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Nutritional and microbial quality of dried larva of *Cirina forda*

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Abstract: The quality of dried larva of pallid emperor moth (*Cirina forda*) sold in various markets of Makurdi metropolis in Nigeria was evaluated. The proximate composition, amino acid profile, mineral contents as well as microbial quality of dried larva of *Cirina forda* were determined using standard methods. The results of proximate composition on dry basis showed that crude protein ranged from 54.36-56.78%; ash, 2.91-3.97%; crude fibre, 11.03-11.15% and moisture, 4.41-5.95% The mineral contents, K, Na, Cu, Fe, Zn, Mn and Mg of the larva samples ranged from 7.67 to 14.39, 0.93 to 1.27, 0.11 to 0.37, 0.37 to 0.56, 0.13 to 0.56, 0.28 to 0.39 and 0.10 to 0.21 mgg^{-1} (dry matter), respectively. Results of the content of amino acids showed the essential amino acids: - isoleucine, leucine, methionine, phenylalanine, threonine, valine and lysine in the ranges of 3.27 to 4.31, 6.55 to 7.57, 2.22 to 2.48, 4.47 to 5.08, 3.22 to 4.18, 3.18 to 4.18 and 5.33 to 6.24g/100g protein respectively. The total viable count of micro organisms in the samples ranged from 2.0 to $3.7x10^3$ cfu/g and the mold counts were <30 cfu/g in all samples. Micro organisms identified included *Escherichia coli*, *Salmonella* spp, *Staphylococus aureus, Proteus* spp, *Micrococcus* spp, *Mucor* spp, *Aspergillus niger* and *Rhizopus* spp. The nutritional content of the dried larva of *Cirina forda* compared favourably with those of conventional foods of animal origin and the microbial load of the dried larva within acceptable range. However, the dried larva should be subjected to further heating such as cooking to destroy the pathogens isolated which will otherwise constitute a health risk to consumers.

Keywords: Quality, Dried Larva, *Cirina forda*

1. Introduction

Edible insects have played an important part in the history of human nutrition in Africa, Asia and Latin America [1] . It has also been used for cultural (rituals) as well as for medicinal purposes throughout much of man's ancient times and the present. Edible insects have been shown to contain appreciable amount of protein of good quality and are highly digestible. [2,3,4].

In some African regions such as Malawi, Zambia and Tanzania, children fight malnutrition by eating flour made out of dried caterpillars. Pregnant and nursing women as well as anaemic patients also eat caterpillar species high in protein, calcium and iron.

Pallid emperor moth (*Cirina forda)* is one of the most widely eaten insects in Southern Nigeria [5] and it has been reported as the third most consumed insect in Benue State, Nigeria [6]. The larva of this insect is a delicacy served as a snack food item or taken with carbohydrate food in Nigeria. It is eaten raw or roasted or dried and added to other foods especially carbohydrate to enhance its nutritional value.

It has been shown that the larva of *Cirina forda* (West wood) has the potential to provide substantial amounts of proteins, minerals and polyunsaturated fatty acids to the diets which are usually deficient in animal protein [7]. Another study in which the larva was processed by boiling, followed by sun drying has shown that such processed larva of *Cirina forda* (Westwood) was neither neurotoxic nor heptatoxic to mice and rats [8].

Although some reports have been written on edible insects, [9,10,11,12] little information is available on the nutritional quality and none on the microbiology of pallid emperor moth consumed in Makurdi metropolis, Nigeria. The result of this study will contribute to the update of the food composition database of the larva as food and aid in the usage for food product formation and development for better utilization.

2. Materials and Methods

2.1. Sample Preparation

Dried *Cirina forda* larvae were purchased from different markets in Makurdi metropolis of Nigeria. The samples were further sun dried to eliminate excess moisture and ground into powdered form using attrition mill. It was kept in air tight, transport plastic containers until required.

2.2. Chemical Analysis

2.2.1. Proximate Analysis

The moisture content, ash, crude fat, crude protein and fibre were determined by methods described by AOAC [12]. The carbohydrate content of the ground larva sample was determined by difference as reported by [14].

