



Review

Insects as food: A review on risks assessments of Tenebrionidae and Gryllidae in relation to a first machines and plants development

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ABSTRACT

The demand for protein food with low environmental impact is currently a major challenge for food production. Insects represent a potential source of good quality food, that is rich in nutrients, cheaper, and easier to produce, with potentially lower environmental impact. Nevertheless, insects could present significant risks for the consumers' safety and for the workers of this emerging production chain. This highlights the need for a specific comprehensive approach that can guarantee safe working conditions and risk-free products. The aim of this review is to summarize the current knowledge pertaining to the potential microbial, chemical, physical, and allergenic risks in the breeding, transformation, and consumption of edible insects. In particular, given the lack of specific studies, the purpose of this paper is to suggest strategies to orient the development of machines and plants in the emerging production chain of insects for food, with ameliorative effects on both finished product and workers' safety. Despite a very appealing nutritional profile that is rich in high quality proteins, lipids, vitamins, minerals, and fiber, insects present several risks, mainly related to microbial contamination, accumulation and production of chemical contaminants like toxins and heavy metals, allergenicity through inhalation or food ingestion by workers and consumers. This paper, according to the findings achieved by the systematic review, suggests strategies, processes, and treatments, which could guide the development of ad hoc machines aimed to guarantee safety in the production chain of insects for food.

1. Introduction

The world population is constantly increasing and it is expected that the world will host 9 billion people by 2050 (Alexandratos & Bruinsma, 2012). Feeding this growing mass of more demanding consumers will necessarily require an increase in food production, particularly in protein sources, which are also the most expensive for the environment. This will inevitably put more pressure on the planet's already limited resources: land, oceans, water, and energy. If food production is to be obtained by means of current techniques, increases in green house gas (GHG) and ammonia emissions, deforestation, soil erosion, loss of plant biodiversity, and water pollution are set to continue. These environmental problems, especially those associated with raising livestock, need urgent attention: according to FAO, livestock is, in fact, the world's largest user of agricultural land (67.3%) (FAOSTAT, 2013). The global demand for meat products is expected to increase by 76.4%

between 2005 and 2050 (from 258 to 455 million tons). Meeting this demand will require innovative solutions (Alexandratos & Bruinsma, 2012; Van Huis, 2015).

Several studies suggest that edible insects may be a viable alternative or supplement to conventional protein sources (Belluco et al., 2013; Mlcek, Borkovcova, & Bednarova, 2014; Payne, Scarborough, Rayner, & Nonaka, 2016; Schlüter et al., 2017; Van Huis, 2015). Edible insects are already popular foodstuffs in many areas, mainly Asia, Africa, Latin America and Oceania. The eaten insects species vary according to the geographical distribution (Patel, Suleria, & Rauf, 2019). Despite Tenebrionidae and Gryllidae families are widely studied in literature, each country has its own niche of insect and tries to valorize such source of protein (e.g. Mopane worm in South Africa (Stack et al., 2003)). Furthermore, the collecting and processing methods are able to influence safety, nutritional values and technological performances of whole and powdered insects (Vandeweyer, Lenaerts, Callens, and Van

Abbreviations: (GHG), Green House Gas; (PCB), Poly-Chlorinated Biphenyl; (OCP), Organo-Chlorine Pesticide; (PBDE), Poly-Brominated Diphenyl Ether; (HFR), Halogenated Flame Retardant; (PFRs), Phosphorous Flame Retardants; (PPE), Personal Protective Equipment; (SERS), Surface-Enhanced Raman Scattering; (FET), Field-Effect Transistor

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Campenhout (2017b), De Gier & Verhoeckx, 2018; Vandeweyer et al. (2017b); EFSA, 2015). Insect eating is estimated to be regularly practiced by at least 2 billion people worldwide (FAO, 2014). Thousands of insect species have been documented in literature as edible, most of them from tropical countries (Jongema, 2017). The most commonly eaten insect groups are beetles, caterpillars, wasps, bees, ants, grasshoppers, locusts, crickets, cicadas, termites, dragonflies, and flies (Van Huis, 2015; Sun-waterhouse et al., 2016). However, Tenebrionidae and Gryllidae families are seen as more suitable for consumption and processing (Marberg, van Kranenburg, & Korzilius, 2017). Furthermore, these families are widely studied in literature (Bednarska et al., 2016; Borremans, Lenaerts, Crauwels, Lievens, & Van Campenhout, 2018; Broekman et al., 2015; Caparros Megido et al., 2018; Fernandez-Cassi, Supeanu, Jansson, Boqvist, & Vagsholm, 2018; Francis et al., 2019; Grau, Vilcinskis, & Joop, 2017; Han et al., 2016; Osimani et al., 2017a; Osimani et al., 2018b; Vandeweyer, Crauwels, Lievens, & Van Campenhout, 2016). In most Western countries, consumer acceptance of entomophagy is still limited by disgust (appearance, taste, sense of dirtiness and danger perception) (Cicatiello, Franco, Pancino, & Blasi, 2016; Shelomi, 2015; Sogari, 2015; Sun-waterhouse et al., 2016; Van Huis, 2015). Nonetheless, even in these regions, insect rearing is increasing and more and more insect-based food products are being studied (Fasolato et al., 2018; Osimani et al., 2018b).

The potential benefits of insect consumption include their nutritional properties: most insects are rich in high quality proteins (with an essential amino acid score ranging from 46 to 96%), good quality lipids (rich in alpha-linolenic acid and linoleic acid), vitamins, minerals (such as calcium, iron, and zinc) and fiber (due to the presence of chitin) (Belluco et al., 2013; Mlcek et al., 2014; Payne et al., 2016; Rumpold & Schlüter, 2013; Schlüter et al., 2017; Sun-waterhouse et al., 2016). These nutritional benefits are well-known and pursued in developing countries, where for a long time, the combination of the richest availability of edible insects and the presence of malnutrition have determined their largely diffused consumption (Garofalo et al., 2017; Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). However, it is important to highlight that the nutritional value of insects varies significantly among species and development stages. Apart from nutritional qualities, the rearing of insects for food is also linked to potential economic, social, and ecological proceeds. Harvesting or farming insects can offer new employment and income-earning opportunities, including some of the poorest segments of society (FAO, 2014). Insect breeding seems to have a lower ecological footprint than livestock production: insects multiply faster, cause lower GHG and ammonia emissions, require less land area and water use, have higher feed conversion efficiency, and can potentially be bred on organic by-products (Garofalo et al., 2017; Klunder et al., 2012; Van Huis, 2015).

Owing to the same properties, insects, like many food products that are rich in nutrients and moisture, also provide a favorable environment for microbial survival and growth (EFSA, 2015; Garofalo et al., 2017; Klunder et al., 2012; Stoops et al., 2016; Van Huis, 2015). Since, as of January 2018, insects are considered as categories of food to which the European Union legislation on novel foods (Regulation (EU) 2015/2283 on novel foods) is applied, more data on the microbial, chemical, and physical safety of edible insects reared or imported in Europe are necessary (European Union, 2015). Despite this, it is important to highlight that insects are not allowed for human consumption in all the European countries. Furthermore, additional microbial hazard data are needed to set up microbiological criteria for edible insects in future, as for other food products (Regulation (EC) N° 2073/2005 on microbiological criteria for foodstuffs) (Belluco et al., 2013; European Commission, 2005; Vandeweyer et al., 2017b). Moreover, little is known about the potential risks derived from the breeding systems, as well as those potentially derived from the transformation processes (in particular physical and allergenic risks for food chain operators and consumers). In fact, this systematic review of the existing bibliography highlights the lack of scientific articles and reviews

regarding machines, plants, and integrated supply chain approaches regarding the production chain of insects for food, thus motivating this work. For this reason, the aim of this review is to summarize the current knowledge related to the potential microbial, chemical, physical, and allergenic risks related to the breeding, transformation, and consumption of edible insects for food.

