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Review

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Abstract: Each year, the food supply chain produces more than 1.3 billion tons of food and agricultural waste, which poses serious environmental problems. The loss of the massive quantity of secondary and primary metabolites retrievable from this resource is a significant concern. What if there is a global solution that caters to the numerous problems arising due to the humongous volume of waste biomass generated in every part of the world? Insects, the tiny creatures that thrive in decaying organic matter, which can concentrate the nutrients present in dilute quantities in a variety of by-products, are an economically viable option. The bioconversion and nutritional upcycling of waste biomass with insects yield high-value products such as protein, lipids, chitin and frass. Insect-derived proteins can replace conventional protein sources in feed formulations. Notably, the ability of the black soldier fly (BSF) or *Hermetia illucens* to grow on diverse substrates such as agri-food industry side streams and other organic waste proves advantageous. However, the data on industrial-scale extraction, fractionation techniques and biorefinery schemes for screening the nutritional potential of BSF are scarce. This review attempts to break down every facet of insect processing and analyze the processing methods of BSF, and the functional properties of nutrients obtained thereof.

Keywords: black soldier fly larvae; fractionation; biorefinery; nutritional upcycling; sustainability

1. Introduction

The land, water and greenhouse gas emissions involved in meat production are becoming unsustainable, and the demand for meat products is expected to reach a whopping 455 million tons by 2050, which is a 76% increase from the meat consumption recorded in 2005/2007 [1]. Eventually, the unsustainable farming practices will reach a breaking point, which could be accelerated by water scarcity, deforestation, and arable land depletion partly due to urbanization and climate change, to name a few. The transition towards a systematic implementation of a circular economy and sustainable practices are part of the solution that can alleviate the burden on the current food production systems. Insect-rearing for food and feed purposes has several advantages: from an environmental standpoint, insect rearing requires less area to produce 1 kg of protein compared to conventional protein sources, reduced greenhouse gas emissions and the bioconversion of waste organic side streams to high-value products [2,3].

Edible insects are species of insects that can be used for human consumption and also as livestock feed as a whole or parts of them. They are considered a promising alternative solution to achieve food safety, security and sustainability in the much anticipated global food crisis. Edible insects provide significant advantages to human nutrition, including high protein, amino acids, lipids, energy and various other micronutrients [4,5]. Among the array of insects that can be domesticated and mass-reared for said applications, an insect with ample favorable properties is the black soldier fly (BSF), *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). Rearing BSF has been proposed since the 1990s for the effective disposal of organic wastes by converting them into a protein and fat-rich biomass (Figure 1) suitable for numerous applications and as a potential alternative to conventional protein sources like soybean and fish meal in animal and aquafeed [6–8]. Industrial rearing, processing, and valorization of BSF for the obtention of proteins, lipids, chitin derivatives, bioactive peptides, organic manure, and other micro, macro-nutrients is an intricate task. It involves several unit operations, extraction and separation mechanisms, fractionation techniques and biorefining schemes for the exhaustive utilization of BSF biomass [9]. The inherent nature of BSF to aggregate select nutrients from the feed substrate [10] can be exploited to retrieve dilute constituents present in the diet. For example, when waste carrot is used in the diet, there is a high possibility that the black soldier fly larvae (BSFL) can accumulate carotenoids in the matrix which, in turn, can be extracted from the lipophilic fraction of the BSFL.

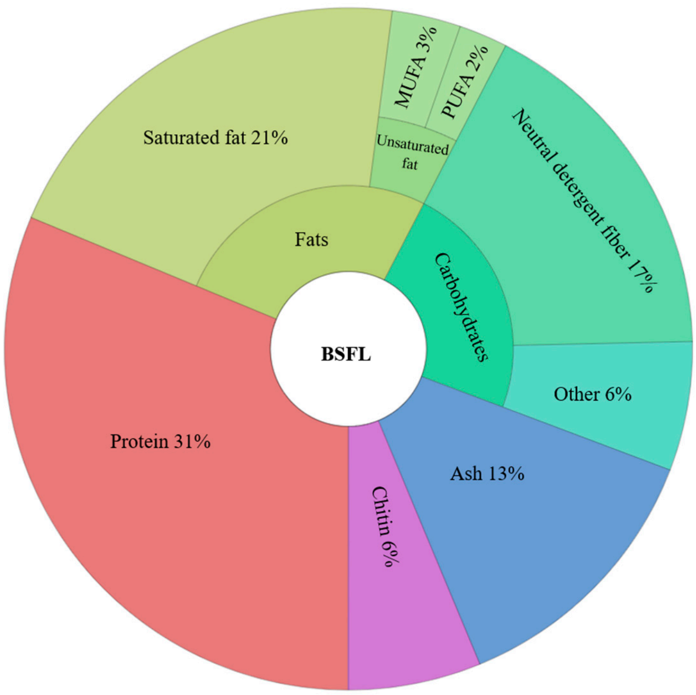

Figure 1. Krona chart of the proximate composition of black soldier fly larvae (BSFL) (adapted from [11]).

This review is a concise compilation of data from previously published articles, patents and conference communications associated with every facet of BSF nutrition, processing and applications with a focus on the extraction, fractionation and biorefining of individual chemical constituents present in black soldier fly larvae. The scope of the review is as follows:

- Insect as biofactories—valorization of waste streams with insect bioconversion, BSFL for waste management and upcycling of nutrients to establish a circular economy is envisaged;
- Biorefining and fractionation—conventional, industrial-scale processing techniques for the retrieval of BSFL constituents with industrial patents as examples and other novel processing mechanisms are deliberated;

- Lipids—the oil content of BSFL reared on different substrates, the fatty acid profile, the lipid class compounds and its application in food, feed and other fields are discussed in detail;
- Proteins—the protein content of BSFL reared on different substrates, properties of partially and highly-defatted protein meal, the nitrogen-to-protein conversion ratio and feed applications;
- Amino acids—the essential amino acid index, apparent and ileal digestibility of various BSFL meal in vitro and in vivo, the limiting amino acids are covered;
- Chitosan—the extraction of chitin and chitin derivatives, the properties and applications of chitin and chitosan are discussed.

The strengths, weaknesses, opportunities and threats (SWOT) analysis for insect processing with three fundamental parameters are proposed. The sustainable development goals achievable with insect rearing are probed. A brief overview of the advances in insect rearing is also provided. Overall, the rationale behind this review is to characterize the vital components involved in insect-based biorefinery for the upcycling of nutrients to establish a sustainable circular economy.

2. Insects as Biofactories: Nutrition Upcycling of Food Wastes

Farm to fork, the journey of food from agricultural fields to the end consumer, is the food supply chain (FSC). Within this framework, the food goes through various segments in the FSC such as agricultural production, postharvest handling and storage, processing and packaging, distribution and consumption. Food wastes and losses occur at every stage, some of which can be prevented and reduced, and the rest of which are inevitably lost. Roughly, one-third of the edible parts accounting for 1.3 billion tons per year of food intended for human consumption is lost or wasted [12]. The extent of food losses and wastes in every segment of select high-, low- and middle-income countries was published [13,14]. The waste can be categorized into cereals, roots and tubers, oilseeds and pulses, fruits and vegetables, milk and egg, meat and fish products. In general, part of the waste is used as feed for livestock, and the rest either ends up in landfill or is used for composting purposes. Novel avenues for waste biomass valorization includes the transformation of waste agro-industrial by-products into platform-chemicals and biofuels. For example, the production of levulinic acid from lemon peels, coffee silverskins and paper waste by means of acid hydrolysis was proposed [15]. Similarly, catalytic production of hydroxymethylfurfural from bread waste with polar aprotic solvent-water mixtures was suggested [16]. The procurement of liquid biofuels such as bio-oil, bio-ethanol, and bio-diesel using chemical and biocatalytic methods from food waste were articulated [17]. The overall aggregate loss percentage of fruits and vegetable, when compared to other food waste categories, is significantly higher in different regions considered. Per-capita fruit and vegetable waste in Asia and Europe was the highest (Figure 2a). The relative loss percentage of roots and tubers was the lowest among all categories compared in Europe (Figure 2b).

Conventional feed production from fruit and vegetable waste involves pressing the biomass to drain out excess water and thermal drying to reduce moisture content and microbial load. In the absence of industrial-scale equipment, sun drying is preferred to dry the waste biomass, which is then pulverized, stored and used as partial replacements of cereal and oilseed crops in feed formulations for poultry and livestock [18]. Valorizing bulky wastes like agricultural by-products and nutritious biowaste like food industry by-products is an intricate, energy-intensive process as it requires large areas to treat and process the biomass. Poor waste management practices such as lack of organic waste sorting and segregation, complex waste collection systems, and the logistics associated with transportation and incentives for disposal in landfills are the current impediments that hinder the value realization of such biomasses [19].

The nutrition upcycling of waste biomass refers to the exploitation of food waste resources to obtain edible high-quality micro and macro-nutrients that can be incorporated in the food chain. Insects are regarded as a sustainable protein source and are perceived as highly nutritious for feed applications. One major area where they can be easily employed is for the valorization of organic waste streams such as agricultural wastes and industrial by-products, and they are increasingly used as a replacement for conventional protein sources, especially in aquaculture. The ability of insects to convert a multitude of food waste sources makes them an ideal candidate for waste management and upcycling of the nutrients. Insects as feed are mediated by two conversion cycles: from organic products to insects, and then from insects (as feed) to production animals. With growing interest in establishing a circular economy worldwide, the upcycling of low-opportunity-cost feed (LCF) is being investigated for the direct incorporation of insects as feed-in production animals [20]. Direct upcycling of LCFs such as food waste, food processing by-products and grass resources into nutritious livestock feed was investigated recently. The authors stipulated that the optimal conversion of LCF available in the European Union (EU) could supply 31 g animal protein per capita per day [21]. Though the direct upcycling of waste biomass nutrients is a direct approach, the diverse components in the waste streams pose a serious challenge in optimizing the nutrient content essential for the growth of livestock. Insect-based bioconversion can ensure the obtention of stable nutrient composition in its biomass, which can be tailored to user-specific requirements and thus be efficiently incorporated in feed formulations. The upcycling of nutrients with insects guarantees a consistent macro-nutrient profile which can be incorporated in feed formulations.

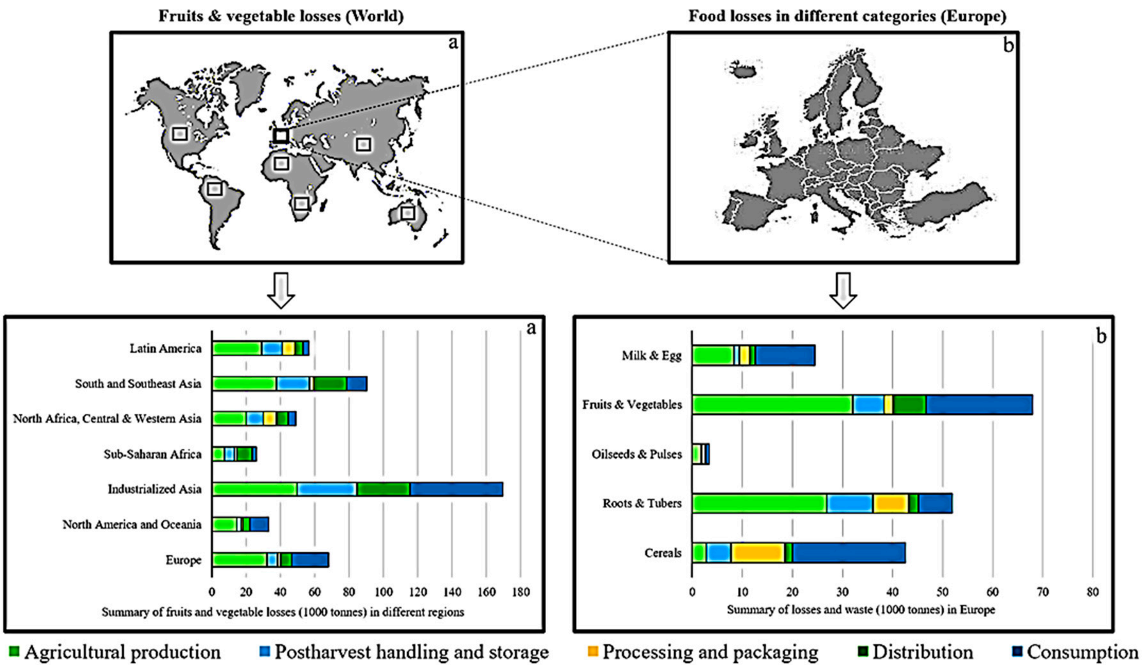

Figure 2. Food wastes: Global and European context. (**a**) global fruit and vegetable losses (**b**) food losses in different categories across Europe (adapted from [13]).

Fruits and vegetable (F&V) wastes are ideal substrates for bioconversion with insects. The average moisture content of fruit wastes such as apple pomace, tomato pomace, pineapple bran and carrot pulp is about 90%, and the protein and lipid content in dry matter is optimal for the rearing of insects, particularly BSF. For instance, based on our internal data, 1000 kg of fruit and vegetable waste (proprietary mixture) converted by BSFL gives approximately 125 kg of fresh BSFL and 250 kg of frass over 14 to 21 days. The proximate analysis of the F&V biomass revealed the protein, lipid, total carbohydrates and ash contents, which were 3.85%, 2.77%, 17.48%, and 2.01%, respectively. The global material balance of nutrient transfer which depicts the upscaled quantity of nutrients is displayed in the infographic (Figure 3). Essentially, the 39 kg of protein and 28 kg of lipids which would have been dumped in a landfill were valorized into high-quality nutrients by BSF (40 kg dry weight) which is suitable for animal, fish feed and pet food. A rough estimate of data collected from the literature suggests that the amount of substrate required to obtain 1000 kg of BSFL might range between

8000 to 20,000 kg depending on the substrate mixture. For example, Cai et al. [22] proposed that a 1000 kg diet substrate containing only mushroom root waste yielded 48–53 kg of BSFL. Meanwhile, when the substrate (60% mushroom waste) was supplemented with auxiliary materials 40% bran or 40% kitchen waste to accelerate the bioconversion, the BSFL yield increased to 68–72 and 129–163 kg, respectively. The average weight of frass estimated was 185 kg, making it the most significant output by volume in insect-based bioconversion processes. The energy required for insect metabolism is obtained intrinsically from the waste substrate it is reared on, giving it a biofactory-like trait.

