Extraction and Isolation of Cricket Protein Isolate with Ammonium Sulfate Addition Method and Its Effect on The Functional Properties of The Proteins

Edward[1], *Thanakorn* Wongprasert[1], *Thasorn* Bunyakanchana[1], *Wasin* Pattaraprachyakul[1], *Panattida* Siripitakpong[1], and *Inthawoot* Suppavorasatit[1,2*]

[1] Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[2] Flavor Science and Functional Ingredients Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand

Abstract. Cricket insect contains a high quality of protein. To be able to use the proteins in food industry, extraction and isolation steps are necessary to elevate the protein content. The objective of this study was to extract and isolate protein of cricket insect and to assess its functional properties. The extraction and isolation steps were carried out by using alkaline extraction-acid precipitation (AEAP) with varying concentrations of ammonium sulfate (0, 20,40, and 60% w/v). It was found that extraction method with 60% ammonium sulphate inclusion showed the highest yield and obtained maximum protein content (92.41%), which could be characterized as cricket protein isolate (CPI). This extraction and inclusion of ammonium sulphate affected physicochemical properties, including water holding capacity, oil holding capacity, emulsifying properties, and foaming properties of CPI. In conclusion, the extraction, isolation, and addition of ammonium sulfate could be used for isolating the CPI containing high protein content and can be further used in food manufactures as an alternative protein in the future.

1 Introduction

The increasing number of world population nowadays has been impacted to many fields of human life, including for demand of food in the future (Santiago et al. 2021). Specifically, demand of protein intake becomes one of most important issues to find alternative protein sources since this compound is needed to be consumed to fulfil human nutritional intake (Friedman 1996). In order to meet this high demand of protein, there have been figured many attempts to find another protein source besides the conventional livestock such as chickens or cows. Insects has become one of most promising alternative protein sources due to its protein content value, which is comparable to traditional livestock (Rumpold and Schlüter 2015). Moreover, the cultivation of insects is more environmental friendly since it is produced fewer gas emissions, more efficient in feed conversion, and lesser water and soil usage (Lucas-González et al. 2019).

Cricket insect becomes one of famous edible insects which has great potency to become new alternative of protein source. This insect has been known for its high quality of protein content (Ndiritu et al. 2017). In terms of nutritional value, the protein content of cricket insect consists of essential amino acids and non-essential amino acids which the amount is adequate to meet the standard for requirement of amino acid for adults and children which states by World Health Organization (WHO) and Food and Agriculture Organization (FAO) (Oibiokpa et al. 2018). Moreover, the proteins with high protein content can be utilized by using its functional properties, such as water holding capacity, oil holding capacity, emulsifying properties, and foaming properties. However, the utilization of the proteins still has some issues regarding to the negative perspective of consumer about the insect itself (de Castro et al. 2018). Furthermore, the off-flavor of cricket that associated with green, earthy, and sweat also becomes another issues which can decrease the acceptance of the cricket insect (Smarzyński et al. 2021). Therefore, processing of cricket insect into another form such as powder form is strongly recommended in order to increase the consumer acceptance of cricket. Cricket powder form has more advantages because it can add into the products easier than other forms (Laroche et al. 2019). Cricket has many components inside not only protein, but also contains carbohydrates, fat, or other vitamins and minerals. Therefore, The defatting step could be used as pre-treatment to reduce the fat content and increasing the extraction of protein content. (Kurdi et al. 2021).

Meanwhile, alkaline extraction-acid precipitation is one of common extraction methods which usually used to extract the protein content. The principle of this method is based on the high solubility of protein on alkaline environment and precipitation of protein molecules by brought it onto its isoelectric point (Jiang et al. 2021). Furthermore, inclusion of ammonium

*Corresponding author: Inthawoot.S@chula.ac.th

sulphate will aid the extraction and isolation of protein content through salting-in/salting-out mechanisms. This existence of salt ion also will affect the functional properties of the protein. Up to now, there are just few publications that conducted research about the cricket protein isolate and its functional properties. There is initiative to contribute research about utilization of cricket insect. Therefore, the aim of this study was to extract and isolate the protein content of cricket insect and to assess its functional properties.

2 Materials and Methods

2.1 Materials

Cricket powder was obtained from Protanica Co Ltd. (Thailand). Sodium hydroxide (NaOH) were purchased from Sigma Aldrich (St Louis, MO, USA). Sodium dihydrogen orthophosphate dihydrate 99% and sodium phosphate dibasic dihydrate 99.5% were obtained from Loba Chemie Pvt. Ltd (Mumbai, India). Ammonium sulfate and hydrochloric acid (HCl) were purchased from Qrëc (Rawang, Malaysia). Dialysis tubing, sodium dodecyl sulfate, and boric acid were purchased from Thermo Fisher Scientific (Waltham, MA, USA). n-Hexane were purchased from Macron Fine Chemical (CV, Pennsylvania, USA)