2. Search strategy

The review considers the EFSA scientific opinion (EFSA, 2015) as the starting point, which is why the years from 2016 onwards (till begin of 2019 included) have been chosen as a time frame. Three databases were explored by means of ad hoc search strings: Science direct, PubMed, and Web of Science. For the microbiological, chemical, and physical risks, a unique search string was used for all the databases, while for the risk related to allergenicity, two different search strings were used: one for Science direct and one for PubMed and Web of Science. The search strings used are as follows:

- “Food AND (Insect* OR “novel food*” OR mealworm* OR cricket* OR entomophagy OR “edible insect*”) AND (microbiota OR “microb* community” OR “microb* count*” OR “microb* load” OR “microb* risk” OR “microb* hazard” OR “microb* saf*” OR “food safety*”)” for microbiological risk,
- “Food AND (Insect* OR “novel food*” OR mealworm* OR cricket* OR entomophagy OR “edible insect*”) AND (“chemical risk*” OR “chemical hazard*” OR “chemical safety” OR radionuclide* OR arsenic OR cadmium OR copper OR zinc OR chrome OR toxin*)” for chemical risk,
- “Food AND (Insect* OR “novel food*” OR mealworm* OR cricket* OR entomophagy OR “edible insect*”) AND (“physical risk*” OR “physical hazard*” OR “physical safety” OR “foreign bod*” OR “breeding substrate*” OR vegetable* OR ran OR flour*)” for physical risk,
- “Food AND (Insect* OR “novel food*” OR mealworm* OR cricket* OR entomophagy OR “edible insect*”) AND (allergen*)” for allergenicity risk on Science direct and “Food AND (Insect* OR “novel food*” OR mealworm* OR cricket* OR entomophagy OR “edible insect*”) AND (allerg*)” on PubMed and Web of Science.

All articles considering insects belonging to the families of *Tenebrio molitor* and *Acheta domesticus*, Tenebrionidae and Gryllidae, respectively, were included. Among all insects species, these families are seen as more suitable for consumption and processing (Marberg et al., 2017). No language restriction was imposed. No restriction regarding the publication status was enforced. All duplications have been excluded from the obtained results. In addition to the studies that mainly deal with the types of risk mentioned above, those in which they are treated in a non-exclusive but exhaustive manner were also included. However, despite the EFSA report (EFSA, 2015) mentioned other kinds of risk (such as hormones), according to the obtained results the authors decided to discuss only microbiological, chemical, physical, and allergenic risks, to which a great amount of literature was found. The search results were first screened by title and abstract reading (excluding, at this step, the articles in the form of abstract and/or index), and then by full text reading. For each of the four types of risk investigated, a flow chart was produced to synthesize the obtained results, which were subsequently summarized in Table 1 (for reviews and book chapters) and Table 2 (for articles).

3. Main findings

3.1. Results of the systematic review

A total of 4593 results were obtained: 1871 for microbiological risk, 639 for chemical risk, 660 for physical risk and 1423 for allergenicity

Table 1
Reviews and book chapters collected with the systematic review.

Author and year	Risk type	Main results
De Castro R. J. S. et al., 2018	Microbiological Allergenic	Enterobacteriaceae and sporulating bacteria were isolated in fresh insects. No pathogen was found. Boiling eliminates the Enterobacteriaceae but not sporulating bacteria. Roasting cannot eliminate Enterobacteriaceae. Arthropods can induce allergic reactions. 238 allergens present in arthropods have been identified (few processes can reduce their allergenicity).
Walia K. et al., 2018	Microbiological	If porridge fortified with cricket powder is boiled prior to consumption, the food safety risk is low for <i>S. aureus</i> ; low to moderate for <i>B. cereus</i> , <i>C. perfringens</i> Type A, <i>C. sakazakii</i> , enterohemorrhagic <i>E. coli</i> , <i>L. monocytogenes</i> , and <i>Salmonella</i> spp., and moderate to serious for <i>C. Perfringens</i> Type C. No outbreaks of <i>B. cereus</i> , <i>C. perfringens</i> , <i>C. sakazakii</i> , <i>L. monocytogenes</i> and <i>Salmonella</i> spp. were recorded. No <i>E. coli</i> , was isolated from cricket powder.
Kouřimská L. & Adámková A., 2016	Microbiological Chemical Allergenic	Insects may produce or contain toxic bioactive compounds, residues of pesticides and heavy metals (e.g. cadmium and lead). The consumption of insects can lead to allergic reactions due to the presence of chitin. If insects are not subjected to fasting, heat treatment, and storage, they can become dangerous from a microbiological point of view.
Marshall D. L. et al., 2016	Microbiological Chemical Physical	Gut of house cricket contains <i>Citrobacter</i> , <i>Klebsiella</i> , <i>Yersinia</i> , <i>Bacteroides</i> , and <i>Fusobacterium</i> . The yellow mealworm hosts <i>Actinobacillus</i> , <i>Propionibacterium</i> , <i>Citrobacter</i> , <i>Serratia</i> , <i>Bacillus</i> , <i>Dermabacter</i> , <i>Brachybacterium</i> , <i>Clavibacter</i> , and <i>Exiguobacterium</i> . Appropriate breeding techniques must be used to control the pathogens dangerous to humans.
Grau T. et al., 2017	Microbiological Chemical Allergenic	Mealworms are not reported to contain pathogens (<i>L. monocytogenes</i> and <i>Salmonella</i> spp.). A short heating treatment or blanching significantly reduces total bacterial counts and enterobacteria counts. No documented evidence of infections during the mass rearing of mealworms, however, this risk cannot be excluded due to similar situations occurring in other insects such as house cricket. The accumulation of hazardous chemicals originating from contaminated insect diets can occur. Regarding the risk of allergies, mealworm proteins cross-react in vitro with IgE in response to tropomyosin. Mealworm allergy can occur in shrimp-allergic patients. A heating treatment and in vitro digestion reduce the allergic reaction but do not eliminate it completely.
Schlüter O. et al., 2016	Microbiological Chemical Allergenic	The transmission of microorganisms can occur mechanically (through contact) or if they resist and multiply inside the insect. Toxins and antinutrients can be absorbed or synthesized by the insect. The cross-reactivity effect of tropomyosin has been confirmed. Tropomyosin and arginine kinase have been identified as cross-reactive proteins. People allergic to crustaceans and dust mites can manifest allergic reactions.
Van der Fels-Klerx H. J. et al., 2018	Microbiological Chemical Allergenic	Potential accumulation of chemical contaminants depends on the type of insect and the growth phase. The substrate and the farming environment strongly influence the microflora and the contamination of insects. Insects are characterized by high counts of total vital bacteria and Enterobacteriaceae. A suitable heating treatment can eliminate a good part of microbiological risk (except for bacterial spores). The pan-allergen tropomyosin was identified in mealworm. Arginine kinase and α -amylase play a role in cross-reactivity between crustaceans and insects.
Fernandez-Cassi X. et al., 2018	Microbiological Chemical Allergenic	The minor risks deriving from the consumption of <i>Acheta domesticus</i> are those related to viruses, prions, fungi, and parasites. The medium risks are high microbial counts, sporulating bacteria, and their growth after heat treatment, bioaccumulation of heavy metals (especially cadmium) and allergenicity. No pathogenic microorganisms such as <i>L. monocytogenes</i> or <i>Salmonella</i> spp. were found; however, species belonging to the genera <i>Clostridium</i> spp., <i>Listeria</i> spp., and <i>Bacillus</i> spp. have been detected.
Grabowski N. T. & Klein G., 2017b	Microbiological	Insects can act as carriers for human pathogens. The exclusion of clinically diseased specimens, hygienic obtention, washing, and thorough heating seem suitable procedures to reduce the risk of food-borne diseases by bacteria. Raw consumption of insects should be evaluated thoroughly. The phyla most present in the intestinal microbiota of insects are <i>Proteobacteria</i> and <i>Firmicutes</i> .
Grabowski N. T. et al., 2017a	Microbiological	Many fungi associated with arthropods do not survive in humans due to body temperature. The prevalence of pathogenic fungi for humans varies from 0 (dragonflies, orthoptera, termites, hemiptera, cockroaches, ephemeral and flies) to above 4 (hemiptera) and 7 (hymenoptera) to 17 (moths) and 71% (cockroaches).
Sun-Waterhouse D. et al., 2016	Chemical Physical Allergenic	Physical risks such as glass, stones, plastics, and metal fragments can be present in insects. Chemical contaminants could be naturally present, synthesized, accumulated or generated during the production process. Some insects may contain cross-reactive allergens: proteins (such as tropomyosin), carbohydrates (such as chitin) and other components that are yet to be identified. Arginine kinases, myosin heavy chains, myosin light chain kinases, hemocyanin, amylase, and sarcoplasmic calcium-binding proteins are other allergens that may be present in edible insects.
Gallo, 2018	Chemical Allergenic	The different chemical contaminants that can contaminate insects are heavy metals, veterinary drugs, hormones, pesticides, toxins, and mycotoxins. Insect consumption can lead to the development of allergic reactions in people allergic to crustaceans, because of the morphological similarity between them and insects.
Liu & Zhao, 2018	Chemical Physical	Physical and chemical risks can occur in insects. Many poisonous substances could be present in Tenebrionidae cockroaches. Some chemical risks can be removed or deactivated by cooking or digestion. Antinutrients of plant origin found in feed can be accumulated in insects.
Varelas & Langton, 2017	Chemical Physical Allergenic	Chemical hazards include substances that are toxic to insects, antibiotics, contaminants and anti-nutrients. Physical hazards derive from parts of insects or from foreign materials, while allergens are associated with enzymes and allergenic substances present in insects.
Van Huis, 2017	Chemical	Yellow mealworms reared on a mycotoxin-contaminated diet (mainly deoxynivalenol) are able to partially excrete them through faeces in an unmetabolized form and the remaining part as metabolized in different compounds.
Marone P. A., 2016	Chemical Physical	Insects can bioaccumulate chemical residues and heavy metals, and produce harmful substances. Insects can contain anti-nutrients that interfere with physiological processes. The major physical risk is suffocation, so insect powder is more suitable and is considered safe compared to the raw and whole insect.
Van Huis A. & Dunkel F. V., 2017	Allergenic	

