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REVIEW

Biological activity and processing technologies of edible insects: a review

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Abstract The burgeoning global population growth has raised concerns regarding the expected increase in the demand for food, which could be partially tackled by identifying novel food sources. To this end, edible insects have recently attracted research interest. Several technologies for utilizing edible insect-derived proteins have been introduced; however, research into their functional utilization is insufficient. Herein, we reviewed the relevant literature on the importance of insects as food sources, extraction of edible insects, the nutritional value of insects, biological activities of components, and their applications in food industries. We summarized the studies primarily focused on the functional utilization of edible insects, suggesting that for successful incorporation and growth of edible insects in food and pharmaceutical industries, strategies to improve the extraction methods are required to explore the biological activity of edible insects. Furthermore, the awareness of edible insects with a focus on their allergens warrants consideration.

Keywords Edible insect · Biological activity · Processing technology · Food resources · Entomophagy

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Introduction

By 2050, the global population is expected to exceed 9 billion, and therefore, the demand for food will proportionally increase, requiring the need to identify new food resources (Kim et al., 2019). Proteins are crucial for physical development and maintenance of health, and they require high energy and opportunity costs for large-scale production (Nyangena et al., 2020; Park et al., 2017). Essential amino acids (EAAs) that play major roles in tissue growth, energy production, immune function, and nutrient absorption are not synthesized in the human body; even if they are synthesized, the amount is minimal, and the requirement must be fulfilled by food consumption. Animal proteins are the most effective source of EAAs (del Hierro et al., 2020). Therefore, the issues associated with agricultural land exploitation for livestock breeding and environmental pollution are expected to intensify (Choi et al., 2017; Kim et al., 2021b). However, there is a limit to the increase in protein demand with traditional livestock production methods, and it is necessary to partially address the demand using alternative meat products (da Silva Lucas et al., 2020; Mintah et al., 2020).

Consequently, the world has started to focus on securing plant-based proteins, cultured meat, and edible insect resources to protect food security (Choi et al., 2020; Kim

et al., 2021a; Park, 2021). In particular, edible insects are emerging as a substitute for high-protein food that can replace traditional animal proteins (Khampakool et al., 2020). In addition, edible insect proteins have the advantage of the requirement of reduced farmland and greenhouse gas emissions compared to traditional animal proteins and exclude the concerns about infectious diseases associated with livestock (Kim et al., 2019; Kwak et al., 2020). Therefore, edible insects have gained increasing research interest as alternative protein sources. In addition, some studies have explored the scope of using edible insects as alternative lipid sources (da Silva Lucas et al., 2020; Kim et al., 2020). Studies have also shown that steps followed in extraction procedures affect the functional properties (Kim et al., 2020) and digestibility (Lee et al., 2021) of insect proteins. Moreover, the biological activities of edible insects have been explored (di Mattia et al., 2019). Several studies have reported the antioxidant (del Hierro et al., 2020), anti-hypertensive (Hall et al., 2018), anti-cancer (Cho et al., 2019), anti-inflammatory (Baek et al., 2018), anti-obesity (Seo et al., 2017), anti-diabetic (Lacroix et al., 2019), and anti-microbial (Rahnamaeian et al., 2015) activities of edible insect proteins. Thus, edible insects are not only highly nutritious but also possess various biological activities. Accordingly, the possibility of their applications as functional food and pharmaceutical materials is increasing, while edible insect-derived proteins are emerging as an alternative resource that can replace existing proteins and lipids in the food industry.

This review summarizes various studies on edible insects that have been conducted worldwide. In particular, we have reviewed studies investigating the use of edible insects as food sources, nutritional value and biological activity of edible insects, and extraction procedure of proteins from edible insects. Finally, we have described the potential applications of edible insects in the food and pharmaceutical industries.

Edible insects as food resources: limitations and safety

Proteins improve the nutritional, technical, and functional properties of food (FAO/WHO/UNU, 1985; Kinsella and Melachouris, 1976). In particular, from a nutritional perspective, the abundance of EAAs has been used as an important index for choosing an alternative source of protein because EAAs are indispensable; however, as they cannot be synthesized in the human body, the diet supplements are the only alternatives (FAO/WHO/UNU, 1985). Owing to the projected increase in food demand, several studies have explored the viable alternatives, of which edible insects have received the most attention, as edible insect proteins have an excellent amino acid profile. It has been reported that mealworms, which are the most widely consumed insects, have an EAA composition similar to that of soybean and bovine casein (Yi et al., 2013). For example, lysine, a limited EAA in rice, wheat, and maize, is present in edible insects; therefore, edible insects are suggested as a suitable alternative source to complement the quality of plant-based food (Ghosh et al., 2017).

Proteins extracted from edible insects exert antioxidant, anti-diabetic, anti-hypertensive, anti-cancer, anti-microbial, anti-inflammatory, and immunomodulatory effects (Nongonierma and FitzGerald, 2017) compared to other food sources (Mintah et al., 2019; Sila and Bougatef, 2016; Zielińska et al., 2017). The bioactive peptides generated from edible insects through different food processing methods, including fermentation, enzymatic or chemical hydrolysis, and digestion processes (Udenigwe and Aluko, 2012), have been shown to enhance these activities (Cicero et al., 2017). Although the aforementioned activities of edible insects have not been fully explored, we speculate that in-depth studies could reveal the potential of edible insect proteins and their hydrolysates as sustainable novel protein sources for human nutrition.

Despite the multitude of beneficial health attributes, there are some limitations to using edible insects as novel food sources. In particular, food neophobia is a critical problem in incorporating edible insects into the food system. Additionally, insects in various idiomatic phrases, proverbs, and adages reflect a negative image, which might be attributed to the negative perception of edible insects (Meyer-Rochow and Kejonen, 2020). Reportedly, people who avoid unaccustomed food are more likely to be disgusted by insect consumption (la Barbera et al., 2018). Several studies have shown that the attitudes of people toward eating insects change positively (Adámek et al., 2018, Barsics et al., 2017); however, a positive attitude does not mean a preference for entomophagy. With these prejudices, Europeans feel daring, interested, and adventurous regarding the consumption of insects (Tuccillo et al., 2020). Moreover, although South Koreans eat edible insects such as grilled crickets, boiled silkworm pupae, or medicine pellet forms, they are reluctant to visit edible insect restaurants (Hwang and Choe, 2020). However, processed protein powder could be an alternative way to consume edible insects as food sources, and consumers would feel less repulsed when consuming insect protein powders rather than whole-form insects (Orkusz et al., 2020). Therefore, insect-based products could serve as a potential alternative to promote the consumption of edible insects.

Regardless of the acceptance of edible insects as food resources, safety associated with edible insects could be a major factor determining their inclusion in the human diet.

