



Edible insects: an overview on farming, from processing procedures to environmental impact, with a glimpse to traditional recipes and to future cultured meat

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With 3 figures and 2 tables

Abstract: In the last decade, the use of insects has grown globally in relation to all sectors of the food chain. Insect farming offers a sustainable alternative to conventional livestock production, with lower environmental impacts and efficient resource use, as shown by Life Cycle Assessments (LCA). However, challenges in scaling production, standardizing processes, and addressing regulatory gaps remain. Continued research and collaboration are essential to fully realize the potential of insects as a sustainable protein source for human consumption. This review analyses some elements related to insect consumption, from the aspects of the hygiene, the different breeding and environmental impact to the description of the processing techniques, also providing the reader with some practical examples related to some world-famous recipes to offer an idea of how insects are perceived as food. The methods used in the processing of edible insects are critical to improving their nutritional content, safety, and palatability. The acceptance of insect-based cuisine can be increased by incorporating insects into traditional foods, thereby minimizing reactions of disgust. In addition, a look to the near future examines the possibilities of cultured meat made from insect cells, which presents encouraging paths toward the creation of sustainable protein, offering a revolutionary strategy that will transform future food production systems toward efficiency and sustainability. This is an absolutely innovative aspect for the production of edible proteins.

Keywords: Entomophagy; food; nutrition; insect industry; insect marketing; hygiene; *Hermetia illucens*

1 Introduction

In recent years, entomophagy, the insect eating by humans, has worldwide attracted great interest from business companies and consumers (van Huis & Tomberlin 2017). Insects can have several benefits as human food, including their high protein, vitamin, and mineral content, and they are also extraordinarily efficient at converting the foodstuffs that they consume into biomass that humans can eat (Hanboonsong et al. 2013). Producers have focused their efforts on creating new products based on both simpler formulation (e.g., whole dried insects, powder forms) and more elaborate insect-based foods (e.g., bars, bread, crackers) (Pippinato et al. 2020). More than 80% of all related published articles in the last five years used the terms “edible insects”. However, despite these many studies conducted on the importance of edible insects as alternative protein sources, the majority of these have

mostly focused on consumer behavior and attitudes (Payne et al. 2016). Over the last decade, there has been an increase of public and private interest in using insects for food and feed (Veldkamp & Gasco 2023). This growing activity has resulted in the creation of several startups throughout the world, as well as the rise of major industrial insect-raising companies capable of generating tons of insects every day (Rivero 2023).

Specialized production processes were created to raise mealworms, crickets, and locusts for human consumption as the concept of using insects for food gained hold (Melgar-Lalanne et al. 2019). Insect rearing companies voluntarily adopted stringent cleanliness standards and tracking systems after realizing the necessity of food safety measures. These procedures agree with the standard guidelines established by the food industry, such as Hazard Analysis and Critical Control Points (HACCP) in the food and feed processing

(Żuk-Gołaszewska et al. 2022). Ensuring the development of this industry in Europe is largely dependent on legislation that addresses sanitary requirements throughout the production process and marketing operations (European Commission 2022). The International Platform of Insects for Food and Feed (IPIFF) published a thorough Guide on Good Hygiene Practices in June 2022, updated in February 2024, in response to the increased demand (IPIFF 2024).

This Guide aims to help companies produce safe insect-based foods while adhering to European Union (EU) standards. Additionally, some insect species like the yellow mealworm (*Tenebrio molitor* L. (Coleoptera: Tenebrionidae)) and the house cricket (*Acheta domesticus* L. (Orthoptera: Gryllidae)) make excellent options for food and feed production due to their high feed conversion ratio and nutritious composition. However, to reduce any possible hazards related to eating insects, it is essential to maintain hygienic and safety requirements during the breeding and processing phases. For example, little literature exists on the fungal toxins, and their role has to be checked in further studies, both for the consumers and people employed in production. Overall, using insects as a sustainable source of protein offers encouraging prospects for tackling issues related to global food security and reducing environmental impact (IPIFF 2024). Nonetheless, the viability and acceptability of insect-based products in the food and feed sectors depend on sustained study and strict respect to hygienic standards (Okaiyeto et al. 2024). In order to completely realize the prospect of insect breeding for sustainable food source, processing methods for edible insects are essential because they enable the development of raw materials that may be used in a variety of culinary applications (Li et al. 2023). These processes fulfill the growing need for sustainable food choices by allowing the transformation of whole insects or their processed forms to meet safety and nutritional criteria (Hassoun et al. 2022). Processing involves using mechanical or chemical methods to alter or preserve insect products so they may satisfy the strict safety and nutritional standards imposed by industry, consumers, and regulatory organizations (Lähteenmäki-Uutela et al. 2021). Insects may be prepared in a variety of ways, including whole, smashed or paste, and protein or fat extracts, by using methods including freeze-drying, sun-drying, boiling, and frying; this allows the insects to be used in a broad variety of food and feed products (Ojha et al. 2021). Processing methods not only increase the shelf life and safety of edible insects, but they also improve their sensory attributes, increasing customer acceptability and palatability (Okaiyeto et al. 2024). Edible insects are high in nutrients, but their incorporation into Western diets is difficult because of their flavor and visual appeal. If, however, insects are included into well-known foods like pasta, bread, and snacks, acceptability and consumption may rise (Liceaga 2022). Because they can be prepared in a variety of ways, including drying, frying, steaming, and roasting, edible insects are used in many different cuisines and culi-

nary traditions across the world (Aguilar-Toalá et al. 2022). Insects, from the toasted grasshoppers of Mexico to the curry insects of Thailand, are considered valuable for their distinct tastes and healthful properties in many cultures (Krongdang et al. 2023). In terms of future prospects, the ability to create cultured meat from insect cells is a frontier in the production of sustainable nutrients (Kumar et al. 2021). A potential way to satisfy the increasing demand for meat worldwide while reducing the negative effects on the environment and the suffering of animals in traditional livestock farming is to produce meat that is made using insect cells (Treich 2021). Insect cell cultures, which make use of tissue engineering techniques, provide prospects for the productive and economical creation of nutrient-rich food items, opening the door for creative solutions to upcoming food-related issues (van Huis 2020).

2 Hygiene

Over the last 10 years there has been a boom in public and private interest in using insects as food and feed. This activity is currently being pursued by hundreds of start-ups throughout the world. Some large industrial insect raising firms capable of generating tons of insects per day have developed too. The academic community interest in this area has also grown rapidly, particularly in the last three years. Public media frequently emphasizes the environmental benefits of employing insects as food and feed. When the idea of utilizing insects for these purposes became popular, several insect rearing enterprises established specialized production lines to raise mealworms, crickets and locusts for human use. These specialized production lines were required since it was recognized that precautions were essential for food safety. Strict hygiene standards, as well as tracking and tracing mechanisms for these food sources, were self-imposed (IPIFF 2024). In general, human and animal meals should be treated for safety, to eliminate possible risks from raw materials during primary production (such as breeding, feeding and harvesting) and ensure safety and for this reason, Hazard Analysis and Critical Control Points (HACCP) are applied during food and feed processing, as a basic requirement of most food industries (Gates 2012; EFSA 2017). HACCP provides for the identification, analysis, and control of physical, chemical, and biological risks (Arévalo et al. 2022). In Europe, the proceedings of legislation and rules addressing hygiene throughout the production process and marketing activities will contribute to the future success of this sector (Hubert 2019). In June 2022, with an update in February 2024, IPIFF provided a detailed Guide called “IPIFF – Guide on Good Hygiene Practices” (Figure 1); the primary goal of the Guide is to assist operators producing insects for food and/or animal feed in achieving consumer and animal health protection through the manufacture of safe goods. The Guide advises insect producers on how to

