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

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The "Food Technology and Safety" working group of the DFG Senate Commission on Food Safety (SKLM) deals with new technologies, which are being developed or used to treat foodstuffs. This statement examines insects as a novel source of proteins, lipids and other substances. The use of insects as a food source is not usual in Europe. The risks associated with such a use have not been sufficiently investigated. On the 22nd of February 2016 the SKLM carried out an initial assessment of the microbial, allergenic, toxicological and food regulatory safety aspects of the production and processing of insects for food use, with a special focus on technological considerations. It also identified gaps in knowledge and areas in which research is needed.

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

1. Introduction

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, if at all. The purpose of this opinion was to identify potential risks in the process chain from the farmed insect to an isolated fraction, with due consideration of different insect species and stages of development and a focus on individual fractions. In particular, the following aspects will be considered:

(i) whether the use and processing of insects to obtain fractions or ingredients give rise to new, previously unknown risks,

(ii) whether these are the same for all insects or vary according to the species and stage of development,

(iii) whether the methods available to minimise or eliminate the risks suffice or whether these need to be modified or new methods need to be developed.

In countries, in which insects are traditionally consumed, there is knowledge – based on everyday experience – regarding the processing, storage and preparation of usually wild-caught insects. However, there is a lack of scientifically based knowledge of the stages of preparation and processing to ensure food safety when these processes are carried out on an industrial scale. Given the potentially diverse use of the products and the resulting wide distribution of insect-derived ingredients in foods, which would reach many different consumers, an assessment of their safety is especially important.

Insects are a widespread food source in many regions of the world [1]. As in the case of arachnids and crustaceans, they belong to the phylum *Arthropoda* (arthropods). A list of insects that are consumed around the world and that are mentioned in scientific publications can be found under the following: http://www.wageningenur.nl/en/Expertise-Services/Chair-groups/Plant-

Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm. Insects of the order *Coleoptera* (beetles and their larvae) account for around 31%, *Lepidoptera* (moths, butterflies and their caterpillars) around 18%, *Hymenoptera* (bees, wasps and ants) around 15% and *Orthoptera* (locusts, such as migratory and desert locusts, crickets and grasshoppers) around 14%. Insects of the order *Diptera* (such as flies and their larvae) represent around 5%. The methods described in the literature for the treatment of gathered whole insects include traditional methods such as boiling, baking, roasting, grilling, smoking, deep-frying, salting, seasoning, marinating and drying [2-5]. In Europe the consumption of insects is not usual, but is attracting growing attention. A selection of insects, which are offered for consumption on a small scale in some European countries or could be used in food production in future, is shown in Table 1.

Insects are nutrient-rich and in some cases have a high protein and fat content when compared to other animal foods (pork, beef and poultry) (Table 2). Protein contents of 60 to 77% (in relation to dry mass) are reported for representatives of the *Orthoptera* order (locusts) [6, 7]. Insects also have high levels of monounsaturated and/or polyunsaturated fatty acids [8]. On average, they contain the essential amino acids identified by the WHO as an important part of a balanced diet [9] and they are rich in micronutrients such as iron, copper, magnesium, manganese, selenium and zinc as well as riboflavin, pantothenic acid, biotin and in some cases folic acid [6]. All insects contain the polysaccharide chitin, a polymer of N-acetyl-D-glucosamine, as a component of the exoskeleton. They also contain enzymes that could be of interest in food processing applications, e.g. cellulolytic and proteolytic enzymes [10, 11]. These data show that insects are not only an alternative source of protein but also contain other substances that could be used in the food industry.

At the present time it is being discussed whether, in addition to their high protein content and favourable 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 [12-15]. Examples of the potential advantages of insect production include reduced land use, high feed conversion efficiency [16, 17] and high fertility, with several life cycles per year. Initial data indicate that insect breeding could produce less greenhouse gases and ammonia per kilogram of mass gained than beef and pork production [18].

In 2013, the Department of Food Safety at the Istituto Zooprofilattico Sperimentale in Venice published a summary on the food safety of insects [19]. This was followed by statements from public authorities in Belgium [20], the Netherlands [21] and France [22] related to the safety aspects of using whole insects as food and animal feed based on a limited selection of currently

relevant species. These statements only examined processing methods with regard to decontamination, mainly focusing on traditional methods such as deep-frying, toasting, drying or freeze-drying. They revealed significant research needs regarding the potential microbial, allergenic and toxicological risks associated with the consumption of whole insects. In October 2015, the European Food Safety Authority (EFSA) published a risk profile for the production and consumption of insects as food and feed [23].

In this opinion of the SKLM, the safety aspects that need to be considered in relation to the fractionation of insects for the production of foods and food ingredients are identified and discussed. It is assumed that the conditions in insect rearing facilities used to produce fractions and ingredients will have to comply with the food safety regulations of the German Food and Feed Code (LFGB) applicable to livestock husbandry (see chapter 4). This includes 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 is not taken into consideration. The risks associated with the use of insects or insect fractions as feed and the production of bee honey are also not taken into account.

2. Technological aspects relating to the use of insects for the extraction of fractions and ingredients

Various fractions could potentially be obtained from insects, e.g. proteins (including enzymes), lipids and polysaccharides. Potential species-specific safety aspects must be considered during the selection of 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; see glossary) depending on the intended use; these include microbial, allergenic and toxicological risks. Quality aspects such as nutritional [24] and sensory properties and processability must also be taken into account.

The properties of the primary material as well as of the desired product affect the use of 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. The larva of the mealworm beetle *Tenebrio molitor*, for example, contains approximately 47% protein and 43% fat, whereas the adult mealworm beetle contains around 65% protein and 15% fat (in relation to dry mass) [25, 26]. In comparison with the other developmental stages, the final larval stage of the black soldier fly (*Hermetia illucens*) contains high amounts of calcium in the form of deposits as calcium carbonate [27], which could affect fractionation.

Insects intended to be consumed are prepared after harvesting (killing, cleaning, classification, if applicable removal of the gut, if applicable cutting up with removal of the head, extremities, antennae and wings, decontamination, preservation) [2-5]. For fractionation, different processes are applied, depending on the primary material and intended use. The choice of processes depends not only on the technological, functional and nutritional properties of the fractions but also on the purity of the fractions that can be achieved. 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, see chapter 3.2) from the fractions. Established processes may have to be modified or new, suitable processes may need to be developed. To ensure microbial safety (see chapter 3.1), additional decontamination steps may be required. Figure 1 shows a flow chart regarding the selection of the type of insect and the processing method to be used. In this context, it is essential to carry out a hazard

analysis (HACCP study[1]) for each product line, taking into account all risks (physical, microbial, allergenic and chemical). For hazards classified as relevant, critical control points and preventive programmes must be established.

2.1. Proteins

Regarding protein extraction from insects, there are differences between insects and conventional sources, such as the binding of insect-specific chitin to structure-providing proteins of the exoskeleton [28-30]. Insect-specific allergens (see chapter 3.3), insect-specific antimicrobial peptides [31-33] and prions that may be taken up by the insect through feed (see chapter 3.1) may also be present. Insect-specific prions have not yet been described. In most cases, the gut cannot normally be removed; therefore, it must be assumed that microbial proteins are co-extracted 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 insects ranges from 5 to 77% with average values between 35 and 61% based on dry matter [6]. The protein content as a percentage of the fresh weight of various edible insects, e.g. the larvae of the Mexican fruit fly (*Anastrepha ludens)* [34], the mealworm beetle (*Tenebrio molitor*), the darkling beetle (*Zophobas mori*) and the wax moth (*Galleria mellonela*) and the adult form of the house cricket (*Acheta domesticus*), ranges from 9 to 25% [24, 35, 36]. Various studies describing the isolation of proteins from selected insect species at the laboratory scale are available [34, 37-40]. 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*; imago) and Dubia roach (*Blaptica dubia*; imago) 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 [37]. Using aqueous extractions at different temperatures and pH values, gelling proteins were isolated from defatted, dried and pulverised beetle species (*Aspongubus viduatus*, *Agonoscelis pubescens*, *Agonoscelis versicoloratus*, *Coridius viduatus*). These proteins had properties similar to conventional gelatine [38, 41]. For proteome analyses of the greenbug (*Schizaphis graminum*), different methods of protein extraction with TCA-acetone, phenol or urea buffer solutions supplemented with detergents were compared [40].

[1] http://www.fda.gov/downloads/food/guidanceregulation/haccp/ucm077957.pdf
http://www.fao.org/docrep/005/Y1579E/y1579e03.htm

Large amounts of co-extracted 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 the present time [42]. A protein concentrate obtained from honeybees with a 10% NaOH extraction and subsequent precipitation was fed to rats and compared with whole pulverised bees. It was observed that the protein concentrate exhibited higher digestibility, measured as the ratio of absorbed to excreted protein. This was attributed to the removal of chitin [39]. Although the extrapolation of these data to humans still has to be verified, it does provide an indication that both the maximum amount of chitin recommended for human consumption in food and protein digestibility in association with chitin should be taken into consideration.

When developing methods to isolate proteins from insects, chitin should be removed as far as possible and the extraction conditions should not alter the amino acid composition in a way that it will reduce the quality of the protein [39]. It must also 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 structures 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. There is a need for research in this area, as no data are currently available.

Enzymes

Because they 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 [43-45], e.g. proteolytic enzymes [46-49], cellulases [11, 50-52], α-amylases [53, 54] and lipases [55]. The genomic analysis of insects and the metagenomic analysis of insect microbiota have allowed researchers to identify genes coding for enzymes with the potential to be used in food technology [44].

The complexity of the insect matrix could make it difficult to extract sufficiently pure enzyme fractions. For their use in food production, it must be established whether the process 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 ensure that the enzymes remain intact and guarantee a complete decontamination of the product. It remains to be established whether possible anti-nutritive properties of certain insect enzymes present a risk when applied in food production (see also chapter 3.2).

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 [56]. 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% [6]. Moreover, a fat content of 4 to 32% has been reported in relation to fresh weight [24].The fatty acid spectrum depends on the species and the stage of development; it is however comparable with other animal lipids [6] and, like these, is affected by the composition of the diet [57]. Essentially, the favourable nutrient composition for each insect species depends on the type and quality of its feed [36, 58].

Lipids can be isolated from insects with standard methods such as extraction with hexane/ petrol ether or supercritical carbon dioxide [59-63].

Only a small amount of data is currently available on the influence of the extraction and thermal treatment on nutritionally relevant substances in insect oils. For example, the extraction conditions can alter the vitamin E content [61]. Thermal treatments resulted in vitamin losses in fat extracts from various insect species [64]. In the larva of the African palm weevil (*Rhynchophorus phoenicis*), various types of thermal treatments and storage affected the properties of the oil, e.g. its free fatty acid content [65].

With regard to the extraction of lipids from insects, it must be assumed that both triglycerides and other endogenous lipophilic substances are co-extracted. One example are ecdysteroids, hormones that control moulting, metamorphosis and reproduction in insects and may potentially elicit pharmacological effects [66, 67]. The levels of these lipid components in insect oils should therefore be investigated.

In addition to endogenous lipophilic substances, lipophilic environmental contaminants such as dioxins [68] represent a potential risk. Lipophilic contaminants which cannot be avoided despite carefully controlled breeding conditions should be minimised 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, a polymer of N-acetyl-D-glucosamine and one of the main component of the exoskeleton [28, 30], and glycogen, which is stored in the cells of the fat body [56] and in the muscles. In nature, chitin occurs in three forms: α, β and γ. In the most common form in nature, the α form, the N-acetyl-D-glucosamine chains are antiparallel, in the β form they are parallel and in the γ form they are both parallel and antiparallel. In insects, chitin occurs in the α form [69]. The level of chitin 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* [70-72]. Chitosan (poly-D-glucosamine) can be manufactured from chitin through deacetylation [73]. This substance can be used in food as a thickening agent, a prebiotic or as an antimicrobial agent. As a semi-permeable coating it can extend the shelf life of fruits, vegetables and other foods by minimising respiration and reducing water losses [74].

