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REVIEW

A Review on Characteristics, Extraction Methods and Applications of Renewable Insect Protein

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ABSTRACT

Due to the expected rise in the world population, an increase in the requirements for quality and safety of food and feed is expected, which leads to the growing demand for new sources of sustainable and renewable protein. Insect protein is gaining importance as a renewable material for several reasons, reflecting its potential contributions to sustainability, resource efficiency, and environmental conservation. Some insect species are known to be able to efficiently convert organic waste into high-value products such as protein, requiring less land and water compared to traditional livestock. In addition, insect farming produces fewer greenhouse gas emissions, contributing to mitigating climate change. Insects are considered as a major potential alternative to animal or plant protein due to their many nutritional benefits, including high protein, mineral, and vitamin contents. On average, the protein content of insects ranges between 35% and 60% dry weight, which exceeds plant protein sources, such as cereal, soybeans, and lentils. As the acceptance of insect protein grows and technologies advance, the food and feed industries continue to explore and expand their applications, offering consumers diverse and sustainable protein choices. In this review, we discuss the recent findings relating to insect protein focusing on its characteristics, extraction methods, applications, and opportunities along with some trade-offs and uncertainties.

KEYWORDS

Circular economy; waste utilisation; protein; insect; *Hermetia illucens*

1 Introduction

As the world population continues to grow, coupled with escalating environmental pollution concerns, there is a rising need for eco-friendly and renewable protein sources. The ongoing struggle for land allocation between food crops and animal feed has persisted for some time. Nearly 50% of available arable land is already committed to feeding the global population, with a third of this land designated for livestock feed production [1]. It was estimated that livestock farming accounts for up to 19.6% of total anthropogenic greenhouse gas emissions [2], particularly, methane, contributing to global warming 28 times greater than carbon dioxide [3]. Meeting the future demand for animal feed and feed ingredients requires innovation towards more sustainable animal diets (Fig. 1). A wide range of novel protein sources including microalgae, macroalgae, and insects can be utilized for feeds.

Insect rearing is a circular-economy process, resulting in waste being converted to an organic fertilizer [4,5], while insects serve as sources of value-added products [6,7]. In addition to environmental and

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socio-economic benefits, insects outperform animals such as poultry or cattle. They can be raised on smaller land areas, leading to a higher yield per hectare compared to conventional crops like soybeans, and emit fewer greenhouse gases and less ammonia per kilogram of meat produced in comparison to pigs and cattle [8]. Moreover, analysts estimate that alternative protein sources will account for 33% of global protein consumption by 2054, 11% of which will be insects [9].

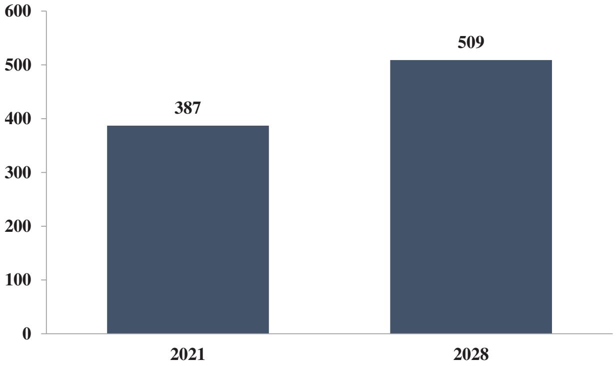

Figure 1: Market forecast of animal feed market (in USD billion) according to research and markets (2023)

In various regions across the globe, insects are already being employed in animal feed for aquaculture and poultry. For instance, many fish species feed on insects; therefore, their use in aquaculture comes naturally. Significant advantages have been linked to incorporating insects into animal nutrition. Insects can be cultivated using low-value agricultural by-products or organic waste from the food industry, resulting in high-quality protein that rivals fish and soybean meals in nutritional value [10]. Insects also serve as a rich source of amino acids, fatty acids, and micronutrients [11–13].

In addition to feed protein, insects represent a promising alternative protein source for the world's growing population. Entomophagy, or human consumption of insects, has already been practiced in many parts of the world, especially in tropical regions. Multiple investigations have illustrated the various functional characteristics of insect proteins in food, including their capacity to create foam, serve as emulsifiers, and contribute to gel formation [14]. It was shown that insect proteins can either be used as food supplements to increase the nutritional quality of food products or as meat and dairy alternatives [15].

Another application of insect protein aims to replace traditional non-biodegradable and non-renewable petroleum sources with renewable and green alternatives, for example, in the production of bioplastics [16,17]. It is known that edible packaging can be prepared using biopolymers, such as carbohydrates, lipids, and proteins, which can be extracted from insects [18,19].

In this article, we review recent work on the use of industrially reared insects as a novel source of renewable protein considering the nutritional composition of insects, methods of insect protein extraction, and its future applications as well as prospects, opportunities, and potential challenges.

2 Insects: Characteristics and Nutritional Composition

Insects are the dominant life forms on the planet whose biomass exceeds the biomass of all invertebrates. Today insect farming is becoming increasingly popular due to the ability of insects to convert organic wastes into useful resources [20]. The nutritional composition of each insect varies depending on the species (Table 1). Some of the variables that could affect the nutritional content of insects include developmental stage, rearing techniques, diets, killing methods, and drying techniques. In addition, insect meal can be a source of high-value bioactive compounds such as peptides with immunostimulatory and anti-microbial effects and biopolymers (chitin, melanin) with potential effects on intestinal health and the immune system [6,21].

Due to the increased application of Black Soldier Fly (*Hermetia illucens* or BSF) larvae in organic waste utilisation (Fig. 2), BSF is becoming the focus of emerging research fronts [22]. The main benefit of *H. illucens* as well as other flies is its use as a tool for waste upcycling, while other insects are reared for feed application only. Furthermore, BSF is more advantageous than other insect species such as mealworms and crickets due to the higher feed conversion rate, short reproductive cycle, and high content of fat, protein, minerals, and vitamins [23,24]. Its survival rate and nitrogen and phosphorus composition are not significantly affected by a change in diet [25].

