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Chemical composition and nutritional value of mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae) different stages as a source of high protein edible insect

Rima, Shahin

Department of Applied Entomology and Zoology, Faculty of Aagriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt.

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Abstract

The mulberry silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) is considered the most commercially reared insect in the world, for its provision of natural silk. The present paper aimed to investigate the importance of this insect from different aspects, as an edible food. Nutritional value of immature stages, larvae, and pupae was determined and compared. The obtained results revealed that a hundred grams of dried pupae contain 53.30% of crude protein, ether extract (19.67%), energy (465.66 kcal), total phenolic (50.80 mg GAE), total flavonoids (55.21mg/CE), antioxidant activity (34.15%), Zn (22.69 mg), Fe (9.19 mg), K (1225.43 mg), P (932.71 mg), essential amino acids (34.94%) and unsaturated fatty acids (80.56%). Comparable with larval crude protein (48.55%), ether extract (8.86%), energy (404.76 kcal), total phenolic (47.01 mg GAE), total flavonoids (48.76 mg/CE), antioxidant activity (24.25%), Zn (16.39 mg), Fe (4.36 mg), K (1034.56 mg), P (659.94 mg), essential amino acids (22.75%) and unsaturated fatty acids (64.04%) contents. Therefore, the mulberry silkworm is strongly recommended as a source of protein and a substitute the conventional animals.

Introduction

The total number of humans on the planet will be over seven billion in 2022 (Glausiusz, 2022), given the current rate of global food production, considerable work remains to be done to fulfil the growing population food demand, especially in many developing countries in Africa and Asia. The expanding population of the world is anticipated to reach about 9 billion people by 2050 (FAO, 2009). Traditional livestock farming will become a less viable way for protein production with the land mass remaining constant, to uncover alternative protein sources and provide the chemical composition of edible insects, more collaboration between entomologists, and nutritionists is required. Taking into consideration, that the global livestock sector has a significant environmental impact, animal excrement and veterinary drugs that leach into land and water make it a serious polluter (Belluco *et al*., 2013).

Insects are classified under the phylum Arthropoda, they have been existing for at least 400 million years, making them one of the largest and oldest terrestrial animals on the planet accounting for up to 80% of the animal kingdom (Gaunt and Miles, 2002). Entomophagy, the utilization of insects as a source of protein, has a number of comparative advantages over livestock, such as, low cost of production with less land and water consumption, high fertility, they have a short life span (45 days on average), and have simple habitat and nutrient requirements, insect protein can be harvested very quickly, it contains more protein per kilogram than fish, pork or chicken (Wu *et al*., 2021). Also, insects are more genetically different than humans, therefore, the diseases that affect insects are considered less threat to infect humans (Oonincx and De Boer, 2012).

One third of the people worldwide, or more than 2 billion people, estimate over 1900 species of insects every day as part of their regular diet. Butterflies and moths, crickets, beetles, termites, ants, grasshoppers, wasps, and bees are among the edible insect species that have been consumed in 113 countries throughout the world (Bernard and Womeni, 2007). Our ancestors have been eating insects a long time ago, for many people it is the only available meal rich in protein, minerals, sugars, and vitamins (Nowak *et al*., 2016).

Silkworms have been the most remarkable and oldest domestic animals, including mulberry and non-mulberry silkworms not only for obtaining silk for weaving fabrics, but also consumed as a delicious food source in Vietnam, Thailand, India, Japan, Korea, and China (Pereira *et al*., 2003; Mishra *et al*., 2003; Zhu, 2004; Ji *et al*., 2016a and Altomare *et al*., 2020). Most importantly silkworm larvae and pupae have been authorized by the Food and Agricultural Organisation (FAO) as a source of protein with high nutritional value (FAO, WHO 2013). In their life cycle, silkworms go through four distinct stages: egg, larva, pupa, and imago. The larva spins a cocoon (Silk) to transform into a pupa, which provides a protective layer to the motionless pupal stage, it reared for its significant medicinal, nutritional, and commercial value (Yang *et al*., 2009 and Ratcliffe *et al*., 2011). The silkworm pupa accounts for 80% of the weight of fresh cocoons or 50% of the dry cocoons and is frequently thrown as waste material after the reeling process (Wu, 2001).

Many people in Egypt suffer from hunger and poverty, we aim this work to shed light on the importance of mulberry silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) which could be an elegant solution to many problems such as, minimizing food insecurity, to expanding sericulture in Egypt and the global food industry.

Therefore, the study here compares and assesses the proximate, amino acid composition, fatty acid profile, mineral, and carbohydrate in larval and pupal Egyptian strain.

Materials and methods.

1. Specimens:

A hybrid univoltine local strain of *B.mori* was provided from the Plant Protection Research Institute, Sericulture Research Department, Agricultural Research Centre, Giza, Egypt. Larvae were fed fresh mulberry leaves four times a day in a laboratory condition of 25±2 °C and 75±5 % relative humidity. The recommended practice of maintenance of hygiene such as disposal of silkworm litter and disease larvae, bed cleaning, was followed regularly (Krishnaswami *et al*., 1971). Samples were picked and gathered from the wondering stage of the last larval instar. The cocoons were cut open and taken the pupae five days old from the day they completed their cocoon spinning and kept at -20 °C until used.

2. Samples preparation:

The *B. mori* larvae and pupae were dehydrated for 10 hours in a hot air oven (X 4733, England), at 60 °C. After that, a laboratory grinder (Moulinex- AR1044) was used to mill the mixture, sieved with a mesh size of 50, and finally packed in polyethylene bags and stored at -20 °C until needed.