The history of entomophagy is longer than that of conventional food resources; however, the emphasis on entomophagy is in progress worldwide, including in the Western society (Tranter, 2013). Several studies have reported food safety hazards and nutritional factors associated with edible insects (Murefu et al., 2019; van Huis et al., 2013). Murefu et al. (2019) reported that processing methods, including boiling, frying, and roasting, could markedly increase the safety of edible insects. In addition, it has been shown that mycotoxins that can cause acute and chronic diseases can be produced in insect guts (Imathiu, 2020; van Huis et al., 2013). The levels of aflatoxin, the most dangerous mycotoxin in edible insects, have been reported to be higher than the regulated limit, which could be due to contamination through open and unhygienic drying conditions (Kachapulula et al., 2018). Furthermore, several studies have reported contamination of various heavy metals in edible insects; however, they have reported no additional hazards compared with conventional animal food sources (Murefu et al., 2019; Poma et al., 2017). In addition, various studies have shown that the amount of antinutrients that imposes several health concerns is not above the regulated limit, and therefore, the potential threat of antinutrients in edible insects is minor (Oibiokpa, 2017; Shantibala et al., 2014). Parasites, pathogenic microorganisms, and pesticide residues in edible insects might be problematic; however, it could be controlled when farming these insects for food but could be a problem when harvesting or hunting wild edible insects (Imathiu, 2020). Moreover, various allergens have been identified in edible insects (de Gier and Verhoeckx, 2018), and allergic reactions mainly involve tropomyosin and arginine kinase (over 70%). However, allergens present in edible insects cannot be controlled well through changes in external factors because they are an endogenous risk factor for health (Murefu et al., 2019). It has also been reported that heating and digestion cannot eliminate the potential allergic reactions of edible insects (Broekman et al., 2015). Furthermore, undeclared allergens could be a threat, even causes of death, to allergen-responsive and sensitive persons (Poms et al., 2004). For instance, people with allergic reactions to crustaceans (arthropods) have shown large potential for cross-reactivity with edible insects (Lopata et al., 2010). Therefore, the edible insect allergens should be identified and declared, and the intake of edible insects as food sources must be carefully considered.

Nutritional value of edible insects

The significance and disadvantages of edible insects and their various nutritional values are listed in Table 1. The primary use of edible insects lies in their protein content, which varies widely from 7 to 91% from fresh matter (Bukkens, 1997; Nakagaki and Defoliart, 1991; van Huis, 2016). These variations might be due to differences in species, breeding, and feeding methods. The feed conversion ratio (FCR), which measures the efficiency of an animal in converting the food provided into the desired output, of edible insects (1.7 kg for 1 kg of cricket) is lower than that of other animals (10, 5, and 2.5 kg for 1 kg of beef, pork, and chicken, respectively). Low FCR is an important factor that renders edible insects an efficient food source with lesser environmental impacts (Govorushko, 2019). Moreover, the amino acid content is also an important factor when choosing a food for protein supply, and most edible insects (> 200 species) contain the recommended level of EAAs. In addition, absorption of digested amino acids is another determining factor for selecting protein sources (Yi et al., 2016). It has been shown that > 50% of insect proteins can be digested by human intestinal conditions in vitro (Nongonierma and FitzGerald, 2017; Yi et al., 2016), indicating that edible insects could serve as an excellent alternative protein source. However, the digestibility values of insect proteins need to be recalculated in some studies because different nitrogen to protein conversion factors (Kp) were used to calculate protein content based on total nitrogen compared to conventional meat sources. The Kp of *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens* larvae is 4.76, that of the proteins extracted from these insects is 5.60, and that of conventional meat sources is 6.25 (Janssen et al., 2017; Kim et al., 2019). The abundant chitin components forming the shell of insects comprise glycoproteins, such as glucosamine, which can be identified using the Kjeldahl method (Janssen et al., 2017).

Lipids are major energy and metabolic activity sources, and essential fatty acid deficiency is associated with protein-energy malnutrition in developing countries (Smit et al., 2004). Edible insects contain ∼ 30% fat on a fresh weight basis and are rich in unsaturated fatty acids, such as linoleic acid and linolenic acid (DeFoliart, 1999). However, lipid content and fatty acid compositions are affected by the environment, sex, diet, stage of development, and species (Bukkens, 1997). Because of abundant lipid content, edible insects are suggested as an attractive alternative source of essential fatty acids (Yi et al., 2013). However, this aspect has been comparatively less explored as most studies have focused on the protein content of edible insects (Paul et al., 2017). Therefore, further studies are required to investigate the safety, application, and utilization methods of various edible insect lipids.

Carbohydrates are not only the primary sources of energy production but are also major constituents of the animal body (Asp, 1993). Although the polysaccharide content in edible insects is lesser than that of proteins and

Table 1 Significance and disadvantages of edible insects as alternative food and their nutritional value

Nutrient[a]	Content (g/100 g)	Significance as an alternative food source	Disadvantages	References
Protein	7–91	Higher feed conversion ratio than other animals Satisfy recommended essential amino acid level for humans High digestibility (> 50%)	Protein nitrogen conversion value is different from conventional animal protein sources because of the high composition of glycoproteins (e.g., chitin)	Govorushko (2019), Janssen et al. (2017), Nongonierma and FitzGerald (2017), van Huis (2016), Yi et al. (2016)
Fat	0.1–30	Rich in unsaturated fatty acids	High diversity according to sex, environment, stage, and species	Bukkens (1997), DeFoliart (1999), Yi et al. (2016)
Carbohydrate	1–10	Significant effect on humoral immunity Wound-healing	Chitin cannot be digested	Long et al. (2007), Xiaoming et al. (2010)
Micronutrient	Under 1	High amount of Fe, Cu, Zn, Ca, Na, Mn, P, vitamin B_1, B_2, B_6, D, E, K, and C	High diversity according to sex, environment, stage, and species	Bukkens (1997), Kim et al. (2019), Xiaoming et al. (2010)

[a]Dry matter was used to determine protein and micronutrient contents, and the fresh matter was used to determine fat and carbohydrate contents

lipids (1–10%), the polysaccharides in silkworm pupae have been reported to have a substantial effect on humoral immunity (Long et al., 2007; Xiaoming et al., 2010). Chitin, the primary component of exoskeletons of insects, is a long-chain polymer of N-acetyl glucosamine, a derivative of glucose used for wound healing and medical film production (Xiaoming et al., 2010).

Micronutrients constitute a smaller part of the human diet; however, their role is critical in maintaining a healthy life (Mlček et al., 2017). In edible insects, metal ions (e.g., iron, copper, zinc, calcium, sodium, and manganese), phosphorus, and vitamins, such as B_1, B_2, B_6, D, E, K, and C, are abundant, and the levels of these nutrients are similar to those in conventional meat sources (Bukkens, 1997; Kim et al., 2019; Xiaoming et al., 2010). However, these values differ widely depending on the species and origin of the analyzed insects. Overall, it can be concluded that environmental differences are essential in determining the nutritional value of edible insects.

Extraction of nutritional and functional components from edible insects

In Western culture, entomophagy is still not generally established, and consumer acceptability of edible insects is low (Yen, 2009). Thus, extracting the nutritional and functional components from edible insects and using them as food ingredients has been suggested to increase consumer acceptability (Sosa and Fogliano 2017). Therefore, protein, fat, and chitin are primarily extracted from edible insects using various methods (Table 2) to be used as functional ingredients in food and nutraceuticals (Gravel and Doyen, 2020; Okagu et al., 2020; Vercruysse et al., 2005).

