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Constraints of HACCP Application on Edible Insect for Food and Feed

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Additional information is available at the end of the chapter

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Abstract

In a near future there is a need to guaranty food security for approximately more than one billion people worldwide. Beyond the population increase other factors contribute to food and feed insecurity such as climate changes, rising costs of animal protein and consumer demands for protein. Edible insects are pointed out as one alternative as they have always been a part of human diets. The concern to guaranty food security cannot be dissociated from food safety and under the *Codex Alimentarius* principles of food hygiene, insects would be comparable to other types of foods of animal origin. The processing and storage of insects and their products should follow the same health and sanitation regulations as for any other food or feed in order to ensure their microbiological and chemical safety. Allergies induced through insects' ingestion should also be considered. This review aims to identify potential hazards related to edible insects' production and transformation. Preventive measures to their control will be presented considering pre-requirements in their production and transformation. An HACCP plan will be described as a study case in insects' transformation, being discussed all constraints regarding implementation.

Keywords: edible insects, potential hazards, preventive measures, HACCP, safety

1. Introduction

Edible insects have gained interest in recent years all over the world as a solution to mitigate the lack of protein in a near future. With more than 9 billion people worldwide in next 20 years, there is a need to guaranty food security. Beyond the population increase, other factors contribute to food and feed insecurity such as climate changes, rising costs of animal protein, and consumer demands for protein. Alternative solutions to conventional

livestock and feed sources urgently need to be found, and edible insects are pointed out as one alternative.

According to the FAO (Food and Agriculture Organization), nearly 2.5 billion humans regularly eat insects in the world. The majority of edible insects are gathered from forest habitats, but in many countries, innovation in mass-rearing systems has begun. Edible insects have always been a part of human diets. They contain high-quality protein, essential amino acids, and vitamins for humans [1].

The concern to guaranty food security cannot be dissociated from food safety, and under the *Codex Alimentarius* principles of food hygiene, insects would be comparable to other types of foods of animal origin [2]. The processing and storage of insects and their products should follow the same health and sanitation regulations as for any other traditional food or feed items in order to ensure food safety. Because of their biological composition, several issues should be considered, such as microbial safety, toxicity, palatability, and the presence of inorganic compounds. Specific health implications should also be observed when insects for feed are reared on waste products such as manure or slaughterhouse waste. Allergies induced through insects' ingestion should be taken into consideration.

This review aims to identify potential hazards related to edible insects' production and transformation. Preventive measures to their control will be presented considering pre-requirements in their production and transformation. A Hazard Analysis Critical Control Points (HACCP) plan will be described as a study case in insects' transformation, being discussed all constraints regarding implementation.

2. Edible insect: intended uses and consumption

Entomophagy is highly rooted in the eating habits of populations from certain regions of the globe [3]. Capture of insects from their natural habitat has been a current practice in African, Asian, and South-American communities [4]. This capture of insects from the nature, without any effort to raise them, can be seen by two completely different points of view. The insects captured in certain ecosystems might result in ecological imbalances with potential negative outcomes [5]. On the contrary, the capture can be strategic to control insect plagues, namely of locusts, resulting in an environmental advantage due to the possibility of reducing insecticides use [6]. Both of those situations are associated with specific populations and connected to the direct capture of insects in the nature. In the so-called developed countries of North hemisphere, the consumption of insects does not have any cultural tradition [7]. Nonetheless, edible insects began to have market penetration in Europe and North America, more as a gourmet product than as a cheap protein source. In fact, edible insects marketed in Europe are very expensive, eventually more expensive than meat [8]. The legal framework of insects and insect-based production in Europe refers to general regulations on food safety, but it can also fall in the category of novel food. In that case, that remains unclear, insects can be considered a novel food, once from the actual regulation "*food consisting of, isolated from or produced from animals or their parts, except for*

animals obtained by traditional breeding practices which have been used for food production within the EU before 1997 and the food from those animals has a history of safe food use within the Union” and “traditional food from a third country (...) with a history of safe food use in a third country” [9] the classification as novel food depends on the assumption that insects were used or not for food in EU before 1997. Nonetheless, the traditional and long use in certain regions allow to consider edible insects as safe for humans.

In traditional consumption regions, due to insects’ nutritional value and consumer demands, a transition has been observed from collection to farm rearing and transition from small domestic productions for self-consumption and local markets to more sophisticated close-cycle farms producing in large scale to a broader market [10]. In order to reduce the production costs and make edible insects price competitive, it is necessary to intensify and automatize the production, since the work cost in Europe and North America is high. Insect farms can be used to produce, virtually, any insect species, provided that it became economically profitable. In an insect farm, all the lifecycle stages should be assured, to guarantee the progeny and continue the production with a new batch [11]. Usually, only a defined stage of the insect’s life cycle stage is used for food (**Figure 1**).

There are thousands of insect species that are used as food around the world. Several very complete inventories of edible species insects have been published in [1, 12, 13]. Facing to the growing interest on insects consumption, the European Food Safety Authority (EFSA) promoted, through the Scientific Committee, an evaluation of *the risk profile related to production and consumption of insects as food and feed* [2]. According to this report, there are only few species that have been produced for food with commercial purposes, both inside or outside Europe:

- **Crickets:** *Acheta domesticus*, *Grillus bimaculatus*, and *Teloegryllus testaceus*.
- **Grasshoppers/locusts:** *Oxya* spp., *Melanoplus* spp., *Hieroglyphus* spp., *Acridia* spp., and *Locusta migratoria*.

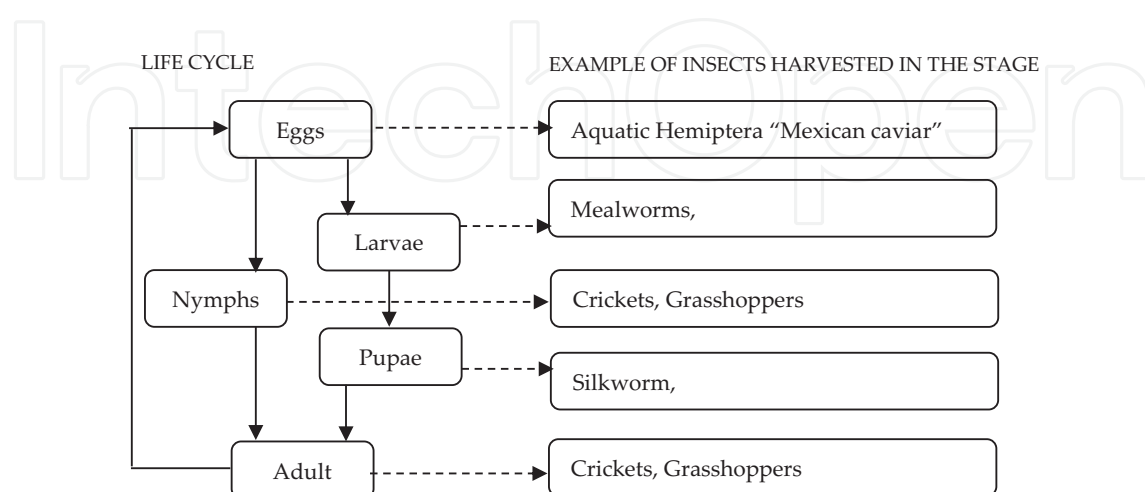


Figure 1. Edible insect’s life cycle stages and examples used as food resulting from a specific stage. Adapted from Ref. [8].

- **Mealworms:** *Tenebrio molitor*, *Alphitobius diaperinus* (lesser mealworm), *Zophobas atratus* (superworm).
- **Silkworm:** *Bombix mori*.

When the production is near to the retail point of consumption, most of these species are harvested and marketed alive or freshly cooked. However, on a larger production, insects must be preserved after harvested.

The culinary use of insects is related to the gastronomic habits of the region and the insect type. They can be fried, sautéed, boiled, roasted, toasted, smoked, and used as ingredient in soups and pizza, among others. Paraphrasing Ref. [14], “the ultimate goal is to elevate certain edible insects to gourmet food status.” That perspective of entomophagy is reflected in the number of cooking books available with recipes to prepare insect. Using the keywords “Cookbook insects,” Amazon.com retrieves about two dozens of results (accessed March 3, 2017). These insects might have different commercial presentations, namely whole insect, chilled, frozen, dried, snacks, or transformed in flour or pastes to be further processed [13].

3. Food production

The intensive production of insects needs to guarantee food safety, shelf-life and diversifications of the product or new presentations to reach different potential target consumers and overcome the neophobia.

