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Original research article

Optimal Ultrasound–Assisted Extraction of Concentrated Protein from Cricket Powder

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ABSTRACT

Edible insects are a valuable protein source, and extracting these proteins is essential for creating customized products with precise compositions. Ultrasound technology offers an environmentally friendly method to improve the extraction process. This study investigated the optimal conditions for protein extraction from cricket powder (*Acheta domesticus*) using ultrasound-assisted extraction. The key parameters analyzed included the solid-to-liquid ratio (S/L), temperature, and extraction time. The compositional analysis revealed that the protein and lipid contents in the cricket powder were 59.37%w/w and 16.78%w/w, respectively. Utilizing the response surface methodology (RSM) with a Box-Behnken design, the optimal conditions for protein extraction were identified: an S/L ratio of 1:6, a temperature of 40 °C, and an extraction time of 30 min at a constant pH of 11. Under these conditions, the protein content obtained was 22.32%w/w. Subsequent freeze-drying of the extracted cricket protein resulted in a powder with a protein content of 69.57%w/w, a yield of 59.55%, and a protein extraction efficiency of 69.78%. Cytotoxicity assessments using the MTT assay on RAW 264.7 macrophages and to mononuclear cells demonstrated that both the raw and freeze-dried cricket powder exhibited no cytotoxic effect. Therefore, the resultant cricket protein powder shows potential for incorporation into functional food products.

Keywords: Cricket powder; Freeze dryer; Nutraceutical property; Response surface methodology; Ultrasound-assisted extraction

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1. Introduction

Edible insects have emerged as a promising protein source for the global population. A comprehensive report analyzing 236 types of edible insects revealed that their protein quality surpasses that of plant and animal proteins, providing all essential amino acids required by humans [1]. According to the Food and Agriculture Organization, the global population is projected to reach 8 billion by 2024, and approximately 9 billion by 2050, intensifying the demand for food and protein resources. Crickets, in particular, have become significant economic insects in Thailand [2].

In 2020, data indicated that 25,218 Thai farmers managed 272,922 cricket ponds, producing a total of 24,563 tons of crickets valued at 274 million baht. Additionally, 0.71 tons were exported, valued at 0.32 million baht. Cricket farming is widespread across Thailand, particularly in the southern provinces of Surat Thani and Nakhon Si Thammarat [3]. In Nakhon Si Thammarat alone, there are 29 cricket farmers [4]. The Thongdam and Sading species are the most commonly raised, with protein content ranging from 60-70% by dry weight and fat content from 10-23% [5]. Chemical composition analysis of Sading crickets revealed moisture, protein, fat, fiber, and ash contents of 12.64%, 60.40%, 16.92%, 12.93% and 3.81%w/w, respectively [6]. Protein is a crucial organic compound in all living organisms, essential in every living cell. Humans obtain protein from both plant and animal sources [7].

Currently, the insect processing industry in Thailand primarily processes insects through frying and freezing, with most products retaining the insects' original shape, causing consumer hesitation and uncertainty. Processing edible insects into powder and extracting proteins or fats for incorporation into other products could enhance consumer to acceptance and consumption [8]. There are three primary methods for protein extraction: thermal, acid or alkali, and enzyme extraction [9]. The resulting proteins can be categorized as protein concentrate, protein isolate, and protein hydrolysate. Concentrated protein extracts, obtained through heat or alkali extraction, are typically used to produce protein concentrates with improved functional properties such as emulsification, foaming, gelation, and solubility [10].

Traditional methods for protein extraction, such as water and alkaline extraction, are time-consuming, low- yield, and are not environmentally friendly. Ultrasound-assisted extraction offers a solution by enhancing efficiency, increasing yield, and reducing extraction time [11]. Entrepreneurs process crickets into cricket powder for use in various food products, such as baked goods, drinks, and snacks. Although cricket powder is high in protein, it also contains significant amounts of saturated fat, which may impact consumer health. Extracting protein or fat from cricket powder results in a concentrated extract suitable for consumers requiring high nutrient levels, especially protein, such as the elderly or individuals engaged in muscle-strengthening exercises [12].