3. General chemical composition:

Proximate analysis of *B. mori* larvae and pupae was performed according to Association of the Official Analytical Chemists (AOAC, 2007) protocols. Briefly, the moisture content was assessed using atmospheric methods and drying at 105°C for four hrs., the micro kjeldahl method was used to determine the crude protein. Diethyl ether was used to extract the crude lipid, then quantified by a Soxhlet extraction method, a direct ashing method at 600°C was used to measure the ash content. The difference was used to compute the carbohydrate content (100 - the sum of moisture, protein, fat, and ash content). The total calorie (energy value) content was calculated according to the approach provided by Crisan and Sands (1978). The Folin-Ciocalteu technique was used to determine the total phenolic content (Singleton and Rossi, 1965). The total flavonoid content was evaluated using the aluminium chloride method as described by (Kiranmai *et al.*, 2011). DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method of Boly *et al*. (2016). All tests were done in triplicate.

4. Minerals content:

Experiments were carried out three times, minerals (Ca, Mg, Mn, Zn, Fe, Cu, Na, K) were measured in ash solution using an Atomic Absorption Spectrometer (AAS) AOAC (2000). Using a spectrophotometer (Alpha-1502; Laxco Inc, Bothell, WA, USA), total phosphorus (P) was colorimetrically measured at 630 nm AOAC (2003).

5. Amino acids composition:

Amino acids were measured using a high-performance Amino Acid Analyzer (Sykam GmbH, Germany) according to the method indicated in AOAC (2012), and the amino acid composition was reported in grams per 100 grams of protein. The essential amino acid (EAA) content to total EAA content in one-gram sample protein was divided by the same EAA content in the reference FAO/WHO (2013) pattern to determine the amino acid score (AAS). According to Khattab (2004), the following regression equations C-BV (%) = 39.55 + 8.89 Lysine were used to obtain the computed biological value of protein.

6. Fatty acid composition:

Fatty acid methyl esters of larval and pupal oil samples were determined using gas chromatography analysis (YL6100GC, Young LIN Instrument Co., Korea), as described by Radwan (1978).

Results and discussion

1. Proximate chemical composition:

In this paper, proximate chemical analysis, total phenolic, flavonoid content, and antioxidant activity of silkworm larvae and pupae is presented (Table 1) as a dry weight basis. The moisture content of the larvae was around 4.32%, which was like the figure reported by Ji *et al.* (2016b), while pupae were around 12.55%, which is higher than the 7.9% reported by Kim *et al*. (2017) makes the larval powder have a better shelf life. The ash content was 9.89-8.17%, in larvae and pupae, respectively, which reflects the quantity of minerals that exist in their bodies. This value was two or three times higher than that mentioned by Pereira *et al*. (2003); Kim *et al*. (2017) and Hirunyophat *et al*. (2021).

The crude protein was the most abundant component of both stages, in which 48.55% and 53.30% were obtained in the larvae and pupae, respectively. The amount of larval crude protein is lower than 66.12%

as reported by Ji *et al*., (2016b), while pupae same as those values of different pupal strains as recorded by Kim *et al*. (2017) and Tomotake *et al*. (2010). Recent studies have evaluated the crude protein content of *Bombyx* larvae and pupae, which was highly variable, ranging from 50 to 70% of the dry weight, this seems to be mainly due to the genetic nature, mulberry strains as a food source, voltinism, metamorphic stages and even within the same stage (Mishra *et al*., 2003; Pereira *et al*., 2003; Ji *et al*., 2016a; Kim *et al*., 2017; Hirunyophat *et al*., 2021 and Karthich Raja *et al*., 2019).

Extensive research on silk protein was carried out, two major proteins exist in the silk gland of the larval stage, fibroin and sericin. Fibroin is a fibrous protein, the main portion of the cocoon (70–80%), previous results showed that fibroin could be digested in the human intestine and enhanced the absorption of important elements, such as Zn, Fe, Mg, and Ca (Sasaki *et al*., 2000 a), reduced the high cholesterol levels in mice (Zhang, 1995). Fibroin has been widely used for medical application areas, including gene therapy, tissue engineering, vascular grafts, biological drug delivery, bone regeneration and wound healing (Zhang *et al*., 2009). While sericin is a hydrophilic globular protein, it accounts for 20-30% of silk protein, it has bioactive properties for utilization in the food and cosmetic industry, as well as in the medical field, such as, anticoagulant, antitumor, antibacterial, and anti-inflammatory drug, anticoagulant, acts in colon health, easing constipation (Zhaorigetu *et al*., 2003; Ogino *et al*., 2006 and Sasaki *et al*., 2000 b).

Silkworm pupa contains many proteins and bioactive peptides, which have variable biological activities including, antitumor, immunity improvement and antioxidant activity (Ni *et al*., 1998; Wang *et al*., 2007; Mishra *et al*., 2003 and Wu *et al*., 2020). Chinese have long history consumed silkworm pupae about 3000 years ago, as food and medicine to alleviate hypertension and fatty liver (Zhang and Zhang, 2001), extended health span and improved resistance to Parkinson's disease (Nguyen *et al*., 2016), decrease blood-glucose levels after one month of consuming silkworm protein with no negative effects and improve immunity (Ryu *et al*., 2013; Xiao *et al*., 2005 and Gui *et al*., 2001).

As stated in Table (1), the larval silkworm generally has a low crude ether extract, being 8.86% on a dry weight basis consistent with Ji *et al.* (2016b) results. In contrast, the crude ether extract of pupa had the second most prominent components 19.67% agreed with Rodriguez-Ortega *et al*. (2016) and Kim *et al*. (2017), and higher than reported by Wu *et al.* (2020). The crude fat of silkworm pupae was in the range of 25-30% on a dry weight basis (Kouřimská and Adámková, 2016 and Longvah *et al*., 2011), the high fat content demonstrates the significance of employing silkworm pupa to produce oil.

