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Characteristics, Functional Properties, and Antioxidant Activities of Water-Soluble Proteins Extracted from Grasshoppers, Patanga succincta and Chondracris roseapbrunner

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Abstract

Water-soluble proteins extracted from two species of grasshoppers, Patanga succincta (WSPP) and Chondracris roseapbrunner (WSPC), were characterized as well as their functional properties and antioxidant activities were investigated. The extraction yield, on a wet weight basis, was 7.35% and 7.46% for WSPP and WSPC, respectively. The most abundant amino acid in both proteins was glutamic acid, followed by aspartic, alanine, and leucine, in that order. The electrophoretic study revealed that proteins with MW of 29, 42, 50, 69, and 146 kDa were the major protein components in WSPP and WSPC. FTIR analysis showed that those proteins remained their structural integrity. The surface hydrophobicity at pH 7 of WSPC was higher than WSPP, but the sulfhydryl group content did not show significant difference between the proteins from two species. Both grasshopper proteins were mostly soluble in strong acidic and alkaline aqueous solutions with a minimum value at pH 4. Those proteins exhibited poor emulsifying properties and foaming capacity, but they had greater foaming stability compared with bovine serum albumin (BSA) ($p<0.05$). WSPC showed greater DPPH• and ABTS•+ scavenging activities and ferric-reducing antioxidant power (FRAP) than did WSPP ($p<0.05$). Therefore, based on characteristics and functional properties, water-soluble proteins from both edible grasshoppers can be used as an ingredient in food applications.

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Research Article

Characteristics, Functional Properties, and Antioxidant Activities of Water-Soluble Proteins Extracted from Grasshoppers, *Patanga succincta* and *Chondracris roseapbrunner*

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Water-soluble proteins extracted from two species of grasshoppers, *Patanga succincta* (WSPP) and *Chondracris roseapbrunner* (WSPC), were characterized as well as their functional properties and antioxidant activities were investigated. The extraction yield, on a wet weight basis, was 7.35% and 7.46% for WSPP and WSPC, respectively. The most abundant amino acid in both proteins was glutamic acid, followed by aspartic, alanine, and leucine, in that order. The electrophoretic study revealed that proteins with MW of 29, 42, 50, 69, and 146 kDa were the major protein components in WSPP and WSPC. FTIR analysis showed that those proteins remained their structural integrity. The surface hydrophobicity at pH 7 of WSPC was higher than WSPP, but the sulfhydryl group content did not show significant difference between the proteins from two species. Both grasshopper proteins were mostly soluble in strong acidic and alkaline aqueous solutions with a minimum value at pH 4. Those proteins exhibited poor emulsifying properties and foaming capacity, but they had greater foaming stability compared with bovine serum albumin (BSA) ($p < 0.05$). WSPC showed greater $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities and ferric-reducing antioxidant power (FRAP) than did WSPP ($p < 0.05$). Therefore, based on characteristics and functional properties, water-soluble proteins from both edible grasshoppers can be used as an ingredient in food applications.

1. Introduction

In 2050, the world population is estimated at more than 9 billion people, resulting in an additional need for food and feed outputs [1]. Conventional sources of protein will not be sufficient for the global human population, and alternative sources such as insects will be required [2]. Approximately 1,900 species of edible insects are traditionally consumed in many parts of the world, for example in Africa, Asia, and Latin America, and are considered as having potential to contribute to the world's food security [3]. In countryside of Northern and Northeastern Thailand, people consume several species of insects including grasshoppers, crickets, beetles, silkworm pupae, and bamboo worm.

Edible insects offer an important source of minerals, lipids, and above all proteins. Edible insects have higher crude protein content and have been reported to be a good source of essential amino acids [4]. Currently, most insect consumption is as a component ingredient of processed foods, and their successful utilization depends on fulfilling one or more functional requirements of good solubility, emulsion/foam capacity and stabilization, and gel formation [5, 6]. Omotoso [7] evaluated the functional properties of the larvae of Pallid Emperor Moth (*Cirina forda*) and found that

they had good solubility and emulsion properties. Yi et al. [8] reported poor foam and gelling properties of five different acid-extracted insect proteins, including those from a cricket (*Acheta domesticus*). Kim et al. [9] determined the effects of adding flour made from defatted mealworm larvae (*Tenebrio molitor*) and defatted silkworm pupae (*Bombyx mori*) and found that the added insect flours increased their cooking yield and hardness on emulsion sausages. Park et al. [10] also found that adding transglutaminase to silkworm pupae flour resulted in improved physicochemical properties of meat batter.

From previous reports, 32 insect species have been evaluated for their nutrition value in Thailand [11]. Their protein content ranged from 6.12 to 25.88 g/100 g wet weight, and their fiber levels ranged from 1.00 to 12.42 g/100 g wet weight [12, 13]. Yang et al. [14] reported polyunsaturated fatty acid content of 6 species of edible insects from Thailand to range from 726 to 2883 mg/100 g and monounsaturated fatty acid content to range from 714 to 5889 mg/100 g. This leads to the fact that edible insects are a potential source of fat and protein, but there is limited information on the characteristics and functional properties of extracted edible protein from specific insects. The aim of this investigation was, therefore, to characterize and compare the functional properties as well as antioxidant activities of water-soluble proteins from two species of grasshoppers (*P. succincta* and *C. roseapbrunner*), commonly found in Thailand, for their potential use as an alternative source of protein in food ingredients.

2. Materials and Methods

2.1. Materials. Frozen Bombay Locust (*P. succincta*) and Spur-throated grasshopper (*C. roseapbrunner*) were obtained from a commercial supplier (Mr. BUC FOOD, Phra Nakhon Si Ayutthaya, Thailand). These two species were selected, because they are well known, cheaper, and easily available in Thailand and are reported to have high protein content [11, 15].

2.2. Samples Preparation. The grasshopper samples were thawed and washed with running portable tap water, rinsed with distilled water, and drained using a plastic sieve for 1 h. The prepared samples were then stored in plastic containers at −20°C until further experiments.

2.3. Protein Extraction. The grasshopper proteins were extracted using an adapted extraction method of Yi et al. [8]. The insects were first blended with cold water (4°C) at a ratio of 1 : 4 w/v for 15 min using a blender (MMB54G5S, BOSCH, Germany) and stirred overnight at 4°C using a magnetic stirrer, to ensure that the proteins were dissolved. The suspension was centrifuged at 12,500 × g for 30 min at 4°C. The upper layer, containing the lipid fraction, and undissolved debris layer were removed. The supernatant or middle layer was collected, freeze-dried, and referred to as "WSPP" and "WSPC" for the protein from *P. succincta* and *C. roseapbrunner*, respectively. Extractions were performed in triplicate, and the protein content of the extracts was measured by the Kjeldahl method [16].

