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An Evaluation of the Nutrients and Some Anti-nutrients in Silkworm, *Bombyxmori* L. (Bombycidae: Lepidoptera)

Olumuyiwa T. Omotoso[*]

Ekiti State University, Ado-Ekiti, Department of Zoology, P.M.B. 5363,Ado-Ekiti. Nigeria

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Abstract

Insects are important as items of aesthetic values, pests and as food. *Bombyx mori* L. is an economically important insect. It is an edible insect that is eaten in the tropics. The larvae of silkworm, *B. mori*, are popular for silk production. Silk is produced when the larvae are ready to pupate. Both the larval and the pupal stages of *B. mori* were analyzed for their nutrient composition, protein solubility, mineral, functional and anti-nutritional factors. The pupal stage had a higher protein content (21.59%) than the larval stage which had 20.79%. The fat content of the larva was 17.57% while that of the pupa was 19.90%. The ash content was higher in the larva (6.34%) than that in the pupal stage (5.50%). The proteins in the larval and pupal stage have two iso-electric points. The results of the mineral analyses showed that mineral salts are persistently higher in the pupal stage than in the larval stage. The functional analyses revealed that the larval stage is highly desirable for chopped meat or powdered food production than the pupal stage. The anti-nutritional content of both stages fall within the tolerance levels with the exception of phytin phosphorus and phytate where higher values were obtained.

Key words: Larval stage, Pupal stage, *Bombyx mori,* Silkworm, Solubility, Tannin, Phytate.

1. Introduction

Arthropods have established intimate relationships with man and his valuables since time immemorial (Banjo *et al.,* 2006; Omotoso and Afolabi, 2007). They are the most diverse of all organisms on earth. While some of them are foes, an appreciable percentage of them are friends in that they are edible, useful for research, and some are used in controlling other organisms, pests, for example.

Members of the class Insecta are important sources of food to many animal species and are also a veritable source of dietary protein and nutrients to man (DeFoliart, 1992). The Silkworm, *Bombyx mori,* is a popular moth which belongs to the order Lepidoptera. This moth is remarkable in producing silk which is used in producing cloths and wigs that are of immense value to man. The rearing of silkworms was reported to have started around 2650 B.C. in China by the Queen of Huang-Di Empire (Satoshi, 2003). The rearing of silkworms consists of a series of tasks which includes the planting of mulberry plants, supplying of mulberry leaves to growing silkworm larvae, cleaning of the rearing beds, mounting of the larvae so that they will spin cocoons and collection and transporting of cocoons. Silkworm rearing requires relatively high temperatures (26 ^{o}C-28 ^{o}C) and high humidity (75%-90%) (Satoshi, 2003; Rahmathulla and Suresh, 2012).

The quests for animal proteins and nutrients have led to the investigation of alternative sources such as insects. Insects are reported to be rich in animal proteins, minerals and fats (Banjo *et al.,* 2006; Omotoso, 2006; Omotoso and Adedire, 2007, 2008). Some of the more popular insects eaten around the world include crickets, grasshoppers, ants, stick insects, beetles and various species of caterpillar. There are 1,417 known species of arthropods that are edible to humans (Yen, 2009). Edible insects are important sources of high protein to rural dwellers and many city dwellers in Nigeria (Fasoranti and Ajiboye, 1993). Among the most important orders of insects consumed in Nigeria are Coleoptera, Hymenoptera, Isoptera, Lepidoptera, Odonata, Orthoptera and they are highly priced (Fasoranti and Ajiboye, 1993). There has been a few studies on the nutrient composition of silkworm *B. mori* (Frye and Calvert, 1989; Finke 2002; Tom *et al.,* 2013). The present paper is carried out to determine the nutrient composition and the nutritive value of the insect as an alternative source of nutrients to man and his livestock.

[*] Corresponding author. e-mail: topeomoth@yahoo.co.uk.

