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Nutritional Potential of *Cirina butyrospermi* found in Benue State, Nigeria

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Abstract

Cirina butyrospermi (caterpillar) was purchased and analyzed for proximate, minerals, amino acids, fatty acids, vitamins, and anti-nutrition compositions to investigate the nutritional potential of the insect. The results of the analyses revealed that Cirina butyrospermi contained higher protein content (48.98%), moderate fat content (19.00%), and carbohydrates (16.53%). It was observed to contain low ash (7.00%), moisture (6.75%) and fiber (1.75%). The predominant minerals found were mainly; potassium (574.27 mg/100g), calcium (125.33 mg/100g), phosphorus (85.56 mg/100g), sodium (55.56 mg/100g), magnesium (48.66 mg/100g) and manganese (35.64 mg/100g). The insect was observed to be rich in essential amino acids with the predominant ones like glutamic acid (12.43%), Aspartic acid (7.57%) and Tryrosine (5.23%). Stearic acid (30.02%), Palmitic acid (27.131%) and Tridecanoic acid (27.13%) were the most abundant fatty acids. Vitamin B_1 and B_2 were present in low quantities (1.68% and 2.12%) respectively). The cirina butyrospermi was found to contain low levels of anti-nutrients (alkaloids 10.45%, tannin 9.60%, saponin 3.20%, Trypsin Inhibitor 1.41%, phytate 0.09% and Oxalate 0.69%). The results of the study indicate that Cirina butyrospermi is a potential source of proteins, lipids, and minerals for human and animal feeds. The low levels of anti-nutritional factors suggest that it is less toxic and can be incorporated into dietary foods and livestock feeds without any adverse health effects.

Introduction

The rapid growth of the world population needs a proportional increase in food production. However, the production of food is not commensurate with the population growth. As such, it is only the privileged class that has foods with adequate nutrients, especially in developing and underdeveloped countries. The rest of the vulnerable populations are faced with hunger and starvation. Even where there is food, the food usually lacks high-quality nutrients proteins. The common sources of animal protein such as meat and eggs are costly and out of reach for the vulnerable populations (Ga³ecki *et al.*, 2021). One of the ways of addressing the nutritional needs of the vulnerable population is by seeking new alternative sources of nutrients that are different from the conventional sources. An example of such an alternative is the utilization of edible insects which are readily available. Babayi, *et al*, (2019), reported that insects could be regarded as future food sources because they are rich in protein, minerals, and vitamins. According to Séré *et al*, (2021), edible insects have been described as sources of protein with an average content ranging from 35 - 61 %.

Adepoju and Daboh (2020), reported that edible insects contained an appreciable amount of proteins of good quality and high digestibility, with beneficial amino acids and fatty acids comparable to conventional livestock and fish. Some of the edible insects that are of nutritional importance are Grasshoppers, Caterpillars, Beetle grubs (sometimes adults), Winged Termites, Cirina forda larvae, etc. Among these edible insects, Caterpillars which are the larvae of butterflies have been found to contain high nutritional value. Michel et al., (2017), reported that the flour of Cirina butyrospermi is usually mixed with cereal flour to combat child malnutrition in Côte d'Ivoire, Malawi, Zambia, and Tanzania. In some of these countries, pregnant and nursing women, as well as anaemic patients, are encouraged to eat caterpillar species which are believed to contain high protein, calcium, and iron. Also, people with wounds are encouraged to eat Cirina butyrospermi hence it is believed to have the ability to heal wounds. It is a good source of essential vitamins, amino acids, and fatty acids (Michel et al., 2017). Morgane et al., (2016), reported that Cirina butyrospermi is a potential source of protein, fat, and minerals for human and animal feeds.