3.1. Process and preservation techniques

Processing of edible insects is now a growing reality in order to accomplish consumer’s demands and the business opportunity. Even with a small market in Europe and North America, there are several manufacturers of edible insect’s products, namely, whole dehydrated insects to be consumed as snacks or ingredients for culinary preparations, and ground as paste or flour used mostly in cookies, snacks, candy, protein bars, hamburgers, and sausages, among others [12, 13, 15–18].

Processing in industries includes operations that are in the frontier between the farming and the processing. While in hot-blooded farm animals, the step of slaughter is a complex operation, in insects industry, that step is simpler and is usually composed by a **fasting** of about 24 hours to reduce the gut content, since it is not possible to remove the gut in almost all of the edible insects, and the labor costs of that operation would be too high. Before slaughter, insects can be **sieved** to eliminate frass. The **slaughter** is made by reducing the temperature (freezing 24 hours, -18°C) or by heat. It is common in some traditional consumers’ regions to cook insects alive, for consumption at home or for selling in local markets. If freezing is chosen, it can be advantageous to improve the operation of **removing legs and wings** [19].

Commercial processing with industrial interests might include three main types of products: dry whole insects, natural, salted or sweetened, or with additional seasonings; pastes obtained by mincing insects, and used to prepare meat-like products, as hamburgers or sausages; flours, used in baking cookies, bread, snacks, and protein bars, among others [13].

The processing steps are basically similar to those used in processing similar products from different raw materials, taking into consideration the specificities of the insects. Once insects have naturally a high microbiota at the beginning of the processing, an initial **cooking** in boiling water during 1–5 min results in a substantial reduction of the microflora. However, it might be associated with nutritional losses [20]. Cooking can be made in a seasoned broth to confer the aimed sensory characteristics of the product [17]. After water drainage, insects are **cooled** and should reach a refrigeration temperature rapidly to avoid the multiplication of survival microorganisms and germination of spores during the cooling. Cooked insects are only pasteurized. Considering its high initial microbiota, they should never be stored at room temperature to avoid spoilage. In products using the **drying** technology, the temperatures used should be adequate to avoid nutritional losses. That operation might be conducted in ovens with temperature varying from 60°C to more than 100°C [21]. During this operation, the insects' water is loss and there is a significant reduction in the microbiota. The hotter is the process, the higher is the microbial reduction, as demonstrated in cooked and dried crickets [22]. Freeze-drying is an interesting alternative to conventional drying, once the water is evaporated directly from the frozen insect, resulting in lesser nutritional and sensory losses, and as all the processes occur at a very low temperature, there is no opportunity for microbial growth [23]. Drying should be controlled to assure that final products have an activity of water enough reduced to inhibit microbial growth. When flours are prepared from dried insects, they are **grinded** to the appropriate size and **sifted**. Further operations made with flours are similar to those using grain flours, having in consideration its technological proprieties [24]. **Minced** products are made from freshly harvested or cooked insects. The raw material is minced to adequate particle size, and the preparation of the product is made, combining it with other ingredients or not, usually to obtain foods similar to those prepared with meat—hamburgers and sausages—aiming to overcome the initial disgusting that the insect itself produces in new consumers [25–27]. Once these products have a high water activity and pH, storage must be done at low temperature, **refrigeration** with a short shelf-life or **freezing**. No matter the processing used, the final product should be correctly **packaged** to avoid recontamination. Considering the high fat levels of most of the edible insects, and particularly the amount of mono and polyunsaturated fatty acids, the package should ideally be made under **modified atmosphere** to avoid or retard lipid oxidation [28]. There are several other processing operations that can be applied to separate fractions with particular nutritional interest [1].

In order to exemplify the processing of edible insects, it is presented as an example of a dry snack prepared from crickets or grasshoppers (**Figure 2(a)**) and flour from mealworms (**Figure 2(b)**).

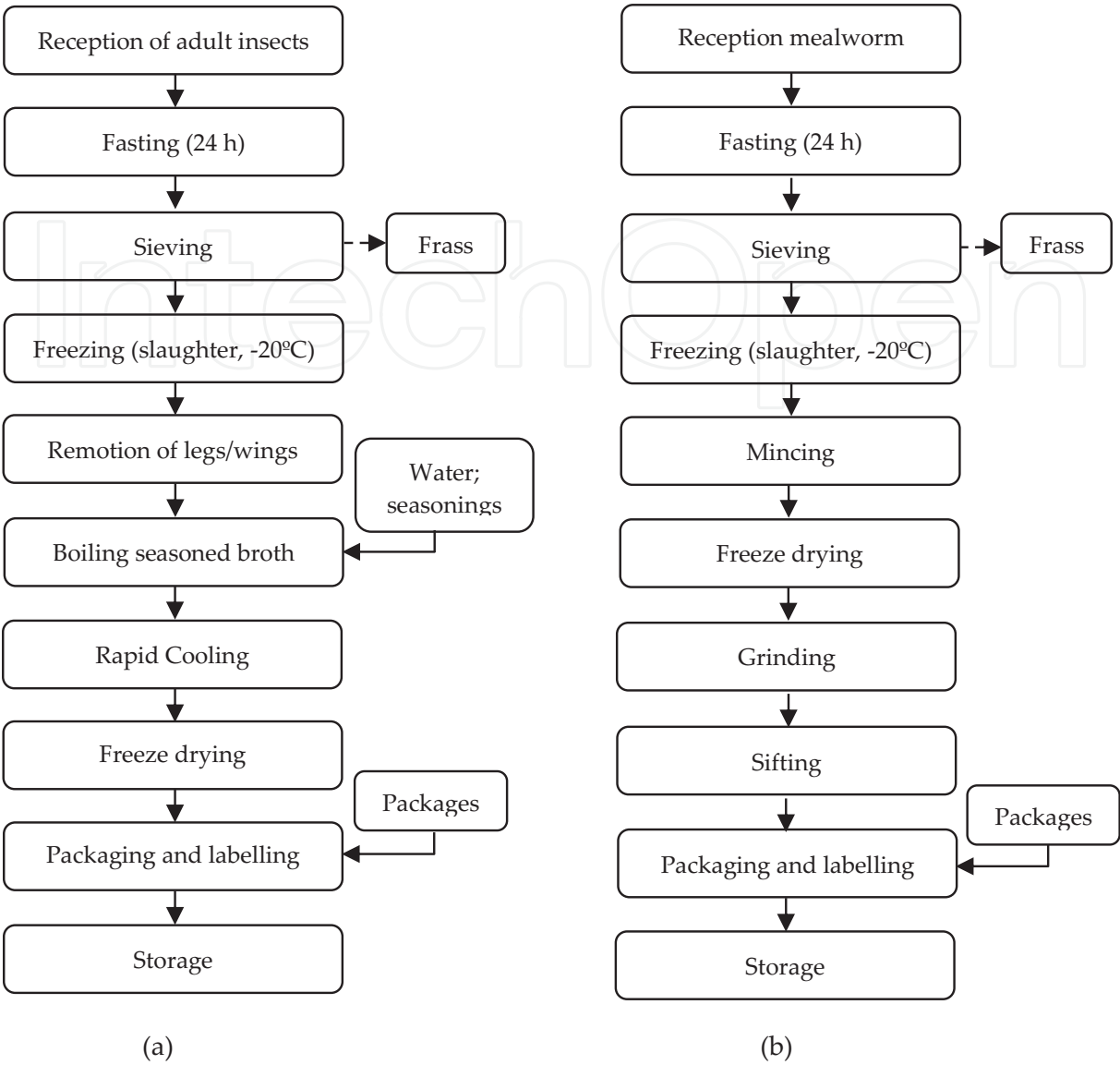


Figure 2. Processing flowchart of (a) cricket/grasshopper dry snacks and (b) mealworm larvae flour. Adapted from Refs. [17, 20, 29].

4. Implementation of an HACCP plan: key to the development of the edible insect sector

The Hazard Analysis Critical Control Points (HACCP) methodology is a systematic tool that identifies specific potential hazards and establishes control systems to ensure the safety of food [30]. The principles preconized on the HACCP methodology are well known, and at food processing level, all the safety management systems were established from *Codex Alimentarius* or on Standards based on it [31, 32]. The identification of biological, chemical, and physical hazards in all materials and process steps is Principle 1. After analysis of the potential hazards identified assessing a consistent risk, according to the preventive measures designed

to control hazards, Principle 2 suggests the identification of adequate critical control points (CCPs) in materials and process steps to control the hazards. Principle 3 is the definition of critical limits for each CCP. Principle 4 is monitoring to assure fulfillment of procedures at each stated CCP. When non-conformities from the outlined limits occur at a CCP, Principle 5 states that corrective action should be immediately applied to restore the control. Actions of verification to evaluate compliance of the HACCP plan are stated in Principle 6. All the procedures planned and implemented for monitoring, corrective actions, and verification performed should be recorded, providing an effective demonstration of the system at work in Principle 7. HACCP plan improvement is needed, and its update is based on any output of the system and when any input to the system changes such as premises, layouts, process, or if new legal or trade requirements are introduced and could have impact on safety. The HACCP plan is specific of a certain product, process, and organization. The use of a generic HACCP model to build specific HACCP plans should be taken as an assisting document that always needs creative adaptation and changing. Pre-requisites need to be fulfilled to accomplish with success the implementation of an HACCP plan. They are mandatory for food safety [33].

