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TITLE PAGE - Food Science of Animal Resources -Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below			
Article Type	Mini-review			
Article Title	Application of edible insects as novel protein sources and strategies for improving their processing			
Running Title (within 10 words)	Edible insects as novel protein sources			
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Special remarks – if authors have additional information to inform the editorial office				
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.			
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was supported by the Main Research Program (E0211200-02) of the Korea Food Research Institute (KFRI) funded by the Ministry of Science and ICT (Republic of Korea).			
Author contributions (This field may be published.)	Conceptualization: Kim TK, Choi YS. Data curation: Jung S, Choi YS. Formal analysis: Kim TK, Cha JY, Choi YS. Investigation: Choi YS. Writing - original draft: Kim TK, Cha JY, Yong HI, Jang HW, Jung S, Choi YS Writing - review & editing: Kim TK, Cha JY, Yong HI, Jang HW, Jung S, Choi YS			
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.			

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9 Application of edible insects as novel protein sources and strategies for improving their
 10 processing

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Abstract

Insects have long been consumed by humans as a supplemental protein source, and interest 12 in entomophagy has rapidly increased in recent years as a potential sustainable resource in the 13 face of environmental challenges and global food shortages. However, food neophobia 14 inhibits the widespread consumption of edible insects, despite their high nutritional and 15 functional value. The own characteristics of edible insect protein such as foaming properties, 16 17 emulsifying properties, gelling properties and essential amino acid ratio can be improved by 18 drying, defatting, and extraction. Although nutritional value of some protein-enriched bread, pasta, and meat products, especially essential amino acid components was increased, 19 replacement of conventional food with edible insects as a novel food source has been 20 hindered owing to the poor cross-linking properties of edible insect protein. This 21 deterioration in physicochemical properties, may further limit the applicability of edible 22 insects as food. Therefore, strategies must be developed to improve the quality of edible 23 insect enriched food with physical, chemical, and biological methods. It was presented that an 24 overview of the recent advancements in these approaches and highlight the challenges and 25 prospects for this field. Applying these strategies to develop insect food in a more familiar 26 27 form can help to make insect-enriched foods more appealing to consumers, facilitating their widespread consumption as a sustainable and nutritious protein source. 28 **Keywords:** edible insects, food resources, entomophagy, protein cross-linking 29

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31 Introduction

Various peptides are linked to form protein structures; when digested in the body, the derived 32 33 amino acids and peptides of proteins are used to support various physiological activities and survival (van Huis et al., 2013). Dietary proteins contain 21 different amino acids, including 34 non-essential and essential amino acids (EAAs), and insufficient EAA consumption could 35 disrupt the balance of the body. Thus, protein intake is essential, but obtaining sufficient 36 dietary protein from conventional food sources could spend too much resources such as soil, 37 water, and energy, and their production and/or harvesting are often harmful to the 38 environment (Post, 2012; Kim et al., 2022b). Moreover, with a limited scope for increasing 39 40 the production of conventional food sources, the increasing global demand for food is difficult to meet (Kim et al., 2019b). Therefore, investigations into identifying various 41 alternatives to conventional food sources have increased, and several solutions have been 42 suggested for the utilization of more efficient food production systems (van Huis et al., 43 2013). To this end, several studies have evaluated the replacement of conventional food 44 sources for obtaining sufficient proteins, including vegetable proteins, in vitro meat, and 45 edible insects as emerging novel protein sources (Post, 2012; van Huis et al., 2013). 46 Similarity in taste and texture to conventional foods is a major deciding factor that influences 47 consumer perception; thus, among potential alternative food sources, edible insects diverge 48 the most from the conventional appearance of food (Kim et al., 2019b). Although numerous 49 people worldwide regularly consume edible insects without processing, many have negative 50 perceptions of insects in general, and therefore cannot be easily convinced to include them as 51 part of their regular diets. Therefore, the promotion and utilization of edible insects in food 52 systems must be improved. 53

To effectively incorporate insect proteins into food systems as high-value-added functional 54 ingredients, these proteins should be thoroughly assessed to best exploit edible insects as 55 dietary protein alternatives. Previous reviews have highlighted the nutritional value, types, 56 pre-treatment technologies, and health benefits of insects available for human consumption 57 58 (Kim et al., 2019b; Liceaga et al., 2022; Mintah et al., 2020a; Mutungi et al., 2019; van Huis et al., 2021). Although application of edible insects on conventional food have been 59 increased, these previous studies have not discussed about advance and limiting the use of 60 edible insects as a food ingredient and improvement strategy. Therefore, in this review, we 61 focused on the research progress to date related to the detailed usage, nutritional information, 62 value, limitations, processing, and application of edible insects and suggested strategies for 63 improving edible insect protein characteristics. 64

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66 Why people have a different attitude toward insects as food by regions and how

67 to regulate edible insects legally

Traditionally, insects were important food resources for early human societies because the 68 hunting of insects was relatively easier than that of other animals (Valadez, 2003). People in 69 various countries and from different cultures continue to routinely consume edible insects; 70 71 insects on the ground are used as food resources for over 80% (Kim et al., 2019b). The 72 consumption of edible insects is higher in human populations living in sub-torrid and torrid zones than in those living in temperate zones (van Huis et al., 2013). These geographic 73 differences in the propensity to consume edible insects as food might be attributed to the 74 differences in the respiratory system of insects. The diffusion of gases could be influenced by 75 the environmental conditions of specific regions, consequently affecting the growth and size 76

of the available insects (Kirkpatrick, 1957). Seasonal changes in the temperate zone are
responsible for the small size and complex assemblages of insects throughout the year,
whereas the consistently high temperature of tropical regions accelerates the diffusion rate of
oxygen, and a stable climate might promote the presence of active insects throughout the year
(van Huis et al., 2013).

