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Characterization, Functionality and Antioxidant Activity of Water-Soluble Proteins Extracted from *Bombyx mori* Linn.

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Abstract

The yield of water-soluble protein extracted from silkworm pupae, *Bombyx mori* Linn. (WSPB) was 3.96% by wet weight basis. The major amino acids found in WSPB were glutamic acid, which was the most abundant, followed by histidine, phenylalanine and glycine in that order. The electrophoretic study revealed that proteins with MW of 37, 64 and 75 kDa were the major protein components in WSPB. Based on FTIR analysis, WSPB remained its structural integrity. The surface hydrophobicity, free and total sulfhydryl group contents were 3.52, 22.17 μmol/g and 23.08 μmol/g, respectively. WSPB was highly solubilized in the pH range of 5-11. WSPB exhibited poor emulsifying properties and foaming capacity but the foam stability was comparable to bovine serum albumin (BSA). WSPB had high antioxidant potential, based on DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP assay. Therefore, protein from silkworm pupae is a potential source of antioxidant and can be served as an ingredient in processed foods to enhance its desired functionality and nutritional value.

Keywords: Characterization, Functionality, Antioxidant activity, Water-soluble protein, Edible insect, *Bombyx mori* Linn.

1. Introduction

Silkworm pupae (*Bombyx mori* Linn.) are considered a good food source for humans because of their high nutritional value [1]. They have been consumed in many Asia countries including Thailand, China, Korea, Japan and India. Silkworm pupae are the main by-product of the silk industry and constitute 60% of dry cocoon weight after extracting threads [2]. The nutritional value of silkworm pupae is rich in lipids (20.1% wt) and protein (12% wt), exhibiting the high levels of essential amino acids such as valine, methionine and phenylalanine [3]. Several studies have shown that silkworm pupae contain 45 to 55% protein (18 amino acids, including 8 essential amino acids) on a dry matter basis. Four kinds of protein components have been identified in silkworm pupae which albumin was found to be the highest at 27.24%, followed by glutelin at 23.72%, prolamine at 11.82% and globulin at 4.21%, respectively [4]. The hydrolysate of albumin was determined with the highest angiotensin-converting enzyme (ACE) inhibiting effect, followed by globulin. Inhibiting ACE could be decreased hypertension, which recognized as a serious risk factor for cardiovascular diseases [4]. This means that it is a good-quality protein source and a good source of bioactive peptides [4, 5].

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Proteins can be added to foods to improve their functional properties such as solubility, emulsifying and foaming properties [6]. Park *et al.* [7] also determined the effects of adding flour made from silkworm pupae to meat batter. They found that combining the flour with transglutaminase improved the physicochemical properties of meat batter. Kim *et al.* [8] found that adding *B. mori* pupae flour to emulsion sausages increased their cooking yield and hardness. *B. mori* pupae have many biomedical advantageous for humans including controlling blood glucose level, improving male sexual function and enhancing memory [9, 10]. Wu *et al.* [1] postulated that animal proteins may have other health benefits, in addition to energy and nutritional functions, including enzyme (ACE) antioxidant activity and free radical-scavenging. Generally, *B. mori* are well known, easily obtained and a favorite in Thailand and are reported to be high in minerals, fatty acids and protein [11, 12]. The aim of this investigation was therefore to study on the characteristics, functional properties and antioxidant activities of water-soluble proteins from silkworm pupae (*B. mori*), in order to determine whether they are a good source of protein that could be extracted and used as a food ingredient.

2. Materials and Methods

2.1 Protein extraction

Frozen silkworm pupae (*B. mori*) was obtained from a commercial supplier (Mr. BUC FOOD, Ayutthaya, Thailand) was blended with cold water (4°C) at a ratio of 1:4 w/v for 15 min using blender (MMB54G5S, BOSCH, Germany) and stirred overnight at 4°C, to ensure that the proteins were dissolved. The suspension was centrifuged at 12,500 g for 30 min at 4°C. After centrifugation, the sample was separated to 3 layers. The top layer was fat layer. The middle layer contained water soluble protein fraction. The bottom layer was undissolved components. Then, the middle layer, containing water soluble protein fraction was collected, freeze-dried and referred to as "water-soluble protein from *B. mori*: WSPB". Extraction was performed in triplicate and the protein content of the extract was determined by Kjeldahl method [13].

2.2 Yield and efficiency of extraction

The yield of WSPB was calculated as a percentage of the weight of WSPB powder in comparison with the weight of *B. mori* before extraction and was calculated as follows:

Yield (%) = (weight of WSPB (g) / weight of sample (g)) × 100.

