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Effect of extraction solution on functional properties of extracted protein from Bombay locust (*Patanga succincta* Johannson, 1763)

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Abstract

Alternative sources of protein are gaining popularity since they require less natural resource input, but still provide a significant amount of protein as compared to traditional protein sources. Insect protein is one of the alternative protein types, and due to its nutritional benefits, research interest in Bombay locust (*Patanga succincta*) (BL) protein has grown. In the present work, we aimed to determine the protein content, yield, molecular weight profile, and functional properties such as protein solubility, emulsion, and foam properties of BL protein extracted using distilled water, salt (NaCl), or alkaline (NaOH) solution, at the concentrations of 0.5, 1.0, or 1.5%. The highest protein extraction yield was alkaline soluble protein (22 - 28%), which was followed by water (16%) and salt (11 - 13%) soluble proteins. The protein powder prepared by 0.5% alkaline extraction had the highest foam capacity (33.33%) and foam stability (12.50%) ($p < 0.05$), but the water soluble protein powder had the highest emulsion activity index (118.3 m^2/g) and emulsion stability index (52.45 min) ($p < 0.05$). These results indicated that the type and concentration of solution could have an impact on the protein extraction yield, molecular weight profile, and functional characteristics.

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Introduction

Currently, research is focused on the development of alternative proteins, such as plant-based proteins, *in vitro* meat products, and insects that can be consumed as meat and seafood substitutes. Due to their nutritional content, edible insects have been considered as an alternative source of protein. Insects have high protein content (40 - 70% dry basis) and excellent polyunsaturated to saturated fatty acid ratio (Gravel and Doyen, 2020). In addition, insects contain high minerals and vitamins, *e.g.*, iron, zinc, copper, magnesium, manganese, phosphorous, biotin, riboflavin, pantothenic, and folic acid (Zielińska *et al.*, 2018). Insects can also be cultivated with fewer resources and investments than any other animals that are used as sources of protein.

Bombay locust (*Patanga succincta*) (BL) is a locust species widespread in Southwest and Southeast Asia. BLs have already been extensively used in Western foods, and up-scaled for human food and pet feed industries (Purschke *et al.*, 2018a). However, a lot of BL consumption has not been that visible due to concerns that the image and unique flavour characteristics of BL might affect consumer acceptability. Therefore, BL has usually been used as raw material, in the form of ground milled flour or powder. BL has high potential to be used in the production of insect-derived ingredients such as protein concentrates and isolates due to its high protein content (65% dry basis) and essential amino acids (Purschke *et al.*, 2018a).

Protein solubilisation and purification are essential stages of the food protein processing procedure. Several solvents, alkaline solution, salt solution, or water have been used for protein solubilisation, and different solubilising solutions have been shown to produce different amino acid profiles, protein quality, and functional properties of the extracted protein. However, insufficient work has been done on the comparison of the solubilisation solution effects on insect protein extraction and the functional properties of the extracted protein (Gravel and Doyen, 2020). Purschke *et al.* (2018a) reported BL protein's composition and functional properties. Only alkaline extracted soluble protein at different

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preparation conditions was studied, and the functional properties such as emulsifying activity, foamability, and stability were found to be comparable to those of egg white protein. Kim *et al.* (2019) reported that the functional properties of proteins derived from edible insects varied with species and solution type. The salt solution soluble protein of *Protaetia brevitarsis seulensis* showed higher protein quality, emulsifying and foaming capacity, and stability than *Tenebrio molitor* and *Allomyrina dichotoma*. In addition, the salt solution improved the functional properties of insect protein (Kim *et al.*, 2019). However, a comparison of extracted soluble protein and functional properties of BL protein extracted using different solutions has not been reported yet. Therefore, in the present work, we investigated the effects of the use of different types and concentrations of extraction solutions (water, alkaline, and salt solution) on the yield, molecular weight profile, and functional properties (solubility, emulsion, and foaming properties) of protein extracted from BL. A further aim was to identify proteins that could be used in different kinds of food products. The highest yield and best functional properties of the protein extracted from BL were also revealed. The present work enhances the body of information about insect proteins and their application in novel foods.

