

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

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24 **Short title**

25 **Lesser mealworm as human foods**  
26

## 27 **Abstract**

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

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

## 49 **1. Introduction**

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

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

66 *Alphitobius diaperinus* is widely distributed throughout the world and is commonly found in  
67 poultry facilities, where it feeds on litter and other organic matter (Rumbos *et al.*, 2019). It is a  
68 pest of stored products that can cause significant economic losses for the food industry. It feeds on  
69 a variety of dry stored products, such as grains, flour, and animal feed. The larvae can tunnel into  
70 grain kernels, reducing the nutritional value and causing spoilage. However, it is regarded as very

71 nutritious. It has been particularly recognized for its high protein and fat content (Janssen, 2017),  
72 as well as its potential for use in animal feed and waste management (Mariod *et al.*, 2017; Volpato  
73 *et al.*, 2016).

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

81 As the global population continues to grow, food security has become a pressing issue, particularly  
82 in developing countries. Insect consumption has the potential to alleviate some of these concerns  
83 by providing a cheap and readily available source of nutrition (Janssen *et al.*, 2017; Van Huis,  
84 2015). Insects are also rich in micronutrients such as iron, calcium, and zinc, which are often  
85 lacking in traditional staple foods such as rice and maize (Mwangi *et al.*, 2018). However, the  
86 widespread adoption of entomophagy as a food source is not without its challenges. Public  
87 perceptions toward insect consumption vary widely, with many Western consumers expressing  
88 disgust at the idea of eating bugs (Tan *et al.*, 2015). Despite these challenges, the potential benefits  
89 of insect consumption are leading many to consider it a novel and future food source. Larvae of *A.*  
90 *diaperinus* have been identified as a promising candidate for mass-rearing due to high nutritional  
91 value and ease of rearing (Rumbos *et al.*, 2019). This species has been successfully used as a feed  
92 source for poultry and fish, as well as a potential ingredient in human food products such as protein  
93 bars and snacks (Rumbos *et al.*, 2019). The mass rearing of *A. diaperinus* has a low environmental  
94 impact because it can be raised on organic byproducts, reducing the need for additional feed  
95 resources (Piña-Domínguez *et al.*, 2022).

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

## 103 **2. European Commission approval for the insect as a novel Food - *Alphitobius diaperinus*** 104 **larvae**

### 105 ***Alphitobius diaperinus* larvae as a novel food**

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



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

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

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

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

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

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

153

154

155



156 *Production process*

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

166 Proper rearing, harvesting, and processing of an insect is vital for industry as well as consumers.  
167 In line to the IPIFF guidelines, the production process of lesser mealworms involves rearing,  
168 harvesting, and processing. Insect rearing methods are crucial to ensure the safety and quality of  
169 the end product. The rearing conditions must be optimal to minimize the risk of contamination by  
170 pathogens and other harmful substances. Besides, the harvesting and processing methods must  
171 also be efficient and hygienic to avoid contamination and ensure the quality of the final product.  
172 Several studies have investigated the impact of different rearing conditions on the chemical  
173 composition and quality of lesser mealworms (Mozaffar *et al.*, 2004; Kotsou *et al.*, 2021; Meijer  
174 *et al.*, 2022). For instance, a study by Kotsou *et al.* (2021) has investigated the impact of  
175 temperature on the growth and development of lesser mealworms and found that a temperature of  
176 27°C was optimal for rearing. So that, the insect can be mass-reared effectively or optimally,  
177 allowing for continuous production to fulfil market demand.

178 *Potential allergenicity*

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

193 *Nutritional value*

194 The chemical composition of lesser mealworms is an essential factor in determining their  
35 suitability as a food source. The composition determines the nutritional value and potential health  
36 risks associated with consuming the product. The major components of lesser mealworms include

197 protein, fat, fiber, ash protein, vitamins, and minerals, making them a highly nutritious food source.  
198 Research has indicated that dried lesser mealworms contained an average of 58-65% crude protein,  
199 which is comparable to other insects approved for human consumption, such as crickets and yellow  
200 mealworms (Rumbos *et al.*, 2019). The study also found that lesser mealworms contained high  
201 levels of essential amino acids, making them a nutritionally valuable food source (Elhassan *et al.*,  
202 2019). Furthermore, another study reported that lesser mealworms contained high levels of iron,  
203 zinc, and calcium, making them a rich source of minerals (van Huis, 2013).

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

## 222 *Safety*

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

## 233 **Status of *Alphitobius diaperinus* larvae as animal feed**

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

237



238 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	<i>Alphitobius diaperinus</i> meal did not affect the growth performance, carcass composition and meat quality of the pig.	Richli <i>et al.</i> , 2023
Rat	300 mg/kg	6 days	<i>Alphitobius diaperinus</i> modulates duodenal and colonic enterohormone release and increases food intake in rats.	Miguéns-Gómez <i>et al.</i> , 2020

239  
 240 Lesser mealworm is also reported to be reared as feed for reptiles, fish and avian pets in the  
 241 Netherlands (Van Huis *et al.*, 2013). Furthermore, it has been reported that, when compared to  
 242 other closely related edible species such as the yellow mealworm *Tenebrio molitor* Linnaeus, 1758  
 243 or the superworm *Zophobas morio* (Fabricius, 1776), *A. diaperinus* may be used more simply to  
 44 provide protein for agricultural animals due to its shorter biological cycle and smaller size, making  
 45 it a better choice as feed in breeding facility (Ricciardi and Baviera, 2016). Despite the potential

246 benefits of using lesser mealworms as animal feed, its regulatory status as a feed ingredient varies  
247 by region. In the United States, the use of insects as animal feed is regulated by the Association of  
248 American Feed Control Officials (AAFCO), and lesser mealworms are not currently listed as an  
249 approved feed ingredient (AAFCO Committees, 2021).

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

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

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

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

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

276



277 Table 2. The list of nations by continent that has consumed *Alphitobius diaperinus* larvae as  
 278 traditional or modern cuisine, along with their cooking style  
 279

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 <i>et al.</i> , 2017
North America	Mexico	Commercial food product and an innovative snack	Ramos-Elorduy and Montesinos, 2007; Van Huis <i>et al.</i> , 2013
Europe	Belgium	Processed food	Stoops <i>et al.</i> , 2017 Tzompa-Sosa <i>et al.</i> , 2023
Europe	Netherlands	Freeze dried	Stoops <i>et al.</i> , 2017
Europe	Germany	Noodles and salad croutons	Nutraingredients, 2018

280

#### 281 4. Bioecology of *Alphitobius diaperinus*

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

#### 287 General morphology

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

#### 297 Distribution

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

#### 302 Life cycle



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

320 The adults (beetles) of *A. diaperinus* are generally oval-shaped and have a length ranging from 5.8  
321 to 6.3 mm (Dunford and Kaufman, 2006). They have shiny brown to black exoskeletons, with the  
322 head deeply tucked into the pronotum. The pronotum, which is about twice as wide as it is long,  
323 has a textured surface with tiny pits or punctures. The elytra, which cover the abdomen, are striated  
324 and can open to enable flight. Adult beetles begin mating shortly after their exoskeletons have  
325 fully hardened, typically within 5-8 days of emerging from the pupal stage. An adult female can  
326 live for four months to a year and lays eggs periodically throughout her adult life (Sammarco *et*  
327 *al.*, 2023). On average, they lay about 3.5 eggs per day, which are usually deposited singly on or  
328 within loose substrate (Rueda and Axtell, 1996). In their lifetime, females typically lay around  
329 2,000 eggs (Dunford and Kaufman 2006).

### 330 **Habitat**

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

337



338 **Diet**

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

343 **As pest and its management**

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

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

358 **5. Nutritional value of *Alphitobius diaperinus* larvae**

359 **Proximate composition**

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

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

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

367  
 368 The protein content of lesser mealworm varies depending on several factors, including their stage  
 369 of development, method of processing, and the conditions under which they are raised (Kotsou *et*  
 370 *al.*, 2021). Lesser mealworm powder has been used as a novel baking ingredient to manufacture  
 371 high-protein, mineral-dense snacks in which the protein content was enriched to 99.3% by  
 372 substituting 30% of wheat flour with lesser mealworm powder (Roncolini *et al.*, 2020). Generally,  
 373 lesser mealworms are considered a good source of protein with a protein content ranging from  
 374 45.10% to 50.54% of their dry weight (Rumbos *et al.*, 2019). Adámková *et al.*, (2016) found that  
 375 the crude protein content of lesser mealworms was 630 g/kg dry matter, which is higher than many  
 376 other protein sources. Compared to traditional protein sources such as beef, pork, and chicken,  
 377 lesser mealworms have a more favourable protein-to-fat ratio (Miguéns-Gómez *et al.*, 2020). This  
 378 means that they provide a relatively high amount of protein per calorie, making them an excellent  
 379 option for individuals looking to increase their protein intake without consuming excessive  
 380 amounts of fat. However, it should be taken into account that the reported protein content is often  
 381 overestimated when a nitrogen-to-protein conversion factor of 6.25 and therefore, Janssen *et al.*  
 382 (2017), proposed a conversion factor for the larvae of *T. molitor*, *A. diaperinus*, and *H. illucens* of  
 383 4.76.

384 Crude fiber refers to the indigestible portion of plant-based foods that pass through the digestive  
 385 system without being absorbed. Insects are not plants, but their exoskeletons contain chitin, a  
 386 polymer of N-acetylglucosamine, which is similar in structure to cellulose and other plant fibers  
 37 (Abidin *et al.*, 2020)). The crude fiber content of lesser mealworm ranges from 5-7% of their dry  
 38 weight, making them a relatively low source of dietary fiber compared to fruits, vegetables, and



389 whole grains (Skotnicka *et al.*, 2021). However, fiber in insects is still beneficial to human health  
390 as it can help promote satiety and regulate bowel movements. Consuming adequate amounts of  
391 dietary fiber is essential for maintaining a healthy digestive system and reducing the risk of chronic  
392 diseases such as heart disease, diabetes, and cancer (Anderson *et al.*, 2009). While lesser  
393 mealworm may not be a significant source of fiber, they can still contribute to an individual's  
394 overall dietary fiber intake when consumed as part of a balanced diet.