Protein extraction

Insect proteins are considered the primary alternative protein resources to meet the growing demand for animal proteins worldwide because of their high nutritional value (Bruinsma 2003). Therefore, various extraction methods, such as water extraction, dry fractionation, and alkaline extraction, are used to extract high-quality proteins from different insects, and new methods (sonication and ultra-high pressure) are being developed (Melgar-Lalanne et al., 2019). The extraction rate and characteristics of insect proteins may vary depending on the insect species, sex, life stage, diet, and geographical origin (Rumpold and Schlüter, 2013a; 2013b). In particular, extraction methods are an important factor that can influence the technical functionalities of insect proteins (Zhao et al., 2016). Previously, Kim et al. (2020) reported that the proteins extracted from three different edible insects (i.e., *T. molitor*, *Allomyrina dichotoma*, and *Protaetia brevitarsis*) showed improved foam capacity by more than 40% and increased stability by more than 40 min through defatting processes with hexane. Dry fractionation diversified the chemical composition of proteins extracted from mealworm larvae (*T. molitor* L.) by increasing the fine fraction (< 500 μm), leading to

Table 2 Extraction methods for each component and their characteristics

Component	Extraction process	Characteristics of the extraction method	Insect species	References
Protein	Alkaline and sonication-assisted extraction	Improvement in foaming and emulsifying abilities	*Schistocerca grega*, *Apis mellifera*	Mishyna et al. (2019)
	Dry fractionation	Diversification of chemical composition	*Tenebrio molitor* L	Purschke et al. (2018a)
	Defatting and sonication	Improvement of the yield	*Tenebrio molitor* L., *Gryllus bimaculatus*, *Bombyx mori*	Choi et al. (2017)
	Defatting and alkaline extraction	Improvement of water and oil absorption properties	*Hermetia illucens*	Mintah et al. (2020)
	Enzymatic hydrolysis	Enhancement of emulsifying activity, foaming ability, and oil binding capacity	*Locusta migratoria* L	Purschke et al. (2018b)
Fat (oil)	Water extraction	Lower lipid yield but higher ω-3 fatty acid contents than Soxhlet and Folch extractions	*Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Blaptica dubia*	Tzompa-Sosa et al. (2014)
	Folch extraction	Higher lipid yield and ω-6 fatty acid contents than Soxhlet and water extractions except for *Blaptica dubia*	*Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Blaptica dubia*	Tzompa-Sosa et al. (2014)
	Supercritical CO_2 extraction	Higher monounsaturated fatty acid contents than Soxhlet extraction	*Acheta domesticus*	Laroche et al. (2019)
	Ultrasound-assisted extraction	Lower acid and peroxide levels and higher polyunsaturated fatty acid contents and thermal stability than Soxhlet extraction	*Clanis bilineata*	Sun et al. (2018)
Chitin	Chemical extraction	Simple and inexpensive	*Pimelia* sp.	Kaya et al. (2016)
	Deep eutectic solvent extraction	Low toxicity and biodegradability	*Hermetia illucens*	Zhou et al. (2019)

improved nutritional and functional properties as ingredients in the food matrix (Purschke et al., 2018a). Moreover, proteins from grasshopper (*Schistocerca gregaria*) and honey bees (*Apis mellifera*) improved the foaming and emulsifying abilities by 10–40% through alkaline and sonication-assisted extractions (Mishyna et al., 2019). In addition, the addition of defatting pretreatment in protein extraction offers the advantage of increasing the protein extraction yield (Choi et al., 2017; Mintah et al., 2020). Overall, it is clear that the changes in the functionalities of insect proteins by the extraction process are closely related to the alteration in surface hydrophobicity, protein charge, and protein composition according to molecular weight.

Lipid extraction

Lipids are the second-largest nutrient component in edible insects after proteins (Xiaoming et al., 2010). In particular, edible insects showed a higher value of essential fatty acids (linoleic acid and α-linolenic acid) than other sources, such as *Eucommia ulmodie*, *Salmo salar*, and *Camellia sinensis* (da Silva Lucas et al., 2020). Therefore, various methods, including water extraction, Folch extraction, Soxhlet method, and supercritical CO_2 extraction, have been used to extract these lipid components. However, unlike proteins, the extraction process does not markedly influence the fatty acid composition, but the types of lipids extracted and the extraction yield are influenced by the lipid extraction process (da Silva Lucas et al., 2020). For instance, the lipid yield from four different edible insects (*T. molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, and *Blaptica dubiawas*) increased from 19 to 60% when obtained by Folch extraction compared with water extraction and Soxhlet extraction, wherein Folch extraction improved the content of ω-6 fatty acids in lipids from *T. molitor*, *A. diaperinus*, and *A. domesticus* (Tzompa-Sosa et al., 2014). Supercritical CO_2 extraction has been reported to isolate thermally sensitive components, decrease the oxidation of solutes, produce solvent-free residues, and employ appropriate solvent characteristics (e.g., non-toxic,

non-explosive, and chemically inert) (Mariod et al., 2010; Purschke et al., 2017; Roy et al., 2006). According to a previous study, oil from *Clanis bilineata* obtained via ultrasound-assisted aqueous extraction exhibits lower peroxide, acid, and *p*-anisidine levels and higher thermal stability and polyunsaturated fatty acid contents compared with oil extracted using the Soxhlet method (Sun et al., 2018). Collectively, these studies indicate that the extraction process can alter the characteristics or types of lipids extracted from edible insects; therefore, it should be carefully selected considering the desired application.

Chitin and chitosan extraction

Currently, the most common sources of chitin are shrimp, shellfish, and crabs. However, extracting chitin from insects can be more advantageous in terms of chemical consumption, extraction method, time, and yield than marine crustaceans (Mohan et al., 2020). Chitin and chitosan, which are non-toxic, biodegradable polymers, are extracted from the exoskeletons of various edible insects. In general, chitin from insects is extracted using a simple and inexpensive chemical extraction process, including demineralization and deproteinization (Mohan et al., 2020). Recently, studies on green extraction methods to overcome the disadvantages of conventional chemical extraction methods—alterations in physicochemical properties, use of expensive chemicals in the purification process, and release of toxic effluent wastewater into the environment—have been emphasized (Dhillon et al., 2013; Huang et al., 2018; Kaur and Dhillon, 2015). For instance, various methods, such as microwave-assisted, phytoextraction, ultrasonic-assisted, and enzymatic methods, have been reported to produce chitin from marine crustaceans (Gartner et al., 2010; Gopal et al., 2019; Hongkulsup et al., 2016; Valdez-Peña et al., 2010). In particular, biological extraction methods by microorganisms, including *Bacillus subtilis*, *Lactobacillus plantarum*, and *Pseudomonas aeruginosa* K-187, have been reported to decrease chitin degradation and impurity levels of extracts (Oh et al., 2000; Rao et al., 2000; Yang et al., 2000). According to Khanafari et al. (2008), the microbial extraction process requires less energy, fewer solvents, less time, and a less complicated procedure than the chemical extraction process. Moreover, the extraction method using deep eutectic solvents (DES) is considered a green alternative because of its low toxicity and cost, ease of synthesis, and biodegradability compared to conventional methods (Paiva et al., 2014; Zhang et al., 2012). Thus, the DES extraction method has been employed to produce chitin from insects (e.g., *Hermetia illucens*) (Zhou et al., 2019), and acid detergent fiber and acid detergent lignin methods have been used for chitin production from *H. illucens* (Brigode et al., 2020). Although further research is needed, it is believed that these green extraction methods have the potential to replace chemical methods.

Biological activities of various edible insect components

Antioxidant activity

Various components of edible insects have been reported to have antioxidant activity, from hydrolysates obtained through various enzyme treatments to peptides obtained through purification and identification processes, extracts using water and organic solvents, and chitosan present in the skin of larvae (Table 3). For example, *Bombyx mori* (silkworm) larvae protein isolate obtained using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin) has been reported to have strong radical scavenging activity (IC_{50} = 57.91 μg/mL) and ferrous ion chelating activity (IC_{50} = 2.03 mg/mL) (Wu et al., 2011). di Mattia et al. (2019) also reported that water- or lipid-soluble extracts of *B. mori* have antioxidant activities, as measured using ferric reducing/antioxidant power (FRAP) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity. The extracts showed higher antioxidant activity than orange juice or olive oil, which are functional food known to regulate human antioxidant networks (Foroudi et al., 2014; Zamora-Ros et al., 2013). Adult *B. mori* (silk moth) hydrolysates obtained using alcalase and alkaline protease also exhibit high radical scavenging activity, as estimated using oxygen radical absorbance capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays (Liu et al., 2017). This study revealed no change in the DPPH radical scavenging activity of these hydrolysates in the gastrointestinal digestion test.