In studies on the chitin extraction from insects, isolation was accomplished in the same way as in the case of marine waste materials containing chitin, such as shrimp shells [69, 73, 75-79].

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 [80], 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 (see chapter 3.3).

2.4. Other components

Except of 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 Germany for use as a food colourant (E 120). The cochineals are bred on the cladodes of various members of the genus Opuntia (a genus in the cactus family) [81]. They are defatted with an organic solvent, e.g. hexane, dried and then finely pulverised. Extraction occurs in a boiling sodium carbonate solution at 95° to 100°C and pH 9 for approximately 30 min. The carmine is precipitated at 100°C and pH 5.5 by the addition of citric acid, aluminium salts (alum) and calcium salts and then dried at 40° to 70°C [82]. Carmine has been described as a trigger for serious allergic reactions [83] (see chapter 3.3).

3. Safety criteria

3.1. Microbial aspects

The microbiota of insects is highly complex [43, 84-86]. Apart from the body surface and the mouthparts, the main habitat for microorganisms is the gut. They colonise the insects in various ways: vertically with the parents' microorganisms through a) the ovary, b) the egg capsule, c) a smear infection during egg laying, and horizontally through diet and environment [87-91]. In recent years, modern metagenomic analyses have greatly increased the knowledge on microbial biodiversity, especially in the insect gut [44, 50, 86-88, 90, 92-105]. It has been shown that the insect microbiota includes many previously unknown species. The number of species varies according to the insect species and its diet.

The use of insects as food entails potential microbiological risks because insects can serve as vectors for microorganisms pathogenic to humans, animals and plants. In this context, one has to discern whether the transmission of microorganisms occurs mechanically through contact with the surface of the insect's body [106-110] or whether the microorganisms are able to persist and multiply inside the insect. Insects may become a natural reservoir for pathogenic microorganisms without themselves becoming sick [111-118]. The pathogens which can be transmitted through insects include viruses [119, 120], Rickettsia [121], bacteria [122], protozoa [123], fungi [124, 125], nematodes and other parasites of the human digestive tract [126, 127]. Insect-pathogenic microorganisms [128] are considered harmless to humans because they have a high degree of tissue tropism (tissue specificity) and can therefore probably only colonise the cells or tissue 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 [129-131]. Insect-specific prions or insects as natural vectors for prions have not yet been described. The transmission of prions to animals and humans by the consumption of insects contaminated by prion-containing feed cannot be ruled out and might be taken into account when deciding on the kind of feed to be used for insect breeding. It has been experimentally demonstrated that grubs of the common flesh fly (*Sacrophaga carnaria*) fed on hamster brain infected with prion proteins PrP^{Sc} (scrapie) can infect healthy hamsters after these ate contaminated fly extracts [132].

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 because of specific environmental or breeding conditions or owing to contact with humans (gathering, harvesting) or other individuals

[85]. Members of the allochthonous microbiota may potentially become permanent commensals of insects. Human-pathogenic and/or opportunistically human-pathogenic and toxin-forming microorganisms of food hygiene 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 known species, which can trigger *inter alia* foodborne diseases (Table 1). These are often zoonotic pathogens, which are key microorganisms in standard food technology processes for food production and preservation and in food infections. Their occurrence in insects is widespread and non-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* [95, 96, 101, 102, 133-140]. *Klebsiella pneumoniae* has been described as the most frequent bacterium in the gut of the Oriental migratory locust (*Locusta migratoria manilensi*) and is classified as autochthonous [50]. Fungi of the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Candida*, which include human-pathogenic, toxin-forming species [98, 103, 104, 124, 136, 137, 141-145] (see also chapter 3.2) and allergenic species (see also chapter 3.3), 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 to 10^{12} bacteria per ml of gut content have been detected in some insect species, depending on the segment of the gut analysed [146]. Microbial biomass accounts for 1 to 10% of the total insect body depending on the insect species [147]. 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 [128]. 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 [86, 88-91, 101, 148-150], 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

[151-153]. The EFSA has proposed a possible classification of substrates for insect breeding with different levels of hazard potential [23]. 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 critical control points during the production and processing of insects [154] and intermediate decontamination stages. Initial data is available on the effects of different processes on the microbiological status of whole insects [20]. No data are available on the assessment of the microbiological risk in comparison to other animal sources.

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 [155, 156] and thiaminases [157]. Toxins and antinutrients should be distinguished according to whether they are absorbed by the insect from the feed or synthesised by the insect itself. Insect breeding conditions should comply with applicable food safety regulations (see chapter 4). Insects selected for the production of food and food ingredients should therefore be kept in such a way as to prevent or minimise the accumulation of externally introduced toxins, drugs or antinutrients.

Some insect species synthesise 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) synthesised 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 (CBP). The toxic effects following consumption include difficulty in swallowing, nausea and vomiting of blood [158-160]. Longhorn beetles may contain toluene. Darkling beetles (Tenebrionidae) produce quinones and alkanes [161], while certain moth species of the genus *Zygaena* contain cyanogenic glycosides [162]. 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 is available on the presence of toxins in potentially edible insect species. A first 90-day feeding trial showed that *Tenebrio molitor* larvae do not lead to adverse effects in rats when fed as dried powder up to the highest dose of 3,000 mg/kg body weight/day [163].

In the few studies on antinutrients in gathered insects, the measured concentrations of substances such as oxalate, tannin and phytate were far below harmful limits [155, 156, 164-166]. Annual seasonal thiamine deficiency in Nigeria has been attributed to heat-stable thiaminases described in the wild-gathered African silkworm (*Anaphe venata*) [157]. Although no relevant data is available, it may be assumed that heat-stable thiaminases are also biosynthesised under controlled breeding conditions. This issue should be investigated if the African silkworm were considered to be used for the production of food and food ingredients.

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, 167]. 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, it is possible that harmful components only present in trace amounts could be enriched together with the actual target components during fractionation, for example cantharidin with protein or toluene with lipids. The toxic potential of antinutrients and the antinutrient content should be minimised by selecting appropriate breeding and processing conditions.

3.3. Allergenic potential

Allergic reactions

Isolated allergic episodes, including anaphylactic reactions [168-170], 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) [171, 172]. For example, pan-allergenic tropomyosin may elicit allergic reactions to *Crustacea* as well as mites and insects (e.g. cockroaches) [173, 174]. This effect was confirmed in a study on cross-reactivity to mealworm larvae (*Tenebrio 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 [175]. 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 sensitisation to these

ubiquitous or pan-allergenic structures and to species-specific allergens is possible. Unexpected allergic cross-reactions may occur due to sensitisation 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 [176, 177], 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* [178], 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.

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 17.02.2016). These are mostly ubiquitous or pan-allergenic proteins, which, in simplified terms, can be categorised 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 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 [179, 180]. 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 is 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, optimised extraction methods and safety risks should be carried out. For example, the colourant carmine has been described as a trigger of severe allergic reactions to food [83]. Carminic acid alone or bound to protein [83] and demonstrably co-extracted IgE-binding proteins from cochineals have been discussed as possible triggers of documented anaphylactic reactions to carmine [181]. The binding of IgE antibodies from carmine allergic individuals to extracted proteins of the cochineal was inhibited *in vitro* by a carmine extract [181]. The results point to allergenic proteins in the cochineal and their presence in carmine. The data do not allow to rule 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 sensitisation studies, for example in *Aspergillus fumigatus* allergic mice and with the chitin-binding allergen Blo t 12 from mites (*Blomia tropicalis*), respectively [182, 183]. Furthermore, chitin-binding vicilins, leguminous storage proteins, have been described in *Enterolobium contortisiliquum* and *Erythrina velutina*[184, 185]. Vicilins have been described as important allergens in other, edible, legumes such as peanuts and soybeans [186, 187]. 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.

Exposure and sensitisation scenarios

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 sensitiation to insects occurs and to what extent such primary sensitiation 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. It has been demonstrated, for example, that heating does not reduce the allergenicity of mealworm proteins but simply modifies the protein solubility [188]. 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 [189].

In contrast, in the case of an isolated inhalant allergy to mites and/or cockroaches, allergens that have not been thermally treated (faeces, dusts) cause a primary sensitisation. 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 (but not eliminated) than IgE cross-reactions of shrimp allergic subjects following thermal treatment and *in vitro* digestion. The profile of the cross-reacting allergens also differed depending on whether the individuals were sensitised 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 [189].

Overall, it can be stated that only a few available food technology processes, such as fermentation and hydrolysis, are able to attain a significant reduction in food allergenicity [190]. Similar results may be assumed in the case of insect allergens which means that new methods for allergen minimisation need to be developed.

Given the high sensitisation 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. Food safety aspects

In Germany, insects, insect parts and insect-derived ingredients intended for human consumption may, as in the case of all other foods, only be placed on the market if they comply with the respective food safety regulations. In this context, these include Regulation (EC) No. 178/2002[2] (known as the basic regulation in food law), Regulation (EC) No. 852/2004[3] and the German Food and Feed Code. In accordance with these regulations it is forbidden to market foods which are not safe or to manufacture or treat foods in such a way that their consumption could be harmful to health.

Insect parts and insect-derived ingredients are novel foods according to Regulation (EC) No. 258/97[4] and may only be marketed in the EU following a health assessment and approval if they were not consumed in a significant degree before the cut-off date, 15th of May 1997.

In some EU Member States, whole insects are also classified as novel foods, while other Member States consider them to be products which do not fall in the scope of Regulation (EC) No. 258/97. Legal clarity is provided by Regulation (EU) 2015/2283[5], which will replace Regulation (EC) No. 258/97 on the 1st of January 2018. In this regulation, whole animals are explicitly covered by the term 'novel foods'. Thus, whole insects will certainly require health assessment and approval before they can be marketed, unless they were consumed in noteworthy quantity in the EU prior to the cut-off date, 15th of May 1997.

Without prejudice to the regulations on novel foods, imports of insects (live or dead), insect parts and insect-derived ingredients into the European Community from third countries are subject to the special import procedures set out in Directive 97/78/EC[6], Directive 91/496/EC[7] and Decision 2007/275/EC[8]. According to these rules, the import of insects from third countries into the

[2] Regulation (EC) No. 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

[3] Regulation (EC) No. 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs

[4] Regulation (EC) No. 258/97 of the European Parliament and of the Council concerning novel foods and novel food ingredients

[5] Regulation (EC) No. 2015/2283 of the European Parliament and of the Council on novel foods

[6] Directive 97/78/EC of the Council laying down the principles governing the organisation of veterinary checks on products entering the Community from third countries

[7] Directive 91/496/EEC of the Council laying down the principles governing the organization of veterinary checks on animals entering the Community from third countries and amending Directives 89/662/EEC, 90/425/EEC and 90/675/EEC

[8] Decision 2007/275/EC of the Commission concerning lists of animals and products to be subject to controls at border inspection posts under Council Directives 91/496/EEC and 97/78/EC

European Community is subject to veterinary checks and must take place by a border inspection post.

