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Evaluation of biological activity and amino acid profile of protein from Thai edible insects

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Abstract

Insects are one of the sources of protein for future foods. In some cultures, insects have been used as a component of traditional medicine. Moreover, some proteins from insects have also shown biological activities related to human health, such as non-communication disease treatment. In this study, the protein content, amino acid profile, and inhibitory activity against a-glucosidase, a-amylase, acetylcholinesterase, and tyrosinase of soluble protein from six species of Thai edible insects were investigated. The protein from six Thai edible insects, including *Gryllus bimaculatus* De Geer (Sample 1), *Omphisa fuscidentalis* (Sample 2), *Bombyx mori* L. (Sample 3), *Brachytrupes portentosus* (Lichtenstein 1796) (Sample 4), *Acheta domesticus* L. (Sample 5), and *Patanga succincta* (Linnaeus) (Sample 6), was extracted and evaluated for its content and biological activity. The results indicated that sample 1 yielded the highest protein content of 84.23±5.15 g/100 g which is more than the protein content of meat and dairy products. A total of eight essential amino acid contents (EAA) were found in all six edible Thai insects. The highest total EAA was obtained from Sample 2 with 37.21±1.82 g/100 g, higher than the criteria established by FAO/WHO/UNU.1985. Sample 3 yielded the highest inhibition percentage of soluble protein from edible insects against α-glucosidase. The same was true for Sample 4 for α-amylase, Sample 1 for acetylcholinesterase, and Sample 2 for tyrosinase. These results indicated the quality and functional properties of protein from six species of Thai edible insects, which may be helpful in their future exploration and application.

1. Introduction

In recent years, population growth has increased continuously. From an estimated 2.5 billion in 1950, the world's population grew to 8.0 billion in mid-November 2022 (United Nations, 2023). The human food supply has been steadily increasing as well. Proteins are one of the main macronutrients for human health, and the protein demand has also been increased sufficiently for population growth (Boland *et al*., 2013). Therefore, the search for alternative proteins is crucial for human consumption. Alternative protein from insects is expected to be the next major source of human food, especially in developing countries (Akhtar and Isman, 2018). People in some countries consume insects and use them as alternative medicine (Kim *et al*., 2019). The protein from insects may be highly qualitative because they contain more proteins than other traditional protein sources, such as meat (Oibiokpa *et al*., 2018).

Some edible insects have been used in traditional medicine (Jideani and Netshiheni, 2017). Termites, one of the most popular insects, have been used as food and in conventional medicine. *Nasutitermes microcephalus*, one of the termite species, has been used to treat asthma, hoarseness, and sinusitis (Figueirêdo *et al*., 2015). The researcher reported that bee venom had been used in several treatments, such as arthritis, rheumatism, pain, cancerous tumors, and skin diseases (Bairagi, 2019). The dry powder of some species of bamboo caterpillars was used for healing wounds (Meyer-Rochow, 2017). Lee *et al*. (2017) reported that the methanol extraction of

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silkworm (*Bombyx mori* L.) affects skin depigmenting by inhibiting melanin synthesis.

A sickness that cannot spread directly from one person to another is considered a non-communicable disease. Parkinson's disease (PD), diabetes, Alzheimer's disease (AD), and other illnesses are examples of non-communicable diseases. One of the treatments for AD is the inhibition of AChE activity (García-Ayllón *et al*., 2011). Although α-glucosidase and α-amylase are the primary enzymes involved in blood glucose measurement, their activity is critical in managing diabetes treatment (Gong *et al*., 2020). Tyrosinase is involved in the neuromelanin production in the substantia nigra. Neuromelanin and Parkinson's disease (PD) pathogenesis are closely related (Li *et al*., 2021). Therefore, inhibiting these enzymes might be a therapeutic option for non-communication diseases.

