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Functional and Nutritional Properties of Protein Concentrates and Isolates from Hercules Beetle Larvae (*Dynestes hercules*)

N.E. Njoku[1], E.C. Chikelu[2], A.E. Uzoukwu[1], I.M. Agunwah[1], C.N. Eluchie[1], S.O. Alagbaoso[1], E.J. Anaeke[1], A.F. Ofoedum[1], V.U. Nneji-Ibekwe[1]

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ABSTRACT

Background: This study focused on the proximate composition, functional qualities and mineral composition of hercules beetle larva (HBL) flour (whole, concentrate and isolate).

Methods: Proximate composition and functional properties were analysed using standard methods.

Result: The result of proximate analysis revealed that the HBL protein concentrate recorded the highest in protein (51.16%), carbohydrate (29.87%), crude fiber (4.73%) and ash (4.95%). HBL protein isolate had the highest in moisture (9.53%) while whole HBL gave the highest value in fat (25.76%); proving its potential adaptability for different ranges of culinary applications and offering improved flavor, texture and stability. The results for functional properties revealed that HBL protein isolate had the highest WAC (3.56 g/g) and OAC (1.95 g/g), highest foaming capacity (37.40%), foaming stability (64.80%), emulsion capacity (97.30 g/g), emulsion stability (95.30 g/g) and water solubility index (44.73 g/g). The results for protein solubility revealed that HBL protein concentrate had the highest at pH1 (83.33%), HBL protein isolate was the highest at pH5 (58.30%), whole HBL recorded the highest value at pH7 (48.56%) and HBL protein concentrate scored the highest at pH10 (88.43%). The results for mineral composition revealed that the potassium was higher in the whole HBL (94.12 mg/100 g).

Key words: Edible insects, Functional, Hercules beetle, Mineral, Protein isolate, Proximate composition.

INTRODUCTION

Edible insects are very nutritious and are a suitable source of the essential nutrients required in human diet (Ayieko *et al.*, 2012). Edible insects comprise of nutrients such as protein, fat, vitamins and minerals (Rumpold and Schlüter, 2013). They also meet amino acid requirements of humans and are high in unsaturated fatty acids (Rumpold and Schlüter, 2013). There are different types of edible insects. They include beetles (*Coleoptera*); caterpillars (*Lepidoptera*); bees, wasps and ants *(Hymenoptera)*; and grasshoppers, locusts and crickets (*Orthoptera*) representing about 31%, 18%, 14 % and 13% of the insect population (Van Huis, 2013). Besides termites and grasshoppers, Hercules beetle larvae rank highest amongst the most consumed species of insects in the world (Chung and Yu, 2010).

Hercules beetle larvae *(Dynestes hercules)* is a species of *rhinoceros* beetle of the order *Coleoptera* and family, *scarabaeidae.* There are many species of hercules beetle larvae such as *D. hercules lichyi, D. hercules morishimai or D. hercules reidi.* (Mbah and Elekima, 2007). However, beetle larvae are also highly perishable and susceptible to microbial spoilage, limiting their shelf life and marketability. Larvae are a right source of fatty acids compared to insects at other development stages (Tang *et al.,* 2020).

Asides their sensory appeal, hercules beetle larvae are consumed due to their nutritional merits. Their proteins and fat content essentially suggest certain behavioral or functional attributes that can be harnessed. These attributes are the essential physiochemical properties of foods that reflect the complex interactions between the structures, molecular conformation, compositions and physicochemical properties of food components with the nature of the environment and conditions in which these are measured and associated (Suresh and Samsher, 2013).

Protein concentrate is the least processed type of protein powder, although going through less processing is usually viewed as a good thing or synonymous with being healthier (Maria, 2020). Protein isolate, on the other hand, is a type of protein powder that has undergone more processing than a concentrate. Processing protein isolate involves separating and collecting the purest protein fractions (Maria, 2020). Isolates are the most refined type

[1]Federal University of Tech. Owerri, Imo State, Nigeria.
[2]School of Chemistry and Material Science, Nanjing University of Information Science and Technology, China.

