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#### **RFVIFW**



# **Edible Insects: Techno-functional Properties Food and Feed Applications and Biological Potential**

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#### **ABSTRACT**

Edible insects as an alternative protein source have gained consumers' attention, leading to new market possibilities. Several investigations have generated and characterized ingredients from insects and assessed their potential application in food, feed, and their biological potential. Insects are a rich source of protein, ranging from 30% to 65%. Insect derived ingredients show great potential to be added to food products. Protein isolates or concentrates, protein hydrolysates and peptides are obtained from edible insects using different methods. Insect protein technofunctional properties include water and oil-holding capacity, emulsifying, solubility and gelling properties. Depending on protein techno-functional properties, food applications can be designed to improve their development in the formulated food. Nowadays, several commercially available food and feed products are formulated including insect ingredients. However, research related to insect-derived peptides biological potential is limited. Indepth biological assays are needed to understand the potential health benefits of insects bioactive components. Potential future research could focus on the technological properties of insect proteins, oriented to the physicochemical interactions with food matrices, including sensory quality, texture and rheological properties of new food products.

#### **KEYWORDS**

Edible insects; protein; techno-functional properties; insect ingredients; insect biological potential

#### Introduction

Edible insects represent a low cost and highly available source of nutrients, principally proteins. There is an important interest in identifying new protein sources for human consumption, lately. Previous research has primarily focused on proteins from two sources: 1) animal sources, such as milk and eggs, and 2) plant sources, such as soy, pea, almond and nuts. However, in both cases, their large production is associated with high environmental impact and low production efficiency.<sup>[1]</sup>

Edible insects could be an alternative source of nutrients with low environmental impact and highprotein content (30-65%). Besides, they can be a good source of lipids (13-33%), even fiber (5-13.6%) and carbohydrates (4.5–23%). [2-4] Some insect species can cause great damage to commercial crops. In México, some insect species such as Mexican grasshopper (Sphenarium purpurascens) have reports of affectation on corn, alfalfa, squash and bean crops, generating great costs for their eradication. Typically, chemical and biological controls are used for insect management, but the first one has a lot of unwanted effects on humans, so harvesting is considered an alternative for its control. [5,6]

Some authors indicate the benefits of insect harvesting to be decrease on required land and water, reduction of greenhouse emissions, higher feed conversions and their protein can be considered of a good quality. [6,7] Other researchers have shown concern about the growing trend in insect harvesting. For instance, the collection of fabricius (Rhynchophorus phoenicis) in Cameroon requires trunk

cuts, in this way damaging the natural ecosystem. Specific regulatory practices and regulations should be considered for maintaining the ecosystem with alterations by protecting specific areas and allowing harvesting for local people to meet protein demand. [6]

Commonly collected insects include Mexican grasshopper (S. purpurascens) and agave red worm (Comadia redtenbacheri). Other insect species can be cultivated on farms including tropical house cricket (Grillodes sigillatus), grasshopper (Achetus domesticus), mealworm (Tenebrio molitor) and silkworm (*Bombyx mori*).<sup>[7,8]</sup>

Different cultures have used insects as an alternative source of nutrients. Entomophagy, defined as the human practice of eating insects, has been practiced for a long time and is uncertain when it began. Evidence of this activity has been found in passages of the Bible and the Koran. It has been proposed that paleolithic humans included insects in their diets. Mesoamerican old texts and paintings show that insect consumption was linked with royalty and insects were used as a tribute to Aztec emperors.[3,8,9]

Nowadays, people from some regions in Africa, Asia and America include edible insects as part of their regular diet. They are considered a rich source of nutrients, such as proteins and lipids. Approximately 2000 different insect species are considered edible around the world. America, followed by Asia and Africa, is the region with the highest number of edible insect species. Commonly consumed insects include Coleoptera (bugs), Lepidoptera (worms) and Orthoptera (crickets).[8,10]

In some regions of Mexico, edible insect consumption is a practice of native Mexicans that continues to actual times. It includes white agave worm (Aegiale hesperiaris), red agave worm (Comadia redtenbacheri), "escamoles" (Liometopum apiculatum), "chapulines" (Sphenarium purpurascens) and "chicatana" (Atta mexicana). Moreover, in Mexico, edible insects can be found either on street markets or gourmet restaurants. [3,11-13]

People from rural areas of Thailand include insects such as beetles in their regular diet. However, there are reports of 194 edible insect species in this country. There is evidence that China had consumed insects since ancient times, and they have documented 324 edible species. In China, insects are used in traditional medicine and consumed directly as regular food or transformed into ingredients.[14-16]

Even though several cultures have consumed insects in the past, nowadays, most Western societies are not comfortable consuming insects. Research studies have found that people associate insects with disease carriers, unhealthy and source of pathogens. [10,17-23] Other studies have researched the willingness of people from different countries to try foods with insect ingredients. For instance, Castro & Chambers (2018) found that countries with a history of entomophagy were the most willing to try insect-based foods, such as Mexico, Peru and China. On the other hand, people from countries such as India and Japan considered insect consumption a health problem, especially linked to allergic reactions. [24] Research shows that men are more willing to try whole-edible insects and their flours in several countries than women. Also, the most disgusting and offensive insects as food ingredients are cockroaches. [10,24] Besides, studies show that consumers are comfortable eating insects when they are included as powder ingredients in conventional food matrices. [25] Tan et al. (2017) related the type of food with the adequate insect incorporation, concluding that a correct food application, such as meat products, could be an acceptable way to introduce insect ingredients in food matrices. [26] Most researchers in the field agree that better knowledge of insects by consumers and a more encouraging regulatory framework for insect ingredients can lead to an optimistic scenario for increasing insect consumption.[27-29]

The most studied insect applications in food are meat products due to their sensory characteristics. Sausage-type products are strongly flavored, which allows adding insect-based ingredients, masking their remnant off-flavors. However, other applications have been explored including snacks, cookies, bakery products and food supplements. [30,31] Besides food ingredients, edible insects have been widely used for animal feed. The addition of edible insects to animal feed formulations is economically viable due to insect low-cost production and fast growth rate. [4,7,15]

Techno-functional properties of high-protein ingredients, such as water-holding capacity (WHC) and oil-holding capacity (OHC), are important attributes considered during food formulation. Mealworm (*T. molitor*) and black soldier fly (*H. illucens*) flours present high WHC and OHC. They could be used as an alternative to vegetable flours in food products. [32] Furthermore, insect proteins from silkworm (*B. mori*) and mealworm (*T. molitor*) have been reported to exert antidiabetic and antioxidant potential using *in silico* and biochemical assays. [33,34]

Companies around the world commercialize edible insects as complete insects and flours. However, it is also common to incorporate them as ingredients in food matrices such as food bars and snacks. Scarce information regarding the role of insect ingredients in food formulations and potential health benefits is available. The objective of this review was to highlight the importance of edible insects as an alternative source of proteins. The techno-functional properties of insect proteins and flours, protein extraction methods and insect ingredient applications are discussed in this work. Besides, food and feed commercial application and potential health benefits of edible insects bioactive components are addressed.