Extraction efficiency was calculated as a percentage of the total protein extracted from *B. mori* of WSPB in comparison with the content of total protein content in *B. mori* in which determined by Kjeldahl method [13]. The extraction efficiency of WSPB was calculated as follows:

Extraction efficiency (%) = (total extracted protein of WSPB (g) / total protein content of *B. mori* (g)) × 100.

2.3 Characterization of WSPB

2.3.1 Amino acid analysis

The amino acid composition of WSPB was determined by The Central Instrument Facility at Mahidol University, Bangkok, Thailand. The analysis was performed using HPLC (Waters Alliance 2695 with heater, Jasco FP2020 fluorescence detector (EX: 250 and EM: 395 nm)) with a Hypersil gold column C18 (4.6×150 mm, 3µm) at 35°C. Amino acid standards (Sigma-Aldrich, USA) were used for calibration.

2.3.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli [14] with slight modifications. The samples (3 g) were mixed with 27 mL of 5% SDS, heated at 85°C for 1 h, then centrifuged at 8,500

g for 5 min using a centrifuge (5804 R Eppendorf, Germany) to remove undissolved debris. The supernatant was collected and mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% (w/v) SDS, 20% (v/v) glycerol and 0.3% (w/v) bromophenol blue) for non-reducing condition and in the presence of 10% (v/v) β-ME for reducing condition. Samples (15 µg protein, determined by Biuret method) were loaded onto a polyacrylamide gel made of 10% separating gel and 4% stacking gel and subjected to electrophoresis at a constant current of 20 mA/gel, using an electrophoresis apparatus (AE-6440, Atto Co., Tokyo, Japan). After electrophoresis, gels were fixed with a mixture of 50% (v/v) methanol and 10% (v/v) acetic acid for 45 min, followed by staining with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid for overnight with constant shaking. Finally, gels were destained with the mixture of 30% (v/v) methanol and 10% (v/v) acetic acid until clear background was obtained. The molecular weight protein standard markers, using Precision Plus Protein™ Unstained Standard (10-250 kDa) (Bio-Rad, CA, USA) were run in the same manner used to estimate the molecular weight of proteins. Gels were imaged using a scanner (MFC-L2700DW, Brother, UK) and band intensities were quantified with the public domain digital analysis software, ImageJ (ImageJ 1.51t, National Institutes of Health, Bethesda, USA).

2.3.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrum of WSPB was determined by Scientific Instrument Centre at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The analysis was performed using a Nicolet Model 6700 FT-IR Spectrometer (Thermo Scientific, Germany). Spectrum was acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–400 cm^{-1} at room temperature. Automatic signals were collected in 32 scans and evaluated against a background spectrum recorded from the clean, empty cell at 25°C.

2.3.4 Determination of surface hydrophobicity

Surface hydrophobicity of the WSPB samples was determined using a fluorescence probe 1-anilino-8-naphathalenesulfonate (ANS) following the method described by Malik *et al.* [15] with some modifications. WSPB was prepared at concentrations in the range of 0.05-0.5 mg/mL with a phosphate buffer (0.1 M, pH 7). 20 µL of ANS (8.0 mM in phosphate buffer 0.01 M, pH 7) was added to 4 mL of WSPB solution, vortexed and kept in the dark for 15 min. Relative fluorescence intensity (RFI) of both the buffer (blank) and each protein solution (from the lowest to the highest concentration) was measured using a fluorescence spectrometer (F-2700, Hitachi, Japan) at 390 nm (excitation wavelength) and 480 nm (emission wavelength), with a scanning speed of 5 nm s^{-1}. RFI of each dilution bank was subtracted from corresponding protein solution with the fluorescence probe ANS to obtain the net RFI. The initial slope of the plot of standardized net RFI values versus % protein concentration was expressed as surface hydrophobicity (H_o).

2.3.5 Determination of free and total sulfhydryl group content determination

The method used for determination of the sulfhydryl group content of WSPB was adopted from Malik *et al.* [15] with some modifications. The protein solution (0.5% w/v) was prepared using a standard buffer pH 8.0 (0.086 M Tris, 0.09 M glycine and 4 mM Na_2EDTA) for free sulfhydryl group determination and a denaturing buffer (standard buffer plus 8 M urea and 0.5% w/v sodium dodecyl sulfate) for total sulfhydryl group determination. The samples were then incubated at room temperature for 30 min. and the mixture was centrifuged (12,500 g for 20 min) prior to collecting the supernatant for determination. To each 4 mL aliquot of supernatant, 0.1 mL Ellman's reagent solution (5,5-dithiobis (2-nitrobenzoic acid): DTNB) (4 mg DTNB/mL buffer) was added, rapidly mixed and allowed to stand for 15 min. The solution was then read at 412 nm in an UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) against a blank. The blank was prepared by mixing 4 mL of the respective buffer with 0.1 mL of Ellman's reagent. In order to calculate micromoles of SH/g of protein, a molar extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} was used.

