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Research Article

Chemical Compositions of Two commonly consumed Insects in Kontagora Local Government Area, Niger State, Nigeria

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ABSTRACT
The practice of entomophagy is culturally acceptable in many parts of Africa including Nigeria. Grasshoppers, *Zonocerus varigatus* and Dung beetle larva, *Aphodius rufipes* are two major insects consumed by people of Kontagora Local Government Area of Niger State, Nigeria. These insects are eaten for nutritional and health benefits and are highly priced as commercial commodities. Proximate, mineral elements and anti-nutrients and amino acids composition of these insects were determined using standard methods. The results showed crude protein content to be 41.6% and 42.3%; fat of 17.5% and 24.2%; ash of 7.2% and 4.3%; fibre of 14.1% and 4.6%; carbohydrate of 13.2% and 18.9% for grasshopper and dung beetle larva respectively. Nutrients like phosphorus, potassium, sodium, calcium and iron are of excellent quantity in both insects. About 44.4% and 66.7% respectively of essential amino acids in grasshopper and dung beetle larvae satisfy human nutritional requirements. The quantity of amino acids lysine, tryptophan and threonine are adequate in grasshopper and dung beetle larvae thereby making them complementary protein for limited cereals diets common to people in this region. Interestingly, a consumption of 97g and 46g of grasshopper and dung beetle larvae respectively could effectively satisfy the daily adult human amino acid requirement. Lastly, anti-nutrients like cyanide and oxalate present in these insects are of tolerable quantity for human consumption. Therefore both insects were recommended as alternatives to conventional sources of animal protein for the human diet.

Keywords: Entomophagy; Amino acid; Proximate; Anti-nutrients; Insects

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INTRODUCTION

The class insecta is one of the most diverse groups of animals. Their diversities in forms and functions have contributed to their roles in relation to other organisms, especially humans in the ecosystem. They have been implicated in the transmission of plants and animal diseases, some are parasites, and pests of crops, while others constitute a nuisance to man. However, their beneficial attributes outweigh all the perceived detriments. One important benefit of insects to man is their edibility and palatability. Human consumption of insects as food is known as entomophagy. It is culturally acceptable in many parts of the world predominantly in Africa, Asia, and Latin America and recently in a few parts of Europe (Olivadese & Dindo, 2023).

Over 2 billion people in the world feed regularly on insects as part of their diets (van Huis, 2022) and over 2200 species of edible insects have been identified to be consumed across 128 countries worldwide (Omuse *et al*., 2024). Among the over 500 species of insects consumed in Africa, 91 species are consumed in West Africa (Kelemu *et al*., 2015), while about 22 species of these belong to one of either order: Isoptera, Orthoptera, Coleoptera, Hemiptera, Hymenoptera and Lepidoptera are consumed in Nigeria (Alamu *et al.,* 2013; Adeoye *et al.,* 2014). Edible insects are rich in proteins (40-75mg/100g dry weight) with high digestibility (77-96%) (Verkerk *et al.,* 2007). The 2013 United Nations Food and Agricultural Organization report confirmed insects to be rich in minerals like potassium, calcium, magnesium, zinc, and iron as well as B vitamins (vanHuis *et al*., 2013). Apart from being eco-friendly, edible insects do not transmit zoonotic diseases and their consumption does not predispose humans to the risk of carcinogenic and cardiovascular diseases.

In Nigeria, apart from their nutritional and health benefits, insects are highly priced commercial commodities. Dry grasshopper, dung beetle larvae and *Cirina forda* larvae are sold in local markets by women to support their family livelihood in major parts of North-Central States of Nigeria. In Kontagora Niger state, 1kg of dry dung beetle larvae is sold for ₦7000 ($5) while 1kg dry grasshopper is sold for ₦6500 ($4). Despite these nutritional and commercial benefits of edible insects, many of those who buy and eat are not fully aware of their nutritional information. They are not also aware of the minimum quantity to be consumed to meet their daily nutritional requirements. This study is therefore aimed at determining the nutritional compositions of dry grasshopper and dung beetle larvae and the minimum quantity to be consumed to satisfy the daily human protein requirement.

MATERIALS AND METHODS

Insect Collection and Preparation

Dry dung beetle larvae and grasshoppers were purchased from the new market. Dirt particles were removed from them and the grasshoppers de-winged. They were separately sundried and grounded into fine particles using an electric blender. Each powder was wrapped into separate aluminium foils and kept in separate air-tight containers bearing respective labels for easy identification before being kept in a refrigerator.