Under German food law, as a special import requirement, imports of insects, insect parts and insect-derived ingredients for human consumption must be accompanied by a certificate in accordance with § 6 Para 2 Clause 2 in conjunction with Appendix 2a of the German Food Import Regulation. In the import certificate, the official veterinarian or inspector in the third country who signs the document must confirm that the products intended for human consumption satisfy the general EU requirements for food safety and food hygiene.

Specific import conditions relating to animal diseases are only defined for live honeybees and bumblebees in Directive 92/65/EC[9] and Regulation (EU) No. 206/2010[10].

[9] Directive 92/65/EEC of the Council laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A (I) to Directive 90/425/EEC

[10] Regulation (EU) No. 206/2010 of the Commission laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements

5. Conclusions

On the one hand, insect-derived fractions may be mixtures whose composition is determined by a shared physicochemical property, e.g. solubility in lipophilic media; on the other hand, single substances may be obtained by using specific purification and isolation methods.

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:

- Microbial, allergenic and toxicological risks must be avoided when selecting insect species, developmental stages and breeding conditions.

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

- 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.

- To assess the allergenic potential of insects and, in particular, of extracted fractions or individual substances, knowledge of potentially allergenic structures is required. The influence of different exposure and sensitisation scenarios and processing technologies on allergenicity should be taken into consideration.

- Insects can synthesise undesired toxic components. This potential may be specific to a particular insect species and may depend on the developmental stage. These components must therefore be minimised during isolation and purification.

- Fractions and food ingredients obtained from insects must be analytically characterised regarding their identity, purity and unavoidable residual levels of potentially harmful

substances. By doing so, it will be possible to compare the obtained data with those of isolates from conventional sources (e.g. plants), for which safety assessments are available.

6. Required research

Based on the criteria defined in chapter 5, the following research needs for the minimisation of microbial, allergenic and toxicological risks have been identified.

- Collection of data on potential microbial, allergenic and toxicological risks related to
  - (i) insect species and stages of development
  - (ii) breeding and cultivation conditions
  - (iii) processing methods

- The suitability of a positive and/or a negative list as well as a QPS (Qualified Presumption of Safety) system similar to that used for microorganisms to select safe insect species / developmental stages for the extraction of fractions and ingredients (http://**www**.efsa.europa.eu/de/efsajournal/pub/4138; 2015) should be examined.

- Examination of the insect species intended for use to establish the presence of safety-relevant microorganisms; examination of the ability of potential decontamination processes to reduce the microbial count resp. to inactivate identified safety-relevant microorganisms.

- Collection of data on
  - (i) the occurrence of allergenic structures in insect species intended to be used for the extraction of insect fractions
  - (ii) exposure and sensitisation in humans
  - (iii) the influence of technological processes on the allergenicity of potentially allergenic structures

- Examination of insect derived ingredients regarding
  - (i) their allergenicity *per se*
  - (ii) their allergenicity depending on the presence or absence of co-extracted endogenous, potentially allergenic protein structures
  - (iii) their immunomodulatory potential, both alone and in complexes with known exogenous allergens, such as vicilins from edible legumes

- Collection of data on
    - (i) the occurrence of insect-specific toxins in the insect species intended for use
    - (ii) the co-extraction or enrichment of toxins and other critical components during the fractionation of insects

- Development of 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 critical control points and potentially required preventive programmes (HACCP concept).

Table 1: Insect species which 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.)* potential vector for *Abbreviata antarctica* (experimental)	[133, 191]
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*	[192]
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 (VS)	[50, 154, 193-195]
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, Klebsiella pneumonia, Klebsiella oxytoca , Escherichia coli, Enterobacter cloacae, Enterobacter agglomerans, Serratia marcescens, Citrobacter sp.), Bacillus cerreus, Clostridium perfringens, Clostridium septicum, Clostridium difficile, Clostridium sporogenes, Clostridium capitovale, Pseudomonas aeruginosa, Acinetobacter sp., Enterococcus sp., Staphylococcus sp., Streptococcus sp., Rhodococcus sp.,*	[196-199]
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, Klebsiella pneumoniae, Citrobacter freundii, Serratia liquefaciens, Serratia sp., Shigella sp., Enterobacter sp., Erwinia sp., Pantoea sp), Aeromonas sp., Pseudomonas aeruginosa, Pseudomonas fluorescens, Clostridium sp., Bacillus sp., Bacillus circulans, Staphylococcus sp., Streptococcus sp., Enterococcus sp., Enterococcus mundtii, Acinetobacter sp., Moraxella sp., Aeromonas hydrophila, Actinobacteria*	[52, 149, 200, 201]
Galleria melonella (Greater Wax Moth)	Lepidoptera (butterflies and moth)	caterpillar	no data on the autochthonous microbiota, *Galleria 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	[202-205]
Imbrasia bellina/Gonimbrasia bellina, (Mopani, Emperor Moth)	Lepidoptera (butterflies and moths)	caterpillar	*Alternaria sp., Aspergillus sp., Chaetomium sp., Drechslera sp., Penicillium sp., Fusarium sp., Mucor sp., Phoma,*	[206]
Imbrasia bellina/Gonimbrasia bellina, (Mopani, Emperor Moth)	Lepidoptera (butterflies and moth)	caterpillar	*Alternaria sp., Aspergillus sp., Chaetomium sp., Drechslera sp., Penicillium sp., Fusarium sp., Mucor sp., Phoma,*	[206]

Alphitobius diaperinus (Litter Beetle)	Coleoptera (beetles)	larva (lesser mealworm)	no data on the autochthonous microbiota vector for *Salmonella enterica*, *Escherichia coli*, *Campylobacter jejuni*, *Acinetobacter sp.* *Aspergillus sp., Infectious Bursal disease virus (IBDV), Marek´s disease virus, Turkey Corona virus, Sporozoa: Coccidia (Eimeria)*	[111-114, 207-216]
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.* vector for *Mycobacterium sp.* (experimental)	[90, 139, 154, 217]
Zophobas atratus (Morio Beetle)	Coleoptera (beetles)	larva	vector for *Mycobacterium sp.* (experimental)	[217]
Atta laevigata (Leaf Cutter Ants)	Hymenoptera (bees, wasps and ants)	imago (adult form)	*Aspergillus fumigatus, Aspergillus sclerotiorum* and *Penicillium* sp., opportunistic human-pathogenic fungi of the genera *Cladophialophora*, *Exophiala*, *Metarhizium*, *Ochroconis* , *Phialophora* and *Penidiella*	[141, 145]
Hermetia illucens (black soldier fly)	Diptera (dipteran, flies)	larva	*Enterobacteriaceae* (*Klebsiella pneumoniae* , *Escherichia 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)*	[88, 218-221]
Musca domestica (Housefly)	Diptera (dipteran, flies)	larva	dependent on rearing conditions frequently transition of allochthonous to autochthonous microbiota: *Enterobacteriaceae* (*Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *Citrobacter* sp., *Shigella* sp., *Morganella* sp., *Proteus* sp., *Providencia* sp.) *Bacillus* sp., *Bacilius cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Enterococcus* sp. fungi as *Aspergillus tamari* and *Alternaria* sp., vector für *Escherichia coli* O157:H7, *Salmonella* sp., *Salmonella typhi*, *Yersinia pseudotuberculosis*, vector for different species of nematodes: round worm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm vector for Circovirus, subtypes of avian influenza virus H5N7 and H7N1, suspected to act as a vector for *Vibrio cholerae*	[95, 106, 109, 110, 126, 138, 222-233]
Blattella germanica (German cockroach)	Blattodea (cockroachs)	imago (adult form)	dependent on rearing conditions frequently transition of allochthonous to autochthonous microbiota: *Enterobacteriaceae (Escherichia coli, Salmonella sp., Klebsiella sp., Klebsiella pneumoniae, Enterobacter sp., Enterobacter aeroginus, Enterobacter cloacae, Serratia sp., Serratia marcesens, Citrobacter sp., Citrobacter freundii, Proteus sp., Proteus mirabilis, Shigella sp.) Enterococcaceae, Enterococcus sp., Staphylococcaceae sp., Staphylococcus aureus, Streptococcus sp., Aeromonas sp., Pseudomonadaceae, Pseudomonas sp., Pseudomonas aeruginosa, Haemophilus sp., Clostrdiales, Candida sp., Mucor sp., Penicillium sp., Aspergillus niger, Aspergillus fumigatus*	[115, 150, 234-237]

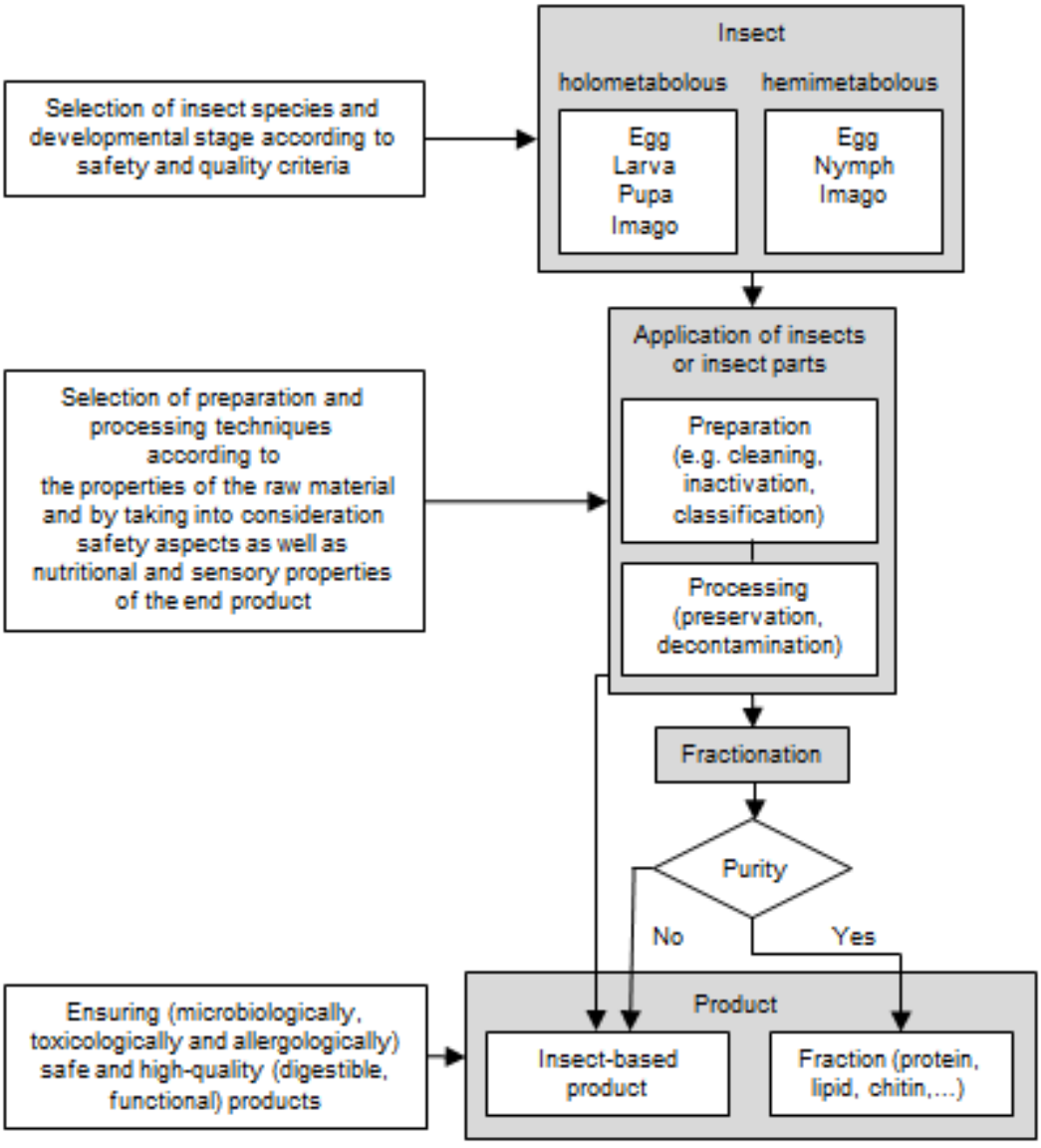
Periplaneta americana (American cockroach)	Blattodea (cockroachs)	imago (adult form)	dependent on rearing conditions: *Enterobacteriaceae (Escherichia coli*, *Escherichia vulneris*, *Salmonella sp., Enterobacter aeroginus, Enterobacter cloacae, Shigella flexneri*, *Klebsiella pneumoniae, Serratia marcesens, Citrobacter freundii*, *Enterobacter cloacae*, *Providencia* sp., *Yersinia pseudotuberculosis*, *Yersinia intermedia*, *Klebsiella sp., Klebsiella oxytoca*, *Klebsiella planticola*, *Salmonella sp.*) *Proteus sp., Proteus mirabilis, Proteus vulgaris, Leclercia adecarboxylata, Rahnella aquatilis, Bacillus sp., Staphylococcus sp., Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus sp., Pseudomonas aeruginosa Aspergillus niger, Mucor sp., Candida sp., Fusarium sp., Penicillium,* round worm (*Ascaris lumbricoides),* whipworm *(Trichuris trichiura), Coccidia, Entamoeba histolytica, Enterobius vermicularis, Schistosoma haematobium, Balantidium coli*	[140, 232, 235, 237, 238]

