# Protein isolation methods from edible insects: A systematic review

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**Abstract.** The development of edible insects, especially Orthoptera orders such as crickets and grasshoppers/locusts, as alternative protein sources continue to grow through various preparations such as flour, concentrates, and isolates to support future food security. The objective of this research was to explore the protein extraction techniques and optimal conditions for producing insect protein concentrates and isolates. A systematic literature review was conducted following the PRISMA guidelines. The formulation of questions using the PICO strategy is population (edible insect, Orthoptera), intervention (edible insect protein extraction method), comparison (extraction pH, extraction time, extraction temperature, precipitation pH), and outcome (protein content and yield). A total of 721 articles were gathered from various reputable sources, and 31 articles that met the study criteria were selected. Based on this literature review, it was determined that the most efficient condition for protein isolation and extraction involved processing at pH 10.5 and 40°C for 60 minutes, followed by precipitation at pH 4–4.5, yielding the highest protein content and recovery. Additionally, modifications such as salting-in to enhance protein solubility, salting-out to increase protein concentration, ultrafiltration for protein purification, and ultrasound treatment to improve protein yield could be applied when necessary.
Keyword: concentrate, edible insect, extraction protein, isolate, isoelectric pH.

## 1 Introduction

Edible insects have emerged as a promising alternative food source for the future and have been increasingly developed as a protein source in recent years. Insects such as *H. illucens*, *B. mori*, *T. molitor*, *Z. morio*, and *A. domesticus*, have protein content equivalent to conventional protein sources, such as beef, chicken, and fish, which is about 19-22% of their wet weight. These insect proteins also contain all the essential amino acids required by humans, with a profile similar to soy protein and slightly below casein [1]. Some communities in the world have even utilized insects as food that is consumed traditionally, such as fried, grilled, or steamed [2], but some people cannot accept all types of insects as food because they are not commonly consumed [3]. Therefore, innovations in processing are necessary, including the development of intermediate food products, which are expected to be adaptable to various food systems.

Intermediate products are processed foods that still require further processing with the addition of other food ingredients, Examples include protein concentrates and isolates. Protein concentrates has a protein content requirement of 65-90%, while protein isolate must have a protein content of at least 90%. Many publications that describe the techniques and methods of making insect protein concentrates and isolates. Generally, the production of protein concentrates and isolates involves extraction under alkaline conditions, followed by precipitation under acidic conditions at a specific pH, known as the isoelectric point, to achieve maximum protein content [4]. In locust species, the isoelectric pH deposition process is carried out at pH 4 [5], while cricket species are carried out at pH 4.5 [6]. Another example is in the yellow mealworm species, the isoelectric point varies from pH 4 [7], pH 4.3 [8], and pH 4.5 [9]. Therefore, it is interesting to study the effect of isoelectric pH conditions on the yield and protein content of these edible insects.

Many modifications can be made to maximize the yield and protein content of edible insects by ultrafiltration, ultrasound, and salt extraction. The protein isolation process of black soldier fly larvae using alkaline extraction followed by ultrafiltration can increase the protein content by 20% compared to the acid-base isolation method [10]. Meanwhile, modification using ultrasound can increase the protein yield of mealworm [8]. Furthermore, insect protein isolation can also be performed with modifications using NaCl and $(NH_4)_2SO_4$ salts [11], [12]. Thus, it is interesting to learn about the advantages, disadvantages, and effect of various types of edible insect protein isolation process methods. Based on the above

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description, this paper aims to examine various edible insect protein extraction techniques through a systematic literature review (SLR) and analyze the characteristics of protein content and yield. In addition, this study will mostly discuss insect species from the Orthoptera order such as crickets and grasshoppers/ locust because these two species are more commonly consumed and widely developed in the Southeast Asian region as food.

## 2 Methods

The SLR stages follow the steps taken by Khan *et al*. (2003), which include the formulation of research questions, collection of study source, quality assessment and selection of study sources, extraction of data findings, synthesis of data findings, and interpretation of data findings [13]. The formulation of questions uses the PICO strategy, namely population, intervention, comparison, and outcome (Table 1). The formulation of questions in this review focused on the study of the effects of various protein extraction processes on protein content and yield of edible insect protein isolates and concentrates.

**Table 1.** Formulation of questions with PICO

| Criteria | Description |
|---|---|
| Population | Edible insect, Orthoptera order |
| Intervention | Edible insect extraction methods |
| Comparison | Extraction time, extraction temperature, extraction pH, precipitation pH (isoelectric pH) |
| Outcome | Protein content and yield of insect protein concentrates/isolates |

The process of searching for study sources was carried out across various scientific journals indexed in reputable electronic databases, including Science Direct, Scopus, and PubMed. There were twelve keywords entered into each electronic database in this literature search process (Table 2). The process of quality assessment and selection of study sources guided by the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA). The software www.covidence.org was used to facilitate the collection, screening and extraction of study sources. In addition, other software tools, such as Publish or Perish and Mendeley, were also utilized to confirm and validate the data, ensuring that the number and identity of articles retrieved were consistent across all platforms. The title and abstract of each article were reviewed, and relevant articles were selected. Each chosen article was then examined and evaluated based on the inclusion and exclusion criteria, as presented in Table 3.

