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Summary

Zusammenfassung

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Review / Übersichtsarbeit

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Insects as feed and human food and the public health risk – a review

Insekten als Futter- und Lebensmittel und Risiken für die öffentliche Gesundheit – eine Übersicht.

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The human consumption of insects (entomophagy) as an alternative and nutritious animal protein is increasing among consumers in Western countries. In order to assess the microbiological and chemical safety of edible insects, in the last decades, several national food safety agencies in the European Union (EU) have attempted to perform risk assessments. More insight was recently provided by the European Food Safety Authority (EFSA) in the published scientific opinion related to production and consumption of insects as food as well as feed, with a focus on human and environmental risks. The aim of this paper is to review the main aspects that revolve around insects as food and feed, such as the production, processing, consumption, current EU legal framework, environmental and nutritional aspects, the risk of biological and chemical hazards associated to insects and to the substrates used in farming, as well as potential for allergies. In addition, the paper identifies main challenges and opportunities on the use of insects feedstuff and foodstuff and provides recommendations for the different stakeholders. In particular, the recommendations highlight the need to conduct more research as regards the feed risk assessment, to develop and validate food safety technological innovation at industrial level, to include insects in the international food safety regulatory framework, and to promote consumers awareness campaigns.

Keywords: entomophagy, risk assessment, EFSA, food safety, feed safety

In der westlichen Welt hat der Verzehr von Insekten (Entomophagie) als alternatives und nahrhaftes Eiweiß tierischen Ursprungs zugenommen. Um die mikrobiologische und chemische Unbedenklichkeit von Insekten abschätzen zu können, haben nationale Behörden der Europäischen Union (EU) in den letzten Dekaden versucht, Risikobeurteilungen zu erstellen. Kürzlich hat die Europäische Behörde für Lebensmittelsicherheit (EFSA) eine wissenschaftliche Stellungnahme für Zucht und Verzehr von Insekten bezüglich der Risiken für Mensch und Umgebung erstellt und damit das Thema vertieft. Zielsetzung dieses Beitrages ist es, die Hauptaspekte zu beleuchten, die im Zusammenhang mit Insekten als Lebens- und Futtermittel wichtig sind, also Zucht, Be- und Verarbeitung, Konsum, gegenwärtiges EU-Regelwerk, Umwelt- und Ernährungsaspekte, das Risiko des Auftretens biologischer und chemischer Gefahren im Umgang mit Insekten und bei der Zucht verwendeter Substrate sowie ihr allergenes Potenzial. Zusätzlich weist der Beitrag die Hauptherausforderungen und -potenziale von Insekten als Lebens- und Futtermittel auf und enthält Empfehlungen für die einzelnen Interessengruppen. Diese Empfehlungen unterstreichen insbesondere die Notwendigkeit einer verstärkten Forschung bezüglich der Futtermittel-Risikobewertung, der Entwicklung und Validierung innovativer, industrieller Technologien zur Lebensmittelsicherheit, der Miteinbeziehung der Insekten in das internationale Lebensmittelrecht und die Förderung von an die Verbraucher gerichteten Medienkampagnen.

Schlüsselwörter: Entomophagie, Risikobewertung, EFSA, Lebensmittelsicherheit, Futtermittelsicherheit

Introduction

Edible insects, a traditional food in many areas of the world, are progressively seen as a viable and alternative nutrient source compared to the conventional livestock, even in industrialized countries due to the higher nutritional benefits, with good-quality fatty acids, protein and micronutrients such as iron, magnesium, and selenium (Akhtar and Isman 2018, van Huis et al. 2013,) high feed conversion efficiency (Nakagaki and DeFoliart 1991) reduced environmental impact (Makkar et al. 2014, Ooninx et al. 2010, van Huis et al. 2013) and high fecundity (Mitsuhashi 2008 Nakagaki und DeFoliart 1991). Considering that in the next 30-40 years, the global system of food production will have to face several challenges, with a global population expected to grow to nine billion people (van Huis et al. 2013, Woods et al. 2010) and with a demand of animal-derived protein increasing at an even higher rate (Godfray et al. 2011), insects might provide adequate protein levels and other nutritional benefits in the diet at a very low environmental cost (http://www.fao.org/fileadmin/templates/est/Investment/Agriculture_at_a_Crossroads_Global_Report_IAASTD.pdf; Pelletier and Tyedmers 2010, Vantomme et al. 2012, Verkerk 2007). A FAO report provides an alarming picture in relation to the chronic hunger that afflicts a billion people in the developing countries, whereas in developed and emerging countries, the availability of food cannot meet the demand that will increase by 50% to 2030 (FAO 2018). While all this highlights an economic and ethical problem of adequacy of production and redistribution of food [as is reflected by the Sustainable Development Goal 2 of the United Nations (UN) program] (<http://www.un.org/sustainabledevelopment/hunger>), insects, due to high nutritional qualities, should be considered a renewable resource to promote sustainable agriculture and to reduce the problem of malnutrition and hunger in many parts of the world. Insect production also fits with the EU priorities established by the Europe 2020 program, which defines the EU's growth strategy for the coming decade and supports smarter and more sustainable growth and efficient resources. Indeed insects can contribute to a circular economy when they are reared on organic side streams (Borello et al. 2017), in line with the EU strategy (EU 2014), also counter the EU animal feed protein deficit (currently mitigated by soymeal and fishmeal), and can help to reduce food waste (PROteIN-SECT 2016; <http://www.proteinsect.eu/>).

This awareness on the feasibility and value of insects as sustainable commodity for food and feed and other applications had recently triggered several research projects with the aim to provide evidence-based results for the safety and efficacy of insect products. The exploitation of this "novel" food opportunity is now supported in the EU by a clearer legal framework than before 2018. In the EU and over the past two years, a number of governmentally funded research projects investigated the potential of insects as feed and food. To name some, PROteINSECT, a 3-year European Commission FP7-funded project (<http://www.proteinsect.eu/>), and GREEINSECT, funded for four years by the Danish International Development Agency (<http://greeinsect.ku.dk/>).

Both represent useful models for exchanging technical information between Europe, Asia, and Africa (Halloran et al. 2015). An ongoing research is investigating the suitability of insect farming systems ("minilivestock")

on board a ship for space travel as well as on a station on Mars or another planet (Katayama et al. 2008) Indeed silkworm moth (*Bombyx mori*) larvae are regarded as an animal protein source and other nutrients for astronauts in the bioregenerative life support system during long-term deep space exploration in the future (Tong et al. 2011, Liang et al. 2014).

Regarding the use of insects from a global perspective, there are two levels of knowledge: traditional and scientific. On one hand, there is an ancient and extensive pool of traditional knowledge on how to use a given species which is typically transmitted orally and within those sectors of society dealing with insects directly, i.e. hunters, gatherers, and salesmen. Over the centuries, they developed strategies to ensure a safe use of insects, but this knowledge seldomly surpassed other sectors of society. On the other hand, there is modern science which has merely started to approach this subject. Before, entomophagy and related issues were recorded mostly by anthropologists and biologists who did not focus on food safety or farming-related aspects (e.g. Meyer-Rochow 1973). Still, the indisputable merit of these reports is having reminded the scientific community on the potential of these animals. Despite some sporadic intents to introduce entomophagy also in Western societies (see below), it is until now that insect production has called the attention of researchers so that, from this point of view, insect product safety is a novel issue. This is enhanced by the possibility that with implementing farming systems for insects, traditional knowledge may no longer apply completely to an otherwise well-known species. However, traditional methods have also been evaluated scientifically (e.g. Acuña Cors 2010, Mbata et al. 2002).

Thus and in contrast to the above advantages, relatively little is known to date on industrial-scale production and processing, as well as food safety of insects and derived products, level of consumption, quality attributes, and risk assessment. Considering the large variety of insect species and the different and not yet standardized methods of rearing and processing, more research is needed on the safety of edible insects at species or family level to guide and support future regulatory measures for production, marketing, and consumption and finally to protect consumers. From the microbiological perspective, few studies have documented actual microbial counts present in/on edible insects and on their microbial community structure (Stoops et al. 2016). The information on level and structure of human consumption is also lacking, with the exemption of tropical countries where about two billion people consume almost 2,000 species of edible insects, attracted by their particular taste and nutritional qualities (Ooninx et al. 2010, Kouřimská and Adámková 2016). In Western societies, despite a timid increasing trend of insect consumption (Verbeke 2015), there is still a stigma or negative perception among consumers over insects used as human food (Stoops et al. 2016). However, most insects, especially edible insects such as grasshoppers, lepidopteran and coleopteran larvae, mostly eat fresh plant leaves or wood and are therefore cleaner and more hygienic than crabs or lobsters which eat carrion (Mitsuhashi 2008).

To promote insects as an alternative human nutrient source, their safety as food and feed is a prominent aspect that still needs to be better investigated in the future. Based on the current lack of relevant data

on microbiological, chemical, and allergenic hazards of insects and related products, more studies are required to define the risk profile of individual insects or of groups of similar insects and get a better knowledge on human exposure, which is a necessary step for the estimation of potential risk for consumers. This paper attempts to address all these aspects, and in the conclusions, key opportunities of the insect sector are identified and specific recommendations formulated, targeting different stakeholders.

From the start on it should be stressed that in terms of production and usage, there is neither “the” insect as there is not “the” mammal nor “the” fish. There are more than 2,000 insect species considered as edible alone, not counting those that may be used exclusively for feedstuff or other, industrial uses. Thus, referring to “insects” is like referring “mammals” or “birds”, i.e. the reader must be aware the strong degree of variability within and among groups and the fact that data is somewhat scarce and scattered. In view of the enormous quantity of insect species and the fact that trivial names may be confounding, scientific names were used whenever available and applicable. The full name is introduced once (e.g. *Acheta domesticus*) and the genus name abbreviated afterwards (*A. domesticus*). To make the text more accessible, the authority and the year of the first description were omitted. When there is no common name available, a combination of the insect family and the closest common term is used. In this way, “crickets” are referring to “crickets of the family Acrididae”, “notodontid caterpillars” to “caterpillars of the family Notodontidae” etc.

History of insects as human food

The class of insects (scientific name *Insecta* Linnaeus, 1758), also called Entoma (segmented) or Hexapoda, belong to the large phylum of *Arthropoda* (*Arthropoda*, comp. of gr. *árthron* ‘articulation’ and *poús, podos* ‘foot’), just like crustaceans, arachnids, milli and centipedes. The human eating of insect is described by the term ‘entomophagy’, from the greek words *éntomon*, meaning insect, and *phageîn*, meaning ‘to eat’. It has to be noted that this definition relates to the dietary consumption of insects by any organism, even though it is commonly used to refer specifically to human consumption of insects. Recently, a new term was coined to indicate a strict use of insects and insect derived products for human consumption ‘anthropoentomophagy’ (Costa Neto and Ramos-Elorduy 2006). Others suggested the term “hexapod-phagy”, which is referring to the eating of insects since they are the only invertebrate animals that have six legs in their adult form. However as described by Evans et al. (2015), the naming might have an important psychological impact on the acceptance of edible insects by Western consumers and needs to be contextualized and reviewed by the scientific community.

Insect consumption by man has been present since the dawn of the human species and insects were one of the main food sources for many cultures. The excavation of the remains of hominid settlements (of the Basin area and the Ozark Plateau in North America and the Shanxi province of China), the analysis of coprolites found in these sites and the discovery of iconographic evidence in caves (Altamira and Araña in Spain, Ariege in France)

have offered a solid proof of the occurrence of this phenomenon since prehistoric times.

Even in ancient times, both Greeks and Romans made use of insects in their diet as evidenced by some passages of Aristophanes’ comedies, Aristotle’s *Historia Animalium* (350 BC) and Pliny the Elder’s *Naturalis Historia* (78 AD). Little is known of the practice of entomophagy in the Middle Ages, but a renewed interest is found from the late Renaissance, with the significant testimonies of the works of Thomas Mouffet, Ulisse Aldrovandi, and Clemente Ferroni (Aldrovandi, 1638). In later centuries and with the spreading of European researchers beyond European borders, traditional entomophagy practices in non-Western cultures were described in numerous books. To give an example, in the Aztec empire it was the custom to devote *áhuautli*, a famous caviar consisting of the eggs of various species of aquatic Hemiptera (bugs) to Xiuhtecuhtli, a central symbol of the Aztec religion. In China, the West and East Indies, and Central America, many species of insects were considered particularly refined and sophisticated foods. This may be due to a particular good taste in connection with a reduced availability, as with other foodstuffs considered as delicatessen.

In 1885, the British entomologist Vincent M. Holt published his small booklet titled “*Why not eat insects?*” in which, although aware of the deep-seated prejudice and strong distaste of entomophagy, he had the most clout in bringing insects to a larger audience, advocating that insect could alleviate the hunger amongst the poor (Holt 1885). Consumption in Europe had almost disappeared over the centuries. The author argued that the only way to persuade Western populations to go back to eating insects was to make them a trendy culinary product. Holt was convinced that prevailing customs were one of the most powerful factors that influence the behavior of Western societies, including the food habit.

The general aversion to consumption of insects in the Western world is opposed to consumption in developing countries, where insects continues to represent a significant part of the human diet. With the publishing of the book “*Insects as human foods*” in 1951 by F.S Bodenheimer, the human entomophagy was granted a more global perspective. But a milestone of the increasing interest in insects as valuable protein sources was the launching of the *The Food Insects Newsletter* in 1988 by the late entomology professor Gene DeFoliart who a decade earlier started investigating the use of edible insects for chicken (DeFoliart et al. 2009). He actively promoted research and public information campaigns on the topic, leading forward-thinking entrepreneurs worldwide to develop insect farming techniques and produce and market insect food-based products (Dunkel and Payne 2016). The movement towards using insects as food ingredients then accelerated considerably with the publication of the forestry paper 171 of the Food Agriculture Organization of the United Nations (van Huis et al. 2013) that served as a catalyst for entrepreneurs entering the industry, for researchers and other academics. A momentum on this path was the conference titled International conference “Insects to Insects” in May 2014, organized by the FAO and Wageningen University, which is currently considered the main research institution aimed to support new entrepreneurs embarked in this sector (https://www.researchgate.net/publication/263548110_Insects_to_Feed_the_World_Conference_SUMMARY_REPORT_

May_2014_edible_Insects_for_food_and_animal_feed). Few other EU countries are at upfront on this topic, e.g. Denmark, Belgium, France, and the UK. On April 2017, a workshop in the UK which attracted nearly 100 delegates, including academic researchers, start-up companies, livestock feed suppliers, and NGOs, addressed the future of insect farming in that country and tried to identify the priorities for heading forward. From 2015 on, the “Insecta” conference has been held on an annual base in Germany, addressing multiple topics in insect production.

