



## **Oryctes owariensis Larvae as Good Alternative Protein Source: Nutritional and Functional Properties**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author EAD designed the study, wrote the protocol and interpreted the data. Authors BA, MDK and SD anchored the field study, gathered the initial data and performed preliminary data analysis. While authors EAD and PLK managed the literature searches and produced the initial draft. All authors read and approved the final manuscript*

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### **ABSTRACT**

In Côte d'Ivoire, the larvae of *Oryctes owariensis* are prized and widely consumed as alternative protein source. The present study was aimed at evaluating the nutritional and functional properties of the larva flour for further food products formulation. Fresh *O. owariensis* larvae were collected from dead trunks of raffia palms at Saïoua (6°29'31" N and 6°15'32" W) in Côte d'Ivoire. The fresh larvae were dried and ground to obtain the crude flour. Chemical composition and functional properties were investigated using standard methods. All results were statistically analysed. The chemical composition revealed that it contains crude protein about 50.64%, crude fat 18.88%,

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moisture 8.41%, ash 7.72%, total carbohydrate 14.33% and energy value of 417.43 kcal. The mineral evaluation in mg/100 g dry weight showed K (1610.50) > Mg (369.75) > P (142.17) > Na (102.25) > Ca (54.51) > Fe (20.26) > Zn (7.89) > Cu (0.91). These results suggest that dried *O. owariensis* larvae can be used in human diet to prevent undernourishment due to protein and some mineral deficiencies. Furthermore, the high in vitro protein digestibility (82.87%) could be an advantage for eating this insect larva. The functional properties showed that it has high water and oil absorption capacity with values of 220.33 and 265.90% respectively. This flour exhibited also good emulsifying and foaming properties making it suitable in formulation of foods such as sausages and bakery products. Dried *O. owariensis* larvae could be utilized as a new feed products of considerable protein, fat and mineral contents. The larva flour shows good functional characteristics for use in many food industries.

**Keywords:** Edible insects; flour; functional properties; nutritional properties; *Oryctes owariensis* larvae; proximate composition.

## 1. INTRODUCTION

Sustainably meeting global food demands is one of humanity's greatest challenges and has attracted considerable attention in the past few years [1]. There is general consensus on agriculture's positive contribution to food security through its role in increasing the availability of affordable food and the incomes of the poor. Within the context of sustainable diet, entomophagy has a significant role to play in assuring food security and improving the livelihood of many peoples in world [2,3].

Eating of insects (entomophagy) has a long history as part of human diets and a large number of insect species are consumed in many parts of the world [4]. For approximately 2.5 billion people, mainly in Africa, Asia and Latin America, eating insects is part of their common diets in a similar way as eating meat or fish.

Insects constitute high quality food for humans and animals and more than 1600 species of insects are eaten worldwide [5,3]. There is a great potential and growing global interest for utilization of insects as food resource to complement the diets of continuously growing populations. Indeed, insects are a highly nutritious and healthy food source with high fat, protein (20 to 70% of dry weight), vitamin, fibre and mineral content [6-8]. The nutritional value of edible insects is function of the wide range of edible insect species. Even within the same group of species, nutritional value may differ depending on the metamorphic stage of the insect, the habitat in which it lives, and its diet. Many edible insects exhibited similar composition of unsaturated fatty acids (omega-3 and 6), protein, vitamin and mineral to that of fish and meat [9,3].

In many parts of Africa, entomophagy was practised as a traditional heritage [10]. The highest diversity of edible insect species was found in the orders Lepidoptera, Orthoptera and Coleoptera. Commonalities were observed across majority of the insects consumed across western, eastern and southern Africa [3]. In humid West African region, where oil and raffia palm tree grow, *Oryctes owariensis* larva (also called grub) is a Coleoptera which is commonly hunted in dead trunks of palms by the villagers. In Côte d'Ivoire, the larva is either eaten raw, boiled, smoked or fried [11]; and may be consumed as part of a meal or as a complete meal [12].

The present study was aimed at evaluating the nutritional and functional properties of the larva flour for further food products formulation. It is well known that functional properties of foods are intrinsic physicochemical characteristics which affect the behaviour of protein in food systems during processing, manufacturing, storage and preparation [13].