(continued on next page)

Table 1 (continued)

Author and year	Risk type	Main results
Pali-Schöll I. et al., 2018b	Allergenic	Cross-reaction between insects and crustacean is possible. Arginine kinase and tropomyosin were found as the major cross-reactive proteins in yellow mealworm. Patients allergic to dust mites and crustaceans can react to foods containing proteins of the yellow mealworm.
Downs M. et al., 2016	Allergenic	Edible insects can cause allergic cross-reactions in patients allergic to shrimp or dust mites. Pan-allergen identified in arthropods are tropomyosin and arginine kinase. Breeders of yellow mealworm developed a primary sensitization to this insect that evolved in food allergy.
De Gier & Verhoeckx, 2018	Allergenic	The most relevant cross-reactivity is between insects and crustaceans. The protein hydrolysis and heating treatments, can reduce allergic reactions.
Van der Spiegel, 2016	Allergenic	Cases of primary allergy to insects and cross-reactivity (related to tropomyosin and arginine kinase) are reported. Heat treatment can lead to a reduction, increase, or no change in the allergenicity. Digestion can also have an effect on allergenicity.
Van Huis, 2016	Allergenic	The consumption of insects can lead to allergic reactions due to cross-reactivity of insect proteins with shrimps and dust mites. An allergen present in the exoskeleton is chitin. Insects can carry anti-nutritional factors with them, but they are minimized or eliminated by cooking.
Pali-Schöll I. et al., 2018a	Allergenic	Insects can generate allergic reactions due to cross-reactivity with the phylum of arthropods. Frying might alter the allergenicity of edible insects.
Ribeiro J. C et al., 2018	Allergenic	A long-term exposure of insect breeders leads to respiratory sensitivity. The potential cross-reactivity has been highlighted in patients allergic to crustaceans and dust mites due to the consumption of mealworm or grasshopper.
		In this systematic review, 25 articles were revised: eight studied the cross-reactivity or co-sensitization between edible insects, crustaceans, and house dust mite, three identified and characterized allergens of edible insects, 10 were cases reports or case series of food allergy caused by insects, three assessed the frequency of anaphylaxis caused by entomophagy, and one assessed the prevalence of allergy among insect-eaters. Many studies report the cross reactivity of the yellow mealworm with crustaceans and dust mites. The heating treatment changes the solubility of mealworms allergens. Boiling or in vitro digestion does not significantly alter the ability of tropomyosin to bind IgE contrary to frying.

risk. After removing duplicates within each database, screening by title and abstract reading, screening by full text reading, and removing duplicates between the three databases, 77 were selected as the sum of the results of all the search strings. By removing the articles in common between the results of the different search strings, the items selected were brought down to 64. Of these 64, two were systematic reviews, 14 were other forms of review, eight were book chapters, one was an epidemiological retrospective cohort study, and 38 were descriptive studies by laboratory researchers (32 on contaminants in insect samples and six on patients' sera). Flow charts developed according to PRISMA statement (Moher, Liberati, Tetzlaff, & Altman, 2009) related to microbiological (Fig. 1a), chemical (Fig. 1b), physical (Fig. 1c), and allergenic risks (Fig. 1d) are shown in Fig. 1. Reviews and book chapters are listed in Table 1 and search articles are listed in Table 2.

3.2. Microbiological risks

The bacterial communities found in mealworms are composed of *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Tenericutes* (Garofalo et al., 2017; Yang et al., 2018), while those present in house crickets (both in whole and in powder form) are *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* (Garofalo et al., 2017). In a study by Garofalo et al. (2017), the microbiological profile of certain insect species has been examined, including mealworm and house cricket. The total mesophilic aerobic count was the highest in powdered cricket ($4.80 \pm 0.06 \log \text{cfu g}^{-1}$) and the lowest in mealworm larvae ($< 2.00 \log \text{cfu g}^{-1}$). Whole crickets were characterized by the higher Lactic Acid Bacteria (LAB) counts, while the lowest values were recorded in mealworm larvae and powdered crickets. Yeast counts were the highest in whole crickets and molds were the highest in powdered crickets and lowest in whole crickets. The Enterobacteriaceae count varied in the selected studies: high in some (Osimani et al., 2018a) while in others low, and, in two cases, below the limit of detection (Fasolato et al., 2018; Garofalo et al., 2017). However, a heat treatment (e.g. blanching or boiling) reduces the Enterobacteriaceae count without sporulating bacteria and bacterial spores (De Castro, Ohara, Aguilar, dos, & Domingues, 2018; Gallo, 2018; Liu & Zhao, 2018; Van der Fels-Klerx, Camenzuli, Belluco, Meijer, & Ricci, 2018; Van Huis, 2016; Van Huis & Dunkel, 2017). *Salmonella* spp. and *Listeria monocytogenes* were not detected in any of the selected

studies (Garofalo et al., 2017; Grabowski & Klein, 2017a; Osimani et al., 2018a, 2017b; Vandeweyer et al., 2016; Vandeweyer et al., 2018; Wynants et al., 2018). Despite the latter two were not detected in the selected studies, this does not mean that insects are safe and free from these pathogens. In fact, their monitoring still remains important for consumers' safety.

Vandeweyer et al. (2017b) studied the effect of refrigerated storage and microwave drying, after a blanching treatment, on microbial load in yellow mealworm larvae. It was observed that, except for spores, blanching leads to a significant reduction of all microbial counts at any time interval. Except for one batch (in which a slight growth of mesophilic and psychrotrophic organisms was observed), no microbial growth occurred during refrigerated storage. After blanching, the chilled conditions allowed mealworms to be stored for at least six days without spoilage, as the numbers remained well below the level considered for food spoilage ($7 \log \text{cfu/g}$). Long drying times after blanching led to large reductions in total counts until a plateau was reached. Drying after blanching resulted in Enterobacteriaceae, LAB, yeast and molds counts below or very close to the detection limit. For bacterial endospore counts, blanching and drying resulted in lower reductions (between 0.3 and $1.0 \log \text{cfu/g}$).

Regarding the fungal biota, Vandeweyer et al. (2016) report a difference between mealworms and crickets: molds were dominant in mealworm larvae and yeasts in crickets. Fungi present in feed may contaminate insects and the major risk concerns those able to produce mycotoxins as *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*, as insects are able to metabolize and accumulate these compounds (Van der Spiegel, 2016). However, good hygiene procedures during the production chain can reduce the risk of fungi in insects (Gallo, 2018). Moreover, a blanching treatment significantly reduces the presence of fungi (Vandeweyer et al., 2017b; (Wynants et al., 2018); Grabowski, Pinggen, Grootaert, & Klein, 2017).

Viruses that are pathogenic for insects are not a risk for humans because they are specific to invertebrates and because of the genetic difference between human and insect. However, although there is no evidence of pathogenic viruses in insect, they may be able to act as mechanical vectors for human pathogenic viruses (Gallo, 2018; Van der Fels-Klerx et al., 2018). EFSA (EFSA, 2015) reports that lower risks regarding house cricket consumption are those related to viruses,

Table 2
Research articles collected with the systematic review.