Furthermore, *Musca domestica* (housefly) hydrolysates obtained using alcalase and neutral proteinase showed hydroxyl, superoxide anion, DPPH radical scavenging activity, reducing power activity, and metal chelating activity (Zhang et al., 2016). Amino acid analysis of the hydrolysates identified several acidic and basic amino acids, including Glu, Asp, Arg, and Lys, which had been shown to contribute to the antioxidant activity of the hydrolysates. According to Li et al. (2017), aromatic and hydrophobic amino acids present in the water extracts of *M. domestica* contribute to the DPPH radical scavenging activity of the extract. Furthermore, it has been reported that chitosan from protein-removed *M. domestica* larvae exerted higher antioxidant activity than ascorbic acid, which was used as a positive control (Ai et al., 2008).

del Hierro et al. (2020) and Messina et al. (2019) confirmed the antioxidant activity of extracts and hydrolysates

from *T. molitor* and *A. domesticus*. Messina et al. (2019) prepared three different enzyme hydrolysates (Protamex, alcalase, and flavourzyme) using *T. molitor* and *A. domesticus*. Comparison of the DPPH radical scavenging activity of each hydrolysate confirmed that the Protamex hydrolysate had the highest antioxidant activity among the *T. molitor* hydrolysates, whereas the alcalase hydrolysate exerted the highest antioxidant activity among the *A. domesticus* hydrolysates. Messina et al. (2019) reported that the higher the degree of hydrolysis, the greater the production of low molecular weight peptides, resulting in higher DPPH radical scavenging activity. del Hierro et al. (2020) produced an extract of *T. molitor* and *A. domesticus* using water, ethanol, and their mixture. They found that DPPH radical scavenging activity increased as the total phenolic compound content of the two edible insect extracts increased. Furthermore, enzyme hydrolysate from *T. molitor* (Zielińska et al., 2017) and water and lipid extracts from *A. domesticus* (di Mattia et al., 2019) have all demonstrated antioxidant activities for scavenging free radicals.

Hall et al. (2018) and Zielińska et al. (2017) have reported the antioxidant activity of the hydrolysates obtained using gastrointestinal enzymes (e.g., pepsin, pancreatin, and α-amylase) of *S. gregaria* and *Gryllodes sigillatus*. The antioxidant activity of the hydrolysates was determined using the radical scavenging assay (DPPH and ABTS), metal chelating assay, and FRAP assay. Zielińska et al. (2017) confirmed that a hydrolysate of *S. gregaria* and *G. sigillatus* with a low molecular weight of $\leq$ 3 kDa exhibited excellent antioxidant activity and confirmed an increase in antioxidant activity according to the heat treatment of the hydrolysates. According to Hall et al. (2018), the antioxidant activity of hydrolysates increased when they were treated with gastrointestinal digestion, suggesting that digestion in vivo can help release the bioactive compounds present in the hydrolysates. Pattarayingsakul et al. (2017) identified antioxidant peptides from *Oecophylla smaragdina* hydrolysates obtained through gastrointestinal digestion followed by their separation and purification using ultrafiltration, size-exclusion chromatography, RP-HPLC, and LC–MS/MS. Finally, they isolated CTKKHKPNC peptide, which showed excellent antioxidant activity (IC_{50} values: 48.2 μM for DPPH assay and 38.4 μM for ABTS assay).

Song et al. (2013) studied chitosan isolated from *Chrysomya megacephala* (blowfly) larvae using chemical methods, including deproteinization, decolorization, decalcification, and deacetylation. Using the DPPH assay, *C. megacephala* chitosan showed excellent antioxidant activity compared to commercial chitosan, with an IC_{50} value of 1.2 mg/mL. This study confirmed that the content of active hydroxyl and amino groups in the chitosan polymer chains is related to the antioxidant activity. Additionally, it was reported that the ease of breaking the inter- and intra-hydrogen bonds due to low molecular weight would have contributed to the high antioxidant activity.

Anti-hypertensive activity

High blood pressure is a well-known major risk factor for cardiovascular diseases (Pattarayingsakul et al., 2017). Angiotensin-converting enzyme (ACE) plays a crucial role in the renin-angiotensin system. ACE converts angiotensin I into angiotensin II, inducing vasoconstriction and increasing blood pressure (Vercruysse et al., 2009a). Therefore, ACE inhibitors have been studied as anti-hypertensive therapeutic agents, and several studies on ACE inhibitory activity have been conducted to investigate the anti-hypertensive activities of edible insects (Table 3).

Studies on hydrolysates and peptides with anti-hypertensive activity have been reported in larvae and pupae of *B. mori* (Vercruysse et al., 2005; Wang et al., 2011; Wu et al., 2011; Wu et al., 2015). Vercruysse et al. (2005) hydrolyzed *B. mori* larvae with pepsin, trypsin, and α-chymotrypsin (gastrointestinal digestion) to obtain enzymatic hydrolysates. In this study, two methods (FAPGG substrate-spectrophotometric method and DTG substrate-HPLC method) were used to determine ACE inhibitory activity. After gastrointestinal digestion, the ACE inhibitory activity was greatly increased. The IC_{50} value decreased from 72.47 (non-hydrolyzed sample) to 0.69 mg/mL (gastrointestinal digestion) via DTG substrate-HPLC method. Similarly, Wu et al. (2011) estimated the ACE inhibitory activities of the hydrolysates obtained via gastrointestinal enzyme digestion using the HHL substrate-spectrophotometric method. The IC_{50} value of the hydrolysate was 8.3 μg/mL, which was close to that of captopril (5.3 μg/mL) used as a positive control. This was expected because of the increased C-terminal aromatic amino acid residue levels of the hydrolysate. It has been shown that α-chymotrypsin (classified as endo-peptidase) used for hydrolysis is known to cleave the C-terminal aromatic amino acid residue. Furthermore, Wang et al. (2011) and Wu et al. (2015) performed enzymatic hydrolysis in *B. mori* pupae. They then purified the hydrolysate following several steps (e.g., ultrafiltration, gel filtration, and RP-HPLC) to obtain specific peptides APPPKK (IC_{50} value: 47 μg/mL) and ASL (IC_{50} value: 102.15 μM) with ACE inhibitory activity. In these studies, a molecular docking analysis was conducted, and as a result, it was confirmed that the peptides were bound to the active site pocket of ACE. Furthermore, Wu et al. (2015), using the Lineweaver-Bruk plot analysis, reported that ASL inhibited ACE in a competitive inhibition pattern.

Furthermore, Vercruysse et al. (2008) and Vercruysse et al. (2009b) produced hydrolysates and peptides from *Spodoptera littoralis* (cotton leafworm) larvae and evaluated ACE inhibitory activity using the HHL substrate-spectrophotometric method. Vercruysse et al. (2008) hydrolyzed the non-water-soluble fraction of *S. littoralis* using gastrointestinal enzymes and identified a tri-peptide, AVF (IC_{50} value: 2,213 μM) with excellent ACE inhibitory activity through sequential separation and purification steps. Additionally, Vercruysse et al. (2009a) reported that when mucosal peptidase was treated after gastrointestinal digestion, the IC_{50} value decreased from 320 to 211 μg/mL, increasing ACE inhibitory activity.

