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

S.A. Siddiqui^{1,2*}, Y.S. Wu^{3,4}, K. Vijeepallam⁵, K. Batumalaie⁶, M.H. Mohd Hatta⁷, H. Lutuf⁸, R.
 Castro-Muñoz^{9*}, I. Fernando^{10*}

¹Technical University of Munich Campus Straubing for Biotechnology and Sustainability,
 Essigberg 3, 94315 Straubing, Germany; ²German Institute of Food Technologies (DIL e.V.),
 Prof.-von-Klitzing Str. 7, 49610 D-Quakenbrück, Germany; ³Centre for Virus and Vaccine
 Research, School of Medical and Life Sciences, Sunway University, Subang Jaya 47500, Selangor,
 Malaysia; ⁴Department of Biological Sciences, School of Medical and Life Sciences, Sunway
 University, Subang Jaya 47500, Selangor, Malaysia; ⁵Faculty of Pharmacy, AIMST University.

University, Subang Jaya 47500, Selangor, Malaysia; ⁵Faculty of Pharmacy, AIMST University,
 Semeling - 08100, Bedong, Kedah, Malaysia; ⁶Sunway College Johor Bahru, Jalan Austin Heights

Utama, Taman Mount Austin, 81100 Johor Bahru, Johor: ⁷Centre for Research and Development.

Asia Metropolitan University, 81750 Johor Bahru, Johor, Malaysia; ⁸Council for scientific and

Asia Metropontali Oniversity, 81750 Johor Baliru, Johor, Maraysia, Council for scientific and industrial research - Oil Palm Research Institute P. O. Box 74, Kade, Ghana; ⁹Department of

15 Sanitary Engineering, Faculty of Civil and Environmental Engineering, Gdansk University of

16 Technology, 80 - 233 Gdansk, G. Narutowicza St. 11/12, Poland; ¹⁰Department of Plant Pest and

17 Diseases, Faculty of Agriculture, Universitas Brawijaya, Malang, East Java, 65145, Indonesia 18

19 Corresponding authors' email:

20 Shahida Anusha Siddiqui (S.Siddiqui@dil-ev.de)

21 Roberto Castro-Muñoz (food.biotechnology88@gmail.com)

22 Ito Fernando (i_fernando@ub.ac.id)

24 Short title

25 Lesser mealworm as human foods

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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 mealworms are considered a good source of protein, with a protein content ranging from 50-65% 35 36 of their dry weight and containing various essential amino acids. Lesser mealworms are also rich in other essential nutrients such as iron, calcium, and vitamins B12 and B6. Furthermore, the 37 hydrolysates of lesser mealworms are known to contain antioxidants, suggesting the therapeutic 38 properties of the insects. To enable and ensure a continuous supply of lesser mealworms, various 39 rearing procedures of the insects and information on optimal environmental rearing conditions 40 have been reported. However, like other edible insects, lesser mealworms are still not commonly 41 consumed in Western countries because of various consumer- and product-related factors. 42 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 environmental impact of food production, and promote a more sustainable and resilient food 46 system for the future. 47

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

49 1. Introduction

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

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

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

nutritious. It has been particularly recognized for its high protein and fat content (Janssen, 2017), 71 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 sources of protein to feed a growing global population. Insects, including A. diaperinus, are highly 76 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 (Cammack et al., 2021). 80

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, 2015). Insects are also rich in micronutrients such as iron, calcium, and zinc, which are often 84 85 lacking in traditional staple foods such as rice and maize (Mwangi et al., 2018). However, the widespread adoption of entomophagy as a food source is not without its challenges. Public 86 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).

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

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

Alphitobius diaperinus larvae as a novel food

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

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In 2018, the European Commission approved the use of insects as food for human consumption, 114 paving the way for the European Union and the others to explore the potential of insects as a 115 sustainable and alternative source of protein (EFSA Panel on Nutrition et al., 2022). Since then, 116 the European Commission has been evaluating applications for the use of insects as novel foods, 117 118 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 production process, microbiological and chemical composition, and potential allergenicity (EFSA 124 Panel on Nutrition et al., 2022). 125

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).

