

Black soldier fly larvae (*Hermetica illucens*) as a sustainable source of nutritive and bioactive compounds, and their consumption challenges

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ABSTRACT

The use of insects as a sustainable source of animal-based food and in the human diet is increasing. Special attention has been given to black soldier fly larvae (BSFL) because this insect can consume organic waste and convert organic matter into high-quality nutrients that can be used for animal feed. In addition, BSFL rapidly reproduce, can convert large volumes of biomass, can be fed with a variety of organic material, and present a high feed conversion rate. Recent studies have also indicated that BSFL have a lower environmental impact than has livestock production, because they use less water and land mass, although life-cycle assessment analysis is required confirm this. Moreover, this insect is a rich source of protein, lipids, and minerals, and may have beneficial medicinal effects due to the presence of bioactive compounds. However, BSFL as well as any other insect species, present some challenges regarding their low consumer acceptance and limited information related to their food safety. Therefore, this review aims to collect information from the current literature regarding aspects related to rearing conditions and characteristics of BSFL as a sustainable source of nutrients. In addition, it will summarise the nutritional profile, the main bioactive compounds, and the challenges with human consumption of BSFL.

Keywords: black soldier fly larvae, consumer perception, food safety, insects, nutraceutical potential, nutritional profile, rearing methods, sustainability.

Introduction

The utilisation of insects as a sustainable and secure source of animal-based food for the human diet has continued to increase in popularity in recent years (Chia *et al.* 2019). Edible insects represent a natural and renewable food resource for humans (Ramos-Bueno *et al.* 2016). In addition, the rapidly growing human population has increased the demand for protein quantities, thus, the consumption of insects as an alternative protein source is considered a future trend that has the potential to improve global food security (de Castro *et al.* 2018; Lu *et al.* 2022a). Across the world, a large population of humans consume insects as a regular part of their diet, including some countries from Latin America, Africa, Asia and Oceania (Mancini *et al.* 2019; Escalante-Aburto *et al.* 2022). Consumer acceptance and perception of edible insects continues to be hindered by prevalent disgust responses and aversion. A recent study conducted by Ros-Baró *et al.* (2022) sought to understand consumer acceptability and perception of edible insects as a novel protein source. The results showed that factors such as disgust, unfamiliarity, and concerns about food safety were the primary drivers behind avoiding insect consumption. Consequently, for the successful integration of edible insects into future diets, it is crucial to educate the public about the numerous health, environmental, and economic advantages they offer. This knowledge can play a pivotal role in fostering a greater willingness among consumers to incorporate edible insects into their regular diet. Owing to the potentially sustainable benefits of farming and utilising insects as the main dietary component, particularly to supplement or change foods and food ingredients made from beef, chicken pork, and

other livestock, are gaining increased attention even in Europe and the United States. In terms of market share, the insect protein market produced a revenue of US\$0.6 billion in 2023 and it is estimated to attain US\$3.1 billion by 2033 at a compound annual growth rate (CAGR) of 17.7% from 2023 to 2033 (Future Market Insights 2023).

Entomophagy is a term for the process of eating insects. Archaeological evidence shows that humankind has evolved into a social, intelligent species by consuming insects for a considerable part of history (Dobermann et al. 2017). The most commonly consumed insects around the world are buffalo worm (*Alphitobius diaperinus*), mealworm (*Tenebrio molitor*), super worm (*Zophobas morio*), house cricket (*Acheta domestica*), and edible silkworm (*Bombyx Mori*) (Yi et al. 2013). Worldwide, nearly 2000 species of insects are considered to be edible across 113 countries, not including the western nations (Tao and Li 2018). Numerous studies have suggested that insects are a rich source of energy, protein, fats, vitamins, and minerals and may have beneficial medicinal effects (Belluco et al. 2013; Chakravorty et al. 2016; Dobermann et al. 2017; Kim et al. 2019). In comparison to livestock such as chicken, beef, and pork, edible insects have qualities such as higher fertility, higher feed conversion efficiency, and potentially lower land, water, and carbon footprint (Gahukar 2016).

Black soldier fly larvae (BSFL) are insects that present a high feed conversion rate, short reproductive cycle, and high content of fat, protein, minerals, and vitamins (Lu et al. 2022a). In addition, BSFL are richer in lipids, protein, vitamin E, and minerals than are other edible insects (Liu et al. 2017). This insect has a dynamic fatty acid profile and is rich in C12:0 fatty acid and the fatty acid profile can be altered by rearing substrate (Sprangers et al. 2017). Moreover, BSFL contain similar or superior concentrations of essential vitamins and minerals, specifically calcium, compared with other edible insects (Sprangers et al. 2017; Wang and Shelomi 2017).

Bioactive peptides are encrypted within the protein sequence and can be released by enzymatic hydrolysis or by a fermentation process. Due to the high protein content in BSFL, bioactive peptides can be generated. The bioactive peptide has been studied among plants and animals alike to understand bioactive properties including inhibition of angiotensin converting enzyme (ACE), anticancer, antimicrobial and antioxidant properties, and many more (Wang et al. 2022). This review will describe the nutritional profile, the main bioactive compounds, and human-consumption challenges of the BSFL.

Black soldier fly: an overview

Black soldier fly (*Hermetica illucens*) is a saprophytic insect that belongs to the subfamily Hermetiinae under the order Diptera (Singh and Kumari 2019; Lu et al. 2022a). This species is distributed worldwide, prominently in tropical and warm temperate regions from Neotropical to Australasian,

Nearctic, Palaearctic, and Afro-tropical regions (Singh and Kumari 2019). Its typical life cycle includes five phases, namely eggs, larvae, pre-pupal, pupal, and adult. Black soldier flies can be consumed in either larva, pupa, or adult stages of their life cycle. BSFL are generally harvested at 15 or 16 days and then converted to feed (Barragan-Fonseca et al. 2017). The feed of black soldier flies can be based on organic wastes such as plant residues, animal manure, food waste, agricultural byproducts, and straw (Lu et al. 2022a). Nutritional changes can be observed in each phase of their life cycle and, thus, the best stage for consumption of black soldier flies is in their larvae form.

Black soldier fly is considered a clean source of food because it destroys most bacteria consumed during digestion. Even if BSFL are subjected to waste as food, they leave an extremely small trace of microorganisms in the body, which reduces the risk of food safety because lesser proportions can be easily reduced with heating/processing (Belluco et al. 2013). Consequently, black soldier fly is an insect that has the ability to convert waste into food. In addition, black soldier fly species can digest food wastes and organic materials in a way superior to any other known species of fly due to the high amylase, lipase, and protease activities in its intestine extracts (Kim et al. 2011). Moreover, BSFL consume approximately twice their body mass, resulting in a positive conversion of biomass. For example, while 10 kg of feed is required to get 1 kg of beef, only 3 kg of feed is required to get 1 kg BSFL with nearly 30–54% protein content (Caligiani et al. 2018).

This species of insect is particularly interesting due to its efficacy in the conversion of organic waste (e.g. cow, pig, and poultry manure, poultry feed, fruit, and vegetable waste) into nutritiously valuable insect protein that might be used as an alternative to expensive protein-rich feed in diet of different animal species (Čengić-Džomba et al. 2020). Therefore, BSFL have been used to feed diverse animals, such as swine (Ipema et al. 2021), poultry (Ndotono et al. 2022), and fish (Goyal et al. 2021), and are being investigated as a potential ingredient to be used in pet food (Bosch and Swanson 2021). BSFL have extensive possibilities of use owing to their rapid reproduction, large biomass conversion, and present a variety of types of feed, and conversion yields (Xia et al. 2021).

Rearing methods

Because BSFL can consume organic waste, they can consume a wide variety of feed, and different feed types will change their nutrient composition and yield. As most of the organic waste streams are a loss in terms of production and economics, they can be used as a rearing substrate for BSFL. According to a recent systematic review (Hopkins et al. 2021), the most studied rearing substrates are grain-based ingredients,

(Tinder *et al.* 2017; Bava *et al.* 2019) and fruit and vegetable ingredients, (Barragan-Fonseca *et al.* 2017; Barbi *et al.* 2020), followed by animal-based ingredients, (Ewald *et al.* 2020; Gold *et al.* 2020) and generic food or kitchen waste (Nguyen *et al.* 2015; Lalander *et al.* 2019).