The edible oil extracted from the pupa of the silkworm has many advantages and uses in medical aspects (Shanker *et al*., 2006 and Wang *et al*., 2013). The experiments showed that when rats consumed pupal oil for several weeks had a remarkable reduction of triglyceride, total cholesterol, and glucose levels (Mentang *et al*., 2011 and Longvah *et al*., 2012), also suggested to control hyperlipidemia by decreasing the level of plasma lipid and lipoprotein in the rat serum (Hu and Chen, 2011).

The total carbohydrate content of silkworm larvae is 32.70%, while it is 18.85 %in the silkworm pupa. These values are lower than those reported by Kim *et al.* (2017), but higher than those reported by Trivedy *et al.* (2010) and Wu *et al.* (2020) and Hirunyophat *et al.* (2021). Chitin is the main component of carbohydrate percent in insects, especially during the larval stage, it can be digested in human gastric juice (Paoletti *et al*., 2007), It has many benefits for the intestine, including, reduced cholesterol levels (Zaccone, 2007) improve immunity and cancer resistance (Hu *et al*., 2005).

Additionally, both stages are a good source of energy, providing 404.76 and 465.66 Kcal/100 g from larvae and pupa, respectively which is compatible with the average values reported by (Kim *et al*., 2017 and Altomare *et al*., 2020). The proportion protein-derived calories were 47.48 to 45.78% for larvae and pupa, respectively.

In larvae, total phenolic content and flavonoid content are lower than in pupae. The same trend was noticed, the radical scavenging activity of larvae (24.25%) was lower than that in pupae (34.15%). The percentage of radical scavenging activity fluctuates between 54-68 in the silkworm (Trivedy *et al*., 2010 and Wannee and Luchai, 2020). These findings backed up the idea that silkworm larvae and pupa could be used as an antioxidant source. Plants like onion shoots (48.9%) and Tamarind (51.30%) have higher antioxidant activity than silkworms, respectively, whereas coriander leaves (26.5); Drumstick leaves (21.8%); curry leaves (24.7%); Allspice leaves (32.6%); and Mint leaves (33.0%) have the same results (Nascimento *et al*., 2017).

Table (1): Nutrient value of silkworm *Bombyx mori* (Larvae and pupae).

Component*	Silkworm larvae	Silkworm pupa
Moisture (%)	4.32±0.31	12.55±0.12
Crude protein (%)	48.55±0.23	53.30±0.43
Crude ether extract (%)	8.86±0.71	19.67±0.09
Total ash (%)	9.89±0.08	8.17±0.02
Total carbohydrate (%)	32.70±0.32	18.85±0.13
Total caloric value (kcal/100g)	404.76±4.92	465.66±5.48
Percentage of protein calories to total cal.	47.48±2.43	45.78±2.59
Total phenolic content (mg GAE/100g)	47.01±0.06	50.80±0.05
Total flavonoids content (mg/CE/100g)	48.76±1.08	55.21±1.65
Radical scavenging activity (%Inhibition)	24.25±1.78	34.15±1.45

***Data as mean ± SD (On dry weight basis).**

2. Minerals contents:

Table (2) shows the contents of assessing minerals for silkworm larvae and pupa, as well as dietary reference values (DRVs) for minerals, which comprise average value adequate intakes (AIs) and reference intake (RIs) ranges. Nine kinds of minerals have been detected, and varying amounts of all minerals in both the larval and pupal stages were obtained. The most abundant minerals in both stages were phosphorus and potassium, with a very low Na/K ratio (0.01), since the high value of this ratio is related to the increase in hypertension (Aburto *et al*., 2013).

In contrast, the amounts of trace minerals as manganese were minimal in both stages. Larvae and pupae have a high level of micro minerals (Fe and Zn) particularly in the pupae where 61.27% and 151.27% of daily requirements (PRI/AI), respectively. Iron and zinc are essential for the immune system and blood production (Talwar *et al*., 1989). A low level of calcium was present in silkworm larvae and pupae because they lack mineralised skeleton, where calcium exists and bind to the chitin (Oonincx and Finke, 2020). The Values of Fe, Ca and Mg in silkworm were about 0.5 to 2.4 times higher than milk, fresh beef, egg, and chicken (Akhtar and Isman, 2018).

This research revealed that the pupal stage mineral concentrations are consistently higher than the larval stages, the same trend was reported by Finke (2002); Omotoso (2015) and Rodriguez-Ortega *et al*. (2016). Silkworm contains all the essential minerals for the regular development and growth of human health (Kim *et al*., 2017; Tang *et al*., 2019 and Zielinska *et al*., 2015). As a result, the silkworm could be appropriate for dietary supplements and should be included in the meals of both adults and children.

Table (2): Minerals content mg/100g of *Bombyx mori* and dietary reference values (DRVs).

Minerals mg/100g	Silkworm larvae	Silkworm pupae	Dietary reference values (DRVs)*
Fe	4.36±0.64	9.19±0.58	15
Zn	16.39±0.92	22.69±0.98	15
Ca	168.35±2.37	109.18±2.85	1000
Mg	189.21±4.87	164.54±4.04	350
Cu	0.67±0.42	1.14±0.04	1.3-1.6
Mn	0.41±0.30	0.62±0.48	5
Na	13.16±0.64	14.20±0.65	2000
K	1034.56±6.65	1225.43±6.47	3500
P	659.94±1.64	932.71±1.73	700

*__Data as mean ± SD (On dry weight basis).__

****European Food Safety Authority (EFSA). Dietary Reference Values for Nutrients Summary Report (2019).**

3. Fatty acid composition:

The fatty acid profile (g/100g) of the composition is presented in Table 3 (% of total fatty acids). Monounsaturated fatty acids (42.15%) made up many of the total detected fatty acids in the larvae, followed by saturated fatty acids (35.96%) and polyunsaturated fatty acids (21.89%). Silkworm larvae lipids have a saturated fatty acid: unsaturated fatty acid ratio of 1: 1.78. Palmitic acid (C16:0) had the largest proportion of saturated fatty acids in silkworm larvae (25.44%), while oleic acid (C18:1) and linolenic acid (C18:3) had the highest quantities of monounsaturated and polyunsaturated fatty acids being 41.94% and 20.13%, respectively. The present results are in accordance with Ji *et al*. (2016a), they investigated that the primary fatty acids found in silkworm larvae lipids were oleic, palmitic and linolenic acids.