Notwithstanding the attempts made by some countries to facilitate the human consumption of insects, the cultural approach towards insects as food, at least in the Western culture lacking a history of entomophagy, have always been characterized by the idea of disgust and fear (Van Itterbeek 2008) since insects are negatively perceived and seen as dirty, pests or vectors of disease, and the insect-eating is considered a practice of primitive peoples. This reaction is described with the term ‘yuck factor’, coined by the bioethicist Arthur Caplan (University of Pennsylvania) (Schmidt 2008). It means the influence of instinctive responses against new technology (technophobic sentiment), that is determined by cultural, personal, and emotional factors (Srivastava et al. 2009). By its origin, disgust towards edible insects is a food neophobia. Thus it starts from being confronted with an item that has so far not been associated with edibility. This leads to heuristics (“educated guess”), comparing the item with other, known items, and judging its edibility from the experiences made by the potential consumer. It may be expected that all human societies group insects into several ‘folk’ categories, e.g. ‘beneficial’ (honeybees), ‘beautiful/bizarre’ (butterflies and other colourful insects) or ‘dangerous’ (pest insects, mosquitoes etc.). In non-entomophageous societies, the category ‘delicious’ is missing, but consumers may develop it if they are interested in the subject, and foster expectations that are fulfilled once the consumer had actually tasted insects (Grabowski 2017).

For this reason and notwithstanding the economic, ecological, and nutritional importance of food insects to human beings, to successfully introduce insect protein into the human food chain, we also need to take into consideration the very nature of our psycho-cultural limitations (Maheu 2011) as this will be a big challenge (Lensvelt and Steenbekkers 2014).

Farming and processing of edible insects for food and feed

Currently, approximately 92% of edible insects are harvested and gathered from the wild (Yen 2015), and worldwide insect farming is still manual. Generally, there are different types of insect production: extensive and intensive, indoor or outdoor (or semicultivation).

In developing countries, the majority of insects consumed by humans are still collected in their natural habitat (traditional entomophagy). Managing a terrain to exploit local insects would be one way of outdoor production which can be intensive or extensive, depending on the species life cycle and its availability. This is mostly widespread in countries with a tropical climate, where the environmental factors such as temperature and humidity play a fundamental role. The advantage

of this “outdoor farming” is the availability of all the elements necessary to perform the entire life cycle naturally, from host plants to adequate hiding areas. However, control on the cycle is minimal and production is optimized by both protection measures adopted by farmers against predators and improvement of food sources (van Itterbeek and van Huis 2012).

In contrast, insect farming in a closed or indoor environment may assure food availability year-around due to biology of certain insect species that are available during certain seasons of months. The advantage (and challenge) of indoor farming is a complete control on the life cycle which in turn means that you have to provide all necessary elements and run the risk that the cycle fails because a vital element was missing.

Essentially, both management systems could be applied to insects with seasonal and all-the-year life cycles alike, although seasonal species kept indoors would also result in facilities used only seasonally.

House crickets (*Acheta domesticus*) and yellow mealworms (*Tenebrio molitor*) are currently the species most frequently farmed for proteins and they are typically used for pet food in Europe, North America, and parts of Asia.

Despite significant advances have been made with artificial rearing diets and controlled conditions, the mass production of insects for human food and animal feed is not currently a common enterprise in most industrialized countries. Indeed, the upscaling to intensive farming still recognizes several constraints and presents some technological challenges as compared with production technology for other farm animals (Dobermann et al. 2017). Critical elements of scale production include the research on insect biology (i.e. life cycle and how to improve it under farming conditions), suitable rearing conditions, and proper diet formulation (Wang et al. 2004, Schneider 2009). Moreover, to meet certain conditions for the industrialization and to make insect farming competitive with the production of meat from livestock, the current system needs the development of a suitable technology to facilitate the automation process, since in insect farms the feeding, collection, cleaning, and rehousing still require manual labour (Kok 1983, Kok et al. 1990, Rumpold and Schlüter 2013). A further limiting factor is the domestication process (Durst and Hanboonsong 2015) as not every type of insect can be raised completely in artificial conditions. So, the industrial production or the mini-farming systems must be limited to certain species. In this context, several basic types of farming according to the substrate may be recognised:

- Xiroculture, i.e. rearing insects on a dry substrate, e.g. crickets, locusts or mealworms. This also includes the silkworm (*B. mori*), a species fully domesticated, which is no more capable to survive without human interference (DeFoliart 1995).
- Hygroculture, i.e. rearing insects on a humid substrate, e.g. fly larvae.
- Aquaculture, e.g. water beetles or water bugs.
- Xyloculture, i.e. rearing insects on a wood substrate, e.g. weevil larvae.

It can be expected that the production parameters and food safety will vary with the farming type; rearing mealworms on cereal flakes and potatoes is prone to produce a different microbiological situation than breeding flies on a humid substrate of food leftovers.

The current criterion to define the industrial scale production of insects is a minimum value of one ton of fresh weight of insects per day. The ideal insect species used for this type of production would have:

- a high deposition rate of eggs and the survival of immature forms, a short development cycle and larval stage, high productivity [i.e. a high conversion rate (kg of biomass increase per kg feed), very high growth rate of body mass per day],
- the ability to live in high-density conditions (kg of biomass per square metre), high resistance to diseases,
- the identification of an ideal feed.

It should be pointed out that these criteria not necessarily have to apply exclusively to insects with traditional usage. In fact, many fly species meet these requirements, a group of insects in which few are consumed traditionally. Species that exhibit the above desired features are primarily the black soldier fly (*Hermetia illucens*) with regards to the production of animal feed, the domestic cricket (*A. domestica*), and the mealworm (*T. molitor*) both for feed and human consumption. The identification of an ideal feed can be a hurdle to the sustainable mass production of insects. The current potential substrates include: animal manures (poultry, pig), organic industrial, and/or domestic wastes (e.g. brewery and supermarket, food processor wastes) and anaerobic digestates. However, not all of these products are currently allowed as animal feedstuffs.

An important factor is to recognize insect species specificity. This is largely accepted in more common livestock species, e.g. in comparing productive performance between cattle, goats, and sheep, in many cases even on race level. Insects, however, are frequently conceived a homogeneous group (see above). One of the areas in which this becomes evident is performance. For example, poultry litter is not a reliable substrate for blue bottleflies (*Calliphora vomitoria*), which develop better on pig manure, but much slower than the housefly (*M. domestica*) or the common green bottle fly (*Lucilia sericata*). In turn, *L. sericata* will develop on pig and poultry manures, but development is slower than *M. domestica*. Indeed suitable waste substrates for rearing of the larvae need to be fully evaluated in terms of the availability and cost of the substrate within the geographical location, the yield of the larvae and potential regulatory requirements (http://www.proteinsect.eu/fileadmin/user_upload/press/final-conf/European_Insect_Production_Systems_in_PROteINSECT.pdf).

Another important aspect to be considered is related to the choice of the farming strategy mostly based on the efficiency and sustainability. If many advocate the wild harvesting as a solution in some localities to contrast the hunger and global food security, others are prone to a full exploitation of industrial mass production as long-term strategy, mainly because the first solution may cause several problems, such as overharvesting, ecological damage, consumers's exposure to environmental and pesticide contamination, pathogens, all risks that can be controlled or eliminated in farmed stock or captive-reared stocks (Gaukar 2016). In regards to processing of insects for human consumption and feed products, the main limitation is the lack of knowledge on the impact of different methods on the safety and quality, once processing reaches beyond simple heat treatments, and legislative and regulatory gaps, mainly

a clearer legal framework in the forms of regulation and guidance developed for producers including the feed sourcing and its standards, welfare, biosecurity, shelf-life, transportation etc.

The level of knowledge on insect (industrial or semi-industrial) farming and processing is related to the level of consumption as practiced in different countries based on the historical and rooted food habit. In West African countries, mainly gathering termites support poultry and fish farming (PROteINSECT 2016). In China, where edible insects have been consumed for more than 2000 years, the industry is well-settled (Feng and Chen 2009, Feng et al. 2018). Mealworms are the most common reared insects with million of tons of dry mealworm larvae produced for export every year (pet food, bird food). The house fly (*M. domestica*) is another important rearing insect in China for producing feed. Two primary production approaches are used: on one hand, insects such as mealworms, cockroaches, and some beetles are fully domesticated and reared completely in captivity; on the other hand, insects such as locusts, wasps, bamboo caterpillars, and dragonflies are only partially raised in captivity or the habitat of the insect is manipulated to increase production (Feng et al. 2017).

In Thailand, an increasing demand for insects as food lead to a more intensive farming compared to the wild collection, in particular the rearing of crickets (*A. domestica*, among others) by individual farmers to provide a valuable source of additional income (Durst and Hanboonsong 2015, Hanboonsong 2008, Hanboonsong et al. 2013).

In the EU, however, the lack of a comprehensive and harmonised legal framework for edible insects have been interfering with the establishment of major insects production units as built in other countries, and insects represent a very small niche market. Yet, despite the restrictions for mass production, insects are a subject of growing interest as an alternative source of raw materials. In the last five years, the insect industry experienced an expansion phase, by moving from the hobby style to industrial scale with integrated process control. Currently the sector is exclusively composed of SMEs and start-up companies with limited capacity so far. The majority of them are dedicated to insect production with fully integrated production steps, from farming up to delivery of insect powder or oil. In the feed sector, black soldier fly (*H. illucens*; BSF), mealworm (*T. molitor*), and lesser mealworm (*Alphitobius diaperinus*) are among the most promising insect species for commercialization as feed (PROteINSECT 2016).

An insect farm can produce a series of final goods (Fig. 1). First, harvested insects may be used as whole animals or homogenised ones. These are the prime materials for derivatives or raw material products, leading to the production of proteins, oils, chitin, or more specific substances (e.g. medicinal compounds). Then, by-products are also obtained from organic waste of insects and processing waste. These by-products are often used as fertilizers in agriculture.

Worldwide the availability of food products containing insects has increased in the last years. Conducting a systematic review of companies selling insects online Muller et al. (2015) interestingly found a remarkable number of them mostly based in North America and Europe, with insects mainly sourced from countries in

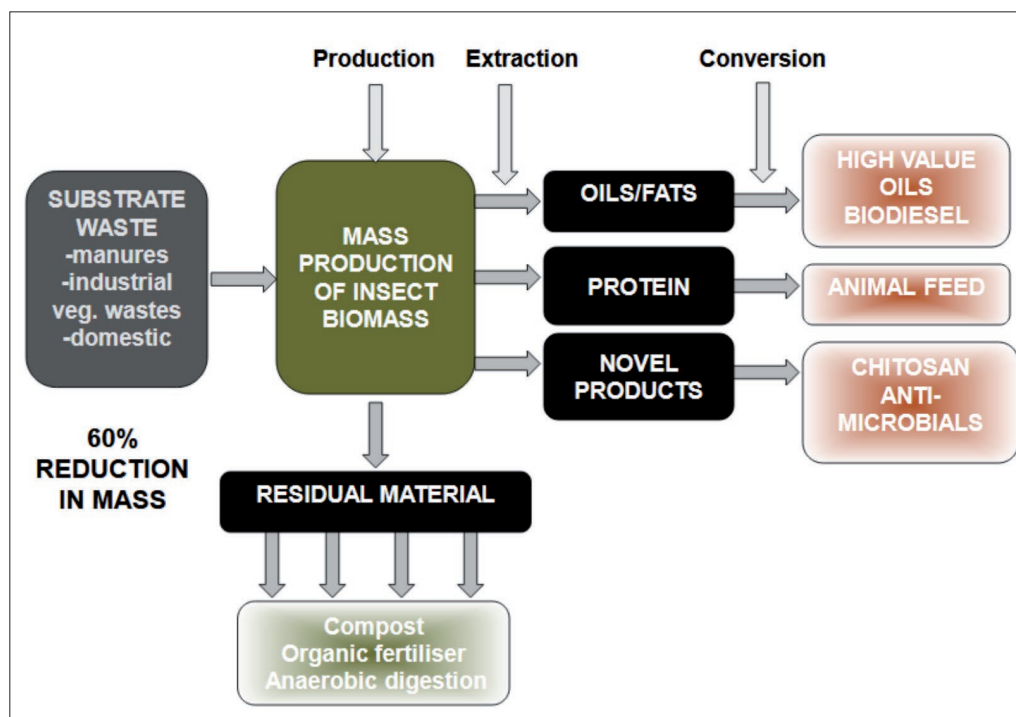


FIGURE 1: Production scheme for insects used not as a foodstuff (based on *ProtelInsect* Presentation by Dr Adrian J. Charlton, *Insect protein Conference, Bruxelles 2016*)

the Northern hemisphere. The source countries usually did not have a long history of insect consumption. Of 98 companies known to offer insects as human food or animal feed, 73 were founded between 2013 and 2015 (Dossey et al. 2016). At this time, there was an incredible growth of small companies and an emergence of few large ones. Only in the US, today there are 27 registered small companies focusing on edible insects (a 200% increase since), and 26 in Europe.

Edible insects consumption

Despite the reluctance to introduce insects in the Western diet, it is estimated the human insect-eating is practiced by at least 2 billion people in 113 countries around the world, particularly in Southeast Asia and the Pacific, sub-Saharan Africa, and Latin America (Hanboonsong, et al. 2013, Pal and Roy 2014). Ramos-Elourdy (2009) reported 2086 insect species consumed by 3071 ethnic groups in 130 countries due to high content of protein and minerals, and also because of their taste and palatability. According to van Huis et al. (2013), the amount of species of edible insects that are already part of human diets varies between approx. 1,000 and 3,000 and takes into account local preferences, sociocultural significance, and region. Currently, the most complete list of edible insects, the majority of which are consumed in the immature forms (larval or nymph), is available online at the University of Wageningen (Netherlands) (https://www.wur.nl/upload_mm/8/a/6/0fdcf700-3929-4a74-8b69-f02fd35a1696_Worldwide%20list%20of%20edible%20insects%202017.pdf). Globally, the order Coleoptera (beetles), which alone represents approximately 40% of all species of insects known to date

account for around 31% of insect species that are commonly consumed by humans, followed by Lepidoptera (moths and butterflies) with around 18%, Hymenoptera (bees, wasps, and ants; around 15%), and Orthoptera (locusts, grasshoppers, and crickets; around 14%). It is difficult to provide a precise estimate of the consumption levels of different species of insects from each country (Yen 2015) because of diets' heterogeneity and the different local names used for the same taxon in different geographical areas. In some cases, only selected instars of an insect species are consumed, e.g. termite imagos, beetle larvae, grubs, and pupas or wasp larvae and pupae. In other taxa, almost all stages are used, e.g. grasshoppers, crickets, and ants (DeFoliart 1989). Some insect taxa are consumed almost everywhere, such as saturniid and other larger moth caterpillars, acridid locusts and grasshoppers, gryllid crickets, cerambycid and other large beetle grubs, formicid ants, and pentatomid and belostomatid bugs, while others are popular only in certain regions, as the adult dragonflies that are consumed mainly in southeast Asia or the alkali flies (*Ephydra hyans*) only by certain Native American communities in the US (Costa Neto and Dunkel 2016). The habit of consuming a given species is traditionally limited by its geographical range and the trading net in which it became involved. The latter can be local, regional, national or even international as in the case of the silkworm (*B. mori*). In contrast, only some edible species already used as pet food are actually produced and traded globally, e.g. some mealworms (*T. molitor*, *Zophobas atratus* [In literature, this species often appears as "*Zophobas morio*" which, however, is now taxonomically incorrect], *Alphitobius* spp.), some locusts (*Locusta migratoria* and *Schistocerca gregaria*) and crickets (*A. domesticus*, *Grylloides sigillatus*, *Gryllus* spp.).

