***Alphitobius diaperinus* larvae (lesser mealworm) as human foods: An approval of the European Commission - A critical review**

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Short title

Lesser mealworm as human foods

Abstract

Due to the increasing threat of climate change and the need for sustainable food sources, human consumption of edible insects or entomophagy has gained considerable attention globally. The larvae of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae), also known as the lesser mealworm, have been identified as a promising candidate for mass-rearing as a food source based the on evaluation on several aspects such as the production process, the microbiological and chemical composition, and the potential allergenicity to humans. As a consequence, the European Commission has recently approved the utilization of lesser mealworms as human foods. Lesser mealworms are considered a good source of protein, with a protein content ranging from 50-65% of their dry weight and containing various essential amino acids. Lesser mealworms are also rich in other essential nutrients such as iron, calcium, and vitamins B12 and B6. Furthermore, the hydrolysates of lesser mealworms are known to contain antioxidants, suggesting the therapeutic properties of the insects. To enable and ensure a continuous supply of lesser mealworms, various rearing procedures of the insects and information on optimal environmental rearing conditions have been reported. However, like other edible insects, lesser mealworms are still not commonly consumed in Western countries because of various consumer- and product-related factors. Ultimately, the European Commission's approval of lesser mealworms as a novel food is a key milestone in the development of the insect food industry. Embracing the consumption of edible insects can help address the challenges of feeding a growing population, mitigate the environmental impact of food production, and promote a more sustainable and resilient food system for the future.

Keywords: edible insects; entomophagy; food source; mass rearing; sustainability

1. Introduction

In recent years, with the increasing threat of climate change and the need for sustainable food sources, the consumption of edible insects has gained considerable attention. Currently, there are over 2,100 edible insect species in the orders Coleoptera (beetles), Lepidoptera (butterflies), Hymenoptera (ants and bees), Orthoptera (grasshoppers), and Hemiptera (true bugs) that have been identified as potential sources of nutrition for humans. However, some edible insects remain without a complete taxonomy identification (Van Itterbeeck and Pelozuelo, 2022). While the consumption of insects has long been part of the diet in many cultures around the world, the concept of entomophagy, or insect consumption, is still relatively new in Western societies (Shelomi, 2015).

Alphitobius diaperinus Panzer, 1797 (Coleoptera: Tenebrionidae), also known as the lesser mealworm during the larval stage or the darkling beetle during the adult stage, has been identified as a promising candidate for mass-rearing as a food source (Kotsou *et al*., 2021; Mitsuhashi, 2016). It includes the wild type and domesticated type, with the latter being the most commonly used for insect farming due to its ease of raising and high protein content (Janssen *et al.*, 2017). The larvae of the insect can be consumed in various ways, from whole insects to processed products such as protein powders and insect-based flours (Melgar-Lalanne *et al.*, 2019).

Alphitobius diaperinus is widely distributed throughout the world and is commonly found in poultry facilities, where it feeds on litter and other organic matter (Rumbos *et al.*, 2019). It is a pest of stored products that can cause significant economic losses for the food industry. It feeds on a variety of dry stored products, such as grains, flour, and animal feed. The larvae can tunnel into grain kernels, reducing the nutritional value and causing spoilage. However, it is regarded as very nutritious. It has been particularly recognized for its high protein and fat content (Janssen, 2017), as well as its potential for use in animal feed and waste management (Mariod *et al.*, 2017; Volpato *et al.*, 2016).

The increasing interest in edible insects as a potential food source is driven by several factors, including concerns over the sustainability of traditional animal farming and the need to find new sources of protein to feed a growing global population. Insects, including *A. diaperinus*, are highly efficient converters of feed into protein, requiring less land, water, and feed than traditional livestock such as cows and pigs (Halloran *et al.*, 2016). In addition, they produce fewer greenhouse gas emissions and are less likely to contribute to the development of antibiotic resistance (Cammack *et al.*, 2021).

As the global population continues to grow, food security has become a pressing issue, particularly in developing countries. Insect consumption has the potential to alleviate some of these concerns by providing a cheap and readily available source of nutrition (Janssen *et al.*, 2017; Van Huis, 2015). Insects are also rich in micronutrients such as iron, calcium, and zinc, which are often lacking in traditional staple foods such as rice and maize (Mwangi *et al.*, 2018). However, the widespread adoption of entomophagy as a food source is not without its challenges. Public perceptions toward insect consumption vary widely, with many Western consumers expressing disgust at the idea of eating bugs (Tan *et al.*, 2015). Despite these challenges, the potential benefits of insect consumption are leading many to consider it a novel and future food source. Larvae of *A. diaperinus* have been identified as a promising candidate for mass-rearing due to high nutritional value and ease of rearing (Rumbos *et al.*, 2019). This species has been successfully used as a feed source for poultry and fish, as well as a potential ingredient in human food products such as protein bars and snacks (Rumbos *et al.*, 2019).The mass rearing of *A. diaperinus* has a low environmental impact because it can be raised on organic byproducts, reducing the need for additional feed resources (Piña-Domínguez *et al.*, 2022).

Edible insects could play a vital role in addressing food insecurity (Janssen *et al.*, 2017). Further research is, however, needed to address concerns related to safety, cultural acceptance, and regulatory approval. With an increasing global demand for protein and growing concerns over the environmental impact of traditional livestock agriculture, the consumption of insects is an avenue worth exploring. This review will provide valuable insights into the potential of the lesser mealworm as a food source and highlight the benefits and challenges associated with the consumption of insects and assess the current status of approval by the European Commission.

2. European Commission approval for the insect as a novel Food - *Alphitobius diaperinus* larvae

Alphitobius diaperinus larvae as a novel food

In recent years, there has been a booming interest in using insects as a source of protein in order to address challenges of rising global food demand and the environmental implications of traditional livestock production (Ardoin and Prinyawiwatkul, 2021; Van Huis, 2013). Insects have been deemed as a highly sustainable source of protein (Rumpold and Schlüter, 2013a). Insect consumption is not an entirely novel idea, and many cultures throughout the world have used insects as part of their diets. However, the use of insects as food for humans is still relatively new in western countries, and there are a number of legal and cultural impediments that will need to be addressed before insects can be generally accepted as a source of food (Van Huis, 2013).

In 2018, the European Commission approved the use of insects as food for human consumption, paving the way for the European Union and the others to explore the potential of insects as a sustainable and alternative source of protein (EFSA Panel on Nutrition *et al.*, 2022). Since then, the European Commission has been evaluating applications for the use of insects as novel foods, including the application for dried lesser mealworm submitted by the company Proti-Farm Holding NV in March 2018 (EFSA Panel on Nutrition *et al.*, 2022). The evaluation of the application for dried lesser mealworm was carried out by the European Food Safety Authority (EFSA), an independent scientific agency that provides scientific advice on food safety to the European Commission, the European Parliament, and European Union (EU) Member States (EFSA Panel on Nutrition *et al.*, 2022). The EFSA evaluated the safety of dried lesser mealworm based on the production process, microbiological and chemical composition, and potential allergenicity (EFSA Panel on Nutrition *et al.*, 2022).

The EFSA concluded that the use of dried lesser mealworm as a food ingredient is safe for human consumption, and that there is no evidence to suggest that it poses a greater risk of allergenicity than other insect species that have already been approved for human consumption (EFSA Panel on Nutrition *et al.*, 2022). The EFSA also found that the production process and microbiological and chemical composition of dried lesser mealworms are similar to those of other insects that have already been approved for human consumption, such as mealworms and crickets (EFSA Panel on Nutrition *et al.*, 2022).

In addition to being safe for human consumption, dried lesser mealworms are also highly nutritious. The EFSA evaluated the nutritional value of dried lesser mealworm and found that it is a rich source of protein, fiber, and essential amino acids, as well as vitamins and minerals such as vitamin B12, iron, and zinc (EFSA Panel on Nutrition *et al.*, 2022). The nutritional composition of dried lesser mealworm is similar to that of other insects that have already been approved for human consumption, such as mealworms and crickets (EFSA Panel on Nutrition *et al.*, 2022).

With the EU's recent approval of dried lesser mealworm as a novel food, the utilization of insects as a viable protein source is set to take off. This shift couldn't come at a better time, as demand for protein continues to grow while traditional livestock production faces its own set of challenges. Insects offer a sustainable alternative, with the potential to minimize the impact of food production on the environment, creating a more wholesome and long-lasting food system. Ultimately, the EU's approval of lesser mealworm as a novel food is a key milestone in the development of the insect food industry, highlighting the potential of insects as a sustainable source of protein for human consumption.

Suitability of *Alphitobius diaperinus* larvae as food for human consumption

A promising future source of alternative protein has been established with the recent approval of dried lesser mealworms for human consumption. Factors such as safety, nutritional value, potential allergenicity, chemical composition and production process were comprehensively evaluated before the decision was made. This section will discuss these parameters in detail and the relevant studies that support the decision to approve lesser mealworms as a novel food.

Production process

Increasing attention is being given to insects as a sustainable alternative to conventional protein sources. Nonetheless, to ensure that they are safe to consume, processing is necessary prior to their use in animal nutrition or consumption by humans. The International Platform of Insects for Food and Feed (IPIFF) guide on good hygiene practices provides an overview of the processing methods that are applied to insects intended for human consumption and animal nutrition. The guide highlights the importance of cleaning, cooking, drying, and freezing insects and maintaining good hygiene practices during all stages of insect processing to ensure the safety of the final product. This includes ensuring that processing equipment is clean and sanitized and that workers follow proper hygiene practices (IPIFF, 2022)

Proper rearing, harvesting, and processing of an insect is vital for industry as well as consumers. In line to the IPIFF guidelines, the production process of lesser mealworms involves rearing, harvesting, and processing. Insect rearing methods are crucial to ensure the safety and quality of the end product. The rearing conditions must be optimal to minimize the risk of contamination by pathogens and other harmful substances. Besides, the harvesting and processing methods must also be efficient and hygienic to avoid contamination and ensure the quality of the final product. Several studies have investigated the impact of different rearing conditions on the chemical composition and quality of lesser mealworms (Mozaffar *et al.*, 2004; Kotsou *et al*., 2021; Meijer *et al*., 2022). For instance, a study by Kotsou *et al.* (2021) has investigated the impact of temperature on the growth and development of lesser mealworms and found that a temperature of 27°C was optimal for rearing. So that, the insect can be mass-reared effectively or optimally, allowing for continuous production to fulfil market demand.

Potential allergenicity

The potential allergenicity of lesser mealworms is another important factor in evaluating their suitability as a novel food. Allergies to insects are not common, but they can occur, and some individuals may be more susceptible than others (Taylor and Wang, 2018). The risk of allergenicity is influenced by the presence of certain allergenic proteins. Leni and Tedeschi (2020) have investigated the allergenic potential of lesser mealworms and found that tropomyosin was identified as the prevalent potential allergen. Furthermore, it was also revealed that lesser mealworms contain peptides that closely resembled the well-known allergens arginine kinase. This similarity indicates that people who are sensitive to house dust mites and crustaceans may be at risk of experiencing cross-reactivity with these insect-derived allergens (Leni and Tedeschi, 2020). Besides, Immunoglobulin E (IgE) serum from patients who were allergic to crustaceans or house dust mites reacted to proteins from lesser mealworms processed in various ways (raw, boiled, lyophilised, and fried) (van Broekhoven *et al.*, 2016). Further, Broekman *et al.* (2017) has discovered that patients allergic to prawns were at a higher risk of food allergy to mealworms and other insects.

Nutritional value

The chemical composition of lesser mealworms is an essential factor in determining their suitability as a food source. The composition determines the nutritional value and potential health risks associated with consuming the product. The major components of lesser mealworms include protein, fat, fiber, ash protein, vitamins, and minerals, making them a highly nutritious food source. Research has indicated that dried lesser mealworms contained an average of 58-65% crude protein, which is comparable to other insects approved for human consumption, such as crickets and yellow mealworms (Rumbos *et al*., 2019). The study also found that lesser mealworms contained high levels of essential amino acids, making them a nutritionally valuable food source (Elhassan *et al.*, 2019). Furthermore, another study reported that lesser mealworms contained high levels of iron, zinc, and calcium, making them a rich source of minerals (van Huis, 2013).

The nutritional composition of lesser mealworms has been extensively studied, and several studies have reported their high nutritional value. Leni and Tedeschi (2020) identified actin, myosin and tropomyosin to be among the most abundant proteins in lesser mealworm. Furthermore, lesser mealworms are reported to be a good source of protein, with levels ranging from 45-60% depending on the stage of development and rearing conditions (van Broekhoven *et al*., 2015). The protein of lesser mealworms is also reported to be of high quality, as lesser mealworms contain all the essential amino acids in adequate amounts (Kurečka *et al.*, 2021; Smola *et al.*, 2023; Tzompa-Sosa *et al.*, 2014; Yi *et al.*, 2013). In fact, the protein content of lesser mealworms was superior to soybean protein (Mariod *et al.*, 2017). Besides, lesser mealworms are also reported to possess a good source of fat, with levels ranging from 20-30% (Mariod *et al.*, 2017). Also, the fat composition of lesser mealworms is also favorable, as they contain high levels of unsaturated fatty acids, particularly oleic and linoleic acids (Anna *et al*., 2016). Moreover, lesser mealworms are also appeared to be good source of vitamins, particularly B vitamins such as thiamin, riboflavin, and niacin (Zhou *et al.*, 2022). Also, Finke (2015) reported that dried lesser mealworms contained high levels of vitamin B12, which is essential for nerve function and DNA synthesis. In fact, a study has been reported that mealworms contained higher levels of iron and zinc than beef (Latunde-Dada *et al.*, 2016), while another study reported that they contained more calcium than milk (Oonincx *et al.*, 2015; Oonincx and de Boer, 2012; Seyedalmoosavi *et al.*, 2022).