Author, year and country	Aim	Type of risk	Main results
Yang S. S. et al., 2018 (USA, China, UK)	To investigate the ability of <i>Tenebrio molitor</i> larvae to digest and biodegrade polystyrene.	Microbiological	Significant taxonomic shifts for mealworms fed with diets of polystyrene plus bran and polystyrene alone. The 99% abundance in the measured bacterial communities were <i>Actinobacteria</i> , <i>Firmicutes</i> , <i>Proteobacteria</i> and <i>Tenericutes</i> . The six most abundant families were Bacillaceae, Enterobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, and Streptococcaceae.
Stoops J. et al., 2017 (Belgium)	To investigate the effect of the production process and the storage conditions (air, 60% CO ₂ /40% N ₂) on the microbial and bacterial counts of a minced meat-like product from yellow mealworm larvae	Microbiological	Fresh living mealworm larvae contain a high number of microorganisms. Both production methods affected the microbiota of the finished products. Modified atmosphere during storage reduces bacterial growth compared to storage in air. The initial load of bacterial spores of both product types remained constant during the entire storage period. After 35 days of storage in air, a statistically significant decrease in OTU richness was noticed for both product types.
Garofalo C. et al., 2017 (Italy)	Assessing the microbial species in some processed marketed edible insects (powdered and whole), through classical microbiological analyses and pyrosequencing.	Microbiological	Relatively low counts of total mesophilic aerobes, Enterobacteriaceae, lactic acid bacteria, <i>Clostridium perfringens</i> spores, yeasts, and molds were found. Food spoilage bacteria were also identified, as well as <i>Spiroplasma</i> spp. in mealworm larvae. <i>Salmonella</i> and <i>L. monocytogenes</i> were not detected. The presence of <i>Listeria</i> spp., <i>Staphylococcus</i> spp., <i>Clostridium</i> spp., and <i>Bacillus</i> spp. was also found through pyrosequencing.
Ong S. Y. et al., 2018 (Malaysia, Germany, Japan)	Recovering PHA from <i>Cupriavidus necator</i> , incorporating the use <i>Tenebrio molitor</i> as partial purification machinery, followed by washing on the fecal pellets with distilled water and sodium hydroxide.	Microbiological	In the gut of mealworms fed with wheat bran and freeze-dried cells a remarkably high relative abundance of <i>Firmicute</i> –89% and 86% respectively. In guts of the mealworms fed with freeze-dried cells, it was as high as 55% of <i>Proteobacteria</i> dominated the gut. The gut of mealworms fed with cells produced from waste animal fats was dominated by <i>Bacillus</i> , the gut of mealworms fed with cells produced from palm olein was dominated by Oxalobacteraceae and the gut of mealworm fed with wheat bran by <i>Enterococcus</i> .
Osimani A. et al., 2017a (Italy)	Assessing the resistance to tetracyclines, macrolide-lincosamide-streptogramin B, vancomycin, beta-lactams, and aminoglycosides in 40 samples of <i>Tenebrio molitor</i> .	Microbiological	Spore-forming bacteria have been detected in both fresh and processed mealworms. Regarding total mesophilic aerobes, mean counts ranged between < 1 and 6.73 log cfu/g. For spore-forming bacteria, viable counts were between < 1 and 4.24 log cfu/g. For lactic acid bacteria, viable counts varied between < 1 and 2.99 log cfu/g.
Osimani A. et al., 2018a (Italy)	To study the bacterial biota characterizing a pilot production chain of fresh <i>Tenebrio molitor</i> larvae.	Microbiological	Larvae and frass were characterized by high loads of Enterobacteriaceae, lactic acid bacteria, and several species of mesophilic aerobes. Spore-forming bacteria were detected to a lesser extent. The combined molecular approach confirmed the low microbial contamination of wheatmeal and allowed the detection of <i>Enterobacter</i> spp., <i>Erwinia</i> spp., <i>Enterococcus</i> spp., and <i>Lactococcus</i> spp. as dominant genera in both larvae and frass. <i>Entomoplasmatales</i> constituted a major fraction of the microbiota of one batch of larvae. From the real-time PCR assays, no sample was positive for either <i>C. burnetii</i> or STEC, whereas <i>P. aeruginosa</i> was detected in one sample of frass. No <i>Salmonella</i> spp. or <i>Listeria monocytogenes</i> were detected.
Osimani et al., 2018b (Italy)	To evaluate cricket (<i>Acheta domesticus</i>) powder in bread-making to obtain bread with enhanced nutritional value.	Microbiological	Cricket powder was characterized by high counts of both LAB and spore-forming bacteria that attested at up to 3.8 log cfu/g and 5 log cfu/g, respectively. Yeasts counts were < 1 log cfu/g.
Wynants E. et al., 2018 (Netherlands)	To characterize the microbial dynamics in the insect, insect feed and substrate, during a production cycle of lesser mealworms (<i>Alphitobius diaperinus</i>)	Microbiological	Enterobacteriaceae, LAB, and fungi dropped significantly to close to or below the detection limit with blanching treatment, except for aerobic endospores. The starvation treatment did not significantly alter the microbial count. <i>Salmonella</i> , <i>L. monocytogenes</i> , <i>Bacillus cereus</i> , and coagulase-positive staphylococci were not detected. Fungal isolates corresponding to the genera <i>Aspergillus</i> and <i>Fusarium</i> were recovered.
Fasolato L. et al., 2018 (Italy)	To investigate the microbial traits of insects available online (mealworms, crickets, mole crickets and silkworms).	Microbiological	Quantities of <i>Staphylococcus</i> , molds, yeasts, LAB, and Enterobacteriaceae was predominantly below the limits of detection. The characterization of strains from the <i>Bacillus cereus</i> group, 17% of the isolated strains presented nine of the 10 toxin targets.
Wynants E. et al., 2017 (Belgium)	To assess the effect on the microbiota of starvation and rinsing at the end of the rearing period of <i>Tenebrio molitor</i> .	Microbiological	The results obtained do not show considerable changes in microbial numbers of mealworm larvae during the starvation period. Metagenetic analyses show an average

(continued on next page)

Table 2 (continued)

Author, year and country	Aim	Type of risk	Main results
Vandeweyer D. et al., 2017b (Belgium)	Study the effect of blanching, followed by chilled storage or microwave drying, on microbial counts of the larvae.	Microbiological	number of OTUs from 21 to 57 per sample. The phyla mainly present were Proteobacteria and Firmicutes. Rinsing and starvation did not have any influence on the microbial counts of larvae. For all total counts, log reductions of 4.4, 6.4 and 5.6 log cycles were observed for blanching times of 10, 20 and 40 s respectively. Long drying times caused strong reductions on total count, until a plateau was reached after 20 min of drying. No major growth was observed during subsequent chilled storage.
Borremans A. et al., 2018 (Belgium)	To apply marination and fermentation processes on larvae of the yellow mealworm <i>Tenebrio molitor</i> .	Microbiological	Marination can increase the shelf life of this products up to seven days. Regarding fermentation, a microbial growth was observed only at the seventh day of fermentation. LAB were the major part of the bacterial community (the other microbial counts were low).
Vandeweyer D. et al., 2016 (Belgium)	To generate an overview of intrinsic parameters (pH, water activity and moisture content) and microbial quality of fresh mealworm larvae and crickets.	Microbiological	The microbial counts of the fresh insects were high. The largest variations were found in numbers of bacterial endospores, psychrotrophs, and fungi. <i>Salmonella</i> spp. and <i>L. monocytogenes</i> were not detected in batches from individual breeders. Comparing mealworms and crickets, mealworms were dominated by molds and crickets by yeasts.
Milanović V. et al., 2016 (Italy)	To investigate the occurrence of 11 transferable antibiotic resistance genes in 11 species of marketed edible insects in order to provide a first baseline for risk assessment.	Microbiological	Microbial counts of total mesophilic aerobes showed a wide variability among samples (10^4 – 10^7 cfu/g). The high microbial load suggest the survival of spore-forming bacteria, due to ineffective boiling or cross-contamination. Tet(K), erm(B), tet(S), and blaZ were the most frequent antibiotic resistance genes, suggesting that insect's consumption might be related to a significant risk.
Li L. et al., 2016 (China)	To study the effects of rearing yellow mealworm (<i>Tenebrio molitor</i> L.) in a closed artificial ecosystem on their associated microbial communities.	Microbiological	Samples from the open environment had higher abundances of <i>Bacillus</i> , <i>Lactococcus</i> , <i>Weissella</i> , <i>Escherichia</i> , and Clostridiaceae, compared with the three samples from the closed ecosystem. In samples from the closed ecosystem <i>Enterococcus</i> and some unclassified Enterobacteriaceae were identified as the predominant bacteria. Some classes that were found in the feed disappeared in the samples of both groups.
Buřler S. et al., 2016b (Germany)	To investigate the protein extractability and techno-functionality of insect flour fractions recovered from <i>Tenebrio molitor</i> and <i>Hermetia illucens</i> .	Microbiological	<i>Tenebrio molitor</i> larvae was highly contaminated with microorganisms. Pureeing, freeze-drying, and grinding of the larvae at low temperatures were found to be inappropriate for production of microbiologically safe insect fractions. Extreme pH conditions apparently do not lead to a reduction in microorganisms but only to a mild effect.
Caparros Megido R. et al., 2018 (Belgium)	To characterize the effects of different cooking methods (boiling, pan-frying, vacuum cooking and oven cooking) on the microbial load and nutritive value of mealworms	Microbiological	Boiling and cooking under vacuum were the most efficient techniques to reduce microbial load while maintaining the high levels of protein and polyunsaturated fatty acids of mealworms. Total aerobic count was higher in crude larvae, followed by larvae cooked in oven, larvae cooked under vacuum, larvae subjected to pan-frying, and larvae subjected to boiling.
Caparros Megido R. et al., 2017 (Belgium)	To characterize the microbial load of edible insects found in Belgium and to evaluate the efficiency of different processing methods in reducing microorganism counts.	Microbiological	Blanching step has led to a reduction of the total aerobic count for all insect species, except for one caterpillar species. Freeze-drying and sterilization treatments were effective in reducing the total aerobic count. Sterilization resulted the most efficient treatment than blanching and freeze-drying.
Grabowski N. T. & Klein G., 2016 (Germany)	To study different drying techniques on cooked edible insects in relation to microbiological quality.	Microbiological	T3 was the most effective of the drying treatments, excepted Bacilli for which T2 was more efficient. Treatment T1 was the less efficient treatment. In cricket, <i>Staphylococci</i> count was in the same range for T1 and T2 and different from T3. Regarding the larva, each treatment was characterized by a different range of <i>Staphylococci</i> count. <i>Bacillus cereus</i> was not isolated in any sample. Treatment T4 resulted the most efficient treatment in the reduction of microbial count.
Grabowski N. T. & Klein G., 2017a (Germany)	To analyze processed insect products using classical food microbiology methods.	Microbiological	All samples were free of <i>salmonellae</i> , <i>L. monocytogenes</i> , <i>E. coli</i> and <i>S. aureus</i> . Dried and powdered insects contained <i>B. cereus</i> , <i>coliforms</i> , <i>Serratia liquefaciens</i> , <i>Listeria ivanovii</i> , <i>Mucor</i> spp., <i>Aspergillus</i> spp., and <i>Cryptococcus neoformans</i> . Three cricket samples and two mealworm samples did not respect the process hygiene criteria imposed by Belgium and the Netherlands.
Stoops J. et al., 2016 (Belgium)		Microbiological	The most abundant operational taxonomic unit in mealworm larvae was <i>Propionibacterium</i> . <i>Proteobacteria</i> ,