To date, in most regions, insects have been consumed as whole by frying, broiling, 82 boiling, steaming, grilling, drying, or other traditional cooking methods. Although some 83 studies have shown a reduction in crude protein, ash, and zinc contents after cooking, these 84 cooking processes may generally enhance the safety, flavor, shelf life, and digestibility of 85 insects (van Huis et al., 2013; Williams et al., 2016). A neophobic response toward insects as 86 food has largely inhibited the broader consumption of edible insects, which is derived from 87 an unfamiliar bias and prejudice that insects are dirty and unclean and that they would have 88 negative effects on health if consumed (Kim et al., 2019b). However, constant marketing and 89 education regarding the nutritional value and consumption benefits of edible insects can 90 gradually affect the willingness of people to consume them (Kim et al., 2019b). 91

92 Regardless of culture or geography, interest in edible insects has been increasing in recent years, especially in western societies, and legislation about the use of insect as food 93 and feed has been established worldwide (Liceaga et al., 2022). The European Union 94 submitted regulation (EU) No 2015/2283 related to edible insects. Acheta domesticus, 95 Gryllodes sigillatus, Alphitobius diaperinus, Hermetia illucens, Apis mellifera, Locusta 96 migratoria, and Tenebrio molitor have been permitted as food for human consumption as of 97 October 2020, and insect food products must pass a safety assessment by the European Food 98 Safety Authority. Moreover, various regulations about the rearing, feeding, slaughtering, and 99 hygiene processing of edible insects have been updated (Lähteenmäki-Uutela et al., 2021). 100

The United States Food, Drug, and Cosmetic Act (United States Code, Title 21) has included the consumption of insects as food and feed since 2013 with no specific set of standards for edible insects beyond following the general Food and Drug Administration standard (i.e., bacterial testing and manufacturing practice certification). Other countries, including Canada, Australia, China, Japan, Thailand, and South Korea, have also updated their regulations related to edible insects in recent years (Lähteenmäki-Uutela et al., 2021).

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¹⁰⁸ Useful information for promoting edible insects as novel protein sources

The critical limitation of conventional food supply systems should be mentioned when 109 promoting edible insects to the population as novel protein sources. The global population 110 has been rapidly increasing, and is expected to reach 9.7 billion in 2050. However, existing 111 conventional food systems cannot provide a sufficient supply to the global population, as 112 such systems incur high environmental costs such as land, water, and air pollution, and loss 113 of biodiversity (Berners-Lee et al., 2018; Post, 2012). Therefore, an alternative food supply 114 115 system must be developed for the present and future generations. Among various alternatives, 116 the consumption of edible insects as food has been emphasized owing to several advantages for the environment and human health (van Huis, 2013). However, people in developed 117 countries generally do not consider insects as food sources because of their negative 118 perception of insects, and the various negative idioms related to insects, spiders, and other 119 invertebrates can inhibit the consumption of edible insects (Meyer-Rochow and Kejonen, 120 121 2020). Thus, reframing to a positive expression of idioms can change this negative perception (Mishyna et al., 2020). 122

Edible insects should be exploited in an effective and positive manner according to 123 precise information derived from experimental data. To encourage edible insect consumption 124 to the public, the following themes are typically included when providing information: 125 population increase, environmental impact, and nutritional value (Kim et al., 2019b). Despite 126 127 these valid reasons, insects are primarily considered to be disease carriers by most consumers (Castro and Chambers, 2019). However, insect-specific pathogenic microorganisms are 128 considered harmless to humans; only contamination with human pathogenic and toxin-129 forming microorganisms during unhygienic rearing, processing, and transport can critically 130 compromise the safety of edible insects (Schluter et al., 2017). Therefore, microbiological 131 criteria should be established according to the standards for general food by each 132 government. In terms of environmental cost, edible insects are a highly efficient food source 133 compared to conventional animal food. Oonincx and de Boer (2012) investigated the 134 environmental impact of the mealworm Tenebrio molitor over their lifecycle. They chose 135 three main factors to determine differences in the environmental impact of food: enteric CH4 136 production, reproduction rate, and feed conversion efficiency. Mealworms exhibited a lower 137 impact than animal sources (pork, chicken, and beef), with advantages of no CH4 emission, 138 high reproduction (one female insect can produce 160 eggs), a short lifecycle, and low feed 139 conversion ratio (mealworm = 2.2, chicken = 2.3, pigs = 4.0, and beef cattle = 2.7-8.8). In 140 addition to mealworms, other species such as black soldier fly larvae, crickets, and 141 142 grasshoppers have taken the center stage as alternative protein sources with a low environmental impact (Bosch et al., 2019; Halloran et al., 2017; Wegier et al., 2018). Edible 143 insects also have high nutritional value, with high levels of EAAs, unsaturated fatty acids, 144 polysaccharides, and micronutrients (Kim et al., 2019b). 145