Safety

The safety of lesser mealworms as a novel food was thoroughly evaluated by the EFSA before approval. The EFSA concluded that dried lesser mealworms are safe for human consumption and do not pose any significant risk to human health (EFSA Panel on Nutrition *et al*., 2022). However, the EFSA also highlighted the need for proper hygiene measures during production and processing to minimize the risk of contamination by pathogens and other harmful substances. Several other studies have also investigated the safety of lesser mealworms as a food source. For instance, ~~Caparros~~ Megido *et al.* (2017) investigated the potential health risks associated with consuming lesser mealworms and found that there is no evidence of any significant risks. In another study, the microbial composition of lesser mealworms was reported to have low levels of pathogenic bacteria, indicating their safety for human consumption (Stoops *et al.*, 2017; Wynants *et al.*, 2018).

Status of *Alphitobius diaperinus* larvae as animal feed

The lesser mealworm has been considered as a potential alternative protein source for animal feed due to its high nutritional value and sustainability. The details of studies using lesser mealworm as animal feed is shown in Table 1.

Table 1. Effects of *Alphitobius diaperinus* larvae meal on farmed animals

Farmed animals	Insect meal inclusion level	Duration of experiments (days)	Main findings	Reference
Broiler chick	10-24 g/kg	9 days	The body weight of chicks feeding on starter feed and larvae was significantly greater than the weight of chicks consuming feed only.	Despins and Axtell, 1995
Turkey poults	Larvae 4.4 g/day	10 days	There was no significant difference between the body weight of poults (2-10 days of age) feeding on larvae and starter feed compared with that of poults consuming feed only.	Despins and Axtell, 1994
Piglets	9%	Feed consumption and fattening performance records started when the animals reached 35 kg. The exact duration was not specified	*Alphitobius diaperinus* meal did not affect the growth performance, carcass composition and meat quality of the pig.	Richli *et al.*, 2023
Rat	300 mg/kg	6 days	*Alphitobius diaperinus* modulates duodenal and colonic enterohormone release and increases food intake in rats.	Miguéns-Gómez *et al.*, 2020

Lesser mealworm is also reported to be reared as feed for reptiles, fish and avian pets in the Netherlands (Van Huis *et al.*, 2013). Furthermore, it has been reported that, when compared to other closely related edible species such as the yellow mealworm *Tenebrio molitor* Linnaeus, 1758 or the superworm *Zophobas morio* (Fabricius, 1776), *A. diaperinus* may be used more simply to provide protein for agricultural animals due to its shorter biological cycle and smaller size, making it a better choice as feed in breeding facility (Ricciardi and Baviera, 2016). Despite the potential benefits of using lesser mealworms as animal feed, its regulatory status as a feed ingredient varies by region. In the United States, the use of insects as animal feed is regulated by the Association of American Feed Control Officials (AAFCO), and lesser mealworms are not currently listed as an approved feed ingredient (AAFCO Committees, 2021).

The regulatory status of lesser mealworms as animal feed may be influenced by factors such as safety concerns and public perception. One concern is the potential for contamination with harmful substances such as pathogens or heavy metals. However, Sánchez-Muros *et al.* (2014) concluded that the use of insects as a sustainable protein rich feed ingredient in diets is technically feasible and opens new perspectives in animal feeding. Besides, Kok (2019) indicated that lesser mealworms are generally safe for use as animal feed. Another concern is the potential for allergenicity in animals, although studies have reported low risk of allergenicity associated with feeding lesser mealworms to animals (German Federal Institute for Risk Assessment (BfR) *et al.*, 2019).

The nutritional value and potential of using lesser mealworms as animal feed have garnered positive results. Despite this, there are concerns surrounding safety and public opinion that have led to discrepancies in its regulatory status. Evaluating the potential of using lesser mealworms as an alternative protein source for animal feed requires further research.

3. Records of *Alphitobius diaperinus* larvae consumption in the world

Lesser mealworms, like other edible insects, are not commonly consumed in many western countries particularly in countries such as Australia, Canada, the entirety of Europe and Russia, New Zealand, and the United States (Payne *et al.*, 2019). Meanwhile, entomophagy is common in many countries around the world, particularly in Africa, Asia, and Latin America (van Huis, 2013). However, specialized insect farms in Europe, particularly in the Netherlands and Belgium, have begun to produce lesser mealworms for human consumption. The lesser mealworms are processed and sold in the form of freeze-dried snacks or processed foods such as pasta, burger patties or snack bars (Van Huis *et al.*, 2013; Foodnavigator, 2018; Nutraingredients, 2018). The lesser mealworms are sometimes marketed as buffalo worms, which might be confusing due to the same term used for larvae of *Alphitobius laevigatus* (Fabricius, 1781) (Marien *et al*., 2022).

Currently, there is limited information on countries consume lesser mealworm as a food source, as it is not a widely accepted food item in most cultures. However, it is consumed in some countries. The details of the countries which consume lesser mealworm are listed in Table 2.

Table 2. The list of nations by continent that has consumed *Alphitobius diaperinus* larvae as traditional or modern cuisine, along with their cooking style

Continent	Country	Style of cooking	References
Europe	Brussels	Mealworms are employed to make the burger meat	Katy, 2018
Europe	Netherlands	Minced meat-like product	Stoops *et al.*, 2017
North America	Mexico	Commercial food product and an innovative snack	Ramos-Elorduy and Montesinos, 2007; Van Huis *et al.*, 2013
Europe	Belgium	Processed food	Stoops *et al.*, 2017 Tzompa-Sosa *et al.*, 2023
Europe	Netherlands	Freeze dried	Stoops *et al.*, 2017
Europe	Germany	Noodles and salad croutons	Nutraingredients, 2018

4. Bioecology of *Alphitobius diaperinus*

Alphitobius diaperinus is a species of beetle that belongs to the family Tenebrionidae (Mariod *et al.*, 2017). This species is a cosmopolitan pest that infests stored grains, poultry, and other animal products (Aalbu *et al.*, 2002). It is considered a major pest in poultry farms worldwide and is known to cause significant economic losses due to damage to poultry feed and disease transmission (Renault and Colinet, 2021). This part of the review will discuss the bioecology of *A. diaperinus*.

General morphology

The adult beetles are small, measuring about 6-8 mm in length, and are typically dark brown to black in color (Alborzi and Rahbar, 2012; Sammarco *et al.*, 2023). The head bears a pair of compound eyes, a pair of antennae, and mouthparts for feeding. The thorax consists of three segments, each bearing a pair of legs (Dunford and Kaufman 2006). The abdomen is composed of ten segments, with the last few segments forming the genitalia and the ovipositor in females (Sammarco *et al.*, 2023). The larvae of *A. diaperinus* are elongated and cylindrical, with a tough, yellow-brown exoskeleton (Sammarco *et al.*, 2023; Dunford and Kaufman, 2006). The morphology of the lesser mealworm is well adapted in a range of environments, including agricultural settings and urban areas, where it can be found in large numbers.

Distribution

Alphitobius diaperinus is believed to have originated in sub-Saharan Africa, but now occurs worldwide (Crippen *et al.*, 2022). The distribution of *A. diaperinus* has been reported in Algeria, Argentina, Australia, Brazil, China, Denmark, France, Greece, India, Pakistan, Poland, and the United States, but likely it has an even broader distribution (Hagstrum *et al.*, 2013).

Life cycle

Alphitobius diaperinus life cycle consists of four stages, i.e., egg, larva, pupa, and adult (Dunford and Kaufman 2006). The eggs are small and white in colour and are usually laid in the substrate, where the larvae will feed. *Alphitobius diaperinus* goes through a series of 8-11 larval stages, which vary in duration depending on the temperature. The time between instars ranges from 10 days at 20°C to 2 days at 30°C (Dunford and Kaufman 2006). When the larvae first emerge, they are a creamy white color and the color darkens as they progress through the instar. After every molting, the color returns to creamy white. The process repeats itself until the third instar where after moulting, a shade of brown is visible, giving it a yellowish-brown appearance (Dunford and Kaufman, 2006). In larval stage they possess three pairs of legs. During their final larval stage, they can reach up to 11 mm in length (Dunford and Kaufman 2006). Before entering the pupal stage, the larvae seek isolation from others and burrow into the substrate. The pupae of *A. diaperinus* are exarate, reaching 6 to 8 mm in length and ranging in colour from creamy white to tan (Dunford and Kaufman, 2006). The entire life cycle of *A. diaperinus,* from egg-laying (oviposition) to the emergence of adult beetles, takes around 34 to 38 days when the temperature is optimal at 30°C. At temperatures below 30°C, the development slows down significantly, and it can take up to 165 days for the eggs to reach adulthood at 20°C. Development ceases completely below this temperature (Rueda and Axtell, 1996).

The adults (beetles) of *A. diaperinus* are generally oval-shaped and have a length ranging from 5.8 to 6.3 mm (Dunford and Kaufman, 2006). They have shiny brown to black exoskeletons, with the head deeply tucked into the pronotum. The pronotum, which is about twice as wide as it is long, has a textured surface with tiny pits or punctures. The elytra, which cover the abdomen, are striated and can open to enable flight. Adult beetles begin mating shortly after their exoskeletons have fully hardened, typically within 5-8 days of emerging from the pupal stage. An adult female can live for four months to a year and lays eggs periodically throughout her adult life (Sammarco *et al.*, 2023). On average, they lay about 3.5 eggs per day, which are usually deposited singly on or within loose substrate (Rueda and Axtell, 1996). In their lifetime, females typically lay around 2,000 eggs (Dunford and Kaufman 2006).

Habitat

Alphitobius diaperinus is a synanthropic species, meaning that it lives in close association with humans. It is commonly found in poultry houses, where it feeds on spilled feed and other organic matter (Aalbu *et al.*, 2002). It is also found in grain storage facilities, feed mills, and other locations where organic matter is present. *Alphitobius diaperinus* is capable of surviving in a wide range of environments, including temperate and tropical climates (Bjørge *et al.*, 2018; Kim *et al.*, 2017; Kotsou *et al.*, 2021).

Diet

The larvae of *A. diaperinus* are omnivorous and feed on a wide range of organic matter, including grain, feed, and animal carcasses. They are capable of surviving on a diet of low-quality feed and are often found in poultry houses where they feed on spilled feed. The adults feed on a variety of organic matter, including grain, feed, and animal carcasses (Ducatelle and Van Immerseel, 2011).

As pest and its management

Alphitobius diaperinus is a major pest of poultry farms worldwide. It causes significant economic losses due to damage to poultry feed and disease transmission (Yeasmin *et al.*, 2014). It has been implicated in the transmission of several diseases, including avian influenza and salmonellosis (Dzik *et al.*, 2022; Mozaffar *et al.*, 2004). It is also known to cause respiratory problems in chickens due to the buildup of fecal dust, which can lead to decreased productivity and increased mortality (Ou *et al.*, 2012).

There are several control measures that can be used to manage *A. diaperinus* infestations. These include cultural, physical, and chemical control measures. Cultural control measures include maintaining good sanitation practices in poultry houses, removing spilled feed and organic matter, and using proper storage methods for grains and feed (Dzik *et al.*, 2022). Physical control measures include trapping adult beetles using sticky traps or light traps and using mechanical devices to remove larvae and pupae from the substrate. Chemical control measures include the use of insecticides to kill adult beetles, larvae, and pupae (Arena *et al.*, 2020). Insecticides can be applied as a spray or dust and should be used in accordance with label instructions.

5. Nutritional value of *Alphitobius diaperinus* larvae

Proximate composition

The nutritional composition of edible insects is difficult to generalise, given that more than 2,100 different species are eaten (Van Itterbeeck and Pelozuelo, 2022b). The proximate composition can be used to evaluate the nutritional value of lesser mealworm as a potential food source for humans or animals. The content in Table 3 shows the proximate nutrient composition of lesser mealworms as reported in several studies.

Table 3. Protein, carbohydrate, fat, ash and various minerals content of *Alphitobius diaperinus* larvae reported in various studies

Nutrient	Amount	Reference
Crude protein	45.10 - 50.54 (% DM)	Rumbos *et al.*, 2019
Carbohydrates	21.8	Sun *et al.*, 2021
Crude lipid	13.4 - 29.0 (% DM)	Rumbos *et al.*, 2019
Ash	3.6 (% DM)	Rumbos *et al.*, 2019
Mineral content	Amount	
Calcium	0.5 ± 0.0 (g/kg DM)	Janssen *et al.*, 2019
Copper	21.9 ± 0.4 (mg/kg DM)	Janssen *et al.*, 2019
Iron	53.5 ± 1.7 (mg/kg DM)	Janssen *et al.*, 2019
Potassium	10.0 ± 0.2 (g/kg DM)	Janssen *et al.*, 2019
Magnesium	1.3 ± 0.0 (g/kg DM)	Janssen *et al.*, 2019
Manganese	5.4 ± 0.3 (mg/kg DM)	Janssen *et al.*, 2019

The protein content of lesser mealworm varies depending on several factors, including their stage of development, method of processing, and the conditions under which they are raised (Kotsou *et al.*, 2021). Lesser mealworm powder has been used as a novel baking ingredient to manufacture high-protein, mineral-dense snacks in which the protein content was enriched to 99.3% by substituting 30% of wheat flour with lesser mealworm powder (Roncolini *et al.*, 2020). Generally, lesser mealworms are considered a good source of protein with a protein content ranging from 45.10% to 50.54% of their dry weight (Rumbos et al., 2019). Adámková *et al.*, (2016) found that the crude protein content of lesser mealworms was 630 g/kg dry matter, which is higher than many other protein sources. Compared to traditional protein sources such as beef, pork, and chicken, lesser mealworms have a more favourable protein-to-fat ratio (Miguéns-Gómez *et al.*, 2020). This means that they provide a relatively high amount of protein per calorie, making them an excellent option for individuals looking to increase their protein intake without consuming excessive amounts of fat. However, it should be taken into account that the reported protein content is often overestimated when a nitrogen-to-protein conversion factor of 6.25 and therefore, Janssen *et al*. (2017), proposed a conversion factor for the larvae of *T. molitor*, *A. diaperinus*, and *H. illucens* of 4.76.