The data were organized in a tabular format with the following columns: number, first author's name, title, journal name, year of publication, indexing database, insect sample name, insect order, country of origin of the insect, protein extraction methods, modification methods, defatting methods, sample weight, sample-to-solvent ratio, extraction time or duration, extraction temperature, extraction pH, settlement pH (isoelectric pH), centrifugation conditions, drying methods, protein content, and yield. Microsoft Excel was used to compile and organize the extracted data. The next step was to interpret these data.

**Table 2.** Keywords used in literature searches

| No. | Keywords |
|---|---|
| 1. | protein extraction AND edible insects |
| 2. | protein extraction AND (Orthoptera OR crickets OR grasshoppers OR locust) |
| 3. | protein extraction AND edible insects AND (precipitation pH OR isoelectric pH) |
| 4. | protein extraction (Orthoptera OR crickets OR grasshoppers OR locust) AND (precipitation pH OR isoelectric pH) |
| 5. | protein extraction AND edible insects AND (protein concentrate OR protein isolate) |
| 6. | protein extraction AND (Orthoptera OR crickets OR grasshoppers OR locust) AND (protein concentrate OR protein isolate) |
| 7. | protein extraction AND edible insects AND (protein concentrate OR protein isolate) AND protein content |
| 8. | protein extraction AND (Orthoptera OR crickets OR grasshoppers OR locust) AND (protein concentrate OR protein isolate) AND protein content |
| 9. | protein extraction AND edible insects AND (protein concentrate OR protein isolate) AND yield |
| 10. | protein extraction AND (Orthoptera OR crickets OR grasshoppers OR locust) AND (protein concentrate OR protein) |
| 11. | protein extraction AND edible insects AND (protein concentrate OR protein isolate) AND techno-functional properties of proteins |
| 12. | protein extraction AND (Orthoptera OR crickets OR grasshoppers OR locust) AND (protein concentrate OR protein isolate) AND techno-functional properties of proteins |

**Table 3.** Inclusion and exclusion criteria

| Inclusion criteria | Exclusion citeria |
|---|---|
| 1. Reputable international journal<br>2. Has full text form<br>3. Original article or research article<br>4. Article published through peer-reviewed stage<br>5. Samples are whole insects or powder<br>6. There is a description of the protein isolation/ extraction method<br>7. There is a description of the extraction pH, temperature and/or time<br>8. There are parameter of protein content and/or yield | 1. Duplication articles<br>2. Book chapters<br>3. Book review<br>4. Review article<br>5. Encyclopedia<br>6. Incomplete data and text<br>7. Title and abstract are not relevant with the topic<br>8. Misaligned study outcomes with the review focus<br>9. Irrelevant intervention methods<br>10. Impropriate research design |

## 3 Result and discussion

Figure 1 shows the result of the SLR using PRISMA. The initial number of articles was 721. The first step was to identify original articles and research articles, which resulted in 352 articles being selected. The next step involved eliminating duplicate articles, resulting in a total of 142 articles. Next, the process of filtering titles and abstracts was carried out using inclusion and exclusion criteria. This process resulted in 58 relevant articles and 84 irrelevant articles. We then conducted a thorough review of all relevant articles and selected 33 articles worthy of further research. The selected articles were then subjected to data extraction by collecting detailed information specifically related to edible insect protein extraction methods.