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Sample

Fresh *O. owariensis* larvae were collected from dead trunks of raffia palms at Saïoua (6°29'31" N and 6°15'32" W) in Côte d'Ivoire. After collection, the larvae were placed in a cooler with ice to keep them fresh and then transported to the laboratory for flour preparation. Fresh larvae (1 kg) were cleaned using distilled water, drained and dried at 65°C in an oven for 72 h. Dried larvae were ground using a porcelain mortar to obtain the crude flour.

## 2.2 Chemical Composition

The dry matters contents were determined by drying in an oven at 105°C during 24 h to constant weight [14]. The crude protein contents were calculated from nitrogen contents (N×6.25) obtained using the Kjeldahl method by AOAC [14]. The crude fat content was determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent [14]. The total ash contents were determined by incinerating in a furnace at 550°C [14]. The carbohydrate contents were determined by deducting the mean values of other parameters that were determined from 100. Therefore % carbohydrate = 100- (% moisture + % crude protein + % crude fat + crude fiber + % ash). Energy value was calculated using standard calculations ([gram of crude protein × 4.0] + [gram of crude fat × 9.0] + [gram of carbohydrates × 4.0]). Minerals including calcium, magnesium, iron, zinc, copper, manganese, sodium and potassium were determined using an Atomic Absorption Spectrophotometer, AAS (Model 372, Perkin-Elmer, Beaconsfield, UK) by wet digestion while phosphorous level was determined using the phosphovanado molybdenate method [14].

## 2.3 Functional Properties

### 2.3.1 Water absorption capacity

The water absorption capacity (WAC) was evaluated according to Phillips et al. [15] method. Two grams (2 g) of flour was weighed into a centrifuge tube and 50 mL distilled water added. The content of the centrifuge tube was shaken for 30 min in a KS 10 agitator. The mixture was kept in a water-bath (37°C) for 30 min and centrifuged (Ditton LAB centrifuge, UK) at 5000 rpm for 15 min. The resulting sediment (M2) was weighed and then dried at 105°C to constant weight (M1). The WAC was then calculated as follows:

$$\text{WAC (\%)} = \frac{M2 - M1}{M2} \times 100$$

### 2.3.2 Oil absorption capacity

For the oil absorption capacity, the method of Beuchat [16] was used. One gram of flour sample was mixed with 10 mL of oil for 30 min in a mixer (Vari-whirl-mixing control set at fast speed). The sample was then allowed to stand at room temperature for 30 min. It was then

centrifuged at 5000 rpm for 30 min, using a spinner (Ditton LAB centrifuge, UK) and the volume of the supernatant noted in a 10 mL graduated cylinder. The density of the oil was determined too. The volume of oil absorbed was multiplied by the density of the oil to determine the weight of oil absorbed.

$$\text{OAC (\%)} = \frac{(V1 - V2) \times P}{W} \times 100$$

Where:

V1 = Initial volume of oil used

V2 = Volume remaining (not absorbed)

P = density of the oil used

W = Weight of sample

### 2.3.3 Foaming capacity and foam stability

The foaming capacity (FC) and stability (FS) of flour was studied according to the method of Coffman and Garcia [17]. Three (3) g of flour was transferred into clean, dry and graduated (50 mL) cylinders. The flour sample was gently levelled and the volumes noted. Distilled water (30 mL) was added to the sample; the cylinder was swirled and allowed to stand for 120 min while the change in volume was recorded every 15 min.

$$\text{FC (\%)} = \frac{V_t - V_0}{V_0} \times 100$$

$$\text{FS (\%)} = \frac{\text{FC}}{\text{FC}_0} \times 100$$

Where  $V_0$  is the original volume of sample (mL),  $V_t$  is the total volume after different times (mL) and  $\text{FC}_0$  is the foam capacity (FC) at 0 min.

