

1 **Review of Improving the Microbiological Safety of Edible Insects using**
2 **Thermal and Non-Thermal Processing Technologies**

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11 **Running title: Processing Technologies for Edible Insects Safety**

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Abstract

28 Edible insects are a promising alternative protein sources in animal-based food and feed industry. However, they
29 can pose a risk to consumer health owing to their potential biological hazards such as pathogens that can cause
30 foodborne illnesses. In this study, the biological hazards of edible insects are considered. Thus, edible insects can be
31 processed using conventional thermal processing or emerging non-thermal processing technologies to inactivate
32 microorganisms. Thermal processing included blanching, boiling, steaming, roasting, and drying. Emerging
33 nonthermal processing technologies, such as high-pressure processing, microfiltration, cold atmospheric pressure
34 plasma, ultrasound, and irradiation can also be applied to edible insects. To ensure the microbiological safety of
35 edible insects during processing, the Hazard Analysis and Critical Control Points (HACCP) model is necessary.
36 Overall, applying appropriate antimicrobial technologies and the HACCP model to edible insect processing can
37 ensure the microbiological safety of edible insects and contribute to further advancements in the insect industry.

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Keywords: Edible insect; Microbial safety; Thermal processing; Non-thermal processing; HACCP

Introduction

40

41 Recently, edible insects have gained considerable global attention as alternative protein sources for both food and
42 animal feed [1]. The global population is expected to increase 9.8 billion people by 2050, and demand for animal-
43 based protein will increase by 70% compared with that for 2020 [2]. International food and sustainability policies,
44 such as the EU farm-to-fork strategy and UN sustainable development goals, highlighted edible insect-based protein
45 as a promising sustainable protein source [1, 3]. Many edible insects have substantial nutritional value, including
46 high proteins, unsaturated fatty acids, fibers, vitamins, and minerals [4]. Moreover, edible insect farming is eco-
47 friendly, because edible insects emit low greenhouse gas levels and use organic waste as feed to reduce the CO₂
48 foodprint [3].

49 Despite these advantages, the edible insect industry should overcome critical limitations, such as consumer
50 acceptance and food safety concerns to meet a broad consumer demand [5]. Similar to other animal-derived foods,
51 edible insects can harbor various biological hazards that pose risks to consumers [6]. They tend to carry high
52 microbial loads owing to the presence of gut microbiota, unsanitary rearing environments, and insufficient
53 processing methods, such as inadequate washing or heat treatment [7]. Insects may carry pathogenic bacteria, such
54 as *Salmonella*, *Staphylococcus*, and *Bacillus* spp., as well as fungi, viruses, and parasites acquired from their
55 environment or feed substrates [6, 8]. Microbial contamination can lead to acute foodborne illnesses if not properly
56 controlled. Therefore, regulators and food scientists have emphasized that edible insect-derived foods must meet
57 microbiological safety standards similar to those of conventional foods [9]. Furthermore, the European Food Safety
58 Authority (EFSA) has examined the risks of insects as food and feed and concluded that potential microbiological
59 hazards depend on the insect species, rearing substrate, and processing technologies used [10]. Although the novel
60 food regulations of the EU now include insects, with several approved species on the market, existing regulatory
61 frameworks still lack provisions that fully address the complexities of insect production [11]. Recent EFSA
62 scientific opinions on insect-based novel foods, including frozen, dried and powdered forms of house crickets and
63 mealworm (2024–2025), further confirm that such products can be considered safe under specified conditions of use,
64 while consistently emphasizing strict hygiene control and the potential for allergenic reactions in susceptible
65 consumers [12, 13]. Public confidence in edible insects depends on rigorous microbiological safety assessments and
66 clear guidelines to ensure that insect-based products are safe for consumption. Notably, the edible insect sector is

67 expected to grow rapidly, with studies predicting a 43% increase in European and North American markets by 2024,
68 making it even more important to actively address microbiological safety and quality issues [8].

69 Appropriate thermal or non-thermal processing technologies are needed to improve the microbial safety of edible
70 insect-based foods. Conventional thermal processing methods, such as blanching, boiling, roasting, and frying, are
71 widely used to reduce microbial loads, dry products, extend shelf life, and enhance flavor attributes in edible insects
72 [14]. Heat processing is applied not only for the improvement of microbial safety, but also for purposes such as
73 drying, extending shelf life, and enhancing flavor; however, excessive heating can also degrade nutrients and
74 negatively impact the sensory qualities of insect products [15]. Meanwhile, non-thermal processing technology is a
75 processing method that does not involve the use of heat, thereby minimizing quality deterioration associated with
76 thermal treatment. Non-thermal processing technologies, such as high-pressure processing (HPP), microfiltration,
77 cold atmospheric pressure plasma (CAPP), ultrasound, and irradiation, inactivate pathogens in insect foods while
78 preserving their nutritional properties [16]. By applying a combination of these technologies, along with strict
79 hygiene controls, the industry aims to produce insect-based foods that are both safe and of high quality.

80 The purpose of this paper is to examine the microbiological hazards associated with edible insects and investigate
81 the microbicidal efficacy of insects using various processing technologies, including thermal and non-thermal
82 processing technologies. Additionally, the Hazard Analysis and Critical Control Points (HACCP) model for
83 microbiological hazards in edible insect processing was reviewed to outline the microbiological safety of edible
84 insects.

85

86 **Biological hazards of edible insects**

87 Various biological hazards, such as viruses, bacteria, fungi, and parasites, must be considered when processing or
88 using edible insects or insect-based food products [6]. Biological hazards are defined by the inherent presence of
89 pathogens and potential for additional contamination risks that may arise during insect rearing or processing [10].

90 According to the EFSA Scientific Committee [10], the biological hazards of edible insects are primarily influenced
91 by the rearing environment and hygiene status of the substrates they consume, such as grain by-products, fruit, and
92 vegetable waste. Additionally, pathogenic bacteria and parasites can be transmitted through insects, and their gut
93 microbiota pose the risk of cross-contamination during rearing and processing [17]. In summary, edible insects may
94 serve as reservoirs or carriers of these hazards depending on their diet and environmental conditions.

95 The potential biological hazards associated with edible insects are summarized in Figure 1. Viruses that infect
96 insects as their hosts generally do not infect humans or vertebrates, and some are considered safe enough for use as
97 agricultural biocontrol agents [18]. However, insect-specific viruses present substantial problems for insect food and
98 feed producers. Additionally, Eilenberg et al. [18] explained that some viruses may enter insect farms through the
99 rearing substrate and have the potential to spread beyond primary production; therefore, preventive processing
100 strategies are necessary. Similarly, parasites have not been observed in farmed insects but have been reported in wild
101 insects and regions where entomophagy is traditionally practiced [10]. Chai et al. [19] suggested that certain
102 trematodes (*Lecithodendridae* and *Plagiorchidae*) might be transmitted through infected insects, potentially causing
103 gastrointestinal infections and inflammation. However, no cases of parasitic infections have been reported in farmed
104 insects, as controlled rearing and processing technologies effectively reduce potential risks and ensure safe
105 consumption.

106 Fungi are another biological hazard associated with edible insects as they can infect insects or be present as
107 contaminants. Entomopathogenic fungi, such as *Metarhizium* species, produce insect-specific toxins that can cause
108 high mortality in farmed insects [20]. Due to insufficient data on their safety for humans and animals, they have not
109 been granted the Qualified Presumption of Safety status [21]. Additionally, edible insects may carry fungi, including
110 molds and yeasts, such as *Aspergillus* and *Penicillium* species, some of which have the potential to produce
111 mycotoxins [7]. Table 1 presents the microbial counts of different raw edible insect species, indicating the presence
112 of yeasts and molds in crickets, mealworms, grasshoppers, and locusts. The detected fungal contamination levels
113 vary depending on species, with mealworms and grasshoppers exhibiting higher yeast and mold counts, reaching 5.7
114 and 8.2 log CFU/g, respectively. In contrast, locust and house crickets showed relatively lower levels, with counts at
115 approximately 5.2 and 4.8 log CFU/g, respectively. These variations indicate that the fungal contamination levels
116 differ among insect species, suggesting that intrinsic biological traits may contribute to these differences.
117 Furthermore, fungal contamination in edible insects varies depending on rearing, processing, and storage conditions
118 [10].

