NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 54 Number 2, December 2023 Pg. 202-207
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Nutritional and Antinutritional Composition of Defatted Emperor Moth (*Cirina forda*) Larvae meal: a nutritional food source rich in amino acids, fatty acids, and minerals

***[1]Edah, B. and [2]Owolabi, O.D.**

[1]Department of Biotechnology/ Fish Nutrition,
Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos
[2]Department of Zoology, University of Ilorin, P.M.B. 1515, Ilorin Nigeria
Corresponding author's email: bernardnda@yahoo.com

Abstract
This study evaluated the nutritional and antinutritional composition of defatted Emperor Moth (*Cirina forda*) larvae meal as well as its functional properties. Freshly harvested larvae were blanched in warm water and oven-dried at 35°C for 72 hours, before milling to a fine powder, and analyzed for their crude fat and fatty acid profile, crude protein and amino acid profile, 9 minerals elements and anti-nutritional contents. Results revealed percentage proximate composition values of *C. forda* before defatting and after defatting as 56.26 ± 0.2 and 65.34 ± 0.02 for crude protein, 17.18 ± 0.15 and 8.69 ± 0.02 for crude fat, 6.16 ± 0.11 and 5.73 ± 0.01 for ash, 9.06 ± 0.03 and 8.42 ± 0.02 for crude fibre and 3.13 ± 0.02 and 4.41 ± 0.00 for carbohydrate respectively. Good levels of mineral contents including, calcium, sodium, magnesium, iron, potassium, manganese, phosphorus, copper and zinc are present in adequate proportions indicating that they are a good source of micro and macro-mineral nutrients. The larvae are also a good source of essential and non-essential amino acids as well as an excellent profile of fatty acids. Antinutritional values of *C. forda* including tannin (average, 281.08 ± 0.04 mg/100g), phytic acid (average, 0.438 ± 0.01 mg/100g) and oxalate acid (average, 35.5 ± 0.07 mg/100g) also fell within tolerable levels, which is a non-toxic level. It was concluded in this research that, *C. forda* larvae contain a good amount of crude protein and a higher amount of it when defatted. They are also an excellent source of macro and micronutrients which can be exploited as an alternative protein source needed in the aquaculture, poultry or livestock feed industries.
Keywords: Emperor moth, defatted, amino acid, and tannin

Introduction

Globally, edible insects have most often been used as foods across different populations and cultures for many years (Papastavropoulou *et al.,* 2022). According to Jideani and Netshiheni, (2017), they form part of the regular food of over two billion people globally, play a vital role in human nutrition, and are also reared for profitable purposes given their high nutrient quality. They offer a host of ecological benefits critical for human survival. Over 1,900 species of insects have been reported as foods (Tiencheu 2017). Flies (Diptera, 2%), termites (Isoptera, 3%), dragonflies (Odonata, 3%), cicadas, leaf hoppers, plant-hoppers, scale insects and true bugs (Hemiptera, 10%), grasshoppers, locusts and crickets (Orthoptera, 13%), bees, wasps and ants (Hymenoptera, 14%), caterpillars (Lepidoptera, 18%), beetles (Coleoptera, 31%), and others (5%) are the most frequently consumed forms of insects across the world (Thakur *et al.,* 2018). Some edible insects have also been reported to contain up to 77% crude protein with a considerable amount of amino acids, fatty acids, minerals and vitamins (Kourimska *et al.,* 2016). The pallid emperor moth, *Cirina forda* is an insect pest of *Vitellaria paradoxa* formerly called *Butyrospernum paradoxum* – the shea-butter tree (Oriolowo *et al.,* 2023). The leaves of the shea tree are what the larvae feed on. An English entomologist named John Obadiah Westwood in 1849 was the first to describe the species (N'Djolosse *et al.,* 2012). *Cirina forda* larvae are widely consumed among rural dwellers in many West African countries however, not much is known about the effect of defatting processes on its nutritional composition. Thus, this research aimed to determine the nutritional and antinutritional composition of defatted *Cirina forda* larvae meal.