395 Lipids, also known as fats, are essential nutrients that provide the body with energy and aid in the  
396 absorption of fat-soluble vitamins. Compared to traditional protein sources such as beef, pork, and  
397 chicken, lesser mealworms have a more favourable protein-to-fat ratio (Miguéns-Gómez *et al.*,  
398 2020), meaning they provide a relatively high amount of protein per calorie. The method of  
399 preparation can also impact the lipid content of lesser mealworm. Roasting or frying the insects  
400 can increase the fat content due to the addition of oils or fats used during cooking while boiling or  
401 steaming can reduce the fat content due to the loss of fat in the cooking water as is the case of *T.*  
402 *moltor* (Mancini *et al.*, 2021). While consuming fat is essential for optimal health, consuming  
403 excessive amounts of fat can lead to weight gain and an increased risk of chronic diseases such as  
404 heart disease and diabetes. Therefore, individuals seeking to increase their protein intake by  
405 consuming lesser mealworm should be mindful of their overall fat intake and consume them in  
406 moderation as part of a balanced diet.

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

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

427 The dry matter content of lesser mealworms is a crucial factor to consider when assessing their  
428 quality and shelf life. Dry matter content refers to the portion of a food product that remains after  
429 removing the water content, and it can influence characteristics like texture, flavor, and microbial  
430 growth (Rawat, 2015). Freshly harvested lesser mealworms generally consist of 25-35% dry  
31 matter, while dried insects may contain as much as 90% dry matter (Turck *et al.*, 2022). Excess  
32 moisture in lesser mealworms can lead to spoilage and bacterial proliferation, impacting their



433 overall quality and safety for consumption (Roncolini *et al.*, 2020). Thus, it's essential to  
434 appropriately store and handle lesser mealworms to maintain their dry matter content within a safe  
435 range. The method of preparation also affects the dry matter content of lesser mealworms (Ortolá  
436 *et al.*, 2022).

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

#### 451 **Amino acid profile**

452 The amino acid profile of the lesser mealworm from various studies is shown in Table 4. The data  
453 reveals significant variations in amino acid content among the different sources. A notable  
454 variability across the studies conducted by Rumbos *et al.* (2019), Hermans *et al.* (2021), Kurečka  
455 *et al.* (2021) reflects potential differences in methodologies, feed composition, rearing conditions,  
456 and possibly the strains of lesser mealworms used. For instance, arginine shows a substantial  
457 difference across the studies, with Kurečka *et al.* (2021) reporting a notably higher content. Some  
458 amino acids are reported in ranges, reflecting potential variability within the study or the use of  
459 different samples. Glutamate content appears to be consistently high across the studies, indicating  
460 a commonality in the nutritional profile of the lesser mealworms. Alanine, aspartic acid, glycine,  
461 proline, and serine are consistently present across all studies, indicating their stable presence in the  
462 amino acid profile of lesser mealworms. These variations in amino acid content could have  
463 implications for the nutritional assessment and utilization of lesser mealworms in various  
464 applications, such as animal feed or human consumption. In other studies, Soetemans *et al.* (2020)  
465 reports on the impact of agri-food side-stream inclusion in the diet of *A. diaperinus* on the larvae  
466 composition. They found that *A. diaperinus* larvae reared on 18 different diets, had a protein  
467 content ranging between 37% and 49%. The most dominant amino acids in the larvae (higher than  
468 32 g/kg DM) were glutamate, arginine, aspartate, alanine, leucine and tyrosine. Differences in the  
469 reported values highlight the need for standardized methods for assessing amino acid content or  
470 understanding the factors influencing these variations. Lesser mealworms are known to be a good  
471 source of essential amino acids. Studies have shown that the protein in lesser mealworms contains  
472 all the essential amino acids (Rumbos *et al.*, 2019), which cannot be produced by the human body  
473 and must be obtained through diet. These essential amino acids include histidine, isoleucine,  
474 leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Lesser mealworms  
475 are particularly rich in lysine, methionine and leucine for which most plant protein feed sources  
476 are usually deficient (Sánchez-Muros *et al.* 2014). The specific amounts of each amino acid in  
477 lesser mealworms may vary depending on factors such as the developmental stage of the insect,

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478 their diet, and how they were reared. However, in general, the lesser mealworm is considered to  
 479 be a good source of protein with a well-balanced amino acid profile. The amino acid profile of  
 480 lesser mealworm is similar to that of meat or dairy protein and comparable to soybean proteins  
 481 (Kurečka *et al.*, 2021). Studies have shown that lesser mealworm powder can be used as a novel  
 482 baking ingredient for manufacturing high-protein snacks, with enriched amino acids content  
 483 (Roncolini *et al.*, 2020).

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

Amino acid	Amount (mg/100 g protein) (Rumbos <i>et al.</i> , 2019)	Amount (mg/100 g protein) (Hermans <i>et al.</i> , 2021)	Amount (mg/100 g protein) (Kurečka <i>et al.</i> , 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

485

486 **Fatty acid profile**

487 Table 5 summarizes the fatty acid composition of lesser mealworms compared to chicken reported  
 488 in some studies. Insects fed on high-fat diets have a higher proportion of both saturated and  
 489 monounsaturated fatty acids. Gharibzahedi and Zeynep (2023) reported that the dominant fatty  
 490 acids in lesser mealworm powders were linoleic acid at 33.66%, oleic acid at 28.97%, palmitic  
 491 acid at 24.98%, stearic acid at 7.23% and  $\alpha$ -linolenic acid at 1.88%. Similar values were reported  
 492 by Roncolini *et al.* (2020). They reported that the fatty acid composition of lesser mealworm  
 493 powder was linoleic acids at 31.5%, oleic acid at 28.5%, palmitic acid at 23.5%, stearic acid at  
 494 7.5%, and  $\alpha$ -linolenic acid at 1.5%. According to Oonincx *et al.* (2020), lesser mealworms diets  
 495 enriched with flaxseed oil during their larval/nymphal stage had the  $\alpha$ -linolenic acid content  
 496 increase by 2.3%-2.7% for each percent of flaxseed oil added and a four percent addition led to an  
 497 increase in the n-3 fatty acid content by 10-20-fold.

38



499 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 <i>et al.</i> , 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
$\alpha$ -linoleic	1.88	1.5

500  
 501 Palmitic acid is a saturated fatty acid that is an important component of cell membranes and is  
 502 involved in many metabolic pathways in the body (Calder, 2015). Stearic acid, unlike palmitic  
 503 acid, has been shown to neutralize blood cholesterol levels and may even have a protective effect  
 504 against heart disease (Calder, 2015). Oleic acid is a monounsaturated fatty acid that is important  
 505 for maintaining healthy cholesterol levels and has been linked to a reduced risk of heart disease  
 506 (Calder, 2015). Linoleic acid is an essential polyunsaturated fatty acid that must be obtained from  
 507 the diet. It is found in high amounts in vegetable oils, nuts, and seeds, and is important for  
 508 maintaining healthy skin and hair and for many other physiological functions (Calder, 2015).  
 509 Linolenic acid is another essential polyunsaturated fatty acid that must be obtained from the diet.  
 510 It is found in high amounts in flaxseed oil, chia seeds, and other plant sources, and is important for  
 511 maintaining healthy brain function, as well as for many other physiological functions (Calder,  
 512 2015). The ability of the human body to absorb fatty acids depends on how well they are broken  
 513 down during digestion and their arrangement in fat molecules (Gharibzahedi and Mohammadnabi,  
 514 2016).

515 **Anti-Nutrient Composition**

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

518 *Chitin*

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





526 *Protease Inhibitors*

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

531 **Reducing Anti-Nutrient Compounds**

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

541

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

543 Edible insects have the potential to serve as healthy, sustainable alternatives to traditional animal-  
544 based foods because of their nutrient contents (Leni et al., 2020; Nowakowski et al., 2022). Insects  
545 are rich in high-quality protein, essential amino acids, fiber, iron, zinc, vitamin B12, and omega-3  
546 fatty acids (Leni et al., 2020; Nowakowski et al., 2022). Insects generally have been found to  
547 contain bioactive compounds such as antioxidants, anti-inflammatory agents, and antimicrobial  
548 agents (Nino et al., 2021). These compounds have been shown to have potential health benefits  
549 such as reducing the risk of chronic diseases like cancer and cardiovascular disease (Nino et al.,  
550 2021). Studies have also found bioactive compounds in insects with characteristics that could  
551 potentially reduce inflammation and improve gut health (Chantawannakul, 2020). Edible insects  
552 or their components also possess antihypertensive properties (Aguilar-Toalá et al., 2022).

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

560 On the antioxidant properties of lesser mealworm, it has been studied for its potential health  
561 benefits. A study investigated the bioactivities of lesser mealworm hydrolysates and found that  
562 they contained bioactive peptides with antioxidant activity (Tejada et al., 2022). According to a  
563 study, extracted oils from lesser mealworm have antioxidant properties (Gharibzahedi and Zeynep,  
564 2023). The study found that the oils contained vitamin D, campesterol,  $\beta$ -sitosterol,  
565 phosphatidylinositol and phosphatic acid, linoleic acid, and hypocholesterolemic or  
566 hypercholesterolemic ratio. Sousa et al. (2020) also found that hydrolysates obtained from *A.*  
567 *diaperinus* possessed antioxidant properties. Antioxidant activity values of  $95.0 \pm 0.8$  and  $95.7 \pm$



568 1.0  $\mu\text{mol}$  Trolox equivalent per g insect have been recorded, however, no antimicrobial or  
569 antidiabetic properties were observed (Sousa *et al.*, 2020).