2.2 Sample preparation

Cricket powder was defatted by using Soxhlet extraction according to Laroche et al. (2019). The extraction and isolation of cricket protein isolate (CPI) were performed by using method of Jiang et al. (2021) with slight modification. Five grams of defatted cricket powder was dispersed in 120 mL of 1.5% NaOH (w/v) and constantly shaken in a water bath (1 h, 80 °C). Next, the sample was cooled to room temperature and centrifuged at 6000 × g for 10 min. Next, the pH of the both supernatant and precipitate would be adjusted to pH 4.5 by using 1 M HCl and 50 mL ammonium sulphate with various concentrations (0%, 20%, 40% and 60%) were added. After that, the mixture will be kept overnight in refrigerator (4°C). The sample was then dialyzed overnight and freeze-dried, and the CPI powder was vacuum sealed in aluminium foil laminated bag and stored at -18°C for further analyses.

2.3 Protein content analysis

Determination of protein content was done by Kjeldahl method (928.08) from AOAC (2002). The conversion factor for protein content determination was 6.25.

2.4 Foaming Properties

Foaming properties, foaming capacity (FC) and foaming stability (FS) were determined by using method of Udomsil et al. (2019) with slight modification. First, 0.5 g of sample was dispersed in 20 mL of distilled water and equilibrated at room temperature (10 min). Next, the protein solution was aerated by homogenizer for 5 min (22,000 rpm). FC was determined by calculation as percentage of increased volume of protein solution after aeration, while FS was expressed by measured the remaining foam (%) after aeration after 30, 60, and 90 min. The calculation of FC and FS were done by using formula from Kunarayakul et al. (2018) with slight modification.

2.5 Water holding capacity and oil holding capacity

The water holding capacity (WHC) and oil holding capacity (OHC) were determined using methods from Zielińska et al. (2018) with some modification. WHC analysis was done by dispersing 0.5 g of sample in 20 mL of distilled water and shook at 250 rpm for 45 min. The dispersion was centrifuged for 15 min (8000 × g) and the WHC was calculated by % weight of the precipitate that obtained after centrifugation step. On the other hands, the OHC was determined by dispersed 0.5 g of sample in 10 mL of vegetable oil and vortexed for 30 s. Next, the dispersion was centrifuged (8000 × g, for 15 min) and the precipitate would be weighed to determine the OHC value of sample.

2.6 Emulsifying Properties

Determination of emulsifying properties, emulsifying activity index (EAI) and emulsifying stability (ES) were determined. These properties were conducted by using methods from Chen et al. (2018) and Meenmanee et al. (2022) with some modification. 0.5 g of sample was dissolved in 10 mM phosphate buffer (pH 7) to a final concentration of 0.1% (w/v). Next the protein suspension (9 mL) was homogenized with 3 mL of vegetable oil. Then, 50 µL aliquot of prepared emulsion system was removed from the bottom of the test tube and diluted in 5 mL 0.1% sodium dodecyl sulfate (SDS) solution and vortexed for five 5 s. The Emulsifying properties would be determined by measuring the absorbance of the dispersion at 500 nm directly and after 10 min. Calculation of EAI and ES were done following methods of Yin et al. (2008).

2.7 Statistical Analysis

Completely randomized design (CRD) was used in all experiments. Data obtained was analyzed by using one-way analysis of variance. Duncan multiple range test (DMRT) was employed in order to determine the significant difference between samples. The analysis in this study was done in triplicates.

3 Results and discussion

3.1 Protein content

Protein content of defatted cricket powder and cricket protein with different extraction methods,

consisted of alkaline extraction-acid precipitation and ammonium sulfate treated at various concentrations (20%, 40%, 60%) were shown in **Table 1**. It was found that the defatting, extraction, and isolation steps could increase total protein content of the samples. Defatting process is one of the pre-treatments before protein extraction, which is employed in order to reduce the lipid content on the food matrix. Cricket insect had been known as an alternative protein source with high lipid content. It was reported that the lipid content of cricket insect was around 16% - 23% (Udomsil et al. 2019). This high lipid content probably caused lipid-protein interactions which possibly could inhibit protein extraction. Thus, defatting step was compulsory to employ in order to enhance the extraction of protein content from food matrix (Gravel and Doyen 2020).

From the result in **Table 1**, the AEAP caused the increase in protein content from around 70% of defatted cricket powder to approximately 83%. This could be explained by the fact that the alkaline chemicals, such as sodium hydroxide (NaOH), could be utilized in order to increase the solubility of protein since the proteins exhibited their higher solubility when the pH was adjusted to alkaline environment than the neutral or acidic pH (Brogan et al. 2021). Hereafter, the acidic chemical, such as hydrochloric acid, could be employed to bring protein compound to its isoelectric point, which was located on pH around 5 (Zielińska et al. 2018). This adjustment would cause the protein charged to be zero and easier for the protein molecules to aggregate and precipitate, which would lead to the increase in protein content of the extracted proteins (Garba and Kaur 2014).