2.2.2. Amino Acid Analysis

The amino acid profile of the ground larva samples was determined by the method described by AOAC [12] in which 2g of the samples was dried to constant weight and defatted with chloroform/methanol (2:1 mixture) using soxhlet apparatus for 15hours. This was followed by nitrogen determination of the sample, hydrolysis of the sample, evaporation in a rotary evaporation and loading (5-10µl) into the Technicom Sequential Multisample Amino Acid Analyser (TSM, model DNA 0209). The net height of each chromogram peak produced by the chart recorder by TSM (each representing an amino acid) was measured. The half height of the peak on the chart was found and width of the peak was then obtained by multiplying the height with the width at half height. The concentration for each amino acid was accordingly obtained from the standard formula.

2.3. Minerals Analysis

The wet ashing method described by AOAC [12] was used to determine the mineral content of *Cirina forda* samples. Here, 2.5g of air dried sample was wet with water, 25ml of nitric acid and the mixture was gently heated to start a sample reaction. After cooling, 10ml of perchloric acid was added and gently heated to concentrate midway. On becoming dark, 2-3ml of nitric acid was added and heating was continued until decomposition was completed as material turned yellowish or colourless. After cooling, 2nd of hydrochloric acid and water as used to prepare fixed volumes of measurement solutions. These diluted clear digest were then charred by combustion and the concentrations of each element was read by the atomic absorption spectrophotometer (Model, Buck 210) using lamps with wavelength that matches that of the corresponding element(s), which were iron, zinc, copper, manganese and magnesium. For the determination of potassium and sodium, the flame photometer (model PF-P7) was used to give direct read-out instead of concentrations. Calibration curves were prepared for each element using standard solutions. The appropriate lamps and correct wavelength for each element were adhered to as specified on the instruction manual of the instrument.

2.4. Microbiological Analysis

Enumeration, isolation and identification of bacteria contaminating the samples was carried out. Total aerobic plate count was done according to procedures described by [14]. Ten grams of each of the ground larva samples was weighed into 90ml of sterile peptone water and 10-fold serial dilution carried out. Aliquot (1ml) of selected dilution was cultured by pour plate method using plate count agar and incubating at 37^{o}C for 48h. Selective media such as Eosin Methylene Blue Agar (EMBA), Salmonella-Shigella Agar (SSA) and Mannitol salt Agar (MSA), were used to enumerate and isolate pathogens such as *E. coli, salmonella* and *Staphylococcus aureus,* respectively. Colonies in duplicate plates were counted and subcultured until pure. Various biochemical tests were used to confirm the identity of the species as outlined in Bergey's Manual of determinative bacteriology [15] yeasts and mould counts were also determined using pour plate method earlier described. Potato Dextrose Agar acidified with 1ml of 10% tartaric acid was used for cultivation and incubation was at 28^{o}C for 3 -5 days. The species of yeasts and moulds were confirmed on the basis of cultural and morphological characteristic.

2.5. Statistical Analysis

Values obtained in the various analyses were subjected to analysis of variance (ANOVA) and Tukey's Least Significance Difference between mean values was determined as outlined by [13].

3. Results

The results of proximate composition of *Cirina forda* on dry weight basis showed very high protein values of 54.36% to 56.78% and fat values ranges from 11.07% to 13.84%. The ash and fibre contents ranged from 2.91 to 3.97% and 11.03 to 11.15%, respectively (Table 1). The moisture was relatively low with values ranging from 4.41 to 5.95 %, while carbohydrate values ranged from 11.09 to 12.84%. All parameters showed significant difference ($P<0.05$) in their mean values except carbohydrates among samples obtained from different locations (Table 1).