Materials and Methods

Purchase of Fresh Emperor Moth (Cirina forda) larvae

Fresh samples of emperor moth larvae (EML) were purchased from the Bida central market of Niger State, Nigeria. Bida Central Market is famous for its display and sales of local farm produce supplied to the market by village dwellers who travel from remote interiors to sell their farm produce. Purchased larvae were stored in a clean ice-chest cooler and transported to the fish technology laboratory section of the Nigerian Institute for Oceanography and Marine Research (NIOMR) for further analysis.

Processing of full-fat Emperor Moth Larvae (EML) into Defatted Emperor Moth Larvae meal

Drying and Defattening of emperor moth larvae

Fresh emperor moth larvae were rinsed thoroughly with clean water, blanched and then oven-dried at 35°C for three days (72 hours) using a laboratory dryer (Germany FP: 240 Model). Dried emperor moth larvae (Plate 1) were subjected to a Defattening process using an automatic oil extractor machine (Model: ZF – 868 Automatic Oil Press Machine). **The oil extraction method involved feeding dry** emperor moth larvae **samples into a barrel through the hopper feeder situated at the top of the machine. In the barrel, the dry insect enters an automatic horizontal screw-press barrel where oil is squeezed out leaving a dry extrudate which is collected in a clean tray at the end of the barrel. The extrudates (coarse** emperor moth larvae **meal) were then milled** using a laboratory milling machine (Model: HK - 860) and passed through a 0.05 mm aperture sieve.

Proximate composition of defatted emperor moth larvae meal

According to AOAC (2000) at the Central Laboratory of the Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria, proximate composition was determined in triplicates. Crude protein was determined by AOAC method 984.13, crude fat by AOAC method 920.39 and crude ash by AOAC method 942.05. The crude fibre was determined according to ISO 6865.2000 (ISO, 17025/2005), while carbohydrate content was determined by the difference in Table 1.

Mineral composition of defatted emperor moth larvae meal

Mineral composition after wet digestion was determined using a flame Atomic Absorption Spectrophotometer AAS (Spectr AA 220, Varian Inc., Australia). Calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe) and copper (Cu) were determined by flame AAS. Sodium and potassium were measured by flame photometer and phosphorus by UV-Vis spectrophotometer. Lanthanum was used to compensate for ionization interferences in the analysis of Ca and Mg.

Fatty acid profile of defatted emperor moth larvae meal

EML samples were analyzed for their various fatty acids at the Central Laboratory of the Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria. Fatty acid methyl esters were extracted from EML samples using the Bligh and Dye method as described by AOAC (2000). Fatty acid methyl esters (FAMEs) were quantitatively measured by gas chromatography, model 8700 (Perkin–Elmer Ltd., Buckinghamshire, England). Fitted with non-bonded bi-scynopropyl siloxane stationary phase, polar capillary column Rt-2560 (100m x 0.25mm) 0.2 µm film thickness (Supelco, PA, USA) and an FID. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. The initial oven temperature was 150°C at the rate of 4 min which was raised to 190°C at a rate of 2 C/min and further to 220°C held for 7 min. The injector and detector temperature were set at 260°C and 270°C, respectively. A sample volume of 1.0 µL was injected. All quantifications were done by a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin–Elmer) as reported earlier by Talpur *et al*. (2008).

Hydrolysis and Amino Acid Analysis by UHPLC-DAD

The amino acid content of defatted dry EML meal was conducted using the Ultra high-performance liquid-chromatography-diode-array (UHPLC-DAD) at the Xell-AG Laboratory, Germany. 6 M HCl was given to the dry samples and used for hydrolysis at 100°C for 24 h. Then, samples were dried *in vacuo* over NaOH. Samples were resolved and used for amino acid measurements. The depicted results were obtained by UHPLC-DAD on an Agilent 1290 system after the derivatization of amino groups. Two different System Suitability Tests (SSTs) were measured along with the samples to verify the calibration. Every sample was measured as a duplicate. Exactly 10 mg of each sample was used for hydrolysis and taken up in 1 ml buffer.