This study extracted the soluble proteins from six Thai edible insects, including *Gryllus bimaculatus* De Geer (Field cricket), *Omphisa fuscidentalis* (Bamboo worm), *Bombyx mori* L. (Silkworm), *Brachytrupes portentosus* (Lichtenstein 1796) (Short tailed cricket), *Acheta domesticus* L. (House cricket), and *Patanga succincta* (Linnaeus) (Bombay locust). Then, the properties of the soluble protein are determined and characterized. We also investigate the biological activity of the soluble proteins from edible insects, such as enzymatic inhibition (α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase).

2. Materials and methods

2.1 Chemical reagents

The chemical reagent including acetylcholinesterase (AChE) from electric eel (*Electrophorus electricus*), acetylthiocholine iodide (ATCI) and 5,5'-dithio-bis(2-nitrobenzoicacid) (DTNB), *p*-nitrophenyl-α-glucopyranoside (4-*p*NPG), maltose, α-glucosidase from *Saccharomyces cerevisiae*, α-amylase from *Aspergillus oryzae,* tyrosinase from mushrooms and 3,4-dihydroxy-l-phenylalanine (L-DOPA), Bradford reagent and coomassie brilliant blue R250. All these chemical reagents are purchased from Sigma-Aldrich (St.Louis, MO, USA).

2.2 Samples preparation

Thai edible insects including *Gryllus bimaculatus* De Geer (Sample 1), *Omphisa fuscidentalis* (Sample 2), *Bombyx mori* L. (Sample 3), *Brachytrupes portentosus* (Lichtenstein 1796) (Sample 4), *Acheta domesticus* L. (Sample 5), and *Patanga succincta* (Linnaeus) (Sample 6) were obtained from TalaadThai market, Pathum Thani, Thailand. The samples were washed with deionized water and then dried in a hot air oven at 40°C for 6 hrs. After that, the insect samples were ground thoroughly to produce a powder with liquid nitrogen (Figure 1). The powder was kept at -20°C until extraction.

2.3 Protein extraction

The soluble proteins of Sample 1, Sample 3, Sample 5, and Sample 6 were extracted with ascorbic acid using the modified method of Kim *et al*. (2019). The insect samples were extracted with hexane, removing the lipids. Two hundred grams of sample powder were mixed with hexane at a ratio of 1:5 (w/v). The mixed solution was shaken for one hr at room temperature, then filtrated with Whatman filter paper No.1. The soluble protein of the filtrated solution was extracted with 0.02% w/v of ascorbic acid (for water-soluble protein), 50 mM phosphate buffer pH 6.8, and 0.58 M NaCl (for salt-

Figure 1. The characteristic of six edible insects' powder after being ground with liquid nitrogen.

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soluble protein). The mixed solution was shaken for 30 mins at room temperature. Then, the mixed solution was centrifuged at 6,000 rpm for 20 mins to discard sample residues. The protein solution was precipitated overnight at 4°C. The precipitated protein was removed from the solution by filtrating with Whatman filter paper No.1. Finally, the precipitated protein was dissolved in a buffer solution (50 mM phosphate buffer, pH 6.8 + 0.50 M NaCl). The method for extracting soluble proteins from Samples 2 and 4 was used, which was similar to the method used for the samples above. But for samples 2 and 4, we added NaCl to the protein solution after protein precipitation overnight at 4°C. This brought the salt concentration up to 40% w/v for Sample 2 and 35% w/v for Sample 4. After that, the precipitated protein was removed from the solution by filtrating with Whatman filter paper No.1. Finally, the precipitated protein was dissolved in a buffer solution (50 mM phosphate buffer, pH 6.8 + 0.50 M NaCl).

2.4 pH and protein concentration determination

After protein extraction, we measured the pH of the protein using a Bench-Top pH meter, model 860031 (Sper Scientific, Scottsdale, USA). The soluble protein concentration was determined by a Bradford protein assay with Bovine Serum Albumin (BSA) as the standard protein. The soluble protein concentration of Thai edible insects was calculated by the calibration curve of BSA ($y = 18.551x + 0.0318$, $R^2 = 0.9933$).