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

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

## 588 7. Harvesting and rearing of *Alphitobius diaperinus*

### 589 The environmental condition in the rearing of lesser mealworm

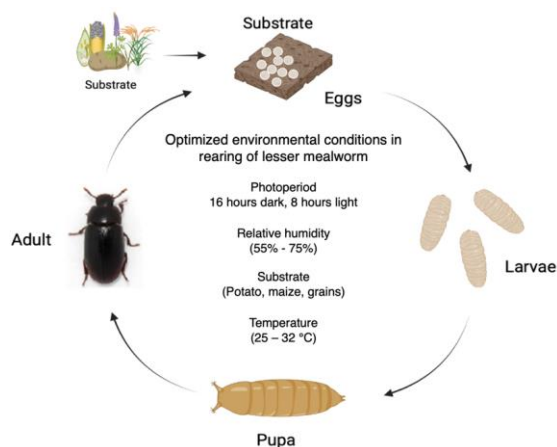
590 One of the important steps that can significantly contribute to the production of edible insects with  
591 high nutritional values is the methods or conditions employed during the rearing activities (Ortiz  
592 *et al.*, 2016). There are several reported methods in the literature that describe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

597 As dietary factors have a significant impact on their growth rates, it is crucial to ensure a proper  
598 nutrition is provided. The larvae of the lesser mealworm can grow on different diets composed of  
599 side-stream materials. Examples of reported diets used in development of lesser mealworm are  
600 Brewers' spent grains, beer yeast, cookies, potato peels, and corn distillers' grain with soluble (van  
601 BroekhovenKotson *et al.*, 201524). Furthermore, diets that contain protein derived from yeast are  
602 deemed more advantageous in terms of larval growth and growth (Van Broekhoven *et al.*, 2016).  
603 On the other hand, using agricultural by-products and side-streams as substrates for insect feeding  
604 could be a sustainable approach to insect rearing. For instance, Rumbos *et al.* (2021) evaluated the  
605 feasibility of rearing the lesser mealworm by utilizing ten by-products obtained from cereal and  
606 legume seed. Among the by-products, lupin and triticale demonstrated the ability to support larval  
607 development from the first instar to pupation. These by-products also exhibited the most  
608 favourable results concerning the growth survival, feed utilization and development time (Rumbos  
39 *et al.*, 2021).

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610 In addition to diet, it is important to consider other factors that can significantly contribute to  
 611 rearing of lesser mealworms. For instance, factors such as temperature and relative humidity levels  
 612 during the rearing process should be emphasized and studied in detail to establish an effective  
 613 mass-rearing system. Kotsou *et al.* (2021) emphasized both factors; biotic and abiotic on rearing  
 614 of the larvae of lesser mealworm and provided multiple variables that could be utilized for mass  
 615 production of this species. They investigated the growth of lesser mealworm by analysing the  
 616 effect of three distinct temperature levels (25, 30, and 32 °C) and two varying degrees of relative  
 617 humidity (55 and 75%). According to the study, temperature played a crucial part in the  
 618 development of the larvae, with the growth and development observed better at higher  
 619 temperatures (30 and 32 °C). However, it was found that the larvae growth was not significantly  
 620 influenced by varying the levels of relative humidity. These findings suggest that *A. diaperinus*  
 621 can be reared within the appropriate temperature and relative humidity range. However, it is crucial  
 622 to note that temperature has a more impact on the development and survival rates of the larvae  
 623 compared to relative humidity (Kotsou *et al.*, 2021). Similarly, previous studies by Renault *et al.*  
 624 (2015) also showed that relative humidity did not significantly affect the survival of *A. diaperinus*,  
 625 as both condition (desiccation, RH 7% and cold, 5 °C) showed similar survival duration (Renault  
 626 *et al.*, 2015). Similar observations were obtained when exposing the lesser mealworm to 10%  
 627 relative humidity. The species showed high resistance to desiccation, with about 50% of  
 628 individuals surviving for 30 days under such conditions. Moreover, some individuals were able to  
 629 survive up to 50 days at 10% ± 2 relative humidity (Engell Dahl and Renault, 2022). Figure 1  
 630 shows the life cycle of the lesser mealworm and the reported optimum conditions used in rearing  
 631 the edible lesser mealworm.



632  
 633 Figure 1. Optimized conditions in rearing of *Alphitobius diaperinus* (created in BioRender.com;  
 634 the image of *A. diaperinus* in adult form was reproduced from Tejada *et al.*, (2022)

635 Kim *et al.* (2017) explored how different temperatures (20 to 35 °C) could affect the development  
 36 of *A. diaperinus*. During the study, the larvae were exposed to these different temperatures while  
 37 maintaining a constant relative humidity of 60%. The results of the study revealed that the time

638 required for larval growth to adult stage was 129.0, 49.8, 40.5, and 31.9 days at temperatures of  
639 20, 25, 30, and 35 °C, respectively. Additionally, the pupal rate was at about 80, 100, 83, and 92%  
640 at temperatures of 20, 25, 30, and 35 °C, respectively. These findings suggested that a shorter  
641 developmental period was observed at higher temperatures (35 °C), but a slightly lower  
642 temperature (25 °C) resulted in a higher pupal rate (100%) (Kim *et al.*, 2017). This finding is in  
643 agreement with the work reported by Kotsou *et al.* (2021), which states that temperature  
644 particularly affects the larvae development. An increase in the rearing temperature to 25 °C  
645 resulted in a significant reduction in pupal development time, which could correspond to a decrease  
646 in infections and rearing costs. Meanwhile, a recent study has shown that temperature levels can  
647 significantly affect the cumulative number of eggs per adult and the cumulative larval hatching  
648 rate of *A. diaperinus*. Optimal reproductive output is achieved when reared within a temperature  
649 range of 25 to 30 °C for 42 days. The study demonstrated that each adult *A. diaperinus* yielded an  
650 average of 73 eggs, with estimated hatchability rates of 69% and 58% at 25 °C and 30 °C,  
651 respectively (Ormanoğlu *et al.*, 2023)

652 In contrast, studies by Bjørge *et al.* (2018) shows that the growth rate of lesser mealworm was high  
653 at 31 °C with 18.3% wet mass growth/day. Furthermore, increasing the temperature to 37 and 39  
654 °C led to the decrease in the growth rate per day. What is interesting is that they found a negative  
655 growth rate per day when the temperature was set to 15 °C. This study suggests that the rate of  
656 growth is highly dependent on temperature. On the other hand, there was a significant variation in  
657 the lipid content (24 to 34% of dry weight) across various temperatures. It can be said that the  
658 variation was good, however, the lipid contents were found to be close to 35% at middle  
659 temperature. In terms of protein content, the lesser mealworm was found to have a high protein  
660 content (60%) at lower temperatures, but a slight increase in temperature resulted in a reduction in  
661 protein content. The lowest protein content was observed at temperatures of 23 and 39 °C, with  
662 values of 48.4 and 48.9%, respectively. It would be interesting to conduct a detailed investigation  
663 on the composition of fatty acid and amino acid, as not all are nutritionally equal, and the works  
664 suggest that the composition of fatty acids, at the very least, is affected by temperature. Moreover,  
665 exploring the influence towards varying the content of lipid and protein in the feed according to  
666 the obtained values here would significantly contribute to the goal of enhancing the quality of the  
667 larvae (Bjørge *et al.*, 2018).

668 In rearing of edible insects, light has been considered as one of the significant factors that can  
669 influence the development of insects and by understanding on how abiotic parameters can affect  
670 insect-rearing is important in order to maximize the potential of lesser mealworms for future meat  
671 consumption (Suppo *et al.*, 2020). One of the abiotic parameters that can significantly influence  
672 the development of insect is photoperiod (Ribeiro *et al.*, 2018). For example, a short photoperiod  
673 can lead to a more significant accumulation of unsaturated fatty acids in this species, which could  
674 enhance the survivability in cold conditions. In fact, the effects of photoperiod on the content of  
675 nutrient are probably indirect, as they act through other processes such as preparation of diapause  
676 (Oonincx and Finke, 2021). In the case of mealworms, they exhibit negative phototropism and  
677 phototaxis (Balfour and Carmichael, 1928), where adults and larger larvae position themselves  
678 below the substrate's surface during daylight and emerge to the surface in darkness (Tyshchenko  
679 and Ba, 1986). For *T. molitor*, recent studies have shown that the highest growth rates, survival  
680 rates, and shortest developmental times were achieved under constant darkness (Eberle *et al.*,  
681 2022). On the contrary, Zim *et al.* (2022) investigated the impact of photoperiod on the rearing of  
682 *T. molitor*, employing photoperiod parameters of 8 hours of light and 16 hours of darkness



683 (8L:16D) and complete darkness (0L:24D). Nevertheless, they concluded that the photoperiod had  
684 no significant effect on pupal development and adult survival. The study revealed that the optimal  
685 photoperiod for larval development was 8L:16D. Therefore, it can be said that while the  
686 photoperiod may influence the development of mealworms, the response to photoperiod tends to  
687 diminish under constant conditions, especially when *T. molitor* becomes arrhythmic (Cloudsley-  
688 Thompson, 1953). To date, there is still a lack of reports in the literature focused on studying the  
689 effects of photoperiod on the growth and development of *A. diaperinus*, or the lesser mealworm.  
690 Several works in the literature have reported the use of a photoperiod of 16 hours of light and 8  
691 hours of darkness (16L:8D) in the development of *A. diaperinus*. Table 6 summarizes the optimal  
692 environmental conditions for the rearing of *A. diaperinus* based on results of various research.

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

Rearing Phase	Environmental Factors	Optimized Conditions	References
Larvae	Temperature	25°C	Rumbos <i>et al.</i> , 2021
	Light	ND	
	Relative Humidity	55%	
	Diet	Wheat bran and yeast (9 : 1)	
	Cage	ND	
Larvae	Temperature	30 and 32°C	Kotsou <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> (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)	

694 ND: Not Determined

695 According to literature (Dossey *et al.*, 2016; Thévenot *et al.*, 2018), insects possess a notable  
696 advantage to grow in crowded areas, which facilitates large-scale production even in closed spaces.37 Typically, small trays manufactured from materials such as wood, polyethylene thermoplastic, or  
38 fiber-reinforced plastic are used to house both larvae and pupae along with a feeding substrate.