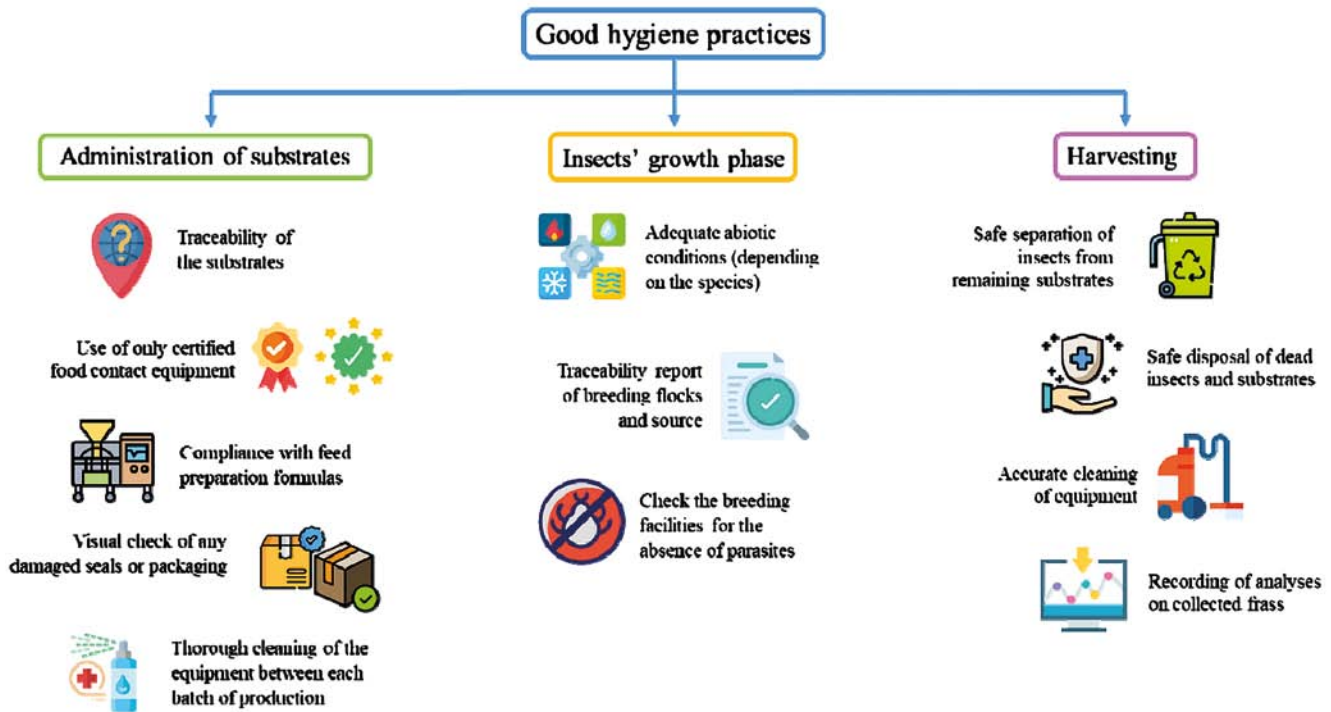


Fig. 1. Summary of 'recommended practices' or 'warning points' associated with insect rearing activities. The image is an infographic illustrating different aspects and considerations in a process or system, organized into three main categories and highlights the importance of thorough planning, quality assurance, regulatory compliance, and continuous monitoring in the lifecycle of a process or system, emphasizing safety, cleanliness, and efficiency at each stage.

apply EU food and feed safety legislation and other related EU requirements (i.e., food and feed labelling, EU animal-by-products, and legislation on transmissible spongiform encephalopathies (TSE)), while also incentivizing them to create a robust food and feed safety management system. To support the knowledge, during the production of the Guide, IPIFF engaged many European organizations of food and feed business sectors, as well as other interested parties, such as the Members of the Advisory Group on the Food Chain and Animal and Plant Health. The Guide covers the production of insects for human consumption or animal feed (including feed for food producing animals, pet food and fur animals) and includes production steps from feeding insects, breeding, killing, and other processing steps, storage, transport, or retail activities, to final delivery of the product to consumers, feed manufacturers, or farmers. However, the Guide does not address the precise processes and/or precautions that operators must take when handling insects/insect products and/or their by-products (i.e. insect frass) intended for 'technical applications' (i.e. non-food and/or feed usage and use of insect fat as biofuel, valorisation of insect frass as organic fertiliser). The Guide identifies elements that require special attention from insect producers, as previously identified by the European Food Safety Authority (i.e. 'the specific production methods, substrates used, harvest stage, insect species and development stage and methods for fur-

ther processing, environmental effects'), in order to achieve compliance with food and feed safety objectives, as defined in applicable EU regulations. Furthermore, the Guide is not intended as a substitute for the current European or national Regulations; consequently, operators should always consult appropriate regulatory requirements for legal compliance. The general health hazards connected with insect intake have previously been addressed in various published risk profiles and scientific opinions (Finke 2015; Schäfer et al. 2016). Because of the enormous diversity within the insect world, there is a need to explicitly target species relevant to European consumers. For example, because of the appealing nutritional composition and efficient feed conversion ratio when compared to other animals, *A. domesticus* is a promising insect. Understanding microbial communities in raised crickets under environmentally controlled circumstances is vital for recognizing future hazard analysis requirements as a key control point for these crickets (Fernandez-Cassi et al. 2020). This species is frequently imported outside the EU, with unclear feeding regimes, transit, packing or manufacturing circumstances, all of which may influence microbial loads and populations. To avoid microbial contamination, it is crucial to use endogenous species in controlled environments. For crickets, the control of such contamination is generally achieved for the following reason: 1) the reared crickets do not appear to carry pathogens with detrimental

effects on farming (i.e. cricket densovirus); 2) any insects that escape from rearing facilities would have a negligible ecological impact on local ecosystems and biodiversity; 3) the crickets can be sourced locally and sustainably, for both ecological and economic benefits.

Tenebrio molitor meal has promise in the food sector as a component in several products due to its excellent nutritional content and extended shelf life (Arévalo et al. 2022). Furthermore, compared to entire insects, its moderate flavor, texture, scent, and color increase acceptance. However, the meal has various potential physical, chemical, and biological concerns including allergies which must be addressed by food safety systems such as HACCP. For the producer of *T. molitor* meal, the majority of concerns occur when they enter in the process as a raw material. Rearing the larvae by themselves, using adequate substrates, can help to control these concerns. However, insect producers should also utilize preventative maintenance to reduce concerns linked with utensils and other equipment, as well as effectively train people to follow Good Manufacturing Practice (GMP) compliant production processes. Also, the establishment of a CCP during *T. molitor* meal manufacturing enables monitoring of factors such as drying time and temperature, particle size, fasting time, packing, and storage. Controlling these factors at the right time guarantees that the defined limits are not exceeded, and that the meal produced is safe for customer consumption (Arévalo et al. 2022).