The anti-hypertensive activity of peptides from *T. molitor* larvae was reported by Dai et al. (2013). In their study, the HHL substrate-HPLC method was used to determine the ACE inhibitory activity of the *T. molitor* alcalase hydrolysate. In addition, tri-peptide (YAN, IC_{50} value: 17 μg/mL) with excellent ACE inhibitory activity was isolated via separation and purification. Using this tri-peptide-containing fraction, an in vivo test was performed on a spontaneously hypertensive rat model, and it was confirmed that systolic blood pressure was reduced in a dose-dependent manner.

In addition, extracts of *M. domestica* (Li et al., 2017) and hydrolysates of *Bombus terrestris* (Vercruysse et al., 2005), *G. sigillatus* (Hall et al., 2018), and *O. smaragdina* (Pattarayingsakul et al., 2017) have been reported to have anti-hypertensive activity.

Anti-cancer and anti-inflammatory activity

Abnormal growth and proliferation of cells in the body are typical characteristics of cancer (Lee and Paik, 2019). Therefore, studies on substances that effectively inhibit the abnormal proliferation of such cancer cells are actively conducted, and several studies using food-derived proteins and peptides have been reported (Chalamaiah et al., 2018; Karami et al., 2019). In addition, inflammation has long been known to be associated with cancer pathophysiology, and it is known that the immune system, consisting of a network of cells, tissues, and organs, can effectively prevent cancer cell growth (Chalamaiah et al., 2018). Cancer immunotherapy has been reported as an important aspect of cancer research and treatment (Lee and Paik, 2019). Therefore, studies are being actively conducted to identify substances with anti-cancer and anti-inflammatory activities in edible insects (Table 3).

Several studies have reported that various components of edible insects have anti-cancer activity. Cho et al. (2019) obtained an ethanol extract from fermented *B. mori*. Briefly, the authors fermented *B. mori* for 3 days using *Aspergillus kawachii* before proceeding with extraction, followed by ethanol extraction to obtain the fermented ethanol extract. The fermented ethanol extract of *B. mori* showed higher inhibition activity of HepG2 hepatocellular carcinoma growth than the unfermented extract. The fermented extract showed increased DNA fragmentation, nucleic condensation, and an apoptotic cell population (Cho et al., 2019). In particular, the fermented extract increased the expression of pro-apoptotic proteins (Bax, and caspase-3, 8, and 9) while inhibiting the expression of the anti-apoptotic protein (Bcl-2), inducing cell apoptosis.

Chernysh et al. (2002) obtained the specific peptide alloferon 1 (HGVSGHGQHGVHG) from *Calliphora vicina* by immunization with a heat-killed bacterial mixture (*Escherichia coli* D31 and *Micrococcus luteus* A270). Alloferon 1 exhibited strong anti-cancer activity by stimulating the natural killer (NK) activity of lymphocytes in mouse spleen or human peripheral blood. It was confirmed that these simulated lymphocytes inhibited the growth of K562 tumor cells. In addition, the authors reported that in mice intranasally administered alloferon 1, interferon synthesis was effectively induced. Furthermore, it has been shown that chitosan isolated from *M. domestica* larvae has anti-cancer activity (Ai et al., 2008). Briefly, chitosan (1 mg/mL) effectively inhibited the growth of HeLa (50.8%) and S-180 (52.9%) tumor cells in an in vitro MTT assay (Ai et al., 2008).

Edible insects that have anti-inflammatory activity include *Polyrhachis dives* (Tang et al., 2015), *Lycorma delicatula* (Baek et al., 2018), *G. sigillatus, T. molitor*, and *S. gregaria* (Zielińska et al., 2017). Tang et al. (2015), using ethanol extraction and chromatography separation, obtained 13 nitrogen-containing non-peptide compounds that showed anti-inflammatory activity. The authors showed that some of these compounds effectively inhibited the proliferation of ConA-stimulated T lymphocytes isolated from mouse spleens. In addition, they claimed that the identified compound not only inhibited the production of TNF-α in RAW 264.7 macrophages induced by lipopolysaccharide (LPS) but also inhibited cyclooxygenase (COX)-1 and Jak3 kinase activities, which are associated with inflammatory responses.

Furthermore, the aqueous fraction of *L. delicatula* has been reported to show anti-inflammatory activity in LPS-induced RAW 264.7 macrophages and ConA-induced splenocytes (Baek et al., 2018). Briefly, the secretion of the pro-inflammatory cytokine, interleukin (IL)-13, was inhibited by the aqueous fraction of *L. delicatula* in splenocytes in a dose-dependent manner. In addition, the increased expression of matrix metalloproteinases (MMPs) in LPS-stimulated RAW 264.7 macrophages was decreased by treatment with *L. delicatula* aqueous fraction. These MMPs are known to induce the elevation of inflammatory mediators (growth factors, inflammatory cytokines, and

adhesion molecules) and exacerbate the inflammatory response (Hong et al., 2015).

Zielińska et al. (2017) reported that hydrolysates obtained from *G. sigillatus, T. molitor,* and *S. gregaria* showed inhibitory activity against lipoxygenase (LOX) and CoX-2. LOX and COX-2 are associated with the production of leukotrienes and prostaglandins, respectively, and overproduction of these substances can lead to dysregulated inflammatory responses (Shrivastava et al., 2017). According to Zielińska et al. (2017), the inhibitory activity of edible insect hydrolysates after the in vitro absorption process was higher than before.

Anti-obesity and anti-diabetic activity

Obesity and diabetes are metabolic disorders that cause various health problems and are the leading causes of death worldwide (Bais and Patel, 2020). In addition, obesity is a major risk factor for type 2 diabetes (Chung et al., 2014). Accordingly, research has been actively conducted to identify substances with anti-obesity and anti-diabetic activities in edible insects (Table 3).

Chung et al. (2014) and Yoon et al. (2015) reported that *A. dichotoma* (Korean horn beetle) has anti-obesity activity. Chung et al. (2014) obtained ethanol extracts of *A. dichotoma* and measured the anti-obesity activity through lipid accumulation and adipocyte-specific gene expression analysis using 3T3-L1 adipocytes. They showed that the treatment with extracts substantially inhibited intracellular lipid accumulation and reduced triglyceride content. Furthermore, extracts of *A. dichotoma* inhibited the expression of CCAAT/enhancer-binding protein (C/EBP)-α, peroxisome proliferator-activated receptor (PPAR), fatty acid synthase, adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL), and stearoyl-coenzyme desaturase-1, which are related to adipogenesis and lipogenesis. Yoon et al. (2015) studied the anti-obesity effect of *A. dichotoma* in vivo using a high-fat diet (HFD) model. Like in vitro studies, administration of *A. dichotoma* was shown to inhibit the expression of PPAR-γ, C/EBP-α, and LPL in HFD-fed mice. In addition, serum analysis showed reduced contents of triglycerides and leptin, which are positively correlated with adiposity (Yoon et al., 2015).

T. molitor larvae have been reported to exert anti-obesity activity by inhibiting adipogenesis through the AMP-activated protein kinase and mitogen-activated protein kinase signaling pathways in 3T3-L1 adipocytes and attenuating the expression of adipocyte-specific marker genes and the body weight gain in an HFD mouse model (Seo et al., 2017).