2.4. Calculation of Extraction Yield and Efficiency. The yields of WSPP and WSPC were calculated as a percentage of the weight of WSPP and WSPC in comparison with the weight of grasshoppers before extraction, as follows:

$$\text{yield}\,(\%) = \left(\frac{\text{weight of WSPP or WSPC}\,(\text{g})}{\text{weight of samples}\,(\text{g})}\right) \times 100. \quad (1)$$

Extraction efficiency was calculated as a percentage of the total protein extracted from *P. succincta* and *C. roseapbrunner* for WSPP and WSPC, respectively, in comparison with that of its protein content, which was determined by the Kjeldahl method [16]. The extraction efficiency of each WSPP and WSPC was calculated as follows:

$$\text{extraction efficiency}\,(\%) = \left(\frac{\text{total extracted protein of WSPP or WSPC}\,(\text{g})}{\text{total protein content of grasshoppers}\,(\text{g})}\right) \times 100. \quad (2)$$

2.5. Physicochemical Characterization

2.5.1. Amino Acid Composition. The amino acid compositions of WSPP and WSPC were determined by the Central Instrument Facility at Mahidol University, Bangkok, Thailand. 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, 3 μm) at 35°C. Amino acid standards (Sigma-Aldrich, USA) were used for calibration.

2.5.2. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was performed according to the method of Laemmli [17] with slight modifications. The samples (3 g) were mixed with 27 mL of 5% (w/v) SDS, heated at 85°C for 1 h, and then centrifuged at 8,500 × g for 5 min at 25°C 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 nonreducing condition and in the presence of 10% (v/v) β-ME for reducing condition. Samples (15 μg protein, determined by the 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 overnight with constant shaking. Finally, gels were destained with the mixture of 30% (v/v) methanol and 10% (v/v) acetic acid until a 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 and 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).

2.5.3. Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra of WSPP and WSPC were determined by Scientific Instrument Centre at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Analysis was performed using a Nicolet Model Nicolet 6700 FT-IR Spectrometer (Thermo Scientific, Germany) from 400 to 4000 cm^{-1} with automatic signal gain at 25°C. The spectra were collected in 32 scans at a resolution of 4 cm^{-1} and evaluated against a background spectrum from a clean empty cell.

2.5.4. Determination of Surface Hydrophobicity (H_0). Surface hydrophobicity of the protein samples was determined using a fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS) following the method described by Malik et al. [18] with some modifications. The protein solution 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 protein 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 spectrophotometer (F-2700 Hitachi, Japan) at 390 nm (excitation wavelength) and 480 nm (emission wavelength), with a scanning speed of 5 $nm \cdot s^{-1}$. RFI of each dilution bank was subtracted from the 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.

2.5.5. Determination of Free and Total Sulfhydryl Group Content. The method used for determination of the sulfhydryl group content of WSPP and WSPC was adopted from Malik et al. [18] 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 4 mM Na_2EDTA) for free sulfhydryl group determination and in the denaturing buffer (standard buffer plus 8 M urea and 0.5% w/v sodium dodecyl sulfate) for total sulfhydryl group determination. The samples were incubated at room temperature for 30 min. The mixture was then centrifuged (12,500 × g for 20 min at 25°C) and the supernatant collected 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 a UV-Vis spectrophotometer (Shimadzu UV 1800, 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 $1.36 \times 10^4\ M^{-1} \cdot cm^{-1}$ was used.

2.5.6. Color Measurement. The colors of WSPP and WSPC were determined using the ColourQuest 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. WSPP and WSPC were filled in a cuvette quartz path length 25 mm, and three observations were measured and expressed as CIE L^* (lightness), a^* (redness), and b^* (yellowness) with 5 readings/sample.

2.5.7. pH Measurement. One gram of WSPP and WSPC 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).

2.6. Evaluation of Functional Properties

2.6.1. Protein Solubility. Protein solubility was measured according to the method of Nalinanon et al. [19] with slight modifications. The solubility of WSPP and WSPC was determined at pH 1 to 11. Briefly, the protein sample (50 mg) 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 the volume was adjusted to 10 mL prior to centrifugation at 6,000 × g for 10 min at 25°C. The supernatant was collected and subjected to protein determination using the biuret method [20]. BSA 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 the protein sample was calculated as follows:

$$\text{relative solubility (\%)} = \left(\frac{\text{protein content in supernatant}}{\text{total protein content in sample}}\right) \times 100. \quad (3)$$

2.6.2. Emulsifying Properties. Emulsion activity index (EAI) and emulsion stability index (ESI) were determined according to the method of Pearce and Kinsella [21] with slight modifications. Two mL of soybean oil and 6 mL of protein solution (5 mg/mL) were homogenized (Model T25 basic; IKA) at 20,000 rpm for 1 min. An aliquot of the emulsion (50 μL) was pipetted from the bottom of the container at 0 and 10 min after homogenization and subsequently diluted 100-fold using 0.1% (w/v) 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:

$$\mathrm{EAI}\left(\mathrm{m}^2/\mathrm{g}\right) = \frac{(2 \times 2.303 \times A \times \mathrm{DF})}{l\Phi C}, \quad (4)$$

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

$$\mathrm{ESI\,(min)} = \left(A_0 \times \frac{t}{\Delta A}\right), \quad (5)$$

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

2.6.3. Foaming Properties. Foaming capacity (FC) and foam stability (FS) of protein samples were determined as described by Nalinanon et al. [19] with slight modifications. Solutions (35 mL) of each sample at 5 mg/mL protein concentration were transferred into a 100 mL cylinder. The solutions were homogenized by a homogenizer (Model T25 basic; IKA) at 16,000 rpm for 1 min at room temperature (~25°C), and the samples were allowed to stand for 0 and 60 min. FC and FS were then calculated using the following equations:

$$\mathrm{FC\,(\%)} = \left(\frac{V_T}{V_0}\right) \times 100, \quad (6)$$

$$\mathrm{FS\,(\%)} = \left(\frac{V_{60}}{V_0}\right) \times 100, \quad (7)$$

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

2.7. Determination of Antioxidant Activities

2.7.1. DPPH Radical Scavenging Activity. DPPH radical scavenging activity was determined by DPPH assay as described by Murakami et al. [22] with slight modifications. Briefly, the reaction mixture contained 5.4 mL of WSPP or WSPC at different concentrations 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 of the resulting solution was measured at 517 nm in a UV-1800 spectrophotometer (Shimadzu, Japan). The control was prepared in the same manner except that distilled water was used instead of the sample. The percentage of $\mathrm{DPPH}^{\bullet}$ radical scavenging activity of the sample was calculated as follows:

$$\text{scavenging activity\,(\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100, \quad (8)$$

where A_{control} is the absorbance of the assay without sample and A_{sample} is the absorbance in the presence of the WSPP or WSPC.

