IJIE Int. J. Indust. Entomol. 43(2) 52-58 (2021)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2021.43.2.52

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)

Study of the organic and mineral composition of living pupae of the wild silkworm *Saturnia pyri* for use as food additives

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Abstract

The article presents the results of the content of the chemical and biochemical composition of the pupa of the wild silkworm *Saturnia pyri* belonging to the family *Saturniidae*, species of *Lepidoptera*. The nutritional value of silkworm *Saturnia pyri* pupae was evaluated, which contained 51% dry matter, 52.50% crude protein, 27.89% fat, 10.50% chitin fibers, 2.5% ash and 27 macro- and microelements and 25 mg alpha tocopherols in 100 g oil. The X-ray fluorescence method was used to determine the content of mineral elements in the pupa of the silkworm *Saturnia pyri*. It was revealed that the pupa of this type of silkworm contains 25 elements, of which the relative amount of K, Mg, Na, Ca, Al is much higher than other elements.

Int. J. Indust. Entomol. 43(2), 52-58 (2021)

Received : 15 Apr 2021
Revised : 26 Apr 2021
Accepted : 06 Oct 2021

Keywords:
Giant peacock moth,
Aristotle's silkworm,
Mineral elements,
Proteins,
Fat,
Fiber,
Ash,
Vitamin E

Introduction

Modern sericulture is an activity in which the products obtained at each stage of silkworm cultivation can be used for different directions of the consumer market. From the experience of world achievements, it is known that after obtaining silk from a cocoon, the remaining pupa is also of industrial interest and serves as a raw material for the manufacture of various medicinal and cosmetic products, food products (oils, seasonings) and is used as feed additives for pets, poultry (Buhroo *et al.*, 2018).

Currently, the most common is the traditional silkworm (*Bombyx mori*). But also, in many countries, non-mulberry species are bred, such as the Chinese oak silkworm (*Antheraea pernyi*), the Japanese oak silkworm (*Antheraea yamamai*), Indian wild species of silkworms (*Samia ricini, Antheraea mylitta, Antheraea roylei*), which belong to the family *Saturniidae* and are primarily used as wild silk producers (Sharma and Gurjar, 1987).

The giant peacock moth or Aristotle's silkworm (*Saturnia pyri*) from the genus *Saturnia*, also belonging to the family *Saturniidae*, has been experimentally hatched by us since 2019 at the Sheki Regional Scientific Center at the National Academy of Sciences of Azerbaijan, in order to study the possibility of breeding these species in laboratory conditions and prospects for use in the silk industry (Shukurlu *et al.*, 2019). In parallel with our study of specific silk of this type, some biochemical

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components of other by-products were also studied (Shukurlu *et al.*,2021).

The silkworm pupae are used in many sectors of the economy, such as agronomy, pharmacology, cosmetology, as well as the food industry, where pupae are used as a source of protein (Li *et al.*, 2017). According to approximate calculations, 1 kg of feed additives from silkworm pupae replaces 2.5 kg of meat. They have long been included in the diet of the population in different countries of the world (Mishra *et al.* 2003; Paul and Dey, 2014) According to some authors, the increased recognition of silkworms as an alternative and useful source of nutrition that does not require large costs can also contribute to the socio-economic development of local peoples, whose main occupation is silkworm breeding (Heuzé *et al.*, 2017; Sheikh *et al.*, 2018). Different types of wild silkworms feed on different fodder plants in different conditions, thus, the index of the content of the chemical composition of their pupae is different (Chieco *et al.*, 2019).

Materials and Methods

Basic preparations

The pupae of giant peacock moth - *Saturnia pyri* were acquired by cutting off fresh *Saturnia pyri* cocoons obtained in our laboratory as a result of the spring and summer feeding season of 2020, which were visually identified a year earlier by associate professor Aliyev Khalid Ali Agha oglu (employee of the Institute of Zoology of the National Academy of Sciences of Azerbaijan and associate professor Baku State University).