The oil of silkworm pupae contains 19.44% saturated fatty acids, 45.69% monounsaturated fatty acids, and 34.87 percent polyunsaturated fatty acids. Oleic acid (C18:1) was the major, accounting for 45.63 percent of total fatty acids, while linolenic acid (C18:3) was the second most common (34.56 %). These results agreed with those obtained by Pereira *et al*. (2003); Yang *et al*. (2009) and Hirunyophat *et al*. (2021). The saturated fatty acid: unsaturated fatty acid ratio of silkworm pupae lipids was 1: 4.14. This ratio is slightly lower than that of typical crude oils like soybean, sesame, and olive oils (Yalcin *et al*., 2012) and greater than that of cotton seed oil (Orsavova *et al*., 2015). It should be noted that both larvae and pupae contain large levels of monounsaturated fatty acids, particularly oleic acid, almost half percent of the oil, which has been shown to be anti-inflammatory, utilized to lower high blood cholesterol levels, cardiovascular and immune diseases (Sales-Campos *et al*., 2013). The consumption of polyunsaturated fatty acids in the human diet has various health benefits for humans. Pupal and larval oil are especially rich in essential linoleic acid, which has been demonstrated to prevent brain stroke (Blondeau *et al*., 2015). Insufficient intake of linolenic acid may lead to kidney, liver, vision, skin, and neurological diseases (Psota *et al*., 2006).

Table (3): Fatty acid content of the larvae and pupae of silkworm *Bombyx mori* (% of total fatty acids).

Fatty acid %	Silkworm larvae	Silkworm Pupae
Capric acid(C10:0)	0.05	-
Myristic acid(C14:0)	-	0.02
Palmitic acid(C16:0)	25.44	17.59
Margaric (C 17:0)	0.03	0.02
Stearic acid(C18:0)	10.04	1.81
Nonadecylic(C 19:0)	0.16	-
Arachidic acid(C20:0)	0.24	-
Myristolic acid(C14:1n9c)	0.04	-
Palmitolic acid(C16:1n9c)	0.17	0.06
Oleic acid(C18:1n9c)	41.94	45.63
Linoleic acid(C18:2n6c)	1.49	0.30
Linolenic acid(C18:3n3)	20.13	34.56
Dihomo-γ-linolenic acid (C20:3)	0.27	-
Eicosapentaenoic(C20:5n-3)	-	0.01
Total (Σ SFA)*	35.96	19.44
Total (Σ UFA)**	64.04	80.56
Total (Σ MUFA)***	42.15	45.69
Total (Σ PUFA)****	21.89	34.87
Saturated:unsaturated ratio	1: 1.78	1: 4.14

*** Saturated fatty acids.**
****Unsaturated fatty acids.**
***** Mono unsaturated fatty acids.**
****** Poly unsaturated fatty acids.**

4. Amino acid composition:

The amino acid content of silkworm larvae and pupa is described in (Table 4). The proportion of EAAs in total amino acids recorded was 22.67 and 34.94 percent for larvae and pupae, respectively. The ratios of EAAs to non-EAAs were (Larvae 0.50) and (Pupae 0.68), which were close to 0.6 suggested reference levels of the FAO/WHO (1973). Silkworm essential amino acid concentration was 2-4 times higher than that of egg, pork, milk, beef, and chicken (Yang *et al*., 2009). The computed biological value of protein in silkworm larvae and pupae were 70.75 and 98.85%, respectively. The amino acid results in Table (4) demonstrated that leucine, lysine and threonine were found in the highest amounts in silkworm larvae among the essential amino acids. Meanwhile, the non-essential amino acids glycine and alanine were the most abundant, followed by serine and tyrosine. In contrast, glutamic acid, aspartic acid, leucine, lysine and proline were the most abundant amino acids in silkworm pupae. The current findings are consistent with prior investigations of larva and pupa amino acid analysis, particularly the main amino acid contents (Ji *et al*., 2016a; Zhou and Han, 2006 and Longvah *et al*., 2011). Interestingly, the amino acid content of the larval stage was completely different from the pupal stage. The presence of enlarged silk glands in mature larvae, which contain the silk fibre, explains these discrepancies. The two primary proteins in silk fibre, fibroin and sericin, have high levels of glycine, alanine, serine, and tyrosine in their amino acid compositions (Mondal *et al*., 2007 and Ji *et al*., 2016a). On the other hand, the major protein of the pupa is storage proteins, which contain a high concentration of necessary amino acids and are largely employed as a source of adult protein synthesis throughout the transformation (Altomare *et al*., 2020).

Table (4): Total amino acid content of the larvae and pupae of silkworm *Bombyx mori* (g/100g protein).