Table 1: The nutritive value variability of the common insect species used as animal feed [12,26–28]

Constituents (% in DM)	*Hermetia illucens* larvae	*Tenebrio molitor* larvae	*Musca domestica* larvae	*Bombyx mori* pupae
Crude protein	43.3	53.3	54.1	54.0
Crude fat	26.3	29.8	21.2	12.0
Ash	12.3	4.2	10.2	4.6
Chitin	4.6	5.9	9.1	18.0

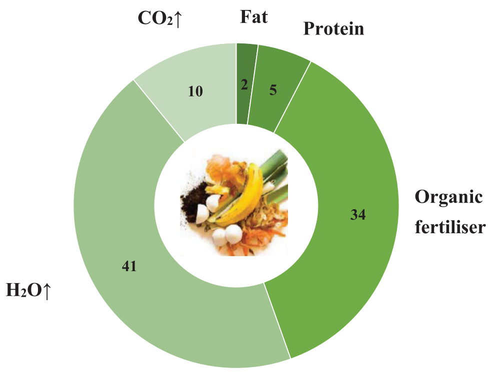

Figure 2: Final products of black soldier fly organic waste treatment (in percent)

- Proteins

Insect bodies are rich in protein–as a rule, the crude protein content of insect meals is high ranging from 42% to 63%, and is similar to soy meal [29]. Proteins' suitability as animal feed relies on their amino acid composition. The high content of essential amino acids also ensures the high biodegradability of materials made out of insect proteins.

An imbalance in amino acids can lead to metabolic issues, necessitating the supplementation of specific amino acids [30]. For instance, fish require ten essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine/cysteine, phenylalanine, threonine, tryptophan and valine, and *H. illucens* larvae contain all these essential amino acids [30]. The representation of essential amino acids in *H. illucens* is very similar to fish meal but the content is lower. As a result, *H. illucens* proteins can replace fish meal in aquaculture providing a more sustainable option. BSF larvae could also be used as feed in poultry and pig breeding due to their high protein content. For example, poultry requires 18%–20% crude protein, while pigs–13%–21% depending on their age [30]. These protein content requirements are hard to maintain by grain feed only.

It was shown that insect proteins satisfy the amino acid composition standards set by the World Health Organisation, demonstrating elevated levels of phenylalanine, tyrosine, tryptophan, lysine, and threonine

[31]. For instance, one of the latest studies by Huang et al. [32] showed that protein extracted from *H. illucens* larvae exhibited a higher ratio of essential amino acids to total amino acids than that of egg protein. The amino acid analysis, therefore, indicates that protein extracted from *H. illucens* larvae can be utilized as an edible animal protein.

- Lipids

In contrast to the amino acid profile, the fatty acid content of insects can vary greatly with the diet. Therefore, it is possible to adjust the desired larval lipid composition depending on the intended purpose of the product. Usually insect fat content is higher at the larva and pupa stages, while at the adult stage, the fat content is relatively lower. Unlike the majority of animal fats, insect oil is liquid at room temperature, which accounts for a considerable number of polyunsaturated fatty acids (e.g., superworm, mealworm and cricket oil) [33,34]. For instance, in BSF they can be promoted when insects are fed on fish discards, coffee silverskin, and seaweeds [35].

Black soldier flies are rich in lipids (up to 49% on a dry matter basis) [29]. The quality of fats from *H. illucens* larvae is high and resembles the fatty acid content of products from plant sources, e.g., palm (kernel) and coconut oil. The high proportion of lauric acid (up to 60%), which is a raw material for many products in the cosmetic, cleaning, and detergent industries, is remarkable.

- Chitin

The mass production of insects can provide an alternative source for chitin and chitosan, its deacetylated derivative, as today the worldwide production of these biopolymers comes largely from crustacean shells. Chitin is present in insects at all stages of ontogenesis and plays an important role in their structure. In addition, in insects, chitin is associated with melanin, which is not found in crustaceans [6]. Melanin is acquired at later stages of insect development and can be extracted in the form of chitin-melanin complexes with enhanced biological activity either as a mechanical mixture (e.g., from bees [36]) or a covalent complex (e.g., from black soldier flies [7,37]).

- Micronutrients

Micronutrients (i.e., minerals and vitamins) are described as the nutrients required by an organism in trace amounts and play an important role in the nutritional value of feed. It was found that insects contain essential micronutrients such as iron, magnesium, calcium, phosphorous, selenium, zinc as well as vitamins A, B, D, E, and K [38].

BSF is considered to be the best source of minerals and calcium [24,39]. *H. illucens* larvae contain higher amounts of essential minerals compared to other insect species including iron, magnesium, calcium, copper, phosphorous, and zinc [40].

- Antimicrobial peptides (AMPs)

The greatest diversity of AMPs is found in insects [41,42]. Insects have a well-developed innate immune system with cellular and humoral defense responses, involving the production of AMPs secreted into the hemolymph [43,44]. AMPs are a class of small peptides that widely exist in nature and are an important part of the innate immune system among living organisms. They have a wide range of inhibitory effects against different microorganisms such as bacteria, fungi, parasites, and viruses [45]. AMPs, acting as potent antibiotics or fungicides, primarily target the cell envelope, notably the cell membrane. They also impact intracellular microbial targets, ultimately causing cell death [46–48]. BSF is a very resilient organism, and its larvae are fed on a variety of decomposing organic substrates typically inhabited by a range of microorganisms. Therefore, it can be expected that *H. illucens* larvae express AMPs and other substances possessing antibiotic activity in order to survive in these unfavorable conditions [30].

3 Methods for Obtaining Protein

There are a limited number of studies devoted to the extraction of insect proteins and their characterization. To date, the most prevalent method for protein extraction in insects is wet fractionation; however, dry fractionation can also be applied.