3 Breeding

The most common method of collecting insects is by gathering them from local natural settings. In line with the customs and cultures of small-scale producers, a wide variety of species at various life stages can be harvested (van Huis & Ooninx 2017). Insects are mostly harvested from fields for domestic use, a process that has no negative influence on the environment and aids in preserving insect supplies over time (Melgar-Lalanne et al. 2019). These farmers have the knowledge and experience needed to identify the host vegetation and timing for growing a particular edible species without harming the ecosystem (Durst & Hanboonsong 2015). Since pre-Hispanic times, traditional insect harvesting has been practiced in Mexico. Interestingly, more than 400 insect species have been collected from the wild, primarily from terrestrial environments, and they were not considered pests but rather as important food sources (Durst & Hanboonsong 2015). Every species is harvested differently depending on its developmental stage (eggs, pupae, larvae, or adult), season (rainy or dry), and location (forest, desert, or agricultural fields) (Melgar-Lalanne, et al. 2019). Due to trophic chain disruptions, unregulated overharvesting is harming the environment at present (Gahukar 2016; van Huis 2022). Efforts should be made to outlaw or control harvesting during breeding seasons and utilizing less damaging harvesting methods

(Durst & Hanboonsong 2015). Some edible wild species have been semi-domesticated where warm, rainy weather is suitable for cultivation (Varelas & Langton 2017). As a worldwide mission, at least for member countries, the Food and Agriculture Organization (FAO) took the initiative to develop a policy and recommended a program to feed people using alternative sources of protein, such as insects (Dust & FAO 2010). The goal is to produce more edible insects in a way that is economically effective, safe, and sustainable considering the rising demand and need for these sources. This document emphasizes the necessity to transition from outside harvesting to indoor farming in terms of technology (Gahukar 2011).

Most insects can be reared in small spaces or containers, they have a short life cycle, can eat agricultural waste instead of grains, can be raised in both urban and rural areas, and can produce short-term financial returns. These factors make it relatively easy and affordable to domesticate edible insects. Due to the economic success, house crickets and yellow mealworms are the two most commonly farmed insects worldwide (Melgar-Lalanne et al. 2019). They have high production densities, low technological needs, and certain life stages do not require sunshine (Hanboonsong et al. 2013). Moreover, the majority of farmed insects may be readily reared in compact, vented plastic containers at high ambient temperatures (up to 30 °C) and relative humidity (up to 70%), while being fed on organic waste. Edible insects are categorized as livestock according to the European Regulation (EC) No. 1069/2009, thus, it is important to abide by edible insect welfare requirements.

Mealworm breeding is fast and inexpensive from a financial perspective (Wang et al. 2011). The egg stage lasts 3 to 9 days, the larval stage is 26 to 76 days, and the pupal stage is 5 to 17 days (Li et al. 2013). Individual growth rate and size are influenced by a variety of parameters, including temperature, humidity, light intensity, feed content, and species density within breeding tanks (Wu et al. 2009). A proper diet is required to maintain good health (Dussutour et al. 2016). *T. molitor* larvae are commonly fed on bran or wheat flour, oat or corn, and protein sources like powdered milk. Fruits and vegetables (carrots, potatoes, lettuce, and chayote) can also be used to supplement the diet with moisture and grain residues (Nguyen et al. 2015). The diet should also contain 5–10% yeast, 80–85% carbohydrates, and vitamin B1 from cereals. The insect can also utilize energy from fatty tissue, absorb minerals from its harder tissues, more efficiently excrete certain components to ease food shortages (nutritional stress) (Adámková et al. 2017). Cannibalism is also a cause of nutritional stress or a lack of breeding space (Wu et al. 2009), which reduces the breeding production. The most popular methods of killing insects include freezing, direct grinding, or cooling followed by boiling. However, it is important to use strategies that minimize animal suffering (van Huis 2013).

Among the mealworms, *Alphitobius diaperinus* P. (Coleoptera: Tenebrionidae) (lesser mealworm) is another species used as food. Currently found everywhere, *A. diaperinus* is thought to have originated in sub-Saharan Africa (Sammarco et al. 2023) and the tropical east African region (Lambkin 2001). This kind of beetle is typically regarded as a pest, especially of grain goods (Spilman 1991; Hosen et al. 2004). Furthermore, because of the favorable humid and warm conditions for their growth, both larvae and adults are frequently found in chicken farms and henhouses, without harming the health of the chickens themselves (Francisco & do Prado 2001). The novel food should be produced in compliance with ISO 22000 regulations, using GMP and hazard analysis critical control point (HACCP) principles. There are three separate stages to the production process: farming, harvesting, and post-harvest processing. Insect breeding and larval rearing are both parts of farming. Depending on the temperature and the availability of food, larvae emerge four to seven days after the eggs are produced and grow through six to eleven larval instars in 40 to 100 days. According to several studies (Strother & Steelman 2001; Renault et al. 2003, 2004; Hosen et al. 2004; Calibeo-Hayes et al. 2005), *A. diaperinus* develops on various diets and in various ambient temperatures and microhabitats. The larvae eat waste, spilled feed, occasionally dead birds, and cracked eggs. For these reasons, lesser mealworms are significant carriers of several parasites and pathogens that affect poultry, including the Newcastle disease, avian influenza, Gumboro disease, the leucosis or Marek's disease virus (Falomo 1986), and the turkey coronavirus (Watson et al. 2000). The formation of particular prion illnesses as a result of consuming food derived by this larval stage is not expected given the vegetable origin of the feed substrates and the absence of prion or prion-related encoding genes in insects (EFSA 2015).

Crickets may be easily raised inexpensively, and they have been regularly used as food for ornamental fish (Taufek et al. 2018). Cricket species, *A. domesticus* and *Gryllus bimaculatus* D. G. (Orthoptera: Gryllidae), are resilient and nutritional (Straub et al. 2019; Bawa et al. 2020). Additionally, both species are routinely raised in large-scale production alongside a variety of other insects (Cortes Ortiz et al. 2016). Crickets adapt rapidly, thus if enough food (grains and vegetables) and water are supplied for a few days after arrival, it is possible to rear healthy insects (Roe et al. 1980). Moreover, this insect is an excellent species for breeding because of its short life cycle (from 60 to 70 days), high reproductive capacity (range of 200–1,500 eggs/female) (Patton 1978), resistance to illness, and ready availability stock from cricket farms. The life cycle of *A. domesticus* includes an incomplete transformation, as happens for insects known as “hemimetabolous,” which implies that when the nymph emerges from the egg, its physiological make-up resembles that of the adult (von Hackewitz 2020). If the correct circumstances are present, the eggs will hatch in 8 days and the juvenile crickets reach adulthood after 6–8 weeks (FAO 2013), and the mating

activity begins 24 to 72 hours after maturation. The harvesting day can often range from day 30 to day 55 on farms (von Hackewitz 2020). The time of development is temperature dependent, and this is the first parameter that should be kept under control because *A. domesticus* is an ectotherm; these animals are not able to adjust their body temperature over time, and, thus, temperature control is essential (Eckert et al. 1988). Temperature also influences the quantity and fertility of eggs laid by crickets. Crickets must have a balanced diet, with high protein content but also with vegetables, grains, carbohydrates and vitamins. Small amounts of fresh fruit and vegetables are needed as sources of hydration. Because of the high protein demand of the house cricket, the price of the feed rises, thus, one of the main challenges in the farming of crickets is the feed (van Huis & Ooninx 2017). Some farmers are utilizing high-protein feed only until the crickets are 20-days old in order to save money. After that, until harvest, lesser protein feed is utilized (von Hackewitz 2020).

Currently, *Locusta migratoria* L. (Orthoptera: Acrididae), also known as migratory locust, is found in Australia, Asia, Africa, and Europe (GBIF Secretariat 2019). Due to density-dependent phase polyphenism, there are two distinct phenotypes of this locust: solitary and gregarious. The adult insect population is mated during farming, and the nymphs are raised. The adult insects are separated from the eggs so that the nymphs can develop independently. The nymphs develop under carefully controlled temperature and humidity in stainless steel containers (Turck et al. 2021). Since these insects are specialized grass-feeders (Raubenheimer & Simpson 2003), they can be fed on plant-derived material. After removal from the substrate and excretions, adults (aged 3 to 5 weeks) are harvested. Following harvest, a minimum 24-hour fast is imposed to give adults time to empty their bowels. The adults are killed during the post-harvest processing by freezing and storage at -18°C (Turck et al. 2021).