119 Although viruses and parasites are generally insect-specific and rarely associated with human infections, bacteria
120 can be direct indicators of food hygiene and contamination levels. The microbial composition of various raw edible
121 insect species, including total viable counts and Enterobacteriaceae, is summarized in Table 1. These microbial
122 groups are hygiene indicator bacteria that represent the overall microbial quality and potential risk of contamination
123 of edible insects [7]. Monitoring these levels is important for evaluating the effectiveness of processing and storage

124 conditions to ensure food safety. Klunder et al. [22] and Caparros Megido et al. [23] have reported high microbial
125 counts in cricket that was 7.2–7.97 log CFU/g. In contrast, Stoops et al. [24] reported that mealworms had a total
126 microbial count of 8.3 log CFU/g, with a large amount of lactic acid bacteria. *Ruspolia differens* (grasshopper)
127 exhibited the highest microbial counts, particularly Enterobacteriaceae and lactic acid bacteria, indicating the need
128 for hygiene measures [25, 26]. For *Locusta migratoria manilensis*, Ng'ang'a et al. [27] reported a relatively low
129 microbial count, whereas Stoops et al. [24] found significantly higher contamination in mealworm larvae, possibly
130 due to physiological differences and higher moisture content, which may promote microbial growth. Moreover,
131 pathogenic microorganisms have been detected in edible insects that can directly cause foodborne illnesses. Stoops
132 et al. [24] reported significant microbial contamination in mealworm larvae and grasshoppers by detecting
133 *Staphylococcus spp.* and *Clostridium spp.* Similarly, Ssepuyya et al. [26] and Ogbalu and Williams [28] identified
134 foodborne pathogens, such as *Salmonella spp.*, Shiga toxin-producing *Escherichia coli*, *Bacillus spp.*, and
135 *Staphylococcus aureus* in edible insects.

136 Ensuring the microbiological safety of edible insects is necessary at every stage of production, processing, storage,
137 and consumption. In particular, the processing step plays a key role in reducing microbial contamination, improving
138 food safety, and extending shelf life [29]. Therefore, evaluating and validating various processing technologies is
139 essential to ensure their effectiveness in microbial reduction and long-term safety of edible insect products.

140

141 **Thermal processing technologies**

142 Conventional thermal processing is commonly used in food preparation and distribution to reduce microbial loads,
143 and ensure food safety and quality for edible insects [30]. These methods include thermal processing, such as
144 blanching, boiling, steaming, roasting, and drying, which effectively reduce microbial loads by destroying cells and
145 inactivating enzyme activities [14]. Although these thermal processes are highly effective for microbial inactivation,
146 they often use high temperatures that may induce negative effects, such as lipid oxidation, protein denaturation, and
147 undesirable changes in color and flavor [30]. Nevertheless, blanching and drying are the two most important
148 conventional processing steps for edible insects because of their effectiveness in microbial reduction and widespread
149 use [14, 15].

150

151 **Blanching**

152 Blanching is a thermal process widely used to reduce microbial loads and inactivate enzymes in food products,
153 including edible insects [14]. It generally involves briefly immersing food in hot water at temperatures between 50
154 and 100 °C, and quickly immersing it in cold water to stop the thermal reaction [31]. In some cases, blanching is
155 increasingly used as a practical and effective method during the slaughter phase of edible insect processing [32].
156 Rodriguez-Rodriguez et al. [31] suggested that blanching and liquid nitrogen immersion are the most effective
157 slaughter methods in terms of protein digestibility and microbial safety, with blanching favoring cost-effectiveness
158 and accessibility. Furthermore, Larouche et al. [30] and Zhen et al. [33] reported that blanching black soldier fly
159 larvae before drying led to reduced microbial load and improved lipid stability. The results of blanching treatments
160 for the reduction of microorganisms in various edible insect species are summarized in Table 2.

161 Mancini et al. [34] applied various blanching conditions on mealworm, focusing on the temperature range of 50 to
162 90 °C and duration of 2.5 to 5 min. The study showed that blanching at 60 °C for 5 min is the most effective method,
163 reducing microbial loads, including Enterobacteriaceae, *Staphylococcus*, lactic acid bacteria, yeasts, molds, and
164 bacterial spores. Blanching at higher temperatures did not significantly enhance microbiological reduction efficacy
165 compared with what was processed at 60 °C. Additionally, Mancini et al. [34] reported that blanching mealworm at
166 60°C for 5 min inhibited enzymatic browning and improved the appearance and acceptability of mealworm-based
167 products.

168 Caparros Megido et al. [23] investigated the microbiological impact of blanching on edible insects in Belgium,
169 such as mealworms and house crickets. Untreated insects exhibited total aerobic counts that significantly exceeded
170 the safety thresholds established for freshly minced meat. Blanching effectively reduced these counts to below the
171 regulatory limits in the two insect species. Klunder et al. [22] found that blanching or boiling mealworm larvae and
172 house crickets substantially decreased Enterobacteriaceae counts. However, bacterial spores in edible insects
173 survived blanching. They observed that a short boiling step was effective in eliminating Enterobacteriaceae,
174 confirming that blanching significantly reduces total microbial counts in edible insects. However, their research
175 suggests the need for complementary methods, such as drying or acidification, to manage bacterial spores that
176 survive blanching.

177 These studies confirmed the effectiveness of blanching as a crucial conventional method for reducing
178 microbiological hazards in edible insects. However, blanching conditions, such as time and temperature, must be
179 carefully selected based on insect species, size, and intended product characteristics to optimize microbial safety
180 without negatively affecting sensory qualities [34].

181

182 **Thermal drying**

183 Drying is an essential post-harvest operation in edible insect processing that can reduce water activity (a_w),
184 suppress microbial growth, and stabilize products for extended storage and further use [35]. Owing to their high
185 moisture content and nutrient-rich composition, including proteins, lipids, and amino acids, insects are highly
186 susceptible to microbial growth [22]. Without proper moisture removal, edible insects can become a source of
187 pathogenic and spoilage microorganisms [36]. Multiple drying technologies have been applied to edible insects,
188 including conventional thermal methods, such as oven and solar drying, and advanced electromagnetic methods,
189 such as microwave and radio frequency drying [37]. The efficiency of these technologies depends on process
190 parameters, insect species, and life stages. A comparative overview of these drying processes, including their
191 experimental conditions and the resulting microbial reductions in different insect species, is provided in Table 3.
192 Solar drying, a traditional drying method that uses sunlight and heat, has significant limitations in industrial-scale
193 edible insect production [38]. Edible insects are exposed to soil and air, therefore, they are vulnerable to external
194 factors, such as cross-contamination [15]. Nyangena et al. [25] reported limited microbial reduction and slight
195 increases in the total viable counts of edible insects after solar drying. In contrast, Nyangena et al. [25] reported that
196 oven drying at 60 °C for 2–3 days significantly reduced total viable counts in all tested edible insect species,
197 including *Hermetia illucens* (black soldier fly), house cricket, *Spodoptera littoralis* (African cotton leafworm), and
198 grasshopper. The total viable counts in edible insects reduced from 0.6 to 2.3 log CFU/g after oven drying. This
199 suggests that oven drying is more effective than solar drying at reducing microbial counts. Furthermore, Bawa et al.
200 [39] demonstrated the efficacy of oven drying at 80 °C based on microbial reduction in house crickets. Specifically,
201 the total aerobic count of house crickets significantly decreased from an initial 7.60 to 4.73 log CFU/g after drying.
202 Additionally, reductions in yeast and molds of house crickets were observed from an initial level of 4.80 to 2.70 log
203 CFU/g, indicating the effective control of spoilage organisms through this drying method. Therefore, oven drying is
204 widely used in the industrial processing of edible insects [36].