Amino Acids	Silkworm larvae	Silkworm pupae
Aspartic acid	4.53	9.42
Threonine	3.81	4.30
Serine	6.12	4.47
Glutamic acid	4.18	11.74
Proline	1.92	6.43
Glycine	11.07	4.23
Alanine	9.55	3.45
Valine	3.21	4.69
Methionine	1.61	2.05
Isoleucine	1.42	3.41
Leucine	3.79	6.74
Tyrosine	5.15	5.86
Phenylalanine	3.69	4.31
Hisitidine	1.63	2.77
Lysine	3.51	6.67
Argnine	2.13	4.96
Cysteine	0.41	1.3
Total (ΣEAA) *	22.67	34.94
Total (ΣNEAA) **	45.06	51.68
C-BV (%) ***	70.75	98.85

***EAA: Essential amino acids**

**** NEAA: Non-essential amino acids.**

***** Computed biological value of protein.**

The essential amino acids were compared to the FAO/WHO (2013) ideal amino acid (Table 5). Silkworm larvae and pupae had amino acid scores of 89.60 and 138.10 percent, respectively, with essential amino acids in pupae exceeding FAO/WHO reference values for adults, which was found to meet the FAO/WHO (2007) recommendations, because of its high amount of necessary amino acids, silkworm pupae protein is termed a complete protein.

Table (5): The essential amino acid content of silkworm *Bombyx mori* larvae and pupae was compared to the FAO/WHO pattern (g/100g protein) and adult amino acid requirements.

Amino Acid	Silkworm larvae	Silkworm pupae	FAO/WHO pattern*	Amino acid score**		Requirements, mg/kg per day***
				Silkworm larvae	Silkworm pupae	
Threonine	3.81	4.30	2.3	165.65	186.96	7
Valine	3.21	4.69	3.9	82.31	120.26	10
Methionine	1.61	2.05	2.3	70	89.13	13
Isoleucine	1.42	3.41	3.0	47.33	113.67	10
Leucine	3.79	6.74	5.9	64.24	114.24	14
Phenylalanine	3.69	4.31	3.8	97.11	113.42	14
Hisitidine	1.63	2.77	1.6	101.88	173.13	8-10
Lysine	3.51	6.67	4.8	73.13	138.96	12
Total EAA	22.67	34.94	25.30	89.60	138.10	

***Pattern for adults FAO/WHO, 2013.**

****The amount of g/100 g protein of EAA sample divided by the same EAA g/100 g protein of the FAO/WHO standard pattern × 100.**

***** Amino acid requirements of adults (FAO/WHO, 2007).**

Mulberry silkworm has astonishing high nutritional values, particularly in the pupal stage. Not only contains high quantity protein, but also high-quality protein and essential amino acids for human daily requirements. It competes with other plants and animals such as chicken, pork, beef, fish, maize and soybeans of protein content and energy. The amino acid profile in larvae and pupae differed, suggesting that they may be used to generate distinct food products. Using protein powder extracted from silkworm in different food products could improve customer acceptance. The second most important component were found in the pupa is fat, which enriched with oleic acid and essential linolenic acid. In addition, silkworm should also be regarded as source of antioxidants and minerals on a commercial scale. Mulberry silkworm has a lot of potential for narrowing the food deficiencies that are widespread in many developing countries.

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References

Aburto, N.J.; Hanson, S.; Gutierrez, H.; Hooper, L.; Elliott, P. and Cappuccio, F.P. (2013): Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. British Medical Journal, 346: 1-19. https://doi.org/10.1136/bmj.f1378.

Akhtar, Y. and Isman, M.B. (2018): Insects as an alternative protein source. In: Yada, R.Y. (ed.) Proteins in Food Processing. Cambridge, UK: Woodhead. pp. 263-288. https://doi.org/10.1016/B978-0-08-100722-8.00011-5.

Altomare, A.A.; Baron, G.; Aldini, G.; Carini, M. and D'Amato, A. (2020): Silkworm pupae as source of high-value edible proteins and of bioactive peptides. Food Science & Nutrition, 8:2652-2661. http://dx.doi.org/10.1002/fsn3.1546.

AOAC (Association of Official Methods of Analytical Chemists) (2000): Official Methods of Analysis 17th Ed., Washington, DC, USA.

AOAC (Association of Official Methods of Analytical Chemists) (2003): Official Methods of Analysis 16th Ed . Arlington, Virginia, USA.

AOAC (Association of Official Methods of Analytical Chemists) (2007): Official Methods of Analysis. 18th Ed. Maryland, USA.

AOAC (Association of Official Methods of Analytical Chemists) (2012): Official Methods of Analysis. No. 994.Chapter 4, p. 9- 13- 19th Ed. USA.

Belluco, S.; Losasso, C.; Maggioletti, M.; Alonzi, C.C.; Paoletti, M.G. and Ricci, A. (2013): Edible insects in a food safety and nutritional perspective: a critical review. Comprehensive Reviews in Food Science and Food Safety 12(3): 296-313. https://doi.org/10.1111/1541-4337.12014.

Bernard, T. and Womeni, H.M. (2007): Entomophagy: Insects as food. In: Vonnie, D. and Shields, C. (eds.) Insect Physiology and Ecology, IntechOpen Publisher, Rijeka, Croatia, pp. 233-253. http://dx.doi.org/10.5772/67384.

Blondeau, N.; Lipsky, R.H.; Bourourou, M.; Duncan, M.W.; Gorelick, P.B. and Marini, A.M. (2015): Alpha-linolenic acid: An omega-3 fatty acid with neuroprotective properties-ready for use in the stroke clinic? BioMed

Research International, 519830. https://doi.org/10.1155/2015/519830.

Boly, R.; Lamkami, T.; Lompo, M.; Dubois, J. and Guissou, I. (2016): DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. International Journal of Toxicological and Pharmacological Research, 8(1): 29-34.

Crisan, E.V. and Sands, A. (1978): Nutritional Value of Edible Mushroom. In: Chang, S.T. and Hayer, W.A. (eds.) Biology and Cultivation of Edible Mushrooms. Academic Press, New York, pp. 137-168. https://doi.org/10.1016/C2013-0-10484-9.