Table 1. *Proximate Composition of Cirina forda on Dry Basis*

% Content	Sample A	Sample B	Sample C	LSD
Crude protein	$56.78^{a}\pm0.002$	$54.36^{b}\pm0.002$	$55.17c\pm0.001$	0.13
Carbohydrate	$11.09^{a}\pm0.024$	$12.84^{a}\pm0.097$	$12.16^{a}\pm0.097$	-
Ash	$3.97^{a}\pm0.001$	$3.76^{b}\pm0.002$	$2.91^{c}\pm0.000$	0.08
Crude fibre	$11.15^{a}\pm0.000$	$11.03^{b}\pm0.000$	$11.07^{a}\pm0.000$	0.05
Fats	$11.07^{a}\pm0.003$	$13.60^{b}\pm0.080$	$13.84^{a}\pm0.000$	1.26
Moisture	$5.95^{a}\pm0.000$	$4.41^{b}\pm0.000$	$4.86^{c}\pm0.000$	0.05

Any two means values bearing the same superscript in the same column are not significantly different ($p<0.05$). Values preceding the means are standard deviations, LSD Least Significantly Differences. Values are means of duplicate determinations.

Sample A: Larva obtained from North Bank Market

Sample B; Larva obtained from Wurukum Market

Sample C: Larva obtained from Modern Market

The larva of *Cirina forda* was observed to contain the elements; Potassium, Iron, Manganese, Sodium and Copper. Potassium had the highest value ranging among samples from 7.67 to 14.39 mgg^{-1}, whereas Magnesium had the least values ranging from 0.93 to 1.27, Iron (0.37 to 0.56), Zinc (0.13 to 0.56), Manganese (0.28 to 0.37) and Copper (0.11 to 0.37) ppm, respectively (Table 2).

Table 2. *Mineral Elements of Cirina forda sold in Makurdi metropolis*

(mg/g)				
Mineral	**Sample A**	**Sample B**	**Sample C**	**LSD**
Potassium	$14.39^{a}\pm0.000$	$7.71^{b}\pm0.005$	$7.67^{b}\pm0.008$	0.13
Sodium	$0.93^{a}\pm0.013$	$1.07^{a}\pm0.005$	$1.27^{a}\pm0.013$	-
Iron	$0.37^{a}\pm0.000$	$0.47^{b}\pm0.000$	$0.56^{c}\pm0.000$	0.01
Zinc	$0.13^{a}\pm0.000$	$0.47^{b}\pm0.000$	$0.56^{c}\pm0.000$	0.02
Copper	$0.11^{a}\pm0.000$	$0.13^{a}\pm0.000$	$0.37^{a}\pm0.000$	-
Manganese	$0.34^{a}\pm0.000$	$0.28^{b}\pm0.000$	$0.39^{c}\pm0.000$	0.03
Magnesium	$0.16^{a}\pm0.000$	$0.21^{b}\pm0.000$	$0.10^{c}\pm0.000$	0.04

Any two means values bearing the same superscript in the same column are not significantly different ($p<0.05$). Values preceding the means are standard deviations, LSD Least Significantly Differences. Values are means of duplicate determinations.
Sample A: Larva obtained from North Bank Market
Sample B; Larva obtained from Wurukum Market
Sample C: Larva obtained from Modern Market