2. Materials and Methods

2.1. Collection and Preparation of Bombyx mori Larvae and Pupae

The larvae and the pupae of silkworm *B. mori*, used for this work, were obtained from the Ministry of Agriculture, Sericulture Department in Ado-Ekiti, Ekiti State of Nigeria. Ado-Ekiti is a town situated in the tropical humid region of Nigeria. The town lies on latitude 7^{o} 38' 0' North of the Equator and longitude 5^{o} 13' 0' East of Greenwich Meridian and 456 meters above the sea level. The larvae were three weeks old and they were handpicked into a plastic container which was used to convey them to the laboratory. They were allowed to stay for 3 h. in the laboratory. The larvae were asphyxiated in a refrigerator for 3 h. and later oven-dried at 60 ^{o}C for 4 h. The pupae were handpicked with their cocoons. The pupae were a week old from the day they completed their cocoon formation. The pupae were oven-dried together with their cocoons at 60 ^{o}C for 4 h. The cocoons were later cut open with a razor blade and the pupae were poured into a container. The dried larvae and the pupae were separately pulverized with a blender and kept in air-tight bottles and put in refrigerator until needed for analysis.

2.2. Nutrient Composition Analysis

The moisture content, ash content, fat content and the mineral content were determined by employing the method explained by AOAC (1990). Sodium and potassium were determined by flame photometric method while phosphorus was determined by the phosphovanado-molybdate reagent method reported by AOAC (2005) using Spectronic 20 Colorimeter. Other minerals, such as magnesium, iron, calcium, zinc, manganese, lead, nickel, copper and cobalt, were determined with Alpha 4 Atomic absorption spectrophotometer. Carbohydrate was determined by difference. The protein content was determined by the method reported by Pearson (1970). Joslyn (1970) method was employed in the determination of the crude fiber content. The protein solubility was determined by Biuret method, using Standard Bovine Serum (BSA).

2.3. Determination of Anti-nutrient Composition

Young and Greaves (1940) method was employed in phytin determination. Oxalate was determined by the method reported by Day and Underwood (1986). Tannin was determined by the method reported by Markkar and Goodchild (1996). Alkaloid was determined by the method reported by Harbone (1973) while the Bohm and Kocipal-Abyazan (1994) method was adopted in flavonoid determination.

2.4. Determination of Functional Properties of Silkworm Protein

The saponin content was determined by adopting the method reported by Obadoni and Ochuko (2001). The methods reported by Coffman and Garcia (1977) were employed in the determination of the least gelation concentration, foaming capacity and foaming stability. Water and oil absorption capacity were determined by Beuchat (1977) methods while emulsion capacity and emulsion stability were determined by Yasumatsu *et al.* (1972) method.

The data collected were subjected to Analysis Of Variance (ANOVA) and where significant differences existed, treatment means were compared at 0.05 significant level using Tukey Test.

3. Results

The result of the nutrient analysis revealed that the ash content in the larval stage was 6.34% while it was 5.50% in the pupal stage. Protein was the second most prominent class of food in silkworm, in which 20.79% and 21.59% were obtained in the larval and pupal stages, respectively (Table 1). The moisture content ranged between 7.92% (in the larva) and 8.26% (in the pupa). Carbohydrate forms the main source of food found in silkworm. The proteins in silkworm are more soluble in acidic medium. The highest solubility obtained in the larval stage was 84.13% in pH 1 and pH 12. In the pupal stage, the highest solubility occurred in pH 1 and pH 11. Each of the developmental stages has two iso-electric points (Figure 1).

Table 1. Nutrient composition of Silkworm, *Bombyx mori*

Parameters (%)	*B. mori*larvae	*B. mori*pupal
Ash content	6.34±0.84	5.50±0.51
Moisture content	7.92±0.98	8.26±0.66
Fat content	17.57±1.51	19.90±1.80
Crude fibre content	6.46±0.21	6.30±0.12
Crude protein content	20.79±2.22	21.59±2.91
Carbohydrate content	40.93±3.20	38.47±4.24

Each value is a mean ± Standard deviation of three replicates (Tukey Test)

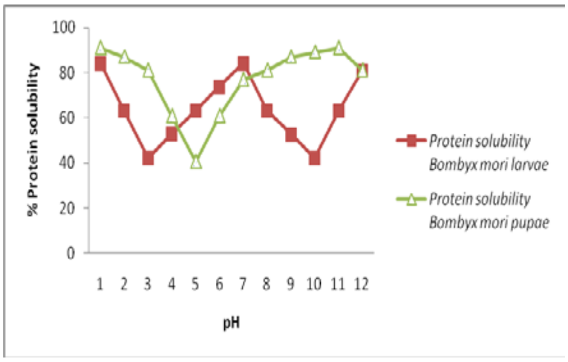

Figure. 1. Percentage Protein solubility of the larval and pupal stages of Silkworm, *B. mori*.