The insects might have different commercial presentations, namely whole insect, chilled, frozen, dried, or processed by mincing and used to prepare meat-like products, as hamburgers or sausages; flours, used in baking cookies, bread, snacks, and protein bars, in some powder or paste and used as ingredient in soups, bakery products, and convenience food. The culinary use of insects is related to the gastronomic habits of the region and the insect type: they can be consumed fried, sautéed, boiled, roasted, toasted, smoked (Fraqueza and Patarata 2017). As mentioned before, entomophagy can be classified in traditional and non-traditional, based on different practice levels for entire countries, ranging from simple gathering and consumption (at times of live insects) to a coordinated market with higher degree of industrialization along with selling of processed and preserved insect products (es. canned saturniid caterpillars in South Africa or dried *Sphenarium* spp. grasshoppers in Mexico; Grabowski and Klein 2017b). In order to provide a general overview, some entomophageous practices are presented subsequently. In Southeast Asian countries, between 150 and 200 species of edible insects are consumed, but only a few have been reared for food, such as crickets and the most popular one, the red palm weevil (*Rhynchophorus ferrugineus*) in Thailand (Durst and Hanboonsong 2015, Hanboonsong et al. 2013). In particular Thailand is recognised as a global edible insects hotspot with the largest thriving insect farming sector and a long tradition of use, especially in rural areas (Durst and Hanboonsong 2015). 150 different insects species, mostly wild-harvested, constitute a vital staple in the diet (Dobermann et al. 2017). Several cricket species dominate the market with almost 2,000 cricket farmers, but palm weevils and bamboo larvae are also farmed and wild-collected (Durst and Hanboonsong 2015, Dossey et al. 2016). Some species of insects, especially aquatic ones, are available year-round, others on a seasonal basis. In South Korea, it is traditional to eat rice-field grasshoppers (*Oxya velox*; *metdugi*; Moon et al. 2009) but also dried silkworm pupae (*B. mori*; *bundaegi*) are popular as they are elsewhere in East Asia and are found in most markets in Seoul. They are also exported for many other international countries (Kang et al. 2012). In China, insects are consumed in many areas by different ethnic groups, and the number of edible insects continue to increase. Recently, the use of insects as food and feed has developed very rapidly. From the last recent published literature review until 2014, 324 species were documented as related to food and feed (Feng et al. 2018). Fried silkworm moth larvae and roasted bee larvae are two common items in food stalls. The silk moth (*B. mori*) pupa is the industrial by-product of the ancient chinese silk industry produced at commercial scale production and sold in many markets, but is also commonly eaten in other Asian countries, including Japan and Thailand. In Japan, where typical food insects are bee brood (*Apis mellifera*) and wasps (*Vespula flaviceps* and *Vespa mandarinia*) larvae, it is common to eat also *zaza-mushi* (mixed larvae and nymphs of aquatic insects), fried grasshoppers (*Oxya* spp.) and cicadas (Nonaka 2010). In Africa, where the consumption of insects in the human diet is very popular and belongs to a rooted tradition, in some areas such as the Central Africa, insects meet more than 50% of animal protein needs with an estimated 96 insect species eaten (Paoletti and Dreon 2005). Mopane worms (saturniid moth *Gonimbrasia belina*) (and termites

are some of the most-consumed edible insects in the sub-Saharan Africa.

In Australia, there are also many different species of edible insects. The best-known is the witchetty grub (*witjuti*), i.e. the larvae of cossid moths such as *Endoxyla leucomochla*, which feeds on the roots of the Witchetty bush (Marshall Cavendish Corporation 2003). For many generations these larvae have been chosen as a key source of protein by the Aboriginal communities. The taste is like scrambled eggs. *Witjuti* is on the menus of Sydneys' sophisticated "Rowntrees" and on a growing list of other restaurants in Australia (Gonçalves Neves 2015).

Another region of the world where the consumption of insects is both widespread and well-documented are the Americas (Milton 1984, Politis 1996, Smith and Paucar 2000). In the USA, there are 54 species of edible insects documented, with several grasshopper species being most popular in Montana but not easily adapted to farming. In regards to processed food insects, powder of house crickets (*A. domesticus*) has been commercially available since 2013. Local eateries already serve crickets, silkworm larvae/pupae, cicadas cooked with rice and vegetables (Gaukar 2011). Silkworm soup and grasshopper tacos are found in some San Francisco, New York, and Washington D.C. restaurants (<https://www.westjetmagazine.com/story/article/insect-focused-restaurants-serving-grasshopper-tacos-poached-bees-cricket-brittle>) as well as cricket protein bars that can be easily found in grocery store shelves, with producers well distributed across the US via New York, Texas, and Arizona. Some restaurants across Canada also already incorporated insects on their menus (<http://www.foodinsectsnewsletter.org/pdfs/Vij%27sArticlebyDGGordon.pdf>).

Nowadays, 547 species of edible insects have been recorded in Mexico (Ramos-Elorduy et al. 2011). Edible insects are renowned delicacies that can be found in local markets and in dedicated restaurants. Most of Mexico's edible insects are caught wild, not farmed, then sold at regional markets or trucked into the cities. The best-known are the *chapulines*, pyrgomorphid grasshoppers of the genus *Sphenarium*, which are mostly appreciated by the producers due to high nutritional properties and reproductive rate, and make considerable revenue per year. They are sold as snacks at local sports events and are becoming revived among foodies (Cohen et al. 2009). Other traditional delicacies are *chinicules* (caterpillars of the cossid moth *Comadia redtenbacheri*), *escamoles* (immature instars of the formicid ant *Liometopum apiculatum*) or *chicatanas* (formicid ants *Atta cephalotes* and *A. mexicana*). These insects and dishes typically received names in indigenous languages, stressing that they have a very long history, but are mostly only confined to the indigenous peoples of that country (Ramos-Elorduy and Pino Moreno 1989). Another typical use is putting caterpillars of agave-parasiting moths (several families and species, among them also *C. redtenbacheri*) in every bottle of *mezcal*, an alcoholic beverage based on agaves. More recently, "modern" insect products also entered the market, e.g. candy-covered mealworms and chocolate-covered locusts. So far, these examples described the situation in countries with prominent entomophageous traditions. In Europe, current traditional entomophagy is very sporadic, and most practices were abandoned over the years. Of the few examples, it is common to eat a certain cheese (*casu marzu*) on the Italian island

Sardinia, ripened with live cheese fly (*Piophilha casei*) maggots (Paoletti and Dreon 2005). They emerge from naturally laid eggs on cheese left out in the open air and by a digestive action cause an advanced level of fermentation. Similar cheeses containing living insects larvae are produced in other Italian regions, on Corsica, in France, and Germany. Zagrobelny et al. (2009) reported that some children in the Carnia region of northeast Italy in the 1940s to the 1960s during early summer used to collect brightly coloured burnet (zygaenid) moths from the genus *Zygaena* in order to consume the low-toxic but very sweet ingluvies (the crop). In times when scarabaeid cockchafers (*Melolontha* spp.) were still numerous (and sometimes considered a pest), a soup based on the adult beetles was consumed in Alsace-Lorraine (Grabowski and Klein 2017b), which should be considered as a strategy to reduce their population and improve human nutrition. These few exceptions, however, confirm the overall assumption that Europe basically lacks an entomophageous tradition. In the European Union (EU), only in recent years, the consumers started to be attracted by insects, more as a gourmet product than as a cheap protein source. In fact, edible insects marketed in Europe are very expensive, eventually more expensive than meat (Rumpold and Schlüter 2013). Considering the vastness of the Wageningen list, only few species have been produced for food with commercial purposes and have the potential to become “common” edible insects in the EU. The EFSA report cites the following species: crickets (*A. domesticus*, *Gryllus bimaculatus*, and *Teleogryllus testaceus*), grasshoppers and locusts (*Oxya* spp., *Melanoplus* spp., *Hieroglyphus* spp., *Acridia* spp., and *L. migratoria*.), mealworms (*T. molitor*, *A. diaperinus*, *Z. atratus*), and silkworms (*B. mori*). Some of these species are already in the market in Belgium and, in part, in the Netherlands (Dossey et al. 2016). The growing interest in consumption of insects clearly emerge from awareness campaigns conducted by traditional media, social media, and by the increasing number of farms devoted to rearing insects (IPIFF 2014). Sometimes, public consumption is limited to restaurants, cooking classes and events such as trading fares, scientific studies, and television shows. Some restaurants serve insect food, including one in the European Parliament, and tastings are offered during different events). In Copenhagen, Denmark, the “Noma” restaurant (voted as the best restaurant in the world), offers edible insects as exploiter cuisine (Dossey et al. 2016). However, the current situation in Europe is complex due the varying degree of regulatory framework, ranging between the possibility of purchasing insects in supermarkets or online and a legal ban accompanied by social taboo. In Germany, there is a growing awareness and interest in entomophagy: an e-commerce of relatively expensive ready-to-eat snacks or (freeze) dried insects for further processing is already set, with providers offering products under self-defined (but not specified) food hygiene standards (Grabowski and Klein 2015). In the UK, 13 companies sell insects in different forms that include cubes of ground-up insects and bags of whole mealworms, crickets and grasshoppers, toasted giant ants and house crickets (http://www.europarl.europa.eu/RegData/etudes/ATAG/2016/583830/EPRS_ATA%282016%29583830_EN.pdf; Dossey et al. 2016). In the Netherlands, insects have been on sale in the supermarket since 2014. Three insect species [yellow mealworm (*T. molitor*) larvae,

lesser mealworm (*A. diaperinus*) larvae, and migratory locusts (*L. migratoria*)] produced and processed specifically for human consumption can be found in specialized shops. Also in Belgium, ten species of edible insects are temporarily tolerated on the market and offered for human consumption as burgers and nuggets as well as vegetable spreads made with mealworms (http://www.afsca.be/denreesalimentaires/circulaires/_documents/2016-04-26_circ-ob_FR_insectes_V2_clean.pdf). Well-renowned supermarket chains offer insect meals and snacks. In Geneva, Switzerland, and in Hannover, Germany supermarket chains have started selling burgers and patties made from insects, a move being billed as a legal first one in Europe. The bug burgers are made of rice, chopped vegetables, spices, and mealworm larvae.

There are also several industrial applications of insects and derivate: a red carmine pigment extracted from the gravid female cochineals (*Dactylopius coccus*), commercially known as E-120, is used for colouring foods (e.g. candies, yoghurt), and beverages.

Use of insects in animal feed

Due to the incessant industrialization of animal husbandry system and in response to the consumer demand for more protein, the need for animal feed has increased in the last two decades. In 2014, the world produced about 980 millions of tons of feed, worth about 460 billion dollars, figures that are going to increase in the future with the increasing meat consumption (Altech Global Feed Survey 2018). Animal feeding is considered the most expensive aspect of animal production and is sadly associated with high ecological footprint (van Huis et al. 2013). The FAO strongly recommended the use of insects as human food and animal feed as a tool for poverty alleviation (<http://www.fao.org/docrep/012/i1380e/i1380e00.pdf>) and estimated that insects have a similar potential market as fishmeal and they could be employed as feed in aquaculture and livestock and also be used in the pet industry (van Huis et al. 2013, FAO 2004).

Currently, the primary source for animal feeding are soy and fish meals. It is acknowledged that the production of soybean is connected with deforestation, soil erosion, extensive use of pesticides, loss of biodiversity and a huge CO₂ footprint (van Huis 2015). In Europe (5% self-sufficiency), 75% of soymeal consumption is imported (15 million tons per year), meeting only 60% of the demand for animal feed protein. Fish meal, which is based on fish cultivated in aquaculture or marine fish species, is a natural, balanced, and highly nutritious feed ingredient predominantly used in the aquaculture, which is the most-developed farming sector in the world consuming around 10% of the world’s fish production as feed (fish meal and fish oils) (https://www.aquaculture-alliance.org/wp-content/uploads/2016/08/Day3_GorjanNikolik_GOAL2015-copy.pdf; PROteINSECT 2016). Fish meal due to problems with over-fishing, progressive depletion of ocean fish stocks and the increasing restrictions on unregulated fishing and catch quotas and environmental pollution, can be regarded as a limited resource. For these reasons this sector – struggled to guarantee a stable production, both quantitatively and qualitatively (AFRIS 2012). In Europe for example, 65% of fishmeal consumption is imported, and accounts

for 10% of the 5 millions of tons of fishmeal produced globally every year. Nowadays, despite fish meal supply is improving and prices stabilising at a lower level, fish meal producers are seriously looking at alternative high-value protein sources to be used as innovative feed ingredient in aquaculture. Among the various alternatives, bacterial, insect-based protein sources and algal oils (krill, microalgae or seaweed) show the greatest potential (<https://research.rabobank.com/far/en/sectors/animal-protein/new-growth-strategies-needed-for-alternative-aquafeed-ingredients.html>; PROteINSECT 2016).

Indeed, focusing on insects as feed ingredients in pig, poultry, and fish farming (at least in the long term), is a need for sustainable future. Despite more knowledge is needed on the chitin functionality, amino acid digestibility, and nutritional values, insects in general could provide much of the proteins animals need at lower price and lower environmental impact (Veldkamp et al. 2012). Moreover some insect species, compared to soy or fish meal, are higher in nutrients and high quality protein (Ramos-Elorduy et al. 1997, Sánchez-Muros et al. 2014). To give an example of the potential of protein yield of insect farming, 2.5 tons of soy crops in one hectare and one year provide 0.9 tons of protein, while 1000 tons of fly larvae in one hectare and in one year has a potential to provide 125-150 tons of protein (PROteINSECT 2016) with 200-fold reduction in land use. Many feeding trials for livestock and aquaculture evaluated the nutrition potential of different species of insects. Generally, control diets contained fishmeal and/or soybean meal. Studies of insect protein values indicate that most tested species had high protein quantities and quality (Ramos-Elorduy et al. 1984, Kourimská and Adámková 2016).

