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REVIEW

Safety aspects of the production of foods and food ingredients from insects

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At present, insects are rarely used by the European food industry, but they are a subject of growing interest as an alternative source of raw materials. The risks associated with the use of insects in the production of foods and food ingredients have not been sufficiently investigated. There is a lack of scientifically based knowledge of insect processing to ensure food safety, especially when these processes are carried out on an industrial scale. This review focuses on the safety aspects that need to be considered regarding the fractionation of insects for the production of foods and food ingredients.

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1 Introduction

Insects are widespread food sources in many regions of the world [1]. A list of insects consumed worldwide, which are cited in scientific publications, can be found under the below mentioned link.[1] In Europe the consumption of insects is uncommon, but is gaining growing attention. A selection of insects, which are already offered for consumption on a small scale in some European countries or might be used in food production in the future, is shown in Table 1.

Insects are nutrient-rich and in some cases have high protein and fat contents when compared to other animal foods (pork, beef, and poultry) [2]. All insects contain the polysaccharide chitin, a polymer of *N*-acetyl-D-glucosamine, as a

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Abbreviation: CCPs, critical control points

[1] http://www.wageningenur.nl/en/Expertise-Services/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm.

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Table 1. Insect species that might be used as food in EU countries

Insect species	Order	Developmental stage being consumed	Relevant pathogenic microorganisms	Literature
Acheta domesticus (cricket)	Orthoptera (long antennae)	Imago (adult form)	*Enterobacteriaceae (Klebsiella sp., Yersinia sp., Citrobacter sp.)*	[107]
Gryllus assimilis (field cricket)	Orthoptera (long antennae)	Imago (adult form)	No data on the autochthonous microbiota	
Gryllus bimaculatus	Orthoptera (long antennae)	Imago (adult form)	No data on the autochthonous microbiota, new species of *Spiroplasma sp.* with 95% identity to *Spiroplasma platyhelix*	[108]
Gryllodes sigillatus (banded cricket)	Orthoptera (long antennae)	Imago (adult form)	No data on the autochthonous microbiota	
Locusta migratoria (migratory locust)	Orthoptera (short antennae)	Imago (adult form)	*Enterobacteriaceae (Klebsiella sp., Klebsiella pneumoniae, Klebsiella oxytoca, Yersinia sp., Enterobacter cloacae*), *Enterococcus sp., Pseudomonas aeruginosa* vector and reservoir for vesicular stomatitis virus	[62, 63, 109–111]
Oxya fuscovittata	Orthoptera (short antennae)	Imago (adult form)	No data on the autochthonous microbiota	
Schistocerca americana (American bird grasshopper)	Orthoptera (short antennae)	Imago (adult form)	No data on the autochthonous microbiota	
Schistocerca gregaria (desert locust)	Orthoptera (short antennae)	Imago (adult form)	*Enterobacteriaceae (Enterobacter sp., Enterobacter liquefaciens, K. pneumonia, K. oxytoca, Escherichia coli, E. cloacae, Enterobacter agglomerans, Serratia marcescens, Citrobacter sp.), Bacillus cerreus, Clostridium perfringens, Clostridium septicum, Clostridium difficile, Clostridium sporogenes, Clostridium capitovale, P. aeruginosa, Acinetobacter sp., Enterococcus sp., Staphylococcus sp., Streptococcus sp., Rhodococcus sp.*	[64]
Achroia grisella (lesser wax moth)	Lepidoptera (butterflies and moths)	Caterpillar	No data on the autochthonous microbiota	
Bombyx mori (silkmoth)	Lepidoptera (butterflies and moths)	Caterpillar, pupa without cocoon	*Enterobacteriaceae (Proteus vulgaris, K. pneumoniae, Citrobacter freundii, Serratia liquefaciens, Serratia sp., Shigella sp., Enterobacter sp., Erwinia sp., Pantoea sp), Aeromonas sp., P. aeruginosa, Pseudomonas fluorescens, Clostridium sp., Bacillus sp., Bacillus circulans, Staphylococcus sp., Streptococcus sp., Enterococcus sp., Enterococcus mundtii, Acinetobacter sp., Moraxella sp., Aeromonas hydrophila, Actinobacteria*	[71, 112, 113]
Galleria melonella (greater wax moth)	Lepidoptera (butterflies and moth)	Caterpillar	No data on the autochthonous microbiota, *G. melonella* is prevalently used as an in vivo infection model for pathogenic bacteria and fungi, because numerous human-pathogenic microorganisms are easy to breed in this species	[114]
Imbrasia belina/Gonimbrasia bellina, (mopani worm, emperor moth)	Lepidoptera (butterflies and moths)	Caterpillar	*Alternaria sp., Aspergillus sp., Chaetomium sp., Drechslera sp., Penicillium sp., Fusarium sp., Mucor sp., Phoma*	[115]