#### **Insect protein extraction**

Insect processing is a new field in food science. There is a need to develop proper methodologies for insect protein and oil extraction. Several scientific publications report different methods for insect flours production, they could be summarized in four steps including drying, defatting, milling and sieving. Once insect flours are obtained, they can be used directly as an ingredient in food formulations or in the production of insect protein concentrates or protein isolates. [14,24,32,35–38]

Several authors have reported methodologies for insect protein extraction aiming to generate protein concentrates and protein isolates (Table 1), which are similar to reported plant protein extraction methods. [42,47,48] Insect protein extraction begins with a drying and milling step to obtain flours, followed by a defatting step, then protein solubilization, mechanical separation of insoluble fractions, protein precipitation using the isoelectric point (pI) method and drying. [14,24,25,32,35,37,38,41,49]

Defatting insect flour is an important step towards achieving the highest protein extraction yield. It has been reported that non-defatted flours presented problems during processing. Furthermore, fat interacts with hydrophobic amino acids diminishing their techno-functional properties, such as the oil-holding capacity, and emulsifying properties.<sup>[32]</sup>

Commonly used methods for insect flour defatting require the use of solvents; hexane is considered to be the first alternative due to its high fat removal capacity (>90%). [32,35,42,44] Green solvents have been used for insect lipid extraction, due to environmental safety and health issues associated with organic solvents. Zhao et al. (2016) reported that ethanol in a 5:1 ratio presented similar defatting efficiency compared to other solvents. [37] Also, Gould et al. (2018) showed that ethanol is a good alternative for complete lipid removal. [45] Ethanol is considered GRAS and has a lower environmental impact and toxicological risk compared to other organic solvents, such as hexane, heptane, and isopropanol. [37]

Alkaline pH has proved to be the most reliable protein extraction method. The defatted insect flour is solubilized in a ratio ranging from 6:1 to 20:1 (v/w), with pH ranging from 9.0 to 11.0 and a final step of drying. [37,38,44] Purschke et al. (2018) obtained a protein yield of 51.7% after an alkaline extraction at pH 9.0 for *L. migratoria*. [41] In the case of *T. molitor*, a 65% of protein dry base (DB) and 59% of yield were obtained after treatments at pH 10.5 and centrifugation at 8,850 rpm. Zhao et al. (2016) obtained 70% of protein recovery using a 0.3 M NaOH (13:1) (v/w), heating at 50°C for 75 min. [37] Mintah et al. (2020) evaluated the effect on the sample/solvent ratio for *Hermetia illucen* protein extraction they found that 20:1 (v/w) ratio presented the highest protein solubility. Furthermore, they concluded that an increase in the solvent ratio leads to lower protein extraction. [46] After the protein extraction with higher pH values, in most cases, a decrease in pH is performed to precipitate them and easily remove the media in which it is solubilized. [37,44,50]

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Insect species	Extraction Method	Protein Recovery	Evaluated properties	Reference
Zophobas morio (Coleoptera:	Protein precipitation with ascorbic acid, centrifugation and freeze-	88.10%	Foamability and foam stability, gel formation	[39]
Tenebrionidae)	dried			
Alphitobius diaperinus (Coleoptera:		91.80%		
l enebrionidae)				
Acheta domesticus (Orthoptera:		89.50%		
Gryllidae)				
<i>Blaptica dubia</i> (Blattodea: Blaberidae)		86.50%		
Locusta migratoria (Orthoptera:	Fat extraction, protein extraction, filtration and freeze-dried	82.30%	Protein solubility, emulsifying activity, foamability and	[40]
Acrididae)			foam stability, gelation properties	5
Gryllodes sigillatus (Orthoptera:	Alkaline extraction (pH 11), centrifugation, precipitation at the isoelectric point centrifugation wash and freeze-dried	ND	Solubility, water holding capacity, oil holding capacity, foaming properties, emulsifying properties.	[38]
Schistocerca gregaria (Orthoptera:		ND		
Actionae) <i>Tenebrio molitor</i> (Coleoptera:		ND		
Tenebrionidae)				
Schizaphis graminum (Hemiptera:	TCA-acetone: Solvent mixing, freezing precipitation, washing with	20.4 mg/g	Electrophoresis (1-D, 2-D)	[54]
Aprilalade)	acetonie, aryling Dhand: Mixing with extraction buffer extraction in phenol precinitation	7.3 mg/a		
	in solvents, washing, drying	9/9!!! 5:		
	Multidetergent: Mixing with Urea and detergents, centrifuged, precipitate	4.79 mg/g		
	With Sulvents, washing, and alyning			[77]
<i>lenebrio molito</i> r (Coleoptera: Tenebrionidae)	Extracted at different pHs (7.0–10.5) the mealworm larvae powder was centrifuged. Soluble proteins were manually separated and freeze-dried.	65 g/100 g dry base	Protein contentProtein recovery	Ē
Locusta migratoria (Orthoptera:	Powder (Migratory locust protein flour) was purchased and treated	ND	Protein solubility, emulsifying activity, foamability and	[42]
Acrididae)	enzymatically (proteases)		roam stability, water and oil binding capacity	[32]
<i>Hermetica illucens</i> (Diptera: Stratiomyidae)	Frozen insect was milled, defatted with hexane and aqueous extraction (distilled water) and centrifugation were performed	QN	Water and oil binding capacity, emulsifying capacity and protein solubility	[35]
<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	twice and the precipitated was freeze-dried	21%		
Tenebrio molitor (Coleoptera:	Insect powder was defatted with hexane (12–48 h) and protein	78%	Yield of defatting and sonication	[32]
Tenebrionidae)	extraction was performed by sonication.			
<i>Gryllus bimaculatus</i> (Orthoptera: Gryllidae)		34%		
Bombyx mori (L epidoptera:Bombycidae)		94%		
Gryllodes sigillatus (Orthoptera: Gryllidae)		5.2- 11.7%	SDS-PAGE, degree of hydrolysis, protein solubility, foamability and foam stability, emulsifying	[25]
	heated, cooled and freeze-dried		properties	

Table 1. (Continued).				
Insect species	Extraction Method	Protein Recovery	Evaluated properties	Reference
<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	Freeze-dried insects were defatted with ethanol, its protein extracted at alkaline conditions, centrifuged, pH adjusted (4.3–4.5), washed, centrifuged and freeze-dried	70%	Water and fat absorption capacity, protein solubility, microstructure	[37]
Anastrepha Iudens (Diptera: Tephritidae)	Fresh larvae were grounded at low temperature, dispersed in water, pH adjusted to 5, centrifuged and filtered	Larvae (100%), Concentrate (68.2%), Isolate (48.5%)	Protein solubility, emulsifying and foaming capacity	[43]
Coridius viduatus (Heteroptera: Dinidoridae) Agonoscelis versicoloratus (Heteroptera: Pentatomida)	1. Mild acid and distilled water. 2. Distilled water and alkali pretreatment. 3. Hot water and alkali treatment	C. viduatus (3%) A. pubescens (3.04%)	Viscoelastic properties of gelatin	[44]
Tenebrionidae)	Lyophilized powder was ground, and lipids removal was performed with ethanol, then protein fraction was dispersed in NaOH (0.25 M), incubated, centrifuged, pH adjusted (4.3–4.5),	46 ± 3%	Interfacial tension, emulsion stability	[45]
Hermetica illucens (Diptera: Stratiomyidae)	Larvae dried by microwave and defatted with ethanol, the protein extraction was performed by solubilization in NaOH (0.25 M) and heated at different temperatures, centrifugated, washed and freeze dried.	47.68– 63.93%	Amino acid content, protein content, color, bulk density, water and oil holding capacities, nitrogen solubility, foaming properties and intrinsic fluorescence.	[46]

ND (Not declared)

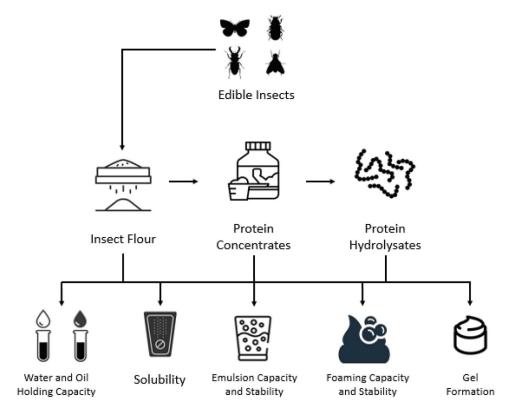


Figure 1. Techno-functional properties of edible commonly used edible insect-derived ingredients.

Alkaline extraction methods show high suitability for industrial scaling, due to the availability and low cost of basic agents such as NaOH.