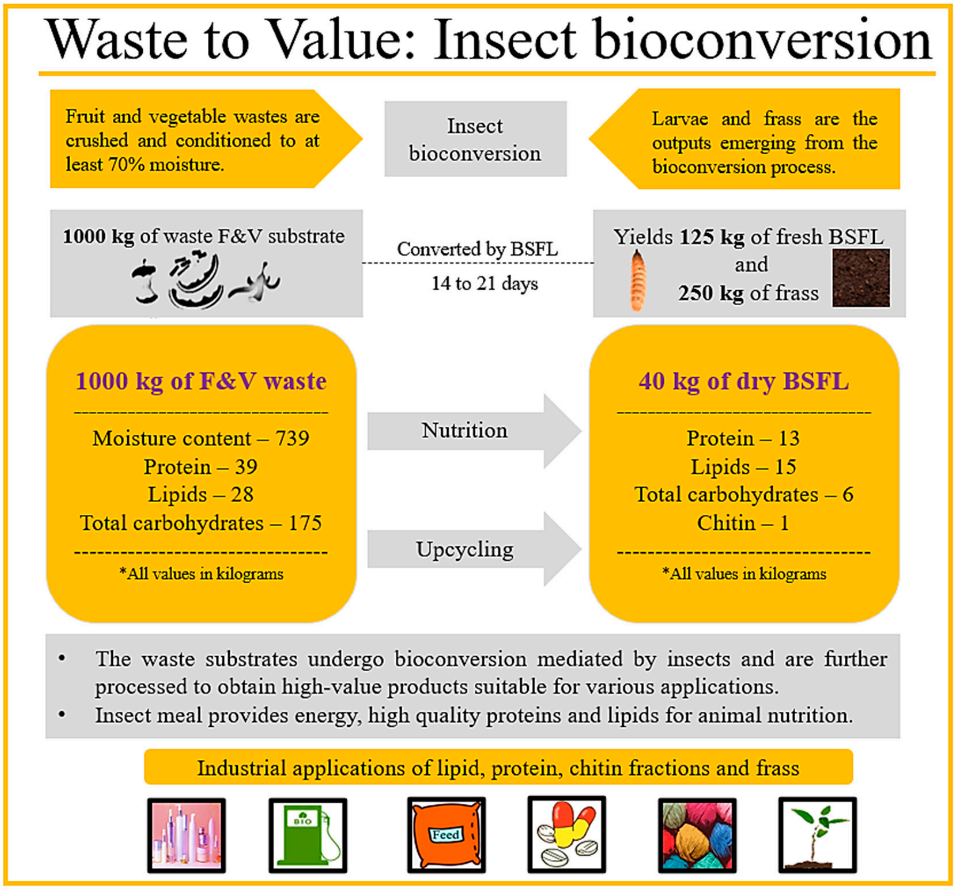

Figure 3. Insect bioconversion: material balance overview.

The typical phases in the upcycling of nutrients from waste to human nutrition can be summarized in four steps: (i) waste biomass—dietary requirements in the form of micro and macro-nutrients for insects are obtained from the waste substrates; (ii) insect bioconversion—larvae convert available nutrients into protein, lipids and chitin, which are retrieved by various fractionation processes; (iii) animal and aquafeed—processed insect-based protein and lipids are incorporated in feed formulations; (iv) human nutrition—fish, poultry and other livestock bred on an insect-based diet are processed in to end products for human consumption. The feasibility of waste valorization into a sustainable nutritional source makes insect an ideal tool for waste management, nutrition upcycling and generating circular economy (Figure 4).

The plasticity and modulation of nutrients in insects by dietary modification is well established and can be tailored to user-specific requirements. Although the crude protein, lipid and carbohydrate concentration may vary depending on the substrate, the underlying fatty acid and amino acid profile do not vary significantly. The feed conversion ratio, specific growth rate, conversion efficiency and all other growth-related parameters of the insects depend on several biotic and abiotic factors [23]. Waste consumption by BSFL was found to be the greatest for fruits and vegetables when compared to standard poultry feed and other waste streams like fish rendering, kitchen waste, pig manure and pig liver [24].

Conflicting reports on protein content in substrate affecting larval growth and its nutritional composition make it tedious to draft or collect optimal nutrition requirement data for insect rearing. For instance, Nguyen et al. [24], in their study, discussed that larvae reared on a high protein content substrate had higher larval protein content, whereas larvae reared on a protein content ranging from 3.5 to 14% had similar larval crude protein [23]. This trend was in line with the findings of Tschirner and Simon [25], who demonstrated that larvae fed on a diet with the lowest crude protein content (8.5% dry matter (DM)) had higher larval protein (52.3% DM). Similar results were achieved where diets with protein content ranging from 9 to 25% had a relatively equivalent larval protein content, which was between the range of 40 and 43% dry matter [26]. The BSFL can cope with the varying nutrient availability in the substrate medium; shortages might lead to the prolonged development time of larvae [27].

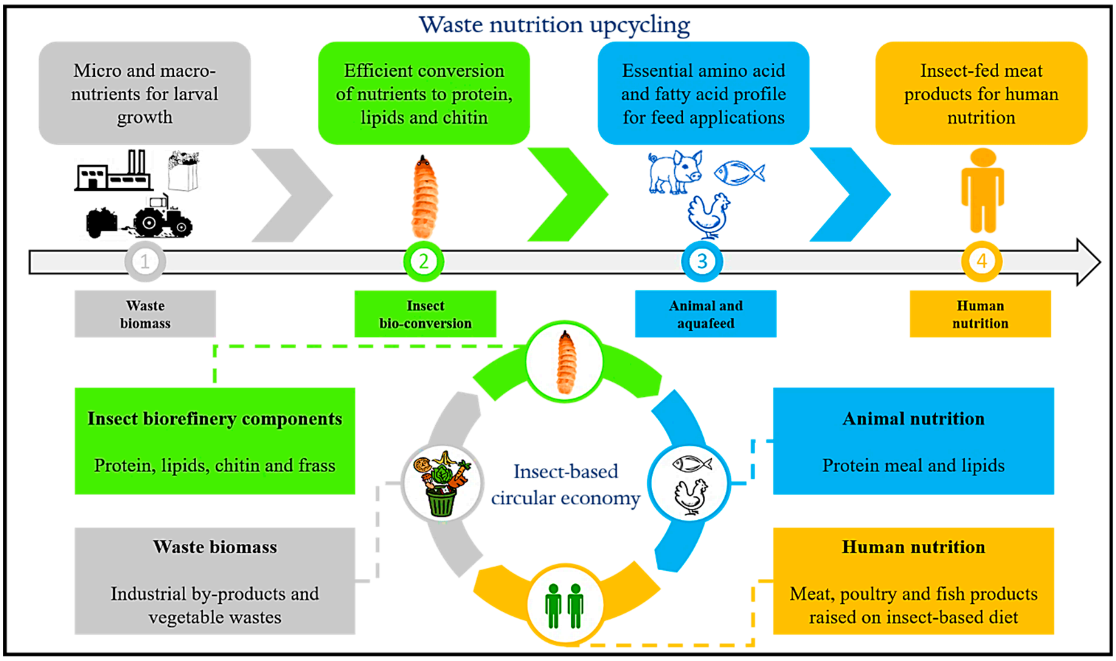

Figure 4. Nutrition upcycling from waste streams and circular economy.

3. Biorefining and Fractionation of BSFL Biomass

3.1. Killing Methods

Once the BSFL are harvested, separated from frass and cleaned, the first unit operation executed in BSFL processing is the killing/devitalizing/euthanizing of the biomass. Though several techniques exist for the slaughtering of insects, swift treatment is preferred for ethical reasons. Based on the end-use or the intended application of the final product, the user can choose a thermal or non-thermal killing method. As a matter of fact, several research articles with a similar theme have been published, which discuss the effect of killing methods on the physical, chemical and biological impact on the BSFL biomass. The effect of several devitalization techniques such as grinding (homogenization at 15,000 rpm), high hydrostatic pressure (600 MPa; 3 min), desiccation (60 °C; 30 min), blanching (boiling water; 40 s), freezing (−20 °C, −40 °C; 1 h), immersion in liquid nitrogen (40 s), asphyxiation (vacuum packing with 100% CO_2 or N_2 flushing; 120 h) on the lipid oxidation, color and microbial load of BSFL was evaluated [28]. Authors Leni et al. [29] found that prepupae killed by blanching exhibited better extractability and displayed improved susceptibility of proteins during enzymatic digestion. Blanching also inhibits browning reaction, as opposed to freezing the BSF biomass. Other benefits of blanching are the prevention of lipolysis and ensuring the stability of the BSFL lipid fraction during prolonged storage under freezing conditions [9,30].

3.2. Fractionation

The fractionation of BSFL biomass can be classified into two types: (1) dry mode and (2) wet mode fractionation. In a typical dry mode fractionation, the BSFL is subjected to an initial drying step to remove the moisture content and subsequently pressed to obtain BSFL meal and oil. Processing condition parameters can be optimized as per user needs and end-product specifications. A simple dry fractionation scheme is represented (Figure 5C), where the BSFL along with frass is fed to the feed hopper which gradually places the mixture in a vibratory screen separator to eliminate frass and debris from the larvae. A water application can also be incorporated to wash the BSFL for the complete removal of physical impurities. Then, the larvae are placed in a conventional oven which serves as a drying and devitalization step. Furthermore, the mechanical pressing of larvae for the obtention of oil and protein meal can be carried out under cold press (ambient temperature, T = 25 °C) or hot press conditions (T > 60 °C). Hot pressing enhances the leaching of oil from dry BSFL and the residual oil content in BSFL meal could be below 10%. A theoretical industrial-scale fractionation scheme is presented (Figure 5D), where moisture removal can be achieved with microwave tunnel drying. Then, the larvae might be pressed, pulverized and electrostatically separated to obtain BSFL protein concentrate.

In the wet mode fractionation of BSFL, the larvae are treated alive, wherein the in-situ water which approximately constitutes two-third of the fresh weight of the larvae is utilized in for the processing. Drying of the fractionated constituents is carried out in the final step. The schematic representation of wet mode fractionation of BSFL is depicted (Figure 5A). The larvae are crushed to obtain pulp and then placed in a holding tank where enzymes (protease) are added in order to hydrolyze the protein. The pulp mixture naturally has three fractions: (a) aqueous (b) lipid and (c) solid fraction after the termination of the enzymatic hydrolysis the pulp mixture is passed through a "Tricanter" to separate aqueous, lipid and solid fractions. The lipid fraction of BSFL at a temperature above 40 °C tends to exist in liquid state, hence the separation is facilitated based on the density difference. The aqueous fraction containing soluble hydrolyzed proteins is spray-dried and thus high-quality BSFL protein without chitin is obtained. The solid fraction, which is essentially made of fibers, insoluble protein and chitin, is oven-dried. The fractionation scheme was inspired based on an industrial patent [31], where the fresh BSFL were directly squashed in a micro-cutter mill (<0.5 mm) and subjected to enzymatic hydrolysis with pepsin for 3–5 h, at 35–65 °C and pH 3–6. The reaction was terminated by heating the mixture to 90 °C and the fats were liquified during this heating process. Physical separation was carried out by the decantation or centrifugation or both and the three fractions are recovered (Figure 6). Enzyme-assisted protein extraction from BSFL with seven different commercial protease enzymes was articulated where the highest protein extraction yield (79%) was obtained with protease from *Bacillus licheniformis* and the degree of hydrolysis was 10.4%. Proteases were able to extract proteins from BSFL in the form of peptides and free amino acids which could be used in food formulations and as a feed supplement [32]. A non-enzymatic approach for wet fractionation is graphically represented (Figure 5B); this process scheme is based on our preliminary trials. Subjecting BSFL to steam for a fixed duration before pulping augments the lipid fraction yield during separation (data not published) when compared to direct crushing of the BSFL. A recent patent [33] with a similar processing scheme was published, where fresh BSFL was ground with hot water (50–70 °C) in a juice press (Angel® Juicer apparatus) to obtain a pulp fraction (rich in chitin) and a liquid fraction (rich in fats and proteins). The pulp fraction was again blended with hot water to ensure maximum extraction of proteins.

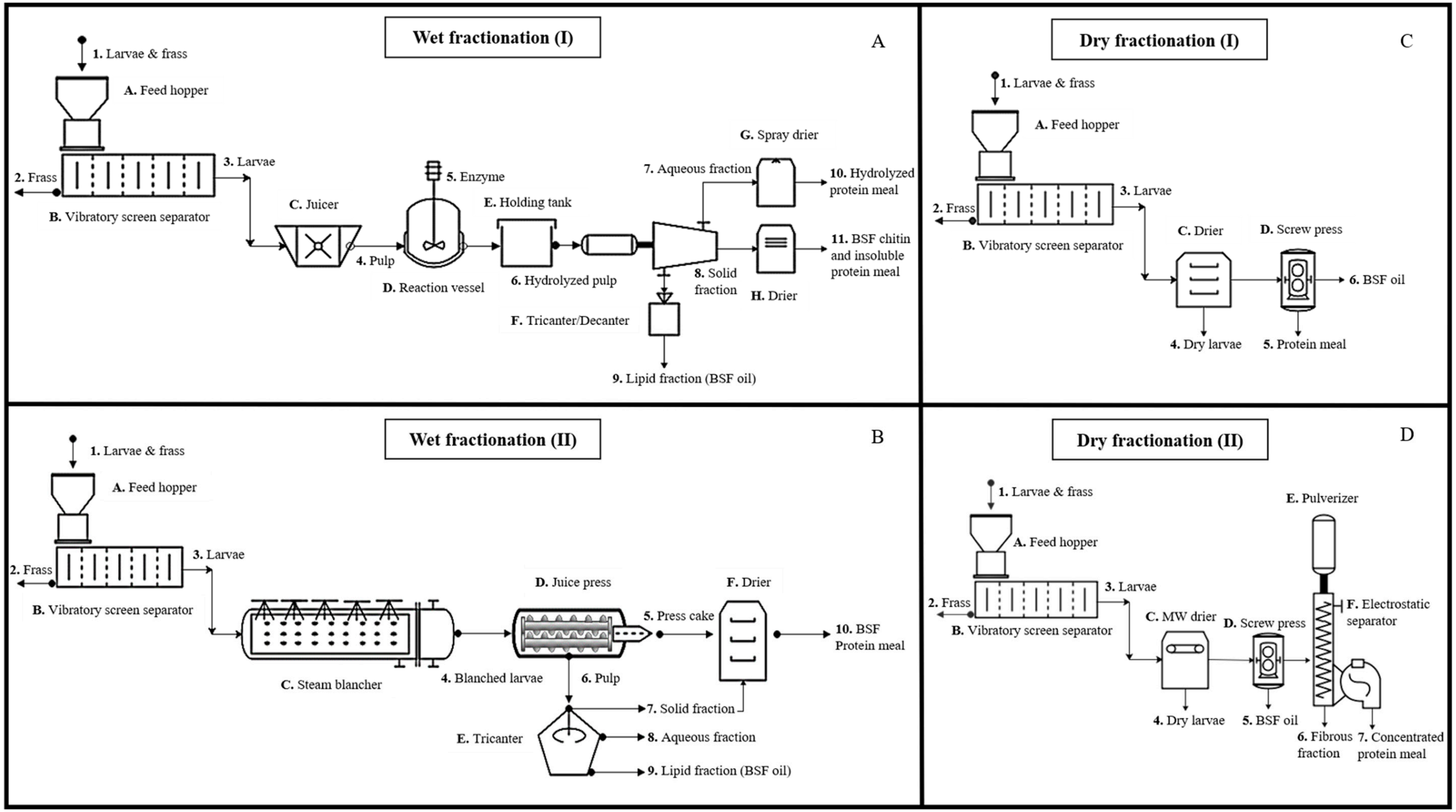

Figure 5. Unit operations in wet and dry fractionation of BSFL: An industrial-scale purview. (**A**) wet fractionation scheme with enzyme application (**B**) wet fractionation scheme with juice press (**C**) converntional dry fractionation (**D**) dry fractionation with an electrostatic separator.