Table 1. Continued

Insect species	Order	Developmental stage being consumed	Relevant pathogenic microorganisms	Literature
Alphitobius diaperinus (litter beetle)	Coleoptera (beetles)	Larva (lesser mealworm)	No data on the autochthonous microbiota vector for *Salmonella enterica*, *E. coli*, *Campylobacter jejuni*, *Acinetobacter sp. Aspergillus sp., Infectious Bursal disease virus, Marek´s disease virus, Turkey Corona virus, Sporozoa: Coccidia (Eimeria)*	[51, 65, 66, 116–118]
Tenebrio molitor (yellow meal beetle)	Coleoptera (beetles)	Larva (mealworm)	*Enterobacteriaceae (Salmonella sp., Erwinia sp., Pantoea sp.), Staphylococcus sp., Haemophilus sp., Clostridium sp., Bacillus sp., Enterococcus sp., Bacillus sp.*)	[45, 63]
Zophobas atratus (morio beetle)	Coleoptera (beetles)	Larva	No data on the autochthonous microbiota	
Atta laevigata (leaf cutter ants)	Hymenoptera (bees, wasps, and ants)	Imago (adult form)	*A. fumigatus, Aspergillus sclerotiorum,* and *Penicillium* sp., opportunistic human-pathogenic fungi of the genera *Cladophialophora*, *Exophiala*, *Metarhizium*, *Ochroconis*, *Phialophora,* and *Penidiella*	[119, 120]
Hermetia illucens (black soldier fly)	Diptera (dipteran, flies)	Larva	*Enterobacteriaceae* (*K. pneumoniae, E. coli, Morganella morganii, Klebsiella sp., Klebsiella granulomatis, Shigella sp., Proteus mirabilis, Providencia rettgeri*, *Providencia stuartii, Citrobacter sp., Enterobacter sp.*) *Enterococcus caccae*, *Clostridium sp., Bacillus sp., Strepptococcus sp., Pseudomonas sp., Staphylococcus sp., Corynebacterium sp., Acinetobacter sp., Wohlfahrtiimonas larvae sp. nov.*potential vector for *Ascaris suum (*experimental*)*	[121]
Musca domestica (housefly)	Diptera (dipteran, flies)	Larva	Dependent on rearing conditions frequently transition of allochthonous to autochthonous microbiota: *Enterobacteriaceae* (*E. coli*, *Enterobacter* sp., *Klebsiella* sp., *Citrobacter* sp., *Shigella* sp., *Morganella* sp., *Proteus* sp., *Providencia* sp.) *Bacillus* sp., *Bacilius cereus*, *Staphylococcus aureus*, *P. aeruginosa*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Enterococcus* sp. fungi as *Aspergillus tamari* and *Alternaria* sp., vector für *E. coli* O157:H7, *Salmonella* sp., *Salmonella typhi*, *Yersinia pseudotuberculosis*, vector for different species of nematodes: round worm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookwormvector for Circovirus, subtypes of avian influenza virus H5N7 and H7N1,suspected to act as a vector for *Vibrio cholerae*	[47, 49, 122, 123]

component of the exoskeleton. They also possess enzymes, e.g. cellulases or proteases, which could be of interest in various different food applications [3, 4]. Therefore, insects not only represent an alternative source of protein but also an alternative source of other substances that could be used by food industry. It is also being discussed whether, in addition to their favorable nutrient profiles, insects may offer ecological and economic benefits over conventional animal production and, therefore, may constitute an alternative or a complement to conventional food sources [5, 6].

Several statements on the food safety of insects were published in the last years [7–10]. They focused on aspects related to the use of whole insects as food and feed and were based on a limited selection of currently relevant species. These statements examined processing methods such as deep-frying, toasting, drying, or freeze-drying, mainly focusing on their decontaminating effects. The authors identified significant research needs regarding the potential microbial, allergenic, and toxicological risks associated with the consumption of whole insects. In 2015, the European Food Safety Authority