Table 2 shows the results of mineral analysis in the larval and the pupal stages. All the essential minerals are present in silkworm. Phosphorus was the highest mineral obtained in both stages. Copper was not discovered in the larval stage but a negligible quantity (0.10%) was obtained in the pupal stage. Iron, zinc, magnesium, calcium, potassium and sodium were obtained in different quantities in both larval and pupal stages. The quantities of trace mineral salts such as cobalt, nickel, copper, manganese and lead are negligible in both stages.

Table 2. Minerals present in Silkworm, *B. mori*.

Parameters (mg/100g)	*B. mori*larvae	*B. mori*pupal
Na	10.52±1.31	11.66±1.22
K	18.65±1.42	22.45±1.72
Ca	20.31±2.78	26.65±3.49
Mg	31.24±3.61	27.53±3.76
Zn	35.63±4.98	37.50±4.64
Fe	5.31±0.72	6.33±0.81
Pb	0.01±0.00	0.02±0.00
Mn	0.02±0.00	0.01±0.00
Cu	ND	0.10±0.00
P	37.66±4.10	41.35±5.82
Ni	0.01±0.00	0.10±0.00
Co	0.33±0.01	0.36±0.01

Each value is a mean ± Standard deviation of three replicates (Tukey Test). ND = Not Detected

The results of the functional properties of the proteins of silkworm is shown in Table 3. Water absorption capacity was 175% in the larvae while it was 115% in the pupa. Oil absorption capacity was higher in the pupal (284.87%) than in the larva (252.18%). Foaming capacity and foaming stability were not detected in both stages. Least gelation in both stages was 6% while emulsion capacity and stability in the larval stage was 75% and 25%, respectively. In the pupal stage, the emulsion capacity was 75% while the emulsion stability was 23%.

Table 3. Functional properties of the proteins in Silkworm, *B. mori*.

Parameters (%)	*B. mori* larvae	*B. mori*pupal
Water absorption capacity	175±8.50	115.00±7.30
Oil absorption capacity	252.18±9.22	284.87±9.45
Foaming capacity	ND	ND
Foaming stability	ND	ND
Emulsion capacity	75.00±3.24	75.00±3.50
Emulsion stability	25.00±2.11	23.00±2.45
Least gelation	6.00±1.02	6.00±1.12

Each value is a mean ± Standard deviation of three replicates (Tukey Test). ND = Not Detected.

Table 4 shows the results of anti-nutrient composition of silkworm. Phytate was the highest anti-nutrient recorded in both the larval stage (72.89%) and pupal stage (110.16%). The amounts of phytin phosphorus in both stages were higher (20.54% in larva and 31.03% in pupa). The quantity of saponin present in the larval stage was lower (6.88%) than the quantity obtained in the pupal stage (7%). Oxalate recorded the lowest quantity of 0.91% in the larval while 1.22% was obtained in the pupal stage. The quantity of flavonoid obtained in the pupal stage (11.54%) was higher that the quantity obtained in the larval stage (11.33%). Alkaloids and tannic acids contents in the larval stage were 8.55% and 1.93% respectively. In the pupal stage, alkaloid was 8.61% while tannic acid was 2.04%.

Table 4. Anti-nutrient composition of Silkworm, *B. mori*.

Constituents	*B. mori*larvae	*B. mori*pupal
Tannic acid (%)	1.93±0.12	2.04±0.24
Saponin (%)	6.88±0.18	7.00±0.88
Alkaloids (%)	8.55±1.26	8.61±1.12
Flavonoid (%)	11.33±1.34	11.54±1.93
Oxalate (mg/g)	0.91±0.07	1.22±0.01
Phytate (mg/g)	72.89±3.72	110.16±9.67
Phytin phosphorus (mg/g)	20.54±2.98	31.03±3.33

Each value is a mean ± Standard deviation of three replicates (Tukey Test).