Additionally, Xia et al. (2013) reported that chitooligosaccharides isolated from *C. bilineata* larvae have hypolipidemic activity. In this study, chitin was extracted from the skin of *C. bilineata* larvae and then hydrolyzed by α-amylase to produce chitooligosaccharides. These chitooligosaccharides reduced weight gain in HFD-fed rats and increased fecal fat and cholesterol contents, indicating that chitooligosaccharides have a strong binding capacity for fat and cholesterol. In addition, via plasma lipid content analysis, it was confirmed that the content of triacylglycerol, total cholesterol, and low-density lipoprotein cholesterol was lowered, whereas high-density lipoprotein cholesterol content was increased. Thus, it was confirmed that chitooligosaccharides derived from *C. bilineata* have an excellent effect on improving blood lipid levels in HFD mice.

Edible insects that have anti-diabetic activity include *M. domestica* (Li et al., 2017), *A. diaperinus* (Lacroix et al., 2019), and *G. sigillatus* (Hall et al., 2018). These three edible insects were shown to inhibit dipeptidyl peptidase (DPP)-IV enzyme activity, a serine protease that hydrolyzes incretin hormones. DPP-IV inhibitors are effective against type 2 diabetes, as they inhibit the breakdown of the incretin hormone, which plays a major role in regulating blood glucose levels (Seshadri and Kirubha, 2009).

Lacroix et al. (2019) obtained hydrolysates of *A. diaperinus* by gastrointestinal digestion followed by hydrolysis using four enzymes (thermolysin, alcalase, flavourzyme, and papain). Among them, it was confirmed that thermolysin hydrolysate showed strong DPP-IV inhibitory activity. In contrast, Hall et al. (2018) first hydrolyzed *G. sigillatus* with alcalase, followed by a gastrointestinal digestion test to obtain the hydrolysate. In this work, the authors confirmed that gastrointestinal digestion conditions enhanced the DPP-IV inhibitory activity of the hydrolysate. Furthermore, Li et al. (2017) conducted water extraction of *M. domestica*, separated the fractions through ultrafiltration, and compared the DPP-IV inhibitory activity according to the molecular weight. It has been reported that the high molecular weight fraction of water extract has a high DPP-IV inhibitory activity. Amino acid analysis revealed that the high molecular weight fraction contained high levels of Ile, Leu, Val, Pro, Gly, Ala, and Phe amino acids. These amino acids identified in other studies reporting DPP-IV inhibitory activity (Xia et al., 2017) are speculated to contribute to the DPP-IV inhibitory activity of *M. domestica*.

Anti-microbial activity and effects on the gut microbiome

The gut microbiota is composed of a wide variety of microbes that interact with the immune system and play an important role in human health (Zong et al., 2020). Changes in the gut microbiota composition are associated with several diseases (Mahnic et al., 2020). Reportedly,

anti-microbial peptide (AMP), a defense system found in all organisms, plays a major role in host-microbe interactions (Zong et al., 2020). Since the isolation of cecropin from *Hyalophora cecropia* in 1980 (Hultmark et al., 1980), multiple AMPs have been isolated from various edible insects (Table 3).

AMPs derived from edible insects were produced through immunization with *E. coli* (Cytryńska et al., 2007; Rahnamaeian et al., 2015). Cytryńska et al. (2007) isolated and purified eight peptides from *G mellonella* larvae and determined their anti-microbial activity against Gram-positive and Gram-negative bacteria. The authors reported that seven of the eight purified peptides showed anti-microbial activity against at least one or more Gram-positive bacteria, whereas, against Gram-negative bacteria, only one purified peptide (Gm cecropin D-like peptide) had anti-microbial activity against *E. coli* D31. In addition, peptides from *Galleria mellonella* inhibited the growth of several yeast and filamentous fungi. Rahnamaeian et al. (2015) studied two AMPs, abaecin and hymenoptaecin, isolated from *B. pascuorum* and *B. terrestris*. The AMPs, when applied individually to *E. coli* D31, did not exert distinct anti-microbial activity; however, the growth of *E. coli* was effectively inhibited when both AMPs were treated simultaneously. These results suggested that anti-microbial activity exerted by the AMPs could be interdependent, where one AMP damaged the bacterial envelope, allowing the other AMP to pass through the membrane and access the intracellular target. In addition, it has been reported that AMPs isolated from *Spodoptera littoralis* (Seufi et al., 2011), *Anopheles gambiae* (Vizioli et al., 2000), and *Papilio xuthus* (Kim et al., 2010) have anti-microbial activity against various Gram-positive and Gram-negative bacteria.

To the best of our knowledge, no previous studies have directly confirmed the effects of AMP-derived edible insects on the gut microbiota. However, a few studies have reported the effects of edible insect ingestion on the gut microbiota. For instance, de Carvalho et al. (2019) studied the effect of *T. molitor* flour on the human gut microbiota using an in vitro digestion model. In this study, the in vitro digestion of *T. molitor* was performed by implementing the digestion process using α-amylase, pepsin, pancreatin, and bile solution and absorption into the small intestine through the dialysis step. The effect of the obtained sample on the gut microbiota was confirmed by measuring the growth of fecal bacteria and the production of organic acids. As a result, it was confirmed that *T. molitor* flour has a positive effect on the growth of *Bacteroidaceae* and *Prevotellaceae*, which can produce propionate, a beneficial organic salt. Additionally, the organic acid analyses performed in this study confirmed that *T. molitor* has a positive effect on the production of short-chain fatty acids (SCFAs), especially acetate and propionate (de Carvalho et al., 2019). These SCFAs help regulate intermediate and peripheral metabolism by acting as signaling molecules between the microbiota and the human host.

Stull et al. (2018), using a crossover trial study, reported that the consumption of *G. sigillatus* increased *Bifidobacterium animalis* growth, a well-known beneficial probiotic that improves gastrointestinal function, prevents diarrhea, and reduces the side effects of antibiotic use. In addition, *G. sigillatus* consumption has been reported to markedly reduce the amount of plasma TNF-α, suggesting that it may help reduce systemic inflammation.

Food application of edible insect

Various species of edible insects are consumed as food in more than 100 countries worldwide. In addition, the species of insects consumed vary from country to country (Kim et al., 2019). Edible insects have historically been cultivated or harvested in the wild and consumed in Asia, South America, Central America, and Africa, where they are considered a part of the traditional diet (Dobermann et al., 2017). However, interest in edible insects has increased in Europe and North America over the past decade (Melgar-Lalanne et al., 2019). Approximately 130 brands and companies have been identified that commercialize edible insect-based food, and several types of processed food products using edible insects, including pasta, chips, snacks, biscuits, chocolate, candy, energy bar, protein bar, protein powder, beer, bread, meatballs, burgers, and sausages, have been reported (Kim et al., 2019; Melgar-Lalanne et al., 2019). As mentioned above, edible insects have a high level of protein and a well-balanced amino acid profile. In addition, it has been confirmed that studies using proteins occupy a large part of research on the biological activities of edible insects. Therefore, it has been reported that protein occupies a large part in applying edible insects to food.