Table 1. Continued

Insect species	Order	Developmental stage being consumed	Relevant pathogenic microorganisms	Literature
Blattella germanica (German cockroach)	Blattodea (cockroaches)	Imago (adult form)	Dependent on rearing conditions frequently transition of allochthonous to autochthonous microbiota: *Enterobacteriaceae (E. coli, Salmonella sp., Klebsiella sp., K. pneumoniae, Enterobacter sp., Enterobacter aeroginus, E. cloacae, Serratia sp., Serratia marcesens, Citrobacter sp., C. freundii, Proteus sp., P. mirabilis, Shigella sp.) Enterococcaceae, Enterococcus sp., Staphylococcaceae sp., S. aureus, Streptococcus sp., Aeromonas sp., Pseudomonadaceae, Pseudomonas sp., P. aeruginosa, Haemophilus sp., Clostrdiales, Candida sp., Mucor sp., Penicillium sp., Aspergillus niger, A. fumigatus*	[73, 124]
Periplaneta americana (American cockroach)	Blattodea (cockroaches)	Imago (adult form)	Dependent on rearing conditions: *Enterobacteriaceae (E. coli*, *Escherichia vulneris*, *Salmonella sp., E. aeroginus, E. cloacae, Shigella flexneri*, *K. pneumoniae, S. marcesens, C. freundii*, *E. cloacae*, *Providenci*a sp., *Y. pseudotuberculosis*, *Yersinia intermedia*, *Klebsiella sp., K. oxytoca*, *Klebsiella planticola*, *Salmonella sp.*) *Proteus sp., P. mirabilis, P. vulgaris, Leclercia adecarboxylata, Rahnella aquatilis, Bacillus sp., Staphylococcus sp., S. aureus, Staphylococcus epidermidis, Enterococcus sp., P. aeruginosa, A. niger, Mucor sp., Candida sp., Fusarium sp., Penicillium,* round worm (*A. lumbricoides),* whipworm *(T. trichiura), Coccidia, Entamoeba histolytica, Enterobius vermicularis, Schistosoma haematobium, Balantidium coli*	[61, 125]

(EFSA) published a risk profile for the production and consumption of insects as food and feed [11].

The purpose of this review was to identify potential risks in the process chain from the farmed insect to an isolated fraction by taking into consideration different insect species and stages of development and by putting a focus on individual fractions. In particular, the following aspects were considered:

(i) whether the use and the processing of insects to obtain fractions or ingredients give rise to new and previously unknown risks,
(ii) whether these are the same for all insects or vary depending on the species and the stage of development,
(iii) whether the methods available to minimize or eliminate the risks suffice or whether these need to be modified or newly developed.

It is assumed that the conditions in insect rearing facilities used to produce fractions and ingredients will comply with the respective food safety regulations applicable to livestock husbandry. These include controlled husbandry and feeding conditions to prevent microbial and chemical contamination. Data on the consumption of whole insects and the risks associated with the capture of insects living in the wild as well as the risks associated with the use of insects or insect fractions as feed and the production of bee honey are not taken into account in this review.

2 Technological aspects related to processing of insects

Depending on the intended use, species-specific safety aspects, e.g. potential microbial, allergenic, and toxicological risks, must be considered when selecting the insect species and their stages of development (in the case of holometabolous insects with a complete metamorphosis: egg, larva, pupa, and adults; in the case of hemimetabolous insects with an incomplete metamorphosis: egg, nymph, and adults). The properties of the primary material as well as those of the desired product may limit the use of certain technological

processing methods. The protein, fat, and chitin contents of the larvae and imagines of an insect species may vary significantly and in turn may greatly influence the processing [12]. Other compounds, e.g. the high amounts of calcium in the final larval stage of the black soldier fly (*Hermetia illucens*), may also affect fractionation [13].

The available food technological isolation and preparation processes need to be examined regarding their capacity to remove undesired insect-specific components and contaminants (toxins and antinutrients) from the fractions. Established processes may have to be modified or may need to be newly developed. To ensure microbial safety, additional decontamination steps may be required. It is essential to carry out a hazard analysis (Hazard Analysis and CCPs study[2]) for each product processing line, thereby taking into account all (physical, microbial, allergenic, and chemical) risks. For hazards classified as relevant, critical control points (CCPs) and preventive programs must be established.

2.1 Proteins

Regarding protein extraction, there are differences between insects and conventional sources, such as the binding of insect-specific chitin to structure-providing proteins of the exoskeleton [14]. Insect-specific allergens, insect-specific antimicrobial peptides [15], and prions, which may be taken up by the insect through contaminated feed, may also be present, whereby insect-specific prions have not yet been described. In most cases, the gut cannot be removed; therefore, it must be assumed that microbial proteins are coextracted with the target protein(s), especially in the case of insects with a high proportion of gut and gut content to total mass.

The protein content of various edible insects ranges from 5 to 77% with average values between 35 and 61% based on dry matter [2]. As a percentage of fresh weight, a protein content of 10–25% has been reported [16]. Different studies describing the isolation of proteins from selected insect species at the laboratory scale are available. A procedure to isolate proteins from mealworm beetle (*Tenebrio molitor*; larvae), darkling beetle (*Zophobas morio*; larvae), lesser mealworm (*Alphitobius diaperinus*; larvae), house cricket (*Acheta domesticus*; adult), and Dubia roach (*Blaptica dubia*; adult) revealed that around 40% of the total protein was found in the filtered residue, around 40% in the centrifuged pellet, and around 20% in the supernatant of the aqueous extraction phase [17]. Using aqueous extractions at different temperatures and pH values, gelling proteins with properties similar to conventional gelatine were obtained from defatted, dried, and pulverized beetle species [18].