Typically, insects can be reared with a combination of various feed sources, including inedible primary biomass and residual agricultural processing materials. Nonetheless, the choice of insect rearing for cultivation needs to adhere to legal standards of food safety and consumer protection (Żuk-Gołaszewska *et al.* 2022). Currently, the main rearing used by the industry for BSFL includes vegetable, dairy, and bakery by-products, spent brewers' grains, and animal feeds (Barrett *et al.* 2023). The inclusion of food surplus, animal by-products manure, and a range of materials such as municipal waste, slaughterhouse waste, meat waste, or fish waste have been proposed. However, to safely utilise these substrates, it is imperative to establish quality-assurance measures (EFSA 2015; FAO 2021). The utilisation of excreta, processed waste containing animal matter, and various other organic side streams as feed for BSFL rearing is prohibited in some countries, such as USA, Canada, and those within the European Union (EU) (Alagappan *et al.* 2022). Regulations regarding the use of BSFL as animal feed state that, by default, insects cannot be directly employed as feed for other farmed animals (Żuk-Gołaszewska *et al.* 2022). The lack of information regarding safety issues associated with the cultivation of BSFL by using organic side streams is a significant factor influencing the development of regulatory frameworks (Alagappan *et al.* 2022).

The hygienic regulations governing insects intended for human consumption are specific in each country. In the USA, BSFL can be reared using approved feed-grade materials, which may include pre-consumer food waste and various by-products from food manufacturing, such as spent brewery grains and other feed-grade materials. In Canada, the authorisation requirements for rearing BSFL vary depending on the insect species, rearing conditions, and the intended livestock species, with each combination typically needing separate authorisation. In the EU regulations, insects can be reared only on substrates of vegetable origin or on materials of animal origin that are explicitly permitted, such as fishmeal and hydrolysed proteins from non-ruminants. In addition, the substrate used for feeding insects must not contain manure, catering waste, or other waste materials (Lähteenmäki-Uutela *et al.* 2021).

The most advantageous substrates based on biomass yield for BSFL composting are those that present a significant portion of easily available carbon and a fairly high protein content to assist larval growth (Lalander *et al.* 2019). In addition, if less quantity of feed is given to the larvae, lesser biomass production is observed and can be controlled by the feed provided. Substrates that contain a high content of easily available carbon, but a low content of nitrogen, reduce the effectiveness of the process, leading to a lower

yield of larval development (Lalander *et al.* 2019). For instance, fruits and vegetables, food waste, and human faeces are considered great options for substrates that provide good conditions for larval growth, and for which BSFL composting would be a good option (Banks *et al.* 2014; Nguyen *et al.* 2015; Barbi *et al.* 2020).

Sustainability

The environmental impact of food production is increasingly being brought to the forefront of sustainability discussions. The majority of debates surround the effect of greenhouse-gas emissions (GHGs) by food production and the reduction of carbon dioxide (CO₂) emissions (Dobermann *et al.* 2017). However, there are two other prime environmental factors heavily ignored that play an indirect role in GHG emission and pollution, namely water and land use. By forecasting, it is predicted that by the year 2025, at least 1.8 billion people will be living in regions with uncertain freshwater supplies and approximately two-thirds of the world population will be in regions with dwindling availability of fresh water and waste (Doreau *et al.* 2012). Freshwater is a finite resource and an estimated 70% is directly or indirectly used by the livestock and agriculture sectors (Doreau *et al.* 2012). Agriculture uses water directly to grow crops and indirectly to grow feed to produce livestock. Out of this, it has been found that the livestock sector uses nearly 70% of available agricultural land worldwide (Ooninx and de Boer 2012). Besides that, livestock production, especially cattle, accounts for a significant portion of the annual GHG emissions, ranging between 15% and 18% (CO₂ equivalent), which directly affects climate change (Boakye-Yiadom *et al.* 2022). Another factor to consider in the livestock sector is the global warming potential associated with parallel pipelines such as transport, slaughter, and storage of meat, which contribute to 17–25% of GHGs, (Ooninx and de Boer 2012; Dobermann *et al.* 2017); however, these factors are also required for insect production processes.

There is a global consensus that the biggest contributor to climate change is the production of GHGs, mainly CO₂, nitrous oxide, and methane, from fossil-fuel energy production and agricultural sector and industrial processes (Dobermann *et al.* 2017). As insects present low emissions of GHG, high feed conversion ratios, and can be produced with low water consumption, they may contribute to food security and be considered a way to help solve the meat crisis (van Huis 2013). However, when insects are reared as a source of food or feed, an impact on the environment is generated. To exemplify, the respiration and metabolism of these insects and their faeces can emit CO₂, CH₄, N₂O, and NH₃ (van Huis and Ooninx 2017). Parodi *et al.* (2021) quantified and compared nutrient balances, nutrient concentrations in residual materials, and emissions of GHG and NH₃ between manure incubated with BSFL and manure without BSFL. It was

observed that more CO₂ (247 vs 148 g/kg of DM manure) and NH₃ (7 vs 4.5 g/kg of DM manure) were emitted with than without BSFL. Methane (CH₄) was indicated as the principal contributor to GHGs, and it was produced at the same levels (1.3 vs 1.1 g/kg of DM manure) in both treatments, while N₂O was negligible in both treatments. The authors suggested that different processes might explain the temporal dynamics and occurrence of CO₂, CH₄, and N₂O emissions observed in this study. Several factors such as type of diet, larval density, and age of starter larvae at the start of the experiment can influence the CO₂ emissions (Parodi et al. 2021). Table 1 shows the gaseous emissions of BSFL when consuming different diets during its rearing.

To assess the environmental impacts of a product during its life cycle, an important tool has been used, named life-cycle assessment (LCA). In the case of food products, LCA measures the production of inputs to the agricultural process until its consumption (e.g. at home or restaurants) and waste disposal (Halloran et al. 2016). The LCA approach was used to assess the global warming potential (GWP) of BSFL in a waste-treatment facility (Mertenat et al. 2019). The authors found that the composting can double the GWP compared with the BSFL treatment facility based on the functional unit of 1 Mg of biowaste (wet weight). The major factor that contributed to the GWP was the residue post-composting (69%) and the electricity needs and source (up to 55%). On the basis of their findings, BSFL biowaste treatment might be considered as an environmental alternative with considerably low direct GHG emissions (47 times lower than from composting) and potentially a high GWP reduction. Another LCA study evaluated the direct emissions of CO₂, CH₄, N₂O and NH₃ from composting of food waste by using BSFL (Ermolaev et al. 2019). It was observed that GHG emissions from composting food waste by using BSFL were lower than those from conventional food waste treatment. The major GHG emitted was from CO₂ from food-waste degradation, with total emissions of 96 g CO₂/kg food waste treated. Emissions of CH₄ and N₂O were equivalent to 0.38 kg CO₂-eq/t food waste treated (assuming GWP over 100 years) and no NH₃ emissions were detected.

In addition, BSFL can produce sustainable compost, because its spent substrate and frass (insect faeces and

exoskeletons) can be converted into biofertiliser (Quilliam et al. 2020). Thus, mineral fertilisers that are harmful to the soil and environment can be replaced by BSFL frass fertiliser, because this has a high concentration of macronutrients (N, P, and K), micronutrients, and organic matter that are easily available for agricultural use (Amrul et al. 2022). However, a post-treatment such as drying is necessary to stabilise the BSFL frass due to the high moisture content (≥60%) of compost (Sarpong et al. 2019). The optimum performance of BSFL frass at three composting stages (freshly produced, composted with forced aeration, and naturally composted in larval rearing chambers) in biochar-based substrate cultivation was evaluated (Song et al. 2021). It was observed that plants cultivated in the composted BSFL frass treatments grew more than those in fresh frass. The LCA also was accessed in terms of GHG emission, and all three types of frasses had a lower global-warming potential than had incineration. It was indicated that the most feasible method was forced aeration when considering compost quality and environmental impact. However, the advantages of using BSFL excrement as a fertiliser still need further research to confirm its low environmental impact.

Insects perform favourably when directly compared with beef, poultry, and pigs. For instance, the total supply-chain emissions of cattle (beef and dairy) production generate 4.6 Gt, which represents a major part of global livestock emissions (Gerber et al. 2013). Other livestock species generate lower levels of emissions when considering the full lifecycle of emissions, such as pigs (0.7 Gt CO₂-eq); poultry (0.7 Gt CO₂-eq); buffalo (0.6 Gt CO₂-eq); and small ruminants (0.5 Gt CO₂-eq) (FAO 2013a, 2013b). In addition, insect cultivation also exhibits a significantly lower ecological footprint than that of field crops (Sándor et al. 2022). Overall, the environmental impact of rearing insects is considerably lower than that of livestock production. It is because insects emit fewer GHGs, and less ammonia than do cattle or pigs, and also need much less land and water (Oonincx and de Boer 2012; van Huis 2020). Thus, BSFL presents advantageous properties, including their ability to recycle nutrients and lower carbon footprint. However, a few specific studies of larger-scale production have reported lesser optimistic values and indicated that numbers are largely

Table 1. Direct gaseous emissions during black soldier fly larvae (BSFL) rearing in different studies (values are means, and are expressed per kg of dry-matter larvae).