Table 2: Content of proximates in selected insects compared to conventional foodstuffs (in [%], if not stated otherwise).

	Water	**Protein**	**Fat**	**Carbo-hydrates**	**Ash**	**Energy [kJ/100g]**
Beef (lean)[1]	75.0	22.3	1.8		1.2	485
Beef (carcasse)[1]	54.7	16.5	28.0		0.8	1351
Pork (lean)[1]	75.1	22.8	1.2		1.0	469
Pork (carcasse)[1]	41.1	11.2	47		0.6	1975
Chicken[1]	75.0	22.8	0.9		1.2	439
Egg (raw)[2]	75.8	12.6	9.9	0.8	0.8	594
Whole wheat flour[2]	11.7	10.7	2.4	74.1	1.0	1426
Silkworm, fresh (*Bombyx mori*)[3-4]	82-87	8.8-9.3	1.2-4	4.4	1.1-1.4	282
House cricket, fresh (*Acheta domesticus*)[3]	69.2	20.5	6.8	0.8	1.1	587
Yellow mealworm, fresh (*Tenebrio molitor*)[3]	61.9	18.7	13.4	0.27	0.9	860
Silkworm,dried (*Bombyx mori*)[3-4]		53.8-69.8	8.1-9.5	25.4	6.4-11.1	1630
House cricket, dried (*Acheta domesticus*)[3]		66.6	22.1	2.6	3.6	1904
Yellow mealworm, dried (*Tenebrio molitor*)[3]		49.1	35.2	7.1	2.4	2258

Sources: [1] [239], [2] [240], [3] [25], [4] [241].

Figure 1: Flow chart for the selection of insect species and processing processes



Glossary

Allochthonous	The allochthonous microbiota refers to all microorganisms that can be found **temporarily** in a certain environment, e.g. in a certain insect species.
Autochthonous	The autochthonous microbiota refers to all microorganisms that can be found **stably** in a certain environment, e.g. in a certain insect species.
CCPs	Critical Control Points
HACCP	The `Hazard Analysis and Critical Control Points´ concept is a preventative food safety system by which every step in the processing, storage and distribution of a food product is scientifically analysed for microbiological, chemical and physical hazards.
Hemimetabolous	Hemimetabolous insects develop from eggs to nymphs and then the adult form (imago).
Holometabolous	Holometabolous insects develop from eggs to larvae or caterpillars, then pupae and finally the adult form.
Imago	The sexually mature adult form of insects, which follows from the juvenile stages after pupation or the final moult.
IgE	Immunoglobulin E
Key microorganisms	Microorganisms which occur in large numbers in a certain disease either alone or with other microorganisms and give a disease its typical pattern. In food production, these are microorganisms (usually those which cause spoilage or zoonotic agents), which occur in certain foods and whose inactivation can be shown to achieve effective sterilisation of the food.
Microbiota	A microbiota is a community of microorganisms which occur in a certain environment, e.g. in a particular insect species, and may be mutually dependent or exist independently of one another due to the same needs.
QPS	Qualified Presumption of Safety
TCA	Trichloroacetic acid
Zoonotic agent	Zoonotic agents (from the Greek "zoon", animal; "nosos", ailment) are pathogens which are transmitted from animals to humans and from humans to animals. In food production this term refers to microorganisms which are transmitted through food.

References

[1] Anankware, P., Fening, K., Osekre, E., Obeng-Ofori, D., Insects as food and feed: a review. *Int J Agric Res Rev* 2015, *3*, 143-151.

[2] Chakravorty, J., Ghosh, S., Meyer-Rochow, V. B., Practices of entomophagy and entomotherapy by members of the Nyishi and Galo tribes, two ethnic groups of the state of Arunachal Pradesh (North-East India). *Journal of ethnobiology and ethnomedicine* 2011, *7*, 5.

[3] Srivastava, S. K., Babu, N., Pandey, H., Traditional insect bioprospecting - As human food and medicine. *Indian Journal of Traditional Knowledge* 2009, *8*, 485-494.

[4] Solomon, M., Prisca, N., Nutritive value of Lepidoptara litoralia (edible caterpillar) found in Jos Nigeria: implication for food security and poverty alleviation. *African Journal of Food, Agriculture, Nutrition and Development* 2012, *12*, 6737-6747.

[5] Acuna, A. M., Caso, L., Aliphat, M. M., Vergara, C. H., Edible insects as part of the Traditional Food System of the Popoloca Town of Los Reyes Metzontla, Mexico. *Journal of Ethnobiology* 2011, *31*, 150-169.

[6] Rumpold, B. A., Schlüter, O. K., Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research* 2013, *57*, 802-823.

[7] Ramos-Elorduy, J., Pino-M, J. M., Correa, S. C., Edible insects of the state of Mexico and determination of their nutritive values. *Anales del Instituto de Biologia Universidad Nacional Autonoma de Mexico Serie Zoologia* 1998, *69*, 65-104.

[8] DeFoliart, G., Insect Fatty Acids: Similar to Those of Poultry and Fish in Their Degree of Unsaturation, but Higher in the Polyunsaturates. *The Food Insects Newsletter* 1991, *4*, 1-4.

[9] WHO, *WHO technical report series* 2007.

[10] Terra, W. R., Ferreira, C., Insect Digestive Enzymes - Properties, Compartmentalization and function. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 1994, *109*, 1-62.

[11] Watanabe, H., Tokuda, G., Cellulolytic Systems in Insects. *Annual review of entomology* 2010, *55*, 609-632.

[12] Lundy, M. E., Parrella, M. P., Crickets are not a free lunch: protein capture from scalable organic side-streams via high-density populations of Acheta domesticus. *PLoS One* 2015, *10*, e0118785.

[13] Oonincx, D. G. A. B., de Boer, I. J. M., Environmental Impact of the Production of Mealworms as a Protein Source for Humans - A Life Cycle Assessment. *Plos One* 2012, *7*, e51145.

[14] Yates-Doerr, E., The world in a box? Food security, edible insects, and "One World, One Health" collaboration. *Soc Sci Med* 2015, *129*, 106-112.

[15] Tabassum, A., Abbasi, T., Abbasi, S. A., Reducing the global environmental impact of livestock production: the minilivestock option. *J. Clean Prod.* 2016, *112*, 1754-1766.

[16] van Huis, A., in: Berenbaum, M. R. (Ed.), *Annual review of entomology* 2013, pp. 563-583.

[17] van Huis, A., van Itterbeeck, J., Klunder, H. C., Mertens, E.*, et al.*, *Edible insects - Future prospects for food and feed security*, FAO, Rome 2013.

[18] Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H.*, et al.*, An Exploration on Greenhouse Gas and Ammonia Production by Insect Species Suitable for Animal or Human Consumption. *Plos One* 2010, *5*.

[19] Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C.*, et al.*, Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review. *Comprehensive reviews in food science and food safety* 2013, *12*, 296-313.

[20] FAFSC, SHC, Brussels 2014.

[21] Opperhuizen, A., Dutch Office for Risk Assessment and Research, Utrecht 2014.

[22] l`Anses, l'Agence nationale de sécurité sanitaire de l'alimentation,de l'environnement et du travail (anses), Maisons-Alfort 2015.

[23] EFSA, Risk profile related to production and consumption of insects as food and feed. *EFSA Journal* 2015, *13*, 4257-4317.

[24] Payne, C. L., Scarborough, P., Rayner, M., Nonaka, K., A systematic review of nutrient composition data available for twelve commercially available edible insects, and comparison with reference values. *Trends in Food Science & Technology* 2016, *47*, 69-77.

[25] Finke, M. D., Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology* 2002, *21*, 269-285.

[26] Nowak, V., Persijn, D., Rittenschober, D., Charrondiere, U. R., Review of food composition data for edible insects. *Food chemistry* 2016, *193*, 39-46.

[27] Johannsen, O. A., Stratiomyiid Larvae and Puparia of the north-eastern States. *Journal of the New York Entomological Society* 1922, *30*, 141-153.

[28] Hamodrakas, S. J., Willis, J. H., Iconomidou, V. A., A structural model of the chitin-binding domain of cuticle proteins. *Insect Biochem Mol Biol* 2002, *32*, 1577-1583.

[29] Khoushab, F., Yamabhai, M., Chitin research revisited. *Mar Drugs* 2010, *8*, 1988-2012.

[30] Willis, J. H., Structural cuticular proteins from arthropods: annotation, nomenclature, and sequence characteristics in the genomics era. *Insect Biochem Mol Biol* 2010, *40*, 189-204.

[31] Bulet, P., Stocklin, R., Insect antimicrobial peptides: structures, properties and gene regulation. *Protein and peptide letters* 2005, *12*, 3-11.

[32] Chernysh, S., Kim, S. I., Bekker, G., Pleskach, V. A.*, et al.*, Antiviral and antitumor peptides from insects. *Proceedings of the National Academy of Sciences of the United States of America* 2002, *99*, 12628-12632.

[33] Yi, H.-Y., Chowdhury, M., Huang, Y.-D., Yu, X.-Q., Insect antimicrobial peptides and their applications. *Applied microbiology and biotechnology* 2014, *98*, 5807-5822.

[34] Valle, F. R. d., Mena, M. H., Bourges, H., An investigation into insect protein. *Journal of Food Processing and Preservation* 1982, *6*, 99-110.

[35] Ghaly, A. E., Alkoaik, F. N., The Yellow Mealworm as a Novel Source of Protein. *American Journal of Agricultural and Biological Sciences* 2009, *4*, 319-331.

[36] Finke, M. D., Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biol* 2015, *34*, 554-564.

[37] Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V.*, et al.*, Extraction and characterisation of protein fractions from five insect species. *Food Chemistry* 2013, *141*, 3341-3348.

[38] Mariod, A. A., Fadul, H., Extraction and characterization of gelatin from two edible Sudanese insects and its applications in ice cream making. *Food Sci Technol Int* 2015, *21*, 380-391.