The sample concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting scavenging activity percentage against sample concentration.

2.7.2. ABTS Radical Scavenging Activity. ABTS radical scavenging activity was determined by ABTS assay as described by Rice-Evans et al. [23] with slight modifications. The ABTS radical ($\mathrm{ABTS}^{\bullet+}$) 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 $\mathrm{ABTS}^{\bullet+}$ solution was diluted with 95% ethanol at a ratio of 1 : 50 (v/v), in order to obtain an absorbance of 0.700 ± 0.020 at 734 nm. A fresh $\mathrm{ABTS}^{\bullet+}$ solution was prepared for each assay. The reaction mixture contained 0.15 mL of WSPP or WSPC at different concentrations and 2.85 mL of $\mathrm{ABTS}^{\bullet+}$ solution. The mixture was incubated at room temperature for 6 min in dark. Then, the absorbance was measured at 734 nm using a UV-1800 spectrophotometer (Shimadzu, Japan). The control was prepared in the same manner except that distilled water was used instead of the sample. The percentage of $\mathrm{ABTS}^{\bullet+}$ scavenging activity of the sample were calculated in the same manner as described in section 2.7.1. The IC_{50} value was the inhibitory concentration at which $\mathrm{ABTS}^{\bullet+}$ were scavenged by 50%.

2.7.3. Ferric-Reducing Antioxidant Power (FRAP). FRAP was determined by the method described by Benzie and Strain [24]. FRAP reagent was freshly prepared by mixing 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mM HCl, 20 mM $FeCl_3$, and 300 mM acetate buffer, pH 3.6 (1 : 1 : 10 v/v/v). A sample (0.1 mL) was mixed with 3 mL 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 UV-1800 spectrophotometer (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 with Trolox ranging from 20 to 120 μg/mL. The antioxidant power of the samples was expressed as μg Trolox equivalent (TE)/g protein.

2.8. Statistical Analysis. Extractions and all results were performed in triplicate ($n = 9$). All data were subjected to analysis of variance (ANOVA), and the differences between means were evaluated by Duncan's multiple range test. 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 Extraction Efficiency. The yield and extraction efficiency of water-soluble protein from grasshoppers, WSPP and WSPC, are shown in Table 1. The proteins from both grasshoppers were simply extracted with distilled water. The yields of the resultant freeze-dried counterparts called WSPP and WSPC were 7.35 ± 0.19 and 7.49 ± 0.19% (wet weight basis), respectively. Clarkson et al. [25] found that protein yield in soluble locust fraction from *L. migratoria* was 9.83%. The difference in extraction yield was probably due to different preparations and extraction procedures. The crude protein contents determined by the Kjeldahl method of *P. succincta* and *C. roseapbrunner* were 15.07 and 17.40% (wet weight basis), respectively (data not

Table 1: Yield, extraction efficiency, and physicochemical characteristics of water-soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC)[†].

	WSPP	WSPC
Yield (%)	7.35 ± 0.19[ns]	7.49 ± 0.19[ns]
Extraction efficiency (%)	48.77 ± 0.37[a‡]	42.86 ± 0.09[b]
pH	6.08 ± 0.01[ns]	6.07 ± 0.00[ns]
Colour		
L^*	47.26 ± 0.17[b]	55.80 ± 0.13[a]
a^*	6.04 ± 0.10[a]	5.48 ± 0.09[b]
b^*	16.80 ± 0.09[b]	18.20 ± 0.08[a]
Free sulfhydryl content (μmol/g)	0.48 ± 0.03[ns]	0.48 ± 0.01[ns]
Total sulfhydryl content (μmol/g)	1.06 ± 0.04[ns]	1.14 ± 0.04[ns]
Surface hydrophobicity (H_0)	15.92 ± 0.21[b]	22.59 ± 0.22[a]

[†]Mean ± SD from triplicate determinations. [‡]Different superscript letters in the same row indicate significant differences ($p < 0.05$). ns = no significant difference.

shown), which is expressed in dry weight to be 65.55 and 71.75%, respectively. The protein content of those grasshoppers was lower than that previously reported in the whole freeze-dried locust and alkali-extracted defatted locusts (65.87 and 82.26% dry weight, respectively) [26]. Clarkson et al. [25] reported that crude protein content of *L. migratoria* was 50.79% (dry weight). The extraction efficiency of WSPP and WSPC were calculated to be 48.77 and 42.86%, indicating high efficacy of protein extraction. Therefore, water-soluble protein might be one of the major protein components in grasshoppers and was suitable for protein extraction by water, an environment friendly method.

3.2. Physicochemical Characteristics of Grasshopper Proteins

3.2.1. pH and Color. The color and pH of WSPP and WSPC are shown in Table 1. WSPP and WSPC exhibited a faintly acidic pH with a value of 6.08 and 6.07, respectively. The color of WSPP and WSPC expressed as L^*, a^*, and b^* values was 47.26, 6.04, and 16.80, respectively, for WSPP and 55.80, 5.48, and 18.20, respectively, for WSPC. The WSPC had a higher value than the WSPP for L^* and b^*, but lower values for a^*, indicating more light green color in WSPC than WSPP. The green coloration could be due to a common green pigment identified in insects called insectoverdin [25]. Goodwin [27] postulated a mixture of two chromoproteins, one yellow component from carotenoids and the other a bile component (blue), creating the green color in the locust. However, a general observation of the visible color of both WSPP and WSPC tended to be brown, which could be attributed to melanin [28].

3.2.2. Surface Hydrophobicity and Sulfhydryl Content. The surface hydrophobicity of WSPC and WSPP was found to be 22.59 ± 0.22 and 15.92 ± 0.21, respectively (Table 1). From the result, surface hydrophobicity of both insect proteins was lower than the previous report of Azagoh et al. [29]. They reported that surface hydrophobicity of *T. molitor* larvae meal was 102.5. Aggregating proteins are more hydrophobic, and hydrophobic zones are buried inside the structure of proteins [30]. The surface hydrophobicity of protein extracted from edible insects has been shown to vary, depending on the extraction protocol [31]. Surface hydrophobicity might also be dependent on the size, conformation, amino acid composition, and sequence of a protein.

The total sulfhydryl content and free sulfhydryl content of WSPC (1.06 and 0.48 μmol/g) was comparable with WSPP (1.14 and 0.48 μmol/g) (Table 1). This was expected from the amount of sulphur-containing amino acids found in both proteins. Differences in sulfhydryl content found in different proteins have been reported. Van der Plancken et al. [32] reported that total sulfhydryl content of untreated egg white solution was 58.5 μM/g protein. Malik et al. [18] found that the free and total sulfhydryl content of sunflower protein isolate was about 7.7 and 80.1 μmol/g. Thus, the quantity of free and total sulfhydryl content might be depended on the source of protein, part of animal, and species of samples.