Large amounts of coextracted chitin may have a negative effect on the digestibility of insect protein. Whether chitin is digested in the human gastrointestinal tract is being controversially discussed at present [19]. A protein concentrate of bees exhibited a higher digestibility in rats than the whole pulverized bees, measured as the ratio of absorbed to excreted protein, and this was attributed to the removal of chitin [20]. Although the extrapolation of these data to humans still has to be verified, it indicates that both the maximum amount of chitin recommended for human consumption in food [21] and protein digestibility in association with chitin should be taken into consideration.

When developing methods to isolate proteins from insects, it must be verified whether extraction methods to obtain proteins from conventional sources are suitable for the insect matrix or whether they need to be modified, and to what extent the native structure and the allergenic potential of the isolated protein fractions are affected. It is likely that the methods will have to be adapted to different insect species.

2.1.1 Enzymes

Because insects have adapted to a variety of habitats and diets, a wide range of digestive enzymes is produced by the insect itself or by members of the gut microbiota [4], e.g. proteolytic enzymes [22], cellulases [3], α-amylases [23], and lipases [24]. The genomic analysis of insects and the metagenomic analysis of insect microbiota allowed to identify genes coding for enzymes with the potential to be used in food technology [4], but up to now no industrial applications of insect enzymes have been reported.

The complexity of the insect matrix could make it difficult to extract sufficiently pure enzyme fractions. For their use in food production, it must first be determined whether the processing steps involved in the isolation and purification of insect enzymes differ from those used for conventional sources. Furthermore, methods to extract enzymes from insects should guarantee a complete decontamination of the product. It remains to be established whether possible antinutritive properties of certain insect enzymes present a risk when applied in food production.

2.2 Lipids

Lipids are found in the fat body surrounding the insect gut, which is the central energy reservoir and an important metabolism site in insects [25]. The average fat content of various edible insect species ranges from 13 to 33% in relation to dry mass, whereby some species reach a maximum fat content of over 70% [2]. Moreover, a fat content of 4–32% has been reported in relation to fresh weight [16]. The fatty acid spectrum depends on the species and the stage of development; nevertheless, it is comparable to that in other animal species [2] and, as in the latter case, is affected by the composition of the diet. Essentially, the favorable nutrient

[2] http://www.fao.org/docrep/005/Y1579E/y1579e03.htm

composition for each insect species depends on the type and quality of its feed [26].

Lipids can be isolated from insects with standard methods such as an extraction with hexane/petrol ether or supercritical carbon dioxide [27]. However, only a limited amount of data is currently available on the influence of the extraction and thermal treatment on nutritionally relevant substances in insect oils [28, 29].

With regard to the extraction of lipids from insects, it must be assumed that both triglycerides and other endogenous lipophilic substances are extracted. One example is ecdysteroids, hormones that control moulting, metamorphosis, and reproduction in insects and may potentially elicit pharmacological effects [30]. The levels of such lipid components in insect oils should therefore be investigated.

In addition to endogenous lipophilic substances, lipophilic environmental contaminants such as dioxins [31] represent a potential risk. Lipophilic contaminants that cannot be avoided despite carefully controlled breeding conditions should be minimized during lipid extraction by making use of appropriate methods (refining).

It must be clarified whether existing and usual methods for the extraction and refining of fats and oils are suitable for removing undesired insect-specific impurities. It may be necessary to adapt standard oil extraction and oil refining technologies to the insect species and their developmental stage.

2.3 Polysaccharides

The polysaccharides present in significant amounts in insects include chitin and glycogen. Glycogen is stored in the cells of the fat body [25] and in the muscles. Chitin, a polymer of *N*-acetyl-D-glucosamine, is the main component of the exoskeleton [14]; its level depends on the insect species and the stage of development.

Chitin is an interesting component for the food industry and is currently extracted from the shells of *Crustacea* [32]. Chitosan (poly-D-glucosamine) can be manufactured from chitin through deacetylation [33]. This substance can be used in food as a thickening, a prebiotic, or an antimicrobial agent as well as a semipermeable coating minimizing respiration and water losses of fruits and vegetables [34].

In studies on the chitin extraction from insects, an isolation was performed in the same way as in the case of marine waste materials containing chitin, such as shrimp shells [33]. However, it is not known whether chitin fractions from insects contain undesired, insect-specific contaminants whose removal would require additional technological processing stages. Given the potential adsorption of heavy metals onto chitin [35], insect breeding would have to take place under appropriately controlled conditions. The allergenic potential of chitin and the binding of allergens to chitin should also be investigated.