Anti-Nutritional Factor Determination

The tannin content was determined by extracting EML samples with a mixture of acetone and acetic acid for 5 h, measuring their absorbance, and comparing the absorbance of the extracts with the absorbance of standard solutions of tannic acid at 500 nm on a Spectronic 20. Oxalate content was determined through the extraction of the samples with water for about 3 hours and standard solutions of oxalic acid were prepared and read on a spectrophotometer (Spectronic 20) at 420 nm. The absorbance of the samples was also read and the amount of oxalate was estimated. Phytic acid was determined according to Sudarmadji *et al.,* (1977) by titrating the aqueous solution of the sample with ferric chloride solution; the trypsin inhibitory activity was determined on casein and the sample absorbance compared with the absorbance of trypsin standard solutions read at 280 nm. All analytic determinations were carried out in triplicate.

Statistical Analysis

All data were measured in triplicates. Microsoft Excel version 2010 and SPSS version.20.0 software was used to analyze the collected data. Analysis of variance with a Type I error rate of 0.05 was performed to identify the significant differences between treatments. Post-hoc LSD tests were deployed to determine where the specific differences occurred.

Results and Discussion

Results

The results of the processing of EML samples into Defatted insect meal, their proximate composition, mineral composition, extruder calibration and extrudate physical properties are presented in; Figure 1 and Table 1, 2, and 3 respectively. After the Defattening process using the automatic oil extractor machine (Model: ZF – 868**)** fat contents in EML meal were reduced by 60% of the initial value.

Mineral Composition and Anti-oxidant Content of EML Meal

The mean mineral content in mg/100g is presented in Table 2. Values are means plus or minus standard deviation.

Anti-Nutritional Factor

The estimations of anti-nutritional factors of defatted EML meal are presented in Table 3. Tannin, phytate and oxalate concentrations in EML meal were not significantly different ($p > 0.05$).

Fatty Acid Profile

The analysis of the fatty acid profile of BSFL was performed at an accredited laboratory; The Central Laboratory of the Nigerian Institute for Oceanography and Marine Research. Table 4.

Amino Acid Profile

EML are good sources of high-quality essential and non-essential amino acids and unlike vegetable proteins which are deficient in lysine, methionine and leucine, EML is rich in these essential amino acids (Hall, 1992) which are the more frequent limiting amino acids. The results of amino acid contents of EML samples are presented in Table 5. The most abundant essential amino acid was Valine (6.20 mg/ml) while the least abundant was methionine (1.7 mg/ml). Glycine and proline were found to be the most abundant and less abundant non-essential amino acids with values of (3.35 mg/ml) and (1.93 mg/ml) respectively.