205 Compared to conventional thermal processing, such as solar and oven drying, microwave and radio frequency
206 drying use electromagnetic energy [40]. However, they require specific equipment, operating costs, and a complex
207 system design compared with those required for solar and oven drying [40, 41]. Nevertheless, these drying methods
208 have advantages, such as reduced processing time and improved microbial reduction [41]. For example, Bawa et al.
209 [39] applied microwave drying at 840 W until the core temperature of house crickets reached 161 to 165 °C. This

210 method led to a reduction in total aerobic counts in house crickets, decreasing from 7.60 to 4.00 log CFU/g.
211 Furthermore, the counts of *B. cereus* in house crickets were reduced from 4.17 to 1.40 log CFU/g. These results
212 suggest that microwave drying provides superior microbial reduction compared to that with conventional oven and
213 solar drying, while also reducing the processing time. Vandeweyer et al. [42] showed that radio-frequency drying
214 significantly reduced the total viable counts of black soldier fly larvae and eliminated detectable levels of *B. cereus*.
215 Compared to microwave drying, radio-frequency drying provides more uniform heating and better penetration,
216 making it suitable for larger volumes of edible insect material [40].

217

218 **Non-thermal processing technology**

219 Non-thermal food processing technologies comprise a range of physical and physicochemical methods that
220 inactivate pathogens and spoilage microorganisms without relying on the high temperatures used in conventional
221 thermal processing [43]. Emerging non-thermal processing technologies include HPP, microfiltration, CAPP,
222 ultrasound, and irradiation. Recently, non-thermal processing technologies have received increasing attention owing
223 to their antimicrobial effects, which enhance microbiological safety while not substantially changing food quality,
224 particularly by avoiding the heat-induced degradation of sensitive compounds [16]. Unlike thermal processing, non-
225 thermal processing technologies operate at or near ambient temperatures, which helps minimize oxidation and
226 nutrient loss, especially in lipid-rich foods, such as edible insects [43]. Lipid oxidation plays a critical role in
227 reducing the shelf life and sensory quality of insect-based foods. By reducing thermal exposure, non-thermal
228 processing technologies can effectively reduce oxidative degradation, thereby helping to extend shelf life while
229 preserving the nutritional and sensory characteristics of food [37, 44].

230 However, there are some limitations to the application of non-thermal processing to edible insects. For example,
231 the regulatory framework for both edible insects and emerging nonthermal processing technologies is still
232 developing. There are limited regulations for applying treatments, such as irradiation or CAPP to insect-based foods,
233 and food safety agencies, such as the FDA have not yet issued formal standards for insect products [11]. This
234 limited regulatory guidance and the need for further validation of the efficacy of these technologies mean that non-
235 thermal processing of edible insects is not currently widely established commercially [11]. Ongoing research and
236 policy development will be crucial to address these hurdles, such as reducing costs, simplifying equipment, and

237 establishing safety standards, to establish the benefits of microbial reduction in edible insects using non-thermal
238 processing technologies on an industrial scale.

239 Freshly harvested insects, such as potential pathogens, often carry high microbial loads. Therefore, edible insect
240 producers require an effective processing step before distribution and consumption [10]. Emerging non-thermal
241 processing technologies have shown the potential to replace conventional thermal steps in insect processing and
242 have been tested for microbial decontamination, enzyme inactivation, and extraction of insect products [37]. The
243 effects of various nonthermal processing technologies on the microbiological safety of different edible insect species
244 are summarized in Table 4. The table summarizes the treatment conditions, sample types, and extent of microbial
245 reduction achieved by each technology, demonstrating their potential to improve food safety without the use of heat.

246

247 **HPP**

248 HPP is a non-thermal processing technology that inactivates microorganisms in food by applying extremely high
249 pressure without significant heating [45]. In HPP, food is usually pre-packaged in water-resistant materials, such as
250 plastic films. It is placed inside a pressure vessel and immersed in a pressurizing medium, typically water, which
251 transmits pressure uniformly throughout the product [46]. The uniform and instantaneous isostatic pressure causes
252 lethal damage to microbial cells by disrupting cell membranes and denaturing essential proteins and enzymes. In
253 practice, HPP treatments typically in the range of 400–600 MPa for a few minutes are sufficient to inactivate most
254 vegetative pathogenic and spoilage microorganisms without substantial heating, whereas bacterial spores are highly
255 pressure-resistant and generally require 600–1200 MPa with heating for effective inactivation [45]. However, the
256 sensory and nutritional qualities of the food are largely preserved [47]. Bacterial spores can survive pressure
257 treatment alone, therefore, complete inactivation of spore-forming bacteria requires the use of combined strategies,
258 such as heat-assisted HPP or another pretreatment. However, it is mainly effective against actively growing bacteria
259 [48]. Bacterial spores can survive pressure treatment alone, therefore, complete inactivation of spore-forming
260 bacteria requires the use of combined strategies, such as heat-assisted HPP or another pretreatment [48].

261 Several studies have investigated HPP for microbial reduction in edible insects and insect-based products.
262 Campbell et al. [49] investigated the effects of HPP on the microbial safety of black soldier fly larvae by applying
263 HPP at 600 MPa for 1.5 or 10 min. The untreated samples exhibited high microbial loads, with total viable counts at
264 7.97 log CFU/g, Enterobacteriaceae at 7.65 log CFU/g, lactic acid bacteria at 6.50 log CFU/g, and yeasts and molds
265 at 5.07 log CFU/g. HPP at 600 MPa for 1.5 min resulted in a minimal reduction in total viable counts in black

266 soldier fly larvae, but it significantly reduced Enterobacteriaceae to 2.09 log CFU/g and showed lactic acid bacteria,
267 yeasts, and molds below detectable levels (<2 log CFU/g). When extended to 10 min, HPP further reduced the total
268 viable counts to 6.67 log CFU/g, while maintaining the effective reduction of other microorganism groups.
269 Campbell et al. [49] suggested that the limited effect of HPP on the total viable counts of black soldier fly larvae
270 was likely due to pressure-resistant spore-forming bacteria. They concluded that HPP alone, at least under these
271 conditions, may not be as cost-effective as heat for large-scale decontamination of black soldier fly larvae unless it
272 confers other benefits, such as better nutrient preservation. These results are similar to those reported by Kashiri et al.
273 [50] who found that HPP at 400 MPa for 2.5 to 7 min can inactivate total aerobic bacteria and *E. coli* O157:H7 in
274 black soldier fly larvae. In their study, treatment at 400 MPa completely inactivated yeasts and molds in black
275 soldier fly larvae. In contrast, the total aerobic bacterial counts in black soldier fly larvae decreased by only
276 approximately 0.3–0.4 log even after the maximum treatment of 400 MPa for 7 min. However, Kashiri et al. [50]
277 also demonstrated that HPP was highly effective against the non-spore-forming bacterium *E. coli* O157:H7 in black
278 soldier fly larvae, achieving a reduction of more than 5 log at 400 MPa for 7 min. HPP can effectively inactivate
279 non-spore-forming bacteria, such as *E. coli*, in edible insects; however, further research is needed to inactivate
280 spore-forming bacteria when applying HPP.