2.3.6 Color measurement

The color of WSPB powder was determined using the Colour Quest XE colorimeter (Hunter Lab., Hunter Assoc. Laboratory, USA). The setting for the illuminant was D_{65} source and the observer was standard 10°. Calibration of the instrument was conducted with black and white calibration tiles. WSPB powder was filled in a cuvette quart path length 25 mm, and three observations were measured and expressed as CIE L^* (lightness), a^* (redness), and b^* (yellowness) with 5 readings/samples.

2.4 pH measurement

WSPB (1 g) was mixed with 9 mL of distilled water and stirred at 100 rpm for 10 min. The pH value of the mixture was measured at room temperature in triplicate using an electronic pH meter (FE-20, Mettler-Toledo Instruments Co., Ltd., Switzerland).

2.5 Determination of protein functional properties

2.5.1 Protein solubility

Protein solubility was measured according to the method of Nalinanon *et al.* [16] with slight modifications. The solubility of WSPB was determined at pH values from 1 to 11. Briefly, 50 mg of WSPB was dispersed in 8 mL of distilled water and the pH of the mixture was adjusted to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 using 1 M HCl or 1 M NaOH. The dispersion was stirred for 30 min at room temperature and then the volume was adjusted to 10 mL and centrifuged at 6,000 g for 10 min. The supernatant was collected and subjected to protein determination using Biuret method. Bovine serum albumin was used as a protein standard. Total protein content in the sample was determined from the soluble portion of the sample in 0.5 M NaOH and relative solubility of protein sample was calculated as follows:

Relative solubility (%) = (protein content in supernatant/ total protein content in sample) × 100

2.5.2 Emulsion activity index (EAI) and emulsion stability index (ESI)

The EAI and the ESI were determined according to the method of Pearce and Kinsella [17], with slight modifications. 2 mL of soybean oil and 6 mL of protein solution (5 mg/mL) were homogenized at 20,000 rpm for 1 min. An aliquot of the emulsion (50μL) was pipetted from the bottom portion of the container at 0 and 10 min after homogenization and subsequently diluted 100-fold using 0.1% sodium dodecyl sulfate (SDS) solution. Each sample was mixed thoroughly for 10 s using a vortex mixer. A_{500} of the resulting dispersion was measured using a spectrophotometer (UV-1800, Shimadzu, Japan). EAI and ESI were calculated as follows:

$$EAI\ (m^2/g) = (2\times 2.303\times A\times DF)/l\Phi C$$

where $A = A_{500}$, DF = dilution factor (100), l = path length of cuvette (m), Φ = oil volume fraction and C = protein concentration in aqueous phase (g/m^3)

$$ESI\ (min) = (A_0\times t/\Delta A)$$

where $\Delta A = A_0 - A_{10}$ and t = 10 min.

2.5.3 Foaming capacity (FC) and foam stability (FS)

FC and FS of WSPB solution was determined as described by Nalinanon *et al.* [16] with slight modification. Sample (35 mL), with 5 mg/mL protein concentration, was transferred into a 100-mL cylinder. The solutions were homogenized at 16,000 rpm for 1 min at room temperature (about 25°C) and the samples were allowed to stand for 0 and 60 min. FE and FS were then calculated using the following equations:

$$FC\ (\%) = V_T/V_0 \times 100$$
$$FS\ (\%) = V_{60}/V_0 \times 100$$

where V_T is total volume after whipping, V_0 is the original volume before whipping, and V_{60} is total volume after leaving at room temperature for 60 min.

2.6 Determination of antioxidant activity

2.6.1 DPPH radical scavenging activity

DPPH radical scavenging activity was measured following the method of Murakami *et al.* [18] with a slightly modification. Briefly, The reaction mixture contained 5.4 mL of WSPB at different concentration and 0.6 mL of 0.8 mM DPPH in 95% ethanol. The mixture was incubated at room temperature for 30 min in dark, and then the absorbance at 517 nm was measured using a spectrophotometer (UV-1800, Shimadzu, Japan). The control was prepared in the same manner excepted that distilled water was used instead of the sample. The percentage of DPPH· scavenging activity of the sample was calculated as:

$$\text{Scavenging activity (\%)} = (1\text{-}A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the assay without sample and A_{sample} is the absorbance in the presence of the WSPB.