Most of these feeding trials have been conducted in developing countries and particularly Asia and Africa with a predominance in aquaculture. In terrestrial livestock, most experiments worked with house flies (*M. domestica*), followed by silkworms (*B. mori*), while species selection was more variable in aquaculture (Sánchez-Muros et al. 2016). Regarding poultry, the available literature confirms the feasibility of partial or total replacement of fish meal with insect meal (Jintasataporn 2012, Makinde 2015, Makkar et al. 2014, Veldkamp and Bosch 2015). Until now, the main research efforts in poultry diets have focused on the black soldier fly, housefly, mealworm, silkworm pupae, earthworm, grasshopper, locust, cricket and *cirina firda* (westwood) (Sohail Hassan Khan 2018). Other studies demonstrated that, the partial or total replacement of soy beans in poultry feeds with insects lead to better feed conversion and growth rate (Marono et al. 2017, Rumpold and Schluter 2013, Loponte et al. 2017, Schiavone et al. 2017). *B. mori* was also the most-studied insect species regarding its potential use in poultry production, for which increased benefits were estimated (Dutta et al. 2012, Ijaiya and Eko 2009), in particular the use of the silkworm pupae (as by-product after the removal of silk thread from the cocoon) in broiler feeding (Jintasataporn, 2012). Indeed the silkworm powder meal has potential to replace the costly and contaminated fish meal, as the protein source, used in poultry industry. There is limited information on the use of insects in pig feeding, but the results of a few tested species (eg. silkworm pupae, house fly and black soldier fly) are promising (Newton et al, 1977, Dankwa et al, 2000, Medhi 2011, Makkar et al. 2014). The use of

insects in fish feed is widely practised by smallholder farms in Africa and Asia (van Huis et al. 2013). Among the many insect species used in aquaculture feeds, black soldier flies (*H. illucens*), housefly (*M. domestica*) larvae, silkworms and mealworms (Tenebrionidae spp.) are the most common ones (Makkar et al. 2014, Magalhaes et al. 2017, Wang et al. 2017).

Despite the difficulties in obtaining clear conclusions on the feeding trials in aquaculture conducted in different parts of the world (because of the huge variety of fish and insects species, diet formulation, ingredients etc.), the results indicate that insect protein could make a bigger and positive impact in aquaculture. Considering the growing commercial interest in insects as animal feed and in order to clarify the future role of insects as valuable substitution in conventional animal feed, more research and feeding trials in different species are needed. This is particularly relevant for the aquaculture sector for which further research is needed to assess *inter alia* the most adequate insects for each fish species, the influence of insect meal on muscle quality, the optimal fish meat substitution percentage etc. Similarly in the poultry sector, there is a need to carefully investigate the impact of insect feed on intestinal morphology, meat quality traits and sensory properties for both consumers acceptance as well as for marketing purposes (Sohail Hassan Khan 2018).

In regard to the use of insects as animal feed in the EU the Commission with Regulation (EU) 2017/893 (in application since July 2017) authorised the use of processed animal protein (PAP) made from insects in feed, but for aquaculture animals only. This issue is further addressed in the chapter "EU insect legal framework".

Environmental impact

The traditional and conventional methods of food production (and particularly those located in the livestock sector) are nowadays considered one of the most significant contributors to serious environmental problems. Livestock is a major threat for the environment due to deforestation, progressive reduction of the global arable land, and the 18% contribution to global greenhouse gases emission (GHGE), with methane (CH₄) and nitrous oxide (N₂O) having greater global warming potential than CO₂ (<http://www.fao.org/newsroom/en/news/2006/1000448/index.html>; <http://www.europarl.europa.eu/climatechange/doc/FAO%20report%20executive%20summary.pdf>; Steinfeld 2012). Feed production, which will have a proportional increase compared to animal production, currently represents 45% of these emissions (Gerber et al. 2013). This is also related to the enhanced global warming (Godfray et al. 2011).

Undoubtedly, increasing population and food consumption are placing an unprecedented demand on agricultural and natural resources. According to Godfray et al. (2011), the demand for animal-derived proteins is expected to increase globally at a rate higher than that of the global population. Just to name meat production, which occupies more than 70% of agricultural land, will rise to 460 million tonnes in 2050, from 226 million tons in 2000 (van Huis et al. 2013). Wu et al. (2014) forecast a projected 72% rise over the next 35 years. In developing countries alone, meat consumption is growing at a rate of 5 percent per year (FAO 2006). To address the dilemma of increasing population and consumption, and to guarantee the future human demand for high-quality

animal derived-protein, the rearing and processing of edible insects come to provide a more valuable, efficient and sustainable solution to this problem. This certainly goes with insect food and feed sectors that are globally and nationally regulated and display internationally agreed quality and safety standards. In contrast to conventional livestock (e.g. chicken, pork or beef), insects require minimal land, less water, emit little GHGE (Oonincx et al. 2010), can be fed on organic waste rather than cultivated grain, and are much more efficient in converting feed to body weight. Thus, the production of 1 kg of crickets (*A. domesticus*) requires 1.7 kg of feed, (and less than 1 L of water and 15m²), whereas 2.5 kg are needed for chicken, 5 kg for pigs, and 10 kg for beef (Collavo et al. 2005). The percentage of actual edible weight is much higher in insects than common breeding animals. Of the same species, up to 80% of the weight is edible and digestible compared to 55 percent for chicken and pigs, and 40 percent for cattle (Nakagaki and DeFoliart 1991). Moreover, insects have a high rate production of body mass. Being cold-blooded animals, they do not need to spend energy for regulating the body temperature (van Huis et al. 2013). Some display high fertility rates with several life cycles per year.

To assess the environmental impacts associated with all stages of a product's life, is necessary to analyze the life cycle, a technique that has been carried out so far only for the mealworms. According to a recent study, mealworm (*T. molitor* and *Z. atratus*) breeding has overall a significantly lower environmental impact than conventional farm animals. Oonincx et al., (2010) quantified the GHG production, energy, and land use area throughout the mealworm production chain. They found that the energy use for the production of 1 kg of mealworm protein was far lower than for beef, comparable with pork, and slightly higher than for chicken and milk. GHGE were much lower than in traditional food animals. It is calculated that the production of 1 kg of meat emits 13.3 kg of CO₂, the same amount emitted by burning of six liters of petrol, while GHGE from mealworm larvae, and similarly for crickets and locusts, are remarkably lower, by a factor of about 100.

In addition, for every hectare required to produce mealworm protein, 2.5 hectares would be required to produce a similar amount of milk protein, between 2 and 3.5 for pork and chicken and 10 for beef.

A further benefit of insects as an alternative animal protein source is the possibility of rearing them on organic side streams of conventional animal farming (e.g. manure, pig slurry, and compost). The most valuable example is the BSF larvae (*H. illucens*), that are reported as feeding on and efficiently converting into insect biomass (more than other insects, eg. crickets and mealworms) a wide range of organic materials such as manure, food waste, fecal sludge, kitchen waste, and so on. This big advantage, which exemplifies the concept of circular economy with the reduction of pollution and costs, make them a sustainable solution for small-scale waste management (Wang and Shelomi 2017, Fisher 2017). They are also grown and recommended for use as animal feed and, considering that they are not toxic (Blum 1994), could be potentially exploited for commercial use in human as food, even though for Western and non-Western consumers alike, insects associated with waste (saprophages) are not seen as edible and they rationally would trigger the disgust factor (Deroy et al.

2015). Many projects focused on rearing and commercial use of BSF. Results of the ENTO-PRISE project of the University of Stirling in Ghana showed that farmed BSF larvae can substitute for high quality imported livestock feed ingredients (e.g. fishmeal, soybean meal), the left-over substrate can be used as a biofertiliser after composting and fresh substrate weight is turned into larvae that can be an ingredient in fish or poultry feed (high protein level). (Maquart et al. 2015). A similar project (AQUAFL) led by the National Institute of Nutrition and Seafood Research (NIFES) in Norway, aimed to utilize the coelopedid kelp flies (*Coelopa* spp.) fed on marine substrates such as seaweed biomass in order to tailor an insect product rich in marine omega-3 fatty acids to supplement existing fishmeal options for sustainable Atlantic salmon aquaculture (<https://www.nifes.no/en/prosjekt/insects-salmon-feed/>). Both projects demonstrated the significant potential for insects to recover resources from organic wastes and provide added value products such as high protein livestock feed and grass biofertilisers. If just 10% of the manure produced in the EU were used to rear fly larvae, this could provide ~1.75 millions of tons of insect protein, which is more than 10% of the annual EU import of soy protein or 50% of protein from global fish meal production (PROteINSECT 2016).

EU insect legal framework

In countries or regions of the world where entomophagy is a common practice, very few regulatory hurdles affect the insect production, marketing, and consumption, with nature conservation and local economy being main issues (Halloran et al. 2015). Differently in Western countries, with less experience and where edible insects are considered as novel food, the lack of a clear legal framework represents a significant limitation for the use of insects. Food safety and consumer protection are the main foci of policy makers. Currently in the US to legally sell whole insects, same standard set for other food for human consumption must be met and the farming, production, and marketing are regulated by the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA). In the EU, the related legislation lagged significantly behind. Adding to that, the scarcity of comprehensive scientific evidences as regards to potential microbiological and chemical hazards and the risk for human consumption, contributed to a certain degree of uncertainty on how to ensure food safety. Consequently in accordance with the precautionary principle, insects have to be proven safe before placing them on the EU market. Basically, insects may be defined as foodstuff according to Regulation (EC) 853/2004 categories "products of animal origin" and, when applicable, "fishery products". With that, EU food legislation applies fully, but unlike other foodstuffs, further details on production, processing, sampling, sample evaluation, official control system etc. have not been provided so far. Being so, official veterinarians lack regulatory provisions and a reliable base to evaluate and, ultimately, certify any food insect enterprise. The first challenge for edible insects entering the European food chain was Regulation (EC) No. 258/97, which was in force until the end of 2017. According to this regulation food and food ingredients, were considered novel

if they were not consumed by human beings to a significant degree in the EU before 15 May 1997 and additionally displayed other markers of novelty, e.g. a novel molecular structure or a newly-developed microorganism. This triggered a debate on definitions, among them the concept of consumption to a “significant degree”, the methods used for assessing consumption data, and the scope of Regulation (EC) No. 258/97, which referred to food “obtained from animals” and not “entire animals”, as is the case for insects and larvae. Some considered insects as novel food, some did not, and some made a difference between entire animals (including products made thereof that retained all the original components, e.g. insect meals) which were not perceived as novel food on one hand, and technically extracted or modified components, e.g. PAP or insect oils, on the other hand, which were in fact considered novel foods. This legal flaw combined with the development of the EU edible insect market, urged the three co-legislators (the Commission, Parliament and EU Council) to adopt in November 25 2015 the Regulation (EU) No. 2283/2015 on novel foods (Novel Food Regulation), which has been applicable since 1 January 2018. The scope of this regulation remains the same as the Regulation (EC) No 258/97: the definition of novel food was not changed in terms of “history of safe use”, “significant degree” nor the “time restriction of 15 May 1997”. The novelty is represented by two new definitions: the first is that of “traditional food from a third country”, which refers to a novel food subgroup as derived from primary production, regardless of whether or not the foods are processed, and have a history of safe food use in a non-EU country; the second one is related to a new category of novel food, which comes to cover “whole insects and their parts. Indeed recital 8 of Regulation 2283/2015/EU states that it is appropriate to review, clarify and update the categories of food which constitute novel foods. Those categories should cover whole insects and their parts.

In doing so, a source of discord arising from Regulation 258/1997 was overcome. In accordance to Regulation (EU) No. 2283/2015, to be authorized as novel food, edible insects shall, on the base of scientific evidence available, not pose a safety risk to human health and not mislead the consumer, especially when the food is intended to replace another food and there is a significant change in the nutritional value. The novelty of this new regulation consists in a more efficient procedure for authorizing ‘novel foods’ to be put in the EU market with shorter deadlines and in the creation of a centralized risk assessment procedure. Two authorization procedures are foreseen: a general authorization procedure and a specific (shorter) authorization procedure for traditional food from a third country. In the first case, the applicant who wants to place insect products for human consumption on the EU market must submit, to the European Commission, a dossier containing detailed information on the product and on scientific evidence demonstrating that it does not pose a risk to human health. The pre-market authorization must be based on risk assessment performed by the European Commission or passed to EFSA for further safety assessment. Article 7 provides that products lawfully placed on the market before 2018 ‘can continue to be sold for at least two years following its entry into application’ (i.e. a transitional period until 2 January 2020). While under Regulation 258/97 authorizations are only granted to

the applicant, the new Novel Food Regulation allows ‘generic’ authorisations to all those producing the product in question (i.e. no obligation to submit a separate dossier for the same product already authorized). This provision facilitates joint application notably by groups of producers covering the same insect species, assuming that similar species would share similar safety risks (due to diet, behaviour, and metabolism). In this context, a sound way of grouping insect species, could be the EFSA Qualified Presumption of Safety (QPS) approach, which was initially developed for microorganisms added to the food chain and now equally applied to botanicals (Belluco et al. 2017). However, the new text also opens the possibility to protect ‘scientific evidence or scientific data supporting the application’ for a five-years period (see article 26) and prevents another operator from benefiting an ‘initial’ authorisation (e.g. in case the authorisation covers the same product) through access to ‘supporting data’. In order to provide implementing rules for the administrative and scientific requirements for applicants, as required by art. 10(1) by 1 January 2018, the Commission recently adopted a draft implementing a regulation that was welcomed by the main associations and organization involved in the related sector (https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2017-3649060_en). In case of importing from a third country, the new Novel Food Regulation also considers specific pathways for insects in the category of Traditional Food from third countries and similar transitional measures. If the “novel” food has a history of safe use, the applicant must to simply notify to the European Commission the intention of placing the food on the market, including the documents providing the history of safety use in the third country. While the Novel Food Regulation was still not amended, some EU Member States and associated countries (Austria, Belgium, Denmark, Finland, Liechtenstein, Netherlands, Norway, and Switzerland) enacted their own interim legislation by means of guidelines to cope with the growing interest on edible insects in their countries (Belluco et al. 2017). These guidelines basically consist of two sections, i.e. connecting a country-specific set of edible insect species to regular food legislation (e.g. by defining them as ordinary livestock as done in Switzerland and, by extension, Liechtenstein) and providing specifications with regard to primary production, processing, surveillance, and trading. These specifications vary strongly in depth, extension, and precision among countries. The Finnish one is very extensive paper intended as a guide for food control authorities, primary producers of insects and businesses producing food from insects (EVIRA 2017). A special case is the UK where, with the occurrence of Brexit, it is unclear whether it will still follow the new EFSA ruling or proceed autonomously with its national Food Standard Agency (FSA) (i.e. no need of EFSA ratification) (Doberman et al. 2017).