Diet	CO ₂ (g)	CH ₄ (mg)	N ₂ O (mg)	N (g)	GWP – CO ₂ eq (g)	References
Pig manure	1956.0	10066.0	6.0	58.0	344.0	Ermolaev et al. (2019)
Food by-products	2750.0	28.0	53.0	1.2	177.0	Mertenat et al. (2019)
Food waste with rice straw	1394.0	14.0	7.0	–	2.5	Pang et al. (2020)
Kitchen food waste	–	5.5	118.0	–	35.0	Parodi et al. (2020)
Restaurant food waste	1750.0	49.0	21.0	–	8.0	Parodi et al. (2021)

Source: Parodi et al. (2021). These values correspond to the emissions during the growth of black soldier fly larvae, and do not account for feed, energy use, and processing-related emissions.

dependent on the type of feed. Because there is currently a non-uniform method for processing insects and variations among species, such equivalent values cannot be specifically assessed.

Nutritional profile

The nutrient content of BSFL will differ due to multiple factors, including the growth stages, nutritional structure ingested, duration of rearing, processing method, and factors such as temperature, humidity, sunlight, moisture content, and pH, among others (Lu *et al.* 2022a). According to the literature, BSFL show a high protein content (27–55%) and may present a large amount of fat content (8–57%). The proximate nutritional composition of BSFL is presented in Table 2.

Protein

The nutritional quality of proteins depends on the availability and digestibility of their amino acid content. Although BSFL, on average, are rich in protein, body and micronutrient composition of BSFL usually depend on the quality and quantity of food given to the BSFL (Seyedalmoosavi *et al.* 2022). For example, it was observed that BSFL reared on substrates supplemented with brewer's yeast, which has

high crude protein and ash contents, presented higher protein content than did those reared on water or yeast plus molasses-supplemented substrates (Chia *et al.* 2020). Another study indicated variations in the crude protein content of 45.3%, 44.6%, and 54.9% when the BSFL was reared with seaweed residues, dairy residues, and mixed vegetable residues respectively (DiGiacomo *et al.* 2019). Large variations across nutrient compositions of different insects can also be seen throughout the course of larval development. For instance, the protein content studied with the effects of stage of growth indicated that it decreases with an increasing age as well as stage, the highest percentage was reported for BSFL in the larvae phase (38.9%), followed by pre-pupae (31.8%) and pupae (31.3%) phases (Smets *et al.* 2020). Besides the rearing and stage of growth, other factors may influence the BSFL protein profile, such as the pre-treatments and processing conditions (e.g. dry, defatted, protein concentrate, or isolate and use of enzymes for protein hydrolysis) (Ravi *et al.* 2020). To exemplify, BSFL full-fat and defatted flours presented high protein content (45.2% and 56.1% respectively); however, the defatting process increased the protein content by ~10% (Zozo *et al.* 2022).

Amino acids

Black soldier fly larvae are rich in protein and, consequently, their amino acid profile is also considered

Table 2. Proximate composition of black soldier fly larvae (BSFL) reported by different studies (% dry basis).

Rearing substrate (RS)/drying method or material	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Chitin (%)	Reference
Different drying methods: spray-dried, oven-dried	39.48–48.20	25.69–38.36	7.26–8.27	7.41–9.96	–	Zulkifli <i>et al.</i> (2022)
Composition of BSFL meal (protein conversion factor of Kp = 4:76)	52.46	9.29	7.80	4.81	9.62	Sándor <i>et al.</i> (2022)
Different dried raw material: dried larvae raw material and dried low-fat larvae raw material	27.54–55.42	9.85–51.53	6.59–8.10	–	3.87–7.21	Traksele <i>et al.</i> (2021)
Different RS: spent barley; spent malted barley; spent malted corn; spent sorghum and barley	29.90–45.70	9.50–49.0	6.70–15.40	–	–	Chia <i>et al.</i> (2020)
Different RS: bread, fish, food waste, fresh mussels, ensiled mussels, rotten mussels, and bread and mussels (10–50%)	32.80–52.60	11.20–57.80	3.90–33.0	–	–	Ewald <i>et al.</i> (2020)
Different RS: okara, maize distillers, brewer's grains	51.20–54.10	–	4.94–11.70	–	–	Bava <i>et al.</i> (2019)
RS: vegetable mix diet (zucchini, apple, potato, green beans, carrot, pepper, orange, celery, kiwi, plum, eggplant)	39.42	35.62	7.08	–	4.02	Cappellozza <i>et al.</i> (2019)
BSF pre-pupae composition	32.00	37.10	19.00	–	9.00	Caligiani <i>et al.</i> (2018)
Vegetable diet: Diet 1, including DDGS, grape pulp, potato peels, bean seeds, cabbage leaves, and old white bread); Diet 2, including DDGS, cabbage leaves, old bread, and cellulose	46.00–47.00	20.00–32.00	–	–	–	Barragan-Fonseca <i>et al.</i> (2017)
Different % substrates: BA0 to BA100 (feeding media with 0–100% brown algae)	33.50–42.30	8.10–33.80	5.10–15.80	–	–	Liland <i>et al.</i> (2017)
BSF pre-pupae reared on food waste (vegetable, meat/fish, bread/pasta/rice)	42.00	35.00	4.56	–	–	Salomone <i>et al.</i> (2017)
BSF pre-pupae reared on food waste	43.70	31.80	6.00	10.10	–	Surendra <i>et al.</i> (2016)
Different RS with % of substrates: spent grains, beer yeast, cookie remains, potato steam peelings	38.30–46.30	24.10–33.50	–	–	–	Oonincx <i>et al.</i> (2015)

DDGS, dried distillers' grains with soluble.

acceptable compared with conventional proteins. The amino acid profile according to different studies in the literature presented in Table 3 indicates that the BSFL protein component is predominantly rich in leucine (5–8%) and lysine (4–7% of protein content) when observing the essential amino acids. It has been found that among different studies, considerable variation was observed in the amino acid profile of BSFL according to its rearing. For instance, essential amino acid concentrations in BSFL reared on swine manure were observed to be extremely similar to those of soybean meal-fed BSFL, being rich in lysine, leucine, phenylalanine, and threonine (Newton et al. 1977). The amino acid profile of BSFL has a variation for feed type and needs to be studied further for potential health benefits. Among the non-essential amino acids, BSFL presents a higher concentration of glutamic acid, and aspartic acid (Table 3).

The amino acid profile of BSFL is similar to the reference standards set out by the WHO (2007), suggesting that they would be a good source of protein for human consumption in the modern food system. Compared with traditional

options of foods such as beef, pork, and poultry, BSFL has a similar profile of amino acids and can be used as a potential alternative source of protein (Bessa et al. 2020). Among all the other amino acids, lysine is of particular interest in developing countries across the world; due to lysine being absent in vegetables and grains, insect consumption can be observed in many countries. Insects can be used as potential sources of lysine because they are cheap and can be included in the staple diet of many people globally (Bessa et al. 2020). While the lysine content in BSFL (54–65 mg/g protein) may be lower than that of beef (83–106 mg/g protein of lysine) or chicken (86–96 mg/g protein of lysine), it still provides a sufficient amount to meet the daily lysine requirement for adults of 45 mg/g protein (WHO 2007; USDA 2019; Bessa et al. 2020).

The amino acid content of the BSFL is not as skewed by external factors as are some of the other nutritional components and only a few changes have been observed; however, studies have suggested that the amino acid content does differ in various life stages of BSFL, with higher concentrations of

Table 3. Proximate amino acid composition of black soldier fly larvae (BSFL) reported by different studies (results expressed in % dry basis).