Discussion

The proximate composition of EML revealed better crude protein values, especially after defatting. The higher values of crude protein after defatting may be due to the reduced effect of crude fat dilution in a given quantity. The values of crude protein obtained are much higher than those reported by Atowa *et al*., 2021 who worked on the nutritional values of *Zonocerus variegatus*, *Macrotermes bellicosus* and *Cirina forda* insects, and Adepoju *et al.,* 2020 who worked on the consumer acceptability and nutrient content of Westwood (*Cirina forda*) larva. *Antinutrients* are compounds that interfere with the absorption of nutrients in a host. In this study, the anti-nutritional content of the EML analyzed was generally low and within acceptable limits. Birkinshaw *et al.,* (2022) reported that high levels of tannins could be detrimental if consumed. Tannins are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids thereby interfering with their bioavailability. The tannin content of EML in this study fell below 76 - 90g/kg DM recommended by WHO, (2003). Pinheiro *et al.,* (2020) reported that high levels of phytate in food reduce the bioavailability of mineral elements like iron, calcium, magnesium, manganese and copper. Brouns (2022) also reported that a phytate diet of 1-6% over a long period may decrease the bioavailability of some mineral elements in monogastric animals. Phytate levels in this study are below 22.10 mg/100g recommended by WHO, (2003). According to Bargagli *et al.,* 2020, oxalate can bind to calcium present in food as it leaves the body thereby rendering calcium unavailable for a normal physiological and biochemical role such as the maintenance of strong bones, teeth, a cofactor in enzymatic reaction, nerve impulse transmission and as a clotting factor in the blood. The oxalate content of EML in this study similarly fell below 200 to 500mg/100g recommended by Pearson, (1973). These findings are similar to the report of Emeka *et al.* (2021) who worked on the nutritional compositions of *Oryctes rhinoceros* larva and *Zonocerus variegatus* and concluded that the antinutrient values of both insects fell within tolerable levels, and subsequently posed no threat to life. The fatty acid composition of EML meal is reported in Table 4. The table shows varying levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of EML with values of 52.59 ± 0.31%, 16.56 ± 0.52% and 30.85 ± 0.22% respectively. In most cases, EML contains more common fatty acids like linoleic acid and palmitic acid and shown to be among the essential and nonessential fatty acids present in their highest proportions among insects. The findings of this study are closely similar to the report of Rapatsa *et al.* 2017 who worked on the aquaculture evaluation of *Imbrasia belina* meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity and, Mohammed *et al.,* 2022, who worked on the effects of aeration rate and feed on growth, productivity and nutrient constituent of black soldier fly (*Hermetia illucens L*.) larvae. The amino acid profile of EML investigated in this study is well within range and similar to the findings of Adepoju *et al*., 2020 who worked on the consumer acceptability and nutrient content of Westwood (*Cirina forda*) larva-enriched *Amaranthus hybridus* vegetable soups, Triani *et al.,* (2021) who worked on the synthesis of protein hydrolysate from the prepupae of *Hermetia illucens* using a papain enzyme and Abduh *et al.,* (2020) who worked on the production of protein hydrolysate and biodiesel from black soldier fly larvae cultured using rotten avocado and tofu residue. Methionine, valine, lysine, arginine and leucine are among the excellent essential amino acids reported for EML in this study. According to Vangsoe *et al.,* (2018), these are essential, branched-chain amino acids that play a very important role in protein synthesis including muscle protein regeneration, blood glucose, and insulin regulation in mammals. The values of other essential amino acids obtained in this study are also comparable to the values reported by Mohammed *et al.,* (2020) and in many cases, exceeded the minimum essential amino acid intake standards set by FAO/WHO 1991 (Muhammad *et al.,* 2022).

Conclusion

This study reveals that *C. forda* larvae contain a good amount of crude protein and a higher amount of it when defatted. All essential amino acids present are also in good proportions. The mineral contents including calcium, magnesium, potassium, iron, sodium and phosphorus are also in adequate amounts indicating they are a good source of macro and micronutrients. The fatty acid profile also revealed an excellent amount of unsaturated fatty acids with a moderate amount of saturated and monounsaturated fatty acids indicating that they can play vital roles in animal fatty acid requirements. The nutritional and antinutritional composition of *C. forda* larvae in this study does not only show its potential use in human nutrition but also the aquaculture, poultry and livestock industries.

References

Abduh, Y., Manurung, R., Putra, R., Permana, A., Tejo, F. and Joseph, R. (2020). Production of Protein Hydrolysate and Biodiesel from Black Soldier Fly Larvae Cultivated using Rotten Avocado and Tofu Residue. *London Journal of Research in Science: Natural and Formal,* 20, 25-38.

Adepoju, O. T. and Daboh, O. O. (2020). Consumer acceptability and nutrient content of Westwood (Cirina forda) larva-enriched Amaranthus hybridus vegetable soups. *African Journal of Food Science*, 14(8), 244-255.

AOAC (2000). Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Bargagli, M., Tio, M. C., Waikar, S. S. and Ferraro, P. M. (2020). Dietary Oxalate Intake and Kidney Outcomes. *Nutrients*, *12*(9), 2673. https://doi.org/10.3390/nu12092673.