281 Ojha et al. [37] applied HPP to mealworm paste, a homogenized insect product that can be used as an ingredient
282 or food product base. In a study by Ojha et al. [37], mealworm paste was treated with HPP at 600 MPa for 5 min and
283 microbial counts were confirmed during refrigerated storage under various packaging conditions. The microbial load
284 of the mealworm paste was significantly reduced after HPP treatment. Specifically, the number of anaerobic
285 mesophilic bacteria in the paste decreased by approximately 2 log CFU/g. Moreover, yeasts and molds were reduced
286 to below detectable levels. However, HPP did not affect mesophilic bacterial spores during storage. These findings
287 are consistent with previous research indicating that HPP is highly effective against actively growing microbial cells
288 and fungi but not effective in inactivating bacterial spores [48, 51, 52]. The application of modified atmospheric
289 packaging, particularly under oxygen-free conditions, successfully suppressed spore growth and delayed spoilage
290 after storage for 14 days. Overall, Ojha et al. [37] concluded that combining HPP with modified atmospheric
291 packaging extends the microbiological safety of mealworm paste products and helps maintain their physicochemical
292 quality during refrigerated storage.

293 Another recent study investigated the use of HPP on processed insect fractions such as protein extracts. Sweers et
294 al. [45] investigated the effects of HPP on the soluble protein fractions of house crickets. In their experiment, cricket

295 protein solutions were treated with HPP at 200, 400, and 600 MPa for 10 min, and the results were compared with
296 those of a traditional blanching treatment (90 °C for 10 min). Total aerobic bacteria in the house cricket soluble
297 protein fractions were significantly reduced by 2 log CFU/mL after HPP at 400 and 600 MPa for 10 min. Moreover,
298 the yeasts and molds in the protein solution were reduced to below detectable levels. Compared to HPP, blanching
299 resulted in a greater reduction in the number of total aerobic bacteria in the soluble protein fractions. However, HPP
300 better preserved the functionality of the protein than blanching. The pressurized proteins retained their secondary
301 structure, and solubility, emulsifying, and foaming capacities compared to those with the blanching treatment. The
302 degree of protein denaturation was significantly lower than that of thermal blanching, which caused enzyme
303 inactivation and loss of functionality. Sweers et al. [45] suggested that although HPP may be less effective in
304 microbial reduction than heat processing, it is a preferable non-thermal processing technology for processing heat-
305 sensitive insect protein ingredients, ensuring both microbial safety and functionality.

306

307 **Microfiltration**

308 Microfiltration is a membrane-based technology that physically removes microorganisms from liquid food
309 streams through size exclusion [53]. It uses a porous membrane which are approximately 0.1–0.2- μ m pore size for
310 sterilization purposes. This process induces a pressure-driven flow to separate suspended microbes and particulates
311 from dissolved nutrients and proteins [54]. Microorganisms and other large particles are retained on the feed side of
312 the membrane, whereas water and soluble components, including proteins, pass through the filtrate [54]. This
313 process, which is commonly researched in the dairy industry, extends shelf life by significantly reducing microbial
314 loads through cold processing, while preserving the sensory and nutritional qualities of the product [53]. By
315 avoiding heat, microfiltration preserves the native structure and quality attributes of food proteins, making it an
316 attractive alternative to thermal treatment [55]. However, because it is a wet fractionation technology, microfiltration
317 is applicable only to fluid or slurry products and not directly to whole insects [54]. Edible insects are processed into
318 liquid extracts or suspensions for microfiltration. Recent studies have investigated the application of microfiltration
319 to soluble protein fractions derived from edible insects as a strategy to achieve microbial safety without inducing
320 protein denaturation.

321 In a recent study by Sweers et al. [54], a microfiltration with a 0.2- μ m membrane was applied to soluble protein
322 fractions of house crickets and lesser mealworm (*Alphitobius diaperinus*). The process achieved complete removal
323 of viable microorganisms from the filtrate. Total viable counts in the insect extracts (pH 3 and 8) decreased from

324 initial levels of approximately 4.6–6.9 log CFU/mL to below detection limits (<1 log CFU/mL) in the microfiltered
325 permeate. This showed a >5–6 log CFU/mL reduction in microbial load, resulting in the sterilization of the protein
326 fraction. These microbial effects correspond with the results from other liquid food systems, showing significant
327 reductions in total bacterial counts following microfiltration. For example, Panopoulos et al. [56] reported that
328 microfiltration with a 1.4- μ m ceramic membrane significantly reduced mesophilic microflora in ovine milk,
329 supporting the broader concept that microfiltration is an effective and mild decontamination technology. Sweers et
330 al. [54] confirmed that no colonies grew on plates containing microfiltered soluble protein fractions of house
331 crickets and lesser mealworms, suggesting that microfiltration ensures microbiological safety. In addition to
332 microbial safety, microfiltration has less of an impact on the nutritional and functional qualities of insect proteins
333 than that with conventional thermal processing. This process imposes no severe heat or chemical stress, therefore,
334 the proteins in the permeate remain largely in their native states [53]. For example, Sweers et al. [54] observed that
335 microfiltered insect protein fractions retained desirable functional properties, such as protein solubility and foaming
336 capacity.

337 However, microfiltration also has several practical limitations when applied to edible insects. Membrane fouling,
338 which can be caused by the deposition and accumulation of solids, lipids, and protein aggregates on or within
339 membrane pores, poses a considerable limitation, as it substantially increases flow resistance and leads to a decline
340 in permeate flux during microfiltration [55]. In a study by Sweers et al. [54], protein recovery of house crickets and
341 lesser mealworms in the permeate was only 14–43%, with more than half of the protein mass being retained on the
342 membrane or in the retentate. This fouling-induced loss implies that a significant portion of the nutrients remained in
343 the retentate, lowering the yield of the microfiltered fraction. Additionally, the microfiltration of insect fractions
344 remains a novel processing approach that has only been explored at the pilot scale and current research is still
345 limited. Specialized equipment and careful control of operating parameters, such as pressure, flow rate, and
346 membrane cleaning are required to handle insect suspensions. As with any membrane process, operational costs,
347 such as membrane replacement, energy for pumping, and throughput limitations, must be evaluated against the
348 benefits.

349

350 **CAPP**

351 CAPP generates partially ionized gases containing energetic electrons, charged ions, UV photons, and reactive
352 chemical species at ambient temperature and pressure [47]. These reactive oxygen and nitrogen species, such as O,

353 O₃, •OH, NO, and NO₂, attack microorganisms by oxidizing cellular components and disrupting vital functions [57].
354 Mechanistically, CAPP generates UV photons that induce direct DNA damage, whereas radicals cause lipid
355 peroxidation of cell membranes and the oxidative modification of proteins and nucleic acids [57]. The presence of
356 certain reactive species is associated with enhanced microbicidal activity. For example, Rumpold et al. [47]
357 observed that nitrogen oxides generated in air-based plasma improved the inactivation of surface bacteria in
358 mealworm. Moreover, CAPP has demonstrated efficacy against bacteria, yeasts, and even hardy forms, such as
359 bacterial spores and viruses, making it a promising technology for improving food safety [58]. However, the
360 inactivation of microorganisms and their mechanisms vary depending on the discharge method, voltage, and power
361 settings of the plasma device [57].

362 The application of CAPP to edible insects resulted in significant reductions in the microbial loads on both whole
363 insects and insect-derived products. Bußler et al. [58] investigated CAPP for insect processing by treating mealworm
364 powder with a surface dielectric barrier discharge-type CAPP. The results showed that the total viable count in the
365 mealworm powder, initially 7.72 log CFU/g, was reduced to 4.73 log CFU/g after 15 min of plasma exposure nearly
366 a 3 log cycle reduction. To compare the efficacy of different types of CAPP, Rumpold et al. [47] applied direct and
367 indirect CAPP to whole mealworm larvae. A high-power remote (indirect) plasma system was applied to the
368 mealworm larvae for 10 min and inactivated to the detection limit on the insect surface, corresponding to a reduction
369 of approximately 7 log CFU/g in total viable counts on the mealworm larval surface. In contrast, a low-power direct
370 jet-type CAPP applied to the larvae had less decontamination effect than indirect plasma owing to the limited
371 plasma penetration and shadowing of microbes. These results suggest that CAPP can be an extremely potent surface
372 sanitizer when exposed to a sufficiently energetic plasma. However, because CAPP primarily acts on the surface, it
373 has little effect on the microbes in the guts of edible insects. Rumpold et al. [56] noted that CAPP did not
374 significantly reduce the overall bacterial load of mealworms when considering the internal microbiota.