The result was expressed as the IC_{50} value. The IC_{50} (concentration providing 50% inhibition) value was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

2.6.2 ABTS radical scavenging activity

ABTS radical scavenging activity was measured following the method of Rice-Evans *et al.* [19] with a slightly modification. The ABTS radical ($ABTS^{\bullet+}$) was produced by reacting 7.4 mM ABTS stock solution with 2.45 mM potassium persulphate at a ratio of 1:1 (v/v). The mixture was allowed to react for 12-16 h at room temperature in the dark. This working solution of $ABTS^{\cdot+}$ solution was diluted with 95% ethanol, in order to obtain an absorbance of 0.700 ± 0.020 at 734 nm. The reaction mixture contained 0.15 mL of WSPB at different concentrations and 2.85 mL of $ABTS^{\cdot+}$ solution. The mixture was incubated at room temperature for 6 min in dark. Then, the absorbance at 734 nm was measured using a spectrophotometer (UV-1800, Shimadzu, Japan). The control was prepared in the same manner excepted that distilled water was used instead of the sample. The percentage of $ABTS^{\cdot+}$ scavenging activity and IC_{50} of the sample was calculated in the same manner as described in section 2.6.1.

2.6.3 Ferric reducing antioxidant power (FRAP)

FRAP was determined by the method described by Benzie and Strain [20]. Briefly, the FRAP reagent was freshly prepared by mixing of 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ solution and 300 mM acetate buffer, pH 3.6 (1:1:10 *v*/*v*/*v*). A sample (0.1 mL) was mixed with 3mL of FRAP reagent and the mixture was left at room temperature for 8 min in the dark. The absorbance was measured at 593 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 20-120 µg. The activity was expressed as µg Trolox equivalent (TE)/g protein.

2.7 Statistical analysis

All results were performed in triplicate. Data were presented as means ± standard deviation and a probability value of <0.05 was considered significant. For pair comparison, T-test was used. SPSS statistic program (SPSS 11.0 for Windows, SPSS Inc., Chicago, USA) was used for data analysis.

3. Results and Discussion

3.1 Yield and characteristics of WSPB

The yield and some physicochemical characteristics of water-soluble protein from *B. mori* (WSPB) are shown in Table 1. The silkworm pupae was simply extracted with distilled water. The yield of the resultant freeze-dried counterpart or WSPB was 3.62% based on wet weight basis. Kim *et al.* [8] reported that the yield of defatted silkworm pupae flour was about 35.84% (dry weight), which composed of ground whole insect without fat. The difference is probably due to different preparation and extraction procedures. According to the total protein content of *B. mori* (6.04%) determined by Kjeldahl method, the extraction efficiency of WSPB was calculated to be 65.62%, indicating high efficacy of protein extraction. The protein extraction method using water in the present work is low cost, environmental friendly and practical of use in large scale of protein extraction.

The pH of WSPB was 6.64, which was slightly higher than that reported for untreated silkworm pupae flour (pH 6.43) [8]. This might be due to the difference in the source or cultivation of the silkworms. The color of WSPB expressed as *L**, *a** and *b** values was 75.21, 2.11 and 24.67, respectively, presenting a bright-light reddish-yellow color. However, a general observation of the visible color of WSPB powder tended to be bright yellow. The predominant pigment of silkworm pupae is melanin, which can be black, brown or yellow in color [21]. Kim *et al.* [8] dried silkworm pupae at high temperature, which might be one cause for the color to be darker (*L** = 42.95) in their experiments compared to this result.

Table 1. Yield and physicochemical characteristics of water-soluble protein from *B. mori* (WSPB)

		WSPB[†]
Yield (%)		3.96±0.14
Extraction efficiency (%)		65.62±2.29
pH		6.64±0.01
Color	*L**	75.42±0.17
	*a**	2.22±0.11
	*b**	24.70±0.13
Free sulfhydryl content (μmol/g)		22.18±0.05
Total sulfhydryl content (μmol/g)		23.08±0.07
Surface hydrophobicity (H_0)		3.52±0.03

[†]Mean ± SD from triplicate determinations.