The mechanism of protein extraction using heat, alkali, and ultrasound involves different processes that enhance the solubility and release of proteins from the source material. Applying heat to the material helps break down the cellular structure and disrupts protein-protein interactions, which makes proteins more accessible for extraction. Heat can also denature proteins, which increases their solubility in the extraction medium. The use of alkali (such as sodium hydroxide) changes the pH of the medium,

which can break ionic bonds and alter protein conformation. This causes proteins to become more soluble in the alkaline solution, facilitating their extraction from the matrix. High-frequency sound waves create cavitation bubbles in the liquid, which expand and collapse, generating localized heat and shear forces. These forces break down the cell walls and disrupt protein-protein interactions, helping to release proteins from the source material into the solution. Therefore, this study aimed to produce cricket powder from popular cricket species in Nakhon Si Thammarat province and extract concentrated protein using ultrasound-assisted extraction. The resulting protein extract can be applied to food products, enhancing their functionality and commercial value.

2. Materials and Methods

2.1 Preparation of cricket powder

Adult crickets (Sading and Thongdam species) were obtained from a farm in Nai Thang, Chian Yai District, Nakhon Si Thammarat province. The crickets were initially soaked in cold water, then strained and rinsed in clean water. They were subsequently washed three times and blanched in boiling water. The crickets were dried in a hot air oven at 80°C for 6 hours, ground using a grinder, and sieved with a 250 μm sieve. The processed cricket powder was stored in sealed aluminum foil bags at 4°C [13]. The powder was then analyzed for quality, including color values (L^*, a^* and b^*) using a Hunter Lab machine, and chemical composition using proximate analysis according to previous methods [14], in preparation for subsequent protein extraction.

2.2 Optimal conditions for extracting protein from cricket powder using ultrasound techniques

Twenty grams of prepared cricket powder (Sading strain with the highest protein content) were used for protein extraction. Distilled water served as the solvent, and the extraction was conducted at a solid-to-liquid (S/L) ratio, temperature, using an ultrasound machine (120 W, 45 kHz) at pH 11.0 [6]. Optimum conditions for protein extraction were determined using statistical programs. Three factors affecting protein extraction were determined: temperature (X_1), sample-to-solvent ratio (X_2), and time (X_3). Each factor was tested at three levels: low (-1), middle (0) and high (+1). The experimental design employed a response surface methodology (RSM) with the Box-Behnken Design technique, setting the protein percentage as the response variable (Y). A total of 17 experimental sets were generated, including five replicates at the center point. Post-extraction, the liquid solution containing the dissolved protein (supernatant) was centrifuged at 4°C at 12,000 rpm for 20 minutes [15]. The clear supernatant was then analyzed for protein percentage to determine the optimal extraction conditions. Subsequently, protein extraction was performed under the optimal conditions in a volume of 1.5 liters. The protein extract (143 g) was freeze-dried, yielding a protein extract with a weight of 85.15 g and a moisture content of less than 5%. The yield percentage and protein extraction rate were calculated as follows:

$$\%\text{yield} = \frac{\text{weight of extract (g)}}{\text{weight of sample (g)}} \times 100,$$

$$\text{Protein extraction rate} = \frac{\text{protein of extract}}{\text{protein of sample}} \times \%\text{yield}.$$

Table 1. Physical and chemical properties of cricket powder.

Species	Physical properties			Chemical properties					
	Color			Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
	L^*	a^*	b^*	(%)	(%)	(%)	(%)	(%)	(%)
Sading (*Acheta domestica*)	46.00 ±0.04a	5.46 ±0.08a	17.25 ±0.25a	3.35 ±0.03b	59.37 ±0.46a	16.78 ±0.15a	1.82±0.63a	3.24 ±0.81a	15.44 ±1.59b
Thongdam (*Gryllus bimaculatus*)	32.21 ±0.18b	5.29 ±0.12b	11.33 ±0.47b	3.63 ±0.02a	55.47 ±0.32b	17.01 ±0.29a	1.98 ±0.42a	1.92 ±0.07b	20.00 ±0.12a

2.3 Efficiency of highly concentrated cricket protein modified in nutraceuticals

- Cytotoxicity assessment: Evaluation of the cytotoxicity effects on mononuclear white blood cells using MTT assay.

- Proliferation analysis: Examination of the extract's impact on the growth and proliferation of mononuclear white blood cells through a proliferation assay.

- T cell analysis: Analysis of CD4+ and CD8+ T cell populations following lymphocyte stimulation with the extracts

3. Results and Discussion Preparation of Cricket Powder

When the Sading and Thongdam crickets were dried in a hot air oven at 80°C for 6 hours, the final moisture content was 3.35% w/w and 3.63% w/w, respectively. The moisture content of both strains was below 10 % w/w, indicating their potential for long-term storage due to reduced degradation. The dried crickets were then ground using a pulverizer and sieved with a sliding screen. The resulting Sading powder was a light brown coarse powder, while the Thongdam powder was darker in color, corresponding to the type of the pollen, as illustrated in Fig. 1.

