Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on the microbiological and allergenic risks associated with the consumption of insects

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Abstract

There is currently significant international interest in promoting and enhancing the consumption of insects. Due to the nutritional properties together with the low ecological and economic impact of production, insect farming and the use of its by-products is turning into a promising food industry which is gradually being developed in Europe, supported by Regulation (EU) 2015/2283 on novel foods. In light of the possible increase in the consumption of food products derived from this type of animal, the Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has been asked to conduct an assessment of the microbiological and allergenic risks associated to eating insects.

Insects are carriers of a highly diverse microbiota. Some of these microorganisms, both from the intestinal contents and from the external surface, are pathogens and may result in food-borne diseases. Technological treatments applied in the food industry, mainly heat treatments (boiling, frying, toasting), help to significantly reduce microbial counts. However, pathogenic spore-forming bacteria may survive these treatments and grow during storage prior to consumption.

The risks of allergy associated with the consumption of insects may be linked to primary allergic reactions following intake or to cross-reactivity due to the presence of pan-allergens in patients already allergic to other invertebrates. Heat treatment reduces, but does not eliminate, all of the allergenicity of some of the proteins responsible for allergenic risk.

Good hygiene practices must be applied during the farming, processing and marketing of insects intended for human consumption in order to control the microbiological hazards. Consequently, Guidelines to Good Hygiene Practices must be prepared to help food business operators to better understand Community legislation on food hygiene, and to apply it correctly and uniformly. In addition, operators who process and/or market insects intended for human consumption should introduce a system based on hazard analysis and critical control points.

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At present, no microbiological criteria have been defined for insects intended for human consumption. It therefore seems advisable to develop specific criteria applicable to this type of food product, considering the product type, the processing and other factors which may affect its quality and microbiological safety.

At home, basic standards of hygiene should be adopted to minimise the risk of cross contamination, the proliferation of potentially pathogenic microorganisms and their survival.

Key words

Edible insects, novel foods, food safety, pathogenic microorganisms, microbiological risk, allergenicity, good hygiene practices.

1. Introduction

1.1 Basis of the request

The new Regulation (EU) 2015/2283 on novel foods is applicable from 1 January 2018 (EU, 2015). The scope of this Regulation includes whole insects and their parts, which were not largely consumed in the European Union before 15 May 1997. In order to authorise a novel food, it is necessary to demonstrate, based on the available scientific tests, that the food does not pose a risk to human health, and one of the aspects to be assessed is the microbiological safety of these novel foods.

In light of the possible increase in the consumption of these animals, the Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has been asked to conduct an assessment of the microbiological and allergenic risks associated with the consumption of insects.

1.2 Species of edible insects

Insects are the most diverse group of animals on Earth, and can be found in almost all terrestrial and aquatic ecosystems, forming a large part of the total animal biomass (Lázaro, 2011). From the Greek *entomos* (insect) and *phagie* (to eat), *entomophagy*, a term used to refer to the human consumption of insects, is a habit that has always formed part of the human diet. However, it might be considered a cultural phenomenon as it is closely linked to the customs and religions of the different peoples of the world. The Old Testament is the oldest written document to describe the ancient custom of eating insects. However, the roots of entomophagy are even older, and several sources indicate that insects have been part of the human diet from immemorial time (Testa et al., 2017).

According to the Food and Agriculture Organisation for the United Nations (FAO) and the World Health Organisation (WHO), the evolution of agriculture and livestock farming is very closely linked to this eating habit, where those societies which have most developed the livestock farming of mammals are the societies who are least accustomed to insects consumption (FAO/WHO, 2013). In addition, entomophagy is linked to rural areas with a natural or subsistence economy through the use of more abundant or easy to obtain resources.

From a food point of view, insects have been consumed for a number of reasons and in different circumstances: in times of famine, as a staple food in the diet (sometimes a delicacy), and they have even been used in association with rites and in traditional medicine (Matallana and Torija, 2006). Their intake is documented from the classical world, in Greece and Rome, to China and India. The Middle Ages in Europe saw a change in eating habits and the consumption of insects disappeared almost completely. Nevertheless, even today they are widely consumed around the world. The FAO/WHO (2013) estimates that more than 2 000 million people in the world are regular insectivores, mainly in the regions of Asia, Africa and America, while in most of Western countries it causes rather aversion. Although interest in edible insects has increased significantly in recent years, their contribution to the total food intake in Europe remains limited. Nonetheless, different international organisations consider that the consumption of insects may have important economic, environmental and nutritional benefits (Testa et al., 2017).

Of all the known species of insects, approximately 2 000 are considered to be edible around the world, and this figure is increasing constantly. However, although there are no available figures for the quantity of insects consumed around the world, according to the FAO/WHO (2013), beetles and caterpillars make up half of the total global consumption of insects, followed by bees, wasps and ants, at 14 % of the total, grasshoppers, locusts and crickets (13 %), Hemíptera (10 %), termites and dragonflies (3 % respectively) and flies (2 %).

Usually, they are harvested wild from the natural environment, processed (boiling, baking or roasting) and eaten (NVWA, 2014) (EFSA, 2015). Eggs, larvae, pupae and adults, that is, all the stages of insect development, are used as food. Unlike vertebrates, insects are usually eaten whole, without removing the digestive tract.

The European Food Safety Authority (EFSA) includes them as a food category in the FoodEx2 food classification and description system. According to the EFSA (2015), the species of insects which are most likely to be consumed in the European Union are flies (*Musca domestica* and *Hermetia illucens*), mealworms (*Tenebrio molitor*, *Zophobas atratus* and *Alphitobius diaperinus*), moths (*Galleria mellonella* and *Achroia grisella*), silkworms (*Bombyx mori*) and crickets, locusts and grasshoppers (*Acheta domesticus*, *Gryllodes sigillatus, Locusta migratora migratorioides* and *Schistocerca americana*). They also include arthropods, such as spiders and scorpions, which strictly speaking are not insects. Table 1 includes the insects and arachnids listed in reports from the EFSA or national organisations of the European Union (Belgium, Holland and Italy) and which, if authorised, may potentially be consumed as human food.

Table 1. Insects and arachnids which are more likely to be consumed in Europe

Common name	Scientific name
House cricket	*Acheta domesticus*
Lesser wax moth	*Achroia grisella*
Litter beetle	*Alphitobius diaperinus*
Black fungus beetle (buffalo worm)	*Alphitobius laevigatua*
European honey bee	*Apis mellifiera*
Leafcutter ant	*Atta laevigata*
Silk worm/moth	*Bombyx mori*
Chinese scorpion	*Buthus martensii*
Blowfly	*Chrysomya chloropyga*
Greater wax moth	*Galleria mellonella*
Emperor moth	*Gonimbrasia belina*
Banded cricket	*Gryllodes sigillatus*
Jamaican field cricket	*Gryllus assimilis*

Table 1. Insects and arachnids which are more likely to be consumed in Europe

Common name	Scientific name
Two-spotted field cricket	*Gryllus bimaculatus*
Field cricket	*Gryllus campestris*
Field cricket	*Gryllus testaceus*
Thailand black tarantula	*Haplopelma minax (nigra)*
Black soldier fly	*Hermetia illucens*
African migratory locust	*Locusta migratora (migratorioides)*
House fly	*Musca domestica*
Locusts/Grasshoppers	*Orthoptera: Mecapoda elongata; Oxya* spp.; *Melanoplus* spp.; *Hieroglyphus* spp.; *Acridia* spp.
Bombay locust	*Patanga succincta*
Palm weevil	*Rhynchophorus ferrugineus*
American grasshopper	*Schistocerca americana*
Desert locust	*Schistocerca gregaria*
Mealworm	*Tenebrio molitor*
Giant mealworm beetle	*Zophobas atratus*

In addition, Regulation (EU) 2017/893 as regards the provisions on processed animal protein (EU, 2017) permits the production of the following species of insects intended for animal feed: black soldier fly (*H. illucens*), common housefly (*M. domestica*), yellow mealworm (*T. molitor*), lesser mealworm (*A. diaperinus*), house cricket (*A. domesticus*), banded cricket (*G. sigillatus*) and Jamaican field cricket (*Gryllus assimilis*). These insects are currently farmed in the European Union and comply with safety conditions. This list may be amended in the future, based on the assessment of the risks posed by the insect species concern to animal health, public health, plant health or the environment.