Crude fiber refers to the indigestible portion of plant-based foods that pass through the digestive system without being absorbed. Insects are not plants, but their exoskeletons contain chitin, a polymer of N-acetylglucosamine, which is similar in structure to cellulose and other plant fibers (Abidin *et al.*, 2020)). The crude fiber content of lesser mealworm ranges from 5-7% of their dry weight, making them a relatively low source of dietary fiber compared to fruits, vegetables, and whole grains (Skotnicka *et al.*, 2021). However, fiber in insects is still beneficial to human health as it can help promote satiety and regulate bowel movements. Consuming adequate amounts of dietary fiber is essential for maintaining a healthy digestive system and reducing the risk of chronic diseases such as heart disease, diabetes, and cancer (Anderson et al., 2009). While lesser mealworm may not be a significant source of fiber, they can still contribute to an individual's overall dietary fiber intake when consumed as part of a balanced diet.

Lipids, also known as fats, are essential nutrients that provide the body with energy and aid in the absorption of fat-soluble vitamins. Compared to traditional protein sources such as beef, pork, and chicken, lesser mealworms have a more favourable protein-to-fat ratio (Miguéns-Gómez *et al.*, 2020), meaning they provide a relatively high amount of protein per calorie. The method of preparation can also impact the lipid content of lesser mealworm. Roasting or frying the insects can increase the fat content due to the addition of oils or fats used during cooking while boiling or steaming can reduce the fat content due to the loss of fat in the cooking water as is the case of *T. molitor* (Mancini *et al.*, 2021). While consuming fat is essential for optimal health, consuming excessive amounts of fat can lead to weight gain and an increased risk of chronic diseases such as heart disease and diabetes. Therefore, individuals seeking to increase their protein intake by consuming lesser mealworm should be mindful of their overall fat intake and consume them in moderation as part of a balanced diet.

The crude ash content of lesser mealworm is an important aspect to consider when evaluating the nutritional value of these insects. Crude ash refers to the inorganic matter remaining after the organic components of food have been burned off. In the case of lesser mealworm, the crude ash content is typically around 2-3% of its dry weight (Soetemans *et al.*, 2020). The crude ash content of lesser mealworm is primarily composed of minerals such as calcium, phosphorus, and potassium (Riekkinen *et al.*, 2022). These minerals play important roles in maintaining bone health, nerve and muscle function, and fluid balance in the body. In addition to minerals, crude ash may also contain trace elements such as iron, zinc, and copper, which are important for various biological processes. While the crude ash content of lesser mealworm may seem relatively low compared to other sources of minerals and trace elements, they can still contribute to an individual's overall nutrient intake when consumed as part of a balanced diet. In addition, insects such as lesser mealworm have been found to have a high bioavailability of minerals, meaning that they are easily absorbed and utilized by the body (Ojha *et al.*, 2021).

Carbohydrates are essential nutrient that provides the body with energy, and they are commonly found in plant-based foods such as fruits, vegetables, and grains. Insects, however, are not a significant source of carbohydrates as they primarily consume a diet of protein and fat. The carbohydrate content of lesser mealworm is typically less than 1% of their dry weight, making them a negligible source of dietary carbohydrates (Cortes Ortiz *et al.*, 2016). Despite their low carbohydrate content, lesser mealworm can still provide a source of energy for the body due to its high protein and lipid content.

The dry matter content of lesser mealworms is a crucial factor to consider when assessing their quality and shelf life. Dry matter content refers to the portion of a food product that remains after removing the water content, and it can influence characteristics like texture, flavor, and microbial growth (Rawat, 2015). Freshly harvested lesser mealworms generally consist of 25-35% dry matter, while dried insects may contain as much as 90% dry matter (Turck *et al*., 2022). Excess moisture in lesser mealworms can lead to spoilage and bacterial proliferation, impacting their overall quality and safety for consumption (Roncolini *et al*., 2020). Thus, it's essential to appropriately store and handle lesser mealworms to maintain their dry matter content within a safe range. The method of preparation also affects the dry matter content of lesser mealworms (Ortolá *et al*., 2022).

Lesser mealworms are also rich in other essential nutrients such as iron, calcium, and vitamins B12 and B6 (Anzani *et al.*, 2020). However, insects typically do not synthesize vitamins; instead, they primarily obtain them by food digestion ~~ingesting other insects~~ that have accumulated these nutrients in their bodies (da Silva Lucas *et al.*, 2020). Micronutrient deficiencies cause approximately one million premature deaths each year (Norheim *et al.*, 2015), demonstrating the need to improve food nutrition and that humans should not only pursue food production but also give due consideration to the nutrition of food. Vitamins and minerals are essential in the metabolic processes of humans and animals, and their deficiency may have adverse health effects (Awuchi *et al.*, 2020). For example, growth retardation, anaemia, inflammatory bowel disease, and other diseases are associated with micronutrient deficiencies (Awuchi *et al.*, 2020). Iron is crucial for producing haemoglobin. Calcium is essential for strong bones and teeth, and vitamins B12 and B6 help maintain healthy brain function and support the nervous system. Consuming *A. diaperinus* as part of a balanced diet can help ensure that an individual meets their daily protein needs (Churchward-Venne *et al.*, 2017).

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Amino acid profile

The amino acid profile of the lesser mealworm from various studies is shown in Table 4. The data reveals significant variations in amino acid content among the different sources. A notable variability across the studies conducted by Rumbos *et a*l. (2019), Hermans *et al*. (2021), Kurečka *et al*. (2021) reflects potential differences in methodologies, feed composition, rearing conditions, and possibly the strains of lesser mealworms used. For instance, arginine shows a substantial difference across the studies, with Kurečka *et al*. (2021) reporting a notably higher content. Some amino acids are reported in ranges, reflecting potential variability within the study or the use of different samples. Glutamate content appears to be consistently high across the studies, indicating a commonality in the nutritional profile of the lesser mealworms. Alanine, aspartic acid, glycine, proline, and serine are consistently present across all studies, indicating their stable presence in the amino acid profile of lesser mealworms. These variations in amino acid content could have implications for the nutritional assessment and utilization of lesser mealworms in various applications, such as animal feed or human consumption. In other studies, Soetemans *et al*. (2020) reports on the impact of agri-food side-stream inclusion in the diet of *A. diaperinus* on the larvae composition. They found that *A. diaperinus* larvae reared on 18 different diets, had a protein content ranging between 37% and 49%. The most dominant amino acids in the larvae (higher than 32 g/kg DM) were glutamate, arginine, aspartate, alanine, leucine and tyrosine. Differences in the reported values highlight the need for standardized methods for assessing amino acid content or understanding the factors influencing these variations. Lesser mealworms are known to be a good source of essential amino acids. Studies have shown that the protein in lesser mealworms contains all the essential amino acids (Rumbos *et al.*, 2019), which cannot be produced by the human body and must be obtained through diet. These essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Lesser mealworms are particularly rich in lysine, methionine and leucine for which most plant protein feed sources are usually deficient (Sánchez-Muros *et al.* 2014). The specific amounts of each amino acid in lesser mealworms may vary depending on factors such as the developmental stage of the insect, their diet, and how they were reared. However, in general, the lesser mealworm is considered to be a good source of protein with a well-balanced amino acid profile. The amino acid profile of lesser mealworm is similar to that of meat or dairy protein and comparable to soybean proteins (Kurečka *et al.*, 2021). Studies have shown that lesser mealworm powder can be used as a novel baking ingredient for manufacturing high-protein snacks, with enriched amino acids content (Roncolini *et al.*, 2020).

Table 4. Amino acids profile of *Alphitobius diaperinus* larvae

Amino acid	Amount (mg/100 g protein) (Rumbos *et al.*, 2019)	Amount (mg/100 g protein) (Hermans *et al.*, 2021)	Amount (mg/100 g protein) (Kurečka *et al.*, 2021)
Arginine	310	160	500
Histidine	200-320	90	380
Leucine	380-430	250	610
Lysine	350-420	180	620
Isoleucine	250-300	90	400
Phenylalanine	250-700	120	460
Methionine	80-150	40	190
Threonine	230-260	120	390
Tryptophan	70	-	-
Valine	340-380	130	510
Alanine	380	230	700
Aspartic Acid	480	210	790
Glycine	270	160	400
Glutamate	710	360	1050
Serine	230	140	310
Proline	320	180	610
Cysteine	-	-	210
Tyrosine	-	-	710

Fatty acid profile

Table 5 summarizes the fatty acid composition of lesser mealworms compared to chicken reported in some studies. Insects fed on high-fat diets have a higher proportion of both saturated and monounsaturated fatty acids. Gharibzahedi and Zeynep (2023) reported that the dominant fatty acids in lesser mealworm powders were linoleic acid at 33.66%, oleic acid at 28.97%, palmitic acid at 24.98%, stearic acid at 7.23% and α-linolenic acid at 1.88%. Similar values were reported by Roncolini *et al.* (2020). They reported that the fatty acid composition of lesser mealworm powder was linoleic acids at 31.5%, oleic acid at 28.5%, palmitic acid at 23.5%, stearic acid at 7.5%, and α-linolenic acid at 1.5%. According to Oonincx *et al.* (2020), lesser mealworms diets enriched with flaxseed oil during their larval/nymphal stage had the α-linolenic acid content increase by 2.3%-2.7% for each percent of flaxseed oil added and a four percent addition led to an increase in the n-3 fatty acid content by 10-20-fold.

Table 5. Fatty acid composition of *Alphitobius diaperinus* larvae reported in some studies

Fatty acids	Amount (% of total fatty acids) (Gharibzahedi and Zeynep, 2023)	Amount (% of total fatty acids) (Roncolini *et al.*, 2020)
Palmitic acid	24.98	23.5
Stearic acid	7.23	7.5
Oleic acid	28.97	28.5
Linoleic acid	33.66	31.5
α -linoleic	1.88	1.5

Palmitic acid is a saturated fatty acid that is an important component of cell membranes and is involved in many metabolic pathways in the body (Calder, 2015). Stearic acid, unlike palmitic acid, has been shown to neutralize blood cholesterol levels and may even have a protective effect against heart disease (Calder, 2015). Oleic acid is a monounsaturated fatty acid that is important for maintaining healthy cholesterol levels and has been linked to a reduced risk of heart disease (Calder, 2015). Linoleic acid is an essential polyunsaturated fatty acid that must be obtained from the diet. It is found in high amounts in vegetable oils, nuts, and seeds, and is important for maintaining healthy skin and hair and for many other physiological functions (Calder, 2015). Linolenic acid is another essential polyunsaturated fatty acid that must be obtained from the diet. It is found in high amounts in flaxseed oil, chia seeds, and other plant sources, and is important for maintaining healthy brain function, as well as for many other physiological functions (Calder, 2015). The ability of the human body to absorb fatty acids depends on how well they are broken down during digestion and their arrangement in fat molecules (Gharibzahedi and Mohammadnabi, 2016).

Anti-Nutrient Composition

Alphitobius diaperinus contain certain anti-nutrient compounds that can interfere with the absorption of nutrients in the human body (Ojha *et al.,* 2021).

Chitin

Chitin is a complex carbohydrate that forms the structural component of insect exoskeletons, including that of lesser mealworms (Elieh-Ali-Komi and Hamblin, 2016). While chitin itself is not necessarily harmful, its presence can interfere with the absorption of certain nutrients due to its indigestible nature (Kipkoech, 2023). Chitin inhibits the availability of nutrients like minerals, amino acids, and vitamins by physically binding to them, making them less accessible for absorption in the digestive tract (Elieh-Ali-Komi and Hamblin, 2016). This can lead to reduced bioavailability of essential nutrients, potentially impacting overall nutritional intake.

Protease Inhibitors

Edible lesser mealworms, like many other insects, contain protease inhibitors (Tejada et al., 2022). These compounds interfere with the activity of proteolytic enzymes in the digestive tract, which are responsible for breaking down proteins into absorbable amino acids (Ojha *et al*., 2019). Reduced protein digestion can affect overall protein utilization.

Reducing Anti-Nutrient Compounds

To mitigate the impact of anti-nutrients in edible lesser mealworms, several strategies can be employed. Heat treatment, such as cooking or roasting, can partially break down chitin and other anti-nutrients, making the beneficial nutrients more accessible for digestion and absorption (Embaby, 2011). Cooking lesser mealworms thoroughly can help to neutralize some of these compounds. Fermentation can also be used to reduce the levels of anti-nutrients. During the fermentation process, certain enzymes and microbes can break down compounds like protease inhibitors, enhancing nutrient availability. Mechanical processing, such as blending or grinding, can help break down the tough exoskeleton of lesser mealworms, potentially increasing the digestibility of chitin and other indigestible components (Embaby, 2011).

6. Nutraceutical and pharmaceutical properties of *Alphitobius diaperinus* larvae

Edible insects have the potential to serve as healthy, sustainable alternatives to traditional animal-based foods because of their nutrient contents (Leni *et al.*, 2020; Nowakowski *et al.*, 2022). Insects are rich in high-quality protein, essential amino acids, fiber, iron, zinc, vitamin B12, and omega-3 fatty acids (Leni *et al.*, 2020; Nowakowski *et al.*, 2022). Insects generally have been found to contain bioactive compounds such as antioxidants, anti-inflammatory agents, and antimicrobial agents (Nino *et al.*, 2021). These compounds have been shown to have potential health benefits such as reducing the risk of chronic diseases like cancer and cardiovascular disease (Nino *et al.*, 2021). Studies have also found bioactive compounds in insects with characteristics that could potentially reduce inflammation and improve gut health (Chantawannakul, 2020). Edible insects or their components also possess antihypertensive properties (Aguilar-Toalá *et al.*, 2022).