Physical and chemical properties shown in Table 1. It was observed that the brightness value (L^*) of the Sading powder was greater than the Thongdam powder. The red (a^*) and yellow (b^*) values were similar for both types of crickets. Regarding the chemical properties of the extracts,

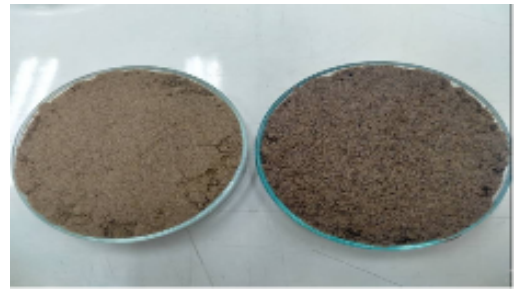

Fig. 1. Appearance of cricket powder (a) *Acheta domestica* (House cricket or Sading) and (b) *Gryllus bimaculatus* (Thongdam).

the Sading powder exhibited a higher protein content (59.37% w/w) than the Thongdam powder (55.47% w/w), with a significant difference noted. The fat was also high and comparable between the two types (16.78-17.01%). According to a previous study, the protein and fat content of cricket powder were 63.43% and 20.86%, respectively [16]. Another study reported protein and fat contents of 71.7% and 10.40%, respectively, in cricket powder [17]. Similarly, one study found a protein content of 53.0%, and fat content values of 60.70% and 23.40%, respectively [18]. The protein content of striped copper crickets ranges from 55% to 70% [19], indicating that protein is the main nutrient component of crickets. These fundings suggest that crickets are a significant source of protein and could be a valuable nutritional resource in the future.

3.1 Optimal conditions for extracting protein from cricket powder using ultrasound techniques

When 20 g of prepared cricket powder was subjected to protein extraction, a sodium hydroxide solution with a concentration of 1.0 N was used as the solvent at a ratio of S/L 1:3 to 1:6, a temperature range of 30-40°C, an extraction time of 10-30 minutes, using ultrasound machine (120 W, 45 kHz). A multiple regression model was developed using the Box-Behnken response surface methodology, incorporating temperature, solid-to-liquid ratio, and extraction time as variables for optimizing protein extraction from cricket powder. The regression equation derived from this model enables the prediction of optimal conditions by examining the relationship between variables. The study considers pairs of variables while maintaining the third variable at a constant value to identify the most suitable conditions from the equation provided.

$$\begin{aligned}\%\text{Protien} = & 21.97 + 0.484X_1 - 1.726X_2 \\ & - 0.1230X_3 - 0.00775X_1^2 \\ & + 0.03964X_2^2 + 0.002118X_3^2 \\ & + 0.00484X_1X_3 - 0.00952X_2X_3,\end{aligned}$$

where X_1 is temperature, X_2 is sample-to-solvent ratio, X_3 is time.

The analysis results indicate that this experiment successfully generated appropriate quadratic equation models through significant statistical evaluation. It was found that data incompatibility had a significant impact. The Variance Inflation Factor (VIF) values ranged between 1 and 5 ($1< VIF <5$), indicating that the variables exhibit a moderate level of correlation, which is considered acceptable. The detailed VIF values and their corresponding statistical significance are presented in Tables 2-3. Therefore, the optimal conditions for extracting protein from cricket powder, as determined by response optimization, were identified as follows: a temperature of 40°C, an S/L ratio of 1:6, and an extraction time of 30 minutes. Under these conditions, the average protein extraction yield from crickets was maximized at 22.32%, with a 95% confidence interval, as illustrated in Fig. 2. These results underscore the effectiveness of response optimization in identifying the most suitable extraction parameters. The limitation a response value of product for extraction temperature, S/L ratio and extraction time is between 30 – 40°C, 1:3 – 1:9, and 10 – 30 minutes, respectively.

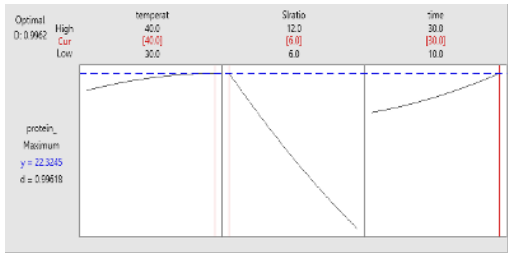

Fig. 2. The optimum conditions by response optimization.