European Food Safety Authority (EFSA) (2019): Dietary Reference Values for Nutrients. Summary Report. EFSA supporting publication, pp. 14-121. https://doi:10.2903/sp.efsa.2017.e15 121.

FAO (Food and Agriculture Organization of the United Nations) (2009): The State of Food and Agriculture: Livestock in the Balance. Rome, Italy.

FAO/WHO (Food and Agriculture Organization and World Health Organization) (1973): Energy and Protein Requirements. FAO/WHO, Rome, Italy.

FAO/WHO (Food and Agriculture Organization and World Health Organization) (2007): Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint FAO/WHO/UNU expert consultation. Technical Report Series 935. World Health Organization, Geneva, pp 206.

FAO/WHO (Food and Agriculture Organization and World Health Organization) (2013): Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Food and Nutrition, FAO, Rome, Italy. pp92.

Finke, M.D. (2002): Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology, 21: 269-285. https://doi.org/10.1002/zoo.10031.

Gaunt, M.W. and Miles, M.A. (2002): An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Molecular Biology and Evolution, 19:748-761.

Glausiusz, J. (2022): Global population is crashing, soaring, and moving. Population in the twenty-first century, Books and arts, Science in culture. Available at: https://www.nature.com/articles/d41 586-022-00926-6.

Gui, Z.; Chen, J.; Chen, W. and Zhuang, D. (2001): Effect of silkworm powder (SP) lowering blood-glucose levels in mice and its mechanism. Science of Sericulture, 27: 114-119 (In Chinese).

Hirunyophat, P.; Chalermchaiwat, P.; Onnom, N. and Prinyawiwatkul, W. (2021): Selected nutritional quality and physicochemical properties of silkworm pupae (frozen or powdered) from two species. International Journal of Food Science and Technology, 56: 3578-3587. https://doi.org/10.1111/ijfs.14985.

Hu, D.; Liu, Q.; Cui, H.; Wang, H.; Han, D. and Xu, H. (2005): Effects of amino acids from selenium-rich silkworm pupas on human hepatoma cells. Life Sciences, 77: 2098-2110. https://doi.org/10.1016/j.lfs.2005.02. 017.

Hu, J.L. and Chen, W.P. (2011): Effect of silkworm chrysalis oil on apo-protein and lipid- metabolizedenzyme level in hyperlipidemia rats. Chinese Traditional and Herbal Drugs, 42(02): 300-306. https://doi.org/10.1088/1009-0630/13/1/25 (In Chinese).

Ji, S.D.; Kim, N.S.; Kweon, H.Y.; Choi, B.H. and Koh, Y.H. (2016a): Nutrient compositions of *Bombyx mori* mature silkworm larval powders suggest their possible health improvement effects in humans. Journal of Asia-Pacific Entomology, 19: 1027-1033. https://doi.org/10.1016/j.asr.2008.12.009.

Ji, S.D.; Kim, N.S.; Kweon, H.Y.; Choi, B.H.; Yoon, S.M.; Kim, K.Y. and Koh, Y.H. (2016b): Nutrition composition differences among steamed and freeze-dried mature silkworm larval powders made from 3 *Bombyx mori* varieties weaving different coloured cocoons. International Journal of Industrial Entomology, 33(1): 6-14. http://dx.doi.org/10.7852/ijie.2016.33.1.6.

Karthick Raja, P.; Aanand, S.; Stephen Sampathkumar, J. and Padmavathy, P. (2019): Silkworm pupae meal as alternative source of protein in fish feed. Journal of Entomology and Zoology Studies, 7(4): 78-85.

Khattab, R.Y.I. (2004): Chemical and technological studies on flaxseed to improve its sensorial and nutritional properties in food. PHD Thesis, Faculty of Agriculture. Alexandria University.

Kim, S.K.; Weaver, C.M. and Choi, M.K. (2017): Proximate composition and mineral content of five edible insects consumed in Korea. CyTA-Journal of Food, 15(1): 143-146. https://doi.org/10.1016/j.foodchem.2011.03.041.

Kiranmai, M.; Kumar, C.M. and Mohammed, I. (2011): Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(3): 254-261.

Kouřimská, L. and Adámková, A. (2016): Nutritional and sensory quality of edible insects. Journal of Nutrition and Food Security, 4:22-26. https://doi.org/10.1016/j.nfs.2016.07.001.

Krishnaswami, S.; Kumararaj, S.; Viyayaraghavan, K. and Kasiviswanathan, K. (1971): Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing, and nitrogen fertilization. Indian Journal of Sericulture, 10:79–89.

Longvah, T.; Mangthya, K. and Ramulu, P. (2011): Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae. Food Chemistry 128(2): 400-403. https://doi.org/10.1016/j.foodchem.2011.03.041.

Longvah, T., Manghtya, K., & Qadri, S. S. (2012). Eri silkworm: A source of edible oil with a high content of α-linolenic acid and of significant nutritional value. Journal of the Science of Food and Agriculture, **92**(9), 1988–1993. https://doi.org/10.1002/jsfa.5572

Mentang, F.; Maita, M.; Ushio, H. and Ohshima, T. (2011): Efficacy of silkworm (*Bombyx mori* L) chrysalis oil as a lipid source in adult Wistar rats. Food Chemistry, 127: 899-904. https:// doi.org/10.1016/j.foodchem.2011.01.045.

Mishra, N.; Hazarika, N.C.; Narain, K. and Mahanta, J. (2003): Nutritive value of non-mulberry and mulberry silkworm pupae and consumption pattern in Assam, India. Nutrition Research, 23: 1303-1311. https://doi.org/10.1016/S0271-5317(03)00132-5.