Phase separation of the liquid fraction to separate proteins and lipids and the further chemical purification of chitin rich pulp was disclosed. Organic acids were used to improve the fractionation of BSFL juice into lipid- and protein-enriched fractions. The authors reported that use of lactic acid in synergy with hydrochloric acid (HCl) proved to be the best condition to obtain high purity (80%) in lipid fractions and acetic acid with HCl or HCl by itself gave the best results for proteins. The results suggested that pH had a significant effect on lipid separation [34], which is vital in wet mode fractionation. Lipids, proteins and chitin were sequentially extracted from the larvae, prepupae and pupae of BSFs with a standard procedure, which proves the feasibility of obtaining valuable biomolecules from BSFs of different life stages reared on low-value organic waste streams with different composition [35]. Systematic approaches for the extraction and fractionation of proteins, lipids, and chitin from BSF prepupa were proposed [30].

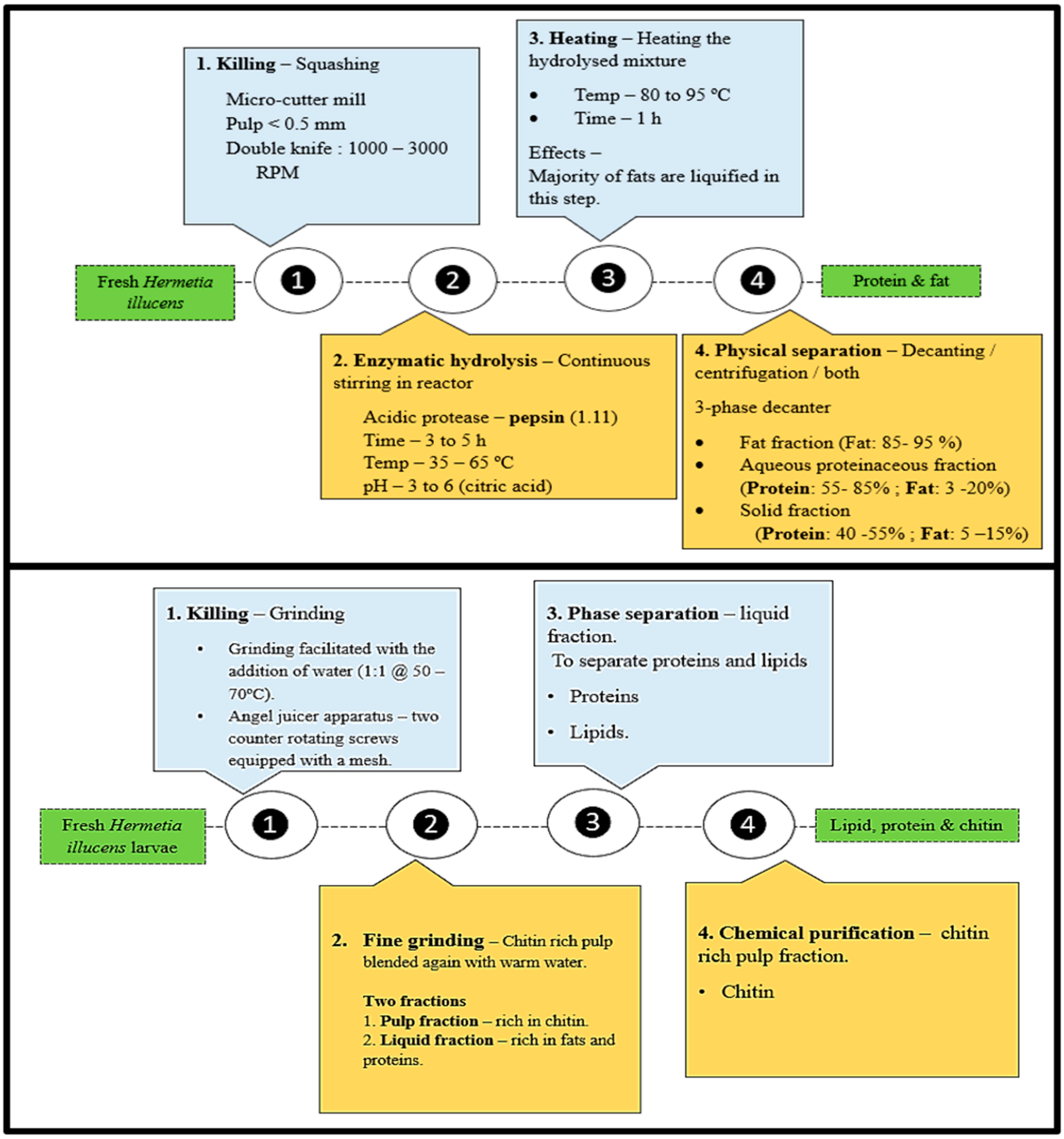

Figure 6. Breakdown of unitary operations in industrial patents.

3.3. Biorefining

A biorefinery is the sustainable processing of biomass into a wide array of marketable products [36]. Insect biorefinery, in particular, BSFL-based biorefining, produces a plethora of products (Figure 7), such as biofuels, enzymes, polymers, resins and animal feed [37]. The concept of cascade utilization of biomass originated from the forestry sector and has been proposed to maximize resource efficiency and reduce greenhouse gas emission; the same can be applied to insect biorefining for exhaustive utilization of various constituents present in them. The principle of cascading biomass use or cascade biorefining would entail a systematic effort, sequentially exploiting the biomass for higher-added-value products [38]. The main products of the economic significance obtainable from BSF rearing are proteins, lipids, chitin and frass. The extraction, separation, and fractionation of these compounds are extensively covered in the earlier sections. A multi-insect-based biorefining system where lignocellulosic biomass (corn stover) was initially converted by yellow mealworm and the residue from the initial treatment was subjected to secondary bioconversion with BSFL for the obtention of biodiesel, and protein was communicated [39]. An integrated biorefinery anaerobic digestion of BSFL biomass for bio-methane production was assessed. Five different BSF-based feedstocks were studied for the bio-methane potential (B0) assays and BSFL reared on food waste had the highest bio-methane potential (B0 = 675 mL CH_4/g volatile solids). The bio-methane potential of BSFL was 1.5–2 times higher than other representative feedstocks, including energy crops and algae [40]. Attributional life cycle analysis of an industrial-scale BSF production plant implied its lower environmental impact when compared to similar sources of animal feed production. The study demonstrated that the different facets of current insect production technology have potential for the production of sustainable protein, lipids and fertilizers when compared to conventional ones. The said advantage was contingent on sourcing waste side-streams that serve as a diet substrate for BSF feed [41].

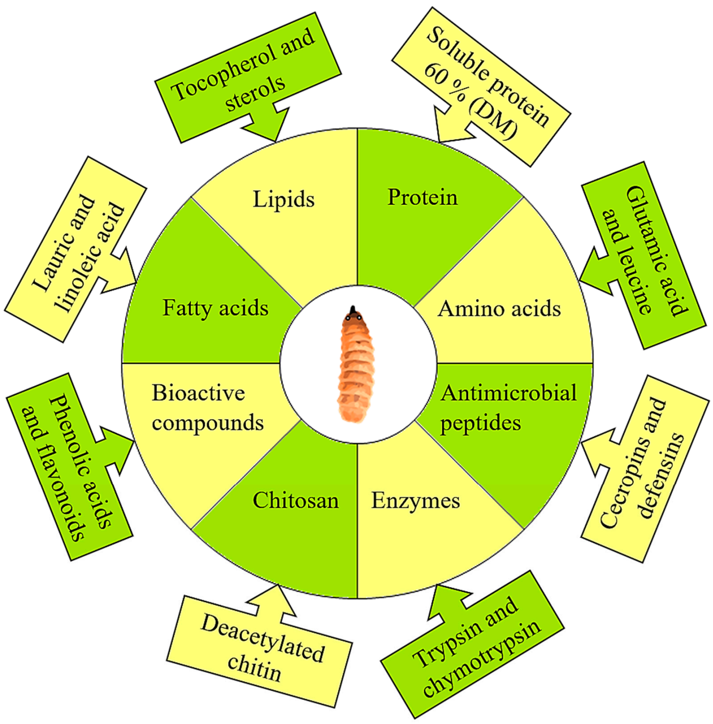

Figure 7. Constituents retrievable via BSFL biorefinery.

3.4. Lipids

Lipids are the dynamic component of macronutrients belonging to BSFL, which can be modulated based on the substrate diet. The crude lipid content of BSFL can range from 8% to 60% dry weight

(Table 1) and the principal lipid class constituent of BSFL oil are triglycerides [30]. Generally, the lipid content is higher in the larval phase and is accumulated as storage fats which serve as the primary energy reserve for the metabolic activities in the adult fly phase. Several studies have articulated the modularity and plasticity of the lipid and fatty acid profile of BSFL in which tailor-made alterations can be effectuated in the lipid fraction by manipulating the substrate diet. Saturated fatty acids (SFA) dominate the fatty acid profile of BSFL oil which is similar to coconut, palm oil, and the principal fatty acids, as detailed in (Table 1). Among the data tabulated, lauric acid, myristic and palmitic are the primary saturated fatty acids, oleic and palmitoleic acid, and linoleic and linolenic acids are the mono- and polyunsaturated fatty acids, respectively, that make up almost 90% of the fatty acid profile of BSFL oil. Larvae reared on eleven different diets composed of mussels, bread, fish and food waste were compared to identify the variation in the fatty acid profile of BSFL oil obtained [42]. The study also found that there were limitations in the uptake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by BSFL as 20–60% of the EPA and DHA remained in the substrate residues. A similar trend was witnessed in the study [43] where BSFL were reared on increasing concentrations of brown algae. The EPA concentration in the larvae increased linearly with the EPA concentrations in the substrate media.

Nevertheless, both the studies discovered that there was a limitation in the extent to which the BSFL can retain these diet-derived fatty acids and witnessed a decrease in the oil yield when drastic diet changes were adopted. The BSFL oil is dominated by saturated triacylglycerides (TAG) with LaLaLa accounting for 27.6% of total TAGs, followed by LaLaM with 16% and LaMM with 15.1%, respectively [44]. Fish waste (discarded round sardinella) as a substrate was used to augment the EPA, DHA and total polyunsaturated fatty acids (PUFAs) content in the lipid fraction of BSFL. The percentage of EPA + DHA observed in the twelve-day old larvae was 12.1%, which was significantly higher than previously reported values [45].

3.4.1. Lipids in Human Nutrition

Lauric acid is a well-established energy substrate for humans and livestock as it is efficiently absorbed and digested [46]. The triacylglycerol of lauric acid is converted to monolaurin, which possesses antiviral, antibacterial and antiprotozoal properties [47]. For the effective utilization of BSFL oil/fat for human nutrition, crude oil has to be processed similar to conventional oil seeds. The classical alkali refining method was employed for BSFL oil refining. Typically, the unit operation involved is (a) degumming to remove the phospholipids, proteins, pigments and carbohydrates, (b) neutralization in order to remove the free fatty acids, phospholipids, sulfur and insoluble matter, (c) bleaching to discard oxidation products, pigments and trace metals, and then finally (d) deodorization to eliminate the free fatty acids and diacylglycerols. The difference between the physicochemical characteristics of crude and refined BSFL oil obtained by mechanical pressing were documented in a recent study [47].

Cold-pressed BSFL oil exhibited higher oxidative stability (50.5 h), which is comparable to coconut (30–250 h) and palm kernel oil (15–100 h), and this can be attributed to the higher amount of saturated fatty acids present in the oil and possibly the high content of Δ5-avenasterol present [44]. Consumers' perception of insect fat (BSFL) as a partial butter replacement for certain bakery products (cake, cookie and waffle) was explored, and the results showed that BSFL fat could replace 25% of the butter in the products studied without changing the overall food experience and liking for the participants. The authors proposed that refined BSFL fat might alleviate the off-flavors perceived in the formulation containing a higher percentage of insect fat used in the formulations [48]. The processing, sustainability and design of insect margarine produced from BSFL fact and mealworm oil were articulated.

Table 1. Fatty acid distribution in BSFL reared on different substrates.

Reference	[49]	[42]	[50]	[50]	[50]	[50]	[43]	[51]	[52]	[9]	[53]		[54]
Fatty Acid (%)/Substrate	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S1**	**S10**		**S11**
C12 (Lauric) [a]	47	51.8	56.24	56.41	56.94	54.18	40.6	23.9	11.85	61.4	42.27	28.1	42.8
C14 (Myristic) [a]	6.5	9.5	9.27	8.46	8.19	7.05	8.5	6.7	2.09	10.2	9.41	3.85	8.12
C16 (Palmitic) [a]	15	12.7	10.26	11.05	8.36	7.95	14.8	16.6	12.69	7.8	13.91	5.78	13.9
C18:1n9 (Oleic) [b]	14	12	7.06	7.15	6.97	6.35	8.8	17.9	54.12	7.8	11.84	4.27	10.4
C16:1(Palmitoleic) [b]	3.1	2.8	2.37	2.24	1.95	2.06	2	2.5	1.26	2.5	2.73	1.65	2.06
C18:2n6 (Linoleic) [c]	9.4	7.7	10.26	10.18	10.74	10.94	17.9	18.6	12.29	7.2	14.29	1.27	12.6
C18:3n3 (Linolenic) [c]	0.8	1.6	-	-	-	-	1.4	1.6	0.44	0.4	1.41	10.3	1.16
Σ Fatty acids	95.8	98.1	95.46	95.49	93.15	88.53	94	87.8	94.74	97.3	95.86	55.22	91.04
Crude lipid	NA	57.8	37.24	36.52	36.18	35.73	33.8	8.1	NA	28.4	32.51	32.97	NA

[a] saturated fatty acid, [b] mono, [c] poly unsaturated fatty acids; NA-Not available. S1-Chicken feed; S2-Bread; S3-Food waste; S4-Pig manure; S5-Chicken manure; S6-Cow dung; S7-Wheat; S8-Brown algae; S9-Crude olive cake; S10-Fruit and vegetable waste; S11-Unknown.