1.3 Insect farms

The farming of insects for human consumption has several advantages over traditional livestock farming: insects convert food into body mass far more effectively, they need less water, release lower greenhouse gases and the energy necessary for their farming is lower. In addition, for most insects, almost all the insect can be used, making their farming even more efficient (FAO/WHO, 2013).

Insect farming has the same characteristics as other animal production systems (EFSA, 2015). Installations must comply with hygiene requirements in order to reduce the possible contamination with pathogenic microorganisms. Contact must be avoided with wild insects and other sources of contamination (Belluco et al., 2013) (NMWA, 2014). Insects need access to water and feed and excrete intestinal content in the form of fine powder waste (EFSA, 2015). This waste can be used as fertiliser (EFSA, 2015).

In European farms, insects are kept in closed environments, such as cages or boxes, where the environment, substrate, water, etc., can be controlled. No hormones, antibiotics or chemical compounds except biocides are used for disinfecting the production environment between batches of insects (EFSA, 2015).

Eggs are introduced onto the substrate manually, mechanically or by natural oviposition directly by the adult insects. The conditions and time necessary for the development of the eggs until reaching the time of harvest, as larvae, pupae or adults depends on the species. For example, mealworms require 8 to 10 weeks at 28-30 ºC and 60 % relative humidity to reach the correct production size, crickets and grasshoppers up to 4 months, but the black soldier fly only needs 12 days (EFSA, 2015).

An active separation process from the substrate is usually required for harvesting the larvae. This may be performed manually, or be more or less automated (EFSA, 2015).

Given the high microbial load in the intestinal tract of this type of animal, it has been suggested that the insects are fasted prior to harvesting and processing in order to ensure that the intestinal tract is empty, together with evisceration (Dobermann et al., 2017). However, some authors have not observed any improvement in the microbiological quality following fasting (Wynants et al., 2017, 2018). In more industrialised systems, which require extensive processing after harvest, this stage is not always included (EFSA, 2015). After harvesting the insects, they may or may not be slaughtered. The most usual form of slaughter is by freezing (EFSA, 2015).

It has been suggested that insect farms emit fewer ammonia and fewer greenhouse gases than conventional farms, and insects are more efficient in converting feed to protein. Insects are able to convert low value organic by-products into high value food or feed (Oonincx and Dierenfeld, 2012) (Van Huis and Oonincx, 2017). In addition, less land is required for the farms, and insect farming is a low-tech, low capital investment activity (FAO/WHO, 2013). Nevertheless, the energy needs of insects farms are high, as being cold-blooded animals their body temperature depends on the ambient temperature, which must be kept relatively high. On the other hand, this also means that the feed consumed by insects is used efficiently for growth, as they do not need to maintain a constant body temperature (Van Huis and Oonincx, 2017). This places Spain in a very favourable position as a potential insect producer, given our climate. It is to be expected that the environmental risks associated with insect farming are comparable to those of other animal production systems (EFSA, 2015).

Insect farming is subject to the prohibitions and exceptions regarding the feeding of animals other than ruminants with products of animal origin as established in Regulation (EC) No 999/2001 (EU, 2001) and the rules established in Regulation (EC) No 1069/2009 (EU, 2009b). Therefore, it is not permitted to feed insects with ruminant proteins, catering waste, meat- and bone-meal and manure. In addition, Regulation (EC) No 767/2009 prohibits the use of faeces in animal feed (EU, 2009a).

1.4 Processing systems

Insects intended for human consumption may be marketed whole, in ground or paste form, or as an extract of protein, fat or chitin. The processing of insects varies depending on the production system and product type.

In many parts of the world, insects are harvested wild from the natural environment. In these cases, traditional processing first includes the removal of foreign bodies and non-edible parts (intestine, wings, legs and head, depending on the species). Next, they are washed with cold or warm water, toasted or cooked and then grounded after drying in the sun, or consumed whole, although sometimes they are eaten raw (Mutungi et al., 2017). They may also be packed in sacks, cans or plastic containers for storage and their subsequent sale.

Where insects are farmed, those which are sold whole are subjected to some sort of processing before distribution in order to reduce the microbial load and to extend their shelf-life. The most usual processes are boiling, freezing and drying. Insects may subsequently be dried and ground, and presented in powder form, or ground without prior treatment or after freezing to present them in paste form. The production process applied depends on the insect in question. It has already been demonstrated that the process applied to a particular insect may not result in a pleasant flavour or texture for another insect (Stoops et al., 2017). These products are normally used as ingredients in other food. A large variety of techniques for mechanical isolation or for extraction with solvents exist for the fragmentation of insects. However, in many cases there is no data available regarding the methodology used (EFSA, 2015).

1.5 Nutritional aspects of insects

Insects are known for having numerous nutritional qualities, particularly associated with their high percentage of protein, high mono- and polyunsaturated fatty acid content, and trace elements including copper, iron, magnesium, manganese, phosphorus, selenium and zinc, and some vitamins such as riboflavin, pantothenic acid, biotin and folic acid. Nevertheless, some authors (Nowak et al., 2016) indicate that the information available about the contribution of insects to the nutrient intake is still too limited to consider them as a source of nutrients or as nutrient rich in accordance with the directives of the *Codex Alimentarius* (FAO/WHO, 2007).

Most of the studies published about the nutritional value of edible insects indicate a high variability depending on the species, the life cycle, the feed and their origin (collected in nature or farmed in a controlled environment). Therefore, the estimated energy value may vary from 293 to 763 kcal per 100 g of dry matter according to the insect in question (Rumpold and Schluter, 2013) (Abbasi, et al., 2016) (Kouřimská and Adámková, 2016).

In general, the protein content is considered to be particularly high in species of Orthoptera (crickets and grasshoppers), with 77 % of protein over dry matter. There are also significant variations within the same order. Within the Hymenoptera, the percentage of protein over dry matter varies from 4.9 % in the *Myrmecosistus melliger* ant to 66 % in the *Atta mexicana* ant.

This is a highly digestible and good quality protein (slightly lower than beef or egg protein), which contain all the essential amino acids, representing 46-96 % of the total quantity of amino acids

(Xiaoming et al., 2010). However, in relation to other foods of animal origin, insects have relatively low proportions of the histidine, lysine and tryptophan amino acids (Sánchez-Muros et al., 2014). It should be noted that the protein content of some species may be overestimated due to the presence of chitin (due to the acetamide group, which provides nitrogen to the total content) in its shell (Lázaro, 2011).