4. Discussion

This study revealed that the larval and the pupal stages of *Bombyx mori* had high nutritional qualities (Table 1). The protein content of the larval stage (20.79%) and the pupal stage (21.59%) were higher than the values reported in *B. mori* (93g/kg)by Finke (2002). Lower protein contents have been reported by Banjo *et al.* (2006) in *Analeptes trifasciata* F. (Cerambycidae: Coleoptera), *Rhynchophorus phoenicis* F (Curculionidae; Coleptera) and *Zonocerus variegatus* L. (Pyrgomorphidae: Orthoptera). Adeyeye and Awokunmi (2010) reported protein values of 258 g/kg and 324g/kg for female and male giant African crickets, *Brachytrypes membranaceus* L. (Gryllidae: Orthoptera), respectively. Protein content of 11.37% was reported in *Epiphora bauhiniae* (Guerin Meneville) (Saturniidae: Lepidoptera). Protein is essential for the development and repair of body tissues of animals. Both the larval and the pupal stages of *B. mori* can adequately supply this essential protein to growing animals. The results of the protein solubility also attested to the fact that the proteins present in each of the developmental stages of *B. mori* contained so many amino acids. There are two iso-electric points in the larval and the pupal stages. The proteins present in each of the stages were more soluble in acidic medium than in alkaline medium (Figure 1). The minimum and maximum solubility in the larval is 42.07% and 84.13%, respectively. However, in the pupal stage, 40.51% was the minimum while 91.15% was the maximum. The moisture content of the developmental stages of silkworm was lower and this indicates that they will have a better shelf-life. The values of moisture content in the stages of *B. mori* were lower than the value reported by Finke (2002) for *B. mori* (827g/kg) and *B. membranaceus* by Adeyeye and Awokunmi (2010). Fat was the second most prominent food component in silkworm stages (17.57% - 19.90%). Fat content of 27% was reported in *E. bauhiniae* by Tom *et al.* (2013). Omotoso and Adedire (2007) reported that fat was the highest food value in the developmental stages of *R. phoenicis* F. (52.40% and 61.45%). Crude fiber which is very important in bowel movement was lower in both stages of *B. mori*. Carbohydrate content is the main food content in *B. mori*. The value of carbohydrate in *B. mori* (38.47% -40.93%) was higher than the value reported in *B. mori* (44g/kg) by

Finke (2002) but lower than the values reported in *Macrotermes notalensis* (Hav.) (Termitidae: Isoptera) (42.8%), *Apismellifera* L. (Apidae: Hymenoptera) (73.6%) and *Brachytrypes spp* (85.3%) by Banjo *et al.* (2006). The ash content was higher in all the stages of development of *B. mori*. However, Finke (2002) reported a lesser quantity in *B. mori* (11g/kg). Ash content of 1% was reported in wild silk moth, *E. bauhiniae* by Tom *et al.* (2013). Ash content is an indication of the quantity of the minerals present in the insect. Both the larval and the pupal stages were rich in minerals which are essential for the normal development and growth of organisms.

The highest mineral salt obtained in the larval and pupal stages of *B.mori* was phosphorus (Table 2). The same trend was reported by Finke (2002) in *B. mori*. The value of 136.4 mg/100g was obtained in *A. trifasciata* by Banjo *et al.* (2006) while Adeyeye and Awokunmi (2010) reported the value of 10880 mg/kg and 10936 mg/kg in male and female *B. membranaceus*, respectively. The highest mineral salt obtained in *R. phoenicis* ranged between 372.50 - 457.50 mg/kg (Omotoso and Adedire, 2007) while in *Cirinaforda* Westwood (Saturniidae: Lepidoptera), the highest mineral salt obtained was phosphorus (215.54 mg/100g) (Omotoso, 2006). The larval and pupal stages of *B. mori* were rich in important mineral salts, such as sodium (Na), potassium (K), calcium (Ca), iron (Fe), magnesium (Mg) and zinc (Zn). The amount of minerals, such as lead (Pb), manganese (Mn), copper (Cu), nickel (Ni) and cobalt (Co), were negligible. The values of minerals reported by Finke (2002) in *B. mori* are consistently higher than all the values reported in this study. Incorporating the larval and pupal stages of *B. mori* in the diets of both adults and children will greatly promote the normal functioning of the systems in the body. Magnesium, copper, zinc, selenium, iron, manganese and molybdenum are important co-factors found in the structure of certain enzymes and are indispensable innumerous biochemical pathways (Soetan *et al.,* 2010). Iron (Fe) is important in blood formation and blood plays a crucial role as a means of communication between the foetus and the mother during pregnancy. A pregnant woman requires 23g of iron per day in her diet (NHMRC, 2005). A pregnant woman can get the required iron content per day (23g) by incorporating 433g of the larvae or 363g of the pupae of silkworm into her diet. Zinc is a component of various enzymes that help maintain structural integrity of proteins and regulate gene expression. Zinc metallo enzymes include ribonucleic acid polymerases, alcohol dehydrogenase, carbonic anhydrase and alkaline phosphatase. The biological function of zinc can be catalytic, structural or regulatory. More than 85% of total body zinc is found in skeletal muscle and bone (King and Keen, 1999). The zinc content of the larvae and the pupae of silkworm are 6 times more than the daily requirements of boys and girls of 9-13 years (6 mg/day).