Table 1. *Protein content of cricket protein powder and cricket protein powder with AEAP and AS methods*

Sample	Protein Content (%)
Cricket Powder	$70.41^{a} \pm 0.92$
AEAP	$83.07^{b} \pm 0.92$
AS 20	$83.53^{b} \pm 0.85$
AS 40	$88.39^{c} \pm 0.78$
AS 60	$92.41^{d} \pm 1.46$

AEAP: alkaline extraction-acid precipitation; AS 20: AEAP +20% ammonium sulfate ; AS 40: AEAP + 40% ammonium sulfate ; AS 60: AEAP + 60% ammonium sulfate. Means ± standard deviation (n=3) with different letters within a column are significantly different ($P \leq 0.05$).

Furthermore, inclusion of ammonium sulfate was also positively affected the extraction step by escalating the total protein content of cricket protein. There were three concentrations of ammonium sulfate utilized in this study that were 20%, 40%, and 60% (w/v). Data displayed in **Table 1** showed the significant difference of protein extraction yields between samples. The protein content of samples extracted with inclusion of ammonium sulfate were increased when increasing in ammonium sulfate content. The result showed that AEAP cricket protein powder had the same protein content with AS20 and not different significantly. Meanwhile, the different protein contents were observed in AS20, AS40, and AS60 samples ($p \leq 0.05$). The gradual inclusion of ammonium sulfate could escalate protein content of cricket protein where 92.41% protein content was obtained by inclusion of 60 percent of ammonium sulfate. This phenomenon could be happened since the addition of salt ion such as ammonium sulfate could promote the salting-in and salting-out mechanism. Put simply, salting-in mechanism could be defined as the phenomenon where the existence of salt ion would interact with the protein and increase its solubility. However, when the amount of salt ion was gradually increased, the salt ion would compete with protein to bind with water molecules. This competition would promote the strong binding between the salt ion with water molecules. At this condition, there would be increasing of exposed of hydrophobic group of protein that drove the protein-protein interactions and led the protein to aggregate and easier to precipitate. This precipitation of protein by using the inclusion of salt ion was known as salting-out phenomenon (Kristianto et al. 2019). This result was in agreements with the study by Zhang et al. (2017), which successfully extracted potato protein using ammonium sulfate extraction. This study obtained protein extraction of potato protein of 83.20% by using 60% ammonium sulfate. In addition, Waglay et al. (2014) also reported increasing of ammonium sulfate from 40% to 60% could significantly increase potato protein isolate from 70.1% to 98.6%, which was higher than the protein content in present study. Furthermore, the precipitation by inclusion of salt ion was explained due to the dissociate of the ionic substances by salt ion that lead to increase of protein surface tension. This event would promote the decrease of hydrogen bonding between protein and water molecules which drive to precipitation, thus the protein content could be increased.

3.2 Foaming Properties

The foaming properties of cricket protein, composing of foaming capacity (FC) and foaming stability (FS) were reported in **Table 2** and **3.** According to Jiang et al. (2021), foaming capacity is reflection of the ability of protein molecules to be dissolved and unfolded rapidly to form a cohesive layer around a gas bubble. Meanwhile, the foaming stability could be defined as ability of protein molecules to form a stable foam that conducted by forming a continuous intermolecular polymer which trapped air. In this study, both FC and FS could not be determined on defatted cricket powder since this sample was unable to form a foaming system. This inability of cricket powder was probably occurred because there the powder still contained lipid in the sample. Lipid content in cricket insect was reported around 16-21% (Stone et al. 2019). This difference of lipid content could be depended on various factors such as insect feed source, stage lives, and age of the cricket itself (Day et al. 2022). Zayas (1997) explained that the lipid content in cricket insect could affect the ability of protein to forming a foam system since lipid molecules has ability to disrupt the protein interactions at air/water interface and inhibited the construction of foam system. Due to this fact, the efficient defatted step was compulsory to employ in order to remove the lipid content from the matrix and

rise the ability to construct the foaming system (Konak et al. 2014). The defatting step incorporated with AEAP step effectivity reflected on the result on this study where the samples that had defatting together with AEAP steps showed ability both in FC and FS.

***Table 2**. Foaming capacity of cricket protein powder and cricket protein powder with AEAP and AS methods*

Sample	Foaming Capacity (%)
Cricket Powder	ND
AEAP	$150.0^{a} \pm 3.16$
AS 20	$207.5^{b} \pm 4.18$
AS 40	$212.5^{c} \pm 2.74$
AS 60	$223.3^{d} \pm 2.58$

AEAP: Alkaline extraction-acid precipitation; AS: ammonium sulfate; AS 20: AEAP +20% ammonium sulfate; AS 40: AEAP + 40% ammonium sulfate ; AS 60: AEAP + 60% ammonium sulfate ; ND: not determined. Means ± standard deviation (n=3) with different letters within a column are significantly different ($P \leq 0.05$).

