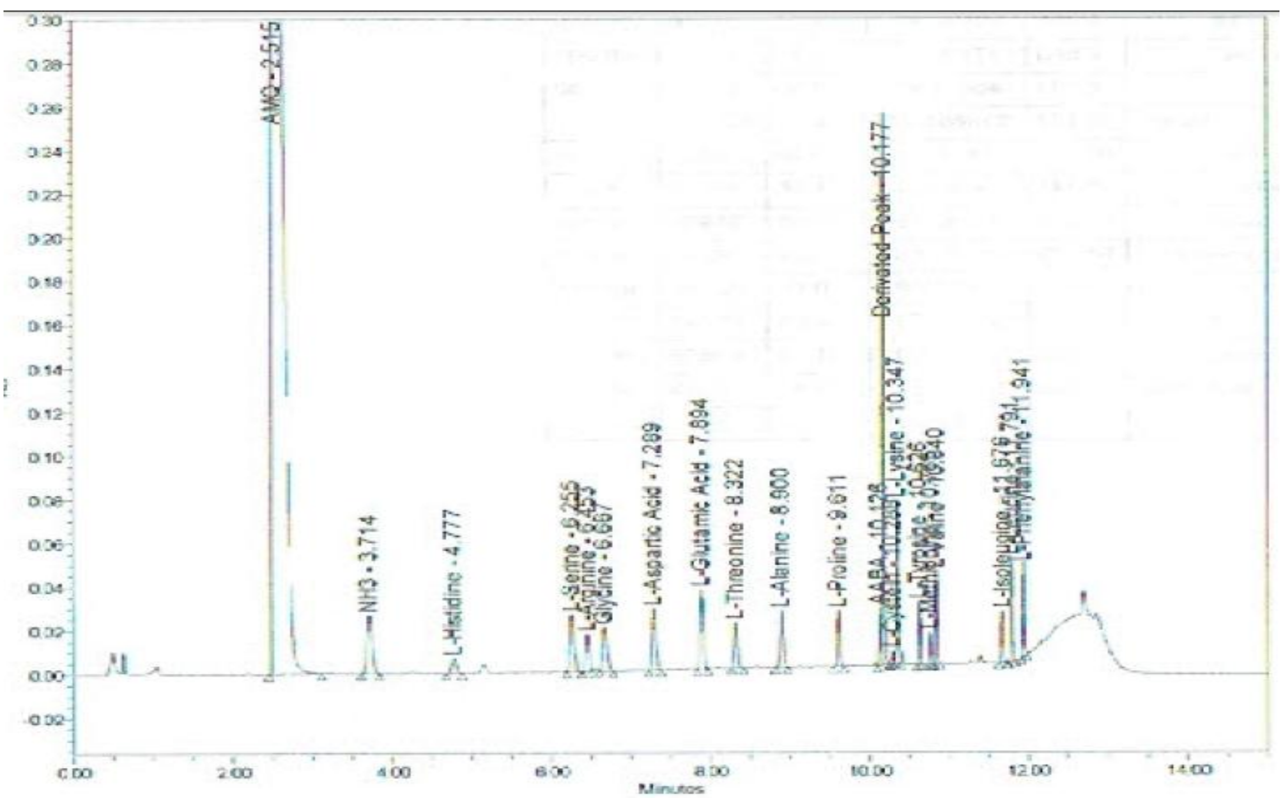

Figure 2: Chromatogram of derivative amino acids from queen termite

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Some research to present protein in the four castes of *M. subhyalinus* is highly digestible; however protein digestibility varies by castes. The queen has the highest digestibility value, while workers and soldiers are the least digestible. The observed difference in the digestibility of the four castes is of biological significance in terms of ecological niche. The queen caste functions mainly in production of eggs and is nourished for that purpose. The recorded highest digestibility value obtained in queen caste may be as a result of abundance of eggs in the body and the nourishment received from the worker caste. The late caste is the potential reproductive in the colony and the body would equally be enriched with nutrient in preparation for the next flight and establishment of new termitaria. Workers accumulate food out of which other members are fed and required to have reserves in the body. Soldier works with workers in search for food and again defends the colony. In the course of these activities, worker and soldier make use of the nutrients more than others in the colony. This may have contributed to the low protein digestibility obtained in them [11].

Queen, soldiers and workers of termites *Macrotermes bellicosus* were analyzed for proximate composition, vitamin and mineral elements and anti-nutrient content. Proximate composition showed that crude protein content of the soldiers (54.68 %) was higher than those workers (25.38 %) and Queen (32.38 %) [12].

Termites can be used as food for non-human primates. Suzuki (1966) described insect eating primarily ants and termites by wild chimpanzees in cites of Tanzania, and other studies were on the eating of insects by chimpanzees, Japanese monkeys and baboons. Termites are eaten in several parts of Nigeria and it is also used for rituals and medicinal purposes. In India termites and even its termitaria have medicinal usages [13] as termites based medicinal companies are established.

Winged termites *Macrotermes falciger* content of crude protein 43.26 % ± 0.03 and major amino acid composition (per 100 g) are arginine, serine, glutamic acid, tyrosine, leusine and histidine 3.01 ± 0.44, 2.08 ± 0.37, 4.68 ± 0.02, 3.44 ± 0.25, 3.16 ± 0.14, 2.65 ± 0.04, respectively [14].Proximate levels of whole *Trinervitermes germinatus*, the raw (fresh), fried and wings showed with the fried sample having the highest value of 41.70 ± 1.48 %. The daily protein requirement of 23.56 % (NRC, 1974) can be substantially augmented by incorporating processed termite meat into children, pregnant and lactating mother's diets or even adults who are malnourished [15].

Roasted *Macrotermes bellicous* contained 36.7 gram protein/100 g portion [16]. Roasting *M. bellicosus* resulted in highly significant reduction in moisture content, with corresponding significant increase in its crude protein, fat, ash and carbohydrate value ($p<0.05$). The roasted insect was also very high in gross energy content. The observed high crude protein and fat content of the insect confirmed the assertion of some authors that insects are good sources of dietary proteins and lipids [10, 17].

Conclusion

This study revealed that termites (*Macrotermes gilvus*) have high nutritional qualities. The result of this study confirms the fact that termites are indeed a good source of protein and amino acids. The consumption of termites should be encouraged. Termites (*Macrotermes gilvus*) provide high quality of proteins and supplement even when dried. Hence, *Macrotermes gilvus* ought to be cultivated with modern techniques in order to increase their commercial value and availability.

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