Proximate Analysis

The proximate compositions for moisture, ash, fibre, crude protein and crude fat were determined as described by AOAC (2005). Total carbohydrate content was determined by the difference as described by Nielsen (2002). The total amount of crude protein, crude fat, moisture and ash of each of the samples was added and subtracted from 100. The value obtained was the percentage carbohydrate content of the samples.

% Carbohydrate = 100 – (% moisture + % protein + % fat)

The metabolisable energy of the sample was calculated using Atwater factors. The value of protein content of each sample was multiplied by 4, that of lipid multiplied by 9 and that of total carbohydrate multiplied by 4. The sum of these values was expressed as Kcal/100g.

Mineral Analysis

Sodium and potassium were determined by flame photometry, phosphorus by phosphovarado-molydate reagent method using spectrophotometry, while minerals like magnesium, iron, calcium, zinc and manganese were determined with an atomic spectrophotometer (AOAC, 2005)

Mineral Analysis

The potassium and sodium content of the samples were assayed by digesting the ash of the samples with perchloric acid and nitric acid and then taking the readings on a digital flame photometer (Bonire, 1990). Phosphorus was determined by Vanado-molybdate colorimetric method (Ologhobo and Fetuga, 1983). Calcium, magnesium, iron zinc, manganese, copper and selenium content of the samples were determined from the digested ash of the samples spectrophotometrically by using an atomic absorption spectrophotometer (AOAC, 2005) and compared with absorption of standards of these minerals.

Phytochemicals or Antinutrients Determination

Phytochemical analysis for the presence of tannins was determined by the method described by Makkar and Goodchild (1996), and the Young and Greaves (1940) method was used for pythin determination. Oxalate was assayed as described by Day and Underwood (1986) while alkaloids and saponin were measured by the method described by Harborne (1973).

Amino Acids Analysis

Amino acid content was determined by ion exchange HPLC chromatography (Benitez, 1989), using the Applied PTH Amino Acid Analyser (Model 120A). 2g of each of the samples was defatted using chloroform/methanol (2:1) (AOAC, 2006) and then hydrolysed at 110^oC under a nitrogen atmosphere for 22hrs with 6M hydrochloric acid. For the determination of tryptophan, 2g of each of the samples were separately hydrolysed with 4.2M sodium hydroxide for 22 hours and were neutralized to P^H 7.0 with 6N of hydrochloric acid. The hydrolysates were injected into the amino acid analyser for separation and characterization. Quantification was obtained by using an external amino acid standard and the results were corrected for the recoveries. All analyses were conducted in triplicate for each sample.

Quality of the Amino Acids

The total amino acid (TAA), total essential amino acid (TEAA), total acid amino acid (TAAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) were determined. The Predicted Protein Efficiency Ratio (P-PER) was determined using the equation developed by Adeyeye (2009).

P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr)

The amino acid score for essential amino acids was calculated according to FAO/WHO (1973)

AAscore = AAAsp/AAARp

Where AAAsp is the amount of limited amino acid in the sample protein (mg/g) while AAARp is the amount of the same amino acid in the reference protein (mg/g).

RESULTS

Table 1 shows the proximate composition of grasshopper and dung beetle larvae. The ash contents are 7.23% and 4.30% respectively. The crude protein contents of the samples are 41.66% and 42.33%. The oil extract is 17.52% and 24.22%, while the carbohydrate contents are 13.20% and 18.90%. Dung beetle larvae have the highest content of crude protein which though not significantly different from that of grasshopper. Dung beetle also has the highest content of oil extract which was significantly different from that of grasshopper. Grasshopper has the highest ash and fibre content.

Table 2 shows the mineral composition of grasshopper and dung beetle larvae. Calcium had the highest concentration of 63.51mg/100g in grasshoppers while phosphorus had the highest concentration of 2860mg/100g in dung beetle larvae. Iron was lowest in grasshoppers while calcium was lowest in dung beetle larvae. Ca^{2+}/P was highest in grasshoppers, K^+/Na^+ was highest in dung beetle larvae and Ca^{2+}/K^+ was lowest in dung beetle larvae. These mineral ratios were significantly higher (P> 0.05) in grasshoppers.

Table 3 shows the anti-nutrient contents of grasshoppers, dung beetle larvae and winged termites. Tannin showed concentrations of 4607.5mg/100g and 924.13mg/100g respectively in grasshopper and dung beetle larvae. Phytate was highest in grasshoppers with a concentration of 1045mg/100g. Cyanide and oxalate were lowest respectively in dung beetle larvae.