4.1. Pre-requisites: good farming practices and good practices for processing insect products

In the sector of edible insects', the implementation of preventive measures should be considered at two levels, primary production and processing. The application of HACCP in the primary production is not obligatory in EU, still it is recommended to apply the general principles established in the food safety regulations. On the other hand, insects processing, which includes all the activities after the harvest, namely slaughter, seasoning, cooking, freezing, drying, mincing, grinding, and packaging, among others, should comply with the HACCP methodology [34].

In the **primary production**, the rearing of insects, the general principles of food safety can be applied through an ensemble of good practices that are commonly designated by good hygiene practices (GHP), good agricultural practices (GAP), good veterinary practices (GVP), or to use the vocabulary currently accepted in the HACCP methodology, pre-requisites program (PRP) [35]. Pre-requisite programs include horizontal measures to reduce the odds of hazards introduction in insects' production chain, avoiding biological, chemical, and physical contamination, including cross-contamination, maintaining the hazards probability of occurrence as low as it is possible to achieve [36]. The applicability of HACCP principles to primary production has several constraints, as elucidated in Ref. [37]. These authors propose to use instead the HACCP principles, the preventive measures (pre-requisites) that are grouped in eight points: Environment; Equipment; Personnel; Inputs; Waste; Storage and Transport; Health Status; Recording and Traceability. These are also applied at processing level with particularities related to premises and equipment hygiene and maintenance programs with high relevance to avoid cross contamination and persistence of pathogens and hazardous chemicals [38, 39].

In the **environment** of production, several aspects should be considered. In the planning of farm location, it should be studied to avoid farm implementation in polluted areas that can be responsible for biological or chemical contamination of insects. When in insects' production,

it is considered to use low-value lands, and it has been referred to use the natural streams and waste streams [11]. Those practices are not recommended due to the possibility of contamination of insects with several types of hazards. Paraphrasing the Annex III of EU Regulation 767 [40] in Europe, it is prohibited due to the interdiction to use *“all waste obtained from the various phases of the urban, domestic and industrial waste water.”* Additionally, it is expected that labor costs for harvesting would be unacceptable [8]. To have a better control on production factors, preventive safety measures, and to reduce the production costs and render the insects' husbandry profitable, the close cycle is recommended [1]. The premises should obey to hygienic design, assuring that contamination is prevented, is built in materials that allow an effective cleaning and disinfection, and avoids pest infestation that might be vehicles of biological hazards [41]. The premises should have adequate sanitary facilities to the personnel. Cleaning and disinfection should be made with a pre-determined frequency and whenever it is necessary. When farms operate with batches in the same life cycle stage, it is common to perform a general cleaning and disinfection in the change of batches. If the farm operates with batches in different stages, cleaning and disinfection must be organized according to the production flow. These practices must be documented, identifying what detergents and disinfectants are used, the concentrations, times of application, as well as the regularity and personnel responsibility [41]. The **equipment** used in insects' husbandry can differ from simple cages in the simplest units, to more complex and sophisticated ones, namely egg separators in reproduction sections, harvest devices, and cold equipment to slaughter the insects. These equipments should comply with general principles of hygiene [35]. **Personnel** should follow personal hygiene practices, use adequate working uniform, and have adequate training and professional attitude. Though insects are phylogenetically very distinct from humans and thus it is highly improbable that any disease is shared between both, they might be vectors of human diseases. Several well-known diseases have a stage of spreading in an insect. These diseases are not probably the major concern, once the insects involved are mostly aphids and mosquitos that are blood suckers and do not have interest for entomophagy. However, insects might be carriers of pathogens that are usually found in warm-blooded animals, like pathogenic Enterobacteriaceae [2]. Thus, the personnel hygiene and the health status of the personnel should be supervised and adequate training should be available to improve the awareness of the workers to the risk factor, they can represent and follow high patterns of good practices [42]. The **inputs** for insects' rearing are probably one of the most sensitive pre-requisite to attend high levels of food safety. **Water and feed** are potential sources of several biological and chemical hazards [37]. Water used at insect farms should be potable to prevent any direct or indirect contamination. Crickets and grasshoppers/locusts are omnivorous, thus they are fed with vegetables and vegetable by-products or formulated feed. To feed mealworms, cereal or cereal by-products or formulated feed is currently used. Other insects have a highly specialized feeding, as silkworms that are nourished only with mulberry leaves [11, 15]. The feed must comply with the safety criteria previewed for any food production animal, in Europe by the Regulation 767 [40]. It is forbidden to use the *“content of digestive and urinary tract of animals slaughtered for human consumption, plant-propagating material, including seeds, treated with plant protection chemical, wood and sawdust treated with preservatives, urban, domestic and industrial waste (solid or liquid), independently of any previous treatment.”* Facing these restrictions, several applications of insect production to take advantage of certain sub-products should be carefully considered. Feeding edible insects with catering and domestic leftovers is not

recommended, or even allowed by law. Also, the use of animal and human manure is not recommended, due to the high potential that feces have to transport agents responsible for numerous diseases [2], and due to the difficulty in assuring that manure curing destroys a significant part of its potential pathogenic microbiota [43]. When high productivity is aimed, feed should be formulated to fulfill the nutritional needs of the insects. There are several references to the use of formulated feed for chicken [11, 44] or specifically formulated feed to the specific insect [1, 45]. These feeds must obey to the parameters required for any preparation used in animal production. As for industry, a particular selection and control should be implemented for suppliers in order to avoid the entrance of hazards in the production or processing system.

The **waste** management from insect production should include a plan to dispose the debris produced by the insects, namely, its frass, and the unused feed and the substrate used as bed. This waste is usually used as fertilizer for plants [11]. **Storage and transport** should be carefully planned. After harvesting, insects can be transported still alive to be sold directly to the consumer or to the processing units. The good hygiene that is required to the premises should also be applied to vehicles used in transport. Any intermediate storage has to be done in adequate facilities, and when insects are dead, temperature control is obligatory. While the insects' **health status** is not seen as a problem for the safety of the edible insects, that situation might change with the intensification of the production. Intensive production results in a higher probability of spreading diseases for the raising animals [46]. The experience from rearing bees has shown that animal-specific diseases might have negative economic outcomes [47]; thus, if the insects' production takes off for higher levels of intensive production, it is predictable that specific diseases might spread in the farms, and administration of veterinary drugs has to be considered, creating a problem with drug residues similar to that we face actually with meat from conventional husbandry [48, 49]. **Recording** of all procedures and measures that have impact on food safety should be made continuously. These records, as well as a clear identification of production batches, are determinant to ensure that **traceability** is achieved [50]. In case of any incident detected after expedition, the traceability will allow a rapid localization of affected products and an efficient recall.

Standard requirements of hygiene for the production, handling, packaging, storing, and distribution of edible insects and resulting products are mandatory to assure a healthy and wholesome supply of such products and the successful construction and implementation of an HACCP program.

4.2. HACCP model for edible insects processing

4.2.1. Potential hazards identification

The identification of biological, chemical, and physical hazards should be listed for all edible insects used as raw material as well all ingredients or accessory material (primary package) used and process steps related to processing. This hazard identification reveals the main concern of the organization to control them. The increasing interest in insects for food and feed does not correspond to the available literature that could be used to identify potential hazards related to those edible insects. More studies will be necessary to understand and validate several clues regarding identification of potential hazards or even emerging potential hazards. The European

Food Safety Agency (EFSA) has been trying to do the identification of hazards regarding edible insects; however, many gaps still exist regarding the occurrence of human and animal pathogens in insects processed for food and feed [2]. That European organism considered that the risk associated with edible insect consumption is similar to other food consumption.