[39] Ozimek, L., Sauer, W. C., Kozikowski, V., Ryan, J. K.*, et al.*, Nutritive Value of Protein Extracted from Honey Bees. *Journal of Food Science* 1985, *50*, 1327-1332.

[40] Cilia, M., Fish, T., Yang, X., McLaughlin, M.*, et al.*, A Comparison of Protein Extraction Methods Suitable for Gel-Based Proteomic Studies of Aphid Proteins. *J Biomol Tech* 2009, *20*, 201-215.

[41] Mariod, A. A., Abdelwahab, S. I., Ibrahim, M. Y., Mohan, S.*, et al.*, Preparation and Characterization of Gelatins from Two Sudanese Edible Insects *Journal of Food Science and Engineering* 2011, *1*, 45-55.

[42] Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N.*, et al.*, Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers* 2012, *87*, 995-1012.

[43] Douglas, A. E., Multiorganismal insects: diversity and function of resident microorganisms. *Annual review of entomology* 2015, *60*, 17-34.

[44] Krishnan, M., Bharathiraja, C., Pandiarajan, J., Prasanna, V. A.*, et al.*, Insect gut microbiome - An unexploited reserve for biotechnological application. *Asian Pac J Trop Biomed* 2014, *4*, S16-21.

[45] Dadd, R. H., *Digestion in insects*, Academic Press, New York, London 2012.

[46] Elpidina, E. N., Goptar, I. A., Digestive peptidases in Tenebrio molitor and possibility of use to treat celiac disease. *Entomological Research* 2007, *37*, 139.147.

[47] Goptar, I. A., Filippova, I. Y., Lysogorskaya, E. N., Oksenoit, E. S.*, et al.*, Localization of post-proline cleaving peptidases in Tenebrio molitor larval midgut. *Biochimie* 2008, *90*, 508-514.

[48] Cristofoletti, P. T., Ribeiro, A. F., Terra, W. R., The cathepsin L-like proteinases from the midgut of Tenebrio molitor larvae: sequence, properties, immunocytochemical localization and function. *Insect Biochem Mol Biol* 2005, *35*, 883-901.

[49] Prabhakar, S., Chen, M. S., Elpidina, E. N., Vinokurov, K. S.*, et al.*, Sequence analysis and molecular characterization of larval midgut cDNA transcripts encoding peptidases from the yellow mealworm, Tenebrio molitor L. *Insect Mol Biol* 2007, *16*, 455-468.

[50] Su, L. J., Liu, H., Li, Y., Zhang, H. F.*, et al.*, Cellulolytic activity and structure of symbiotic bacteria in locust guts. *Genetics and molecular research : GMR* 2014, *13*, 7926-7936.

[51] Genta, F. A., Dillon, R. J., Terra, W. R., Ferreira, C., Potential role for gut microbiota in cell wall digestion and glucoside detoxification in Tenebrio molitor larvae. *J Insect Physiol* 2006, *52*, 593-601.

[52] Anand, A. A., Vennison, S. J., Sankar, S. G., Prabhu, D. I.*, et al.*, Isolation and characterization of bacteria from the gut of Bombyx mori that degrade cellulose, xylan, pectin and starch and their impact on digestion. *J Insect Sci* 2010, *10*, 107-127.

[53] Kaur, R., Kaur, N., Gupta, A. K., Structural features, substrate specificity, kinetic properties of insect alpha-amylase and specificity of plant alpha-amylase inhibitors. *Pestic Biochem Physiol* 2014, *116*, 83-93.

[54] Kouadio, E. J. P., Konan, H. K., Tetchi, F. A., Kouakou, D. B., Kouamé, L. P., Novel α-Amylases Amy A1 and Amy A2 from digestive tract of tropical house cricket Gryllodes sigillatus (Orthoptera: Gryllidae): hydrolysis and transglycosylation reactions. *Agriculture and Biology Journal of North America* 2012, *3*, 198-207.

[55] Weidlich, S., Hoffmann, K. H., Woodring, J., Secretion of lipases in the digestive tract of the cricket Gryllus bimaculatus. *Arch Insect Biochem Physiol* 2015, *90*, 209-217.

[56] Arrese, E. L., Soulages, J. L., Insect fat body: energy, metabolism, and regulation. *Annual review of entomology* 2010, *55*, 207-225.

[57] Oonincx, D. G. A. B., *Feed conversion efficiency, survival, crude protein content and lipid composition of four insect species on diets composed of organic by-products. In: Insects as food and feed: Nutrient composition and environmental impact.* , Ageningen University, Wageningen, NL 2015.

[58] van Broekhoven, S., Oonincx, D. G., van Huis, A., van Loon, J. J., Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *J Insect Physiol* 2015, *73*, 1-10.

[59] Wu, S., Supercritical carbon dioxide extraction of oil from Clanis bilineata (Lepidoptera), an edible insect. *African Journal of Biotechnology* 2012, *11*, 4607-4610.

[60] Pan, W.-J., Liao, A.-M., Zhang, J.-G., Dong, Z., Wei, Z.-J., Supercritical Carbon Dioxide Extraction of the Oak Silkworm (Antheraea pernyi) Pupal Oil: Process Optimization and Composition Determination. *International Journal of Molecular Sciences* 2012, *13*, 2354-2367.

[61] Mariod, A. A., Abdelwahab, S. I., Gedi, M. A., Solati, Z., Supercritical Carbon Dioxide Extraction of Sorghum Bug (Agonoscelis pubescens) Oil Using Response Surface Methodology. *Journal of the American Oil Chemists Society* 2010, *87*, 849-856.

[62] Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. S., Lakemond, C. M. M., Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Research International* 2014, *62*, 1087-1094.

[63] Mustafa, N. E. M., Mariod, A. A., Matthaeus, B., Antibacterial activity of Aspongopus viduatus (melon bug) oil. *Journal of Food Safety* 2008, *28*, 577-586.

[64] Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., Ayieko, M. A., Effect of Processing Methods on the In Vitro Protein Digestibility and Vitamin Content of Edible Winged Termite (Macrotermes subhylanus) and Grasshopper (Ruspolia differens). *Food and Bioprocess Technology* 2010, *3*, 778-782.

[65] Tiencheu, B., Womeni, H. M., Linder, M., Mbiapo, F. T.*, et al.*, Changes of lipids in insect (Rhynchophorus phoenicis) during cooking and storage. *European Journal of Lipid Science and Technology* 2013, *115*, 186-195.

[66] Lafont, R., Dinan, L., Practical uses for ecdysteroids in mammals including humans: And update. *Journal of Insect Science (Tucson)* 2003, *3*, 1-30.

[67] Dinan, L., Lafont, R., Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals. *Journal of Endocrinology* 2006, *191*, 1-8.

[68] Nordentoft, S., Cederberg, T. L., Fischer, C. H., Bjerrum, L., *Proceedings of the 34th International Symposium on Halogenated Persistent Organic Pollutants* 2014.

[69] Zhang, M., Haga, A., Sekiguchi, H., Hirano, S., Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx Mori) pupa exuvia. *International Journal of Biological Macromolecules* 2000, *27*, 99-105.

[70] Aranaz, I., Mengibar, M., Harris, R., Panos, I.*, et al.*, Functional Characterization of Chitin and Chitosan. *Current Chemical Biology* 2009, *3*, 203-230.

[71] Shahidi, F., Abuzaytoun, R., Chitin, chitosan, and co-products: chemistry, production, applications, and health effects. *Advances in Food and Nutrition Research* 2005, *49*, 93-137.

[72] Shahidi, F., Synowiecki, J., Isolation and characterization of nutrients and value-added products from snow crab (Chinoecetes opilio) and shrimp)Pandalus borealis) processing discards. *Journal of Agricultural and Food Chemistry* 1991, *39*, 1527-1532.

[73] Zhang, A.-J., Qin, Q.-L., Zhang, H., Wang, H.-T.*, et al.*, Preparation and Characterisation of Food-Grade Chitosan from Housefly Larvae. *Czech Journal of Food Sciences* 2011, *29*, 616-623.

[74] Galed, G., Fernandez-Valle, M. E., Martinez, A., Heras, A., Application of MRI to monitor the process of ripening and decay in citrus treated with chitosan solutions. *Magn Reson Imaging* 2004, *22*, 127-137.

[75] Kaya, M., Baran, T., Asan-Ozusaglam, M., Cakmak, Y. S.*, et al.*, Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta). *Biotechnology and Bioprocess Engineering* 2015, *20*, 168-179.

[76] Kaya, M., Baran, T., Erdogan, S., Mentes, A.*, et al.*, Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata). *Materials Science & Engineering C-Materials for Biological Applications* 2014, *45*, 72-81.

[77] Kaya, M., Erdogan, S., Mol, A., Baran, T., Comparison of chitin structures isolated from seven Orthoptera species. *International Journal of Biological Macromolecules* 2015, *72*, 797-805.

[78] Liu, S., Sun, J., Yu, L., Zhang, C.*, et al.*, Extraction and Characterization of Chitin from the Beetle Holotrichia parallela Motschulsky. *Molecules* 2012, *17*, 4604-4611.

[79] Paulino, A. T., Simionato, J. I., Garcia, J. C., Nozaki, J., Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers* 2006, *64*, 98-103.

[80] Bailey, S. E., Olin, T. J., Bricka, R. M., Adrian, D. D., A review of potentially low-cost sorbents for heavy metals. *Water Research* 1999, *33*, 2469-2479.

[81] Dapson, R. W., The history, chemistry and modes of action of carmine and related dyes. *Biotechnic & Histochemistry* 2007, *82*, 173-187.

[82] IDRC, International Development Research Centre, Ottawa, Canada 1999.

[83] Wuthrich, B., Kagi, M. K., Stucker, W., Anaphylactic reactions to ingested carmine (E120). *Allergy* 1997, *52*, 1133-1137.

[84] Engel, P., Moran, N. A., The gut microbiota of insects - diversity in structure and function. *FEMS microbiology reviews* 2013, *37*, 699-735.

[85] Dillon, R. J., Dillon, V. M., The gut bacteria of insects: nonpathogenic interactions. *Annual review of entomology* 2004, *49*, 71-92.

[86] Yun, J. H., Roh, S. W., Whon, T. W., Jung, M. J.*, et al.*, Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Appl Environ Microbiol* 2014, *80*, 5254-5264.

[87] Dematheis, F., Kurtz, B., Vidal, S., Smalla, K., Microbial communities associated with the larval gut and eggs of the Western corn rootworm. *PLoS One* 2012, *7*, e44685.

[88] Jeon, H., Park, S., Choi, J., Jeong, G.*, et al.*, The intestinal bacterial community in the food waste-reducing larvae of Hermetia illucens. *Curr Microbiol* 2011, *62*, 1390-1399.

[89] Montagna, M., Chouaia, B., Mazza, G., Prosdocimi, E. M.*, et al.*, Effects of the diet on the microbiota of the red palm weevil (Coleoptera: Dryophthoridae). *PLoS One* 2015, *10*, e0117439.

[90] Jung, J., Heo, A., Park, Y. W., Kim, Y. J.*, et al.*, Gut microbiota of Tenebrio molitor and their response to environmental change. *Journal of microbiology and biotechnology* 2014, *24*, 888-897.

[91] Belda, E., Pedrola, L., Pereto, J., Martinez-Blanch, J. F.*, et al.*, Microbial diversity in the midguts of field and lab-reared populations of the European corn borer Ostrinia nubilalis. *PLoS One* 2011, *6*, e21751.

[92] Zheng, L., Crippen, T. L., Singh, B., Tarone, A. M.*, et al.*, A survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. *Journal of medical entomology* 2013, *50*, 647-658.