Other methods such as precipitation using solvents and detergents showed low protein recovery and further studies are needed to evaluate their feasibility. Some authors have reported the use of ultrasound-assisted extraction of insect matrices for protein solubility. The results showed an improvement in protein extraction; however, more research must be performed to optimize protein conditions for maximum protein yield. [24,35,44]

Some authors have researched protein composition of edible insects. For instance, Bubler et al. (2016) evaluate the effect of different extraction methods on protein composition. It was found that an increase in extraction pH significantly affected the low molecular weight proteins obtained (98.9%). It was suggested that extreme conditions, such as high pH and the use of solvents (i.e. hexane), can lead to a partial and even complete proteolysis. [32]

Ten fractions were obtained of T. molitor, from 10 to 250 kDa SDS-PAGE bands. In this study, it was suggested that low-molecular-weight bands could be from cuticle proteins, chymotrypsin-like proteinases, other enzymes, B-glycosidase, among others. Higher molecular weight proteins could be linked to vitellogenin-like protein. [32]

Another study related the extraction method with the protein profile obtained in the wheat aphid, Schizaphis graminum. The most suitable alternatives for protein extraction and characterization were obtained with TCA-acetone and multidetergente methods. A wide molecular weight profile was found, with values ranging from 6.5 to over 200 kDa. [24]

Crickets have been studied likewise. A research showed that tropical house cricket (G. sigillatus) has proteins of different molecular weight, ranging from 14.4 to 212 kDa. On the other side, protein hydrolyzates of the same insect showed low molecular weight bands in SDS-PAGE tests; indicating that alcase is an adequate option for successful hydrolysis. [25] Other researchers have indicated that a combination of proteases (papain, Flavourzyme, Neutrase and Alcalase) with longer hydrolysis times can lead to a higher quantity of low-molecular-weight proteins. This can even be related to an increase in solubility, due to the interaction of exposed amino acids to the media. [42]

Previously, Purschkle, et al. (2018) obtained the molecular weight protein profile of L. migratoria. The protein size ranged from <6 kDa to 100 kDa. In the same work, the authors found tubulin peptide fragments and Tropomyosin. [40] These results were similar to the ones obtained for T. molitor, which also reported tropomyosin. This protein is responsible for allergic reactions in crustaceans. [51]

Insect protein identification has been scarcely studied, and an adequate recognition of specific proteins can lead to a better understanding of their future food and feed applications.

# **Techno-functional properties**

Techno-functional properties of proteins are important during food formulation or traditional ingredients substitution. In the case of edible insect proteins, few studies are available compared to traditional protein sources, such as plant proteins, due to the differences in methodologies and aims of the researches. However, some of the most studied edible insect protein techno-functional properties are solubility, emulsion capacity and stability, foamability, gel formation, water-holding capacity and oil-holding capacity (Fig. 1). [25,32,37,42,49] Solubility is an important attribute in beverages and food powders. Good emulsion capacity and stability are ideal for the meat industry; sausage formulation uses strong emulsifiers to avoid water losses. Foaming is an important characteristic for desserts and shakes. Appropriate matrix formation as a gel is necessary for dairy products such as cheese or cream to obtain the right texture and reduce syneresis. [52] The understanding of the techno-functional properties of insect proteins is important to promote their incorporation in a wider range of food products.

#### **Protein solubility**

Protein solubility is a critical functional property during food processing and new product development. Solubility refers to the arrangement of polar and non-polar groups of the protein allowing them to interact with water molecules and remains in solution. [25,53] Different factors affect the solubility of insect proteins including pH, salt concentration and the amino acid composition. [42] Insect flours, such as grasshopper (A. domesticus) and yellow mealworm (T. molitor) are highly soluble at pH 9.0 and 10.0, respectively. Furthermore, their isoelectric points were reported at pH 3.0 and 4.0, respectively. Similar behavior has been reported in pea protein, with low solubility near the isoelectric point and higher solubility at higher pH, such as 8 and 9[32,52]

Insect protein products and flours show similar solubility behavior compared to plant proteins, increasing their solubility at alkaline pH compared to acidic pH. This is due to the high concentration of acidic amino acids of insect proteins. [42] The solubility of yellow mealworm (T. molitor) and black soldier fly (H. illucens) flours has been reported at different conditions and pH, ranging from 3% to 95%. [32] Mexican fruit fly (Anastrepha ludens) protein concentrate showed a minimum solubility at pH 5.0 (8%) and a maximum solubility at pH 10.0 (95%) which is consistent with other edible insect proteins. [43] Migratory locust (Locusta migratoria) protein concentrate did not follow typical protein solubility behavior. Its solubility decreased at alkaline pH, and this behavior has been reported in proteins with a heterogeneous amino acid profile. [40] Compared to common proteins, such as soy (~40%) and bean protein concentrates (70-80%), it is found that some edible insect proteins can lead to a higher solubility if an adequate extraction method is performed.<sup>[1]</sup>

Protein hydrolysis is an important process that increases protein solubility. The reduction of the protein molecular weight during the production of protein hydrolysates and peptides increases protein solubility. Hydrolysates of tropical banded cricket (Gryllodes sigillatus) showed an increase in protein solubility due to the unfolding of the protein and the exposure of hydrophilic functional groups.



Likewise, an increment in protein solubility was observed after the hydrolysis of *G. sigillatus* proteins. The increase in solubility from 20% to 55% could be attributed to functional groups of protein fractions and peptides that were able to interact with water molecules. [25,41]

#### Water and oil-holding capacity

The water-holding capacity (WHC) is the amount of water that can be absorbed by food polymers (i.e. proteins and carbohydrates). This can be influenced by various factors, such as amino acid profile, protein conformation, hydrophobicity, pH, temperature, protein concentration, among others. [54] T. molitor flours and protein isolates are the most studied insect-based ingredients. Several authors have reported WHC values ranging from 0.8 g/g to 3.95 g/g. [32,37,38] Similar results have been reported for B. mori flours and protein isolates (Zielińska et al., 2018). [38] This research group concluded that due to differences in protein profile, each insect protein should be evaluated individually. Yellow mealworm (T. molitor) protein isolate WHC (3.95 g/g) was higher compared to insect flour (1.29 g/g). Other insects such as desert locust (Schistocerca gregaria) present similar WHC in protein isolates and flours (2.18 and 2.31 g/g, respectively). [38] Nevertheless, plant proteins seem to still have higher WHC values compared to insects, with results from studies from 1.5 to 6 g/g. [1]

Insect protein hydrolysates exert good water-holding capacity; this could be due to water molecules that interact with the hydrophilic amino acid residues of the proteins. Also, it is being reported that flours have greater WHC compared to protein concentrates. T. molitor flour presented higher WHC (0.8 g/g) compared to its protein concentrate (0.4 g/g). This could be due to the effect of carbohydrates and other polymers on insect flours. [32,41]

The oil-holding capacity (OHC) is described as the physical entrapment of lipids in the food polymers (proteins and carbohydrates) and it is affected by the concentration of non-polar or hydrophobic amino acids present in the protein chain. [37,38] Insect protein concentrates, protein isolates and flours present different OHC. B. mori flour OHC ranged from 2.5 to 2.8 g/g. T. molitor presented an OHC of 1.71 g/g for insect flour and 3.95 g/g for insect protein isolate. [38,44] Similarly, Bubler et al. (2016) reported that protein isolates of T. molitor presented higher OHC compared to insect flours and defatted flours. [32] Enzymatic treatments have been shown to enhance the OHC. For instance, T. molitor presented an OHC of 2.33 g/g after enzymatic hydrolysis. [48]

Plant proteins have lower OHC compared to insect proteins, as the obtained values for the first ones range from 1 to 2 g/g compared to some insects, such as T. molitor with OHC of 2-4 g/g. [1,37,44] This behavior can be attributed to the amino acid composition of each specie.