Regarding potential microbial hazards, it is known that insects have their own microbiota and can serve as vectors for microorganisms pathogenic to humans [51]. Intrinsic microbiota of insects includes Enterobacteriaceae (*Proteus*, *Escherichia*), *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Micrococcus*, *Lactobacillus*, and *Acinetobacter* [52–54]. These families/genera can be related with specific pathogens for humans, while others are considered non pathogens in healthy people. In **Table 1** are listed **microbiological hazards** on edible insects

Insects	Potential hazards	Causes	Scientific evidence	Preventive measures
<i>Tenebrio molitor</i> (yellow meal beetle) <i>Schistocerca gregaria</i> (desert locust) <i>Bombyx mori</i> (silkworm) <i>Acheta domesticus</i> (cricket) <i>Locusta migratoria</i> (whole locust),	<i>Salmonella</i> , <i>E. coli</i> , <i>Staphylococcus aureus</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>Aeromonas hydrophila</i> <i>Bacillus cereus</i> , <i>Clostridium perfringens</i> , <i>Cl. septicum</i> , <i>Clostridium difficile</i> , <i>Cl. sporogenes</i> , <i>Listeria</i> spp.	Rearing conditions, farming practices, handling	[51, 62–65]	PRP
<i>Locusta migratoria</i> (migratory locust)	Vesicular stomatitis virus		[19, 66]	
<i>Imbrasia bellina</i> / <i>Gonimbrasia bellina</i> , (mopani worm, emperor moth) <i>Atta laevigata</i> (leaf cutter ants)	<i>Aspergillus fumigatus</i> , <i>Aspergillus sclerotiorum</i> , <i>Penicillium</i> , <i>Fusarium</i> , <i>Cladosporium</i> , and <i>Phycomycetes</i>		[4, 59, 67, 68]	
Aquatic insects Insect larva	Cercaria and metacercaria Nematodes <i>Dicrocoelium dendriticum</i> ; <i>Plagiorchis</i>		[21, 69]	
<i>Acheta domesticus</i> (small crickets) <i>Locusta migratoria</i> (locusts) <i>Hyboschema contractum</i> (Rhino beetles) <i>Gryllotalpidae</i> (mole crickets)	Antibiotic-resistant genes E.g., <i>tet(M)</i> <i>tet(O)</i> <i>tet(S)</i> <i>tet(K)</i> , <i>erm(B)</i> , <i>blaZ</i>	Farming practices, improper use of antibiotics, fail on biosecurity	[70]	PRP Farm biosecurity
<i>Edible insects in general</i>	Mechanical or biological vectors of prions	Rearing conditions, farming practices, handling	[71–74]	PRP

Table 1. Identification of biological hazards associated with raw materials/ingredients reported, principal causes, and preventive measures.

and the respective scientific evidence. *Campylobacter*, verotoxigenic *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* may be present in non-processed insects and their occurrence should be assessed. The detection of some pathogens in some edible insect has been reported (**Table 1**), while the presence of others was not yet demonstrated. The prevalence of some of these pathogens is lower when compared with other sources of animal protein, for example, *Campylobacter* does not replicate in the intestinal tract of the insects [55–57].

Insects can have virus pathogenic to their self and are considered virus vectors implicated in many plant and animal diseases. The Rhabdovirus causing vesicular stomatitis in animals and humans has been reported on edible insects (**Table 1**). However, there is also a lack of information related to the likelihood of human viruses such as norovirus, rotavirus, and Hepatitis E and A, being passively transferred from feedstock through residual insect gut contents [2].

Insects can harbor protozoa implicated in animal and human diseases. Several human parasitic diseases implicate insects as infected vectors for the transmission, for example, of *Trypanosoma* and *Leishmania*. The parasitic foodborne diseases related to edible insects are not well documented. It was reported that the trematode *Dicrocoelium dendriticum* (family *Dicrocoeliidae*) is a parasitic zoonotic agent that potentially infects humans through insect consumption. There are evidences suggesting the possible foodborne transmission of parasites (trematodes) belonging to the families *Lecithodendridae* and *Plagiorchiidae* [58]. Despite the reported occurrence of parasites in the insects and the relationship between sporadic human parasitic disease and insect consumption, there are no data on the occurrence of parasites in farmed insects. Insects may also be carriers of fungi and yeasts with potential hazard to animals and humans. Yeasts and fungi were found in considerable amounts in fresh, freeze-dried as well as in frozen insects (*T. molitor* and *Locusta migratoria*) in Ref. [2].

The importance of proper processing, handling, drying, and storage was stressed regarding aflatoxins in some commercial lots of mopane worms (*Gonimbrasia belina*: *Saturniidae*) [4]. From the same species, dried under laboratory conditions, some fungi were isolated (*Aspergillus* spp. and *Penicillium* spp.), among which are mycotoxigenic species [59]. In general, any hazard from fungi associated with insects produced for food and feed or introduced during farming, processing, and storage could be mitigated by hygienic measures in the entire production chain.

Three main issues with prion-related risks from insects were reported by EFSA [2]: insect-specific prions, insects as mechanical vectors of animal/human prions, and insects as biological vectors of prions (i.e., involving replication of animal/human prions within insects).

The **chemical hazards** identified in **Table 2** could result from substances synthesized by the insect itself or substances accumulated by the insect from its environment or feed. Not all insect species are therefore edible or may be edible depending on the stage of the life cycle [19]. Toxins and antinutritional factors should be assessed in order to select insects for feed and food. The main chemical hazards present on edible insects are related with farming practices and rearing conditions that should comply with the applicable food safety regulations. Edible insects entering in the production of food and food ingredients should be kept in such a way to prevent or minimize the accumulation of externally introduced toxins, drugs, or antinutrients. Chitin, a constituent of the insect exoskeleton, and chitosan, one of its derivatives,

should be regarded as antinutritional factors and also as a potential allergenic substance with complex effect on the immune system. Another serious concern related to edible insects is related to allergic reactions induced by substances present on edible insects; however, there are a very limited number of studies available [21]. Allergens identified and listed (**Table 2**) should be indicated on the label of edible insect's products.

The use of veterinary drugs should be planned in insect farms to reduce the mortality, especially associated with bacterial or parasitic infections. The information related to the presence of veterinary drug residues in insect is very scarce. The use of chloramphenicol, a broad-spectrum antibiotic prohibited in animal production [60], was referred to treat a disease in silkworms (*Bombyx mori*). Other biocides should be considered as potential hazards since the control of insects' diseases in intensive production will probably be needed.

Insects might accumulate hazardous chemicals, including heavy metals, dioxins, and flame retardants. Data on hazardous chemicals in reared insects and in insect-based food are scarce. Recently, chemical hazards on edible insect and their detection were shown [61]. Apart from the environmental contaminations and the possibility for several chemicals to accumulate in the farmed insect, the levels of contamination were relatively low and concentrations were similar or lower than those measured in commonly eaten animal products, such as meat, fish, and eggs.

Insects have a high-quality amino acid profile with high contents of phenylalanine and tyrosine [8]. These amino acids could give rise to the formation of biogenic amines tyramine and histidine associated with aminogenic microorganism and inadequate conditions of storage. Also, contamination with molds could increment the potential hazard micotoxins (Aflatoxins, Beauvericin; Enniatin A; Enniatin A1) described by several authors [19].

Physical hazards are dense contaminants or foreign bodies that might cause injury to the consumer. Edible insects are not especially prone to be a vector of physical hazards. During edible insects' processing, a recontamination by foreign bodies from the process (metal and plastic), as with any other processed food, could occur. Generally, all the edible insects might have hard parts: elytra, rostrums, and wings; and leaves and soil could be considered hazards, particularly for unused consumers. The prevention of its occurrence can be achieved by a strict compliance with the pre-requisites plan.

Tables 1 and 2 list the main biological and chemical hazards, respectively, identified in edible insects. Physical hazards are not listed once this production does not have particular physical hazards, beyond those previously described. **Table 3** presents the potential hazards associated with several processing operations that can be used in insects processing, namely, freezing, freeze-drying, dehydration, boiling, deep-frying, toasting, cooling, acidification, packaging, and end product storage, as well as the preventive measures that can be applied to prevent its occurrence in the final product. In the tables, PRP refers to the Pre-Requisite Plan, as discussed in item 4.1.