Table 3. *Amino acids profile of Cirina forda sold in Makurdi metropolis*

g/100g protein				
Amino acid	**Sample A**	**Sample B**	**Sample C**	**LSD**
Lysine	$5.64^{a}\pm0.000$	$5.33^{b}\pm0.000$	$6.24^{c}\pm0.000$	0.04
Histidine	$2.57^{a}\pm0.004$	$2.32^{b}\pm0.002$	$2.40^{b}\pm0.000$	0.09
Arginine	$6.78^{a}\pm0.004$	$5.48^{b}\pm0.026$	$5.02^{c}\pm0.000$	0.20
Aspartic	$8.65^{a}\pm0.029$	$8.69^{a}\pm0.000$	$9.43^{b}\pm0.002$	0.17
Cystine	$1.45^{a}\pm0.062$	$1.28^{a}\pm0.000$	$1.07^{b}\pm0.000$	-
Valine	$3.18^{a}\pm0.010$	$4.18^{b}\pm0.000$	$4.05^{c}\pm0.001$	0.12
Glycine	$3.80^{a}\pm0.016$	$4.07^{b}\pm0.000$	$2.72^{b}\pm0.000$	0.44
Alanine	$4.31^{a}\pm0.007$	$3.63^{b}\pm0.002$	$4.34^{a}\pm0.000$	0.13
Threonine	$4.18^{a}\pm0.023$	$3.94^{b}\pm0.003$	$3.22^{c}\pm0.000$	0.18
Cerine	$3.03^{a}\pm0.004$	$3.31^{b}\pm0.000$	$2.91^{c}\pm0.004$	0.08
Glutamic	$14.34^{a}\pm0.058$	$13.41^{b}\pm0.000$	$12.24^{c}\pm0.000$	0.28
Croline	$3.12^{a}\pm0.002$	$2.34^{b}\pm0.004$	$2.74^{c}\pm0.002$	0.11
Methionine	$2.35^{a}\pm0.030$	$2.38^{a}\pm0.093$	$2.22^{a}\pm0.000$	-
Isoleucine	$3.27^{a}\pm0.007$	$3.37^{b}\pm0.000$	$4.31^{b}\pm0.000$	0.09
Leucine	$7.51^{a}\pm0.000$	$7.26^{b}\pm0.002$	$6.55^{c}\pm0.000$	0.05
Tyrosine	$2.74^{a}\pm0.000$	$3.205^{b}\pm0.003$	$3.53^{c}\pm0.002$	0.08
Phenyalanine	$5.08^{a}\pm0.007$	$4.90^{b}\pm0.000$	$4.57^{c}\pm0.000$	0.10

Any two means values bearing the same superscript in the same column are not significantly different ($p<0.05$). Values preceding the means are standard deviations, LSD Least Significantly Differences. Values are means of duplicate determinations.
Sample A: Larva obtained from North Bank Market
Sample B; Larva obtained from Wurukum Market
Sample C: Larva obtained from Modern Market

The amino acid profile of larva of *Cirina forda* shows the presence of isoleucine, leucine, methionine, phenylalanine, threonine, valine, lysine at varying amount and other non-essential amino acids at low but measurable levels (Table 3). The essential amino acids of *Cirina forda*, its daily requirement as well as the mass (g) of the larva required for the daily minimum requirements are reported in Table 4.

The microbial load of the larva revealed total viable count of 2.0 to 3.7 x 10^3 cfu/g and total fungal count of <30 cfu/g (Table 5). Predominant organisms isolated from the larva included *Escherichia coli, Salmonella* spp, *Staphylococcus aureus, Micrococcus* sp, *Proteus* sp, and the fungi *Aspergillus niger, Mucor* sp and *Rhizopus* sp (Table 6 and Table 7).

Table 4. *Essential amino acid profile of Cirina forda and daily requirement*

Amino acid	**Average acid content (mg/100g)**	**DMR (mg)**	**Minimum mass (g) of insect required for DMR**
Isoleucine	365	450-700	123
Leucine	711	620-1100	87
Methionine	235	550-1100	234
Phyenylalanine	485	220-1100	45
Threonine	378	310-500	82
Valine	380	650-800	171
Lysine	574	500-800	87

DMR- Daily Minimum Requirement

Table 5. *Microbial load of dried larva of Cirina forda*

Samples from various locations	**Total viable count (cfu/g)***	**Total fungal count(cru/g)**
North Bank Market	$3.7x10^3$	<30
Wurukum Market	$2.0x10^3$	<30
Modern Market	$2.3x10^3$	<30

*Mean value of duplicate determination

Table 6. *Mould Isolates of Cirina forda sold in Makurdi metropolis*

Samples bought different locations	**Isolates**	**Growth rate/days**	**Cfu/g**
North Bank Market	*Mucor* sp	2-3	<30
Wurukum Market	*Aspergillus niger*	3	<30
Modern Market	*Rhizopus* and *Mucor spp*	3	<30

Table 7. *Cutural and Biochemical characteristics of bacterial isolates*

Isolate Code	**NB_1, WM_1**	**NB_2, WM_3**	**MM_2, NB_3**	**WM_2,**	**MM_1**
Gram's reaction	-	-	+	+	-
Catalase	+	+	+	+	+
Oxidase	-	-	-	+	-
Coagulase	-	-	+	-	-
Indole	+	-	-	-	+
VP	-	-	+	-	-
MR	+	+	+		+