Item	Amino acid composition (%)					
Essential amino acids						
Histidine	2.8	3.7	1.5	3.0	2.8	3.3
Isoleucine	2.4	5.0	3.0	5.1	3.9	4.2
Leucine	3.6	7.9	5.0	7.9	6.4	6.6
Lysine	3.6	7.4	3.9	6.6	6.2	5.9
Methionine	1.1	1.9	1.3	2.1	1.7	1.6
Phenylalanine	2.1	4.8	3.2	5.2	4.0	3.6
Threonine	1.9	4.4	2.8	3.7	3.9	3.9
Tryptophan	–	1.4	0.6	0.5	–	–
Valine	3.1	6.6	4.0	–	5.8	5.7
Non-essential amino acids						
Alanine	3.1	7.3	–	7.7	6.2	7.8
Arginine	2.6	5.2	3.3	5.6	4.6	4.8
Aspartic acid	5.1	10.5	–	11.0	9.4	8.2
Cysteine	0.2	1.2	–	0.1	–	0.9
Glutamic acid	6.1	13.3	–	10.9	10.3	11.8
Glycine	0.3	5.8	–	–	4.6	5.6
Proline	2.9	6.1	–	6.6	5.3	2.2
Serine	2.1	4.4	–	3.1	4.0	4.3
Tyrosine	3.1	5.7	–	6.9	5.7	5.1
Reference	Zulkifli et al. (2022) ^A	DiGiacomo and Leury (2019) ^B	Sándor et al. (2022)	Barragan-Fonseca et al. (2017) ^C	Liland et al. (2017) ^D	Tschirner and Simon (2015) ^E

^AResult expressed as %DM, the value of BSFL spray-dried (SPR).

^BResult originally expressed as mg/g, based on dry larvae average harvested at 16 days.

^CResult expressed as %DM and g/16 g nitrogen.

^DResult expressed as %crude protein, the value of BA0 (insect larvae grown on pure plant-based medium).

^EResult expressed as %crude protein, the value of larvae control.

essential amino acids occurring in larvae, usually between 4 and 6 days old (Liu *et al.* 2017). Moreover, the amino acid content is also significantly affected by processing parameters, such as the killing method and heat treatment. Killing by freezing is observed to activate enzymatic pathways, which results in the loss of cysteine and lysine, whereas blanching has no negative effect on the amino acid profile. It has been found that cysteine and lysine are involved during the enzymatic browning process. Thus the losses in freeze-dried larvae can be explained (Leni *et al.* 2019).

Protein digestibility

According to the literature, the protein digestibility of insects is considered high, because, depending on the species, digestibility higher than 80% has been found. For instance, the *in vitro* crude protein digestibility (N digestibility) has been assessed in mealworms (85.0%) (Caparros Megido *et al.* 2018), houseflies (93.3%), yellow mealworms (92.5%), black soldier flies (87.7%) (Bosch *et al.* 2016), and house crickets (91.7%) (Bosch *et al.* 2014).

The insect-derived product digestibility depends on the insect species, rearing substrates, the species consuming the insects, and processing methods and conditions, such as time, and temperature (Campbell *et al.* 2020). Besides these parameters, a limiting factor for improved protein digestibility in insects is the chitin content. Chitin is a non-digestible fibre, composed of a polymer of N-acetylglucosamine with β -(1/4) linkages, which is not decomposed and not absorbed in the small intestine (Traksele *et al.* 2021; Sándor *et al.* 2022). In fact, a study investigated the effect of chitin on nutrient digestibility in fish feed with BSFL with different chitin contents. It was found that the fish species tested (Nile tilapia and rainbow trout) could digest chitin, but its digestibility decreased with a higher dietary chitin inclusion level, indicating that chitin could act as an anti-nutrient (Eggink *et al.* 2022). However, while chitin is commonly regarded as indigestible, its digestibility depends on the enzyme repertoire of the consuming species (Rodríguez-Rodríguez *et al.* 2022). Chitin, the primary structural polysaccharide found in insect exoskeletons, is broken down by the enzyme chitinase (Fontes *et al.* 2019). In addition, acidic chitinase and gastrointestinal bacteria are also believed to supplement the chitinases in the digestion of chitin (Tabata *et al.* 2018). Consequently, the efficiency of chitin utilisation by monogastric animals is often a subject of debate and is closely related to the presence or absence of chitinolytic enzymes (Fontes *et al.* 2019).

Acidic chitinase was found to be highly expressed in mouse, chicken, and pig stomach tissues, and it can digest chitin in the respective gastrointestinal tracts (Tabata *et al.* 2018). In addition, some studies have confirmed the presence and activity of chitinolytic enzymes in various organs of fish species (Fontes *et al.* 2019). The inclusion of 4.5% BSFL as a partial substitute for soybean meal in piglet diets (replacing 25% of soybean meal) improved nutrient digestibility and

growth performance in piglets (Liu *et al.* 2023). Additionally, the authors suggested that the piglets appear to possess the ability to degrade chitin, releasing the nutrients encapsulated within it and producing chito-oligosaccharides that can act as prebiotics, further enhancing their health. A recent study evaluated the apparent digestibility coefficients of different nutrients, including chitin in African catfish hybrid juveniles that were fed with defatted BSFL, yellow mealworm (MW), or fully fat blue bottle fly (BBF) meals (Sándor *et al.* 2022). Even though digestibility of insect protein has been considered a hurdle, the authors in this study found that the chitin digestibility was relatively high for BSFL (96.1%) and significantly differed from that of other meals (MW, 44.9%; and BBF, 77.8%). Therefore, BSFL were found to be well digestible for catfish and it has been suggested that up to 30% of the catfish diet can be replaced with BSFL meal without causing difficulties in the digestibility and utilisation of nutrients. Differences in the protein digestibility of BSFL, which were reared on various substrates such as maize distillers, brewer's grains, or soymilk by-products, were observed using an *in vitro* digestion model for monogastric animals (Galassi *et al.* 2021). The diet had a significant impact on the *in vitro* crude protein digestibility, with the highest values being recorded for BSFL reared on maize distillers (87.8%). Intermediate values were observed for brewer's grains and okara-fed BSFL, while the lowest digestibility was noted for hen-fed BSFL (82.7%), indicating that the BSFL diet also may affect the digestibility.

Over the past decades, protein quality in human populations has been evaluated primarily by indirect methods, such as *in vitro* assays and animal or human metabolic studies. However, to ensure accuracy and broad applicability, these methods should encompass the fundamental parameters of protein quality, including absolute and relative quantities of dietary ileal indispensable amino acid, protein digestibility, and amino acid bioavailability (FAO 2013c). To determine the protein quality, a novel, and comprehensive method has been applied, namely, digestible indispensable amino acid score (DIAAS), which is calculated on the basis of the value of true ileal indispensable amino acid (IAA) digestibility (Huang *et al.* 2019). The accuracy of this method extrapolating data from one species to another, such as from animal trials to humans, relies on the resemblance in metabolic processes and gastrointestinal physiology. Growing pigs have often been preferred as a model for mimicking digestion in adult humans (FAO 2013c).

The current DIAAS calculations rely on crude protein (total nitrogen \times 6.25), which may overestimate the protein content in insects, leading to an underestimation and potential misclassification of their protein quality. Therefore, the utilisation of an *in vitro* digestibility model is essential in product development to enhance the protein quality of edible insects (Hammer *et al.* 2023). This method is primarily determined by the most limiting digested IAA within the protein source; therefore, the composition of IAA in a

protein source has the greatest impact on its DIAAS value (Herreman et al. 2020). For example, milk-based protein materials were demonstrated to be of higher quality than were plant- and insect-based materials by DIAAS (Komatsu et al. 2023). Furthermore, given the substantial variation in reported IAA composition in edible insects, it is expected that the digestibility of their protein content will also vary (Hammer et al. 2023). The *in vitro* and *in vivo* digestibility of the proteins of the oven-dried BSFL were investigated (Traksele et al. 2021). In the *in vitro* experiments, digestibility of 48% was observed for the dried larvae protein and 75% for the low-fat larvae. Regarding the *in vivo* digestibility in rats, the DIAAS value was determined to be 73%. Thus, the authors suggested that BSFL possesses an easily digestible amino acid, so this insect can be used as a highly bioavailable protein source for human nutrition. Another study determined the *in vitro* digestibility by DIAAS and it was >75% for BSFL proteins that were conventionally dried, and microwave dried (Huang et al. 2019). Therefore, the authors also indicated that the BSFL protein could be identified as being of 'good' quality.

