# *Bombyx mori* Silkworm Pupae Protein Isolate and Its Application on High Protein Powdered Milk

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## Abstract

*High protein food consumption is a new trend in society with busy life to fulfill the balance of daily protein need. A product with high protein content is high protein powdered milk (HPPM) for instance which commonly use high protein fortificant added through its processing. Bombyx mori silkworm pupae (BSP), by-product of silk spinning industry, has potential to be developed as an alternative for cheaper high protein fortificant. Previous research had isolated BSP protein to obtain protein isolate then fortified it into powdered milk. The continuing study is needed to produce pure protein isolate in order to produce HPPM with better characteristics. The two inventions applied in this protein isolation method were defatting and modifying pH on protein extraction and precipitation process. Hexane was used to extract powdered pupae fat. Protein extraction by NaOH 2N until pH 11 and precipitation by HCl 2N until pH 4.1 were used in this research to make sure all the protein fractions of BSP were dissolved in alkaline condition and precipitated as below its isoelectric pH. The objective of this research was to study the properties of Bombyx mori silkworm pupae protein isolate (BSPPI) and its application on HPPM. The results showed that defatting process could decrease 60.87% fat content of powdered pupae. The modified protein isolation method was able to produce pure BSPPI with 81.84% protein content. Fortification of 20% BSPPI showed very high significant difference ($P<0.01$) on protein content and protein digestibility of powdered milk, 40.44% and 95.15% respectively.*

*Keywords: Bombyx mori silkworm pupae, high protein powdered milk, protein isolate*

## Introduction

Protein is a second majority compound of human body component after waterneeded forhuman growth process. Therefore, the adequacy of daily protein is very important.High protein food consumption is a new trend in society with busy life to fulfill the balance of daily protein need. A product with high protein content is high protein powdered milk (PM) for instance which commonly use high protein fortificant added through its processing.Fortificant sources commonly used are whey and casein. Both are very expensive fortificant and still depend onimport supply.

*Bombyx mori s*ilkworm pupae is by-product of silk spinning industry which have been used as foods in some countries to provide nutritional benefits because it containshigh and balanced nutrients. Pupae is also known as a better protein content than soy, fish, and beef protein (Trivedy *et al*., 2007) as well as essential amino acidssuch as BCAA (branched chain amino acids) i.e lysine, leucine, and valine(Tomotake *et al*.,2010). BCCA are widely used as food supplement because of its effectiveness to build muscle and improve its endurance. However, silkworm pupae is generally used as fish feed ingredients and broiler chickens in Indonesia (Rangacharyulu *et al*., 2003).

Previous research had utilized silkworm pupae as flour for soups and cream cracker ingredient. Artanti (2009) and Miyatani (2008) stated that silkworm pupae-based product contains high protein and high protein digestibility. Khan *et al.*(2011) carried out an invention to improve *Bombyx mori* silkworm pupae-based product acceptability by isolating *Bombyx mori* silkworm pupae proteinprior tofortification to milk. However, further research is needed to find more precise method of protein isolation to produce pure *Bombyx mori* silkworm pupae protein isolate (BSPPI)in order to produce powdered milkwith better characteristics.The objective of this research wasto study the properties of BSPPI and its application on high protein powdered milk.

## Materials and Methods

The main ingredients used were fresh cow's milk (from dairy farm of Animal Sciences Faculty, Bogor Agricultural University), *Bombyx mori*silkworm pupae (from Rumah Sutera Alam, Ciapus–Bogor, West Java Province), NaOH 2N, HCl 2N, distilled water, and Whatman filter paper. The main equipments used were refrigerator, centrifuger, pH meter, digital scales, spray drier, evaporator, homogenizer, and blender.

*Manufacturing of BSPPI (modified Wang et al.,2010) and Fortified Powdered Milk*

The manufacturing of BSPPI was divided into two stages; manufacturing of

powdered pupae, defatting of powdered pupae, isolating ofBSPPI, and drying of BSPPI. Fresh pupae was pealed and washed by submerging into clean water. The water was changed repeatedly until it was clear. After the pupae were clean, they were boiled in 15 minutes and dried by 60 °C oven for 24 hours. Once the pupae were dried, it was powdered using blender. After obtaining the powdered pupae, the fat was removed by hexane. The defatted powdered pupae protein was extracted using NaOH 2N until pH 11 and left for one hour to dissolve the protein completely. As soon as the protein was dissolved, the residue was removed by centrifuge on 6,000 g-force. The dissolved protein was then precipitated by decreasing the pH until 4.1 using HCl 2N until the protein reached its isoelectric point and was left for 15 hours to precipitate protein completely. The protein precipitate was teh obtained by centrifuge on 6,000 g-force. The last step was cleaning by distilled water and centrifuge on 6,000 g-force for 3 times. BSPPI was then dried by spray drier (inlet 180 $^{o}$C, outlet 80 $^{o}$C) to produce powdered BSPPI.Powdered BSPPIwas then added into powdered milk (PM)with 5 treatments (0%, 5%, 10%, 15%, and 20%).The fortified powdered milk was analysed based on chemical properties and protein digestibility.