The results of the functional properties of silkworm show that the meat of the insect will be good and useful in food industries (Table 3). Functional properties of proteins in food are the physic-chemical properties which govern the behavior of protein in foods. The insect can be easily incorporated into confectioneries and comminuted foods, such as pie, cake and buns. The higher water absorption capacity shows that the meat of the insect will be very useful in confectionery industries that deal with baking processes such as bread bakeries. The higher oil absorption capacity also shows that the meat of the insect will be useful in cake and pie making industries since they will easily bind to greater quantities of oil. There are no reported works on the functional properties of insects that this result can be compared with except the work of Omotoso and Adedire (2008) in which higher water absorption capacity (93.33%) and higher oil absorption capacity (112.33%) were reported in *R. Phoenicis*. The foaming capacity and stability were not detected. This shows that the oil derived from silkworm may not be useful in food industries where foaming is essential to cause the rising of the food. The values of emulsion capacity (75%), stability (23%-25%) and least gelation concentrations (6%) are encouraging, showing that the silkworm meats are good candidates for food industries. Forming gels at 6% shows that the meat of this insect will be very good in canned food as the food will stay together in the can.

The highest anti-nutrient content of silkworm was phytate (72.89 mg/g-110.16 mg/g), followed by phytin phosphorus (20.54-31.03mg/g) while oxalate was the least (0.91 mg/g-1.22mg/g). The low levels of oxalate, tannic acid, saponin, alkaloid and flavonoids obtained in the developmental stages of silkworm are indications that the insect is very safe for human and livestock consumption (Table 4). Oxalates are naturally occurring substances found in plants, animals and humans (Rahman *et al.,* 2013). Oxalates combine with calcium and magnesium to form insoluble Ca and Mg oxalates which lead to low serum Ca and Mg levels as well as to renal failure because of the precipitation of these salts in the kidneys (kidney stones) (Rahman *et al.,* 2013). There are a few relatively rare health conditions that require strict oxalate restrictions. These conditions include absorptive hypercalciuria type II, enteric hyperoxaluria and primary hyperoxaluria (Rahman *et al.,* 2013). Saponins are also other naturally occurring compounds that are widely distributed in all cells of legumes (Shi *et al.,* 2004). Saponin has some beneficial effects on human. Such effects include promoting the immune system so as to protect it against cancer, lowering cholesterol levels, lowering the risks of contacting cancer, lowering blood glucose response, inhibiting dental caries and inhibiting platelets aggregation in humans (Shi *et. l.,* 2004). Flavonoids are ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves (Theoharis, 2000). Fruits and vegetables are the main dietary sources of flavonoids for humans, together with tea and wine (Yao *et al.,* 2004). Wink (2013) reported that legumes produce more nitrogen containing secondary metabolites, such as alkaloids and amines, non-protein amino acids, cyanogenic glucosides and peptides, than other plants. Flavonoids were reported to inhibit mast cell secretions in mammals (Theoharis, 2000). The importance of flavonoids in humans include antioxidative activity, free-radical scavenging capacity, coronary heart disease prevention and anti-cancer activity (Yao *et al.,* 2004). Silkworm has better nutritive values than pulses and legumes which contain higher levels of tannins, oxalates and phytic acids that are known to affect protein digestibility and absorption. Similar observations of lower anti-nutrient values were reported by Omotoso and Adedire (2008) in *R. phoenicis*. All the anti-nutrients reported in the developmental stages of silkworm could have been from the leaves (Mullberry leaves) they eat. Mullaney *et al.* (2000) reported that non-ruminant animals lack the required digestive phytase which removes phosphate from phytic acid, phytate and phytin phosphorus. Thus, the anti-nutrients are generally not bioavailable to non-ruminant animals.

5. Conclusion

Both the larval and the pupal stages of silkworm *B. mori* are rich in proteins and other food nutrients that are of importance to man and his livestock. These developmental stages (larvae and pupae) are also rich sources of most essential mineral salts, which are needed for the normal development of humans. It is a fact that these developmental stages are good candidates of food processing industries where higher oil and water absorption capacities are required.

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