In addition, the lipid composition, which may vary between 10 % (African migratory locust *L. migratoria*) and 60 % (*G. mellonella* caterpillar), is characterised by a high content of unsaturated fatty acids (higher than 60 %) and in particular the oleic, linoleic and α-linolenic acids, all of which have been proven to have a positive effect on human health. In general, the lipid content is higher in the larval stages than in adult insects (Santurino-Fontecha et al., 2016). Recent studies confirm that insects may be considered as food sources of fatty acids with a high nutritional value thanks to the balanced composition of SFAs (saturated fatty acids), MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids) (Aman et al., 2017) (Sampat et al., 2017).

With regard to micronutrients, insects have a highly variable content of vitamins and minerals depending on the species and genera, and on the metamorphic stage and diet. Rumpold and Schluter (2013) reported that except for the larvae of the fly *M. domestica*, insects are poor in calcium, potassium and sodium. However, they have high quantities of phosphorus, magnesium, zinc and iron and adequate quantities of manganese and copper for meeting the daily requirements of humans. In general, the iron and calcium content is higher than that of beef, pork and chicken. The caterpillar of the *G. belina* moth is noted for its iron content (31-77 mg/100 g of dry matter) and the larvae of the palm weevil *Rhynchophorus phoenicis* for their content in zinc (26.5 mg/100 g of dry matter).

In addition, the consumption of 100 grammes of insects may cover the daily requirements of an adult in riboflavin, pantothenic acid and biotin. The Orthoptera and Coleoptera have a particularly high folic acid content, and the yellow beetle *T. molitor* and the cricket *A. domesticus* have a high vitamin B12 content. However, they are considered to be a major source of the vitamins A and C, niacin and thiamine. In addition, the vitamin E content is low except in the species *Drosophila melanogaster* and *Microcentrum rhombifolium* (Oonincx and Dierenfeld, 2012).

Lastly, some studies claim that chitin may be considered as dietary fibre, making edible insects a very important source of fibre, especially those species with hard exoskeletons (Belluco et al., 2013).

In light of the above, the WHO and the FAO openly recommend the consumption of insects as a possible path to solving nutritional deficiencies in developing countries and in particular, as feed for livestock (FAO/WHO, 2013).

1.6 Antimicrobial resistance

Some studies indicate that insects may be vectors of bacteria, carriers of certain genetic determinants of antimicrobial resistance. In addition, the possibility has been put forward that the intestinal tract of insects acts as an *in vivo* scenario for the plasmid horizontal transfer of antimicrobial resistance between bacteria (Crippen and Poole, 2009) (Anacarso et al., 2016).

However, little information is available in the scientific literature in relation to the presence and distribution of resistance genes in species of edible insects. Nevertheless, significant percentages have been observed of the presence of antimicrobial resistance genes widely used in primary production, such as the tetracyclines, macrolides, aminoglycosides and last resort antibiotics such as vancomycin. Osimani et al. (2017) observed a high prevalence of the gene *tet*M which codes resistance to tetracycline in the mealworm *Tenebrio molitor L*., which is higher in samples marketed in Europe (100 %) than in the samples from Thailand (80 %). They also detected the presence of the macrolide resistance gene *erm*(B) in 57.5 % of the samples, and of *van*A, which provides resistance to vancomycin, in 90 % of samples from Belgium and in 10 % of those produced in France, while the gene *aac-aph* which provides resistance to aminoglycosides was observed in 40 % of the samples produced in Holland and Belgium and in 20 % of those from Thailand. The authors link these findings to the usual microbiota identified in the mealworm (*Lactobacillus, Pseudomonas* spp*., Escherichia coli, Klebsiella* spp. and *Enterococcus*). In addition, Milanović et al. (2016) found a high incidence of genetic determinants of tetracycline and macrolide resistance in 11 species of edible insects, observing a different pattern depending on the country of origin (Holland and Thailand). This is probably due to differences in the regulation of the use of biocides established in each country, which may exercise selective pressure on the microorganisms present in the insects, favouring the appearance of resistance to antimicrobial substances.

Moreover, it has also been recently suggested that the intestinal content of insects might be a source of new antimicrobial compounds (Akbar et al., 2018).

These studies, although limited, verify the need to propose the cautious use of antimicrobial substances in the production of insects intended for human consumption, and to address the assessment of this potential risk for public health associated with their consumption.

1.7 Consumption of insects in Europe and in third countries

Most of the edible species which have been catalogued are found in Latin America, Asia and Africa, coinciding with the location of the majority of regular consumers of insects.

There is a long history of entomophagy of man's adaptation to his environment in these regions. From the "cultivation" of insect eggs (mainly diptera and grasshoppers) by the Aztecs in standing water and lakes to obtain a sort of caviar (*ahuauhlte*), to the methods used to collect weaver ants whose larvae stages have always been highly appreciated for both their nutritional and medicinal qualities in Southeast tropical China, in Bangladesh, India, Malaysia and Sri Lanka. Numerous methods are also employed in Africa for the collection of termites whose larvae stages are preferred for consumption.

Mexico is one of the main countries with a traditional insect consumption. Although this has declined in recent years, the frequency of consumption is estimated to vary from several times a week to once a month depending on the seasons of the agricultural harvest. The most frequently consumed species include the chapuline (Orthoptera of various species of crickets and grasshoppers including *Sphenarium purpurascens*) in maize *tortillas* with avocado and chilli, escamoles (larvae of the ant *Liometopum apiculatum*) in *tacos* or *tortillas*, jumiles (stink bugs from the *Pentatomidae* genus), fly larvae or ahuauhtle (the afore-mentioned Aztec caviar) and Maguey worms. The latter are the larvae stage of the *Comadia redtenbacheri* or *Aegiale hesperalis* butterflies harvested on the leaves of *Agave hesperalis*, and eaten in *tortillas* or used, together with the larvae of the weevil *Scyphophorus acupunctatus* (Curculionidae) to ferment the well-known Mezcal, a distilled alcoholic beverage made from the leaves of *Agave americana* in the state of Oaxaca.

In some countries such as Mexico and the south of Asia, the gastronomy associated with insects is currently found as a tourist attraction with more than 150 edible species on offer.

Unlike the entomophagic societies, Western culture, although there are records of entomophagy in the West, are considered enthomophobic, with widely disparate reactions and feelings, ranging from curiosity to absolute rejection. However, in countries such as the United States, Japan and the European Union, interest in the consumption of insects is re-emerging through restaurants offering exotic cuisine, markets which sell insects and some businesses which farm and prepare them for wholesale or directly to the consumer. These products include ants, butterfly caterpillars and bee larvae covered in chocolate; *chapulines*, silkworms and Maguey worms, bees, and even scorpions in Japan, fried or prepared in syrup. Products prepared with insect by-products are also available, including bread made with cricket flour which is sold in Finland or energy bars, made with cricket flour sold in Spain.

They are also found in processed food products. This is the case of the natural carmine red colorant E 120, which is commonly used in dairy and meat products, and which is obtained from the extract of the dried bodies of the females of the cochineal *Dactylopius coccus*.