Providing this positive information can also help increase the willingness of 146 consumers to pay for edible insects. Consumers generally have a positive attitude toward 147 tasting insects when they are first provided brief information regarding the various 148 advantages of edible insects for the environment and human health (Barsics et al., 2017). 149 150 Placentino et al. (2021) served insect-based protein bars to professional athletes, and found that providing brief information regarding the environmental and nutritional benefits of 151 edible insects enhanced the willingness of the athletes to taste the bar. The authors further 152 expected that the consumption of edible insects by professional athletes would promote 153 broader consumer engagement. The sensory experience and appearance of edible insects can 154 also be important factors in improving the taste of edible insects (Mishyna et al., 2020). As an 155 unfamiliar appearance can reduce the willingness to taste edible insects, processing to modify 156 the appearance has been attempted by the food industry (Castro and Chambers, 2019). 157 Processed edible insects have been used as food resources after drying, defatting, and 158 extraction. 159

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Processing methods for obtaining high-quality protein from edible insects 161 Conventional proteins derived from slaughtered animals can be categorized by their source 162 such as meat, blood, organs, or skin; the physicochemical properties of the proteins clearly 163 164 differ across these categories, which determine their applicability in various purposes. However, such categorization of edible insects is more difficult because of their small size. 165 Therefore, fractionation is the most important factor in insect protein processing because of 166 the difficulty in separating the skeleton and protein parts of the insect body (Purschke et al., 167 2018a). In this section, we provide an overview of the key processes that should be 168

considered for enhancing the physicochemical properties of proteins from edible insects. A
summary of the key processing methods of protein enhancement in edible insects is provided
in Table 1.

172 Drying methods

Drying is a useful and traditional method for increasing the efficiency of food systems by reducing the weight and increasing the shelf life of the product. However, drying processes that reduce the moisture content, inhibit microbial growth, and alter the enzymatic activity and chemical reactions can ultimately change the quality of food (Mujumdar and Jangam, 2011).

178 More efficient and hygienic drying methods have been developed in recent years, which have applicability in the food industry for obtaining high-quality edible insect powder 179 or extracted protein. Drying can be performed using a heat pump at a temperature lower than 180 that used with a conventional hot-air oven because of the recovered heat cycle. For improving 181 the performance of heat pump drying, the development systems have been divided into four 182 groups: heat pump system, external heating mode, drying gas, and operating mode 183 (Mujumdar and Jangam, 2011). The energy used for drying can be reduced with a 184 combination of two or more technologies (Hnin et al., 2018). Owing to its simplicity and 185 hygiene, solar-assisted drying could be a useful method in this regard, which utilizes an 186 auxiliary air heater, forced convection, and chemical heat pump (Hnin et al., 2018). 187

When comparing the effects of solar-drying and oven-drying on the moisture content and microbial count of edible insects, the moisture content was not significantly different between the two methods, whereas the microbial count was found to be higher with solardrying than with oven-drying methods (Nyangena et al., 2020). Therefore, microbial hazards

should be considered when using solar-drying methods. Infrared radiation can also be used to 192 dry food materials, which can be combined with hot-air drying, microwave drying, or freeze-193 drying technologies (Antal, 2015). In infrared-radiated samples, the diffusion rate increases 194 without increasing the surrounding air temperature. This technology is therefore widely used 195 196 in the food industry owing to its advantages, such as uniform heating, high heat transfer rate, and cost-efficiency. The effect of the hybrid drying method combining infrared drying and 197 freeze-drying was reported to be similar to that of the single freeze-drying method (Antal, 198 2015). Infrared-assisted freeze-drying of *Protaetia brevitarsis* reduced the energy 199 consumption by more than 90%, and the protein content and appearance score were higher 200 than those obtained with freeze-drying and oven-drying (Khampakool et al., 2020). 201 Microwaves have various advantages such as a rapid drying speed and easy control of drying 202 conditions; however, non-uniform drying, low penetration depth, and damage in texture are 203 critical drawbacks of using microwaves as a drying technology (Mujumdar and Jangam, 204 2011). Although minor changes were observed between microwave-dried and freeze-dried T. 205 molitor, sensory inspection requires improvement (Lenaerts et al., 2018). In addition to the 206 207 aforementioned drying methods, modified atmosphere drying and methods involving superheated steam, impinging steam, contact sorption, pulsed electric field, osmotic 208 dehydration, fluid bed, belt conveyor, ultrasound, and ultrasonic waves can be used to dry 209 food materials, including edible insects (Hnin et al., 2018). 210