In the literature, protein isolation is usually performed by the following steps: homogenization, defatting, protein solubilization, isoelectric precipitation of the proteins, protein resolubilisation and drying [49]. Nevertheless, the parameters for each step may vary depending on the insect and its life stage and should be adjusted accordingly.

The aim of the homogenization process is to convert insects to a smaller particle size to increase the surface area between the insect particles and the extraction solvent. This leads to a more efficient process of protein extraction.

Defatting may be carried out by a solvent extraction step. Different lipid removal processes have been described in the literature such as petroleum ether [50–53], hexane [54,55], ethanol or hexane: isopropanol (3:2 (v/v)) [56], supercritical CO_2 [57]. However, the use of organic solvents during the lipid removal step may result in losses of protein due to their affinity for the solvent. An alternative defatting method was applied to edible insect homogenates consisting of centrifugation of insect homogenates at 4°C and removal of the upper layer of lipid fraction from the supernatant [57].

The prevailing technique involves combining alkaline solubilization with isoelectric precipitation, although certain studies may choose either alkaline or acid solubilization separately. To summarise, in the alkaline solubilisation coupled with the isoelectric precipitation method, insect meal is initially solubilised under alkaline conditions to dissolve proteins. Subsequently, centrifugation is employed to separate the soluble proteins, with the resulting solution collected while discarding the pellet. Following this, the pH is adjusted to the isoelectric point to induce protein precipitation.

According to the literature, either water-soluble or non-water-soluble proteins can be isolated from insects. To obtain water-soluble proteins, alkaline extraction procedure is performed: the insect homogenate is adjusted to pH values higher than 7.0 to solubilize proteins in the aqueous phase [58–62]. This creates a high overall charge, which results in electrostatic repulsion allowing proteins to be soluble in aqueous phases.

To extract non-water soluble proteins, a centrifugation step on insect homogenates is conducted, and the pellet containing the non-water soluble protein fraction is retained [63,64]. For instance, the collection of various protein fractions from *B. mori* pupae was described by a sequential extraction using water, 5% NaCl, 0.1 M NaOH, and 70% ethanol solutions [65]. Very few studies, however, have characterized obtained insect protein isolates. Also, specific methods of extracting protein isolates are hard to scale-up and do not appear economically viable for commercial feed applications.

Alternatively, enzymatic hydrolysis can be carried out either directly on insect homogenates or insect protein isolates to generate bioactive peptides (BAPs) or to improve the techno-functional properties of the proteins. In some cases, proteins may be denatured by physical means, e.g., ultrasound prior to enzymatic hydrolysis [52,66].

In order to generate protein-derived BAPs, mammalian digestive enzymes and microbial enzymes are commonly used. The method of sequential hydrolysis with pepsin, trypsin and chymotrypsin (simulated gastrointestinal digestion) was applied to hydrolyze various insect species such as *B. mori* larvae and pupae [53,67], *S. littoralis* [63,64], adult *A. annulipes*, *S. gregaria* and *L. migratoria*, *B. dubia*, *G. portentosa* as well as *T. molitor* and *Z. morio* [68,69].

Plant-derived enzyme preparations during insect protein hydrolysis are rarely used. An example is papain hydrolysis of *B. mori* chrysalises [57]. Some papers do not disclose the origin of the enzyme preparation.

Another study explores a range of commercial enzymes of vegetal (papain), bacterial (PBL, dispase) and animal (pepsin, trypsin, pancreatin) origin to obtain protein hydrolysates from *Alphitobius diaperinus* and *Hermetia illucens* larvae [70].

The potential of microbial (lactic) fermentation of *H. illucens* biomasses with two different strains (*L. rhamnosus* and *L. plantarum*) is studied by Luparelli et al. [71].

Several protein extraction techniques can be applied including chemical and enzymatic methods (Table 2). Most of the studies focus on protein extraction from *H. illucens* larvae. In the study by Mshayisa et al. [61] two chemical techniques, alkaline solution and isoelectric precipitation (using 1 M NaOH and 1 M HCl) and alkaline extraction (using 1 M N NaOH), were investigated to extract protein concentrates from defatted *H. illucens* larval flour. The results indicated that alkaline and acid precipitation extractions of *H. illucens* larval concentrates led to improved nutritional and functional properties. The crude protein of *H. illucens* larval protein concentrate obtained by alkaline and acid precipitation was 73.35%, whereas for alkaline extraction the value was 68.47%.

Table 2: Advantages and disadvantages of different protein extraction methods

Extraction method	Advantages	Disadvantages
Chemical extraction (alkaline solubilisation with acid precipitation)	• Simple • Cost-effective • Suitable for large-scale production	• May lead to protein denaturation • Multiple steps involved • Environmental pressure
Enzymatic extraction (enzymatic hydrolysis)	• May be very specific without introducing contaminants • Gentler on proteins reducing the risk of denaturation • Eco-friendly	• Harder to scale-up (requires optimisation) • Can be time-consuming • Enzymes can be expensive increasing the overall cost of protein extraction • Not all proteins are easily digested

Batish et al. [72] examined two methods: chemical extraction using 1 M NaOH and precipitation with 10% trichloroacetic acid solution in acetone as well as enzymatic hydrolysis with Alcalase, papain and pepsin. Enzymatic hydrolysis lasted for 2 h, and each mixture's enzyme was deactivated by heating the solution in a water bath afterward. The heated suspension underwent centrifugation resulting in three distinct phases: a semisolid bottom phase containing insoluble protein and chitin, an intermediate liquid phase with protein hydrolysates, and a top liquid with the lipid fraction [72]. The intermediate supernatant phase was isolated and subjected to freeze-drying. The effects of both methods on the functional properties, antioxidant activity, amino acid composition, and protein structure of *H. illucens* larval protein were evaluated. Even though enzymatic hydrolysis offered a sustainable processing method, it reduced the functional properties in *H. illucens* hydrolysates.