Nutraceuticals are products derived from food sources that offer extra health benefits in addition to the essential nutritional value found in foods. They are non-specific biological therapies used to promote general well-being, control symptoms, and prevent malignant processes (Chelladurai *et al.*, 2022). Examples of nutraceuticals include garlic, omega-3 (found in fish), soybeans, ginger, minerals, vitamins, dietary fiber, hydrolyzed proteins, fortified foods, enriched foods. Nutraceuticals can be nutrient-rich foods or medicinally active foods or specific components of particular foods (Chelladurai *et al.*, 2022) .

On the antioxidant properties of lesser mealworm, it has been studied for its potential health benefits. A study investigated the bioactivities of lesser mealworm hydrolysates and found that they contained bioactive peptides with antioxidant activity (Tejada *et al.*, 2022). According to a study, extracted oils from lesser mealworm have antioxidant properties (Gharibzahedi and Zeynep, 2023). The study found that the oils contained vitamin D, campesterol, β-sitosterol, phosphatidylinositol and phosphatic acid, linoleic acid, and hypocholesterolemic or hypercholesterolemic ratio. Sousa *et al.* (2020) also found that hydrolysates obtained from *A. diaperinus* possessed antioxidant properties. Antioxidant activity values of 95.0 ± 0.8 and 95.7 ±

1.0 µmol Trolox equivalent per g insect have been recorded, however, no antimicrobial or antidiabetic properties were observed (Sousa *et al.*, 2020).

On the antimicrobial properties of edible lesser mealworms, there is limited research. An antimicrobial activity assay against bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*) and also of inhibitory activity of the enzyme α-glucosidase, demonstrated that *A. diaperinus* had no antimicrobial capacity or inhibitory effect of the enzyme (Sousa et al, 2020). However, insects, in general are a good source of antimicrobial peptides and compounds that can be screened against multidrug-resistant bacteria (Mudalungu *et al.*, 2021). Some insects have been found to produce antimicrobial peptides that can inhibit bacterial growth (Saadoun *et al.*, 2022). Hence it is possible that *A. diaperinus* may contain similar antimicrobial peptides and compounds as other insects, however, further research is needed to ascertain this.

On potential anti-inflammatory properties, although there is no direct evidence of the anti-inflammatory properties of *A. diaperinus* specifically, some studies have shown that edible insects, have health benefits such as anti-inflammatory properties. Insects are rich in bioactive compounds such as peptides and ethanol extracts that have been shown to have anti-inflammatory effects both in vitro and in vivo (Aguilar-Toalá *et al.*, 2022). It is possible that *A. diaperinus* may contain bioactive compounds with similar effects. Further research is, however, needed to fully understand the mechanisms behind the potential health benefits of consuming lesser mealworm and to ensure their safety and quality as a food source.

7. Harvesting and rearing of *Alphitobius diaperinus*

The environmental condition in the rearing of lesser mealworm

One of the important steps that can significantly contribute to the production of edible insects with high nutritional values is the methods or conditions employed during the rearing activities (Ortiz *et al.*, 2016). There are several reported methods in the literature that describe the important parameters that need to be considered, such as nutrition (dietary) and environmental conditions, as well as production facilities that can contribute to the production of insects with sufficient quantities and high nutritional values. In this section, the current methods and technologies used in insect-rearing are discussed in detail.

As dietary factors have a significant impact on their growth rates, it is crucial to ensure a proper nutrition is provided. The larvae of the lesser mealworm can grow on different diets composed of side-stream materials. Examples of reported diets used in development of lesser mealworm are Brewers' spent grains, beer yeast, cookies, potato peels, and corn distillers' grain with soluble (van BroekhovenKotsou *et al.*, 201521). Furthermore, diets that contain protein derived from yeast are deemed more advantageous in terms of larval growth and growth (Van Broekhoven *et al.*, 2016). On the other hand, using agricultural by-products and side-streams as substrates for insect feeding could be a sustainable approach to insect rearing. For instance, Rumbos *et al.* (2021) evaluated the feasibility of rearing the lesser mealworm by utilizing ten by-products obtained from cereal and legume seed. Among the by-products, lupin and triticale demonstrated the ability to support larval development from the first instar to pupation. These by-products also exhibited the most favourable results concerning the growth survival, feed utilization and development time (Rumbos *et al.*, 2021).

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In addition to diet, it is important to consider other factors that can significantly contribute to rearing of lesser mealworms. For instance, factors such as temperature and relative humidity levels during the rearing process should be emphasized and studied in detail to establish an effective mass-rearing system. Kotsou *et al.* (2021) emphasized both factors; biotic and abiotic on rearing of the larvae of lesser mealworm and provided multiple variables that could be utilized for mass production of this species. They investigated the growth of lesser mealworm by analysing the effect of three distinct temperature levels (25, 30, and 32 °C) and two varying degrees of relative humidity (55 and 75%). According to the study, temperature played a crucial part in the development of the larvae, with the growth and development observed better at higher temperatures (30 and 32 °C). However, it was found that the larvae growth was not significantly influenced by varying the levels of relative humidity. These findings suggest that *A. diaperinus* can be reared within the appropriate temperature and relative humidity range. However, it is crucial to note that temperature has a more impact on the development and survival rates of the larvae compared to relative humidity (Kotsou *et al.*, 2021). Similarly, previous studies by Renault *et al.* (2015) also showed that relative humidity did not significantly affect the survival of *A. diaperinus*, as both condition (desiccation, RH 7% and cold, 5 °C) showed similar survival duration (Renault *et al.*, 2015). Similar observations were obtained when exposing the lesser mealworm to 10% relative humidity. The species showed high resistance to desiccation, with about 50% of individuals surviving for 30 days under such conditions. Moreover, some individuals were able to survive up to 50 days at 10% ± 2 relative humidity (Engell Dahl and Renault, 2022). Figure 1 shows the life cycle of the lesser mealworm and the reported optimum conditions used in rearing the edible lesser mealworm.

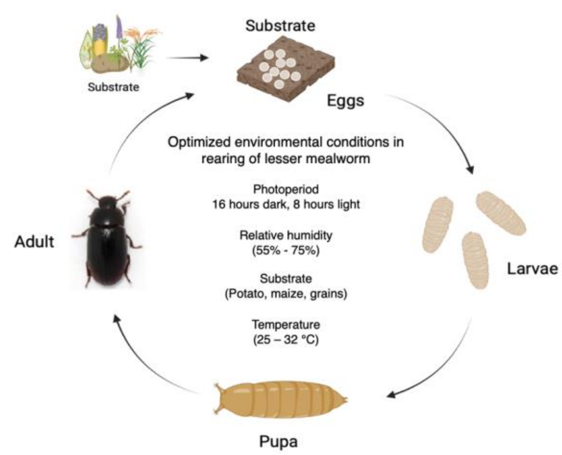


Figure 1. Optimized conditions in rearing of *Alphitobius diaperinus* (created in BioRender.com; the image of *A. diaperinus* in adult form was reproduced from Tejada *et al.,* (2022)

Kim *et al.* (2017) explored how different temperatures (20 to 35 °C) could affect the development of *A. diaperinus*. During the study, the larvae were exposed to these different temperatures while maintaining a constant relative humidity of 60%. The results of the study revealed that the time required for larval growth to adult stage was 129.0, 49.8, 40.5, and 31.9 days at temperatures of 20, 25, 30, and 35 °C, respectively. Additionally, the pupal rate was at about 80, 100, 83, and 92% at temperatures of 20, 25, 30, and 35 °C, respectively. These findings suggested that a shorter developmental period was observed at higher temperatures (35 °C), but a slightly lower temperature (25 °C) resulted in a higher pupal rate (100%) (Kim *et al.*, 2017). This finding is in agreement with the work reported by Kotsou *et al.* (2021), which states that temperature particularly affects the larvae development. An increase in the rearing temperature to 25 °C resulted in a significant reduction in pupal development time, which could correspond to a decrease in infections and rearing costs. Meanwhile, a recent study has shown that temperature levels can significantly affect the cumulative number of eggs per adult and the cumulative larval hatching rate of *A. diaperinus*. Optimal reproductive output is achieved when reared within a temperature range of 25 to 30 °C for 42 days. The study demonstrated that each adult *A. diaperinus* yielded an average of 73 eggs, with estimated hatchability rates of 69% and 58% at 25 °C and 30 °C, respectively (Ormanoğlu *et al.,* 2023)

In contrast, studies by Bjørge *et al.* (2018) shows that the growth rate of lesser mealworm was high at 31 °C with 18.3% wet mass growth/day. Furthermore, increasing the temperature to 37 and 39 °C led to the decrease in the growth rate per day. What is interesting is that they found a negative growth rate per day when the temperature was set to 15 °C. This study suggests that the rate of growth is highly dependent on temperature. On the other hand, there was a significant variation in the lipid content (24 to 34% of dry weight) across various temperatures. It can be said that the variation was good, however, the lipid contents were found to be close to 35% at middle temperature. In terms of protein content, the lesser mealworm was found to have a high protein content (60%) at lower temperatures, but a slight increase in temperature resulted in a reduction in protein content. The lowest protein content was observed at temperatures of 23 and 39 °C, with values of 48.4 and 48.9%, respectively. It would be interesting to conduct a detailed investigation on the composition of fatty acid and amino acid, as not all are nutritionally equal, and the works suggest that the composition of fatty acids, at the very least, is affected by temperature. Moreover, exploring the influence towards varying the content of lipid and protein in the feed according to the obtained values here would significantly contribute to the goal of enhancing the quality of the larvae (Bjørge *et al.*, 2018).

In rearing of edible insects, light has been considered as one of the significant factors that can influence the development of insects and by understanding on how abiotic parameters can affect insect-rearing is important in order to maximize the potential of lesser mealworms for future meat consumption (Suppo *et al.*, 2020). One of the abiotic parameters that can significantly influence the development of insect is photoperiod (Ribeiro *et al.*, 2018). For example, a short photoperiod can lead to a more significant accumulation of unsaturated fatty acids in this species, which could enhance the survivability in cold conditions. In fact, the effects of photoperiod on the content of nutrient are probably indirect, as they act through other processes such as preparation of diapause (Oonincx and Finke, 2021). In the case of mealworms, they exhibit negative phototropism and phototaxis (Balfour and Carmichael, 1928), where adults and larger larvae position themselves below the substrate's surface during daylight and emerge to the surface in darkness (Tyshchenko and Ba, 1986). For *T. molitor*, recent studies have shown that the highest growth rates, survival rates, and shortest developmental times were achieved under constant darkness (Eberle *et al.*, 2022). On the contrary, Zim *et al.* (2022) investigated the impact of photoperiod on the rearing of *T. molitor*, employing photoperiod parameters of 8 hours of light and 16 hours of darkness

(8L:16D) and complete darkness (0L:24D). Nevertheless, they concluded that the photoperiod had no significant effect on pupal development and adult survival. The study revealed that the optimal photoperiod for larval development was 8L:16D. Therefore, it can be said that while the photoperiod may influence the development of mealworms, the response to photoperiod tends to diminish under constant conditions, especially when *T. molitor* becomes arrhythmic (Cloudsley-Thompson, 1953). To date, there is still a lack of reports in the literature focused on studying the effects of photoperiod on the growth and development of *A. diaperinus*, or the lesser mealworm. Several works in the literature have reported the use of a photoperiod of 16 hours of light and 8 hours of darkness (16L:8D) in the development of *A. diaperinus*. Table 6 summarizes the optimal environmental conditions for the rearing of *A. diaperinus* based on results of various research.

Table 6. Environmental factors in rearing of *Alphitobius diaperinus*

Rearing Phase	Environmental Factors	Optimized Conditions	References
Larvae	Temperature	25°C	Rumbos *et al.*, 2021
	Light	ND	
	Relative Humidity	55%	
	Diet	Wheat bran and yeast (9 : 1)	
	Cage	ND	
Larvae	Temperature	30 and 32°C	Kotsou *et al.*, 2021
	Light	ND	
	Relative Humidity	55% and 75%	
	Diet	Wheat bran and yeast (9 : 1)	
	Cage	48 cm × 28 cm × 10 cm	
Egg	Temperature	20, 25, 30 and 35 °C	Kim *et al.*, 2017
	Light	16 Light; 8 Dark	
	Relative Humidity	60%	
	Cage	ND	
Larvae	Temperature	35°C	
	Light	16 Light; 8 Dark	
	Relative Humidity	60%	
	Cage	ND	
Pupae	Temperature	35°C	
	Light	16 Light; 8 Dark	
	Relative Humidity	60%	
	Cage	ND	
Larvae	Temperature	31%	Bjørge *et al.* (2018)
	Light	Natural light	
	Relative humidity	ND (Not controlled directly)	
	Diet	Mixtures of yeast, wheat, rye grain and pea	
	Cage	7 cm × 4 cm × 4 cm (Aluminium)	

ND: Not Determined

According to literature (Dossey *et al.*, 2016; Thévenot *et al.*, 2018), insects possess a notable advantage to grow in crowded areas, which facilitates large-scale production even in closed spaces. Typically, small trays manufactured from materials such as wood, polyethylene thermoplastic, or fiber-reinforced plastic are used to house both larvae and pupae along with a feeding substrate.