3.2.3. Protein Patterns. Protein patterns and molecular weight distribution of WSPP and WSPC analyzed by SDS-PAGE using 10% separating gel under reducing and nonreducing conditions are shown in Figure 1. There was a wide range of molecular weights in the WSPP and WSPC ranging from lower 20 kDa to 250 kDa. Six major groups of protein bands under reducing condition were found to be more than 250 kDa,75–150 kDa, 50–75 kDa, 37–50 kDa, 25–37 kDa and less than 25 kDa with the observed WSPP protein bands at 24 kDa, 29 kDa, 45 kDa, 52 kDa, 61 kDa, 69 kDa, 124 kDa and 146 kDa being abundant. WSPC protein bands were abundant at 22 kDa, 29 kDa, 35 kDa, 37 kDa, 42 kDa, 47 kDa, 50 kDa, 61 kDa, 69 kDa and 146 kDa. Under reducing condition, WSPC found more protein bands than WSPP, and 50–75 kDa and 37–50 kDa were generous protein bands. Five major groups of protein bands under nonreducing condition were found to be 100–150 kDa, 50–75 kDa, 37–50 kDa, 25–37 kDa and less than 25 kDa with the observed WSPP protein band intensity 24 kDa, 29 kDa, 42 kDa and 61 kDa being abundant. WSPC protein bands were abundant at 22–23 kDa, 27 kDa, 42 kDa, 61 kDa and 146 kDa. Purschke et al. [26] reported that protein concentrate from *Locusta migratoria* included proteins in the range of 6–100 kDa. The characteristic bands (40, 50 and 100 kDa) of *L. migratoria* protein contained muscle protein tropomyosin and high amounts of tubulin, which are responsible for the formation of the microtue bules [26]. Zielińska et al. [33] reported that raw locust *S. gregaria* had protein band with molecular weight range between 29.0 and 44.3 kDa and 97.2 kDa, while boiled locust found low intensity of protein band at range 29.0–44.3 kDa and baked locust disappeared protein band. The differences in protein patterns under reducing and nonreducing conditions might be depended on species of insects, extraction method, and insects processing. Furthermore, protein functionalities and antioxidant activities are also governed by molecular weight distribution, amino acid composition and amino acid sequence of the protein itself [19].

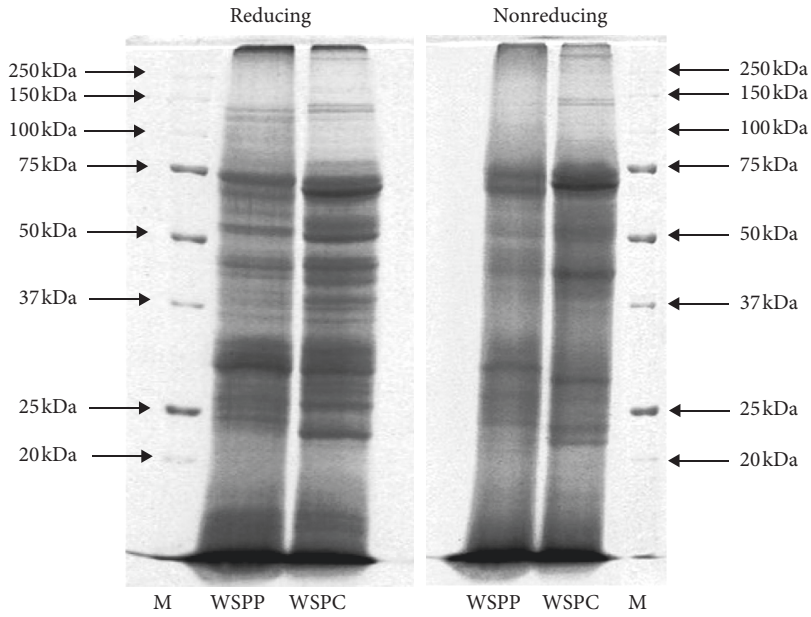

Figure 1: SDS-PAGE patterns of water-soluble protein from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) under reducing and nonreducing conditions.

3.2.4. Amino Acid Composition. The amino acid compositions of WSPP and WSPC are presented in Table 2. Generally, both grasshopper proteins had similar amino acid profile. WSPP and WSPC composed of 15 amino acids of which 7 essential amino acids and 8 nonessential amino acids. Methionine, tryptophan and cysteine were not detectable, possibly due to they had very low ratio or were not present in the proteins studied. The predominant essential amino acids were leucine and lysine which are similar to those reported for *Locusta migratoria* [26], *Tenebrio molitor* larvae [29] and silkworm larvae protein isolates [34]. The amount of essential amino acids in WSPP and WSPC were sufficient to meet the adult nutritional requirements based on the FAO/WHO guidelines [1]. The predominant nonessential amino acids were glutamic acid, aspartic acid, alanine and arginine. Glutamic acid, aspartic acid, leucine and alanine were the major amino acid found in both WSPP and WSPC with descending amount in order. Glutamic acid was the most abundant amino acid in locust and grasshopper [35, 36]. These results are similar to those reported by Makkar et al. [35] for grasshoppers (glutamic acid 4.5–15.2 g/16 g nitrogen). These differences might be due to the differences in the extraction method, species and source of insects. In general, protein functionality and bioactivity govern by its amino acid composition as well as amino acid sequence [37]. Both WSPP and WSPC contained higher amount of hydrophobic amino acids than hydrophilic amino acids. It has been shown that hydrophobic amino acids and one or more residues of histidine, proline, methionine, cysteine, tyrosine, tryptophan and phenylalanine can enhance the activities of the antioxidant peptides [5]. However, although both WSPP and WSPC had similar amino acid profile, they might have same or different molecular properties, functionalities and bioactive potentials which also depending on their amino acid sequence.

Table 2: Amino acid composition of water-soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) (residues/1000 residues).

Amino acids	WSPP	WSPC
Essential amino acids		
Histidine	22	23
Isoleucine	48	44
Leucine	86	81
Lysine	76	74
Methionine	ND	ND
Phenylalanine	44	43
Threonine	49	49
Tryptophan	ND	ND
Valine	59	60
Nonessential amino acids		
Alanine	85	88
Arginine	83	79
Aspartic/asparagine	98	98
Cysteine	ND	ND
Glutamic/glutamine	156	151
Glycine	62	63
Proline	55	57
Serine	52	50
Tyrosine	27	39
Hydrophobic amino acids*	439	436
Hydrophillic amino acids**	382	387

ND is not detectable. *Hydrophobic amino acids include isoleucine, leucine, methionine, phenylalanine, valine, alanine, glycine, and proline. **Hydrophillic amino acids include serine, threonine, cysteine, aspartic/asparagine, glutamic/glutamine, and tyrosine.

