Chemical physical characteristics of *Bombyx mori* L C301 pupae under heat shock treatment

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Abstract. *Bombyx mori* pupae, the remaining of silk thread spinning contains a fairly high protein. Protein is not resistant to heat temperatures. Heat shock application causes failure of pupae formation and will affect the content of pupae organic compounds. Heat shock was given for 3 hours at 42\(^\circ\)C when the larvae in instar IV, then the larvae were rearing until they become cocoons. Pupae was removed from the cocoon, then pupae morphology was observed. Analysis of pupae sampling includes protein, total fat and carbohydrates based on SNI 01-2891-1992. The failure of pupae formation was indicated by the blackish brown pupae and the irregular shape of the cocoon, while the pupae without heat shock was light brown. The content of silkworm pupae organic compounds that were given a heat shock was 80.4% protein. Total fat was 2.13% and carbohydrates were 6.58%. Heat shock did not affect the level of protein in pupae.

1. Introduction

Silkworm growth requires optimum temperature \([1,2]\), hence high temperatures become one of the environmental factors that can cause stress to the growth of silkworms. Silkworms rearing at 28\(^\circ\)C can cause higher dry matter consumption but reduce feed conversion efficiency \([3]\). High temperatures affect the biochemical and physiological reactions of silkworms \([4]\). The optimum temperature for normal growth of silkworms is between 20-28\(^\circ\)C and the desired temperature for the maximum productivity is 23-28\(^\circ\)C. Temperatures above 30\(^\circ\)C directly affect the health of silkworm \([5,6]\). The Rearing temperature in the tropics exceeds the optimum temperature of silkworm growth.

Rearing of silkworm is an agro-industrial activity. When silkworms enter the pupae phase, cocoons form. Producing silk was done by killing the cocoons; boiling, drying or soaking it in NaOH before producing enzymes. Pupae is produced in large quantities and is a major by-product of spinning or silk thread production \([7]\). One kg raw silk contains 8 kg of wet pupae (2 kg dried pupae) \([8]\). In silk-producing regions, the release of large numbers of pupae can cause serious environmental problems \([9]\). Utilization of this source for feed and food or other production such as chitin, protein, oil and fatty
acids (α-linolenic acid) is a way to reduce the environmental impact of the silk thread industry. Pupae can be used as a source of conventional protein [10].

Silkworm pupae contain proteins with high nutritional value. The crude protein content ranges from 50% DM to more than 80% DM. The content of lysine (6-7% protein) and methionine (2-3% of protein), this includes a very high value. However, the actual protein (calculated as the number of amino acids) in silkworms turned out to be only in accordance with 73% of crude protein [11], which is explained by the presence of chitin, because this component contains nitrogen. However, the chitin content in the pupae is relatively low, around 3-4% DM [11,12]. The presence of chitin and insoluble proteins due to fiber, and a DMF value of 6-12% [11,13]. This silkworm pupae is classified as mineral poor (DM 3-10%).

In Korea pupae is used as street food made from silkworm cocoons [14]. These snacks are boiled or steamed then served in paper cups with sticks [15]. Pupae proteins are also efficient for wound or wound dressing, hepatoprotective and antiapoptosis activities, antigenotoxicity, regulation of blood glucose and lipids, anticancer agents, and so on. Therefore, silkworm cocoons can be utilized as dietary supplements and their extraordinary proteins open up new dimensions for biomedical science [16]. Does rearing of silkworms at hot temperatures will affect the content of organic compounds in the pupae?

2. Methods
The material used was silkworm Bombyx mori L. C301 instar IV which begins with hatching, rearing and treatment. Egg seeds were obtained from the center of silk Alam CantirotoTemanggung, Jawa Tengah. The research procedure was carried out through several stages, namely egg hatching, silkworm rearing, cocoon harvesting, pupae collecting, morphological observation and analysis of pupae organic compounds.

Heat shock treatment was carried out on instar IV larvae for 3 hours with a heat shock temperature of 42°C. The heat shock treatment was carried out on the initial instar IV caterpillar, by means of the caterpillar placed in a basket that was covered with paper and still given mulberry leaf feed and put into the oven. After 3 hours the caterpillar was removed from the oven then put back into the rearing room until the caterpillar coooning [17]. Protein content analysis was using the Kjeldahl method, fat content using the Soxhlet method, carbohydrate levels using the formula carbohydrate by difference (SNI 01-2891-1992).