The characteristics of the pupae meet the standard criteria. No preliminary killing of chrysalis and drying of cocoons was carried out. After the pupae were freed from their cocoons, they were washed with distilled water and transferred to a thermostat at 105°C until constant weight. After drying, the samples were placed for 30-45 min in a desiccator for cooling, before weighing they were ground into a fine powder in an agate mortar.

Measurement and characterization

The moisture content in the pupae was determined to the last weight by drying them at a temperature of (105 ± 2) °C, according to the given method (Bradley, 2010). The percentage of ash and fat was calculated by the method of Marshall (2010). Method of Min and Ellefson (2010) was used to determine the percentage of fatty oil in pupae.

The protein content in the pupae of *Saturnia pyri* was determined according to the Kjeldahl method — by multiplying a certain nitrogen content by the Jones coefficient — the conversion of nitrogen into protein (Mariotti *et al.*, 2008). It is known that the total organic nitrogen in food will represent nitrogen mainly from proteins and to a lesser extent from all organic nitrogen-containing non-protein substances. In the Kjeldahl procedure, proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. Total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The formed borate anions are titrated with a standardized acid, which is converted into nitrogen in the sample. The result of the analysis is the content of *chitin* protein in food, since nitrogen also comes from non-protein components (Chang, 2010).

Vitamin E was determined by thin layer chromatography on "Silufol" (Czech Republic) plates with a fixed layer of silica gel (Interstate standard 30417-2018, 2020; Hodisan *et al.*, 2008).

Determination of *chitin fiber* was carried out according to the method of Kurschner and Haneck (1930) and Kozina (2012). The content of mineral elements was analyzed at the National Center for Nuclear Research CJSC (Baku, Azerbaijan) by the X-ray fluorescence method for Omega 4000 ("Innov-X," head office: Massachusetts, U.S.A., CEO: Don Sackett) (Hutton *et al.*, 2014).

Results and Discussion

The Eri silkworm (*Samia ricinii*) is a traditional food source in northeastern India, where it is grown primarily for silk and food production. Nutrient analysis showed that the approximate composition of mature silkworm larvae and pupae reared on castor bean and tapioca was comparable and provided a good source of protein (16%), fat (8%) and minerals (Longvah *et al.*, 2011). Therefore, the biochemical parameters of the pupa of the Eri silkworm (*Samia ricinii* (Longvah *et al.*, 2011) or *Pilosamia ricini* (Mazumdar, 2019)) were taken for comparison with the pupae of the Giant peacock silkworm (*Saturnia pyri*).

For comparison, let's pay attention to theoretical calculations, the component composition of Bombyx mori silkworm pupae. Calculations show that the mass content of lipids in the composition of the silkworm pupa is about 12.1-27.4, proteins - 59.8-75.1, chitin - 3.5-4.7, ash - 2.7-5.6 and humidity ~10 percent (Avazova *et al.*, 2020).

Table 1. Chemical composition of the pupa of *Saturnia pyri* silkworm grown on cherry leaves, silkworm *Samia ricini* grown on castor bean leaves (Longvah et al., 2011), and silkworm *Bombyx mori* grown on mulberry leaves (Avazova et al., 2020).

Items, %	Pupae (Dry weight) *Saturnia pyri*	Pupae (Dry weight) *Samia ricinii* (Longvah *et al.*, 2011)	Pupae (Dry weight) *Bombyx mori* (Avazova *et al.*,2020)
Moisture	5.50 ± 0.28	8.50 ± 0.21	~10,00
Protein	52.50 ± 0.63	54.60 ± 0.56	59,8-75,1
Fat	27.89 ± 0.32	26.20 ± 0.35	12,1-27,4
Ash	2.50 ± 0.05	3.80 ± 0.67	2,7-5,6
Chitin fiber	10.50 ± 0.03	3.45 ± 0.06	3,5-4,7

Table 1 shows the quantitative content of certain biochemical values in the silkworm pupa of *Samia ricinii*, *Saturnia pyri* and *Bombyx mori*. As can be seen from this table, the dry solids content of raw protein is high enough to make the wild silkworm pupa *Saturnia pyri* - a good addition to protein food.