Fatty acids

The BSFL lipidic fraction is highly variable (8–57%) as can be observed in Table 2. In general, the lipid content is higher in the larval phase, and lipid is accumulated as storage fats, which perform as the primary energy reserve for the metabolic activities in the adult fly phase (Ravi et al. 2020). Besides that, older larvae usually show an increase in saturated fatty acids and decreasing concentrations of unsaturated fatty acids (Liu et al. 2017). Adult flies have not been studied for their fatty acid profile but BSFL

contains 58–72% saturated fatty acids (SFA) and 19–40% mono and polyunsaturated fatty acids of the total lipid content (Kroeckel et al. 2012; Makkar et al. 2014). When comparing the fat content with other insects, BSFL contain a high amount of fat, particularly saturated fatty acids (Ramos-Bueno et al. 2016). Among the SFA, the most common fatty acid found in BSFL are lauric acid (C12:0), palmitic acid (C16:0), and oleic acid (C18:1 n-9) (Ewald et al. 2020). Regarding the monounsaturated fatty acids (MUFA), oleic (C18:1n-9) is the most prevalent, while the main polyunsaturated fatty acid (PUFA) is linoleic acid (C18:2n-6) (Cullere et al. 2018). The fatty acid content of BSFL, according to different studies, is presented in Table 4.

According to the literature, the diet may affect the nutritional composition of BSFL, including the fat content and also the fatty acid profile (Barragan-Fonseca et al. 2017; Spranghers et al. 2017). For instance, a study found a fat content ranging from 12.9% DM fat in BSFL reared in seaweed waste to 40.0% DM fat when reared on dairy waste (a mixture of Greek yogurt, full fat-milk powder, and tasty cheese) (DiGiacomo et al. 2019). Concerning the fatty acid profile, BSFL fed with fish offal and brown algae were found to incorporate greater amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (St-Hilaire et al. 2007; Liland et al. 2017). Another study observed that BSFL fed fish offal presented 43% more lipid than did the controls fed cow manure only, and nearly 3% of this lipid was omega-3 fatty acids (EPA, DHA, and alpha-linolenic acid (ALA)) (St-Hilaire et al. 2007). The BSFL fatty acid composition was evaluated through 11 different diets containing mussels, bread, fish, and food waste (Ewald et al. 2020). It was observed that the diet affected the fatty acid composition

Table 4. Proximate fatty acid composition of black soldier fly larvae (BSFL) reported by different studies (results expressed in % dry basis).

Item	Fatty acid composition (%)				
Saturated fatty acids					
C 12:0 (Lauric)	17.9	51.8	11.9	40.6	42.6
C 14:0 (Myristic)	5.2	9.5	2.1	8.5	6.9
C 16:0 (Palmitic)	20.7	12.7	12.7	14.8	11.1
C 18:0 (Stearic)	3.0	1.5	1.4	2.5	1.3
Monounsaturated fatty acids					
C 16:1n-7 (Palmitoleic)	1.8	2.8	1.3	2.0	–
C 18:1n-9 (Oleic)	9.3	12.0	54.1	8.8	12.3
Polyunsaturated fatty acids					
18:2n-6 (Linoleic)	16.2	7.7	12.3	17.9	3.6
18:3n-3 (Linolenic)	2.0	1.6	0.4	1.4	0.7
Reference	Zulkifli et al. (2022) ^A	Ewald et al. (2020) ^B	Starcevic et al. (2019) ^C	Liland et al. (2017) ^D	St-Hilaire et al. (2007) ^E

^ABSFL treated by spray-drying (SPR).

^BBSFL diet: bread.

^CBSFL diet: crude olive cake (COC, 100%).

^DBSFL diet: pure plant-based medium (BA0).

^EBSFL diet: fish offal (50%) and cow manure (50%).

and the larval weight. Additionally, they indicated that it is possible to incorporate n-3 fatty acids such as EPA (C20:5) and DHA (C22:6) from the diet into the larval fat, but when the larvae increase in weight, the percentage of these fatty acids reduces. Thus, the authors indicated that BSFL can be used as a substitute for vegetable oils in food and feed, and to produce biofuel. Although diet has been suggested to impart strong influences on fatty acid profiles, the development of fatty acid biosynthesis should also be taken into consideration to explain the overall fatty acid composition (Barragan-Fonseca *et al.* 2017). In addition, it is unknown which fatty acids the larvae produce by themselves, or to what extent the fatty acid composition of the diet influences the final fatty acid composition of the BSFL (Ewald *et al.* 2020).

Besides the diet, pre-treatment given to the larvae biomass and the applied extraction technique also may affect the fatty acid profile. For instance, Almeida *et al.* (2022) tested different fatty extraction methods (decoction, microwaves, maceration, and ultrasound), by using different solvents (water, acetone, n-hexane). Results showed that the different extraction techniques and solvents produced the following ranges of fatty acid: lauric acid, 37–62%; palmitic acid, 11–14%; linoleic acid, 4–17%; and oleic acid, 4–13%. The extractions using organic solvents provided higher concentrations of PUFA, and higher concentrations of lauric acid were achieved through aqueous extractions, providing 41–62% in the extract. Moreover, the killing method also has a significant effect on the lipid composition of the larvae. Freezing BSFL enhances the free fatty acids released,

owing to the inactivation of lipase activity on unsaturated fatty acids (Leni *et al.* 2019).

Mineral concentration

Regardless of the life cycle (larvae, pre-pupae, and pupae), BSFL are considered a good source of mineral elements, with calcium being the most prevalent mineral (Smets *et al.* 2020). The high calcium content in BSFL was reported in different studies (Table 5) (Liland *et al.* 2017; Campbell *et al.* 2020; Smets *et al.* 2020). This might be partly explained by the fact that the body of BSFL secretes calcium carbonate (CaCO₃), which may account for the high calcium and ash content (Newton *et al.* 1977). Similarly, in the last phase of the lifecycle (adult phase) calcium concentrations have been observed to be 0.03% due to most of the calcium present in the exoskeleton of the adult fly (Newton *et al.* 1977). Other macro minerals such as potassium, phosphorus, magnesium, and sodium present variation over the different developmental stages, while trace concentrations of the micro minerals including manganese, iron, zinc, and copper do not exhibit large variation among life stages (Smets *et al.* 2020). Table 5 shows the macro- and micro-mineral concentrations of BSFL in different studies.

Black soldier fly larvae have great concentrations of macro- and micro-minerals, as can be observed in Table 5. For instance, BSFL reared on agro-industrial substrates are promising alternatives to fishmeal and soybean meal in terms of mineral content (Chia *et al.* 2020). The high concentrations of Ca, P, and other essential micro minerals such as Fe and Zn depict a high potential of the larvae as a feed component

Table 5. Proximate mineral composition of BSFL according to different studies (results expressed as dry basis).

Item	Mineral concentration (g kg ⁻¹)				
Macro minerals					
Calcium (Ca)	18.50	35.60	3.50	2.40	28.72
Magnesium (Mg)	2.90	3.30	2.20	1.80	2.46
Potassium (K)	6.90	9.10	1.30	10.00	5.94
Phosphorus (P)	4.40	7.00	4.80	4.60	4.04
Sodium (Na)	–	1.60	0.80	4.80	0.60
Micro minerals					
Copper (Cu)	–	0.09	0.90	0.006	0.01
Iron (Fe)	–	0.14	0.40	–	0.11
Manganese (Mn)	0.12	–	0.30	0.05	0.24
Zinc (Zn)	0.11	0.33	1.00	0.04	0.07
Reference	Campbell <i>et al.</i> (2020) ^A	Smets <i>et al.</i> (2020) ^{B,C}	Shumo <i>et al.</i> (2019) ^D	Liland <i>et al.</i> (2017) ^E	Spranghers <i>et al.</i> (2017) ^F

^AReared on brewer's by-product.

^BMineral profile after sequential extraction.

^COriginally, the micro nutrients were given as mg/100 g DM.

^DReared on spent grain.

^EBA0 (insect larvae grown on pure plant-based medium).

^FReared on vegetable waste.

in livestock feed (Chia et al. 2020). Thus, the rearing type affects the mineral concentration extensively because insects do not synthesise minerals, but they are found in the body through bioaccumulation. However, some toxic and harmful elements (such as Ba, Hg, and Mo) might also bioaccumulate in BSFL, which would implicate safety issues for feed and food production of this insect (Lu et al. 2022a). Therefore, it must be recommended that heavy metal-rich diets are not given to the larvae because they can cause heavy metal toxicity if consumed due to the mineral bioaccumulation.