Reportedly, edible insect proteins positively affect the quality and nutritional aspects of meat products by being applied as an additive to various meat products. Kim et al. (2016) and Kim et al. (2017) manufactured emulsified meat products containing edible insect proteins. The authors reported that sausages containing edible insect protein could be manufactured without a substantial difference in quality characteristics compared to existing products. In addition, Scholliers et al. (2020) reported that cooked sausages made by partially replacing meat with insect proteins had positive effects on cooking loss. Park et al. (2017) reported that meat batter formulated with *B. mori* powder had improved nutritional value and reduced cooking loss. However, the addition of transglutaminase

Table 3 Functional bioactive components from edible insects

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
Antioxidant	*Bombyx mori* (Larvae)	Hydrolysates (IC_{50} = 57.91 μg/mL for DPPH; 2.03 mg/mL for ferrous chelating)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin)	DPPH assay Ferrous ion chelating assay Reducing power assay	Wu et al. (2011)
	Bombyx mori (Adult)	Hydrolysates	Hydrolysis using enzyme mixture (alcalase and alkaline protease)	DPPH assay ORAC assay	Liu et al. (2017)
	Bombyx mori (Not described)	Water extracts, lipid extracts	Extraction with hexane and then residue extraction with water	ABTS assay FRAP assay	di Mattia et al. (2019)
	Schistocerca gregaria (Adult)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	DPPH assay ABTS assay Ferrous ion chelating assay Reducing power assay	Zielińska et al. (2017)
	Musca domestica (Larvae)	Water extracts	Extraction with water (decoction method)	DPPH assay	Li et al. (2017)
	Musca domestica (Larvae)	Hydrolysates	Hydrolysis using alcalase or neutral proteinase	DPPH assay Superoxide anion radical assay Hydroxyl radical assay Ferrous ion chelating assay Reducing power assay	Zhang et al. (2016)
	Musca domestica (Larvae)	Chitosan (IC_{50} = 373 μg/mL for DPPH)	Extraction with chemical method (deproteinization, decolorization, decalcification, and deacetylation)	DPPH assay Reducing power assay Ferrous ion chelating assay	Ai et al. (2008)
	Gryllodes sigillatus (Adult)	Hydrolysates	Hydrolysis using alcalase and then further digestion using gastrointestinal enzymes (pepsin, bile salt, pancreatin)	DPPH assay ABTS assay FRAP assay Ferrous ion chelating assay	Hall et al. (2018)
	Gryllodes sigillatus (Adult)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	DPPH assay ABTS assay	Zielińska et al. (2017)

Table 3 continued

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
				Ferrous ion chelating assay Reducing power assay	
	Tenebrio molitor (Larvae)	Extracts	Ultrasound-assisted extraction or pressurized-liquid extraction using ethanol and ethanol: water (1:1) mixture	DPPH assay	del Hierro et al. (2020)
	Tenebrio molitor (Larvae)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	DPPH assay ABTS assay Ferrous ion chelating assay Reducing power assay	Zielińska et al. (2017)
	Tenebrio molitor (Not described)	Hydrolysates	Hydrolysis using Protamex or flavourzyme or alcalase	DPPH assay	Messina et al. (2019)
	Acheta domesticus (Adult)	Extracts	Ultrasound-assisted extraction or pressurized-liquid extraction using ethanol and ethanol: water (1:1) mixture	DPPH assay	del Hierro et al. (2020)
	Acheta domesticus (Not described)	Water extracts, lipid extracts	Extraction with hexane and then residue extraction with water	ABTS assay FRAP assay	di Mattia et al. (2019)
	Acheta domesticus (Not described)	Hydrolysates	Hydrolysis using Protamex or flavourzyme or alcalase	DPPH assay	Messina et al. (2019)
	Chrysomya megacephala (Larvae)	Chitosan (IC_{50} = 1.2 mg/mL)	Extraction with chemical method (deproteinization, decolorization, decalcification, and deacetylation)	DPPH assay	Song et al. (2013)
	Oecophylla smaragdina (Larvae)	Peptide CTKKHKPNC (IC_{50} = 48.2 μM for DPPH; 38.4 μM for ABTS)	Digestion using gastrointestinal enzyme (pepsin and trypsin) and purification with ultrafiltration, size-exclusion chromatography, and RP-HPLC	DPPH assay ABTS assay	Pattarayingsakul et al. (2017)

Table 3 continued

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
Anti-hypertensive	*Spodoptera littoralis* (Larvae)	Peptide AVF (IC_{50} = 2,123 μM)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and chymotrypsin)	ACE inhibitory activity assay using HHL substrate-spectrophotometric method	Vercruysse et al. (2008)
	Spodoptera littoralis (Larvae)	Hydrolysates Sample with gastrointestinal digestion (IC_{50} = 320 μg/mL), sample with gastrointestinal digestion and mucosal peptidase (IC_{50} = 211 μg/mL)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin) and then hydrolysis using mucosal peptidase	ACE inhibitory activity assay using HHL substrate-spectrophotometric method	Vercruysse et al. (2009a, b)
	Bombyx mori (Larvae)	Hydrolysates (IC_{50} = 0.69 mg/mL)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin)	ACE inhibitory activity assay using FAPGG substrate-spectrophotometric method DTG substrate-HPLC method	Vercruysse et al. (2005)
	Bombyx mori (Larvae)	Hydrolysates (IC_{50} = 8.3 μg/mL)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin)	ACE inhibitory activity assay using HHL substrate-spectrophotometric method	Wu et al. (2011)
	Bombyx mori (Pupae)	Peptide APPPKK (IC_{50} = 47 μg/mL)	Hydrolysis using acidic protease and purification with Sephadex gel filtration	ACE inhibitory activity assay using HHL substrate-HPLC method	Wang et al. (2011)
	Bombyx mori (Pupae)	Peptide ASL (IC_{50} = 102.15 μM)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin) and purification with ultrafiltration, gel filtration chromatography, and RP-HPLC	ACE inhibitory activity assay using HHL substrate-spectrophotometric method	Wu et al. (2015)
	Bombus terrestris (Adult)	Hydrolysates (IC_{50} = 4.96 mg/mL)	Digestion using gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin)	ACE inhibitory activity assay using FAPGG substrate-spectrophotometric method DTG substrate-HPLC method	Vercruysse et al. (2005)
	Musca domestica (Larvae)	Water extracts (IC_{50} = 430 μg/mL)	Extraction with water (decoction method)	ACE inhibitory activity assay using the fluorescent substrate-spectrophotometric method	Li et al. (2017)
	Gryllodes sigillatus (Not described)	Hydrolysates	Hydrolysis using alcalase and then further digestion using gastrointestinal enzymes (pepsin, bile salt, and pancreatin)	ACE inhibitory activity assay using HHL substrate-spectrophotometric method	Hall et al. (2018)
	Tenebrio molitor (Larvae)	Peptide YAN (IC_{50} = 17 μg/mL for ACE inhibition)	Hydrolysis using alcalase and purification with Sephadex gel filtration and RP-HPLC	ACE inhibitory activity assay using HHL substrate-HPLC method	Dai et al. (2013)