Hermetia illucens L. (Diptera: Stratiomyidae) (also known as the “Black Soldier Fly”, BSF) is a Diptera whose larvae have the extraordinary ability to feed on organic substrates, including manure (Franco et al. 2022), animal waste, fruits and vegetables (Scala et al. 2020; Scieuzo et al. 2022), and bioconvert them into larval biomass rich in proteins, lipids, chitin and its derived chitosan (Triunfo et al. 2021), and AMPs (antimicrobial peptides) (Moretta et al. 2020; Scieuzo et al. 2023). BSF is not a pest and can be reared and harvested without special infrastructure. Although, also without a clear legislation about, the BSF farms produce larvae advised for use as animal feed, there are local regulatory constraints on how this can be accomplished. Larvae might theoretically be processed and transformed into a textured protein with a strong flavour for commercial usage in human diets. Their capacity to turn waste and by-products into food gives them a significant advantage over other insects, creating value and completing nutrient loops while lowering costs and pollution (Wang et al. 2019). It is challenging to

locate evidence of BSF ingestion by humans (Mitsuhashi 2016). As ethnographers are not often entomologists and that locals are not always inclined to use the scientific name of an insect when eating it, this prevents precise identification of the species being consumed (Ramos-Elorduy et al. 1997; Mitsuhashi 2016). BSF larvae are suitable for human eating in terms of nutrition (protein mainly) but, based on our current notions of which fatty acids are deemed healthy, BSF larvae have one of the least healthy fatty acid profiles when compared to other insects (Franco et al. 2024). Larval amino acid and fatty acid profiles can vary according to feeding substrates (Spranghers et al. 2018; Ushakova et al. 2019), so it is possible to calibrate the nutritional needs of the consumers. According to Chia et al. (2018), temperature and diet, the two most important environmental factors, can affect not only the rate of insect development and seasonal and daily cycles (Logan et al. 1976), but also various aspects of insect biology, such as immature survival, adult life span, growth, fecundity, fertility, sex ratio, and population growth parameters (Gabre et al. 2005; Schneider 2009). Development, for example, might take two weeks to several months depending on the substrate type, and the resultant larvae can have a protein concentration varying from 10 to 40% of body weight (Ooninx et al. 2015). Optimal temperature and humidity conditions par insect species are summarized in Table 1.

4 Environmental impact

Livestock provides 25% of the total protein content eaten by humans, making animal-based food items an essential source of protein in diets as a whole. Livestock also has a large negative influence on the environment, contributing to 10–12% of all anthropogenic CO₂ emissions. Moreover, one of the human activities with the greatest environmental impact is food production. Agriculture alone is responsible for 30% of the world's greenhouse gas emissions and 70–85% of its water impact (2.5 times more than global

transport). Alternative solutions, that meet the demand for food through sustainable production systems, are also increasing due to the expanding worldwide need for protein sources. The projection of a 70% increase in food production by 2050 emphasizes the significance of environmental effects associated with meat production (Tomlinson 2013). The creation of numerous meat alternatives, including plant-based, dairy-based, and animal-based options, strives to reduce the negative effects of livestock on the environment (Smetana et al. 2019). In this perspective, insect farms are an efficient alternative. In traditional farming, insects are much more effective at bioconverting organic matter into animal protein and nutritional energy than their vertebrate competitors. Using insects as food and feed has a lot of potential as a sustainable option in future food systems. Indeed, in terms of amino acids, lipids, minerals, and vitamins, the high nutritional quality of edible insects is on equal level with that of animal products. According to several studies (Ooninx & de Boer 2012; van Huis 2013; Siegrist & Hartmann 2019), meat substitutes have a lower environmental impact than traditional meat. LCA (Life Cycle Assessment) is a tool for assessing the environmental effects of products and services while taking into account the entire life cycle of the pertinent system or product. Environmental effects on food items can be traced from the production of agricultural inputs to consumption in homes and restaurants, as well as trash disposal. Although there are several other environmental impact assessment techniques, LCA is the most comprehensive and it is utilized for most food items and supply chains and has been embraced as the methodological foundation for environmental declaration schemes. The products must perform the same function, which is specified and quantified in the functional unit, in order to be comparable. The amount of edible portions (e.g., 1 kg of the edible fraction) or animal protein could serve as the functional unit for insect production systems (e.g., 1 kg of protein). For human toxicity, the subcategories classified are global warming, human health, human carcinogenic toxicity, human noncarcinogenic toxicity, ionizing radiation, ozone formation, stratospheric ozone depletion, water consumption and fine particulate matter formation. The impact of mealworm production on resources is also better; mealworm protein production has a lower resource impact than edible pig protein, with a resource impact value of 7.53 USD per kg of mealworm protein and 59.04 USD per kg of pork protein (Table 1). This is because the inputs used in the mealworm industry are designed to minimize the impact of depletion of resources. Moreover, according to estimates, compared to the 2–5 hectares needed for pig farming to provide the same quantity of protein, insect farms only need one hectare of land (Alexander et al. 2017). Finding substitute feed sources using surpluses and waste from crop production, while also keeping in mind that high-protein feeds produce the most effective conversion ratios, is another strategy to reduce the effects associated with feed production. Similar data were obtained by Halloran et al.

Table 1. Optimal temperature and humidity regime conditions for the rearing of the insect species addressed in section *Breeding*. TM: *T. molitor*, ADi: *A. Diaperinus*, AD: *A. domesticus*, LM: *L. migratoria*, HI: *H. illucens*.

Insect species	Optimal temperature (°C)	Optimal RH (%)	Reference
TM	25–28	70	Soares Araújo et al. 2019
ADi	30–33	90	Dunford & Kaufman 2006
AD	30–32	60–70	von Hackewitz 2020
LM	34–42	70	Hamilton 1950
HI	30	70	Chia et al. 2018

(2015) in their comparison of the production of broilers and cricket; with the exception of ozone depletion and resource depletion, broiler production had the worst effects across the board when using 1 kg of edible mass as the functional unit (Halloran & Flore 2018). Particularly, broiler production increased the potential for acidification from 0.12 Mole of H-eq to 0.08 Mole of H-eq on the current cricket farm, the global warming potential from 3.90 kg of CO₂-eq to 2.57 kg of CO₂-eq, the freshwater ecotoxicity from 35.45 CTUe (comparative toxic unit) to 26.41 CTUe, and the potential for terrestrial eutrophication from 0.49 Mole of N eq to 0.40 Mole of N eq. The Global Warming Potential (GWP) of the current cricket farm scenario is comparable to two LCAs of mealworm production, with an equivalent of 2.7 kg CO₂-eq. per kg of fresh weight (Oonincx & de Boer 2012), 2.84 and 3.02 CO₂-eq. per kg of fresh weight (Oonincx & de Boer 2012; Smetana et al. 2015a). The phase of feed production was likewise identified as one of the main hotspots in these two investigations. In accordance with the abovementioned studies, Salomone et al. (2017) investigated the various environmental impacts for different phases of a BSF breeding in Italy. Observing the single impact categories, the results shown that, considering the total impact related to GWP (30.2 kg CO₂-eq – 100% of total contribution), the contributions of transport of input materials phase and the larval frass and dried larvae production phase are 5.4 kg CO₂-eq (18% of total contribution) and 22.5 kg CO₂-eq (75% of total contribution), respectively, while the phases of substratum production and egg and larvae production contribute 2.1 kg CO₂-eq (7% of total contribution) and 0.2 kg CO₂-eq (0,7% of total contribution), respectively.