[93] Fang, W., Fang, Z., Liu, Z., Yuan, J.*, et al.*, Phylogenetic analysis of bacterial community in the gut of American cockroach (Periplaneta americana). *Wei Sheng Wu Xue Bao* 2013, *53*, 984-994.

[94] Broderick, N. A., Raffa, K. F., Goodman, R. M., Handelsman, J., Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Appl Environ Microbiol* 2004, *70*, 293-300.

[95] Gupta, A. K., Nayduch, D., Verma, P., Shah, B.*, et al.*, Phylogenetic characterization of bacteria in the gut of house flies (Musca domestica L.). *FEMS Microbiol Ecol* 2012, *79*, 581-593.

[96] Gupta, A. K., Rastogi, G., Nayduch, D., Sawant, S. S.*, et al.*, Molecular phylogenetic profiling of gut-associated bacteria in larvae and adults of flesh flies. *Med Vet Entomol* 2014, *28*, 345-354.

[97] Schauer, C., Thompson, C., Brune, A., Pyrotag sequencing of the gut microbiota of the cockroach Shelfordella lateralis reveals a highly dynamic core but only limited effects of diet on community structure. *PLoS One* 2014, *9*, e85861.

[98] Liu, N., Zhang, L., Zhou, H., Zhang, M.*, et al.*, Metagenomic insights into metabolic capacities of the gut microbiota in a fungus-cultivating termite (Odontotermes yunnanensis). *PLoS One* 2013, *8*, e69184.

[99] Otani, S., Mikaelyan, A., Nobre, T., Hansen, L. H.*, et al.*, Identifying the core microbial community in the gut of fungus-growing termites. *Mol Ecol* 2014, *23*, 4631-4644.

[100] Kautz, S., Rubin, B. E., Russell, J. A., Moreau, C. S., Surveying the microbiome of ants: comparing 454 pyrosequencing with traditional methods to uncover bacterial diversity. *Appl Environ Microbiol* 2013, *79*, 525-534.

[101] Tang, X., Freitak, D., Vogel, H., Ping, L.*, et al.*, Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. *PloS one* 2012, *7*, e36978.

[102] Bansal, R., Hulbert, S., Schemerhorn, B., Reese, J. C.*, et al.*, Hessian fly-associated bacteria: transmission, essentiality, and composition. *PLoS One* 2011, *6*, e23170.

[103] Suh, S. O., Nguyen, N. H., Blackwell, M., Yeasts isolated from plant-associated beetles and other insects: seven novel Candida species near Candida albicans. *FEMS Yeast Res* 2008, *8*, 88-102.

[104] Suh, S. O., McHugh, J. V., Pollock, D. D., Blackwell, M., The beetle gut: a hyperdiverse source of novel yeasts. *Mycological research* 2005, *109*, 261-265.

[105] Yu, C., Liu, C., Wang, X., Zhao, J.*, et al.*, Streptomyces polyrhachii sp. nov., a novel actinomycete isolated from an edible Chinese black ant (Polyrhachis vicina Roger). *Antonie Van Leeuwenhoek* 2013, *104*, 1013-1019.

[106] Chaiwong, T., Srivoramas, T., Sueabsamran, P., Sukontason, K.*, et al.*, The blow fly, Chrysomya megacephala, and the house fly, Musca domestica, as mechanical vectors of pathogenic bacteria in Northeast Thailand. *Tropical biomedicine* 2014, *31*, 336-346.

[107] Graczyk, T. K., Knight, R., Tamang, L., Mechanical transmission of human protozoan parasites by insects. *Clinical microbiology reviews* 2005, *18*, 128-132.

[108] Lima, W. R., Marques, S. G., Rodrigues, F. S., Rebelo, J. M., Ants in a hospital environment and their potential as mechanical bacterial vectors. *Revista da Sociedade Brasileira de Medicina Tropical* 2013, *46*, 637-640.

[109] Wasala, L., Talley, J. L., Desilva, U., Fletcher, J., Wayadande, A., Transfer of Escherichia coli O157:H7 to spinach by house flies, Musca domestica (Diptera: Muscidae). *Phytopathology* 2013, *103*, 373-380.

[110] Forster, M., Klimpel, S., Sievert, K., The house fly (Musca domestica) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Vet Parasitol* 2009, *160*, 163-167.

[111] McAllister, J. C., Steelman, C. D., Skeeles, J. K., Reservoir competence of the lesser mealworm (Coleoptera: Tenebrionidae) for Salmonella typhimurium (Eubacteriales: Enterobacteriaceae). *J Med Entomol* 1994, *31*, 369-372.

[112] McAllister, J. C., Steelman, C. D., Skeeles, J. K., Newberry, L. A., Gbur, E. E., Reservoir competence of Alphitobius diaperinus (Coleoptera:Tenebrionidae) for Escherichia coli (Eubacteriales:Enterobacteriaceae). *J Med Entomol* 1996, *33*, 983-987.

[113] Strother, K. O., Steelman, C. D., Gbur, E. E., Reservoir competence of lesser mealworm (Coleoptera: Tenebrionidae) for Campylobacter jejuni (Campylobacterales: Campylobacteraceae). *J Med Entomol* 2005, *42*, 42-47.

[114] Skov, M. N., Spencer, A. G., Hald, B., Petersen, L.*, et al.*, The role of litter beetles as potential reservoir for Salmonella enterica and thermophilic Campylobacter spp. between broiler flocks. *Avian diseases* 2004, *48*, 9-18.

[115] Menasria, T., Moussa, F., El-Hamza, S., Tine, S.*, et al.*, Bacterial load of German cockroach (Blattella germanica) found in hospital environment. *Pathogens and global health* 2014, *108*, 141-147.

[116] Ghosh, A., Akhtar, M., Holderman, C., Zurek, L., Significance and survival of Enterococci during the house fly development. *J Med Entomol* 2014, *51*, 63-67.

[117] Zurek, L., Ghosh, A., Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits. *Appl Environ Microbiol* 2014, *80*, 3562-3567.

[118] Kampen, H., [What makes an insect a vector?]. *Berl Munch Tierarztl Wochenschr* 2009, *122*, 451-457.

[119] Hogenhout, S. A., Ammar el, D., Whitfield, A. E., Redinbaugh, M. G., Insect vector interactions with persistently transmitted viruses. *Annual review of phytopathology* 2008, *46*, 327-359.

[120] Whitfield, A. E., Falk, B. W., Rotenberg, D., Insect vector-mediated transmission of plant viruses. *Virology* 2015, *479-480C*, 278-289.

[121] Parola, P., Davoust, B., Raoult, D., Tick- and flea-borne rickettsial emerging zoonoses. *Veterinary research* 2005, *36*, 469-492.

[122] Wales, A. D., Carrique-Mas, J. J., Rankin, M., Bell, B.*, et al.*, Review of the carriage of zoonotic bacteria by arthropods, with special reference to Salmonella in mites, flies and litter beetles. *Zoonoses and public health* 2010, *57*, 299-314.

[123] Koura, E. A., Kamel, E. G., A study of the protozoa associated with some harmful insects in the local environment. *Journal of the Egyptian Society of Parasitology* 1990, *20*, 105-115.

[124] Davari, B., Khodavaisy, S., Ala, F., Isolation of fungi from housefly (Musca domestica L.) at Slaughter House and Hospital in Sanandaj, Iran. *Journal of preventive medicine and hygiene* 2012, *53*, 172-174.

[125] Phoku, J. Z., Barnard, T. G., Potgieter, N., Dutton, M. F., Fungi in housefly (Musca domestica L.) as a disease risk indicator-A case study in South Africa. *Acta Trop* 2014, *140*, 158-165.

[126] Adenusi, A. A., Adewoga, T. O., Human intestinal parasites in non-biting synanthropic flies in Ogun State, Nigeria. *Travel Med Infect Dis* 2013, *11*, 181-189.

[127] Azambuja, P., Garcia, E. S., Ratcliffe, N. A., Gut microbiota and parasite transmission by insect vectors. *Trends Parasitol* 2005, *21*, 568-572.

[128] Eilenberg, J., Vlak, J., Nielsen-LeRoux, C., Cappellozza, S., Jensen, A. B., Diseases in insects produced for food and feed. *Journal of Insects as Food and Feed* 2015, *1*, 87-102.

[129] Almuzara, M. N., Palombarani, S., Tuduri, A., Figueroa, S.*, et al.*, First case of fulminant sepsis due to Wohlfahrtiimonas chitiniclastica. *Journal of clinical microbiology* 2011, *49*, 2333-2335.

[130] Rebaudet, S., Genot, S., Renvoise, A., Fournier, P. E., Stein, A., Wohlfahrtiimonas chitiniclastica bacteremia in homeless woman. *Emerging infectious diseases* 2009, *15*, 985-987.

[131] Lacey, E. D., *Manual of Techniques in Insect Pathlogy*, Academic Press Elsevier Science, Wapato, USA 1997.

[132] Post, K., Riesner, D., Walldorf, V., Mehlhorn, H., Fly larvae and pupae as vectors for scrapie. *Lancet* 1999, *354*, 1969-1970.

[133] Ulrich, R. G., Buthala, D. A., Klug, M. J., Microbiota Associated with the Gastrointestinal Tract of the Common House Cricket, Acheta domestica. *Appl Environ Microbiol* 1981, *41*, 246-254.

[134] Bansal, R., Hulbert, S. H., Reese, J. C., Whitworth, R. J.*, et al.*, Pyrosequencing reveals the predominance of pseudomonadaceae in gut microbiome of a gall midge. *Pathogens* 2014, *3*, 459-472.

[135] Tagliavia, M., Messina, E., Manachini, B., Cappello, S., Quatrini, P., The gut microbiota of larvae of Rhynchophorus ferrugineus Oliver (Coleoptera: Curculionidae). *BMC Microbiol* 2014, *14*, 136.

[136] Banjo, A. D., Lawal, O. A., Adeduji, O. O., Bacteria and fungi isolated from housefly (Musca domestica L.) larvae. *African Journal of Biotechnology* 2005, *4* 780-784.

[137] Banjo, A. D., Lawal, O. A., Adeyemi, A. I., The Microbial Fauna Associated with the Larvae of Oryctes Monocerus. *Journal of Applied Sciences Research* 2006, *2*, 837-843.

[138] Nazni, W. A., Seleena, B., Lee, H. L., Jeffery, J.*, et al.*, Bacteria fauna from the house fly, Musca domestica (L.). *Tropical biomedicine* 2005, *22*, 225-231.

[139] Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., Nout, M. J. R., Microbiological aspects of processing and storage of edible insects. *Food Control* 2012, *26*, 628-631.

[140] Akbari, S., Oshaghi, M. A., Hashemi-Aghdam, S. S., Hajikhani, S.*, et al.*, Aerobic Bacterial Community of American Cockroach Periplaneta americana,a Step toward Finding Suitable Paratransgenesis Candidates. *J Arthropod Borne Dis* 2015, *9*, 35-48.

[141] Duarte, A. P., Attili-Angelis, D., Baron, N. C., Forti, L. C., Pagnocca, F. C., Leaf-cutting ants: an unexpected microenvironment holding human opportunistic black fungi. *Antonie Van Leeuwenhoek* 2014, *106*, 465-473.

[142] Pagnocca, F. C., Rodrigues, A., Nagamoto, N. S., Bacci, M., Jr., Yeasts and filamentous fungi carried by the gynes of leaf-cutting ants. *Antonie Van Leeuwenhoek* 2008, *94*, 517-526.