The study aimed at identifying the processing parameters (solid fat content and color), environmental impact and product design properties of lipids derived from the insects. The substitution of lipids (up to 75%) with insect fat without negative effects on spreading abilities was identified. The substitution of 75% of lipids in margarine resulted in higher environmental impact product when compared to regular margarine [55]. Insect-based milk and ice creams (Gourmet grubb, ENTOMILK^{TM}) have found their way to the market as a dairy-free option for consumers. The application of fats from insects in human nutrition is rare due to the legal restrictions in Europe when classifying part of insects or ingredients containing insects as a novel food according to regulations (EU 2015/2283). Once the insect-product-specific regulations are established, several other avenues for the incorporation of insect fat, in particular, BSFL fat, can be explored. Meanwhile, the data concerning BSFL fat look promising for human nutrition applications. Examples for conventional extraction of lipids are hydraulic pressing, solvent extraction and heat extraction.

Freezing is not ideal as it does not destroy or inhibit the endogenous lipase action enzymes which are responsible for the hydrolysis of triacylglycerides (TAGs) into intermediary constituents like diglycerides and fatty acids, thereby altering the lipid profile of the larvae. In fact, direct freezing tends to activate lipase enzymes. The lipase enzyme activity of the gut extracts from BSF was 7.75 U/g, which is the highest when compared to other endogenous enzymes such as the α-amylase, protease and trypsin-like protease present in the gut of BSF [56]. Blanching, as a killing method for the devitalization of BSFL, plays a pivotal role in preventing the hydrolysis of TAGs in the lipid fraction [9,30]. Though the utilization of solvent (n-hexane, ethanol) for the extraction of lipids from BSFL may not be the industrial norm, research works concerning the defatting of insects engage in the use of n-hexane. Green alternatives for replacing petroleum-derived hexane solvent for degreasing insect larvae have been the subject of few research articles. Degreasing fresh BSFL was compared using an array of solvents (petroleum ether, n-hexane, isopropanol, ethyl acetate and aqueous ethyl acetate). Ethyl acetate and aqueous ethyl acetate (90%) exhibited higher degreasing rates of 28.71% and 29.04%, respectively, when compared to control n-hexane [57]. The crude oil yield increased when 2-methyloxolane was employed for defatting freeze-dried BSFL when compared with reference n-hexane, and resulted in relatively better antioxidant properties without any deleterious effect on the protein fraction of the defatted flour [9].

3.4.2. Lipids in Animal Nutrition

Lipids constitute one-third of the dry matter of BSFL, and the possible utilization of the BSFL fat fraction in animal nutrition is rather scarce. The replacement of soybean oil with BSFL fat in the diet for finisher broiler chicken was considered and 100% substitution resulted in overall satisfactory meat quality in terms of nutritional and sensory profile. The only drawback was the enrichment of the saturated fatty acids which might negatively affect the nutritional profile of the chicken meat. The total SFA content in the control of the chicken leg meat fed with 0% BSF fat was 25.8%, for 50% BSF fat substitution the SFA content increased to 35.1%, and for 100% BSF fat the value was 44.2%, indicating a strong correlation between the dietary and meat fatty acid profile [58]. Histomorphological investigations of finisher chickens fed BSFL oil suggested that BSFL fat could completely replace soybean oil without any adverse effects on growth performance, hematological parameters, serum biochemical indices, intestinal morphology or histological features.

Interestingly, the dietary difference in the intake of lauric and myristic acid did not elicit a positive growth performance. Instead, the parameters were equivalent to the control diet [59]. The use of BSFL fat to replace soybean oil as a dietary ingredient for rabbits revealed similar traits wherein the proportion of total SFA increased and those of MUFA and PUFA reduced. The nutritional quality parameters like n-3 fatty acids, PUFA/SFA and Σn-6/Σn-3 FA ratios are indicators of fat quality for human consumption, and for meat-fed BSFL oil the values were well within the optimal values [60]. The replacement of canola oil with BSFL fat in salmonid feeds had no negative impacts on the fish responses. The fatty acid composition of fish reflects those of the diet adapted; BSFL oil incorporation might not influence the immune system ability of fishes, particularly in Jian carp, as observed by authors Li et al. [61]. The possible addition of BSFL oil to a tune of 25 g/kg diet in Jian carp without compromising the growth performance was illustrated.

Dietary inclusion of BSFL oil was capped at 10% of feed ingredients for rainbow trout was asserted, and the determination of the maximum threshold to which insect oil can be included could be the subject of subsequent studies [62]. The apparent digestibility coefficients (ADC) of fatty acids were high (ADC > 95%) in Atlantic salmon fed an insect-based diet, where the lipid fraction was BSFL oil, which is rich in medium-chain fatty acids like lauric acid. The high content of lauric acid in the insect-based diets led to inhibited lipid storage in the fish liver [63]. The partial or total replacement of soybean oil with supercritical CO_2 BSFL fat extract applied in young turkey poult had no adverse effect on the growth performance, nutrient digestibility or selected internal organ weights and lengths. Additional benefits like antimicrobial activity and support to immune responses can be attributed to BSFL fat [64]. Another study [65] probing the effect of BSFL fat inclusion in nursery pigs diet elucidated the improved growth performance of the recipient, attesting to the fact that BSFL oil is a promising, high-energy alternative feed ingredient that can efficiently replace plant-based oils in animal nutrition.

3.4.3. Non-Food Applications of BSFL Lipids: Biodiesel

A renewable and environment-friendly liquid fuel with a better ecological footprint than conventional petroleum-based fossil is biodiesel. Conventional oil crop feedstocks for biodiesel production are limited and expensive. Hence, alternative feedstock such as oleaginous yeast, fungi and insect-based biomass are attractive for this application and have been the subject of several research works. The bioconversion of organic waste such as animal, residential, commercial and institutional materials into lipids, and the subsequent processing of lipids to obtain biodiesel, can be materialized with BSFL. The unit operations to facilitate the synthesis of biodiesel from BSFL biomass is depicted (Figure 8).

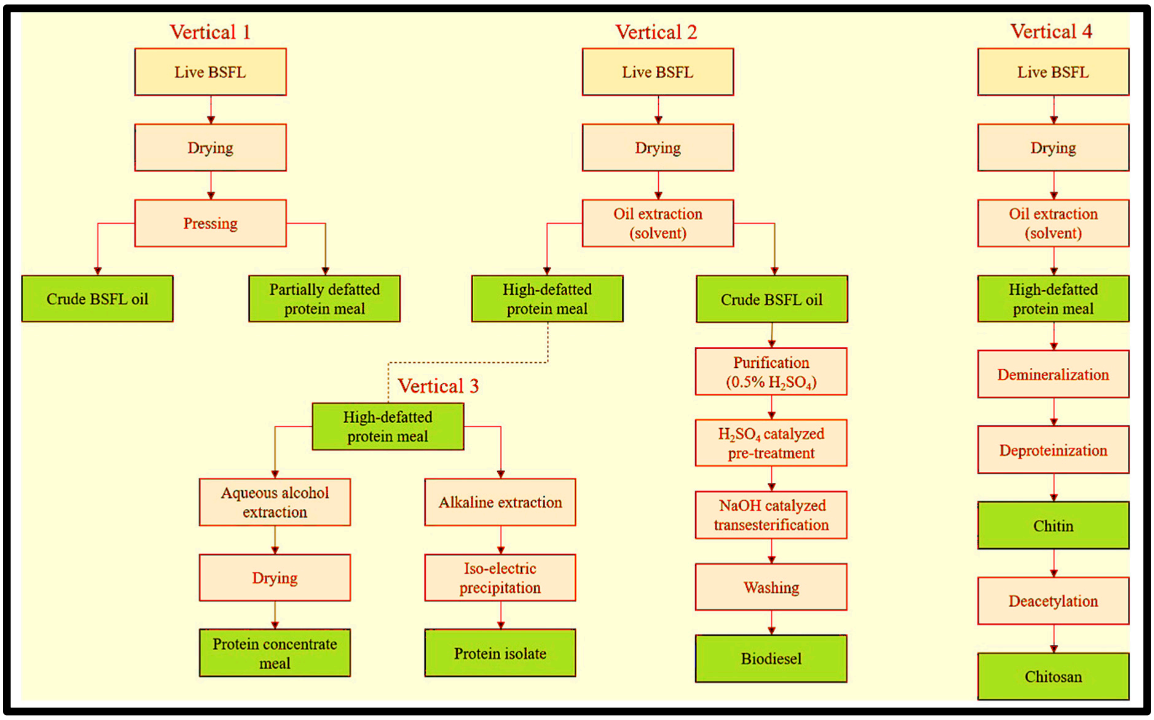

Figure 8. Flow chart of BSFL oil, protein, biodiesel and chitin extraction.

Lipids constitute ~30% of the BSFL biomass, which could serve as a valuable non-food feedstock for biodiesel production. To establish an effective model for the transformation of BSFL lipids to biodiesel, the BSFL biomass reared on various waste substrates should have a higher yield. In order to test this hypothesis, animal wastes like cattle, pig and chicken manure were used for BSFL rearing and the crude lipid obtained was converted to biodiesel through acid-catalyzed esterification and alkaline-catalyzed transesterification. The biodiesel thus obtained met all the criteria of European biodiesel standard (EN14514), and the fuel properties such as density, viscosity, ester content, flash point, and cetane number were comparable with rapeseed biodiesel [66]. Direct transesterification was accomplished by mixing dry BSFL, methanol, cosolvent (acetone, chloroform, petroleum ether or n-hexane) and sulfuric acid in a sealed reactor. The highest biodiesel yield was obtained was 94.1% with the following parameters: solvent dosage (2 mL/g of biomass), methanol dosage (4 mL/g of biomass), acid catalyst dosage (0.6 mL/g of biomass), temperature (120 °C) and reaction time (90 min). The solvent n-hexane was identified as the ideal cosolvent and the biodiesel yield obtained by the direct transesterification process was relatively better than the conventional process [67]. Solid residual fraction (degreased restaurant waste) was used as a substrate for BSFL rearing. To ensure an efficient conversion of free fatty acids into biodiesel, a two-step approach with an acid-catalyzed esterification step and an alkaline-catalyzed transesterification step was followed, which generated a biodiesel yield of 93.1% and the fuel properties were on par with the specification [68]. The enzyme-assisted lipid extraction of BSFL for biodiesel production was investigated and different protease enzymes (protamex, flavourzyme, papain, champzyme and bromelain) were evaluated for their efficacy in lipid separation. The fat yield obtained with protamex enzyme was 18.17%, and hence was used for predicting optimal conditions. The higher lipid yield with enzyme pretreatment was attributed to the improved release of lipids due to tissue and cell membrane degradation [69]. The enzyme-mediated transesterification of BSFL fat was studied, in which various lipase enzymes were compared in terms of the reaction yield of biodiesel. The study employed canonical methods to predict the optimal reaction conditions for determining maximum biodiesel yield and suggested the following conditions: methanol:fat molar ratio, enzyme loading rate (Novozym 435), reaction temperature and time of 6.33:1, 20%, 26 °C and 9.48 h, respectively [70]. The properties of biodiesel obtained from BSFL lipids along with the European reference standard is summarized in (Table 2). A brief review covering various aspects of different insect species and their utilization for biodiesel production was communicated [71].

Table 2. Properties of biodiesel from BSFL reared on different substrates.

Biodiesel Properties	Units	EN14214	[66]	[72]	[68]	[69]	[70]
Density	Kg/m^3	860–900	885	872	860	875	872
Viscosity at 40 °C	mm^2/s	1.9–6.0	5.8	4.5	4.9	5.3	5.2
Sulfur content	wt.%	0.05	NR	NR	ND	0.04	ND
Ester content	%	96.5	97.2	97.2	96.9	98.7	98.3
Water content	mg/kg	<0.03	0.03	NR	0.02	NR	0.03
Flash point	°C	120	123	121	128	121	121
Cetane index	-	48–60	53	NR	58	50	50
Acid number	mg KOH/g	<0.8	1.1	0.8	0.6	<0.5	<0.8

NR-Not reported. ND-Not defined.

3.4.4. Non-Food Applications of BSFL Lipids: Cosmetic Applications

Insects which are rich in lipids might be ideal for producing high-quality chemical constituents suitable for cosmetic applications. Insect fats (*Hermetia illucens*, *Locusta migratoria*, *Acheta domesticus*) were evaluated for their usability in skincare products, primarily for hand cream formulations. They were found to be effective, except for BSFL fat, as it was disproportionately higher in lauric acid when compared to other medium-chain C16 and C18 fatty acids [73]. A BSF-based skincare product was patented recently, where the commercial-oil-component in the formulation was replaced with purified BSFL oil, and the product is being marketed internationally [74]. A new industrial joint venture (Bug Bacon., Cambodia and Sibu Sea Berry therapy., Midvale, UT, USA) has resulted in the very first ento-vegan skincare product (Point68 illucens) made from BSFL oil, sea buckthorn and other plant oils. Such product commercialization unlocks new avenues for the incorporation of BSFL-based constituents for diverse applications, especially as a cosmetic ingredient.

Sodium lauryl sulfate (SLS) is a surfactant and a versatile ingredient used as the main component in detergent personal care formulations. For example, it is found in hair care products (shampoo, conditioner), lotions (hand cream, sunscreen) and grooming products (hand sanitizer, shaving cream). Sodium laureth sulfate or sodium lauryl ether sulfate is a surface-active agent with milder sensitivity when compared to SLS and a preferred ingredient in cosmetic products. Two industrial-scale processes are suitable for SLS production; the Oxo process is optimal when petroleum feedstocks like oil are used as the initial raw material. In the Oxo process, hydrogen and carbon monoxide are reacted initially with an olefin ($R\text{-}CH{=}CH_2$), which yields an aldehyde ($R\text{-}CH_2\text{-}CH_2\text{-}CHO$), and then the aldehyde is reduced to provide the corresponding fatty alcohol. The Ziegler process is an alternative approach where ethylene (CH2=CH2) is reacted with triethyl aluminum to obtain an alkyl aluminum, which is subsequently oxidized into an aluminum alcoholate and finally hydrolyzed under acidic conditions [75]. Coconut and palm kernel oil are traditional agricultural feedstocks used for SLS production as they are rich in medium-chain fatty acids (C12, C14, C16 and C18). Therefore, BSFL can serve as a practical alternative feedstock, as its fatty acid profile is relatively close to the feedstocks mentioned above.