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Table 2 (continued)

Author, year and country	Aim	Type of risk	Main results
	To characterize the microflora of fresh edible mealworm larvae and grasshoppers in a quantitative and qualitative way.		<i>Firmicutes</i> , and <i>Actinobacteria</i> were found as dominant phyla in yellow mealworm. Regarding the structure of bacterial community, in the yellow mealworm a total of 50 different OTUs was found, the most abundant OUT was that of <i>Propionibacterium</i> sp.
Vandeweyer D. et al., 2018 (Belgium)	To investigate the microbial dynamics, from industrial rearing to processing and storage, of the tropical house cricket reared for human consumption.	Microbiological	None of the investigated food pathogens were found. Some possible mycotoxin-producing fungi were isolated from the crickets. A postharvest heat treatment reduced microbial numbers, but an endospore load of 2.4 log cfu/g remained. An increase in microbial counts was observed for the dried and smoked/dried crickets. The microbial numbers remained constant over a six-month storage period, whether frozen or at ambient temperature.
Vandeweyer D. et al., 2017a (Belgium)	To increase the knowledge on the microbial quality of edible insects by comparing the bacterial community composition of mealworms and crickets.	Microbiological	The bacterial community composition of the investigated crickets was similar among different companies and between both crickets species. Mealworms communities were dominated by <i>Spiroplasma</i> and <i>Erwinia</i> species, while crickets were abundantly colonized by (<i>Para</i>) <i>bacteroides</i> species. Only a few OTUs could be associated with potential human pathogens.
Osimani A. et al., 2017b (Italy)	To provide insight into the proximate composition and microbial diversity of some processed edible insects marketed in the European Union.	Microbiological	It was seen a great variation in microbial counts. <i>L. monocytogenes</i> and <i>Salmonella</i> spp. were not detected. Cricket powder was characterized by significantly higher viable counts for all microorganisms investigated. It was seen a great microbial diversity in quantitative and qualitative terms among all products analyzed.
Bužler S. et al., 2016a (Germany)	To investigate the possible use of the CAPP technology for the decontamination and modification of flour produced from mealworms.	Microbiological	Following CAPP treatment, the total microbial load of <i>Tenebrio</i> flour of 7.72 log ₁₀ cfu/g was reduced to 7.10 (1 min), 6.72 (2.5 min), 5.79 (5 min), 5.19 (7.5 min), 5.21 (10 min) and 4.73 (15 min) log ₁₀ cfu/g. In conclusion, the CAPP treatment seems to be effective for the decontamination of mealworm flours.
Han. S. R et al., 2016 (Korea)	To evaluate subchronic toxicity, including potential hypersensitivity, of freeze-dried powdered <i>T. molitor</i> larvae in male and female Sprague-Dawley rats.	Chemical + Allergenic	The administration of <i>Tenebrio molitor</i> larvae freeze-dried did not lead to adverse or toxic effects in rats, including allergologic evaluation.
Poma G. et al., 2017 (Belgium)	To provide a comprehensive overview of the residual levels of different chemical compounds, in insects and insect-based food intended for human consumption, and to compare these levels to those measured in common animal products.	Chemical	In the two larvae were found PCBs levels of 26.5 and 33.8 pg/g ww, whereas in insect-based products values were higher. PBDEs levels were low. The untargeted screening analysis revealed the presence of vinyltoluene, tributylphosphate and pirimiphos-methyl. The levels of Cu and Zn in insects were similar to those measured in meat and fish in other studies, whereas As, Co, Cr, Pb, Sn levels were relatively low in all samples. Results suggest the possibility to consume these insect species with no additional hazards in comparison to the more commonly consumed animal products.
Van der Fels-Klerx H. J. et al., 2016 (The Netherlands)	To investigate the accumulation of cadmium, lead and arsenic provided to larvae of <i>Tenebrio molitor</i> and <i>Hermetia illucens</i> , using contaminated feed.	Chemical	Bioaccumulation was seen in all treatments in <i>Hermetia illucens</i> larvae and for three arsenic treatments in <i>Tenebrio molitor</i> larvae. Development of <i>Tenebrio molitor</i> larvae was not influenced by the treatment applied. Results showed that yellow mealworm larvae can accumulate arsenic in the body, instead of cadmium and lead, of which concentrations were found higher in feed.
Camenzuli L. et al., 2018 (Belgium, The Netherlands)	To investigate the potential accumulation of mycotoxins in the lesser mealworm (<i>Alphitobius diaperinus</i>) and black soldier fly (<i>Hermetia illucens</i>) larvae.	Chemical	No differences were observed between larvae reared on mycotoxins individually or as a mixture with regard to either larvae development or mycotoxin accumulation/excretion. For all applied treatments, the concentration values of mycotoxins and their metabolites in <i>Alphitobius diaperinus</i> larvae were below the detection limit.
Bednarska A. J. & Świątek Z., 2016 (Poland)	To compare the sequestration processes of cadmium and zinc in <i>Tenebrio molitor</i> larvae and to assess the potential availability of the metals to higher trophic levels.	Chemical	The concentration of cadmium in the flour affected the concentration of cadmium in the larval subcellular fraction, whereas the concentration of zinc only affected the zinc concentration in S2 and G fractions. Larvae sequestered approximately 30% of cadmium and zinc in the S1 fraction, which is important for the transport of metals to higher trophic levels.
Chomchai & Chomchai, 2018 (Thailand)	To investigate the connection between histamine poisoning and insect consumption.	Chemical	Out of 227 students, 18 developed illness, which included flushing, pruritus, urticarial rashes, headache, nausea, vomiting, diarrhea, dyspnea and bronchospasm. Three students begin to develop the symptoms within 1 h and half after ingestion, the other 25 after 2–7 h. The highest attack rate was associated with the consumption of locusts and silkworms. Histamine poisoning can therefore also occur with the consumption of insects.