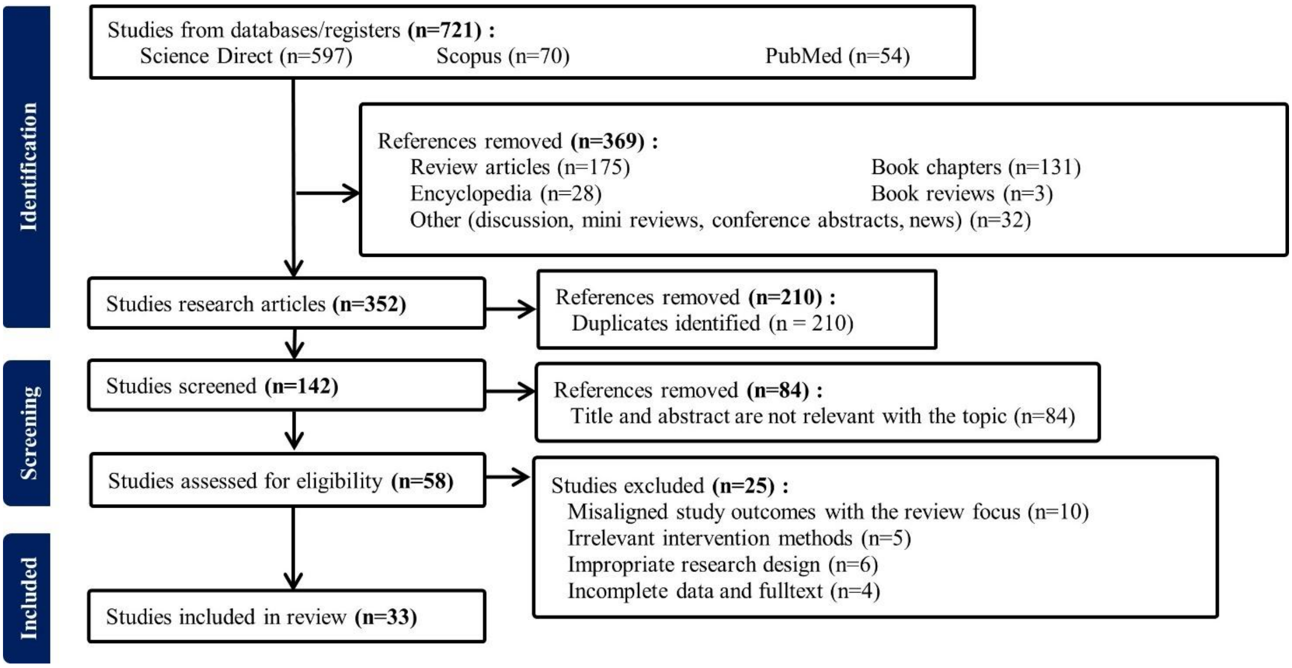


**Fig. 1.** PRISMA results

**Table 4.** Various species of edible insects

| Insect name | Insect country of origin | Protein content of raw materials (%) db | Number of article |
|---|---|---|---|
| Order: Ortoptera | | | |
| Grasshoppers (*P. beltrani*) | Mexico | not available | 1 |
| Grasshoppers (*S. gregaria*) | Israel | 38 | 1 |
| Cricket (*A. domesticus*) | Netherlands; Canada; Poland; Kenya | 53-64 | 4 |
| Cricket (*G. assimilis*) | Poland; Brazil | 59 | 2 |
| Cricket (*G. bimaculatus*) | Korea | not available | 1 |
| Cricket (Gryllidae) | USA | 63.43 | 1 |
| Order: Coleoptera | | | |
| Beetle adults (*A. diaperinus* L.) | Netherlands | not available | 1 |
| Beetle larvae (*P. brevitarsis*) | Korea | 47-61 | 5 |
| Beetle larvae (*A. dichotoma*) | Korea | 49 | 3 |
| Beetle larvae (*R. phoenicis*) | Cameroon | not available | 1 |
| Mealworm (*T. molitor*) | Netherlands; Canada; Korea; France; India; China; Germany; Grecee | 41-71 | 14 |
| Mealworm (*A. diaperinus* L.) | Germany; United Kingdom | 59 | 3 |
| Superworm (*Z. morio*) | Netherlands; Poland | 49 | 2 |
| Order: Diptera | | | |
| Black soldier fly larvae (*H. illucens*) | Italy; Netherlands; S. Africa; Korea; Tunisia | 37-44 | 5 |
| Order: Lepidoptera | | | |
| Silkworm (*B. mori*) | Thailand; Korea | not available | 2 |
| Waxworm (*G. mellonella*) | United Kingdom | 32 | 1 |
| Order: Blattodea | | | |
| Cockroach (*B. dubia*) | Netherlands | not available | 1 |
| Order: Hymenoptera | | | |
| Honey bee (*A. mellifera*) | Israel | 22 | 1 |

### 3.1 Profile of edible insect species

Research on insects as an alternative protein source has highlighted their significant potential to meet future human protein needs. Each insect species varies in protein content, depending on factors such as species, geographical conditions, and processing methods. Table 4 presents a summary of the protein content of various edible insect species, along with the number of studies that have conducted protein isolation or extraction processes for each species.

Overall, mealworms *(T. molitor*) were the most researched insect with 14 studies spread across different countries, including the Netherlands, India, Canada, Korea, France, China, Germany and Greece. This reflects the high interest in the potential of mealworms as an alternative protein source. This is because mealworms have a fairly high protein content of 41-71% (db), so the chances of getting isolates are greater. In the Coleoptera order, apart from mealworms, beetle larvae such as *P. brevitarsis* from Korea also attracted attention with five studies that varied in protein content between 47-61% (db).