Table 4 shows the amino acid compositions of grasshopper and dung beetle larvae. About 44% and 66.6% of the essential amino acids in grasshopper and dung beetle respectively satisfy the FAO/WHO recommendation for human consumption. Amino acid, isoleucine was limiting in grasshoppers while phenylalanine was limiting in dung beetle larvae. The essential to non-essential amino acid ratios were 0.86 and 0.69 respectively in grasshopper and dung beetle larvae. Their amino acid scores were 0.52 and 0.59 respectively in grasshopper and dung beetle larvae.

Table 5 shows the recommended daily intake of the samples that can satisfy the essential amino acid requirements of adult humans. The values in brackets indicate the amount of the sample in grams to be consumed daily to meet the daily human essential amino acids needs. A minimum consumption of either 96.55g of grasshopper or 45.45g of dung beetle larvae will sufficiently cater for adult human essential amino acids requirements.

Table 1: Proximate composition of *Z. varigatus* and *A. rufipes* larvae (%)

	Z. varigatus	*A. rufipes* larva
Moisture	6.35 ± 0.06^a	5.87 ± 0.07^a
Ash	7.23 ± 0.05^a	4.30 ± 0.04^b
Crude protein	41.66 ± 0.05^a	42.33 ± 0.85^a
Fiber	14.07 ± 0.09^a	4.59 ± 0.03^b
Crude fats	17.52 ± 0.06^a	24.22 ± 0.85^b
Carbohydrate	13.20 ± 0.13^a	18.90 ± 0.78^b
Energy (Kcal)	376.66 ± 0.03^a	459.65 ± 15.47^{b}Kcal

Values are the mean ± SD of three observations

Means in the same row with the same superscript are not significantly different (T-test, P < 0. 05)

Table 2: Elemental composition of *Z. varigatus* and *A. rufipes* larva (mg/100g)

Minerals	*Z. varigatus*	*A. rufipes* larva
Iron	10.96 ± 0.05^a	34.38 ± 1.43^b
Phosphorus	7.12 ± 0.04^a	2860.48 ± 7.80^b
Calcium	63.51 ± 0.25^a	29.22 ± 0.76^b
Sodium	18.86 ± 0.08^a	593.10 ± 3.78^b
Potassium	23.18 ± 0.14^a	1060.42 ± 7.87^b
Zinc	15.02 ± 0.13	ND
Magnesium	31.41 ± 0.12^a	32.43 ± 1.07^a
K^+/Na^+	1.23	1.79
Ca^{2+}/P	8.92	0.01
Ca^{2+}/Mg^{2+}	2.02	0.90
Ca^{2+}/K^+	2.74	0.03

Values are the mean ± SD of three observations

Means in the same row with the same superscript are not significantly different (T-test, P < 0. 05)

Table3: Anti-nutrient composition of *Z. varigatus* and *A. rufipes* larva (mg/100g)

Anti-nutrient	*Z. varigatus*	*A. rufipes* larva
Cyanide	59.72 ± 2.65[a]	0.73 ± 0.02[b]
Oxalate	90.76 ± 0.45[a]	4.06 ± 0.09[b]
Phytate	1045.93 ± 18.59[a]	295.28 ± 1.66[b]
Tannins	4607.50 ± 12.53[a]	924.13 ± 5.05[b]
Saponnins	ND	450.30 ± 1.64

Values are the mean ± SD of three observations

Means in the same row with the same superscript are not significantly different (T-test, P < 0. 05)

Table 4: Amino acid Composition of *Z. varigatus* and *A. rufipes* larva (g/100g protein)

Amino acid	*Z. varigatus*	*A. rufipes*[a]	*FAO/WHO (RDA)
Leucine	5.19 ± 0.05	5.95 ± 0.02	6.60
Lysine	6.29 ± 0.04	5.93 ± 0.02	5.80
Isoleucine	1.45 ± 0.05	3.08 ± 0.03	2.80
Phenylalanine	4.09 ± 0.05	3.72 ± 0.02	6.30
Tryptophan	2.28 ± 0.08	2.20 ± 0.01	1.10
Valine	2.23 ± 0.04	4.53 ± 0.02	3.50
Methionine	1.99 ± 0.04	2.23 ± 0.02	2.50
Proline	9.14 ± 0.05	4.68 ± 0.01	9.13
Arginine	8.01 ± 0.03	5.16 ± 0.02	8.00
Tyrosine	8.27 ± 0.05	5.33 ± 0.02	6.30
Histidine	7.58 ± 0.06	2.36 ± 0.01	1.90
Cysteine	0.61 ± 0.03	0.48 ± 0.04	2.50
Alanine	3.12 ± 0.03	4.85 ± 0.02	3.11
Glutamic acid	5.61 ± 0.05	12.19 ± 0.01	5.60
Glycine	9.94 ± 0.03	6.29 ± 0.02	7.12
Threonine	7.13 ± 0.03	8.36 ± 0.03	3.40
Aspartic acid	5.32 ± 0.04	4.75 ± 0.02	5.30
Serine	5.26 ± 0.04	6.16 ± 0.05	9.93
∑TAA	97.51	88.29	
%TEAA	45.04(46.2%)	36.16 (41.0%)	
%TNEAA	52.47(53.8%)	52.13 (59.0%)	
∑TEAA/∑TNEAA	0.86	0.69	
P-PER	1.02	1.67	
AA_{score}	0.52	0.59	