One study reported the use of a standard tray measuring 65 × 50 × 15 cm^3 for fattening the yellow mealworm (*T. molitor*) as this size was easy to manage and could prevent adult larvae from escaping (Dossey *et al.*, 2016). Based on the method according to the design from an EU pilot mill with the aim of producing 17 tons of fresh *T. molitor* larvae annually, the insects can be successfully reared at a density of 5 larvae cm^{-2} (Thévenot *et al.*, 2018). Although *T. molitor* and *A. diaperinus* come from different species, they belong to a similar family. Hence, they may share a similar bioecology. Therefore, the rearing system and conditions applied for *T. molitor* may be suitable for rearing *A. diaperinus*. This was also demonstrated by Rumbos *et al*. (2021), who reared both *T. molitor* and *A. diaperinus* using the same rearing system and facility. Figure 2 displays a design for rearing boxes arranged in multilevel shelves, which can minimize the space required for the production of mealworm. In certain cases, stackable boxes are recommended to be used. By utilizing multilevel shelves that cover the entire surface area of the rearing space, it may be feasible to produce several thousand tons of larvae per year (Dossey *et al.*, 2016). Within the breeding area, cages are used to house adults and are equipped with food and water sources and the boxes used for rearing larvae and breeding adults are often similar in design. Dividers can be also utilized to optimize the space (Dossey *et al.*, 2016). Moreover, it is important to limit oviposition sites to specific locations within the breeding cages to facilitate the collection of eggs. According to the rearing method adopted by Deruytter *et al.* (2021), the yellow mealworms were reared at the following conditions; relative humidity (60 ± 3%) and temperature (27 ± 1°C), using plastic crates (60 x 40 cm; inner surface up to 2000 cm^2). This method is expected to be suitable for rearing the lesser mealworm since both yellow mealworms and lesser mealworms belong to a similar family. For semi-industrial production of up to 45,000 larvae per tray, lesser mealworm larvae could be reared in open, stacked plastic trays with the size of 40 x 60 x 12 cm. The trays were kept at a temperature of 28 °C to 32 °C with a humidity of 60% and above (Gianotten *et al.*, 2020). Another reported study used a container with 19 cm × 11.5 cm; 950 cc for rearing all the three different stages (larvae, pre-pupae and beetles) of *A. diaperinus* (Meijer *et al.*, 2022).

Figure 2. Example of the multilevel shelves used in the process of rearing insect (reproduced with permission from Ortiz *et al.*, 2016)

Challenges associated with entomopathogens

Insect infections are not uncommon in mass rearing facilities. Previously, traditional insect rearing systems have been long-suffering from the impact of diseases such as build-up of microbial and viral pathogens (Maciel-Vergara *et al.*, 2021). However, there is still a lot of insect-borne diseases that remains to be discovered as well as the pathogens they are associated with (Leger, 2021). This emphasizes the challenge posed by the emergence of infectious diseases in the insect rearing facilities, particularly in the production of insects as novel foods (Rumpold and Schlüter, 2013b).

Insects can be infected by various pathogens, including fungi, bacteria, viruses, and microsporidia, which can cause serious diseases in the insects. These viruses (RNA or DNA-based genomes) have raised major concerns among farmers, particularly those involved in large-scale rearing facilities (Bertola and Mutinelli, 2021). The majority of viruses have host-specificity; however, there is one exception known as invertebrate iridescent virus 6 (IIV-6), which has been found to cause an infection in several hosts (i.e.; Orthoptera and Blattodea) (Maciel-Vergara *et al.*, 2021). These viruses have been reported to cause epizootics in insect-farming facilities, posing a significant threat to the entire production stock (Maciel-Vergara and Ros, 2017). In the worst case, these problems can lead to the shutdown of the affected rearing facilities. For example, contamination by fungi such as *Penicillium* spp. during the production of spore in one of the production facilities in Brazil has led to the closure of the farm (Li *et al.*, 2010) .

There are various types of entomopathogens that may infect *A. diaperinus* and these entomopathogens must be thoroughly identified and characterized to prevent and control the transmission of the diseases. Entomopathogens such as fungi, bacteria, nematodes and viruses have been reported in the literature to be affecting the rearing of *A. diaperinus*, For example, species of entomopathogenic nematodes such as *Heterorhabditis bacteriophora*, *Heterorhabditis megidis*, *Steinernema affine* and *Steinernema carpocapsae* have been reported to cause high mortality of larvae of *A. diaperinus* (Kucharska *et al.*, 2018). Meanwhile, *Beauveria bassiana*, a fungus that grows naturally in soils, has been pathogenic to the species of *A. diaperinus* (Kucharska *et al.*, 2018). These kinds of infections could cause major consequences in the mass-rearing facilities, ranging from asymptomatic infection to the collapse of the entire colony. As there is no cure for viral infections in edible insects, preventative measures are the only available options to contain the infection. This clearly shows the importance of biosecurity in maintaining disease-free in the production facilities (Bertola and Mutinelli, 2021). Moreover, a proper hygienic practice is highly required to ensure zero infection of entomopathogens.

Challenges associated with microbes contamination

According to the IPIFF, approximately 6,000 tones of insect proteins were produced in European countries in 2019. Additionally, it is estimated that up to 3 million tons of edible insects will be produced in 2030 (Niyonsaba *et al.*, 2021). The rapid development of edible insect industry has prompted regulatory bodies such as the European Food Safety Agency (EFSA) to initiate microbiological risk assessments and research on food safety (Niyonsaba *et al.*, 2021). As clear from the literature, many reared insects may have been contaminated by various kinds of microorganisms (Smith *et al.*, 2022; Vandeweyer *et al.*, 2021). For example, in the case of *A. diaperinus*, they have been reported to harbour a wide variety of viral, bacterial, and parasitic pathogens as well as poultry-specific and zoonotic viral that can also be transmitted to humans (Smith *et al.*, 2022). While it is unlikely that insects to be consumed in their raw form, it is still important to identify the microorganisms present in them as it may require specific processing treatments to ensure food safety before being declared safe for human consumption (Vandeweyer *et al.*, 2021). Another challenge that needs to be addressed is the development of antimicrobial resistance, which could result in various other health problems (Gwenzi *et al.*, 2021). It can be said that the risk of transmitting zoonotic infections to humans and livestock from insects may be lower compared to birds and mammals (Lange and Nakamura, 2021). However, considering the examples of zoonotic infections such as the coronavirus disease which have caused pandemics with significant economic and political impacts (Hatta *et al.*, 2023), it is crucial to take preventative measures during the production of edible insects.

For the large-scale production of lesser mealworms for human consumption, it is important to thoroughly study and evaluate the microbial dynamics that occur during the production cycle (Table 7). Wynants *et al*. (2018) conducted a characterization of microbial numbers and diversity during the production cycle of lesser mealworms. The results revealed a high count in substrate, feed, faeces, and exuviae compared to larvae. Likewise, the bacterial diversity was found to be reduced during larval rearing, with only certain bacterial species exhibiting a competitive advantage within the insect gut and becoming dominant. Therefore, a blanching treatment was conducted after harvesting the larvae, resulting in a reduction of microbial count. However, the number of aerobic endospores remained at 4.0 log cfu/g. Furthermore, fungal isolates corresponding to the genera *Aspergillus* and *Fusarium* were recovered in this study. Thus, it cannot be ruled out that mycotoxins were present. These findings enhance the understanding of microbial dynamics and food safety aspects involved in edible insect production.

Table 7. Microbial characteristics of lesser mealworm-based products

Pathogen	Phase	Source	Origin	Microbial Counts	References
Bacterial spores; Lactic acid bacteria; Yeasts and moulds	Larvae	Market	Belgium	2.0 - 3.6 log cfu/g	Stoops *et al.*, 2017
Fungal isolates (*Aspergillus* and *Fusarium*)	Larvae	Proti-Farm	Netherland	4.0 log cfu/g	Wynants *et al.*, 2018
Bacillus and *Paenibacillus* genera.	Larvae	Market	Netherland	< 1 and 4.49 log cfu/g (Lactic acid bacteria) 2.24 - 2.35 log cfu/g. (Yeast)	Roncolini *et al.*, 2020

ND: Not Determined

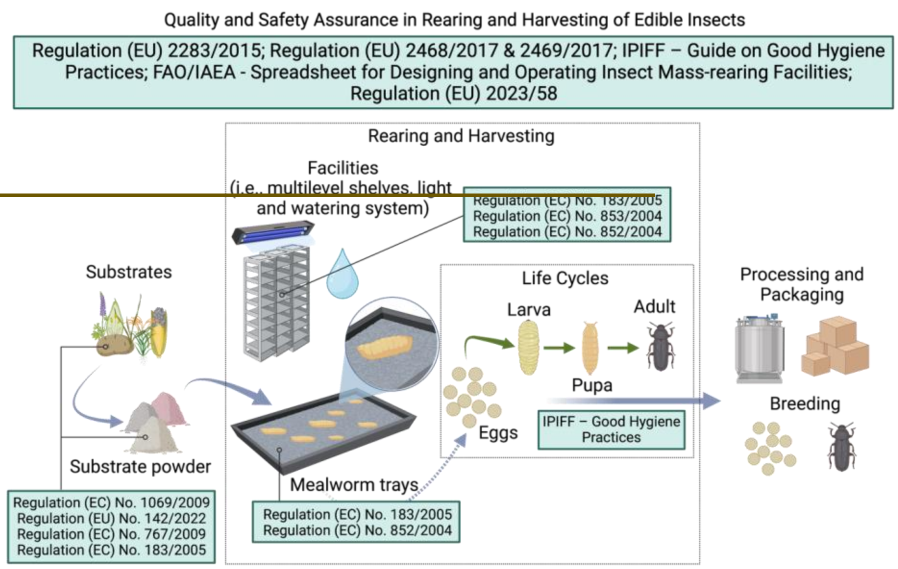
Microorganisms such as Enterobacteriaceae and lactic acid bacteria have been found in both fresh and processed products of lesser mealworm. Stoops *et al.* (2017) investigated the presence of aforementioned microbial in minced-meat product prepared from lesser mealworm powder (Stoops *et al.*, 2017). The production process for lesser mealworm larvae was designed with modified atmospheric conditions during storage, and the study recommended that this design could reduce bacterial growth compared to storing the larvae in normal air conditions. However, the design process is still not capable or reduce the number of microbes to almost zero. Although the numbers of microbes are low, the regulations and framework governing the commercialization of edible insects require the absence of specific food pathogens. Further research and development are required before these products can be sold in the market.

Another challenge that has been associated with microbial contamination is the problem with spore-forming bacteria that has been found in the edible insects (Garofalo *et al.*, 2019; Walia *et al.*, 2018). For instance, in the case of *Bacillus cereus*, the counts of aerobic spore-forming bacteria can be considered high if their presence is detected within the range of 1.6 – 8.1 log cfu/g (25% of the analysed samples) (Walia *et al.*, 2018). This foodborne pathogen has been acknowledged to cause intoxication at high doses, such as when the bacterial counts exceed 5 log cfu/g. Although Wynants *et al.* (2018) detected the presence of this bacterium in lesser mealworm, its count was found to be below the detection limit of <100 cfu/g (Wynants *et al.*, 2018).

In a recent study, Roncolini *et al.* (2020) examined the microbial growth in lesser mealworm during the preparation of fortified foods, which utilized lesser mealworm as a novel baking ingredient. The study involved the preparation of snacks such as sourdough, dough, breads, and rusks, where a powder containing a mixture of lesser mealworm and 10% - 30% of wheat flour was used as an ingredient. The microbiological analysis showed that spore-forming bacteria, specifically *Bacillus* and *Clostridium*, were detected, with the highest microbial count recorded at 1.45 ± 0.17 log cfu/g (Roncolini *et al.*, 2020). Although the microbial counts suggest that the lesser mealworm may not be a suitable substrate for the growth of these microorganisms, it is still important to monitor the presence of spore-forming bacteria in the species.

Quality and safety assurance in rearing edible insects

In EU, rules concerning insect-based products fall under the Regulation 2015/2283 (European Union, 2015) on Novel Foods and implementation of Regulations 2017/2468 and 2017/2469 (European Union, 2017a), and these regulations stated that Novel Foods cover all the type of insects that will be used for foods. Before being sold in market, authorization must be obtained from the Commission effective 1 January 2018. Therefore, it is necessary to ensure the quality and safety of these insects before they can be marketed and commercialized especially as novel foods. The focus of this section is on the rearing of edible insects, encompassing the legislative and regulatory frameworks, as well as the standards and guidelines at national and international levels that govern the utilization of insects as food and feed. Figure 3 shows the regulation and guidelines in rearing and harvesting of edible insects (primary production) according to EU/EC and IPIFF as well as by the joint Food and Agriculture Organization and International Atomic Energy Agency (FAO/IAEA) (FAO/IAEA, 2012).



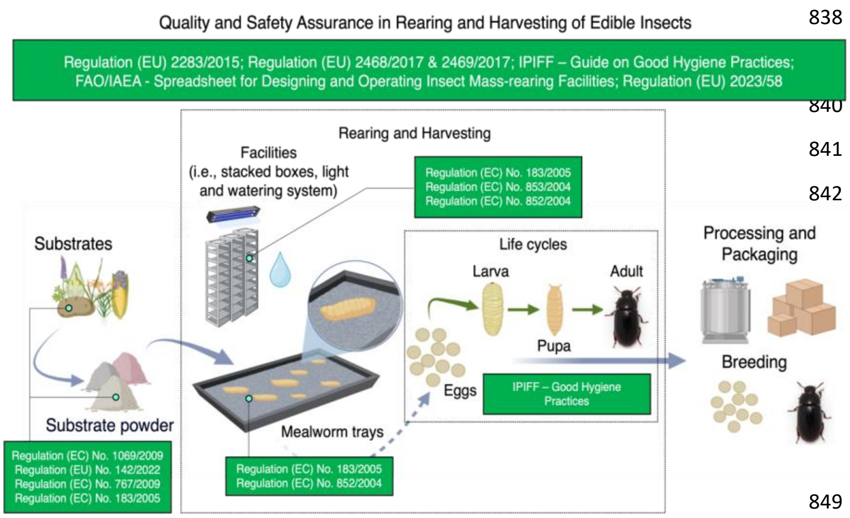


Figure 3. Rearing and harvesting of edible insect with guidelines according to national and international regulations (EU/EC/FAO) (Created in BioRender.com)

The growing interest in the utilization of insects in feed and food in Europe over the past decade has been due to their high nutritional value as a food source, which offers significant health benefits, as well as the notable advancements in the legislative perspective (Mancini *et al.*, 2022). Additionally, the lifting of longstanding bans, such as Regulation EU No. 2017/893 (European Union, 2017b) amending Regulations EC 999/2001 and EU No. 142/2011, has contributed to this trend. In addition, insects are not only a rich source of nutrients but also considered climate-friendly, as they require less space and water to grow and develop compared to chicken, pig, or cattle, as well as having a diet mainly consisting of organic products (Van Huis *et al.*, 2013). In order to maintain quality and safety standards in the rearing of edible insects and to establish new guidelines for the rearing process, there are several reviews and published opinions available in the literature (Lange and Nakamura, 2021; Rumpold and Schlüter, 2013b). Some of these opinions have highlighted data gaps in the field. As a result, current studies are concentrating on identifying food safety hazards that are specifically associated to insects that are raised under controlled condition. This has resulted in the comprehensive understandings and knowledge in providing edible insects that are safe for human consumption.