The other hand, the Orthoptera order, which includes grasshoppers and crickets, has been the focus of nine studies conducted across various countries, including the Netherlands, Brazil, Canada, Kenya, Korea, Poland, Mexico, and Israel. Crickets have a protein content of 53-64% (db), while grasshoppers have a protein content of 38% (db). Locusts are a type of insect that is commonly consumed with various processing such as fried or grilled. The utilisation of locusts as an alternative source of protein is particulary interesting to be explored because such species of locusts are pests of rice plants so their presence is often eliminated by farmers in Indonesia.

Other insects such as Black Soldier Fly larvae (*H. illucens*) from the order Diptera have five studies. This insect has a protein content of 37-44% (db). The order Lepidoptera represented by silkworm and waxworm had a more limited number of studies, namely two studies on silkworm (*B. mori*) and one study on waxworm (*G. mellonella*). The order Blattodea was only represented by cockroaches (*B. dubia*) with one study, while the order Hymenoptera recorded one study on honeybees (*A. mellifera*). Overall, these studies reflect a global trend that focuses more on processing insects as food intermediates in the form of protein isolates and concentrates.

### 3.2 Overview of Edible Insect Protein Isolation Process

Insect protein isolation is generally carried out using alkaline extraction followed by acidic precipitation (AEAP), but some studies have also made various modifications such as salting-in, salting-out, ultrasound and ultrafiltration (Figure 2). The process of insect protein isolation and extraction generally consists of three stages: raw material preparation, deffating and extraction. The raw material preparation stage begins with killing the insects by freezing them. Most studies do not explain how to kill the insects and some choose to directly use commercial insect meal. The next process is washing and cleaning the insect meat from dirt or contaminants using running water. The clean insects are then freeze dried. This drying method was chosen because it does not damage the protein. The dried insects are then ground and sieved. The result of this first stage process is insect flour.

The insect meal will then go through a defatting process with the addition of a solvent. The majority of studies use hexane as the solvent in the deffating process because it is considered more effective. Besides hexane, methanol, ethanol, petroleum ether, and ethyl acetate can also be used. The ratio of insect meal: solvent is generally 1:5 b/v. According to a report by Kim *et al*. (2021), the protein content of insect flour deffated with hexane is higher than that of ethanol and methanol solvents [14]. Insect flour that has been given a solvent, then stirred for 60 minutes at room temperature. After that, a centrifugation process was carried out to separate the precipitate and supernatant. The resulting precipitate was then taken for deffating process again. According to Zielinska *et al.* (2022) and Anusha *et al.* (2023), this process can be repeated twice, but according to Lee *et al*. (2021) and Kim *et al*. (2020), this process can be repeated up to five times to maximise the insect fat removal process [5, 14, 15, 16].

The third stage is the protein extraction process under alkaline conditions followed by precipitation under acidic conditions. This process begins by mixing non-fat insect meal and solvents such as distilled water. Furthermore, the pH adjustment of the extraction is generally pH 10-11. Then centrifugation is carried out. The resulting supernatant is then taken to adjust the pH of precipitation under acidic conditions. Generally, it is done at pH 4.3-4.5, but there are several types of insects that are deposited at pH 4 and pH 5 (Table 5). After that, the centrifugation process is carried out again. The precipitate obtained is then taken to be dried by the freeze drying method. The precipitate that has turned into powder is called protein isolate or concentrate. If the protein content is >90%, it is a protein isolate, but if the resulting protein content is 70-90%, it is a protein concentrate.

### 3.3 Conventional Isolation Process

#### 3.3.1 Effect of Extraction and Precipitation pH on protein content and yield

The effect of pH on protein extraction results from insects is very important, because pH affects protein solubility, molecular structure, and precipitation efficiency. Alkaline extraction methods generally use a pH between 10 and 11 to dissolve proteins from insects before the acid precipitation process. At alkaline pH, proteins tend to be more soluble because the protein structure is denatured, which facilitates the release of proteins from the cellular matrix. In species such as *P. beltrani* and *S. gregaria* extracted at pH 10, the yield of extracted protein ranged from 52.9 to 73%. However, *G. assimilis* showed higher results with a protein content of 95% at pH 10.5. This suggests that increasing the pH slightly above 10 can increase the extraction efficiency, although too high a pH can lead to excessive protein denaturation and damage to the protein structure. At pH 11, some species such as *A. domesticus* and *G. assimilis* showed fairly consistent and high protein contents of around 75-76%, with yields varying from 40.32 to 51.9%. In *T. molitor*, extraction at pH 11 produced 80.08% protein with a yield of 71.40%. This indicates that a more alkaline pH (pH 11) tends to produce proteins with higher quality and better yield than lower pH.