Twenty grams of cricket extract powder were used to extract protein under the ideal conditions determined by RSM (Fig. 2). Distilled water was used as the solvent with an S/L ratio of 1:6. The extraction process was conducted at a temperature of 40°C for 30 minutes, utilizing ultrasound (120 W, 45 kHz) at pH 11. The resulting solution was centrifuged at 12,000 rpm and 4°C, yielding 1.5 liters of protein extract solution. This solution was then freeze-dried to produce cricket protein powder, as depicted in Fig. 3.

Upon analyzing the protein content of the freeze-dried cricket protein extract powder, it was determined to be 69.57%, with a yield of 59.55% and a protein extrac-

Table 2. Analysis of variance.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	207.732	25.966	743.76	0.000
Linear	3	191.393	63.798	1827.36	0.000
X_1	1	0.538	0.538	15.41	0.001
X_2	1	187.525	187.525	5371.30	0.000
X_3	1	3.330	3.330	95.37	0.000
Square	3	1.541	0.514	14.72	0.000
X_1^2	1	0.316	0.316	9.05	0.006
X_2^2	1	1.072	1.072	30.71	0.000
X_3^2	1	0.188	0.188	5.38	0.029
2-Way Interaction	2	1.262	0.631	18.07	0.000
X_1X_3	1	0.528	0.528	15.13	0.001
X_2X_3	1	0.734	0.734	21.02	0.000
Error	25	0.873	0.035		
Lack-of-Fit	4	0.804	0.201	61.33	0.000
Pure Error	21	0.069	0.003		
Total	33	208.605			

Table 3. Regression equation of RSM of S/L ratio and time for protein extraction from cricket powder.

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	17.1637	0.0780	220.02	0.000	
X_1	0.1933	0.0492	3.93	0.001	1.11
X_2	-3.6087	0.0492	-73.29	0.000	1.11
X_3	0.4562	0.0467	9.77	0.000	1.13
X_1^2	-0.1937	0.0644	-3.01	0.006	1.01
X_2^2	0.3568	0.0644	5.54	0.000	1.01
X_3^2	0.2118	0.0913	2.32	0.029	1.14
X_1X_3	0.2422	0.0623	3.89	0.001	1.11
X_2X_3	-0.2855	0.0623	-4.58	0.000	1.11

tion rate of 69.78%. These findings align with another study [20], which looked at the potential of protein fractionated from crickets for pasta formulation, reporting an extraction yield of 64.3%, a total protein content of 48.8% in PBS extract powder, and an extraction efficiency of 75.2%. Similarly, it was found that using ultrasound extraction of cricket protein powder yielded an extraction rate of 37% with an extraction time of 15 minutes [21]. When compared with soy protein concentrate, commonly used as a meat substitute in the meat industry, which has a protein content between 62-69%, the research results are comparable. Additionally, the protein extraction yield and protein purity of copper-striped crickets was reported to range between 31.0-38.9% and 58.3-78.5%, respectively [22].

The physical and chemical properties of freeze-dried cricket protein extract powder with optimal conditions are presented in Table 4 When comparing cricket powder and freeze-dried cricket protein extract, it was found that the color value and protein content increased, while fat, fiber, and ash content decreased. Similar results have been reported regarding the physicochemical and techno-functional characterization of soluble proteins extracted by ultrasound

Fig. 3. Cricket protein extract powder from freeze dry with distilled water as a solvent.

Table 4. Physical and chemical properties of freeze-dried cricket protein extract powder.

Properties	Cricket powder	Freeze-dried cricket extract powder
Physical properties		
L^*	46.02±0.23	69.58±0.01
a^*	5.44±0.08	6.53±0.02
b^*	17.33±0.25	23.88±0.14
Chemical properties		
Moisture content(%)	3.34±0.03	7.56±0.12
Protein (%)	58.32±0.81	68.10±0.00
Fat (%)	16.80±0.15	0.03±0.02
Fiber (%)	2.09±0.15	0.73±0.15
Ash (%)	3.39±0.81	0.48±0.06
Carbohydrate(%)	16.06±1.72	23.10±0.28

from the cricket *Acheta domesticus* [23]. It was found that total protein increased from 45.75 to 53.85%, while fat and carbohydrate content decreased. Glutamic acid and glutamine were the most abundant acids in cricket [17].