3. Results And Discussion
Analysis of pupae physical character morphologically can be seen in Figure 1. The morphological characteristics of the pupa were visible in blackish brown colour with imperfect pupa condition, dry and hardened. Most of the pupae was dead, while the pupae without heat shock was light brown and alive. Heat shock treatment will affect the growth and development of silkworms which will eventually cause failure in the formation of cocoon and pupae [17]. The failure of pupae formation will affect the content of organic pupae compounds.

Figure 1, the morphological conditions of the pupae were broken and fragile with a hardened body texture (Figure 1 b). Pupae skin without heat shock was denser and brighter in colour. The morphological changes of the pupae also cause changes in the content of organic compounds (Figure 2). Figure 2, it can be seen that the silkworm pupae organic compounds that were given heat shock has 80.4% protein, 2.13 5 total fat and 6.58% carbohydrate.
Figure 1. Morphological characteristics of silkworm pupae (*Bombyx mori*) a. control pupae skin b. pupa skin with heat shock treatment

![Morphological characteristics of silkworm pupae](image)

Figure 2. The content of organic compounds in Silkworm (*Bombyx mori* L.) pupae with heat shock treatment

![Content of organic compounds](image)

There was a decrease compared to the content of organic compounds in the pupae without heat shock, namely; 7% fat and 29.62%, while protein levels increase. Pupae protein content without heat shock was 58.93%. The chemical composition and nutritional quality of Antheraea pupae protein has 71.9% crude protein and 20.1% fat [18]. Pupae silkworm is a new source of high-quality protein, lipids, and α-glucosidase inhibitors [19]. The ecdysone hormone titer has the highest value on the instar IV of *Bombyx mori* larvae [20]. In the larvae-pupa extraction process, females have 60% protein from total body fat protein (80% dissolved protein), while males have 20% of the body's total protein fat [21]. Silkworm cocoons contain 55.60% of total protein and 32.2% fat [16]. (Table 1).
Table 1. The content of organic compounds (%) of Silkworm (*Bombyx mori* L.) pupa with heat shock treatment

| No. | Organic compounds | B. mori with Heat Shock | B. mori (Kumar *et al.*, 2015) | Samia ricinii |
|-----|-------------------|-------------------------|--------------------------------|--------------|
| 1.  | Protein           | 80.4                    | 55.60                          | 16           |
| 2.  | Total Fat         | 2.13                    | 32.20                          | 8            |
| 3.  | Carbohydrate      | 6.58                    |                                |              |

Eri silkworm (*Samia ricinii*) is a traditional food source in northeastern India. *Samia ricinii* is used to produce silk and food. Nutritional analysis showed that the proximate composition of silkworms and pupae was a good source of protein (16 g%), fat (8 g%) and minerals (Table 1). The high protein content in pupae causes the pupa as a waste from the yarn spinning process to be used as a source of protein for animal feed. Silkworm pupae flour can be used from 25 - 75% to replace fish meal protein [22]. Pupae is used as the future production of recombinant eukaryotic protein [23]. In recent years, research has focused on various biomedical applications of silkworm pupae proteins. Cocoon proteins work efficiently in wound dressing, hepatoprotective and antiapoptotic activity, antigenotoxicity, regulation of blood glucose and lipids, anticancer agents, etc. Therefore, silkworm cocoons can be used as food supplements and the enormous protein opens up a new dimension for biomedical science [16]. Proteins from silkworm cocoons have been considered as a source of high-quality protein that is renewable and contains all the amino acids needed by the human body [18].

The vitamin content, especially B-complex and certain essential sugars, proteins, amino acids, minerals and others are responsible for the growth and development of silkworms, *B. mori* [24]. Silkworm pupae are used as animal feed, organic fertilizers, food ingredients, and traditional medicines in several Asian countries, namely Korea, China, Thailand, Japan and India. Silkworm pupae are optional food products and began to be commercialized in different foods and industrial medicine to meet the nutritional needs and hidden treatment of various human diseases. Pupa (*Bombyx Mori*) protein is also used as an alternative protein source for fish (Aquaculture) [25]. Muga and Eri silkworm pupae can be used as natural antioxidants in food products [26].

4. Conclusion

Heat shock treatment caused the failure of pupae formation and the content of silkworm pupae organic compounds which were given hot shock treatment namely; protein 80.4%, total fat 2.13% and carbohydrates 6.58%. Heat shock did not affect the level of protein in pupae.

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