211 Defatting methods

Defatted edible insects have higher nutritional value and functionality than raw edible insect proteins, such as improved foaming capacity and emulsifying properties (Kim et al., 2020a). In the food industry, hexane is frequently used to remove fat components from food such as

soybean, because of its higher efficiency than other organic solvents (Russin et al., 2011). 215 However, because the hexane solvent originates from fossil fuels, a discontinuous supply and 216 unstable price might impose a limitation for its economical use. Therefore, alternatives to 217 hexane that are amenable to the assessment of environmental and health risks need to be 218 219 developed. In addition, such alternatives should satisfy the following conditions: continuous supply, health and environment safety, high fire resistance, and economic and industrial 220 efficiency (Russin et al., 2011). Kim et al. (2020c) suggested that ethanol could be considered 221 an alternative to hexane to defat edible insects and improve protein quality. Moreover, Russin 222 et al. (2011) suggested that aqueous extraction could replace hexane extraction. However, 223 some other technologies are applied when defatting from insects using aqueous extraction 224 because the defatting yield of this extraction method was the lowest when compared organic 225 solvent extraction (Laroche et al., 2019). Ultra-sonication, protein precipitation, micro- or 226 ultrafiltration, membrane isolation methods, and enzyme hydrolysis could be suggested to 227 enhance the efficiency of aqueous extraction (Russin et al., 2011). The supercritical carbon 228 dioxide extraction method was developed in the early 1980s; however, this technology 229 appears to be more useful for oil extraction than protein extraction (Russin et al., 2011). 230 Wiese and Snyder (1987) demonstrated that defatting performed after drying or grounding 231 provides a higher oil yield than flakes in soybean; therefore, it is recommended to perform 232 drying or grounding before defatting (Gravel and Doyen, 2020). 233

234 Extraction methods

Extraction has been conducted for enhancing the functional properties of proteins. Protein solubility could be a key factor and serves as an excellent index of quality, as it is related to other functional properties (Zayas, 2012). The solubility of proteins in the solubilized buffer

is determined by the distinction between the isoelectric point of the protein, which in turn is 238 affected by the pH and ionic strength of the buffer (Zayas, 2012). In addition, protein 239 structure and properties such as the amino acid composition, amino acid sequence, polar and 240 nonpolar contents, and molecular weight could be used for determining the proportions of 241 242 hydrophobic and hydrophilic residues that affect protein solubility (Zayas, 2012). Therefore, by controlling the solubility with different extraction methods, the yield of proteins extracted 243 from different food sources can be changed. Although protein extraction from edible insects 244 has not been explored extensively to date, it has garnered increased interest recently. Yi et al. 245 (2013) studied the effect of aqueous extraction on the protein quality of five different 246 mealworms, and their supernatant yield was approximately 20%. Kim et al. (2020a) 247 compared the changes in protein technical function following protein extraction by 248 centrifugation and suggested that centrifugation could be useful for separating soluble and 249 insoluble proteins. Both these studies used 0.02% ascorbic acid to prevent oxidation during 250 extraction. However, a recent study showed that alkaline conditions can solubilize proteins 251 more effectively than what is possible with ascorbic acid because of their strong ionic 252 strength (Kim et al., 2019a). Furthermore, dissolving the extracted protein in an alkaline 253 buffer enhances its function (Yi et al., 2013). In-salt methods can enhance the ionic strength 254 of edible insects and increase the functional properties of the extracted proteins (Kim et al., 255 2019a). Following the dissolution of proteins in solution, the protein can be collected via 256 257 precipitation by adjusting the pH to the isoelectric point, centrifugation, or the salting-out method. Additionally, hydrolysis of insect proteins has been explored as a method to improve 258 the functional properties of edible insects (Purschke et al., 2018b). Freeze-drying or 259 membrane filtration can also be used for the purification of extracted proteins (Gravel and 260 Doyen, 2020). 261

263 Quality characteristics of the protein in processed edible insects

The physicochemical properties of proteins can be modified via the aforementioned steps to enhance the nutritional value and functional properties of edible insects. Regardless of insect biotic factors, chemical or mechanical modification can improve the value of edible insects for people who rely on edible insects as novel food resources.

268 Nutritional aspects

Edible insects have high nutritional value and low environmental costs in terms of protein 269 production (Bosch et al., 2019; Kim et al., 2019b; Oonincx and de Boer, 2012). Bussler et al. 270 (2016) increased the crude protein yield from two different edible insects (T. molitor and 271 Hermetica illucens) by defatting and increased the protein content through protein isolation. 272 Mintah et al. (2020a) estimated the chemical composition of black soldier fly larvae using a 273 Box–Behnken design with the Box–Wilson methodology to optimize the processing 274 275 conditions. The optimal extraction time, temperature, and alkaline solution ratio to obtain the highest protein yield were 59.43 min, 52.23°C, and a 24.85-fold alkaline solution, 276 respectively. These optimized conditions yielded a protein content of over 80% and enhanced 277 278 the utility of edible insect proteins. An increase in protein content was also reported in another previous study on insect-processing; however, the authors indicated the possibility of 279 overestimation of the protein content in edible insects (Janssen et al., 2017). The crude 280 protein content was estimated using various nitrogen-to-protein conversion factors (Kp) 281 derived from the ratio of protein nitrogen to total nitrogen, and the average Kp value of three 282 283 different edible insect larvae (4.76 before extraction, 5.60 after extraction) was lower than the conventional Kp value (6.25). Thus, the non-protein fraction from insects, which includes 284

nitrogen, can cause protein content overestimation, and removal of the non-protein fraction 285 can increase the Kp value of edible insects (Janssen et al., 2017). Kim et al. (2020a) reported 286 that the insect protein content can be increased by defatting and extraction. The protein 287 content was increased to a maximum of 40% after defatting and extraction, and the >80% 288 289 chitin content in edible insects was removed following extraction. Among various components, regulating or enhancing the protein content is the main challenge in promoting 290 the widespread consumption and nutritional value of edible insects; amino acid profiling and 291 digestibility have also been used to estimate the value of edible insects as novel protein 292 sources. A few fresh edible insects cannot meet the amino acid requirements for humans; 293 however, the general processed edible insects can meet the requirements of human nutrition 294 and high digestibility, which are comparable with those obtained from animal meat (van Huis 295 et al., 2021). 296