Hazard analysis and assessment performed by the food industry are qualitative using a bidimensional model based on hazards severity and probability of occurrence [30], but a quantitative analysis can only be performed when detailed studies based on knowledge of

Insects	Potential hazards	Causes	Scientific evidence	Preventive measures
<i>Oecophylla smaragdina</i> (Hymenoptera: Formicidae); <i>Odontotermes</i> sp. (Isoptera:Termitidae); <i>Coptotermes gestroi</i> (soldier termites, Rhinotermitidae); <i>Cirina forda</i> (Lepidoptera: Saturniidae)	Antinutritional factors: phytic acid, oxalates, hydrocyanic acid, tannins, thiaminase	Species-specific	[75–77]	Labeling
<i>Bombyx mori</i> (silkworm pupae); <i>Ophiocordyceps sinensis</i> (caterpillar fungus), <i>Rhynchophorus ferrugineus</i> (red palm weevil), <i>Tenebrio molitor</i> (yellow meal beetle) <i>Locusta migratoria</i> (locust)	Allergens: myosin, troponin, α-amylase, tropomyosin, arginine Kinase, hémocyanine, Hexamérine, -amylase, Arginine kinase, chitinase, glutathion-S-transférase, triose, phosphate isomérase, trypsine, Chitin, pollen, Histamine		[23, 69, 78, 79]	
<i>Lytta vesicatoria</i> (Spanish fly) Tenebrionidae (darkling beetles) moth species (<i>Zygaena</i>)	Toxic substances: cantharidin, amonoterpene (2,6-dimethyl-4,10-dioxatricyclo-[5.2.1.02,6]decane-3,5-dione), quinones, and alkanes cyanogenic glycosides		[19]	PRP selection of edible insects
All edible insects	Micotoxins: Aflatoxins, Beauvericin; Enniatin A; Enniatin A1	Rearing conditions, farming practices,	[19]	PRP
<i>Tenebrio molitor</i> (yellow meal beetle)	Pesticides (ex. clopyralid, benzoquinons), persistent organic pollutants	Farming practices, improper use of pesticides	[80]	
All edible insects	Dioxins, polychlorinated non-ortho and mono-ortho biphenyls (dioxin like PCBs), organochlorine compounds (OCPs), poly- brominated diphenyl ethers (PBDEs),		[61, 81]	
All edible insects	Heavy metals: cadmium, lead, arsenic, zinc, copper	Farming practices, improper use of antibiotics, and fail on biosecurity	[61, 82–85]	
<i>Bombyx mori</i> (silkworms) <i>M. domestica</i>	Veterinary drug residues (ex. chloramphenicol, 4,4'-dinitrocarbanilide (nicarbazin))		[86, 87]	

Table 2. Identification of chemical hazards associated with raw materials/ingredients reported, principal causes, and preventive measures.

Processing steps	Potential hazards	Causes	Preventive measures
Reception of raw material	All hazards mentioned for all edible insects	Fails on temperature; without safety specification for purchase	Selection and control of suppliers, definition of safety criteria for purchase, temperature of reception
Reception of seasoning (herbs and spices) and additives	Biological: Pathogenic microorganisms: <i>Salmonella</i> , <i>L. monocytogenes</i> ; <i>S. aureus</i> , <i>E. coli</i> (VTEC) <i>Clostridium perfringens</i> spores; and <i>Aspergillus flavus</i> Chemical: Pesticides (aldrin, linden, etion), herbicides, dioxins, heavy metals (selenium), additives legal requirement for toxic dose, micotoxins (Aflatoxins) Physical: Soil, stones, wood, and plastic fragments		
Freezing (slaughter)	Biological: Pathogenic microorganisms multiplication present on raw material Chemical: None Physical: None	Fails on temperature, improper hygiene, and maintenance of equipment	Correct temperature and time, preventive maintenance plan for equipment
Bleaching	Biological: no inactivation of pathogenic microorganisms present on raw material due to fails on temperature/time Chemical: Heavy metals Physical: None	Improper maintenance of equipment, incorrect binomial temperature, and time for bleaching, improper water	Correct temperature and time, preventive maintenance plan for equipment, potable water
Boiling	Biological: no inactivation of pathogenic microorganisms present on raw material Chemical: Heavy metals Physical: None	Improper maintenance of equipment, incorrect binomial temperature and time for boiling, improper water	
Rapid cooling	Biological: recontamination with pathogenic microorganisms (spores germination) and growth (<i>Cl. perfringens</i> and other pathogenic <i>Bacillaceae</i> , <i>L. monocytogenes</i> , <i>Salmonella</i>) Chemical: Heavy metals Physical: None	Fails on temperature, long time for cooling, improper hygiene, and maintenance of equipment, improper water	Corrected temperature and time for cooling, GHP, potable water, hygiene program for equipment and premises, preventive maintenance plan for equipment and premises
Storage under Refrigeration	Biological: Pathogenic microorganisms multiplication present on raw material Chemical: Histamine, Micotoxins: Aflatoxins, Beauvericin; Enniatin A; Enniatin A1 Physical: None	Fails on temperature, incorrect relative humidity, improper practices of hygiene and maintenance of equipment, long time of storage	Corrected temperature and relative humidity, GHP, hygiene program for equipment and premises, preventive maintenance plan for equipment and premises

Processing steps	Potential hazards	Causes	Preventive measures
Mincing	Biological: Pathogenic microorganisms multiplication/contamination Chemical: Heavy metals from water Physical: Metals particles		GHP, potable water, hygiene program for equipment and premises, preventive maintenance plan for equipment and premises
Freeze drying	Biological: Pathogenic microorganisms multiplication present on raw material Chemical: None Physical: None	Improper maintenance of equipment, incorrect relative humidity, fail on aw reduction	Corrected air velocity and relative humidity, preventive maintenance plan for equipment and premises
Grinding	Biological: Pathogenic microorganisms multiplication Chemical: None Physical: Metals particles	Improper practices of hygiene and maintenance of equipment, improper practices of processing with long time of grinding	Preventive maintenance plan for equipment and premises
Packaging finished products and labeling	Biological: Pathogenic microorganisms contamination (<i>Aspergillus fumigatus</i> , <i>Aspergillus sclerotiorum</i> , <i>Penicillium</i> , <i>Fusarium</i> ; <i>L. monocytogenes</i> ; <i>Salmonella</i>) Chemical: Packaging migration contaminants: ink, bisphenol A and phthalates, Allergens not identified in the label Physical: Metals	Improper packaging material, allergens, improper sealing, incorrect labeling	Selection and control of suppliers, GHP, GMP, control of good sealing, detection of metals on real time, preventive maintenance plan for equipment, correct labeling, GMP
Storage end product	Biological: Recontamination with pathogenic microorganism and growth of <i>Aspergillus fumigatus</i> , <i>Aspergillus sclerotiorum</i> , <i>Penicillium</i> , <i>Fusarium</i> Chemical: Tiramine, Histamine, Micotoxins: Aflatoxins, Beauvericin, Enniatin A; Enniatin A1 Physical: None	Storage conditions Fails on temperature/humidity Long time of storage, fails on GHP and GMP	GHP, GMP Correct time/temperature/humidity, shelf-life validation

Table 3. Identification of potential hazards associated with the processing operations potentially used in insect processing, principal causes, and preventive measures.

likelihood of exposure to a specific hazard are available. The analysis of the hazards identified on edible insects should be performed; however, the occurrence of several hazards is not well documented. This could be a major constraint in the planning of a safety management system for an organization related to edible insects processing, since the hazards analysis and risk might not be adequately established. Preventive measures are actions taken to control the hazards. These include physical, chemical, or biological factors or other hurdles required to control a hazard likely to occur at particular stages of the processing of insects' products. The occurrence of hazards identified in an organization depends on preventive measures

already implemented. Several preventive measures may be required to control a specific hazard. Likewise, there are hazards that can be effectively controlled by one single preventive measure. The control of some preventive measures designed to eliminate or reduce potential hazards may be assured by validated pre-requisites programs (PRP), also named Good Hygiene and Good Processing Practices (GHP/GMP). From the hazards analysis, according to their occurrence and severity, only some corresponding to a high or moderate risk level will be questioned for the identification of critical control points.

4.3. Identification of critical control points on edible insects processing

A critical control point (CCP) is a step or procedure where control can be applied and a hazard can be prevented, eliminated, or reduced to acceptable levels. The determination of a CCP in a process should use the safety knowledge about edible insects, only for real and likely occurring hazards and where preventive measures are available for their control. The *Decision Tree* for raw materials and processing steps is an algorithm to help finding the adequate CCPs for each considered hazard [32]. Critical control points require strict monitoring, which implies costs. So, CCP should be in the minimum number if it is possible, no more than three or four in each plan. After identification of all CCPs, it is needed to decide how their control will be done and establish the criteria for acceptance. The absolute tolerance at a CCP is known as a critical limit [88]. In **Table 4**, some of the (potential) identified CCPs on insects processing are summarized. An effective monitoring operation of CCPs is fundamental to assure product safety and should be preconized by defining critical limits of parameters that must be under control.