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

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

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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 and Feed (IPIFF) guide on good hygiene practices provides an overview of the processing methods 160 that are applied to insects intended for human consumption and animal nutrition. The guide 161 162 highlights the importance of cleaning, cooking, drying, and freezing insects and maintaining good hygiene practices during all stages of insect processing to ensure the safety of the final product. 163 164 This includes ensuring that processing equipment is clean and sanitized and that workers follow proper hygiene practices (IPIFF, 2022) 165

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 et al., 2022). For instance, a study by Kotsou et al. (2021) has investigated the impact of 174 175 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 individuals may be more susceptible than others (Taylor and Wang, 2018). The risk of allergenicity 181 182 is influenced by the presence of certain allergenic proteins. Leni and Tedeschi (2020) have investigated the allergenic potential of lesser mealworms and found that tropomyosin was 183 184 identified as the prevalent potential allergen. Furthermore, it was also revealed that lesser mealworms contain peptides that closely resembled the well-known allergens arginine kinase. This 185 186 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

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

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protein, fat, fiber, ash protein, vitamins, and minerals, making them a highly nutritious food source. 197 Research has indicated that dried lesser mealworms contained an average of 58-65% crude protein, 198 which is comparable to other insects approved for human consumption, such as crickets and yellow 199 mealworms (Rumbos et al., 2019). The study also found that lesser mealworms contained high 200 201 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 tropomyosin to be among the most abundant proteins in lesser mealworm. Furthermore, lesser 206 mealworms are reported to be a good source of protein, with levels ranging from 45-60% 207 depending on the stage of development and rearing conditions (van Broekhoven et al., 2015). The 208 209 protein of lesser mealworms is also reported to be of high quality, as lesser mealworms contain all the essential amino acids in adequate amounts (Kurečka et al., 2021; Smola et al., 2023; Tzompa-210 Sosa et al., 2014; Yi et al., 2013). In fact, the protein content of lesser mealworms was superior to 211 212 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, and niacin (Zhou et al., 2022). Also, Finke (2015) reported that dried lesser mealworms contained 217 high levels of vitamin B12, which is essential for nerve function and DNA synthesis. In fact, a 218 study has been reported that mealworms contained higher levels of iron and zinc than beef 219 (Latunde-Dada et al., 2016), while another study reported that they contained more calcium than 220 milk (Oonincx et al., 2015; Oonincx and de Boer, 2012; Seyedalmoosavi et al., 2022). 221

222 Safety

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223 The safety of lesser mealworms as a novel food was thoroughly evaluated by the EFSA before approval. The EFSA concluded that dried lesser mealworms are safe for human consumption and do not pose any significant risk to human health (EFSA Panel on Nutrition et al., 2022). However, 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 lesser mealworms and found that there is no evidence of any significant risks. In another study, 230 the microbial composition of lesser mealworms was reported to have low levels of pathogenic bacteria, indicating their safety for human consumption (Stoops et al., 2017; Wynants et al., 2018).

233 Status of Alphitobius diaperinus larvae as animal feed

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

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

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

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

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

approved feed ingredient (AAFCO Committees, 2021).

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

257 mealworms to animals (German Federal Institute for Risk Assessment (BfR) et al., 2019).

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

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

Lesser mealworms, like other edible insects, are not commonly consumed in many western 263 countries particularly in countries such as Australia, Canada, the entirety of Europe and Russia, 264 New Zealand, and the United States (Payne et al., 2019). Meanwhile, entomophagy is common in 265 many countries around the world, particularly in Africa, Asia, and Latin America (van Huis, 2013). 266 However, specialized insect farms in Europe, particularly in the Netherlands and Belgium, have 267 begun to produce lesser mealworms for human consumption. The lesser mealworms are processed 268 and sold in the form of freeze-dried snacks or processed foods such as pasta, burger patties or 269 270 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).

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

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

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

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281 4. Bioecology of Alphitobius diaperinus

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

287 General morphology

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

Distribution 297

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

າງ2 Life cycle

Alphitobius diaperinus life cycle consists of four stages, i.e., egg, larva, pupa, and adult (Dunford 303 and Kaufman 2006). The eggs are small and white in colour and are usually laid in the substrate, 304 305 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 are a creamy white color and the color darkens as they progress through the instar. After every 308 309 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 Kaufman, 2006). In larval stage they possess three pairs of legs. During their final larval stage, 311 312 they can reach up to 11 mm in length (Dunford and Kaufman 2006). Before entering the pupal stage, the larvae seek isolation from others and burrow into the substrate. The pupae of A. 313 *diaperinus* are exarate, reaching 6 to 8 mm in length and ranging in colour from creamy white to 314 tan (Dunford and Kaufman, 2006). The entire life cycle of A. diaperinus, from egg-laying 315 (oviposition) to the emergence of adult beetles, takes around 34 to 38 days when the temperature 316 is optimal at 30°C. At temperatures below 30°C, the development slows down significantly, and 317 it can take up to 165 days for the eggs to reach adulthood at 20°C. Development ceases completely 318 319 below this temperature (Rueda and Axtell, 1996).

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

330 Habitat

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

338 Diet

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

342 organic matter, including grain, feed, and animal carcasses (Ducatelle and Van Immerseel, 2011).