Firmansyah et al. [73] carried out enzymatic hydrolysis of defatted *H. illucens* larvae using a bromelain enzyme. Defatted *H. illucens* larvae were dissolved in a phosphate buffer solution, followed by the addition of the enzyme. The hydrolysis reaction occurred in a water bath shaker for 3, 13.5 or 24 h, with termination of the reactions achieved through sample heating. For remaining *H. illucens* larvae samples, complete hydrolysis was achieved by applying 6 M HCl at 110°C for 24 h to determine the total free amino acid content [73]. Protein hydrolysate from *H. illucens* larvae was separated via centrifugation and freeze-dried. The authors reported that the obtained protein hydrolysate had a yield of 10.70% (on a weight basis), while the protein concentration varied between 240–310 µg/mL.

As the acceptance of insect protein grows and technologies advance, the food and feed industries continue to explore and expand their uses, offering consumers diverse and sustainable protein choices. The application of insect proteins will be dealt with in the following section.

4 Applications

4.1 Animal Feed

Insect protein is commonly used as an ingredient in animal feed for poultry, livestock, and aquaculture. It provides a sustainable and protein-rich alternative to traditional feed sources, contributing to healthier and more efficient animal growth. Insect protein is also increasingly incorporated into pet food formulations, offering a nutrient-dense and eco-friendly protein source for companion animals.

As a rule, whole or powdered dried insects are applied as a feed source for animals. In some cases, the biomass can be separated by physical methods such as pressing or chemical methods such as extraction with organic solvents. These treatments yield protein and lipid fractions, and in certain instances, a chitin fraction [74]. The isolated protein can be subjected to a finer grinding and drying by convection, obtaining a fat-free meal that is used asalternative to fish meal [75]. However, this treatment can lead to the destruction of part of the protein by prolonged exposure to heat as well as the presence of chitin in the final product [76].

Table A1 summarises the literature data on insects investigated as a source of protein with a potential application in animal diets. Most studies (approximately 46%) apply insects in a partially defatted form, obtained through mechanical removal of fats by continuous screw press [77–79] and supplied from a commercial source. In some studies, chemical treatments using Soxhlet [80], solvent, e.g., ethyl alcohol [81,82] or enzymatic hydrolysis, e.g., using proteases and chitinases [83] were performed, which are likely to result in higher efficiency of fat (and other impurities) removal but can incur additional economic costs.

Dried powdered form (approximately 34%) is the second most frequently used form of insects for feed [84–86].

Whole dried insects (approximately 20%) are less often applied, however, still result in a significant number of studies, particularly in birds [87,88]. The least frequent form of application is live insects [89].

Insects are mostly used as a feed additive to animal diets [79,86,90–92] rather than a single component of feed and usually replace fish meal and soy protein. The application of insect proteins as a feed source will be dealt with in the following section.

4.1.1 Inclusion of Insects in Animal Diet

As demonstrated in Section 2, the nutritional properties of insects are very high, and they can successfully substitute for many traditional ingredients used in the production of feed such as fish meal and soy. Black soldier flies *Hermetia illucen*s, yellow mealworms *Tenebrio molitor*, common housefly *Musca domestica*, and silkworms *Bombyx mori* have been recognised as major species for the commercial production of feed [25,93].

The highest proportion of research articles are devoted to the investigation of *H. illucens* as a future feed for animals, which could be explained by the increased use of BSF technology to upcycle agriculture and food industry waste into high-quality insect ingredients (Table 2). However, the majority of studies examine the partially defatted or dried powdered larvae as a feed source for animals rather than extracting a protein fraction. It should be noted that insects contain other substances such as lipids, chitin and melanin which was discussed earlier in Section 2. These can affect animal health and digestion (e.g., chitin) and should be taken into account.

The second largest proportion of articles is dedicated to the yellow mealworm (*Tenebrio molitor*). *T. molitor* is an insect species with fast growth and reproduction rates feeding on bread and grains. *T. molitor* has a high protein content (47%–63%) and a moderate lipid content (30%–41%), and its nutrient composition matches with animal requirements [29,94,95].

T. molitor is usually applied at the larval stage, *H. illucens*–at larval, prepupal and rarely pupal stages, while *B. mori* is used as pupae, *G. sigillatus* and *B. lateralis*–as imagoes (Table 2).

A large number of studies investigated insect protein in the diets of fish [96–98], poultry [87,89,99], pigs [100,101], and pets [102–104]. These findings demonstrate that insects can be applied as a universal feed source for various animals.

4.2 Food Industry

Insect-derived protein powders are gaining popularity as a nutritious and sustainable source of protein for human consumption. Some examples include nutritional supplements, functional foods, sports nutrition and bakery/confectionary products. Most of the papers focus on insect proteins as either supplements of food products to increase their nutritional quality or the reformulation of products aimed at the reduction or replacement of meat or milk proteins [105].

Table 3 summarises some key applications of insect protein in the food industry. In the largest proportion of studies (approximately 67%) insects are dried and ground into a fine powder. This insect powder or flour can be blended with traditional flours to create bakery goods or pasta with enhanced nutritional profiles [106–109].

Whole insects or defatted insects (e.g., *T. molitor*, *Z. morio*) can be incorporated into a wide range of processed foods, including burgers, sausages contributing to their protein content or used as meat substitutes [110–112].