Fossil depletion was the second-largest impact noted by Smetana et al. (2015b) and it is comparable to the significant energy use as determined by Oonincx & de Boer (2012). According to research by Miglietta et al. (2015), mealworms need 4.3 m³ of water per kilogram of edible mass, which is roughly ten times more water than the present cricket farm in Thailand. The feed production is mostly responsible for mealworms' water impact. Another important function that the bioconverter insects, particularly the BSF larval stage, can contribute to improve is the disposal of food waste, of any organic matter. The primary goal of food waste management methods is to reduce waste, but finding innovative ways to value waste is also a valid and possibly beneficial option. The bioconversion process through BSF larvae is a very appealing option, given that it could be a valuable solution to different issues: food waste management, the rising demand for feed, and the competition between land use for food or energy crops (Zheng et al. 2012; Salomone et al. 2017). Organic waste from the agrifood chain is used to produce a valuable insect biomass in the form of protein and fat sources to be used in the animal feed industry. The use of the LCA approach in biorefinery solutions is quickly becoming essential (Olofsson & Börjesson 2018).

Studies on the quantification of the emissions that insects create are currently insufficient in number. Indeed, there is a lack of experimental data on emissions from insect production, and what little data there is only applies to a relatively small subset of insect species. Indeed, only two studies on the Greenhouse Gas emissions from insect species have been published (Hackstein & Stumm 1994; Oonincx et al. 2010). Compared to meat products, insects are thought to be a less harmful source of protein for the environment. The impact of insect rearing is greatly influenced by the species, production method, and nutrition because some of these factors result in more emissions than others (Oonincx et al. 2015). Despite extensive coverage of insect-related topics in literature, there is still a scarcity of data on an industrial scale. For accurate comparisons with conventional protein sources and the creation of industrial recommendations, industrial scale is crucial. According to Halloran et al. (2015), Salomone et al. (2017), and Thévenot et al. (2018), most analyses of economic viability and environmental impact are carried out for small pilot or small industrial scale of production with a rate of 0.02–1 ton of dry insect biomass processed per day. Additionally, the majority of research depends on incomplete and aggregated data rather than a consequential LCA method, which has the ability to detect changes in the market system brought on by new technologies and goods (van Zanten et al. 2018; Larrea-Gallegos et al. 2019).

Compared to the production of fish protein, the environmental impact of insect protein is significantly lower. Fish farming, or aquaculture, can also have substantial negative environmental effects, including habitat destruction, water pollution, and high feed conversion ratios. For instance, the production of farmed fish such as salmon requires substantial inputs of feed derived from wild fish and agricultural crops, which intensifies overfishing and deforestation issues (Naylor et al. 2000; Hall et al. 2015). Based on Pelletier et al. (2009), Henriksson et al. (2014) and Parker et al. (2021), insect protein production (mealworms and crickets) generally has a lower or comparable GWP per kg of protein compared to fish protein production (salmon, tilapia, and catfish). This suggests that insects could be a more environmentally sustainable source of protein. Furthermore, the water footprint of insect protein production is substantially lower than that of fish protein production. The efficient use of water in insect farming is crucial in a time when water scarcity is becoming an increasingly critical global issue (Miglietta et al. 2015).

5 Processing procedures for edible insects

Utilizing insect breeding for the production of raw materials opens avenues also for sustainable food sourcing, enabling the processing of whole insects or their processed forms to serve various culinary purposes and applications, thus foster-

ing innovative solutions for future food production, addressing the growing demand for sustainable food options.

Processing means “to modify or preserve something by performing a series of mechanical or chemical processes on it (Dossey et al. 2016)”, and each process must be examined by the consumer, industry, and regulatory body to see if it meets its nutritional and safety criteria. In addition, processing is necessary for most foods in general to improve quality, palatability, and to improve food safety. Cooking, for example, is a form of processing (Dossey et al. 2016). In 2024, the global edible insect market for human consumption is expected to reach a value of US\$ 653.7 million and is forecasted to reach a valuation of US\$ 1.1 billion by the 2034 (Global Market Insights 2024).

One important aspect in the processing of insects is to remove the insect dejections which is typically achieved using a commonly used mechanical sieve, in particular with a mesh size 3–5 mm, which is specifically chosen to allow the smaller dejections to pass through while retaining the larger insects. The insects are fit for subsequent procedures for human food production since this separating procedure guarantees that they are cleaner and devoid of trash. Because it strikes a compromise between removing dejections efficiently, easily and preserving the insects themselves, the mesh size is essential (Peng et al. 2022).

Insects are killed by freeze-drying, sun-drying, or boiling after being harvested in the wild or grown in a domesticated context. They can be processed and ingested in a variety of ways, such as whole insects, ground or paste, and protein, fat, or chitin extracts for supplementing food and feed products. Insects can also be cooked alive and eaten (FAO 2017). In particular, frying improves the sensory quality of food by forming aromatic compounds, appealing colors, crust, and texture. Cooking insects may also improve their safety, acceptability, palatability, and digestibility (Caparros Megido et al. 2018). Although nutritional values are important, the product must also be palatable from a sensory standpoint in order to be consumed consistently. Because the consumption of insects as food is uncommon in the Western world, consumers must be persuaded not only of the nutritional benefits, but also of taste and overall sensory appeal (The World Bank 2017). Depending on the species, life stages of insects, and customary cuisines of different countries, insects can be cooked in a variety of ways, including hot air drying, oven broiling, roasting, pan frying, deep frying, boiling, steaming, and microwaving (Melgar-Lalanne et al. 2019). Oven broiling, in particular, gives insects the desirable aroma of steamed corn. Flavor, taste, and texture of edible insects change from one product to another. For this reason, some books on recipes with edible insects describe these features: for example mealworms, crickets and grasshoppers have an intense aroma of cereal, nuttiness, and wood, a less pronounced aroma of broth and an intense flavor of nut, cereal, and umami and slightly less intense flavor of vegetables; ants have an intense sourness like lemon; termites have a pronounced aroma of

nuttiness and broth, with notes of cereal, wood, and soy sauce (Evans et al. 2017; van Huis & Dicke 2014). Because each bug has its own distinct flavor, they are suitable for inclusion in various types of foods (Carcea 2020).

6 Dried whole insects

In tropical nations, insects are commonly eaten whole; however, some insects, such as grasshoppers and locusts, require portions of the body to be removed, i.e., wings and legs, to reduce the risk of intestinal constipation that could be possibly caused by ingestion of the large spines on the insect tibia (Food and Agriculture Organization of the United Nations 2013). Fluidized bed drying, freeze-drying, hot air oven drying, microwave drying, smoke drying, torrefaction, and solar drying are all techniques used to dry intact edible insects and extend shelf life (Melgar-Lalanne et al. 2019). The freeze-drying method involves extracting ice from a sample and drying it via sublimation (Grabowski & Klein 2017). The product is frozen, then the pressure is reduced and heat is added to allow the frozen water in the substance to sublimate. One advantage of freeze drying is that it preserves heat-sensitive components, because it is carried out at a low temperature (Klunder et al. 2012). However, compared to most other drying processes, this approach has low productivity and high cost. Solar drying, often known as sun drying, is one of the most traditional drying procedures used for intact edible insects (Kröncke et al. 2019). Because of the minimal energy input and ease of implementation, it is typically used at the household level. The theory behind sun drying is that solar radiation heats the sample as well as the surrounding air, increasing the rate at which water evaporates from the insects. One problem related to the application of this method is that it can vary depending on weather and sunlight conditions (Mutungi et al. 2019). Smoke drying of whole edible insects is another traditional method. The insects are exposed to smoke generated by pyrolysis of wood. Typically, this procedure is combined with salting, and the entire process consists of a smoke chamber that incorporates the steps of salting, drying, heating, and smoking. Whole edible insects are also dried using microwaves. Microwaves penetrate the insects during microwave drying and are converted into heat, which evaporates the contained water. Microwave drying has the advantage of taking less time to dry insects than freeze-drying or oven drying. Microwave drying, like oven drying, can denature proteins and impact the functional qualities of the resulting components (Kröncke et al. 2019).