The use of insects in animal feed has less challenging regulations to overcome since Regulation (EU) N°893/2017 amending the TSE Regulation, partially uplifted the feed ban rules regarding the use of insect PAPs for aquaculture animals, PAPs are currently limited to seven insect species, including three types of crickets, two types of mealworm, and two fly species. Despite the feeding of non-ruminant PAP to aquaculture animals was already allowed by Regulation (EU) No 56/2013 amending the TSE Regulation, this opening could not

be applied to insects due to the wording used, which refers to slaughterhouses, while insect larvae are not slaughtered before being processed into PAP. Indeed the use of insects as feed material has become an emerging issue in the EU with the Commission actively engaged in defining the safety concept for insects as feed (https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_marketing_concept-paper_insects_201703.pdf). A further good prospect is the plan to give green light soon for insects protein to be used also in feed for poultry (<https://agriorbit.com/door-opening-in-the-eu-for-insect-protein-for-poultry/>) and full stop pigs (However, many potential feedstuffs for insect remain banned such as manure, slaughterhouse offals, catering waste. Another aspect that still needs to be addressed by the regulatory framework and could raise ethical concern is the animal welfare in insects. Indeed, at the moment with the up-scaling of insects rearing, the scientific knowledge on farm conditions compatible with well-being is lacking (Erens et al. 2012). However, if we consider that most insect species used for farming naturally live in large groups in small amounts of space (in fact, this is one of the major selection criteria for farming them in the first place), raising large amounts of insects in small space in the industrial farming could mirror the natural condition and presumably the microlivestocks would not be stressed from overcrowding. Still, maximum densities will have to be assessed in order to avoid cannibalism which has been occurring in some species. Then, there is a debate if insects, as animals in biological sense, possess consciousness, if we lack the full understanding of the way insects experience any pain or if they indeed do at all (Erens et al. 2012). This is relevant for the animal welfare legislation, which is based on the principle that animals are sentient creatures. And this leads to the question: what is the humane way to kill an insect? Today the most widespread industrial killing method is by chilling insects to freezing temperature, which causes the insects to enter a state of sleep much like a coma. Generally, depending on the species, after 2-3 days of extended period of being frozen the insects die without regaining consciousness. This method compared with the one used for traditional livestock, is believed to induce a lower pain level than other methods (Dossey et al. 2016).

At present, the living and killing conditions of farmed insects are not regulated at EU level, since they are not covered by (vertebrate-based) regulations on animal welfare, transportation, and slaughter. This void is clearly raising ethical concerns in the current debate on animal welfare of insects used as food and feed (De Goede et al. 2013, Dumitras et al. 2015, Erens et al. 2012, Gjerris et al. 2016, Knutsson 2016).

Another issue which appears as a legal paradox is related to the definition provided by the Regulation (EU) N° 2017/893 which considers insects bred for the production of PAP as “farmed animals”. By definition, farmed animals have to be killed in a certified slaughterhouse, with a welfare officer present. In turn, killing in slaughterhouses implies immobilization and exsanguination. This lead fish farms to use chicken offal instead of insects to feed their animals (Kupferschmidt 2015). For the future, a clearer definition would be beneficial. Another aspect that needs to be clarified with the purpose of safety requirements and official control system, is the likely inclusion of insects among food of animal origin as defined in the sections of Annex II of

Reg. 853/2004, such as those of frogs and snails, and the obligations of producers to comply with rules and principles of general food law [Regulation (EC) No. 178/2002], the Food hygiene [Regulation (EC) No. 852/2004], and feed law as for Regulation (EC) No. 183/2005 on feed hygiene, closing the gaps mentioned at the beginning of this section. Despite the existing legal gaps, we can certainly say that, *vis-a-vis* the increasing trend that recognizes insects as an important, efficient and sustainable source of food and feed protein in Europe, the new Novel Food Regulation assigns to edible insects a clearer legal status than before 2018. This legislative action is a remarkable step forward to providing, on one hand, insect producers, suppliers and sellers with a better EU regulatory environment to plan their investment and marketing activities and, on the other hand, consumers with a safe and nutritious food product. Finally, official veterinarians will profit from this and further regulations in order to provide a sound and just service to all sectors engaged.

Risk assessment of insects as human food

The actual forecast indicates that in the near future, insects will be a relevant part of modern diet in Western societies, and food safety issues will have to be dealt with by practitioners, scientists, regulators and consumers. In this context, it is once again important to contemplate both traditional and modernized entomophagy.

By trial and error, methods have been developed to traditionally make use of insects as food resources, even species which are basically toxic. In the majority of cases, insects are consumed after heating (cooking, roasting, frying, etc.). This inactivates microorganisms and thermo-labile toxins (Menzel and D’Aluisio 1998). In fact, foodborne diseases or other pathological conditions appearing after traditional consumption of insects are usually related to ignoring these traditional food safety rules. To give an example, a beriberi-like vitamin deficiency was observed in Western Africa in patients that consumed large amounts of seasonally-available, raw notodontid caterpillars (*Anaphe venata*). They contain a thiaminase which is inactivated by boiling which in turn is the traditional way of consuming these caterpillars. By consuming them against traditional rules, the problem arose (Adamolekun 1993).

In modernised entomophagy, safety-related problems will appear just as with other foodstuffs. Modernisation in this sense also means uncoupling primary production from processing, from transport, and from trading, and this conveys more risks of contamination or fails in the food production chain. Thus it is thought that insect food safety is based on some common issues which are directly related to the insect (e.g. microbiology or venoms) plus specifications depending on whether insects are handled traditionally or submitted to modernised production systems.

The risk assessment of insects and derived products has not been comprehensively investigated, primarily due to a lack of scientifically based knowledge of insect processing especially on an industrial scale (Schlüter et al. 2017). However attempts were made recently in the EU. According to a Italian summary on the food safety of insects (Belluco et al. 2013), three EU countries by

then had addressed food safety of insects as food or feed in the framework of risk assessment, namely, Belgium (FASFC 2014), the Netherlands (<https://zenodo.org/record/439001>), and France (ANSES 2015).

In order to merge the scattered information on food safety of edible insects, the Commission asked EFSA to assess the microbiological, chemical, and environmental risks arising from the production and consumption of insects as food and feed (including pet food). The EFSA scientific opinion adopted in October 2015 (EFSA 2015) is not a risk assessment *per se*, but rather a risk profile for an indicative list of insects based on a list of hazards commonly found in other protein sources and based on data stemming from peer-reviewed scientific literature, Member states's assessments, and stakeholders' information. At that time, there are very limited and uncertain information on the risks associated with individual families or species of insects, details of the manufacturing processes used, environmental impact of different farming systems, and lack of human consumption data. Table 2 shows the paucity of data that currently affects each phase of the risk assessment of insect production and consumption. EFSA considered main biological hazards (bacteria, viruses, parasites, fungi, prions) and chemical hazards (heavy metals, toxins, veterinary drugs, hormones, and others) as well as allergenicity and environmental hazards. The results indicate that the risk of consuming insects is similar to other food protein sources. Moreover, conventional animal feed materials used as substrate for insect production are assumed to have similar microbial hazards of feed administered to other animals, while insects fed on substrates based on ruminant by-products need to be evaluated to control the risks of prions. The occurrence of chemical hazards in edible insects is not well-characterized, but the likely level of contamination depends to a large extent of the level of contamination of the substrate. Specifically the litter can accumulate heavy metals from their substrates, in particular cadmium.

The EFSA opinion highlighted a pronounced lack of data regarding microbiology, virology, parasitology, and toxicology of edible insects, and identified key factors that have an impact on occurrence and levels of these hazards in food and feed products derived from insects, namely: the production methods, the substrate used, the stage of harvest, the insect species, development stage, and methods for further processing. The opinion included also recommendations for further research and studies to reduce the scientific uncertainties that currently hamper the possibility to carry out a full risk assessment for certain insect species.

Prions

In regards to the TSE risk, prions are not able to replicate in insects as they do in mammals, due to the absence of PrP-encoding genes. This is the reason why insects at the present state of knowledge cannot be considered as biological vectors and amplifiers of prions. However, various studies suggested the possible role of insects (e.g. flies) as mechanical vectors of infectious prions if insects are raised on contaminated substrates (EFSA 2015). Hence as stated by EFSA, a contaminated substrate could play a relevant role for insects farmed for use as food and feed, if by-products from ruminants (e.g. certain tissues from ruminants because of BSE-related risks) were used (which is in fact why these tissues are

currently excluded from the food and feed). However, while insects fed on substrates of non-human and non-ruminant origin should not pose any additional risk compared to the use of other food or feed, those fed on substrates that include protein of human (e.g. human manure and sewage sludge) and animal origin need to be evaluated with the purpose of controlling the risks of prions (e.g. the risk would be much higher for material of ruminant origin). The effective control would be assured by submitting the material used as substrate to adequate thermal treatments prior using it.

Viruses

The many entomopathogenic viruses may cause disease or lead to mortality and colony collapse (Belluco 2009). However, most of these viruses cannot be transmitted to humans or other vertebrates such as farm animals and birds due to their species-specificity, even though they are taxonomically related to vertebrate viruses (King et al. 2012). Yet, they can act as passive or mechanical carriers of human and farm animal viruses (Wanarathana et al. 2013).

Some members of a few entomopathogenic families such as Iridoviridae, Parvoviridae, Flaviridae, Dicistroviridae, and Reoviridae also occur in food and feed insects, which may be important for colony health, requiring further assessment. A special attention must be paid to densovirus (Parvoviridae) and to the dicistrovirid *Picornavirales* viruses that cause infection respiratory paralysis in crickets. Both virus types have close relatives in humans (human parvovirus B19, polio, and hepatitis A virus), and might show their zoonotic potential by crossing the vertebrate/invertebrate border. Other viruses like arboviruses (or arthropod-borne viruses) that cause disease in humans (e.g. dengue, West Nile disease, Rift Valley fever etc.) or in farm animals can successfully replicate in their invertebrate vectors and for this reason are capable of crossing the species barrier (EFSA 2015). However, these vectors are mosquitoes which are not considered among the edible insects.

As stated by EFSA (2015), there is evidence that vertebrate viruses that are able to survive in the substrates used for insects produced for food and feed might be considered a hazard for vertebrates including humans. The related risk could be controlled if a proper substrate and a safe processing, e.g. by applying thermal and other effective preservation methods, are used.

Bacteria

Insects both collected in nature or raised on farms show a vast array of micro-organisms (microbiota) spanning from symbiotic to mutualist to pathogenic. Thus, some of them are vital symbionts for the insect, while others are entomopathogens (pathogenic bacteria affecting insect only), used sometimes in the biological control of pest insects (e.g. *Bacillus thuringiensis*). Once the insect dies, they are part of the spoilage microbiota. For other species, they can also be pathogenic, sometimes even zoonotic. There are two types of micro-organisms to be considered as potential risks: intrinsic microbiota harboured by the insect itself (digestive tract, and exoskeleton) and external microbiota present in the substrate, feedstuff, and litter (van Huis et al. 2013).

Intrinsic and external microbial flora

From the taxonomical point of view, it appears that the microbiota of a given insect species is composed of

TABLE 1: Examples of shortage of data for risk assessment of insect production and consumption

Risks assessment steps	Uncertainty and lack of data (some examples)
Hazard identification	<ul style="list-style-type: none"> • Biological hazard: information on the probability of transferring human viruses such as norovirus, rotavirus, hepatitis A from feed and intestinal contents of insect • Chemical risk: use of veterinary drugs for the treatment of insects bred for food or feed
Hazard characterization	<ul style="list-style-type: none"> • Ability of the insects to act as mechanical vectors
Exposure assessment	<ul style="list-style-type: none"> • Levels of human and animal consumption • Studies on the presence of human pathogenic bacteria in insects and animals processed for food and feed • Studies on the chemical contamination in insects and in the different substrates
Risk characterization	<ul style="list-style-type: none"> • Lack of epidemiological data on human food-borne outbreaks or allergies incidents caused by the consumption of insects or their products

micro-organisms which are common to all insects plus specific elements common in the different taxa, from order down to species (Douglas 2015, Engel and Moran 2013, Grabowski et al. 2017, Muthukalingan et al. 2014, Yun et al. 2014).

Within the gut, the bacterial communities vary immensely in total size, composition, locations and functions and the number of species is related to the insect species, its instar, and its diet. In recent years, the metagenomic analyses have shed a light on the microbial biodiversity present in the insect guts, with the identification of many previously unknown species (Gupta et al. 2014, Liu et al. 2013). The diversity of the gut flora is greater in omnivorous than in strictly herbivorous or carnivorous insects (Grabowski and Klein 2017b). Moreover the presence of some pathogens, e.g. enterococci, also depends on the season (Martin and Mundt 1972).

Since insects are genetically very different from higher vertebrates and their microbiota frequently lack cross-reactivity between mammals and insects (interfering thus with the replication of many human-pathogenic micro-organisms in insects), most of microorganisms (including pathogens) present in or on insects are considered by many to be safe for humans or are not involved in food spoilage or foodborne disease (Banjo et al. 2006, van Huis et al. 2013). Zoonotic bacteria and fungi were detected in or on insects. Still, these findings usually appear separately, meaning that one and the same pathogen was encountered in humans and insects, but no transmission after ingesting prepared insects has been documented to the knowledge of the authors (Grabowski et al. 2017b).

This blends into the external microbiota, the second source of micro-organisms emanating from insect production. Industrialized insect farming and processing's environment might be a source of the same bacteria (including pathogens) that affect other food production systems. This means that food safety of insects and derived products recognizes similar problems like other conventional food sectors, with some specific conditions related to different rearing, processing, storage, and transportation that affect the occurrence of microbiological and chemical hazards (eg. toxic metal elements and pesticide residues; Feng et al. 2018). In this way, insects (specially larvae) might serve as mechanical vectors through the body surface (Chaiwong et al. 2014, Lima et al. 2013, Vega and Kaia 2012) and become a natural reservoir for pathogenic microorganisms, without themselves becoming sick (McAllister et al. 1994).

The way insects are reared in the same company or between different companies might influence also the bacterial community composition and the related quality. Vandeweyer et al. (2017b) observed remarkable

differences between mealworm (*T. molitor*) and crickets rearing companies and related production cycles. For the mealworm, they found more variation in terms of microbial quality between companies, while crickets showed a high similarity among different companies, even between both cricket species investigated (*A. domesticus* and *G. sigillatus*). With respect to food safety, they noted a likely association with potential human pathogens such as *Cronobacter* spp. or spoilage bacteria such as *Pseudomonas* spp.

So, substrates used to feed insects and the farming environment can be a key entrance point for contaminations. Substrates can be feed materials authorized as feed for food producing animals, by-products from animals fit for human consumption at slaughterhouses, or manure, each one bearing different levels of hazard potential, including biological (bacteria and prions) and chemical hazards in non-processed insects. It is evident that a clean insect farm or processing plant with well-implemented good hygiene practices can reduce microbiological contamination of the facility. However, care must be taken not to eliminate those parts of the microbiota which fulfill important physiological tasks in the insects (Tanada and Kaya 1993).

Microbial profiles of raw insects

Research on insect microbiology is currently developing, and in these first stages, results are sometimes hard to compare because of differences regarding insect species, instar, processing, and analysis and evaluation methods.