Table 3: Application of insect protein in the food industry

Food type	Insect species	Processing form	Protein content	Reference
Alternative meat products	*Acheta domesticus*	Insect flour	61.4% (full-fat insect flour), 68.5% (low-fat insect flour)	[113]
	Alphitobius diaperinus	Full-fat larvae	15.2%	[112]
	Tenebrio molitor	Defatted insect flour	77.5% (freeze-dried *T. molitor* flour), 75.0% (microwave-dried *T. molitor* flour)	[111]
	Tenebrio molitor, *Alphitobius diaperinus*	Full-fat larvae	n/a*	[114]
	Tenebrio molitor, *Bombyx mori*	Defatted and hydrolysed *T. molitor* larvae and *B. mori* pupae	75.4% (*T. molitor*), 73.5% (*B. mori*)	[115]

(Continued)

Table 3 (continued)

Food type	Insect species	Processing form	Protein content	Reference
		flours, untreated *T. molitor* larvae and *B. mori* pupae flours		
	Zophobas morio	Full-fat larvae	3.3%	[116]
	Zophobas morio	Full-fat larvae	3.4%	[110]
Bakery and confectionary	*Acheta domesticus*	Insect flour	69.1%	[108]
	Acheta domesticus	Insect powder	n/a	[117]
	Acheta domesticus	Insect powder	48.9%	[118]
	Alphitobius diaperinus	Full-fat insect powder	58.4%	[119]
	Bombyx mori	Insect powder	n/a	[109]
	Gryllus assimilis	Insect powder	62.8%	[120]
	Hermetia illucens	Dried powdered prepupae	n/a	[106]
	Hermetia illucens, *Acheta domestica*, *Tenebrio molitor*	Insect flours	45.1% (*H. illucens*), 56.6% (*A. domestica*), 48.8% (*T. molitor*)	[121]
	Nauphoeta cinerea	Insect flour	63.6%	[122]
	Schistocerca gregaria	Insect powder, defatted insect powder	35.3% (*S. gregaria* powder), 39.1% (defatted *S. gregaria* powder)	[123]
	Tenebrio molitor	Insect powder	54.2%	[124]
	Tenebrio molitor, *Alphitobius diaperinus*	Powdered larvae	54.1% (*Tenebrio molitor*), 59.6% (*Alphitobius diaperinus*)	[125]
	Tenebrio molitor, *Alphitobius diaperinus*, *Acheta domesticus*	Insect flours	49.9% (*T. molitor*), 55.7% (*A. diaperinus*), 62.5% (*A. domesticus*)	[107]
Dairy alternatives	*Bombyx mori*, locust	*B. mori* pupae powder, locust powder	60.7% (*B. mori*), 43.2% (locust)	[126]
	Tenebrio molitor	full-fat larvae	20.1%	[127]

Note: *not available.

Several studies have been conducted to assess the safety and food safety of insect protein, ensuring its acceptability and compliance for human consumption [128,129]. In a recent comprehensive review by J.C. Ribeiro et al. the authors delved into scientific advancements related to the allergic risks associated with consuming insects as food [129]. The study identified two primary risk groups susceptible to developing food allergies linked to insect consumption: individuals allergic to crustaceans and those consistently exposed to edible insects. Cross-reactivity, predominantly mediated through tropomyosin, was noted as a potential cause for allergic reactions, with *T. molitor* tropomyosin capable of eliciting allergic responses in animal models [130]. Moreover, the research demonstrated that individuals consistently exposed to *T. molitor* may become sensitized and subsequently develop a food allergy to this insect [131]. The study also identified different allergens (larval cuticle protein and cockroach allergen-like protein) depending on the route of sensitization [132]. These findings contribute valuable insights to enhance our understanding of the safety aspects surrounding insect protein consumption.

4.2.1 Biopackaging

The manufacturing of plastics, particularly from petroleum-based sources is known to contribute to environmental, health, and sustainability concerns, leading to climate change. Hence, bioplastics, derived from renewable biomass sources, offer several benefits compared to traditional petroleum-based plastics. Insect proteins are explored for their potential in developing biodegradable plastics and bio-packaging.

Zhang et al. [133] explored the locust (*Locusta migratoria*) as a new protein source to develop edible film by solvent casting. Dehydrated adults of *L. migratoria* locusts were powdered and defatted by hexane. Protein extraction was performed by the standard alkaline dispersion and acid precipitation method. The authors reported that the optimal range of glycerol for locust protein film formation was 30%–50%. The FTIR and XRD analyses showed that there was good compatibility between protein and glycerol due to the hydrogen bonding interactions. The results revealed that the barrier and mechanical properties of edible insect films were similar to the unmodified cereal protein, which highlights the potential use of insect protein in food packaging and the future development of green biomaterials.

Zhang et al. [134] also developed a novel antimicrobial edible packaging based on a grasshopper protein/soy protein isolate blend by solution casting using xylose as a crosslinker and cinnamaldehyde as an antimicrobial agent. Insect protein was extracted by the alkaline dispersion and acid precipitation method. The authors stated that this novel insect protein-based composite film can be applied as a potential edible antimicrobial film for active packaging.

Qoirinisa et al. [135] produced edible films using grasshopper gelatine extracted by the alkaline dispersion and acid precipitation method using NaOH and HCl, respectively. The extraction process produced 55.2% raw gelatine powder from the dry weight of grasshoppers. The results revealed high foaming properties and high solubility of insect protein. It was concluded that insect protein can be applied in the manufacturing of edible films as environmentally friendly packaging.

Another study [136] explored the potential of the black soldier fly reared on food waste to develop bio-packaging film for food products. Protein (42%–44%) and chitin (9%–11%) contents of *H. illucens* prepupae were determined. The film was produced with 4% (w/v) black soldier fly prepupae flour in 1% (w/v) chitosan solution. The physical and mechanical properties of insect bio-packaging such as tensile strength, antioxidant activity, and water activity were evaluated. The study concluded that black soldier fly prepupae were a promising source for producing sustainable bio-packaging films for the food sector.

These diverse applications highlight the versatility and potential of insect protein across various sectors, providing solutions to challenges related to food security, environmental sustainability, and resource efficiency.