3.5. *Proteins*

Insects are an attractive alternative for the conventional plant, animal and microbial protein sources. Academia and industry alike are working to solve the knowledge gaps and to address the uncertainties regarding insect protein as they are a sustainable and nutrient-rich source for food and feed. The nutritional quality of proteins is determined based on their essential amino acid (AA) content, digestibility and bioavailability. Several factors influence the quality of the insect protein; the pre-treatments and processing conditions play a particularly vital role in BFSL proteins. The BSFL-derived protein meal for any feed composition can be formulated from live BSFL, dried BSFL, partially or highly defatted BSFL flour, BSFL protein concentrate, BSFL protein isolate and enzyme hydrolyzed BSFL proteins. The unit operations involved in typical protein extraction from BSFL biomass are disclosed (Figure 8). Extracting insect protein and fortifying it in food and feed compositions could be an efficient way to enhance acceptability among customers. The techno-functional properties of protein from five insect species, namely *Tenebrio molitor* (TM), *Zophobas morio*, *Acheta domesticus*, *Blaptica dubia* and *Alphitobius diaperinus* were investigated. Apart from the protein purity of the extracts, the functional properties of proteins like gel formation were reported in that study [76].

3.5.1. Protein Extraction

Protein extraction is predominantly carried out in an alkaline medium where the biomass is mixed with an alkaline solution or buffer. Akin to conventional protein extraction, BSFL proteins can be extracted in similar conditions. Defatting of the insect biomass can increase protein recovery as the lipids do not interfere during the protein extraction process. The direct extraction of protein from the insect flour can be tedious partly due to its high lipid content, and simplest of unit operation like centrifugation or decantation causes involuntary leaching of lipids into the solvent medium. A unique feature regarding insect proteins (BSFL, TM) is their high solubility in the acidic region, precisely at pH 2, which is equivalent to or greater than solubility at alkaline conditions in some cases. Increasing the temperature from 20 to 60 °C resulted in improved protein recovery for defatted BSFL flour [77]. A stepwise BSF prepupae protein extraction based on the Osborne fractionation protocol was established by Caligiani et al. [78] in which sequential aqueous, salt (0.5 M NaCl), alcohol (70% ethanol) and alkali (0.1 M NaOH) extraction was executed for the recovery of albumin, globulin, prolamin and glutelin fraction, respectively. The protein solubility curve for BSF at different life stages as a function of pH was elucidated and the maximum protein solubility for all samples appeared in the basic range of 10 to 12. The isoelectric pH for precipitation of solubilized protein in alkaline medium was identified within the pH range of pH 4–4.5 [35]. The possible explanation for the limited overall yield of protein from BSFL when compared to other insects like *Tenebrio molitor* and *Acheta domesticus* could be the presence of insoluble proteins in the matrix. Another plausible contention could be the occurrence of sclerotization, a phenomenon in insects where the chitin and proteins are cross-linked to form stabilized structures to harden the exocuticle, which might inadvertently result in poor solubility of proteins. Melanin, which is found in insect cuticles, is responsible for the characteristic brownish or black color of the BSFL protein fraction [79]. Enzymatic oxidation results in the transformation of cuticular proteins into melanin-proteins mediated by the polyphenol oxidases (PPO) enzyme where the oxidation of phenolic compounds also facilitates the enzymatic browning [80]. Polyphenols in insects also act as substrates for oxidative enzymes contributing to the immune response, wound healing and sclerotization of the cuticle [81].

3.5.2. The Kp Conundrum

The cumulative amino acid content of the BSFL biomass reveals the actual protein content. The current nitrogen estimation methods like Kjeldahl and Dumas results in an overestimation of protein due to the presence of non-protein nitrogen like chitin, nucleic acids and phospholipids in the BSFL matrix. The nitrogen to protein conversion factor (Kp) value of 6.25 is considered the industrial norm for protein estimation. However, the presence of glucosamines contributes to the overall nitrogen content, thereby resulting in augmented protein values. Authors Janssen et al. [82] calculated a Kp value of 4.76 for whole BSF larvae and 5.6 for BSFL protein extracts, respectively. Though the above-mentioned Kp values can be considered for preliminary proximate BSFL composition analysis, the validity of the suggested Kp value for other diverse BSFL-based products that are used for feed formulations needs further scrutiny. For instance, products like defatted flour (partial or complete), protein concentrate, protein isolate and enzyme hydrolysates, which have a varied range of lipids and chitin in them, might need detailed amino acid analysis for accurate determination of the protein content, thereby estimating the Kp value for further analysis.

3.5.3. Essential Amino Acids and Their Digestibility

For a better understanding of the nutritional quality of BSFL protein in insect-based feed formulations and its impact in the recipient diets, the determination of the bioavailability of proteins in the feed formulations is evaluated based on their essential amino acid score, standardized, apparent and ileal digestibility. Unlike the fatty acid profile, the amino acid distribution in BSF is rather similar regardless of its geographical origin and diet (Table 3).

Glutamic and aspartic acids are the principal amino acids, whereas leucine, lysine and isoleucine are the primary essential amino acids found in BSFL. Contrary to the diet-based lipid modulation for BSFL, no validated model exists for enhancing the protein content by altering the diet substrate. The amino acid profiles of BSF larvae grown on four different substrates—chicken feed, vegetable waste, biogas digestate, and restaurant waste—were similar, although the substrate's amino acid composition varied substantially [26], which proves that the amino acid profile of BSFL is diet-independent. In the study by Cummins et al. [83], the authors examined the possibility of the partial or complete replacement of marine fish meal in diets of Pacific white shrimp (*Litopenaeus vannamei*) and found that BSFL meal inclusion exceeding 7% had a diminishing performance, as the deficiency in essential amino acids had negative impact on the growth. The optimal range of BSFL meal inclusion was within the range of 7–20% and the limiting order of essential amino acids in BSFL, when compared to fish meal, were methionine, lysine, threonine, phenylalanine, tryptophan and arginine. Perhaps the exhaustive defatting step should be considered, as the BSFL meal had a relatively high residual lipid content (11.81%), whereas the corn protein concentrate and soybean meal tested in the study had 4.89% and 2.05%, respectively. The current limitations can be overcome by fortifying the feed mix with the further addition of limiting amino acids.

Table 3. Essential amino acid profile of BSFL reared on different substrates.

Reference	[49]	[50]	[50]	[50]	[50]	[84]	[84]	[84]	[44]	[44]	[52]	[54]	[78]	[85]
Substrate	**S1**	**S2**	**S3**	**S4**	**S5**	**S4**	**S6**	**S7**	**S8**	**S9**	**S1**	**S10**	**S10**	**S10**
EAA	g/kg CP	g/kg DM				mg/g DM			% CP		g/kg DM	% BSFM	% DM	% BSFM
Arginine	57.1	20.9	21.1	20.6	20.1	1.1	5	2.5	4.5	6.5	20.5	1.64	1.96	3
Histidine	33.4	14.2	13.9	13.9	13	3.5	3.3	4.7	2.8	2.7	31.6	0.47	1.17	1.9
Isoleucine	51.9	18.4	18.3	17.7	17.5	1.6	2.6	1.8	3.9	3.8	15.6	2.24	1.47	2.2
Leucine	86.3	28.6	28.1	28.1	27.4	3	2.9	3.7	6.4	6.2	27.1	3.3	2.7	4.2
Lysine	71.2	24	24.6	24.1	23.4	4.1	4.7	4.7	6.2	5.6	23.2	1.96	2.3	3.8
Methionine	21.8	8	8.1	7.9	7.5	6.1	7.9	7.4	1.7	1.4	22.4	0.62	0.6	1.2
Phenylalanine	38.9	18.1	18.5	18.3	17.2	1.9	4.6	2.4	4	3.2	18.6	1.69	1.3	2.3
Threonine	46.4	19.1	19.5	18.4	19.2	-	-	-	3.9	3.9	18.4	1.93	1.49	2.4
Valine	72.1	19.7	19.5	18.4	19.2	7.2	1.2	9.3	5.8	5.5	18.7	3.58	2.4	3.7
Crude protein	476	436.9	428.3	417.1	412.5	411	330	413	40	41.3	39.2	43.4	32	56.01
Crude lipid	118	372.4	365.2	361.8	357.3	301	343	310	33.8	8.1	28.4	NA	37.1	11.81

CP: Crude protein; DM: Dry matter; BSFM: Black soldier fly meal; NA: Not available. S1: Chicken meal; S2: Food waste; S3: Pig manure; S4: Chicken manure; S5: Cow dung; S6: Kitchen waste; S7: Spent grain; S8: Wheat; S9: Brown algae; S10: Unknown.

The apparent ileal digestibility coefficients of essential amino acids in the BSFL meal determined by different assays are summarized (Table 4). The mean digestibility coefficients were within the range of 0.64–0.88. The standardized ileal digestibility (SID) contents of crude protein fed to broilers, particularly the SID contents of lysine, methionine and threonine, were 2.85%, 0.82% and 1.98% dry matter, which were higher than or comparable with the values obtained for soybean meal, fermented soybean meal and fish meal [86,87].

Table 4. Amino acid digestibility of BSFL meal estimated by different assays.

Essential Amino Acids	Amino Acid Digestibility of BSFL Meals				
Reference	**A [88]**	**B [88]**	**C [89]**	**D [90]**	**E [62]**
Arginine	0.79	0.8	0.91	0.83	0.92
Histidine	0.64	0.63	0.83	0.81	0.89
Isoleucine	0.83	0.87	0.87	0.45	0.88
Leucine	0.84	0.89	0.86	0.76	0.84
Lysine	0.8	0.8	0.9	0.46	0.9
Methionine	0.83	0.78	0.93	0.42	0.85
Phenylalanine	0.82	0.86	0.9	0.63	0.86
Threonine	0.73	0.77	0.88	0.75	0.87
Tryptophan	-	-	0.93	-	0.96
Valine	0.9	0.91	0.75	0.64	-
Mean	0.8	0.81	0.88	0.64	0.89

A—Apparent ileal digestibility coefficients for partially defatted BSFL in broilers. B—Apparent ileal digestibility coefficients for highly defatted BSFL in broilers. C—Amino acid digestibility using precision-fed cecectomized rooster assay. D—Apparent ileal digestibility coefficients of amino acid for BSFL in broilers. E—Apparent digestibility coefficient of amino acids in BSFL meal.

The mean values of apparent ileal digestibility coefficients (AIDC) of essential amino acids of partially defatted BSFL meal and highly defatted BSFL meal for broilers were 0.80 and 0.81, respectively. The study also found that high defatting of BSFL facilitated improved amino acid levels in the meal when compared to other insect meal rich in amino acids [88]. The protein quality of BSF during its different life stages was evaluated by Do et al. [89], and the AA digestibility was highest in 14-, 18- and 23-day old larvae. Among the EAA, threonine, methionine and tryptophan were the first-limiting amino acids of BSFL based on digestible indispensable AA score (DIAAS)-like values for dogs and cats. The overall protein and amino acid profile of BSFL of all ages were of high quality, and BSFL can potentially be a source of high-quality protein for pet foods.

3.5.4. The Molecular Weight Distribution of BSFL Proteins

The electrophoretic separation of BSFL proteins aids in the identification of protein bands obtainable from the soluble protein fraction. Meanwhile, data on the molecular weight distribution of proteins can be used for the selection of suitable purification processes like ultrafiltration or membrane separation. Two significant bands characterized by the molecular weight of 14.3 and 80.5 kDa are the abundant proteins recovered from defatted BSFL. Modifying the pH of the protein extraction system determines the composition of the protein extract. For example, increasing the pH of the extraction system to 2 led to a gradual decrease in the low molecular weight (LMW) protein fraction, whereas increasing the pH of the extraction system to 12 increased the LMW protein fraction to 98.9% [77]. The molecular weight of an abundant protein extracted from defatted BSFL using a citric acid-disodium phosphate buffer was found near 75 kDa and all of the soluble proteins were within the range of 25 and 75 kDa [9]. No deleterious effect on the defatted protein flour was detected when solvents like n-hexane and 2-methyloxolane were used for defatting purposes at 55 °C. Equivalent isolated protein fractions from BSFL were reported, wherein abundant proteins were found at 75 kDa resolved by SDS-PAGE; the study acknowledged the depletion of protein bands in BSFL after bio-decomposition [91].

3.5.5. BSFL Protein Meal as Feed

Fish meal (FM) is a primary protein source which is typically used in aquafeeds for its well-balanced amino acid composition, high digestibility and palatability [92]. Insect meals are marketed as a suitable replacement for fish meal in aquaculture feed formulations as they have similar protein content, and depleting fish stocks fuels the fluctuation in their price and availability [11,26]. Several dedicated review articles have extensively covered the effects of insect inclusion in diets of animal, livestock, fish and pet food nutrition [8,93–99]. Recently, authors Gasco et al. [100], in their comprehensive review titled "Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality", consolidated the effects of insect meal inclusion in fishes, shellfish, shrimps, broiler chickens, broiler quails, broiler ducks, laying hens, pigs (weaning, growing), and rabbits. Makkar et al. [8] outlined the applications of insects like mealworm, locusts, grasshoppers, crickets and silkworm prepupae meal as an alternative protein ingredient for animal feed and nutrition. Hence, research articles published after the timeline (2019) are considered in this section of the review.

The critical attribute of commercial aquaculture feed is its complementary mixture of ingredients that collectively supply high-quality digestible protein, energy and essential nutrients. This aspect was put to the test to assess whether BSFL meal could fit the criteria for the diets of Atlantic salmon, and the digestibility coefficients were over 75% for the majority of micro- and macro-nutrients. The growth study determined a threshold of 200 g/kg inclusion of BSFL meal for Atlantic salmon [85]. In the diets of European sea bass, replacement of fish meal with dry BSFL meal up to 50% was reported, and no deleterious effects on the proximate fish-body composition, hemato-biochemical variables and overall of growth of the fish were observed. In fact, the authors worked out the economic advantage of replacing fishmeal with dry BSFL meal and suggested 7.8%, 8.7% and 15.6% cost reduction per ton fish gain for inclusion levels of 25%, 35% and 50%, respectively [101]. There are numerous factors that influence the optimal substitution or replacement threshold of fishmeal with BSFL meal, which varies considerably (25% to 100%). The key parameters that affect the inclusion rate in diet formulations are the BSFL meal quality, which again depends on the rearing and processing (drying, defatting) conditions, the species of fish, etc. The total replacement of fish meal with BSFL meal did not compromise the gut health of Atlantic salmon [102]. The administration of BSF-based diets (25 and 50% BSF full-fat prepupae meal) from larvae to adult in zebrafish (*Dani rerio*) was investigated, and no major negative effects inhibiting the growth of the fish were observed. As evidenced by previous studies, full-fat BSF prepupae meal, which is high in saturated fats, caused hepatic steatosis in zebrafish and gut histological analysis on intestine samples revealed no signs of inflammation [103].

