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Rachelle El Hajj, Houcine Mhemdi, Colette Besombes, Karim Allaf, Victor Lefrançois, et al.. Edible Insects' Transformation for Feed and Food Uses: An Overview of Current Insights and Future Developments in the Field. *Processes*, 2022, 10 (5), pp.970. 10.3390/pr10050970 . hal-03828642

HAL Id: hal-03828642

<https://univ-rochelle.hal.science/hal-03828642>

Submitted on 12 Dec 2023

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Review

Edible Insects' Transformation for Feed and Food Uses: An Overview of Current Insights and Future Developments in the Field

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Abstract: The integration of insect-derived extracts in feed and food products has become a field of growing interest in recent years. In this review, we collect different studies carried out on edible insects' transformation processes and focus on the various treatment operations, extraction technologies, and solvents used in different processing steps. We include an overview of current insights into the different steps of the transformation process: insect reception, killing methods, pretreatments, storage, delipidation, protein extraction, as well as chitin and chitosan extraction. Finally, we reflect on the most important future challenges of this sector.

Keywords: edible insects; lipids; chitin; chitosan; proteins; extraction; biomolecules



Citation: El Hajj, R.; Mhemdi, H.; Besombes, C.; Allaf, K.; Lefrançois, V.; Vorobiev, E. Edible Insects' Transformation for Feed and Food Uses: An Overview of Current Insights and Future Developments in the Field. *Processes* **2022**, *10*, 970. <https://doi.org/10.3390/pr10050970>

Academic Editor: Pasquale Crupi

Received: 4 April 2022

Accepted: 8 May 2022

Published: 12 May 2022

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1. Introduction

Nowadays, generating sufficient food for the world's population, which is expected to reach 9 billion by 2050, is one of the greatest challenges facing the food industry [1]. To meet this need, new sustainable agricultural techniques for food production [2] as well as alternative sustainable protein sources for the food and feed industries must be investigated [3]. In other words, there is a massive need to change traditional farming practices and to find new protein sources that have lower environmental impacts (e.g., water consumption, greenhouse gas emissions, etc.) [4] than conventional sources (e.g., cattle, sheep, pig, and poultry livestock). In this regard, plant-based meat alternatives, single-cell proteins (i.e., mycoprotein, yeasts, fungi, bacteria, photosynthetic microalgae, and cyanobacteria), macroalgae (i.e., red seaweed), insects, and cultured meat are all being explored as alternative proteins and future foods [5–7]. Among these alternative protein sources, cultured meat has the highest environmental impact and cost due to high-energy requirements for the medium and meat-growing processes, followed by mycoprotein-based food, which has high-energy needs and agricultural feed-growing activities. Insect-based and soy meal-based alternatives have the lowest impact, because of the use of efficient processing and growing methods, as well as the usage of by-products and side streams [8].

The number of edible insect species consumed worldwide in 2017, at their different life stages, is estimated to be around 2000, spread across Asia, Australia, Central, and South America, and narrowly across Europe and North America [9]. However, insects are currently gaining attention in Europe as a promising alternative protein source that can answer the growing demand for animal protein products while being low cost, nutritious, safe, and environmentally friendly [10,11].

The most consumed insect species in the world, in decreasing order, are beetles (*Coleoptera*, 31%); caterpillars (*Lepidoptera*, 17%); ants, bees, and wasps (*Hymenoptera*, 15%); grasshoppers and locusts (*Orthoptera*, 13%); true bugs (*Hemiptera*, 11%); dragonflies (*Odonata*, 3%); termites (*Isoptera*, 3%); flies (*Diptera*, 2%); cockroaches (*Blattodea*, 2%); others 3% [9]. *Coleoptera*, *Lepidoptera*, and *Diptera* are mainly consumed in the larval stage, while *Orthoptera*, *Blattodea*, *Hymenoptera*, *Hemiptera*, and *Isoptera* are consumed in the adult stage [2].

Entomophagy, i.e., the consumption of insects is frowned upon in most Western countries. It is worthy to mention that most people are averse to the idea of eating insects and they link the practice with primitive behavior. In addition to basic human perceptions, the origins of disgust may be traced back to culture (i.e., “taste is culture”), which has a significant impact on eating habits. The laws that define what is edible are impacted by culture, which is influenced by the environment, history, community organization, human endeavor, mobility, and economical status [12]. On the one hand, low levels of readiness and intention to try insects have been linked to the fear of trying new foodstuffs (food neophobia) [13]. Curiosity, on the other hand, appears to be one of the most powerful motivators for individuals to try insects, according to a recent study [14]. Hence, despite being very nourishing, as well as rich in proteins, minerals, lipids, and vitamins, insects have been found to be disliked, and commonly, not accepted as a human food source in most developed countries [15]; this psychological barrier to entomophagy acceptance is highly influenced by metamorphological life-stage, type of gastronomic preparation, and level of insect visibility [16].

Therefore, to increase consumer acceptance, insect proteins can be extracted in the form of protein flour, and “hidden” in familiar processed foods (such as bread, meat, pasta, and pizza) [17] or used as a feed source for animals such as poultry, fish, and pigs (indirect entomophagy) [15].

Insect usage as an alternative food source is not only nutritious but also has a positive impact on the environment and the economy [2]. Insect larvae can be raised by consuming agricultural bio-waste and food waste, and converting them into protein and fat; this is the “circular economy” concept [18]. The circular economy has become one of the most central concerns in the last decade, emphasizing the development of sustainable and resource-efficient policies for long-term socioeconomic and environmental advantages. The circular economy approach seeks to break the linear pattern of production and consumption in industrial production systems by implementing circular or “closing the loop” processes [19]. Thus, due to their ability to transform organic matter into protein, insects can help to solve the problem of food waste [20]. Commercial insect rearing is a viable method for reintroducing nutrients into the food chain in the form of protein-rich animal feed, human food, and fertilizer [19]. However, insects can only be fed with substrates that are eligible as feed materials for farmed animals, according to the animal by-products EU regulation (1069/2009) [21].

Insect farming presents many other advantages over conventional livestock farming including better feed-conversion efficiency, lower water pollution, higher reproduction rates, lower greenhouse gas emissions, and minimal land area use. To produce the same protein quantity obtained from 1 ha of mealworm, it requires 2 to 3.5 ha for chickens or pigs and 10 ha for cattle [2].

Insects contain around 35–61% (DM) of protein and 15–40% of fats. They also contain fiber, non-protein nitrogen, and ash, in no specific order, depending on the insect species (Figure 1) and developmental stages [22]. Insect composition may vary within the same species, and these variations can originate from many factors such as origin, processing treatments, measuring methods, as well as some extrinsic factors such as feeding and rearing conditions [22,23].