2.5 Amino acid profile and protein quality

The amino acid profile and the amino acid content were determined using an amino acid analyzer (L-8900: Hitachi, Japan). Briefly, 5 mg of the protein sample was dissolved in 1.0 mL of 6 N HCl. The reaction was hydrolyzed at 110°C for 22 hrs. The hydrolysate sample was transferred to a round bottle to concentrate the protein using an evaporator. Then the hydrolysate was filtered using a 0.20 µm syringe filter. Finally, amino acids in the protein hydrolysate were identified by the amino acid analyzer. The standard amino acids were obtained from Sigma-Aldrich (USA).

2.6 Enzyme inhibitory assay

The inhibitory potential of protein samples against a-glucosidase, a-amylase and tyrosinase was determined by the modified methods of Nanok and Sansenya (2021), and the inhibitory potential of the protein samples against acetylcholinesterase was determined by the modified methods of Pohanka *et al*. (2011).

For a-glucosidase and a-amylase inhibitory activities, 4-*p*NPG and maltose were used as the substrates for the a-glucosidase and a-amylase activity assays, respectively. In the a-glucosidase activity reaction, including 10 µL of 1 mM 4-*p*NPG, 4 µL of 0.5 mg/mL a-glucosidase, 10 µL of protein sample (3.0 mg/mL) and 50 mM phosphate buffer pH 6.8. Then the reaction was incubated at 37°C for 20 mins. After that, the enzyme activity was stopped with 50% Na_2CO_3. The release of the *p*-nitrophenolate group was measured at 405 nm using a UV/Vis spectrophotometer. In the a-amylase activity reaction, include 10 µL of 1 mM maltose, 5 µL of 0.5 mg/mL a-amylase, 10 µL of protein sample (3.0 mg/mL) and 50 mM phosphate buffer at pH 6.8. The reaction was incubated at 37°C for 25 mins. After that, the enzyme activity was stopped by heating it in boiling water for 5 mins. The detection of glucose-release was performed by a peroxidase-glucose oxidase assay at 475 nm using a UV/Vis spectrophotometer.

For the tyrosinase activity assay, the reaction mixture, including 10 µL of 0.10 mg/mL tyrosinase, 10 µL of protein sample (3.0 mg/mL), and 50 mM phosphate buffer pH 6.8 was incubated at 37°C for 15 mins. Then 10 µL of 5.0 mM L-DOPA (in 5% DMSO) was added to the reaction. The mixed reaction was incubated at 37°C for 25 mins. The production of dopachrome was measured at 492 nm using a UV/Vis spectrophotometer.

For the acetylcholinesterase activity assay, in the reaction, 10 µL of 1.0 mg/mL acetylcholinesterase, 10 µL of protein sample (3.0 mg/mL) and 50 mM Tris-HCl buffer pH 6.8 were incubated at 37°C for 20 mins. Then 10 µL of 10 mM ATCI and 10 µL of 1 mM DTNB were added to the reaction. After that, the mixed reaction was incubated at 37°C for 20 mins. Finally, the product was measured at 405 nm using a UV/Vis spectrophotometer.

The inhibition percentage of protein samples against a-glucosidase, a-amylase, tyrosinase and acetylcholinesterase activity was calculated by $[(A - B)/A] \times 100$ (A = absorbance without sample; B = absorbance with sample).

2.7 Statistical analysis

The experiments, including protein content, amino acid content, and enzymatic inhibitory activity, were performed in triplicate. The determinations are reported as the mean ± standard deviation (SD). Statistical differences were determined by a one-way analysis of variance (ANOVA), and the differences were considered significant at *P* values less than 0.05.

3. Results

3.1 Protein content and the pH of Thai edible insect protein

The protein content of six Thai edible insect species

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is shown in Table 1. The protein concentration of Thai edible insects was shown to range from 37.73±0.83 g/100 g to 84.23±5.15 g/100 g. The highest protein concentration was obtained from Sample 1 (84.23±5.15 g/100 g). However, the protein yields of Sample 6 (81.02±5.11 g/100 g) were similar to the protein yields of Sample 1. The protein content of two of these samples was not significantly different ($P > 0.05$). The lowest protein content of Thai edible insects was obtained from Sample 4 (37.73±0.83 g/100 g).