3.2.5. Fourier Transform Infrared Spectra. The FTIR spectra of WSPP and WSPC are depicted in Figure 2. The result showed that WSPP and WSPC had five characteristic amide bands representing amide *A* (3200–3300 cm^{-1}), amide *B* (2900–3200 cm^{-1}), amide *I* (1600–1700 cm^{-1}), amide *II*

(1500–1600 cm^{-1}) and amide *III* (1200–1400 cm^{-1}), which confirms those previously reported in collagen from skins of young and adult Nile perch (*Lates niloticus*) by Muyonga et al. [38]. The major peaks of WSPC were found at wavenumbers of 3268.80, 2924.28, 1623.80, 1515.21, and 1398.25 cm^{-1} for amide *A*, amide *B*, amide *I*, amide *II* and amide *III*, respectively, whereas WSPP were found at wavenumbers of 3259.16, 2921.09, 1621.87, 1514.87 and 1400 cm^{-1}, respectively. Ramappa et al. [39] reported that the major absorption peaks in FTIR spectra of various silkworm pupae powder were also ranged in amide regions of 1630–1680 (N-H bending), 1600–1650 (C=O stretching) and 1500–1570 (N-H bending). Amide *A* corresponds to the stretching vibrations of N–H group, amide *B* corresponds 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 [40]. Amide *II* representing N–H bending vibrations coupled to C–N stretching vibrations. 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 [38]. Those typical amide bands of the protein corresponded to particular stretching and bending vibrations of the protein backbone [41]. Amide *I* arises from α-helix (1650–1658 cm^{-1}) and β-sheet (1638 cm^{-1}, 1687 cm^{-1}), while N-H bending vibrations coupled to C-N stretching vibrations attributed to amide *II* [42]. For amide *III*, it was obtained from a complex mix of α-helix (1290–1340 cm^{-1}) and β-sheet (1181–1248 cm^{-1}) along with random coil (1255–1288 cm^{-1}) [42]. As a result, both WSPP and WSPC remained their structural integrity after extraction. However, the FTIR spectra of WSPP and WSPC were generally similar but the differences in a few characteristic peaks were detected (Figure 2), indicating slight differences in the structure, amino acids and functional groups of proteins [43].

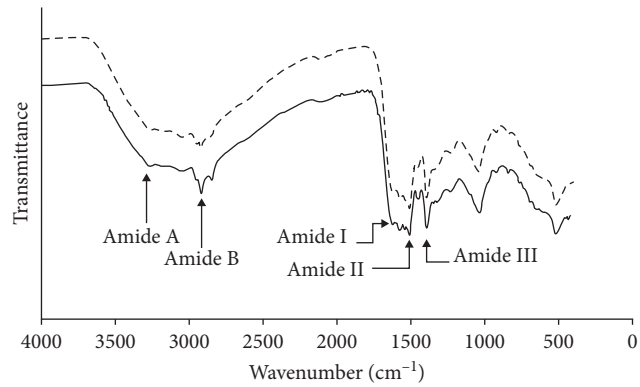

Figure 2: Fourier-transform infrared spectra of water-soluble protein from *P. succincta* (WSPP) (—) and *C. roseapbrunner* (WSPC) (---).

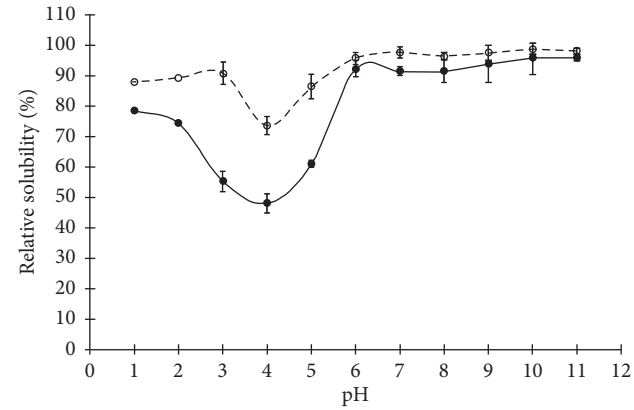

Figure 3: Relative solubility (%) of water-soluble protein from *P. succincta* (WSPP) (—) and *C. roseapbrunner* (WSPC) (---) as affected by different pHs.

3.3. Functional Properties

3.3.1. Relative Solubility. The relative solubility (%) of WSPP and WSPC in the pH range of 1–11 was depicted in Figure 3. The result showed that WSPC generally had higher relative solubility than WSPP in all pHs tested. WSPP and WSPC were highly solubilized more than 85% in the pH range of 6–11, indicating that the protein in grasshopper can be solubilized at neutral to alkaline pH. At strong acidic condition (pH 1–2), the solubility of grasshopper proteins was relatively high with relative solubility of 73–78% and 88–90% for WSPP and WSPC, respectively. However, the solubility of those proteins decreased with increasing pH to 3 and 4. The lowest relative solubility was found at the pH 4 for both proteins, indicating their isoelectric pH (p*I*). This was due to a reduction in electrostatic repulsive forces between the proteins, leading to protein aggregation [29]. These results correspond well with the protein from migratory locusts *S. gregaria* [44]. The p*I*s of migratory locust [26], edible meal worm and black soldier fly [45] were also found at pH 4. Additionally, the solubility of insect proteins was similar to those reported for several legumes, animal proteins and protein isolates [26, 29, 41, 45, 46].

3.3.2. Emulsifying Properties. Emulsifying and foaming properties of WSPP and WSPC are shown in Table 3. The emulsion activity index (EAI) and emulsion stability index (ESI) of WSPP and WSPC ranged from 29.23 to 36.69 m^2/g and 15.67 to 33.34 min, respectively. The EAI and ESI of those proteins were significantly lower than bovine serum albumin (BSA) ($p < 0.05$). Low emulsifying properties found in both insect proteins might be due to they contained high amount of low molecular weight components. When peptides are shorter and less globular, they will form less stable protein layers around the oil droplets that offer less resistance to coalescence or Ostwald ripening [47]. Adebowale et al. [48] reported an adequate emulsification but poor stability in whole giant African cricket (*Gryllidae* sp.) powder. In contrast, both high emulsion formation and stability in moth (*Cirina forda*) larva and silkworm (*Bombyx Mori*) powders have been report by [49]. Although WSPC exhibited greater EAI and ESI than WSPP, they had poor emulsifying properties when compared with BSA and

Table 3: Emulsifying and foaming properties of water-soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) and bovine serum albumin (BSA)[†].