In general, consumers are concerned about the safety and how it may affect their consumption, specifically in terms of potential infectious agents such as viruses and bacteria, as well as bioaccumulation of toxic chemicals that could be harmful to humans. For example, Meijer *et al*. (2022) investigated the potential bioaccumulation and impact on growth or survival of *A. diaperinus* after exposure to a range of selected insecticides including chlorpyrifos ethyl, spinosad, tebufenozide, and piperonyl butoxide. The levels of the selected insecticides were spiked within the legally permissible limits in the EU. It was found that the bioaccumulation did not occur in the larvae as the concentrations of the insecticides were below the quantification limit. However, the use of Spinosad has shown the significant reduction in the total yield. Spinosad is allowed to be utilized in agriculture, however, its usage raises concerns about the safety of reared insects. Therefore, it is recommended that further studies be conducted on the safety and quality of the reared insects with respect to the amount of accumulated insecticides. According to the report by EFSA (2017), the potential hazards to human and animal health were found to be dependent on the techniques used in the rearing and processing of insects. In the majority of European countries, particularly in the area where insects are reared, they are typically farmed in controlled environments that fulfilled the proper sanitation procedures. This helps to mitigate certain hazards, such as microbiological contamination (Rumpold and Schlüter, 2013b). However, the safety (and quality) of edible insects can vary depending on the environment in which they are reared and harvested (Raheem *et al.*, 2019). As a result, frameworks governing insects-based food have been developed in the past 20 years (Lange and Nakamura, 2021). Furthermore, the type of insect species for farming must adhere to legal regulations pertaining to both food and consumer safety. For example, the insect-based food must comply with legal regulations aimed at preventing and eradicating bovine spongiform encephalopathy (BSE). These ordinances also prohibited the use of insects for the feeding of other farmed animals (Żuk-Gołaszewska *et al.*, 2022).

To ensure the safety of animal feeds during the rearing process especially within the EU region, the producers or operators may refer to several issued regulations according to European Commission (EC) for compliance with the safety and quality objective as shown in Table 8. The production of insects should be managed efficiently to achieve optimal yields and profits while meeting the requirements of food safety (Żuk-Gołaszewska *et al.*, 2022). In addition, the production of novel foods requires the adoption of efficient management systems, which include good breeding and hygiene practices, and the Hazard Analysis and Critical Control Points (HACCP) system (Awuchi, 2023). Moreover, the regulations impose hygiene and biosecurity standards that must be fulfilled by all farm buildings and production facilities.

Table 8. Regulations issued by the European Commission to ensure the safety of animal during production of insects.

Regulations	Remarks
Commission Regulation (EC) No. 1069/2009	Operators are required to separate animal by-products of different categories from each other under this regulation
Commission Regulation (EU) No. 142/2011	Enforcing measures to comply with public and animal health regulations related to animal by-products
Commission Regulation (EC) No. 999/2001	Regulations pertaining to the prevention, control, and elimination of specific transmissible spongiform encephalopathies
Commission Regulation (EC) No. 1137/2014 amending Annex III of Regulation (EC) No. 853/2004	Management of specific organs and tissues from livestock meant for human consumption
Commission Regulation (EC) No. 767/2009	Replaced and updated several measures regarding the marketing, labelling, and composition of animal feed
Commission Notice (EU) 2018/C 133/02	Recommendations for utilizing food that is not suitable for human consumption as feed for animals

In general, insect breeding facilities should be designed to prevent cross-contamination from other farming sites. Meanwhile, regular monitoring must be performed by the producers or operators of any rearing facilities in order to identify and address issues such as dust contamination and leaks at any facility. Furthermore, producers must ensure that pest management and eradication systems are implemented to ensure safety and quality during food and feed production. Insect farms should be equipped with safety systems to protect against external sources of pests and prevent insects from escaping from facilities. Production facilities must adhere to relevant standards, including the elimination of food residues, removal of unnecessary equipment and materials, and provision of organic waste containers to maintain good facility conditions (Ortiz *et al.*, 2016). These measures are in accordance with the draft of Regulation (Article 5) and Regulation (EC) No 1069/2009 (European Union, 2009a), which state that the substrate used for feeding insects should not include manure, catering waste, or other types of waste. However, as of October 2020, the draft Regulation has not been put into effect. The next section will discuss the quality and safety measures related to the rearing and harvesting of edible insects, as outlined by the standards issued by the IPIFF.

Rearing facilities

It is recommended that the location for rearing insects to be equipped with basic services (electricity, watering system, and waste management) (Regulation (EC) No. 853/2004) (European Union, 2004a). In addition, the building should be located at a distance from neighbouring facilities that could potentially lead to contamination, such as areas with chemicals, rivers, or flood-prone regions. Additionally, it should be located away from areas with high levels of airborne microorganisms and exposure to loud noise, both of which could have a negative impact during the rearing process (Żuk-Gołaszewska *et al.*, 2022) . Furthermore, the operators must ensure that there are proper measures in cleaning and disinfecting to reduce the hazard risk and prevent contamination, and other potential adverse impact to preserve the quality of the reared insects (Annex II and relevant articles of Regulation (EC) No 183/2005) (European Union, 2005). As per the same regulation, it is required that the facilities have sufficient natural or artificial lighting. Apart from lighting, the design and construction of ceilings and overhead fixtures should also prevent the accumulation of dirt and the formation of moulds, which could potentially impact the safety and quality of the reared insects. To ensure a clean air and avoid mechanical airflow from contaminated area, ventilation system is required to be installed in the facilities, in accordance with Annex II, Chapter I of Regulation (EC) No 852/2004 (European Union, 2004b).

Watering systems

According to the guidelines issued by IPIFF for the farming of edible insect, watering system is one of the prerequisites in building rearing facilities. A sufficient supply of potable water is required to ensure no major problems occur during the production process. Moreover, there should be an adequate quantity of pressurized water available at an appropriate temperature. In addition, the water should be ensured to be free from any contamination, and the supply of potable water must adhere to national regulatory standards. Finally, the water used in plant operations for cooling and processing procedures, must meet the necessary quality and microbiological standards based on its intended usage (IPIFF, 2022).

Sanitary of facilities

As per the Annex II of Regulation (EC) No 183/2005 (European Union, 2005) and Annex II, Chapter V of Regulation (EC) No 852/2004 (European Union, 2004b), the facilities and equipment employed during the mixing and/or manufacturing process must undergo regular inspections in accordance with the manufacturer's written protocols. The metering devices utilized in feed manufacturing must be suitable for the weights or volumes to be measured, and they should undergo regular accuracy testing. Moreover, the mixers used in feed manufacturing should have the capability to produce homogeneous mixtures and dilutions that are appropriate for the process. It is necessary to thoroughly clean and disinfect all equipment that comes into contact with food. Finally, it is essential to maintain materials in good condition to minimize the risk of contamination.

Feeding process

When selecting substrates for the rearing process, it is recommended that producers consider criteria such as nutritional composition, potential hazards to the insects, and the ease of removal. Moreover, the impact on the intended insect species, such as growth, weight, and feed conversion ratio, must be taken into account. It is essential to note that the properties of substrates are important parameters for the development and ensuring safe growth conditions for the insects. Typically, the lesser mealworm is reared on dry substrates (xiroculture). In the EU, insect producers are required to obtain feed materials for farmed animals that are approved as substrates in accordance with the regulations outlined in Regulation (EC) No 1069/2009 (European Union, 2009a) and Regulation (EU) No 142/2011 (European Union, 2011). Additionally, the use of prohibited materials listed in Regulation (EC) No 767/2009 - Annex III (European Union, 2009b) is not permitted. In addition, the use of any substrate mixed with insect frass in subsequent production cycles is prohibited under Article 9(g) of Regulation (EC) No 1069/2009 (European Union, 2009a) since insect frass is classified as 'Category 2' material. On the other hand, if substrates are obtained from outside the production facility, producers must obtain the substrates from registered suppliers or approved feed business operators, as per the regulations outlined in Regulation (EC) No 183/2005 (Article 5(6)) (European Union, 2005). Furthermore, the producers are encouraged to search for alternative nutritious substrates which are low-cost and sustainable.

Harvesting activities

The harvesting process involves collecting the larvae upon the completion of the rearing process. Generally, insects are extracted from their chambers and removed from the substrate. Insects with a holometabolous life cycle, such as mealworms, are harvested at the full-grown larvae stage. It is important to note that the harvesting techniques may differ among insect species, depending on their breeding behaviour. For instance, mealworm larvae usually remain in the growing substrate before the separation step (sieving), whereas black soldier fly larvae tend to naturally migrate from moist to dry environments. This step allows for manual or mechanical separation (Rumbos *et al.*, 2021).

Specific measures are recommended for the conditions under which the sieving machine method is applied during the harvesting process. For example, the size of the sieve should be suitable for efficiently separating the insects from the frass and any residual substrate, using either a one- or two-step process. Sorting techniques are recommended to effectively eliminate foreign materials, such as plastics or metals. Additionally, the sieving equipment must be thoroughly cleaned and sanitized to reduce the risk of microbiological contamination. In special cases, such as dealing with volatile faeces, producers are required to separate the faeces from the larvae in a designated and closed space. The residue of the feeding substrate must be disposed of properly, and operators are encouraged to monitor the microbiological status of the disposed substrate through sampling measures (IPIFF, 2022).

8. Consumer acceptance of *Alphitobius diaperinus* larvae as food

It is noted that the current techniques and methods employed in the production process, from the initial stages to the final product placement, are still inadequate and somewhat fragmented to fully facilitate the commercialization of insect-based food products (Kauppi *et al.*, 2019). Considering the matter from a global context, roughly two billion people currently incorporate insects into their daily diets (Van Huis *et al.*, 2022). To date, the literature indicates that there are approximately 2,111 species of insects that have been documented as edible (Guachamin-Rosero *et al.*, 2023). Although most of the reported edible insects are safe to be consumed, eating insects is not a common practice, especially among most Westerners. Hence, the consumer acceptance of insect-eating in this section will be thoroughly discussed for most of western countries especially countries within European Union.

Previous research focusing on consumer acceptance of edible insects has produced a variety of results, with their acceptability depending on various factors (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). There are several reports discuss the factors, justifications, and strategies that can be employed to encourage the wider adoption of insect-based foods by Western consumers (Ardoin and Prinyawiwatkul, 2021; Kauppi *et al.*, 2019; Onwezen *et al.*, 2021). Gaining insight into the elements that drive consumer acceptance or rejection of insect-based food can enhance the efficiency of future research and development, particularly in enhancing our understanding of the commercialization of potential edible insects. However, there is insufficient data regarding consumer needs, behaviours, experiences, and preferences to effectively engage them with insect-based products. In addition, there is a limitation in understanding consumer acceptability towards edible insects due to fragmented scientific literature. For instance, there are cases in which current research findings contradict with the findings from the past (House, 2016). The following section discusses consumer acceptance of edible insects, specifically the lesser mealworm, from a broader perspective based on the adapted framework illustrated in Figure 4. The adapted framework for consumer research findings discussed is based on a conceptual framework developed by Kauppi *et al.* (2019) and divided into two main categories: findings about the consumer (1) and the product (2). For the consumer, the consumer acceptance will be discussed according to the sociodemographic factor (culture, household composition, location and social acceptability) and individual factor (motivations, taste orientation, gender, and age). Meanwhile, for the product, the consumer acceptance will be discussed according to product-related factor (availability, product placement, sociability and ethical aspect) and product properties (taste properties, form, price, and nutritional properties). The summary of the consumer acceptance based on both of "The Consumer" and "The Product" elements are presented in Table 9.