Values are mean of triplicate measurements, *FAO/WHO (1991), [a](Oriolowo *et al.,* 2020)

Table 5: Daily recommended intake of protein samples which satisfy the body's amino acids requirements

	Recommended Daily Intake[b]		g/100g of protein	
Amino acid	**mg/Kg body weight**	**g/70kg body weight**	***Z. varigatus***	***A. rufipes***
Leucine	39.0	2.70	5.19 (52.02)	5.95 (45.38)
Lysine	30.0	2.10	6.29 (33.39)	5.93 (35.41)
Isoleucine	20.0	1.40	1.45 (**96.55**)*	3.08 **(45.45)***
Phe + Tyr	25.0	1.75	12.36 (14.16)	9.05 (19.34)
Theonine	15.0	1.05	7.13 (10.56)	6.16 (17.05)
Met + Cys	15.0	1.05	2.60 (40.38)	2.73 (38.46)
Valine	26.0	1.82	2.23 (8161)	4.53 (40.18)
Tryptophan	4.0	0.28	2.28 **(**12.28**)**	2.20 (12.72)
Histidine	10.0	0.70	7.58 (9.23)	2.36 (29.66)
∑Essential	184.0	12.58		

RDI Standard FAO/WHO (2007)**[b]**

Value in parentheses with an asterisk* represents the minimum recommended daily intake in grams of samples for adult humans

DISCUSSION

The moisture contents obtained for insects in this study ranged between 5.87-8.43% and this is considered appropriate for their good shelf life. Spoilage due to proteolytic, lipolytic and microbial proliferation is reduced at a moisture level below 15% (Kaneko, 1976). Grasshopper, and dung beetle larvae are rich in crude protein content with 41.67 and 42.33 respectively. Similar results were reported for toasted grasshopper, *Ruspolia dfferens* by Ochieng *et al.* (2022), sundried dung beetle larva, *Aphodius rufipes* by Bamayi *et al.* (2019). These insects' crude proteins are higher than beef (22.3%), chicken (22.25%) and pork (22.0%) based on mass (Probst, 2018). Consumption of these insects could contribute to improved dietary protein quality and gradual replacement of intake of convectional animal proteins among rural dwellers in Nigeria (Banjo *et al.* 2006). The ash content of grasshoppers in this study was higher than those reported by Geoffrey *et al.* (2017) and Ocieng *et al.* (2022) for edible grasshoppers, *Ruspolia nitidula* and *Ruspolia dfferens* respectively. The ash content of *A. rufipes* in this study was lower than those reported by Bamayi *et al.* (2019). The ash content of food is an index of its mineral content and the amount of minerals the food can supply to the body when eaten (Braide and Nwaoguikpe, 2011).

The dietary fibre of 14.1% in grasshoppers was higher than those reported by Ocieng *et al.* (2022). A good intake of dietary fibre has tremendous health benefits such as lowering the risks of developing coronary heart disease, stroke, hypertension, diabetes, obesity and gastrointestinal diseases (Anderson *et al.,* 2019). The crude fat content from grasshoppers was lower than those reported by *Ocieng et al.* (2022) and Geoffrey *et al.* (2017) for *Ruspolia differens* and *Ruspolia nitidula* respectively. Crude fat obtained from *A. rufipes* was higher than the value reported for sundried dung beetle larvae (Bamayi *et al.*, 2019). Lipids are vital in the structural and biological functioning of cells, transport of essential soluble vitamins, providing energy and maintaining body temperature (Anhwange *et al.*, 2016). These insects have excellent mineral contents which can complement the nutritional needs of adult humans. Iron contents ranged between 10.96-34.38mg/100g and consumption of 100g of either of the insects will satisfy the Recommended Daily Intake (RDI) of 8-15mg per day (Hellwig *et al.*, 2006). Adequate intake of iron in humans will prevent anaemia, a common nutritional deficiency. *A. rufipes*has adequate amount of potassium which could satisfy the Recommended Daily Intake of 400-4700mg/day when adequately consumed. A sufficient amount of potassium in the diet can protect against heart disease, hypoglycaemia, diabetes, obesity and kidney diseases (Akullo *et al.,* 2018). Grasshopper has a sufficient amount of zinc which satisfies the Recommended Daily Intake of 3.0-8.0mg/day (Hellwig *et al.,* 2006). Zinc is involved in many areas of metabolism and its deficiency includes, impaired growth, alopecia, diarrhoea, delayed sexual maturity and impotence, altered immune function etc.