In Europe, until the entrance of the new Regulation (EU) 2015/2283 on novel foods, some Member States of the European Union permitted the marketing of certain species of insects for human consumption. For example, in Belgium up to 10 species of edible insects were marketed (FASFC, 2014). This trade was subject to the application of good hygiene practices, traceability, labelling and self-checking system all along the food chain. The mealworm (*T. molitor*), the litter beetle (*Alphitobius diaperinus*) and the migratory locust (*Locusta migratoria*) are farmed in Holland (Belluco et al., 2013). Insects are also found in other countries of the European Union including Austria, Denmark, Finland and the United Kingdom. It is interesting to note that a scientific journal specifically dedicated to the role of insects in human and animal food and feed has recently been published (*Journal of Insects as Food and Feed*; http://www.wageningenacademic.com/loi/jiff), as a result of the first *"Insects to feed the world"* Conference held in Holland in May 2014.

Apart from the fashion for exotic cuisine, at present there is a real interest at international level to promote and value the nutritional properties of insects. The report "Edible insects: future prospects for food and feed security'' by the FAO/WHO, published in 2013, analyses the possibilities offered by insects as an important alternative for the future of humanity and indicates that their potential as food and, in particular, as feed is open to investigation.

2. Principal microorganisms and food-borne pathogenic parasites present in insects

Insects are carriers of a very diverse microbiota associated with their life-style and the farming and processing conditions. Given their habitats, cold blood, and composition, significant differences are expected as regards the microbiological and parasitic risks, compared to the farming of conventional animals. It is necessary to distinguish between the risk of purchasing wild edible insects, which may be very different to the inherent risks of consuming farmed insects. In addition, certain associated diseases summarised below are produced as a result of accidental intake (EFSA, 2015). The microorganisms intrinsically associated with insects may be present in their digestive tract or on their external surface. The microbiota of the digestive tract is essential for their metabolism, behaviour and survival and remains in the insect, even when access to food is limited, prior to harvest, in order to void the intestinal tract. Some of these microorganisms, both from the intestinal tract and from the external surface may be pathogenic (EFSA, 2015).

The different studies conducted on the microbiological risks of insects arrive at different conclusions. Important factors which increase the risk of microbial contamination would appear to be deficient hygiene and inadequate conditions of harvesting, drying, transport, storage and distribution (Testa et al., 2017). The farming of insects allows the production conditions to be controlled, thus reducing the risk of pathogenic microorganisms. Wild insects are more exposed to possible contamination (NVWA, 2014). These conditions are particularly relevant in underdeveloped and developing countries, where inadequate hygiene conditions have been recorded during handling, processing, storage and marketing (Braide et al., 2011).

The literature contains several microbiological studies conducted on different species which are used in human food or may potentially be used. Many of these studies have been performed in Africa and South America, as these are regions in which there is a traditional consumption of certain species (Amadi et al., 2005) (Agabou and Alloui, 2010) (Braide et al., 2011) (Hernández-Flores et al., 2015).

2.1 Bacterial pathogens

The study of insect microbiology has been addressed from two principal approaches: conventional microbiology (based on cultivation) or populational analysis (independent of cultivation). The cultivation-based studies indicate a high bacterial load, with high mesophilic aerobic counts in the majority of cases, at 10^6 CFU/g (Amadi et al., 2005) (Braide et al., 2011) (Klunder et al., 2012) (Stoops et al., 2016) (Garofalo et al., 2017) (Grabowski and Klein, 2017a) (Vandeweyer et al., 2017a). It is necessary to remember that these studies have been conducted on different species of insects which have been subjected to diverse treatments and have not always been processed in the same way.

High counts have been found in the digestive tract of insects, reaching levels of 2.8×10^{10} CFU/ml (Cazemier et al., 1997), being an important source of contamination (Rumpold et al., 2014) (Grabowski and Klein, 2017a). The bacteria isolated in the digestive tract of insects include: *Streptococcus, B. subtilis, E. coli, Enterobacter liquefaciens, Klebsiella pneumoniae* and *E. cloacae* (Dillon and Charnley, 2002). The microbiota of the feed appear to determine the microbiota present in the insect, although some species show a competitive advantage in the digestive tract of the insect and become dominant (Wynants et al., 2018). It should be noted that the insects are often consumed whole, without evisceration.

In the study carried out by Vandeweyer et al. (2017a) on three species of insects, high pH values were observed (between 6.4 and 6.7), together with high values of water activity (0.96). These high values for pH and water activity are suitable for the growth of a wide range of microorganisms.

The presence of certain pathogenic bacteria has been demonstrated regularly. The presence of *Salmonella* spp., *Campylobacter* spp., *E. coli* O157:H7, *Staphylococcus aureus, Bacillus cereus* has been recorded (Wales et al., 2010) (Belluco et al., 2013) (Rumpold and Schlüter, 2013) (Dobermann et al., 2017) (Grabowski and Klein, 2017a) (Mutungi et al., 2017). In addition, it should be noted that certain insects are considered to be vectors of *Salmonella* spp. and *Campylobacter* spp. (Belluco et al., 2013). The spores of pathogenic bacteria, resistant to the processing treatments generally applied to insects are of particular concern, as these are able to grow during storage (NVWA, 2014). In this respect, it has recently been demonstrated that spore-forming bacteria survive at levels of 10^4 CFU/g after boiling, while the remaining bacterial groups were significantly affected (Vandeweyer et al., 2017b) (Wynants et al., 2018).

Significantly different results have been obtained in the studies conducted on the presence of pathogenic bacteria in insects. Some studies observe the absence of the pathogenic bacteria studied while others have detected the presence of pathogenic bacteria and mycotoxin-producing moulds (Braide et al., 2011) (Belluco et al., 2013) (Dobermann et al., 2017) (Grabowski and Klein, 2017a) (Wynants et al., 2018). Nevertheless, it should be remembered that the presence of pathogenic bacteria in insects which may be intended for human consumption depends on the insect species, the developmental stage, the farming conditions (production system, food and environment), their processing and handling, and even the conditions of consumption (FASFC, 2014) (EFSA, 2015). This fact may explain the differences found in the literature.

The insects are farmed at relatively high temperatures, therefore if the pathogenic bacteria indicated are present in the production environment or in the food, they may multiply. Consequently, these pathogenic bacteria may be present in non-processed insects intended for human consumption (NVWA, 2014).

Given the huge variety of edible insects, it is important to consider the species. Different studies find microbiological differences among insect species, and within the same species a huge variability was found from the microbiological point of view. The production system and food also play an important role. Moreover, depending on the insect species, they are used for consumption in different developmental stages (larvae, pupae, adults). In addition, the insects may be sold whole, or as their parts, and even as by-products used as a source of proteins. All these factors may affect microbiological safety (EFSA, 2015) (Vandeweyer et al., 2017a).

Data on the histamine content in insects, although present as a neurotransmitter, has not been found in the literature (Elias and Evans, 1983). Nor has data been found on the free histidine content or the presence of decarboxylase-producing bacteria. The histidine composition in insects ranges between 15.7 and 27 mg/g of protein (Rumpold and Schlüter, 2013), suggesting that the risk due to biogenic amines is very low.

Culture-independent microbiological studies reveal a huge microbial diversity, which also differs according to the insect species and the farming systems (Jung et al., 2014) (Stoops et al., 2016) (Garofalo et al., 2017) (Wynants et al., 2018). In addition, these studies reveal the presence of potentially pathogenic microorganisms (*Listeria* spp., *Clostridium* spp., *Staphylococcus* spp. or *Bacillus* spp.) (Garofalo et al., 2017). The conclusions which can be reached from these studies are that the microbial communities vary according to the insect species, the diet and farming conditions and the capacity of the microorganisms to adapt to the different environmental conditions occurring in the insect (Jung et al., 2014) (Garofalo et al., 2017).