### 2.3.4 Emulsifying activity and emulsion stability

The method of Beuchat [16] was used. Two gram of flour sample and 50 mL distilled water were blended at room temperature for 30 sec in Philips blender at 1600 rpm. After complete dispersion, vegetable oil (Gino) was added continuously in 10 mL portions from a burette. Blending continued until the emulsion breakpoint (where a separation into two layers/phases) was observed. The emulsion capacity was expressed

as mL of oil emulsified per gram of sample and was expressed as %:

$$EC (\%) = \frac{V_F \times 100}{V \times W}$$

Where:

W = weight of sample  
 $V_E$  = Volume of emulsion layer E  
 V = Total volume of mixture

For the emulsion stability, the emulsion so prepared was then allowed to stand in a 250 mL graduated cylinder over time and the volume of the emulsion layer read. The stability was measured in terms of the amount of oil that was retained in the emulsion layer and given by:

$$ES (\%) = \frac{V_{ET} \times 100}{V}$$

$V_{ET}$  = Emulsion volume at Time (T)  
 V = total volume of the mixture

### **2.3.5 In vitro protein digestibility**

The in vitro protein digestibility of the sample was measured according to the method of Sauders et al. [18]. About 250 mg of the sample was suspended in 15 mL of 0.1N HCl containing 1.5 mg pepsin (1: 10,000) in a 100 mL conical flask. The mixture was incubated at 37°C for 3 hours. The mixture was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 mL of 0.2M phosphate buffer (pH 8) containing 0.005 M sodium azide. The mixture was incubated at 37°C for 24 hours. About 10 ml of 10% trichloroacetic acid (TCA) were added to the mixture to stop the reaction. The mixture was then centrifuged at 5000 rpm for 5 min. About 5 mL of the aliquots from the supernatant were pipetted and analyzed for nitrogen content [14]. Protein digestibility was determined according to the equation.

Protein Digestibility % =

$$\frac{N \text{ in supernatant- enzyme N}}{N \text{ in Sample}} \times 100$$

### **2.4 Statistical Analysis**

All experiments in this study are reported as means of three replicate analyses. One-way analysis of variance (ANOVA) was carried out to compare the mean values of *O. owariensis* flour.

Differences in the mean values were determined using Duncan's multiple range tests (SAS, 1990).

## **3. RESULTS AND DISCUSSION**

### **3.1 Chemical Composition**

The chemical composition and energy value of *O. owariensis* investigated are shown in Table 1. The moisture content was 8.41%. This value was very lower than those of *Macrotermes bellicosus* (48.45%), *Oryctes rhinoceros* (16.7%) and *Rhyncophorus pheonicis* (11.3%) [19,20]. This low value suggests that dry *O. owariensis* larvae are not likely to be susceptible to microbial growth and subsequent spoilage.

The high ash content (7.72%) of *O. owariensis* indicates that these insect larvae are good sources of mineral elements. The ash content of the sample is of nutritional importance as a previous report indicated that when leaves are to be used as food for humans, they should contain about 3.0% ash [21]. The ash content reported in this study is higher than those of fourteen edible insects eaten in south-western Nigeria [22].

As far as concerned proteins, edible insects have been shown to have higher protein content, on a mass basis, than other animal and plant foods such as beef, chicken, fish, soybeans, and maize [23]. The crude protein content of dry *O. owariensis* larvae which was 50.64% is higher than values reported for others edible insects such as for *Cirina forda* larva (20.0%) [24], *Oryctes boas* (26%), *R. phoenicis* (28.42%), *Analeptes trifasciata* (20.1 %), *Macrotermes notalensis* (22.1%), *Anaphe recticulata* (23%) from Nigeria [22].

The carbohydrate content of the studied edible insect was relatively low, with value of 14.33%. Similar values which ranged from 7 to 20% have been reported in some insects in Nigeria [25]. Recent research has revealed that insects have considerable amounts of polysaccharides that can enhance the immunity function of the human body [26]. Furthermore, such diets lower the intake of calories, resulting in expected weight loss [27].

Fats are important nutritive elements in the human body. They are the main energy source, can reduce consumption of protein and help detoxification. They are also essential in diets as they increase the palatability of foods by

absorbing and retaining their flavours [28]. In this study, a crude fat content of 18.88% was obtained. Similar results have been reported in other African edible insects, with lipid contents ranging from 1.5 to 31.4% [22].