Corresponding Author: A.F. Ofoedum, Federal University of Tech. Owerri, Imo State, Nigeria.
Email: ofoedum.arizona.edu@gmail.com
ORCIDS: 0000-0001-9935-5853

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of protein product with the highest concentration of protein; although they lack dietary fiber (Garba and Kaur, 2014, Ofoedum *et al.,* 2025). Despite the potential benefits of using hercules beetle larvae as a protein source, there is still scarce information on the isolates and concentrates produced from hercules beetle larvae. Consequently, information on the functional behavior of its isolates and concentrates is also scarce. This work is aimed to investigate the functional and nutritional properties of protein concentrate and isolate from hercules beetle larvae (*Dynestes hercules*).

MATERIALS AND METHODS

Raw material procurement

Two kilogram of live hercules beetle larva were purchased from a commercial dealer in Bayelsa State, Nigeria and transported in a bucket full with sugar cane. The fully developed Hercules beetle larva is shown in Plate 1.

Place and duration of research

The analyses were carried out in National Root and Crop Research Institute Umudike, Abia State and Department of Food Sci. and Tech., Federal University of Technology, Owerri, Nigeria and it took a period of 11 months from May, 2024 to January, 2025.

Preparation of whole larva flour

Two kilogram of live larva of hercules beetle were properly washed with water. They were drained and dried in a moisture oven Model (DHG-9101-05A) at 50°C for 42 hrs as shown in Plate 2. The dried larva was cooled and milled using an attrition mill (Model 368 Corona) to obtain the oily coarse palm whole weevil larva flour as shown in Plate 3.

Preparation of defatted hercules beetle larva concentrates and isolates

Five hundred grams of whole larvae flour were properly defatted by using N-hexane (1:10 w/w) for 3 hours to obtain the defatted hercules beetle larva protein concentrate. The isolate was prepared by suspending 500 grams of live larva of hercules beetle flour in hydrochloric acid to leach out the protein isolates. Notably, the pH level of this mixture stood at 5.8 prior to the subsequent step. Sodium hydroxide was introduced, bringing the pH level to a balanced 7 to obtain the protein isolate. The whole Hercules beetle larva flour concentrates and the flour isolates were shown in Plate 3, 4 and 5 respectively.

Proximate analysis of the samples

The standard method of AOAC (2010) was adopted to evaluate the proximate compositions of the whole, protein concentrate and protein isolate samples.

Functional properties of palm weevil larva

The different functional properties which include the Water and oil absorption capacity, water solubility, foam capacity and stability, emulsion capacity and protein solubility were determined by the methods of Cheng *et al*. (2016); Sathe and Salunkhe (1981) and Sathe *et al.* (2002).

Determination of mineral composition

Mineral composition of potassium, calcium, sodium, iron and zinc was determined using a PerkinElmer Model 290 Atomic Absorption Spectrometer using standard methods of AOAC (2010).



Plate 1: Fully developed Hercules beetle larva.



Plate 2: Hercules beetle larva undergoing oven drying process.



Plate 3: Whole Hercules beetle larva flour.

Statistical analysis

The data generated from the analysis were subjected to analysis of variance using SPSS version 2023 to separate the mean, standard deviation and significance differences at 95% confidence level.

RESULTS AND DISCUSSION

Proximate compositions of the edible insect flours

The results of the proximate composition of the hercules beetle larva (HBL) flours, were provided on Table 1. The moisture, ash, protein, crude fats, crude fibre and carbohydrates contents were all measured.



Plate 4: Hercules beetle larva flour concentrate.



Plate 5: Hercules beetle larva flour isolate.

The moisture contents of the samples ranged from (7.16%) for HBL protein concentrate to (9.53%) for the HBL protein isolate and (7.25%) for whole HBL. HBL protein isolate gave the highest value for moisture with the value (9.53%). The relatively low moisture content of samples showed the HBL protein concentrate will have longer shelf life due to its low moisture content. Also, the relatively low moisture contents of the samples will inhibit the development of contaminating micro-organisms whose growth and activities are favoured by the level of moisture (Ugwu, 2014).

The results of the crude proteins indicated that the HBL protein concentrate has the highest (51.16%) followed by the HBL protein isolate at (43.09%) and whole HBL at (38.52%).

Other edible insect larva has been found to have varying protein contents. For example, *Huhu* grubs (26.2-30.5%) (Kalve *et al.,* 2022). The result showed that consumption of the samples will help to furnish adequate number of amino acids to the body (Obasi, 2000; Ofoedu, *et al.,* 2021).