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Table 2 (continued)

Author, year and country	Aim	Type of risk	Main results
Bosch G. et al., 2017 (The Netherlands)	To evaluate the tolerance and accumulation of aflatoxin B1 in two insect species to determine whether they could be used to retain condemned mycotoxin contaminated crops in the food chain.		Both insects tolerated the presence of aflatoxin B1 in feed up to the highest level of 0.415 mg/kg. Feed contaminated with aflatoxin B1 did not affect survival and body weight in both insects, indicating a high tolerance. The residues of yellow mealworm contained aflatoxin M1 when larvae were fed with levels of aflatoxin B1 of 0.023, 0.084 mg/kg or more, with levels of aflatoxin B1 excreted from 0.9 to 1.7%. This suggests that aflatoxin B1 was partially transformed in the yellow mealworm in aflatoxin M1.
Van Broekhoven et al., 2017 (The Netherlands)	To study the degradation and excretion of Fusarium toxin deoxynivalenol in <i>Tenebrio molitor</i> reared with wheat flour naturally contaminated with mycotoxins.	Chemical	No deoxynivalenol derivatives were found in samples. Deoxynivalenol was not present at detectable levels either in the larvae harvested directly or for those held fasting for 24 h. The results show that diets containing deoxynivalenol do not affect the mortality or weight of the larvae, which is partially excreted in the frass and does not remain in an unmetabolized form in the body.
Broekman H. C. H. P. et al., 2017a (The Netherlands)	To address the risk of mealworm sensitization and allergy in subjects without shrimp allergy.	Allergenic	All four subjects had developed an allergy during professional or domestic mealworm breeding. The two professional breeders had inhalant allergy symptoms when they entered the room where they were raised but did not develop symptoms when they ate them (sporadically). A domestic breeder after two years of exposure developed rhinoconjunctivitis that evolved into dyspnea.
Broekman H. et al., 2016 (The Netherlands)	To investigate the potential allergenicity of mealworm in shrimp-allergic patients.	Allergenic	The DBPCFC test confirmed allergy to mealworm in 13 subjects out of 15. The symptoms were oral allergy (7/13), urticaria (6/13), nausea (8/13), abdominal cramps (4/13), vomiting (1/13) and dyspnea (2/13). It has been concluded that allergy to this insect is highly probable shrimp-allergic patients with potentially serious outcome.
Hall F. et al., 2018 (USA)	To evaluate the effect of enzymatic hydrolysis on the bioactive properties of cricket protein hydrolysates before and after simulated gastrointestinal digestion.	Allergenic	All sera reacted to tropomyosin in unhydrolyzed cricket and in protein hydrolysates with a hydrolysis degree of 15–50%. There was no reaction in the case of cricket protein hydrolysates with a degree of hydrolysis from 60 to 85%. Cricket protein hydrolysates had the greatest bioactive potential and less reactivity to tropomyosin than other cricket protein hydrolysates and control.
Francis F. et al., 2019 (Belgium)	To investigate arginine kinase known as allergens mainly from shrimps and to investigate the cross-reactivity of these allergens taken from <i>A. domesticus</i> and <i>T. molitor</i> .	Allergenic	Positive responses were obtained with sera from <i>Acheta domesticus</i> samples for both protein extracts and purified arginine kinase, while a diversity of sample bands from protein extracts from <i>Tenebrio molitor</i> . Regarding arginine kinase, low specific allergenicity was here illustrated and discussed in relation to the development of safe insect consumption by humans.
Van Broekhoven S. et al., 2016 (The Netherlands)	To assess IgE cross-reactivity of protein, isolated from three edible mealworm species, with sera from patients allergic to either crustaceans or house dust mite, and to determine the effect of processing and in vitro digestion of whole mealworms on IgE-reactivity.	Allergenic	Results obtained reveal that IgE from crustaceans and house dust mite allergic patients showed cross-reactivity to mealworm tropomyosin or α -amylase, hexamerin 1B precursor and muscle myosin. The major protein identified to cross-react with IgE from crustacean allergic patients was tropomyosin. Heat processing as well as in vitro digestion did diminish, but not eliminate, house dust mite or tropomyosin IgE cross-reactivity. Tropomyosin allergenicity appeared decreased in fried samples and disappeared in fried in vitro digested samples.
Broekman H. C. H. P. et al., 2017b (The Netherlands, Italy)	To investigate the allergenic potential of edible insects in both the shrimp and primary mealworm allergic population.	Allergenic	The most IgE-binding proteins were tropomyosin and arginine kinase. Subjects with primary mealworm allergy showed sensitization to the tested insect extracts. Tropomyosin and arginine kinase were identified in all insect extracts and many subjects have shown reactivity to these two allergens. From the results obtained, it can be concluded that shrimp-allergic patients are probably at risk not only for food allergy to mealworm but also from other insects.

prions, fungi, and parasites (Fernandez-Cassi et al., 2018). There is no evidence of the presence of parasites in bred insects, but it can be assumed that this risk can be eliminated under controlled breeding

conditions: in fact, in controlled breeding, the elements needed to complete their lifecycle are lacking, and processing (freezing and cooking) can contribute in the elimination of this risk (Gallo, 2018).

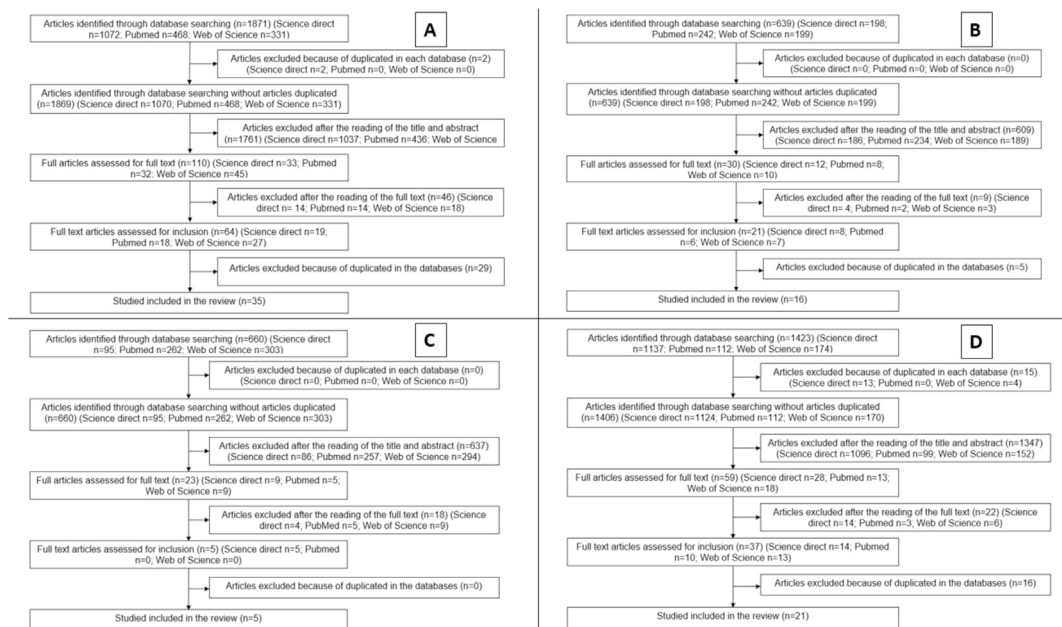


Fig. 1. Flow charts regarding the selection of papers on microbiological (a), chemical (b), physical (c), and allergenic (d) risks obtained according to the results of the systematic review.

The prions of insects have not been described and no diseases caused by them have ever been observed (Grau et al., 2017; Van der Fels-Klerx et al., 2018). The risk related to prions is linked to the substrate: if the substrate is contaminated, the insects may be able to act as mechanical vectors in their transmission to humans (Gallo, 2018; Van der Fels-Klerx et al., 2018; Van der Spiegel, 2016).

3.3. Chemical risks

Chemical contaminants, like toxins, can be absorbed by insects from the feed or the insects themselves can synthesize these compounds (Schlüter et al., 2016). It is reported that darkling beetles, from the Tenebrionidae family, produce quinones and alkanes, but the risk arising from these substances needs to be better investigated (Schlüter et al., 2016). However, it is important to highlight that darkling beetles are not usually consumed as food. In a study by Poma et al. (2017), the levels of different chemical contaminants in different insects and insect products were analyzed. Poly-Chlorinated Biphenyl (PCB) levels were higher in cricket croquettes (1712 pg/g ww) than in *Alphitobius diaperinus*-based food (783 and 156 pg/g ww), *Alphitobius diaperinus* larvae (33.8 pg/g ww) and *Tenebrio molitor* larvae (26.5 pg/g ww). In the products mentioned, the level of Organo-Chlorine Pesticide (OCP) was higher in *Tenebrio molitor* larvae (156 pg/g ww). Poly-Brominated Diphenyl Ether (PBDE) and Halogenated Flame Retardant (HFR) were below the limit of quantification, except for one *Alphitobius diaperinus*-based food (16.2 pg/g ww). A total of six Phosphorous Flame Retardants (PFRs) were detected and their concentration in samples were higher than the other compounds analyzed, which might be due to their use in treatment processes and packaging.