Functional properties	WSPP	WSPC	BSA
Emulsion activity index (EAI) (m^2/g)	29.23 ± 0.79[c‡]	36.96 ± 0.59[b]	295.24 ± 2.30[a]
Emulsion stability index (ESI) (min)	15.67 ± 0.18[c]	33.34 ± 4.37[b]	38.55 ± 0.77[a]
Foaming capacity (FC) (%)	8.57 ± 4.04[c]	25.71 ± 4.04[b]	81.23 ± 7.27[a]
Foam stability (FS) (%)	98.72 ± 1.81[a]	86.41 ± 2.78[b]	67.41 ± 2.95[c]

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

protein from locusts *S. gregaria* [44]. Emulsifying properties of other proteins have been determined and reported. Tirgar et al. [50] reported that EAI and ESI of flaxseed protein were 46.5 m^2/g and 12.51 min, respectively. Hall et al. [51] reported EAI of 27 to 32 m^2/g for cricket (*G. sigillatus*) protein hydrolysates. EAI and ESI of WSPP were similar to that found by Nalinanon et al. [19], who reported EAI and ESI for protein hydrolysate at concentration of 0.5% from ornate threadfin bream (*Nemipterus* sp.) muscle and DH10-30% using skipjack tuna (*Katsuwonus* sp.) pepsin digestion were 29.9 to 30.3 m^2/g and 14.1 to 18.6 min, respectively. WSPC exhibited greater emulsifying properties than WSPP. This might be due to a greater surface hydrophobicity of WSPC had a positive effect on emulsification (Table 1). The ability of a protein to rapidly lower free energy of a newly created interface is controlled by (1) how rapidly it can adsorb to the interface and (2) how rapidly and easily it can undergo conformational rearrangement and reorientation at the interface [52]. 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 [44]. The lower ESI of WSPP and WSPC than BSA which could be attributed to partial denaturation of proteins and change in the distribution of molecular charge that exposes hydrophobic amino acid [44].

3.3.3. Foaming Properties. Foam capacity (FC) and foam stability (FS) of WSPP and WSPC are shown in Table 3. WSPP and WSPC exhibited 8.57, 25.71% and 86.41, 98.72% for FC and FS, respectively. WSPC showed higher FC than WSPP, although the FC of those proteins were low when compared with BSA (81.23%) ($p < 0.05$). The result of FC from WSPC was similar to those reported by Adebowale et al. [48], for whole giant cricket (*Gryllidae* sp.) powder that had an FC of 6%, and Omotoso [7], who reported the FC from *Cirina forda* larvae powder as 7.1%. However, WSPC had lower FS than WSPP. The FS of WSPP and WSPC (~80%) was significantly higher than that of BSA (67.41%) ($p < 0.05$), indicating the excellent capacity to stabilize foam against collapse. Generally, foam collapse takes place by any of these three mechanisms including (1) disproportionation of bubbles; (2) coalescence of bubbles due to instability of thin film between them; and (3) drainage of water from the surface of the bubbles down to the liquid layer, thereby leading to the removal of protein from film around the bubble [52]. Zielińska et al. [44] reported the FC of protein preparations with alkali extraction from locusts (*S. gregaria*) as 32% and FS was 6.17%. The differences between FC and FS of proteins may be due to the factors influencing foam formation including hydrophobic amino acid content, surface hydrophobicity, location of hydrophobic amino acid residues on the protein surface, presence of thiol groups, cations and anions, carbohydrates, and lipids [44]. Johnson and Zabik [53] explained that intermolecular protein-protein interaction enhances the cohesive nature of the film, 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 [53]. Additionally, Nalinanon et al. [19] suggested that low MW peptide could not maintain well-ordered orientation of the molecule at the interface.

3.7. Antioxidant Activities. Antioxidant activities as determined by ABTS, DPPH, and FRAP assays of WSPP and WSPC are shown in Table 4. WSPP and WSPC exhibited strong scavenging activities with IC_{50} of 204.67 and 176.31 μg/mL for DPPH radical and 81.97 and 69.12 μg/mL for ABTS radical, respectively. Zielińska et al. [54] reported that the antiradical activities against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ for the hydrolysates obtained after digestion of locusts *L. migratoria* were found to be 67 μg/mL and 25.9 μg/mL of their IC_{50}, respectively. These reports found less effective on $DPPH^{\bullet}$ scavenging activity when compared with the present result (IC_{50} of 176.31 to 204.67 μg/mL). Additionally, Zielińska et al. [33] reported that the protein hydrolysates prepared by alkali extraction from locusts (S. gregaria) exhibited IC_{50} for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities were 28.5 and 16.6 μg/mL, respectively. With high ABTS radical-scavenging activity, it was postulated that antioxidative compounds were most likely hydrophilic [19]. Also, with high DPPH radical-scavenging activity, the results obtained suggest that the grasshopper proteins contained amino acids or peptides that were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. The protein from various species of edible insects might give a different scavenging activity on $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. It might be depended on molecular weight of protein or peptide as well as its amino acid composition. Zhang et al. [55] postulated that peptides with large molecular weight have less antioxidant activity. Nalinanon et al. [19] found that peptides in hydrolysates from ornate threadfin bream muscle with various degree of hydrolysis (DH) might differently scavenge two different radicals, ABTS and DPPH radicals. In addition, the presence of hydrophobic sequences in the peptides could interact with

Table 4: Antioxidant activity of water-soluble protein from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC)[†].

Antioxidant assays	WSPP	WSPC
DPPH (IC_{50} μg/mL)	204.67 ± 2.02[a‡]	176.31 ± 2.02[b]
ABTS (IC_{50} μg/mL)	81.97 ± 0.07[a]	69.12 ± 0.26[b]
FRAP (μg TE/g protein)	22.26 ± 0.45[b]	27.59 ± 0.06[a]

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

lipid molecules and could scavenge by donating protons to lipid-derived radicals [5].

As per FRAP assay, WSPP and WSPC also presented reducing power on Fe^{3+} of 22.26 and 27.59 μg TE/g protein. Similar results were obtained by Xia et al. [56] who reported that the antioxidant properties of the barley glutelin hydrolysates had ferric-reducing power values of 24.0 μg Fe^{2+}/mg, respectively. Bousopha et al. [57] 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 [58]. 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 [59].

4. Conclusion

The proteins from edible insects, *P. succincta* and *C. roseapbrunner*, could be effectively extracted by water. Based on physicochemical and functional properties as well as antioxidant activities, water-soluble proteins from both insects are beneficial for human nutrition and had potential use as a food ingredient. This opens up the possibility for these edible insect proteins to be used as in suitable food applications.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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References

[1] FAO, *Food and Agriculture Organization of the United Nations, How to feed the World in 2050*, 2009.