3.2 Efficiency of highly concentrated cricket protein modified in nutraceuticals

Cricket protein extract toxicity was evaluated using the MTT assay to determine cytotoxicity and cell viability in RAW 264.7 macrophages and 10,000 mononuclear cells/well. Concentrations of 31.25, 62.50, 125, 250, and 500 μg/mL were used to assess the protein's toxic effects on both cell types. The results of the study found that crickets, ground Sading cricket powder, and freeze dried Sading cricket powder did not affect the toxicity to cells, as shown in Tables 5-6. However, this study was only conducted in cells; toxicity studies in laboratory animals, both acute and long-term, as well as further studies in humans are still required.

Table 5. Physical and chemical properties of freeze-dried cricket protein extract powder.

Conc. (μg/mL)	%Cell viability					
	Cricket powder			Freeze dried		
31.25	102.5	99.4	98.7	103.5	98.2	100.8
62.5	99.2	98.5	103.4	99.2	102.7	98.5
125	100.7	96.2	102.5	96.4	104.2	100.6
250	98.2	104.1	100.3	103.1	97.5	99.4
500	102.4	96.5	99.7	102.5	96.3	98.5

Table 6. Cricket protein effects on mononuclear cells by MTT assay.

Conc. (μg/mL)	%Cell viability					
	Cricket powder			Freeze dried		
31.25	98.1	104.4	100.3	97.3	104.3	99.2
62.50	103.6	97.3	99.5	102.5	97.4	98.8
125	96.7	101.6	102.8	101.6	103.5	97.3
250	101.3	98.7	96.5	96.7	102.2	97.4
500	96.9	102.5	97.7	97.2	96.5	101.7

The effect of cricket extracts on mononuclear white blood cell proliferation was studied using the [3H] thymidine incorporation method. It was found that the extract at a concentration of 31.25 - 500 μg/mL was able to stimulate cells to increase proliferation, as shown in Table 7. Stimulation of mononuclear cells by the extract revealed that cricket protein at concentrations of 500 and 250 μg/ml could stimulate CD4+ white blood cells, while the concentration of 500 μg/ml could stimulate CD8+ white blood cells when compared to the positive control, Staphylococcal enterotoxin B (SEB). Protein sources have been shown to be important for immune cell

proliferation and effector molecules. The amino acids arginine, glutamine, and tryptophan have been found to play a role in the immune response and are found in cricket proteins [24]. Glutamine has been recognized as important for the "maintenance of normal immune function". Glutamine regulates the proliferation of lymphocytes, neutrophils, and macrophages via the signal transduction pathway kinase (JNK) and activator protein (AP)-1, and regulates the production of cytokines such as IL-6, IFN-γ, and TNF-α [25]. In addition, activated B and T cells have an increased requirement for glutamine for the production of IFN-γ and IL-2, and for the proliferation of T cells. Furthermore, whey protein consumption has been reported to be associated with higher CD4+ and C8+ lymphocyte counts, total white blood cells, and increased IFN-γ production by concanavalin A-stimulated spleens in mice [26].

4. Conclusion

This study successfully identified the optimal conditions for protein extraction from cricket powder (*Acheta domesticus*) using ultrasound-assisted extraction, highlighting an S/L ratio of 1:6, a temperature of 40°C, and an extraction time of 30 minutes at pH 11. The extracted protein demonstrated a substantial increase in concentration following freeze-drying, achieving a protein content of 69.57% w/w and a high extraction efficiency. Importantly, cytotoxicity assessments confirmed the safety of both raw and processed cricket powders, showing no adverse effects on RAW 264.7 macrophages and mononuclear cells. These findings underscore the potential of cricket protein powder as a viable ingredient for functional food products, offering an environmentally sustainable and nutritionally rich protein source. Cricket protein powder shows potential for incorporation into functional food products. Therefore, the extracted substance should be further tested in food products.

Table 7. Cricket protein that affects proliferation of mononuclear cell white blood cells.

Conc. (μg/mL)	%Cell viability					
	Cricket powder			Freeze dried		
31.25	25.1	30.7	31.2	41.5	45.1	46.1
62.50	52.4	60.4	57.1	62.4	65.7	70.1
125	96.2	99.5	101.7	108.4	107.2	99.8
250	112.3	125.3	126.4	130.5	134.5	129.3
500	170.2	165.2	145.7	170.5	172.4	164.2

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References

[1] Panto T. Edible insects: nutritional value and processing for use. Food. 2020;50:1-8.

[2] Chansom T. Effects of different foods on growth, reproduction rate and protein content of black gold cricket (Gryllus bimaculatus de Geer). [MS Thesis]. Bangkok: Kasetsart University; 2020.