699 One study reported the use of a standard tray measuring  $65 \times 50 \times 15 \text{ cm}^3$  for fattening the yellow  
700 mealworm (*T. molitor*) as this size was easy to manage and could prevent adult larvae from  
701 escaping (Dossey *et al.*, 2016). Based on the method according to the design from an EU pilot mill  
702 with the aim of producing 17 tons of fresh *T. molitor* larvae annually, the insects can be  
703 successfully reared at a density of 5 larvae  $\text{cm}^{-2}$  (Thévenot *et al.*, 2018). Although *T. molitor* and  
704 *A. diaperinus* come from different species, they belong to a similar family. Hence, they may share  
705 a similar bioecology. Therefore, the rearing system and conditions applied for *T. molitor* may be  
706 suitable for rearing *A. diaperinus*. This was also demonstrated by Rumbos *et al.* (2021), who reared  
707 both *T. molitor* and *A. diaperinus* using the same rearing system and facility. Figure 2 displays a  
708 design for rearing boxes arranged in multilevel shelves, which can minimize the space required  
709 for the production of mealworm. In certain cases, stackable boxes are recommended to be used.  
710 By utilizing multilevel shelves that cover the entire surface area of the rearing space, it may be  
711 feasible to produce several thousand tons of larvae per year (Dossey *et al.*, 2016). Within the  
712 breeding area, cages are used to house adults and are equipped with food and water sources and  
713 the boxes used for rearing larvae and breeding adults are often similar in design. Dividers can be  
714 also utilized to optimize the space (Dossey *et al.*, 2016). Moreover, it is important to limit  
715 oviposition sites to specific locations within the breeding cages to facilitate the collection of eggs.  
716 According to the rearing method adopted by Deruytter *et al.* (2021), the yellow mealworms were  
717 reared at the following conditions; relative humidity ( $60 \pm 3\%$ ) and temperature ( $27 \pm 1^\circ\text{C}$ ), using  
718 plastic crates (60 x 40 cm; inner surface up to 2000  $\text{cm}^2$ ). This method is expected to be suitable  
719 for rearing the lesser mealworm since both yellow mealworms and lesser mealworms belong to a  
720 similar family. For semi-industrial production of up to 45,000 larvae per tray, lesser mealworm  
721 larvae could be reared in open, stacked plastic trays with the size of 40 x 60 x 12 cm. The trays  
722 were kept at a temperature of  $28^\circ\text{C}$  to  $32^\circ\text{C}$  with a humidity of 60% and above (Gianotten *et al.*,  
723 2020). Another reported study used a container with 19 cm  $\times$  11.5 cm; 950 cc for rearing all the  
724 three different stages (larvae, pre-pupae and beetles) of *A. diaperinus* (Meijer *et al.*, 2022).

725



726

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

729



730 **Challenges associated with entomopathogens**

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

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

748 There are various types of entomopathogens that may infect *A. diaperinus* and these  
749 entomopathogens must be thoroughly identified and characterized to prevent and control the  
750 transmission of the diseases. Entomopathogens such as fungi, bacteria, nematodes and viruses have  
751 been reported in the literature to be affecting the rearing of *A. diaperinus*. For example, species of  
752 entomopathogenic nematodes such as *Heterorhabditis bacteriophora*, *Heterorhabditis megidis*,  
753 *Steinernema affine* and *Steinernema carpocapsae* have been reported to cause high mortality of  
754 larvae of *A. diaperinus* (Kucharska *et al.*, 2018). Meanwhile, *Beauveria bassiana*, a fungus that  
755 grows naturally in soils, has been pathogenic to the species of *A. diaperinus* (Kucharska *et al.*,  
756 2018). These kinds of infections could cause major consequences in the mass-rearing facilities,  
757 ranging from asymptomatic infection to the collapse of the entire colony. As there is no cure for  
758 viral infections in edible insects, preventative measures are the only available options to contain  
759 the infection. This clearly shows the importance of biosecurity in maintaining disease-free in the  
760 production facilities (Bertola and Mutinelli, 2021). Moreover, a proper hygienic practice is highly  
761 required to ensure zero infection of entomopathogens.

762 **Challenges associated with microbes contamination**

763 According to the IPIFF, approximately 6,000 tones of insect proteins were produced in European  
764 countries in 2019. Additionally, it is estimated that up to 3 million tons of edible insects will be  
765 produced in 2030 (Niyonsaba *et al.*, 2021). The rapid development of edible insect industry has  
766 prompted regulatory bodies such as the European Food Safety Agency (EFSA) to initiate  
767 microbiological risk assessments and research on food safety (Niyonsaba *et al.*, 2021). As clear  
768 from the literature, many reared insects may have been contaminated by various kinds of  
769 microorganisms (Smith *et al.*, 2022; Vandeweyer *et al.*, 2021). For example, in the case of *A.*  
770 *diaperinus*, they have been reported to harbour a wide variety of viral, bacterial, and parasitic  
771 pathogens as well as poultry-specific and zoonotic viral that can also be transmitted to humans  
772 (Smith *et al.*, 2022). While it is unlikely that insects to be consumed in their raw form, it is still  
773 important to identify the microorganisms present in them as it may require specific processing





774 treatments to ensure food safety before being declared safe for human consumption (Vandeweyer  
 775 *et al.*, 2021). Another challenge that needs to be addressed is the development of antimicrobial  
 776 resistance, which could result in various other health problems (Gwenzi *et al.*, 2021). It can be said  
 777 that the risk of transmitting zoonotic infections to humans and livestock from insects may be lower  
 778 compared to birds and mammals (Lange and Nakamura, 2021). However, considering the  
 779 examples of zoonotic infections such as the coronavirus disease which have caused pandemics  
 780 with significant economic and political impacts (Hatta *et al.*, 2023), it is crucial to take preventative  
 781 measures during the production of edible insects.

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

794 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 <i>et al.</i> , 2017
Fungal isolates ( <i>Aspergillus</i> and <i>Fusarium</i> )	Larvae	Proti-Farm	Netherland	4.0 log cfu/g	Wynants <i>et al.</i> , 2018
<i>Bacillus</i> and <i>Paenibacillus</i> genera.	Larvae	Market	Netherland	< 1 and 4.49 log cfu/g (Lactic acid bacteria) 2.24 - 2.35 log cfu/g. (Yeast)	Roncolini <i>et al.</i> , 2020

795 ND: Not Determined

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

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

814 In a recent study, Roncolini *et al.* (2020) examined the microbial growth in lesser mealworm  
815 during the preparation of fortified foods, which utilized lesser mealworm as a novel baking  
816 ingredient. The study involved the preparation of snacks such as sourdough, dough, breads, and  
817 rusks, where a powder containing a mixture of lesser mealworm and 10% - 30% of wheat flour  
818 was used as an ingredient. The microbiological analysis showed that spore-forming bacteria,  
819 specifically *Bacillus* and *Clostridium*, were detected, with the highest microbial count recorded at  
820  $1.45 \pm 0.17$  log cfu/g (Roncolini *et al.*, 2020). Although the microbial counts suggest that the lesser  
821 mealworm may not be a suitable substrate for the growth of these microorganisms, it is still  
822 important to monitor the presence of spore-forming bacteria in the species.

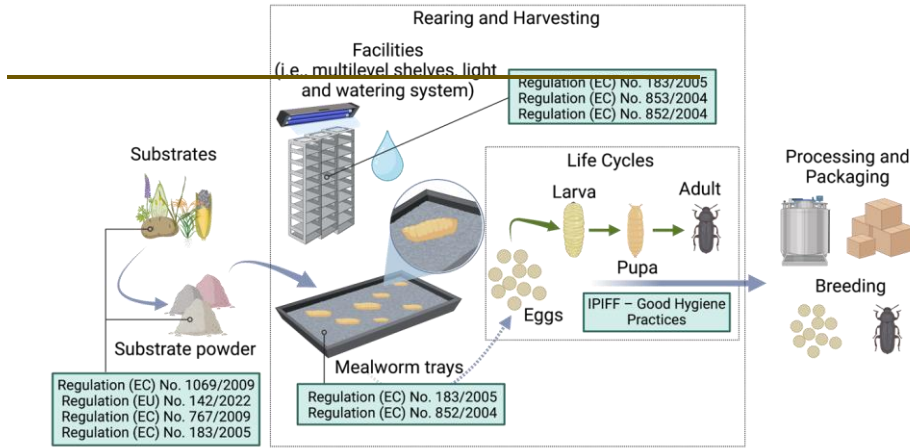
### 823 **Quality and safety assurance in rearing edible insects**

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



Quality and Safety Assurance in Rearing and Harvesting of Edible Insects

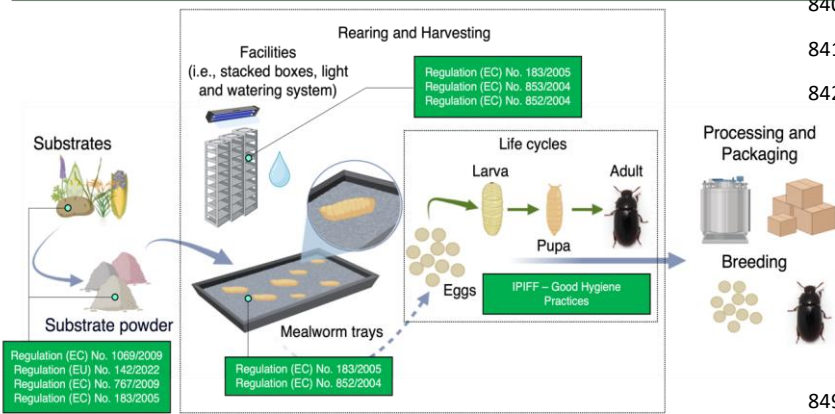
Regulation (EU) 2283/2015; Regulation (EU) 2468/2017 & 2469/2017; IPIFF – Guide on Good Hygiene Practices; FAO/IAEA - Spreadsheet for Designing and Operating Insect Mass-rearing Facilities; Regulation (EU) 2023/58



836  
837

Quality and Safety Assurance in Rearing and Harvesting of Edible Insects

Regulation (EU) 2283/2015; Regulation (EU) 2468/2017 & 2469/2017; IPIFF – Guide on Good Hygiene Practices; FAO/IAEA - Spreadsheet for Designing and Operating Insect Mass-rearing Facilities; Regulation (EU) 2023/58



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849

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



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

867 In general, consumers are concerned about the safety and how it may affect their consumption,  
868 specifically in terms of potential infectious agents such as viruses and bacteria, as well as  
869 bioaccumulation of toxic chemicals that could be harmful to humans. For example, Meijer *et al.*  
870 (2022) investigated the potential bioaccumulation and impact on growth or survival of *A.*  
871 *diaperinus* after exposure to a range of selected insecticides including chlorpyrifos ethyl, spinosad,  
872 tebufenozide, and piperonyl butoxide. The levels of the selected insecticides were spiked within  
873 the legally permissible limits in the EU. It was found that the bioaccumulation did not occur in the  
874 larvae as the concentrations of the insecticides were below the quantification limit. However, the  
875 use of Spinosad has shown the significant reduction in the total yield. Spinosad is allowed to be  
876 utilized in agriculture, however, its usage raises concerns about the safety of reared insects.  
877 Therefore, it is recommended that further studies be conducted on the safety and quality of the  
878 reared insects with respect to the amount of accumulated insecticides. According to the report by  
879 EFSA (2017), the potential hazards to human and animal health were found to be dependent on  
880 the techniques used in the rearing and processing of insects. In the majority of European countries,  
881 particularly in the area where insects are reared, they are typically farmed in controlled  
882 environments that fulfilled the proper sanitation procedures. This helps to mitigate certain hazards,  
883 such as microbiological contamination (Rumpold and Schlüter, 2013b). However, the safety (and  
884 quality) of edible insects can vary depending on the environment in which they are reared and  
885 harvested (Raheem *et al.*, 2019). As a result, frameworks governing insects-based food have been  
886 developed in the past 20 years (Lange and Nakamura, 2021). Furthermore, the type of insect  
887 species for farming must adhere to legal regulations pertaining to both food and consumer safety.  
888 For example, the insect-based food must comply with legal regulations aimed at preventing and  
889 eradicating bovine spongiform encephalopathy (BSE). These ordinances also prohibited the use of  
890 insects for the feeding of other farmed animals (Żuk-Gołaszewska *et al.*, 2022).