Mondal, M.; Trivedy, K. and Irmal Kumar, SN. (2007): The silk proteins, sericin and fibroin in silkworm, Bombyx mori Linn., A review. Caspian Journal of Environmental Sciences, 5:63-76.

Nascimento, K.O.; Reis, I.P. and Augusta, I.M. (2017): Total phenolic and antioxidant capacity of flower, leaf and seed of *Moringa oleifera*. International Journal of Food and Nutrition Research, 1: 1-6.

Nguyen, P.; Kim, K-Y.; Kim A-Y; Kim, N-S.; Kweon, H.Y.; Ji, S-D. and Koh, Y.H. (2016): Increased health span and resistance to Parkinson's disease in Drosophila by boiled and freeze-dried mature silkworm larval powder. Journal of Asia-Pacific Entomology, 19: 551-561. https://doi.org/10.1016/j.aspen.2016.05.003.

Ni, H.; Chen, H. X.; Yang, Y. Y. and Tao, L. (1998): Studies on extraction and preparation technique of silkworm chrysalis (*Bombyx mori* L.) pupa chitin and chitosan. Journal of Hubei University (Natural Science Edition), 20(1): 94-96. (In Chinese)

Nowak, V.; Persijn, D.; Rittenschober, D. and Charrondiere, U.R. (2016): Review of food composition data for edible insects. Food Chemistry, 193: 39-46. https://doi.org/10.1016/j.foodchem.2014.10.114.

Ogino, M.; Tanaka, R.; Hattori, M.; Yoshida, T.; Yokote, Y. and Takahashi, K. (2006): Interfacial behavior of fatty-acylated sericin prepared by lipase-catalyzed solid-phase synthesis. Bioscience, Biotechnology and Biochemistry, 70(1): 66-75. https://doi.org/10.1271/bbb.70.66.

Omotoso, O.T. (2015): An Evaluation of the nutrients and some anti-nutrients in silkworm, *Bombyx mori* L. (Bombycidae: Lepidoptera). Jordan Journal of Biological Science, 8(1): 45-50.

Oonincx, D.G.A.B. and De Boer, I.J.M. (2012): Environmental impact of the production of mealworms as a protein source for humans-a life cycle assessment. PloS One 7(12): e51145. https://doi.org/10.1371/journal.pone.0051145.

Oonincx, D.G.A.B. and Finke, M.D. (2020): Nutritional value of insects and ways to manipulate their composition. Journal of Insects as Food and Feed, 7(5):1-22. https://doi.org/10.3920/JIFF2020.0050.

Orsavova, J.; Ladislava ,M.; Ambrozova, J.; Vicha, R. and Mlcek, J. (2015): Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. International Journal of Molecular Sciences, 16: 12871-12890. https://doi.org/10.3390/ijms160612871.

Paoletti, M.G.; Norberto, L.; Damini, R. and Musumeci, S. (2007): Human gastric juice contains chitinase that can degrade chitin. Annals of Nutrition and Metabolism, 51: 244-251. https://doi.org/10.1159/000104144.

Pereira, N.R.; Ferrarese-Filho, O.; Matsushita, M. and DeSouza, N.E. (2003): Proximate composition and fatty acid profile of *Bombyx mori* L. chrysalis toast. Journal of Food Composition Analysis, 16: 451-457. https://doi.org/10.1016/S0889-1575(03)00016-4.

Psota, T.L.; Gebauer, S.K. and Kris-Etherton, S.K. (2006): Dietary Omega-3 Fatty Acid Intake and Cardiovascular Risk. The American Journal of Cardiology, 98(4): 3-18. https://doi.org/10.1016/j.amjcard.2005.12.022.

Radwan, S.S. (1978): Coupling of two-dimensional thin layer chromatography with gas chromatography for the quantitative analysis of lipid classes their constituent fatty acid. Journal of Chromatographic Science, 16: 538-542.

Ratcliffe, N.A.; Mello, C.B.; Garcia, E.S.; Butt, T.M. and Azambuja, P. (2011): Insect natural products and processes: New treatments for human disease. Insect Biochemistry and Molecular Biology, 41(10): 747-769. https://doi.org/10.1016/j.ibmb.2011.05.007.

Rodriguez-Ortega, A.; Pino-Moreno, J.M.; Angeles-Campos, S.K.; Garcia-Perez, A.; Barron-Yanez, R.M. and Callejas-Hernandez, J. (2016): Nutritional value of larvae and pupae of silkworm (*Bombyx mori*) (Lepidoptera: Bombycidae). Revista Colombiana de Entomologia, 42(1): 69-74 (In Spanish).

Ryu, K.S.; Lee, H.S.; Kim, K.Y.; Kim, M.J.; Sung, G.B.; Ji, S.D. and Kang, P.D.(2013): 1-Deoxynojirimycin content and blood glucose-lowering effect of silkworm (*Bombyx mori*) extract powder. International Journal of Industrial Entomology, 27: 237-242. https://doi.org/10.7852/IJIE.2013.27.2.237.

Sales-Campos, H.; Reis de Souza, P.; Crema Peghini, B.; Santana da Silva, J. and Ribeiro Cardoso, C. (2013): An overview of the modulatory effects of oleic acid in health and disease. Mini Reviews in Medicinal Chemistry, 13(2): 201-210. https://doi.org/10.2174/1389557511313020003.

Sasaki, M., Yamada, H. and Kato, N. (2000 a): Consumption of silk protein, sericin elevates intestinal absorption of zinc, iron, magnesium and calcium in rats. Nutrition Research, 20: 1505-1511.

Sasaki, M.; Yamada, H. and Kato, N. (2000 b): A resistant protein, sericin improves atropine-induced constipation in rats. Food Science and Technology Research, 6(4): 280-283.