CCP and location (<i>Principle 2</i>)	Parameters to establish critical limits (<i>Principle 3</i>)	Monitoring procedures and frequency (<i>Principle 4</i>)	Corrective actions (<i>Principle 5</i>)	Verification procedures (<i>Principle 6</i>)
Drying or freeze drying	Relative humidity/air velocity/temperature duration according to the process preconized Weight loss (related with aw). aw < 0.60	Measure humidity/ air velocity/ temperature/time of the operation; Take a sample to measure the weight loss to achieve the desired aw per batch	Increase the drying period; reprocess	Take a sample to measure water activity in 5% of the batches produced according to a plan of sampling
Packaging	0% of metal particles 0% of fail on sealing package	100% packages screened on the metal detector 5% batch package sealing control	Reject packages with metal hazards and not sealed; Review the equipment	Calibration of metal detector with control packages with known metal samples size
Labelling	Allergenic hazard presence should be communicated and be clearly identified in all the labeling	Trained workers should inspect all the packages for the presence of label	Packages without label with incorrect information should be drawn from the line to be labeled again	A random sample of packages should be drawn to check for integrity and readability of the label

Table 4. Example of an HACCP plan with possible CCP for mealworm flour.

4.4. Critical limits establishment for CCP and monitoring actions

Critical limits must be associated with a measurable factor that can be routinely monitored according to a fixed schedule. These critical limits are established based on published data (scientific literature, in-house and supplier records, and regulatory guidelines), experimental data, expert advice, and mathematical modeling [32] and designated to be monitored at CCPs. Monitoring procedures assure that they have been respected. Regarding some technological parameters associated with the manufacture of edible insect products, the available information is scarce. The steps of edible insects' processing for flour or snacks (**Figure 2**) relate with preventive measures that control identified potential hazards and are recognized CCPs for several technological processes. Chilling, freezing, and freeze-drying or drying will have an impact on pathogenic microbiota control, avoiding its multiplication, while blanching, boiling, and other thermic treatment or culinary treatment will be effective and destructive against pathogenic microbiota. All these steps are to be controlled by objective parameters that allow to check on-line all these CCPs, giving the opportunity of immediate correction or corrective measures if a fail is detected. The monitoring frequency depends on the nature of the CCP, the type of monitoring procedure, and the amount of production [88]. The validation of the binomial temperature/time for an effective lethal effect of microorganisms should be considered in practice, being considered a real evidence of the control.

Some chemical hazards identified (mycotoxins and biogenic amines) at processing steps such as storage can be avoided in controlling the producing microorganisms. Aminogenic microorganisms can be avoided when good hygiene practices are effectively implemented. For flour production, some bacteriostatic additives might be introduced, as well as antioxidants. If chemical additives are used, it should be considered as a potential hazard and be controlled at the weighing. This step can be considered a CCP or eventually a good practice.

Monitoring data prove that the process is under control and provide a pool of data that, after trend analysis, give outputs to improve the implemented system. Particularly if the data correspond to fails occurring on the implemented safety system, the cause should be analyzed to a better establishment of corrective actions.

4.5. Corrective actions

The corrective actions are those that are previewed to implement when a deviation occurs, detected by the CCP monitoring, and include those related to the adjustment of the process to bring it back under control and those related to the amount of product that might not be complying with the safety requirements. These actions can include the segregation of any suspect product and holding it for the period of time needed to study the risk it might represent. All this information will lead to different decisions: rejection and destruction of product, product rework, and product release. All these actions must be kept on record. In **Table 4**, some examples related to corrective actions in a dry freezing process for insect flour process are given.

4.6. Verification actions

The HACCP plan must be revised when any modification in production or equipment occurs. The HACCP team is responsible for the revision based on internal and external audit reports, records of corrective actions, and client complaints. Revision will contribute to a continual improvement of the plan.

5. Concluding remarks

Production of insects and insect-based products is a current practice in several regions of the globe with rooted habits of its consumption. In Europe and North America, these products began to explore a potential market of alternative foods. Safety issues related to insects' rearing and processing are a major concern, once due to their composition they are prone to be contaminated with pathogens and hazardous chemicals. Application of HACCP to the production and processing should be performed as for any other animal products used for human consumption. In the primary production of insects, the implementation of a robust pre-requisites plan will guarantee the reduction of the likelihood of incidents involving spreading microorganisms responsible for foodborne diseases or chemical compounds harmful for the consumer. In the processing of edible insects, once the operations used are similar to those used in other industries, the safety concerns are similar to those. In both primary production and processing, the rigorous application of HACCP principles relies on a deep knowledge on the hazards associated with the products and processes. Notwithstanding the generous amount of scientific information published in the last few years on the potential hazards associated with edible insects, the major constraint that we still face is the lack of solid information, related to the occurrence of biological and chemical hazards, how can that situation changes in contexts of production intensification, as well as surveillance or epidemiologic data on foodborne diseases having edible insects as vehicle of the hazard. That information, which is still scarce, will allow the food safety team of the insect industry to conduct an adequate and correctly supported hazard analysis that is the fundamental to the success of HACCP plan and its implementation. Additionally, the direct transposition of critical control points and respective critical limits, particularly those related to biologic hazards, from other industries to the insect processing might also have some risks, once the behavior of pathogens might be different, due to substantial differences in its composition. Thus, validation of control measures considered in CCP should be seriously considered in the insect industry.

The expected growth of insect business has several hurdles to overcome. Probably, the acceptance by new consumers is the biggest one. The rigorous compliance with the food safety rules, through the application of HACCP, will result in safe products, allowing the consumer to profit from its excellent nutritional value and from the sensorial experience.

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References

- [1] van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, et al. Edible Insects: Future Prospects for Food and Feed Security. Rome: FAO; 2013. p. 201
- [2] EFSA. Risk profile related to production and consumption of insects as food and feed EFSA Scientific Committee. EFSA Journal. 2015;**13**(October):1-60
- [3] Shelomi M. The meat of affliction: Insects and the future of food as seen in expo. Trends in Food Science & Technology. [Internet]. 2016;**56**:175-179. Available from: <http://dx.doi.org/10.1016/j.tifs.2016.08.004>
- [4] Schabel H. Forest insects as food: A global review. In: FAO, editor. Forest Insects as Food: Humans Bite Back. Bangkok; 2010. pp. 37-64
- [5] Halloran A, Vantomme P. The Contribution of Insects to Food Security, Livelihoods and the Environment [Internet]. FAO. 2017. Available from: <http://www.fao.org/docrep/018/i3264e/i3264e00.pdf> [Accessed: January 8, 2017]
- [6] Cerritos R, Cano-Santana Z. Harvesting grasshoppers *Sphenarium purpurascens* in Mexico for human consumption: A comparison with insecticidal control for managing pest outbreaks. Crop Protection. 2008;**27**(3-5):473-480
- [7] Deroy O, Reade B, Spence C. The insectivore's dilemma, and how to take the West out of it. Food Quality and Preference. 2015;**44**:44-55
- [8] Rumpold BA, Schlüter OK. Potential and challenges of insects as an innovative source for food and feed production. Innovative Food Science and Emerging Technologies [Internet]. 2013;**17**:1-11. Available from: <http://dx.doi.org/10.1016/j.ifset.2012.11.005>
- [9] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. Official Journal of the European Union. 2015;**11.12.2015**: L327/1
- [10] Halloran A, Roos N, Flore R, Hanboonsong Y. The development of the edible cricket industry in Thailand. Journal of Insects as Food and Feed. 2016;**2**:91-100
- [11] Halloran A, Bruun S. Life cycle assessment of edible insects for food protein: A review. Agronomy for Sustainable Development [Internet]. 2016;**36**:1-13. Available from: <http://dx.doi.org/10.1007/s13593-016-0392-8>