Bioactive properties

Cultures that consume insects mostly associate them with various health benefits beyond common nutrition (Hartmann and Siegrist 2017). These health benefits are related to the presence of natural bioactive compounds that exert a therapeutic action leading to improved health and reduced risk of disease. Foods that contain these active compounds or substances that provide nutritional and functional benefits to the body are known as nutraceuticals (Ordoñez-Araque and Egas-Montenegro 2021). The bioactive compounds found in BSFL include the presence of chitin, medium-chain fatty acids (C6-12), and antimicrobial peptides (Surendra et al. 2020). In addition, recent studies found a simultaneous multi-bioactivity in BSFL extracts with potential as an antioxidant and as an inhibitor of pancreatic lipase; and a novel polysaccharide from BSFL capable of activating the innate immunity of mammalian macrophages (Fariz Zahir Ali et al. 2019; Navarro del Hierro et al. 2021). The nutraceutical benefits of chitin and its derivatives, lauric acid, antimicrobial and antioxidant peptides present, and other novel bioactive compounds in BSFL are reviewed.

Chitin and chitosan

Next to proteins and lipids, BSFL also contain valuable biopolymer chitin. Chitin and its main derivative chitosan have great economic value due to their properties and several potential applications (Smets et al. 2020). Chitin is a non-digestible oligosaccharide present in various food products, mainly in seafood and others such as insects with exoskeletons. It can be used as a potential healthy product for the host and could easily be metabolised by gut microbiota. Studies on the dietary supplementation of chitin conducted on weaning pig, because of the gut microflora similar to humans, found that it improved the gut barrier function and increased the populations of *Bifidobacterium* spp. and *Lactobacillus* spp., and left the *Escherichia coli* counts unaffected in the colon (Yang et al. 2012). However, various studies have reported that supplementation of chitin and its derivatives significantly increases the growth and colonies of *Escherichia* spp. in rats (Shang et al. 2017), but decreases the comparative

proliferation of *Lactobacillus* in young pigs (Yang et al. 2012). Moreover, the addition of chitin and other resistant starch mixtures has been shown to significantly reduce metabolic disorders by the proliferation of gut microflora, lipid absorption, and metabolism in the gut, as well as the thickening of the mucosa layer of the colon of rats (Shang et al. 2017).

Some studies have indicated the betterment of gut microflora by chitin and its use as a prebiotic (Selenium et al. 2018); when consumed with different food fractions, chitin and other dietary fibres have the ability to reduce LDL-cholesterol concentrations in the blood (Caparros Megido et al. 2018). Ailments such as glucose intolerance, dyslipidemia, reduced insulin secretion, and others have been observed to be alleviated in rats treated with a fat-rich diet (Zheng et al. 2018). Chitin and its derivatives additionally have other bioactive properties such as anticancer, antimicrobial as well as antifungal properties, and a bacteriostatic effect on the Gram-negative bacteria *E. coli*, *Vibrio cholerae* and *Shigella dysenteriae* (Piccolo et al. 2017).

Chitosan is a naturally occurring derivative of chitin and has been extensively researched for its ability to decompose in the gut (Udayangani et al. 2017). Moreover, it has also been found that chitosan is biocompatible with the gut, does not cause any harmful reactions, has low toxicity, and possesses mutagenic properties (Xu et al. 2020). Scientific studies conducted on different animals have found that chitosan has properties such as wound healing, immunostimulation, antioxidant properties, antitumor properties, cholesterol reduction, and antibacterial, antifungal, and anti-inflammatory properties greater than those of chitin. Moreover, another study conducted on humans found that chitosan can reduce the risk of arthritis, reduce diabetes, and also lower serum cholesterol concentrations (Lee et al. 2002). Thus, chitin can be extracted from BSFL to produce chitosan, which can be used in pharmaceuticals and nutraceuticals due to its interesting health benefits. A 2-week study conducted on adult healthy males found that 3–6 g per day of oral supplementation of chitosan significantly reduced the serum cholesterol and significantly increased the high-density lipoprotein cholesterol (Maezaki et al. 1993). Thus, chitosan shows beneficial results on human health. However, the long-term effects of chitosan on the human body have not yet been studied and need to be investigated for it to be used as a pharmaceutical product.

According to previous studies, digestion of fatty acids and their absorption, along with the ability to reduce bile acid synthesis, have been the proposed method via which chitosan provides lipid-lowering effects in the body (Zhou et al. 2006; Xia et al. 2011). Moreover, oral supplementation of chitosan has been shown to improve digestion as well as the overall health of humans (Shang et al. 2017). Thus, due to its interesting ability as a prebiotic, chitin and its derivatives need to be studied as a holistic component of BSFL.

Lauric acid

Lauric acid is a 12-carbon fatty acid. As can be observed in Table 4, concentrations of lauric acid (C 12:0) may vary from about 12% to 52% in BSFL, depending on the rearing and extraction methods. Because this insect has a high content of fatty acids and is rich in lauric acid, it shows potential applicability in the pharmaceutical and cosmetic industry as skin care (Almeida *et al.* 2022). Black soldier fly larvae present a similar content of lauric acid as found in coconut oil, which is applied in skincare products, mainly due to the presence of this fatty acid (Almeida *et al.* 2020). Thus, a blend of fatty acid and its derivatives present in the BSFL lipid fraction is commonly utilised as emollients, emulsifiers, and stabilisers of disperse systems in cosmetic formulations (Almeida *et al.* 2022). In addition, lauric acid has been investigated regarding its potential as a preservative, given its significant antiviral and antibacterial activities, and this fatty acid has demonstrated prebiotic effects on the microbiota of livestock (Mohana Devi and Kim 2014; Anzaku *et al.* 2017).

The mechanism of action regarding the antimicrobial properties of fatty acids remains unclear. It is suggested that due to the amphiphilic nature of fatty acids at high concentrations, they may act as a detergent and aid the solubilisation of the lipids in the membranes, causing cell lysis (Churchward *et al.* 2018). The lauric acids have demonstrated antimicrobial effects on a wide variety of microorganisms, including *Campylobacter* spp., *Clostridium* spp., *Salmonella* spp. and *E. coli*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Chlamydia trachomatis*, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, and *Helicobacter pylori* (Khoramnia *et al.* 2013; Churchward *et al.* 2018; Çenesiz and Çiftci 2020). Thus, the BSFL rich in fatty acids, especially lauric acid, can be considered as an alternative not only to conventional protein in animal diets but also to in-feed antibiotics used for promoting growth, and preventing and curating gastrointestinal diseases (Decuypere and Dierick 2003).

The prebiotic effects of dietary supplementation with medium-chain fatty acids (MCFA) along with probiotic in weanling pigs has been evaluated (Mohana Devi and Kim 2014). These authors suggested that the presence of MCFA (C 6:0 to C 12:0), such as lauric acid, can improve growth performance, improve nutrient digestibility, and increase biochemical profiles, in a way comparable to antibiotic treatments. Therefore, BSFL can be an alternative to in-feed antibiotics owing to the presence of a high content of MCFA.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are natural antibiotics that can kill or inhibit several microorganisms (Alencar-Silva *et al.* 2018). Antimicrobial peptides from a broad range of insects have been demonstrating effective microbicidal properties against microbial-related diseases (Elhag *et al.* 2017).

The antimicrobial peptides derived from BSFL have excellent potential as alternatives to antibiotics for prophylaxis and treatment of diseases in animals, since they present broad antimicrobial properties and a lower tendency to induce resistance (Xia *et al.* 2021). Black soldier fly larvae have been reported with high expression of AMPs and other substances with activity against drug-resistant pathogens, being a promising source of AMPs due to their remarkable ability to live in hostile environments and to feed on decomposing substrates, which are abundant in microbial colonies (Almeida *et al.* 2020; Moretta *et al.* 2020). However, currently, the exact mechanism of production of AMPs in BSFL is still unknown.