The fat content in the pupa of *Saturnia pyri* is 27.89%, where it is mostly unsaturated fatty acids, which have important physiological functions according to previous studies by scientists (Rao, 1994).

In fig. 1 shows the oil from 4 batches of pupae (pupa oil) of the silkworm *Saturnia pyri*, obtained by us, which has a dark yellow color and a specific odor. Experiments have shown

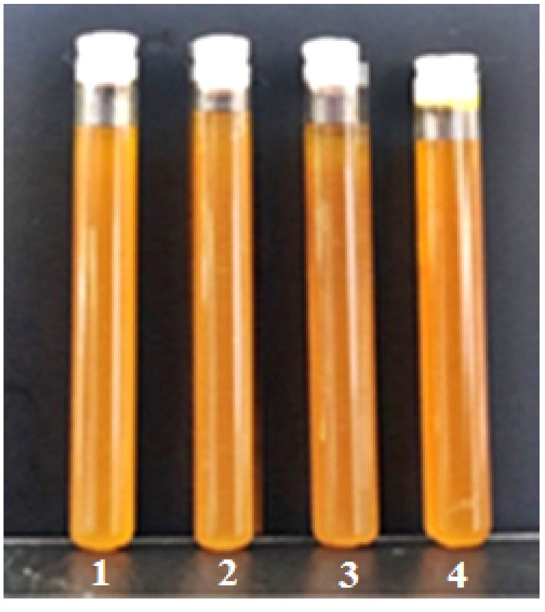

Fig. 1. The oil from the pupae (*chrysalis oil*) of the silkworm *Saturnia pyri:* 1 – female; 2 – male; 3 – larger; 4 - smaller

that the oil obtained from a female (sample 1), a male (sample 2), larger (sample 3), and smaller (sample 4) pupae does not particularly differ in color or smell. The average melting point of the chrysalis oil is 26.0°C, the density is 930 kg/m^3.

Vitamin E (or *tocopherol*) is a fat-soluble vitamin, meaning it dissolves in fats. In nature, there are 8 different compounds that represent vitamin E (alpha, beta, gamma, delta tocopherols). The most commonly known form is alpha tocopherol. In addition, it is the most active vitamin.

Vitamin E is a physiological antioxidant, the intake of which is directly related to the consumption of polyunsaturated fats. These lipids are involved in the formation of cell membranes and proteins that carry fats into the blood. They are very sensitive to the effects of oxygen, and vitamin E is a reliable protection for them. Since fat must be supplied to our bodies with food on a daily basis, it is important that the diet also contains a sufficient amount of vitamin E (Nielsen *et al.*, 2001).

Vitamin E is an antioxidant that protects cells from free radicals and carcinogens. It is also necessary for the normal functioning of the immune system, the cardiovascular system, promotes vasodilation, strengthens the walls of capillaries, and prevents thrombus formation. Vitamin E plays an important role in the process of regeneration and reproduction (Eiichi, 2002).

To determine vitamin E in the oil of the pupa of the *Saturnia pyri* silkworm, the amount of tocopherol was calculated. To establish the mass fraction of this vitamin, the content of α-tocopherol was calculated according to the calibration graph using the method of thin layer chromatography. Fig. 2 shows the chromatography of tocopherol in oil of the pupa of *Saturnia pyri* on a silica gel plate. This chromatography shows that only alpha-tocopherol is present in the oil - the natural and most biologically active form of all tocopherols.

Thus, we calculated the content of natural vitamin E in the form of acetate – 25 mg per 100 g of oil.

Minerals play a key role in the life of living organisms. They are found in food as organic and inorganic compounds.