The physical and functional properties of insect-based extruded products, in which insect flour (BSF) and corn flour-based extrudates were compared. The extrudates formulated with insect flour displayed higher water solubility index and lower water absorption and oil absorption indices when compared to corn-based extrudates. These encouraging results could potentially qualify BSFL flour as a fortification agent that can be added to augment the protein content of ingredients to produce extruded products [104].

3.6. Chitin

Chitin [poly(β-(1→4)-N-acetyl-D-glucosamine) is a natural polysaccharide, and the second most abundant biopolymer available after cellulose. Chitin is a structural component (ordered crystalline microfibrils) in the exoskeleton of arthropods or the cell walls of fungi and yeast [105]. Chitin and chitosan (a deacetylated derivative of chitin) are used in diverse applications. The myriad functionalities of chitin and chitosan include their application in the textile industry as a sizing agent, in sewage sludge treatment as a flocculant, in biomedicine as composites for wound dressing, in pharmaceuticals as a drug delivery agent, and in the paper industry and cosmetic formulations [106–108]. Chitin in insects, particularly its concentration in different life stages of BSF, varies significantly and is mostly diet-independent. The physicochemical structure of chitin isolated from *Hermetia illucens* was reported in 2016 [109], in which the chitin extraction from larvae and imago was executed by the conventional method (Figure 8) where acid and alkaline solutions were used for demineralization and deproteinization purposes. The study claimed that the larval chitin had a complex, convex structure, whereas the surface of chitin obtained from BSF larvae was smooth and the fibers were distributed parallelly. Chitin from both larvae and imago were present in α crystalline form, a typical configuration in insect chitins [109].

One of the under-utilized biomass streams generated while insect rearing is the exuviae, which is left over after pupation. It is essentially the leftover exoskeleton when the adult fly emerges from pupae. Generally, the exuviae along with the dead adult flies (after mating) are mixed with frass as a carbon source for composting. The physicochemical structure analysis of chitin extracted from exuviae and imago was elucidated in a recent study [110]. The chitin content of exuviae and imago was 9 and 23% dry body weight. The authors found that the physical characteristics of the chitin varied, and the exuviae chitin was amorphous and non-porous.

Meanwhile, the imago chitin was mesoporous and had a higher surface area. The production of chitosan from BSFL exoskeletons was presented where the biomass was subjected to demineralization, deproteinization, deacetylation, and showed that the processing conditions were based on commercial chitin purification methods applicable for crab shells or crustacean shells. The processing parameters of demineralization with formic acid, washing with water were optimized and the deproteinization parameters (temperature, the molarity of NaOH and time) were optimized by the design of the experiment approach [111]. The extrapolation of data from such studies can be used to predict the chitin yield and their physicochemical properties at pilot or industrial scale. A systematic approach for extraction and fractionation of proteins, lipids and chitin by a sequential procedure from BSF prepupae was proposed by Caligiani et al. [78]. An interesting alternative to conventional extraction of chitin was recommended in which natural deep eutectic solvents (NADES) were selected for the decalcification and deproteinization of defatted BSFL. It was reported that improved decalcification was observed with NADESs of choline chloride-lactic acid (ChLa) and betaine-urea (BeU). The best efficiency for deproteinization was achieved with betaine-urea (BeU) as a solvent at 80 °C [112].

3.7. Properties and Applications of BSF Chitin

The elemental composition analysis of chitins from larvae and adults of BSF divulges the carbon (C), hydrogen (H) and nitrogen (N) distribution in the biomass. As per Waśko et al. [109], BSF pupal exuviae possess 35% C, 5% H, 3.7% N and the distribution in BSF imago is slightly different with 32% C, 4.8% H and 3.9% N. According to the elemental analysis results of Purkayastha and Sarkar. [110], BSF imago had 39.7% C, 5.5% H, 6% N and the BSF exuviae had 43.7% C, 5.8% H, and 6.14% N, respectively.

The Fourier transform infrared (FTIR) spectroscopy analysis of BSF chitin, typically existing in α crystal form reveals bands in the vicinity of 1650, 1620 and 1550 cm^{-1} [110]. The infrared spectra of chitin extracted from larvae and adult had three significant amide bands at 1654, 1617 and 1550 cm^{-1} corresponding to amide I of C=O, amide II of N–H and amide III of C–N stretching [109]. Similarly, the larvae chitin vibration peaks of found at 1655, 1621 and 1556 cm^{-1}, prepupae peaks at 1651, 1621 and 1556 cm^{-1}, pupae peaks at 1650, 1621 and 1552 cm^{-1}, and adults at 1650, 1621 and 1553 cm^{-1} were reported [113].

The X-Ray diffraction (XRD) study of both BSF exuviae and imago chitin showed intense peaks at 9.3° and 19.8° and small peaks at 23° and 26°, respectively [110]. In the case of larvae and imago chitin examined by Waśko et al. [109], sharp peaks were reported at 19°, 22°, 24°, and 30° and one weak peak at around 9°. Determination of chitin crystallinity obtained from BSF at various developmental stages presented two peaks at 9°, 19° and four weak peaks around 12°, 21°, 23°, and 26° [113]. The difference in the data on the X-Ray diffraction analysis of BSF chitin can be attributed to the crystallinity indexes (CrI) of the chitin samples. For instance, based on the CrI values, it was inferred that the chitin from BSF imago was less amorphous compared to BSF exuviae chitin. Although the development stages of BSF do not influence the physicochemical structure of chitin, the crystallinity of chitin at different developmental stages exhibits specific differences. It was also observed that the CrI values of chitin increase gradually at developmental stages [113].

Thermogravimetric analysis (TG) of chitin from BSF reveals that the decomposition occurs in two steps: evaporation of water is the first one at a temperature range of 0–122 °C, and the decomposition of saccharide backbones at 122–450 °C is the second one. Overall, the maximum thermal degradation temperature (DTG_{max}) for larvae chitin was found to be at 389 °C, and 387 °C was the DTG_{max} for imago chitin [109]. Comparable results were reported by Wang et al. [113], where the first stage of mass loss was due to water witnessed at 0–150 °C and the second stage of mass loss was attributed to the decomposition of sugar structure at 150–400 °C. The DTG_{max} of BSF larvae, prepupae, pupa and adult chitin were 372, 373, 371 and 372 °C, respectively. Similarly, the DTG_{max} for chitin from BSF imago and exuviae were recorded at 363 and 371 °C, respectively [110].

A fascinating study focusing on the circular manufacturing of chitinous bio-composites via the BSF-based bioconversion of urban refuse was published recently [114]. In that study, chitosan isolated from BSF from two geographical locations (BSF_{IT}-Italy and BSF_{SG}-Singapore) was characterized and cast into ~250-μm-thick films and were subjected to mechanical tests. The mechanical properties of BSF_{IT} and BSF_{SG} were as follows: ultimate tensile strength 31.86 and 33.31 MPa; Young moduli 1.09 and 1.15 GPa, respectively. The values were in the range of tensile strength values for shrimp chitin and commodity plastics. A solid biosorbent prepared from BSF exuviae was utilized for the removal of bromophenol blue dye. Moreover, it was found that the adsorbent showed an average efficiency of 99% in reuse tests (five cycles), thus enabling it as a potential technology that can be implemented in textile industries for the removal of anionic dyes from effluents [115].

3.8. Minerals

The mineral content of BSFL reared on different substrates is tabulated (Table 5). Based on the data collected, it is apparent that the mineral concentrations of BSFL are substrate-specific and there is no unilateral trait for assimilation of minerals in BSF. For instance, prepupae reared on waste streams such as food waste, pig manure, chicken manure and cow dung had higher concentrations of calcium, potassium, phosphorus and magnesium but were dominated by the calcium content [50]. BSFL reared on similar diets like chicken manure, kitchen waste and spent grains had a distinctly different mineral profile, with relatively higher concentrations of sodium, potassium and cobalt [84]. In the case of incremental seaweed inclusion in the substrate the specific mineral uptake by BSFL was evidenced. For example, manganese remained stable in larvae despite varying concentrations in the substrate media. Meanwhile, concentrations of the minerals phosphorus and copper increased in the BSFL matrix, although they were present in lower amounts in the substrate. Overall, the authors concluded that the retention of minerals in the larvae decreased with more seaweed inclusions in the media with a sole exception of phosphorus [43]. The importance of mineral content in fish feed was outlined by Irungu et al. [116]. The fish feed formulations containing cricket and BSFL meal were compared in terms of mineral supplementation to the fish diet and both the insect meals were found to be ideal replacements for freshwater shrimp meal in the diet formulations [116]. As pointed out by Cohen. [117] and emphasized by many other studies published recently, the mineral nutrition in insects is the most poorly understood aspect of insect nutrition. Further studies on the mineral uptake and retention in BSFL biomass with respect to diet and life stages could perhaps shed some light on the biomechanism involved.

Table 5. Mineral content of BSFL reared on different substrates.

Reference	[50]	[50]	[50]	[50]	[84]	[84]	[84]	[118]	[43]	[43]	[116]	[54]	[85]
Substrate	**S1**	**S2**	**S3**	**S4**	**S3**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S10**	**S10**
Minerals	g/kg dry matter												
Ca (Calcium)	19.48	23.71	21.94	34.71	3.2	2	1.7	0.08	8.4	30	1.97	9.77	27.6
K (Potassium)	6.17	6.04	6.71	7.53	4.9	5.7	4.4	9.9	10.2	21.3	13.85	11.54	14
P (Phosphorus)	3.62	3.94	4.14	4.76	3.9	4.1	4.6	19.2	6.8	11.3	8.58	8.33	8.6
Mg (Magnesium)	2.71	2.35	2.82	2.11	4	3.3	3.5	4.1	2.1	6.2	3.54	2.51	3.9
Fe (Iron)	0.42	0.36	0.28	0.39	0.6	2.2	0.3	0.5	0.21	0.35	0.85	0.3	-
Na (Sodium)	0.59	0.51	0.47	0.39	2.4	2	2.6	2.2	1	12.3	22.55	1.81	1.9
Mn (Manganese)	0.13	0.17	0.16	0.2	1.4	0.9	1.1	0.2	0.19	0.17	1.76	0.17	0.26
Zn (Zinc)	0.1	0.11	0.14	0.18	0.3	0.3	0.3	0.3	0.07	0.15	0.58	0.1	0.14
Cu (Copper)	0.01	0.01	0.01	0.01	0.4	0.2	0.5	-	0.01	0.01	1.61	0.02	0.02

S1-Food waste; S2-Pig manure; S3-Chicken manure; S4-Cow dung; S5-Kitchen waste; S6-Spent grain; S7-Solid aquaculture waste; S8-Wheat; S9-Brown algae; S10-Unknown.

3.9. Functional and Bioactive Peptides

Insects have antimicrobial substances, which are produced on the surface or within their digestive tract giving it the ability to protect itself against microbial infections. As they lack an adaptive immune system, they are unable to synthesize antibodies. AMPs are synthesized in the fat body of insects in response to microbial invasion of the hemolymph, thus playing a vital role in its innate immunity [119]. The presence of antimicrobial peptides (AMPs) in hemolymphs was first detected in 1974 and in recent decades several types of antimicrobial substances have been identified [120]. The AMPs are generally small basic proteins with a molecular weight in the range of 3–14 kDa and contain 12–100 amino acid residues [121]. Methanolic extracts of BSFL displayed antibacterial activity against Gram-negative bacteria, whereas no activity was found when tested against Gram-positive bacterial strains in vitro [122]. The potent immune system of BSF was put to the test; a study focused on eliciting target specific (*Helicobacter pylori*) antibacterial peptide was communicated. Larvae challenged with *Escherichia coli* to elicit anti-*H. pylori* peptides, which resulted in the identification of four active peptides with average masses of approximately 4.2 kDa, thus proving BSFL's ability to produce inducible antimicrobial peptides and its potential as a target for further bioprospecting [123]. Hemolymph from BSFL immunized with *Lactobacillus casei* (probiotic) were fractionated to identify specific peptides responsible for antibacterial activity and the fraction HP/F9 (screened by high-performance liquid chromatography (HPLC)) with inhibitory effects on the growth of bacteria was tested for in vitro cytotoxicity. The peptides did not exhibit a significant level of cytotoxicity; the results support the fact that BSFL can be used to produce AMP with unique properties, pharmacological action and complete cytocompatibility on bacteria and cells [124]. A novel cecropin-like peptide with a molecular weight of 4.84 kDa was isolated from BSFL and characterized. In silico analysis of the peptide suggested that it belonged to the cecropin superfamily of AMPs characterized as cationic, linear, α-helical, and amphipathic polypeptides [125].

A novel member of the attacin family from BSFL comprising 169 amino acids and with antibacterial activities against *E. coli* and methicillin-resistant *Staphylococcus aureus* was reported [119]. Nutritional immunology of BSFL was elucidated by inferring the diet-dependent antimicrobial activity of BSFL extracts against four bacterial species. It was shown that BSFL expresses an expanded spectrum of AMPs, many of which were induced by inoculating the larvae feed with high bacterial loads [126]. Seven new gene fragments of AMPs obtained from BSFL were named cecropinZ1, sarcotoxin1, sarcotoxin (2a), sarcotoxin (2b), sarcotoxin3, stomoxynZH1, and stomoxynZH1(a). Such findings indicate that BSFL could be an affluent source for the production of new AMPs [127]. Hydrolyzed BSFL proteins with alcalase enzyme demonstrated the highest antioxidant activity and it was found that low molecular weight protein fractions (<3 kDa) exhibited the highest radical scavenging capacities [128]. The structures of important biomolecules present in BSFL are illustrated (Figure 9).