343 As pest and its management

Alphitobius diaperinus is a major pest of poultry farms worldwide. It causes significant economic
 losses due to damage to poultry feed and disease transmission (Yeasmin *et al.*, 2014). It has been
 implicated in the transmission of several diseases, including avian influenza and salmonellosis

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).

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

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

Nutrient	Amount	Reference
Crude protein	45.10 - 50.54 (% DM)	Rumbos et al., 2019
Carbohydrates	21.8	Sun et al., 2021
Crude lipid	13.4 - 29.0 (% DM)	Rumbos et al., 2019
Ash	3.6 (% DM)	Rumbos et al., 2019
Mineral content	Amount	
Calcium	$0.5\pm0.0~(g/kg~DM)$	Janssen et al., 2019
Copper	$21.9\pm0.4~(mg/kg~DM)$	Janssen et al., 2019
Iron	$53.5 \pm 1.7 \text{ (mg/kg DM)}$	Janssen et al., 2019
Potassium	$10.0\pm0.2~(g/kg~DM)$	Janssen et al., 2019
Magnesium	$1.3 \pm 0.0 \; (g/kg \; DM)$	Janssen et al., 2019
Manganese	$5.4 \pm 0.3 \text{ (mg/kg DM)}$	Janssen et al., 2019

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

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

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

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

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., 2020), meaning they provide a relatively high amount of protein per calorie. The method of 398 preparation can also impact the lipid content of lesser mealworm. Roasting or frying the insects 399 can increase the fat content due to the addition of oils or fats used during cooking while boiling or 400 401 steaming can reduce the fat content due to the loss of fat in the cooking water as is the case of T. 402 molitor (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 and muscle function, and fluid balance in the body. In addition to minerals, crude ash may also 413 contain trace elements such as iron, zinc, and copper, which are important for various biological 414 processes. While the crude ash content of lesser mealworm may seem relatively low compared to 415 other sources of minerals and trace elements, they can still contribute to an individual's overall 416 417 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).

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

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

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

437 Lesser mealworms are also rich in other essential nutrients such as iron, calcium, and vitamins B12 and B6 (Anzani et al., 2020). However, insects typically do not synthesize vitamins; instead, 438 439 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 need to improve food nutrition and that humans should not only pursue food production but also 442 give due consideration to the nutrition of food. Vitamins and minerals are essential in the metabolic 443 processes of humans and animals, and their deficiency may have adverse health effects (Awuchi 444 445 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 (Churchward-Venne et al., 2017). 450

451 Amino acid profile

452 The amino acid profile of the lesser mealworm from various studies is shown in Table 4. The data reveals significant variations in amino acid content among the different sources. A notable 453 variability across the studies conducted by Rumbos et al. (2019), Hermans et al. (2021), Kurečka 454 455 et al. (2021) reflects potential differences in methodologies, feed composition, rearing conditions, and possibly the strains of lesser mealworms used. For instance, arginine shows a substantial 456 difference across the studies, with Kurečka et al. (2021) reporting a notably higher content. Some 457 458 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, proline, and serine are consistently present across all studies, indicating their stable presence in the 461 462 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 content ranging between 37% and 49%. The most dominant amino acids in the larvae (higher than 467 32 g/kg DM) were glutamate, arginine, aspartate, alanine, leucine and tyrosine. Differences in the 468 reported values highlight the need for standardized methods for assessing amino acid content or 469 470 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 all the essential amino acids (Rumbos et al., 2019), which cannot be produced by the human body 472 473 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 are usually deficient (Sánchez-Muros et al. 2014). The specific amounts of each amino acid in 76 lesser mealworms may vary depending on factors such as the developmental stage of the insect, 77

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

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Fatty acid profile

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

499	Table 5 Fatty	v acid com	position of A	Inhitohius dia	merinus 1	arvae report	ed in some s	tudies
433	Table J. Patt	y actu com	position of A	p_{moons} and	$_{\mu}$	arvac report	cu m some s	tuuitos

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
α -linoleic	1.88	1.5

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501 Palmitic acid is a saturated fatty acid that is an important component of cell membranes and is involved in many metabolic pathways in the body (Calder, 2015). Stearic acid, unlike palmitic 502 503 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 for maintaining healthy cholesterol levels and has been linked to a reduced risk of heart disease 505 (Calder, 2015). Linoleic acid is an essential polyunsaturated fatty acid that must be obtained from 506 the diet. It is found in high amounts in vegetable oils, nuts, and seeds, and is important for 507 maintaining healthy skin and hair and for many other physiological functions (Calder, 2015). 508 509 Linolenic acid is another essential polyunsaturated fatty acid that must be obtained from the diet. It is found in high amounts in flaxseed oil, chia seeds, and other plant sources, and is important for 510 511 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

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

518 Chitin

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

Protease Inhibitors 526

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).