Materials and methods

Materials

Frozen Bombay locusts were purchased from Mr. Bucfood Co., Ltd. (Ayutthaya, Thailand). All chemical reagents were of analytical grade. Sodium hydroxide, sulphuric acid 96%, and potassium sulphate were purchased from Carlo Erba (Chaussée du Vexin, France). Sodium chloride, hydrochloric acid, and methyl red were purchased from Merck (Darmstadt, Germany). Commercial grade hexane was purchased from Sac Science-Eng (Bangkok, Thailand).

Sample preparation

The legs and antennae of locusts were removed since these are non-edible. Then, the locusts were rinsed and dried before mincing. Locusts were minced using a blender (VK-569, Vprogress, Bangbon, Bangkok) at 4,000 rpm for 5 min. The locust was then defatted twice with hexane at a 1:8 (w/v) ratio. The mixture was stirred at 800 rpm for 20 min at 4°C. The defatted locust was filtered off using a piece of filter cloth, and dried in a fume hood for 15 h. The defatted locust was sieved and kept in an aluminium bag at -20°C for further analyses.

Protein extraction procedure

The defatted locust was dispersed in distilled water, or NaCl solution (0.5, 1.0, or 1.5%), or NaOH solution (0.5, 1.0, or 1.5%) at a ratio of 1:4 (w/v). Then, each mixture was stirred at 4°C for 24 h. Each supernatant was collected after centrifugation (12,000 *g*, 4°C, 30 min) (5804 R, Eppendorf, Hamburg, Germany). The pH of the supernatants was adjusted to 4.2 using 1.0 M HCl, which was the isoelectric pH (pI) of the locust protein determined in a previous study, and the precipitates were collected after centrifugation (12,000 *g*, 4°C, 30 min). The resulting protein precipitates were the water, salt, and alkali soluble protein. Then, 10% of each protein precipitate was dissolved in distilled water, and pH was adjusted to 7.0 to neutralise the pH of the solution before transfer to a dialysis membrane occurred. Each protein solution was stirred in distilled water for 12 h, and dried using a freeze-dryer (LD-0.5, Tungkru, Bangkok, Thailand). The resulting BL derived protein powders were the water soluble protein (WS-BL), salt soluble protein (SS-BL), and alkali soluble protein (AS-BL). These protein powders were stored in an aluminium vacuum pack at -18°C until further analyses.

Determination of yield and protein concentration

Protein concentration was determined following the Biuret method (Chatsuwan *et al.*, 2018). Briefly, 1 mL of sample solution was mixed with 4 mL of Biuret reagent. Then, the mixture was incubated at room temperature for 30 min, and the absorbance was observed at 540 nm. Bovine serum albumin was used as the protein standard**.** The yield of each soluble protein was determined as a percentage by comparing the weight of fresh crude protein after precipitation to the weight of fresh locust (before freeze-drying) using Eq. 1:

$$\text{Yield (\%)} = \frac{\text{Weight of crude protein after precipitation (g)}}{\text{weight of fresh locust (g)}} \times 100 \quad \text{(Eq. 1)}$$

Molecular weight and protein distribution analysis

The molecular weight and protein distribution of each soluble protein powder were determined

using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Kim *et al.* (2019) with minor modification. Briefly, the concentration of each soluble protein powder was calculated using the Biuret method. The protein concentration was adjusted to 6 mg/mL by dissolving each powder in sample buffer and heating to 100°C for 5 min. After cooling, each sample (10 µL) was loaded into 10% acrylamide gel. After running the electrophoresis, each gel was soaked in a fixing solution for 10 min before being stained overnight at room temperature with Coomassie brilliant blue R250. After that, de-staining using 10% acetic acid and 30% methanol every 10 min took place. The SDS-PAGE gel image was transferred to an ImageJ program (a public domain Java image processing program inspired by NIH Image) to calculate the intensity distribution percentage of each molecular weight as compared to other molecular weights.