7 Granular forms with grinding/milling

The most difficult challenge in the expansion of acceptability of edible insects is reducing reactions of disgust by people. Thus, the acceptance of insect-based cuisine

can be increased by ‘hiding’ insects in traditional foods. Researchers have employed cereal-based meals like bread, bakery items and pasta, which are popular around the world and widely accepted by the public, as a carrier to introduce varied percentages of insect flours to increase nutrition (Carcea 2020). In these preparations, the amount of insects varies and is largely tied to the type of product. The insect content as an ingredient is classified as follows: > 90%, between 90 and 10%, and less than 10% (Pippinato et al. 2020). Soybeans, for example, are frequently converted into tofu and other meat substitutes: meat is processed into hamburgers and hot dogs, and fish is processed into famous meals like fish fingers (Food and Agriculture Organization of the United Nations 2013). Edible insects can, likewise, be processed into more palatable forms in the same way: they are frequently formulated into a paste or powder and added to foods. Drying, grinding, or milling the insects is a simple approach to obtain powder (Choi et al. 2017). Chili paste made from crushed and pulverized giant bugs (*Lethocerus indicus* L. (Hemiptera: Belostomatidae)) is a common main ingredient in Thailand and the Lao People’s Democratic Republic (known locally as “nam phik” and “jaew maeng da”, respectively): this as a practical example that granular or paste version of the insects are more acceptable in societies where customers are not accustomed to consuming whole insects (FAO 2021). Dehydrating or roasting whole insects, then grinding them into a fine powder called flour, is how most insect flours are made (Liceaga 2021). The potential use of insect powders as a novel protein source for gluten-free products is an intriguing feature of insect powders in relation to bakery products (Nissen et al. 2020). A fine particle size dry powder would be the best insect-based component format for most products (Bußler et al. 2016). Powders: 1) offer longer shelf life (typically more than a year, depending on production/processing method, packaging, and storage environment), 2) can be blended effectively with a variety of other ingredients without compromising texture or structural integrity of the product, 3) can have the mildest flavor and scent, as well as the lightest color, depending on the processing procedure, 4) are the easiest technique to put bug into a product without “noticing the insect”, which is great for market acceptability, and 5) are appropriate for most food equipment (Dossey et al. 2016).

8 Extracted insect nutrients

Insects have nutrients that can be extracted and added to diets (Hajj et al. 2022). Insect proteins, in particular worm proteins, for example, are isolated with the aim of adding them to food products to increase nutritional value (Zhao et al. 2016). Since insects have not been significant in Western food culture, consumers may be hesitant to accept insects as a viable protein source. Extracting insect proteins for human food products could be a good way to gain cus-

tomer acceptance (Yi et al. 2013). However, supplementing meals with insects requires a thorough knowledge of the characteristics of the extracted proteins: amino acid profile, thermal stability, solubility, gelling, foaming and emulsifying ability (Lock et al. 2016). By separating the extracted protein groups according to solubility, they can be used for specific foods (Smetana et al. 2015b). Enzymatic procedures to obtain proteins with particular chain lengths are another option. Fluidized bed chromatography and ultrafiltration are protein separation technologies (Dong et al. 2020). These innovative food processing methods and others like microwave, pulsed-electric field, ultrasound and high hydrostatic pressure alone or coupled with enzymatic hydrolysis can reduce protein allergenicity of food proteins, compared to bioactive peptides extracted using conventional heating, including edible insect products, in order to increase product quality, reducing the molecular weight and enhancing the bioavailability and solubility of nutrients (Dong et al. 2020). The extracted insect proteins could be used in animal feed, depending on economic feasibility (Smetana et al. 2015a; van Huis et al. 2017). Protein extraction is demanding (Smetana et al. 2015b), with high costs, legislation, limited production capacity and initial plant costs as significant hurdles to overcome (Ojha et al. 2021). Thus, insect-based proteins remain expensive and need process optimization for large-scale production (Fortune Insects Insights 2024). Moreover, according to Chen et al. (2010), unsaturated fatty acids promote healthy human growth, prevent the skin, and decrease the production of thrombi and blood platelet clotting. Conversely, consuming dietary saturated fat has been connected to an increase in low-density lipoprotein (LDL) cholesterol, which is linked to a higher risk of cardiovascular disease. Randomized clinical trials and epidemiological research have shown that replacing saturated fat with polyunsaturated fat is advantageous for coronary heart disease (Siri-Tarino et al. 2010). Another potential issue regards the amount of chitin in the exoskeleton of insects; indeed, chitin, an insoluble polysaccharide, is the most prevalent type of fiber found in insects (van Huis 2013). Edible insects, particularly those with hard exoskeletons, have a high fiber content because of chitin, although having the potential to reduce protein digestion, can also have a function as dietetic fiber (Muzzarelli et al. 2001; Bukkens & Paoletti 2005). Nutritional value of edible insects is shown in Table 2.

9 Drying pastes, slurries, and liquids forms

Liquids, as well as slurries or pastes, are the next most suitable insect-based ingredient formats. Liquids, especially if the insect material is finely powdered, mix well with other ingredients and products, and can be used in a variety of food processing equipment (Dossey et al. 2016). They can be pasteurized and even exported as an ingredient, albeit liquid shelf life is always significantly shorter than a dry

Table 2. Nutritional values of the most consumed edible insects (Giaccone 2005; Bukkens & Paoletti 2005). ZM: *Zophas morio*, GM: *Galleria mellonella*, TM: *T. molitor*, AD: *A. domesticus*. Data for proteins, fibers and lipids are reported as percentage of dry matter. Data for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) are reported as percentage from total lipids.

	ZM	GM	TM	AD
Proteins	44.0%	40.0%	57.0%	69.0%
Fibers	4.7%	3.4%	5.9%	8.0%
Lipids	45.0%	54.0%	30.0%	21.0%
SFA	39.5%	30.2%	28.8%	33.8%
MUFA	39.7%	48.6%	39.7%	25.8%
PUFA	16.5%	19.6%	28.8%	35.2%

product (Dossey et al. 2016). Insects can be dried in whole form or as pastes, slurries or liquids, but, in the latter case, the drying process becomes less efficient, more expensive, and time-consuming when the product contains more water (Noyens et al. 2021). Slurries, liquids, and pastes also have a more uniform composition, resulting in a more consistent dry product (Mermelstein 2015). Drying liquids, pastes, and slurries into powders or meals can be done in a variety of ways: drum drying, spray drying, fluid bed drying, tray drying, freeze-drying, boiling, roasting, vacuum drying, and dehydrating are some options. A product (liquid, paste, or slurry) is produced and propelled into the air as a spray or mist using either spray atomization (with a high-pressure spray nozzle) or rotary atomization (where material is spun or “flun” into the air at a rapid velocity to create fine droplets of spray) (Nasr et al. 2002). As the water is rapidly removed, the spray or mist is suspended and cycled in warm dry air and falls to the bottom of the spray drying device (Dossey et al. 2016). The water is removed from the droplets/particles at the end of the procedure, leaving just the solid nonvolatile (and non-water) components (Dossey et al. 2016). As a result, fine solid particles are formed. Freeze-drying insects in their complete, unground form, as well as paste, slurry, or liquid forms, is another possibility (Dossey et al. 2016). The best approach for retaining the chemical and other qualities of any substance, biological material, or food is freeze-drying (Dossey et al. 2016). Freeze-drying, however, is costly, takes time, is inefficient, and necessitates access to highly specialized and energy-intensive (electricity-intensive) equipment that is not available in many parts of the world (Tarkan 2015).