Reduced protein digestion can affect overall protein utilization. 530

531 **Reducing Anti-Nutrient Compounds**

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

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542 6. Nutraceutical and pharmaceutical properties of Alphitobius diaperinus larvae

543 Edible insects have the potential to serve as healthy, sustainable alternatives to traditional animalbased foods because of their nutrient contents (Leni et al., 2020; Nowakowski et al., 2022). Insects 544 are rich in high-quality protein, essential amino acids, fiber, iron, zinc, vitamin B12, and omega-3 545 fatty acids (Leni et al., 2020; Nowakowski et al., 2022). Insects generally have been found to 546 547 contain bioactive compounds such as antioxidants, anti-inflammatory agents, and antimicrobial agents (Nino et al., 2021). These compounds have been shown to have potential health benefits 548 such as reducing the risk of chronic diseases like cancer and cardiovascular disease (Nino et al., 549 550 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).

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

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

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568 1.0 umol Trolox equivalent per g insect have been recorded, however, no antimicrobial or antidiabetic properties were observed (Sousa et al., 2020). 569

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 the enzyme α -glucosidase, demonstrated that A. diaperinus had no antimicrobial capacity or 573 574 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 can inhibit bacterial growth (Saadoun et al., 2022). Hence it is possible that A. diaperinus may 577 contain similar antimicrobial peptides and compounds as other insects, however, further research 578 is needed to ascertain this. 579

On potential anti-inflammatory properties, although there is no direct evidence of the anti-580 581 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 in vitro and in vivo (Aguilar-Toalá et al., 2022). It is possible that A. diaperinus may contain 584 bioactive compounds with similar effects. Further research is, however, needed to fully understand 585 586 the mechanisms behind the potential health benefits of consuming lesser mealworm and to ensure their safety and quality as a food source. 587

588 7. Harvesting and rearing of Alphitobius diaperinus

The environmental condition in the rearing of lesser mealworm 589

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

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

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



Figure 1. Optimized conditions in rearing of Alphitobius diaperinus (created in BioRender.com;

the image of A. diaperinus in adult form was reproduced from Tejada et al., (2022)

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

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required for larval growth to adult stage was 129.0, 49.8, 40.5, and 31.9 days at temperatures of 638 20, 25, 30, and 35 °C, respectively. Additionally, the pupal rate was at about 80, 100, 83, and 92% 639 640 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 particularly affects the larvae development. An increase in the rearing temperature to 25 °C 644 645 resulted in a significant reduction in pupal development time, which could correspond to a decrease in infections and rearing costs. Meanwhile, a recent study has shown that temperature levels can 646 647 significantly affect the cumulative number of eggs per adult and the cumulative larval hatching rate of A. diaperinus. Optimal reproductive output is achieved when reared within a temperature 648 range of 25 to 30 °C for 42 days. The study demonstrated that each adult A. diaperinus yielded an 649 average of 73 eggs, with estimated hatchability rates of 69% and 58% at 25 °C and 30 °C, 650 651 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 °C led to the decrease in the growth rate per day. What is interesting is that they found a negative 654 growth rate per day when the temperature was set to 15 °C. This study suggests that the rate of 655 656 growth is highly dependent on temperature. On the other hand, there was a significant variation in the lipid content (24 to 34% of dry weight) across various temperatures. It can be said that the 657 variation was good, however, the lipid contents were found to be close to 35% at middle 658 659 temperature. In terms of protein content, the lesser mealworm was found to have a high protein content (60%) at lower temperatures, but a slight increase in temperature resulted in a reduction in 660 protein content. The lowest protein content was observed at temperatures of 23 and 39 °C, with 661 values of 48.4 and 48.9%, respectively. It would be interesting to conduct a detailed investigation 662 663 on the composition of fatty acid and amino acid, as not all are nutritionally equal, and the works suggest that the composition of fatty acids, at the very least, is affected by temperature. Moreover, 664 exploring the influence towards varying the content of lipid and protein in the feed according to 665 666 the obtained values here would significantly contribute to the goal of enhancing the quality of the larvae (Bjørge et al., 2018). 667

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

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680 ^31 683 (8L:16D) and complete darkness (0L:24D). Nevertheless, they concluded that the photoperiod had

no significant effect on pupal development and adult survival. The study revealed that the optimal photoperiod for larval development was 8L:16D. Therefore, it can be said that while the

photoperiod nor have development was oblight. Therefore, it can be said that while the photoperiod may influence the development of mealworms, the response to photoperiod tends to

diminish under constant conditions, especially when *T. molitor* becomes arrhythmic (Cloudsley-

Thompson, 1953). To date, there is still a lack of reports in the literature focused on studying the

effects of photoperiod on the growth and development of *A. diaperinus*, or the lesser mealworm.