Table 3 continued

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
				In vivo test using SHR model	
	Oecophylla smaragdina (Larvae)	Peptide FFGT (IC_{50} = 19.5 μM), LSRVP (IC_{50} = 52.7 μM)	Digestion using gastrointestinal enzymes (pepsin and trypsin) and purification with ultrafiltration, size-exclusion chromatography, and RP-HPLC	ACE inhibitory activity assay using the 3HB-GGG substrate-spectrophotometric method	Pattarayingsakul et al. (2017)
Anti-cancer	*Bombyx mori* (Larvae)	Extracts	Fermentation with *Aspergillus kawachii* and extraction with ethanol	The following assay using HepG2 human hepatocellular carcinoma cells (Sulforhodamine B assay, cell cycle analysis, Annexin V staining assay, DNA fragmentation analysis, western blot analysis)	Cho et al. (2019)
	Calliphora vicina (Larvae)	Peptide HGVSGHGQHGVHG	Immunization with heat-killed *Escherichia coli* D31 and *Micrococcus luteus* A270 as well as isolation peptide from the hemolymph	NK activity assay using K562 tumor cells In vivo interferon induction test using mice	Chernysh et al., (2002)
	Musca domestica (Larvae)	Chitosan	Extraction with chemical methods (deproteinization, decolorization, decalcification, and deacetylation)	MTT assay using HeLa human cervical carcinoma and mouse sarcoma-180 tumor cells	Ai et al. (2008)
Anti-inflammatory	*Schistocerca gregaria* (Adult)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	LOX, COX-2 inhibitory activity assay using spectrophotometric method	Zielińska et al. (2017)
	Gryllodes sigillatus (Adult)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	LOX, COX-2 inhibitory activity assay using spectrophotometric method	Zielińska et al. (2017)
	Tenebrio molitor (Larvae)	Hydrolysates	Digestion using gastrointestinal enzymes (α-amylase, pepsin, pancreatin, and bile extract)	LOX, COX-2 inhibitory activity assay using spectrophotometric method	Zielińska et al. (2017)
	Polyrhachis dives (Not described)	13 nitrogen-containing non-peptide compounds	Extraction with ethanol and isolation with various chromatography	Anti-proliferation assay using T lymphocytes isolated from mouse-erived spleens	Tang et al. (2015)

Table 3 continued

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
				TNF-α production assay using RAW 264.7 macrophages	
				COX, Jak3 inhibitory activity assay	
	Lycorma delicatula (Adult)	Extracts	Extraction with water	IL-13 production assay using ConA-activated splenocytes	Baek et al. (2018)
				MMP expression assay using LPS-activated RAW 264.7 macrophages	
Anti-obesity	*Tenebrio molitor* (Larvae)	Extracts	Extraction with ethanol	The following assays using 3T3-L1 adipocytes were performed: Oil red O staining assay, triglyceride content analysis, PCR, and western blot analysis	Seo et al. (2017)
				In vivo test using high-fat diet-induced obese mouse	
	Allomyrina dichotoma (Larvae)	Extracts	Extraction with ethanol	The following assays using 3T3-L1 adipocytes were performed: Oil red O staining assay, triglyceride content analysis, PCR, and western analysis	Chung et al. (2014)
	Allomyrina dichotoma (Larvae)	Powder	Freeze-drying and grinding	In vivo test using high-fat diet-fed mouse	Yoon et al. (2015)
	Clanis bilineata (Larvae)	Chitooligosaccharides	Extraction with chemical method from larvae skin (deproteinization, dehydration, and deacetylation) and then hydrolysis using α-amylase	In vivo test using high-fat diet-fed rat	Xia et al. (2013)
Anti-diabetic	*Musca domestica* (Larvae)	Water extracts (IC_{50} = 3.52 mg/mL)	Extraction with water (decoction method)	DPP-IV inhibitory activity assay using Gly-Pro-pNA substrate-spectrophotometric method	Li et al. (2017)
	Alphitobius diaperinus (Not described)	Hydrolysates	Digestion using gastrointestinal enzymes (pepsin and pancreatin) and then hydrolysis using thermolysin or alcalase or flavourzyme or papain	DPP-IV inhibitory activity assay using Gly-Pro-AMC substrate-spectrophotometric method	Lacroix et al. (2019)
	Gryllodes sigillatus (Not described)	Hydrolysates	Hydrolysis using alcalase and then further digestion using gastrointestinal enzymes (pepsin, bile salt, and pancreatin)	DPP-IV inhibitory activity assay using Gly-Pro-pNA substrate-spectrophotometric method	Hall et al. (2018)
Anti-microbial	*Bombus terrestris and Bombus pascuorum* (Not described)	Peptides	Immunization with *E. coli* and isolation peptide from the hemolymph	*E. coli* growth inhibition assay	Rahnamaeian et al. (2015)
	Galleria mellonella (Larvae)	Peptides	Immunization with viable *E. coli* D31 and isolation peptide from the hemolymph	Colony counting assay	Cytryńska et al. (2007)

Table 3 continued

Biological activity	Edible insects (stage)	Sample	Sample preparation	Experimental method	References
				Radial diffusion assay	
Gut microbiome	*Tenebrio molitor* (Not described)	Hydrolysates	Digestion using gastrointestinal enzyme (α-amylase, pepsin, pancreatin, and bile solution) and dialysis	In vitro gut microbiota simulation model (bacterial composition analyzed by fluorescence in situ hybridization and organic acid production analyzed by GC)	de Carvalho et al. (2019)
	Gryllodes sigillatus (Not described)	Powder	Drying and roasting	Clinical crossover trial test (twenty healthy adults aged 20–48 participated)	Stull et al. (2018)

further improved the texture and physicochemical properties of meat batter, suggesting the combination of silkworm pupae and transglutaminase as a new nutritional and functional source.

Preparation of processed food products using edible insect powder as an ingredient is a widely used method to avoid the reluctance of consuming whole insects (Ordoñez-Araque and Egas-Montenegro, 2021). In addition, because of its high-protein content, low-fat content, rich dietary fiber, and rich minerals such as calcium, phosphorus, and iron, many studies have confirmed their potential as additives or substitutes for grain-based food (Osimani et al., 2018). For instance, Haber et al. (2019) produced bread with added *S. gregaria* powder, which increased the nutritional value and reported no marked difference in color, texture, and taste parameters compared to conventional bread. Similarly, Biró et al. (2019) reported that buckwheat pasta prepared from flour enriched with *B. mori* powder showed higher protein content and masked the disliked flavor imparted by buckwheat. Furthermore, Azzollini et al. (2018) prepared a snack with added *T. molitor* powder, which showed improved nutritional properties and increased digestibility of the snack. Collectively, these studies demonstrated that the addition of edible insects increased the nutritional value of the product and had compensatory advantages for existing problems. These studies (Azzollini et al., 2018; Biró et al., 2019; Haber et al., 2019) suggest that determining the appropriate amount of edible insects to add is of paramount importance to prepare a product with fortified nutrition without compromising its physicochemical properties.

In conclusion, edible insects are a promising novel protein source globally, as they have excellent nutritional composition, especially amino acids. As described in this review, when edible insects are appropriately processed, including protein, lipid, chitin, and chitosan extraction processes, they can be used in various fields as an excellent food resource. Recent studies have also reported various biological activities of edible insects, including antioxidant, anti-hypertensive, anti-cancer, anti-inflammatory, anti-obesity, anti-diabetic, and anti-microbial activities. According to the functional properties of these edible insects, they could also serve as potential novel medicinal resources.

However, despite the considerable value of edible insects, further research is needed to use edible insects in the food and pharmaceutical industries. First, the consumer safety of insect proteins must be considered. Safety problems, such as allergies to edible insects, have been reported, but in-depth studies to decipher the specific causes and control methods are still needed. Second, functional components of edible insects, such as bioactive peptides, need to be identified, and their biological activity should be validated by performing adequate in vitro and in vivo experiments prior to determining their biological activity in humans. Finally, it is necessary to establish a system to standardize the use of edible insects in the food and pharmaceutical industries. If the above problems are solved, in the future, the edible insect industry will expand.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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