297 Functional properties

In the food industry, protein-based materials such as meat, fish, eggs, milk, and plant 298 proteins are widely processed using various methods. The major protein functionalities in 299 food include solubility, water/oil holding capacity, foaming properties, emulsifying 300 properties, and gelling properties (Zayas, 2012). Previous studies have shown that the 301 addition of edible insect flour could increase the nutritional value of food, however, it 302 resulted in a decrease in the physicochemical properties (Azzollini et al., 2018; Bessa et al., 303 2019; Biró et al., 2019; da Rosa Machado and Thys, 2019; Duda et al., 2019; Haber et al., 304 2019). These characteristics can be enhanced by the removal of non-protein ingredients. 305 Furthermore, modification of insect protein functionality has been investigated (Gravel and 306 Doyen, 2020). Fractionation processing has been used for enhancing insect protein 307

functionalities (Bussler et al., 2016; Gravel and Doyen, 2020; Kim et al., 2020a; Kim et al., 308 2019a; Mintah et al., 2020a; Purschke et al., 2018b). The increase in protein content 309 increased the protein functionality of T. molitor, P. brevitarsis, and Allomyrina dichotoma 310 (Kim et al., 2020a). Following fat removal, the foaming capacity increased more than three-311 312 fold and the foam stability increased rapidly. Chitin removal had a more critical impact on the emulsifying properties than fat removal. Enzymatic hydrolysis can also be used to enhance 313 the protein functionality of edible insects (Gravel and Doyen, 2020; Purschke et al., 2018b). 314 Purschke et al. (2018b) studied the effect of enzymatic hydrolysis on the functional properties 315 of Locusta migratoria L. Insect was hydrolyzed by various enzyme (alcalase, neutrase, 316 flavourzyme, and papain), enzyme-substrate ratio (0.05-1.0%), heat pre-treatment (60-80 °C; 317 10-60 min), and hydrolysis time (0-24 h). They confirmed that changes in functional 318 properties of hydrolyzed insect were different by hydrolysis condition. Protein solubility, 319 foam properties, emulsifying activity, and oil-binding capacity increased with a decrease in 320 the protein molecular weight (decrease from 25–75 kDa to 10–15 kDa) after enzymatic 321 hydrolysis. However, Dion-Poulin et al. (2020) found no significant difference in protein 322 functionality following enzymatic hydrolysis by Alcalase® (3%, 80 °C, 15 min) but reported 323 that emulsifying activity decreased. Mintah et al. (2020b) pre-treated soldier flies with 324 enzymatic hydrolysis (alkaline protease; 9000 U/g, 50 °C, 90 min) using ultrasound, and the 325 pre-treated hydrolysates had a high molecular weight distribution below 1000 Da with 326 changes in the secondary structure of proteins (increased α -helix and decreased β -sheet), 327 consequently improving the dispersibility, turbidity, and particle size. Enzymatic 328 polymerization has been shown to increase protein functionality. Transglutaminase is an 329 effective and typically used polymerizing enzyme in the food industry. Kim et al. (2020b) 330 reported the effect of reaction time on the protein functionality of P. brevitarsis, in which a 331

reaction time of over 90 min could induce excessive protein aggregation and decrease in
functionality. Therefore, regulation of the non-protein components and structural
characteristics of insect proteins should be conducted to improve the protein functionality of
insects.

336

337 Application of insect protein in foods

The high nutritional value of edible insects in terms of their protein and fat content can 338 339 help promote their incorporation into conventional dishes. The quality characteristics of insect-enriched foods have been estimated with various methods. Some researchers have 340 focused on the nutritional value, while others have mainly focused on the physical 341 characteristics of insect-enriched food. Detailed information of these studies and the various 342 methods employed are provided in Table 2. Here, we summarize the effects of these methods 343 on enhancing or reducing the value and food quality of insect-enriched food from the aspects 344 of nutritional and physicochemical properties. 345

346 Nutritional enhancement

Edible insects are a good source of nutrition with several advantages, and their 347 supplementation in various foods has been shown to enhance the nutritional value of foods. 348 For example, protein, fat, fiber, and dietary fiber content was found to be higher in bread 349 containing crickets (Acheta domesticus) than in control bread (Osimani et al., 2018). 350 Cinereous cockroaches also increase the nutritional value of bread by enhancing the protein 351 content and unsaturated fatty acid composition (de Oliveira et al., 2017). Similarly, bread 352 353 enriched with defatted powder of grasshopper (Schistocerca gregaria) had higher protein, fat, and dietary fiber content (Haber et al., 2019). Moreover, supplementation with T. molitor 354

improved the nutritional value of bread by increasing the EAA, free amino acid, and protein 355 content, without significant differences in the nutritional quality of lipids (Roncolini et al., 356 2019). Crude fiber and protein content of egg pasta was increased using mealworm and 357 grasshopper powder supplementation (Cabuk and Yilmaz, 2020). Cricket powder and 358 359 silkworm powder increased the protein content of durum wheat pasta and buckwheat pasta, and the energy value also increased with the addition of insects (Biró et al., 2019; Duda et al., 360 2019). Various carbohydrate-based foods, such as snacks, protein bars, energy bars, rice, 361 buns, and biscuits, have been enriched with edible insects (Adámek et al., 2018; Alemu et al., 362 2016; Azzollini et al., 2018; Niaba et al., 2013; Tao, 2016). These studies suggest that the 363 protein and EAA content of carbohydrate-based foods could be improved by supplementation 364 with edible insect protein powder, consequently enhancing the nutritional value of edible 365 insects. 366