***Table 3.** Foaming stability of cricket protein powder and cricket protein powder with AEAP and AS methods*

Sample	Foaming Stability (%)			
	0 min	30 min	60 min	90 min
Cricket Powder	ND	ND	ND	ND
AEAP	$150.0^{Da} \pm 3.16$	$32.5^{Ca} \pm 2.74$	$11.^{7Ba} \pm 2.58$	$6.^{67Aa} \pm 2.58$
AS 20	$207.5^{Db} \pm 4.18$	$77.^{5Cb} \pm 2.74$	$29.^{2Bb} \pm 2.04$	$24.^{2Ab} \pm 2.04$
AS 40	$212.5^{Dc} \pm 2.74$	$115.^{8Cc} \pm 4.92$	$49.^{2Bc} \pm 3.76$	$27.^{5Ac} \pm 2.74$
AS 60	$223.3^{Cd} \pm 2.58$	$218.^{3Bd} \pm 2.58$	$218.^{3Bd} \pm 2.58$	$212.^{5Ad} \pm 2.74$

AEAP: Alkaline extraction-acid precipitation; AS: ammonium sulfate ; AS 20: AEAP +20% ammonium sulfate ; AS 40: AEAP + 40% ammonium sulfate ; AS 60: AEAP + 60% ammonium sulfate; ND: not determined. Means ± standard deviation (n=3) with different letters within a column are significantly different ($P \leq 0.05$).

In addition, there was a report by Gravel et al. (2021) that mentioned the defatting process by using hexane as a solvent could decrease the lipid content of yellow mealworm (*Tenebrio molitor*) from 28.5% to 0.04% and drastically escalated both the FC and FS of yellow mealworm protein. From **Table 2**, Cricket protein in every treatment was different significantly. AEAP samples was obtained the lowest FC and AS20, AS40 and AS 60 samples showed that the FCs were increased to be 207, 212, and 223 percent, respectively. The FS (**Table 3**) was also increased in the same trends as FC in every 30, 60, and 90 minutes. Furthermore, it was found that gradual increasing of ammonium sulfate concentration had positive impact to foaming properties of cricket protein isolate. The value of each sample was significantly different where inclusion of 60 percent of ammonium sulfate, which had the highest value for FC and FS (90 min) as 223.3% and 212.5%, respectively. This result was higher compared with the result by Jeong et al. (2021) who reported the FC and FS of cricket protein concentrate that defatted by using hexane which were only 61.1% and 73.9% (30 min), respectively. Mishyna et al. (2019) also reported the similar result where alkaline extraction could also increase foaming properties of protein that extracted from edible grasshopper, which was up to 90% for FC and 74.1% for FS (120 min). Increment of foaming properties after alkaline extraction could be explained with high protein content and high protein solubility. Alkaline treatment also possibly changed the protein characteristics such as partial protein unfolding that correlated to ability of protein to undergo rapid conformational changes at the interface and rapid adsorption at the air-water interface (Mishyna et al. 2019). Moreover, previous literatures from Adenekan et al. (2018) reported the isolation of pigeon pea protein with ammonium sulfate and found that the foaming properties of pigeon pea protein isolate were improved from 18.20% to 35.10% and 25.30% to 55.73% for FC and FS. This increment was possibly related to escalate of protein solubility and exposure of hydrophilic residues (Jiang et al. 2021). Moreover, it was found that the FS was decreased over the resting time. The alkaline extraction with inclusion of ammonium sulfate was found not only increase the FC, but also affected the FS of the samples. It was reflected from data shown in **Table 3** where AS60 sample displayed the highest FS value compared to other samples. This result was in agreements with Zhang et al. (2017) who stated that the increment of salt concentration could increase the stability of foaming system of potato protein isolate. This could be further explained by higher protein content after extraction would cause more initial protein introduced into the foaming system (Cui et al. 2020). Furthermore, another study by Yuliana et al. (2014) also reported the increase of FS value of cashew nut shell protein from 56.03% to 76.91% at 60 min when increasing in salt ion concentration. It was explained that addition of salt ion to protein affected the FS since the salt ion could increase surface activity and solubility of soluble protein.

3.3 Oil holding capacity and water holding capacity

Oil holding capacity (OHC) and water holding capacity (WHC) were two important parameters in food applications that strongly affected by the protein native state, protein composition, the pH condition, and ionic strength of the protein (Mishyna et al. 2021). The definition of OHC could be understood as the physical entrapment of oil where the mechanism of oil absorption was involving capillarity interaction that would be allowed the retained of oil that absorbed (Zielińska et al. 2018). Meanwhile, the definition of WHC by Zayas (1997) was an ability to hold the water molecules which added during application of many food processing like heating and centrifugation from three-dimensional structure of protein.