As can be seen from Table 2, pupae of the silkworm *Saturnia pyri* mostly accumulate mineral substance, containing elements K, Mg, Na, Ca, Al. It is known that sodium and potassium regulate water-salt metabolism. In addition, the Na/K ratio in the pupae of *Saturnia pyri* is low (0.08). This is interesting from a nutritional point of view, since the consumption of sodium chloride and food with a high Na/K ratio can cause an increase in blood pressure. The magnesium ion plays a huge role in the life

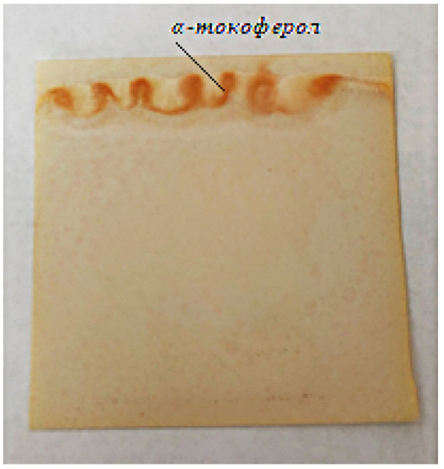

Fig. 2. Chromatography of vitamin E in oil of the pupae of the wild silkworm *Saturnia pyri* on a plate with silicogel

of a living organism, participating in the metabolism of proteins, carbohydrates, and phosphorus (Zhou and Han, 2006b).

Calcium (Ca) is a plastic material for bones, a blood coagulation factor, normalizes the activity of the heart and muscles, is a part of the nucleus and membranes of cells, cellular and tissue fluids, and activates a number of enzymes and hormones (Pravina *et al.*, 2013). Muscular and mental activity of a person depends on intake of phosphorus (P) (Takeda *et al.*, 2004), iron (Fe) (Abbaspour *et al.*, 2014) into the human body, which are important elements for almost all living organisms, since they are involved in a wide range of metabolic processes, including oxygen transport, synthesis of deoxyribonucleic acid (DNA) and transport electrons.

Manganese (Mn) is an essential nutrient for intracellular activity; it acts as a cofactor for various enzymes including arginase, glutamine synthetase (GS), pyruvate carboxylase, and Mn superoxide dismutase (Mn-SOD). Through these metalloproteins, Mn plays a critical role in development, digestion, reproduction, antioxidant defense, energy production, immune response, and regulation of neuronal activity (Chen *et al.*, 2018).

Cobalt and molybdenum increase the intensity of bioenergetic processes and protective reactions of the body. Cobalt (Co) is an essential trace mineral for the human body and can be present in organic and inorganic forms. The organic form is an essential component of vitamin B_{12} and plays a very important role in the formation of amino acids and some proteins in nerve cells, as well as in the creation of neurotransmitters, which are necessary for the proper functioning of the body (Czarnek *et al.*, 2015).

Table 2. Mineral contents of *Saturnia pyri* pupae

Micro- and macro elements in pupae		The relative number to the mass of a dry weight of pupae, mg/g
Kalium	K	3.761
Natrium	Na	3.113
Magnesium	Mg	7.019
Calcium	Ca	1.016
Aluminum	Al	1.564
Siliceous	Si	0.611
Phosphorus	P	0.342
Sulfur	S	0.076
Barium	Ba	0.031
Lead	Pb	0.003
Iron	Fe	0.036
Copper	Cu	0.032
Zinc	Zi	0.042
Titanium	Ti	0.045
Vanadium	V	0.003
Chromium	Cr	0.009
Manganese	Mn	0.013
Nickel	Ni	0.022
Gallium	Ga	0.001
Zirconium	Zr	0.011
Tin	Sn	0.001
Strontium	Sr	0.006
Yttrium	Y	0.003
Niobium	Nb	0.011
Rubidium	Rb	0.002

Molybdenum (Mo) is a trace element that acts as a cofactor for at least four enzymes: sulfite oxidase, xanthine oxidase, aldehyde oxidase, and a component that reduces mitochondrial amidoxime (Novotny, 2011); it also participates in the synthesis of amino acids.

Zinc (Zn) is part of a number of enzymes and insulin. That is why zinc plays a central role in cell growth, differentiation, and metabolism (Brown *et al.*, 2001).

Sulfur (S) is a part of some amino acids (cystine, methionine) that form proteins - methionine, cysteine, cystine, homocysteine, homocystine, and taurine (Parcell, 2002).