375 Pina-Perez et al. [59] applied surface microdischarge CAPP to house cricket powder and observed significant
376 reductions with a minimal impact on sensory quality. The number of total mesophilic bacteria in house cricket
377 powder was lowered by approximately 1.6 log CFU/g and the Enterobacteriaceae count by 1.9 log CFU/g. The
378 CAPP treatment was maintained at a low intensity to avoid oxidative damage to the lipids of the cricket powder.
379 Accordingly, no significant increase in triglyceride degradation or free fatty acids was observed at 22 mW/cm.
380 However, colonies of *B. cereus*, *B. subtilis*, and *B. megaterium* which are spore-forming bacteria, were isolated from
381 house cricket powder, and their spores survived plasma exposure, even at the highest power density applied. This

382 finding is similar to that of earlier reports showing that bacterial endospores are the most CAPP-tolerant
383 contaminants in dry foods. For example, Kim et al. [60] found that CAPP alone achieved a 2.5 log CFU/g reduction
384 of *Aspergillus flavus* spores on red pepper powder, but inactivation of *B. cereus* spores required applying the plasma
385 treatment with a blanching at 90°C.

386 In addition, applications in edible insects and studies on dry food matrices have demonstrated the efficacy of
387 CAPP. Although CAPP can induce rapid microbial inactivation on surfaces, further reductions are limited due to
388 factors, such as shadowing, matrix shielding, and limited penetration depth [62]. In an experiment on sprouting
389 seeds, Butscher et al. [62] achieved up to 3.4 log CFU/g reduction of *E. coli* on cress seed surfaces with atmospheric
390 dielectric barrier discharge plasma, but could not completely sterilize the seeds because of the rough seed coat
391 surface-sheltering bacteria. Similar structural barriers in insect powder or whole insects may protect microbes from
392 embedded sites [47]. Active species of CAPP act primarily on the surface, internal microorganisms, such as those in
393 insect guts, may remain unaffected. Additionally, bacterial spores demonstrate resistance to CAPP, which requires a
394 combination of strategies to achieve complete inactivation. Therefore, optimizing the treatment parameters and
395 integrating CAPP with complementary technologies, such as thermal steps or fluidization systems, is essential to
396 overcome the current limitations and enable safe application in the edible insect food industry.

397

398 **Ultrasound**

399 Ultrasound treatment is a non-thermal food-processing technology that uses high-power sound waves above the
400 human hearing range to inactivate microorganisms [62]. In food processing, power ultrasound (20–100 kHz) induces
401 acoustic cavitation by causing rapid formation and collapse of microscopic bubbles in a liquid medium [63].
402 Cavitation generates intense conditions that physically disrupt microbial cells by damaging their membranes and
403 internal structures [64]. The collapse of cavitation bubbles produces shock waves and shear forces that can damage
404 cell walls and membranes, creating pores, and causing cell lysis [63]. This process simultaneously triggers a
405 chemical reaction. Extreme differences in pressure and temperature lead to the ultrasonic breakdown of water
406 molecules and formation of reactive free radicals, such as hydroxyl radicals and hydrogen peroxide, which oxidize
407 cellular components [64].

408 The potential of ultrasound for microbial reduction in edible insects was demonstrated. Bogusz et al. [62]
409 investigated the use of ultrasound as a pre-treatment for *Zophobas morio* (superworm). In their study, fresh larvae

410 were sonicated in a water bath at 37 kHz for 30 min with the water at 50°C, then freeze-dried. This ultrasound
411 treatment achieved a reduction of approximately 2 log CFU/g in the total viable count of the superworms. However,
412 spore-forming bacteria were largely unaffected by treatment, with their levels remaining at approximately 1.3 to 1.6
413 log CFU/g. Nevertheless, effectiveness of ultrasound for microbial inactivation has been demonstrated in numerous
414 foods. Ultrasound pasteurization of fruit juices has been reported to inactivate *E. coli* by approximately 3–5 log
415 cycles with minimal heat, particularly when applying mild heat or longer sonication times [65]. In meats and animal
416 products, ultrasound is studied for surface decontamination and inhibits bacterial growth on poultry and beef by
417 damaging cell membranes, although penetration into tissue is limited [66]. However, the degree of inactivation
418 varies depending on the product and organism. Studies on the effects of ultrasound on the microbiological safety of
419 edible insects are limited. Therefore, research on the application of ultrasound to edible insects is required.
420 Moreover, bacterial spores in various food matrices often show only minimal log reduction under ultrasound alone
421 [64]. Current research emphasizes optimizing ultrasound parameters, such as frequency, intensity, time, and
422 temperature, and combining ultrasound with other hurdles to enhance its lethality against resistant targets [63].

423

424 **Irradiation**

425 Irradiation is a food processing method that uses ionizing radiation to inactivate microorganisms and enhance
426 food safety [67]. Irradiation involves the use of gamma-rays, electron beams, and X-rays. Gamma rays are emitted
427 from radioactive isotopes, such as cobalt 60 or cesium 137, and high-energy electron beams are generated by
428 particle accelerators. X-rays are produced by striking a metal target with high-energy electrons [68]. These methods
429 differ in radiation sources, penetration depths, and suitability for different food types. Ionizing radiation inactivates
430 microorganisms via direct and indirect mechanisms. The direct effect involves the destruction of microbial DNA,
431 whereas the indirect effect occurs when radiation interacts with water molecules and generates reactive species, such
432 as hydroxyl radicals ($\bullet\text{OH}$), which contribute to microbial inactivation [69]. Importantly, they do not leave residues
433 or render food radioactive, as confirmed by international agencies. Moreover, irradiation does not affect nutritional
434 quality or sensory attributes when applied properly [69]. Joint FAO/WHO/IAEA expert committees have concluded
435 that foods irradiated with up to a dose of 10 kGy are safe for consumption and even higher doses can be used for
436 specific purposes without toxicological risks [68]. Many countries permit the irradiation of various foods to enhance
437 their safety and shelf life, although consumer acceptance depends on clear communication of its benefits and safety
438 [68].

439 Irradiation substantially reduces the microbial load across a range of insect species. Peguero et al. [67] showed
440 that low-energy electron beams achieved a 4 log CFU/g reduction in *E. coli* in dried black soldier fly larvae and a 4
441 log CFU/g reduction in the total viable counts of yellow mealworm products. Similarly, Chen et al. [70] reported
442 that gamma irradiation doses of 10 and 15 kGy on freeze-dried queen bee larval powder completely eliminated total
443 viable bacteria, reducing the initial 4.96 log CFU/g to undetectable levels. A lower dose of 5 kGy produced a 2 log
444 cycle reduction in the total viable cell count, indicating that higher doses are required to achieve pasteurization in
445 insect products. In addition to microbial safety, irradiation can induce physicochemical changes in insect
446 components.

447 Despite the extensive use of irradiation in many food sectors, including spices, fruits, meat, and seafood, its
448 application to edible insects remains largely unexplored in regulatory practices. While over 60 countries permit the
449 irradiation of various food products, none explicitly approved irradiation for the microbial decontamination of edible
450 insect products [21]. Promising laboratory-scale results have demonstrated a 3–5 log CFU/g microbial reduction
451 with minimal quality impact [67, 70, 71]. Future research should focus on developing standardized irradiation
452 protocols for edible insects. This includes defining the optimal dose ranges that maximize microbial inactivation
453 while minimizing adverse effects on sensory attributes, such as flavor, texture, and color, which can improve
454 consumer acceptance [70].