As other earth-dwelling animals, raw insects contain elevated bacterial and fungal counts on both the animal surface and inside the gastro-intestinal tract. In some farmed, raw animals, the total viable counts (TVC), mostly Gram-negative (e.g. faecal and total coliform bacteria) and Gram-positive bacteria (e.g. *Micrococcus* spp., *Lactobacillus* spp., and *Staphylococcus* spp.) are similar in the different species, ranging overall from 4.0 to 7.0 log cfu/g, although higher levels have also been reported, e.g. TVC in fresh mealworms (7.0 – 8.0 log cfu/g). Silkworm pupae (*B. mori*) yielded less micro-organisms, as pupation usually goes along with an elimination of all internal microbiota along with a re-arrangement of body tissues, making pupae by themselves microbiologically sterile (Barbehenn and Kristensen 2003), and eventual bacterial counts the result of contamination from outside. Generally the microbial load of fresh mealworms after rearing is high, with TVC generally being about 7 to 8 log cfu/g (Grabowski et al. 2014, Klunder et al. 2012, Stoops et al. 2016). Raw edible insects sold in Germany showed a high degree of variety of bacterial counts between species, referred to TVC, Enterobacteriaceae, staphylococci, yeast, mold and bacilli. TVC ranged from

5.7 (*A. mellifera*) to 7.5 log cfu/g (*A. domesticus*), staphylococci ranged from negative (butterworm *Chilecomadia moorei*, a cossid moth) to 6.1 log cfu/g (*T. molitor*). Bacilli were detected at high levels from 3.5 (*A. domesticus*) to 7.2 log cfu/g (*C. moorei*). Also Enterobacteriaceae counts were elevated. Differences were noted also between living insects and dead ones, with the latter having higher counts. In this study none of the samples (n = 39) contained *Salmonella* spp. or *Escherichia coli*, and *Bacillus cereus* was absent in all samples (Grabowski and Klein 2017a). However this spore-forming bacterium merits a special attention because it has been isolated frequently with high bacterial counts in fresh samples (Belluco et al. 2013) although it was found in concentrations <100 cfu/g (correct) in 93% of the samples (NVWA 2014). Giaccone (2005) carried out the microbiological analyses of five insect species with rearing potential, namely superworm (*Z. atratus*), yellow mealworm (*T. molitor*), wax moth (*Galleria melonella*), butterworm (*C. moorei*), and house cricket (*A. domesticus*). Neither *Salmonella* spp. nor *Listeria monocytogenes* were detected in the tested samples.

In a study conducted in Africa by Klunder et al. (2012) on farmed mealworms (*T. molitor*) and house crickets (*A. domesticus*), and wild-harvested large gryllid crickets (*Brachytrupes* spp.), fresh insects showed a high microbiological load, principally composed of Enterobacteriaceae and heat-resistant spore-forming bacteria. The authors recorded TVC of approx. 7.0 log cfu/g and Enterobacteriaceae counts of 4.0 to 6.0 log cfu/g, which are typical values for foodstuffs that were harvested from or stored on soil.

Megido et al. (2017), in a study on edible insects from the Belgian market [i.e., European farmed *T. molitor* and *A. domesticus* and Congolese wild-harvested, smoked termites (*Macrotermes* spp.) and saturniid caterpillars (*Cirina forda*)], found that fresh mealworms had a high TVC (approx. 8 log cfu/g) in accordance with other studies showing a level from 7.7 to 8.3 log cfu/g. Similarly the TVC of fresh house crickets showed a level of 7.2 log cfu/g, which is consistent with the results of Klunder et al. (2012).

Regarding opportunistic pathogens, bacilli (*Bacillus thuringiensis*, *B. licheniformis*, *B. pumilis*), and *Pseudomonas aeruginosa*, were encountered in Mediterranean field crickets (*G. bimaculatus*) and superworms (*Z. atratus*). The latter can also affect immunocompromised patients during nosocomial infections (Grabowski and Klein 2015, 2016a).

Microbial profiles of processed insects

The microbiological properties of Botswanan saturniid processed caterpillars (*phane*; *G. belina*) were studied extensively by Mpuchane et al. (2000). Total aerobicial counts (TAC) varied from 1×10^2 – 2×10^3 cfu/g for laboratory-processed *phane* to 1×10^4 – 2×10^8 cfu/g for *phane* crafted conventionally. Another study contemplated raw and processed *Gryllus assimilis*, *L. migratoria*, *T. molitor*, and *Z. atratus* in Germany. All samples were negative (below detection limit) for salmonellae, *L. monocytogenes*, *Clostridium perfringens*, *Staphylococcus aureus* (massive coagulase-negative staphylococci though), and *Campylobacter* spp. TAC ranged between 4.0 and 6.7 lg cfu/g, Enterobacteriaceae between 2.0 to 6.0 lg cfu/g, *E. coli* between 1.0 and 3.7 lg cfu/g, and coagulase-positive staphylococci between 2.0 and 3.0 lg cfu/g. The microbial pattern was species-specific and processed insects showed lower counts than raw and unheated insects with

a significant differences (Grabowski and Klein 2016a). Klunder et al. (2012) demonstrated that boiling insects in water for a few minutes eliminated Enterobacteriaceae and reduced other counts significantly (e.g. TBC: <1.7 to 2.5 lg cfu/g, varying with the species), while crushing increased them, possibly because of the release of bacteria from the gut. Spores were found to survive this process and with favourable conditions (°C and moisture) could germinate and the bacteria grew, causing food spoilage. Megido et al. (2017) evaluated the efficiency of different processing methods (i.e., blanching, freeze-drying and sterilization) in reducing microorganism counts and confirmed that fresh insects, but also smoked insects from non-European trades, need a cooking step, at least composed of a first blanching step, before consumption. In Belgium, the Federal Agency for the Safety of the Food Chain postulates that a heating step, such as blanching, is necessary to reduce microbial numbers on insects before they are placed on the market (Ngonlong et al. 2016). The effect of blanching was further studied by Vandeweyer et al. (2017b) who noted considerable reductions on TBC, Enterobacteriaceae, lactic acid bacteria (LAB), yeasts, moulds, and psychrotrophs, except for aerobic endospores. The effect of treatment was also corroborated by a previous research (Grabowski and Klein 2016a), finding that, as for other food-stuffs of animal origin, the heat treatment inactivates most pathogenic microorganisms. However, the kind of heat treatment also seems to be an important factor of influence. When submitting crickets (*G. bimaculatus*) and superworms (*Z. atratus*) to four different drying techniques (after boiling), none of the samples yielded salmonellae, *L. monocytogenes* nor *E. coli*. However, bacterial counts (TBC, Enterobacteriaceae, staphylococci, bacilli, yeasts and moulds) varied strongly displaying species- and treatment-specific patterns and the combination of various temperatures during the drying process resulted in the most effective way to reduce most bacterial and fungal counts. Still, TAC remained high more pronounced in crickets than in superworms while foodborne pathogens but *S. aureus* were eliminated by all treatments. In this way, processing changed the microbiota species-specifically (Grabowski and Klein 2016b). A similar set of microbiological parameters – in fact mainly those proposed by Belgian and Dutch authorities – was applied to processed insects originating from various parts of the world. The results suggested an influence by the product type, allowing to define two product classes in terms to bacterial counts. One class comprises cooked and deep-fried products and is characterised by low bacterial counts, while the other class contains milled and (freeze-)dried insect products that display markedly higher counts. Still, each product type revealed a microbiological profile of its own. Again, samples were negative for salmonellae, *L. monocytogenes*, *E. coli* and *S. aureus*, but dried and powdered insects contained *B. cereus*, coliforms, *Serratia liquefaciens*, and *Listeria ivanovii* (Grabowski and Klein 2016a). The presence of emerging opportunistic pathogens showed the need to establish effective, species-specific drying procedures to ensure food safety with a maximum of food quality.

Evaluation

These results, along with those obtained in raw insects, show a very heterogeneous picture which could be expected considering the vast array of insect species, processing methods, and products. To compare it with

more common situations, reviewing the microbiome of insects and products made thereof is like reviewing the one of mammals and the foodstuffs made from them, e.g. reaching from steak tartare and blubber to sausages and canned corned beef.

Following Regulation (EC) 2073/2005 with its division of criteria into process hygiene and food safety criteria, it was seen that basically, bacterial counts in raw insects are high and may be reduced by appropriate heating steps, at least in the case of some microbiological parameters. If processing is insufficient, bacterial counts start to increase again during storage. Stoops et al. (2016) explain increased bacterial counts with the presence of gut bacteria. Traditionally, many harvested insects are either degutted, left to fast for a certain time, or fed more "pleasant" feeds before killing and processing. This is basically done to improve taste (Menzel and D'Aluisio 1998), but it was thought that particularly fasting would also add to the reduction of bacterial counts, a theory which by now was rejected. Possibly, high bacterial counts in insect meals may be attributed to breaking the natural barrier of the gastrointestinal tract, allowing thus gut bacteria to spread and colonize the entire product.

In any way, decontamination is mandatory in terms of process hygiene. Typically, heating procedures are applied, but other methods (high pressure, plasma, irradiation, microwave etc.) are also subject of research (e.g. Vandeweyer et al. 2017). Results so far showed that any decontamination has to be evaluated carefully with regard to species specificity.

In relation to food safety criteria, two kinds of pathogens should be considered, at least in the initial stages of insect production, i.e. those pathogens which have been found separately on both insect (intrinsic) and human being (Grabowski and Klein 2017a, Grabowski et al. 2017), and the "classic" food pathogens that originally play no major role in the insect but contaminate it along the production chain, although there may be overlapping between these two categories. The occurrence of bacteria potentially pathogenic for vertebrates on insects has been the focus of many recent studies, but still there is much more to be investigated due to the many different edible species that are produced and consumed, each one with proper biological cycle and farming conditions. In relation to the zoonotic pathogens that are typically involved in food-borne diseases, such as *E. coli* and *Salmonella* spp., (the risk assessment conducted by EFSA concluded that their presence and in particular *Salmonella* spp., *Campylobacter* spp., verotoxigenic *E. coli* in non-processed insects, compared to other animal protein sources, might be equal or lower depending on the substrate used and the rearing and specific management conditions (EFSA 2015). Hence the risk of infection can be modulated by a combination of the substrates and the processing steps between farming and consumption. The main species isolated from insects belongs to the following genera: *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Bacillus*, and *Clostridium*, or belong to the Enterobacteriaceae, such as *Escherichia*, *Enterobacter*, *Salmonella*, *Klebsiella*, *Serratia*, *Shigella*, *Yersinia*, and *Acinetobacter* (Agabou and Alloui 2010, Giaccone 2005, Amadi et al. 2005). Having limited data collection to farm insects only, the EFSA list of traditional foodborne pathogens mentioned *E. coli*, *Salmonella* spp. and *Aspergillus* spp.. *Klebsiella pneumoniae* has been described as the most frequent bacterium in the

gut of the Oriental migratory locust (*Locusta migratoria manilensis*), and the subspecies *pneumoniae* was isolated from the pentatomid bug *Nezara viridula* (Medrano and Bell 2017), which apart from being edible is a significant vector of cotton boll-rot pathogens. It is worth to note that *Klebsiella* is a recognized source of nosocomial and community-acquired human pneumonia infections and a multi-drug resistance species (Calbo et al. 2011).

As for other foodstuffs, the possibility to detect pathogens in insects is hampered by the limit of classical culturing, which expectedly yields less data than microbiome analysis conducted with molecular biology techniques. While next-generation sequencing technologies, such as Illumina, Solexa, and 454 amplicon pyrosequencing have changed the scenario of metagenomic sequencing (Muthukalingan et al. 2014) and have been used to study microbial communities in diverse environments and food products, surprisingly they have not yet been applied to investigate the microbial quality of fresh edible insects. Belda et al. (2011), in a research on the microbiome in the crambid European corn borer (*Ostrinia nubilalis*) found a series of pathogens not encountered so far in other insects like *Brucella abortus*, *B. melitensis*, and *Streptococcus pyogenes*. Garofalo et al. (2017), with the aim to elucidate the microbiota associated with edible insects, analysed microbial species occurring in some processed marketed edible insects, namely *A. domesticus*, *L. migratoria*, and *T. molitor* through classical microbiological analyses and pyrosequencing. They found a great bacterial diversity and variation among insects. Along with low counts of total mesophilic aerobes, Enterobacteriaceae, *C. perfringens* spores, yeasts and moulds, pyrosequencing allowed the detection of several gut-associated bacteria, some of which may act as opportunistic pathogens in humans. Although viable pathogens such as *Salmonella* spp. and *L. monocytogenes* were not detected, the presence of *Listeria* spp., *Staphylococcus* spp., *Clostridium* spp. and *Bacillus* spp. was confirmed. Moreover, insect gut metagenomic methodologies allow for discovery of novel genes, protein and enzymes that can have a potential in industry applications (Muthukalingan et al. 2014, Krishnan 2014).

Insect guts are a reservoir of antibiotic resistance genes with the potential for dissemination. Tetteh-Quarcoo et al. (2013) and Wannigama et al. (2014) found cockroaches (*Periplaneta americana* and *Blattella germanica*) from food-handling facilities, households, and a hospital being carriers of antibiotic resistance traits. Tian et al. (2012) by applying a metagenomic approach to screen for antibiotic resistance in bacteria from the gut of adult honeybees detected an accumulation of mobile genes coding for resistance to tetracycline and oxytetracycline that were closely related to genes from human-pathogenic strains. Allen et al. (2009) characterized the antibiotic resistome in cultured isolates from microbial community in midguts gypsy moth larvae (*Lymantria dispar* L.) and found a novel beta-lactamase that confer resistance to *E. coli*. Lowe and Romney (2011) isolated vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* from five human bedbugs (*Cimex lectularius* L.) in Vancouver, Canada. Despite the above larvae are not edible, these studies provide interesting evidences that insects may act as environmental reservoirs of antibiotic-resistant bacteria. More recent studies found the presence of antibiotic resistance genes in several

species of marketed edible insects (Milanovic et al. 2016, Osimani et al. 2017). All these studies suggest a prudent use of antimicrobial compounds in insect farming to prevent selection and transmission of antibiotic-resistant bacteria (and their genes) associated with this novel food.

Fungi

Fungi are frequent on the surface and in guts of insects that feed on wood or detritus, where they likely play a part in digestion (Engel and Moran 2013). Like other components of the microbiota, some of them can be pathogenic to insects by means of specific toxins and cause mortality. Some species are used in the food industry as biocontrol agents for insect pests. Despite incidence of invasive fungal infections is rising especially in immunocompromised individuals (oncology patients and transplant recipients) (Low and Rotstein 2011, Chian-Yong and Rotstein 2011), diseases associated with entomopathogenic fungi are seen occasionally, and in general these insect-pathogenic specific fungi have a very good safety record both for vertebrate animals as well as for the environment (EFSA 2015). As seen with bacteria, the insect mycobiome is made of genera and taxon-specific fungi. Among fungi found in insects, potentially human-pathogenic ones account for 31% of all listed genera and species (Grabowski et al. 2017a).