Lead (Pb) levels do not exceed the maximum lead levels (0.5 mg/kg) set for a number of foods in world standards (Suldina, 2016).

Realizing the extent to which silkworm cocoon byproducts are used through appropriate methods is an immediate pursuit of income optimization. In terms of nutritional value, pupae are suitable for human consumption, food for poultry, fish, rabbits, pigs, and dogs. In recent years, the *Bombyx mori* silkworm pupae have been listed by the Chinese Ministry of Health as a "new food resource used as common food" (Zhou and Han, 2006a).

The preliminary results presented in this article show that the pupae of the silkworm *Saturnia pyri* have great potential for obtaining various types of active dietary supplements, medicinal components that can be useful in dietetics, cosmetologists, pharmacologists, etc. in our country. Considering that our planned volume of industrial feeding of *Saturnia pyri* does not give us the opportunity to talk about such large-scale production of feed additives yet, at this stage, we need to conduct a detailed analysis of the pupae of *Saturnia pyri* for the presence of specific, valuable nutrients and non-nutrients, secondary metabolites with medicinal potential. This will allow us to expand the boundaries of research in the field of national nutritional science and biomedicine, which is a crucial task of modern science.

The pupae of the silkworm (*Bombyx mori*) were studied, which, after unwinding the cocoons, are sent to the waste processing workshop, are processed raw with a moisture content of up to 200% and contain various impurities that must be removed. Such raw pupae are squeezed out of excess water in a centrifuge at a temperature of 85-95^{o}C. After drying, the pupae are sorted to remove rotten and moldy pupae and worms do not turn into pupae. Removes fibers and other contaminants. After that, the dried pupae are crushed and poured through the holes of the sieve, and the pulp remaining in the sieve is presented in the form of entangled fibers and this is removed. Shredded pupae with a moisture content of 9% or less are packed in paper bags. Measurements carried out on samples of these bags show that dry silkworm pupae contain ~60% crude protein and ~29% fat and are a high-quality source of insect protein with a rich balanced content of essential amino acids: valine, phenylalanine and sulfur-containing methionine and cysteine. It was found that fat contains the most acids such as oleic (34.9%), palmitic (29.6%), linolenic (11.4%), linoleic (11.8%) and stearic (10.5 %).

So far, in our region, *Bombyx mori* pupae are a waste material often discarded in the open environment or used as fertilizer and mainly used in poultry and fish farming. But we know that it can be extracted to yield valuable oil used in industrial products such as paints, varnishes, pharmaceuticals, soaps, candles, plastic and biofuels (Trivedy *et al*., 2008). The extracted meal is sometimes used for the production of chitin, the long-chain polymer of N-acetylglucosamine which is the main component of the exoskeleton (Suresh *et al.*, 2012). Silkworm pupae have long been eaten by humans in Asian silk-producing countries and are considered as a delicacy in regions of China (Luo, 1997), Japan (Mitsuhashi, 1997), Thailand (Yhoung-Aree *et al.*, 1997), India (Longvah *et al.*, 2011), and elsewhere. Due to its high protein content, silkworm pupae meal has been found suitable as a livestock feed, notably for monogastric species (poultry, pigs, and fish), but also for ruminants (*Trivedy et al.*, 2008).

Conclusions

It was found that the pupae of the silkworm *Saturnia pyri* contain 51,0% dry matter, 52.5% crude protein, 27.9% fat, 10.5% chitin fiber, 2.5% ash. It has been established that the oil of the pupae (*chrysalis oil*) of the silkworm *Saturnia pyri* contains 25 mg of α-tocopherol per 100 g of oil, the natural and most biologically active form of all tocopherols. It was revealed that pupae of the silkworm *Saturnia pyri* accumulate K, Mg, Na, Ca, Al to a large extent, and the *Al* content is within the normal range, not higher than 2 *mg* per 100 *g* of pupa. The accumulation of Ti, Fe, Cu, Ba, Zn in the pupa is evenly distributed.

Acknowledgment

This study was conducted in accordance with the requirements of the State Program for the development of cocoons and natural silk production in the Republic of Azerbaijan in 2018-2025.

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