A recent study extracted and identified the bioactive compounds in the BSFL to detect its antioxidant and antibacterial properties by investigating the metabolic pathway of UV light-induced BSFL (Lu *et al.* 2022b). The authors found that the bioactive components in BSFL after the treatment consisted mainly of peptides, alkaloids, and fatty acids. Thus, they can be applied as an antioxidant as well as in cosmetics, due to their antioxidant and antibacterial activities. A new AMP of 40 amino acids (defensin-like peptide4) has been identified (Park *et al.* 2015). This AMP was constitutively induced and exclusively purified from the immunised haemolymph of *H. illucens* larvae. As a result, the authors found that this AMP exhibits bactericidal activity in Gram-positive bacterial strains. A bioinformatic study of antimicrobial peptides in BSFL identified a set of candidate peptides that could serve as a starting point for subsequent functional characterisation of *H. illucens* AMP (Moretta *et al.* 2020). The results led to the identification of 57 peptides, 13 of which were predicted as endowed with antimicrobial activity. Besides that, the authors also identified multifunctional peptides, 22 with antimicrobial and anticancer activity, eight with antimicrobial and antiviral activity, two with antimicrobial and antifungal activity, and seven with antimicrobial, anticancer, and antiviral activity. Another study assessed the effectiveness of a range of *H. illucens* AMPs against various human pathogens and a human cell line. Furthermore, the study delved into the characteristics of two AMPs, namely cecropins (Hill-Cec1 and Hill-Cec10), by examining their haemolytic properties, time-to-kill kinetics, membrane-permeabilisation effects, and anti-biofilm activity. These specific cecropin peptides exhibited activity against Gram-negative pathogens, including *Pseudomonas aeruginosa* (Van Moll *et al.* 2022).

Antioxidant peptides

Antioxidants are bioactive compounds that inhibit or reduce damage caused by the harmful action of free radicals or non-radical reactive species (de Castro *et al.* 2018). Some short-chain peptides and free amino acids can actively scavenge reactive oxygen species and free radicals; thus, they are known to present antioxidant activity (Mouithys-Mickalad *et al.* 2020).

There is an increased interest in search of antioxidant peptides from edible insects, because these active compounds may help prevent cell damage, limit free-radical damage, and can be used to develop cosmetic products, enhancing the economic benefits of the whole system of food waste and insect high-value products (de Castro et al. 2018; Lu et al. 2022b). However, the mechanism by which these peptides exert their antioxidant action is not fully understood.

Some studies have reported that the presence of hydrophobic and aromatic amino acids such as histidine, methionine, tyrosine, lysine, and cysteine in peptides increases the antioxidant activity by single electron transfer, hydrogen atom transfer, and metal-chelating ability (Zielińska et al. 2017). One study reported that insect peptides with lower molecular weights have a higher scavenging ability due to the ability to connect freely by the hydrogen atom transfer mechanism and impart antioxidant activity (Zielińska et al. 2017). Thus, it can be assumed that the high radical-scavenging activities of protein hydrolysates are due to the presence of low-weight molecules such as dipeptides and tripeptides and synergistically show antioxidant activity.

The effects of the protein hydrolysate from *H. illucens* using bromelain as an enzyme on its antioxidant activity were evaluated (Firmansyah and Abduh 2019). The protein hydrolysate from BSFL had an antioxidant activity of up to 77% and the amino acid composition of the protein hydrolysate was also determined and mainly consisted of lysine, leucine, and valine. The effects of chemical protein extraction, and enzymatic hydrolysis with different proteases (alcalase, papain, and pepsin), on the antioxidant activity of BSFL protein were assessed (Batish et al. 2020). The results showed that, under two-step hydrolysis, alcalase produced protein hydrolysates with a higher degree of hydrolysis (18%), greater antioxidant activity (~3 µmol/mg), and amino acid compositions with higher concentrations of hydrophobic amino acid (alanine, isoleucine, leucine, phenylalanine, proline, tyrosine, and valine). Therefore, the authors suggested that BSFL could be used for feed and food development using enzymatic hydrolysis.

Other novel bioactivities

Pancreatic lipase is responsible for the digestion and absorption of dietary fat through the hydrolysis of the triacylglycerols to glycerol and fatty acids. The inhibition of this enzyme avoids the breakdown of dietary fat into fatty acids, leading to reduced absorption in the gut, which may be a useful approach to the control of hyperlipidemia and obesity (Zhang et al. 2015). The inhibition of the pancreatic lipase by edible insects is a quite novel bioactivity that was reported for the first time for non-defatted extracts of *T. molitor* and *A. domesticus* (Navarro del Hierro et al. 2020). However, the specific mechanism related to the hypolipidemic activity of insects has not been elucidated. The effect of defatting and extraction solvent after ultrasound-assisted

extraction on the antioxidant and pancreatic lipase inhibitory activities of extracts from BSFL were studied (Navarro del Hierro et al. 2021). The results indicated an antioxidant activity of *H. illucens* of 20%. Regarding the inhibitory activity on lipase, both *H. illucens* extracts (ethanol and methanol) showed interesting bioactivity against this enzyme, with 70% enzymatic inhibition. This study presented for the first time the inhibitory activity against pancreatic lipase by *H. illucens* extract.

Polysaccharides are carbohydrate compounds that contain more than 10 monosaccharides linked by glycosidic bonds (Wang 2020). Polysaccharides may present different biological properties, such as anti-tumour, immunomodulatory, anti-diabetes, anticoagulant, antiviral, and antioxidant properties (Liu et al. 2015). However, functional polysaccharides from insects have been neglected when compared with those from plants, fungi, and bacteria (Fariz Zahir Ali et al. 2019). So far, a few studies in insects have discovered novel bioactive polysaccharides termed dipterose-BC (*Bactrocera cucurbitae* larvae), dipterose-BSFL (*H. illucens* larvae), silkrose-AY (*Antheraea yamamai*, pupae), and silkrose-BM (*B. mori* pupae) (Ali et al. 2022). A novel bioactive polysaccharide was identified in BSFL as a molecule that activates the mammalian innate immune response (Fariz Zahir Ali et al. 2019). The authors suggested that the bioactive polysaccharide, dipterous-BSFL, has immunomodulatory potential by activating the host innate immune system, which allows it to be a novel immunomodulator for implementation as a functional food supplement in poultry, livestock, and farmed fish. Thus, this novel bioactive compound has potential benefits as a functional feed ingredient for agriculture and aquaculture.

Major challenges for insect consumption

Although insects as food present several advantages, their production faces two major challenges that are related to the low consumer acceptance and the current limitation regarding food safety, as well as clear legislation to regulate this new foodstuff (de Castro et al. 2018). The main challenges, including insects as a food product, consumer acceptance and food safety concerns, and current legislation are reviewed.

Insects as food products

In the western world, insects are consumed as ingredients in pasta (with mealworm or cricket flour), protein bars, cricket flours, snack packs, insect candies, and biscuits, among others. There are more than 2000 species of insects that are considered edible worldwide. Most of these edible insect species are beetles (31%), caterpillars (18%), wasps, bees, and ants (15%), crickets, grasshoppers and locusts (13%), true bugs (11%), termites, dragonflies, flies, and others (12%) (van Huis 2016). These edible insects can be

consumed as eggs, larvae, pupae, or adults, although some insects are not edible at all stages of development. Due to sensory characteristics, insects are largely studied to be applied in food as meat products (Villaseñor *et al.* 2022). They might be incorporated into gastronomy through different forms of cooking, such as fried, stewed, cooked, steamed, boiled, or roasted, being properly heat-treated. They also can be consumed directly as snacks (e.g. nuts or vegetable chips) or can be used as ingredients in different culinary preparations (e.g. salads or pizzas) (Ordoñez-Araque and Egas-Montenegro 2021). Besides that, insects also have been studied regarding their protein techno-functional properties to produce high-protein ingredients (Villaseñor *et al.* 2022).

As a high-protein ingredient, functional properties of BSFL protein have been evaluated. Bußler *et al.* (2016) investigated the protein techno-functionality of insect flour fractions recovered from *H. illucens*. It was observed that BSFL flour could be used as an alternative to vegetable flours in food products due to its high water-holding capacity and oil-holding capacity. Another study evaluated the technological and nutritional parameters of baked products by adding fortified flour with BSFL pre-pupae (Montevecchi *et al.* 2021). An improvement in bread texture was observed, which was attributed to satisfactory results of physicochemical and rheological characteristics. Moreover, an increase in the content of essential amino acids in the doughs was noted, and this increased concentration was maintained throughout the production steps and resulted in an efficient fortification of the baked products.

Concerning flavour and mouthfeel, the texture of BSFL is not considerably different from that of other larvae and is often compared to fish meal and oil, albeit with an earthier flavour (Wang and Shelomi 2017). Currently, there is no study on the sensory profile of BSFL-derived food. However, as sensory properties are one of the most important aspects determining consumer acceptance, it is crucial to understand the functional properties of BSFL, which will allow for the optimisation of processing parameters to ensure that products are of good quality (Bessa *et al.* 2020; Wang *et al.* 2022). The BSFL are already used and recommended for animal feed, and from a nutritional point of view, they are adequate for human consumption (Wang *et al.* 2022). However, despite all the favourable nutritional content of BSFL as a possible protein alternative, they have been limited to their use as an animal feed (Bessa *et al.* 2020).