Birkinshaw, A., Kreuzer, M., Sutter, M., Reidy, B. and Terranova, M. (2022). Effects of early herbage cutting and vine leaves on methane emission, urine N losses and the milk fatty acid profile of dairy cows, *J. Dairy Sci*. 105(9): 7416–31.

Brouns, F. (2022). Phytic Acid and Whole Grains for Health Controversy. *Nutrients*, 14(1):25. doi: 10.3390/nu14010025. PMID: 35010899; PMCID: PMC8746346.

Chidiebere, O. A., Benedict, C. O., Ekene, C. U., Angelina, O. A., Okezie, E., et al. (2021). Nutritional values of Zonocerus variegatus, Macrotermes bellicosus and Cirina forda insects: Mineral composition, fatty acids and amino acid profiles. Elsevire Publication. *Scientific African*, 12 (2021) e00798. https://doi.org/10.1016/j.sciaf.2021.e00798.

Emeka, G.A et al, (2021). Nutritional compositions of two edible insects: Oryctes rhinoceros larva and Zonocerus variegatus. *Heliyon*, 7(3), e06531, ISSN 2405-8440. https://doi.org/10.1016/j.heliyon.2021.e06531. (https://www.sciencedirect.com/science/article/pii/S2405844021006344)

FAO/WHO (1991). Protein Quality Evaluation (Report of Joint FAOWHO Expert Consultation. FAO Food and Nutrition Paper 51). FAOWHO, Rome, Italy.

Hall, G. M. (1982). Silage from tropical fish 3. Lipid behaviour. *J. Food Technol*., 21, 45-54.

Jideani, A. and Netshiheni, K. (2017). Selected Edible Insects and Their Products in Traditional Medicine, Food and Pharmaceutical Industries in Africa: Utilisation and Prospects. 10.5772/intechopen.68330.

Kourimska, L. and Adamkova, A. Nutritional and sensory quality of insects, *NFS J.,* 4 (2016) 22–26, doi: 10.1016/j.nfs.2016.07.001.

Muhammad, Y. A et al., (2022). Effects of aeration rate and feed on growth, productivity and nutrient composition of black soldier fly (*Hermetia illucens L.*) larvae. *Journal of Asia-Pacific Entomology,* 25:101902.). Contents lists available at ScienceDirect Journal of Asia-Pacific Entomology journal homepage: www.elsevier.com/locate/jape https://doi.org/10.1016/j.aspen.2022.101902.

N'Djolosse, K. et al. (2012). Inventory of insects associated with shea trees (Vitellaria paradoxa) (Sapotaceae) in central and northern Benin. *International Journal of Tropical Insect Science*. 32. 10.1017/S1742758412000240.

Oriolowo, O. B., Ande, A. T. and Azeez, M. O (2023). Accumulation and utilization of food reserves in diapause-destined Pallid emperor moth, *Cirina forda* (Westwood).

Papastavropoulou, K., Xiao, J. and Preston, C. (2022). Edible insects: Tendency or necessity (a review). eFood, 4(1), e58.https://doi.org/10.1002/efd2.58

Pinheiro, B. et al., (2020). Phytate and iron bioavailability in legumes. *Acta Portuguesa de Nutrição*. 48-51. 10.21011/apn.2020.2209. DOI: 10.21011/apn.2020.2209.

Thakur, A. et al., (2017). Entomophagy (insects as human food): a step towards food security - *Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India*. DOI: 10.13140/RG.2.2.29644.72327.

Tiencheu, B. and Womeni, H. (2017). Entomophagy: *Insects as Food*. 10.5772/67384.

Triani, R., Alfianny, R., Manurung, R. and Abduh, M.Y. (2021). Synthesis of protein hydrolysate from the prepupae of Hermetia illucens using a Papain Enzyme. *Res. J. Appl. Sci. Eng. Technol.*, 18 (1), 12–19. https://doi.org/10.19026/rjaset.18.6059.