The total sulfhydryl content and free sulfhydryl content of WSPB were 23.08 and 22.18 μmol/g, respectively. It was suggested that WSPB may have a good reducing power as a function of –SH group. The surface hydrophobicity (H_o) of WSPB was 3.52±0.03, which was lower than previously reported by Azagoh *et al.* [22] who found surface hydrophobicity of the mealworm beetle larvae (*Tenrbrio molitor*) to be 102.5. Higher surface hydrophobicity was also previously found in soy protein isolate [23] and soluble protein from *T. molitor* larvae meal [22]. They explained that aggregating proteins are more hydrophobic and hydrophobic zones are buried inside the structure of proteins. Elias *et al.* [24] proposed that amino acids in protein including those with sulfhydryl groups (methionine and cysteine) or aromatic ring (tryptophan, tyrosine, and phenylalanine) contain a hydrogen atom that can interact with free radicals. With high free sulfhydryl content and low surface hydrophobicity, WSPB could be easily extracted into water fraction and might also ready to modify its structure to have better functionality as desired.

3.2 Amino acid composition

The amino acid composition of WSPB is presented in Table 2. WSPB composed of 15 amino acids of which 7 are essential amino acids and 8 nonessential amino acids. The predominant essential amino acids were histidine, lysine, threonine and valine and the predominant nonessential amino acids were glutamic acid, proline, glycine and tyrosine. Glutamic acid, histidine, proline and glycine were the major amino acid found in WSPB with descending amount in order. Rao [25] also reported that glutamic acid was the most abundant amino acid in silkworm larvae. These results are similar to those reported by Wu *et al.* [1] for *B. mori*, although, the level of amino acid content was lower when compared to those reports of Rao [25] and Longvah *et al.* [26]. These differences might be due to differences in the extraction method and source of insects. Generally, protein functionality and bioactivity govern by its amino acid composition as well as amino acid sequence [27]. In addition, the total hydrophobic amino acid, including isoleucine, leucine, methionine, phenylalanine, valine, alanine, glycine and proline of WSPB was calculated to be 333 residues/1000 residues, indicating that WSPB had slightly low molecular hydrophobicity.

Table 2. Amino acid composition of water-soluble protein from *B. mori* (WSPB) (residues/1000 residues)

Amino acids	WSPB (residues/1000 residues)
Essential amino acids	
Histidine	129
Isoleucine	29
Leucine	36
Lysine	54
Methionine	ND
Phenylalanine	23
Threonine	53
Tryptophan	ND
Valine	50
Nonessential amino acids	
Alanine	52
Arginine	37
Aspartic/Asparagine	62
Cysteine	ND
Glutamic/Glutamine	210
Glycine	66
Proline	77
Serine	59
Tyrosine	53

ND is not detectable.

3.3 SDS-PAGE

Protein pattern and molecular weight distribution of WSPB were analyzed by SDS-PAGE using 10% separating gel under reducing and non-reducing conditions as shown in Figure 1. There was a wide range of molecular weights in the WSPB ranging from lower 20 kDa to 250 kDa. Four major groups of protein bands under reducing condition were found to be 50-75 kDa, 37-50 kDa, 25-37 kDa and less than 25 kDa with the observed protein bands at 37 kDa, 45 kDa, 64 kDa, 75 kDa and 80 kDa being abundant. Four major groups of protein bands under non-reducing condition were found to be over 150 kDa, 50-100 kDa, 37-50 kDa and less than 25 kDa with the observed protein band intensity 37 kDa, 64 kDa and 75 kDa being abundant. The protein patterns of WSPB under non-reducing condition were difference from reducing condition. The absence of protein bands with molecular weight over 250 kDa and lower band intensity of three major bands in WSPB under non-reducing condition and with the presence of protein bands with 80 kDa, 54 kDa and 45 kDa under reducing condition indicated that WSPB contained disulfide bonds. Wang *et al.* [4] reported four major protein components in silk worm pupae, including albumin (97.4 kDa, 61.4 kDa, 44.4 kDa and 26.7 kDa), glutelin (200 kDa and 15 to 60 kDa), globulin (130.0 kDa and 26.8 kDa) and prolamin (15.3 to 46 kDa).