891 To ensure the safety of animal feeds during the rearing process especially within the EU region,  
892 the producers or operators may refer to several issued regulations according to European  
893 Commission (EC) for compliance with the safety and quality objective as shown in Table 8. The  
894 production of insects should be managed efficiently to achieve optimal yields and profits while  
895 meeting the requirements of food safety (Żuk-Gołaszewska *et al.*, 2022). In addition, the



896 production of novel foods requires the adoption of efficient management systems, which include  
897 good breeding and hygiene practices, and the Hazard Analysis and Critical Control Points  
898 (HACCP) system (Awuchi, 2023). Moreover, the regulations impose hygiene and biosecurity  
899 standards that must be fulfilled by all farm buildings and production facilities.

900 Table 8. Regulations issued by the European Commission to ensure the safety of animal during  
901 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

902

903 In general, insect breeding facilities should be designed to prevent cross-contamination from other  
904 farming sites. Meanwhile, regular monitoring must be performed by the producers or operators of  
905 any rearing facilities in order to identify and address issues such as dust contamination and leaks  
906 at any facility. Furthermore, producers must ensure that pest management and eradication systems  
907 are implemented to ensure safety and quality during food and feed production. Insect farms should  
908 be equipped with safety systems to protect against external sources of pests and prevent insects  
909 from escaping from facilities. Production facilities must adhere to relevant standards, including  
910 the elimination of food residues, removal of unnecessary equipment and materials, and provision  
911 of organic waste containers to maintain good facility conditions (Ortiz *et al.*, 2016). These  
912 measures are in accordance with the draft of Regulation (Article 5) and Regulation (EC) No  
913 1069/2009 (European Union, 2009a), which state that the substrate used for feeding insects should  
914 not include manure, catering waste, or other types of waste. However, as of October 2020, the draft  
915 Regulation has not been put into effect. The next section will discuss the quality and safety  
916 measures related to the rearing and harvesting of edible insects, as outlined by the standards issued  
917 by the IPIFF.

#### 918 *Rearing facilities*

919 It is recommended that the location for rearing insects to be equipped with basic services  
920 (electricity, watering system, and waste management) (Regulation (EC) No. 853/2004) (European  
921 Union, 2004a). In addition, the building should be located at a distance from neighbouring facilities  
922 that could potentially lead to contamination, such as areas with chemicals, rivers, or flood-prone  
923 regions. Additionally, it should be located away from areas with high levels of airborne



924 microorganisms and exposure to loud noise, both of which could have a negative impact during  
925 the rearing process (Żuk-Gołaszewska *et al.*, 2022) . Furthermore, the operators must ensure that  
926 there are proper measures in cleaning and disinfecting to reduce the hazard risk and prevent  
927 contamination, and other potential adverse impact to preserve the quality of the reared insects  
928 (Annex II and relevant articles of Regulation (EC) No 183/2005) (European Union, 2005). As per  
929 the same regulation, it is required that the facilities have sufficient natural or artificial lighting.  
930 Apart from lighting, the design and construction of ceilings and overhead fixtures should also  
931 prevent the accumulation of dirt and the formation of moulds, which could potentially impact the  
932 safety and quality of the reared insects. To ensure a clean air and avoid mechanical airflow from  
933 contaminated area, ventilation system is required to be installed in the facilities, in accordance with  
934 Annex II, Chapter I of Regulation (EC) No 852/2004 (European Union, 2004b).

#### 935 *Watering systems*

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

#### 944 *Sanitary of facilities*

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

#### 955 *Feeding process*

956 When selecting substrates for the rearing process, it is recommended that producers consider  
957 criteria such as nutritional composition, potential hazards to the insects, and the ease of removal.  
958 Moreover, the impact on the intended insect species, such as growth, weight, and feed conversion  
959 ratio, must be taken into account. It is essential to note that the properties of substrates are  
960 important parameters for the development and ensuring safe growth conditions for the insects.  
961 Typically, the lesser mealworm is reared on dry substrates (xiroculture). In the EU, insect  
962 producers are required to obtain feed materials for farmed animals that are approved as substrates  
963 in accordance with the regulations outlined in Regulation (EC) No 1069/2009 (European Union,  
964 2009a) and Regulation (EU) No 142/2011 (European Union, 2011). Additionally, the use of  
55 prohibited materials listed in Regulation (EC) No 767/2009 - Annex III (European Union, 2009b)  
56 is not permitted. In addition, the use of any substrate mixed with insect frass in subsequent





967 production cycles is prohibited under Article 9(g) of Regulation (EC) No 1069/2009 (European  
968 Union, 2009a) since insect frass is classified as 'Category 2' material. On the other hand, if  
969 substrates are obtained from outside the production facility, producers must obtain the substrates  
970 from registered suppliers or approved feed business operators, as per the regulations outlined in  
971 Regulation (EC) No 183/2005 (Article 5(6)) (European Union, 2005). Furthermore, the producers  
972 are encouraged to search for alternative nutritious substrates which are low-cost and sustainable.

#### 973 *Harvesting activities*

974 The harvesting process involves collecting the larvae upon the completion of the rearing process.  
975 Generally, insects are extracted from their chambers and removed from the substrate. Insects with  
976 a holometabolous life cycle, such as mealworms, are harvested at the full-grown larvae stage. It is  
977 important to note that the harvesting techniques may differ among insect species, depending on  
978 their breeding behaviour. For instance, mealworm larvae usually remain in the growing substrate  
979 before the separation step (sieving), whereas black soldier fly larvae tend to naturally migrate from  
980 moist to dry environments. This step allows for manual or mechanical separation (Rumbos *et al.*,  
981 2021).

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

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

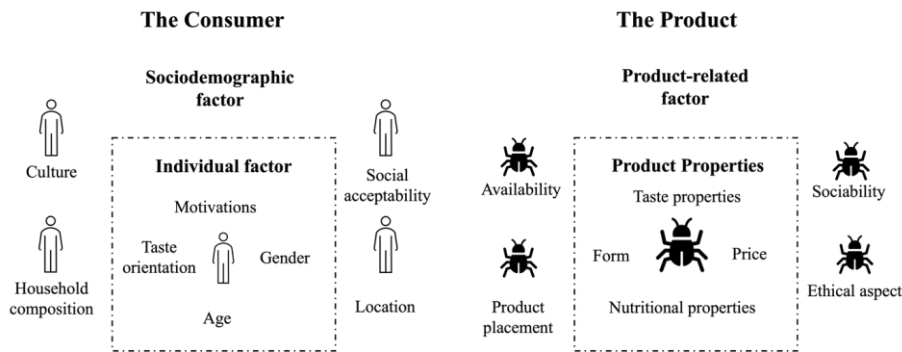
993 It is noted that the current techniques and methods employed in the production process, from the  
994 initial stages to the final product placement, are still inadequate and somewhat fragmented to fully  
995 facilitate the commercialization of insect-based food products (Kauppi *et al.*, 2019). Considering  
996 the matter from a global context, roughly two billion people currently incorporate insects into their  
997 daily diets (Van Huis *et al.*, 2022). To date, the literature indicates that there are approximately  
998 2,111 species of insects that have been documented as edible (Guachamin-Rosero *et al.*, 2023).  
999 Although most of the reported edible insects are safe to be consumed, eating insects is not a  
1000 common practice, especially among most Westerners. Hence, the consumer acceptance of insect-  
1001 eating in this section will be thoroughly discussed for most of western countries especially  
1002 countries within European Union.

1003 Previous research focusing on consumer acceptance of edible insects has produced a variety of  
1004 results, with their acceptability depending on various factors (Kauppi *et al.*, 2019; Kröger *et al.*,  
1005 2022). There are several reports discuss the factors, justifications, and strategies that can be  
1006 employed to encourage the wider adoption of insect-based foods by Western consumers (Ardoin  
1007 and Prinyawiwatkul, 2021; Kauppi *et al.*, 2019; Onwezen *et al.*, 2021). Gaining insight into the  
1008 elements that drive consumer acceptance or rejection of insect-based food can enhance the  
1009 efficiency of future research and development, particularly in enhancing our understanding of the





1010 commercialization of potential edible insects. However, there is insufficient data regarding  
 1011 consumer needs, behaviours, experiences, and preferences to effectively engage them with insect-  
 1012 based products. In addition, there is a limitation in understanding consumer acceptability towards  
 1013 edible insects due to fragmented scientific literature. For instance, there are cases in which current  
 1014 research findings contradict with the findings from the past (House, 2016). The following section  
 1015 discusses consumer acceptance of edible insects, specifically the lesser mealworm, from a broader  
 1016 perspective based on the adapted framework illustrated in Figure 4. The adapted framework for  
 1017 consumer research findings discussed is based on a conceptual framework developed by Kauppi  
 1018 *et al.* (2019) and divided into two main categories: findings about the consumer (1) and the product  
 1019 (2). For the consumer, the consumer acceptance will be discussed according to the  
 1020 sociodemographic factor (culture, household composition, location and social acceptability) and  
 1021 individual factor (motivations, taste orientation, gender, and age). Meanwhile, for the product, the  
 1022 consumer acceptance will be discussed according to product-related factor (availability, product  
 1023 placement, sociability and ethical aspect) and product properties (taste properties, form, price, and  
 1024 nutritional properties). The summary of the consumer acceptance based on both of “The  
 1025 Consumer” and “The Product” elements are presented in Table 9.