Zinc was the most abundant metal in samples, followed by copper Poma et al. (2017). In a study on contaminated substrates with different heavy metal levels, it was observed that *Tenebrio molitor* larvae can accumulate arsenic but not cadmium and lead (Van der Fels-Klerx, Camenzuli, Van Der Lee, & Ooninx, 2016). Regarding mycotoxin contamination, the results of Bosch, van der Fels-Klerx, De Rijk, and Ooninx (2017) suggest that aflatoxin B1 is rapidly catabolized and excreted by yellow mealworm larvae, either by conversion into aflatoxin M1 or simply as aflatoxin B1. This highlights a significant risk related to bioaccumulation. Moreover, the study reported by Van

Broekhoven, Gutierrez, De Rijk, De Nijs, and Van Loon (2017) shows that *Tenebrio molitor* larvae are able to metabolize deoxynivalenol because the remaining fraction of this compound, i.e. the portion excreted through faeces, has not been detected. Cooking or digestion can remove or deactivate some chemical hazards (Liu & Zhao, 2018). However, collecting insects for the production of food and food ingredients must be done in such a way as to prevent or minimize the accumulation of toxins, drugs, and antinutrients coming from outside (Schlüter et al., 2016). In particular, the most critical steps regard the breeding phase, which is crucial to control the chemical risk, and the transformation process.

3.4. Physical risks

In none of the selected articles is the physical risk treated specifically for yellow mealworm larvae or house cricket. Only general information is given for all insects. Choking is the greatest physical hazard associated with insects consumption: in fact, the insect body is inhomogeneous because it has appendages like spines or cuticles, which make insects a foreign texture to those with which Western palates are accustomed (it is particularly hazardous for elderly people and children, owing to greater difficulty in ingestion) (Marone, 2016). For this reason, powdered insects are considered safer than whole insects and it is also the form that mostly facilitates consumer acceptance (Marone, 2016). The control of physical hazards when the risk is identified as a part or an ingredient of the food, in our case represented by the whole insect or a part of it, is particularly difficult; the best way to prevent it is to follow good manufacturing practices (e.g. IPIFF guide on Good Hygiene Practices) and increase the awareness of employees in controlling all the steps of the food chain (Marshall, Dickson, & Nguyen, 2016). However, according to the degree of risk exposure, the adoption of personal protective equipment (PPE), such as filtering facepiece respirator, could protect the workers during the processing phases.

3.5. Allergenic risks

Based on the number of results obtained and the fact that most of the articles confirm the likelihood of developing food allergy to insects, it can be stated that the risk of allergic reactions related to the

consumption of insects is significant. People allergic to crustaceans and house dust mites can develop allergic reactions to insects due to the close taxonomic relationship between insects and arthropods, which helps to predict allergens (Downs, Johnson, & Zeece, 2016). The major allergens that cause cross-reactivity in insects are tropomyosin and arginine kinase, but there are also other allergens such as sarcoplasmic calcium-binding protein, myosin light chain, troponin C, sarcoplasmic endoreticulum calcium ATPase, hemocyanin, and phospholipase (Downs et al., 2016). In a study by Broekman et al. (2017a), it has been observed that mealworm breeders (both domestic and professional) develop inhalant allergy and food allergy to this insect, and binding IgE were found towards allergens as tropomyosin, arginine kinase, myosin light chains, and myosin heavy chains. In shrimp-allergic patients, mealworm consumption shows different symptoms like oral allergy, urticaria, nausea, abdominal cramping, vomiting, and dyspnea (Broekman et al., 2016). More in general, insect food allergy can occur in different ways (from a mild localized reaction to anaphylactic shock) and symptoms are subdivided into cutaneous, gastrointestinal, and respiratory (De Gier & Verhoeckx, 2018).

Regarding cricket allergenicity, Hall, Johnson, and Liceaga (2018) studied cross-reactivity between human shrimp-allergic sera and tropomyosin in crickets (*Gryllosides sigillatus*) at different degrees of hydrolysis: cricket protein hydrolysates with a degree of hydrolysis of 60–85% had the lowest reactivity to tropomyosin compared to those with a degree of hydrolysis of 15–50% and to the unhydrolyzed cricket. Processing can affect insect allergenicity. In fact, tropomyosin allergenicity seems to be decreased in fried samples and disappeared in fried and in vitro digested samples (Van Broekhoven, Bastiaan-net, de Jong, & Wichers, 2016). Apart from proteins, there is also an allergen of insects among carbohydrates, namely chitin. Chitin is a fiber present in the exoskeleton of insects (also in crustaceans) (Van der Spiegel, 2016). The effect of processing on allergens can be understood from studies devoted to closely related arthropod species, as there is not much information regarding insect-specific allergens (Downs et al., 2016). However, data from recent literature about the effect of thermal treatment are contradictory because highlight that the allergenicity may decrease, increase, or remain the same (Broekman et al., 2015; De Gier & Verhoeckx, 2018; Van Broekhoven et al., 2016). Contradictory results may be explained by the fact that processing changes the solubility of proteins (De Gier & Verhoeckx, 2018). Although digestion has an effect on food allergenicity due to the acidic environment, protein degradation does not always occur completely and sometimes does not occur at all (De Gier & Verhoeckx, 2018).

4. Discussion

From the selected studies chosen by means of the systematic process of review emerges the exigence of developing machines and processes to reduce the microbiological, chemical, physical, and allergenic risks related to emerging production chain of insects for food. This is necessary in order to lay the foundations for the development of a specifically designed supply chain of insects for food, which can guarantee the safety and health for workers and consumers.

4.1. Strategies for reducing the microbiological risks

The microbiological content of *Tenebrio molitor* and *Acheta domesticus* (fresh, processed, or stored) was evaluated in the study by Klunder et al. (2012), along with the effect of certain processes on it. The effect of boiling was observed to completely eliminate Enterobacteriaceae after 5 min of treatment. In crickets, however, prolonging the boiling time to 10 min does not affect the level of the remaining bacteria (Klunder et al., 2012). Boiling and cooking under vacuum were seen to be the most efficient treatments for reducing the microbial load in mealworms (Caparros Megido et al., 2018). Based on the obtained results, Klunder et al. (2012) recommend applying boiling before storing

insects in the refrigerator.

Another heat treatment that can be applied to insects is blanching. In the study by Vandeweyer et al. (2017b), it is shown to lead to a significant reduction of microbial counts. Particularly for blanching times of 10, 20, and 40 s, log reductions for total counts of 4.4, 6.4, and 5.6 log cycles were observed (no relationship between the magnitude of the reduction and blanching times was observed). Blanching followed by refrigerated storage can preserve mealworms without spoilage for at least six days (Vandeweyer et al., 2017b). In the same study, it was observed that a blanching treatment followed by microwave drying represents a pasteurization treatment because of the killing of vegetative cells but not of the spores. Moreover, blanching preceded by roasting leads to a strong reduction in the number of Enterobacteriaceae (FASFC, 2014), although Klunder et al. (2012) observe that roasting alone is not able to completely eliminate Enterobacteriaceae. No treatment was found to be capable of significantly reducing the count of sporulating bacteria and bacterial spores: in the reviewed studies, no one was able to eliminate them with heat treatment (De Castro et al., 2018; Gallo, 2018; Liu & Zhao, 2018; Van der Fels-Klerx et al., 2018; Van Huis, 2016; Van Huis & Dunkel, 2017).

After heat treatments, insects may be frozen or freeze-dried: these two treatments are applied to obtain an extension of the shelf life of the product, but it was observed in a preliminary study that they barely influence the total aerobic bacterial count of the insects (FASFC, 2014). The crushing of the larvae leads to the release of microorganisms contained in the intestine and to their distribution across the whole insect; this leads to an increase in viable bacteria count (Klunder et al., 2012). According to Rumpold and Schlüter (2013), a crushing step before thermal treatment (boiling or roasting) does not improve the efficacy but results in a higher microbial content in comparison with the whole insect. However, confirmation studies which test several time-temperature ratios and different methods of heating application (e.g. direct and indirect heating) are necessary to validate this statement. One possibility to reduce the contamination resulting from the microbiota of insect gut, is to adopt a starvation period before insect harvest. However, the effect of a starvation period of 48 h has been studied; there is no significant effect of reducing the microbial content of insect gut (Wynants et al., 2017). Further studies are needed to clarify the effect of starvation in edible insects.

Fermentation of insects is also documented. It is used to increase the shelf-life and microbial safety of ground insects (FASFC, 2014). It was observed that fermentation deactivates Enterobacteriaceae and maintains spore-forming bacteria under 10^3 cfu/g (Klunder et al., 2012). During fermentation, spores were unable to germinate and grow (Klunder et al., 2012). Borremans et al. (2018) studied fermentation of insects, specifically in *Tenebrio molitor* larvae; their results suggest that to obtain rapid acidification with the aim to inhibit undesirable microorganisms, a starter culture should be added. In this study, marination of insects was also investigated; it was observed that it can prolongs the shelf life of fresh mealworms for at least seven days due to a reduction of pH which inhibits microbial growth during storage (Borremans et al., 2018).