The **Table 4** displays the data of OHC and WHC of cricket protein isolate. It was shown for OHC data there was a significant difference between OHC values of cricket powder with the other samples that had been undergone the extraction and isolation steps. The extraction and isolation steps that employed in this study was positively affected the OHC of cricket protein isolate. The OHC value of cricket powder was increased drastically from 1.35 g/g to 4.97 g/g when it was treated with 20% inclusion of ammonium sulfate. This result was higher compared to result reported by Ogunwolu et al. (2009) for cashew nut protein (3.4 mL/g) that

extracted with the same method. Based on study conducted by Mao and Hua (2012), the escalating of OHC was probably occurred due to partial denaturation during extraction and isolation step. As mentioned in the methods section, this study was utilized the alkaline chemical (NaOH) and acid chemical (HCl) to adjust the pH into extremely alkaline (around pH 11) and acidic condition (pH 4.5). The usage of extreme pH during extraction and isolation step would probably cause partial denaturation of protein and more exposition of hydrophobic amino acid groups of cricket protein isolate that would lead to improvement of oil holding ability.

Table 4. *Oil holding capacity and water holding capacity of cricket protein powder and cricket protein powder with AEAP and AS methods*

Sample	OHC (g oil/g sample)	WHC (g water/g sample)
Cricket Powder	$1.35^{a} \pm 0.23$	$2.42^{d} \pm 0.13$
AEAP	$4.69^{c} \pm 0.08$	$0.34^{a} \pm 0.09$
AS 20	$4.97^{d} \pm 0.07$	$0.44^{b} \pm 0.02$
AS 40	$4.28^{b} \pm 0.08$	$0.35^{a} \pm 0.01$
AS 60	$4.75^{c} \pm 0.10$	$0.54^{c} \pm 0.03$

AEAP: Alkaline extraction-acid precipitation; AS: ammonium sulfate; AS 20: AEAP +20% ammonium sulfate; AS 40: AEAP + 40% ammonium sulfate; AS 60: AEAP + 60% ammonium sulfate. Means ± standard deviation (n=3) with different letters within a column are significantly different ($P \leq 0.05$).

Moreover, Sun et al. (2022) stated that the inclusion of salt ion would also affect the OHC value since the existence of salt ion such as ammonium sulphate could distract the zeta potential of protein solution and allow for exposition of big number of hydrophobic groups which would increase OHC of cricket protein isolate.

On the contrary, difference trend was depicted in **Table 4** for WHC value of cricket protein isolate. It was found that extraction and isolation that used in this study were drastically decreased the WHC value. The result was quite lower when compared to study by Hu et al. (2017) that mentioned the WHC value of walnut protein was 5 g/g. This result was probably correlated involvement of heat treatment during defat, extraction and isolation step of cricket protein isolate. Zayas (1997) explained the ability of protein to retain water molecules could be reduced by application of heat treatment since it caused the unfolding of polypeptide chain and transition of globular conformation to random coil that resulted in reduction of polar amino acid availability to bind water molecules. Furthermore, inclusion of ammonium sulfate as salt ion also played an important role for decreasing of WHC of cricket protein isolate. Ogunwolu et al. (2009) explained that the system at low salt concentration, hydrated salt ions would be weakly bound to group charged of proteins and binding of ions to proteins causing no any hydration shell of charged groups on protein. Nevertheless, as the salt ions increases, more water molecules would be bounded with salt ions and led to intermolecular interactions among protein molecules that caused dehydration of the protein and reduction of WHC value. This result was in agreements with Yuliana et al. (2014) who reported the WHC of defatted cashew nut shell decreased from 2.04 cm^3 H_2O/g to 1.73 cm^3 H_2O/g after further increased of NaCl concentration.

3.4 Emulsifying properties

Emulsification is one of functional properties of protein which can be described as the ability of protein to absorb and stabilize oil-water interfaces. Various factors such as movement of a protein from aqueous phase to a newly formed interface, unfolding, crosslinking and other conformational rearrangement over time were involved in a formation of stabilized emulsion by protein molecules. Protein that are soluble, flexible, amphiphilic, and has small molecular size tend to be more effective emulsifiers (Day et al. 2022).

Table 5. *Emulsifying properties of cricket protein powder and cricket protein powder with AEAP and AS methods*

Sample	EAI (m^2/g)	ES (min)
Cricket Powder	$11.26^{d} \pm 0.38$	$30.66^{b} \pm 2.72$
AEAP	$8.09^{b} \pm 0.24$	$24.49^{a} \pm 3.76$
AS 20	$8.07^{b} \pm 0.57$	$32.92^{b} \pm 6.14$
AS 40	$5.49^{a} \pm 0.61$	$21.52^{a} \pm 2.21$
AS 60	$9.33^{c} \pm 0.85$	$21.56^{a} \pm 3.81$

AEAP: Alkaline extraction-acid precipitation; AS: ammonium sulfate; AS 20: AEAP +20% ammonium sulfate ; AS 40: AEAP + 40% ammonium sulfate ; AS 60: AEAP + 60% ammonium sulfate. Means ± standard deviation (n=3) with different letters within a column are significantly different ($P \leq 0.05$).