Cirina butyrospermi is an insect that is eaten in many countries like South Africa, Zambia, Zimbabwe, Cote d'Ivoire, Togo, Mali, Ghana, Democratic Republic of Congo, Mozambique, Namibia, and Nigeria. In Nigeria, *Cirina butyrospermi* is eaten as a delicacy by many communities (Banjo *et al.*, 2006). It also serves as a source of income for most communities. In Benue State, although the eating of *Cirina butyrospermi* is a common practice, there is a dearth of information about the chemical composition of the insect. Also, considering the nutritional potential of the insect, regular consumption will supplement the protein needs of the less privileged. This study, therefore, considered the analysis of the chemical composition of *Cirina butyrospermi* in Benue State pertinent with the aim of ascertaining the nutritional profile of the insect.

Materials and Methods Sample collection

Samples of fresh *Cirina Butyrospermi* were purchased at Ikpayongo market in Gwer East Local Government of Benue State, Nigeria in August, 2019. The insects were identified and authenticated by an Entomologist in the Department of Biological Sciences, Benue State University Makurdi. The sample was then dried at 45 °C to constant weight ground into powder using mortar and pestle and stored in an air-tight container for further analysis

Determination of Proximate Composition

Percentage moisture content was calculated by loss in weight on drying the sample in an oven at 105 °C for 2 h. Fats content was calculated by drying the fats after extraction in a Soxhlet using petroleum ether (40 - 60) °C to constant weight. Crude protein was determined by the Kjeldahl method and the total protein content was calculated by determining the product of total nitrogen and the protein conversion factor (6.25). Ash content was determined by combusting the samples at 550 °C for 4 h using a porcelain crucible placed in a muffle furnace. Carbohydrate content was determined by deference [AOAC, 2012].

Determination of Mineral elements

The flame photometry method reported by AOAC (2012) was used to determine the sodium and potassium contents of the sample. A corning 405 flame photometer was used. Calcium, Fe, Mg, Zn, Cu, Pb, Ni, and Cr were determined using Alpha 4 atomic absorption spectrophotometer (AAS). Phosphorus content was determined by employing the method reported by Vanado Molybate and read on CECIL CE 3041 colorimeter (AOAC, 2012). All determinations were in triplicates.

Determination of Amino acids

The dried sample was hydrolyzed with 200 iL of constant boiling 6M HCl and 40 iL of phenol through vapor-phase hydrolysis. The sample was dried in an oven at 112-116 $^{\circ}$ C for 20 - 24 h. After

completion of hydrolysis, excess HCl was cleaned off and the tubes were vacuum-dried for 90 minutes. The plant samples were then reconstituted with 100 iL of 20 mM boiling HCl (Musadiq et al., 2019, Igbabul et al., 2014). The reconstituted 20 iL samples were derivatized with an AccQ-Fluor reagent kit (WAT052880- Waters Corporation, USA). AccQ-Fluor borate buffer (60 iL) was added to the sample tube with micro pipette and vortexed. Thereafter, 20 iL of AccQ-Fluor reagent was added and immediately vortexed for 30 sec. and the contents were transferred to maximum recovery vials. The vials were heated for 10 min in a water bath at 55 °C before the separation of amino acids using the reverse phase-high pressure liquid chromatography (HPLC) (Buck Scientific BLC10/11 USA (Musadiq et al., (2019).

Determination of Essential Fatty Acids

Ten (10) g ground of cirina butyrospermi flour sample was poured into a thimble and placed into a soxhlet extractor. The thimble and its contents were then introduced into the soxhlet extractor and mounted into a round-bottomed flask and the extraction apparatus was set up with the flask connected to a water condenser. The solvent (petroleum ether) was heated; boiled, evaporated, condensed and dropped into the thimble it extracted the oil into the round-bottomed flask. This process was allowed to go on repeatedly for about 4 - 6 h after which the thimble was removed and the amount of petroleum ether) remaining in the flask was recovered and the oil extract was left in the flask. The oil was transferred into an air-tight container for further analysis. The lipid portion was then saponified derivatives to fatty acid methyl esters with boron trifluoride methanol and determined by gas chromatography using Flame Ionised Detector (FID). This method determines the percentage area of the fatty acids and the absolute concentration of specific fatty acids estimated using fatty acids' internal standards (Michel et al., 2017)