Cold atmospheric pressure plasma is a treatment that can be applied without the use of heat. Bußler et al. (2016a) studied the effect of cold atmospheric plasma on the microbial load of *Tenebrio molitor* flour. It led to a reduction in the microbial content, the magnitude of which increased with the prolongation of treatment time: the initial microbial load of $7.72 \log_{10}$ cfu/g was reduced to $7.10 \log_{10}$ cfu/g after 1 min and to $4.73 \log_{10}$ cfu/g after 15 min of treatment (Bußler et al., 2016a). According to present knowledge, the most interesting strategies to reduce the microbial contamination would include the addition of blanching machine in the production chain, followed by refrigeration in the case of whole insect conservation, or microwave treatment before processing. However, after the blanching and/or microwaves treatments, which reduce the microbial load, the fermentation with selected strains of yeasts and LAB might significantly reduce the microbiological

risk, exalting the efficacy and efficiency of the blanching and/or microwaves treatments.

4.2. Strategies for reducing the chemical risks

Regarding chemical contaminants, EFSA (2015) reports that processing conditions (e.g. heating or freeze-drying) have only minimal effect on them. The best way to control the levels of chemical contaminants is fractionation because they are accumulated in specific insect fractions (EFSA, 2015). For the prevention of chemical risk, it is important to control insect breeding substrates, feed, and the environment in which insects are bred, because of the ability of insects to accumulate chemical contaminants. In addition to breeding, a check could be applied for the presence of chemical contaminants during the process. For the rapid detection of chemical contamination, several methods can be used: immunodetection (which is based on the interaction between an antigen and an antibody, it is used in pesticide detection, for example), chemistry method (in presence of metallic ion, organic agrochemical of phosphorous hydrolyze giving rise to a hydrolysis product that leads to color change) and biosensor (the concentration of the contaminant is converted into a measurable electrical signal) (Tang, Lu, Zhao, & Wang, 2009). For heavy metal analysis, the common techniques employed are different types of spectrometry (Zukowska & Bizuk, 2008); however, these methods require a laborious phase of sample preparation and are not possible in time restrictions.

Recently, nanotechnology is often used to provide sensors for heavy metal detection in the environment, such as fluorescent sensors, plasmonic sensors, surface-enhanced raman scattering (SERS) sensors, optofluidic sensors, electrochemical sensors, field-effect transistor (FET) sensors (Li, Gou, Al-Ogaidi, & Wu, 2013). Due to the fact that the accumulation of heavy metals in insects comes mainly from feed, these sensors could be used for heavy metal detection in the breeding environment. Apart from feed and breeding, control should be maintained throughout the production process because chemical contaminants could be introduced during unit operations, for example migration from the machines used. From this point of view, in the choice of the machines to be included in the production process, the evaluation of the usability of the machines is important. An example of the migration of chemical contaminants in food during a unit operation, specifically the grinding, is reported by Yahaya, Aremu, and Abdullahi (2012): in ground food samples, an increase in iron content was observed, so it was recommended to subject the grinding discs to heat treatment to minimize metal contaminants in ground foods. In accordance with the findings, possible strategies intended to reduce the chemical risk could start by preventive approach in the breeding phase, followed by careful fractionation and finally rapid detection strategies during the critical control points of the process with nanosensors.

4.3. Strategies for reducing the physical risks

As previously mentioned, the major physical hazard present in insects is choking: the way to limit this risk is to grind insects (Marone, 2016). For an optimal control of physical risks, critical points should be identified throughout the production process. In particular, the HACCP system should be focused on the most critical phases (sorting, grinding, and packaging) to guarantee safe working conditions and product free from foreign bodies. Moreover, unit operations are crucial so it would be advisable to use machines that prevent the introduction of foreign bodies, such as closed grinders, with significant reduction in the inhalation risk for the workers. To verify the good functioning of the most critical unit operations, of the HACCP plan and of the identified critical points, the installation of instruments capable of detecting the presence of foreign bodies during the process and in the product might be useful. The presence of foreign bodies such as metals and glass can be verified by installing metal detectors or X-ray machines (Marshall et al., 2016).

The operators involved in the production must follow correct personal hygiene practices (in particular the good manufacturing practices in force (e.g. IPIFF guide on Good Hygiene Practices)) and wear suitable clothing and protections to prevent the introduction of foreign bodies and the risk of inhalation.

In agreement with the scarceness of data collected, with the utilization of an effective insect-sorting machine, it is possible to effectively reduce the risk of foreign-body contamination in the product. An important focus should regard the physical risk related to the workers' activities. In particular, the selection of machines optimized in ergonomics and usability terms, as automated as possible, would reduce the cutting and contusion risks along the production chain of insects for food. Furthermore, in order to guarantee workers safety, another important focus might regard the adoption of specific PPE, such as filtering facepiece respirator, which could protect the workers from inhalation risk during the processing phases.

4.4. Strategies for reducing the allergenic risks

As previously reported, there are in literature contradictory data about the effect of thermal treatment on the food allergenicity of insects (De Gier & Verhoeckx, 2018). In fact, EFSA (EFSA, 2015) also reported that the allergenicity of certain compounds can be triggered or blocked through heat treatment that alters the structure of proteins (Fernandez-Cassi et al., 2018). Although, the understanding of the effect of processing on insect-allergens can be found in studies devoted to closely related arthropod species (Downs et al., 2016), there are some studies that investigate allergenicity. Broekman et al. (2015) were the first to study the effect of heat treatment on mealworm allergenicity: they observed that the IgE-binding capacity and IgE cross-linking functionality of mealworm allergens were not lowered by the processing, while the solubility of proteins was strongly influenced. As the heat treatment seems to increase the solubility of tropomyosin, it was hypothesized that this phenomenon happened when the bonds with proteins were broken or when the formation of soluble aggregates took place during the treatment (Broekman et al., 2015). Tropomyosin allergenicity, in the study by Van Broekhoven et al. (2016), decreased in fried samples and disappeared in fried and in vitro digested samples. On the other hand, heating causes the arginine kinase to unfold, exposing the hydrophobic amino acids and leading to the formation of larger protein aggregates that, in most cases, are insoluble (Broekman et al., 2015).

Regarding the allergenicity of crickets, in a study by Hall et al. (2018), it was observed that the enzymatic hydrolysis of proteins had an effect on tropomyosin allergenicity: in fact, cricket protein hydrolysates with a major degree of hydrolysis showed the lowest reactivity of tropomyosin. Given the consistency of information in the references, it is advisable not to encourage people who are allergic to crustaceans and house dust mite to consume insects. It is, therefore, essential that the information accompanying the product must be clear: on the label, it should be reported a warning addressed to people allergic to crustaceans and house dust mites, informing them of the allergenic risk that they run in case of consumption of the product. However, frying and enzymatic hydrolysis seems to be the most interesting strategies to reduce the tropomyosin allergenicity. Finally, further investigations are needed to assess the effect on workers' health connected with the risk of developing allergenicity due to inhalation during the grinding unit operation and in others phases of the production chain (Broekman et al., 2017a). In particular, a clarification is necessary regarding the effects on workers' health to understand if single exposure is sufficient to produce damages on health or if prolonged exposure is needed.

5. Conclusions & future trends

The growing demand of protein food with reduced environmental impacts is currently a big challenge for food production. Insects represent a potential frontier of good quality food, rich in nutrients and

easy to be produced, at cheaper costs and with possible lower environmental impact. Nonetheless, it is fundamental for the consumers' safety to conduct prospective studies on the risks raised by them, since in the majority of the EU Countries, as well as in the Northern part of America, their use is too recent to have solid evidences on the impact on health, as well as on the effectiveness of the processes used to control the described risks. As for all the novelties, insects will also require specific surveillance systems that can promptly record any unfavorable episode for consumers' health, which can address the private and public control to improve the safety of the whole production chains, so as to ensure safe products on the market. Many other primary studies are necessary to untie these nodes, but this review is a preliminary proof that authorized novel foods can, at least partially, represent a possible answer to the increasing demand of protein food by a population that is forecast to grow significantly and whose food demand will not be reduced in the future. This paper suggests strategies to orient the development and improvement of specific machines for the emerging production chain of insects for food, with ameliorative effects on workers and finished products safety. In particular, this review aims to lay down the foundations for the development of the production chain of insects for food which should be based on integrated supply chain approaches, specific self-regulations and HACCP plans, development of ad hoc machines, and defined processes flowcharts that might lead to a specific European Regulation for edible insects.

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