10 Boiling and steaming

When insects are cooked by boiling or vacuuming, they become soft and juicy (Caparros Megido et al. 2018).

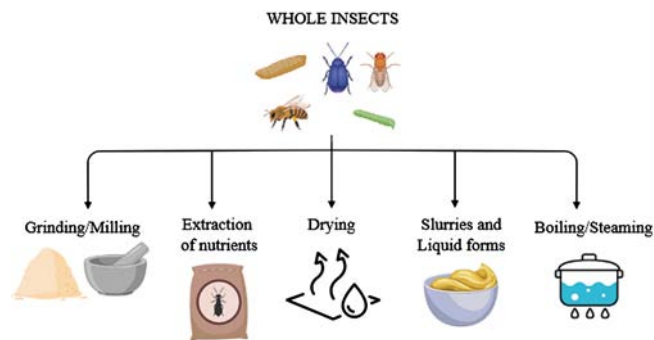


Fig. 2. Scheme on processing procedures for edible insects. The figure represents various processing methods for whole insects, including grinding/milling, nutrient extraction, drying, creating slurries and liquid forms, and boiling/steaming.

Boiling and steaming mealworms keep the larvae looking and feeling fresh, with steamed maize, and boiled mushroom flavors define larvae cooked by boiling and steaming (Baek et al. 2019).

Blanching is also useful for insects. It involves placing a food in boiling water for a short time, removing it, and then plunging it into ice water or placing it under cold running water to terminate the thermal process. This process is useful to lower bacteria load and inactivate degradative enzymes that cause food spoilage in most commercialized edible insects, on both an industrial and artisanal scale (Melgar-Lalanne et al. 2019).

11 Added fresh to food products and recipes

Fresh mealworms can be used to make processed foods like burgers by adding them whole to beef (Elhassan et al. 2019). Insects are also valuable for food formulations like meatballs or burger patties because they are ‘unseen as insects’ and hence more acceptable to European customers (Caparros Megido et al. 2018). Insect-based ingredients are also sold for the manufacture of cookies, chocolates, tortilla-style chips, and other snacks (Melgar-Lalanne et al. 2019), as well as being added to a variety of goods, including energy drinks (Hartmann & Siegrist 2017, 2018) (Figure 2).

12 Some recipes from the world

Mexico is the Latin American country with the longest tradition of eating insects. Grasshoppers and crickets (also known as “chapulines”) are frequently toasted or roasted in a skillet without oil until crispy; they are harvested from late spring until early winter, when the rainy season begins (Cohen et al. 2009). Lemon, salt, and chili are fre-

quently added after toasting, when the insects are still hot. The water used to boil the insects can be acidified with lemon juice or vinegar, and flavoring elements like garlic and onion added. During boiling, the insects turn a reddish color and aromatic compounds form. Chapulines are used in a variety of traditional foods, including tacos (corn tortillas filled with insects and hot sauce), hot chili sauces (chili sauce with chapulines (Hernández-Álvarez et al. 2014)), and “chiles relleno” (chiles stuffed with chapulines). Chapulines are frequently served with alcoholic beverages as a snack (Cohen et al. 2009). In Thailand, about 200 species of insects are consumed, and they are prepared in a variety of ways other than the traditional (roasting, frying, and steaming), including curried, dipping (combined with chili paste), and salted (Halloran et al. 2015; Raheem et al. 2019). There is no tradition of cooking insects in Western countries except for the use of insects as food colorants, particularly cochineal (*Dactylopius coccus* C. (Hemiptera: Dactylopiidae)). Cochineal dye, made from the dried bodies of female cochineal insects, has been used for centuries as red colourants for food industry (Deveoglu 2020).

In Italy, Casu marzu, a Traditional Agri-food Product (TAP) of Sardinia, consists of a sheep’s milk cheese whose sensory characteristics originate (during the ripening period) from the development of the of the small larvae of *Piophilidae* L. (Diptera: Tephritidae) (Regione autonoma della Sardegna 2014). Once the cheese has become creamy, it is consumed by lifting the lid and removing the product with a spoon. The TAPs are products whose processing methods, preservation, and maturation have been consolidated over time according to traditional rules and are included in a list prepared by the Ministry of Agricultural Food and Forestry Policies at the indication of the regions. Regulated initially by the decree of 18 July 2000 and the sixteenth revision of the national list of traditional agri-food products published in the Official Gazette of the Italian Republic No 143 of 21 June 2016 this cheese already has its own promotion committee (based in Ossi, Sardinia) of companies, public bodies, producers and the University of Sassari, that supports obtaining of the Protected Designation of Origin (PDO), requiring a production specification based on compliance with specific health and hygiene requirements, such as that of breeding *P. casei* in a controlled environment. Many Japanese individuals raise wasps in their homes. The wasps are given meat, fish, and sugar water, and their nests are placed in a safe location. The larvae and pupae are gathered for food in the fall. The majority are cooked in soy sauce with sugar and sake (rice wine) and served with cooked rice. Canned wasp larvae and pupae are available in Japanese supermarkets and are considered a high-priced delicacy (van Huis & Dicke 2014). Termites are regarded as a unique and nutritious diet in the tropics, as they are rich in protein, amino acids and fatty acids, iron, calcium, and other micronutrients. Termites are frequently fried in their own fat, and the remaining oil can be utilized to cook other meat dishes. Otherwise, termites can

be steamed after being wrapped in banana leaves or dried for storage after cooking. In Kenya, powdered, sundried termites are used to replace up to 5% of the wheat flour in cakes (Gordon 2013). A popular strong liquor in Latin America is made with a 2-inch (5 cm) “worm”, a caterpillar, at the bottom of the bottle. Mezcal is a distilled beverage made entirely of Mexican agave plants with a minimum alcohol concentration of 45%. Mezcal is mostly manufactured in the state of Oaxaca, where thirty different species of agave are utilized to create the different flavors. Tequila, on the other hand, is only 51% agave and does not come with a caterpillar. The mezcal caterpillars are essentially an agave pest (*Comadia redtenbacheri* L. (Lepidoptera: Cossidae)). They eat the plant stems and leaves and are not regulated with pesticides; instead, they are harvested and used in traditional Oaxacan cuisine. They are not just found at the bottom of mezcal bottles, but they are also regarded as a delicacy: fried and served with guacamole on a tortilla, or ground and mixed with tomatoes and chilies in a spicy sauce (van Huis & Dicke 2014). A curious dish is Mexican-style Escamoles, known as Mexican caviar or insect caviar: fried edible larvae and pupae of the ant *Liometopum apiculatum* L. (Hymenoptera: Formicidae) and seasoned with spices and vegetables and often served with tacos (Ramos-Elorduy 1998).

13 A look into the near future: cultured meat from insect cells

While the utilization of insects in the food industry represents an innovative approach to addressing nutritional requirements while reducing environmental impact, another frontier lies in the potential production of cultured meat derived from insect cells. This emerging technology offers promising possibilities for sustainable protein production, leveraging the biological efficiency of insects to meet the growing global demand for meat while mitigating the environmental challenges associated with traditional livestock farming. In recent years, increasing attention has been paid to the identification of alternative sources for the production of edible proteins. One possible solution is the use of insect cell lines to produce cultivated meat (CM, also known as cell-based or cultured meat) via tissue engineering methods (Giglio et al. 2024) (Figure 3).