2.4 Other components

Except for carmine, there is currently no knowledge regarding other insect-derived components that are or could be applied in the food industry. Carmine is extracted from gravid female cochineals (*Dactylopius coccus*) and is approved in the European Union (EU) for use as a food colorant (E 120). The cochineals are bred on the cladodes of various members of the genus *Opuntia* (a genus in the cactus family) [36]. After defatting with organic solvent, the cochineals are extracted at an elevated pH, and carmine is isolated by precipitation under acidic conditions [37]. Carmine has been described as a trigger for serious allergic reactions [38].

3 Safety criteria

3.1 Microbial aspects

The microbiota of insects is highly complex [39–42]. Apart from the body surface and the mouthparts, the main habitat for microorganisms is the gut. They colonize the insects in various ways: vertically with the microorganisms of the parents through (i) the ovary, (ii) the egg capsule, (iii) a smear infection during egg laying, and (iv) horizontally through the diet and the environment [42, 43]. In recent years, modern metagenomic analyses have greatly increased the knowledge on microbial biodiversity, especially in the insect gut [4, 42, 44–48].

The use of insects as food entails potential microbiological risks because insects can serve as vectors for microorganisms pathogenic to humans, animals, and plants. One has to discern whether the transmission of microorganisms occurs mechanically through contact with the surface of the insect body [49, 50] or whether the microorganisms are able to persist and multiply inside the insect without themselves becoming sick [51]. The pathogens that can be transmitted through insects include viruses [52], rickettsia [53], bacteria [54], protozoa [55], fungi [56], nematodes, and other parasites of the human digestive tract [57]. Insect-specific pathogenic microorganisms [58] are considered harmless to humans because they have a high degree of tissue tropism (tissue specificity) and can therefore probably only colonize the cells or tissues of insects. So far, no insect-specific pathogenic microorganisms harmful to human health have been described, except for a few representatives of the rickettsia genus [59]. Insect-specific prions or insects as natural vectors for prions have not yet been described. The transmission of prions to animals and humans by consumption of insects contaminated by prion-containing feed cannot be ruled out [60] and might be taken into account when deciding on the kind of feed to be used for insect breeding.

Generally a distinction is made between microorganisms of the autochthonous microbiota, which always occur in all individuals of an insect species, and microorganisms of the allochthonous microbiota, which occur sporadically

and are present either because of specific environmental or breeding conditions or owing to contact with humans or other individuals [41]. Human-pathogenic and/or opportunistically human-pathogenic and toxin-forming microorganisms of food hygienic and/or medical relevance are found in the autochthonous as well as the allochthonous microbiota. As far as is currently known, they are limited to the species capable of triggering inter alia foodborne diseases (Table 1). Their occurrence in insects is widespread and not specific. They belong to the genera *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Pseudomonas, Bacillus,* and *Clostridium* or belong to the *Enterobacteriaceae*, such as *Escherichia*, *Enterobacter*, *Salmonella*, *Klebsiella*, *Serratia*, *Shigella,* or *Yersinia* [47, 66]. Fungi of the genera *Aspergillus, Penicillium, Alternaria,* and *Candida*, which include human-pathogenic, toxin-forming species [67,68] as well as allergenic species, are also part of the microbiota on the surface and in the gut of insects.

Since in virtually all insect species it is not possible to remove the gut with its microbiota, the ratio of gut content to total mass is of particular interest in relation to the preparation of insects as food. The volume of the intestinal tract varies from 0.05 to 2 mL depending on the species. Average bacterial densities of 10^6–10^{12} bacteria per milliliter of gut content have been detected in some insect species, depending on the segment of the gut analyzed [69]. The microbial biomass accounts for 1–10% of the total insect body and depends on the insect species [70]. When processing insects to produce fractions or extract enzymes, it must therefore be assumed that ab initio there is an unavoidably high microbial contamination, which needs to be taken care of with appropriate processing steps, e.g. heating processes.

It is likely that a contamination with human-pathogenic and toxin-forming species of the allochthonous microbiota can be made manageable by means of controlled breeding conditions, but the high density of insect monocultures represents an additional difficulty [58]. It must also be noted that different feed substrates can alter the species spectrum of the gut microbiota as well as the proportion of individual species depending on the insect species and developmental stage [42, 45, 71, 72], which in turn may increase the microbial count of human-pathogenic microorganisms. The composition of the microbiota also changes during the complex individual development of insects and can be influenced by diet and environmental conditions [73, 74]. EFSA has proposed a possible classification of substrates for insect breeding with different levels of hazard potential [11]. Human-pathogenic microorganisms, which cannot be avoided even under controlled breeding conditions, must be given special attention and must be inactivated by adequate processes.