#### **Emulsion capacity**

The emulsion capacity (EC) is the maximum amount of lipids that can be linked to an amphiphilic molecule under certain conditions. In the case of proteins, the ratio between hydrophilic and hydrophobic amino acids and the secondary structure of the protein are key factors for EC. The exposure of hydrophobic amino acids after protein denaturation allows their interaction with lipid molecules, enhancing its EC. [32,40] Protein hydrolysis using enzymatic treatments (Alcalase®, papain, Neutrase®) improves the emulsion capacity. L. migratoria protein increased their EC after enzymatic hydrolysis. The increment in EC was due to the breakdown of large protein molecules and the exposure of the hydrophobic amino acids. [40] Also, tropical banded cricket (G. sigillatus) protein after enzymatic cleavage leads to a higher EC (27-32%). [45] Protein hydrolysis must be controlled because when it is excessive, emulsifying properties are reduced, due to interfacial absorptivity and new molecular rearrangements of the generated protein fractions and peptides. [40]

pH is one of the most important parameters for emulsion capacity of proteins. Also, ionic strength, temperature, protein concentration and proportion of amino acids affect considerably this functional property. In the case of *L. migratoria*, the highest EC (55.19%) was observed at pH 5.0 and 3% NaCl. At different NaCl concentrations, insect flours and protein isolates showed high emulsion capacity and stability (up to 50%). *T. molitor* flours and protein showed EC values ranging from 65% to 66%, respectively. [41] Other insects, such as *L. migratoria* presented a wide range of EC which ranged from 7.5 to 55% in a pH-dependent manner. [40,42] In general, insect protein isolates present high EC; however, some insect proteins, such as *A. ludens* (Mexican fruit fly larvae), did not present EC properties. This could be due to amino acid composition and protein structure. [14]

#### **Foamability**

The foam formation mechanism involves the migration, unfolding, and reorganization of molecules at the air–water interface to reduce surface tension. <sup>[25,49]</sup> The importance of foamability is associated with its potential to improve texture, consistency, and appearance of foods. <sup>[37]</sup> Some of the factors that affect foam formation are protein surface hydrophobicity, specifically the presence of thiol groups and hydrophobic amino acids. Amphiphilicity is required in a protein to exert good foaming properties. *L. migratoria* flours showed no foam formation at acidic pH (<3.0) compared to pH 5.0 which presented the highest foam formation (200%). Protein hydrolysis and pH around 5.0 favors foam formation due to small peptides interactions. However, this property was improved at alkaline pH, this could be due to repulsive forces generated at pH higher than the protein pI. <sup>[42]</sup>

Other insects, such as *Zophobas morio* formed foams at different pH (3.0, 7.0 and 10.0) with a stability of 6 min. *A. domesticus* formed foams at pH 3.0 and *Blaptica dubia* at pH 5.0. Acidic pH promotes foam formation in some edible insect proteins; however, pH close to pI neutralizes the charge of some of the amino acid residues, decreasing the foam formation. [39]

Tropical banded cricket (*G. sigillatus*) protein hydrolysates prepared using a 0.5% E/S ratio (Alcalase, 30 min) presented a higher foaming capacity (155%) compared to a non-hydrolyzed control. This insect protein showed higher foaming capacity compared to other insect proteins such as pallid emperor moth (*Cirina forda*). However, the foam stability among treatments showed a reduction of 60% after 60 min. [17]

#### **Gel formation**

The gelation capacity is a measurement of the protein's ability to aggregate and form a gel, primarily due to disulfide bonds and hydrophobic interactions. The primary factors affecting gelation capacity are protein concentration, pH, ionic strength, processing, and protein denaturation. [39,40]

Limited investigation on edible insects gelling properties has been performed, mainly using protein concentrates. Yi et al. (2013) studied different edible insect protein concentrates and indicated that at low protein concentration (<3%) it was not possible to form a gel. [39] However, protein concentrations of up to 30% at neutral and alkaline pH formed gels for yellow mealworm (*T. molitor*), at lower levels for mealworm (*A. diaperinus*), superworm (*Z. morio*), house cricket (*A. domesticus*) and orange-spotted roach (*B. dubia*). Moreover, the authors reported that acid conditions were not favorable to form gels due to the closeness to the protein pI. Under these conditions, aggregates are formed upon denaturation as a result of weak electrostatic interactions between proteins. On the other hand, at alkaline pH gels are easily formed with low protein concentration. For *L. migratoria* at pH 7.2, gels were formed at 4% and 20% of protein concentration. [40] Proteins with good gel formation capacity are important during food product development in the replacement of traditionally used proteins in food formulations.

#### **Insect-based food applications**

New food trends include sustainable sources of protein for food applications. Edible insects could be a good alternative due to their low environmental impact, the reduced space required for growth. Also, insect harvesting from crops could help to diminish some farmer's economic problems.<sup>[5,8]</sup>

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Table 2. Insect based applications in the look industry.	lood industry.			
Insect	Application	Other Ingredients	Evaluated Parameters	Reference
Tenebrio molitor (Coleoptera: Tenebrionidae)	Extruded snacks	Wheat flour	Nutritional analysis, microstructure, texture, simulation gastrointestinal digestion	[65]
<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	Fermented Seasoning Sauce	Toasted rice flour, koji (Aspergillus oryzae)	Toasted rice flour, koji ( <i>Aspergillus</i> Proximate analysis, color, browning, sensory analysis oryzae)	[99]
<i>Nauphoeta cinerea</i> (Blattodea: Blaberidae)	Wheat Bread	Wheat flour, sucrose, yeast, vegetable fat, water.	Proximate analysis, crumb hardness, crumb color, sensory evaluation	[43]
Acheta domesticus (Orthoptera: Gryllidae)	Meat emulsion	Lean pork, fatback, ice	Chemical composition, technological properties (pH, protein solubility, cooking yield, and texture)	[67]
<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	Frankfurters	Pork ham, fatback, ice	Proximate composition, cooking loss, color, emulsion stability, protein solubility, texture, sensory evaluation	[88]
Sphenarium purpurascens (Orthoptera: Pyrgomorphidae)	Extruded snacks	Corn flour	Water holding capacity, oil holding capacity	[69]
Bombyx mori (Lepidoptera: Bombycidae)	Meat batter	Pork lean meat, fatback	Proximate analysis, color, pH, cooking loss, apparent viscosity, texture	[48]
Bombyx mori (Lepidoptera: Bombycidae)	Emulsion Sausages	Pork ham muscle, fatback	Proximate composition, pH, color, protein solubility, emulsion capacity, gelforming capacity	[67]
Ephemeroptera Diptera Isoptera	Crackers, muffins, meatloaf, sausages	QN.	Sensory analysis	[70]
Coridius viduatus (Heteroptera: Dinidoridae) Agonoscelis versicoloratus (Heteroptera: Pentatomida)	lce-Cream	Ice-cream mix, milk, cream, gelatin, sugar	Sensory evaluation	[44]
Hermetica illucens (Diptera: Stratiomyidae) Acheta domesticus (Orthoptera: Gryllidae) Tenebrio molitor (Coleoptera: Tenebrionidae)	Bread	Wheat flour, salt, compressed yeast	Texture, volume, color, rheological properties	[71]
ND (Not declared)				



Sensory evaluation of edible insects is an interesting topic that has been addressed lately. Some authors have described the texture of insects from soft and juicy, to hard and crispy. [55,56] And this trait depends on the insect cooking method. Caparros et al. (2016) indicate that cooking and frying achieve a crispy texture, rather than boiling cooking that promotes a juicy insect texture, which is not very palatable.

Regarding flavor, some authors have described the flavor profile of different insects. Ramos-Elorduy (1998) in her book "Creepy Crawly Cuisine: The Gourmet Guide to Edible Insects" describes categories of insects and an overall taste. For instance, ants and termites were described as sweet, almost nutty. Beetles tasted such as whole-wheat bread, cockroaches presented a mushroom flavor. Other insects such as the dragonfly were described as fishy notes and mealworms showed nutty flavor. [56] Termites flavor agrees with Nigerian reports, which reported a nutty flavor for termite (Macrotermes nigeriensis).<sup>[57]</sup>

Insects, such as stinkbugs have been described with apple flavor and the African population indicated this insect has a chili flavor. [56,58] Honeybee has been evaluated and described in terms of milky, sweet and chewy savory flavors, although these traits depend on the location of the insect. [36,59] Volatile organic compounds were identified, finding that most of these molecules were odorless alkanes and alkenes, which are used as a defense mechanism. [36] Even though there are some reports on sensory evaluation of whole-edible insects, efforts must be directed to the evaluation of flavor, odor, and texture in food applications.