Shanker, K.S.; Shireesha, K.; Kanjilal, S.; Kumar, S.V.; Srinivas, C.; Rao, J.V. and Prasad, R.B. (2006): Isolation and characterization of neutral lipids of desilked eri silkworm pupae grown on castor and tapioca leaves. Journal of Agricultural and Food Chemistry 54(9): 3305-3309. https://doi.org/10.1021/jf060581x.

Singleton, V. L. and Rossi, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16 (3):144–158.

http://www.ajevonline.org/cgi/content/abstract/16/3/144. doi: 10.5344/ajev.1965.16.3.144.

Tang, C.; Yang, D.; Liao, H.; Sun, H.; Liu, C.; Wei, L. and Li, F. (2019): Edible insects as a food source: a review. Food Production, Processing and Nutrition, 1:8. https://doi.org/10.1186/s43014-019-0008-1.

Tomotake, H.; Katagiri, M. and Yamato, M. (2010): Silkworm pupae (*Bombyx mori*) are new sources of high-quality protein and lipid. Journal of Nutritional Science and Vitaminology, 56: 446-448. https://doi.org/10.3177/jnsv.56.446.

Trivedy, K.; Ramesh, M.; Nirmal Kumar, S. and Qadri, S.M.H. (2010): Major nutritional component of silkworm (*Bombyx mori* L:) powder. Indian Journal of Sericulture, 49(2): 210-214.

Wang, G. J.; Yin, W. F.; Wang, J.; Tang, J. L. and Huang, Y. P. (2007): Effect of polysaccharide of silkworm pupa on immunological function in mice. Journal of Jiangsu University (Medicine Edition), 17(5): 373-375. https://doi.org/10.13312/j.issn.1671-7783.2007.05.001 (In Chinese).

Wang, J.; Zhang, J.L and Wu, F.A. (2013): Enrichment process for α-linolenic acid from silkworm pupae oil. European Journal of Lipid Science and Technology, 115 (7): 791-799. https://doi.org/10.1002/ejlt.201200324.

Wannee, S. and Luchai, L. (2020): 1-Deoxynojirimycin and polyphenolic composition and antioxidant activity of different native Thai silkworm (*Bombyx mori*) larvae. Journal of King Saud University-Science, 32: 2762-2766. https://doi.org/10.1016/j.jksus.2020.06.012.

Wu, N.; Wang, X.; Xu, X.; Cai, R. and Xie, S. (2020): Effects of heavy metals on the bioaccumulation, excretion, and gut microbiome of black soldier fly larvae (*Hermetia illucens*). Ecotoxicology and Environmental Safety, 192: 110323. https://doi.org/10.1016/j.ecoenv.2020.110323.

Wu, X.; He, K.; Velickovic, T.C. and Liu, Z. (2021): Nutritional, functional, and allergenic properties of silkworm. Food Science & Nutrition, 9(2): 1-11. https://doi.org/10.1002/fsn3.242.

Wu, Y.M. (2001): Study on nutrient ingredients of yellow blood silkworm. Guangdong Academy of Agricultural Sciences, 4: 31-33 (in Chinese).

***Xiao*, H.; Si, X.; Luo, C.; Cui, W. and Li, R. (2005):** Comparative study of silkworm powder compound on lowering both blood glucose level and blood fat level. Science of Sericulture, 31: 171-174. (In Chinese).

Yalcin, H.; Toker, O. and Dogan, M. (2012): Effect of oil type and fatty acid composition on dynamic and steady shear rheology of vegetable oils. Journal of Oleo Science, 61:(4) 181-187. https://doi.org/10.5650/jos.61.181.

Yang, Y.; Tang, L.; Tong, L. and Liu, H. (2009): Silkworms culture as a source of protein for humans in space. Advances in Space Research, 43:1236-1242. https://doi.org/10.1016/j.asr.2008.12.009.

Zhang, F. and Zhang, Z. (2001): Study on edible insect resources and their exploitation and utilization. Resource Science, 23: 21-23 (In Chinese).

Zhang, X.; Wang, X.; Keshav, V.; Wang, X.; Johanas, G.T.; Leisk, G.G. and Kaplana, D.L. (2009): Dynamic culture conditions to generate silk-based tissue-engineered vascular grafts. Biomaterials, 30: 3213-3223. https://doi.org/10.1016/j.biomaterials.2009.02.002.

Zhang, Y. (1995): Study on new approach of silk fibroin utilization. Chinese Bulletin of Entomology, 32: 254-255 (in Chinese).

Zhaorigetu, S.; Yanaka, N.; Sasaki, M.; Watanabe, H. and Kato, N. (2003): Silk protein, sericin, suppresses DMBA-TPA-induced mouse skin tumorigenesis by reducing oxidative stress, inflammatory responses, and endogenous tumor promoter TNF-α. Oncology Reports, 10(3): 537-543. https://doi.org/10.3892/or.10.3.537.

Zhou, J. and Han, D. (2006): Proximate, amino acid and mineral composition of pupae of the silkworm *Antheraea pernyi* in China. Journal of Food Composition Analysis, 19: 850-853. http://dx.doi.org/10.1016/j.jfca.2006.04.008.

Zhu, L.S. (2004): Exploitation and utilization of the silkworm *Antheraea pernyi*. Northern Sericulture, 25(101): 32-33. https://doi.org/10.3969/j.issn.1673-9922.2004.02.014.

Zielinska, E.; Baraniak, B.; Karas, M.; Rybczynska, K. and Jakubczyk, A. (2015): Selected species of edible insects as a source of nutrient composition. Food Research International, 77:460-466. https://doi.org/10.1016/j.foodres.2015.09.008.