Regarding the ash contents in the samples whole flour from HBL, HBL protein isolate and HBL protein concentrate ranged from (4.15%) for protein isolate to (4.95%) for the concentrate, ash content shows the level of minerals present in a particular food sample. The ash contents of the samples was significantly different (≤0.05). The concentrate recorded the highest ash contents when compared among the samples. Also, the ash content value obtained in this work is higher when compared to the range (0.64% to 0.92%) reported by (Adedoke, 2003).

The crude fibre was relatively higher in HBL protein concentrate with a mean score of 4.73% than the whole (4.61%) and HBL protein isolate (4.02%). Also, the crude fibre content of the samples differed significantly (p≤0.05). Crude fibre contents of the samples is of good value. It helps in the removal of waste products from the body, thereby preventing constipation and many health disorders.

Investigation on the crude fats showed that whole HBL recorded more fat contents (25.76%) compared to HBL protein concentrate (2.12%) and HBL protein isolate (11.03%). Fat content of the Hercules samples ranged from (2.12%) for the sample concentrate to (25.76%) for the (whole flour). The fat content of the samples were significantly different (p≤0.05). The sample whole flour had the highest fats value which could be attributed to the non-

Table 1: Proximate Compositions of whole, concentrate and isolate of hercules beetle larva.

Samples	Moisture (%)	Crude protein (%)	Crude fibre (%)	Fat (%)	Ash (%)	Carbohydrate (%)
WF HBL	$7.25^{b}\pm0.04$	$38.52^{c}\pm0.02$	$4.61^{b}\pm0.01$	$25.76^{a}\pm0.01$	$4.72^{b}\pm0.05$	$19.12^{c}\pm0.01$
HBL protein isolate	$9.53^{a}\pm0.25$	$43.09^{b}\pm0.01$	$4.02^{c}\pm0.02$	$11.03^{b}\pm0.02$	$4.15^{c}\pm0.02$	$28.13^{b}\pm0.01$
HBL protein concentrate	$7.16^{c}\pm0.35$	$51.16^{a}\pm0.01$	$4.73^{a}\pm0.02$	$2.12^{c}\pm0.01$	$4.95^{a}\pm0.01$	$29.87^{a}\pm0.02$
LSD	0.0279	0.01122	0.01247	0.01277	0.01122	0.01656

Means having different superscripts along the same columns are significantly different (p≤0.05).

Key; HBL = Hercules beetle larva flour.

removal of fat from the sample. Fat content of any food sample aids in the improvement of mouth feel and flavor.

The carbohydrates contents of the larva flour was greater in the HBL protein concentrate (29.87%) and HBL protein isolate (28.13 %) compared to whole HBL (19.12%). This result implies that the HBL protein concentrate is better sources of energy than the other samples and can be used in various food formulations to provide energy and improve texture.

Functional properties of whole flour, protein isolate and concentrate of hercules beetle larva

The functional properties of different hercules beetle larva flours were provided in the Table 2. The water and oil absorption capacity WAC/OAC, foam stability and capacity, emulsion stability and capacity, protein solubility and water solubility index were all measured.

The product's texture and flavor are directly influenced by its capacity to absorb and hold on to water and oil. High protein contents, protein structures, amino acids composition and surface hydrophobicities are some of the inherent factors affecting water-holding capacity (Vanqa *et al.,* 2022). It is true that flours with high water absorption capacities contain higher amounts of hydrophilic ingredients such polysaccharides and proteins that are high in polar amino acid content.

The WAC recorded in the result of HBL protein isolate (3.56 g/g) was found to be higher than the HBL protein concentrate (3.07 g/g) and lastly whole HBL (2.78 g/g). This high-water absorption capacity of the HBL protein isolate indicates the ability of a sample to absorb enough water during its preparation.

The OAC of the result indicated that the PWL protein isolate has the highest (1.95 g/g) followed by the PWL protein concentrate at (1.65 g/g) and whole PWL (1.41 g/g). The high oil absorption capacity of the samples indicates the ability of the sample to absorb enough oil. Oil absorption capacity is of great importance since fat as a flavor retainer and increases the mouth feel of foods (Aremu *et al.,* 2017).

The foaming capacity of the HBL protein isolate (37.40% at 30 min) was found to be highest than the whole HBL (8.11% at 30 min) and HBL protein concentrate (6.520% at 30 min). The results obtained in this study for all samples are lower compared to what is reported for other edible insects such as *A. mellifera* larvae (43.30-45% at 60 min) and that of *S. gregaria* (grasshopper) protein extract (90% at 60 min) (Mishyna *et al.,* 2019). This investigation shows that the whole, concentrate and HBL protein isolate may not be acceptable for use in food systems that demand a high percentage of foam, such as cake and ice cream. The foam capacity of the samples indicates the ability of the sample to absorb enough oil; thus it measures the amount of interfacial area that can be created by whipping (Aremu *et al.,* 2017).