Determination of functional properties

Protein solubility

The protein solubility of the freeze-dried protein powders was determined according to Klompong *et al.* (2007) with a slight modification. The WS-BL, SS-BL, and AS-BL powders (0.8 g) were dissolved in distilled water (8 mL). Samples of each mixture were adjusted for pH over the range of 1.0 to 11.0 using 1.0 M HCl or 1.0 M NaOH. Each mixture was stirred for 30 min at room temperature (25 - 27°C). Then, each supernatant was collected after centrifugation (10,000 *g*, 4°C, 15 min). The protein content of each supernatant was measured using the Biuret method, and compared to the protein content of the whole soluble mixtures.

Emulsifying properties

A sample of each of the WS-BL, SS-BL, or AS-BL (0.6 g) powder was dissolved in distilled water (6 mL). Soybean oil (2 mL) was added, and then each mixture was homogenised (20,000 rpm, 1 min) using a homogeniser (Tissuemizer Ultra-turrax T-25, Janke and Kunkel IKA Labortechnik, Germany). Then, 50 µL of each emulsion that had formed was diluted with 1% sodium dodecyl sulphate (5 mL), and the absorbances of the mixtures at 0 and 10 min were measured at 500 nm. Emulsifying activity index (EAI) and emulsifying stability index (ESI) were calculated according to Pearce and Kinsella (1978) and Arsa and Puechkamutr (2022), using Eq. 2 and Eq. 3 respectively:

$$\text{EAI}\left(\frac{\text{m}^2}{\text{g}}\right) = \frac{2 \times 2.303 \times \text{Absorbance measured at initial time}}{(1-\text{mass of protein (g)}) \times \text{Volume fraction of oil in the emulsion}} \quad \text{(Eq. 2)}$$

$$\text{ESI (min)} = \frac{\text{Absorbance of the emulsion at initial time} \times 10}{\text{Absorbance of the emulsion at initial time} - \text{Absorbance of the emulsion at 10 min}} \quad \text{(Eq. 3)}$$

Foaming properties

A sample of each WS-BL, SS-BL, or AS-BL (0.2 g) powder was dissolved in 20 mL of distilled water. Each mixture was homogenised (20,000 rpm, 1 min) in a 50 mL measuring cylinder. Then, the foam volumes at 0 and 60 min were measured. Foaming capacity (FC) and foaming stability (FS) were calculated following Aydemir and Yemenicioğlu (2013) and Arsa and Puechkamutr (2022), using Eq. 4 and Eq. 5 respectively:

$$\text{FC (\%)} = \frac{\text{Volume after homoginiztion} - \text{Volume prior homoginization}}{\text{Volume before homoginized}} \times 100 \quad \text{(Eq. 4)}$$

$$\text{FS (\%)} = \frac{\text{Foam volume after 60 min}}{\text{Initial foam volume}} \times 100 \quad \text{(Eq. 5)}$$

Statistical analysis

All measurements were performed in triplicate. The experimental data were evaluated using a completely randomised study design. Data were analysed by One-way analysis of variance and Duncan's new multiple range test procedures to separate means, and differences were reported as significant at $p < 0.05$, using SPSS version 12.0 (SPSS Inc., Chicago, USA).