*Protein Content Assay*

Protein content was tested by Kjeldahl method (AOAC, 2007). This method consists of three stages i.e digestion, distillation, and titration.

*Protein Digestibility by in vitro Assay (Anderson et al., 1969)*

The digestibility of protein in fortified powdered milk was determined using the multienzyme method. A number of 250 mg powdered milk added with 15 ml HCl 0.1 N contained 1.5 mg pepsin and were incubated at 37 $^{o}$C for 3 hours. The solution was neutralized using NaOH 0.5 N and added with 4 mg pancreatine solution in phosphate buffer 0.2 M pH 8.0 which contained 0.005 M sodium azyde. Solution of the enzyme mixture were incubated at 37 $^{o}$C for 24 hours. The residuewas obtained by centrifuge on 2,500 g-force for 5 minutes. The protein in residue indicated total number of undigestible protien. Total number of protein before and after this treatment were measured by Kjeldahl method (AOAC, 2007). Apparent Protein Digestibility (APD) was calculated by equation below:

APD (%)= (Total protein-undigestible protein)/(Total Protein) ×100%

*NaCl Content Assay (Modified Volhard's Method)*

Ash obtained from 5000 mg sample ($W_{sample}$) were dissolved with nitric acid. The solution was then added by distilled water until its volume 100 ml.The solution was incubated at room temperature for 24 hours. Briefly, 10 ml of the solution ($V_{sample}$) was then added by nitric acid 4N, silver nitrate solution 0.1N,and ferric ammonium sulfate 40% with 5ml for each. The solution was then homogenized and

titrated with potassium thiocyanate 0.1006N until permanent orange of its color. NaCl content was calculated based on formula below :

$$\text{NaCl (\%)} = \frac{(V_{blanko} - V_{sample}) \times CF \times N_{KSCN} \times 58.50}{W_{sample}} \times 100\%$$

Explanation :
Coefficient Factor (CF)= 10, Volume of Blanko ($V_{blanko}$)= 5.05 ml

*Statistical Analysis*

This research used a completely randomized design with5treatments of BSPPI fortification (0%, 5%, 10%, 15%, and 20%) to powdered milk. All experiments and measurements were performed in triplicate and duplicate respectively and the means reported. The results were expressed as mean values ± standard deviation (n = 3). Analysis of variance (ANOVA), followed by Tukey or Kruskal Wallis test, were used for statistical comparisons among treatments, with a value of $P < 0.05$ indicating significant difference. All data were analyzed using Statistix 8.0 statistical software package.

## Results and Discussion

*Characteristic of BSPPI*

Basic material usedas aprotein fortificant in this research was BSPPI. BSPPIwas obtained through modifiedmethodtoincrease thepurity of proteinisolatecomparedwith previous researchmethod. The inventionsof this research are defatting implemented to powdered prior to protein isolation, pHvariations usagein proteinisolation step,an dwashingforremoving any chemical residue in isolate.

Table 1. Physical and Chemical Profile of BSPPI

| Parameters | Content (%) |
|---|---|
| Protein | 81.84 ± 0.62 |
| Protein digestibility | 94.59 ± 0.43 |
| Fat | 6.22 ± 0.10 |
| Moisture | 7.16 ± 0.03 |
| Ash | 3.68 ± 0.69 |
| Yield | 29.94 |

Table 2. Powdered Pupae Fat Content Before and After Defatting Process

| Sampels | Content (%) |
|---|---|
| Powdered pupae | 26.43 |
| Defatted powdered pupae | 10.34 |

Defatting is a very important step in this isolation processbecause fatis thesecondlargestcomponent ofpowdered pupae (in dry basis) after protein. Proteinandfatbeforedefatting were 60.75% and26.43%respectively (Table 2)(Khan *etal*., 2011). The resultsshowed that defatting decreasedfat content of powdered pupae

60.87±0.19%. The inventions of this research i.edefatting and pHvariations usage were able to producepure BSPPIwith high purity that contains protein about 81.84% and with less fat content, 6.22% (Tabel 1).It means that protein isolation process used in this researchcould separate the fat remained in the sampel.Washing process also successfully washed NaCl remaining after chemical reaction from NaOH and HCl during isolation process. Based on the result of NaCl assay, powdered milk with or without BSPPI were not significantly different (P>0.05) (Figure 1a).