[2] E. Zielińska, B. Baraniak, M. Karaś, K. Rybczyńska, and A. Jakubczyk, "Selected species of edible insects as a source of nutrient composition," *Food Research International*, vol. 77, pp. 460–466, 2015.

[3] A. van Huis, "Potential of insects as food and feed in assuring food security," *Annual Review of Entomology*, vol. 58, no. 1, pp. 563–583, 2013.

[4] S. Belluco, C. Losasso, M. Maggioletti, C. C. Alonzi, M. G. Paoletti, and A. Ricci, "Edible insects in a food safety and nutritional perspective: a critical review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 12, no. 3, pp. 296–313, 2013.

[5] M. Chalamaiah, B. Dinesh kumar, R. Hemalatha, and T. Jyothirmayi, "Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review," *Food Chemistry*, vol. 135, no. 4, pp. 3020–3038, 2012.

[6] M. Garcés-Rimón, M. Sandoval, E. Molina, R. López-Fandiño, and M. Miguel, "Egg protein hydrolysates: new culinary textures," *International Journal of Gastronomy and Food Science*, vol. 3, pp. 17–22, 2016.

[7] O. T. Omotoso, "Nutritional quality, functional properties and anti-nutrient compositions of the larva of Cirina forda (Westwood) (Lepidoptera: saturniidae)," *Journal of Zhejiang University SCIENCE B*, vol. 7, no. 1, pp. 51–55, 2006.

[8] L. Yi, C. M. M. Lakemond, L. M. C. Sagis, V. Eisner-Schadler, A. van Huis, and M. A. J. S. van Boekel, "Extraction and characterisation of protein fractions from five insect species," *Food Chemistry*, vol. 141, no. 4, pp. 3341–3348, 2013.

[9] H. W. Kim, D. Setyabrata, Y. J. Lee, O. G. Jones, and Y. H. B. Kim, "Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages," *Innovative Food Science and Emerging Technologies*, vol. 38, pp. 116–123, 2016.

[10] Y. S. Park, Y. S. Choi, K. E. Hwang et al., "Physicochemical properties of meat batter added with edible silkworm pupae (*Bombyx mori*) and transglutaminase," *Korean Journal for Food Science of Animal Resources*, vol. 37, no. 3, pp. 351–359, 2017.

[11] J. Yhoung Aree, P. Puwastien, and G. A. Attig, "Edible insects in Thailand: an unconventional protein source?," *Ecology of Food and Nutrition*, vol. 36, no. 2–4, pp. 133–149, 1997.

[12] U. Klinhom, C. Rasrirattana, and S. Jitjamnong, *An Investigation of Some Nutritive Value, Some Parasites and Some Toxic Components of Edible Insects in Northestern Thailand*, Srinakharinwirot University, Mahasarakham, Thailand, 1984.

[13] A. Lewvanich, S. Chunram, N. Chareontesprasit, and Y. Hanboonsong, *Diversity of Edible Insects in the North And North-East of Thailand in Biodeversity in Thailand: Biodiversity Research and Training Program*, 1999.

[14] L. F. Yang, S. Siriamornpun, and D. U. O. Li, "Polyunsaturated fatty acid content of edible insects in Thailand," *Journal of Food Lipids*, vol. 13, no. 3, pp. 277–285, 2006.

[15] Y. Hanboonsong, T. Jamjanya, and P. B. Durst, *Six-Legged Livestock: Edible Insect Farming, Collection and Marketing in Thailand*, Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand, e-book. http://www.fao.org/3/a-i3246e.pdf, 2013.