455

456 **HACCP model for microbiological hazards in edible insect** 457 **processing**

458 The HACCP system is an internationally recognized approach for ensuring food safety through the identification
459 and control of hazards at specific stages of food production [10]. HACCP involves identifying specific hazards and
460 instituting controls at critical points in the production process to eliminate or reduce these hazards to safe levels.
461 Microbial hazards are a major concern in edible insect processing, as insects are consumed as a whole, including the
462 gut, which can carry a high microbial load [72]. Pathogenic bacteria, including *Salmonella spp.*, *L. monocytogenes*,
463 and *E. coli*, as well as heat-resistant spores of *Bacillus* and *Clostridium* species, are among the most critical
464 microbiological hazards associated with edible insect products [10]. It is important to distinguish between thermal
465 processing and drying, as both involve heat, but serve different purposes in microbial control. Thermal processing

466 primarily aims to eliminate pathogens through direct heat exposure. In contrast, drying, including oven drying,
467 microwave drying, and freeze-drying, primarily reduces water activity (a_w) and prevents microbial growth. High-
468 temperature oven drying can reduce the microbial load to some extent, but it does not serve as the primary pathogen
469 elimination step. Therefore, it is classified separately in the HACCP analysis [10].

470 The HACCP model applied to edible insect processing, detailing microbial hazards, critical control points (CCPs),
471 and corresponding control measures, is presented in Table 5. These control points are essential to reduce
472 microbiological risks and ensure compliance with food safety standards. As summarized in Table 5, microbial safety
473 during edible insect processing is primarily maintained through two CCPs: thermal processing and drying. Thermal
474 processing, such as blanching, is a key step in eliminating bacterial pathogens. Blanching insects at 100°C for at
475 least 5 min significantly reduces microbial loads, with over 12 log CFU/g reductions in pathogens, including
476 *Salmonella* and *L. monocytogenes* [72, 73]. However, bacterial spores, including those of *B. cereus* and *Clostridium*
477 *perfringens*, demonstrate greater resistance to heat, requiring supplementary control measures in later processing
478 stages [10]. The second CCP, drying, plays a crucial role in microbial stability by reducing the water activity below
479 0.60, thereby preventing microbial growth during storage [72].

480 Although the identified CCPs significantly mitigate microbial hazards, prerequisite programs, such as Good
481 Hygiene Practices (GHP) and Good Manufacturing Practices are essential at the non-CCP stages to prevent
482 recontamination. Preprocessing steps, such as harvesting and washing, do not qualify as CCPs but must still be
483 carefully managed. For instance, sieving and washing insects with potable water can reduce external contaminants
484 but does not eliminate pathogens inherent to the gut microbiota [10]. In summary, microbial safety during edible
485 insect processing relies on a combination of preventive and critical control measures. Maintaining proper hygiene in
486 the early stages of insect production, including controlled feeding and rearing conditions, helps minimize pathogen
487 introduction, but does not eliminate all risks [74]. To ensure food safety, processors establish CCPs, such as heat
488 treatment to destroy pathogens and drying to enhance shelf stability [10]. These CCPs require careful monitoring of
489 the temperature, time, and moisture levels to maintain their effectiveness. Additionally, strict sanitation and hygiene
490 handling practices are essential to prevent recontamination. International food safety frameworks, including the
491 Codex Alimentarius and other regulatory guidelines, provide the foundation for HACCP implementation in edible
492 insect processing [73]. Recent studies have consistently identified thermal processing and drying as the key steps in
493 microbial control. By adopting HACCP principles and focusing on scientifically established CCPs, the edible insect
494 industry can produce foods that are both nutritious and microbiologically safe.

495

496

Challenges and Conclusion

497 Edible insects have substantial potential as a sustainable food and feed source. However, there are several
498 challenges to improving the microbiological safety of edible insects. Conventional thermal processing and emerging
499 non-thermal processing technologies can significantly reduce the microbial load in edible insects. However, high
500 microbial loads of edible insects are often carried out during processing, which requires more accurate and effective
501 decontamination steps [75]. For example, Van Campenhout and Eilenberg [75] suggested that contaminated rearing
502 substrates, such as Salmonella-infected flour could directly transmit pathogens into edible insects. Therefore, insects
503 should be reared and harvested under hygienic conditions to minimize the total microbial load during processing. In
504 summary, the initial microbial and chemical qualities of insects can be improved through improved farm and
505 substrate management. Achieving low starting counts, in turn, makes each processing step more effective.

506 Furthermore, there is a limitation to single processing for reducing the high initial microbial loads of edible
507 insects. Many previous studies have shown the efficacy of single processing; however, further studies are needed on
508 the microbicidal efficacy of multi-hurdle processing, which involves a combination of several processing
509 technologies. For example, Cruz-Garcia et al. [76] suggested that drying alone fails to suppress microbes to safe
510 levels; however, pretreating insects before drying considerably enhances microbial reduction. Similarly, combining
511 HPP and CAPP with mild heating or modified atmosphere packaging in multi-hurdle processing can inactivate
512 resistant spores in seafood that a single processing technology cannot fully eliminate [77]. This indicates the need
513 for a multi-hurdle processing approach to ensure effective decontamination. Moreover, the chemical safety of edible
514 insects must be considered to ensure microbiological safety. Recent studies have shown the presence of heavy metal
515 contamination in insect foods, suggesting that thermal processing, fermentation, or irradiation can help manage these
516 toxins [76]. However, standardized data are still required to determine how each processing technology affects
517 chemical hazards. For example, the EFSA noted that the extent to which contaminants are transferred from feed to
518 insects remains largely unknown [10]. In addition, this approach requires validation on a commercial scale; however,
519 research has been limited to laboratory or pilot-scale studies.

520 To use edible insects as an alternative protein source, it is crucial to improve feed and rearing practices, and
521 validate multi-hurdle processing technologies on a commercial scale. The application of strict hygiene controls,
522 validated processing methods, and insect-specific safety regulations are instrumental in ensuring consumer safety

523 and advancing the edible insect industry. These measures will help to establish the potential of edible insects as
524 sustainable and safe sources of dietary proteins.

525

526

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529

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Tables and Figures

740

741 **Table 1. Microbial counts of raw edible insects by species**

Edible insect	Species	Microbial counts	Referencess
Cricket	<i>Acheta domesticus</i>	<ul style="list-style-type: none"> ◦ Total viable counts: 7.2 log CFU/g ◦ Enterobacteriaceae: 4.2 log CFU/g ◦ Bacterial spores: 3.6 log CFU/g 	[22]
		<ul style="list-style-type: none"> ◦ Total aerobic counts: 7.97 log CFU/g ◦ Yeasts and molds: 4.80 log CFU/g 	[23]
Mealworms	<i>Brachytrupus sp</i>	<ul style="list-style-type: none"> ◦ Total viable counts: 6.7 log CFU/g ◦ Enterobacteriaceae: 4.4 log CFU/g ◦ Bacterial spores: 4.4 log CFU/g 	[22]
		<ul style="list-style-type: none"> ◦ Total aerobic counts: 8.58 log CFU/g ◦ Yeasts and molds: 4.70 log CFU/g 	[23]
Grasshopper	<i>Tenebrio molitor</i>	<ul style="list-style-type: none"> ◦ Total viable aerobic counts: 8.3 log CFU/g ◦ Enterobacteriaceae: 7.6 log CFU/g ◦ Lactic acid bacteria: 7.6 log CFU/g ◦ Bacterial endospores: 3.5 log CFU/g ◦ Yeasts and molds: 5.7 log CFU/g 	[24]
		<ul style="list-style-type: none"> ◦ Total viable counts: 9.1 log CFU/g ◦ Yeasts and molds: 8.2 log CFU/g 	[25]
Grasshopper	<i>Ruspolia differens</i>	<ul style="list-style-type: none"> ◦ Total viable counts: 8.9 log CFU/g ◦ Enterobacteriaceae: 7.3 log CFU/g ◦ Lactic acid bacteria: 8.6 log CFU/g ◦ Aerobic bacterial endospores: 4.3 log CFU/g 	[26]