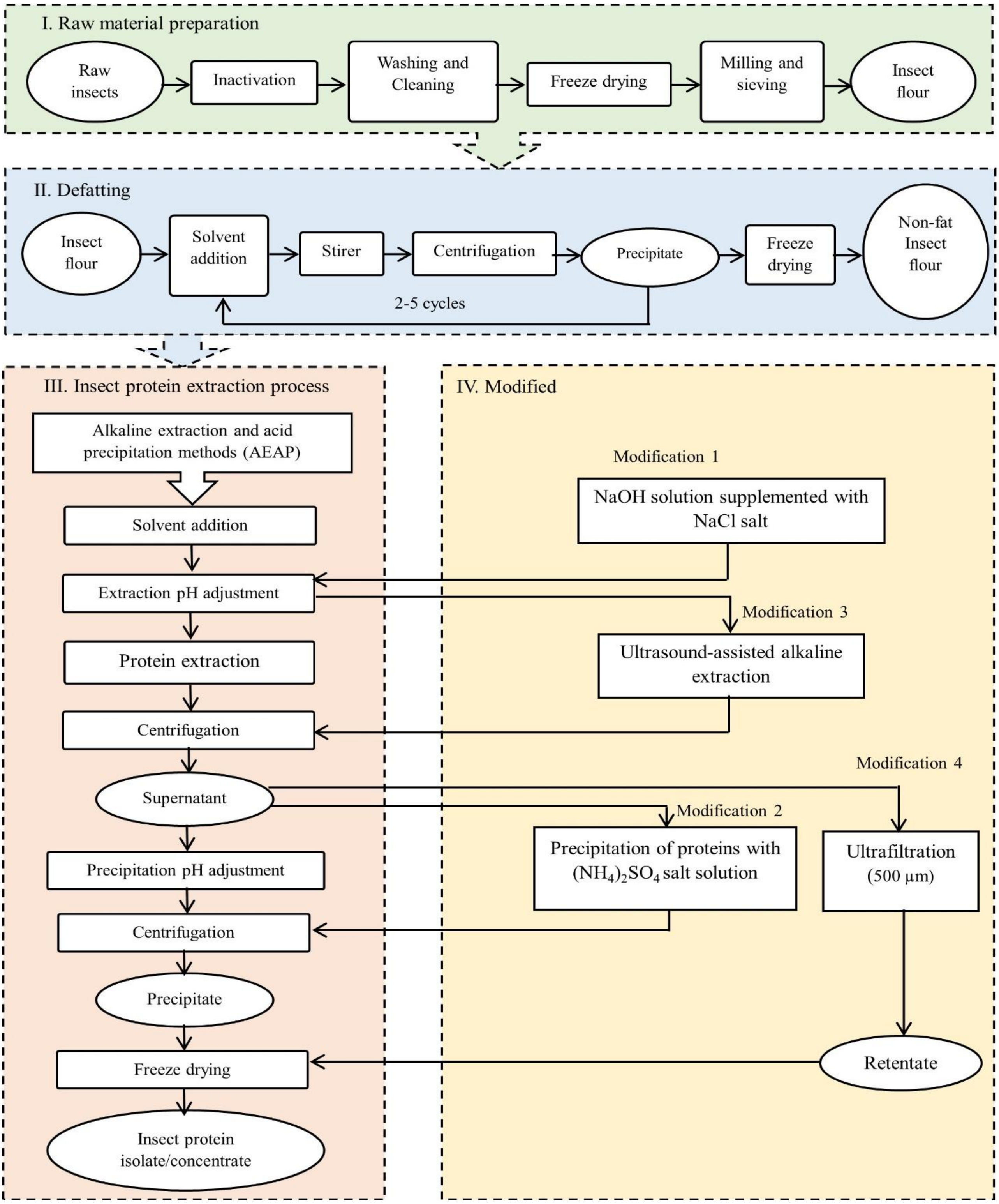


**Fig. 2.** Insect protein isolation and extraction process

After the protein is dissolved at an alkaline pH, an acid precipitation process is performed to precipitate the protein at the isoelectric point (pI) pH of the protein, usually at pH 4-4.5. At this pH, proteins have the lowest solubility, so they precipitate and can be separated from other components such as fats and carbohydrates. Every protein has an isoelectric point where its net charge becomes zero. At this pH, proteins tend to precipitate because the attraction between protein molecules increases, which reduces their solubility [4]. Therefore, precipitation is often performed at a pH close to the isoelectric point of the target protein.

Most of the studies in the table show that precipitation is carried out at a pH of around 4 to 4.5, which is the optimal pH for precipitating insect proteins. For example, in *T. molitor*, precipitation at pH 4.3-4.5 resulted in protein content of about 64-80%, depending on the temperature and extraction time. In *A. domesticus* and *T. molitor*, acid precipitation at pH 4.3-4.5 resulted in protein yields varying from 31 to 81.6%, indicating that in addition to precipitation pH, other factors such as temperature and extraction duration also affect the final yield.