Furthermore, edible insects can be added to animal protein-based foods as substitutes 367 or ingredients. Replacing 10% of lean meat with pre-treated mealworms and silkworms in 368 emulsified sausage was shown to significantly increase its protein and mineral content (Kim 369 370 et al., 2016). Similarly, silkworm (*Bombyx mori*) pupae added to pork meat batter was reported to increase the protein content of the meat product (Park et al., 2017). Thus, these 371 studies indicated that the addition of insect flour could increase the protein content of various 372 foods. However, because the black soldier fly (*H. illucens*) has a lower protein content than 373 374 pork, insect-based Vienna-style sausages had a lower protein content than pork-based sausages (Bessa et al., 2019). 375

376

377 Enhancement of physicochemical properties

Proteins have various functional properties, which may affect the final physicochemical 378 properties of food. Gluten-free food may contain alternative proteins that can form a structure 379 similar to that of gluten with a good gas-holding capacity (Moore et al., 2004). Cricket 380 powder showed acceptable enrichment in gluten-free products, and bread enriched with 381 382 insects had better functional results than those enriched with lentil and buckwheat (da Rosa Machado and Thys, 2019). Bread enriched with 5% cinereous cockroach flour showed no 383 significant difference from standard wheat bread, and it was classified as good-quality bread. 384 However, the hardness of bread increased when the replacement ratio was more than 10% (de 385 Oliveira et al., 2017). When wheat flour was replaced with grasshopper flour, the specific 386 volume and hardness decreased. Furthermore, bread enriched with defatted insect flour had 387 higher springiness than that prepared with non-defatted insect flour (Haber et al., 2019). The 388 springiness of bread is closely related to its gas-holding capacity. Defatted edible insects have 389 better foaming capacity and stability than non-defatted edible insects (Crowley et al., 2000; 390 Kim et al., 2020a). These studies further demonstrated that the addition of edible insects 391 decreased the specific volume. For example, bread supplemented with mealworm powder has 392 a higher specific volume and softer texture than standard bread (Roncolini et al., 2019). The 393 authors described that these advantages of mealworm might be attributed to the stabilization 394 effect of melted fat on expanding gas cells (Pareyt et al., 2011). Therefore, further studies are 395 required to determine the effect of insect lipids on bread quality. In pasta enriched with 396 397 crickets, cooking loss decreased, whereas the optimal cooking time and firmness increased (Duda et al., 2019). Silkworm powder increased the water absorption rate and hardness, and 398 decreased the optimal cooking time (Biró et al., 2019). Furthermore, the excessive addition of 399 edible insects induced limited expansion of snacks, which may be due to the increased 400

401 amounts of fat particles (Azzollini et al., 2018). Therefore, the species and fat content of insects should be carefully selected for improving the quality of carbohydrate-based foods. 402 In meat products, insect flour as a protein replacement could increase the cooking 403 yield and textural properties of meat emulsion sausage owing to an increase in the ionic 404 405 strength (Choi et al., 2017; Kim et al., 2016). Silkworm pupae increased the viscous properties, hardness, gumminess, chewiness, and pH, and decreased the cooking loss value 406 when added as an ingredient in pork meat batter (Park et al., 2017). Vienna-style sausages 407 composed of black soldier fly larvae exhibited similar hardness and cohesiveness values on 408 day 1; however, after 14 days, these values decreased more rapidly than those in pork meat-409 based sausages (Bessa et al., 2019). 410

411

412 Suggesting strategies for improving edible insect protein characteristics

Aforementioned, edible insects processing such as drying, defatting, and protein extraction 413 could increase protein functionality and use of edible insects can be expanded. However, 414 there are clear indications that edible insect proteins as food ingredients exhibit poor 415 maintenance of form and textural properties, suggesting that the application of edible insects 416 mimicking conventional food may be difficult. Therefore, protein modification and 417 improvement of cross-linking are suggested for their efficient use in the edible insect 418 industry. The protein modification could be done with various methods. Protein cross-linking 419 reactions during processing occur under various conditions such as alkaline conditions, 420 heating, oxidizing agents, Maillard reaction, lipid cross-linking, and enzymatic reactions 421 (Singh, 1991). When protein denatured, disulfide cross-linking in protein-protein interaction 422 is the most frequent reaction occurring in food processing. Cysteine residues produce 423

disulfide cross-links and gelling properties could be affected by this bond. In addition, protein
denaturation exposed hydrophobic residues and protein functionality can be increased with
protein denaturation (Zayas, 2012). However, excessive denaturation under non-suitable
condition could aggregate protein and deteriorate quality characteristics of protein based food
(Nikbakht Nasrabadi et al., 2021).