The pH of six Thai edible insect species (Sample 1 to Sample 6) was also shown in Table 1. The pH range of six samples was displayed between 5.83 and 6.63. The pH identity of all samples of Thai edible insects is lower than pH 7.0, indicating acidity.

Table 1. The protein content and the pH identity of Thai edible insect protein

Samples	Protein Content (g/100 g)	pH
Sample 1	84.23±5.15^{a}	6.63
Sample 2	73.58±0.88^{b}	5.97
Sample 3	72.36±1.07^{b}	6.51
Sample 4	37.73±0.83^{d}	5.83
Sample 5	43.85±0.67^{c}	6.37
Sample 6	81.02±5.11^{a}	6.10

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different between the protein content of Thai edible insect ($P < 0.05$).

3.2 Amino acid profile and protein quality

The amino acid profile and amino acid content of six samples of insect-soluble protein are shown in Tables 2 and 3. The eight EAA, including His, Ile, Leu, Val, Lys, Met, Phe, and Thr, were identified from all edible insect samples. Leu seems to have a higher content than other essential amino acids. For more observation, the total EAA content of Sample 2 (*Omphisa fuscidentalis*) has shown the highest amount (37.21±1.82 g/100 g) followed by Sample 4 (*Brachytrupes portentosus* (Lichtenstein 1796)), Sample 1 (*Gryllus bimaculatus* De Geer), and Sample 6 (*Patanga succincta* (Linnaeus)) with the contents of 18.26±1.87, 17.26±1.23, and 15.98±1.38 g/100 g, respectively.

The total amount of non-essential amino acids (non-EAA) in six proteins from edible insects is the same as the total amount of EAA. The three samples, including Sample 2 (29.95±1.28 g/100 g), Sample 6 (28.24±2.30 g/100 g), and Sample 1 (27.55±2.19 g/100 g), had the highest total non-EAA content, while Sample 4, with a content of 19.55±1.03 g/100 g, had a lower total non-EAA content compared to Samples 1, 2, and 6. However, Sample 4 has a higher total non-EAA content than Sample 5 (6.59±0.15 g/100 g) and Sample 3 (5.09±0.34 g/100 g).

3.3 Inhibitory activity of protein against diabetes related -enzymes

The inhibitory potential of insect insoluble proteins against α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase was presented in the inhibition percentage and shown in Table 4. The results reveal that Sample 4 against α-amylase activity (47.52±1.03%) yields the highest inhibitory efficiency. Furthermore, the highest inhibition percentages against α-glucosidase, acetylcholinesterase, and tyrosinase were obtained from Sample 3 (30.67±1.21%), Sample 1 (45.16±2.59%) and Sample 2 (17.64±1.17%) respectively. Moreover, the insect-soluble protein seems to have higher inhibition activity against acetylcholinesterase than other enzymes.

4. Discussion

The pH of Thai edible insect proteins (Sample 1 to Sample 6) lies between 5.83 to 6.63. When comparing between six species, Sample 1 (*Gryllus bimaculatus* De

Table 2. The essential amino acid profile and amino acid content of insect soluble protein