Results and discussion

Yield and protein concentration

The yield and protein concentration of each soluble protein from the locust are shown in Figures 1A and 1B. Alkaline extraction produced significantly the highest yield (22 - 28%), followed by water (16%) and salt (11 - 13%) extraction. Yield increased as the concentration of alkaline and salt solution were increased. As a result, the concentration of 1.5% solution of both alkaline and salt soluble protein yielded more than 0.5 and 1.0% solutions. Plant protein solubilisation in previous work also

yielded similar results (Osman and Simon, 1991; Karaca *et al.*, 2011). The high pH of the alkaline extraction increased the protein charge at the surface, and promoted electrostatic repulsion between protein and protein-water interactions, thus resulting in increased protein solubility and extraction yield (Lam *et al.*, 2016; Singhal *et al.*, 2016). Furthermore, as seen in Figure 2's SDS-PAGE profile, the alkaline soluble protein had a larger molecular weight than did the other soluble proteins. After a centrifugation step, these proteins precipitated considerably more easily than did the water and salt soluble proteins, thus resulting in the higher yield of the alkaline extraction. The maximum protein concentration of alkaline extraction (9 - 12 mg/g) ($p < 0.05$) in Figure 1B confirmed this. The alkaline soluble protein extraction produced the highest yield and protein concentration as compared to other components.

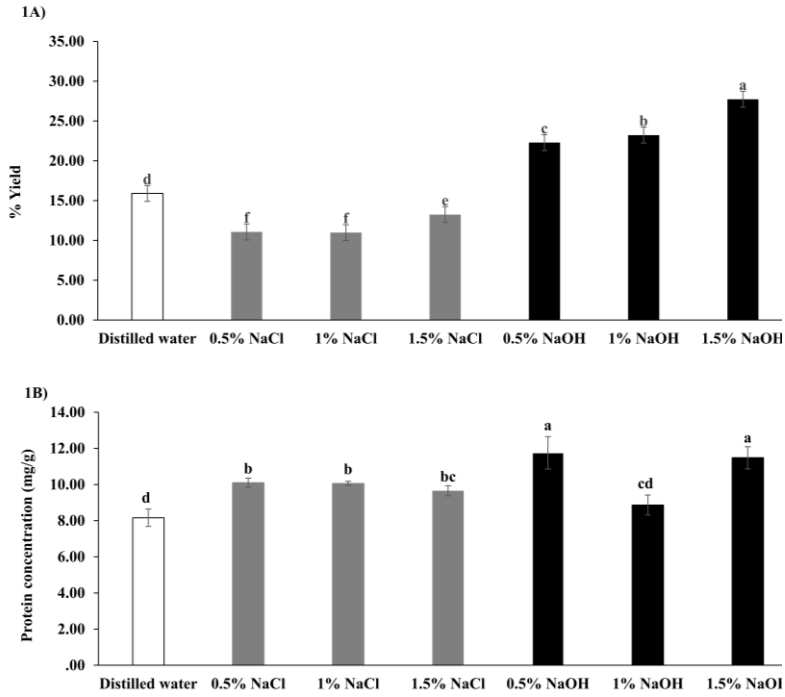

Figure 1. Yield **(A)** and protein concentration **(B)** of soluble proteins extracted from different extraction solutions. Different lowercase letters indicate significant differences at $p < 0.05$**.**

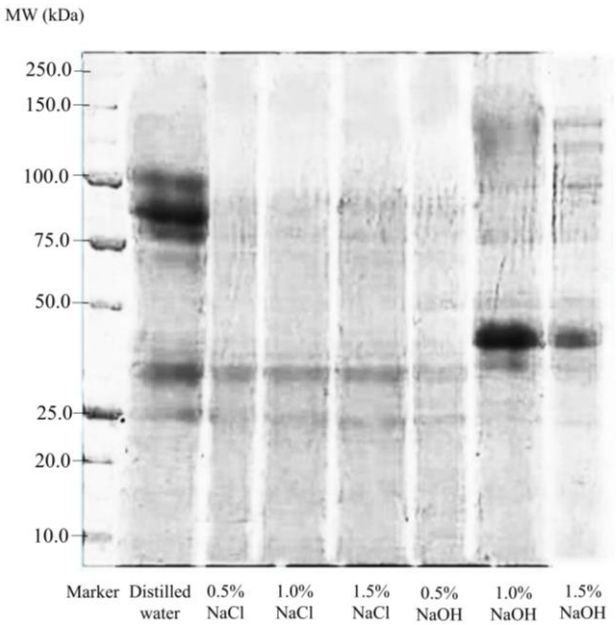

Figure 2. Molecular weight and protein distribution of soluble protein powders prepared from different extraction solutions.