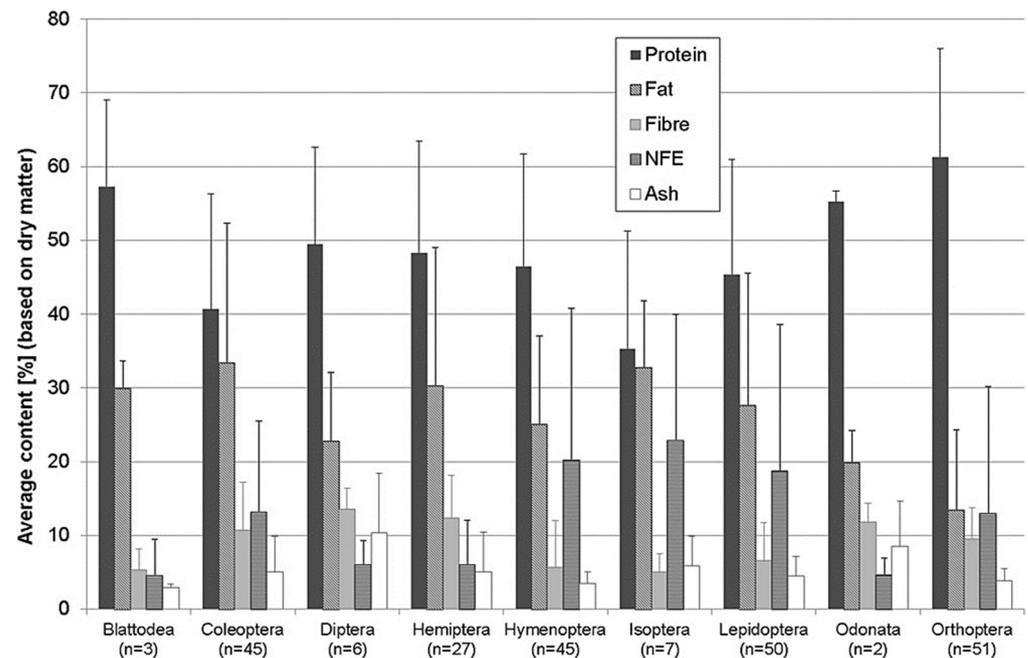


Figure 1. Average nutrient contents (%) (based on the dry matter) of edible insects belonging to the same order. n, number of insect samples obtained from literature; NFE, nitrogen-free extract [22].

The literature analysis shows a lack of reviews dealing with the transformation processes of edible insects. In the current context marked by the growth of the insect transformation field, there is a need for a review that brings together all of the published works. Moreover, it is essential to provide to readers a document that covers all aspects of these works, as well as a critical analysis, to open the door to future developmental opportunities based on the field's challenges and areas that require improvement. This review sheds light on the use of innovative and emerging technologies in receiving, killing, pretreating, and processing edible insects to obtain extracts that can be used in feed and food. Particularly, it focuses on comparing lipid, protein, and chitin extraction, as well as transformation process methods from insects using different conventional and emerging technologies.

2. Transformation Processes for Edible Insects

2.1. Raw Materials and Sample Preparation

The most commonly used edible insect species in protein extraction processes are presented in Table 1.

Table 1. The most commonly used edible insect species in protein extraction processes.

Order	Family	Genus	Species	Common Name	Stage	References
Coleoptera	Tenebrionidae	<i>Tenebrio</i>	<i>molitor</i>	Yellow mealworm	Larvae	[1,10,24–27]
	Tenebrionidae	<i>Alphitobius</i>	<i>diaperinus</i>	Lesser mealworm	Larvae	[15,25,28]
	Tenebrionidae	<i>Zophobas</i>	<i>morio</i>	Superworm	Larvae	[28]
Orthoptera	Gryllidae	<i>Gryllodes</i>	<i>sigillatus</i>	Tropical house cricket	Adult	[26,27]
	Acrididae	<i>Schistocerca</i>	<i>gregaria</i>	Desert locust	Adult	[27,29]
	Gryllidae	<i>Acheta</i>	<i>domesticus</i>	House cricket	Adult	[15,26,28]
	Gryllidae	<i>Gryllus</i>	<i>bimaculatus</i>	Two-spotted cricket	Adult	[24]
Blattodea	Blaberidae	<i>Blaptica</i>	<i>dubia</i>	Dubia cockroach	Adult	[15,28]

Table 1. Cont.

Order	Family	Genus	Species	Common Name	Stage	References
Lepidoptera	Sphingidae	<i>Clanis</i>	<i>bilineata</i>	Two-lined Velvet hawkmoth	Larvae	[30]
	Bombycidae	<i>Bombyx</i>	<i>mori</i>	Silkworm moth	Pupae	[24]
Diptera	Stratiomyidae	<i>Hermetia</i>	<i>illucens</i>	Black soldier fly	Larvae and prepupae	[3,10,25]
Hymenoptera	Apidae	<i>Apis</i>	<i>melifera</i>	Honey bee	Larvae and pupae	[29]

Insects can be either collected in the wild, cultivated indoors, or issued as by-products from other productions (e.g., *Bombyx mori* from silk production) [23]. In general, farmed insects are reared in specific conditions, mainly by following a specific diet, aiming at achieving the highest feed conversion ratio, reducing mortality, accelerating their development, and improving their nutritional composition. They can be fed on plant resources (e.g., wheat, wheat bran, cereal, oats, soy, rye, corn, chicken mash, and organic young wheat plants), fruits and vegetables (e.g., carrots), microbial resources (e.g., beer yeast), premix, and NaCl [1,15,27–29]. By-products and organic wastes can also be used in the diet as complements [31].

Upon collection, to clear their digestive tracts, insects should be fasted between 24 and 48 h, and then sieved to separate them from frass before processing [10,15,27–29,32], yet this step is not mandatory [33]. Afterward, many different ways of reception, storage, and pretreatments can be applied to the insects before their transformation. Generally, insects are received from the supplier alive, frozen, or dried (lyophilized, microwave dried, etc.), and then they are stored and pretreated accordingly (Figure 2).

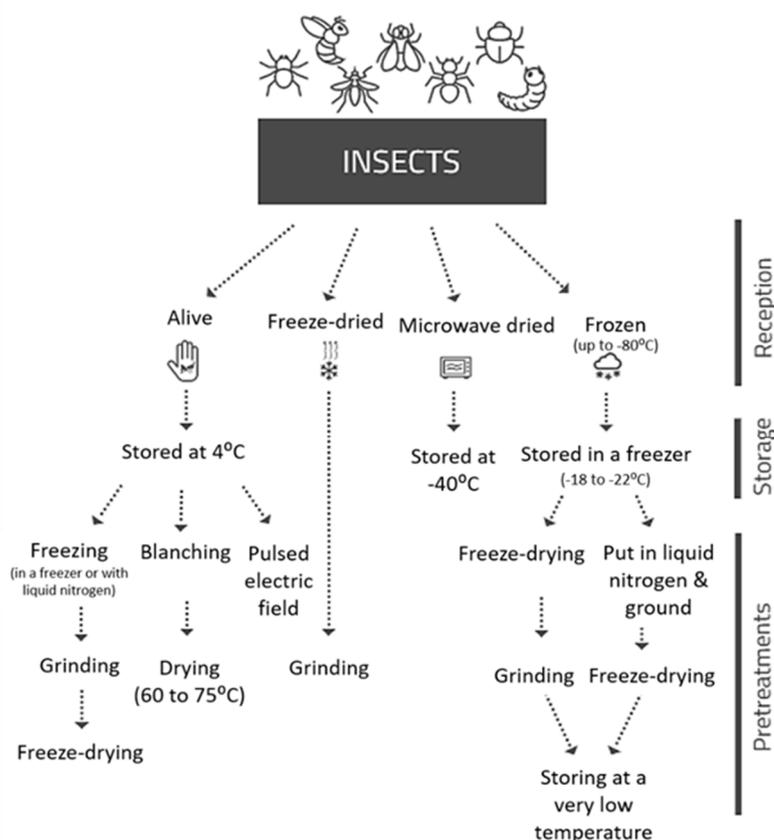


Figure 2. Different methods of reception, storage, and pretreatments of edible insects.