Determination of Vitamins

(a) Determination of water-soluble vitamins. The water-soluble vitamins (vitamin B group) were extracted by the method adopted by Adepoju and Daboh (2020). Exactly 2 g of the ground sample was placed in 25 mL of $0.1 \text{ M H}_2\text{SO}_4$ solution and

incubated for 30 mins at 121 °C. The content was cooled and adjusted to pH 4.5 with 2.5M sodium acetate, and 50 mg Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No. 4 filter paper, and the filtrate was diluted with 50 mL of distilled water and filtered again through a micropore filter (0.45 5ØBm). Twenty microliters of the filtrate were injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards. Standard stock solutions for thiamine, riboflavin, pyridoxine, and cobalamin were prepared as reported by Rokayya et al., (2014). Chromatographic separation was achieved on a reversed phase- (RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 5ØBm) through the isocratic delivery mobile phase $(A/B 33/67; A: MeOH, B: 0.023 M H_2PO_4, pH =$ 3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature (Rokayya et al., 2014)

(b) Determination of Ascorbic Acid (Vitamin C)

Ascorbic Acid was extracted according to a modification of a published method (Rokayya et al., 2014). Ground sample (4 g) was added to an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was then filtered through a Whatman No. 4 filter paper, and the sample was extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of l-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a linear range based on four measured concentration levels. Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH = 4.9, B: acetonitrile: water [50:50]) at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature (Rokayya et al., 2014) **Determination of Anti-nutrition**

Determination of Oxalate (Titration Method,

Agbai, et al, 2021)

Two (2) g of the sample was suspended in 190 mL of distilled water in a 250 mL volumetric flask. 10 mL of 6 M HCl was added and the suspension was digested at 100 °C for 1 hour, the solution was cooled and made up to 250 mL mark before filtration. Duplicate portions of the filtrate were measured into conical flasks and two drops of methyl red indicator were added. Then the NH₂OH solution was added (drop-wise) until the test solution changed from pink to faint yellow colour. Each portion was heated at 90 °C for 40 minutes and 10 mL of 5% CaCl, solution was added while being stirred constantly. The solution was left overnight at 25 °C and filtered again. 10 mL of 20% (v/v) H₂SO₄ solution was measured and poured into the solution. The filtration was heated until near boiling and then titrated against 0.05 M standardized KMnO₄ solutions to a faint pink colour which persisted for 30 seconds The oxalate content was calculated using the formula:

Oxalate /100glmg =
$$\frac{TV \times 0.00223}{Weight of sample} \times 100$$

Determination of Alkaloid

Five (5) g of the sample was weighed into a 250 mL beaker and 50 mL of 20% acetic acid in ethanol was added and allowed to stand for 4 hours at 25 °C. It was then filtered and the filtrate was concentrated using a hot plate to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until a precipitate was complete. The solution was filtered using a pre-weighed filter paper; the residue on the filter paper was dried in the oven at 80 °C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed (Agbai, *et al*, 2021),

Determination of saponin

5 g of the sample was weighed into a 250 mL conical flask 50 mL of 20% of acetic acid in ethanol was added and allowed to stand in a water bath at 50 °C for 24 hours. This was filtered and the extract was concentrated using a hot plate to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until

a precipitate was complete. The solution was filtered using a pre-weighed filter paper; the residue on the filter paper is the saponin and was dried in the oven at 80 °C. The saponin content was calculated and expressed as a percentage of the weight of the sample analysed (Igidi and Edene, 2014).

% of saponin content =weight of filter paper with Residualweight of filter paper______x100 Weight of sample

Determination of phytate

The method used was the one adopted by Lucas and Markakes (1975). Exactly 0.2 g of the sample was weighed into a 250 mL conical flask. 50 mL of 20% HCl was added and allowed to stand for 3 hours. The solution was then filtered and 25mL of distilled water was added to the filtrate in a 250 mL conical flask. 5 mL of 0.03 ammonium thiocyanate solution was added as the indicator and titrated with standard iron (III) chloride solution which contains 0.00195 g iron per 1mL. The phytate content was calculated.