Molecular weight and protein distribution

The molecular weight and protein distribution of the WS-BL, SS-BL, and AS-BL powders are presented in Figure 2. Across the different solutions, many bands in the range of 10 - 250 kDa were seen for the locust protein. Proteins with a high molecular weight of about 150 kDa were abundant in the 1% AS-BL, whereas the WS-BL contained lower molecular weight proteins with a molecular weight range of 10 -100 kDa. The same range of molecular weights was found in the same solution at different concentrations of extracting agent (0.5, 1.0, or 1.5%). The molecular weight and protein distribution of the WS-BL was similar to that found by Chatsuwan *et al.* (2018), who analysed the water soluble proteins extracted from *Patanga succincta*. The major molecular weights were estimated at 25, 40, 41 - 42, 50, 65, and 75 - 100 kDa in WS-BL, SS-BL, and AS-BL powders. Andersen *et al.* (1995) reported that the molecular weight of 14 - 32 kDa was insect cuticle proteins, which are the exoskeleton of insects. Therefore, the well-defined at 25 kDa (~7% distribution) represented cuticle proteins in locust that were found by Brogan *et al.* (2021). Three bands found at molecular weight 40, 50, and 100 kDa (~32% distribution) revealed the tubulin peptide fragments detected in the protein concentrate of water soluble proteins of migratory locust (*Locusta migratoria*) (Purschke *et al.*, 2018a). The molecular weight of 41 kDa (~3% distribution) was arginine kinase, which is commonly detected in insects, and 42 kDa (~3% distribution) was actin that is considered muscle protein (Andersen *et al.*, 1995; Srinroch *et al.*, 2015). Hexamerin was identified at 65 kDa (~5% distribution), and it was found to be an allergen from raw and fried Bombay locust (*Patanga Succincta*) (Phiriyangkul *et al.*, 2015). The estimated molecular weight of 75 kDa (~19% distribution) was haemocyanin, which is an oxygen transport protein commonly found in crustaceans and insects (Srinroch *et al.*, 2015; Brogan *et al.*, 2021).

In food product applications, the peptide size is a crucial factor in the functional qualities of locust protein. Proteins with smaller peptide sizes or lower molecular weights were reported to have higher solubilities and could potentially act as emulsifiers that were absorbed at the water-oil interface. However, the short chain peptides could not stabilise the oil droplets in the emulsion system, and thus could not act as stabilisers (Wu *et al.*, 1998).

Protein solubility

The protein solubilities of the WS-BL, SS-BL, and AS-BL powders prepared from different solutions are presented in Figure 3. Protein solubility has generally been regarded as a crucial characteristic since it is related to other functional properties of protein such as emulsifying, foaming, and gelling properties (Purschke *et al.*, 2018b). Protein solubility is influenced by a number of factors, the most important of which is pH. The locust protein powder in the present work had a protein solubility of 15 - 98%. This result was similar to that of the locust protein prepared by alkaline extraction in the study of Purschke *et al.* (2018a). The solubility of locust protein was higher than that of other insect proteins,