If they are received alive, they should be stored at 4 °C until further processing (with or without fasting). Fresh insects can be killed in various methods, including freezing with liquid nitrogen or freezing at −20 °C and then grinding and freeze-drying, blanching in boiling water [3,28,30,34], or by applying pulsed electric fields (PEF) as both a killing method and a pretreatment at once [35]. Alles et al. (2020) [32] studied the effect of PEF on insect larvae (*Hermetia illucens*) as a killing method and a pretreatment to enhance drying rate and oil extraction. This study proved that applying PEF as a pretreatment at high levels (≥ 3 kV/cm, 5 kJ/kg) induced insects' mortality and increased oil yield slightly, and that levels of 2 to 3 kV/cm and 20 kJ/kg reduced drying time by 30%. Another study by Psarianos et al. (2022) [34] found that at 4.90 kJ/kg, PEF treatment improved the functional properties of house cricket flour as well as the separation of interesting molecules. The flour's oil binding capacity, emulsifying capacity, and antioxidant activity were increased by 19.53, 22.06, and 45.79%, respectively, while the extraction yields of protein and fat were increased by 18 and 40%, respectively.

If insects are received in a lyophilized form, they should first be ground, and then transformed [26]. If they are received after microwave drying, they should be stored at −40 °C until processing [24]. When they are received frozen, either with liquid nitrogen or in a freezer, they should be stored between −18 and −22 °C, and then pretreated. The pretreatment can be freeze-drying followed by grinding or liquid nitrogen followed by grinding and freeze-drying. In all cases, the samples should be stored at a very low temperature until use [1,10,15,24,25,27,29].

Leni et al. (2019) [3] studied the effect of two killing methods on the browning and the quality of protein fraction of black soldier fly (BSF, *H. Illucens*) prepupae. By comparing the color of *H. Illucens* killed by blanching and freezing, it was confirmed, visually and with absorbance measurements, that the insects killed by freezing have a darker appearance than the blanched insects. This is explained by the fact that steam-blanching inactivates the enzymes responsible for color changing, thus, controlling the enzymatic browning. In addition, a comparison of the intensity of some metabolites of *H. Illucens* prepupae killed by freezing and blanching was done. This comparison showed that the samples killed by freezing exhibited numerous energy metabolisms, including glucose hydrolysis, lactic acid synthesis, citric acid consumption, and lipolysis. These transformations were not visible in samples killed by steam blanching because, unlike freezing, blanching is a rapid killing method that results in the thermal inactivation of enzymes involved in metabolic activities. Moreover, concerning the total amino acid profile, Leni et al. (2019) [3] proved that killing by freezing induced partial degradation of Cys and Lys, which was not seen in steam-blanched insects. Cys and Lys are essential amino acids that are associated with the insect melanization process as well as protein aggregations. Thus, proteins from blanched BSFs require less intense conditions for extraction and present a better nutritional profile than frozen BSFs. Hence, blanching is found to be a better killing method than freezing since it results in the inactivation of energy metabolisms and avoids browning.

Moreover, these facts reveal the importance of insect-killing methods in further processing steps such as lipid and protein extractions and their incorporation in food and feed products as well as the final product's nutritional profile.

In light of that, when implementing new pretreatment/killing techniques, their effect on the larval enzymatic activities and energy metabolisms must be considered.

2.2. Extraction Processes

2.2.1. Insect Delipidation

Before proceeding to protein extraction, fat removal is recommended for insects, as they generally have a high lipid content. They can be primarily pressed either in a continuous screw press or in a hydraulic discontinuous press, which removes part of their water and fat content [1,35], and then they are dried and ground for further fat removal steps [1].

Defatting may be done in many ways; the most widespread methods are solvent extraction, accelerated solvent extraction (ASE), Soxhlet extraction, Folch extraction, supercritical CO₂ extraction, and aqueous extraction.

Solvents used for lipid solvent extraction are mainly hexane (100%), ethanol (99.5%), or a mixture of hexane and isopropanol (ratio 3/2) [1,10,24,29,33,36]. This method (Figure 3) consists of dissolving insect powder in the solvent 1:5 (*w/v*), and then stirring on a magnetic stirrer for 1 h. After sedimentation, the solvent-fat mixture is decanted, and the whole process is repeated until clarity of the solvent. Afterward, residual solvent is removed by evaporation overnight [10,29]. Alternatively, after dissolving insect powder in solvent, the mixture can be vortexed/shaken for 15 min, then centrifuged, and finally, solvent removal is done using a rotary evaporator, and the remaining insoluble product is dried to a powder form by passing it through nitrogen gas. An additional step of fine grinding of the defatted insect powder can produce defatted insect flour that should then be stored at $-20\text{ }^{\circ}\text{C}$ until usage [36]. High hydrostatic pressure (HHP)-assisted fat extraction was studied by Bolat et al. (2021) [37] on mealworm and cricket powders, to improve the defatting process. The HHP study was carried out at a pressure of 500 MPa for 15 min at two different temperatures, i.e., $30\text{ }^{\circ}\text{C}$ and $40\text{ }^{\circ}\text{C}$, using the original powder/solvent ratio. According to the findings, HHP and temperature seemed to have no influence on fat extraction; however, the functional properties and antioxidant activities of the studied insects were significantly affected by both parameters.

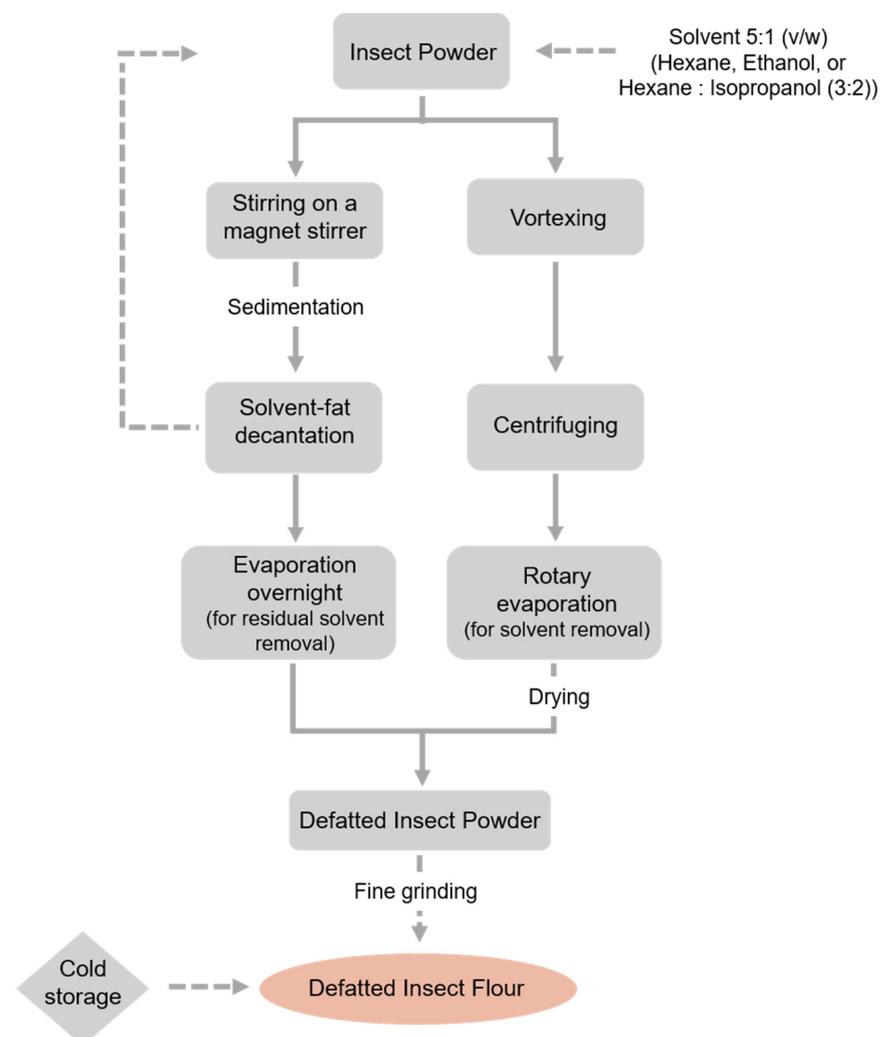


Figure 3. Flow diagram for fat solvent extraction.