It can be assumed that the methods employed to process insects for the extraction of protein and lipid fractions or enzymes will remove the microbial contamination of the raw material. Nevertheless, it may be necessary to establish CCPs during the production and processing of insects [63] and intermediate decontamination stages. Initial data are available on the effects of different processes on the microbiological status of whole insects [8].

3.2 Chemical and toxicological aspects

The selection of insect species suitable for consumption must also include a consideration of toxins and antinutrients, such as oxalate, tannin, phytate [75, 76], and thiaminases [77]. Toxins and antinutrients should be distinguished according to whether they are absorbed by the insect from the feed or synthesized by the insect itself. Insect breeding conditions should comply with the applicable food safety regulations (see Section 4). Insects selected for the production of food and food ingredients should therefore be kept in such a way as to prevent or minimize the accumulation of externally introduced toxins, drugs, or antinutrients.

Some insect species synthesize substances, which are toxic to humans. One example is cantharidin, a monoterpene (2,6-dimethyl-4,10-dioxatricyclo-[5.2.1.02,6]decane-3,5-dione) synthesized by the Spanish fly (*Lytta vesicatoria*), a beetle that belongs to the oil beetle family, and various other beetles. It is bound to proteins, which are therefore referred to as cantharidin-binding proteins. The toxic effects following consumption include difficulty in swallowing, nausea, and vomiting of blood [78]. Longhorn beetles may contain toluene. Darkling beetles (Tenebrionidae) produce quinones and alkanes [79], while certain moth species of the genus *Zygaena* contain cyanogenic glycosides [80]. The risk potential of such substances needs to be investigated. The same applies to toxins that may be formed by microorganisms in the insect gut, for example toxins of the genera *Bacillus*, *Clostridium,* and *Aspergillus* (Table 1). No data are available on the presence of toxins in potentially edible insect species. A first 90-day feeding trial showed that *T. molitor* larvae do not lead to adverse effects in rats when fed as dried powder up to the highest dose of 3000 mg/kg body weight/day [81]. The traditional consumption of insects in certain parts of the world is taken as an indication that the consumption of insects does not present a health risk [1, 82]. There may be specific cases in which the existing knowledge on the traditional use of insects in certain countries is comprehensive enough to serve as a basis to demonstrate a "history of safe use." However, so far this point has not been scientifically or systematically investigated. Particularly as a result of processing steps, it is possible that harmful components only present in trace amounts could be enriched together with the actual target components during fractionation. The toxic potential of antinutrients and the antinutrient content should be minimized by selecting appropriate breeding and processing conditions.

3.3 Allergenic potential

3.3.1 Allergic reactions

Isolated allergic episodes, including anaphylactic reactions [83–85], have been documented in the medical literature in connection with the consumption of insects. Pan-allergenic structures have been identified in arthropods (*Arthropoda*), which include insects (*Insecta*, e.g. bees, beetles, locusts, and cockroaches), arachnids (*Arachnida*, e.g. mites), and crustaceans (*Crustacea*, e.g. shrimps, crabs, and lobsters). Similarly, pan-allergenic structures have been described in molluscs (Mollusca) [86, 87]. For example, pan-allergenic tropomyosin may elicit allergic reactions to *Crustacea* as well as mites and insects (e.g. cockroaches) [88, 89]. This effect was confirmed in a study on cross-reactivity to mealworm larvae (*T. molitor*) in patients with inhalant and food allergy to mites and *Crustacea*, respectively. Tropomyosin and arginine kinase were identified as cross-reactive proteins. It is therefore possible that people who are allergic to *Crustacea* and house dust mites will also experience an allergic reaction to foods containing proteins from mealworm larvae [90]. Very recently, a double-blind placebo-controlled food challenge with mealworm protein demonstrated that the majority of subjects allergic to shrimps are allergic to mealworm [91]. Other ubiquitous or pan-allergenic structures (see below) may also result in a possible allergic cross-reaction to arthropods and therefore edible insects in atopic subjects. Furthermore, a primary sensitization to these ubiquitous or pan-allergenic structures and to species-specific allergens is possible. Unexpected allergic cross-reactions may occur due to sensitization to pan-allergenic structures, as the allergic consumer cannot directly identify the source of the allergen. This problem becomes even more significant when potentially allergenic fractions, for example the protein fraction, are extracted and used as an ingredient in compound foods. Considering the relatively high frequency of inhalation allergies to house dust mites, flour mites, and cockroaches in the general population when compared to classic food allergies [92, 93], a much larger proportion of the population could be affected by possible cross-reactions between mite and insect pan-allergens. The possible contamination of insects with pathogenic molds with known allergenic potential, such as *Aspergillus* and *Penicillium*, or pathogenic yeast, such as *Candida* [94], should be taken into account as a secondary trigger of allergic reactions, i.e. not directly due to the insect. Measures would therefore have to be taken to ensure that cultivated insects were free from organisms with allergenic potential.