Amino acid	Essential amino acid content g/100 g (EAA)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
His	0.65±0.05^{b}	3.65±0.34^{a}	0.08±0.01^{c}	0.46±0.08^{b}	0.19±0.04^{c}	0.72±0.06^{a}
Ile	2.15±0.17^{b}	6.07±0.13^{a}	0.18±0.02^{c}	2.73±0.42^{b}	0.21±0.07^{c}	2.38±0.37^{bc}
Leu	3.50±0.49^{c}	15.52±0.51^{a}	0.49±0.08^{d}	7.55±1.05^{b}	0.33±0.04^{d}	6.15±0.17^{d}
Val	1.85±0.05^{c}	2.05±0.17^{c}	0.20±0.05^{d}	3.43±0.32^{a}	0.65±0.06^{d}	2.57±0.60^{b}
Lys	2.39±0.31^{b}	4.18±0.87^{a}	0.27±0.04^{d}	1.05 ±0.14^{c}	0.31±0.08^{d}	2.26±0.29^{b}
Met	4.01±0.66^{a}	1.25±0.25^{b}	0.040±0.02^{c}	0.0021±0.001^{c}	0.022±0.01^{c}	0.0020±0.001^{c}
Phe	1.62±0.08^{b}	2.84±0.35^{a}	0.082±0.01^{c}	2.46±0.45^{a}	0.097±0.02^{b}	0.84±0.15^{b}
Thr	1.09±0.11^{b}	1.65±0.05^{a}	0.17±0.03^{d}	0.57±0.06^{c}	0.23±0.04^{d}	1.05±0.08^{b}
Total EAA	17.26±1.23^{b}	37.21±1.82^{a}	1.52±0.21^{c}	18.26±1.87^{b}	2.03±0.04^{c}	15.98±1.38^{b}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different between the essential amino acid content of Thai edible insect ($P < 0.05$).

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Table 3. The non-essential amino acid profile and non-essential amino acid content of insect soluble protein.

Amino acid	Non-essential amino acid content g/100 g (non-EAA)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Ala	3.76±0.47^{b}	3.92±0.53^{b}	0.93±0.12^{c}	6.90±0.25^{a}	0.63±0.07^{c}	7.33±0.53^{a}
Arg	1.26±0.07^{b}	2.06±0.29^{a}	0.21±0.03^{c}	0.11±0.02^{c}	0.22±0.03^{c}	1.88±0.11^{a}
Asp	5.18±0.03^{b}	6.37±0.17^{a}	0.53±0.02^{f}	2.01±0.05^{d}	0.77±0.05^{e}	4.55±0.05^{c}
Cys	0.71±0.11^{a}	0.66±0.07^{a}	0.17±0.02^{c}	0.16±0.02^{c}	0.14±0.01^{c}	0.29±0.02^{b}
Glu	8.79±0.23^{b}	9.93±0.08^{a}	0.94±0.09^{d}	4.25±0.07^{c}	0.99±0.1^{d}	4.44±0.51^{c}
Gly	3.20±0.81^{a}	1.94±0.15^{b}	0.94±0.08^{c}	3.15±0.35^{a}	0.43±0.08^{c}	3.52±0.50^{a}
Pro	1.49 ±0.17^{cd}	1.72±0.14^{bcd}	1.17±0.17^{d}	2.13±0.37^{b}	2.07±0.13^{bc}	3.28±0.60^{a}
Ser	1.59 ±0.04^{c}	2.63 ±0.06^{a}	0.15 ±0.03^{f}	0.70 ±0.06^{d}	0.51 ±0.04^{e}	1.59 ±0.04^{c}
Tyr	1.56 ±0.47^{a}	0.72 ±0.28^{b}	0.03 ±0.01^{c}	0.13 ±0.02^{c}	0.83 ±0.06^{b}	0.67 ±0.06^{b}
Total AA	27.55±2.19^{a}	29.95±1.28^{a}	5.09±0.34^{c}	19.55±1.03^{b}	6.59± 0.15^{c}	28.24±2.30^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different between the essential amino acid content of Thai edible insect ($P < 0.05$).

Table 4. The inhibition potential of soluble proteins of Thai edible insect against α-glucosidase, α-amylase, acetylcholinesterase and tyrosinase.