Several works in the literature have reported the use of a photoperiod of 16 hours of light and 8 hours of deduced (101, 8D) in the development of A dimensions. Table (communicate the actional

hours of darkness (16L:8D) in the development of *A. diaperinus*. Table 6 summarizes the optimalenvironmental conditions for the rearing of *A. diaperinus* based on results of various research.

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

Table 6. Environmental factors in rearing of Alphitobius diaperinus 693

ND: Not Determined

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

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699 One study reported the use of a standard tray measuring $65 \times 50 \times 15$ cm³ for fattening the yellow 700 mealworm (T. molitor) as this size was easy to manage and could prevent adult larvae from escaping (Dossey et al., 2016). Based on the method according to the design from an EU pilot mill 701 702 with the aim of producing 17 tons of fresh T. molitor larvae annually, the insects can be successfully reared at a density of 5 larvae cm⁻² (Thévenot et al., 2018). Although T. molitor and 703 A. diaperinus come from different species, they belong to a similar family. Hence, they may share 704 705 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 both T. molitor and A. diaperinus using the same rearing system and facility. Figure 2 displays a 707 design for rearing boxes arranged in multilevel shelves, which can minimize the space required 708 for the production of mealworm. In certain cases, stackable boxes are recommended to be used. 709 By utilizing multilevel shelves that cover the entire surface area of the rearing space, it may be 710 feasible to produce several thousand tons of larvae per year (Dossey et al., 2016). Within the 711 breeding area, cages are used to house adults and are equipped with food and water sources and 712 the boxes used for rearing larvae and breeding adults are often similar in design. Dividers can be 713 also utilized to optimize the space (Dossey et al., 2016). Moreover, it is important to limit 714 715 oviposition sites to specific locations within the breeding cages to facilitate the collection of eggs. According to the rearing method adopted by Deruytter et al. (2021), the yellow mealworms were 716 717 reared at the following conditions; relative humidity ($60 \pm 3\%$) and temperature ($27 \pm 1^{\circ}$ C), using plastic crates (60 x 40 cm; inner surface up to 2000 cm²). This method is expected to be suitable 718 719 for rearing the lesser mealworm since both vellow mealworms and lesser mealworms belong to a similar family. For semi-industrial production of up to 45,000 larvae per tray, lesser mealworm 720 721 larvae could be reared in open, stacked plastic trays with the size of 40 x 60 x 12 cm. The trays were kept at a temperature of 28 °C to 32 °C with a humidity of 60% and above (Gianotten et al., 722 2020). Another reported study used a container with 19 cm \times 11.5 cm; 950 cc for rearing all the 723 three different stages (larvae, pre-pupae and beetles) of A. diaperinus (Meijer et al., 2022). 724



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

730 Challenges associated with entomopathogens

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

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 infection in several hosts (i.e.; Orthoptera and Blattodea) (Maciel-Vergara et al., 2021). These 742 viruses have been reported to cause epizootics in insect-farming facilities, posing a significant 743 744 threat to the entire production stock (Maciel-Vergara and Ros, 2017). In the worst case, these problems can lead to the shutdown of the affected rearing facilities. For example, contamination 745 746 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 been reported in the literature to be affecting the rearing of A. diaperinus, For example, species of 751 752 entomopathogenic nematodes such as *Heterorhabditis bacteriophora*, *Heterorhabditis megidis*, Steinernema affine and Steinernema carpocapsae have been reported to cause high mortality of 753 larvae of A. diaperinus (Kucharska et al., 2018). Meanwhile, Beauveria bassiana, a fungus that 754 755 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.

Challenges associated with microbes contamination

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

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774 treatments to ensure food safety before being declared safe for human consumption (Vandewever 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 compared to birds and mammals (Lange and Nakamura, 2021). However, considering the 778 examples of zoonotic infections such as the coronavirus disease which have caused pandemics 779 780 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 (Table 7). Wynants et al. (2018) conducted a characterization of microbial numbers and diversity 784 during the production cycle of lesser mealworms. The results revealed a high count in substrate, 785 feed, faeces, and exuviae compared to larvae. Likewise, the bacterial diversity was found to be 786 787 reduced during larval rearing, with only certain bacterial species exhibiting a competitive advantage within the insect gut and becoming dominant. Therefore, a blanching treatment was 788 789 conducted after harvesting the larvae, resulting in a reduction of microbial count. However, the number of aerobic endospores remained at 4.0 log cfu/g. Furthermore, fungal isolates 790 corresponding to the genera Aspergillus and Fusarium were recovered in this study. Thus, it cannot 791 792 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.

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

794 Table 7. Microbial characteristics of lesser mealworm-based products

ND: Not Determined

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Microorganisms such as Enterobacteriaceae and lactic acid bacteria have been found in both fresh 796 797 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 edible insects require the absence of specific food pathogens. Further research and development 804 805 are required before these products can be sold in the market.