The fungi most commonly isolated include genera such as *Aspergillus*, *Penicillium*, *Candida*, *Fusarium*, *Cladosporium*, and fungi previously subsumed under the nowadays obsolete class *Phycomycetes* (Simpanya et al. 2000). Data on fungi in processed insects is scarce. Mpuchane et al. (2000) studied the fungal population inside dried saturniid caterpillars of *mopane* (*G. belina*). With 47% of positive findings, *Aspergillus* spp. was the most common fungus present in this product. Contents of aflatoxin ranged between 0 and 50 µg/kg. *Penicillium* spp., *Chaetomium* spp., and *Fusarium* spp. were isolated sporadically from this product. Amadi et al. (2005) analysed fresh emperor moth (*Bunaea alcinoe*) caterpillars, and obtained fungal counts 2.1×10^6 and 1.3×10^6 cfu/g on the skin and in the intestines, resp. *Mucor* spp., *Aspergillus* spp., *Penicillium* spp., and *Cryptococcus neoformans* were found in dried and powdered insects (Grabowski and Klein 2015, 2016a). Also Braide et al. (2011) investigated into the microbiological status of processed caterpillar of *Bunaea alcinoe*. A total of 9.5×10^6 cfu/g of fungi was isolated, namely: *Aspergillus*, *Penicillium* and *Fusarium*, and one species of yeast, *Saccharomyces cerevisiae*. Some fungi (*Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.) and yeasts are considered in both the Belgian regulation and EFSA scientific opinion that regard fungi as relevant risk factors for the consumer due to the production of mycotoxins, e.g. *Alternaria* spp. (alternariol, alternariol monomethyl ether, altenuene, tenuazonic acid, altertoxin-I), *Aspergillus* spp. (aflatoxins B1, B2, G1, G2, ochratoxin A, ochratoxin B, sterigmatocystin), and *Penicillium* spp. (patulin, penicillic acid; Jay et al. 2005). Mpuchane et al. (1996) found levels of aflatoxins varying from 0 – 50 µg/kg of product, while the maximum safe level set by FAO is 20 µg/kg. The possible contamination of insects with pathogenic moulds with known allergenic potential, such as *Aspergillus* spp. and *Penicillium* spp. or pathogenic yeasts such as *Candida* spp. should be taken into account as a secondary trigger of allergic reactions, i.e. not directly due to the insect (SKLM 2016).

Yeasts were found in considerable amounts in fresh, freeze-dried as well as in frozen insects such as mealworm and the migratory locust (*T. molitor* and *L. migratoria*; FASFC 2016). As with other foodstuffs, the risks by arthropod-associated fungi will depend on the production level and the task of the individual along the food chain (e.g. hunter-gatherers or people producing arthropods for private consumption, animals are gathered/reared and sold on local markets, either raw or processed). Several steps between producers and the end-consumer may be occurring. The longer this chain becomes, the more the risk of human pathogens/spoilage increases (Grabowski et al. 2017). The fungi can contaminate insects from leaves and soil and during processing re-contamination can occur due to unhygienic conditions of drying and storage. Hence, it is important to adopt hygienic measures in the entire production chain, such as working on dry culture media, periodically removing the faeces and frequently changing feed. Routine disinfection of farming beds and residual materials after each growing cycle are also indispensable.

Parasites

Parasites that use insects as intermediate or temporary hosts, can pose a risk to humans when insects are consumed raw or insufficiently cooked (Belluco 2009, NVWA 2014, Chai et al. 2009, Hinz 2001).

As regards to protozoa, *Entamoeba histolytica* and *Giardia lamblia*, two potential foodborne and waterborne pathogens, were isolated in cockroaches and in some species of flies. Two other groups of cockroaches, i.e. American (*Periplaneta* spp.) and German cockroaches (*B. germanica*), can also harbor *Sarcocystis* spp. and *Toxoplasma* spp. (Graczyk et al. 2005).

As with viruses, insects can serve as vectors for severe parasitoses, e.g. Chagas disease (*Trypanosoma cruzi*), but the vectors (reduviid bugs; Pereira et al. 2010) are not considered edible.

Chemicals

For the chemical risk associated to insect consumption, different factors related to the production methods, substrate, stage of harvest and insect species need to be considered. Different feedstock and insect combinations may lead to different risks. Chemical contaminants can originate from the natural or artificial sources or can be produced by insect metabolism (venoms, toxins; see above) and found in insect derived food and feed products. These chemicals include environmental contaminants (e.g. heavy metals, dioxins), mycotoxins, and plant biocides used to clean facilities and equipment or veterinary drugs to treat certain diseases. Examples might include bioaccumulation (metals and environmental contaminants), concentration of natural contaminants (mycotoxins), and transfer of toxic residues (e.g. pesticides). The main factors influencing the rate of bioaccumulation of metals in insects are the species, the metal in question, and the growth stage, with larvae showing higher concentrations than adults (Lindqvist 1992). The bioaccumulation is less likely to occur in insects with a short life cycle than in insects that are reared over a longer time period, but data are lacking to conclude on the extent of accumulation in comparison with food producing animals (Ooninx and de Boer 2012). So far, not many chemical studies have been conducted to evaluate the toxicity of whole insects or their proteins, not even pertaining a particular instar.

Available studies show that transfer of heavy metals from substrates (e.g. organic matter, plants) to insects is apparently the most important route of contamination. To confirm the role of substrates as the main source of chemical contamination of insects, some studies found that mealworm larvae fed on organic soil matter bioaccumulate cadmium and lead in different parts of the body, mainly in fat, cuticle, reproductive organs, and digestive system (Diener et al. 2011, Lindqvist and Block 1995). Poma et al. (2017) found that in composite samples of several species of edible insects (greater wax moth, migratory locust, mealworm beetle, buffalo worm), the organic chemical mass fractions were relatively low (e.g. PCBs and dioxin compounds), generally lower than those measured in common animal products, and the levels of Cu and Zn were similar to those measured in meat and fish in other studies. These results indicate that no additional hazards are in insect in comparison to the more commonly consumed animal products. Handley (2007) documented that species caught in fields are more likely to contain pesticides or heavy metals than those collected in dense forests. *Chapulines*, pyrgomorphid grasshoppers (*Sphenarium purpurascens*) harvested in regions like southeast Mexico (Oaxaca), have been found to contain high concentrations of lead from nearby mines. Other insect-related chemical hazards are metabolic steroids (including testosterone and dihydrotestosterone) found in dytiscid beetles that can potentially cause growth retardation, hypofertility, masculinization in females, edema, jaundice, and liver cancer (Belluco et al. 2013). It should be that the technological treatments used in insect production have minimal effects on the concentrations of chemical contaminants which contrasts to their efficacy to meet biological hazards. As for other food-producing animals, but to a likely minor extent, another concern is the antibiotic-resistance as result of inappropriate and excessive use of antimicrobials or other veterinary drugs in insect farming. The related creation of antibiotic-resistant populations of microbes is a risk for both the environmental exposure and human consumption. This can be the case after decades of routine treatment of honeybee colonies with oxytetracycline for control of larval pathogens with the potential spreading of resistance genes (Tian et al. 2012). Most of the drugs are used for emergency treatment of diseases caused by bacteria, fungi or microsporidia (Eilenberg et al. 2015). In the specific case of silkworm farming, one of the most used antibiotics added to artificial diet to fight against frequent bacterial infections is chloramphenicol. However, due to the limited time of treatment, the risk would be lower compared to treatments in other livestock species. Treating production insects has become a debate, and some countries with insect guidelines forbid the treatment, e.g. Switzerland. Currently in the EU, the veterinary (residue) drug legislation does not contain provisions for insects. The only insect product in which maximum residue limits have been set for a few veterinary drugs is honey (Regulation (EU) No 37/2010). Among the risks exposed so far, the chemical and pharmacological ones appear one of the least-attended. Much research will be needed to develop not only appropriate treatment regimes for the different species in view of production diseases, but also adequate test systems that ensure insect products are placed on the market without iatrogenic inhibitory substances that are differentiated from intrinsic inhibitory substances secreted by the insect itself.

Allergenic potential of edible insects

Insects and insect-based products, just like crustaceans, arachnids, and shellfish can cause allergic reactions like eczema, rhinitis, conjunctivitis, angioedema and bronchial asthma by bite, contact or inhalation (Barre et al. 2014). Even anaphylactic shocks in humans caused by consumption of insects have been documented. The allergic reactions might develop in individuals already sensitized to insects or through cross-reacting allergen or *de novo* sensitization without prior exposure. Laboratory research has established cross-reactivity between dust mites and crustaceans in 80% of those who are allergic to crustaceans. The insect allergen causing the cross-reaction has been identified as tropomyosin which is also found in dust mites and crustaceans. The same type of allergens, usually glycoproteins, are found in molluscs and insects, and may also lead to cross-reactions. The relatively close phylogenetic relationships between the different phyla of arthropods with the existence of B epitopes in some common allergens (pan-allergens) can explain the cross allergy between edible insects and other arthropods, mites and other arachnids, and shellfish (Verhoeckx et al. 2014).

The cross-reactivity is also based on other allergens such as triosephosphate isomerase, tubulin and arginine kinase. The latter is known to be an enzyme responsible for allergic cross-reaction between different crustaceans, mites, the silkworm (*B. mori*), and cockroaches (*P. americana*, *B. germanica*; Verhoeckx et al. 2014, Liu et al. 2009). It is important to note that the risk of allergic reactions to chitin increases in those people who, through long-term exposure to an allergen in sufficient quantities, have developed a sensitivity (van Huis et al. 2013). Chitin, a natural polysaccharide of glucosamine, is present in insects, as well as in the exoskeleton of crustaceans and in the cell wall of lower organisms such as fungi, is also suspected to cause severe allergic reactions in susceptible patients. In addition, chitin may have antinutrient properties due to the potential negative effects on protein digestibility (Belluco et al. 2013). Recently, EFSA stated that the intake of 5g of chitin-glucan from shellfish means no health risk to humans (EFSA 2010). However, a contrasting view on this subject hypothesizes that the protein from chitin-containing organisms are responsible for the allergic reaction rather than chitin itself. The allergenic activity of foods derived from insects can be enhanced during the production process by revealing hidden allergens or concentrate allergens already present (EFSA 2015). It is therefore critically important that the potential for allergies is labelled very clearly on all foods made from insects and foods containing insects. Today the Liquid chromatography-Mass Spectrometry (LCMS/MS) enables the identification of known allergens including tropomyosin, arginine kinase, and myosin light chain and bioinformatics are able to search for orthologues of allergens where insect genomes are available. A high homology may indicate allergenic potential (PROteINSECT 2016). A less dramatic view on chitin is offered by findings that, by inducing non-specific host resistance against infections of bacteria and viruses, it has a potential for boosting immune system functioning by modulating the immune response depending on the administration route and size of the chitin particles (Lee et al. 2008, Muzzarelli 2010, van Huis et al. 2013) and this might make it a promising alternative to antibiotics (van Huis et al. 2013).

TABLE 2: Potential hazards associated with the rearing and processing of insect as human food

PHASE	HAZARDS (B = biological; C = chemical; P = physical)
Environment	Human pathogens, pesticides, heavy metals, mycotoxins
Insect rearing and reception of raw material	B: <i>Salmonella</i> spp., <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus</i> (<i>E. faecalis</i> , <i>E. faecium</i> , <i>Aeromonas hydrophila</i> , <i>Bacillus cereus</i> , <i>Clostridium</i> (<i>C. perfringens</i> , <i>C. septicum</i> , <i>C. difficile</i> , <i>C. sporogenes</i> , <i>Listeria</i> spp., mechanical or biological vectors of prions C: pesticides, herbicides, dioxins, heavy metals (selenium), additives (legal requirement for toxic dose), mycotoxins P: soil, stones, wood, plastic fragments
Processing	
Killing	B: bacteria* multiplication present on raw material
Bleaching and boiling	B: no inactivation of pathogens present on raw material due to fails on temperature/time C: heavy metals
Rapid cooling	B: recontamination with pathogens (spore germination) and growth (<i>C. perfringens</i> , and other pathogenic <i>Bacillaceae</i> , <i>L. monocytogenes</i> , <i>Salmonella</i> spp.) C: heavy metals
Storage under refrigeration	B: recontamination or growth of sporulated bacteria C: histamine, mycotoxins (aflatoxins, beauvericin); enniatin A and A1
Mincing	B: bacteria multiplication/contamination C: heavy metals from water P: metals particles
Freeze drying	B: recontamination or growth of sporulated bacteria
Grinding	B: bacteria multiplication P: metals particles
Packaging finished products and labeling	B: pathogen contamination (e.g. <i>Aspergillus</i> spp., <i>L. monocytogenes</i> , <i>Salmonella</i> spp.) C: ink, bisphenol A and phthalates, allergens not identified in the label P: metals
Storage end product	B: recontamination with pathogenic microorganism and growth of <i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Fusarium</i> spp. C: tyramine, histamine, mycotoxins (aflatoxins, beauvericin)

* Bacteria: includes pathogens and food spoilers

Food safety risk management of edible insects and consumers risk

The risk management interventions to reduce or eliminate the food safety risk (e.g. microbiological and chemical hazards) associated to human consumption of insects or parts thereof are not different compared to other food stuffs. A safe final product is the result of the proper implementation of preventive measures at primary production level that include control of quality and safety of raw insects, the substrates used for insect farming as well as proper implementation of good hygiene practices (GHPs) through the remaining steps such as production, processing, storage, and transport.

Essentially, the same food chain-integrated concept used for other foodstuffs would work with the insect industry, with specific risk interventions applied to each level of the production chain. The adoption of GHPs can contribute to the control of cross-contamination during rearing and processing, while the implementation of a Hazard Analysis and Critical Control Points (HACCP) system as science-based and systematic preventive tool (van Huis et al. 2013) can be used to identify specific hazards and to monitor them at critical points, where they can likely occur, during insects processing, which includes all the activities after the harvest, namely killing, cooking, freezing, drying, mincing, grinding, and packaging, among others (see Table 2).

Unlike the complex hot-blooded farm animals slaughter, the production steps in insects industry are simpler and usually composed by a fasting of about 24 hours to reduce the gut content to increase palatability (see above). The killing is made by reducing the temperature (freezing 24 hours, $-18\text{ }^{\circ}\text{C}$) or by heat, while the processing steps are basically similar to those used in processing conventional food products, taking into consideration the specificities of the insects (Fraqueza and Patarata 2017).

The risk exposure of consumers is expected to change in relation to the different levels, with higher risk associated to the consumption of unprocessed insects and lower risk at the end of the food chain, where the majority of insects are processed in a way to eliminate the human pathogens.