Isolate Code		NB_1, WM_1	NB_2, WM_3	MM_2, NB_3	WM_2,	MM_1
Spore		-	-	-	-	-
Motility		+	+	-	-	+
Citrate		-	+	+		-
	Lactose	AG	A	A	-	-
	Maltose	AG	A	A	-	A
Sugar Fermentation	Glucose	AG	A	A	-	AG
	Mannitol	AG	-	A	-	-
	Sucrose	AG	A	A	-	A
Probable organisms		*E.coli*	*Salmonella*	*Staph* *aureus*	*Micro-*	*coccus sp* *Proteus sp*

Key to Table 7
NB =North Bank Market
WM=Wurukum market
MM= Modern market
+= Positive
-=Negative
A=Acid production
G=Gas production

4. Discussion

The dried larva of *Cirina forda* had high protein and fat values (Table1). The crude protein content of 55.44% falls within the protein range of 55–60% previously reported for various forms of lepidopterous edible insects from the state of Mexico [4] and that of *Cirina forda* (55.5%) reported by [10]. However, the value was higher than the protein value (33.12%) of *Cirina forda* reported by [7]. This protein content obtained for *Cirina forda* in this study falls within the range of 23-60% recommended as daily protein requirement. Although whole insects as a source of protein are of somewhat lower quality than vertebrate animal products because of the indigestibility of chitin, the consumption of the caterpillars can supplement to a substantial degree some of the nutrients inadequate in cereals. Insect proteins have been recommended as a supplement for high cereal diets and weaning foods for infants FAO/UN [16]

The high amount of fat recorded (Table 1) is essential in diets as it increases the palatability of foods by absorbing and retaining their flavours. Fats are also vital in the structural and biological functioning of cells and help in the transportation of nutritionally essential fat-soluble vitamins. The low moisture content of the samples is an indication that the samples will have good storage stability if properly packaged. It may have also contributed to the reduction in microbial growth in the samples, possibly extending the shelf-life of the product.

The larva of *Cirina forda* is observed to be a good source of mineral elements with values for zinc and iron exceeding that reported by [7]. The caterpillars studied by [12], also proved to be excellent source of iron. Since *Cirina forda* is rich in iron, the blood building element, it would be desirable for human and animal consumption in anaemic conditions. Iron deficiency is a major problem in women's diet in the developing world (Africa), particularly among pregnant women and vegetarians everywhere are known to be at risk of zinc deficiency. Therefore the high content of iron and zinc in many edible insects including *Cirina forda* and potassium levels of the larva (Table 2) approximately 10:1. This favourable ratio renders the larva of *Cirina forda* a potential component of diets for the management of hypertension, since it has been documented that potassium intake is useful for lowering blood pressure by antagonizing the biological effect of sodium [17]. The magnesium level of *Cirina forda* was below its RDA, hence the larva would not be recommended for diet of people in need of magnesium. The nutritional value of the insect as determined by the amino acid composition shows that *Cirina forda* larva contains all the essential amino acids needed for human growth in nearly optimal proportions, hence can be considered as good quality or complete protein. However, the levels of the amino acids followed the general trend for insects of being low in methionine/cysteine, but high in lysine and threonine.

The total aerobic plate count of about 10^3cfu/g obtained in the samples is within the acceptable limit (10^4cfu/g) [18]. It is also lower than the total bacterial counts of 10^5-10^6 cfu/g observed in smoked meat product ("suya") [19]. Contamination of the samples by pathogens could have resulted during processing and handling. [19] also smoked meat product 'suya'.

5. Conclusion

The results of this study showed that the nutritional content of dried larva of *Cirina forda* compares favourably with those of conventional foods of animal origin. Hence, it has the potential to provide substantial amounts of protein, essential amino acids and minerals to the diet of low income people in the area of study (Makurdi metropolis), whose diets are usually deficient in animal protein, thereby alleviating their problem of protein malnutrition. The microbial load of the dried larva was within acceptable range. However, observed contamination with pathogens such as *Salmonella*, *E. coli* and *Staphylococcus aureus* could constitute health risk to consumers. The dried larva should therefore be subjected to further heating such as cooking before consumption.

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