Phytic acid =
$$\frac{TV \times 0.00195 \times 1.19}{2} \times 100$$

Determination of Tannin

The Follins-Dennis titration method as described by Pearson (1976) was adopted. To 5 g of the sample in a conical flask, 50 mL of petroleum ether was added and covered for 24 hours at 25 °C. The solution was then filtered and allowed to stand for 15 minutes until the petroleum ether evaporated. It was re-extracted by soaking in 25 mL of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate was collected. 15 mL of NH₄OH was added to the filtrate to precipitate the alkaloids. The solution was heated with a hot plate to remove some NH₄OH still in the solution. The remaining volume was measured. Exactly 2.5 mL of this volume was taken and 10 mL of ethanol was added to it. It was titrated with 0.1 M NaOH using phenolphthalein as an indicator until a pink endpoint was reached. Tannin content was then calculated.

% Tannin acid content = $\frac{C_1 \times 100}{Weight \ of \ sample}$ where C_1 = concentration of sample.

Determination of Trypsin Inhibitor

One (1) g of the sample was dispersed in 50 mL of 0.5 M NaOH solution. The mixture was stirred for 30 min at room temperature and filtered. 2 mL

of the standard trypsin was added to 8 mL of the substrate of the sample. The absorbance of the mixture was taken at 410 nm using distilled water as blank. The trypsin inhibitor was determining using this formula:TP(mg/100g) = $\frac{Ca \times total \ vol.(mL) \times dilution \ (100)}{wt \ of \ sample \times 1000} \dots \ (3.13)$

Ca = conc. of the sample in mg/L from the calibration curve (mg/L) Umeobika et al 2015).

Results and Discussion

The results of the proximate composition of *Cirina* butyrospermi (Table 1.0) indicate moisture content to be 6.75%. This value is below the acceptable value of 10% for food products. According to Archileo and Willian (2006), when the moisture content of a food material exceeds the safe limit, storing the food product becomes very difficult, and it may develop moulds and fungi, leading to rot. The low moisture content of the insect revealed that if dried properly could be stored for a long time without spoilage. The result also compared favourably with that of Michael *et al*, (2017), who reported the moisture content of *C. butyrospermi* to be 7.92%.

The ash content was found to be 7.00%. This value is higher than the values reported by Michael *et al*, (2017), and Séré *et al* (2021), but lower than the 8.6% reported by Anankware *et al.*, (2021). Any fresh food that has ash content of 5% is considered to be a good source of minerals. The ash content recorded here is an indication that the insect is a source of minerals (Aunyachulee and Chutinan 2020).

The crude protein of the insect was recorded to be 48.98%. Pirjo et al. (2018), reported that the recommended protein content for a product is 16%. The high value of protein shows that the insect could be a source of supplementary protein which could aid in the building and repairing of body tissues for both man and animal. Crude fat was found to be 19.00%. This is an indication that the insect is also a good source of fat. The crude fibre content was found to be 1.75%. The low crude fibre content of the insect indicates that it is a poor source of fibre. Carbohydrate content of C. butyrospermi was found to be 16. 53 %. The low amount of carbohydrate content implies that the insect is a proteinous food material. The result also compared favourable with results obtained by Séré et al (2021) and Morgane et al., (2016).

The mineral composition of C. butyrospermi (Table 2.0) indicate the mean values of calcium, potassium, sodium, magnesium, zinc iron cupper manganese and selenium to be 125.33, 574.27 55.56, 48.66, 5.12, 7.15, 0.56, 35.64 and 0.03 mg/ 100g respectively. The insect was discovered to contain different concentrations of minerals that complement the mineral requirements of humans from traditional diets. Michel et al., (2017), reported that the consumption C. butyrospermi gives a nutritional plan with an adequate amount of minerals in the body. The presence of magnesium for example will aid the reaction of many enzymes since magnesium is a cofactor for many enzyme reactions in the body. It is also essential for the synthesis of carbohydrates, lipids, nucleic acids and proteins etc. Calcium aid blood clotting process and is essential for the formation of bones and teeth.