The foaming capacity was observed in the samples after 30 minutes of producing the foam. The whole HBL was at (44.10% at 30 min), HBL protein concentrate at (46.80% at 30 min) and HBL protein isolate at (64.80% at 30 min). This result was lower than that reported for sago grub protein extract (88.8% at 120 min) (Kalve *et al.,* 2022) and *S. gregaria* protein extract (74.1% at 120 min) (Mishyna *et al.,* 2019). The result showed that heating a globular protein in the HBL samples to achieve partial denaturation will enhance foaming properties. HBL protein isolate had the highest foam stability. Foam stability percentage depends on source of the protein isolate.

The emulsion capacity of the HBL protein isolate (97.30 g/g at 30 min) found to be highest than the whole HBL (94.60 g/g at 30 min) and HBL protein concentrate (91.50 g/g at 30 min). Enhancing texture, shelf life and ingredient distribution in foods and cosmetics, the HBL protein isolate has the maximum emulsion capacity and will produce stable emulsions. The higher emulsion stability of HBL protein isolate (95.30 g/g at 30 min) compared to whole HBL (82.1 g/g) and HBL protein concentrate (65.2 g/g) indicates its superior ability to maintain emulsions over time, providing improved texture, shelf life and ingredient distribution in food, cosmetic and industrial applications. The emulsion capacity of the samples indicates the ability of sufficiently soluble proteins to migrate to the water or oil interface and environmental conditions, like pH and ionic strength which influences the emulsifying capacity of proteins by affecting their solubility and hence their conformation and functional properties.

The water solubility index results show that HBL protein isolate (44.73 g/g) has the highest solubility, followed by whole HBL (42.61 g/g) and HBL protein concentrate (40.72 g/g). This suggests that HBL protein isolate is easily dissolved in water, boost the integration of active ingredients in drinks, powdered supplements and quick mixes, as well as improve hydration and dispersibility.

Table 2: Functional properties of whole flour, protein isolate and concentrate of hercules beetle larva.

Samples	Bulk density (g/ml)	WAC (g/g)	OAC (g/g)	Foam capacity (%)	Foam stability (%)	Emulsion capacity (%)	Emulsion stability (%)	Water solubility index (%)
Whole HBL	$0.406^{a}\pm0.01$	$2.78^{c}\pm0.05$	$1.41^{c}\pm0.01$	$8.11^{b}\pm0.11$	$44.1^{c}\pm0.32$	$94.6^{b}\pm0.01$	$82.1^{b}\pm0.02$	$42.61^{b}\pm0.02$
Isolate HBL	$0.36^{b}\pm0.00$	$3.56^{a}\pm0.02$	$1.95^{a}\pm0.05$	$37.4^{a}\pm0.01$	$64.8^{a}\pm0.02$	$97.3^{a}\pm0.11$	$95.3^{a}\pm0.01$	$44.73^{a}\pm0.02$
Conent HBL	$0.31^{c}\pm0.05$	$3.07^{b}\pm0.01$	$1.65^{b}\pm0.05$	$6.52^{c}\pm0.01$	$46.8^{b}\pm0.30$	$91.5^{c}\pm0.11$	$65.2^{c}\pm0.02$	$40.72^{c}\pm0.02$
LSD	0.0047	0.0128	0.0061	0.01305	0.02194	0.01414	0.01414	0.01678

The "abc" means with different superscripts along the same column are significantly different ($p \leq 0.05$).

Table 3: Protein solubility index of whole flour, protein isolate and concentrate of Hercules beetle larva.

Samples	pH_1	pH_5	pH_7	pH_{10}
Whole HBL	$70.43^{c}\pm0.30$	$51.34^{c}\pm0.32$	$48.56^{a}\pm0.15$	$82.40^{b}\pm0.36$
Isolate HBL	$78.46^{b}\pm0.37$	$58.30^{a}\pm0.10$	$42.62^{c}\pm0.36$	$80.30^{c}\pm0.20$
Concentrate HBL	$83.33^{a}\pm0.21$	$55.40^{b}\pm0.17$	$47.36^{b}\pm0.37$	$88.43^{a}\pm0.21$
LSD	0.2494	0.18076	0.2567	0.2177

The “abc” means having different superscripts along the same columns differ significantly ($p\leq0.05$).