Table 5 shows the data of emulsion activity index (EAI) and emulsion stability (ES) of cricket proteins. The EAI data depicted there was a decreasing trend where defatted cricket powder as the raw material had the highest EAI value compared to other samples. the EAI value of cricket powder of this study (11.26 m^2/g) was similar with the EAI value of cricket reported by Dion-Poulin et al. (2020) which was around 11.86–13.32 m^2/g.

It was found that extraction and isolation step were negatively affected by decreased the EAI value of the cricket protein isolate. This phenomenon was probably caused by the involvement of heat treatment during defat and extraction step. On the material and methods section, it was mentioned that the Soxhlet method was used for defatting process which was processed at high temperature. According to Gould and Wolf (2018), they noticed that utilization of heat treatment with a temperature up to 90 °C would change the microstructure of protein and affect to degree of flocculation and thus increase the droplet size. The heat application would also cause protein denaturation and conformation changes which led to increase in surface hydrophobicity and increased the protein-protein interactions. The result in present study was also similar to the study reported by Lee et al. (2019). They reported the emulsion activity and ES of yellow mealworm protein were decreased significantly after heat treatment (95 °C) from 46.46 to 34.94 and 87.59. to 69.39%, respectively. Heat treatments would promote intermolecular interaction among proteins that led to droplet flocculation and coalescence.

On the other hands, difference trend was found on ES value of cricket protein isolate. Inclusion of ammonium sulfate up to 20% would increase the ES, while further increase of ammonium sulfate concentration caused the decrease in the ES. This unique trend could be explained since the addition salt ion at low concentration would reduce the droplet size and increase the solubility of the proteins which resulted in escalating of ES. Nevertheless, high concentration of added salt ion would drive to protein-protein interactions and thus reduced the protein-oil interactions (Cui et al. 2014).This result was similar with the study by Idris et al. (2003), Hu et al. (2017) and Lawal et al. (2005). Moreover, emulsifying properties of cricket protein isolate were also depended on the types of proteins in the CPI. According to Brogan et al. (2021), cricket protein consisted of actin, myosin, and arginine-kinase. These types of protein could be classified as fibrous protein. It was also known that actin and myosin could contribute good emulsion capacity and ES. In particular, myosin itself could be categorized as one of fibrous protein types which capable to form highly elastic gel matrices and cohesive, rigid fat globule membrane in emulsifying system due to its well-balanced hydrophilicity – hydrophobicity and a large, and long fibrous structures. However, fibrous protein was also sensitive to heat treatment. Xu et al. (2020) mentioned that heat treatment could cause aggregation which made protein became insoluble and just only could allow for a few protein molecules to be adsorbed at oil /water interface. This could be another reason why the EAI value was decreased after extraction step to obtain AEAP sample (treated with 80 °C for 1h). Nevertheless, different trend was shown on ES value where the extraction step caused an increase in the ES. Li et al. (2022) stated that the extraction by using extreme pH could cause the protein to be unfolded, inducing structural changes, which would lead to greater stability of emulsion system.

4 Conclusion

The effect of defatting, extraction, isolation, and inclusion of various concentration of ammonium sulfate on the yield and functional properties of cricket protein isolate (CPI) were investigated. The defatting, extraction and isolation process has been proven that they could improve the extraction yield of cricket protein. The water holding capacity (WHC), oil holding capacity (OHC), emulsifying properties, and foaming properties of cricket protein isolate were affected by these steps. Moreover, the inclusion of ammonium sulphate as salt ion could affect the extraction yield and functional properties of cricket protein isolate. The inclusion of 60 percent ammonium sulfate increased the extraction yield and enhanced the foaming properties and OHC of cricket protein isolate. These results provide an important information for potential of CPI to become one of promising alternative protein sources in the future.

References

Adenekan, M. K., Fadimu, G. J., Odunmbaku, L. A. and Oke, E. K. (2018) Effect of isolation techniques on the characteristics of pigeon pea (*Cajanus cajan*) protein isolates. *Food science & nutrition, 6*(1), pp. 146-152.

AOAC (2002) Crude protein in meat. 928.08. *Official methods of analysis*.

Brogan, E. N., Park, Y.-L., Matak, K. E. and Jaczynski, J. (2021) Characterization of protein in cricket (*Acheta domesticus*), locust (*Locusta migratoria*), and silk worm pupae (*Bombyx mori*) insect powders. *LWT-Food Science and Technology, 152*, pp. 112314.

Chen, J., Mu, T., Zhang, M., Goffin, D., Sun, H., Ma, M., Liu, X. and Zhang, D. (2018) Structure, physicochemical, and functional properties of protein isolates and major fractions from cumin (*Cuminum cyminum*) seeds. *International Journal of Food Properties, 21*(1), pp. 685-701.

Cui, Z., Chen, Y., Kong, X., Zhang, C. and Hua, Y. (2014) Emulsifying properties and oil/water (O/W) interface adsorption behavior of heated soy proteins: Effects of heating concentration, homogenizer rotating speed, and salt addition level. *Journal of agricultural and food chemistry, 62*(7), pp. 1634-1642.