In physically, heating processing is used most widely. Unfolded protein by thermal energy 429 could improve functionality (Zayas, 2012). With technological development, various heating 430 methods are used to heat food such as ohmic heating, microwave heating, radio frequency, 431 and infrared irradiation (Nikbakht Nasrabadi et al., 2021). However, heating under alkaline 432 conditions induces racemization that causes the production of de-hydro amino acids 433 (lysinoalanine and lanthionine), which cannot be digested, thereby reducing the nutritional 434 value of food (Gerrard, 2002). Moreover, high pressure can be used to accelerate the cross-435 linking reaction and protein aggregation. Food processed under a high-pressure conditions 436 has a soft texture; however, it is not easily broken. Pressure levels and time are important 437 factors in determining the textural properties of food (Totosaus et al., 2002). Above these 438 technologies, irradiation, pulsed-electric filed, extrusion, and cold atmospheric plasma have 439 been used to improve quality characteristics of protein based food (Azzollini et al., 2018; 440 Nikbakht Nasrabadi et al., 2021; Ojha et al., 2021). 441

Chemically, grafting technology, which involves the reaction of protein and sugar
(Maillard reaction), can improve the functional properties of proteins. However, among the
various compounds produced during the Maillard reaction, compounds that improve the
functional properties of reacted proteins by reducing sugar have not yet been identified
(Gerrard, 2002). Phosphorylation has been used to modify protein based materials to keep
nutritional value (Liu et al., 2020). Catalyzed covalent bonds between phosphate and protein

change protein structure and protein functionality can be improved (Nikbakht Nasrabadi et 448 al., 2021). Physicochemical properties of pH-shifted edible insects were improved and it also 449 improved gel properties of myofibril gel (Kim et al., 2022a). In addition, because edible 450 insects contain abundant chitin contents, acetylation modification could be improved 451 452 functionality of insects (Kim et al., 2019b; Nikbakht Nasrabadi et al., 2021). Enzymatic reactions may be a useful way to enhance the textural properties along with 453 food safety. Disulfide cross-linking can be achieved through enzymatic systems such as 454 disulfide isomerase and rearrangement of low-molecular-weight sulfhydryl compounds, and 455 sulfhydryl oxidase can catalyze the formation of disulfide bonds (Singh, 1991). Lysine 456 residues can cross-link with each other, and this reaction can enhance or retain the nutritional 457 value of protein by transglutaminase (Gerrard, 2002). Hydrocolloids can be used to enhance 458 the properties of insect proteins. Owing to their high viscosity, thickening, emulsifying, 459 stabilization, and gelling properties, hydrocolloids can maintain the shape of protein extracts, 460 and the processes to manufacture food mimics using edible insects could be increased (Saha 461 and Bhattacharya, 2010). Various ingredients containing proteins and polysaccharides have 462 high hydrophilic properties. Starch; xanthan; and various gums such as guar gum, locust bean 463 gum, and cellulose derivatives can be used as thickening ingredients, while alginate, gelatin, 464 agar, carrageenan, and pectin act as gelling agents (Saha and Bhattacharya, 2010). 465

466

467 Conclusion

Although edible insects with excellent nutritional value have been consumed widely in
developing countries, food neophobia and prejudice against insects limit their consumption as
food sources on a global scale. Therefore, the food industry has attempted to change the

471 appearance of insects through grinding or extraction before adding them to conventional

472 food. Unfortunately, despite the enriched nutritional value of edible insect-supplemented

473 food, the textural and gelling properties are reported to deteriorate. Therefore, there is a need

to develop more consumer-friendly insect food to increase the consumption of edible insect

- 475 proteins as a sustainable alternative to conventional food.
- 476

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- 652 Tables
- Table 1. Edible insect-processing methods following harvesting and their advantages and
 disadvantages.
 Table 2. Nutritional effects of the application of edible insects to conventional food

Methods	Advantages	Disadvantages
Drying process		
Sun-drying	- Economical	- High temperature differential
	- Easy to dry	- Rewetting
		- Contamination by animals, wind, or dust
		- Long drying period
Freeze-drying (lyophilization)	- High nutritional value	- High cost
	- Conservation of protein quality	- Lipid oxidation
Oven-drying	- Short drying period	- Protein deterioration
	- High accuracy	
Heat pump drying	- High energy efficiency in cost	- High cost and difficult to install
	- Lower protein deterioration than that with	- Low efficiency under cold conditions
	oven-drying	
Solar-assisted drying	- More hygienic than sun-drying	- Microbial hazard

Table 1. Edible insect-processing methods following harvesting and their advantages and disadvantages.