2.2 Viral pathogens

There is a large variety of viruses which may be pathogenic for insects. However, only on a very few occasions do insects act as vectors of viruses able to infect vertebrates. These arthropod viruses are called Arboviruses. They successfully replicate in vectors such as flies, mosquitoes or ticks, they are able to cross the species barrier, replicate efficiently in vertebrates and cause disease in humans (such as Dengue fever, the West Nile disease, Rift Valley yellow fever, haemorrhagic fever or Chickungunya) or farm animals (such as Schamallenberg). Nevertheless, there is no evidence that these viruses occur in insects used as food or feed (EFSA, 2015).

Insects may also act as passive vectors of human and farm animal viral diseases. It has been suggested on a number of occasions that, for example, flies may passively transmit the avian flu virus (EFSA, 2015). It has also been suggested that some viruses may survive in animal manure used as a substrate for fly farming (EFSA, 2015). Nevertheless, in any of these cases, the use of a suitable substrate for the farming of the insects or an effective processing would mitigate the risk of the possible transmission of these viruses.

2.3 Fungi

The fungal microbiota analysis also revealed very varied results in the counts, ranging from less than 2 log CFU/g to 5.7 log CFU/g (Simpanya et al., 2000) (Stoops et al., 2016) (Garofalo et al., 2017) (Vandeweyer et al., 2017b) (Wynants et al., 2018). Some isolated insect moulds belong to species with mycotoxigenic potential (*Aspergillus, Penicillium, Fusarium*) and the presence of aflatoxins was also detected in samples of insects, in concentrations which sometimes reached 50 µg/kg (Mpuchane et al., 1996), far higher than the maximum established limits in the European Union for aflatoxins, which may not exceed 15 µg/kg in the most permissive case (EU, 2007). Charlton et al. (2015) detected Beauvericin mycotoxins (synthesised by the entomopathogenic fungus *Beauveria bassiana*), eniantin A and eniantin A1 (synthesised by *Fusarium* spp.) in examples of *Musca domestica*. Another notable aspect is the huge quantity of new fungal species found in the digestive tract of insects, unknown to date, which in the case of yeasts is estimated to be almost 50 % (Suh et al., 2005).

2.4 Parasites

The risks of disease from parasites associated with the intake of insects are scarcely documented. The literature describes various examples of insects as vectors for parasitosis, although it should be noted that many of the descriptions are anecdotal.

As regards single-cell parasites, Chagas disease in South America caused by *Trypanosoma cruzi* with triatoma as vectors affects more than 10 million people. In addition, *Toxoplasma gondii* has been found in cockroaches and some diptera (Graczyk et al., 2005), and therefore its transmission via these insects cannot be dismissed. *Entamoeba histolytica* and *Giardia lamblia* have also been found in cockroaches and in the common fly, and other protozoa (Kinfu and Erko, 2008) (Oyeyemi et al., 2016) (Mutungi et al., 2017).

There is a review on the risk of various types of foodborne trematodosis, but it does not specify insects as the authoritative cause in all cases. It is, in fact, more associated to consumption habits in the endemic regions (Chai et al., 2009). *Dicrocoelium dendriticum* is a trematode which affects grazing livestock. However, it has been associated with infection in humans as a result of the accidental intake of ants in Kyrgyzstan (Jeandron et al., 2011). In eastern Asia, the trematodes *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* are able to cause infection in their cercarial and metacercarial forms, and are found in the nymph stage (naiad) and adult stage of the dragonfly and the damselfly (Belluco et al., 2013).

The cestoda *Hymenolepsis diminuta* uses a number of insects as intermediate hosts, including the mealworm *Tenebrio molitor*. The consumption of the infected insects transmits the parasite, typically to the final host (rodents), but also to livestock and humans (Šhostak, 2014).

In countries such as Nigeria and Ethiopia, it has been possible to isolate the nematodes *Trichuris trichiura*, *Ascaris lumbricoides, Enterobius vermicularis, Taenia* spp. in cockroaches and in the common fly (Kinfu and Erko, 2008) (Oyeyemi et al., 2016). The nematode *Gongylonema pulchrum* uses beetles and cockroaches as intermediate hosts in Iran. Some cases have been specifically reported associated to the intake of these insects (Wilson et al., 2001).

Intestinal myiasis is associated with the accidental intake of eggs or larvae of the common fly and other diptera, of which some are used in animal farming (ANSES, 2015).

No data is presently available regarding parasitic disease caused by the intake of farmed insects (EFSA, 2015). As initial sterile states of these farmed insects are used, the acquisition of parasites from the environment is unlikely (Belluco et al., 2013). Other authors indicate that prior experience of the appearance of new microbiological risk in the farming of other insects indicates that new risks cannot be dismissed, as the invertebrate pathogens are more frequently found in mass breeding systems (Grau et al., 2017).

2.5 Prions

Prions are the cause of a group of lethal neurodegenerative pathologies characteristic of mammals, also known as Transmissible Spongiform Encephalopathies. Molecularly they are formed of a functional protein which has lost its normal function, acquiring the capacity to change the conformation of the normal form to pathological and to auto-replicate. The scientific literature includes references to different types of prions, specifically of mammals, of yeast and filamentous fungi (Wickner et al., 2004) and of a marine mollusc of the genus *Aplyssia* (Bussard, 2005). However, to date no related prions or proteins specifically from insects have been identified and, as they lack the genes that code them, the possibility that the PrP mammalian prion protein might replicate in the insects is dismissed. Some published studies support this theory by inoculating prions from sheep infected with typical and atypical Scrapie in transgenic and non-transgenic Diptera *D. melanogaster* (Post et al., 1999) (Thackray et al., 2012, 2014). The transgenic specimens able to express the coding gene for the ovine

PrP protein developed accelerated neurotoxic effects, while the wild specimens did not develop any type of response. In addition, the appearance of a protein of resistance against PrP^{Sc} (the ovine PrP protein) was observed in the transgenic individuals. Other studies are available on the expression of mammalian PrP proteins in transgenic *D. melanogaster* (Gavin et al., 2006) (Sartori et al., 2010).

Although edible insects cannot act as biological vectors of human or animal prions, a number of studies suggest the possibility that they behave as mechanical vectors for these prions. Post et al. (1999) detected the presence of the PrP^{Sc} protein in dead adult specimens of the Diptera *Sarcophaga carnaria*, responsible for human and animal myiasis, after feeding fly larvae with hamster brains infected with Scrapie. In addition, clinical symptoms were observed and the detection of PrP^{Sc} in 50 % of the healthy rodents fed with internal organs of infected larvae and pupae, concluding that the larvae of *S. carnaria*, as they fed on a contaminated source, might be passively colonised with the prion responsible for Scrapie, and that all the phases of their life cycle may act as a passive vector for the prion, even when dead, which is transmitted when consumed by a vertebrate, thus permitting the development of the pathology. In addition, Lupi (2006) concluded that the ocular, cutaneous, intracerebral and spinal myiasis, due to *Hypoderma bovis* or *Oestrus ovis*, are linked to the development of human prion diseases and to the transmission of prion encephalopathy which affects wild cervids in North America, and Corona et al. (2006) linked the larvae of *O. ovis* carriers of PrP^{Sc} with the transmission of Scrapie in sheep.