The energy value of 417.43 kcal obtained in this study is expected from a protein-rich sample. Edible insects have been shown to have higher protein content, on a mass basis, than other animal and plant foods such as beef, chicken, fish, soybeans, and maize [23]. Ramos-Elorduy et al. [29] reported the nutritional value of 78 species of edible insects in Mexico, with protein values ranging from 15-81%, and calorie content ranging from 293-762 kcal/100 g.

The in vitro protein digestibility of 82.87% recorded (Table 1) is much higher than reported values of 58.05% for *O. monoceros* larva [30] and 64% for some edible insects evaluated in Mexico [31]. Generally, insect proteins are known to be of good digestibility containing some essential amino acids in appreciable amounts and the limiting amino acids can easily be supplemented with plant based protein sources.

**Table 1. Chemical composition of dry *O. owariensis* larvae**

Parameters	Values (%)
Moisture	8.41±0.06
Dry matter	91.59±1.8
Crude proteins	50.64±1.1
Carbohydrates	14.33±0.23
Crude fats	18.88±1.3
Ash	7.72±0.04
Energy value (kcal/100g de MS)	417.43±2.6
In vitro protein digestibility	82.87±0.7

Values are mean ± standard deviation of three measurements (n = 3)

### 3.2 Mineral Element Composition

The mineral element composition of dry *O. owariensis* larvae as presented in Table 2 shows that potassium (1610.50 mg/ 100 g) was the most predominant mineral followed by magnesium (369.75 mg/ 100 g), phosphorus (142.17 mg/ 100 g), sodium (102.25 mg/ 100 g), calcium (54.51 mg/ 100 g) and iron (20.26 mg/ 100 g) while zinc, copper and manganese occurred in trace amounts. Consumption of 100 g dry larva would provide 253%, 100%, 92%, 71%, 34% and 342% of the Recommended Dietary Allowances of iron, copper, magnesium, zinc, potassium and manganese respectively

[32]. Hence, insects could be used in diet to prevent mineral deficiency diseases especially in developing countries where deficiency diseases such as anaemia are predominant among preschool children and pregnant women. This often results in morbidity in children and maternal death [33].

**Table 2. Mineral element composition of dry *O. owariensis* larvae**

Minerals	Values (mg/100 g)	Intake recommendation for 25-year-old males (mg per day)*
Sodium	102.25±0.63	1500
Potassium	1610.50±0.82	4700
Calcium	54.51±0.21	1000
Phosphorus	142.17±0.34	700
Magnesium	369.75±0.57	400
Iron	20.26±0.74	8
Manganese	7.88±0.16	2.3
Copper	0.91±0.01	0.9
Zinc	7.89±0.62	11

Values are mean ± standard deviation of three measurements (n = 3).

\*Dietary reference intakes (DRIs): recommended dietary allowances and adequate intakes, minerals, Food and Nutrition Board, Institute of Medicine, National Academies

Also, these results confirm that, edible insects are undeniably rich sources of iron and their inclusion in the daily diet could improve iron status and help prevent anaemia in developing countries. Indeed, in these countries, one in two pregnant women and about 40 percent of preschool children are believed to be anaemic. Health consequences include poor pregnancy outcomes, impaired physical and cognitive development, increased risk of morbidity in children and reduced work productivity in adults. Anaemia is a preventable deficiency but contributes to 20 percent of all maternal deaths [33].

### 3.3 Functional Properties

#### 3.3.1 Water and oil absorption capacity

As suggested by Barbut [34], Hydration or rehydration is the first and perhaps most critical step in imparting desirable functional properties to proteins in a food system. Interactions of water and oil with flours are very important in food transformation because of their effects on the

flavour and texture of foods. Intrinsic factors affecting water binding properties of food flours with relatively high protein contents include amino acid composition, protein conformation and surface polarity/hydrophobicity [34]. Water absorption capacity describes flour – water association ability under limited water supply. The results of water and oil absorption capacity of *O. owariensis* larvae flour are shown in Table 3. The studied flour had high water absorption capacity (WAC) of 220.33%. The dry *O. owariensis* larvae may have contained more hydrophilic constituents, which gave rise to higher water absorption capacity (WAC). The lower moisture content of these dry larvae also enhanced its WAC. Water absorption of flour is dependent mainly on the amount and nature of the hydrophilic constituents and to some extent on pH and nature of the protein [35]. The result of this study suggests that flour from *O. owariensis* larvae would be useful in foods such as bakery products which require hydration to improve handling characteristics [34].