[143] McFrederick, Q. S., Mueller, U. G., James, R. R., Interactions between fungi and bacteria influence microbial community structure in the Megachile rotundata larval gut. *Proc Biol Sci* 2014, *281*, 20132653.

[144] Haeder, S., Wirth, R., Herz, H., Spiteller, D., Candicidin-producing Streptomyces support leaf-cutting ants to protect their fungus garden against the pathogenic fungus Escovopsis. *Proc Natl Acad Sci U S A* 2009, *106*, 4742-4746.

[145] Guedes, F. L., Attili-Angelis, D., Pagnocca, F. C., Selective isolation of dematiaceous fungi from the workers of Atta laevigata (Formicidae: Attini). *Folia microbiologica* 2012, *57*, 21-26.

[146] Cazemier, A. E., Hackstein, J. H. P., Op den Camp, H. J. M., Rosenberg, J., van der Drift, C., Bacteria in the Intestinal Tract of Different Species of Arthropods. *Microb Ecol* 1997, *33*, 189-197.

[147] Douglas, A. E., Multiorganismal insects: diversity and function of resident microorganisms. *Annual review of entomology* 2015, *60*, 17-34.

[148] Colman, D. R., Toolson, E. C., Takacs-Vesbach, C. D., Do diet and taxonomy influence insect gut bacterial communities? *Mol Ecol* 2012, *21*, 5124-5137.

[149] Liang, X., Fu, Y., Tong, L., Liu, H., Microbial shifts of the silkworm larval gut in response to lettuce leaf feeding. *Applied microbiology and biotechnology* 2014, *98*, 3769-3776.

[150] Perez-Cobas, A. E., Maiques, E., Angelova, A., Carrasco, P.*, et al.*, Diet shapes the gut microbiota of the omnivorous cockroach Blattella germanica. *FEMS Microbiol Ecol* 2015, *91*, 1-14.

[151] Vojvodic, S., Rehan, S. M., Anderson, K. E., Microbial gut diversity of Africanized and European honey bee larval instars. *PLoS One* 2013, *8*, e72106.

[152] Singh, B., Crippen, T. L., Zheng, L., Fields, A. T.*, et al.*, A metagenomic assessment of the bacteria associated with Lucilia sericata and Lucilia cuprina (Diptera: Calliphoridae). *Applied microbiology and biotechnology* 2015, *99*, 869-883.

[153] Moll, R. M., Romoser, W. S., Modrzakowski, M. C., Moncayo, A. C., Lerdthusnee, K., Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. *J Med Entomol* 2001, *38*, 29-32.

[154] Stoops, J., Crauwels, S., Waud, M., Claes, J.*, et al.*, Microbial community assessment of mealworm larvae (Tenebrio molitor) and grasshoppers (Locusta migratoria migratorioides) sold for human consumption. *Food microbiology* 2016, *53*, 122-127.

[155] Ekop, E. A., Udoh, A. I., Akpan, P. E., Proximate and anti-nutrient composition of four edible insects in Akwa Ibom State, Nigeria. *World Journal od Applied Science and Technology* 2010, *2*, 224-231.

[156] Shantibala, T., Lokeshwari, R. K., Debaraj, H., Nutritional and antinutritional composition of the five species of aquatic edible insects consumed in Manipur, India. *Journal of insect science* 2014, *14*, 1-10.

[157] Nishimune, T., Watanabe, Y., Okazaki, H., Akai, H., Thiamin is decomposed due to Anaphe spp. entomophagy in seasonal ataxia patients in Nigeria. *J Nutr* 2000, *130*, 1625-1628.

[158] Karras, D. J., Farrell, S. E., Harrigan, R. A., Henretig, F. M., Gealt, L., Poisoning from "Spanish fly" (cantharidin). *Am J Emerg Med* 1996, *14*, 478-483.

[159] Till, J. S., Majmudar, B. N., Cantharidin poisoning. *South Med J* 1981, *74*, 444-447.

[160] Tagwireyi, D., Ball, D. E., Loga, P. J., Moyo, S., Cantharidin poisoning due to "Blister beetle" ingestion. *Toxicon* 2000, *38*, 1865-1869.

[161] Brown, W. V., Doyen, J. T., Moore, B. P., Lawrence, J. F., Chemical Composition and Taxonomic Significance of Defensive Secretions of Some Australian Tenebrionidae (Coleoptera) *J. Aust. ent. Soc.* 1992, *31*, 79-89.

[162] Zagrobelny, M., Dreon, A. L., Gomiero, T., Marcazzan, G. L.*, et al.*, Toxic Moths: Source of a Truly Safe Delicacy. *Journal of Ethnobiology* 2009, *29*, 64-76.

[163] Han, S. R., Lee, B. S., Jung, K. J., Yu, H. 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 : RTP* 2016, *77*, 206-212.

[164] Omotoso, O. T., Nutritional quality, functional properties and anti-nutrient compositions of the larva of Cirina forda (Westwood) (Lepidoptera: Saturniidae). *J Zhejiang Univ Sci B* 2006, *7*, 51-55.

[165] Ekpo, K. E., Nutrient composition, functional properties and anti-nutrient content of Rhynchophorus pheonicis (F) larva. *Annals of Biological Research* 2010, *1*, 178-190.

[166] Ajayi, O. E., Biochemical analyses and nutritional content of four castes of subterranean termites, Macrotermes subhyalinus (Rambur) (Isoptera: Termitidae): differences in digestibility and anti-nutrient contents among castes. *International Journal of Biology* 2012, *4*, 54-59.

[167] DeFoliart, G., Insects as food: Why the Western attitude is important. *Annual review of entomology* 1999, *44*, 21-50.

[168] Ji, K. M., Zhan, Z. K., Chen, J. J., Liu, Z. G., Anaphylactic shock caused by silkworm pupa consumption in China. *Allergy* 2008, *63*, 1407-1408.

[169] Yew, K. L., Kok, V. S., Exotic food anaphylaxis and the broken heart: sago worm and takotsubo cardiomyopathy. *Med J Malaysia* 2012, *67*, 540-541.

[170] Choi, G. S., Shin, Y. S., Kim, J. E., Ye, Y. M., Park, H. S., Five cases of food allergy to vegetable worm (Cordyceps sinensis) showing cross-reactivity with silkworm pupae. *Allergy* 2010, *65*, 1196-1197.

[171] Leung, P. S., Chow, W. K., Duffey, S., Kwan, H. S.*, et al.*, IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *J Allergy Clin Immunol* 1996, *98*, 954-961.

[172] Kamath, S. D., Abdel Rahman, A. M., Komoda, T., Lopata, A. L., Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. *Food Chem* 2013, *141*, 4031-4039.

[173] Hemmer, W., Insects as a cause for allergic reactions. *Denisia* 2010, 381-409.

[174] Reese, G., Ayuso, R., Lehrer, S. B., Tropomyosin: An invertebrate pan-allergen. *International Archives of Allergy and Immunology* 1999, *119*, 247-258.

[175] Verhoeckx, K. C. M., van Broekhoven, S., den Hartog-Jager, C. F., Gaspari, M.*, et al.*, House dust mite (Der p 10) and crustacean allergic patients may react to food containing Yellow mealworm proteins. *Food and Chemical Toxicology* 2014, *65*, 364-373.

[176] Calderon, M. A., Linneberg, A., Kleine-Tebbe, J., De Blay, F.*, et al.*, Respiratory allergy caused by house dust mites: What do we really know? *J Allergy Clin Immunol* 2015, *136*, 38-48.

[177] Pomes, A., Arruda, L. K., Investigating cockroach allergens: aiming to improve diagnosis and treatment of cockroach allergic patients. *Methods* 2014, *66*, 75-85.

[178] Fukutomi, Y., Taniguchi, M., Sensitization to fungal allergens: Resolved and unresolved issues. *Allergol Int* 2015, *64*, 321-331.

[179] Commins, S. P., Platts-Mills, T. A., Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1,3-galactose (alpha-gal). *Curr Allergy Asthma Rep* 2013, *13*, 72-77.

[180] Platts-Mills, T. A., Schuyler, A. J., Tripathi, A., Commins, S. P., Anaphylaxis to the carbohydrate side chain alpha-gal. *Immunol Allergy Clin North Am* 2015, *35*, 247-260.

[181] Chung, K., Baker, J. R., Baldwin, J. L., Chou, A., Identification of carmine allergens among three carmine allergy patients. *Allergy* 2001, *56*, 73-77.

[182] Dubey, L. K., Moeller, J. B., Schlosser, A., Sorensen, G. L., Holmskov, U., Chitin enhances serum IgE in Aspergillus fumigatus induced allergy in mice. *Immunobiology* 2015, *220*, 714-721.

[183] Zakzuk, J., Benedetti, I., Fernandez-Caldas, E., Caraballo, L., The influence of chitin on the immune response to the house dust mite allergen Blo T 12. *Int Arch Allergy Immunol* 2014, *163*, 119-129.

[184] Moura, F. T., Oliveira, A. S., Macedo, L. L., Vianna, A. L.*, et al.*, Effects of a chitin-binding vicilin from Enterolobium contortisiliquum seeds on bean bruchid pests (Callosobruchus maculatus and Zabrotes subfasciatus) and phytopathogenic fungi (Fusarium solani and Colletrichum lindemuntianum). *J Agric Food Chem* 2007, *55*, 260-266.

[185] Macedo, L. L., Amorim, T. M., Uchoa, A. F., Oliveira, A. S.*, et al.*, Larvicidal effects of a chitin-binding vicilin from Erythrina velutina seeds on the mediterranean fruit fly Ceratitis capitata. *J Agric Food Chem* 2008, *56*, 802-808.

[186] Burks, A. W., Cockrell, G., Stanley, J. S., Helm, R. M., Bannon, G. A., Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995, *96*, 1715-1721.

[187] Holzhauser, T., Wackermann, O., Ballmer-Weber, B. K., Bindslev-Jensen, C.*, et al.*, Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *J Allergy Clin Immunol* 2009, *123*, 452-458.

[188] Broekman, H., Knulst, A., den Hartog Jager, S., Monteleone, F.*, et al.*, Effect of thermal processing on mealworm allergenicity. *Mol Nutr Food Res* 2015, *59*, 1855-1864.

[189] van Broekhoven, S., Bastiaan-Net, S., de Jong, N. W., Wichers, H. J., Influence of processing and in vitro digestion on the allergic cross-reactivity of three mealworm species. *Food Chem* 2016, *196*, 1075-1083.

[190] Verhoeckx, K. C., Vissers, Y. M., Baumert, J. L., Faludi, R.*, et al.*, Food processing and allergenicity. *Food Chem Toxicol* 2015, *80*, 223-240.

[191] King, C., Jones, H. I., Tay, C. Y., Arthropod intermediate hosts of Abbreviata antarctica (Nematoda: Physalopteridae) in Australia. *The Journal of parasitology* 2013, *99*, 708-711.

[192] Nai, Y. S., Su, P. Y., Hsu, Y. H., Chiang, C. H.*, et al.*, A new spiroplasma isolate from the field cricket (Gryllus bimaculatus) in Taiwan. *J Invertebr Pathol* 2014, *120*, 4-8.

[193] Zhao, Y., Zhang, Z. H., Wang, G. J., Identification of the intestinal bacteria in Locusta migratoria manilensis and their antagonism to Metarhizium anisopliae. *Plant Protection* 2008, *34*, 69-72.

[194] Shi, W., Guo, Y., Xu, C., Tan, S.*, et al.*, Unveiling the mechanism by which microsporidian parasites prevent locust swarm behavior. *Proc Natl Acad Sci U S A* 2014, *111*, 1343-1348.