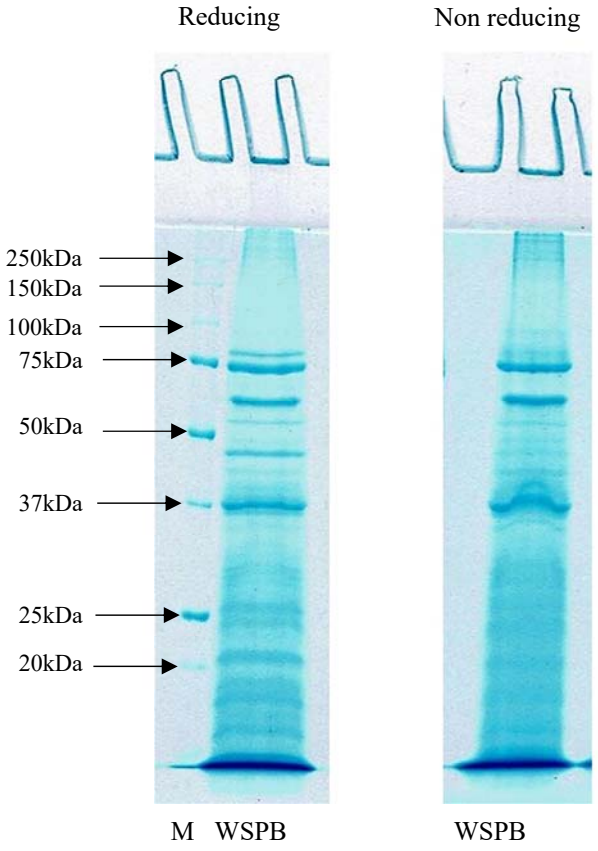

Figure 1. SDS-PAGE patterns of water-soluble protein from *B. mori* (WSPB) under reducing and non-reducing conditions. M denotes standard molecular weight protein markers.

3.4 Fourier-transform infrared (FTIR) spectrum of WSPB

The FTIR spectrum of WSPB is depicted in Figure 2. The result showed that WSPB had three characteristic amide bands representing amide *B* (2900–3200 cm^{-1}), amide *I* (1600–1700 cm^{-1}) and amide *III* (1200–1400 cm^{-1}). This result was in accordance with those previously reported in silkworm pupae protein modified by ultrasound or micronization techniques [28]. The major peaks were found at wavenumbers of 2924.47, 1609.58, and 1398.30 cm^{-1} for amide *B*, amide *I* and amide *III*, respectively. Amide *B* corresponded to asymmetric stretch vibration of =C–H as well as $–NH_3^+$ and amide *I* bands originated from C=O stretching vibrations coupled to N–H bending vibrations, CN stretch and CCN deformation [29]. Amide *III* represented the combination peaks between N–H deformation and C–N stretching vibrations and was involved with the triple helical structure of protein [30]. As a result, WSPB remained its structural integrity after extraction.

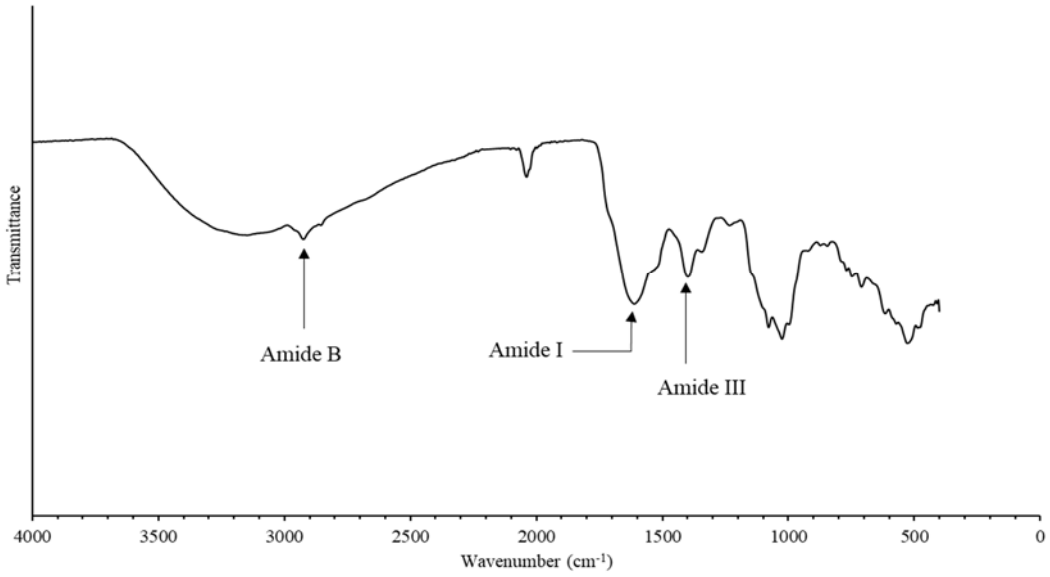

Figure 2. Fourier transform infrared spectrum of water-soluble protein from *B. mori* (WSPB)