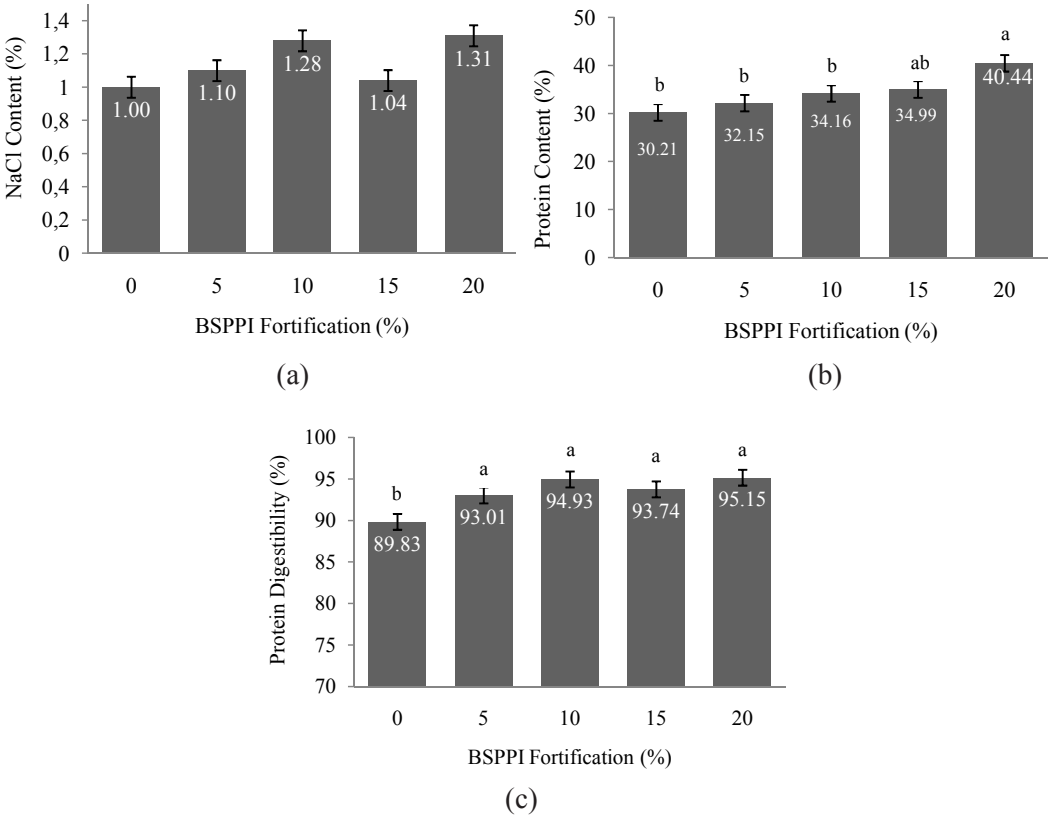


Figure 1. Effect of BSPPI fortification to NaCl content (a), protein content (b), and protein digestibility (c)of powdered milk. Explanation : Different superscript (a, b) shows very high significant difference(P<0.01)

The result showed that fortification of BSPPI into powdered milk increased 1.94%-10.23% of protein content (Figure 1b). The quality of BSSPI-fortified powdered milk had fulfilled standard of powdered milk protein content, more than 23% (NSC, 2006).Fortification of 20% BSPPI showed very high significant increase (P<0.01) on protein content.Fortification of BSPPI increased protein digestibility of powdered milk, 3.18%-5.32%, with very high significant increase (P<0.01) on 5% fortification (Figure 1c). This increasing was caused by high protein digestibility of BSPPI, 94.59% (Table 1). High protein claim could be provided to powdered milk

fortified by 20% of BSPPI because it had fulfilled 20% ofNRV (nutrition reference value) per 100 g of protein (CAC, 2001)

## Conclusions

Defatting process decreased 60.87% fat content of powdered pupae. The modified protein isolation method was able to produce pure BSPPI with 81.84%, 94.59%, 7.16%, 3.68% of protein content, protein digestibility, water, and ash respectively. Fortification of 20% BSPPI showed very high significant increase ($P<0.01$) on protein content and protein digestibility of powdered milk, 40.44% and 95.15% respectively.

## Acknowledgement

The authors would like to give an acknowledgement to Rumah Sutera Alam at Ciapus Bogor-West Java province for facilitating us with *Bombyx mori*pupaeand Directorate of Higher Education of National Education and Culture, Ministry of Republic of Indonesia for providing fund to establish this research.

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