To date, there are no scientific studies to link species of edible insects to the role of vector or carrier of animal or human prions. The recent report from the EFSA (2015) on the hazards posed by insects for human and animal food and feed indicate that this hazard may be associated with the use of substrates for farmed insects which include proteins derived from animal by-products, in particular protein of human or ruminant origin. Although the hazard is estimated to be comparable and not higher than other non-processed sources of protein of animal origin, further studies will be necessary if manure, kitchen waste, or even human manure are used for feeding the insects. The EFSA also extends their conclusion to proteins derived from insects, as the transformation of insects may reduce the presence of biological hazards even more.

3. Risks associated with allergenicity

There is still too little knowledge about allergic reactions following the intake of insects, although in recent years some research groups have begun to study the epidemiology and predictability of allergic reactions, particularly in patients who are already allergic to other arthropods. Reactions caused by primary sensitivity to insects can be distinguished from the hypothetically predictable reactions due to cross-reactivity in patients who are already allergic to other arthropods. In 2017, Ribeiro et al. published a systematic review of the risks of allergy produced by edible insects, analysing articles describing, on the one hand, primary allergic reactions to insects and on the other hand the possibility of cross-reactivity and co-sensitivity among edible insects, shellfish and house dust mites (Ribeiro et al., 2017). This is currently the most complete and updated review in this area. The review and reports from official bodies (in particular (ANSES, 2015) (EFSA, 2015) also include other lateral aspects linked with the intake of insects, and which are therefore briefly mentioned here.

The capacity of insects to sensitise and/or cause allergic reactions is well-known and depends on the path of exposure. Most cases of insect allergy are caused by stings from insects belonging to the order Hymenoptera, although bites by blood-sucking insects, poisonous spines or hairs and defensive secretions can also elicit allergic reactions (Ribeiro et al., 2017).

Additionally, insects or volatile substances emanating from them can also act as aeroallergens. The professional exposure of workers in contact with insects may cause eczema, contact urticaria or respiratory symptoms such as rhino conjunctivitis and bronchial asthma. In these scenarios, it is possible to confirm an allergic mechanism due to aeroallergens or contact allergens among insect farm workers, farmers or bakers (contamination of the flours). Specifically, the sensitisation due to inhalation of the mealworm or migratory locust has been documented (*Locusta migratoria*) (Ribeiro et al., 2017). Fungi from the *Aspergillus, Penicillium, Alternaria* and *Candida* genera are able to sensitise by inhalation and form part of the microbiota on the surface of insects and at the same time be a cause of occupational allergy (Schlüter et al., 2017).

The relevance of the sensitisation via inhalant allergens is due to the possibility of causing, in a second stage, a clinical relevance with possible allergic reaction after intake, not only of the insect in question, but also of others, due to cross-reactivity. Thus, one publication describes the allergy due to the intake of mealworms in persons with prior inhalant allergy due to the same insect as a result of professional or domestic contact. These patients are described due to their primary sensitivity to this insect without an allergy to shellfish, although one of the patients was sensitive to dust mite (Broekman et al., 2017). Preventive measures include adequate ventilation and the use of protective clothing and masks, as well as the exclusion of allergic personnel from these tasks.

As regards the possibility of allergies due to cross-reactivity, this area is hypothetical rather than clearly demonstrated. However, the first *in vitro* studies carried out recently have been able to demonstrate cross-reactivity between seafood (in particular shellfish, but also molluscs) and edible insects, and between shellfish and house dust mite. Both sensitisations to dust mite or shellfish are of relevance, in particular, due to their epidemiological importance. Allergies to shellfish are among the most frequently occurring food allergies, while allergies to dust mite in many parts of the world are the most frequent cause of respiratory allergies (rhinoconjunctivitis and bronchial asthma). Studies of possible cross-reactivity have been carried out in particular for edible insects expected to be of relevance in the European Union: the mealworm (*T. molitor*), field crickets (*Gryllus bimaculatus* and *G. campestris*), the house cricket (*A. domesticus*), locusts (*Patanga succinta* and *Mecopoda elongata*), the litter beetle (*A. diaperinus*) and the giant mealworm (*Z. atratus*) (Ribeiro et al., 2017). In one study, the majority of sera from patients allergic to dust mite or shrimp tropomyosin recognised *in vitro* the extracts of the mealworm. The same authors were able to demonstrate a predictability of cross-reactivity *in silico*, based on a high probability of cross-reactivity if there is 35 % identity over a sliding window of 80 or more amino acids (Verhoeckx et al., 2014). The same research group published the only study of possible clinical relevance of these findings. 15 shrimp-allergic patients were provoked (double-blind and controlled) with mealworm. 87 % of the provocations were positive, with the threshold dose for the appearance of objective symptoms found in equal to or lower doses than the quantity of protein from this insect contained in snacks produced at present (Broekman et al., 2016). These findings were interpreted as surprising in the review by Ribeiro et al. (2017), as the high percentage referred to as the clinical relevance of cross-reactivity is higher than the 75 % of the possible clinically relevant cross reactions between different species of shellfish, which are always taxonomically closer. An extension of this study with the same patients demonstrated the cross-reactivity (*in vitro*) of shrimps with other insects tested: the giant mealworm *Zophobas morio*, the litter beetle *A. diaperinus*, the wax moth *G. mellonella*, the black soldier fly *H. illucens* (all in final larvae stage), and the house cricket *A. domesticus* and the locust *L. migratoria migratorioides*. Thus cross-reactivity was demonstrated with four different orders (Coleoptera, Lepidoptera, Díptera, Orthoptera and different life stages) (Broekman et al., 2017). The field cricket has also been studied, finding the recognition of two bands with a set of sera from shrimp-allergic patients in its extract. Other studies demonstrated *in vitro* cross-reactivity between the silkworm and cockroaches on the one hand and shrimps on the other.

With respect to the allergens responsible for cross-reactivity between edible insects, dust mite and shellfish, tropomyosin and arginine-kinase should first be noted but also others including α-tubulin, β-tubulin, actin, troponin, fructose-biphosphate aldolase, myosin light chain, paramyosin, hexamerin, hemocyanin.

The findings of conserved sequences of the allergenic proteins and hence cross-reactivity are not unexpected if you consider that the invertebrates in question are taxonomically close in relation to humans, whereas the distance between humans and the invertebrates studied is sufficiently distant to easily induce an immune response. Nevertheless, it should be noted that the *in vitro* studies and the *in-silico* tests must be confirmed *in vivo* (provocation tests or accumulated experience of cases described).

Although the intake of insects may be accidental, the publications on allergic reactions to insects are related to voluntary intake. The majority of the literature refers to cases and studies in Asia, Africa and South America. The species reported as the cause of allergic reactions are grasshoppers/locusts, silkworm larvae, *Cordyceps sinensis*, a fungal parasite of insects such as the silkworm pupae (*Bombyx mori*), the emperor moth caterpillar (*Gonimbrasia belina*), the mealworm, the giant mealworm (*Zophobas morio*) some cicadas, bee pupae and larvae, the moth *Clanis bilineata* and the red palm weevil (*Rhynchophorus ferrugineus*). On the whole, the epidemiology of the allergic reactions is unknown and to date they have been particularly described in regions where their consumption is more frequent.