- [12] Nowak V, Persijn D, Rittenschober D, Charrondiere UR. Review of food composition data for edible insects. *Food Chemistry*. 2016;**193**:39-46
- [13] Sun-Waterhouse D, Waterhouse GIN, You L, Zhang J, Liu Y, Ma L. Transforming insect biomass into consumer wellness foods: A review. *FRIN* [Internet]. 2016;**89**:129-151. Available from: <http://dx.doi.org/10.1016/j.foodres.2016.10.001>
- [14] Johnson D. The contribution of edible forest insects to human nutrition and to forest management. In: *Forest Insects as Food: Humans Bite Back*. Bangkok: FAO; 2010. pp. 4-22
- [15] Kim H, Setyabrata D, Jae Y, Jones OG, Brad YH. Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages. *Innovative Food Science and Emerging Technologies* [Internet]. 2016;**38**:116-123. Available from: <http://dx.doi.org/10.1016/j.ifset.2016.09.023>
- [16] Stoops J, Vandeweyer D, Crauwels S, Verreth C, Boeckx H, Borght M Van Der, et al. Minced meat-like products from mealworm larvae (*Tenebrio molitor* and *Alphitobius diaperinus*): Microbial dynamics during production and storage. *Innovative Food Science and Emerging Technologies* [Internet]. 2017;**41**:1-9. Available from: <http://dx.doi.org/10.1016/j.ifset.2017.02.001>
- [17] Thailand Unique [Internet]. 2017. Available from: www.thailandunique.com [Accessed: February 10, 2017]
- [18] Entomarket [Internet]. 2017. Available from: www.edibleinsects.com [Accessed: February 10, 2017]
- [19] Schlüter O, Rumpold B, Holzhauser T, Roth A, Vogel RF, Quasigroch W, et al. Safety aspects of the production of foods and food ingredients from insects. *Molecular Nutrition & Food Research* [Internet]. 2016;**0**:1-16. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/mnfr.201600520/full%5Cnwww.mnf-journal.com>
- [20] van der Spiegel M, Noordam MY, van der Fels-Klerx HJ. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive Reviews in Food Science and Food Safety*. 2013;**12**(6):662-678
- [21] ANSES (French Agency for Food E and OH and S). Opinion on the use of Insects as Food and Feed and the Review of Scientific Knowledge on the Health Risks Related to the Consumption of Insects [Internet]. 2015. Available from: <https://www.anses.fr/fr/system/files/BIORISK2014sa0153EN.pdf> [Accessed: January 7, 2017]
- [22] Grabowski NT. Microbiology of cooked and dried edible Mediterranean field crickets (*Gryllus bimaculatus*) and superworms (*Zophobas atratus*) submitted to four different heating treatments. *Food Science and Technology International*. 2017;**23**:17-23
- [23] Han S, Lee B, Jung K, Yu H, Yun E, Sam J, et al. Safety assessment of freeze-dried powdered *Tenebrio molitor* larvae (yellow mealworm) as novel food source: Evaluation of 90-day toxicity in Sprague-Dawley rats. *Regulatory Toxicology and Pharmacology* [Internet]. 2016;**77**:206-212. Available from: <http://dx.doi.org/10.1016/j.yrtph.2016.03.006>

- [24] Kinyuru JN, Kenji GM. Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (*Macrotermes subhyllanus*) and grasshopper (*Ruspolia differens*). Food and Bioprocess Technology. 2010;**3**:778-782
- [25] Tan HSG, Fischer ARH, Tinchin P, Stieger M, Steenbekkers LPA, van Trijp HCM. Insects as food: Exploring cultural exposure and individual experience as determinants of acceptance. Food Quality and Preference. 2015;**42**:78-89
- [26] Shan H, Tan G, Fischer ARH, Van Trijp HCM, Stieger M. Tasty but nasty? Exploring the role of sensory-liking and food appropriateness in the willingness to eat unusual novel foods like insects. Food Quality and Preference [Internet]. 2016;**48**:293-302. Available from: <http://dx.doi.org/10.1016/j.foodqual.2015.11.001>
- [27] Hartmann C, Siegrist M. Becoming an insectivore: Results of an experiment. Food Quality and Preference [Internet]. 2016;**51**:118-122. Available from: <http://dx.doi.org/10.1016/j.foodqual.2016.03.003>
- [28] Usub T, Lertsatitthakorn C, Poomsa-ad N. Food and bioproducts processing. Food and Bioproducts Processing [Internet]. 2009;**88**(2-3):149-160. Available from: <http://dx.doi.org/10.1016/j.fbp.2009.04.002>
- [29] Bußler S, Rumpold BA, Fröhling A, Jander E, Rawel HM, Schlüter OK. Cold atmospheric pressure plasma processing of insect flour from *Tenebrio molitor*: Impact on microbial load and quality attributes in comparison to dry heat treatment. Innovative Food Science and Emerging Technologies [Internet]. 2016;**36**:277-286. Available from: <http://dx.doi.org/10.1016/j.ifset.2016.07.002>
- [30] Codex Alimentarius. Recommended International Code of Practice General Principles of Food Hygiene; WHO & FAO. Rome, Italy. 2003. p. 31
- [31] NACMCF (National Advisory Committee on Microbiological Criteria for Foods). HAZARD analysis and critical control point principles and application guidelines. Journal of Food Protection. 1997;**61**:762-775
- [32] Mortimore S, Wallace C. HACCP—A Practical Approach. New York: Springer-Verlag; 2013. p. 475
- [33] Arvanitoyannis I, Kassaveti A. HACCP and ISO 22000—A comparison of the two systems. In: Arvanitoyannis I, editor. HACCP and ISO 22000—Application to Foods of Animal Origin. Chichester, UK: John Wiley & Sons; 2009. pp. 3-45
- [34] Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union. 2004; **2004-04-30**:L139/1
- [35] Codex Alimentarius. Animal Food Production. Rome: WHO/FAO; 2009. p. 246
- [36] ISO 22000. Food Safety Management Systems—Requirements for Any Organization in the Food Chain. International Organization for Standardization; Genève, Switzerland. 2005. 53 p

- [37] Cerf O, Donnat E, Working H. Application of hazard analysis—Critical control point (HACCP) principles to primary production: What is feasible and desirable? *Food Control*. 2011;**22**:1839-1843
- [38] Henriques A, Telo Da Gama L, Fraqueza M. Assessing *Listeria monocytogenes* presence in Portuguese ready-to-eat meat processing industries based on hygienic and safety. *Food Research International*. 2014;**81**:81-88
- [39] Henriques A, Telo Da Gama L, Fraqueza M. Tracking *Listeria monocytogenes* contamination and virulence in the ready-to-eat meat-based food products industry according to the hygiene level. *International Journal of Food Microbiology*. 2017;**242**:101-106
- [40] Regulation (EC) No. 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed. *Official Journal of the European Union*. 2009;**2009-09-01**:L229/1
- [41] Copa, Cocega. EU Guide to Good Hygiene Practice (GGHP) for the Primary Production of Foodstuffs [Internet]. Brussels: European Farmers European Agri-Cooperatives; 2016. p. 34. Available from: <http://www.copa-cogeca.eu/> [Accessed: December 12, 2016]
- [42] Ko W. Evaluating food safety perceptions and practices for agricultural food handler. *Food Control* [Internet]. 2010;**21**(4):450-455. Available from: <http://dx.doi.org/10.1016/j.foodcont.2009.07.005>
- [43] Sahlström L. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*. 2003;**87**:161-166
- [44] Hanboonsong Y. Edible insects and associated food habits in Thailand. In: FAO, editor. *Forest Insects as Food: Humans Bite Back*. Bangkok; 2010. pp. 173-182
- [45] van Zyl C. Cost-effective culturing of *Galleria mellonella* and *Tenebrio molitor* and entomopathogenic nematode production in various hosts. *African Entomology*. 2015;**23**:361-375
- [46] Belluco S, Losasso C, Maggioletti M, Alonzi CC, Paoletti MG, Ricci A. Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive reviews in food science and technology*. 2013;**12**:296-313
- [47] Chantawannakul P, de Guzman LI, Li J, Williams GR. Parasites, pathogens, and pests of honeybees in Asia. *Apidologie*. 2016;**47**(3):301-324
- [48] Niewold TA, van Essen GJ, Nabuurs MJ, Stockhofe-Zurwieden N, van der Meulen J. A review of porcine pathophysiology: A different approach to disease. *The Veterinary Quarterly*. 2000;**22**(4):209-212
- [49] Fraqueza MJ, Martins A, Borges AC, Fernandes MH, Fernandes MJ, Vaz Y, et al. Antimicrobial resistance among *Campylobacter* spp. Strains isolated from different poultry production systems at slaughterhouse level. *Poultry Science*. 2014;**93**(6):1578-1586
- [50] Bosona T, Gebresenbet G. Food traceability as an integral part of logistics management in food and agricultural supply chain. *Food Control* [Internet]. 2013;**33**(1):32-48. Available from: <http://dx.doi.org/10.1016/j.foodcont.2013.02.004>