1026  
 1027 Figure 4. Factors that influence consumer acceptance of insect-based products (the framework is  
 1028 adapted from Kauppi *et al.*, 2019)

1029

1030 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.



1032 **The consumer**

1033 *Sociodemographic circumstances*

1034 Insects are commonly viewed in a negative way, and they are frequently associated with terms like  
1035 'dirty,' 'unhealthy,' and 'disease vectors' (Kröger *et al.*, 2022). Although edible insects have been  
1036 declared safe and various campaign as well as promotion have been carried out to improve their  
1037 public perception, studies show that many Westerners are still reluctant to include insects in their  
1038 diet due to negative perceptions (Ardoin and Prinyawiwatkul, 2021; Kauppi *et al.*, 2019; Kröger  
1039 *et al.*, 2022; Onwezen *et al.*, 2021). While surveys have shown that improving consumer  
1040 knowledge and education about insects as a food source can increase willingness to try them, food  
1041 choice motivation (FCM) can be a complex process as it involves various predictors, such as  
1042 cognitive, cultural, demographic, geographical, social norm, and situational factors (Dagevos,  
1043 2021; Kröger *et al.*, 2022; Lammers *et al.*, 2019).

1044 When it comes to culture, insects have long been associated with the feelings of disgust among  
1045 Westerners (Looy *et al.*, 2014). Numerous reports have consistently demonstrated that disgust has  
1046 a negative effect on the acceptance of insect-based food (Kröger *et al.*, 2022). However, in contract  
1047 to this report, several reports showed that consumer interest in insect consumption has been  
1048 improving recently. For example, a study by House (2016) in Netherland demonstrated that  
1049 nobody is refusing the insect consumption due to disgust factor but more to the factors of price,  
1050 taste, and availability. The survey focused on the convenience foods made from insects produced  
1051 by the Belgian company, Damhert Nutrition. The products, such as burgers and nuggets were made  
1052 from vegetables and contained 13% to 15% of the larvae of the lesser mealworm. On the other  
1053 hand, a focus group study conducted in the Western part of Denmark revealed that Western  
1054 cultures could be open to insect consumption if certain criteria are fulfilled. The study presented  
1055 prospective consumers with various insect-containing products made from flour containing edible  
1056 insects such as the larvae of lesser mealworm and yellow mealworm. The survey found that  
1057 consumers were interested in having more insect-based recipes, nutritional information, and lower  
1058 prices. Moreover, they expressed a preference for insects that can be used as main ingredients in  
1059 meals rather than just snacks and suggested that insects should be incorporated into familiar  
1060 products such as meat or bread. Finally, in order to promote regular consumption of insects, it is  
1061 recommended that prices to be set at an affordable level for consumers (Bryning *et al.*, 2020).

1062 In another study, Ortolá *et al.* (2022) developed biscuits using flours from *T. molitor* and *A.*  
1063 *diaperinus* and assessed their physical and sensory properties. A panel of 30 testers from Spain,  
1064 aged 18 to 65 years, participated in the survey. The physicochemical analysis indicated that the  
1065 biscuits had high protein content, in compliance with Regulation (EC) No. 1924/2006 (European  
1066 Union, 2006). However, many of the panelists found the biscuits too dark and not crunchy enough.  
1067 One of the suggestions by the panelists was to increase the sweetness as it could potentially  
1068 enhance its appeal. Based on this study, it can be suggested that understanding the nutritional value  
1069 of edible insects, previous exposure to insect-based foods, and a desire for new sensations could  
1070 increase the consumer acceptance among Westerners (Ortolá *et al.*, 2022).

1071 Meanwhile, Austria, Belgium, the Netherlands, and France have experienced higher levels of  
1072 consumer acceptance of edible insects due to their wider incorporation into the food industry as a  
1073 novel food (Yi *et al.*, 2013). According to Lammers *et al.* (2019), 15.9% of German participants  
74 were willing to consume unprocessed insects, while Verbeke (2015) found that 16.3% of Belgian  
75 participants were open to incorporating insects as a food source into their diets (Verbeke, 2015).



1076 Kostecka *et al.* (2017) also reported that approximately 37% of participants in Poland found  
1077 products with processed insects, such as insect flour, to be acceptable for introduction in the market  
1078 (Kostecka *et al.*, 2017). Based on the study by Mazurek *et al.* (2022), consumer acceptability can  
1079 be significantly influenced by the form of the insects, specifically their flavour, and the way in  
1080 which they were presented. The studies used lesser mealworm powder as a flavour in preparing  
1081 wheat pancakes. The acceptability of insects among consumers was evaluated among people in  
1082 Poland. Based on the conducted survey, as the proportion of insect composition increased, scores  
1083 for all parameters decreased. Despite positive responses towards the idea of entomophagy in  
1084 general, these studies indicated that people still showed hesitation in incorporating insects into  
1085 their diets. Hence, it can be said that not all consumers in European countries are ready for insect-  
1086 based food, and additional factors need to be considered before commercialization. Factors such  
1087 as food disgust, neophobia, and seeking sensation have been identified as predictors of the  
1088 acceptance of edible insects, and these factors will be discussed further in the product section  
1089 (Mazurek *et al.*, 2022).

1090 Social and cultural norms are also the important factors when it comes to accepting insects as novel  
1091 foods (Kröger *et al.*, 2022; Tzompa-Sosa *et al.*, 2023). As demonstrated by Ros-Baró *et al.* (2022),  
1092 the perception of insect eating as a primitive practice was not a significant barrier to the  
1093 consumption of insects (Ros-Baró *et al.*, 2022). Insect preparations are often viewed as delicacies  
1094 in Western countries, but they are typically seen as basic or emergency food sources elsewhere  
1095 (Tzompa-Sosa *et al.*, 2023). In addition, there are concerns regarding the potential existence of  
1096 pathogenic microorganisms and heavy metals, as well as the possibility of allergic reactions from  
1097 consuming insects (Vandeweyer *et al.*, 2021). In this case, the EFSA, on July 6, 2022, has issued  
1098 a positive opinion on the safety of the lesser mealworm as novel foods, according to Regulation  
1099 (EU) 2015/2283 (EFSA Panel On Nutrition *et al.*, (2022). Moreover, in January 2023, the  
1100 commission authorized the marketing of lesser mealworm under Regulation (EU) 2023/58  
1101 (European Union, 2023). There are few legal frameworks that consider insects as food (Grabowski  
1102 *et al.*, 2020) and this suggests that promoting edible insects as a food source to populations  
1103 unfamiliar with entomophagy would require greater efforts in sensitization and awareness-raising  
1104 to communicate their benefits and safety (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). The  
1105 acceptability of consuming insect-based food can also depend on household composition,  
1106 including who a person eats with and how well their current eating habits align with this new food  
1107 choice (House, 2016). However, information about these predictors is limited.

#### 1108 *Individual factor*

1109 Numerous studies conducted on Western populations have shown that men are generally more  
1110 open in consuming insect-based foods compared to women (Kröger *et al.*, 2022; Tzompa-Sosa *et*  
1111 *al.*, 2023). In contrast, survey conducted among Belgium consumers show that gender appears to  
1112 have no effect on consumer acceptance towards products prepared from mealworms (Caparros  
1113 Megido *et al.*, 2014). The study also demonstrated that the prospect of cooking a non-conventional  
1114 and "fun" food like insects could increase their willingness to cook and consume them.

1115 According to Verbeke (2015), younger males in Western countries who are less attached on meat,  
1116 more receptive to new food experiences, and concerned about the environmental consequences of  
1117 their dietary choices are the ideal candidates for the initial adoption of insects as an innovative and  
18 environmentally friendly protein source. In contrast, research conducted on younger Australians,  
19 comprising Millennials and Generation Z, has demonstrated a lack of interest in substituting meat



1120 with edible insects. This perspective is primarily attributed to factors such as neophobia and disgust  
1121 towards insects, as well as a perception of insects as a threat to traditional masculinity. Moreover,  
1122 despite being aware of the nutritional benefits of consuming insects, consumers do not seem to be  
1123 influenced in considering them as a food alternative (Sogari *et al.*, 2019). It can be said that  
1124 although knowledge about entomophagy may have an impact on consumer acceptance, the level  
1125 of education may not be a significant factor (Ardoin and Prinyawiwatkul, 2021; Kröger *et al.*,  
1126 2022; Tzompa-Sosa *et al.*, 2023). Similar to meat, insects can also be subjected to dietary  
1127 restrictions based on nutritional or animal welfare grounds. It is anticipated that individuals  
1128 following a vegetarian or vegan diet may have a greater reluctance to consume insects (Kröger *et*  
1129 *al.*, 2022; Onwezen *et al.*, 2021).

1130 The acceptance of insects as food can be significantly influenced by emotions (Kröger *et al.*, 2022).  
1131 Most of the studies reported in the literature have focused on emotions associated with disgust or  
1132 fear. However, there is one comprehensive study on the influence of emotions in general terms.  
1133 For example, the feeling of happiness, satisfaction, or pride when thinking about eating insects.  
1134 These are defined as positive emotions, while negative emotions can be defined as the feeling of  
1135 anger, sadness, or even guilt when thinking about eating insects (Kröger *et al.*, 2022). According  
1136 to Onwezen *et al.* (2019), positive emotions have been shown to positively affect consumer  
1137 acceptance, while negative emotions do not have a significant effect.

1138 In Western countries, the concept of entomophagy frequently evokes negative emotional reactions,  
1139 with disgust being one of the most common and prominent ones. Several studies that have been  
1140 reported in the literature indicate that the feeling of disgust has become a barrier to insect  
1141 consumption (Kröger *et al.*, 2022). For example, a survey on consumer response in Australia  
1142 revealed that the emotion of disgust is the main barrier to consumer acceptance of insects as food.  
1143 Majority of the participants showed negative associations with the idea of regarding insects as  
1144 food. The survey revealed the use of negative words, such as 'disgust', 'detestation', 'revulsion',  
1145 'dislike', 'vomit', and 'neophobia', in reference to the insect-based product (Sogari *et al.*, 2019).