	- Improved drying efficiency when combined	- Additional cost
	with other methods	- Relatively humidity under 60%
	- Easy to dry	- Climate conditions affect drying
	- Saving energy	efficiency
Infrared radiation	- Uniform heating	- Distance control
	- High heat transfer rate	
	- High cost-efficiency	
Microwave	- Rapid drying speed	- Non-uniform drying
	- Easy to control drying conditions	- Low penetration depth
		- Damage in texture
Modified atmosphere drying	- Browning reaction or any oxidation could be	- Requirement of the replacement of
	prevented	oxygen with nitrogen or carbon dioxide
Superheated steam	- Low explosion hazard	- High operating pressure needed for high
	- Protection from oxidation	drying rate
	- Microbial safety	- Low drying rate with large equipment

	- Constant drying rate	
Impinging steam	- High drying rate in thin products	- Less uniform texture
	- Uniform drying	
Contact sorption	- Easy way to assist	- Food safety of absorbents
		- Requirement of another drying process
		to dry absorbents
Pulsed electric field	- Microbial safety	- High initial cost
	- Prevent nutrients	- No effects on spores or enzymes
	- Short treatment time	- Only suitable for liquid
		- Low energy efficiency
Osmotic dehydration	- Improvement of quality and prevention of quality	- Increase in saltiness or sweetness
	deterioration	- Decrease in acidity
	- Low energy cost	
	- Flavor retention	
	- Reduces enzymatic browning reaction	

Fluid bed	- High drying rate	- High energy consumption
	- High thermal efficiency	- Non-uniform quality
	- Low maintenance cost	- Agglomeration of flour
	- Reduced contact time	- Flammable
	- Easy to control	
Defatting process		
Hexane	- High efficiency	- Environmental implications
	- Low protein denaturation	- Limited quantity
		- Health risk
		- Highly flammable
Alcohols	- Sustainable	- Higher protein denaturation than hexane
	- Familiarity	- Low efficiency
		- High cost
Aqueous extraction	- Low pollution	- The lowest efficiency
	- Non-toxic	

	- Low cost	- High level of residual lipids and their
	- Minimal protein denaturation	oxidation
Supercritical CO ₂	- Rapid oil extraction	- Not suitable for protein extraction
Extract process		
pH and salt	- Easy to control	- Different protein solutions by pH and salt
Temperature	- Easy to extract protein	- Irreversible protein
Sonication	- Increased efficiency	- Additional cost
		- Changes of temperature
Membrane filtration	- Easy to control	- Long time period
	- Purification of protein is possible	

662 **Sources:** Drying (Antal, 2015; Hnin et al., 2018; Khampakool et al., 2020; Lenaerts et al., 2018; Mujumdar and Jangam, 2011; Nyangena et al.,

663 2020), Defatting (Gravel and Doyen, 2020; Kim et al., 2020a; Russin et al., 2011; Wiese and Snyder, 1987), Extraction (Gravel and Doyen,

664 2020; Purschke et al., 2018b; Yi et al., 2013; Zayas, 2012).

Compared food				
type	Added insects	Enriched food quality	Devalued food quality	References
5) F C				
Wheat bread	Acheta domesticus	- Protein, fat, fiber ash,	- Specific volume	(Osimani et al., 2018)
		and energy value	- Microbial characteristics	
		- Essential		
		leavening amino		
		acid		
		- Essential fatty acid		
		- Firmness		
	Locusta	- Protein, fat, fiber, and ash content	Specific volumeOverall acceptability	(Althwab et al., 2021)
	migratoria	- Essential amino acid	1 7	
Gluten-free bread	Gryllus assimilis	- Protein, fat, ash, and	- Specific volume	(da Rosa Machado and
		lipid content	- High porosity and cell density	Thys, 2019)
		- Hardness, chewiness		

666Table 2. Nutritional effects of the application of edible insects to conventional food

Wheat bread	Tenebrio molitor	-	Protein and essential	-	Firmness	(Roncolini et al., 2019)
			amino acids	-	Sensory evaluation	
		-	Specific volume			
	Schistocerca	-	Protein, fat, ash, fiber	-	Hardness, springiness	(Haber et al., 2019)
	gregaria			-	Specific volume	
Pasta	Cricket powder	-	Protein, fat, ash, and	-	Cooking time	(Duda et al., 2019)
			energy value			
		-	Cooking loss			
		-	Firmness			
Buckwheat pasta	Silkworm powder	-	Protein	-	Energy value	(Biró et al., 2019)
		-	Cooking time	-	Acidity over storage	
Extruded cereal	T. molitor	-	Protein	-	Poor expansion	(Azzollini et al., 2018)
snack		-	Digestibility	-	Porosity	
		-	Pore density	-	Pore size	
		_	Pore well thickness			

	Sphenarium	-	Protein, ash Browning index	-	Carbohydrate value Whiteness index	(Ramírez-Rivera et al.,
	purpurascens	-	browning index	-	winteness index	2021)
Meat emulsion	T. molitor	-	Protein, fat, and ash	-	Cooking loss	(Choi et al., 2017)
				-	Protein solubility	
				-	Emulsion stability	
				-	Hardness	
				-	Viscosity	
Emulsion sausage	T. molitor and	-	Protein, fat, and	-	Protein solubility	(Kim et al., 2016)
	Bombyx mori pupae		minerals	-	Lipid oxidation	
		-	Cooking yield			
		-	Hardness, springiness,			
			cohesiveness,			
			gumminess, and			
			chewiness			
Meat batter	Bombyx mori pupae		Protein, fat, and ash	-	Lightness	(Park et al., 2017)

	- Viscosity
	- Cooking loss
	- Hardness, springiness,
	gumminess, and
	chewiness
Jerky type	 Amino acid value Water activity Drying yield Shear force Rehydration ratio