Amarender et al. (2020) [36] carried out a comparison study between defatting insects by solvent extraction using hexane and ethanol. The results confirmed that ethanol, as compared with hexane, resulted in a higher lipid extraction rate. Therefore, it is a good alternative, and it is also preferred in the food industry over hexane since it is a green solvent. Alternatively, the influence of several defatting solvents (aqueous, methanol, ethanol, and n-hexane) on the characteristics and functional properties of isolated proteins from *Protaetia brevitarsis* larvae was studied by Kim et al. (2021) [38]. In terms of protein functional qualities such as foam and emulsion characteristics, as well as essential amino acid index, the findings suggested that hexane extract was the best solvent for protein processing. Whilst ethanol extract had lower protein content, quality, and stability than hexane extract, foam and emulsion capacity were comparable to hexane extract after defatting with ethanol.

An alternative intensified method of solvent extraction is accelerated solvent extraction (ASE) [1], so-called pressurized solvent extraction (Figure 4). This process uses the same solvents previously cited and it is performed under high pressure (up to 140 bars.) and at an elevated temperature (up to 200 °C). The extractor is developed in a manner that it is capable of withstanding high pressures, which allows an increase in temperature above the boiling point of the used solvent and maintains the solvent in a liquid form at a high temperature. This method has several advantages such as improved solvent diffusivity, less solvent consumption, accelerated operation time, as well as higher extraction yield and rate [39].

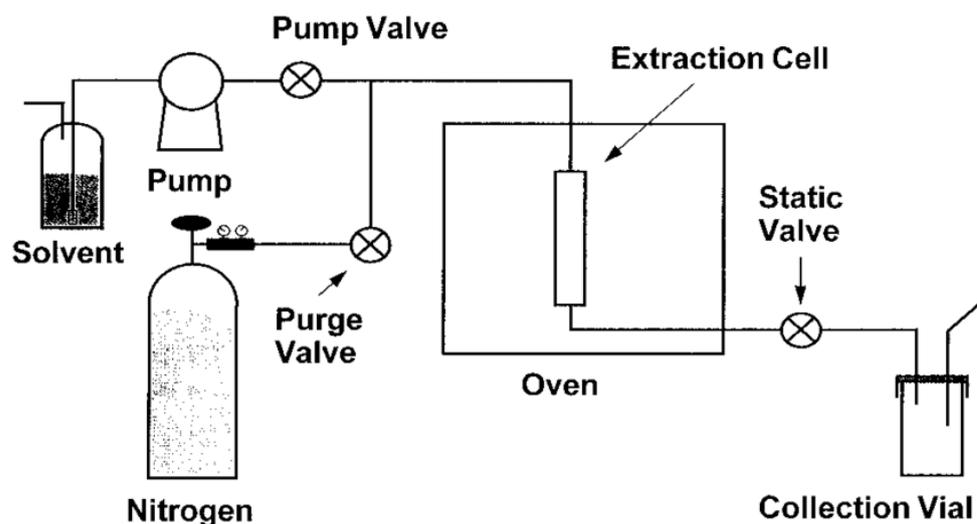


Figure 4. Schematic diagram of an accelerated solvent extraction (ASE) system [40].

Soxhlet extraction is one of the most often used methods for lipid extraction. It employs organic solvents such as hexane, diethyl ether, petroleum ether, acetone, and ethanol. This is a specific solvent extraction method usually performed in a Soxhlet apparatus for 6 h (Figure 5).

Afterward, the residual solvent is evaporated either under a nitrogen N_2 stream at a temperature between 45 and 60 °C for 1.5 to 3 h (depending on the solvent) or using a rotary evaporator [3,15,25–28].

A comparison study for the five organic solvents on ground cricket samples was carried out by Ribeiro et al. (2019) [26] to determine the specific best conditions of Soxhlet fat extraction. The quantity of extracted fat was determined gravimetrically. The results are reported in Table 2 and indicated that: ethanol had the highest crickets' fat extraction followed by acetone, with no detected differences between diethyl ether, ether petroleum, and hexane. Mainly, crickets defatted using ethanol had between 61.3% (*G. sigillatus*) and 70.1% (*A. domesticus*) less lipids than when defatted using the other solvents. These results were comparable to a study conducted by Laroche et al. (2019) [41], who concluded that

the highest fat extraction rate (22.7% *w/w*) of *A. domesticus* and *T. molitor* was produced by ethanol and that hexane was the least efficient solvent. However, they also concluded that ethanol was less effective at extracting nonpolar fatty acids, according to fatty acid profiles obtained by gas chromatography. Therefore, the defatting method should be chosen based on the fat composition profile of the edible insects.

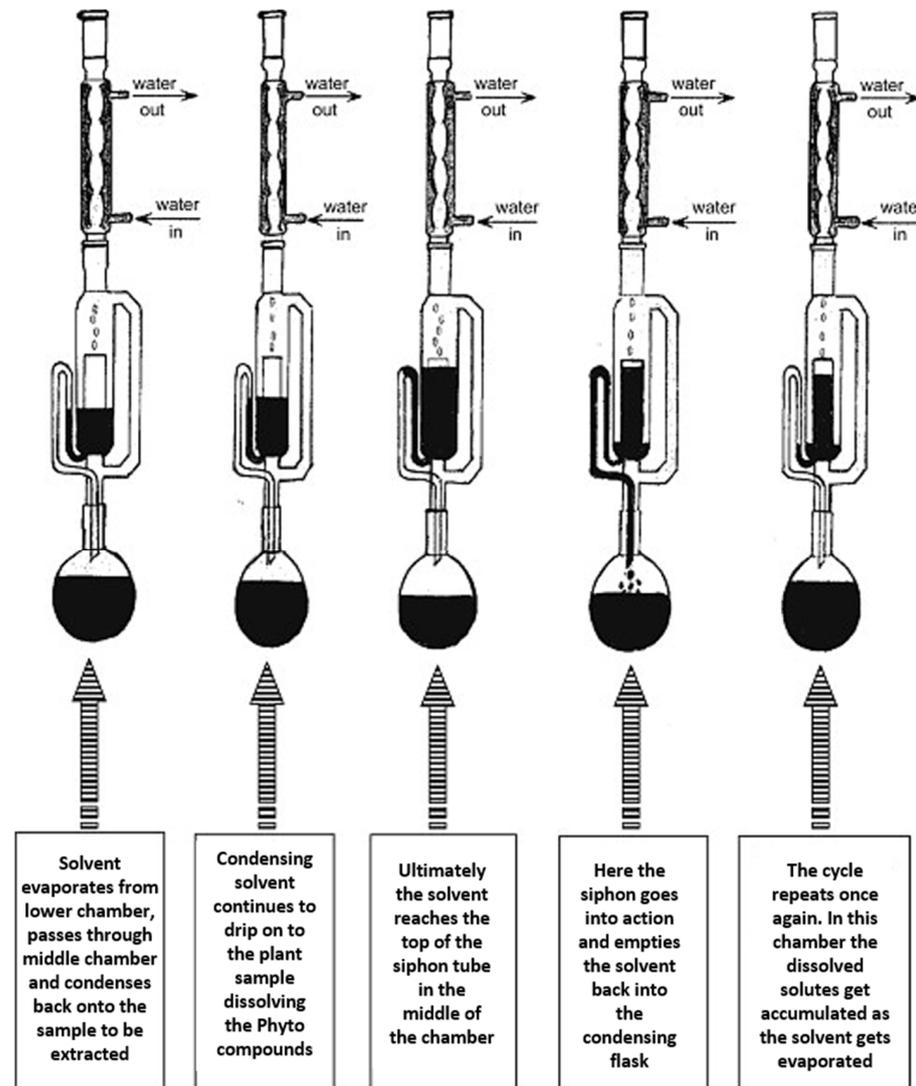


Figure 5. Schematic diagram showing the Soxhlet extraction technique [39].