CM is meat produced from animal stem cells by replicating the process of cells growing and dividing in the body to obtain a product with the same nutritional and organoleptic attributes as traditional meat. In 2002, the first experimental evidence of CM was published, demonstrating that cultivated fish cells might help the growth of a goldfish muscle explant. In 2013, Dr. Mark Post’s team, at Maastricht University, made a widely publicised cheeseburger containing the first documented taste of CM (Post 2012). Cells, cell culture media, scaffolds, and bioreactors are required for the development of CM. In practice, cells are expanded in bioreactors

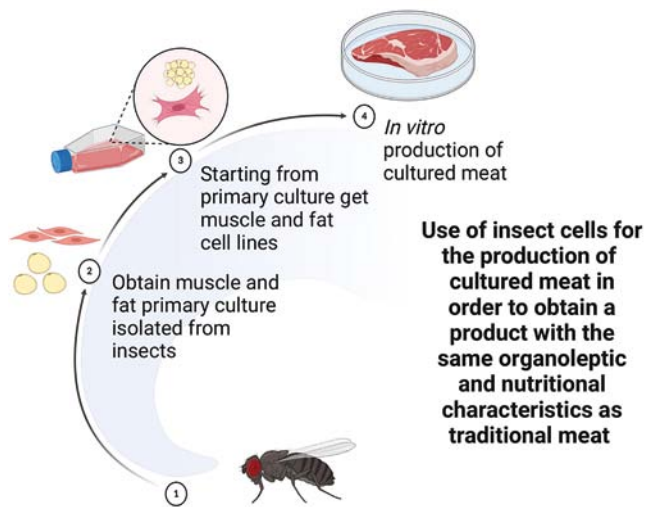


Fig. 3. Schematic representation of different steps for the *in vitro* cultured meat production, starting from muscle and fat cells of insect embryo, to achieve organoleptic and nutritional characteristics similar to traditional meat. The steps include: (1) isolating muscle and fat primary cultures from insects, (2) obtaining muscle and fat primary cultures, (3) culturing these primary cultures to develop muscle and fat cell lines, and (4) producing cultured meat *in vitro*.

(devices used to grow cells) with nutrients and growth signals and incorporated into a scaffold that mimics the texture of traditional meat products. This workflow begins with the source of cells, which can be obtained in one of two ways: the first, and most common, is to perform a tissue biopsy on the animal of interest or to use post-mortem tissues; the second option, is to use a source of pluripotent stem cells (Reiss et al. 2021). For the generation of CM, cells from a range of species have been considered, primarily bovine, porcine, and avian. The necessity for suitable growth conditions for a variety of cell types and the high cost of media are two technological obstacles that prevent the development and expansion of cell culture (Rubio et al. 2019b). Cells originating from less common species may be able to overcome these obstacles. Numerous properties of insect cells suggest their potential for large-scale production, including easier adaptation to suspension and serum-free culture, lower volume and simpler culture media requirements, and favourable growth conditions (including growth in suspension, at room temperature without CO₂, more adaptable to changing environments, avoiding the need for serum or expensive growth factors) when compared to mammalian cells (Geden & Hogsette 1994; Global Market Insights 2021). Animal-derived meat is primarily composed of muscle and fat tissue; thus, muscle and fat cells must be cultivated to create CM products. Finding a robust source of insect muscle and fat cells is a substantial obstacle to the synthesis of insect tissues for food. Primary myoblasts immortalised from *Drosophila*

melanogaster L. (Diptera: Drosophilidae) and contractile myotubes from *Manduca sexta* L. (Lepidoptera: Sphingidae) have been described (Baryshyan et al. 2012; Dequéant et al. 2015). Insect muscle cells were isolated and cultivated using *D. melanogaster* and *M. sexta* embryos as well as *M. sexta* pupae of second stage (Simcox et al. 2008; Dequéant et al. 2015). In contrast to primary embryonic myoblasts and pupal myoblasts, which are capable of both proliferation (extended growth to generate many cells) and differentiation (to form specific cell types like muscle or fat for foods), the genetically immortalised adult muscle precursor cells of *D. melanogaster* have a high proliferative ability but a restricted differentiation potential (Dequéant et al. 2015; Rubio et al. 2019a). Since there is evidence that insect myoblasts need direct contact with neurons for complete development, the lack of support cell types in the early cultures may be the cause of the limited differentiation of immortalised insect muscle cells. The ability to regulate the proliferation and differentiation of cultured muscle cells is crucial for large-scale manufacturing. Significant progress has been achieved despite the fact that the hormonal pathways involved in the development of insect muscles are not as well understood as those in humans and other animals. For instance, it has been shown that ecdysteroids, which are steroid hormones, govern muscle growth (Tischler et al. 1989). Low quantities of 20-hydroxyecdysone enhance myoblast proliferation in *M. sexta*, but amounts beyond the critical threshold limit myoblast development. The analogue of juvenile hormone, methoprene, inhibits the ability of large doses of ecdysteroids to induce proliferative arrest and differentiation. The hormonal regulators of the differentiation process may be used to control cell proliferation during production (Rubio et al. 2019b).

While muscle provides the majority of meat biomass, fat is essential for flavour and nutrition in cellular agriculture. Numerous insect species have significant levels of omega-3 and omega-6 essential fatty acids. Insect fat body tissue contains proteins and carbohydrates in addition to lipids. It is necessary to cultivate both the muscle and fat tissue of insects in order to provide nutritious and tasty food. Other insect cells may be generated *in vitro* utilizing cells from the insect's fat body (Chapman 2012). The accumulation and release of nutrients by fat cells may prolong the *in vitro* survival and contraction of muscle cells for months without modifying the media (Baryshyan et al. 2012). In a similar manner, procedures conditioned by the fat cells may improve *in vitro* embryonic cell growth. One of the first types of insect tissue to be cultured *in vitro* for the study of protein synthesis was the fat body (Nowock et al. 1975; Raikhel et al. 1997). Fat body cells produce essential proteins such as vitellogenin, the precursor protein of the egg yolk, and growth hormones that bind to proteins (Nowock et al. 1975; Wyatt 1988). Numerous papers detail the process of isolating insect fat cells. The majority of treatments use advanced-stage larvae and ethanol or sodium hypochlorite to

sterilise the insects. Adipose tissue is taken from the abdomen and homogenised into a culture medium or buffer solution. In most treatments, explants were generated without tissue digestion. A number of media formulations, including basal media with or without fetal bovine serum and antibiotics, have been used to develop fat body cells. Most of the techniques included reseeded the medium every one to two weeks and culturing the cells between 25 and 27 °C. In contrast to the enzymatic procedures often applied to vertebrate cells, passage may usually be accomplished with a slight mechanical perturbation. One study established a cell line from *Spilarctia seriatopunctata* L. (Lepidoptera: Erebidae) that grew in suspension, as opposed to the majority of cell lines that are grown as adherent culture (Mitsuhashi 1984). There is evidence that fat-tissue-specific cells develop slowly at the beginning but may form continuous lines, and a fat body cell line generated from *Mamestra brassicae* L. (Lepidoptera: Noctuidae) was passaged 100 times over the course of nine months, 26 days after isolation. The replication rates of fat cell lines varied from 48 to 72 hours. Fat cells may be preserved temporarily at 5 °C (Mitsuhashi 1981). The innovative concept of CM enables the manufacturing of meat without the need for animals by employing tissue engineering techniques. In terms of economics, health, animal welfare, and the environment, CM production may be better than conventional meat production. CM has the ability to significantly minimise animal suffering while also satisfying customers' nutritional requirements. The in vitro meat production technology is predicted to be both time and energy efficient compared with animal-derived meat, requiring only a few weeks as compared to many months or years. Producing CM will also reduce land for raising animals. The use of insect cell cultures in cell farming shows potential as a means of overcoming technological limitations and generating nutrient-rich food in a more cost-effective approach. The durability of insect cell culture techniques, simplicity of immortalization, high density proliferation, serum-free nature, and flexibility of suspension culture with respect to mammalian cells make them important candidates for inclusion into meat cultures and other innovative products.

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