Day, L., Cakebread, J. A. and Loveday, S. M. (2022) Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology, 119*, pp. 428-442.

de Castro, R. J. S., Ohara, A., dos Santos Aguilar, J. G. and Domingues, M. A. F. (2018) Nutritional, functional and biological properties of insect proteins: Processes for obtaining, consumption and future challenges. *Trends in Food Science & Technology, 76*, pp. 82-89.

Dion-Poulin, A., Laroche, M., Doyen, A. and Turgeon, S. L. (2020) Functionality of cricket and mealworm hydrolysates generated after pretreatment of meals with high hydrostatic pressures. *Molecules, 25*(22), pp. 5366.

Friedman, M. (1996) Nutritional value of proteins from different food sources. A review. *Journal of agricultural and food chemistry, 44*(1), pp. 6-29.

Garba, U. and Kaur, S. (2014) Protein isolates: Production, functional properties and application. *International journal of current research and review, 6*(3), pp. 35-45.

Gould, J. and Wolf, B. (2018) Interfacial and emulsifying properties of mealworm protein at

the oil/water interface. *Food Hydrocolloids, 77*, pp. 57-65.

Gravel, A. and Doyen, A. (2020) The use of edible insect proteins in food: Challenges and issues related to their functional properties. *Innovative Food Science & Emerging Technologies, 59*, pp. 102272.

Gravel, A., Marciniak, A., Couture, M. and Doyen, A. (2021) Effects of hexane on protein profile, solubility and foaming properties of defatted proteins extracted from *Tenebrio molitor* larvae. *Molecules, 26*(2), pp. 351.

Hu, H., Fan, T., Zhao, X., Zhang, X., Sun, Y. and Liu, H. (2017) Influence of pH and salt concentration on functional properties of walnut protein from different extraction methods. *Journal of Food Science and Technology, 54*, pp. 2833-2841.

Idris, W. H., Babiker, E. E. and El Tinay, A. H. (2003) Fractionation, solubility and functional properties of wheat bran proteins as influenced by pH and/or salt concentration. *Food/Nahrung, 47*(6), pp. 425-429.

Jeong, M.-S., Lee, S.-D. and Cho, S.-J. (2021) Effect of three defatting solvents on the techno-functional properties of an edible insect (*Gryllus bimaculatus*) protein concentrate. *Molecules, 26*(17), pp. 5307.

Jiang, Y., Zhu, Y., Zheng, Y., Liu, Z., Zhong, Y., Deng, Y. and Zhao, Y. (2021) Effects of salting-in/out-assisted extractions on structural, physicochemical and functional properties of *Tenebrio molitor* larvae protein isolates. *Food Chemistry, 338*, pp. 128158.

Konak, Ü. İ., Ercili-Cura, D., Sibakov, J., Sontag-Strohm, T., Certel, M. and Loponen, J. (2014) CO2-defatted oats: Solubility, emulsification and foaming properties. *Journal of cereal science, 60*(1), pp. 37-41.

Kristianto, H., Prasetyo, S. and Sugih, A. K. (2019) Pemanfaatan ekstrak protein dari kacang-kacangan sebagai koagulan alami. *Jurnal Rekayasa Proses, 13*(2), pp. 65-80.

Kunarayakul, S., Thaiphanit, S., Anprung, P. and Suppavorasatit, I. (2018) Optimization of coconut protein deamidation using protein-glutaminase and its effect on solubility, emulsification, and foaming properties of the proteins. *Food Hydrocolloids, 79*, pp. 197-207.

Kurdi, P., Chaowiwat, P., Weston, J. and Hansawasdi, C. (2021) Studies on microbial quality, protein yield, and antioxidant properties of some frozen edible insects. *International Journal of Food Science, 2021*, pp. 1-7.

Laroche, M., Perreault, V., Marciniak, A., Gravel, A., Chamberland, J. and Doyen, A. (2019) Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods, 8*(11), pp. 572.

Lawal, O., Adebowale, K., Ogunsanwo, B., Sosanwo, O. and Bankole, S. (2005) On the functional properties of globulin and albumin protein fractions and flours of African locust bean (*Parkia biglobossa*). *Food Chemistry, 92*(4), pp. 681-691.

Lee, H.-j., Kim, J.-h., Ji, D.-S. and Lee, C.-H. (2019) Effects of heating time and temperature on functional properties of proteins of yellow mealworm larvae (*Tenebrio molitor L.*). *Food science of animal resources, 39*(2), pp. 296-308.

Li, L., Zhao, X. and Xu, X. (2022) Trace the difference driven by unfolding-refolding pathway of myofibrillar protein: Emphasizing the changes on structural and emulsion properties. *Food Chemistry, 367*, pp. 130688.

Lucas-González, R., Fernández-López, J., Pérez-Álvarez, J. A. and Viuda-Martos, M. (2019) Effect of drying processes in the chemical, physico-chemical, techno-functional and antioxidant properties of flours obtained from house cricket (*Acheta domesticus*). *European Food Research and Technology, 245*(7), pp. 1451-1458.