The severe cases of allergy described, the anaphylactic reactions deserve an approximation and special mention: in China 17.3 % of the food-induced anaphylactic shocks were caused by the intake of insects, the most frequent species being the grasshopper. In China, allergic reactions from the intake of the silkworm are estimated to amount to 1 000 cases per year (Ji et al., 2008). In Thailand, food-induced anaphylaxis amounted to 19.4 % due to the intake of the same species. Seven cases of anaphylaxis following the intake of fried grasshoppers and locusts were reported during 2 years in a Thai hospital (Pener, 2014). In another approach, in Laos, the prevalence of allergic reactions in the insect-consuming population was 7.6 %, without giving details of the species concerned. There are no fatal cases recorded in the literature. The document published by the ANSES (*Agence* *nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail*) calculates the risk of reaction due to cross-reactivity as 100 000 people in France, in a hypothetical risk (ANSES, 2015). This number appears to be very high and should be re-assessed as entomophagy is introduced as a mode of food in Europe.

Chitin is a carbohydrate which forms part of the cell walls of fungi and the exoskeletons of arthropods. It has properties which increase the immune response or encourage an inflammatory allergic response. The human being has chitinases, but there is often a deficiency of this enzyme and consequently this substance is considered indigestible. Dissected insects contain up to 10 % chitin (Arbia et al., 2013). Nevertheless, a daily portion of 45 g of lyophilised insects with an average content of 6 % chitin does not pose a health concern (EFSA, 2010). Body parts with chitin may build up in the intestine and cause intestinal obstruction (FAO/WHO, 2013). Grasshopper legs and wings should be removed before consumption.

In conclusion, analysis of the literature highlights the possibility of cross-reactivity due to the presence of pan-allergens in patients already allergic to other invertebrates, with a higher risk of reaction if the allergic reaction is due to shellfish. Primary sensitisation to edible insects does not appear to be associated to the sensitisation to pan-allergens, probably due to sensitisation to more specific allergens of the insect in question. The true epidemiology of allergic reactions to edible insects is not known. It would appear that patients with primary sensitisation to any insect are able to tolerate a number of other insects (Broekman et al., 2017). Nevertheless, lack of experience due to the limited entomophagous customs in Europe calls for caution when attempting to forecast allergenic hazards. The documents consulted recommend that, at minimum, consumers with prior allergies to shellfish be advised of the possibility of allergic reactions to insects. To date, there is no treatment for insects prior to their intake which guarantees the elimination of the allergenic effects.

4. Expected effect of processing on the microbiological quality of the insects and their allergenicity

The type of processing applied to insects intended for human consumption has a major influence on their microbiological safety and quality (Belluco et al., 2013) (Grabowski and Klein, 2017b). In general, different studies have revealed a high microbial load in insects, highlighting high mesophilic aerobic microorganisms, *Enterobacteriaceae* and spore counts (Braide et al., 2011) (Klunder et al., 2012) (Caparros Megido et al., 2017) (Grabowski and Klein, 2017a).

A number of studies have assessed the effect of heat treatment on the microbiological quality of the insects for human consumption. Klunder et al. (2012) demonstrated that boiling insects *Tenebrio molitor* at 100 ºC (5-10 minutes) reduces the total Enterobacteriaceae and mesophilic aerobic microorganisms counts from 10^7 to less than 10 CFU/g. Moreover, these authors found that oven drying at 90 ºC for 110 minutes reduces the total mesophilic count to 2 or 3 log cycles and the Enterobacteriaceae count from 3 to 5 log cycles. Roasting for 10 minutes preceded by scalding also produced a significant reduction in the Enterobacteriaceae count. High mesophilic aerobic microorganisms counts and mould and yeast counts have been observed in smoked insects, and therefore prior boiling in water at 100 ºC, for 10 minutes is recommended in the case of the mealworm and 5 minutes for the domestic cricket (Caparros Megido et al., 2017). To the contrary, spore-forming bacteria are able to survive these heat treatments, germinating during the process and proliferating during the conservation period prior to consumption. This risk is reduced in the case of insects associated with lactic fermentation processes due to the effect of the pH on bacterial growth and germination. Similar results in relation to heat treatment have been obtained more recently by Stoops et al. (2017), by Vandeweyer et al. (2017b) and by Wynants et al. (2018). Adámek et al. (2018) concluded that the most ideal procedure for the long-term conservation of insects was boiling in water followed by drying at 103 ºC for 12 hours and hermetic packing of the product.

In addition, it has been confirmed that the process of grinding raw insects increases the microbial load and decreases the effectiveness of heat treatments in comparison to when these treatments are applied to whole insects.

Grabowski and Klein (2017b) indicated differences in microbiological quality according to the type of product tested. These authors observed higher microbiological counts in dried and powdered insects than in those which were fried and boiled. The groups tested by these authors were: mesophilic aerobic microorganism, Enterobacteriaceae, moulds and yeasts, *Staphylococcus* spp. and *Bacillus* spp. All the samples analysed of processed insects revealed the absence of *Salmonella, L. monocytogenes, E. coli* and *Staphylococcus aureus*. However, these authors detected the presence of *B. cereus, Listeria ivanovii, Mucor* spp., *Aspergillus* spp. and *Penicillium* spp. in samples of dried and powdered insects. The dried and powdered insects which were tested exceeded the values established in the hygiene criteria for processes applicable to meat products, the criteria which have been proposed for insects. Consequently, these authors recommend the application of heat treatment prior to their consumption.

B. cereus has been observed in dried and powdered insects, in some cases reaching populations of 6.0 log CFU/g (Grabowski and Klein, 2017b). Powdered insects may be used in other food products and be subject to heat treatments. Given that *B. cereus* has high heat resistance, it is able to survive pasteurisation. Consequently, special attention should be given to the control of *B. cereus* in dried products.

Different methodologies exist for drying the insects. The hygiene conditions, and the different time-temperature measures produce different microbial counts (Mpuchane et al., 2000) (Grabowski and Klein, 2017a). This demonstrates the importance of hygiene measures during the drying process.

The effect of high hydrostatic pressures has been studied for mealworms. Treatments of 600 MPa have proven effective in the microbial inactivation. Similarly, heat treatment in water at 90 ºC for 15 minutes is effective (Rumpold et al., 2014).

As with any food, the handling of the insects during the process is a factor which increases the risk of their contamination. The presence of *Staphylococcus* spp. has been recorded in insects subject to heat treatment and is attributed to contamination during post-treatment handling (NMWA, 2014).

The storage and packing of insects is an important factor to be considered in order to guarantee the safety of the insects (Braide et al., 2011). In relation to packaging, this should take place in modified atmospheres in order to reduce humidity and microbial proliferation. In this way the shelf life of the product can be extended (Braide et al., 2011) (Stoops et al., 2017). Lastly, the storage conditions of the processed insects is a risk factor for the multiplication of agents which have survived the treatments applied, in particular spore-forming microorganisms.

It is important to carry out studies in order to establish the shelf life of the insects sold. The shelf life of 52 weeks indicated by some manufacturers of insects processed for human consumption has not been documented with any scientific basis (NMWA, 2014). However, there are studies on mealworm paste which conclude a shelf life of 3-7 days at 2-7 ºC, of 7 days if vacuum packed and 14 days if the packaged product is pasteurised (FASFC, 2014).