**Table 5.** Various conditions for insect protein isolation by alkaline extraction and acid precipitation methods

| Insect species | Extraction conditions | | | precipita-tion pH | Protein content of precipitate (%) | Protein extraction yield (%) | References |
|---|---|---|---|---|---|---|---|
| | pH | t (min) | T (°C) | | | | |
| Grasshoppers (*P. beltrani*) | 10 | 60 | rt | 4 | 73 | - | [5] |
| Grasshoppers (*S. gregaria*) | 10 | 60 | 40 | 4 | 52.9±1.3 | 24.2±1.2 | [18] |
| Cricket (*A. domesticus*) | - | 60 | 40 | 4.3-4.5 | 78.5±2.0 | 31.0±4.0 | [19] |
| Cricket (*A. domesticus*) | 11 | 60 | rt | 4.5 | 75.35±0.53 | 40.32 | [6] |
| Cricket (*G. assimilis*) | 11 | 60 | rt | 4.5 | 76.02±0.53 | 51.9 | [6] |
| Cricket (*G. assimilis*) | 10.5 | 60 | 40 | 4.5 | 95 | - | [20] |
| Beetle larvae (*R. phoenicis*) | 10 | 60 | 4 | - | - | 53.6±5.6 | [21] |
| Mealworm (*T. molitor*) | - | 60 | 40 | 4.3-4.5 | 75.4±0.5 | 33.2±0.6 | [19] |
| Mealworm (*T. molitor*) | 10 | 60 | 60 | 4 | 68.2±0.3 | - | [7] |
| Mealworm (*T. molitor*) | - | 60 | 40 | 4.3-4.5 | 64.19±0.18 | 81.60±0.42 | [16] |
| Mealworm (*T. molitor*) | 11 | 60 | 50 | 4.5 | 80.08±2.96 | 71.40±3.26 | [9] |
| Mealworm (*T. molitor*) | 10 | 60 | 25 | 4.3-4.5 | 75.75±0.34 | 39.56±0.68 | [8] |
| Mealworm (*T. molitor*) | 10 | 60 | 45 | 4.4 | 75.1±0.16 | - | [22] |
| Mealworm (*T. molitor*) | - | 60 | 80 | 4.5 | - | 22.26±0.46 | [11] |
| Mealworm (*T. molitor*) | 10 | 60 | 45 | - | 80.8±2.2 | - | [23] |
| Mealworm (*A. diaperinus* L.) | - | 60 | 40 | 4.3-4.5 | 46±3.00 | - | [24] |
| Superworm (*Z. morio*) | 11 | 60 | rt | 4.5 | 70.51±0.49 | 32.14 | [6] |
| BSF larvae (*H. illucens*) | 11 | 180 | 25 | 4 | 76.02±0.72 | - | [10] |
| BSF larvae (*H. illucens*) | 10 | 30 | 25 | 4.5 | 73.35±0.88 | - | [25] |
| BSF larvae (*H. illucens*) | 10 | 60 | 40 | 4.3-4.5 | 61.1 | - | [26] |
| BSF larvae (*H. illucens*) | 8 | 2880 | 30 | - | 46.4±5.0 | - | [27] |
| Silkworm (*B. mori*) | 8 | 2880 | 30 | - | 61.7±4.0 | - | [27] |
| Waxworm (*G. mellonella*) | 10 | 60 | rt | 4.5 | 87.61 | 28.07 | [28] |
| Honey bee (*A. mellifera*) | 10 | 60 | 40 | 5 | 39.6±2.1 | 27.5±1.1 | [18] |

Description: t = time duration; T = temperature; rt = room temperature

### 3.3.2 Effect of extraction temperature on protein content and yield

Based on Table 5, the extraction temperature was carried out at various conditions, namely room temperature (rt), 40, 45, 50, 60, and 80°C. Insect species such as *A. domesticus* and *G. assimilis* are extracted at room temperature. Although processing at room temperature tends to be more energy-efficient and practical, extraction yield and protein content are not always maximised. For example, *A. domesticus* extracted at pH 11 at room temperature yielded a protein content of 75.35 and a yield of 40.32%, while *G. assimilis* showed a protein content of 76.02% with a yield of 51.9% [6]. This shows that room temperature can produce good protein content, but the yield is relatively lower compared to processes at higher temperatures.

Most species, including *S. gregaria* and *T. molitor*, are extracted at temperatures around 25 to 40°C. At this temperature, there is a balance between relatively high protein content and better yield compared to room temperature. For example, *T. molitor* extracted at 40°C with a precipitation pH of 4.3-4.5 gave a yield of 81.60% [16], which is one of the highest yields in the table. This shows that extraction at moderate temperatures can produce proteins with good quality and high yields.

Extraction at high temperatures, such as that performed on *T. molitor* (50°C) and other *T. molitor* at 60°C, results in high protein content, e.g. 80.08% at 50°C. However, an increase in temperature does not always mean higher yields. For example, *T. molitor* extracted at 60°C yielded a protein content of 68.2% with no yield information, while another *T. molitor* extracted at 25°C and precipitation pH of 4.3-4.5 gave a higher yield of 39.56%. This suggests that very high temperatures may not always be optimal in producing high-quality protein, but can be effective for certain species or under certain conditions.