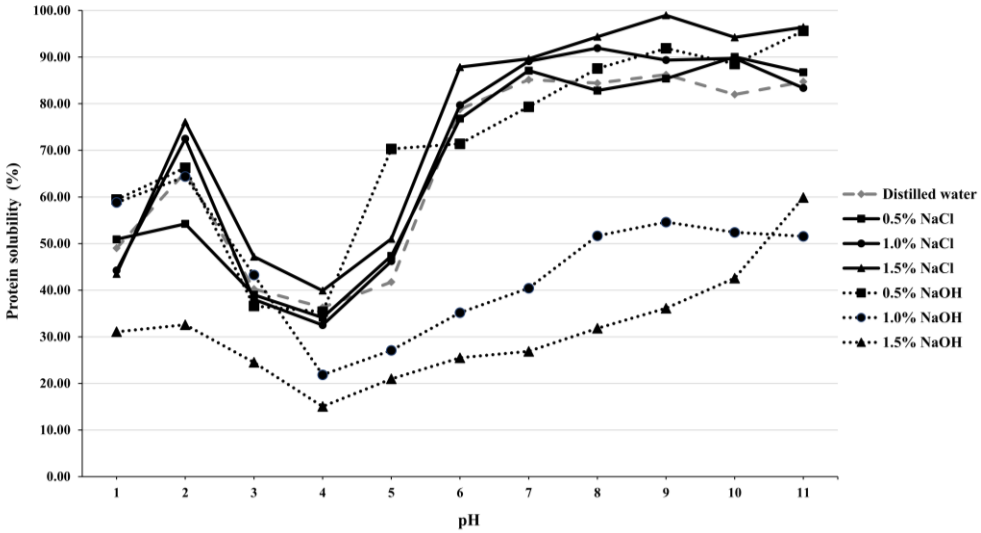

Figure 3. Protein solubility of soluble protein powders prepared from different extraction solutions.

e.g., tropical banded cricket (5 - 25%), house cricket (12 - 23%), or mealworm larvae (3 - 45%) (Bußler *et al.*, 2016; Hall *et al.*, 2018). The profiles of protein solubility *versus* pH of several soluble locust protein powders in the present work resembled the U-shaped curve seen for other insect proteins (Zhao *et al.*, 2016; Purschke *et al.*, 2018a). This was probably due to the protein solubility decreasing when pH decreased, or because the pH was acidic (pH 1 - 3). Moreover, over a broad pH range (pH 5 - 11), solubility improved from 30 - 50% to 60 - 90%, depending on the sample. This might have been due to the electrostatic repulsion produced by the amino acid side chains and hydrogen bond formation with the solvent (Flores *et al.*, 2010). In the present work, locust protein had the lowest solubility (15 - 40%) at pH 4. From our previous work, locust protein aggregated and precipitated at around pH 4.2, which was the pI (isoelectric point) of locust protein. At the pI, locust soluble protein had higher protein solubility (15 - 40%) as compared to the plant proteins reported in other studies, *e.g.*, soy protein (2 - 5%) or corn gluten (5 - 9%) (Franzen and Kinsella, 1976; Tschimirov *et al.*, 1983). The protein solubility of SS-BL protein powder increased as the NaCl concentration increased, with 1.5% NaCl yielding the maximum solubility. This was due to salting-in phenomena, in which the solubility of the solute increased as the ionic strength increased. Therefore, the concentration of NaCl used in the present work (0.5 - 1.5%) was not able to increase the salting-out of soluble locust protein. Furthermore, Chatsuwan *et al*. (2018) and Purschke *et al*. (2018a) reported that water extracted soluble locust protein included more hydrophilic amino acids, *e.g.*, asparagine, serine, threonine, and glutamic acid or glutamine than did the alkaline extracted soluble protein. As a result, the solubility of WS-BL was higher than that of AS-BL.

Emulsifying properties

The EAI and ESI values of the WS-BL, SS-BL, and AS-BL powders prepared using various solutions are shown in Figures 4A and 4B. The highest significant EAI value was found in WS-BL (118 m^2/g), followed by 1.0% AS-BL (84 m^2/g) and 1.0% SS-BL (72 m^2/g) ($p < 0.05$). The molecular weight and protein distribution of WS-BL ranged from 10 - 100 kDa on SDS-PAGE, which was lower than the molecular weight of AS-BL and SS-BL. A protein with a smaller molecular weight moves quickly to the interface of water and fat droplets according to Kim *et al.* (2019) and Gravel and Doyen (2020), thus resulting in good emulsion formation or a high EAI value. However, protein composition also plays a role in emulsification capacity. Moreover, as mentioned in the previous section, the protein solubility of WS-BL at pH 7.0 was greater than 80%. This resulted in WS-BL having a higher dispersibility and EAI value, followed by 1.0% SS-BL, both of which had more soluble protein than the others (Figure 3).