3.5 Functional properties of WSPB

The solubility of WSPB in the pH range of 1-11 was depicted in Figure 3. The result showed that WSPB was highly solubilized more than 80% in the pH range of 5-11, indicating that the protein in silkworm pupae can be solubilized at neutral to alkaline pH. At acidic pH, the solubility of WSPB was generally low (< 40%). The lowest solubility was found in the pH range of 3-4, indicating isoelectric pH (pI) of WSPB. Wang *et al.* [4] reported that the pIs of albumin, globulin, glutelin and prolamin of silk worm protein were 2.5, 2.7, 4.0 and 4.5, respectively. This was due to a reduction in electrostatic repulsive forces between the proteins, leading to protein aggregation [22]. This effect is similar to those reported for several legumes, animal protein and protein isolates [22, 31, 32].

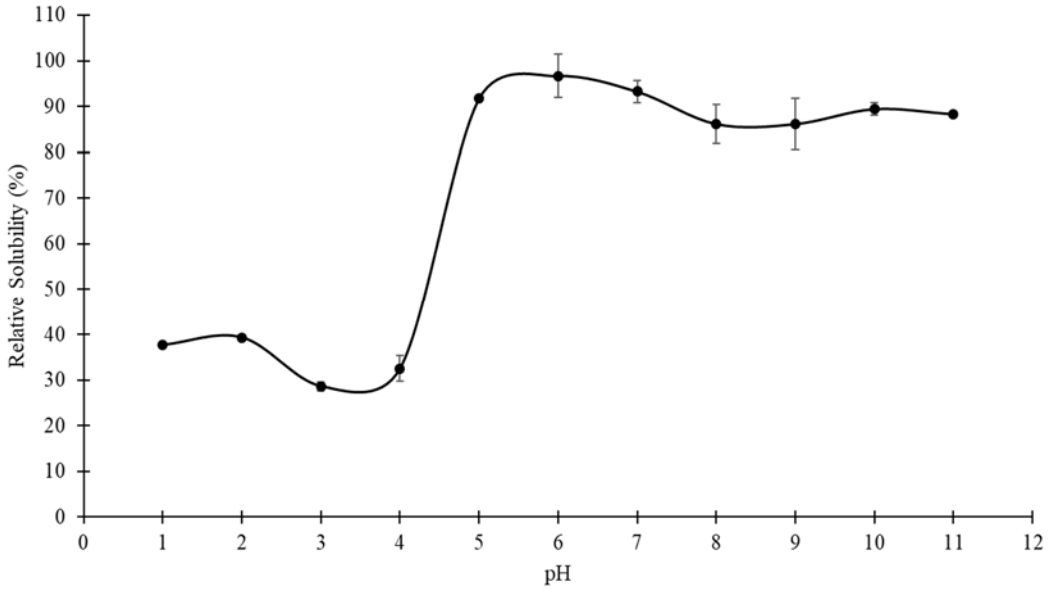

Figure 3. Relative solubility (%) of water-soluble protein from *B. mori* (WSPB) as affected by different pHs.

Emulsifying and foaming properties of WSPB are shown in Table 3. The emulsion activity index (EAI) and emulsion stability index (ESI) of WSPB were 25.09 m^2/g and 21.15 min, respectively. The EAI and ESI of WSPB were significantly lower than bovine serum albumin (BSA) ($p<0.05$). The differences between the emulsion activity and emulsion stability are related to the amphiphilicity of the protein surface, protein contents (soluble and insoluble) and other components [6].

Table 3. Emulsifying and foaming properties of water-soluble protein from *B. mori* (WSPB) and bovine serum albumin (BSA) [†]

Functional properties	WSPB	BSA
Emulsion activity index (EAI) (m^2/g)	$25.09\pm1.34^{b*}$	295.24 ± 2.30^{a}
Emulsion stability index (ESI) (min)	21.15 ± 0.22^{b}	38.55 ± 0.77^{a}
Foam capacity (FC) (%)	9.29 ± 1.01^{b}	81.23 ± 7.27^{a}
Foam stability (FS) (%)	93.46 ± 0.06^{a}	67.41 ± 2.95^{b}

[†] Mean ± SD from triplicate determinations.
[*] Different superscript letters in the same row indicate significant differences ($p<0.05$).