Insect composition includes different molecules such as proteins, lipids, carbohydrates, minerals, and fibers. Chitin, a long-chain polymer of N-acetylglucosamine, is the most usual insoluble fiber in insects and is the main component of the insects exoskeleton. [60-63] The chitin content of edible insects depends on the development stage. [8] This fiber does not present direct allergic activity but has been reported to present immune-stimulating properties that can be linked to allergic reactions if consumed at high concentrations. Besides, chitin has poor digestibility which can decrease its nutritional value. [64] In lower concentrations, it has proven to be beneficial to human health, as it acts as a prebiotic for gut bacteria. [61,63]

Several authors have evaluated the use of edible insect ingredients in food formulations, principally in meat products such as sausages (Table 2). One of the most used edible insect ingredients is the yellow mealworm flour (Tenebrio molitor) due to its high protein (50-55% DB) and fat content (30-35% DB). [35,65] Also, house cricket (A.domesticus) was added to a meat emulsion to substitute lean pork and backfat. It showed that a replacement of 10% of backfat with the insect flour could increase the protein content and could be considered a good alternative as a non-meat-based ingredient. [67]

Other studies have reported different insect ingredients in meat products due to their strong flavor that could mask insect taste. Choi et al. (2017) used dried T. molitor larvae to substitute pork ham in Frankfurters. [68] Different treatments, including diverse percentages of mealworm flour, were used ranging from 5% to 30%. They reported that 10% insect substitution presented similar properties compared to pork ham, such as emulsion stability and overall acceptability compared to regular Frankfurters. Other studies showed that the addition of insect ingredients into meat-based products increased the nutritional value by increasing the protein content and showed good sensory characteristics compared to the original product. [29,68]

Research performed on the effect of edible insect ingredients over nutritional and sensory characteristics is extensive. However, the impact of ingredient substitution on techno-functional properties is scarce. Meat products, such as sausages and meat emulsions present different techno-functional behavior when fat is replaced by insect ingredients. [48,67,68]

The texture is one of the sensory attributes primarily affected by food products added with edible insect ingredients. A low concentration of insect protein ingredients (i.e. yellow mealworm powder or house cricket flour) can improve hardness and gumminess. Conversely, an increase in insect concentration from 10% up to 25% negatively affects texture parameters, increasing the chewiness due to more elasticity associated with the formation of protein networks in meat products. [48,67] Waterbinding properties of meat products are also affected by the addition of insect ingredients. Insect flour

Table 3. Patents including edible insect ingredients.

Publication	Publication			
number	Year	Title	Inventors	Edible insect
EP3262958	2018	Preparation made from Insect Larves and Method for the Production Thereof	Boeckx Hilde, <i>et al</i>	Morio worms (Zophobias morio), mealworms (Tenebrio molitor), lesser mealworms (Alphitobius diaperinus), wax moth (Galleria mellonella), silk moth (Bombux mori)
CN106923059 106,819,662	2017	High-protein nutritive crab feed Method for producing insect proteins and grease by efficient conversion of crop straws through multi-stage	Du Fengzhen Zhen Longyu, <i>et al</i>	Bombyx mori Hermetia illucensTenebrio molitor
CN106615935	2017	cooperation of insects Traditional Chinese medicine composition used for anti-hirtic-free breading of free-range shirkens	Zhou Suqing	Worm
WO2017066880	2017	Edible insect derived products and processes for the	Lee Cadesky	Cricket (Acheta domesticus), mealworm (Tenebrio molitor), black soldier fly
CN106962275A CN106901057	2017 2017	Inanuature and use interest Black swine culturing method Chicken feed prepared from mulberry pomace and	但珠明 Yu Huailong	iarvae ( <i>tremeta macens</i> ) and wax moth larvae ( <i>borneyx mon).</i> Worms, yellow mealworm, aphids and bees Insect powder
CN106833865	2017	preparation method thereof Non-solout type high-efficient extracting method for anaryor incart linds	Wang Cunwen, et al	QN .
CN106819630	2017	Fish Feed	Li Liurong	Insect powder
CN106689785	2017	Palatable and disease-resisting feed for gooses		Worm protein
CN106666144	707	Milking sow feed containing insect powder and preparation method of milking sow feed	Yang Huansheng, et al	Musca domestica, Hermetia illucens and Chrysomya megacephala
CN106615628	2017	Piglet conservation feed containing insect powder and preparation method of piglet conservation feed	Yang Huansheng,	Musca domestica, Hermetia illucens and Chrysomya megacephala
CN106615800	2017	Creep feed with insect powder for piglets and method for	Yang Huansheng,	Musca domestica, Hermetia illucens and Chrysomya megacephala
CN106561978	2017	prepaining creep leed Cheap snake feeding feed and fabrication method thereof Animal metrition enhancer and amplication thereof	et al Pan Shengwen Hii Wenfeng <i>et al</i>	Tenebrio molitor Tanabrio molitor and Hermetia Illurens
CN106260548	2017	Compound snake feed	Zhang Songbo	Bombyx mori
CN106551175	2017	Special feed for prawn culture	Yi Dajun	Musca domostica
CN 106551188	/107	reed capable of increasing the deficious degree of siming and preparation method thereof	auo cnengii, <i>et ai</i>	Musca domesuca and suck insect
CN106260771	2017	Feed formula special for improving meat quality of fried	Luo Shihua	Green insect
CN106360067	2017	gartan siminips Quail feed	Luo Rongjuan	Gicada pupae
CN106360082	2017	ılturing	Yu Jie, Lan Peijian	Musca domestica, Tenebrio molitor and red worms, snails and cockroaches
WU201/01/635	7107	reed additive, Use thereof and Method of Feeding Poultry	Damian Jozепак, et al	Snellfordelia lateralis, Hermetia IIIucens, Gryllodes Sigiliatus and Gryllus assimilis
CN106387452 KR1020160134939	2017 2017	Meat pigeon feed Feed Additive for Fish Farming Using Insects	Luo Rongjuan Kang Seung-ho,	Worm Tenebrio molitor, Gryllus bimaculatus.
US20170318855	2017	Method and System for Spray Drying Insects	<i>et al</i> Mott, Gabe, <i>et al</i>	ON ON
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Musca domestica, Cryptotermes domesticus, Lucilia sericata, Tenebrio molitor katydids, crickets)Diptera (flies)Lepidoptera (moths and butterflies) Hermetia illucens, Musca domestica, Zophobas morio, Tenebrio molitor Blattodea (roaches, cockroaches)Orthoptera (grasshoppers, locusts, Bombyx mori, Tenebrio molitor, black mites, locusts and scorpions Cockroaches, grasshoppers, green worms, and grass mites Grasshoppers, cockroaches, and silkworm pupae Edible insect Musca domestica and Hermetia illucens Clanis bilineata tsinatauica Clanis bilineata tsingtauica Clanis bilineata tsingtauica Sphenarium purpurascens Earthworm, cockroach Tenebrio molitor Hermetia illucens Musca domestica Tenebrio molitor Fenebrio molitor Peng Changan, et al Alejandro Barragán D. Papadoyianis, Уланова Рузалия, Chen Yangcheng; Tarique Arsiwalla, Chen Chen, et al Dong Xiao, et al Gene W. Bunch Huang Yanhua, Gao Xueli, et al Ocaña, et al Su Zhaoshang Su Zhaoshang Su Zhaoshang Inventors Song Fumei Chen Wei Li Zhihua Li Zhihua Cai Xisen et al et a et al Production and processing of insects for transformation into Ernest High-protein nutritious noodles and making method thereof Pig full-price compound feed containing insect protein and Method to Convert Insects or Worms into Nutrient Streams Edible insect albumen powder and the production process Sphenarium purpurascens Ch. y Glycine max cubierto con itopenaeus vannamei compound feed prepared based on conversion of hermetia illucens larvas of kitchen waste Amaranthis hypochondriacus adicionado con harina de High-protein noodles and production method thereof Health-care food for female pregnancy preparation Blue peafowl feed and processing method thereof Pig feed capable of improving fragrance of pork Method for extracting Tenebrio molitor oil Preparation method of fiber health food protein meal for fish and animal diets and Compositions Obtained Thereby Milk Substitute Production Method pig meat after feeding thereof Healthcare feed for finless eels Health food for male fertility and application thereof Dry Pet Food **Dry Cat Food** Poultry Feed chocolate. Fish Food Publication 2016 2016 2016 2016 2016 2017 2017 2016 2016 2016 2016 2016 2016 2015 2015 2014 2012 2008 1997 Year US20080075818 **JS20150374005** MXMX/A/2009/ RU0002557402 CN105475660 CN106172759 CN106173466 CN105614030 CN106172812 CN107410707 CN107348349 CN106107127 CN106071388 CN106036246 CN105995122 CN105942197 CN106174487 CN106118847 CN101116471 US5618574A **Publication** 001344 number