The oil absorption capacity (OAC) was 265.90%. This value was found to be much higher than those reported for full fat and defatted flours from *Imbrasia oyemensis* larvae [36]. The higher OAC suggested the presence of apolar amino acids in *O. owariensis* flour [37]. Oil absorption capacity is attributed mainly to the physical entrapment of oils. It is an indication of the rate at which protein binds to fat in food formulations [13]. Akubor and Eze [38] shown that OAC is useful in formulation of foods such as sausages and bakery products [38] and this shows that the studied flour would be useful in this respect. Fat acts as a flavour retainer and increases the mouth feel of foods. Fat increases the leavening power of the baking powder in the batter and improves the texture of the baked product [38].

### **3.3.2 Emulsifying properties**

The emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domains. Food emulsions are thermodynamically unstable mixtures of immiscible liquids. The formation and stability of emulsion is very important in food systems such as salad dressing [39,40]. As shown in Table 3 the emulsion activity and stability were 29.97 and 104.84% respectively. The results obtained were much higher than those reported by Ekpo et al.

[41] for *Imbrasia belina* larvae flour. The capacity of proteins to enhance the formation and stabilization of emulsion is important for many applications in cakes, coffee whiteners, and frozen desserts. In these products varying emulsifying and stabilizing capacities are required because of different compositions and stresses to which these products are subjected [42].

**Table 3. Functional properties of *O. owariensis* larvae flour**

<b>Parameters</b>	<b>Values (%)</b>
Emulsifying activity	29.97±0.12
Emulsion stability	104.84±1.23
Water absorption capacity	220.33±1.5
Oil absorption capacity	265.90±1.31
Foaming capacity	17.87±0.16
Foam stability	12.36±0.03

\* Values are mean ± standard deviation of three measurements (n = 3)

### **3.3.3 Foaming capacity and foam stability**

The foaming capacity (FC) of a protein refers to the amount of interfacial area that can be created by the protein and foam stability (FS) refers to the ability of protein to stabilize against gravitational and mechanical stresses [43]. Foam formation and foam stability are a function of the type of protein, pH, processing methods, viscosity and surface tension. The studied flour showed foaming capacity of 17.87% and foaming stability of 12.36% (Table 3). These values are greater than those reported for some insect flours by some workers: *I. belina*, 11.8% [41] and *R. phoenicis*, 10% [44]. Akubor and Chukwu [45] reported that foams are used to improve the texture, consistency and appearance of foods. Graham and Phillips [46] linked good foamability with flexible protein molecules, which reduces surface tension. Low foamability on the other hand can be related to highly ordered globular proteins, which resists surface denaturation. Better foamability suggest that proteins are the ability to (i) adsorb rapidly at air water interface during bubbling, (ii) undergo rapid conformational change and rearrangement at the interface. For the stability of the foam, it is necessary that proteins form a cohesive viscoelastic film via intermolecular interactions [43].

#### 4. CONCLUSION

To sum up this report, it may be concluded that dried *O. owariensis* larva is an excellent source of nutrients mainly consisting of protein, fats and minerals. This edible insect was found to be very rich in minerals especially iron, copper, magnesium, zinc, potassium and manganese. Therefore, it can be used in diet to prevent undernourishment due to protein and some mineral deficiencies. Furthermore, the high in vitro protein digestibility could be an advantage in the consumption of *O. owariensis* larva. Consequently, the insect larva could be used for formulation of new food products with considerable protein value. As regards functional properties, flour from dried *O. owariensis* larva showed high water and oil absorption capacity indicating its usefulness in formulation of foods such as sausages and bakery products. Also, this flour exhibited good emulsifying and foaming properties making it suitable for nutritional and industrial applications.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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