The processing treatments have some effect on allergenicity, as with other foods, which cannot always be predicted. As regards the capacity of recognition of the IgE of shrimp-allergic patients with *in vitro* methods, the heat treatment of the mealworm does not change the binding capacity of the IgE (Broekmanet al., 2017). This was confirmed in another study which analysed the binding capacity of IgE compared to tropomyosin more specifically (Van Broekhoven et al., 2016), while frying at 180 ºC did eliminate it. The increase of allergenicity of the glyceraldehyde-3-phosphate dehydrogenase of the Bombay locust (*Patanga succinta*) has also been demonstrated (Phiriyangkul et al., 2015). It should be noted that the very stability of the proteins in the case of extreme temperatures and digestive enzymes is one of the characteristics of food-borne allergens. For tropomyosin, the stability of the epitopes during lyophilisation is also known (Van Broekhoven et al., 2016). It can be concluded that heat treatment reduces, but does not eliminate, all of the allergenicity of, at least, some of the proteins responsible for allergenic risk.

5. Good hygiene practices related to the consumption of insects

For the control of microbiological hazards in insects intended for human consumption, adequate hygiene measures must be applied during the breeding, processing and marketing processes. The raw consumption of insects may be linked to the food-borne diseases described, therefore adequate culinary treatment or freezing is recommended prior to consumption. The study at industrial level of the different treatments (lyophilisation, freezing, heat treatments, etc.) used on insects for the prevention of these diseases is considered necessary. The different studies carried out show that the concentration of hygiene indicator microorganisms is extremely variable and may be very high in insects which have not received any type of heat treatment; however, the most frequently applied heat treatments (boiling, frying, toasting) produce a significant reduction in the microbial counts (NVWA, 2014).

In the European Union the sector dedicated to edible insects, as is the case in any other food sector, must comply with a set of obligatory hygiene requirements established in Regulations 852/2004 (EU, 2004a) and 853/2004 (EU, 2004b). These include the requirement to prepare, apply and maintain a procedure based on the principles of Hazard Analysis and Critical Control Points (HACCP) system. In addition, it must satisfy the requirements of Regulation (EC) No 178/2002 (EU, 2002) on traceability. Consequently, Guides to Good Hygiene Practices should be prepared to help the sector to better understand Community legislation on food hygiene, and to apply it correctly and uniformly, together with Guides for the Application of the Principles of the HACCP system, which help the sector to apply this system with consideration for its nature and characteristics.

At present, there are no defined microbiological criteria for insects intended for human consumption. Although the following food safety criteria have been proposed for insects sold for human consumption: absence of *Salmonella* in 10 grammes, and *Listeria monocytogenes* less than 100 CFU/g (NMWA, 2014). The process hygiene criteria applicable in the European Union for foods of animal origin includes the following counts, with the highest maximum limit M as indicated: aerobic colonies (5 x 10^6 CFU/g in minced meat), Enterobacteriaceae (100 CFU/g in egg products), *E. coli* (5 000 CFU/g in meat products), coagulase positive staphylococci (10^5 CFU/g in raw milk-based cheeses), *Campylobacter* spp. (1 000 CFU/g in broiler carcasses) and alleged *Bacillus cereus* (500 CFU/g in dried infant formulae), in addition to the absence of *Salmonella* in carcasses. As regards food safety criteria, they consider *Listeria monocytogenes* (less than 100 CFU/g during the product shelf life), *Salmonella* and *Cronobacter* spp. (absence in 25 g and 10 g, respectively), Staphylococcal enterotoxins (not detected in 25 g) and histamine (less than 400 mg/kg) (EU, 2005). In light of the large number of potential insect species which might be destined to human consumption and the variety of possible methods of processing, the development of specific criteria applicable to insects is advisable, taking into account the product type, the processing method and other factors which might affect the microbiological quality.

One aspect of great relevance is adequate labelling. The labelling of marketed insects must indicate the adequate conditions of storage and preparation, together with tips to consumers on practices at domestic level to reduce risks, including instructions on the elimination of certain parts, such as cricket wings or legs, to improve the culinary experience and avoid risks of asphyxia, the message "wash before use" or instructions for cooking (FASFC, 2014) (EFSA, 2015). An inadequate cooking temperature may destroy vegetative pathogenic microorganisms, but may encourage sporulation of *B. cereus*. Therefore, special attention must be paid to the preparation of insects by the consumer, recommending boiling for 5 or 10 minutes, or oven drying at 90 ºC for 15 minutes. In the report written by the FASFC (2014) the application of heat (cooking) prior to consumption is considered essential.

The label should also warn of the possibility of allergic reactions in patients who are allergic to shellfish. This information must be available to the consumer to allow them to proceed correctly when handling these insects and therefore reduce any risks.

In many cases, depending on the processing type to which the insects have been subjected, they should be stored refrigerated. The temperature and shelf life of the product must be indicated.

At home, the following basic standards of hygiene should be adopted to minimise the risk of cross-contamination, the proliferation of potentially pathogenic microorganisms and their survival:

- Do not consume insects of dubious origin or that have been produced for animal feed.
- Insects for human consumption must be purchased from authorised establishments and must be suitably packaged and labelled in accordance with current legislation.
- The special conditions of conservation of the edible insects indicated on the label must be observed.
- Do not consume insects beyond the best before date indicated by the manufacturer.
- Do not consume raw insects as these may be contaminated by microorganisms.

- Raw insects should be boiled in water for 5 or 10 minutes, or oven dried at 90 ºC for 15 minutes prior to consumption.
- After cooking the insects must not be left at room temperature as this may cause the proliferation of microorganisms.
- If they cannot be consumed immediately, the left-overs to be kept must be maintained in a cool atmosphere and eaten as soon as possible. These left-overs must be heated to temperatures of more than 65 ºC prior to consumption.
- Cooked insects must be stored in closed containers.
- Cooked insects must be stored separately to raw food or objects (knives, chopping boards, work surfaces, tea towels, etc.) which have previously been in contact with raw food, as they may be re-contaminated by contact.
- Edible insects must only be handled with clean hands, utensils and on clean surfaces.
- Only use drinking water for handling at home.

Conclusions of the Scientific Committee

Insects are carriers of a large variety of microorganisms, some of which may be pathogenic for human. Hygienic conditions during insect farming and production have a notable effect on the risk of the presence and proliferation of pathogenic microorganisms. The presence of certain pathogenic bacteria has been regularly demonstrated, in particular spore-forming bacteria, able to resist the processing treatments and to grow during the subsequent storage.

The effects of insect processing on allergenicity are not foreseeable. Some of the allergens responsible for allergic reactions are stable to lyophilisation and heat treatment. To date, there is no processing for insects prior to their intake which guarantees the elimination of the allergenic effects.

The processing applied to insects intended for human consumption has a significant influence on their microbiological safety and quality. Depending on the type of processing applied, heat treatment is recommended prior to consumption.

It would be important to establish the most suitable preservation methods for the different species of insect intended for human consumption and to assess the impact on the microbiological safety and quality. Similarly, studies of shelf life would be of interest together with a risk assessment associated with their consumption.

The preparation of Guides for Good Hygiene Practices together with Guides for the Application of the Principles of the HACCP system should be encouraged, to help the edible insect sector to introduce self-control systems in accordance with their production and processing characteristics, and to promote the information provided to the consumer on the purchasing, handling and preservation of edible insects and specific recommendations for consumption for certain population groups.

The definition and application of specific microbiological criteria applicable to insects and their by-products is necessary, considering the product type, the processing and other factors which may affect their quality and microbiological safety.

For insects intended for human consumption, adequate labelling is required which includes instructions on the storage and preparation conditions and a warning about possible allergic reactions.

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