[195] Nunamaker, R. A., Lockwood, J. A., Stith, C. E., Campbell, C. L.*, et al.*, Grasshoppers (Orthoptera: Acrididae) could serve as reservoirs and vectors of vesicular stomatitis virus. *J Med Entomol* 2003, *40*, 957-963.

[196] Dillon, R., Charnley, K., Mutualism between the desert locust Schistocerca gregaria and its gut microbiota. *Research in microbiology* 2002, *153*, 503-509.

[197] Hunt, J., Charnley, K. A., Abundance and distribution of the gut flora of the desert locust, Schistocerca gregaria. *Journal of Invertebrate Pathology* 1981, *38*, 378-385.

[198] Stevenson, J. P., The normal bacterial flora of the alimentary canal of laboratory stocks of the desert locust, Schistocerca gregaria Forskal. *J Invertebr Pathol* 1966, *8*, 205-211.

[199] Stevenson, J. P., Motile streptococci from the desert locust, Schistocerca gregaria. *J Invertebr Pathol* 1966, *8*, 258-259.

[200] Cappellozza, S., Saviane, A., Tettamanti, G., Squadrin, M.*, et al.*, Identification of Enterococcus mundtii as a pathogenic agent involved in the "flacherie" disease in Bombyx mori L. larvae reared on artificial diet. *J Invertebr Pathol* 2011, *106*, 386-393.

[201] Xiang, H., Li, M. W., Zhao, Y., Zhao, L. P.*, et al.*, Bacterial community in midguts of the silkworm larvae estimated by PCR-DGGE and 16S rDNA gene library analysis. *Acta Entomologica Sinica* 2007, *50*, 222-232.

[202] Evans, B. A., Rozen, D. E., A Streptococcus pneumoniae infection model in larvae of the wax moth Galleria mellonella. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2012, *31*, 2653-2660.

[203] Fedhila, S., Buisson, C., Dussurget, O., Serror, P.*, et al.*, Comparative analysis of the virulence of invertebrate and mammalian pathogenic bacteria in the oral insect infection model Galleria mellonella. *J Invertebr Pathol* 2010, *103*, 24-29.

[204] Glavis-Bloom, J., Muhammed, M., Mylonakis, E., Of model hosts and man: using Caenorhabditis elegans, Drosophila melanogaster and Galleria mellonella as model hosts for infectious disease research. *Adv Exp Med Biol* 2012, *710*, 11-17.

[205] Mylonakis, E., Galleria mellonella and the study of fungal pathogenesis: making the case for another genetically tractable model host. *Mycopathologia* 2008, *165*, 1-3.

[206] Simpanya, M. F., Allotey, J., Mpuchane, S. F., A mycological investigation of phane, an edible caterpillar of an emperor moth, Imbrasia belina. *J Food Prot* 2000, *63*, 137-140.

[207] Bates, C., Hiett, K. L., Stern, N. J., Relationship of Campylobacter isolated from poultry and from darkling beetles in New Zealand. *Avian diseases* 2004, *48*, 138-147.

[208] Crippen, T. L., Sheffield, C. L., Esquivel, S. V., Droleskey, R. E., Esquivel, J. F., The acquisition and internalization of Salmonella by the lesser mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae). *Vector Borne Zoonotic Dis* 2009, *9*, 65-72.

[209] Hazeleger, W. C., Bolder, N. M., Beumer, R. R., Jacobs-Reitsma, W. F., Darkling beetles (Alphitobius diaperinus) and their larvae as potential vectors for the transfer of Campylobacter jejuni and Salmonella enterica serovar paratyphi B variant Java between successive broiler flocks. *Appl Environ Microbiol* 2008, *74*, 6887-6891.

[210] Leffer, A. M., Kuttel, J., Martins, L. M., Pedroso, A. C.*, et al.*, Vectorial competence of larvae and adults of Alphitobius diaperinus in the transmission of Salmonella enteritidis in poultry. *Vector Borne Zoonotic Dis* 2010, *10*, 481-487.

[211] Roche, A. J., Cox, N. A., Richardson, L. J., Buhr, R. J.*, et al.*, Transmission of Salmonella to broilers by contaminated larval and adult lesser mealworms, Alphitobius diaperinus (Coleoptera: Tenebrionidae). *Poultry science* 2009, *88*, 44-48.

[212] Zheng, L., Crippen, T. L., Sheffield, C. L., Poole, T. L.*, et al.*, Evaluation of Salmonella movement through the gut of the lesser mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae). *Vector Borne Zoonotic Dis* 2012, *12*, 287-292.

[213] McAllister, J. C., Steelman, C. D., Newberry, L. A., Skeeles, J. K., Isolation of infectious bursal disease virus from the lesser mealworm, Alphitobius diaperinus (Panzer). *Poultry science* 1995, *74*, 45-49.

[214] Goodwin, M. A., Waltman, W. D., Transmission of Eimeria, Viruses, and Bacteria to Chicks: Darkling Beetles (Alphitobius diaperinus) as Vectors of Pathogens. *J Appl Poult Res* 1996, *5*, 51-55.

[215] Watson, D. W., Guy, J. S., Stringham, S. M., Limited transmission of turkey coronavirus in young turkeys by adult Alphitobius diaperinus (Coleoptera: Tenebrionidae). *J Med Entomol* 2000, *37*, 480-483.

[216] Crippen, T. L., Zheng, L., Sheffield, C. L., Tomberlin, J. K.*, et al.*, Transient gut retention and persistence of Salmonella through metamorphosis in the lesser mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae). *Journal of applied microbiology* 2012, *112*, 920-926.

[217] Fischer, O. A., Matlova, L., Dvorska, L., Svastova, P.*, et al.*, Beetles as possible vectors of infections caused by Mycobacterium avium species. *Vet Microbiol* 2004, *102*, 247-255.

[218] Lee, J. K., Lee, Y. Y., Park, K. H., Sim, J.*, et al.*, Wohlfahrtiimonas larvae sp. nov., isolated from the larval gut of Hermetia illucens (Diptera: Stratiomyidae). *Antonie Van Leeuwenhoek* 2014, *105*, 15-21.

[219] Zheng, L., Crippen, T. L., Holmes, L., Singh, B.*, et al.*, Bacteria mediate oviposition by the black soldier fly, Hermetia illucens (L.), (Diptera: Stratiomyidae). *Sci Rep* 2013, *3*, 2563.

[220] Lalander, C., Diener, S., Magri, M. E., Zurbrugg, C.*, et al.*, Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)--from a hygiene aspect. *The Science of the total environment* 2013, *458-460*, 312-318.

[221] Lalander, C. H., Fidjeland, J., Diener, S., Eriksson, S., Vinneras, B., High waste-to-biomass conversion and efficient Salmonella spp. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development* 2015, *35*, 261-271.

[222] De Jesus, A. J., Olsen, A. R., Bryce, J. R., Whiting, R. C., Quantitative contamination and transfer of Escherichia coli from foods by houseflies, Musca domestica L. (Diptera: Muscidae). *Int J Food Microbiol* 2004, *93*, 259-262.

[223] Farag, T. H., Faruque, A. S., Wu, Y., Das, S. K.*, et al.*, Housefly population density correlates with shigellosis among children in Mirzapur, Bangladesh: a time series analysis. *PLoS Negl Trop Dis* 2013, *7*, e2280.

[224] Fotedar, R., Banerjee, U., Samantray, J. C., Shriniwas, Vector potential of hospital houseflies with special reference to Klebsiella species. *Epidemiol Infect* 1992, *109*, 143-147.

[225] Joyner, C., Mills, M. K., Nayduch, D., Pseudomonas aeruginosa in Musca domestica L.: temporospatial examination of bacteria population dynamics and house fly antimicrobial responses. *PLoS One* 2013, *8*, e79224.

[226] Rahuma, N., Ghenghesh, K. S., Ben Aissa, R., Elamaari, A., Carriage by the housefly (Musca domestica) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya. *Annals of tropical medicine and parasitology* 2005, *99*, 795-802.

[227] Sola-Gines, M., Gonzalez-Lopez, J. J., Cameron-Veas, K., Piedra-Carrasco, N.*, et al.*, Houseflies (Musca domestica) as Vectors for Extended-Spectrum beta-Lactamase-Producing Escherichia coli on Spanish Broiler Farms. *Appl Environ Microbiol* 2015, *81*, 3604-3611.

[228] Wang, Y. C., Chang, Y. C., Chuang, H. L., Chiu, C. C.*, et al.*, Transmission of Salmonella between swine farms by the housefly (Musca domestica). *J Food Prot* 2011, *74*, 1012-1016.

[229] Zurek, L., Denning, S. S., Schal, C., Watson, D. W., Vector competence of Musca domestica (Diptera: Muscidae) for Yersinia pseudotuberculosis. *J Med Entomol* 2001, *38*, 333-335.

[230] Nielsen, A. A., Skovgard, H., Stockmarr, A., Handberg, K. J., Jorgensen, P. H., Persistence of low-pathogenic avian influenza H5N7 and H7N1 subtypes in house flies (Diptera: Muscidae). *J Med Entomol* 2011, *48*, 608-614.

[231] Fotedar, R., Vector potential of houseflies (Musca domestica) in the transmission of Vibrio cholerae in India. *Acta Trop* 2001, *78*, 31-34.

[232] Bouamamaa, L., Sorlozano, A., Laglaoui, A., Lebbadi, M.*, et al.*, Antibiotic resistance patterns of bacterial strains isolated from Periplaneta americana and Musca domestica in Tangier, Morocco. *Journal of infection in developing countries* 2010, *4*, 194-201.

[233] Szalanski, A. L., Owens, C. B., McKay, T., Steelman, C. D., Detection of Campylobacter and Escherichia coli O157:H7 from filth flies by polymerase chain reaction. *Med Vet Entomol* 2004, *18*, 241-246.

[234] Elgderi, R. M., Ghenghesh, K. S., Berbash, N., Carriage by the German cockroach (Blattella germanica) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospitals and households in Tripoli, Libya. *Annals of tropical medicine and parasitology* 2006, *100*, 55-62.

[235] Pai, H. H., Chen, W. C., Peng, C. F., Cockroaches as potential vectors of nosocomial infections. *Infect Control Hosp Epidemiol* 2004, *25*, 979-984.

[236] Salehzadeh, A., Tavacol, P., Mahjub, H., Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran. *Journal of vector borne diseases* 2007, *44*, 105-110.

[237] Wannigama, D. L., Dwivedi, R., Zahraei-Ramazani, A., Prevalence and Antibiotic Resistance of Gram-Negative Pathogenic Bacteria Species Isolated from Periplaneta americana and Blattella germanica in Varanasi, India. *J Arthropod Borne Dis* 2014, *8*, 10-20.

[238] Isaac, C., Orue, P. O., Iyamu, M. I., Ehiaghe, J. I., Isaac, O., Comparative analysis of pathogenic organisms in cockroaches from different community settings in Edo State, Nigeria. *Korean J Parasitol* 2014, *52*, 177-181.

[239] Heinz, G., Hautzinger, P., in: (RAP), F. R. O. f. A. a. t. P. (Ed.), FAO Regional Office for Asia and the Pacific (RAP), Bangkok 2007.

[240] Saxholt, E., Christensen, A. T., Møller, A., Hartkopp, H. B.*, et al.*, Department of Nutrition, National Food Institute, Technical University of Denmark 2009.

[241] Finke, M. D., Estimate of chitin in raw whole insects. *Zoo Biology* 2007, *26*, 105-115.