Another challenge that has been associated with microbial contamination is the problem with 806 spore-forming bacteria that has been found in the edible insects (Garofalo et al., 2019; Walia et 807 808 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 $1.6 - 8.1 \log \text{cfu/g}$ (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).

In a recent study, Roncolini et al. (2020) examined the microbial growth in lesser mealworm 814 815 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 rusks, where a powder containing a mixture of lesser mealworm and 10% - 30% of wheat flour 817 818 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 mealworm may not be a suitable substrate for the growth of these microorganisms, it is still 821 822 important to monitor the presence of spore-forming bacteria in the species.

823 Quality and safety assurance in rearing edible insects

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

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

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The growing interest in the utilization of insects in feed and food in Europe over the past decade 852 853 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 Union, 2017b) amending Regulations EC 999/2001 and EU No. 142/2011, has contributed to this 856 trend. In addition, insects are not only a rich source of nutrients but also considered climate-857 858 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 order to maintain quality and safety standards in the rearing of edible insects and to establish new 860 861 guidelines for the rearing process, there are several reviews and published opinions available in the literature (Lange and Nakamura, 2021; Rumpold and Schlüter, 2013b). Some of these opinions 862 have highlighted data gaps in the field. As a result, current studies are concentrating on identifying 863 food safety hazards that are specifically associated to insects that are raised under controlled 864 condition. This has resulted in the comprehensive understandings and knowledge in providing 865 edible insects that are safe for human consumption. 866

In general, consumers are concerned about the safety and how it may affect their consumption, 867 specifically in terms of potential infectious agents such as viruses and bacteria, as well as 868 869 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 larvae as the concentrations of the insecticides were below the quantification limit. However, the 874 use of Spinosad has shown the significant reduction in the total yield. Spinosad is allowed to be 875 utilized in agriculture, however, its usage raises concerns about the safety of reared insects. 876 Therefore, it is recommended that further studies be conducted on the safety and quality of the 877 878 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 quality) of edible insects can vary depending on the environment in which they are reared and 884 885 harvested (Raheem et al., 2019). As a result, frameworks governing insects-based food have been developed in the past 20 years (Lange and Nakamura, 2021). Furthermore, the type of insect 886 species for farming must adhere to legal regulations pertaining to both food and consumer safety. 887 888 For example, the insect-based food must comply with legal regulations aimed at preventing and eradicating bovine spongiform encephalopathy (BSE). These ordinances also prohibited the use of 889 insects for the feeding of other farmed animals (Żuk-Gołaszewska et al., 2022). 890

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

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production of novel foods requires the adoption of efficient management systems, which include
good breeding and hygiene practices, and the Hazard Analysis and Critical Control Points
(HACCP) system (Awuchi, 2023). Moreover, the regulations impose hygiene and biosecurity

standards that must be fulfilled by all farm buildings and production facilities.

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

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

Rearing facilities

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

microorganisms and exposure to loud noise, both of which could have a negative impact during 924 925 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 Annex II, Chapter I of Regulation (EC) No 852/2004 (European Union, 2004b). 934

Watering systems 935

According to the guidelines issued by IPIFF for the farming of edible insect, watering system is 936 one of the prerequisites in building rearing facilities. A sufficient supply of potable water is 937 938 required to ensure no major problems occur during the production process. Moreover, there should be an adequate quantity of pressurized water available at an appropriate temperature. In addition, 939 940 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).

Sanitary of facilities 944

As per the Annex II of Regulation (EC) No 183/2005 (European Union, 2005) and Annex II, 945 Chapter V of Regulation (EC) No 852/2004 (European Union, 2004b), the facilities and equipment 946 947 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

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

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production cycles is prohibited under Article 9(g) of Regulation (EC) No 1069/2009 (European 967 Union, 2009a) since insect frass is classified as 'Category 2' material. On the other hand, if 968 969 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 moist to dry environments. This step allows for manual or mechanical separation (Rumbos et al., 980 981 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 sanitized to reduce the risk of microbiological contamination. In special cases, such as dealing 987 988 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 are encouraged to monitor the microbiological status of the disposed substrate through sampling 990 measures (IPIFF, 2022). 991

992 8. Consumer acceptance of Alphitobius diaperinus larvae as food

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

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

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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 (2). For the consumer, the consumer acceptance will be discussed according to the 1019 sociodemographic factor (culture, household composition, location and social acceptability) and 1020 individual factor (motivations, taste orientation, gender, and age). Meanwhile, for the product, the 1021 consumer acceptance will be discussed according to product-related factor (availability, product 1022 1023 placement, sociability and ethical aspect) and product properties (taste properties, form, price, and nutritional properties). The summary of the consumer acceptance based on both of "The 1024 Consumer" and "The Product" elements are presented in Table 9. 1025



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

Factors				
	Culture	Westerners are not open to the concept of entomophagy,		
Sociodemographic Factor	Culture	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		
	Social acceptability	as Australia. 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.		
	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.		
Individual Factor	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.		
	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.		
Product Related Factor	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.		
	Taste properties	Consumers prefer an insect-based meal with a neutral taste.		
Product Properties	Form	Prepare insect-based foods with an appealing appearance, such as incorporating edible insects into wheat flour.		
rioudel riopetites	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.		