Regarding control on the farm, the essential steps to be considered are: breeding (adult colony and egg production), production (insect growth management and feedstuff), separation (separating animals from residues); processing, store, transport, and sale (<http://ipiff.org/good-hygiene-practices/>). Each step might be affected by species-specific food safety risk factors that must be identified and described in the operator's own control system and managed/modulated taking into account the nature of operation, level of automation of the production process, training of staff on health hazards. In the self-monitoring plan, the operator clarifies how they supervise and comply with the rules related to the safety and quality of the insect they process.

Similarly to other foodstuffs, the operators have to ensure that the activities and the insects produced fulfil the legal requirements and that food safety is not compromised. Self-monitoring and its thorough documentation is one of the basic tools demanded by the authorities once they evaluate any food-related business. The EU food legislation does not currently cover the specific requirements for insects. Only basic hygiene requirements set up in the regulation on the hygiene of foodstuffs (Regulation (EC) No 852/2004) might be applied to the primary production. Another concern is the definition of "primary production" in insects. In Switzerland, primary production covers all the steps from oviposition until killing, heating, and freezing, so that no living insect leaves a Swiss farm. In Denmark, primary production stops at the moment of selling living insects. In addition, the general food regulation (EC) No

178/2002 applies when it comes to define the principles of responsibility of operators and traceability, record keeping, cooperation between operators in the food chain and with authorities to manage potential risk of products under their control.

As seen in section “Risk assessment of insects”, the microbiological safety of the insect and derived products is influenced by an intrinsic microbiota and the external microbiological (cross-)contamination during breeding, processing, storing and preservation phases. A marked variety of preservation methods are available, but high safety and quality can be assured only by specific measures that take into account the biological makeup of different insect species.

Reducing the microbial load in raw insects by improving husbandry conditions or traditional methods before and during harvest represents a difficulty due to the high density of insect monocultures. Moreover, as explained earlier, different feed substrates can affect the composition of gut microbiota as well as the proportion of individual species depending on the insect species and developmental stage (Montagna et al. 2015, Perez-Cobas et al. 2015, Yun et al. 2014). The composition of the microbiota also changes during the complex individual development of insects and can be influenced by diet and environmental conditions (Singh et al. 2015). The processing must include control of the gastrointestinal contents. This is typically done by letting them fast. Degutting, as done traditionally to larger caterpillars and grubs, will be a challenge for technification, just as peeling common shrimps (*Crangon crangon*) mechanically was. As mentioned before, gastrointestinal depletion is more associated with improving the taste rather than that reducing the microbial risk. Since the total removal of gut with its microbiota cannot be assured, the ratio of gut content to total mass is of particular interest in relation to the preparation of insects as food (SKLM 2016). Taking the unavoidably high microbial contamination risk in raw insects it becomes clear that modernised entomophagy has to adopt GHP and should also follow the entomophageous tradition which foresees a heating step before consumption in order to eliminate pathogens such as salmonellae, *E.coli* and bacilli. In such cases and like with other foodstuffs, hygienic handling is equally important to prevent the potential risk of re-contamination and cross-contamination. If the insects are to be sold unheated and frozen (like e.g. in Finland), there has to be instructions on the package advising the insects to be heated before use. At more operational levels, one way to manage food safety risk during processing could be to identify a step in the HACCP system, to which a process hygiene criterion applies (i.e., a given microbiological parameters and its corresponding evaluation scheme), e.g. after the heat treatment, since it usually results in microbial inactivation or growth inactivation (Jay et. al 2005). In term of risk exposure and with a more social and biological perspective, it can be argued that for evaluating an individual or community risk, much more information is necessary on the cycle-related occurrence of pathogens. In fact, some of them are only present in certain developmental stages (instars) and absent in others; e.g. the autosterilisation during pupation and seasonal occurrence of certain bacteria as mentioned before. The traditional entomophagy as practised on different levels of technification, ranging from simple gathering and consumption by one and the same person to coor-

inated market for entire countries, might contribute to a different exposure and diversification of risk. Indeed in some parts of the world with traditional entomophagy, few cases of raw consumption were reported. However, this food habit seems to be limited to certain species under very specific circumstances in which experience demonstrated that there are no risks. Most animals, however, are heated, especially those that would be toxic and in which heating inactivates these toxins. Is the case of consumption of raw arthropods, which is also part of the entomophageous tradition worldwide (Chung 2010, Costa-Neto and Ramos-Elorduy 2006, Grabowski and Klein 2017b, van Huis et al. 2013, Yen 2010, Yen and Ro 2013). The results of food surveys conducted among the population in Thailand and Nigeria revealed that 93% and 30% of respondents respectively, admitted to consume raw insects (Hanboonsong et al., 2001, Adeoye et al. 2014). In China, uncooked, raw larvae are also consumed in some areas (Feng et al. 2018). In Mexico, pentatomid stink bugs (*jumiles*), and in particular the species *Atizies taxcoensis*, are among the most treasured delicacies that are typically eaten live and are prized for their powerful anise-like flavour and cinnamon finish (<https://www.theguardian.com/world/2013/jul/23/mexico-insect-cuisine-sustainable-food>). However, no data on the food safety of these products have been published yet. On one hand, it may be presumed that no human pathogens may be transmitted by these species or human cases were not notified. On the other hand, consumers may not be acting reasonably and ignore the risk, like Europeans sometime do with raw milk. In any case, these raw-consumed species are not available in Europe, and eating insects alive must be discouraged because of ethical reasons. One of the risk management tools to assure food safety available to operators and control authorities is the monitoring of food microbiological criteria to assess the hygiene of the process and the safety of the final products. As explained in the previous chapters, the only currently available evaluation schemes are those for foodstuffs in general as contained in Regulation (EC) 2073/2005 (process hygiene and food safety criteria) and, more specifically, those of the national guidelines. The latter, however, were taken from other food matrices, and comparative studies showed that process hygiene criteria were not met by certain product classes. In their guidelines, Belgium and the Netherlands published process hygiene and food safety criteria in the fashion of Regulation (EC) 2073/2005. They were not developed genuinely from insects, but taken from meat preparations and seafood, and while food safety criteria are usually met, process hygiene criteria often fail to comply despite GHPs, which makes these criteria debatable (Grabowski 2017). After that, Austria, Finland, Liechtenstein, and Switzerland also addressed these issues in their guidelines. All in all, criteria for edible insects vary markedly among countries. Evidence-based data from different species and products is necessary for EU harmonisation. It seems that criteria should be formulated according for the product type [cooked or deep-fried vs. meals and (freeze)-dried] and, if possible, to the insect species (Grabowski and Klein 2016a). As soon as uniform microbiological criteria are developed at EU level for insects and derived products, food business operators can include sampling in their self-monitoring activities for demonstrating the safety of their foodstuffs and control authorities might proceed to official

sampling of this new food category to verify that the sampling regarding the microbiological safety functions properly, and supply information on the microbiological quality and potential risk of the products on the market. Relevant associations of insect producers and processors as well as national food authorities have been very active in the last years in providing practical risk management tools for insects operators based on field experience and promoting best hygiene practices and shared standards within the insect industry. These future hygiene practices will have to cover, among others, the management of insect feed, practices in insect rearing, processing activities, storage, transport and delivery, environment, and quality practices.

Recommendations

According to the road-map drawn up during the consultation of FAO experts in 2012 (Expert Consultation Meeting on Assessing the Potential of Insects as Food and Feed in Assuring Food Security in Rome; <http://www.fao.org/docrep/015/an233e/an233e00.pdf>) and the recent DG SANTE strategic safety concept document for insects, the sustainable and safe production of insect in the future relies on the key role and related policy of both national and international organizations dealing with nutrition and food security, as well as committed NGOs (non-governmental organizations). Based on the knowledge gaps affecting the different components of the insect food and feed chain, proposal of solutions and related recommendations must be targeted to the following subjects: regulators, policy makers and health authorities, veterinarians, food insect operators, researchers, industry, and consumers and restaurateurs:

Regulators

- A clearer and more comprehensive global (FAO) and international (EU) legal framework for insects as food and feed is a condition *since qua non* to overcome the regulatory barriers to the establishment of an edible insect sector and to facilitate investments in the sector, both domestic and industrial farming. The planned inclusion of recommended edible insect species into the Codex Alimentarius and Regulation (EC) 853/2004 would be a useful step for setting international standards for the industry. The recent Commission Regulation (EU) No. 2283/2015 on novel food provides a blue print for similar legislative solutions in other countries.
- Since insects proved to be a sustainable sources of animal feed to reduce the dependence on feed derived from wild fish or soy, there is a need to explore the usage of feed products derived from insects for animal farming. For this aspect, an active role of the major national and international organizations is required to explain the benefits linked to insects farming in terms of low threat to our environment, higher sustainability compared to conventional productions, and most importantly to develop global policies to guarantee safe farming, production, processing and consumption of insects as human food and animal feed. The recent EU regulation allowing the use of insect processed animal proteins (PAPs) for aquaculture animals is a promising opportunity. Additionally, the food safety still needs to be intensively investigated.

Policy-makers and health authorities

- Promote more studies on global, regional, and local levels on the health value and nutritional benefits of edible insects. Currently, the information available on the health value of insects primarily is related to protein, amino acids, fat, and fatty acid contents and on comparisons of nutritive content between insects and other conventional animal foods. There are no data on digestibility of insects in humans.
- Standardize the methods for determining the nutritional values of the various species of insects and safety and quality criteria for insect protein products.
- Understand the dynamics of the food industry production chains and the international trade of insects and insect-based products and the economic contribution to local economies.
- Launch consumer information campaigns to overcome the cultural aversion of consumers in Western countries towards edible insect as nutritious and resource efficient food and to facilitate the inclusion of insects in the human diet. Consumer acceptance as psycho-cultural limitation is a multi-dimensional issue that requires further investigation. Effective media and public awareness campaigns on the safety, nutritional, environmental and sustainability benefits, in a context of economic crisis and food hunger are needed.

Veterinarians

- Recognize the potential that insect farming provides for veterinarians. In fact, whenever insects are farmed to whatever goal (food, feed, industrial use), veterinarians must be involved because they have the basic skills to handle a given life cycle in order to generate high-quality products. Their knowledge on housing, hygiene, and disease management makes veterinary practitioners an indispensable ally to the insect farmer, just as it has been case with more common livestock.
- For public health veterinarians, develop sound evaluation schemes for insects along their production chain, from evaluating insect farming and processing facilities (including competence of their operators) to assessing and evaluating quality and safety of the products originated in them.
- The necessary knowledge is currently missing. This can be overcome by additional trainings and by including insects in regular university teaching (as already happening in some part of Europe, e.g. Germany).
- The welfare issues related to insect stress and diseases need to be investigated since this is an important point to avoid uncontrolled development of pathogens in the rearing environment.

Food insect operators

- Acquire the necessary skills and knowledge to run livestock farms or food-processing businesses. The basics are already present in farmers, bee-keepers, aquaculture operators, and feed insect breeders. Additional knowledge must be obtained regarding the specifications of the targeted insect species.
- Understand that insect farming is a novel situation for all stakeholders involved. Being so, it will require mutual patience, comprehension, and the will to cooperate. In time, teething troubles will be overcome, but this settling can only develop with these soft skills.

Researchers

- As seen in this review, there are many different areas of research that need future attention and financial support. Understanding the basic biology of edible insects and their characteristics with regard to their farming and domestication is one central aspect. This knowledge would also help to elucidate the likely occurrence of different hazards through the different instars and support risk assessment studies. Thus, research targeting microbiological (intrinsic and external) and chemical risks must be intensified. The same is the case for reaction on the different insect matrices towards processing, also in terms of these hazards. Like with other livestock, research will have to include on-farm scenarios, the influence of rearing and processing conditions on that insects's microbiota, to the impact of handling and correct storage decontamination treatments on product safety.
- Development of adequate treatments for insect production diseases, e.g. immunostimulants.
- From the other side, research should also be dedicated to the understanding of the different farming methods for different insects, the industrial (automation) technologies, and advanced methods during processing with the aim to fine-tune risk management strategies to protect consumers.
- Finally, the impact of entomophagy (benefits and challenges) on consumers should also be analysed. Little is known of the potential of insects as functional food, and considering the vast amount of combinations of insect species vs. feeding, there is much to study for future generations.

Industry

- Introduce automation technologies and develop processing methods to ensure the economic production with the development of more effective and safer large-scale farming methods for different insects and their production systems.
- The development and optimization of fly larvae production methods for use in both developed and developing countries at small and large scale and the determination of the optimal design of insect-based animal feed production systems utilising the results of a comprehensive life cycle analysis.
- The determination of the optimal design of insect-based animal feed production systems utilising the results of a comprehensive life cycle analysis.

Consumers and restauranters

- Understand that farmed insects represent a valuable addition to the menu rather than any kind of substitute.
- Aversion towards insects is a cultural issue which can be overcome by means of information, a personal interest, the opportunity to taste them, and the culinary curiosity to include them in everyday dishes. Eventually and from a global perspective, insects are a foodstuff, nothing more, and nothing less.
- See that insects represent a class of products by their own. Since they clearly taste differently than meat, they are no true "meat substitutes".
- Realize that each insect species tastes differently, thus opening plenty of novel uses of insects in the kitchen.

Conclusions

To cope with an increasing human population (expected to reach 9,6 billion in 2050) and the related per capita demand of animal derived protein (Pelletier and Tyedmers 2010), among the various alternatives, edible insect could be a more efficient, nutritious and sustainable food compared to the conventional livestock production and can also address the persistent problem of human food insecurity in some regions and countries (Gaukar 2011). Indeed, there is a general optimism derived from forward-thinking, scientists, and food futurists, who accept the idea that sustainability-minded humanity will increasingly exploit edible insects as alternative protein for human food and animal feed (Dunkel and Payne 2016). However, although the insect farming and processing sectors are constantly emerging (particularly in developing countries), sensitive constraints discussed in this paper remain that limit their full exploitation. There is a need of a greater attention for the insect industry with financial support from governments to provide the means to move insects-based foods from the laboratory to the market. A multi-disciplinary approach is needed to catch the substantive benefit of insect as human food on a global level. The sector also needs engineers to design new rearing systems to remove/recycle waste biomass and to produce insect biomass for food and feed, while coping with different environments, insect species, legal constraints.

In conclusion, future policies to guarantee safe farming, production, processing, and consumption of insects as human food and animal feed will have to be mainly relying on the microbiological and chemical risks assessment studies and on a close cooperation between regulators, producers, researchers and consumers, towards the common objectives to promote the necessary technological innovations in the sector, include insects in the food law, stimulate consumer preferences, and ultimately bring to the tables of consumers in every part of the world a food product (insects and their derivatives) that is safe, nutritious, tasty as well as sustainable and eco-friendly.

Conflict of interest

We declare no conflict of interest.

Ethic approval

No animals were killed for this review paper. Consuming living insects is addressed in the paper but clearly discouraged.

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