5 Challenges

Despite the aforementioned advantages and predicted increased demand for insect protein as a potential food and feed source, little is known about possible risks. Several studies and reviews have pointed out potential challenges. These include concerns about chemical and microbiological safety, allergenicity in animals and humans, deficiencies in specific amino acids, and issues related to digestibility and taste, most of which are not yet fully characterized or understood [11,137–140].

Before incorporating insects into food products and animal feed, it is crucial to establish their safety. The growing concern is the accumulation of heavy metals in the feed, which poses a significant food safety risk, potentially harming animals and endangering human health by introducing toxic substances into the food chain [141–143]. This could be explained by the presence of chitin, which is an effective sorbent. As insects can grow on agricultural waste materials containing chemical contaminants such as pesticides and veterinary drug residues, it is important to evaluate the safety of insects in novel foods and animal feeds.

The breeding and rearing of insects might also have an impact on biodiversity, thus, the introduction of non-native species should be subjected to a risk assessment procedure [144]. Considering the utilization of suitable technologies for managing, processing, and storing insects after harvesting is essential. Such technologies may need adjustments to accommodate this new ingredient while ensuring the same level of safety in terms of identifying hazards, assessing risks, and tracing origins, all without compromising efficiency and product quality. Up-scaling of insect-rearing facilities into economically viable businesses is one of the major challenges.

Another issue is related to the reactions of farmers, stakeholders, and consumers towards the use of insects, which are likely to determine the future success of using insect-based feed for different species and acceptance of foods obtained from animals raised on insect-based feed. Several studies have investigated the acceptance of insects in animal feed and food products [145–147].

Achieving cost competitiveness in the commercialization of insect proteins presents a complex challenge that varies across different geographical regions. The initial investments in infrastructure, technology, and research for insect farming can be substantial, impacting the overall economic viability. Scaling up production to meet market demands without compromising quality further intensifies the challenge, requiring optimized breeding practices, efficient feed formulations, and automation technologies. Balancing these factors is crucial to achieving a competitive cost structure compared to traditional protein sources. Additionally, fluctuations in market prices for feedstocks and energy prices, uncertainties in regulatory compliance costs, and variations in consumer acceptance can impact the overall cost dynamics. Successful commercialization hinges on strategic investments, continuous optimization, and collaborative efforts across the value chain to enhance the cost-effectiveness of insect protein production and make it a sustainable and economically viable alternative in the global protein market [148].

An additional concern is the legal regulation of insects for food and feed applications, which differs across the world. For instance, in the European Union (EU), insects that are bred for either human consumption or animal feed are categorized as ‘farmed animals’ according to Regulation (EC) No. 1069/2009. This classification has specific implications for obtaining permission to use a feed (organic resource or substrate) for these farmed animals. Across the EU, all feed, including insects, must adhere to general rules, ensuring safety and preventing direct adverse effects on the environment or animal welfare, as stipulated in Regulation (EC) No. 767/2009 and Regulation (EC) No. 178/2002. Additionally, there are regulations governing feed hygiene (Regulation (EC) No. 183/2005) and setting maximum limits for certain undesirable substances in animal feed (Directive, 2002/32/EC).

The rapid development of research on the use of insect-based feed ingredients for farmed animals over the past decade led to the authorization of insect-processed animal protein in feed for farmed fish in 2017 (Regulation (EU) 2017/893). Currently, 7 insect species, including black soldier fly, common housefly, yellow mealworm, lesser mealworm, house cricket, banded cricket and field cricket, are permitted as feed for aquaculture, poultry, and swine animals in the EU. Nevertheless, there are no restrictions on the use of fats derived from insects. In some countries, e.g., China or South Korea, no limitation is applied.

Nowadays, insects are being explored intensively as future foods, and the EU has recently authorized four insect species intended to be marketed as a snack or as a food ingredient, in a number of food products: *Tenebrio molitor* larva (Regulation (EU) 2021/882 and Regulation (EU) 2022/169); *Locusta migratoria* (Regulation (EU) 2021/1975); *Acheta domesticus* (Regulation (EU) 2022/188); *Alphitobius diaperinus* (Regulation (EU) 2023/58).

6 Conclusions and Future Perspectives

In this review, insects were considered a novel source of renewable protein. The conventional natural resources used to produce food and feed are not enough to satisfy the growing demand for proteins due to the expected rise in world population and urbanization as well as the limited availability of land, fertilizers, energy, and water. Furthermore, the global prices of fish meal, maize, and soybean meal have significantly increased over the last few decades. The use of insects in food and feed has become a potential solution to these constraints.

This review of literature data demonstrates the use of insects as high-quality feed, food additives, and biop-ackaging materials. The majority of studies have focused on whole insects or defatted powdered meals when referring to insect protein, leaving behind chitin and other compounds present in insects, which can affect the properties of the final product.

Importantly, in the face of climate change, a shift to insect-based food and feed offers a sustainable solution for recycling biowaste and reducing greenhouse gas emissions. In addition, insect farming can create new jobs contributing to improved food security and income by providing a cheaper source of feed and organic fertilizer, reducing food-feed competition, and diversifying income-generating opportunities for insect-producing farmers and other actors.

Even though the potential of insects in food and feed is clear, the question of how to effectively rear and process insects into commercial products remains a challenge. The chemical and microbial safety, allergenicity, amino acid deficiency, digestibility, and taste of insect proteins are not yet fully understood. The safety assessment of insects in novel foods and feeds is still incomplete. Additionally, insect farming may have an impact on biodiversity. The acceptance of insects by farmers, stakeholders, and consumers may influence the future success of insect feed. Therefore, further research on protein extraction from insects is required. As the nutrient composition of insects varies with the type of insect, its stage in the life cycle, rearing conditions, and the extraction method should be evaluated and adjusted accordingly.