The WS-BL had the greatest ESI value (52.45 min), which was also significant. This could have been due to the high protein solubility of WS-BL, followed by SS-BL and AS-BL, respectively. Moreover, according to Chatsuwan *et al.* (2018) and O'Sullivan *et al.* (2016), the hydrophobic amino acids of the water soluble proteins isolated from *Patanga succincta* were at higher level than the hydrophilic amino acids. The amino acids with high hydrophobicity reduced droplet break-up and enhanced protein adsorption at the interface, thus resulting in better emulsion stability. According to Wu (2001), high ionic strength solution decreased both the EAI and ESI values of corn gluten meal. This was in agreement with our observation that both the EAI and ESI values of SS-BL were significantly lower than those of WS-BL. As a result, the EAI and ESI values were influenced by more than just molecular weight and protein solubility. The emulsifying properties were also affected by hydrophobicity, hydrophilicity, and amino acid content (Gravel and Doyen, 2020).

Foaming properties

The FC and FS values of the WS-BL, SS-BL, and AS-BL powders prepared from several solutions are shown in Figures 5A and 5B. In general, the foaming property of a protein is a functional characteristic that helps to keep air bubbles at the air-liquid interface. It is influenced by the structure and distribution of proteins (Gravel and Doyen, 2020). In locust soluble protein powder prepared from low concentration (0.5%), the FC and FS showed a comparable trend. AS-BL presented higher FC (55%) and FS (27.5%) than WS-BL and SS-BL, respectively ($p < 0.05$). This was due to the insoluble protein chitin fraction at 25 kDa (Kim *et al.*, 2019), which has been shown to disrupt the foam stability of WS-BL and SS-BL (Yi *et al.*, 2013).

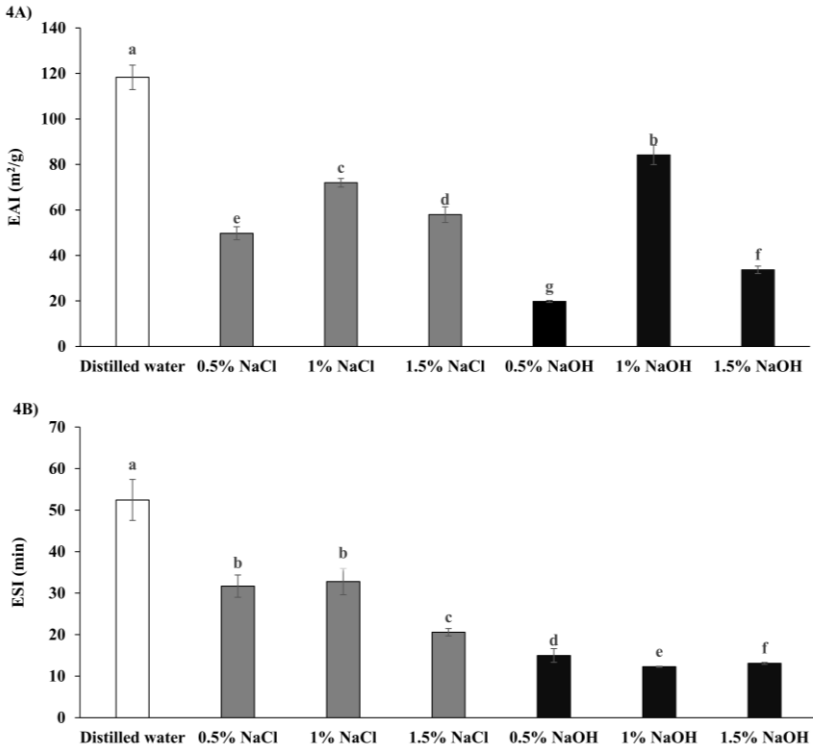

Figure 4. EAI **(A)** and ESI **(B)** values of soluble protein powders prepared from different extraction solutions. Different lowercase letters indicate significant differences at $p < 0.05$.