[16] A.O.A.C., *Official Methods of Analysis*, The Association of official analytical chemists, Washington, DC, USA, 16th edition, 2000.
[17] U. K. Laemmli, "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature*, vol. 227, no. 5259, pp. 680–685, 1970.
[18] M. A. Malik, H. K. Sharma, and C. S. Saini, "High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: effect on physicochemical and functional properties," *Ultrasonics Sonochemistry*, vol. 39, pp. 511–519, 2017.
[19] S. Nalinanon, S. Benjakul, H. Kishimura, and F. Shahidi, "Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna," *Food Chemistry*, vol. 124, no. 4, pp. 1354–1362, 2011.
[20] A. G. Gornall, C. J. Bardawill, and M. M. David, "Determination of serum proteins by means of the biuret reaction," *Journal of Biological Chemistry*, vol. 177, no. 2, pp. 751–766, 1949.
[21] K. N. Pearce and J. E. Kinsella, "Emulsifying properties of proteins: evaluation of a turbidimetric technique," *Journal of Agricultural and Food Chemistry*, vol. 26, no. 3, pp. 716–723, 1978.
[22] M. Murakami, T. Yamaguchi, H. Takamura, and T. M. Atoba, "Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds," *Journal of Food Science*, vol. 69, no. 1, pp. FCT7–FCT10, 2004.
[23] C. Rice-Evans, N. Miller, and G. Paganga, "Antioxidant properties of phenolic compounds," *Trends in Plant Science*, vol. 2, no. 4, pp. 152–159, 1997.
[24] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.
[25] C. Clarkson, M. Mirosa, and J. Birch, "Potential of extracted locusta migratoria protein fractions as value-added ingredients," *Insects*, vol. 9, no. 1, p. 20, 2018.
[26] B. Purschke, H. Tanzmeister, P. Meinlschmidt, S. Baumgartner, K. Lauter, and H. Jäger, "Recovery of soluble proteins from migratory locust (*Locusta migratoria*) and characterisation of their compositional and techno-functional properties," *Food Research International*, vol. 106, pp. 271–279, 2018.
[27] T. W. Goodwin, "The biochemistry of locust pigmentation," *Biological Reviews*, vol. 27, no. 4, pp. 439–460, 1952.
[28] P. J. Wittkopp and P. Beldade, "Development and evolution of insect pigmentation: genetic mechanisms and the potential consequences of pleiotropy," *Seminars in Cell and Developmental Biology*, vol. 20, no. 1, pp. 65–71, 2009.
[29] C. Azagoh, F. Ducept, R. Garcia et al., "Extraction and physicochemical characterization of *Tenebrio molitor* proteins," *Food Research International*, vol. 88, pp. 24–31, 2016.
[30] J. R. Wagner, D. A. Sorgentini, and M. C. Añón, "Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 8, pp. 3159–3165, 2000.
[31] J. Gould and B. Wolf, "Interfacial and emulsifying properties of mealworm protein at the oil/water interface," *Food Hydrocolloids*, vol. 77, pp. 57–65, 2018.
[32] I. Vander Plancken, A. Van Loey, and M. E. G. Hendrickx, "Changes in sulfhydryl content of egg white proteins due to heat and pressure treatment," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 14, pp. 5726–5733, 2005.
[33] E. Zielińska, B. Baraniak, and M. Karaś, "Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects," *Nutrients*, vol. 9, no. 9, p. 970, 2017.
[34] Q. Y. Wu, J. Q. Jia, G. X. Tan, J. L. Xu, and Z. Z. Gui, "Physicochemical properties of silkworm larvae protein isolate and gastrointestinal hydrolysate bioactivities," *African Journal of Biotechnology*, vol. 10, no. 32, pp. 6145–6153, 2011.
[35] H. P. S. Makkar, G. Tran, V. Heuzé, and P. Ankers, "State-of-the-art on use of insects as animal feed," *Animal Feed Science and Technology*, vol. 197, pp. 1–33, 2014.
[36] V. M. Ruiz, H. Sandoval-Trujillo, T. Quirino-Barreda, K. Sánchez-Herrera, R. Díaz-García, and C. Calvo-Carrillo, "Chemical composition and amino acids content of five species of edible Grasshoppers from Mexico," *Emirates Journal of Food and Agriculture*, vol. 27, no. 8, pp. 654–658, 2015.
[37] S. Nalinanon, S. Benjakul, and H. Kishimura, "Biochemical properties of pepsinogen and pepsin from the stomach of albacore tuna (*Thunnus alalunga*)," *Food Chemistry*, vol. 121, no. 1, pp. 49–55, 2010.
[38] J. H. Muyonga, C. G. B. Cole, and K. G. Duodu, "Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*)," *Food Chemistry*, vol. 85, no. 1, pp. 81–89, 2004.
[39] V. K. Ramappa, P. Dev, S. Shankar, and U. Shanker, "Analysis of chemical compounds in different mulberry and non mulberry silkworm pupae powder by FTIR and EDX," *International Journal of Scientific and Innovative Research*, vol. 4, no. 1, pp. 120–135, 2016.
[40] J. Bandekar, "Amide modes and protein conformation," *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, vol. 1120, no. 2, pp. 123–143, 1992.
[41] A. Barth, "Infrared spectroscopy of proteins," *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, vol. 1767, no. 9, pp. 1073–1101, 2007.
[42] M. Du, J. Xie, B. Gong et al., "Extraction, physicochemical characteristics and functional properties of Mung bean protein," *Food Hydrocolloids*, vol. 76, pp. 131–140, 2018.
[43] S. E. Glassford, B. Byrne, and S. G. Kazarian, "Recent applications of ATR FTIR spectroscopy and imaging to proteins," *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, vol. 1834, no. 12, pp. 2849–2858, 2013.
[44] E. Zielińska, M. Karaś, and B. Baraniak, "Comparison of functional properties of edible insects and protein preparations thereof," *LWT-Food Science and Technology*, vol. 91, pp. 168–174, 2018.
[45] S. Bußler, B. A. Rumpold, E. Jander, H. M. Rawel, and O. K. Schlüter, "Recovery and techno-functionality of flours and proteins from two edible insect species: meal worm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae," *Heliyon*, vol. 2, no. 12, article e00218, 2016.
[46] R. Horax, M. S. Vallecios, N. Hettiarachchy, L. F. Osorio, and P. Chen, "Solubility, functional properties, ACE-I inhibitory and DPPH scavenging activities of Alcalase hydrolysed soy protein hydrolysates," *International Journal of Food Science and Technology*, vol. 52, no. 1, pp. 196–204, 2017.
[47] X. Guan, H. Yao, Z. Chen, L. Shan, and M. Zhang, "Some functional properties of oat bran protein concentrate modified by trypsin," *Food Chemistry*, vol. 101, no. 1, pp. 163–170, 2007.

[48] Y. A. Adebowale, K. O. Adebowale, and M. O. Oguntokun, "Evaluation of nutritive properties of the large African cricket (*Gryllidae* sp)," *Pakistan Journal of Scientific and Industrial Research*, vol. 48, no. 4, p. 274, 2005.

[49] O. T. Omotoso, "An evaluation of the nutrients and some anti-nutrients in silkworm, *bombyxmori* L.(Bombycidae: Lepidoptera)," *Jordan Journal of Biological Sciences*, vol. 8, no. 1, pp. 45–50, 2015.

[50] M. Tirgar, P. Silcock, A. Carne, and E. J. Birch, "Effect of extraction method on functional properties of flaxseed protein concentrates," *Food Chemistry*, vol. 215, pp. 417–424, 2017.

[51] F. G. Hall, O. G. Jones, M. E. O'Haire, and A. M. Liceaga, "Functional properties of tropical banded cricket (*Gryllodes sigillatus*) protein hydrolysates," *Food Chemistry*, vol. 224, pp. 414–422, 2017.

[52] W. Panpipat and M. Chaijan, "Functional properties of pH-shifted protein isolates from bigeye snapper (*Priacanthus tayenus*) head by-product," *International Journal of Food Properties*, vol. 20, no. 3, pp. 596–610, 2017.

[53] T. M. Johnson and M. E. Zabik, "Ultrastructural examination of egg albumen protein foams," *Journal of Food Science*, vol. 46, no. 4, pp. 1237–1240, 1981.

[54] E. Zielińska, M. Karaś, and A. Jakubczyk, "Antioxidant activity of predigested protein obtained from a range of farmed edible insects," *International Journal of Food Science and Technology*, vol. 52, no. 2, pp. 306–312, 2017.

[55] L. Zhang, J. Li, and K. Zhou, "Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation," *Bioresource Technology*, vol. 101, no. 7, pp. 2084–2089, 2010.

[56] Y. Xia, F. Bamdad, M. Gänzle, and L. Chen, "Fractionation and characterization of antioxidant peptides derived from barley glutelin by enzymatic hydrolysis," *Food Chemistry*, vol. 134, no. 3, pp. 1509–1518, 2012.

[57] S. Bousopha, S. Nalinanon, and C. Sriket, "Production of collagen hydrolysate with antioxidant activity from Pharaoh cuttlefish skin," *Chiang Mai University Journal of Natural Sciences*, vol. 15, no. 2, pp. 151–162, 2016.

[58] A. Zambrowicz, M. Pokora, E. Eckert et al., "Antioxidant and antimicrobial activity of lecithin free egg yolk protein preparation hydrolysates obtained with digestive enzymes," *Functional Foods in Health and Disease*, vol. 2, no. 12, pp. 487–500, 2012.

[59] J. Y. Je, K. H. Lee, M. H. Lee, and C. B. Ahn, "Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis," *Food Research International*, vol. 42, no. 9, pp. 1266–1272, 2009.