		<ul style="list-style-type: none"> ◦ Yeasts and molds: 6.4 log CFU/g 	
	<i>Locusta migratoria manilensis</i>	<ul style="list-style-type: none"> ◦ Total viable counts: 5.9 log CFU/g ◦ Enterobacteriaceae: 5.7 log CFU/g ◦ Aerobic bacterial endospores: 6.3 log CFU/g ◦ Yeasts and molds: 5.2 log CFU/g 	[25]
Locust	<i>Locusta migratoria manilensis</i> larvae	<ul style="list-style-type: none"> ◦ Total viable counts: 8.2 log CFU/g ◦ Enterobacteriaceae: 7.4 log CFU/g ◦ Lactic acid bacteria: 8.1 log CFU/g ◦ Aerobic bacterial endospores: 3.6 log CFU/g ◦ Yeasts and molds: 5.2 log CFU/g 	[24]

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743 **Table 2. Blanching treatment to inhibit microbes in edible insects**

Insect species	Life stage	Blanching condition	Main findings	Reference
Mealworm (<i>Tenebrio molitor</i>)	Larvae	Submerged in water at 50–90 °C for 2.5–5 min	<ul style="list-style-type: none"> ◦ Microbial reduction and browning prevention are most effective at 60°C for 5 min. ◦ Higher temperatures (70–90 °C) further reduced microbes but caused darker color changes. 	[34]
House cricket (<i>Acheta domesticus</i>)	Adult	Submerged in hot water (99 °C) for 4 min	<ul style="list-style-type: none"> ◦ Effectively reduced microbial, yeast, and mold counts. ◦ Total aerobic counts: 7.97 → 4.39 log CFU/g (3.58 log reduction) 	[23]
Mealworm (<i>Tenebrio molitor</i>)	Larvae	Submerged in boiling water for 10 min	<ul style="list-style-type: none"> ◦ Total viable counts: 7.7 → <1.7 log CFU/g (≥ 6.0 log reduction) 	
House cricket (<i>Acheta domesticus</i>)	Adult	Submerged in boiling water for 5 min	<ul style="list-style-type: none"> ◦ Total viable counts: 7.2 → 2.5 log CFU/g (4.7 log reduction) 	[22]
Large cricket (<i>Brachytrupus sp.</i>)	Adult	Submerged in boiling water for 10 min	<ul style="list-style-type: none"> ◦ Total viable counts: 6.7 → 2.8 log CFU/g (3.9 log reduction) 	

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745 **Table 3. Drying processing technologies to inhibit microbes in edible insects**

Drying processing	Insect species	Life stage	Drying condition	Main findings	Reference
Solar drying	Black Soldier Fly (<i>Hermetia illucens</i>)	Pre-pupae	50–60 °C, 15–25% relative humidity for 2–3 days, solar dryer with polyethylene sheet	Total viable counts	7.7 → 7.8 log CFU/g
	House cricket (<i>Acheta domesticus</i>)	Adult			8.3 → 8.8 log CFU/g
	African cotton leafworm (<i>Spodoptera littoralis</i>)	Larvae			7.0 → 7.4 log CFU/g
	Grasshoper (<i>Ruspolia differens</i>)	Adult			9.1 → 9.2 log CFU/g
Oven drying	Black Soldier Fly (<i>Hermetia illucens</i>)	Pre-pupae	Oven drying at 60 °C for 2-3 days	Total viable counts	7.7 → 6.2 log CFU/g
	House cricket (<i>Acheta domesticus</i>)	Adult			8.3 → 6.8 log CFU/g
	African cotton leafworm (<i>Spodoptera littoralis</i>)	Larvae			7.0 → 6.4 log CFU/g
	Grasshoper (<i>Ruspolia differens</i>)	Adult			9.1 → 6.8 log CFU/g
	House cricket (<i>Acheta domesticus</i>)	Adult	Oven drying at 80 °C until ≤ 5% moisture content	<ul style="list-style-type: none"> ◦ Total aerobic counts: 7.60 → 4.73 log CFU/g ◦ <i>Bacillus cereus</i>: 4.17 → 3.27 log CFU/g ◦ Yeasts and molds: 4.80 → 2.70 log CFU/g 	[39]

Microwave drying	House cricket (<i>Acheta domesticus</i>)	Adult	Microwave drying at 840 W until $\leq 5\%$ moisture content, core temperature 161–165 °C	<ul style="list-style-type: none"> ◦ Total aerobic counts: 7.60 → 4.00 log CFU/g ◦ <i>Bacillus cereus</i>: 4.17 → 1.40 log CFU/g ◦ Yeasts and molds: 4.80 → 1.57 log CFU/g 	[39]
Radio frequency drying	Black Soldier Fly (<i>Hermetia illucens</i>)	Larvae	RF drying at 27.12 MHz for 90 min, core temperature 130 °C	<ul style="list-style-type: none"> ◦ The total viable counts decreased significantly. ◦ Effective for pathogen elimination (<i>Bacillus cereus</i> not detected after radio frequency drying.) 	[42]

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747 **Table 4. Effects of novel non-thermal processing technologies on the microbiological aspects of edible insects**

Technologies	Insect species	Sample type	Treatment condition	Main findings	Reference	
High pressure	Black Soldier Fly (<i>Hermetia illucens</i>)	Larvae	600 MPa for 1.5 min	<ul style="list-style-type: none"> Total viable counts: 7.97 → 7.28 log CFU/g Enterobacteriaceae: 7.65 → 2.09 log CFU/g Lactic acid bacteria: 6.50 → < 2 log CFU/g Yeasts and molds: 5.07 → < 2 log CFU/g 	[49]	
			600 MPa for 10 min	<ul style="list-style-type: none"> Total viable counts: 7.97 → 6.67 log CFU/g Enterobacteriaceae: 7.65 → < 2 log CFU/g Lactic acid bacteria: 6.50 → < 2 log CFU/g Yeasts and molds: 5.07 → < 2 log CFU/g 		
				400 MPa for 2.5 to 7 min	<ul style="list-style-type: none"> Yeasts and molds: > 5 log reduction for all time Total aerobic counts: ~ 0.35 log reduction Inoculated <i>E. coli</i> O157:H7: > 5 log reduction 	[50]
	Mealworm (<i>Tenebrio molitor</i>)	Paste	600 MPa for 5 min	<ul style="list-style-type: none"> Anaerobic mesophilic bacteria reduced by approximately 2 log Yeasts and molds reduced to below the detection limit No effect on mesophilic bacterial spores 	[37]	
	House Cricket (<i>Acheta domesticus</i>)	Soluble protein fractions (pH 3)	200, 400, 600 MPa for 10 min	<ul style="list-style-type: none"> Total aerobic counts: 4.6 → 2.6 log CFU/mL (400 and 600 MPa) Yeasts and molds: Reduced below the detection limit 	[45]	