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Reference	[73]	[74]	[75]	[76]	<u>[</u>	[78]	[57]	[62]	[26]	[80]	[81]	[82]
Evaluated Parameters	Proximate analysis, palatability trial, digestion trial	Weight gain, feed consumption, feed efficiency, mortality rate, length	Proximate analysis, growth performance, feed efficiency, plasma metabolites, digestibility coefficients, activity of specific enzymes	Proximate analysis, Growth performance (mortality, dry matter intake, weight gain, specific growth rate, feed conversion, protein efficiency, feeding rate)	Proximate analysis, growth performance, hematological and serum parameters	Proximate analysis, apparent digestibility, apparent metabolizable, energy, amino acid apparent ileal digestibility	Growth performance, morphometric analysis, organoleptic traits, proximate analysis	Hepatosomatic index, intraperitoneal fat ratio, muscle ratio, muscle composition, fatty acid composition	Proximate analysis, growth performance, feed utilization, weight gain, mortality rate	Chemical composition, growth performance, somatic index, apparent nutrient digestibility, digestive enzyme activity, total bile acids level	Proximate composition, floatability, expansion ratio, surface area and volume, bulk density, pellet durability index, water absorption index and water solubility index, water stability and sinking behaviour.	Proximate analysis, diameter, and floatability of pellets.
Other Ingredients	Soybean, corn, swine grease, mineral premix, vitamin premix	Fat, poultry by-product, soybean, milled rice, distiller dried grains with solubles alfalfa, gluten, and wheat middlings	Gluten (corn, wheat), fish oil, vitamin and mineral premix, agar, soybean, binder, taurine, calcium diphosphate, rellulose	Gluten (corn, wheat), Vitamin and mineral premix, Starch, Fish oil	Corn, soybean, gluten, vitamin-mineral premix	Maize, soybean, soybean oil, dicalcium phosphate, calcium carbonate, sodium chloride, sodium bicarbonate, vitamin, and mineral premix	Herring, gelatinized starch, fish oil, vitamin, and mineral premix, carboxymethylcellulose	Anchovy, gluten, soybean, gem gel, fish oil, poultry fat, vitamin and mineral premix, taurine, dicalcium, phosphate, amino acids	Fish meal, groundnut cake soybean, corn, vitamin/ mineral premix, palm oil, chromic oxide, salt	Fish meal, soy protein concentrate, wheat gluten, fish oil, rapeseed oil, Vitamin, and mineral premix	Sunflower cake, maize germ, wheat pollard, dried freshwater shrimps and dried cassava chunks	Wheat flour, vitamin and mineral premix.
Application	Meal supplement for ewine	Feed for channel catfish and blue tilania	Feed for European	Feed for European Seabass	Free-range chickens meal	Broiler chickens meal	Blackspot sea bream meal	Rainbow trout meal	African catfish fingerlings meal	Atlantic salmon meal	Extruded aquafeed pellets	Fish feed formulation
Insect	Hermetica illucens (Diptera: Stratiomyidae)	Hermetica illucens (Diptera:	Hermetica illucens (Diptera: Stratiomyidae)	Tenebrio molitor (Coleoptera: Tenebrionidae)	<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	Tenebrio molitor (Coleoptera: Tenebrionidae) Hermetica illucens (Diptera: Stratiomyidae)	<i>Tenebrio molitor</i> (Coleoptera: Tenebrionidae)	Hermetica illucens (Diptera: Stratiomyidae)	Zonocerus variegatus L. (Orthoptera: Pvrgomorphidae)	Hermetia illucens (Diptera: Stratiomvidae)	Hermetia Illucens (Diptera: Stratiomyidae) Acheta domesticus (Orthoptera:	Gryllidae) Hermetia Illucens (Diptera: Stratiomyidae)

addition has been reported to increase pH in the final product compared to meat. An increment in pH in meat product formulations could increase water loss during the cooking and drying process. [48,68]

Ayieko et al. (2010) prepared different food products including crackers, muffins, meatloaf and sausages using lake flies from families Chaoboridae and Chironomidae and termites from Termitidae family. Results showed that formulation with insect flours required the incorporation of food additives to increase the product sensory characteristics. Furthermore, insect flours addition needs to be adapted depending on the product to improve the techno-functional and sensory profile. For instance, mayfly is recommended to be used on meat products due to its strong flavor. [70] Besides, using insect flours in bakery products is a common food application. Bread made with 5% of H. illucens, A. domestica and T. molitor flours showed an increase in protein and lipid content compared to wheat control. Regardless of the displeasing perception of cockroach, fortification of bread with N. cinereal flour showed an excellent sensory profile and good acceptance among consumers. [43,71]

Other food products such as snacks and ice-cream have been formulated with a percentage substitution of their regular ingredients with insect-based ingredients. For instance, extruded snacks using with up to 15% of Sphenarium purpurascens flour showed acceptable sensory characteristics and good process effectivity. [69,72] Also, T. molitor flour addition improved extruded snack technological properties. Microstructure, expansion and pore structure were the characteristics that presented the best results. Mariod et al. (2014) extracted gelatin of melon bug (Coridius vidautus) and sorghum bug (Agonoscelis versicoloratus) and used it as an ingredient for ice-cream production. Results showed a less appealing product compared to a commercial product. [46,65]

In extruded snacks, it is reported that the addition of insect ingredients can modify the technofunctional properties. Azzollini et al. (2018), indicated that porosity and pore size decreased with an increase in the concentration of yellow mealworm flour in the food formulation. On the other hand, Cuj-Laines et al. (2017) reported that water absorption capacity increased when Mexican grasshopper protein concentrate was added to extruded snacks. This could be due to a higher amount of proteins to interact with the water molecules. [65,69] The effect of edible insect flours on bakery products has been widely studied. However, their physicochemical and sensory characteristics are principally affected. [43,71] Water absorption capacity tends to decrease in the bakery products formulated with insect flours. Gonzalez et al. (2018) conclude that the addition of A. domestica and H. illucens flours decreased the water absorption capacity in the formulated product. This could be attributed to the particular composition of these particular insect proteins (low hydrophilic amino acids). Furthermore, bread crust color is affected by the incorporation of insect flours, for instance, darker bread is generated when adding insect flours such as N. cinerea, A. domestica and H. illucens. [43,71]

One important factor that should be taken into consideration is the adequate amino acid profile of edible insects. Ramos-Elorduy, et al. (1997) evaluated edible insects from Oaxaca region in Mexico, finding that the highest protein quality score was to Hoplophorion monograma (79%), followed by Scyphophorus acupunctatus (72%) and Polistes instabilis (72%). It was noted that this score depends on the concentration of amino acids that fulfill the requirements indicated by the World Health Organization (WHO). Interesting information regarding this study is that a large number of insects lack or contain limited concentrations of tryptophan. [2]

The incorporation of edible insect ingredients into food products as flours represents a challenge. Different studies suggest that low concentrations of insect flours could be incorporated into bakery and meat products without important sensory modifications. Higher concentrations can lead to modification of the final product sensory profile, including the color of the crust in bread and hardness and chewiness in meat products. [43,48,65,67,71] The substitution of wheat flour with gluten-free flours such as edible insect flours complicates bread and bakery product formulation. [29,43,71] Furthermore, consumers tend to dislike food products formulated with edible insect ingredients when they realize they are included in the food formulation. [29,43,67,71] This could be considered the biggest challenge to overcome in edible insect food products. More information related to edible insect importance should be diffused among consumers to increase edible insect ingredients overall acceptability.

 Table 5. Commonly consumed edible insect pictures.