Table 2. Ether extract content (g/100 g DM \pm s.d.) of whole and defatted *Acheta domesticus* and *Gryllobates sigillatus* determined with different solvents. ^{a,b,c}—Represents in each column homogeneous groups in accordance with the Turkey's test, within each cricket species ($p < 0.050$). Results are means of three (defatted) and four (whole) replications [26].

Solvent	<i>A. domesticus</i>		<i>G. sigillatus</i>	
	Whole	Defatted	Whole	Defatted
Acetone	24.6 \pm 1.02 ^b	4.7 \pm 1.99 ^{ab}	23.5 \pm 0.27 ^b	5.8 \pm 1.93 ^{ab}
Diethyl ether	20.8 \pm 1.17 ^c	4.4 \pm 0.55 ^{ab}	20.8 \pm 0.96 ^c	6.5 \pm 2.28 ^b
Ethanol	28.2 \pm 1.63 ^a	1.4 \pm 0.74 ^a	28.4 \pm 1.10 ^a	2.3 \pm 0.65 ^a
Ether petroleum	21.3 \pm 0.31 ^c	4.1 \pm 0.66 ^{ab}	20.2 \pm 0.36 ^c	6.9 \pm 0.50 ^b
Hexane	18.9 \pm 1.65 ^c	5.5 \pm 2.47 ^b	20.8 \pm 0.97 ^c	4.6 \pm 1.02 ^{ab}

Regarding the Folch extraction, it consists of diluting the insects with dichloromethane/methanol (1:1, v/v), and then shaking the mixture for 20 s. After that, it is sonicated for 10 min before being shaken on a rotary shaker for 2 h. Next, a specific quantity of demineralized water is added to induce phase separation, and the mixture is centrifuged for 20 min. At this point, an upper phase with non-lipid compounds and a lower phase containing lipids with the organic solvents are obtained (Figure 6). The upper phase is removed using a glass pipette and the lower one is filtered using a paper filter. Later, the organic solvents are evaporated in a rotary evaporator [15].

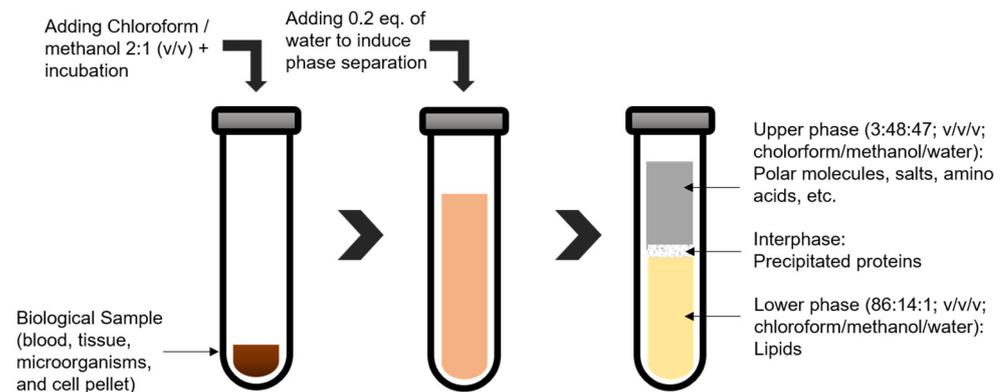
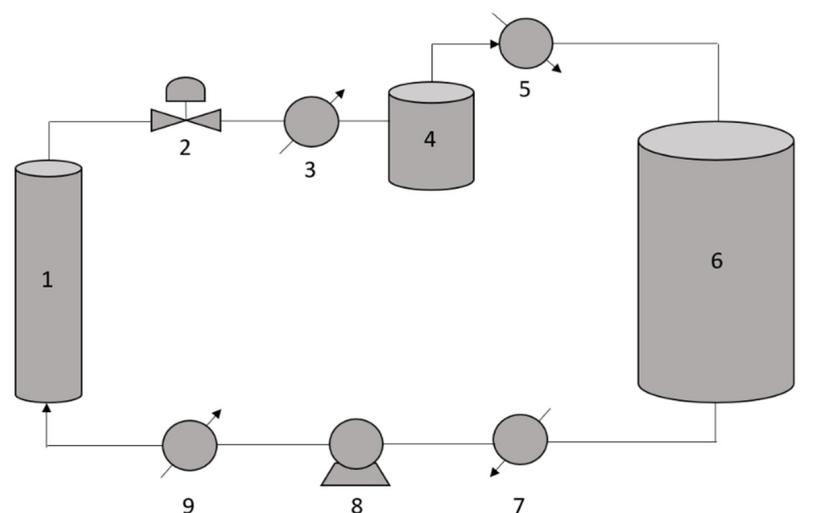


Figure 6. Workflow according to the Folch's extraction method [42].

Moreover, supercritical CO_2 (SC- CO_2) fat extraction (Figure 7) is considered to be a promising eco-friendly alternative to the conventional solvent extraction methods. This operation presents several advantages such as safety because of the use of nonorganic solvents, high product quality due to the moderated operating temperature (lower than 60°C), an extract free of residues, and selective extraction [43]. Wu (2012) studied the optimum conditions for supercritical CO_2 fat extraction from *Clanis bilineata*. He concluded that the highest extraction yield (97%) for 20 g of dried CB meat was achieved at a temperature and pressure of 35°C and 25 MPa, respectively, with a supercritical CO_2 flow rate of 20 L/min and in an extraction time of 60 min [44]. However, Laroche et al. (2019) [41] proved that the SC- CO_2 extraction method, despite being sustainable, was less efficient than Soxhlet extraction, and therefore, proposed its optimization using co-solvents.



1-Extraction vessel, 2- Pressure reduction valve, 3-Vaporizer, 4-Separator, 5-Condenser, 6-Receiver, 7-Precooler, 8-Pump, 9-Preheater

Figure 7. General flow diagram of a supercritical fluid extraction process from solid material [39].

Finally, for the aqueous extraction, 200 g of frozen insects are dissolved in 600 mL of demineralized water and mixed for 1 min. Afterward, the mixture is sonicated for 15 min, then sieved through a 350 μm stainless steel filter sieve, and centrifuged for 30 min at 4 $^{\circ}\text{C}$. By this stage, the mixture is divided into three phases: an upper lipid phase, the supernatant in the middle phase, and the pellet in the lower phase. The lipid phase undergoes a second centrifugation at 40 $^{\circ}\text{C}$ which results in another three phases: a transparent anhydrous lipid extract at the top, a thin cream layer in the middle, and finally water and water-soluble compounds in the lowest phase [15].

Tzompa-Sosa et al. (2014) [15] conducted a comparison study among Soxhlet lipid extraction (SLE), Folch lipid extraction (FLE), and aqueous lipid extraction (ALE), on four insect species. They calculated the extracted lipid content (g/100 g of fresh insects) for each method and compared the yields (%) of aqueous and Soxhlet lipid extraction relative to Folch lipid extraction using the following formulas: ALE/FLE and SLE/FLE (Table 3).