Sample	% Inhibition at 0.30 mg/mL			
	α-Glucosidase	α-Amylase	Acetylcholinesterase	Tyrosinase
Sample 1	11.04±0.56^{d}	2.10±0.83^{c}	45.16±2.59^{a}	16.37±1.86^{a}
Sample 2	9.70±0.58^{d}	2.61±1.29^{c}	15.60±3.77^{c}	17.64±1.17^{a}
Sample 3	30.67±1.21^{a}	11.09±1.91^{b}	20.32±1.50^{b}	10.35±1.56^{b}
Sample 4	15.57±0.78^{c}	47.52±1.03^{a}	2.45±1.51^{d}	11.87±3.17^{b}
Sample 5	10.20±0.30^{d}	3.40±0.42^{c}	24.24±2.06^{b}	11.54±0.30^{b}
Sample 6	19.62±0.94^{b}	2.77±1.03^{c}	20.89±0.62^{b}	2.77±1.03^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different between the essential amino acid content of Thai edible insect ($P < 0.05$).

Geer) and Sample 4 (*Brachytrupes portentosus* (Lichtenstein 1796)) get the highest and lowest pH values, respectively. The soluble protein extracted from *Tenebrio molitor* shows a pH value lower than 7.0 (Kim *et al*., 2019). This soluble protein is extracted with 0.02% ascorbic acid, similar to our extraction method (soluble protein of a Thai edible insect was extracted with 0.02% ascorbic acid). Moreover, protein solubility has been affected by many factors such as pH, salinity, and temperature because of changes in the net charge of the protein (Mishyna *et al*., 2019). Amarender *et al*. (2020) reported that the protein content of crickets (Gryllidae) has the highest yields at lower pH compared with higher pH. This study indicated that the solubility of the proteins was found to be highly dependent on the pH during the extraction process. Our findings imply that the water-soluble protein may be appropriate for extraction at lower pH levels, as was previously described.

The soluble proteins of six Thai edible insects range from 37.73±0.83 g/100 g to 84.23±5.15 g/100 g. The highest and the lowest soluble protein contents are obtained from Sample 1 (*Gryllus bimaculatus* De Geer) and Sample 4 (*Brachytrupes portentosus* (Lichtenstein 1796)) respectively. The crude protein contents of mealworm larva (46±1.0 g/100 g) and cricket nymph (56±3.1 g/100 g) (Adámková *et al*., 2017) have similar contents to the protein content of 43.85±0.67 g/100 g of Sample 4 (*Acheta domesticus* L.). The protein content of traditional protein sources such as pork shoulder, beef sirloin, and chicken drumstick has been reported as 16.89, 20.1, and 17.8 g/100 g, respectively (Orkusz, 2021). The work of Payne *et al*. (2016) also reports that the protein content of many insect species is significantly higher than that of meat products. The protein contents of six Thai edible insects, according to our results, are also more significant than those from meat products and might be sufficient for human health.

The amino acid composition, especially EAA, has determined the protein quality. The eight essential amino acids were identified from six species of Thai edible insects (Table 2). The range of total EAA contents of six insect species is 1.52±0.21 g/100 g to 37.21±1.82 g/100 g, which is similar to the total AA content (5.09±0.34 g/100 g to 29.95±1.28 g/100 g). According to the previous results, the amino acid composition of edible insects is vastly different among the species. The total EAA content represents 46–96% of the total amount

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of amino acids (Akhtar and Isman, 2018). However, the total EAA content has been recommended by FAO/WHO/UNU 1985 (Kim *et al*., 2019) is 27.1 g/100 g, and the total EAA content of our report (Thai edible insects) is lower than this value except for Sample 2 (*Omphisa fuscidentalis*). Some species of Cricket (*Gryllus assimilis*) from Nigeria show some EAA content, such as Met (2.29±0.04 g/100 g), Ile (3.36±0.06 g/100 g), similar to the Met (4.01±0.6 g/100 g) and Ile (2.15±0.17 g/100 g) content of Sample 1 (*Gryllus bimaculatus* De Geer) of our result. Moreover, some amino acid content of Grasshopper (*Melanoplus foedus*) such as Leu (6.99±0.08 g/100 g) has similar content to the Leu (6.15±0.17 g/100 g) of Sample 6 (Grasshopper; *Patanga succincta* (Linnaeus)) (Oibiokpa *et al*., 2018). The previous report and our results indicate that the EAA contents of edible insects have a widely different range among the species. However, some of the EAA contents of the six samples are similar to the EAA content recommended by FAO/WHO/UNU 1985, especially for Sample 2. The EAA content of His, Ile, and Leu is higher than this EAA content. Therefore, the soluble proteins in Sample 2 might be more qualitative than those in other samples.