WSPB exhibited 9.29% and 93.46% for foam capacity (FC) and foam stability (FS), respectively. The FC of WSPB was low when compared with BSA (81.23%) ($p<0.05$). This might be due to the variation of molecular weights of protein component in WSPB, interrupting the formation of protein film at the lamellae of air bubble. The result was similar to those reported by Adebowale *et al.* [33] for whole giant cricket (*Gryllidae* sp.) powder that had a FC of 6%, and Omotoso [34] who reported the FC from *Cirina forda* as 7.1%. However, FS of WSPB was significantly higher than that of BSA

(67.41%) ($p<0.05$). Johnson and Zabik [35] explained that intermolecular protein-protein interaction enhances the cohesive nature of the film, therefore imparting stability and elasticity to the membrane. This interaction appears to be dependent on the presence of a high ratio of nonpolar/polar side chains in the protein [35] which was found in WSPB, according to its amino acid component. Zielińska *et al.* [6] reported the FC of *Gryllidae sigillatus* flour as 41% and FS was 34.67%. The differences between FC and FS of proteins may be due to their different compositions in different species and their different conformational characteristics [6]. The differences in FS is also probably due to components such as carbohydrates, which reduces protein-protein interactions and leads to formation of weak interfacial membranes that are unable to stabilize the foams [36].

3.6 Antioxidant activities

Antioxidant activities as determined by ABTS, DPPH and FRAP assays of WSPB are shown in Table 4. WSPB exhibited strong scavenging activity on DPPH and ABTS radicals with IC_{50} of 43.11 and 16.57 µg/mL, respectively. Wu *et al.* [1] reported that protein hydrolysates from larval instars of silkworm, obtained after digestion with gastrointestinal proteases, had $DPPH^{\bullet}$ scavenging capacity (IC_{50}) of 57.91 µg/mL. Pachiappan *et al.* [37] reported that silkworm pupae powder had $DPPH^{\bullet}$ scavenging capacity (IC_{50}) of 60.58 µg/mL. These reports found less effective on $DPPH^{\bullet}$ scavenging activity when compared with the present result (IC_{50} of 43.11 µg/mL). In contrast, the results had low potential on DPPH inhibition when compared with methanolic silkworm pupae extract from muga silkworm (*Antheraea assamensis*) (IC_{50} of 25.83 µg/mL as reported by Deori *et al.* [38]. Additionally, Zielińska *et al.* [36] reported that the antiradical activity against $DPPH^{\bullet}$ for the hydrolysates obtained after digestion of five edible insects ranged from 19.1 to 76.3 µg/mL. The antiradical activity against $ABTS^{\bullet+}$ (IC_{50}) ranged from 4.6 to 25.9 µg/mL [36]. The more stable products could be formed and the radical chain reaction terminated from these peptides since they are electron donors that could react with free radicals [39]. These results indicate that insect protein could be a good source of antioxidant peptides.

Table 4. Antioxidant activity of water-soluble protein from *B. mori* (WSPB)

Antioxidant assays	WSPB[†]
DPPH (IC_{50} µg/mL)	43.11±0.11
ABTS (IC_{50} µg/mL)	16.57±0.04
FRAP (µg TE/g protein)	54.20±0.13

[†] Mean ± SD from triplicate determinations

As per FRAP assay, WSPB also presented reducing power on Fe^{3+} of 54.20 µg TE/g protein. Bousopha *et al.* [40] reported that collagen hydrolysate from Pharaoh cuttlefish skin with 10-30% DH had ferric reducing power values of 23.50 to 26.50 µmolTE/g protein. The increase or decrease in ferric reducing power for protein hydrolysates may be related to the exposure of electron-dense amino acid side chain groups, such as polar or charged moieties during hydrolysis [41]. Chalamaiah *et al.* [42] reported that the reducing power of three carp roe protein hydrolysates increased with increasing concentrations. Compounds with higher reducing power were shown to have a better ability to donate electrons or hydrogen and serve as a significant indicator of their potential for use as an antioxidant [29]. The antioxidant activities of $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP scavenging ability, appear to be dependent on the molecular weight of the peptide [42]. In addition, those with a low-molecular weight had lower antioxidant activity. This suggests that the hydrolysates obtained from some edible insect protein can be used as compounds that are able to donate electrons and thus show antioxidant activity.

4. Conclusions

B. mori could be a good source of protein. Its water-soluble protein fraction exhibited beneficial physicochemical and functional properties as well as high antioxidant activity. This edible insect protein can be used as an alternative food ingredient in many food applications.

5. Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

6. Acknowledgements

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