Table 3. Total lipid quantity extracted from four insect species after aqueous, Soxhlet, and Folch extraction expressed on the basis of live weight. Extraction yields of aqueous and Soxhlet extraction relative to Folch extraction (mean \pm S.D., N = 3) [15].

Insect Species	Extracted Lipid (g/100 g Fresh Insects)			Yield (%) (ALE/FLE)	Yield (%) (SLE/FLE)
	Aqueous	Soxhlet	Folch		
<i>T. molitor</i>	7.8 \pm 0.4 ^A	12.7 \pm 2.4 ^B	12.9 \pm 0.2 ^B	60.3 \pm 0.4	98.4 \pm 2.4
<i>A. diaperinus</i>	5.5 \pm 1.0 ^A	10.7 \pm 0.5 ^B	9.4 \pm 1.0 ^B	58.3 \pm 1.4	113.5 \pm 1.1
<i>A. domesticus</i>	1.6 \pm 0.1 ^A	6.0 \pm 0.3 ^B	8.0 \pm 1.1 ^C	19.2 \pm 1.1	74.8 \pm 1.1
<i>B. dubia</i>	3.1 \pm 0.3 ^A	7.6 \pm 0.2 ^B	7.5 \pm 0.3 ^B	40.9 \pm 0.4	100.5 \pm 0.4

Statistical analysis: one-way ANOVA-LSD. Figures with different letters between rows are significantly different ($p < 0.05$). ALE—aqueous lipid extracts; SLE—Soxhlet lipid extracts; FLE—Folch lipid extracts.

Their results show that lipid extraction (g/100 g in fresh insects) of all species ranged between 1.6 and 7.8% for the ALE, between 6 and 12.7% for SLE, and between 7.5 and 12.9% for FLE. Concerning the comparison to the Folch extraction method: SLE/FLE reached almost 100% for *T. molitor*, *A. diaperinus*, and *B. dubia*, and 74.8% for *A. domesticus*; regarding ALE/FLE, the values were much lower (between 19 and 60.3%). These findings indicate that Soxhlet and Folch extraction methods provide nearly the same lipid extraction efficiencies, with the highest quantities extracted, which is not the case for the aqueous extraction [15].

In short, after insect pressing, which extracts a high amount of lipids and water in a low-energy manner, solvent extraction is needed. Although ethanol has been found to be the most efficient solvent for extracting lipids from insects, it is the least effective at extracting nonpolar fatty acids. Therefore combining a nonpolar and a polar solvent, such as hexane/ethanol and hexane/propan-2-ol, might be a solution to improve the lipid extraction yield. When it comes to the SC-CO₂, coupling it with a co-solvent such as ethanol might be a solution to improve its efficiency in an environmentally friendly way.

2.2.2. Insect Protein Extraction

Insect proteins are extracted by diffusion in water either in an alkaline or an acidic environment, possibly assisted by the sonication technique.

Alkaline aqueous extraction of the soluble proteins (Figure 8) consists of adding distilled water to defatted insects and adjusting the pH of the solution to 10 using sodium hydroxide (0.1 or 1 N NaOH) with constant stirring (magnetic stirrer) and at a constant temperature (between 45 and 90 $^{\circ}\text{C}$), from 30 min to 1 h, maintaining the pH at 10. Next, the solution is centrifuged, the supernatant is collected [1] and undergoes isoelectric protein precipitation at pH 4.5 to 5, by adding 0.1 M of hydrochloric acid (HCl) [27,29,33,45]. Re-extraction at either pH 2 [10] or pH 10 [33] of the pellet followed by isoelectric precipitation might be conducted at this stage. Precipitated proteins from both extractions are collected and go through another centrifugation, to be washed with distilled water.

The obtained proteins are frozen, freeze-dried, and then ground and preserved at low temperature [1,10,27,33,45]. Sonication can be applied instead of the magnetic stirrer for a sonication-assistant extraction [29].

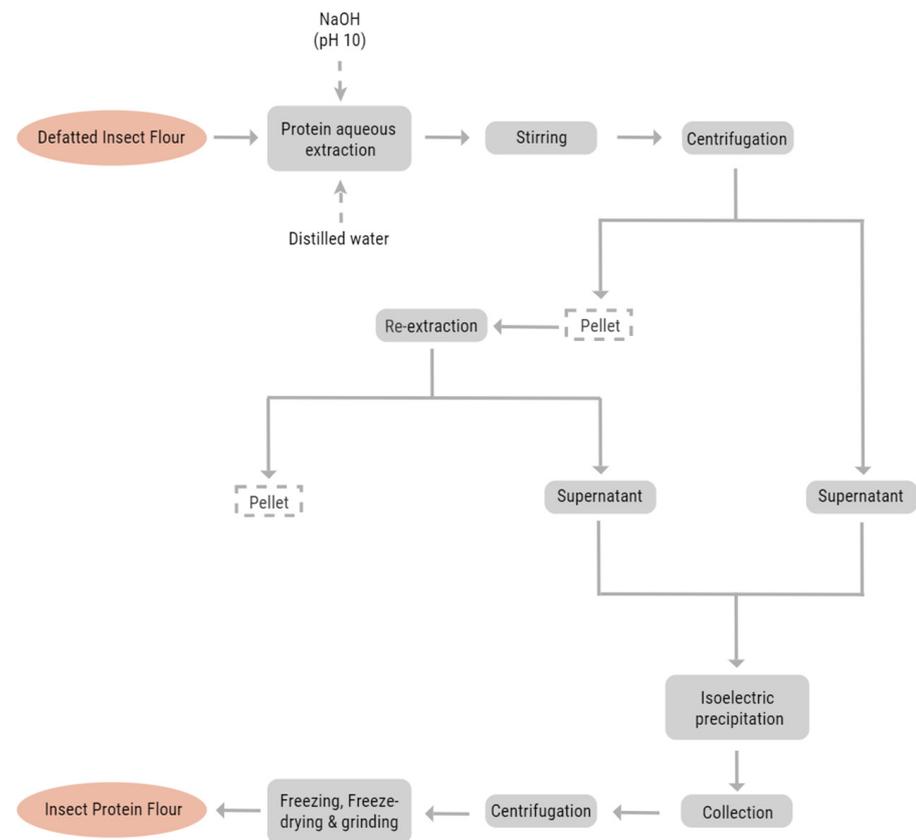


Figure 8. Schematic representation of insect protein extraction from defatted insect flour.

On the one hand, Zhao et al. (2016) [33] worked on finding the most optimal alkaline extraction conditions for yellow mealworms. After a study based on the fractional factorial experimental design, they found the following optimal conditions: 0.25 M NaOH solution as extraction solvent, liquid/solid ratio of 15 mL/g, $T = 40\text{ }^{\circ}\text{C}$, and $t = 1\text{ h}$.

On the other hand, an acidic aqueous extraction was performed as an alternative method of aqueous extraction. It consisted of adding ascorbic acid to the solution instead of NaOH followed by vortexing and finally centrifuging. The supernatant and gel layer were collected, and a second extraction was conducted on the pellet, followed by vortexing and centrifuging, to obtain a protein extract, which was frozen and freeze-dried [36].