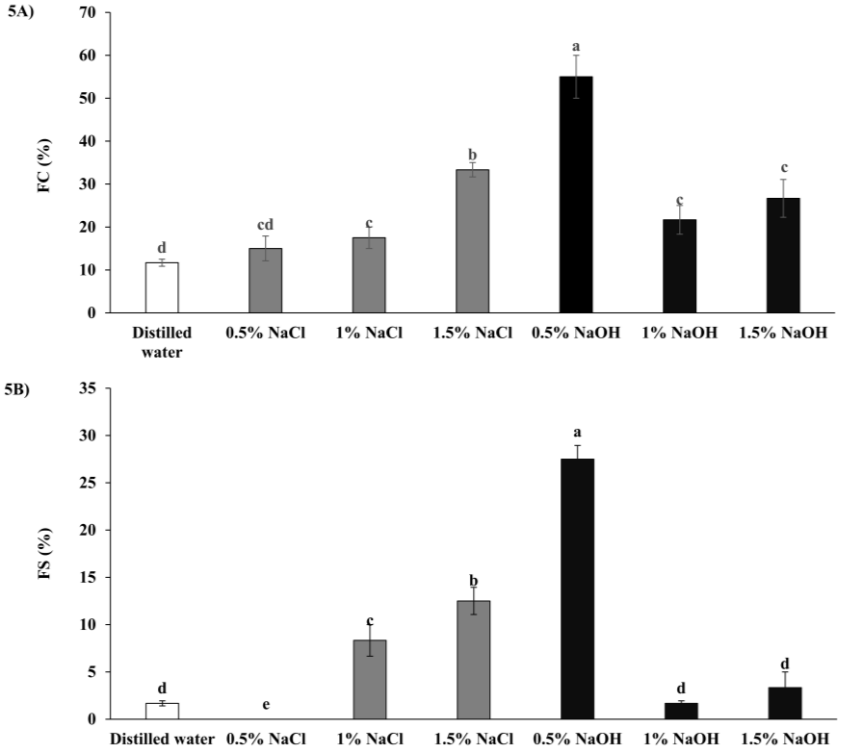

Figure 5. FC **(A)** and FS **(B)** values of soluble protein powders prepared from different extraction solutions. Different lowercase letters indicate significant differences at $p < 0.05$.

At 1.0 and 1.5% concentrations, however, the FS of SS-BL was greater than that of AS-BL. This might have been due to the effect of ionic strength, which improved foaming behaviour as NaCl concentration increased (Purschke *et al.*, 2018b). Kim *et al.* (2019) investigated the effect of ionic strength on foaming stability. They found that the salt soluble protein isolated from *Tenebrio molitor* and *Protaetia brevitarsis seulensis* had better foam stability than water soluble protein. Furthermore, the hydrophobic amino acid content, which was higher in salt soluble protein extracted from *Tenebrio molitor* and *Protaetia brevitarsis seulensis* than in water soluble protein (Panpipat and Chaijan, 2016; Zielińska *et al*., 2018), increased foam stability (Kim *et al.*, 2019).

Conclusion

Alkaline soluble protein (22 - 28%) was the highest yield extracted from BL, followed by water (16%) and salt (11 - 13%) soluble proteins. The highest protein solubility was found in 1.5% salt soluble protein powder. The highest FC and FS were found in 0.5% alkaline soluble protein powder. The highest EAI and ESI values were found in water soluble protein powder ($p < 0.05$). These findings suggested that the functional properties of soluble protein from BL varied, and could be modified by concentration and solution type. Furthermore, it was demonstrated that protein from BL can be employed in a variety of culinary products, depending on the product attributes and properties required.

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