Table 1.0: Proximate Composition of CirinaButyrospermi (g/100g dry weight

Parameters	% Composition	
Moisture	6.75±1.77	
Ash	$7.00{\pm}028$	
Protein	48.98±1.83	
Fat/oil	19.00 ± 0.00	
Fibre	1.75 ± 1.06	
Carbohydrates	16.53 ± 6.05	
T 7 1	$1 \rightarrow CD(2)$	

• Values are expressed as mean ± S.D (n=3)

Table 2.0: Mineral Composition of CirinaButyrospermi (mg/100g)

Elements	Concentrations (mg/100g)
Calcium	125.33 ± 0.01
Potassium	574.27±0.02
Sodium	55.56 ± 0.01
Magnesium	48.66 ± 0.04
Zinc	$5.12{\pm}0.01$
Phosphorous	85.56±0.01
Iron	$7.15{\pm}0.00$
Cupper	0.56±014
Manganese	35.64±0.01
Selenium	$0.03{\pm}0.00$

• Values are expressed as mean ± S.D (n=3)

Parameters	Concentrations (g/100g)
Lysine*	3.21±0.01
Histidine*	3.54 ± 0.02
Isoleucine*	2.01±0.01
Methionine*	0.86±0.01
Phenylalanine*	3.75 ± 0.00
Threonine*	1.75 ± 0.00
Tryptophan*	3.72±0.01
Valine*	3.87±0.01
Alanine	1.77±0.01
Arginine	2.12±0.01
Aspartic acid	7.57±0.01
Cysteine	2.00 ± 0.00
Glutamic acid	12.43±0.01
Glycine	3.13±0.01
Proline	1.71 ± 0.01
Serine	2.80±0.01
Tyrosine	5.23±0.01

Table 3.0: Amino acid composition of CirinaButyrospermi (g/100g)

Essential amino acid (*), Values are nean \pm S.D (n=3

Table 4.0: Fatty acid composition of oil extract

 from *Cirina Butyrospermi*

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Fatty acids	Composition (%)
14- pentadecenoic acid	1.04 ± 0.40
Archidinic acid	1.06 ± 0.01
Lauric acid	1.04 ± 0.22
Lignoceric acid	2.33 ± 0.01
Linoleic acid	7.32 ± 0.12
Linolenic acid	21.08 ± 0.22
Myristic acid	1.18 ± 0.20
Oleic acid	$24.05{\pm}~0.14$
Palmitic acid	27.13 ± 0.11
Stearic acid	30.02 ± 0.30
Tridecanoic acid	27.13 ± 0.21
V_{2}	

Values are mean \pm S.D (n=3)

Table 5.0: Vitamin C	Composition of	ľ
Civing Dutynognowni	(ma/100a)	

Cirina Duiyrosperna (mg/100g)	
Parameters	Concentrations
	(mg/100g)
Thiamine (Vitamin B1)	1.68 ± 0.62
Riboflavin (vitamin B2)	2.12±0.01
Pyridoxine (Vitamin B6)	2.6±0.02
Cobalamin Vitamin B12	4.2±0.03
Ascorbic Acid (Vitamin C)	1.82 ± 0.04

• Values are expressed as mean ± S.D (n=3)

Table 6.0: Anti-nutritional values of

 Cirina Butyrospermi (mg/100g)

Parameters	Concentrations
	(mg/100g)
Oxalate	0.69 ± 0.02
Alkaloid	10.45 ± 0.07
Saponin	$3.2{\pm}0.00$
Phytate	0.09 ± 0.00
Tannin	9.60±0.14
Trypsin inhibitor	$1.91{\pm}0.01$

• Values are expressed as mean \pm S.D (n =3)

Kieliszek and B³a¿ejak (2016) reported that selenium is essential micronutrient needed in trace amount for the proper functioning of organisms. It protects the cells against excess hydrogen peroxide during metal detoxification, and also regulates the immune and reproductive systems. It also ensures the proper functioning of the thyroid gland and also induces the occurrence of the selenoprotein synthesis which is a process involving the antioxidant defense mechanism of organisms. Other elements like manganese, sodium, copper iron and potassium are also necessary for the proper functioning of the body systems.