Otherwise, proteins are extracted by sonication using an ultrasonic generator set to 20 kHz for 15 to 20 min, with 75% AMPL (amplitude), and pulsed every 3 s with equal resting intervals. Then, samples are filtered using a stainless-steel filter of 1 mm, and filtrates are collected, freeze-dried, and stored at cold for further usage [24,46]. The effect of the ultrasound-assisted process (UAP) on the yield, functional, antioxidant, and molecular properties of protein isolated from Bombay locusts (*Patanga succinta* L.) was studied by Kingwascharapong et al. (2021) [47]. They stated that to avoid undesirable effects on the protein's molecular structure, the right UAP condition should be assessed. For Bombay locust, UAP at 60% amplitude for 20 min was identified as being a good condition for protein extraction with minimal side effects.

Laroche et al. (2019) [41] conducted a study on the influence of the defatting methods on proteins purity. This study proved that the lipid extraction method affected protein extraction and ethanol-Soxhlet defatting produced a higher protein purity in *A. domesticus*

as compared with other solvents such as petroleum ether, ethyl acetate, hexane, and even as compared with supercritical CO₂ extraction.

Bußler et al. (2016) [10] indicated that during the protein extraction process, protein solubility was extremely dependent on the pH. Usually, protein solubility of defatted insect flour is higher in pH 2 and 11, and it decreases as it approaches the isoelectric point (pI) around pH 4 (Figure 9) [27,33]. On this line, Amarender et al. (2020) [36] conducted a comparative analysis of the extraction of defatted cricket proteins in an alkaline (NaOH) and an acidic solution (Ascorbic acid). The results (Table 4) led to the following conclusion: extraction in an acidic medium (pH from 2 to 3) gave an extract with higher protein content (69.69%) than the one issued from the extraction in an alkaline medium of pH 13 (61.75%). This can be explained by the high solubility of cricket proteins in an acidic solution rather than an alkaline solution. A study done by Brogan et al. (2021) [48] determined that proteins can be successfully separated from insects through pH solubility precipitation and that this method results in isolates with good nutritional and functional qualities.

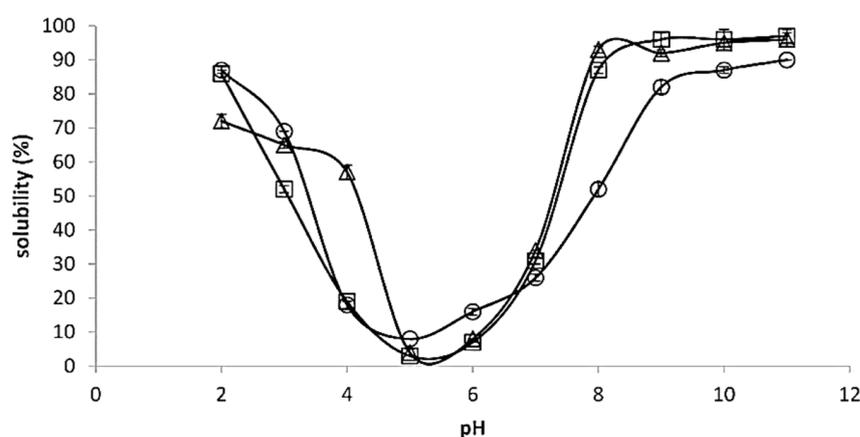


Figure 9. Protein solubility (%) with changes at pH 2–11. *Tenebrio molitor* (square), *Gryllosides sigillatus* (triangle), and *Schistocerca gregoria* (circle) [27].

Table 4. Extraction yield, true protein content, and extraction rate of protein from ethanol defatted cricket powder [36].

Sample	Extraction Yield (%) of Defatted Cricket Powder	True Protein (%)	Extraction Rate of Protein (%)
Defatted protein extract (ascorbic acid)	87.75 ± 1.53 ^a	69.69	82.95
Defatted protein extract (NaOH)	80.78 ± 0.17 ^b	61.75	67.66

^{a,b}—Represents in each column homogeneous groups.

The protein content of insects is generally determined using the Kjeldahl method. This method gives the total nitrogen content, and then a protein-to-nitrogen conversion factor (Kp) of 6.25 is used to calculate the protein content [1] (equivalent to 0.16 g nitrogen per gram of protein). However, scientists have assumed that using the Kp factor of 6.25 has led to a large overestimation of protein content and this is due to the presence of non-protein nitrogen (NPN) mainly as chitin-bound nitrogen in the exoskeleton, nucleic acid, and inorganic nitrogen.

To avoid this overestimation, Janssen et al. (2017) [25] conducted a study on three insect species, to calculate the Kp factors to be used for whole insect and protein extracts derived from insects. First, they determined the exact protein content, by calculating the amino acid composition of insects and concluding from it the total amino acid content, which is equivalent to the total true protein content. Then, they calculated the adjusted Kp factors that should be used for insects to be able to determine the true protein contents

based on the nitrogen content using the Kjeldahl method. They proposed to use an insect-specific Kp factor of 4.76 for protein content in the whole larvae (Figure 10) and 5.60 for soluble proteins extracted from insects. The last one is significantly higher than the Kp of the whole larvae and this is due to the elimination of NPN during the extraction steps. In addition, the right estimation of protein content in the whole insect will lead to an increase in protein extraction yield (%) due to the removal of initial protein overestimation.

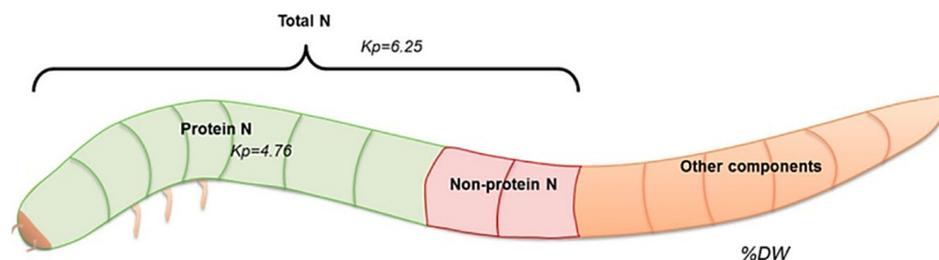


Figure 10. Insect larvae nitrogen-to-protein conversion factors [25].

Kim et al. (2020) [49] studied the effect of protein extraction steps, i.e., grinding, defatting, and extraction, on the essential amino acid composition and changes in protein qualities and functionalities. They determined that the essential amino acid index (EAAI) increased during extraction, and that protein functionalities such as foam capacity and stability increased as well, which might be due to the removal of chitin and hydrophobic amino acids. They also indicated a slight increase in the emulsion capacity and a decrease in emulsion stability with the extraction steps for the most studied insect species. Moreover, Zielińska et al. (2018) [27] studied the water holding capacity (WHC g/g) and the oil holding capacity (OHC g/g). They noticed a higher WHC as well as OHC in protein extracts than in whole insects. These results prove that protein extraction is beneficial in improving protein qualities and functionalities of edible insects, and, these properties, in addition to insects' solubility in a wide pH range (Figure 9), are very important for the use of protein extracts in food and feed applications.

This being said, protein extraction is often carried out in an extremely alkaline or acidic environment, using HCl, NaOH, and ascorbic acid, and could be enhanced by sonication. However, natural alternatives to currently used chemicals, such as the use of ionic liquids (ILs), notably natural deep eutectic solvents (NADES), should be considered.

2.2.3. Chitin and Chitosan Extraction

Chitin is a biopolymer found in the exoskeleton of arthropods (i.e., insects, arachnids, and crustaceans). It is chemically a linear polymer of N-acetylglucosamine units, a cellulose derivative, and classified into three types: α , β , and γ [50,51]. Since it is insoluble in most solvents due to its compact structure, chemical treatments are needed to obtain a more soluble substance called chitosan [51].