Common name	Scientific name	Picture	Reference
Black soldier fly	Hermetia illucens		[71]
House cricket	Acheta domestica		[71]
Mealworm	Tenebrio molitor	1(1(	[71]
Migratory locust	Locusta migratoria	ATTAIN	[86]
Silkworm	Bombyx mori		[26]
Mexican fruit fly	Anastrepha ludens	1 1 4 4 4 8 C	[50]



Table 5. (Continued).

Common name	Scientific name	Picture	Reference
Morio Worm/King Worm	Zophobas morio	THE REAL PROPERTY OF THE PARTY	[87]
Dubia roach	Blaptica dubia		[88]
Mexican grasshopper	Sphenarium purpurascens		Own picture

Due to the growing interest in edible insects and their applications, several companies and research centers have performed intensive investigations on edible insects. One clear example is the growth in patent publications using insects, according to the World Intellectual Property Organization (WIPO) (Table 3). Food applications are limited compared to feed applications; however, some products have been developed, such as a nutritive bar added with "chapulín" (S. purpurascens) flour or noodles enriched with protein from a mixture of insects. Companies in Mexico use grasshoppers and crickets (Orthoptera) to obtain flours and bakery products. Other enterprises in the United States are taking advantage of house cricket (A. domesticus) to produce complete insect snacks, flours, and protein bars.

Table 6. Biological potential of different insects.

Reference	[51]				[83]		[28]	[83]	[68]	[06]	(Continued)
Dose	B terrestris (1.253 mg/mL)	S. gregaria (8.157 mg/mL)	S. littoralis (0.591 mg/mL)	8. mori (0.588 mg/mL)	10 g air-dry material		5% of cricket flour	0.08 to 0.24 mg/mL	0.2– 1 mg/mL	DPPH: 0.25–6.25 mcg/mL. Hydroxyl and superoxide radical: 1.25–15 mcg/mL Antioxidant enzymes: 1.25–10 mcg/mL Cell culture: 0.4–1.2 mcg/mL)	
Assay Method	Spectrophotometric method using FAPGG as substrate. HPLC method using DTG as substrate				In vitro method: Simulation of the dog's digestive processes in the stomach, small and large intestine		Antioxidant: FRAP and ORAC, In vitro digestion: 5% of cricket flour Gastric bioreactor	ORAC and DPPH	Superoxide and hydroxyl radical scavenging activity, DPPH, reducing power and metal chelating activity	DPPH, Hydroxyl radical, superoxide radical, activities of antioxidant enzyme (rGST and rCAT). The antioxidant potential using THP-1 monocytes.	
Bio-functionality	ACE inhibitory activity				Protein quality (food supplementation) and fermentation characteristics of their indigestible fractions		Acheta domesticus Thermal processing of Antioxidant capacity, In vitro (Orthoptera: cricket flour digestibility Gryllidae)	Antioxidant activity	Antioxidant activity	Antioxidant activity	
Studied component	2 Enzymatic hydrolyzates of insect protein vs nonhydrolyzed	extract			Clean and ground insect powder		Thermal processing of cricket flour	Peptides from enzymatically hydrolyzed male silkmoth	Housefly larvae protein Antioxi hydrolysates	Aquous extract	
Insect	Spodoptera littoralis (Lepidoptera: Noctuidae)	Bombyx mori (Lepidoptera: Rombycidae)	Schistocerca gregaria (Orthoptera: Acrididae)	Bombus terrestris (Hymenoptera: Apidae)	Hermetica illucens (Diptera: Stratiomyidae) Musca domestica (Diptera: Muscidae)	(Coleoptera: Tenebrionidae)	Acheta domesticus (Orthoptera: Gryllidae)	<i>Bombyx mori</i> (Lepidoptera: Bombycidae)	<i>Musca domestica</i> (Diptera: Muscidae)	<i>Vespa affinis</i> (Hymenoptera: Vespidae)	

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Reference	[91]					[92]	[49]	[84]	[33]	[4]
Dose	BD: 5.21 mg/mL	GP: 5.62 mg/mL	LM: 5.88 mg/mL	ZM: 1.88 mg/mL	AA: 1.68 mg/mL	1 & 2 mg/mL	IC50: 102.15 µМ	IC50: 21.7 µM	IC50: 37.1 mg/mL	N/A
Assay Method	ABTS and DPPH, Iron and copper chelating activity					Expression levels of PPAR-y, C/EBPa with real time PCR. Expression levels of lipogenesis	spedit genes Skebr-1 C, LPL, SCD I & FAS. Molecular docking	Molecular docking	α-Glucosidase inhibitory activity	ABTS and DPPH
Bio-functionality	Antioxidant activity					Anti-adipogenic and antiobesity effects	ACE inhibitory activity	ACE inhibitory activity	Antidiabetic potential	Antioxidant activity
Studied component	Peptides obtained by in vitro	digestion				Ethanol extract	Silkworm pupa hydrolysate	Silkworm pupa hydrolysate	Silk Cocoon hydrolysate	Sericin extracted from Antioxidant activity cocoons
Insect	Blaptica dubia (Blattodea:	Gromphadorhina portentosa	(blattodea: Blaberidae) <i>Locusta migratoria</i> (Orthoptera:	Acrididae) <i>Zophobas morio</i> (Coleoptera:	Tenebrionidae) Amphiacusta annulipes (Orthoptera:	Gryllidae) Tenebrio molitor (Coleoptera:	lenebrionidae) Bombyx mori (Lepidoptera: Rombycidae)	Bombyx mori (Lepidoptera: Rombycidae)	Bombyx mori (Lepidoptera:	Bombyx mori (Lepidoptera:

Absorbance Capacity), **DPPH** (2,2-diphený-1-picrylhydrazyl), **rGST** (recombinant glutáthlóné S-transferase), **rCAT** (recombinant catalase) **ABTS** (2,2'-azino-bis(3-ethylbenzothlazolline-6-sulphonic acid)), **PPAR-y** (Peroxisome proliferator-activated receptor gamma), **C/EBPa** (CCAAT-enhancer-binding proteins), **PCR** (Polymerase chain reaction), **SREBP-1 C** (Sterol regulatory element-binding transcription factor 1), **LPL** (lipoprotein lipase), **SCD1** (Stearoyl-CoA desaturase-1), **FAS** (fatty acid synthase). Notes: ACE (Angiotensin-I converting enzyme), FAPGG (3-(2-Furylacryloyl)phenylalanyl-glycyl-glycine), DTG (dansyltriglycine), FRAP (Ferric-reducing ability of plasma), ORAC (Oxygen Radical



## **Insect-based feed application**

The animal feed industry has used insects as an inexpensive source of protein and other nutrients. Especially, the aquaculture industry has experienced an important growth in recent years. One of the major challenges that this industry faces is the high demand for specialized feed products. Plant-based feedstuffs are becoming unsustainable resources. Besides, marine feed sources are scarce and expensive. Therefore, the use of insect ingredients could be an adequate source of nutrients for aquaculture feed (Table 4). [36,62,74-76,79,80] It is worth mentioning that most research related to the use of edible insect ingredients in feed formulations do not focus on the texture characteristics or techno-functional properties of feed products. However, good acceptance is taken into consideration when meals are formulated.[75]

Black soldier fly (H. illucens) larva is one of the most studied insect species as a feed ingredient. This insect is used for replacing other protein sources. Insect meal could replace up to 50% of regular ingredients without affecting fish growth or sensory quality of the fillets. For catfish and blue tilapia, a 100% larvae feed resulted in insufficient protein intake for normal growth; therefore, a 10% replacement exhibited better results compared to 100%. [4,74] European bass trials showed that inclusion of 20% to 25% of H. illucens and T. molitor powder could be used with a significant improvement in growth performance, feed utilization and digestibility. [75,76] Besides, 60% of *H. illucens* in combination with fish oil can be supplemented in Atlantic salmon diet without changes in body composition.<sup>[80]</sup>