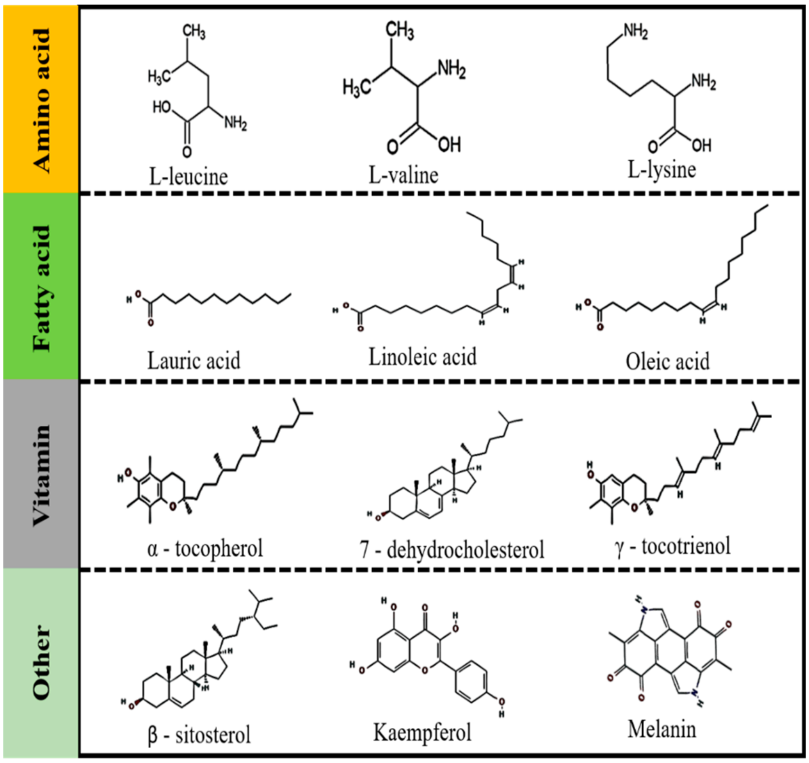

Figure 9. Structures of select chemical constituents present in BSFL.

3.10. Frass

Frass is the combination of undigested leftovers of the substrates and organic refuse which are excreted by insects. They are arguably the most abundant by-product resulting from insect rearing. The expanding industry of insects for food and feed produces a large quantity of frass (compost-like material) which can be a good source of nitrogen (N) and phosphorus (P). As claimed by Schmitt and Vries. [129], there are three distinct advantages for the production and application of insect frasses: (i) higher economic value when compared to conventional compost materials; (ii) modification of soil microbiome due to the presence of insect derivative product like chitin that can substantially benefit the plant growth; and (iii) lowered greenhouse gas emission than conventional composting methods [41,130,131]. Data on frass compositions are rarely reported and the composition of the insect frass changes during composting with other carbon-rich sources. Mushroom root waste with 70% moisture supplemented with auxiliary material bran (40%) and kitchen waste (40%) to accelerate conversion yielded 48–53, 68–72, 129–163 kg of live BSFL and 170, 190, 200 kg of organic fertilizer, respectively [22]. A liquid biofertilizer from BSF frass composting was suggested as an alternative to conventional fertilizers for sugarcane farms in Indonesia [132]. To unlock the true potential of BSF frass as soil amendment and fertilizer, they have to carefully composted to achieve a standard N, P and K ratio. Random use of frass before composting could lead to the stunted growth of crops [133,134]. A recent review on the potential benefits of using BSF frass as a soil amendment and their environmental impact reduction was published [129].

4. BSFL Rearing

Insect farming has been touted as a viable alternative when compared to conventional animal or livestock farming as it requires low land and water requirements. The principle unit operation in BSF rearing is the pre-processing of waste feed substrate. A simple lab-scale processing of BSF and the different processing verticals involved is represented (Figure 10). Once the waste materials are sourced, they are transported to the facility and pre-treated before being fed to the larvae. Reducing the particle size of the substrate aids in the homogenous distribution of nutrients in the diet and expedites the larval growth. A detailed, step-by-step guide for BSF rearing and biowaste processing by the Eawag–Swiss federal institute of aquatic science and technology was published [135]. Typically, the hatchlings (larvae exiting the eggs) are reared on a standard diet for a fixed period and then transferred to the waste substrate. The supplementation of feed during the larval growth depends on larval density, initial feed load and the depletion of initial substate, and is industry-specific.

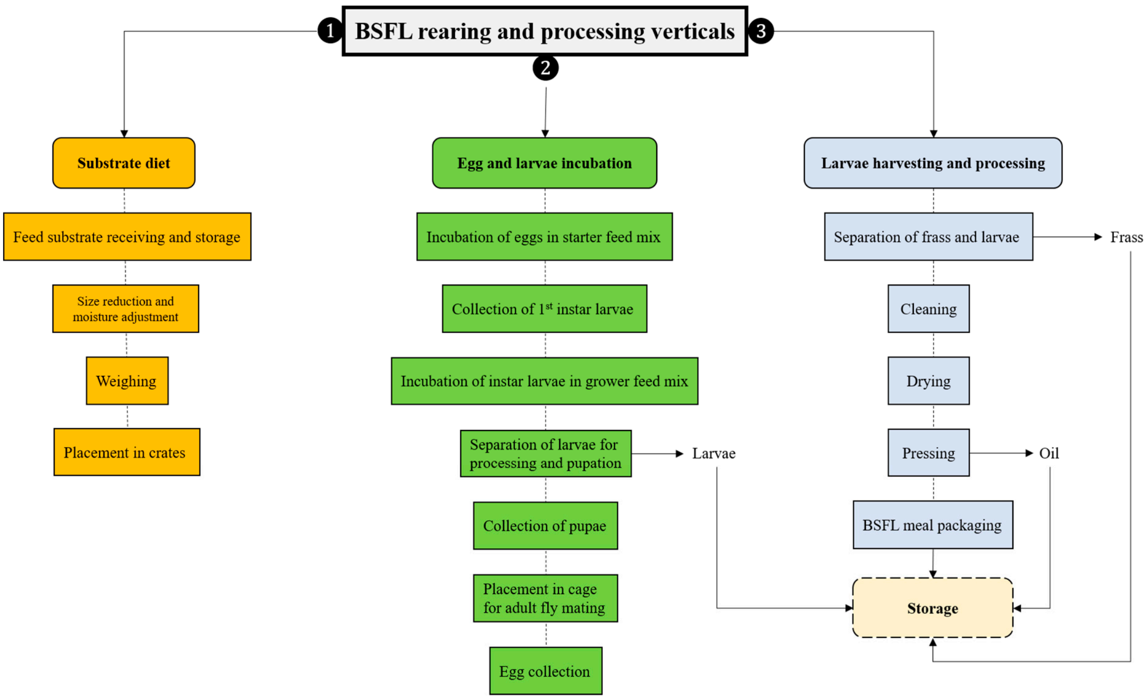

Figure 10. General overview of verticals in BSF rearing and processing.

Several factors that influence the growth performance of BSFL are disclosed (Figure 11). The factors affecting the growth of BSF can be placed into three categories: physical, chemical and others. For instance, Meneguz et al. [136] found that BSFL is capable of resisting and manipulating acidic (pH = 4) and basic (pH = 9.5) environments. The mortality was not affected by the substrate pH, whereas the feeding system influenced the larval growth. Batch-fed BSFL grew faster due to the availability of surplus feed. Meanwhile, the daily fed BSFL weight gain was reduced.

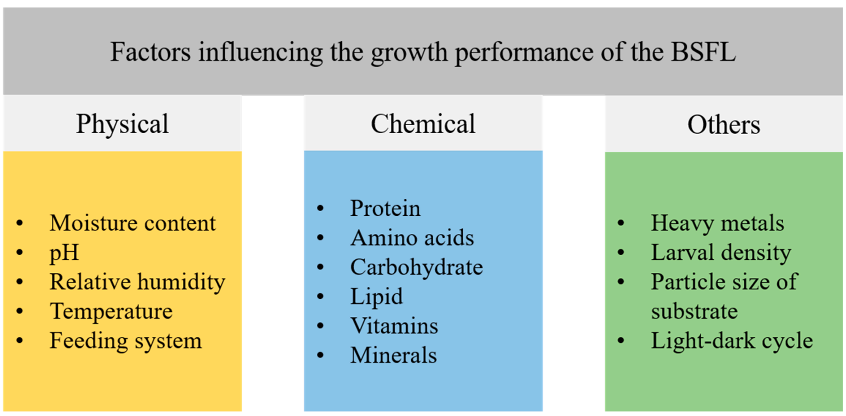

Figure 11. Factors influencing BSFL growth.

These findings could be important for mass rearing production units with large quantities of waste. Temperature is an important abiotic factor that directly and indirectly impacts several aspects of insect life-history traits. The highest percentage of BSF egg eclosion was recorded at 30 °C (80%), the larval survival rate was highest at 35 °C (92%), the highest percentage of prepupal survivorship was observed at 35 °C (79%) and the highest fecundity of BSF was observed at 30 °C [137].

Insect development is directly proportional to temperature, at lower temperatures, the rate of development is inhibited, and at high temperatures the rate of development increases. Holems et al. [138] demonstrated that egg eclosion and adult emergence success increases with increasing relative humidity. Larvae reared on a Gainesville diet with a moisture content of 70% developed faster, grew larger and required less food on those reared on 55% moisture. Larvae reared on a balanced diet with 21% of protein and 21% of carbohydrate and at 70% moisture developed the fastest on the least amount of food and demonstrated the highest survivorship to the prepupal stage [139], highlighting the importance of essential nutrient required for larval growth. Authors Lalander et al. [140] contended that robust BSFL growth could be achieved on a variety of substrates provided they have sufficient volatile solids and protein content. The minimum protein content in substrates for successful rearing was 35% (DM) and the minimum total volatile solids were 87%. More data on essential nutrient requirement for insect diets are available in the book "insect diets" [second edition] [117]. BSFL reared on a diet spiked with heavy metals like cadmium and lead negatively influenced the larval growth (reduced larval mass) and increased mortality. The presence of mycotoxins and pesticides in the diet did not alter the growth performance of the larvae [141].

Co-conversion or co-digestion in BSF rearing is the inoculation of specific micro-organisms with the substrate diet to facilitate better process performance (bioconversion rate, feed conversion ratio, specific growth rate, waste reduction potential etc.) of the larvae. There are surplus data that substantiate the application of microorganisms as a co-conversion agent or a pretreatment technique for unraveling the nutrient potential of the substrate in sustainable BSFL rearing. The ideal approach for better utilization of the substrate in BSFL rearing is to breakdown the substrate biomass into macromolecular components such as protein, carbohydrate and lipids. That way, a baseline can be established to understand the minimum requirement of essential nutrients required for successful insect rearing. The principal challenge that the industry encounters is the fluctuation in the nutritional profile of BSFL that arises due to the variation in the substrate employed for BSFL rearing.

For example, Somroo et al. [142] found that adding *Lactobacillus buchneri* (L3-9) to the soybean curd increased the larval biomass weight, bioconversion rate, protein and fat content. Moreover, BSFL reared on a substrate with *L.buchneri* had a higher concentration of some amino acids than the control groups. Similarly, the co-conversion strategy for better valorization of rice straw and restaurant solid waste with microbes (Rid-X) was performed. The conversion rate of cellulose, hemicellulose, lignin, protein and lipid were higher with BSFL and Rid-X when compared to BSFL only [143]. A novel approach by adding exo-microbes in the form of bacterial consortium powder to ferment coconut endosperm waste as a pre-treatment for diet substrate enhanced the protein content of BSFL. Fermenting the substrate spurred the growth of acid-producing bacteria like *Clostridium* sp. and *Bacillus* sp. The presence of these mo's could rapidly hydrolyze the carbohydrates in the feed substrate, making it available for BSFL [144]. Several other new ideas, perspectives and patents [145–148] focusing on the optimization of BSF breeding have been communicated attesting the growing interest in industrial-scale BSF rearing.

5. Legislation and Safety Aspects of BSF

A detailed review of the legal framework and regulatory guidelines on the use of insects as feed in the European Union, North America, and some Asian countries was summarized [3]. According to the European legislation [149], the production of processed animal protein (PAP) from insects is considered as farmed animals and subject to the feed ban and animal feeding rules. Hence, the use of ruminant proteins, catering waste, meat-and-bone meal, and manure as feed for insects is prohibited [150]. Meanwhile, seven species of insects: house cricket (*Acheta domesticus*), tropical house cricket (*Gryllodes sigillatus*), field cricket (*Gryllus assimilis*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), black soldier fly (*Hermetia illucens*) are permitted for use as animal feed with strict regulations on the substrates allowed in the insect diets. With the exception of species that might impart adverse effects on plant, animal and human health, terrestrial invertebrates are considered as appropriate materials for feed in all their life stages [3]. Recently, the office for risk assessment and research (BuRO) of the Netherlands food and consumer product safety authority (NVWA) outlaid the possibility of breeding insects on former foodstuff (FF) containing meat ingredients. Pre-requisites such as the treatment of FF to ensure its safe usability have to be considered to eliminate possible chemical, microbial cross-contamination. The International collaborations among the key industry players in the insect sector have paved way for the inception of several non-government and non-profit organizations. In the European Union, insect-based firms can enroll as members in "International Platform of Insects for Food and Feed (IPIFF)". Currently, IPIFF has 64 members and has put forth numerous guidelines, suggestions and opinion papers related to insect processing. One such proposal is the "IPIFF Guide on Good Hygiene Practices for European Union producers of insects as food and feed", which encourages the implementation of said practices in their member institutions. The usability of BSF-based products for animal- and aquafeed adopted from IPIFF's website is given below (Figure 12). Food safety issues arising due to the inclusion of insects for feeds and foods can be grouped into chemical, microbiological, and allergenic hazards. Heavy metal bioaccumulation in insects has to be monitored to ensure that they are within the maximum permissible limit in the insect-based feed composition. A thorough review considering several factors such as chemical hazards, heavy metals and arsenic, mycotoxins, veterinary drugs and hormones, pesticide residues, dioxins, dioxin-like polychlorinated biphenyls, polyaromatic hydrocarbons, microbiological hazards, viruses, bacteria, parasites, prions, and allergens in association with insect safety was documented [151]. Heavy metal accumulation, especially cadmium and arsenic, in the BSFL biomass is a major concern. Estimating the initial concentration of toxic metals in the substrate can help predict their presence in the final larvae collected for food and feed purposes. Data on the bioaccumulation factor of heavy metals in different life stages of BSF suggest that metals like copper, iron, mercury, magnesium, selenium, zinc, and molybdenum are accumulated in the biomass. For the first time, the concentrations of chosen non-essential elements Ba, Bi, Ga, which undergo bioaccumulation, and elements Al, Si, which do not undergo bioaccumulation in BSF was reported [152]. According to Bosch et al. [153] BSFL and TM exhibit high aflatoxin B_1 tolerance and does not accumulate in BSFL biomass. Similar research work on the tolerance of BSFL to mycotoxins like aflatoxin B_1, deoxynivalenol, ochratoxin A and zearalenone was elucidated [154]. Data on the behavior of aflatoxin M_1 when dairy-based substrates are used for BSFL rearing have to be examined. Another essential parameter to be considered regarding the safety of BSFL as animal feed is the presence of anti-nutritional factors, which could impede the bio-availability and absorption of essential nutrients when ingested in large quantities.