Currently, chitin and chitosan are gaining high interest in various industrial fields such as the chemical, pharmaceutical, and food industries. This attention is due to their antimicrobial, emulsifying, thickening, and stabilizing properties, in addition to being biodegradable, non-toxic, wound healing promoters, hemostatic, hypolipidemic, enhancers for the immune system, and most importantly, very abundant in the environment [50,51].

A study carried out by Kaya et al. (2016) [50], revealed that insects' chitin content increased progressively with their developmental stages. Chitin contents of *Vespa crabro* (wasp) larvae, pupa, and adult were found to be respectively 2.2, 6.2, and 10.3% (dry basis), which indicated that an intense production in chitin takes place between the larvae and pupa stage.

Chitin is found in the exoskeleton in form of chitin nanofibrils wrapped with proteins, and the chitin-protein fibers are incorporated in a protein-mineral matrix [52]. Thus, to obtain pure chitin, an extraction process is required. Chitin is commonly isolated from entire insects or by-products issued from the insect protein extraction process. [53].

Chemical chitin extraction consists of two essential steps: demineralization and deproteinization. For demineralization, samples are treated with HCl at a high temperature, to remove minerals, then, they are filtered/centrifuged and rinsed with distilled water several times. Sediments collected from the previous step undergo the deproteinization step: they are heated in NaOH to remove proteins, and the extract is filtered and washed with distilled water until the pH becomes neutral, and then dried. The extract obtained is designated as the isolated chitin in the form of light brown powder that requires decolorization to remove pigments and impurities. This step is done by passing the sample through a mixture of chloroform, methanol, and distilled water in the ratio of 1/2/4 at ambient temperature and stirring, then rinsing in distilled water, and finally drying [50,53–55].

Alternatively, to overcome chemical extraction problems such as the deterioration of the quality and the physicochemical properties of chitin by concentrated acids and bases, and high temperatures, a biological extraction of chitin is possible. This method uses enzymes (proteases, e.g., alcalase) from proteolytic bacteria for deproteinization and acid from acid-producing bacteria (like lactobacillus) for demineralization, which produces a chitin extract of high quality (Figure 11). However, this method as compared with the chemical method is less efficient in demineralization and deproteinization, therefore, it is still limited to a laboratory scale [56].

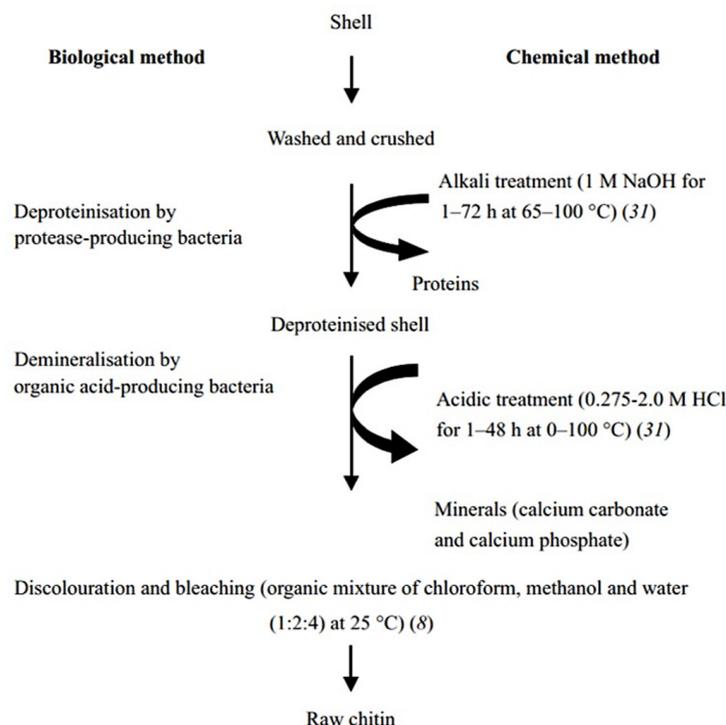


Figure 11. Chitin recovery by chemical and biological methods [56].

At this point, extracted chitin has to be deacetylated to obtain chitosan, which, unlike chitin, is soluble in acidic solutions. This is done by treating chitin with NaOH at 100 °C for 3 h, washing with water until achieving a neutral pH, and then drying in an oven for 24 h [53,57].

3. Future Challenges for the Sector

To achieve an eco-efficient production system, as well as development and transformation of insect larvae, several key points must be addressed by further researches. The major relevant key points are the following:

- Study the integration of the insect rearing step in an ecological and economic space and the valorization of various agricultural wastes and by-products from the agri-food

industry (peels, grape pomace, downgraded fruits and vegetables, etc.) by using them as potentially effective raw materials for bioconversion by insects [19];

- Establishing environmental assessments of the two main phases of the insect sector, i.e., the production of insect larvae [58] and the industrial transformation of the larvae into lipid, flour, and protein isolates, by comparing several scenarios of feeding and transformation of insects;
- Water and energy are the main resources used in the transformation of insects. Therefore, a Pinch analysis must be done along with a technical-economic study to reduce the use of these resources through energy integration by using new ways and techniques of extraction. In this context, substantial interest should be paid to the use of emerging technologies to: intensify transfer phenomena, obtain purified extracts, preserve product quality, avoid its oxidation, increase production yield, and reduce both energy and chemical consumption. To do this, low-energy pretreatments such as pulsed electric fields (PEFs) can be applied to insects to permeabilize their cells and facilitate the extraction steps and fractionation downstream. This pretreatment can be used as a killing method at once [35]. Another innovative technology that might be used in this context is the instant controlled pressure drop DIC. It can be used as a pretreatment to intensify the extraction steps, such as DIC-assisted solvent or press extraction of lipids, which have been studied in various cases of pulses and oleaginous grains and seeds [59,60], or to intensify the drying process and preserve the quality of products [61]. DIC-autovaporisation can also be studied as a highly effective desolventation way;
- Innovative and less energy-consuming dehydration techniques should be adopted, such as concentration by the superheated steam while preserving the quality of proteins [62];
- Improving the efficiency of the SC-CO₂ extraction process, especially with the use of co-solvents [41].

4. Conclusions

With the rapid growth in world population, farming and processing of edible insects are considered to be, nowadays, one of the promising solutions to answer the mounting demand for food and feed supply as a new sustainable alternative for protein sources. Recently, many studies have focused on the importance of insect consumption, but the insect processing sector is still in its initial stages.

In this review, we have focused on the current insect processing methods from reception to final products: lipids, proteins, and chitin/chitosan. The collective results implicate that blanching is an effective killing method that preserves larval quality and appearance. Lipid extraction is usually done in two steps: pressing and solvent extraction. The optimal solvent has been shown to be ethanol, which is noteworthy since it is a green solvent; nevertheless, to extract both polar and nonpolar fatty acids, an improvement might be considered. As for the extraction of proteins, isoelectric precipitation is the most widely used and successful method; however, natural alternatives might be explored to reduce the need for chemicals in the process. Finally, using emerging technologies to intensify the processing steps might be an efficient way to improve the whole process while staying environmentally friendly, cost-effective, and producing high-quality final products.

Bottom line, more studies on industrial-scale farming and processing cost, as well as on processing steps optimization and the use of novel processing technologies, are required to formulate functional and accepted insect-based ingredients that can promote global entomophagy the right way.

Author Contributions: Writing, R.E.H.; review and editing, all the authors; project administration and funding acquisition, H.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work is a part of the project RAFINSECT (2022-2025) funded by the region Hauts de France.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the region Hauts de France for the financial support.

Conflicts of Interest: The authors declare no conflict of interest.

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