Edible insect ingredients in feed formulation represent a good source of certain amino acids, such as alanine and arginine, compared to plant-based products. Magalhaes et al. (2017) and Belghit et al. (2018) reported that Hermetia illucens flour is an adequate source of essential amino acids for fish growth. [80,83] Even though edible insect ingredients have been successfully added to aquaculture feed, there are some disadvantages, such as the lack of polyunsaturated fatty acids (PUFA), which are important nutrients in fish metabolism. [57,80]

Beyond the nutritional impact of insect ingredients on fish nutrition and growth, some authors have found a reduction in cholesterol levels in some fish species. This could be due to the presence of chitin in insect ingredients. Furthermore, chitin has been shown to be an effective prebiotic material for bifidobacterium in some fish species. [75,79]

There is scarce research related to the physicochemical and techno-functional properties of insect feed. Most studies use whole insects or ground meals with little or no processing. Pellet formation is a common process to elaborate fish meals. Pellet formation process could enhance nutrients digestibility and bioavailability. [81,84] Characteristics such as floatability, moisture content, bulk density are important during aquaculture feed development. Tze et al. (2017) evaluated fish meal pellets added with black soldier fly (H. illucens) at 10% and 50% substitution. Pellet size and protein ratio affected floatability, bigger pellets with lower protein showed the lowest floatability. [84] Besides, extruded fish meals formulated by adding black soldier fly (H. illucens) and common cricket (A. domestica) did not present significant effect on pellet floatability, expansion rate, surface, and volume. Also, the moisture content is a very important parameter that affects feed floatability. Extruded pellets with high water content show high floatability values, expansion rate, surface area and volume. [81]

H. illucens were used in swine feed supplementation, 6% to 8% replacement of the larvae meal was recommended, contributing to the amino acid profile necessary for swine growth. Furthermore, broiler chickens have been fed with other edible insects, such as T. molitor and H. illucens due to their digestible amino acid content, showing no negative effect on their productive performance or morphological features. [64,77,78] On the other hand, Bosch et al. (2016) obtained the amino acid profile for black soldier fly (H. illucens), housefly (M. domestica) and yellow mealworm (T. molitor) and evaluated their bioavailability and fermentation of indigestible fractions using a simulated dog digestive model. They found that H. illucens and T. molitor contain more valine compared to M. domestica. This study also showed that the fermentation of T. molitor residues showed the best results compared to other insects. Therefore, dog food added with T. molitor could be a nutritious alternative for dog meals.[85]

China is one of the countries with a high number of reported edible insect species. This country is leading edible insect patenting, especially on feed applications (Table 3). T. molitor and H. illucens are common insects used in feed applications. Also, insects such as M. domestica and cockroaches are used in the feed industry. In general, insect flours are used in combination with other ingredients including gums, flours from other sources, maltodextrin, etc. Some inventors have used up to 10% of insect powders in the developed products. Images of commonly consumed and utilized insects are presented in Table 5. Members of the Orthoptera order (A. domestica, L. migratoria and S. purpurascens), Coleoptera order (Z. morio and T. molitor), Diptera order (A. ludens and H. illucens), Lepidoptera order (B. mori) and Blattodea order (B. dubia) are widely used worldwide in food and feed applications as well as consumed directly dried or fried.

# Biological potential of insect protein

The biological potential of edible insect proteins has been scarcely studied. However, some authors have reported on the biological potential of insect-derived protein hydrolysates (Table 6). Silkworm pupae (B. mori) peptides showed the potential to block the angiotensin-I converting enzyme (ACE) using molecular docking tools. Peptide sequence ASL (Alanine-Serine-Leucine) showed an IC50 of 102.15 µM for ACE inhibition. Peptide sequence GNPWM (Glycine-Asparagine-Proline-Tryptophan-Methionine) presented an IC<sub>50</sub> of 21.70  $\mu$ M for ACE inhibition. [49,84]

Lee et al. (2011) reported the antidiabetic potential of edible insect protein hydrolysates. They evaluated the inhibition of  $\alpha$ -glucosidase enzyme using biochemical assays. [33] This enzyme plays an important role in complex carbohydrates degradation to monosaccharides. B. mori protein was hydrolyzed to obtain short-length peptides. Two of the peptides, GQY (Glycine-Glutamine-Tyrosine) and GYG (Glycine-Tyrosine-Glycine) showed good inhibition potential for  $\alpha$ -glucosidase with an IC<sub>50</sub> of 2.7 mg/mL and 1.5 mg/mL, respectively. Additionally, the anti-obesity potential of insect-based ingredients was evaluated using in vitro and in vivo models. T. molitor ethanol extract reduced the expression of lipogenesis-specific genes such as Sterol Regulatory Element-Binding Transcription Factor-1 (SREBP-1), lipoprotein lipase (LPL), stearoyl-CoA desaturase (SCD1) and fatty-acid synthetase (FAS).[4]

Several studies have performed antioxidant biochemical assays on flour, protein hydrolysates and peptides from different edible insects. For instance, A. domesticus flour presented antioxidant capacity in (ferric reducing antioxidant power), FRAP and oxygen radical absorbance capacity (ORAC) assays. Thermally treated flour and untreated flour obtained similar results. Concluding that using A. domesticus flour for bakery applications could present health benefits against free radicals. [69] B. mori proteins and peptides antioxidant potential have been evaluated. Silkworm protein, sericin, presented inhibition for ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. This could be due to the high hydrophilic amino acid content of this protein (up to 76%) that could have acted as electron donors. Furthermore, the protein hydrolysates and peptides from the complete B. mori proteins showed antioxidant potential in ORAC and DPPH assays. Liu, et al. (2017) studied the purified hydrolysates of B. mori, obtaining low molecular weight proteins. In most of their samples, it ranged from 3 to 30 kDa. Samples treated with gastrointestinal digestion obtained consistent protein fractions, while samples treated with proteases were hydrolyzed to peptides and amino acids. [4,33] Protein fractions and peptides obtained from B. mori were rich in glutamine, which has been linked to an antioxidant effect due to the carboxyl side, which can interact with metal ions. [83]

Other edible insect species have shown antioxidant potential. For example, housefly (Musca domestica) larvae hydrolysate and lesser banded hornet (Vespa affinis) aqueous extracts presented antioxidant potential against DPPH radical. [50,90] Peptides obtained from gastrointestinal digestion from dubia roach (B. dubia), Madagascar hissing cockroach (Gromphadorhina portentosa), migratory locust (L. migratoria), king worm (Zophobas morio) and cricket (Amphiacusta annulipes) presented

higher copper chelating activity, DPPH and ABTS radicals inhibition, compared to non-digested proteins.[91]

In most cases, protein extraction with specific proteases, such as α-amilase, pepsin, pancreatin and commercial enzymes (i.e. Flavourzyme, Alcalase, Neutrase) has shown a wide hydrolysis of the protein and short length peptides, from 3 to 50 kDa. These low molecular weight molecules can interact easily, due to the exposed amino acidic sites, with free radical and metal ions, giving this way their antioxidant activity.[34,83,91]

## Concluding remarks

Edible insects could be consumed directly or processed as ingredients in food and feed products. Edible insects represent a good source of proteins with good amino acid composition. Nowadays, entomophagy has gained popularity; however, cultural issues limit their consumption. Edible insect protein could be used as an alternative food ingredient. Nevertheless, actual protein extraction methods limit its exploitation due to low extraction yields. Insect protein techno-functional properties provide important information needed during new food product development. The addition of insect ingredients into food matrices must consider insect protein techno-functional properties, the percentage of substitution of regular ingredients and sensory attributes of final products. The correct application of insect-based ingredients in food matrices is key to achieve higher acceptance of final products. Further studies are needed for each insect species, including protein profile, protein structure, protein amino acid sequences and techno-functional properties. The biological potential of edible insect protein and peptides has been reported, including antidiabetic, antihypertensive, antiobesity and antioxidant potential. Current scientific studies are preliminary and explorative, and more in-depth research is needed to support their biological potential and to elucidate their potential mechanism of action. Insect farming should be considered as the most viable option for edible insect production since collecting implies chemical and microbiological risks. Edible insect research must continue, focusing on high-scale farm production, analytical characterization, techno-functional properties and biological potential. This could generate a better understanding of edible insects and expand their potential applications in the food and feed industry.

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