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Appendix

Table A1: Different insect species as a source of feed protein for animals

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
Aquaculture				
African catfish *Clarias gariepinus*	*Hermetia illucens*	Partially defatted larvae	42.6%	[77]
Barramundi *Lates calcarifer*	*Hermetia illucens*	Full-fat larvae	45.0%	[149]
European perch *Perca fluviatilis*	*Hermetia illucens*	Partially defatted larvae	55.3%	[150]
	Hermetia illucens, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*,	Powdered defatted *H. illucens* larvae, defatted *M. domestica* larvae, highly defatted *T. molitor* larvae, defatted	55.5% (*H. illucens*), 58.8% (*M. domestica*), 74.3% (*T. molitor*), 55.3% (*A. diaperinus*), 71.0% (*A. domesticus*),	[98]

(Continued)

Table A1 (continued)

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
	Gryllodes sigillatus, *Gryllus assimilis*	*A. diaperinus* larvae, *A. domesticus* adults, *G. sigillatus* adults, *G. assimilis* adults	71.0% (*G. sigillatus*), 66.8% (*G. assimilis*)	
European seabass *Dicentrarchus labrax*	*Hermetia illucens*	Partially defatted prepupae	55.8%	[151]
	Tenebrio molitor	Full-fat larvae	51.9%	[152]
Gilthead seabream *Sparus aurata*	Chironomids	Dried powdered larvae	58.6%	[84]
	Tenebrio molitor, Hermetia illucens and Musca domestica	Full-fat *T. molitor* larvae, partially defatted *H. illucens* larvae, full-fat *M. domestica* larvae	61.0% (*T. molitor*), 67.0% (*H. illucens*), 58.5% (*M. domestica*)	[97]
Hybrid tilapia Nile x Mozambique, *Oreocromis niloticus* x *O. mozambique*	*Hermetia illucens*	Larval frass	18.5%	[153]
Japanese seabass *Lateolabrax japonicus*	*Hermetia illucens*	Partially defatted larvae (solvent extraction)	55.4%	[81]
Jian carp *Cyprinus carpio*	*Hermetia illucens*	Defatted larvae (Soxlet method)	40.7%	[80]
Largemouth bass *Micropterus salmoides*	*Hermetia illucens*	Full-fat larvae and prepupae	35.3% (larvae), 42.7% (prepupae)	[154]
	Hermetia illucens	Zymolytic larvae pulp (enzymatically hydrolysed with proteases and chitinases)	30.6%	[83]
	Tenebrio molitor	Powdered larvae	78.3%	[92]
Meagre *Argyrosomus regius*	*Acheta domesticus*	Full-fat meal	62.2%	[155]

(Continued)

Table A1 (continued)

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
Nile tilapia *Oreochromis niloticus*	*Hermetia illucens*	Partially defatted larvae	60.8%	[91]
	Nauphoeta cinerea, *Gromphadorhina portentosa*, *Gryllus assimilis*, *Zophobas morio*, *Tenebrio molitor*	Powdered *N.cinerea* adults, *G. portentosa* adults, G. *assimilis* adults, *Z. morio* larvae, *T. molitor* larvae	64.8% (*N.cinerea*), 69.9% (*G. portentosa*), 62.1% (G. *assimilis*), 49.9% (*Z. morio*), 47.8% (*T. molitor*)	[96]
Pacific white shrimp *Litopenaeus vannamei*	*Hermetia illucens*	Partially defatted larvae (solvent extraction)	52.0%	[82]
	Tenebrio molitor	Powdered larvae	55.6%	[156]
	Tenebrio molitor	Partially defatted larvae	74.8%	[157]
Pearl gentian grouper *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*	*Hermetia illucens*	Dried full-fat larvae	35.2%	[158]
Pikeperch *Sander lucioperca*	*Hermetia illucens*	Partially defatted larvae	54.5%	[159]
Rainbow trout *Oncorhynchus mykiss*	*Hermetia illucens*	Partially defatted larvae	39.4%	[78]
	Hermetia illucens	Partially defatted larvae	55.3%	[90]
	Hermetia illucens	Dried powdered prepupae	n/a*	[160]
	Hermetia illucens	Dried powdered prepupae	30.8%	[161]
	Hermetia illucens	Partially defatted prepupae	48.6%	[162]
	Hermetia illucens	Partially defatted pupae	n/a	[163]
	Tenebrio molitor	Powdered larvae	67.1%	[164]
	Tenebrio molitor	Partially defatted larvae	44.3%	[165]
Red drum *Sciaenops ocellatus*	*Hermetia illucens*	Dried powdered larvae	43.3 and 44.8% (depending on the diet)	[85]

(Continued)

Table A1 (continued)

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
Red hybrid tilapia	*Hermetia illucens*	Dried powdered larvae	43.2%	[166]
Rice field eel *Monopterus albus*	*Hermetia illucens*	Full-fat larvae	32.1%	[167]
Sea trout *Salmo trutta*	*Tenebrio molitor*, *Hermetia illucens*, *Gryllodes sigillatus*, *Blatta lateralis*	Powdered *T. molitor* larvae, *H. illucens* larvae, *G. sigillatus* adults, *B. lateralis* adults	56.3% (*T. molitor*), 40.4% (*H. illucens*), 61.3% (*G. sigillatus*), 54.6% (*B. lateralis*)	[168]
Siberian sturgeon *Acipenser baerii*	*Hermetia illucens*	Full-fat larvae	35.0%	[169]
	Hermetia illucens	Highly defatted larvae	62.5%	[170]
	Hermetia illucens	Highly defatted larvae	62.5%	[171]
Totoaba *Totoaba macdonaldi*	*Hermetia illucens*	Partially defatted larvae	53.0%	[172]
Turbot *Psetta maxima*	*Hermetia illucens*	Partially defatted pupae	47.6%	[173]
Yellow catfish *Pelteobagrus fulvidraco*	*Hermetia illucens*	Dried powdered larvae	47.0%	[174]
	Tenebrio molitor	n/a	n/a	[175]
Zebrafish *Danio rerio*	*Hermetia illucens*	Full-fat prepupae	n/a	[176]
Poultry				
Barbary partridge *Alectoris barbara*	*Tenebrio molitor*, *Hermetia illucens*	*T. molitor* larvae, Partially defatted *H. illucens* larvae	51.9% (*T. molitor*), 61.3% (*H. illucens*)	[177]
Broiler chickens	*Hermetia illucens*	Partially defatted larvae	60.8%	[178]
	Hermetia illucens	Dried powdered larvae	41.1%	[86]
	Tenebrio molitor	Dried powdered larvae, fresh insects	50% (dried), >20% (fresh)	[89]
	Tenebrio molitor	Full-fat larvae	52.4%	[179]
	Tenebrio molitor, *Hermetia illucens*	Dried powdered larvae	52.4% (*T. molitor*), 36.9% (*H. illucens*)	[180]