3.3.2 Allergenic structures

The main allergenic structures in insects are (glyco)proteins, which include the insect venom allergens (e.g. phospholipase A, hyaluronidase). In arthropods, 239 individual allergens are currently registered according to the requirements of the Allergen Nomenclature Sub-Committee of the World Health Organization and the International Union of Immunological Societies (www.allergen.org, last accessed on February 17, 2016). These are mostly ubiquitous or pan-allergenic proteins, which, in simplified terms, can be categorized as muscle proteins (e.g. tropomyosin, myosin, actin, troponin C), cellular proteins (e.g. tubulin), circulating proteins (e.g. hemocyanin, defensin), and enzymes (e.g. arginine kinase, triosephosphate isomerase, α-amylase, trypsin, phospholipase A, hyaluronidase). About half of the current arthropod database entries relate to allergens in insects, although so far there has been no systematic investigation of the different stages of insect development. It is therefore unclear to what extent the developmental stages of insects contribute to allergenicity.

In addition to (glyco)proteins, further allergenic or immunomodulatory substances are known in arthropods. For example, immunogenic glycostructures, some with anaphylactic potential, have been described. It has been demonstrated that the allergenic glycoepitope galactose-α-1,3-galactose (α-Gal) can trigger anaphylactic reactions [95, 96]. For example, the bite of the tick or tick larva *Amblyomma americanum* (Arthropoda, Arachnida) can cause sensitivity to α-Gal with anaphylactic cross-reactions to the meat of non-primates, in which α-Gal is a blood group substance. There are currently no data on the presence of allergenic α-Gal in edible insects.

Regarding the use of individual (micro)components from insects, research on their levels in insects, optimized extraction methods, and safety risks should be carried out. For example, the colorant carmine has been described as a trigger of severe allergic reactions to food [38]. Carminic acid alone or bound to protein [38] and coextracted IgE-binding proteins from cochineals have been discussed as possible triggers of documented anaphylactic reactions to carmine [97]. The binding of IgE antibodies from individuals, which are allergic to carmine, to extracted proteins of the cochineal was inhibited in vitro by a carmine extract [98]. The results point to allergenic proteins in the cochineal and their presence in carmine. The data do not allow ruling out the possibility that carminic acid may contribute to the described effects.

Chitin has also been described as another molecular structure with immunomodulatory potential. There is evidence to suggest that chitin can enhance the formation of allergen-specific IgE antibodies, which play a central role in the pathomechanism of immediate hypersensitivity reactions. This has been demonstrated in murine sensitization studies, for example in mice allergic to *Aspergillus fumigatus* and with the chitin-binding allergen Blo t 12 from mites (*Blomia tropicalis*) [98, 99]. Furthermore, chitin-binding vicilins, leguminous storage proteins, have been described in *Enterolobium contortisiliquum* and *Erythrina velutina* [100, 101]. Vicilins have been described as important allergens in other edible legumes such as peanuts and soybeans [102, 103]. It is unclear whether complexes of chitin and known allergenic vicilins from dietetically relevant legumes possess an immunomodulatory

potential to increase the formation of allergy-mediating antibodies in a similar way as described for chitin-binding Blo t 12 from mites.

3.3.3 Exposure scenarios for sensitization and elicitation

The different types of exposure, i.e. injection, inhalation, skin contact, and ingestion, are described for allergies or cross-allergies to insects. Possible exposures to insect venom, which is known to cause allergic reactions up to an anaphylactic shock after injection, occur in connection with the processing of whole insects, if the venom is not inactivated by the processing. However, up to now, allergic reactions including anaphylactic reactions following the consumption of insects have only been described in individual cases.

There is still considerable uncertainty as to what extent primary sensitization to insects occurs and to what extent such primary sensitization can lead to allergic reactions. Similarly, there is a need to clarify possible cross-sensitivities to arthropod species and, especially, their clinical relevance and the associated exposure scenarios, for example inhalation versus ingestion. Since *Crustacea* are usually heated before consumption, it must be assumed that these allergens and, thus, also cross-reactive insect allergens possess a certain degree of thermostability. For example, allergic reactions in shrimp-allergic patients occurred upon challenge with blanched mealworm protein [91]. The most important conclusion from this study was that a mealworm allergy with a potentially severe outcome is highly likely in shrimp-allergic patients. Furthermore, it has been demonstrated that heating does not reduce the allergenicity of mealworm proteins but simply modifies the protein solubility [104]. Another study involving three different mealworm species documented that the IgE cross-reaction of *Crustacea*-allergic subjects to mealworm tropomyosin was reduced but not eliminated after thermal treatment and in vitro digestion [105].