In addition to considering individual minerals, it is important to consider their interrelationships or ratios. Mineral ratios are important in determining nutritional adequacy because they can predict metabolic dysfunction (Olagbemide, 2015). The high Ca/P ratio recorded in grasshoppers in this study makes it nutritionally beneficial. A food is considered "appropriate" if the Ca/P ratio is above one and "poor" if the ratio is less than 0.5. A Ca/P ratio above two enhances the absorption of calcium from the small intestine (Niemann *et al.,* 1992). These insects also have a good K/Na ratio, a K/Na ratio of above one is recommended for healthy human living. Their intake as a diet could reduce blood pressure and the risk of cardiovascular diseases (Parez and Chang, 2014). Grasshopper also has a good Ca/K ratio otherwise known as thyroid ratio. A ratio of above 16 and below 2 is associated with mental and emotional disturbance (ARL, 2012).

It is always recommended to evaluate the anti-nutrient contents of phytophagus insects when they are being considered as food (Berenbaum, 1993). The cyanide obtained in this study was lower than the value reported for *A. rufipes* by Bamayi *et al.* (2019). The cyanide contents of grasshopper and dung beetle larvae were lower than the 200mg/100g recommended by the National Research Council, NRC (NRC, 1974). The oxalate content obtained in the two insects under study is moderate. The value of 4.06mg/100g obtained in dung beetle larva was lower than 180mg/100g reported for dung beetle larva by Bamayi*et al.* (2019). Oxalates are naturally occurring in plants and animals (Rahman *et al.*, 2013) and they combine with calcium and magnesium to form insoluble Ca and Mg oxalate which reduces serum calcium and magnesium levels. However, the consumption of moderate oxalate could reduce blood cholesterol (Savage, 2000). The phytate content obtained for a dung beetle in this study was lower than the 790.8mg/100g reported for similar insects (Bamayi *et al.,* 2019), while phytate obtained for a grasshopper (1045.9mg/100g) was higher than the values obtained for oven dried grasshopper (Adeduntan, 2005). Phytic acid binds with other nutrients like protein and essential mineral elements such as iron, calcium and zinc to form complexes, thus reducing their availability to the body (Francis *et al.,* 2001).

Grasshopper and dung beetle larvae have 46.2% and 41.0% essential amino acids respectively. These values satisfy the dietary recommendation of at least 39% for all categories of humans (WHO/UNO, 1985). They also satisfied the recommended value of 0.6 essential to non-essential amino acids ratio for human diets (FAO/WHO, 1973). The essential amino acids lysine, tryptophan, histidine and threonine are adequate in grasshoppers while lysine, isoleucine, tryptophan, valine, histidine and threonine are abundant in dung beetle (FAO/WHO, 1991). Lysine helps in the synthesis of carnitine, which plays an important role in the production of hormones, antibiotics and enzymes. A deficiency in lysine could lead to a lack of niacin which results in pellagra (Fagbenro *et al.*, 2005). Histidine helps produce histamine, which takes part in allergic and inflammatory reactions. The amino acid tryptophan is a precursor of the neurotransmitter, serotonin which acts as a relaxant and alleviates insomnia, and migraine reduces anxiety and promotes immune functions (Oriolowo *et al.,* 2020). Threonine is an important precursor in the formation of bones, cartilage, hairs, teeth and nails. Leucine is a regulator of protein turnover, transporter of nitrogen in the brain and translator regulator; arginine regulates enzyme activities and signal transducer; while glutamine is a substrate for protein synthesis, hepatic and renal gluconeogenesis and control of acid-base balance (Pencharz, 2012).

CONCLUSION

The findings from this study revealed that grasshopper and dung beetle larvae are rich sources of dietary protein, fats, fibre, minerals, and essential amino acids and have tolerable anti-nutrient content. They can supplement the convectional animal protein sources such as fish, beef and chicken. They are therefore recommended for human consumption.

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