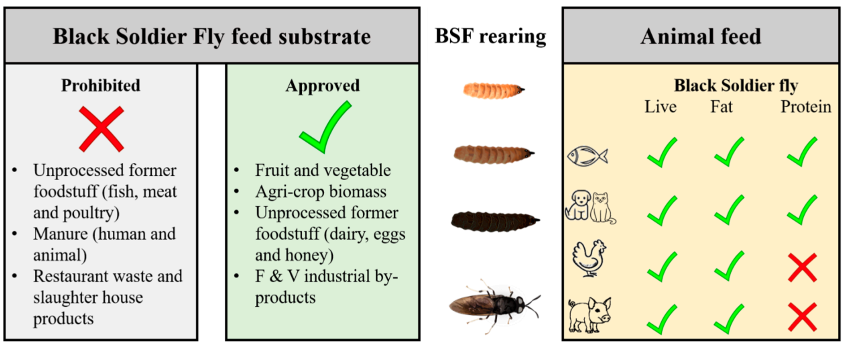

Figure 12. Substrates approved for BSF rearing (adapted from the International Platform of Insects for Food and Feed (IPIFF)).

Anti-nutritional factors (ANF), mainly phytates, tannins, and oxalates, reduce the availability of nutrients, principally minerals and proteins, when present in feed formulations. The phytate-mineral complexation is higher when the availability of calcium in the feed is high, which in turn results in the incomplete precipitation of phytate, leading to the co-precipitation of other minerals present in the diet. The dietary presence of phytates can form insoluble complexes, mainly with cations of minerals such as calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe), thereby limiting the bioavailability of these minerals [155]. Somroo et al. [142] disclosed that the tannin, oxalate, and phytate contents of BSFL developed on soybean curd residue were 13%, 1.2%, and 125.1%, respectively.

6. Conclusion and Prospects

6.1. SWOT Analysis for Insect Processing

The SWOT analysis undertaken is BSF-centric; certain aspects of it can be extrapolated for other insect species as well, and the critical points are highlighted (Figure 13). Three main components (substrate, production and BSFL characteristics) in insect-processing that heavily influence the global utilization of BSF were chosen to represent the whole sector. All observations and inferences suggested are based on the data from the previously documented literature and specific topics covered in this review.

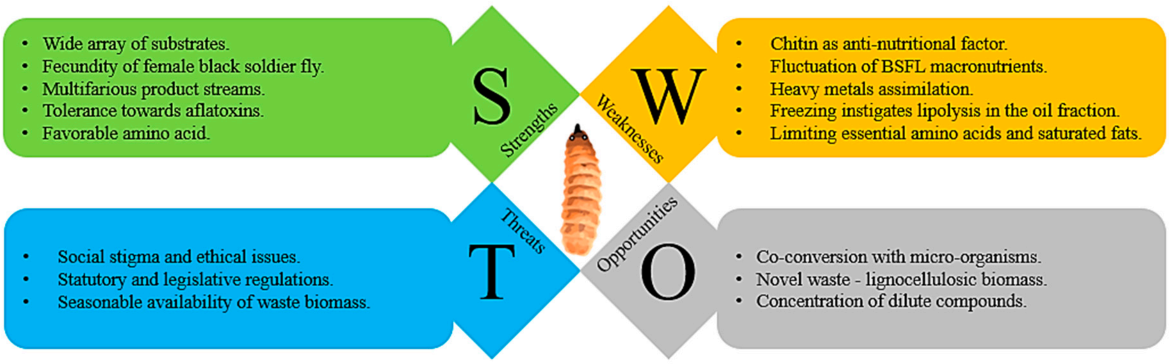

Figure 13. Strengths, weaknesses, opportunities, and threats (SWOT) analysis of BSFL rearing and processing.

The ideas proposed are the subjective opinion of the authors, and hence no references are provided in this section. A comprehensive review with SWOT analysis and insights into insect and fish by-products as sustainable alternatives for animal nutrition was reported recently [156].

6.1.1. Strengths

Substrate—BSFL can be reared on a wide array of substrates. Regardless of origin, any vegetable, fruit, honey, egg and dairy products can be used to formulate feed diets for BSFL. This vegetable origin diet restriction is only applicable for insects reared with the intent to produce animal feed;

Production—Fecundity of female BSF allows rapid scale-up, as the adult female fly can lay up to 800 eggs. BSFL rearing yields multifarious product streams such as protein meal, lipid, chitin, and frass, which are all marketable products contributing to revenue generation;

BSFL characteristics—The bioreactor like characteristic BSFL can be exploited to concentrate chemical constituents of interest by manipulating the diet. BSFL is tolerant towards aflatoxins and is not accumulated in their biomass. Favorable amino acid and fatty acid profiles make BSFL an ideal replacement for fish and soybean meal in animal and aquafeeds.

6.1.2. Weaknesses

Substrate—Although waste substrates can be used for BSFL rearing, the nutritional threshold of the diet substrate has to be maintained to achieve uniformity in the end product. Variation in the nutritional profile of BSFL might lead to alterations in processing conditions and adds to testing costs to determine the proximate composition;

Production—Freezing of BSFL leads to lipolysis of the lipid fractions, hence freezing as the primary devitalization step should be avoided. Wet mode fractionation with or without enzymes requires substantial capital investment to accommodate the increased unitary operations;

BSFL characteristics—Chitin in the defatted meal is of significant concern, and when present in low quantities they are beneficial; in higher quantities, they create undesirable effects like digestion problem, gut inflammation in animal and aquafeed. BSFL tends to assimilate heavy metals, so if they are present in large quantities in the substrate, it could negatively impact the biomass composition. Limiting essential amino acids and high saturated fatty acid content might be a cause of concern in downstream applications of BSFL biomass.

6.1.3. Opportunities

Substrate—Bioconversion or co-conversion of the waste substrate with specific strains of micro-organisms enhances nutrient absorption in BSFL and results in better feed conversion ratio. Novel waste resources like lignocellulosic biomasses can be used as a substrate with appropriate pre-treatments. Products of animal origin and former foodstuffs after biological treatments could be a potential source. Near-infrared spectroscopy can be used to detect the proximate composition of substrate diets;

Production—Automation in insect rearing is gaining traction, and automated systems to monitor rearing parameters and data collection to identify stress points in the production cycle could boost output. Green and innovative extraction techniques like ultrasound, microwave and supercritical fluid can be incorporated to enhance the yield of select macro and micronutrients;

BSFL characteristics—Constituents present in limited quantities or, if they are spread out within the vast volume of substrate, they can be concentrated by means of selective rearing. Genetic modification of BSFL to obtain preferred composition (enhanced protein, altered amino acid profile and lowered saturated fats) could be the subject of future studies.

6.1.4. Threats

Substrate—Seasonal availability of fruits and vegetable waste might lead to changes in the nutritional composition of BSFL diet, which would alter the end product's nutritional profile. The statutory and legislative framework restricts the use of products of animal origin as feed for BSFL, which limits the options to vegetable-origin substrates.

Production—Repeated in-breeding of BSF over a long period might negatively impact their immunity and the long-term implications of such have to be studied. Supplementing existing BSF colonies with wild-caught BSF could help to alleviate the problem;

BSFL characteristics—Social stigma associated with consumption of insects and the palatability of BSFL-based feed products in animals might raise concerns. Ethical issues concerning the euthanization and processing of insect should be addressed. Overestimation of protein should be avoided. The allergenic potential of insects warrants additional research.

6.2. Insect Rearing: Sustainable Development Goals Perspective

In 2015 the United Nations (UN) general assembly set out 17 sustainable development goals (SDG) for a future global society based on sustainability principles. The panoramic vision entails the ecological, economic and social dimensions of the sustainability, providing principles and a reference for national and local policy [157]. The SDGs proposed were (a) no poverty, (b) zero hunger, (c) good health and well-being, (d) quality education, (e) gender equality, (f) clean water and sanitation, (g) affordable and clean energy, (h) decent work and economic growth, (i) industry, innovation and infrastructure, (j) reduced inequalities (k) sustainable cities and communities, (l) responsible consumption and production, (m) climate action, (n) life below water, (o) life on land, (p) peace, justice, and strong institutions and (q) partnerships for the goals. Based on data available in the literature and the immense potential of insect (BSFL), we postulate that insect rearing and the products obtained thereof can directly meet eight out of the seventeen SDGs and indirectly promote the rest (Figure 14).

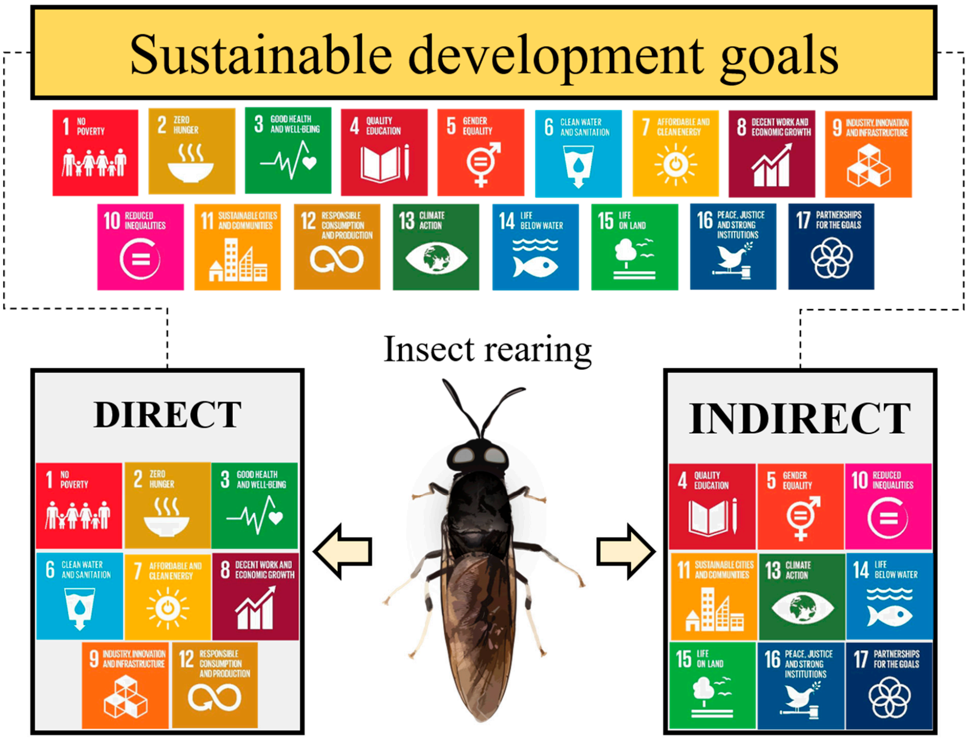

Figure 14. Insect rearing: Sustainable development goals perspective.

The direct benefits of insect rearing and processing in terms of meeting the SDGs are profound. For instance, insect rearing can aid farmers by generating additional revenue, also rather than investing in expensive post-harvest treatments, the farmers can use the excess or waste produce as substrates for BSFL rearing. Similarly, industries can valorize their waste side streams by opting to rear insects and thus establish a circular economy. Lipids and proteins from BSFL can be used as animal feed and work its way up in the food chain, thereby helping in the eradication of poverty and hunger. As discussed, the nutritional immunology and bioprospecting of AMPs from BSFL can contribute to good health and well-being. Chitin derivatives from BSFL can be used in water treatment plants, thereby complimenting the clean water and sanitation SDG. Biodiesel production from the lipids of BSFL and biogas from intermediate compounds of BSFL can be used for the application of affordable and clean energy. The practical simplicity of rearing insects in the micro-, small- and medium-scale can provide numerous job opportunities to skilled and unskilled laborers entering the job market annually. The ubiquitous applications of proteins, lipids and chitin extractable from BSFL promote industrial innovation and new infrastructures, as industry players adopt state-of-the-art techniques to obtain high-quality products. The inherent advantage of using waste substrates for BSFL production compliments the responsible consumption and production SDG. Insect rearing indirectly facilitates several SDGs, promoting women entrepreneurs in the field of insect farming, building smart and self-sustainable cities and lowering greenhouse gas emissions, thereby reducing the carbon footprint are some examples.

The growing interest in edible insects which, according to a new market research report published by Meticulous Research®, is expected to reach USD 7.96 billion by 2030 and the insect for animal feed market is projected to reach a value of USD 1.39 billion by 2024. This highlights the transition of industries' reliance on conventional protein sources that have detrimental effects on the planet to a sustainable protein source like insects that ensures not only economic viability but also boosts the feasible circular economy. BSF, in itself, is a bio-factory that capitalizes on waste resources to provide a myriad of products with great industrial significance. Protein meal from BSFL has been successfully integrated as an alternative to fish meal in aquafeeds. Once the regulatory guidelines are established, it is only a matter of time before poultry, livestock and animal feed formulators can begin incorporating BSF-based products. The facts gathered from several research works that are discussed in detail in this review paint a vivid picture of the future potential of insect-based components in multiple industries. Challenges do exist in BSFL processing, like the high percentage of saturated fats present in the lipid fraction of BSFL and high chitin content in defatted protein meal.

Nevertheless, precise solutions can be devised to circumvent these problems. For example, altering the fatty acid in substrate diet could change the fatty acid profile of BSFL, and specific nutritional tailoring can be achieved as far as the lipid fraction is concerned. Fractions of BSF with a high chitin content can be treated with chitinase enzyme to breakdown the chitin to chitooligosaccharides. Perhaps, chitinase enzymes can be added to feed formulations, which could help the recipient digest the chitin without any discomfort. Wet-mode fractionation can be adopted in the processing line of BSF to remove the chitin-rich fraction. Insect proteins, particularly BSFL proteins, are robust with a good essential amino acid score and very few limiting amino acids, and thus offer dynamic solutions in the highly competitive alternative protein market for food and feed applications.

Though the idea of 100% BSF-based ingredients for feed is possible, in reality, the complete replacement of fishmeal or soybean meal with BSFL remains far-fetched. Studies have shown the species-specific threshold limit in the inclusion levels of BSF for feed applications. Therefore, new biorefining schemes, separation and fractionation techniques for the extraction of functional components from BSFL has to be explored. Lipids from BSFL have diverse applications; they can be used as a fat source for animal feed, butter and margarine replacer in food, biodiesel, and as a cosmetic ingredient. The multifarious applications of chitin and chitosan derivatives in wastewater treatment, textiles, drug delivery, biofilm and composites are an added advantage in BSF processing. Naturally present bioactive antimicrobial peptides and the potential for bioprospecting to produce novel peptides are prospective areas for future research. Further research on mass production, extraction, isolation, fractionation, cascading biorefinery and characterization of the nutrients in insects on an industrial scale could solidify insect-based products as a mainstream alternative to conventional sources. Furthermore, optimizing the diet of insects from various organic side-streams, industrial waste with co-conversion and pre-treatments could unlock new avenues in modern insect rearing.

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