### 3.3.3 Effect of extraction time on protein content and yield

Extraction time is one of the important factors as it affects the amount of protein that can be extracted and also its quality. Most studies use an extraction time of about 60 minutes, which seems to be a common standard in insect protein extraction using alkaline methods. At this duration, many insect species are capable of producing high protein content, although yields vary. *S. gregaria*, for example, was extracted for 60 minutes at 40°C and pH 10, resulting in a protein content of 52.9% and a yield of 24.2% [18]. This suggests that while 60 minutes is sufficient time to extract protein, the quality of the extracted protein (in terms of protein content) could be lower than other species, possibly due to a combination of other factors such as temperature or pH. *A. domesticus* extracted for 60 minutes at pH 11 and room temperature gave a higher protein content of 75.35%, with a yield of 40.32%. This suggests that 60 minutes can provide optimal results if supported by other extraction conditions, such as a higher alkaline pH. In general, an extraction duration of 60 minutes seems to be efficient enough for most species to produce adequate protein content, as seen in *T. molitor* and *G. assimilis*, which produced protein content in the range of 70-80% with the same duration.

## 3.4 Modified Isolation Process

### 3.4.1 Salt Extraction

The salting-in method uses salt at a low concentration to increase protein solubility. In the research of Jiang *et al*. (2021), salting-in using NaCl was shown to increase protein solubility by adding charged ions to the solution [12]. These ions interact with the protein surface, thereby reducing the forces between protein molecules that can cause aggregation. Thus, the protein remains dissolved in the solution and prevents it from precipitating. In the salting-in method, the protein content obtained is quite high, although not as high as in the salting-out method, due to the more soluble nature of the protein. In contrast, the salting-out method involves adding a high concentration of salt, such as ammonium sulfate, which causes the protein to precipitate. This salt increases the ionic strength of the solution and thus reduces the solubility of the protein by attracting water molecules that normally surround the protein, causing the protein to clump and precipitate [29].

Gkinali *et al*. (2021) compared several extraction methods, including salting-in with NaCl, alkaline extraction, and isoelectric precipitation. Of these three methods, alkaline extraction yielded the highest protein content, reaching 75.1%, with a yield that was also highest at over 60%. While the salting-in method with NaCl produced slightly lower but still significant protein levels, with the main advantage of high protein solubility at acidic pH and good oil binding capacity [22].

Research by Jiang *et al*. (2021) also showed significant differences in yield and protein content based on salting-in and salting-out methods. The salting-out method with ammonium sulfate produced the highest protein yield, reaching 39.54%, with higher emulsification activity and foaming capacity. Meanwhile, the salting-in method produced high protein content, but slightly lower than salting-out, but still gave good results in terms of protein solubility.

### 3.4.2 Ultrafiltration

The ultrafiltration method significantly increased the extracted protein content compared to other methods such as isoelectric precipitation. In the study of Berthelot *et al*. (2024), the use of ultrafiltration-diafiltration successfully increased the protein content from 57.29 to 72.40% [30]. This happens because ultrafiltration separates large molecules such as proteins from small molecules such as sugars, salts, and free amino acids, resulting in proteins with higher purity.

Ultrafiltration is known to be effective in purifying proteins because the process uses a membrane to filter out molecules of a certain size. In the study of Miron *et al*. (2023), ultrafiltration yielded proteins with a purity of 96.42%, much higher than the isoelectric precipitation method which only reached 76.02% [10]. This process enables a more efficient separation between proteins and contaminants or other non-protein molecules, such as fats and sugars.

Although ultrafiltration increases protein content and purity, it tends to produce lower yields compared to other methods. In the same study by Miron et al. (2023), ultrafiltration only produced a yield of 24.30%, lower than isoelectric precipitation which had a yield of 37.22%. This is because ultrafiltration focuses on purification, so some proteins may be lost during the filtration process.

### 3.4.3 Ultrasound

Based on several articles that have been analyzed, sonication has a significant influence on protein content and yield in the protein extraction process from insects. Sonication, or ultrasonically-assisted extraction, works by breaking the insect cell walls through high-frequency sound waves, which allows for a more effective protein release. In the study of Mishyna *et al*. (2018), the sonication method was shown to increase the protein content in *S. gregaria* locusts by 57.5% and in *A. mellifera* honeybees by 55.2%.