Vangsoe, M. T., Thogersen, R., Bertram, H. C., Heckmann, L. L. and Hansen, M. (2018). Ingestion of Insect Protein Isolate Enhances Blood Amino Acid Concentrations Similar to Soy Protein in A Human Trial. *Nutrients*, *10*(10), 1357. https://doi.org/10.3390/nu10101357,

WHO. (2003). The World Health Report: 2003: shaping the future. World Health Organization. https://apps.who.int/iris/handle/10665/42789.

Plate 1: Dried Emperor moth (*Cirina forda*) Larvae

Table 1: Proximate Composition of EML Samples Before and After Defattening

Parameters	% Moisture	% Crude protein	% Total Ash	% Crude Fibre	% CHO
Before Defattening	8.21 ± 0.04	56.26 ± 0.2	6.16 ± 0.11	9.06 ± 0.03	3.13 ± 0.02
After Defattening	7.41± 0.10	65.34 ± 0.02	5.73 ± 0.01	8.42 ± 0.02	4.41 ± 0.00

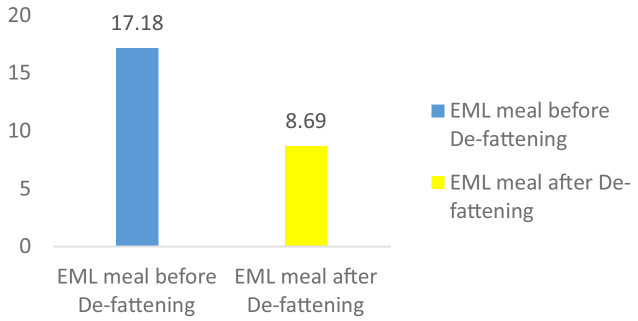

Fig 1: Percentage crude fat content of EML before and after Defattening

Table 2: Mineral Composition of dried EML meal

Parameters (mg/100g)	EML meal
Calcium	87.37 ± 007
Magnesium	141.25 ± 0.37
Phosphorus	223.25 ± 0.37
Potassium	968.18 ± 0.14
Sodium	536.9 ± 0.35
Manganese	3.22 ± 0.26
Iron	37.48 ± 0.29
Copper	0.56 ± 0.01
Zinc	7.39 ± 0.02
Anti-oxidant	57.61 ± 0.21

Values in the same row denoted by the same superscript are not significantly different at (p>0.05)

Table 3: Anti-Nutritional Factor Content of Defatted EML

Anti-nutritional factors	***Concentration***
Tannin (mg/100g)	281.08 ± 0.04
Phytic acid (mg/100g)	0.438 ± 0.01
Oxalate acid (mg/100g)	35.5 ± 0.07

Table 4: Percentage of Fatty Acids Composition of BSFL

Fatty Acids	EML meal
Total Fatty Acid Composition	100
Saturated Fatty Acid (SFA)	52.59
Monounsaturated Fatty Acid (MUFA)	16.56
Polyunsaturated Fatty Acid (PUFA)	30.85
PUFA/SFA	0.59
n-3 (Omega-3 Fatty acid)	4.05
n-6 (Omega-6 Fatty acid)	2.02
n-6/n3	0.5

WHO, 2005 (n-6/n-3 ≤ 5:1), (PUFA/SFA > 0.4); HMSO, 2001 (n-6/n-3 ≤ 4:1)

Table 5: Amino Acid Profile of EML

Essential Amino Acid Profile (EAA)	EML, (g/100g) protein	Non-Essential Amino Acid Profile (Non-EAA)	EML, (g/100g) protein
Arginine	6.2	Ala	2.52
Histidine	3.2	Asn	N/A
Ile	4.6	Asp	3.56
Leu	2.24	Cit	N/A
Lys	6.4	Cys-Cys	N/A
Met	3.2	Eta	N/A
Phe	5.2	Gln	N/A
Thr	4.2	Gly	2.67
Tyr	6.1	HyPro	N/A
Val	5.6	Orn	N/A
		Ser	1.85
		Trp	N/A
		Cys	N/A
		Glu	3.12
		HyPro	N/A
		Pro	1.91
		Tau	N/A

Note: EML= Emperor moth larvae