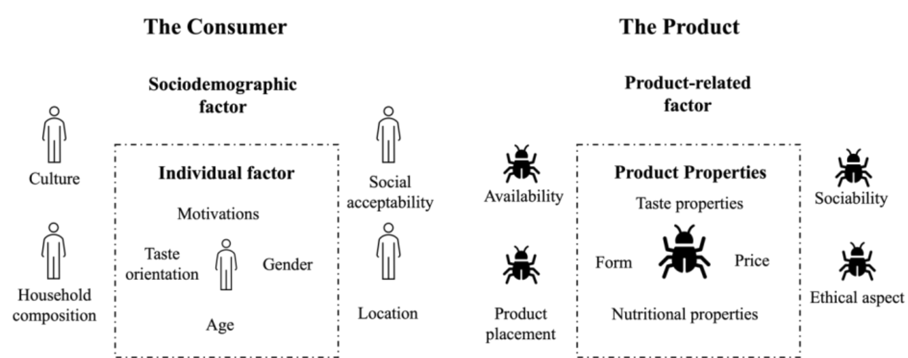


Figure 4. Factors that influence consumer acceptance of insect-based products (the framework is adapted from Kauppi *et al.*, 2019)

Table 9. Summary of the factors affecting consumer acceptance towards entomophagy

Factors		
Sociodemographic Factor	Culture	Westerners are not open to the concept of entomophagy, as they classify the concept as a form of disgust.
	Location	Austria, Belgium, the Netherlands, and France show higher levels of consumer acceptance of edible insects due to their wider incorporation into the food industry as a novel food compared to other Western countries such as Australia.
	Social acceptability	Eating insects might be viewed as a primitive practice and typically regarded as primary or emergency food sources.
	Household composition	In-home eating and family relationship may influence consumer acceptance of edible insects.
Individual Factor	Motivation	The influence of health benefits has no effect on the consumers' willingness to try the product.
	Gender	Men are more open to consuming insect-based foods compared to women.
	Age	Younger males in Western countries who are less attached to meat are willing to eat insects. However, the younger generation in Australia demonstrated a lack of interest in eating insect-based food.
	Taste orientation	A survey in Denmark showed that consumers prefer insects to be used as main ingredients in meals rather than just snacks while another survey commented to increase the sweetness of products.
Product Related Factor	Sociability	Consumer acceptance can be improved by expert recommendations and the experiences of peers.
	Ethical aspect	Consumers may question the methods used to rear and kill insects for human consumption while vegans view insect consumption as immoral and irresponsible.
	Product placement	The lesser mealworm-based product is sold in over 800 stores in Austria. Marketing techniques such as using celebrity endorsements and peer-to-peer advertising have been suggested to raise public awareness of insect-consumption.
	Availability	A survey towards Dutch consumers showed that the unavailability of the products in the market might stop their intention to purchase and move towards other sustainable foods.
Product Properties	Taste properties	Consumers prefer an insect-based meal with a neutral taste.
	Form	Prepare insect-based foods with an appealing appearance, such as incorporating edible insects into wheat flour.
	Price	An affordable or cheap price for an insect-based product may significantly influence the consumers' purchase behaviour
	Nutritional factor	Consumers may favour insect-based products with high nutritional values.

The consumer

Sociodemographic circumstances

Insects are commonly viewed in a negative way, and they are frequently associated with terms like 'dirty,' 'unhealthy,' and 'disease vectors' (Kröger *et al.*, 2022). Although edible insects have been declared safe and various campaign as well as promotion have been carried out to improve their public perception, studies show that many Westerners are still reluctant to include insects in their diet due to negative perceptions (Ardoin and Prinyawiwatkul, 2021; Kauppi *et al.*, 2019; Kröger *et al.*, 2022; Onwezen *et al.*, 2021). While surveys have shown that improving consumer knowledge and education about insects as a food source can increase willingness to try them, food choice motivation (FCM) can be a complex process as it involves various predictors, such as cognitive, cultural, demographic, geographical, social norm, and situational factors (Dagevos, 2021; Kröger *et al.*, 2022; Lammers *et al.*, 2019).

When it comes to culture, insects have long been associated with the feelings of disgust among Westerners (Looy *et al.*, 2014). Numerous reports have consistently demonstrated that disgust has a negative effect on the acceptance of insect-based food (Kröger *et al.*, 2022). However, in contract to this report, several reports showed that consumer interest in insect consumption has been improving recently. For example, a study by House (2016) in Netherland demonstrated that nobody is refusing the insect consumption due to disgust factor but more to the factors of price, taste, and availability. The survey focused on the convenience foods made from insects produced by the Belgian company, Damhert Nutrition. The products, such as burgers and nuggets were made from vegetables and contained 13% to 15% of the larvae of the lesser mealworm. On the other hand, a focus group study conducted in the Western part of Denmark revealed that Western cultures could be open to insect consumption if certain criteria are fulfilled. The study presented prospective consumers with various insect-containing products made from flour containing edible insects such as the larvae of lesser mealworm and yellow mealworm. The survey found that consumers were interested in having more insect-based recipes, nutritional information, and lower prices. Moreover, they expressed a preference for insects that can be used as main ingredients in meals rather than just snacks and suggested that insects should be incorporated into familiar products such as meat or bread. Finally, in order to promote regular consumption of insects, it is recommended that prices to be set at an affordable level for consumers (Brynning *et al.*, 2020).

In another study, Ortolá *et al.* (2022) developed biscuits using flours from *T. molitor* and *A. diaperinus* and assessed their physical and sensory properties. A panel of 30 testers from Spain, aged 18 to 65 years, participated in the survey. The physicochemical analysis indicated that the biscuits had high protein content, in compliance with Regulation (EC) No. 1924/2006 (European Union, 2006). However, many of the panelists found the biscuits too dark and not crunchy enough. One of the suggestions by the panelists was to increase the sweetness as it could potentially enhance its appeal. Based on this study, it can be suggested that understanding the nutritional value of edible insects, previous exposure to insect-based foods, and a desire for new sensations could increase the consumer acceptance among Westerners (Ortolá *et al.*, 2022).

Meanwhile, Austria, Belgium, the Netherlands, and France have experienced higher levels of consumer acceptance of edible insects due to their wider incorporation into the food industry as a novel food (Yi *et al.*, 2013). According to Lammers *et al.* (2019), 15.9% of German participants were willing to consume unprocessed insects, while Verbeke (2015) found that 16.3% of Belgian participants were open to incorporating insects as a food source into their diets (Verbeke, 2015).

Kostecka *et al.* (2017) also reported that approximately 37% of participants in Poland found products with processed insects, such as insect flour, to be acceptable for introduction in the market (Kostecka *et al.*, 2017). Based on the study by Mazurek *et al.* (2022), consumer acceptability can be significantly influenced by the form of the insects, specifically their flavour, and the way in which they were presented. The studies used lesser mealworm powder as a flavour in preparing wheat pancakes. The acceptability of insects among consumers was evaluated among people in Poland. Based on the conducted survey, as the proportion of insect composition increased, scores for all parameters decreased. Despite positive responses towards the idea of entomophagy in general, these studies indicated that people still showed hesitation in incorporating insects into their diets. Hence, it can be said that not all consumers in European countries are ready for insect-based food, and additional factors need to be considered before commercialization. Factors such as food disgust, neophobia, and seeking sensation have been identified as predictors of the acceptance of edible insects, and these factors will be discussed further in the product section (Mazurek *et al.*, 2022).

Social and cultural norms are also the important factors when it comes to accepting insects as novel foods (Kröger *et al.*, 2022; Tzompa-Sosa *et al.*, 2023). As demonstrated by Ros-Baró *et al.* (2022), the perception of insect eating as a primitive practice was not a significant barrier to the consumption of insects (Ros-Baró *et al.*, 2022). Insect preparations are often viewed as delicacies in Western countries, but they are typically seen as basic or emergency food sources elsewhere (Tzompa-Sosa *et al.*, 2023). In addition, there are concerns regarding the potential existence of pathogenic microorganisms and heavy metals, as well as the possibility of allergic reactions from consuming insects (Vandeweyer *et al.*, 2021). In this case, the EFSA, on July 6, 2022, has issued a positive opinion on the safety of the lesser mealworm as novel foods, according to Regulation (EU) 2015/2283 (EFSA Panel On Nutrition *et al.*, (2022). Moreover, in January 2023, the commission authorized the marketing of lesser mealworm under Regulation (EU) 2023/58 (European Union, 2023). There are few legal frameworks that consider insects as food (Grabowski *et al.*, 2020) and this suggests that promoting edible insects as a food source to populations unfamiliar with entomophagy would require greater efforts in sensitization and awareness-raising to communicate their benefits and safety (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). The acceptability of consuming insect-based food can also depend on household composition, including who a person eats with and how well their current eating habits align with this new food choice (House, 2016). However, information about these predictors is limited.

Individual factor

Numerous studies conducted on Western populations have shown that men are generally more open in consuming insect-based foods compared to women (Kröger *et al.*, 2022; Tzompa-Sosa *et al.*, 2023). In contrast, survey conducted among Belgium consumers show that gender appears to have no effect on consumer acceptance towards products prepared from mealworms (~~Caparros~~ Megido *et al.*, 2014). The study also demonstrated that the prospect of cooking a non-conventional and "fun" food like insects could increase their willingness to cook and consume them.

According to Verbeke (2015), younger males in Western countries who are less attached on meat, more receptive to new food experiences, and concerned about the environmental consequences of their dietary choices are the ideal candidates for the initial adoption of insects as an innovative and environmentally friendly protein source. In contrast, research conducted on younger Australians, comprising Millennials and Generation Z, has demonstrated a lack of interest in substituting meat with edible insects. This perspective is primarily attributed to factors such as neophobia and disgust towards insects, as well as a perception of insects as a threat to traditional masculinity. Moreover, despite being aware of the nutritional benefits of consuming insects, consumers do not seem to be influenced in considering them as a food alternative (Sogari *et al.*, 2019). It can be said that although knowledge about entomophagy may have an impact on consumer acceptance, the level of education may not be a significant factor (Ardoin and Prinyawiwatkul, 2021; Kröger *et al.*, 2022; Tzompa-Sosa *et al.*, 2023). Similar to meat, insects can also be subjected to dietary restrictions based on nutritional or animal welfare grounds. It is anticipated that individuals following a vegetarian or vegan diet may have a greater reluctance to consume insects (Kröger *et al.*, 2022; Onwezen *et al.*, 2021).

The acceptance of insects as food can be significantly influenced by emotions (Kröger *et al.*, 2022). Most of the studies reported in the literature have focused on emotions associated with disgust or fear. However, there is one comprehensive study on the influence of emotions in general terms. For example, the feeling of happiness, satisfaction, or pride when thinking about eating insects. These are defined as positive emotions, while negative emotions can be defined as the feeling of anger, sadness, or even guilt when thinking about eating insects (Kröger *et al.*, 2022). According to Onwezen *et al.* (2019), positive emotions have been shown to positively affect consumer acceptance, while negative emotions do not have a significant effect.

In Western countries, the concept of entomophagy frequently evokes negative emotional reactions, with disgust being one of the most common and prominent ones. Several studies that have been reported in the literature indicate that the feeling of disgust has become a barrier to insect consumption (Kröger *et al.*, 2022). For example, a survey on consumer response in Australia revealed that the emotion of disgust is the main barrier to consumer acceptance of insects as food. Majority of the participants showed negative associations with the idea of regarding insects as food. The survey revealed the use of negative words, such as 'disgust', 'detestation', 'revulsion', 'dislike', 'vomit', and 'neophobia', in reference to the insect-based product (Sogari *et al.*, 2019).

To determine how health benefits influence consumer behaviour towards insect consumption, Poortvliet *et al.* (2019) conducted a study on 134 Dutch consumers to measure their willingness to try insect meat. In this study, participants were shown pictures of hamburgers with a description indicating that the burger was produced from either ground beef (for the bovine meat type condition) or a combination of ground lesser mealworms and locusts (for the insect meat type condition). The researchers found that uncommon products such as shish kebabs were more preferred over common products like burgers. However, the study did not reveal any significant difference in preference between the two meat types (bovine meat and insect meat). According to this study, the influence of health benefits and the factor of disgust had no effect on the consumers' willingness to try the product. This finding revealed the factor on why insect-based products are not as popular as bovine meat products. However, the study also highlights an interesting point that health benefits could potentially be a significant driver for the acceptance and adoption of insect consumption. When developing marketing strategies, it is important to include the health features of insect products such as nutritional content and safety considerations. Additionally, attention should be given to packaging design, labelling, and other elements of product presentation that can affect consumers' perceptions and willingness to try these novel food products (Poortvliet *et al.*, 2019).

The product

Product-related circumstances

The term 'product-related circumstances' refers to the social, practical, and contextual factors that are associated with insect-based food products (Kauppi *et al.*, 2019). While the price and taste of an insect-based burger may influence repeat consumption, it is important to consider the effect of social, practical, and contextual factors as well (Kröger *et al.*, 2022). In addition, the consumer acceptance can be significantly increased by expert recommendations and the experiences of peers (Berger *et al.*, 2019). According to Caparros Megido *et al.* (2014), if consumers can associate insect-based food products with familiar flavours, they are willing to purchase and prepare them at home. This shows that, from a practical view, edible insects have the potential to become a commonly used food ingredient among European populations.

As interest in edible insects continues to grow, it raises questions about how consumers perceive the welfare of insects (Delvendahl *et al.*, 2022). This issue has been a matter of public concern since the nineteenth century, but it has become increasingly relevant in recent years. However, to this day, it is still unclear how consumers perceive the welfare of farmed insects. The ethical implications of consuming insects are likely to have an impact on consumers, and this concern is expected to grow as consumers start to question the methods used to raise and kill insects for consumption (Kauppi *et al.*, 2019). Notably, the criteria for insect welfare may vary from those established for vertebrate welfare. It could be argued that establishing guidelines for insect welfare may prove challenging given the diverse habitats and dietary needs of insects. Furthermore, there is an ongoing debate about whether insects possess consciousness and experience pain. Some researchers have recommended treating insects as sentient beings and rearing them in natural living conditions (Delvendahl *et al.*, 2022). Nevertheless, consumers' views and understanding regarding this matter have received little consideration thus far.

Halonen *et al.* (2022) conducted a survey on the ethical aspects of insect consumption, and they found that attitudes towards the ethics of consuming insects in Finland are heavily influenced by whether the respondent is a semi-vegetarian, vegetarian, or vegan. The survey revealed that 72% of semi-vegetarians were open to incorporating insects into their diet, whereas most vegetarians (56%) and vegans (71%) considered the consumption of insects to be ethically unacceptable (Halonen *et al.*, 2022). Meanwhile, Elorinne *et al.* (2019) reported that vegetarians hold the most favourable views on consuming edible insects, and both omnivores and vegetarians perceive entomophagy as a wise solution to global nutrition challenges. In contrast, vegans view insect consumption as immoral and irresponsible (Elorinne *et al.*, 2019).