The amino acid profile of C. *butyrospermi* is presented in Table 3.0. Seventeen amino acids were analysed include the essential amino acids; lysine, histidine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine. The non-essential amino acids were; alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline serine, and tyrosine. The amino acids were discovered to exist in different concentrations, which could complement the protein needs met by traditional protein sources. The composition of amino acids in C. *butyrospermi* compared favourably with results reported by (Yusuf *et al.*, 2016, Michel *et al.*, 2017 and Séré *et al.*, 2021).

The result of the fatty acids profile revealed the presence of both saturated and unsaturated fatty acids in varying concentrations (Table 4.0). Linoleic and oleic acids were found to have the highest concentrations 21.08% and 24.05% respectively among the unsaturated fatty acids. Whereas palmitic acid and tridecanoic acid were observed to be the predominant saturated fatty acids with values 27.13% and 27.43% respectively. According to Nagy and Tiuca (2017), fatty acids are important to the human body, they serve as a source of energy and are constituents of cellular membranes. As part of phospholipids, they aid fluidity, flexibility, and permeability of the membrane and also ensure the passive transport through the membrane and interconnection with other proteins in intra and intercellular ways.

The vitamin content of C. butyrospermi indicates the presence of vitamin B_1 , B_2 , B_6 , B_{12} , and vitamin C with vitamin B₁₂ (cobalamin) being the highest (4.2 mg/100g) while vitamin B_1 was the lowest (1.68 mg/100g) Table 5.0. The concentrations of vitamins observed in all cases were found to be above the recommended daily intake, which ranges from 0.12-0.4 mg/100g, except for vitamin C (Anatol et al., 2019). All the Vitamins analysed are water-soluble vitamins that dissolve in water upon entering the body system. This class of vitamins is immediately absorbed into the bloodstream upon entering the body system. The human body does not store the excess amount of these vitamins for later use and as such, they need to be replenished regularly in our diet from time to time. The deficiency of any of these vitamins will result in a clinical syndrome that may lead to severe morbidity and mortality.

The concentrations of anti-nutritional factors (Tables 6.05) indicate the amount of alkaloid, tannins, saponins, trypsin inhibitors, oxalate and phytates to be 10.45%, 9.60%, 3.20%, 1.91%, oxalate 0.69%, and 0.09% respectively. The values of oxalate, phytate and tannins obtained are higher than the values obtained by (Ifie et al., 2011) for Oryctes monoceros larva. Oibiokpa et al (2017), however, reported lower values of 0.48%, 0.09% and 1.2% for tannins, phytates and saponins respectively. The results also compared favourably with the results obtained by Omotoso (2006) for Cirina forda. Alkaloids cause gastrointestinal upset and neurological disorders especially when taken in excess. Tannin interacts with salivary proteins and glycoproteins in the mouth and renders the tissues astringency to taste which gives tannin the medicinal value in preventing diarrhoea and dysentery and controlling hemorrhage. When tannins polymerize, they form a protective barrier against microbial attack. Trypsin inhibitor forms complexes with trypsin to reduce its proteolytic activity which in turn reduced the availability of amino acids and growth. Phytate depresses the growth of chicks fed with phytatecase in the diet by forming a complex with zinc making the latter unavailable. Phytate forms a complex with protein

by actions of cations such as Zn, Ca or Mg thereby reducing the bio-availability of such minerals (Umeobika *et al.*, 2015). The low anti-nutritional factors of the insect are an indication of the nutritional potential of the insect.

Conclusion

The study's findings showed that C. butyrospermi, discovered in Benue State, Nigeria, could potentially be a source of nutrients such as protein and lipids. This caterpillar is high in amino acids, fatty acids, and minerals such as potassium, calcium, sodium, magnesium, phosphorus, and manganese. As a result, the insect could serve as a supplement to traditional sources of protein, fat, and minerals for both human and animal consumption.

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