1030 Table 9. Summary of the factors affecting consumer acceptance towards entomophagy

The consumer 1032

Sociodemographic circumstances 1033

Insects are commonly viewed in a negative way, and they are frequently associated with terms like 1034 'dirty,' 'unhealthy,' and 'disease vectors' (Kröger et al., 2022). Although edible insects have been 1035 declared safe and various campaign as well as promotion have been carried out to improve their 1036 public perception, studies show that many Westerners are still reluctant to include insects in their 1037 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, 2021; Kröger et al., 2022; Lammers et al., 2019). 1043

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

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

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

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Kostecka et al. (2017) also reported that approximately 37% of participants in Poland found 1076 products with processed insects, such as insect flour, to be acceptable for introduction in the market 1077 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 insectbased food, and additional factors need to be considered before commercialization. Factors such 1086 as food disgust, neophobia, and seeking sensation have been identified as predictors of the 1087 acceptance of edible insects, and these factors will be discussed further in the product section 1088 1089 (Mazurek et al., 2022).

Social and cultural norms are also the important factors when it comes to accepting insects as novel 1090 foods (Kröger et al., 2022; Tzompa-Sosa et al., 2023). As demonstrated by Ros-Baró et al. (2022), 1091 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 pathogenic microorganisms and heavy metals, as well as the possibility of allergic reactions from 1096 consuming insects (Vandeweyer et al., 2021). In this case, the EFSA, on July 6, 2022, has issued 1097 a positive opinion on the safety of the lesser mealworm as novel foods, according to Regulation 1098 (EU) 2015/2283 (EFSA Panel On Nutrition et al., (2022). Moreover, in January 2023, the 1099 commission authorized the marketing of lesser mealworm under Regulation (EU) 2023/58 1100 1101 (European Union, 2023). There are few legal frameworks that consider insects as food (Grabowski et al., 2020) and this suggests that promoting edible insects as a food source to populations 1102 unfamiliar with entomophagy would require greater efforts in sensitization and awareness-raising 1103 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.

Individual factor

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

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

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with edible insects. This perspective is primarily attributed to factors such as neophobia and disgust 1120 towards insects, as well as a perception of insects as a threat to traditional masculinity. Moreover, 1121 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 following a vegetarian or vegan diet may have a greater reluctance to consume insects (Kröger et 1128 1129 al., 2022; Onwezen et al., 2021).

The acceptance of insects as food can be significantly influenced by emotions (Kröger et al., 2022). 1130 Most of the studies reported in the literature have focused on emotions associated with disgust or 1131 1132 fear. However, there is one comprehensive study on the influence of emotions in general terms. For example, the feeling of happiness, satisfaction, or pride when thinking about eating insects. 1133 These are defined as positive emotions, while negative emotions can be defined as the feeling of 1134 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.

In Western countries, the concept of entomophagy frequently evokes negative emotional reactions, 1138 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 try insect meat. In this study, participants were shown pictures of hamburgers with a description 1148 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 condition). The researchers found that uncommon products such as shish kebabs were more preferred over common products like burgers. However, the study did not reveal any significant difference in preference between the two meat types (bovine meat and insect meat). According to this study, the influence of health benefits and the factor of disgust had no effect on the consumers' willingness to try the product. This finding revealed the factor on why insect-based products are not as popular as bovine meat products. However, the study also highlights an interesting point that health benefits could potentially be a significant driver for the acceptance and adoption of insect consumption. When developing marketing strategies, it is important to include the health features of insect products such as nutritional content and safety considerations. Additionally, attention should be given to packaging design, labelling, and other elements of product presentation that can affect consumers' perceptions and willingness to try these novel food products (Poortvliet et al., 2019).

The product 1164

Product-related circumstances 1165

The term 'product-related circumstances' refers to the social, practical, and contextual factors that 1166 are associated with insect-based food products (Kauppi et al., 2019). While the price and taste of 1167 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 insect-based food products with familiar flavours, they are willing to purchase and prepare them 1172 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.

As interest in edible insects continues to grow, it raises questions about how consumers perceive 1175 the welfare of insects (Delvendahl et al., 2022). This issue has been a matter of public concern 1176 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 established for vertebrate welfare. It could be argued that establishing guidelines for insect welfare 1182 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.