In contrast, in the case of an isolated inhalant allergy to mites and/or cockroaches, allergens that have not been thermally treated (feces, dusts) cause a primary sensitization. The question regarding a possible cross-reaction with edible arthropod species should therefore be examined in the context of the food technology process used. For example, in vitro IgE cross-reactions of house dust mite-allergic subjects to mealworm proteins were reduced to a greater extent than IgE cross-reactions of shrimp-allergic subjects following thermal treatment and in vitro digestion, but were not eliminated. The profiles of the cross-reacting allergens also differed depending on whether the individuals were sensitized to shrimps or house dust mites. It was concluded that the consumption of mealworms that were only thermally treated represents a risk to individuals, who are allergic to shrimps and/or house dust mites [105].

Overall, it can be stated that only a few available food technology processes, such as fermentation and hydrolysis, are able to achieve a significant reduction in food allergenicity [106]. Similar results may be assumed in the case of insect allergens, which means that new methods for allergen minimization need to be developed.

Given the high sensitization potential associated with arthropods (e.g. shrimps, mites, cockroaches), it has to be assumed that the increased consumption of insects or insect-based products will be associated with a rise in the frequency of allergic reactions to insects.

4 Regulatory aspects

Insect parts and insect-derived ingredients are novel foods according to Regulation (EC) No. 258/97 and may only be marketed in the EU following a safety assessment and approval if they were not consumed to a significant degree before the cutoff date, May 15, 1997. Legal clarity is provided by Regulation (EU) 2015/2283, which will replace Regulation (EC) No. 258/97 on January 1, 2018. In this regulation, whole animals are explicitly covered by the term "novel foods." Thus, whole insects will require safety assessment and approval before they can be marketed, unless they were consumed in noteworthy quantity in the EU prior to May 15, 1997. Without prejudice to the regulations on novel foods, imports of insects (live or dead), insect parts, and insect-derived ingredients into the EU from third countries are subject to the special import procedures set out in Directive 97/78/EC, Directive 91/496/EC, and Decision 2007/275/EC. According to these rules, the import of insects from third countries into the EU is subject to veterinary checks and must take place by a border inspection post.

5 Conclusions and research needs

The objective of this review was not to elaborate a guidance document offering a complete set of recommendations on the risk assessment of insects used for foods or food ingredients. The purpose was to demonstrate that there are significant knowledge gaps regarding the safety-related and technological aspects of the processing of insects. To ensure the quality and safety of fractions or ingredients derived from insects, the following criteria must be taken into account:

(i) Microbial, allergenic, and toxicological risks must be avoided when selecting insect species, developmental stages and breeding conditions. The presently scattered knowledge does not yet allow generic assessment approaches. However, the suitability of a positive and/or a negative list as well as a Qualified Presumption of Safety system similar to that used for microorganisms to select safe insect species/developmental stages for further processing should be examined (http://www.efsa.europa.eu/de/efsajournal/pub/4138; 2015).

(ii) For the extraction of fractions and food ingredients, insects must be cultivated under defined husbandry and feeding conditions to prevent undesired components (pathogenic microorganisms, toxins, allergens, antinutrients, etc.) being absorbed/enriched from the feed or the environment.

(iii) Relevant insect species must be examined regarding the presence of safety-relevant microorganisms. Microorganisms must be inactivated by means of suitable process steps after harvesting. A critical aspect in the selection and/or development of methods is the effective killing of the gut microbiota, as it is not possible to remove the gut from most insects. Further decontamination steps may be necessary depending on the product line.

(iv) Insect-derived fractions and ingredients have to be examined regarding (i) their allergenicity per se; (ii) their allergenicity depending on the presence or absence of coextracted endogenous, potentially allergenic protein structures; and (iii) their immunomodulatory potential, both alone and in complexes with known exogenous allergens, such as vicilins from edible legumes. The influence of different exposure scenarios on sensitization and elicitation, and the impact of processing technologies on allergenicity must be taken into account.

(v) Fractions and food ingredients obtained from insects must be analytically characterized regarding their identity, purity, and their residual levels of potentially harmful substances. The potential of insects to synthesize undesired components may be specific to a particular insect species or may depend on the developmental stage. These components must be thoroughly screened and minimized during isolation and purification steps.

(vi) Criteria to assess the suitability of technological processes for the extraction of safe insect fractions and insect ingredients with due consideration of hazard analyses, identification of CCPs, and potentially required preventive programs (Hazard Analysis and CCPs concept) must be developed.

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