Table 4: Mineral composition of Hercules beetle larva (mg/100 g).

Samples	Ca	K	Na	Fe	Zn
WF	49.64	94.12	42.06	3.16	4.75

Keys: HBL= Hercules beetle larva; LSD = Least significant difference.

Protein solubility index

The results of the protein solubility index of Hercules beetle larva were provided in the Table 3. The solubility index of the samples was carried out at pH1, pH5, pH7 and pH10. Protein solubility index at pH1 is defined as the concentration of protein in saturated solution that is in equilibrium with a solid phase, either in crystalline or amorphous, under a given set of conditions. Protein solubility index at pH1 ranged from 70.43% for the whole flour, 78.46% for the protein isolate and 83.33% for the concentrate. Protein solubility index of the samples were significantly different ($p\leq0.05$).

Protein solubility index at pH7 is at neutral, 48.56% for the whole flour, 42.62% for the protein isolate and 47.36% for the concentrate. Protein solubility index of the samples at pH7 were significantly different ($p\leq0.05$). The protein solubility index of the samples at pH7 shows the samples are low solubility index. Protein solubility index at pH10 ranged from 51.34% for the whole HBL, 58.30% for the HBL protein isolate and 55.40% for the HBL protein concentrate. Protein solubility index of the samples were significantly different ($p\leq0.05$). According to Ofoedum *et al* (2023), protein solubility is important during food production because it defines the type of food that can be produced solid or liquid, the type of phase that can be stabilized (oil, air) and the type of processing operation needed.

Mineral composition of the edible insect flours

The results of mineral compositions of whole HBL are provided in the Table 4. The mineral evaluated were calcium, potassium, sodium, iron and zinc. Mineral contents of Hercules beetle whole flour revealed that its calcium contents was (49.64 mg/100 g), potassium content was (94.12 mg/100 g), sodium content was (42.06 mg/100 g), iron content was (3.16 mg/100 g) and zinc content was (4.75 mg/100 g). The potassium was higher in the whole HBL (94.12 mg/100 g).

Result revealed calcium ranging at (49.64 mg/100 g) for whole HBL. The calcium content compares well with that reported by Koffi *et al.* (2017) on *R. Phoenicis* larva (225.66 mg/100 g. The composition of potassium in whole HBL was (94.12 mg/100 g), these results were compared with the (92 mg/100 g) in *R. Phoenicis* larva (Koffi *et al.,* 2017; Olawuni *et al.,* 2023; Odimegwu *et al.,* 2024). The high potassium contents of hercules beetle larva whole flour sample may not be attributed to metabolic changes in the larva during sample preparation.

The sodium contents of the whole HBL was (42.06 mg/100 g). The value was greater than the (30 mg/100 g) observed in the larva of *Anaphe veneta* (Meyer-Rochow *et al.*, 2021).

Iron composition in whole HBL was (3.16 mg/100g). The iron content is also comparable to the (12.69 mg/100g) obtained for *R. Phoenicis* larva (Koffi *et al.*, 2017).

The composition of zinc in whole HBL was (4.75 mg/100 g). The mean value of zinc from the hercules beetle larva whole flour samples were greater than values reported by Koffi *et al.* (2017) on *R. Phoenicis* larva (2.68 mg/100 g).

CONCLUSION AND RECOMMENDATION

The potential of Hercules beetle larvae as flexible protein sources by effectively producing whole flour, isolates and concentrates from them. To meet the varied nutritional needs and consumer preferences, the food industry can employ these distinct types of protein in a variety of ways. The comprehensive investigation has illuminated their proficiency in several procedures such as proximate, functional and mineral properties. Additionally, the study has evaluated the functional characteristics of isolates, concentrates and complete proteins from hercules beetle larvae. By highlighting the distinct benefits and constraints of every protein form, this comparative analysis has made it possible to make well-informed judgments about how to use them in various food products including baked goods, baby foods, drinks and protein powders or milkshakes. Its conversion into flour would also increase the shelf life and make packing and delivery simpler, hence, the recommendation.

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Informed consent

All animal procedures for this experiment were approved by the committee of Experimental Animal Care and Handling techniques were approved by the University authority.

Conflict of interest

No conflicts of interest exist.

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