Consumer attitudes regarding edible insects

Food neophobia is the term used to define the disliking of new food products. The food neophobia scale (FNS) is a scale used by modern-era clinical scientists who study food and people's choices with food. As several new foods are being developed and an ever-expanding food list FNS has become a common scale to rate the disliking of new foods on the basis

of appearance and pre-conceived notions. Insects have been a media headline as the new-age alternative protein that has caused public backlash due to the disgust observed in the consumers mainly from the western or western-influenced nations. For example, a study evaluated the consumer acceptance of insects in New Zealand (Clarkson *et al.* 2018). The findings indicated that participants were both disgusted and intrigued about entomophagy, with common barriers including culture, food neophobia, disgust sensitivity, lack of necessity, and knowledge. And motivational drivers for the participants were novelty, health, sustainability, and/or nutrition.

Overall, it has been understood that insect proteins and the use of insects in the human diet have been considered a taboo, and it is negatively correlated to people's choices of food (Gahukar 2011; Clarkson *et al.* 2018; van Huis 2018). Behind this disgust lies the fact that they are considered to have pathogenic properties and are mostly generated from waste, which is not the case for the majority of commercially produced insects. Disgust is a universal emotion; yet, it has been observed only among a certain category of people (Gahukar 2011; Clarkson *et al.* 2018; van Huis 2018). The disgust among cultures is not evident among the African and oriental cultures, where entomophagy is a common practice.

It is stated that the fear of new foods can also be linked to risk avoidance. Moreover, as insects are generally associated with being dirty, the feeling of risk avoidance comes into the picture to have a safe food product for consumption (Clarkson *et al.* 2018). Furthermore, these two factors are closely linked to people's negative experiences and views of entomophagy. However, food futurists believe that sustainability minded humanity will gradually include insects as an alternative protein (Wang and Shelomi 2017). For instance, the acceptance by Belgian consumers of edible insects and their potential to become a usual food ingredient in Western European populations was studied (Caparros Megido *et al.* 2014). The authors indicated a slight neophobia, but people agreed to evaluate insect preparations. The results show that consumers are ready to buy and cook insects at home if they can associate them with a familiar flavour. Hence, it can be expected that in the near future, due to progressive mindsets, people may be willing to use insects as a means of food.

Food safety

Food safety is highly important and must be considered for any new food source. Even though insects present a great nutritional profile, and sustainable advantages over conventional meat, studies regarding food safety are very limited. However, this can be of critical importance to meeting society's approval (Belluco *et al.* 2013). The main safety concerns related to insect consumption are

microbiological, parasitological, and allergenic (van Huis 2016; de Castro et al. 2018). In the context of edible insects, their food safety risks are related to (1) toxicity from the insect itself, (2) toxicity from toxic substances or human pathogens during the insect's life cycle, and (3) possible allergic reaction to the insect by consumers (van Huis 2016).

High microbial contents have been detected among edible insect species, with pathogenic bacteria such as *E. coli*, *Bacillus cereus*, and *S. aureus* being considered of particular concern (Garofalo et al. 2019). It has been indicated that some factors have an influence on the microbial load, such as feed, farming environment, and post-harvesting processes (blanching) (Klunder et al. 2012). When considering BSFL specifically for human food, growing BSFL on organic waste may lead to increased concerns for their use in food due to the presence of potential contaminants that could accumulate from the feed (Bessa et al. 2021). However, it is known that BSFL do not accumulate or concentrate on pesticides or mycotoxins (Wang and Shelomi 2017). Concerning microbial contamination, BSFL demonstrate an ability to significantly reduce *Enterobacteriaceae* colonies and *Salmonella* spp. in different feed sources (Lalander et al. 2015). This might be related to the BSFL being natural decomposers (Bessa et al. 2021).

Parasites represent another potential hazard in relation to insect consumption (Belluco et al. 2013). Parasites can be transmitted between definitive hosts by ingestion with contaminated food and water and are able to survive in the external environment for months to years (Müller et al. 2019). If BSFL are reared with non-defined waste containing potentially infectious pathogens, there is a high risk of disease transmission to the animal that consumes it. The risk of passive transmission of animal parasites by feeding contaminated rearing substrates to BSFL and pre-pupae was evaluated (Müller et al. 2019). It was found that the parasites studied (*Eimeria tenella*, *Eimeria nieschulzi*, and *Ascaris suum*) were not eliminated or inactivated by the BSFL. Thus, the authors indicated that the risks of disease transmission to animals and humans are not reduced when untreated BSFL larvae are used as feed for food-producing animals. Therefore, to avoid this, some pre-treatments such as washing, heat treatment, and/or ammonia sanitisation can be applied.

The Arthropoda family contains crustaceans, insects, arachnids, and myriapods that have proteins in them that are susceptible to causing an allergic reaction in some individuals (de Gier and Verhoeckx 2018). Food allergies are usually caused by (glyco)proteins such as arginine kinase, glyceraldehyde 3-phosphate dehydrogenase, haemocyanin, and tropomyosin (EFSA 2015; de Gier and Verhoeckx 2018). Tropomyosin is a protein found in our muscles, yet it can cause allergic reactions in some individuals. This protein was identified as the major allergen in BSFL and crustaceans, indicating that consumers who are allergic to tropomyosin in

crustaceans are more likely to experience allergies when consuming BSFL (Bessa et al. 2020). Moreover, allergies can also occur if you come in contact with an individual working at an insect-rearing station (Barragan-Fonseca et al. 2017). Bessa et al. (2021) assessed the safety of consuming BSFL directly for human consumption. The research explored whether the type of feed provided to BSFL, and the method of slaughter (blanching or freezing) had any impact on bacterial load, heavy metal accumulation, and allergen content, thereby affecting the overall food safety of BSFL. The findings indicated that the method of slaughter significantly influenced the microbial load and heavy metal content in BSFL. Although heavy metals were detected, they remained within permissible limits as defined by legal standards. Additionally, cross-reactive allergens, specifically tropomyosin and arginine kinase, were identified in BSFL samples. The authors highlighted the importance of considering the type of food supplied to BSFL and implementing appropriate decontamination steps, such as blanching, to ensure the safety of BSFL for human consumption.

According to the FAO (2021), there is a lack of insect-specific legislation, standards, labelling, and other regulatory instruments in most countries related to the production and commercialisation of insects in food- and feed-supply chains. Thus, the legislation in countries where insects are not usually consumed as human food or animal feed products tends to include insects as impurities or pests that contaminate food (FAO 2021). The risk profile related to the production and consumption of insects as food and feed, according to the European Food Safety Authority, includes food substrates and the handling and storage of cultivated insects, rather than the species themselves from insects; heavy metals have been shown to accumulate in some insect species, but published data to draw additional conclusions are lacking; and the use of other substrates currently not permitted for insect feeding, such as post-consumer food waste and secondary organic fertiliser, should be specifically evaluated (Finke et al. 2015). Regarding current regulations, based on EFSA evaluation, tree different insects, namely mealworm (*T. molitor*), migratory locust, grasshopper (*Locusta migratoria*), and house cricket (*A. domesticus*), have received authorisation from the Commission as novel foods for human consumption in EU. Additionally, there are more novel food applications for insects undergoing safety evaluation by EFSA, including the use of larvae of *H. illucens* (Liguori et al. 2022). Consequently, it is expected that insect species authorised under the novel food regulation emerge as source of alternative protein in the human diet.

Conclusions

The use of insects as an alternative protein source is considered a future trend and a feasible strategy to improve

global food security in a more environmentally friendly way. The BSFL consume organic waste and convert it into nutrition. However, depending on the rearing, the nutrient composition and yield of BSFL may change. Depending on the diet, BSFL present a high protein content (up to 30%), may present a large amount of fat (up to 10%), and a high amount of calcium (up to 20%). The main bioactive compounds found in BSFL include chitin, lauric acid, and antimicrobial and antioxidant peptides, and the novel active compounds found in this insect are inhibitors of pancreatic lipase, and bioactive polysaccharides. Thus, BSFL can be applied as a nutraceutical, food ingredient, prebiotic, in-feed antibiotic for animals, and in the cosmetic industry. However, the low consumer acceptance and the scarce information regarding their food safety and legislation are still the major challenges for their consumption, which need to be overcome.

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Data availability. The data that support this study are available in the article.

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