1146 To determine how health benefits influence consumer behaviour towards insect consumption,  
1147 Poortvliet *et al.* (2019) conducted a study on 134 Dutch consumers to measure their willingness to  
1148 try insect meat. In this study, participants were shown pictures of hamburgers with a description  
1149 indicating that the burger was produced from either ground beef (for the bovine meat type  
1150 condition) or a combination of ground lesser mealworms and locusts (for the insect meat type  
1151 condition). The researchers found that uncommon products such as shish kebabs were more  
1152 preferred over common products like burgers. However, the study did not reveal any significant  
1153 difference in preference between the two meat types (bovine meat and insect meat). According to  
1154 this study, the influence of health benefits and the factor of disgust had no effect on the consumers'  
1155 willingness to try the product. This finding revealed the factor on why insect-based products are  
1156 not as popular as bovine meat products. However, the study also highlights an interesting point  
1157 that health benefits could potentially be a significant driver for the acceptance and adoption of  
1158 insect consumption. When developing marketing strategies, it is important to include the health  
1159 features of insect products such as nutritional content and safety considerations. Additionally,  
1160 attention should be given to packaging design, labelling, and other elements of product  
1161 presentation that can affect consumers' perceptions and willingness to try these novel food products  
1162 (Poortvliet *et al.*, 2019).



1164 **The product**

1165 *Product-related circumstances*

1166 The term 'product-related circumstances' refers to the social, practical, and contextual factors that  
1167 are associated with insect-based food products (Kauppi *et al.*, 2019). While the price and taste of  
1168 an insect-based burger may influence repeat consumption, it is important to consider the effect of  
1169 social, practical, and contextual factors as well (Kröger *et al.*, 2022). In addition, the consumer  
1170 acceptance can be significantly increased by expert recommendations and the experiences of peers  
1171 (Berger *et al.*, 2019). According to Caparros-Megido *et al.* (2014), if consumers can associate  
1172 insect-based food products with familiar flavours, they are willing to purchase and prepare them  
1173 at home. This shows that, from a practical view, edible insects have the potential to become a  
1174 commonly used food ingredient among European populations.

1175 As interest in edible insects continues to grow, it raises questions about how consumers perceive  
1176 the welfare of insects (Delvendahl *et al.*, 2022). This issue has been a matter of public concern  
1177 since the nineteenth century, but it has become increasingly relevant in recent years. However, to  
1178 this day, it is still unclear how consumers perceive the welfare of farmed insects. The ethical  
1179 implications of consuming insects are likely to have an impact on consumers, and this concern is  
1180 expected to grow as consumers start to question the methods used to raise and kill insects for  
1181 consumption (Kauppi *et al.*, 2019). Notably, the criteria for insect welfare may vary from those  
1182 established for vertebrate welfare. It could be argued that establishing guidelines for insect welfare  
1183 may prove challenging given the diverse habitats and dietary needs of insects. Furthermore, there  
1184 is an ongoing debate about whether insects possess consciousness and experience pain. Some  
1185 researchers have recommended treating insects as sentient beings and rearing them in natural living  
1186 conditions (Delvendahl *et al.*, 2022). Nevertheless, consumers' views and understanding regarding  
1187 this matter have received little consideration thus far.

1188 Halonen *et al.* (2022) conducted a survey on the ethical aspects of insect consumption, and they  
1189 found that attitudes towards the ethics of consuming insects in Finland are heavily influenced by  
1190 whether the respondent is a semi-vegetarian, vegetarian, or vegan. The survey revealed that 72%  
1191 of semi-vegetarians were open to incorporating insects into their diet, whereas most vegetarians  
1192 (56%) and vegans (71%) considered the consumption of insects to be ethically unacceptable  
1193 (Halonen *et al.*, 2022). Meanwhile, Elorinne *et al.* (2019) reported that vegetarians hold the most  
1194 favourable views on consuming edible insects, and both omnivores and vegetarians perceive  
1195 entomophagy as a wise solution to global nutrition challenges. In contrast, vegans view insect  
1196 consumption as immoral and irresponsible (Elorinne *et al.*, 2019).

1197 In addition to ethical considerations, pricing is another important factor that can have a significant  
1198 impact on consumer acceptance. Furthermore, pricing can also influence repeat consumption  
1199 (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). A survey conducted by House (2016) showed that price  
1200 was a significant factor in their acceptability and repeat consumption. Consumer perception of the  
1201 price of Insecta, a burger made from 13 - 15% of lesser mealworm powder, was deemed relatively  
1202 expensive in Belgium, as per the findings of those studies. For instance, the cost of a pack  
1203 containing two insect-based burgers was approximately €4, which was higher than most  
1204 comparable vegetarian products (€2 - €3) and meat products (€1 - €3). Around 36% of the  
1205 participants regarded the insect food as too expensive to purchase, while almost half of them (45%)  
1206 recognized the relatively elevated cost but did not consider it as problem for future purchases.

1207 While price alone would not stop the majority (64%) of people from purchasing, it was frequently  
1208 acknowledged as one of several intersecting factors that impeded future purchases.

1209 In addition to price, it is crucial to ensure that edible insects are guaranteed to be of high quality  
1210 and meet food safety standards. It is noted that the lesser mealworm has been granted novel food  
1211 status by the EU as of January 2023, following its safety approval by the EFSA (European Union,  
1212 2023). Despite lesser mealworm not being extensively commercialized in European countries yet,  
1213 various studies have demonstrated a growing interest in this insect. Consequently, it may be  
1214 recommended to boost the population or production of lesser mealworms to enhance the  
1215 predictability and availability of insects (Van Huis *et al.*, 2022). For example, a survey conducted  
1216 by House (2016) among Dutch consumers found that the unavailability of the products in the  
1217 market had hindered their intention to purchase (House, 2016). This finding was in agreement with  
1218 the studies by Shelomi (2015) who reported that low availability of products resulted in consumers  
1219 purchasing less frequently than they would have preferred, leading to passive rejection of the  
1220 products by potential consumers (Shelomi, 2015).

1221 The growing interest in edible insects over the past decade has led to the emergence of several  
1222 business owners and enterprises actively involved in insect production. In European countries, the  
1223 insect-based production industry primarily focused on breeding insects for biocontrol purposes or  
1224 animal feed production. These activities were often carried out in zoological gardens (Mancini *et al.*,  
1225 2022). These days, the situation is different and there are now several operators in the insect  
1226 feed business (iFeedBOs) who also engage in food production activities as well as some operators  
1227 in the insect food business (iFoodBOs). To date, lesser mealworms have been applied in a variety  
1228 of products across Europe, including Zirp, which is sold in over 800 Billa stores in Austria. Other  
1229 examples include cereal bars, Issac shakes, and gourmet burgers made from mealworm that are  
1230 featured in several Danish restaurants. The company has been expediting the commercialization  
1231 of its products in additional European markets (Ynsect, 2021). According to Collins *et al.* (2019),  
1232 incorporating insect-based foods into human diets not only benefits the environment but also  
1233 makes good business sense. They also emphasized the significance of utilizing different marketing  
1234 techniques, such as using celebrity endorsements and peer-to-peer advertising. Developing  
1235 marketing and advertising strategies for insect-based products and raising public awareness about  
1236 the entomophagy is crucial. This can be accomplished by launching educational campaigns aimed  
1237 at farmers to promote awareness of the advantages of edible insects as a substitute for traditional  
1238 livestock rearing (Collins *et al.*, 2019; Žuk-Gołaszewska *et al.*, 2022).

### 1239 *The product properties*

1240 Due to the unfamiliarity of entomophagy among Westerners, improving familiarity has often been  
1241 emphasized as a critical factor in reducing neophobia and increasing acceptance (Kröger *et al.*,  
1242 2022). There are various studies that emphasized the significance of the process of familiarization  
1243 and increasing exposure as a means of reducing and overcoming feelings of disgust and fear  
1244 associated with the consumption of insects (Kauppi *et al.*, 2019; Kröger *et al.*, 2022). It is proven  
1245 that testing insects as food and exposing people to insect-based foods can help reduce neophobia  
1246 and increase acceptability (Kröger *et al.*, 2022). Therefore, the acceptability of a new food product  
1247 can be heavily influenced by its taste. This was also in agreement by Caparros-Megido *et al.* (2016),  
1248 which the taste and appearance significantly influenced the participants' overall liking of burgers  
49 prepared from mealworms. Meanwhile, a study conducted by Brynning *et al.* (2020) obtained  
50 similar results, which suggested that the protein, fat, and chitin composition of insect flour made





1251 from lesser mealworm should be refined and separated to achieve a more neutral taste. This could  
1252 potentially enhance the usability of insect flour as an ingredient in food. Moreover, Mazurek *et al.*  
1253 (2022) revealed that the attitudes of potential consumers in Poland towards entomophagy and  
1254 consuming pancakes with addition of edible insects were promising. Nonetheless, despite the  
1255 positive attitude, most respondents were reluctant to taste the test samples due to the addition of  
1256 insect in meal. The pancake flour used in the study was mixed with 10-30% of lesser mealworm.  
1257 The study showed that the primary factor that influenced the overall sensory acceptability was the  
1258 taste. It can be said that insect-based products have potential for introduction in Western society.  
1259 However, some improvements may be required, such as refining the taste, altering recipes, and  
1260 modifying product structure.