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

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

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

1209 In addition to price, it is crucial to ensure that edible insects are guaranteed to be of high quality and meet food safety standards. It is noted that the lesser mealworm has been granted novel food 1210 1211 status by the EU as of January 2023, following its safety approval by the EFSA (European Union, 2023). Despite lesser mealworm not being extensively commercialized in European countries yet, 1212 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 by House (2016) among Dutch consumers found that the unavailability of the products in the 1216 market had hindered their intention to purchase (House, 2016). This finding was in agreement with 1217 the studies by Shelomi (2015) who reported that low availability of products resulted in consumers 1218 1219 purchasing less frequently than they would have preferred, leading to passive rejection of the products by potential consumers (Shelomi, 2015). 1220

1221 The growing interest in edible insects over the past decade has led to the emergence of several business owners and enterprises actively involved in insect production. In European countries, the 1222 insect-based production industry primarily focused on breeding insects for biocontrol purposes or 1223 animal feed production. These activities were often carried out in zoological gardens (Mancini et 1224 1225 al., 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), incorporating insect-based foods into human diets not only benefits the environment but also 1232 makes good business sense. They also emphasized the significance of utilizing different marketing 1233 techniques, such as using celebrity endorsements and peer-to-peer advertising. Developing 1234 1235 marketing and advertising strategies for insect-based products and raising public awareness about 0 1236 the entomophagy is crucial. This can be accomplished by launching educational campaigns aimed at farmers to promote awareness of the advantages of edible insects as a substitute for traditional 1237 livestock rearing (Collins et al., 2019; Żuk-Gołaszewska et al., 2022). 1238

The product properties

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

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from lesser mealworm should be refined and separated to achieve a more neutral taste. This could 1251 potentially enhance the usability of insect flour as an ingredient in food. Moreover, Mazurek et al. 1252 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. However, some improvements may be required, such as refining the taste, altering recipes, and 1259 1260 modifying product structure.

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

As lesser mealworms are rich in protein, a high amount (>40%) of substitution may cause the product appearance to become darker, thereby potentially affecting consumer acceptance 1282 (Brynning et al., 2020). In another study, Gantner et al. (2022) evaluated the physicochemical properties of bread incorporated with 5, 10, and 15% of mealworms. The study revealed that a higher addition of mealworms would significantly reduce the intensity of the bread flavor, even at 1284 levels of 10% and 15% addition. This effect was particularly showed in the intensity of the bitter 1285 taste and nutty flavor of the bread samples with a higher amount of mealworms (15%). Sensory 1286 evaluation indicated that the incorporation of mealworms significantly affected the visual 1287 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 amount, as any quantity beyond that threshold would negatively affect the physicochemical 1291 properties and overall sensory quality of the bread. 1292

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

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mealworm as insect flour, was demonstrated by Brynning et al. (2020). The study surveyed 1296 individuals from the Western part of Denmark and found that the main predictor for improving 1297 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.

The food industry is increasingly favoured towards selecting innovative and sustainable raw 1306 materials to produce nutrient-fortified foods, in response to the growing consumer trend for such 1307 1308 products (Hassoun et al., 2022). One way to fortify foods is by adding a specific molecule during the processing stage, while another approach is to use ingredients that are naturally high in the 1309 desired nutrient (Roncolini et al., 2020). In this perspective, using edible insects as food 1310 ingredients can be a promising strategy for fortifying conventional foods, especially to increase 1311 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 integrated into recognizable food items such as bread or meat products. 1316

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 amino acids was observed, with the fortification of histidine reaching up to 129% in rusks. The 1322 addition of lesser mealworm powder has enriched the minerals such as iron (Fe), potassium (P), 1323 1324 and zinc (Zn). Considering the possibility of producing insect-based rusks on an industrial scale, the aforementioned product can be categorized as level 4 on the Technology Readiness Level scale.

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

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

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appearance, and the use of flours has been found to be the most accepted format (Ros-Baró et al., 1340 2022). Recently, Brynning et al. (2020) developed a non-snack food product based on three 1341 different insect species which are house cricket, lesser mealworm, and yellow mealworm. The 1342 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 and vitamin levels. These findings suggest that insect flour could be utilized as a primary 1350 component in the preparation of main meals with some modification in the development kitchen. 1351

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

1364 9. Conclusion and future perspectives

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

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

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

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.

1388 Funding

Financial support from Nobelium Joining Gdańsk Tech Research Community (contract number
 DEC 33/2022/IDUB/1.1; NOBELIUM nr 036236) is gratefully acknowledged.

1391 Author Contributions

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

1398 **Conflict of interest**

1399 The authors declare no conflict of interest.

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33

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