- [51] Grabowski NT, Klein G. Bacteria encountered in raw insect, spider, scorpion, and centipede taxa including edible species, and their significance from the food hygiene point of view. *Trends in Food Science & Technology*. 2017;**63**:80-90
- [52] Agabou A, Allou i N. Importance of *Alphitobius diaperinus* (Panzer) as a reservoir for pathogenic bacteria in Algerian broiler houses. *Veterinary World*. 2010;**3**:71-73
- [53] Amadi E, Ogbalu O, Barimalaa I, Pius M. Microbiology and nutritional composition of an edible larva (*Bunaea alcinoe* Stoll) of the Niger Delta. *Journal of Food Safety*. 2005;**25**:193-197
- [54] Braide W, Oranusi S, Udegbumam L, Oguoma O, Akobundu C, Nwaoguikpe R. Microbiological quality of an edible caterpillar of an emperor moth. *Journal of Ecology and the Natural Environment*. 2011;**3**:176-180
- [55] Strother K, Steelman C, Gbur E. Reservoir competence of lesser mealworm (Coleoptera: Tenebrionidae) for *Campylobacter jejuni* (Campylobacterales: Campylobacteraceae). *Journal of Medical Entomology*. 2005;**42**:42-47
- [56] Templeton JM, De Jong A, Blackall P, Mifflin J. Survival of *Campylobacter* spp. in darkling beetles (*Alphitobius diaperinus*) and their larvae in Australia. *Applied and Environmental Microbiology*. 2006;**72**:7909-7911
- [57] Wales A, Carrique-Mas J, Rankin M, Bell B, Thind B, Davies R. Review of the carriage of zoonotic bacteria by arthropods, with special reference to Salmonella in mites, flies and litter beetles. *Zoonoses Public Health*. 2010;**57**:299-314
- [58] Chai J, Shin E, Lee S, Rim H. Foodborne intestinal flukes in South-east Asia. *The Korean Journal of Parasitology*. 2009;**47**:69-102
- [59] Simpanya M, Allotey J, Mpuchane S. A mycological investigation of phane, an edible caterpillar of an emperor moth, *Imbrasia belina*. *Journal of Food Protection*. 2000;**63**:137-140
- [60] Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union*. 2010;**20.1.2010**:L15/1
- [61] Poma G, Cuykx M, Amato E, Calaprice C, Francois J, Covaci A. Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. *Food and Chemical Toxicology* [Internet]. 2017;**100**:70-79. Available from: <http://dx.doi.org/10.1016/j.fct.2016.12.006>
- [62] Dillon R, Charnley K. Mutualism between the dessert locust *Schistocerca gregaria* and its gut microbiota. *Research in Microbiology*. 2002;**153**:503-509
- [63] Jung J, Heo A, Park YW, Kim YJ, Koh H, Park W. Gut microbiota of *Tenebrio molitor* and their response to environmental change. *Journal of Microbiology and Biotechnology*. 2014;**24**(7):888-897
- [64] Stoops J, Crauwels S, Waud M, Claes J, Lievens B, Van Campenhout L. Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. *Food Microbiology*. 2016

- [65] Garofalo C, Osimani A, Milanovi V, Taccari M, Cardinali F, Aquilanti L, et al. The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. *Food Microbiology*. 2017;**62**:15-22
- [66] Hogenhout S, Ammar D, Whitfield A, Redinbaugh M. Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*. 2008;**46**:327-359
- [67] Duarte A, Attili-Angelis D, Baron N, Forti L, Pagnocca F. Leaf-cutting ants: An unexpected microenvironment holding human opportunistic black fungi. *Antonie Van Leeuwenhoek*. 2014;**106**:465-473
- [68] Guedes F, Attili-Angelis D, Pagnocca F. Selective isolation of dematiaceous fungi from the workers of *Atta laevigata* (Formicidae: Attini). *Folia Microbiology (Praha)*. 2008;**57**:21-26
- [69] Chen X, Feng Y, Chen Z. Common edible insects and their utilization in China: Invited review. *Entomology Research*. 2009;**39**:299-303
- [70] Milanović V, Osimani A, Pasquini M, Aquilanti L, Garofalo C, Taccari M, et al. Getting insight into the prevalence of antibiotic resistance genes in specimens of marketed edible insects. *International Journal of Food Microbiology*. 2016;**227**:22-28
- [71] Post K, Riesner D, Walldorf V, Mehlhorn H. Fly larvae and pupae as vectors for scrapie. *Lancet*. 1999;**354**:1969-1970
- [72] Thackray A, Muhammad F, Zhang C, Denyer M, Spiropoulos J, Crowther D, et al. Prion-induced toxicity in PrP transgenic *Drosophila*. *Experimental and Molecular Pathology*. 2012;**92**:194-201
- [73] Thackray A, Di Y, Zhang C, Wolf H, Pradl L, Vorberg I, et al. Prion-induced and spontaneous formation of transmissible toxicity in PrP transgenic *Drosophila*. *Biochemical Journal*. 2014;**463**:31-40
- [74] Thackray A, Zhang C, Arndt T, Bujdoso R. Cytosolic PrP can participate in prion-mediated toxicity. *Journal of Virology*. 2014;**88**:8129-8138
- [75] Nishimune T, Watanabe Y, Okazaki H, Akai H. Thiamin is decomposed due to *Anopheles* spp. entomophagy in seasonal ataxia patients in Nigeria. *The Journal of Nutrition*. 2000;**130**:1625-1628
- [76] Mathew T, Ndamiso M, Shaba E, Mustapha S, Muhammed S, Adamu A. Phytochemical, physicochemical, anti-nutritional and fatty acids composition of soldier termites (*Coptotermes gestroi*) from Paikoro local government, Niger state, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology*. 2013;**7**:71-75
- [77] Chakravorty J, Ghosh S, Megu K, Jung C, Meyer-rochow VB. Entomology nutritional and anti-nutritional composition of *Oecophylla smaragdina* (Hymenoptera: Formicidae) and *Odontotermes* sp. (Isoptera: Termitidae): Two preferred edible insects of Arunachal Pradesh, India. *Journal of Asia-Pacific Entomology [Internet]*. 2016;**19**(3):711-720. Available from: <http://dx.doi.org/10.1016/j.aspen.2016.07.001>

- [78] Ji K-M, Zhan Z-K, Chen J-J, Liu Z-G. Anaphylactic shock caused by silkworm pupa consumption in China. *Allergy*. 2008;**63**:1405-1410
- [79] Barre A, Velazquez E, Delplanque A, Bienvenu F, Bienvenu J, Benoist H, et al. Les allergènes croissants des insectes comestibles. *Rev française d'allergologie* [Internet]. 2016;**56**(7-8):522-532. Available from: <http://dx.doi.org/10.1016/j.reval.2016.10.008>
- [80] Houbraken M, Sprangers T, De Clercq P, Cooreman-algoed M, Couchement T, De Clercq G, et al. Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. *Food Chemistry* [Internet]. 2016;**201**:264-269. Available from: <http://dx.doi.org/10.1016/j.foodchem.2016.01.097>
- [81] Gaylor M, Harvey E, Hale R. House crickets can accumulate polybrominated diphenyl ethers (PBDEs) directly from polyurethane foam common in consumer products. *Chemosphere*. 2012;**86**:500-505
- [82] Green K, Broome L, Heinze D, Johnston S. Long distance transport of arsenic by migrating Bogong moths from agricultural lowlands to mountain ecosystems. *The Victorian Naturalist* 2001;**118**:114-116
- [83] Vijver M, Jager T, Posthuma L, Peijnenburg W. Metal uptake from soils and soil-sediment mixtures by larvae of *Tenebrio molitor* (L.) (Coleoptera). *Ecotoxicology and Environmental Safety*. 2003;**54**:277-289
- [84] Handley M, Hall C, Sanford E, Diaz E, Gonzalez-Mendez E, Drace K, et al. Globalization, binational communities, and imported food risks: Results of an outbreak investigation of lead poisoning in Monterey County, California. *American Journal of Public Health*. 2007;**97**:900-906
- [85] Zhuang P, Zou H, Shu W. Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. *Journal of Environmental Sciences*. 2009;**21**:849-853
- [86] Cappellozza S, Saviane A, Tettamanti G, Squadrin M, Vendramin E, Paolucci P, et al. Identification of *Enterococcus mundtii* as a pathogenic agent involved in the "flacherie" disease in *Bombyx mori* L. larvae reared on artificial diet. *Journal of Invertebrate Pathology* [Internet]. 2011;**106**(3):386-393. Available from: <http://dx.doi.org/10.1016/j.jip.2010.12.007>
- [87] Inglis D, Sikorowski P. Microbial contamination and insect rearing. In: Schneider J, editor. *Principles and Procedures for Rearing High Quality Insects*. Mississippi State University; 2009. pp. 325-340
- [88] Fraqueza M, Barreto A. HACCP. In: Toldra, Hui Y, Astiasaran I, Sebranek J, Talon R, editors. *Handbook of Fermented Meat and Poultry*. Oxford: Wiley-Blackwell; 2005. pp. 513-534