(Continued)

Table A1 (continued)

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
	Tenebrio molitor, *Zophobas morio*	Dried powdered larvae	n/a	[181]
	Tenebrio molitor, *Zophobas morio*	Dried powdered larvae	47.0% (*T. molitor*), 49.3% (*Z. morio*)	[182]
	Bombyx mori, *Tenebrio molitor*, *Zophobas morio*	Dried powdered *B. mori* pupae, *T. molitor* larvae, *Z. morio* larvae	58.0% (*B. mori*), 63.7% (*T. molitor*), 45.8% (*Z. morio*)	[99]
Broiler quail	*Hermetia illucens*	Dried powdered larvae	23.5 and 23.7% (depending on the diet)	[183]
Cockatiels *Nymphicus hollandicus*	Cockroach *Gromphadorhina portentosa*	Crushed adults	57.8%	[184]
Japanese quail chicks	*Spodoptera littoralis*, *Bactrocera zonata*	Dried powdered larvae	51.2% (*S. littoralis*), 58.1% (*B. zonata*)	[185]
Laying chickens	*Hermetia illucens*	Dried full-fat larvae	41.1%	[186]
Laying hens	*Hermetia illucens*	Partially defatted larvae	55.6%	[187]
	Hermetia illucens	Partially defatted larvae	55.6%	[188]
	Hermetia illucens	Dried full-fat larvae and prepupae	14.6% (larvae), 16.5% (prepupae)	[189]
Laying quail	*Hermetia illucens*	Partially defatted larvae	51.8%	[79]
Meat-type ducks (Cherry Valley)	*Periplaneta americana, Hydrous cavistanum, Tenebrio molitor, Zophobas morio, Bactrocera dorsalis, Hermetia illucens, Musca domestica, Achroia grisella, Bombyx mori, Philosamia ricini, Acheta domesticus, Gryllotalpa africana, Gryllus bimaculatus, Gryllus testaceus, Locusta migratoria, Patanga succincta.*	Dried powdered *P. americana* pupae, *H. cavistanum* adults, T. *molitor* larvae, *Z. morio* larvae, *B. dorsalis* larvae, *H. illucens* prepupae, *M. domestica* larvae, *A. grisella* larvae, *B. mori* larvae, *B. mori* pupae, *P. ricini* pupae, *A. domesticus* adults, *G. africana* adults, *G. bimaculatus* adults, *G. testaceus* *adults*, *L. migratoria* adults, *P. succincta* adults	64.4% (*P. americana*), 41.9% (*H. cavistanum*), 53.0% (T. *molitor*), 42.0% (*Z. morio*), 45.2% (*B. dorsalis*), 37.9% (*H. illucens*), 54.8% (*M. domestica*), 37.6% (*A. grisella*), 61.2% (*B. mori* larvae), 50.4% (*B. mori* pupae), 64.5% (*P. ricini*), 52.8% (*A. domesticus*), 54.3% (*G. africana*), 53.3% (*G. bimaculatus*), 40.2% (*G. testaceus*), 58.5% (*L. migratoria*), 63.3% (*P. succincta*)	[190]
Muscovy ducks *Cairina moschata*	*Hermetia illucens*	Partially defatted larvae	56.7%	[191]

(Continued)

Table A1 (continued)

Animal species	*Insect species*	*Processing form*	*Protein content*	*Reference*
Turkeys	*Hermetia illucens*	Full-fat larvae	40.4%	[87]
	Tenebrio molitor, *Hermetia illucens*	Dried powdered larvae	47.0% (*T. molitor*), 42.4% (*H. illucens*)	[192]
Other animals				
Chinese soft-shelled turtles	*Hermetia illucens*	Partially defatted larvae	46%	[193]
Dogs	*Hermetia illucens*	Partially defatted larvae	21.6%	[104]
	Hermetia illucens	Larvae meal	61.8%	[103]
	Hermetia illucens	Partially defatted larvae	53.6%	[102]
	Hermetia illucens, *Musca domestica*, *Tenebrio molitor*	Dried powdered larvae	65.0% (*H. illucen*), 71.6% (*M. domestica*), 69.0% (*T. molitor*)	[194]
Finishing pigs	*Hermetia illucens*	Dried powdered prepupae	35.0%	[195]
	Hermetia illucens	Dried powdered prepupae	35.0%	[196]
Growing pigs	*Hermetia illucens*	Dried powdered larvae	46.6%	[197]
	Hermetia illucens	Partially defatted larvae	60.8%	[100]
	Alphitobius diaperinus, *Tenebrio molitor*, *Acheta domesticus*, *Gryllodes sigillatus*, *Hermetia illucens*	Dried powdered insects	62.1% (*A. diaperinus*), 50.4% (*T. molitor*), 68.1% (*A. domesticus*), 56.0% (*G. sigillatus*), 44.6% (*H. illucens*)	[101]
Rabbits	*Tenebrio molitor*, *Acheta domesticus*	*T. molitor* larvae, *A. domesticus* adults	53.1% (*T. molitor*), 64.2% (*A. domesticus*)	[88]

Note: *not available.