Microfiltration	Lesser mealworm (<i>Alphitobius diaperinus</i>)	Soluble protein fractions (pH 3,8)	Microfiltration cell (UHP-90K)	<ul style="list-style-type: none"> Total viable counts before microfiltration: 6.00 log CFU/mL (pH 3), 6.91 log CFU/mL (pH 8) After microfiltration: No microorganisms detected (<1 log CFU/mL) 	[54]
	House crickets (<i>Acheta domesticus</i>)			<ul style="list-style-type: none"> Total viable counts before microfiltration: 4.62 log CFU/mL (pH 3), 5.38 log CFU/mL (pH 8) After microfiltration: No microorganisms detected (<1 log CFU/mL) 	
Cold atmospheric pressure plasma	Mealworm (<i>Tenebrio molitor</i>)	Powder	8.8 kVPP at a frequency of 3.0 kHz (15 min)	<ul style="list-style-type: none"> Total viable counts: 7.72 → 4.73 log CFU/g 	[58]
		Larvae	Power consumption in the range of 1.2 kW at a frequency of 2.45 GHz for 10 min	<ul style="list-style-type: none"> Total viable counts reduction up to 7 log cycles 	[47]
	House Cricket (<i>Acheta domesticus</i>)	Powder	Surface micro discharge plasma power density: 8.7–22.0 mW/cm ² for 5 min	<ul style="list-style-type: none"> Initial microbial load: Total mesophilic bacteria (1.9×10^6 CFU/g), Enterobacteriaceae (1.1×10^6 CFU/g) Reduction achieved: Total mesophilic bacteria (1.4 log reduction), Enterobacteriaceae (1.9 log reduction) 	[59]

				◦ <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> spores were resistant to treatment.	
Ultrasound	Superworm (<i>Zophobas morio</i> F.)	Larvae	Sonication at a frequency of 37 kHz for 30 min	◦ Total viable counts: 3.24 → 1.48 log CFU/g	[62]
Irradiation	Honey bee larvae (<i>Apis mellifera</i>)	Powder	Sterilized through gamma irradiation (10 and 15 kGy)	◦ Total viable counts: 4.96 → 0 log CFU/g	[70]

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749 **Table 5. HACCP model for microbial control in edible insect processing**

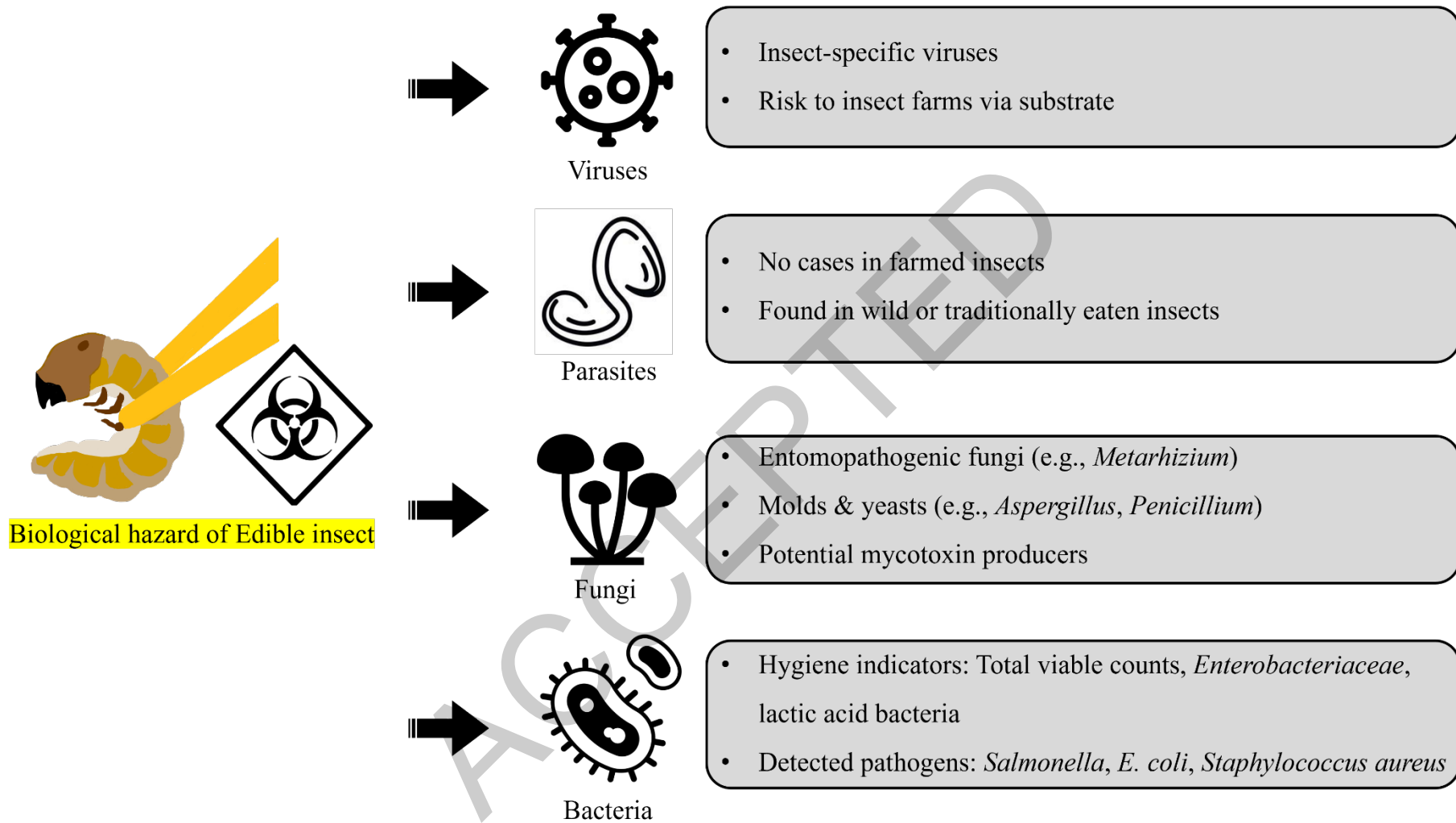
Processing step	Potential Microbial Hazards	Critical Control Points (CCP)	Control Measures	Monitoring Procedures	References
Rearing and Harvesting (pre-process)	<ul style="list-style-type: none"> High microbial load on insects from gut and rearing media Environmental contaminants (soil microbes, fecal bacteria) 	No (managed by GHP)	<ul style="list-style-type: none"> Good Hygiene Practices (GHP) on the farm to minimize initial contamination (clean feed, water, habitat) Optionally starve insects ~24–48 h to the empty gut (reduces feces in a product, though not proven to reduce bacteria) 	Feed and environment testing for pathogens	[8, 74]
Receiving and Pre-Processing (post-harvest handling)	<ul style="list-style-type: none"> Microbial growth if harvested insects are held at room temperature Cross-contamination from equipment or water during any rinsing 	No (managed by GHP)	<ul style="list-style-type: none"> Chill or process insects quickly after harvest to prevent bacterial overgrowth If washing/rinsing insects, use potable water and food-grade sanitizers as needed; ensuring clean equipment 	<p>Check holding time/temperature</p> <p>Monitor rinse water quality</p>	[10, 74]

Thermal processing (blanching)	<ul style="list-style-type: none"> ◦ Survival of pathogens if thermal treatment is insufficient (e.g. <i>Salmonella</i>, <i>Listeria</i>, <i>E. coli</i> O157 H7, etc.) ◦ Heat-resistant spores (<i>Bacillus</i>, <i>Clostridium</i>) 	Yes	<ul style="list-style-type: none"> ◦ Apply a validated thermal process (blanching in boiling water for ≥ 5 min) 	Thermometer checks: monitor water/steam temperature (continuous or each batch)	[73, 74]
Drying	<ul style="list-style-type: none"> ◦ Survival of pathogens if thermal drying is used but not hot enough/long enough ◦ Mold or bacterial growth on product if final moisture or water activity (a_w) is too high 	Yes	<ul style="list-style-type: none"> ◦ Dry to a specified moisture/a_w level that prevents microbial growth (e.g. $a_w < 0.60$) 	Measure water activity or moisture of finished batches	[10. 72]

Packaging	<ul style="list-style-type: none"> ◦ Post-process contamination during packing (from handlers, packaging materials, or environment) 	No (addressed by GHP)	<ul style="list-style-type: none"> ◦ Aseptic or sanitary packaging conditions 	Packaging line inspections: ensure the hygiene protocol is followed	[74]
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752 **Fig. 1. Potential biological hazards in edible insects.**