[3] Economic Insect Promotion Group, Office of Agricultural Product Promotion and Management. Insect situation: Crickets [Internet]. 2022 [cited 2024 May 10]. Available from: http://www.agriman.doae.go.th.

[4] Center for the Promotion of Agricultural Technology in Economic Insects, Chumphon Province. Report on information on economic insect farmers by province in 2018; 2018.

[5] Udomsil N, Imsoonthornruksa S, Gosalawit C, Ketudat-Cairns M. Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). Food Sci Technol. 2019;25(4):597-605.

[6] Chooklin S. Effects of Nakhon upland brown rice flour and cricket powder on quality of extruded snacks. RMUTSV Research Journal. 2023; 15(3): 702-15.

[7] Rattanapanon N. Food chemistry. Bangkok: Odeon Store; 2014.

[8] Jino T, Suruang S. Chemical composition and optimum conditions for extracting concentrated protein from commercial crickets. TJST. 2020;9:64-73.

[9] Panyam D, Kilara A. Enhancing the functionality of food proteins by enzymatic modification. Trends Food Sci Technol. 1996;7(4):120-5.

[10] Kristinsson H, Rasco B. Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr. 2000;40(1):43-81.

[11] Duangjarus N, Chaiworapuek W, Ritthiruangdej P, Sae-tan S, Charoensiddhi S. Ultrasound-assisted extraction for the recovery of proteins from duckweed (Wolffia globosa) using response surface methodology. In: The 59th KU Annual Conference, Kasetsart University, Bangkok; 2021.

[12] Lertrat K, Pamanothai A, Sanan Chokloi S. The path to creating healthy food innovations. Khon Kaen: Khon Kaen Printing; 2018.

[13] Chooklin S, Muadsri T, Chairik A, Chusuwan A, Nuthong T, Thong-in N. Development of extruded native brown rice supplemented with cricket protein powder using a twin-screw extruder. In: Report of the 59th Kasetsart University Academic Conference, Kasetsart University, Bangkok; 2021.

[14] Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of AOAC International. 17th ed. Gaithersburg: AOAC International; 2000.

[15] Mahmood T, Yang PC. Western blot: technique, theory, and troubleshooting. N Am J Med. 2012;4(9):429-34.

[16] Rashmi VA. Functional insect protein extracts for food applications. M.S. Thesis, University of Central Oklahoma; 2019.

[17] Udomsil N, Imsoonthornruksa S, Gosalawit C, Ketudat-Cairns M. Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). Food Sci Technol. 2019;25(4):597-605.

[18] Laroche M, Perreault V, Marciniak A, Gravel A, Chamberland J, Doyen A. Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. Foods. 2019;8(572):1-11.

[19] Rumpold BA, Schulter OK. Nutritional composition and safety aspects of edible insects. Mol Nutr Food Res. 2013;57(5):802-23.

[20] Pasini G, Cullere M, Vegro M, Simonato B. Potentiality of protein fractions from the house cricket (*Acheta domesticus*) and yellow mealworm (*Tenebrio molitor*) for pasta formulation. LWT-Food Sci Technol. 2022;164(2):1136-8.

[21] Choi BD, Wang NAK, Auh JH. Defatting and sonication enhances protein extraction from edible insects. Korean J Food Sci Anim Resour. 2017;37(6):955-61.

[22] Laroche M, Perreault V, Marciniak A, Gravel A, Chamberland J, Doyen A. Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. Foods. 2019;8(572):1-11.

[23] Cruz-Lopez SO, Escalona-Buendia HB, Martinez-Arellano IM, Dominguez-Soberanes J, Alvarez-Cisneros YM. Physicochemical and techno-functional characterization of soluble proteins extracted by ultrasound from the cricket Acheta domesticus. Heliyon. 2024;10:1-15.

[24] Hachimura S, Totsuka M, Hosono A. Immunomodulation by food: Impact on gut immunity and immune cell function. Biosci Biotechnol Biochem. 2018;82:584-99.

[25] Iddir M, Brito A, Dingeo G, Fernandez Del Campo SS, Samouda H, La Frano MR, Bohn T. Strengthening the immune system and reducing inflammation and oxidative stress through diet and nutrition: Considerations during the COVID-19 crisis. Nutrients. 2020;12:1562.

[26] Ford JT, Wong CW, Colditz IG. Effects of dietary protein types on immune responses and levels of infection with Eimeria vermiformis in mice. Immunol Cell Biol. 2000;78:380-5.