The inhibitory effects against α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase of soluble proteins from 6 species of Thai edible insects are shown in Table 4. The results reveal that the highest α-glucosidase and α-amylase were obtained from Sample 3 (30.67±1.21%) and Sample 4 (47.52±1.03%). Samples 1 and 2 show the highest inhibitory activity against acetylcholinesterase and tyrosinase, with inhibition percentages of 45.16±2.59% and 17.64±1.17%, respectively. Lee *et al*. (2011) reported that the silk peptide from the cocoon of *Bombyx mori* had an α-glucosidase inhibitory activity with an IC_{50} of 484.5±14.5 mg/mL. Zhang *et al*. (2016) also showed that the selected peptides of silkworm pupae from the database had shown strongly inhibited α-glucosidase. Our results indicate that the soluble protein extracted from Sample 3 (silkworm pupae; *Bombyx mori*) yields the highest inhibitory activity against α-glucosidase. This soluble protein also has an inhibition percentage of 11.09±1.91% against α-amylase. Two edible cricket species, Sample 1 (Cricket; *Gryllus bimaculatus* De Geer) and Sample 4 (Giant cricket; *Brachytrupes portentosus* (Lichtenstein 1796)) showed the highest acetylcholinesterase and α-amylase inhibitory activity. Zielińska *et al*. (2020) reported that the chemical synthesis peptide from crickets (*Gryllodes sigillatus)* showed α-glucosidase inhibitory activity with an IC_{50} of 18.37±0.4 μg/mL. Previous reports and our results indicate that the protein from some cricket species has inhibitory activity against diabetes enzymes. Moreover, these species have a variety of proteins that function as inhibitors for some enzymes, such as α-glucosidase, α-amylase, and acetylcholinesterase. The highest tyrosinase inhibitory activity of the soluble protein was obtained from Sample 2 (Bamboo caterpillar; *Omphisa fuscidentalis*). The researcher reported that sericin from the cocoon (*Bombyx mori*) had tyrosinase inhibitory activity (Kato *et al*., 1998). While the soluble protein from Sample 3 (silkworm pupae; *Bombyx mori*) had an inhibition percentage of 10.35±1.56% against tyrosinase. Our results and previous reports suggest that the proteins from these species' caterpillar state and pupae or cocoon state have tyrosinase inhibitory activity. Still, the potential depends on the different species. Thus, the results indicated that the protein from six edible insects with inhibition potential against α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase might be applied to non-communication disease treatments such as PD, AD, and diabetes. Moreover, edible insects are a source of protein and bioactive compounds for human health.

5. Conclusion

Our results show the biological properties of soluble proteins from six samples of Thai edible insects. The highest soluble protein content was obtained from Sample 1 (84.23±5.15 g/100 g), and the pH range of soluble protein (six samples) was 5.83–6.63. Eight essential amino acids were found in all six samples. While Sample 2 has the highest total EAA content (37.21±1.82 g/100 g). The highest inhibition potential against α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase were obtained from Sample 3 (30.67±1.21%), Sample 4 (47.52±1.03%), Sample 1 (45.16±2.59%) and Sample 2 (17.64±1.17%) respectively. The results suggest that six Thai edible insects have high-quality proteins, and these proteins contain biological activities related to human health. Moreover, the protein of six Thai edible insects might be applied for non-communication disease treatments such as PD, AD, and diabetes.

Conflict of interest

The authors declare no potential conflicts of interest.

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