Mao, X. and Hua, Y. (2012) Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia L.*). *International journal of molecular sciences, 13*(2), pp. 1561-1581.

Meenmanee, S., Rattananukrom, A., Thaiphanit, S. and Suppavorasatit, I. (2022) Improvement of solubility, foaming, and emulsification properties of coconut (Cocos nucifera L.) protein by non-enzymatic deamidation. *Lwt, 153*, pp. 112493.

Mishyna, M., Keppler, J. K. and Chen, J. (2021) Techno-functional properties of edible insect proteins and effects of processing. *Current Opinion in Colloid & Interface Science, 56*, pp. 101508.

Mishyna, M., Martinez, J.-J. I., Chen, J. and Benjamin, O. (2019) Extraction, characterization and functional properties of soluble proteins from edible grasshopper (*Schistocerca gregaria*) and honey bee (*Apis mellifera*). *Food Research International, 116*, pp. 697-706.

Ndiritu, A. K., Kinyuru, J. N., Kenji, G. M. and Gichuhi, P. N. (2017) Extraction technique influences the physico-chemical characteristics and functional properties of edible crickets (*Acheta domesticus*) protein concentrate. *Journal of Food Measurement and Characterization, 11*(4), pp. 2013-2021.

Ogunwolu, S. O., Henshaw, F. O., Mock, H.-P., Santros, A. and Awonorin, S. O. (2009) Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale L.*) nut. *Food Chemistry, 115*(3), pp. 852-858.

Oibiokpa, F. I., Akanya, H. O., Jigam, A. A., Saidu, A. N. and Egwim, E. C. (2018) Protein quality of four indigenous edible insect species in Nigeria. *Food Science and Human Wellness, 7*(2), pp. 175-183.

Rumpold, B. A. and Schlüter, O. (2015) Insect-based protein sources and their potential for human consumption: Nutritional composition and processing. *Animal Frontiers, 5*(2), pp. 20-24.

Santiago, L. A., Fadel, O. M. and Tavares, G. M. (2021) How does the thermal-aggregation behavior of black cricket protein isolate affect its foaming and gelling properties? *Food Hydrocolloids, 110*, pp. 106169.

Smarzyński, K., Sarbak, P., Kowalczewski, P. Ł., Różańska, M. B., Rybicka, I., Polanowska, K., Fedko, M., Kmiecik, D., Masewicz, Ł. and Nowicki, M. (2021) Low-Field NMR study of shortcake biscuits with cricket powder, and their nutritional and physical characteristics. *Molecules, 26*(17), pp. 5417.

Stone, A. K., Tanaka, T. and Nickerson, M. T. (2019) Protein quality and physicochemical properties of commercial cricket and mealworm powders. *Journal of Food Science and Technology, 56*, pp. 3355-3363.

Sun, Y., Zhong, M., Wu, L., Huang, Y., Li, Y. and Qi, B. (2022) Effects of ultrasound-assisted salt (NaCl) extraction method on the structural and functional properties of Oleosin. *Food Chemistry, 372*, pp. 131238.

Udomsil, N., Imsoonthornruksa, S., Gosalawit, C. and Ketudat-Cairns, M. (2019) Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). *Food Science and Technology Research, 25*(4), pp. 597-605.

Waglay, A., Karboune, S. and Alli, I. (2014) Potato protein isolates: Recovery and characterization of their properties. *Food Chemistry, 142*, pp. 373-382.

Xu, Y., Dong, M., Tang, C., Han, M., Xu, X. and Zhou, G. (2020) Glycation-induced structural modification of myofibrillar protein and its relation to emulsifying properties. *LWT-Food Science and Technology, 117*, pp. 108664.

Yin, S.-W., Tang, C.-H., Wen, Q.-B., Yang, X.-Q. and Li, L. (2008) Functional properties and in vitro trypsin digestibility of red kidney bean (Phaseolus vulgaris L.) protein isolate: Effect of high-pressure treatment. *Food Chemistry, 110*(4), pp. 938-945.

Yuliana, M., Truong, C. T., Huynh, L. H., Ho, Q. P. and Ju, Y.-H. (2014) Isolation and characterization of protein isolated from defatted cashew nut shell: Influence of pH and NaCl on solubility and functional properties. *LWT-Food Science and Technology, 55*(2), pp. 621-626.

Zayas, J. F. (1997) *Functionality of proteins in food,* Berlin, German: Springer science & business media.

Zhang, D. Q., Mu, T. H., Sun, H. N., Chen, J. W. and Zhang, M. (2017) Comparative study of potato protein concentrates extracted using ammonium sulfate and isoelectric precipitation. *International Journal of Food Properties, 20*(9), pp. 2113-2127.

Zielińska, E., Karaś, M. and Baraniak, B. (2018) Comparison of functional properties of edible insects and protein preparations thereof. *LWT-Food Science and Technology, 91*, pp. 168-174.