Meanwhile, in the study of Choi *et al*. (2017), *T. molitor* insects extracted protein with modified sonication for 20 minutes had a protein content of 63% [31]. Research by Zhang *et al*. (2023) also highlighted that protein extraction from *T. molitor* using the alkali-assisted ultrasonic method can increase the protein yield to 60.04% after 30 min of sonication. This study confirmed that alkali-assisted sonication increased the protein yield of *T. molitor* by 60.04%. Sonication treatment for 60 min leads to a decrease in particle size, an increase in surface hydrophobicity, and an increase in the content of free amino and sulfihydryl groups in *A. diaperinus* L. protein isolates [32]. Sonication accelerates the release of proteins from the insect cellular matrix, so that more proteins can be extracted in a shorter time. However, prolonged sonication can lead to decreased yields, as excessive ultrasonic energy can damage the protein structure and reduce the extraction effectiveness.

**Table 6.** The advantages, disadvantages, and effect of various edible insect protein extraction methods

| Extraction methods | Advantages | Disadvantages | Effect (in Order Orthoptera) | Ref |
|---|---|---|---|---|
| Alkaline extraction-isoelectric precipitation | Effective in extracting high-purity protein | Use of chemicals potentially harmful to the environment | Affects protein structure and solubility, which can impact texture and food applications | [5] |
| | Allows separation of protein based on its isoelectric point | Requires identification of isoelectric point | Increases protein purity up to 78.5% in crickets | [19] |
| | Produces highly soluble protein with good functional properties | May cause protein denaturation, reducing its fuctionality | In cricket protein, it shows increased solubility and better emulsifying properties compared to grasshoppers | [6] |
| | Simple and relatively inexpensive method | Extreme pH processing may damage some nutrients | Enhances foaming capacity and emulsion stability but may cause darker color due to protein-phenolic interactions | [25] |
| Salt Extraction | More environmentally friendly extraction method | Requires optimization of salt concentration for maximum efficiency | Produces protein with higher stability but lower purity compared to acid-base methods | [19] |
| | Improves foam and emulsion stability in cricket protein | Lower extraction efficiency compared to alkaline methods | Using 0.3–0.5 M NaCl in cricket protein extraction shows better foam stability than without salt | [20] |
| Ultrafiltration | Does not require hazardous chemicals | Requires specialized and expensive equipment | Produces high-purity insect protein suitable for functional food applications | [19] |
| | Separates protein based on molecular size, resulting in high purity | Potential loss of small protein fractions | Reduces non-protein compounds such as chitin | [1] |
| | Produces high-purity protein | Slower process compared to other methods | Effectively separates water-soluble protein from crickets and grasshoppers while retaining better nutrition | [33] |
| Ultrasonication | Can improve extraction efficiency by disrupting cellular structure | May cause protein structure changes due to cavitation | Increases extraction yield up to 19.4% for grasshoppers, enhancing protein solubility | [25] |
| | Enhances protein solubility and emulsification | May cause excessive protein denaturation if duration or intensity is too high | Improves foaming and emulsifying capacity of cricket protein | [33] |
| | Relatively fast process and reduces chemical usage | Risk of protein damage due to high heat or pressure | Increases emulsion stability up to 85.5%, enhances foaming capacity, and protein coagulation | [31] |

According to Table 6, alkali extraction followed by isoelectric precipitation is widely used in the isolation process of Orthoptera insect proteins because it can produce high-purity protein [5]. Another method, salt extraction, is considered a more environmentally friendly alternative as it does not involve hazardous chemicals [19]. This method can enhance foam and emulsion stability, particularly in cricket proteins, although its purity is lower compared to the acid-base method [20]. Ultrafiltration is another commonly used technique, as it does not require chemicals and can effectively isolate high-purity protein fractions [1]. However, despite its effectiveness, this method requires specialized and expensive equipment. Ultrasonication has also been explored as a fast extraction technique and reduces the use of chemicals. This method works by disrupting cellular structures, thereby improving extraction yield and protein solubility [25]. Further research on combining these four extraction methods is of interest, with the expectation of enhancing protein yield and quality in insect-based proteins.

## 4 Conclusion

Based on the results of this review, pH 10-11 for alkaline extraction gave good protein extraction yield, with the highest protein content reaching 95% at pH 10.5 (*G. assimilis*) and the highest yield reaching 81.6% at pH 10 (*T. molitor*). Acidic precipitation conditions around pH 4-4.5 also showed consistent effectiveness to precipitate protein after alkaline extraction process. Higher extraction temperatures generally accelerate the extraction process but can cause protein denaturation, while low temperatures tend to protect the protein structure but result in lower yields. Salting-in modification helps to keep the proteins dissolved, while salting-out is very effective in increasing the protein content as the precipitated proteins can be efficiently separated from the solution. Ultrafiltration has a positive effect on increasing protein content and purity, and preserving protein structure. However, it tends to produce lower yields than isoelectric precipitation methods. The choice of ultrafiltration is suitable if the main focus is to obtain proteins with high quality and good purity. Modification with ultrasound/sonication can be done to improve the protein yield.

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