In addition to ethical considerations, pricing is another important factor that can have a significant impact on consumer acceptance. Furthermore, pricing can also influence repeat consumption (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). A survey conducted by House (2016) showed that price was a significant factor in their acceptability and repeat consumption. Consumer perception of the price of Insecta, a burger made from 13 - 15% of lesser mealworm powder, was deemed relatively expensive in Belgium, as per the findings of those studies. For instance, the cost of a pack containing two insect-based burgers was approximately €4, which was higher than most comparable vegetarian products (€2 - €3) and meat products (€1 - €3). Around 36% of the participants regarded the insect food as too expensive to purchase, while almost half of them (45%) recognized the relatively elevated cost but did not consider it as problem for future purchases.

While price alone would not stop the majority (64%) of people from purchasing, it was frequently acknowledged as one of several intersecting factors that impeded future purchases.

In addition to price, it is crucial to ensure that edible insects are guaranteed to be of high quality and meet food safety standards. It is noted that the lesser mealworm has been granted novel food status by the EU as of January 2023, following its safety approval by the EFSA (European Union, 2023). Despite lesser mealworm not being extensively commercialized in European countries yet, various studies have demonstrated a growing interest in this insect. Consequently, it may be recommended to boost the population or production of lesser mealworms to enhance the predictability and availability of insects (Van Huis *et al.*, 2022). For example, a survey conducted by House (2016) among Dutch consumers found that the unavailability of the products in the market had hindered their intention to purchase (House, 2016). This finding was in agreement with the studies by Shelomi (2015) who reported that low availability of products resulted in consumers purchasing less frequently than they would have preferred, leading to passive rejection of the products by potential consumers (Shelomi, 2015).

The growing interest in edible insects over the past decade has led to the emergence of several business owners and enterprises actively involved in insect production. In European countries, the insect-based production industry primarily focused on breeding insects for biocontrol purposes or animal feed production. These activities were often carried out in zoological gardens (Mancini *et al.*, 2022). These days, the situation is different and there are now several operators in the insect feed business (iFeedBOs) who also engage in food production activities as well as some operators in the insect food business (iFoodBOs). To date, lesser mealworms have been applied in a variety of products across Europe, including Zirp, which is sold in over 800 Billa stores in Austria. Other examples include cereal bars, Issac shakes, and gourmet burgers made from mealworm that are featured in several Danish restaurants. The company has been expediting the commercialization of its products in additional European markets (Ynsect, 2021). According to Collins *et al.* (2019), incorporating insect-based foods into human diets not only benefits the environment but also makes good business sense. They also emphasized the significance of utilizing different marketing techniques, such as using celebrity endorsements and peer-to-peer advertising. Developing marketing and advertising strategies for insect-based products and raising public awareness about the entomophagy is crucial. This can be accomplished by launching educational campaigns aimed at farmers to promote awareness of the advantages of edible insects as a substitute for traditional livestock rearing (Collins *et al.*, 2019; Żuk-Gołaszewska *et al.*, 2022).

The product properties

Due to the unfamiliarity of entomophagy among Westerners, improving familiarity has often been emphasized as a critical factor in reducing neophobia and increasing acceptance (Kröger *et al.*, 2022). There are various studies that emphasized the significance of the process of familiarization and increasing exposure as a means of reducing and overcoming feelings of disgust and fear associated with the consumption of insects (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). It is proven that testing insects as food and exposing people to insect-based foods can help reduce neophobia and increase acceptability (Kröger *et al.*, 2022). Therefore, the acceptability of a new food product can be heavily influenced by its taste. This was also in agreement by ~~Caparros~~ Megido *et al.* (2016), which the taste and appearance significantly influenced the participants' overall liking of burgers prepared from mealworms. Meanwhile, a study conducted by Brynning *et al.* (2020) obtained similar results, which suggested that the protein, fat, and chitin composition of insect flour made from lesser mealworm should be refined and separated to achieve a more neutral taste. This could potentially enhance the usability of insect flour as an ingredient in food. Moreover, Mazurek *et al.* (2022) revealed that the attitudes of potential consumers in Poland towards entomophagy and consuming pancakes with addition of edible insects were promising. Nonetheless, despite the positive attitude, most respondents were reluctant to taste the test samples due to the addition of insect in meal. The pancake flour used in the study was mixed with 10-30% of lesser mealworm. The study showed that the primary factor that influenced the overall sensory acceptability was the taste. It can be said that insect-based products have potential for introduction in Western society. However, some improvements may be required, such as refining the taste, altering recipes, and modifying product structure.

On the other hand, it is important to highlight that there is a recommended upper limit (%) for incorporating insect flour as a wheat flour substitute. Exceeding this limit could affect the bread quality, especially the carbohydrate content (Skotnicka *et al.,* 2022). Lesser mealworms consist of approximately 60% protein (Roncolini *et al.,* 2020), and several studies (Brynning *et al.,* 2020; Mazurek *et al.,* 2022; Roncolini *et al.,* 2020; Skotnicka *et al.,* 2022) have demonstrated that up to 30% of lesser mealworms were added to replace the wheat flour for the production of bread and rusk. However, it is essential to note that increasing the amount of lesser mealworm could lead to a decline in bread quality due to the reduction in carbohydrate content (Brynning *et al.,* 2020). This is supported by studies conducted by Skotnicka *et al.,* (2020), which demonstrated that increasing the protein content in the pancakes led to a reduction in carbohydrate content ($p < 0.05$). Meanwhile, a study by Roncolini *et al.,* (2020) revealed that a 30% substitution of lesser mealworm enriched the protein content of rusk by up to 99.3%, suggesting that 30% was the optimum amount for substitution. Additionally, the analysis of mineral composition in the rusk showed that incorporating 30% of lesser mealworm provided the recommended daily intake of minerals such as Fe, Zn, Mg, and Ca. For instance, the recommended daily intake for Fe falls between 7 and 58 mg per day. The mineral composition analysis of the prepared rusk (with 30% lesser mealworm substitution) indicated Fe contents ranging from 28 to 33 mg/kg. Consequently, consuming at least 200 g per day of rusk with 30% substitution for lesser mealworm would be suitable to meet the recommended daily intake for this mineral.

As lesser mealworms are rich in protein, a high amount (>40%) of substitution may cause the product appearance to become darker, thereby potentially affecting consumer acceptance (Brynning *et al.,* 2020). In another study, Gantner *et al.* (2022) evaluated the physicochemical properties of bread incorporated with 5, 10, and 15% of mealworms. The study revealed that a higher addition of mealworms would significantly reduce the intensity of the bread flavor, even at levels of 10% and 15% addition. This effect was particularly showed in the intensity of the bitter taste and nutty flavor of the bread samples with a higher amount of mealworms (15%). Sensory evaluation indicated that the incorporation of mealworms significantly affected the visual appearance, flavor, odor, and overall sensory quality of the bread. Based on the results, it can be concluded that the maximum amount of insect addition is dependent on the type of insect used. For example, in the case of the lesser mealworm, it can be said that 30% would be the maximum amount, as any quantity beyond that threshold would negatively affect the physicochemical properties and overall sensory quality of the bread.

Various studies have demonstrated that price can influence the consumer acceptance of edible insects (Brynning *et al.*, 2020; Mazurek *et al.*, 2022; Roncolini *et al.*, 2020). An example of a study exploring the influence of price on the development of non-snack insect food, using lesser mealworm as insect flour, was demonstrated by Brynning *et al.* (2020). The study surveyed individuals from the Western part of Denmark and found that the main predictor for improving insect-based products was to lower the price. The participants were asked about what price range would be considered acceptable for food products made from them. In the survey, participants were instructed to select an insect product and guess its sales price. The actual price was 30 - 50% higher than their estimates, which came as a surprise to them. Pricing was identified as the primary challenge in the adoption of insect products in the future. When asked to choose between plant-based and insect-based alternatives to meat, they preferred plant-based products due to their lower cost. Therefore, in the future, it is recommended that producers evaluate their pricing strategy and decide whether to prioritize competition, value, or marketing over the current cost-based approach.

The food industry is increasingly favoured towards selecting innovative and sustainable raw materials to produce nutrient-fortified foods, in response to the growing consumer trend for such products (Hassoun *et al.*, 2022). One way to fortify foods is by adding a specific molecule during the processing stage, while another approach is to use ingredients that are naturally high in the desired nutrient (Roncolini *et al.*, 2020). In this perspective, using edible insects as food ingredients can be a promising strategy for fortifying conventional foods, especially to increase consumer interest in nutrient-fortified foods. Based on study by Brynning *et al.*, (2020), they showed that consumers are looking for insect-based products that go beyond snacks and can be used as elements of main meals. Furthermore, they also look for nutritional details regarding these products. In terms of the form of insect-based products, they also suggested that insects be integrated into recognizable food items such as bread or meat products.

In a different study, Roncolini *et al.* (2020) used lesser mealworm powder as a novel baking ingredient to produce protein and mineral-rich snacks. The lesser mealworm was substituted for wheat flour in amounts ranging from 10% to 30% to increase the protein and mineral content of crunchy snacks. The study found that when 30% of the lesser mealworm was added, the protein content was enriched up to 99.3% in rusks. Furthermore, a substantial rise in the level of essential amino acids was observed, with the fortification of histidine reaching up to 129% in rusks. The addition of lesser mealworm powder has enriched the minerals such as iron (Fe), potassium (P), and zinc (Zn). Considering the possibility of producing insect-based rusks on an industrial scale, the aforementioned product can be categorized as level 4 on the Technology Readiness Level scale.

Another factor that can significantly influences consumer acceptance is the textural properties. There are several studies reported on the addition of edible insects can affect the textural properties of the product (Kröger *et al.*, 2022). For example, García-Segovia *et al.* (2020) evaluated the effect of adding insect-based protein in the manufacturing of bread. The wheat flour used in production was lesser mealworm powder and the consumer acceptance of the prepared bread was measured. According to the survey, they found that the textural properties were significantly affected when insect-based flour was used. However, despite the effect of insect-based flour on textural properties, the prepared bread received a high liking score for attributes related to overall acceptance, indicating that textural properties did not significantly impact consumer liking (García-Segovia *et al.*, 2020).

According to several aforementioned studies, lesser mealworm flour can be produced by incorporating them into wheat flour and this can serve as an ingredient in various food products, including energy bars, bread, pasta, noodles, and more. Considering factors such as disgust, neophobia, and food safety, it is important to prepare insect-based foods with an appealing appearance, and the use of flours has been found to be the most accepted format (Ros-Baró *et al.*, 2022). Recently, Brynning *et al.* (2020) developed a non-snack food product based on three different insect species which are house cricket, lesser mealworm, and yellow mealworm. The flavour profiles of the three insect flours were analysed using quantitative descriptive sensory analysis. According to their findings, insect flour has a distinct flavour profile characterized by three main taste notes: Protein/meat, cereal/bread, and mature/old. They also observed that among the three insect flours tested, Tenebrio had the most neutral taste. The studies on consumer acceptance were conducted on individuals residing in the western region of Denmark. The study indicated that consumers favoured incorporating insect flour into meat products or bread instead of wheat flour. In addition, it is recommended that the insect flour to be utilized to boost protein and vitamin levels. These findings suggest that insect flour could be utilized as a primary component in the preparation of main meals with some modification in the development kitchen.

Finally, integrating environmental and sustainability features into insect packaging is crucial for marketing and appealing to consumers (Wade and Hoelle, 2020). One survey (Brynning *et al.*, 2020) showed that consumers may favour the design that looked cheaper. Furthermore, the presence of pictures of insects on the packaging was found to have a deterrent effect, as consumers preferred insect images to be in the form of drawings or illustrations. In term of naming, considerations should be taken during production by using "IN" as a prefix to a familiar name instead of "insect" in order to provide a more modern touch. To enhance consumer preference, the production company may opt to use specific insect names like Lesser Mealworm or *Alphitobius diaperinus* instead of using general terms like "insect." This helps the consumers to know the exact type of insect present in the product. In conclusion, customizing the packaging design to align with the sustainable image of insects may prove to be a crucial approach in persuading consumers to accept insect-based products.

9. Conclusion and future perspectives

The European Commission's recent approval of *A. diaperinus* larvae (lesser mealworm) as a novel food has opened up new opportunities for sustainable food production and consumption. A comprehensive review of the scientific literature on this topic suggests that the insect has the potential to become an important source of protein and other essential nutrients for human diets.

Records of lesser mealworm consumption in various parts of the world suggest that it has been a traditional food source in many cultures for centuries. This insect's bioecology and nutritional value make it a promising candidate for human consumption. It is easy to rear, has a short life cycle, and can consume a wide range of organic matter, including agricultural by-products and food waste. Furthermore, lesser mealworm is rich in protein, vitamins, and minerals, including calcium, phosphorus, and iron. Studies have shown that edible lesser mealworm also has nutraceutical and pharmaceutical properties. The insect contains bioactive compounds that may have beneficial effects on human health, such as antioxidant and antimicrobial properties.

Consumer acceptance of edible lesser mealworm remains an important consideration. Although lesser mealworm has been consumed for centuries in some cultures, it may be challenging to introduce it to new markets. However, studies suggest that consumer acceptance can be improved through effective communication and education. Consumers are more likely to accept lesser mealworm as a food source if they understand its nutritional value, safety, and environmental benefits. Further research is needed to explore more about the potential health benefits of lesser mealworm and to develop effective strategies for introducing it to new markets such as focusing on environmental, economic and social sustainability or tackling the safety concerns in the new markets. Several effective strategies exist for introducing edible lesser mealworms to new markets. One approach is to craft a go-to-market strategy. Another approach is to consider success factors before entering the market.

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Author Contributions

S.A.S. - Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization, Data Curation, Project administration, Investigation, Supervision. Y.S.W. - Writing - Review and Editing. K.V. - Writing - Original Draft. K.B. - Writing - Original Draft. M.H.M.H. - Writing - Original Draft. H.L. - Writing - Original Draft. R.C.-M. – Funding, Validation. I.F. - Writing - Original Draft, Writing - Review and Editing.

Conflict of interest

The authors declare no conflict of interest.

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