1261 On the other hand, it is important to highlight that there is a recommended upper limit (%) for  
1262 incorporating insect flour as a wheat flour substitute. Exceeding this limit could affect the bread  
1263 quality, especially the carbohydrate content (Skotnicka *et al.*, 2022). Lesser mealworms consist of  
1264 approximately 60% protein (Roncolini *et al.*, 2020), and several studies (Brynnning *et al.*, 2020;  
1265 Mazurek *et al.*, 2022; Roncolini *et al.*, 2020; Skotnicka *et al.*, 2022) have demonstrated that up to  
1266 30% of lesser mealworms were added to replace the wheat flour for the production of bread and  
1267 rusk. However, it is essential to note that increasing the amount of lesser mealworm could lead to  
1268 a decline in bread quality due to the reduction in carbohydrate content (Brynnning *et al.*, 2020). This  
1269 is supported by studies conducted by Skotnicka *et al.*, (2020), which demonstrated that increasing  
1270 the protein content in the pancakes led to a reduction in carbohydrate content ( $p < 0.05$ ).  
1271 Meanwhile, a study by Roncolini *et al.*, (2020) revealed that a 30% substitution of lesser mealworm  
1272 enriched the protein content of rusk by up to 99.3%, suggesting that 30% was the optimum amount  
1273 for substitution. Additionally, the analysis of mineral composition in the rusk showed that  
1274 incorporating 30% of lesser mealworm provided the recommended daily intake of minerals such  
1275 as Fe, Zn, Mg, and Ca. For instance, the recommended daily intake for Fe falls between 7 and 58  
1276 mg per day. The mineral composition analysis of the prepared rusk (with 30% lesser mealworm  
1277 substitution) indicated Fe contents ranging from 28 to 33 mg/kg. Consequently, consuming at least  
1278 200 g per day of rusk with 30% substitution for lesser mealworm would be suitable to meet the  
1279 recommended daily intake for this mineral.

1280 As lesser mealworms are rich in protein, a high amount (>40%) of substitution may cause the  
1281 product appearance to become darker, thereby potentially affecting consumer acceptance  
1282 (Brynnning *et al.*, 2020). In another study, Gantner *et al.* (2022) evaluated the physicochemical  
1283 properties of bread incorporated with 5, 10, and 15% of mealworms. The study revealed that a  
1284 higher addition of mealworms would significantly reduce the intensity of the bread flavor, even at  
1285 levels of 10% and 15% addition. This effect was particularly showed in the intensity of the bitter  
1286 taste and nutty flavor of the bread samples with a higher amount of mealworms (15%). Sensory  
1287 evaluation indicated that the incorporation of mealworms significantly affected the visual  
1288 appearance, flavor, odor, and overall sensory quality of the bread. Based on the results, it can be  
1289 concluded that the maximum amount of insect addition is dependent on the type of insect used.  
1290 For example, in the case of the lesser mealworm, it can be said that 30% would be the maximum  
1291 amount, as any quantity beyond that threshold would negatively affect the physicochemical  
1292 properties and overall sensory quality of the bread.

1293 Various studies have demonstrated that price can influence the consumer acceptance of edible  
34 insects (Brynnning *et al.*, 2020; Mazurek *et al.*, 2022; Roncolini *et al.*, 2020). An example of a study  
35 exploring the influence of price on the development of non-snack insect food, using lesser





1296 mealworm as insect flour, was demonstrated by Brynning *et al.* (2020). The study surveyed  
1297 individuals from the Western part of Denmark and found that the main predictor for improving  
1298 insect-based products was to lower the price. The participants were asked about what price range  
1299 would be considered acceptable for food products made from them. In the survey, participants  
1300 were instructed to select an insect product and guess its sales price. The actual price was 30 - 50%  
1301 higher than their estimates, which came as a surprise to them. Pricing was identified as the primary  
1302 challenge in the adoption of insect products in the future. When asked to choose between plant-  
1303 based and insect-based alternatives to meat, they preferred plant-based products due to their lower  
1304 cost. Therefore, in the future, it is recommended that producers evaluate their pricing strategy and  
1305 decide whether to prioritize competition, value, or marketing over the current cost-based approach.

1306 The food industry is increasingly favoured towards selecting innovative and sustainable raw  
1307 materials to produce nutrient-fortified foods, in response to the growing consumer trend for such  
1308 products (Hassoun *et al.*, 2022). One way to fortify foods is by adding a specific molecule during  
1309 the processing stage, while another approach is to use ingredients that are naturally high in the  
1310 desired nutrient (Roncolini *et al.*, 2020). In this perspective, using edible insects as food  
1311 ingredients can be a promising strategy for fortifying conventional foods, especially to increase  
1312 consumer interest in nutrient-fortified foods. Based on study by Brynning *et al.*, (2020), they  
1313 showed that consumers are looking for insect-based products that go beyond snacks and can be  
1314 used as elements of main meals. Furthermore, they also look for nutritional details regarding these  
1315 products. In terms of the form of insect-based products, they also suggested that insects be  
1316 integrated into recognizable food items such as bread or meat products.

1317 In a different study, Roncolini *et al.* (2020) used lesser mealworm powder as a novel baking  
1318 ingredient to produce protein and mineral-rich snacks. The lesser mealworm was substituted for  
1319 wheat flour in amounts ranging from 10% to 30% to increase the protein and mineral content of  
1320 crunchy snacks. The study found that when 30% of the lesser mealworm was added, the protein  
1321 content was enriched up to 99.3% in rusks. Furthermore, a substantial rise in the level of essential  
1322 amino acids was observed, with the fortification of histidine reaching up to 129% in rusks. The  
1323 addition of lesser mealworm powder has enriched the minerals such as iron (Fe), potassium (P),  
1324 and zinc (Zn). Considering the possibility of producing insect-based rusks on an industrial scale,  
1325 the aforementioned product can be categorized as level 4 on the Technology Readiness Level scale.

1326 Another factor that can significantly influences consumer acceptance is the textural properties.  
1327 There are several studies reported on the addition of edible insects can affect the textural properties  
1328 of the product (Kröger *et al.*, 2022). For example, García-Segovia *et al.* (2020) evaluated the effect  
1329 of adding insect-based protein in the manufacturing of bread. The wheat flour used in production  
1330 was lesser mealworm powder and the consumer acceptance of the prepared bread was measured.  
1331 According to the survey, they found that the textural properties were significantly affected when  
1332 insect-based flour was used. However, despite the effect of insect-based flour on textural  
1333 properties, the prepared bread received a high liking score for attributes related to overall  
1334 acceptance, indicating that textural properties did not significantly impact consumer liking  
1335 (García-Segovia *et al.*, 2020).

1336 According to several aforementioned studies, lesser mealworm flour can be produced by  
1337 incorporating them into wheat flour and this can serve as an ingredient in various food products,  
38 including energy bars, bread, pasta, noodles, and more. Considering factors such as disgust,  
39 neophobia, and food safety, it is important to prepare insect-based foods with an appealing



1340 appearance, and the use of flours has been found to be the most accepted format (Ros-Baró *et al.*,  
1341 2022). Recently, Brynning *et al.* (2020) developed a non-snack food product based on three  
1342 different insect species which are house cricket, lesser mealworm, and yellow mealworm. The  
1343 flavour profiles of the three insect flours were analysed using quantitative descriptive sensory  
1344 analysis. According to their findings, insect flour has a distinct flavour profile characterized by  
1345 three main taste notes: Protein/meat, cereal/bread, and mature/old. They also observed that among  
1346 the three insect flours tested, Tenebrio had the most neutral taste. The studies on consumer  
1347 acceptance were conducted on individuals residing in the western region of Denmark. The study  
1348 indicated that consumers favoured incorporating insect flour into meat products or bread instead  
1349 of wheat flour. In addition, it is recommended that the insect flour to be utilized to boost protein  
1350 and vitamin levels. These findings suggest that insect flour could be utilized as a primary  
1351 component in the preparation of main meals with some modification in the development kitchen.

1352 Finally, integrating environmental and sustainability features into insect packaging is crucial for  
1353 marketing and appealing to consumers (Wade and Hoelle, 2020). One survey (Brynning *et al.*,  
1354 2020) showed that consumers may favour the design that looked cheaper. Furthermore, the  
1355 presence of pictures of insects on the packaging was found to have a deterrent effect, as consumers  
1356 preferred insect images to be in the form of drawings or illustrations. In term of naming,  
1357 considerations should be taken during production by using "IN" as a prefix to a familiar name  
1358 instead of "insect" in order to provide a more modern touch. To enhance consumer preference, the  
1359 production company may opt to use specific insect names like Lesser Mealworm or *Alphitobius*  
1360 *diaperinus* instead of using general terms like "insect." This helps the consumers to know the exact  
1361 type of insect present in the product. In conclusion, customizing the packaging design to align with  
1362 the sustainable image of insects may prove to be a crucial approach in persuading consumers to  
1363 accept insect-based products.

## 1364 9. Conclusion and future perspectives

1365 The European Commission's recent approval of *A. diaperinus* larvae (lesser mealworm) as a novel  
1366 food has opened up new opportunities for sustainable food production and consumption. A  
1367 comprehensive review of the scientific literature on this topic suggests that the insect has the  
1368 potential to become an important source of protein and other essential nutrients for human diets.

1369 Records of lesser mealworm consumption in various parts of the world suggest that it has been a  
1370 traditional food source in many cultures for centuries. This insect's bioecology and nutritional  
1371 value make it a promising candidate for human consumption. It is easy to rear, has a short life  
1372 cycle, and can consume a wide range of organic matter, including agricultural by-products and  
1373 food waste. Furthermore, lesser mealworm is rich in protein, vitamins, and minerals, including  
1374 calcium, phosphorus, and iron. Studies have shown that edible lesser mealworm also has  
1375 nutraceutical and pharmaceutical properties. The insect contains bioactive compounds that may  
1376 have beneficial effects on human health, such as antioxidant and antimicrobial properties.

1377 Consumer acceptance of edible lesser mealworm remains an important consideration. Although  
1378 lesser mealworm has been consumed for centuries in some cultures, it may be challenging to  
1379 introduce it to new markets. However, studies suggest that consumer acceptance can be improved  
1380 through effective communication and education. Consumers are more likely to accept lesser  
1381 mealworm as a food source if they understand its nutritional value, safety, and environmental  
32 benefits. Further research is needed to explore more about the potential health benefits of lesser  
33 mealworm and to develop effective strategies for introducing it to new markets such as focusing



1384 on environmental, economic and social sustainability or tackling the safety concerns in the new  
1385 markets. Several effective strategies exist for introducing edible lesser mealworms to new markets.  
1386 One approach is to craft a go-to-market strategy. Another approach is to consider success factors  
1387 before entering the market.

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1393 Original Draft, Writing - Review and Editing, Visualization, Data Curation, Project administration,  
1394 Investigation, Supervision. Y.S.W. - Writing - Review and Editing. K.V. - Writing - Original Draft.  
1395 K.B. - Writing - Original Draft. M.H.M.H. - Writing - Original Draft. H.L. - Writing - Original  
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#### 1398 **Conflict of interest**

1399 The authors declare no conflict of interest.

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