10. Food safety and preservation

Food safety, processing and preservation are closely related. Insects, like many meat products, are rich in nutrients and moisture, providing a favourable environment for microbial survival and growth (Klunder *et al.*, 2012). Traditional processing methods, such as boiling, roasting and frying, are often applied to improve the taste and palatability of edible insects and have the added advantage of ensuring a safe food product. Cultural preferences and organoleptic (sensory) aspects play important roles in chosen preservation methods. Although a wide variety of modern preservation methods is available, specific measures to ensure a high-quality and safe food item may be required for different insect species, depending on their biological makeup. Determining optimal preservation methods will be a critical factor in the commercialization of edible insects on a global scale, be it for food or feed. In this chapter the main focus is on food; however, the same also applies for feed.

The Hazard Analysis Critical Control Points (HACCP) system, a science-based and systematic tool, identifies specific hazards and establishes control systems to ensure the safety of food (FAO/WHO, 2001a). Its focus is preventative in nature, rather than relying mainly on end-product testing. HACCP is recognized worldwide as a system for quality assurance, identifying, evaluating and controlling physical, chemical and biological hazards throughout the production process. The system can be applied across the food chain, from primary production to final consumption.

As well as increasing food safety, the application of HACCP can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety. For these reasons, the adoption of HACCP throughout the insect supply chain will be a determining factor in the success and development of the edible insect sector. According to FAO, "any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments" (FAO/IAEA, 2001).

Although it has been stated that no significant health problems have arisen from the consumption of edible insects (Banjo, Lawal and Songonuga, 2006b), consumer confidence is arguably strongly correlated with the perceived safety of a given product. In this vein, the application of pesticides on insects destined for the food sector raises important issues, both for nutritional security and participation in the global market. It is well documented that species caught in fields, for example, are more likely to contain pesticides or heavy metals than those collected in dense forests. Chapulines (*Sphenarium purpurascens*) – red grasshoppers typically harvested in regions like Oaxaca, Mexico – have been found to contain high concentrations of lead from nearby mines (Handley, 2007). Many countries in Africa do not have policies governing the use of chemicals in fields in areas where villagers collect edible insects. Most of the time, collection takes place with little knowledge of the consequences that eating chemically treated insects might have (Ayieko *et al.*, 2012) (for more information on this topic, see Chapter 12). However, food-safety issues are important not only for insects collected in the wild but also for farmed insects.

10.1 PRESERVATION AND STORAGE

Insects are often consumed quickly after harvesting. Some insects are commercialized and transported within countries or beyond national borders for sale in distant markets; this is not uncommon between the Lao People's Democratic Republic and Thailand, for example. Live insects, after washing, are typically transported in ice coolers shortly after collection. Refrigeration is also recommended for fried and boiled insects.

Insects can be preserved and traded after (sun-)drying – a typical method used in processing the mopane caterpillar, for example (Allotey and Mpuchane, 2003) (Box 10.1). The dry environments typically found in places where sun-drying is common practice limits the growth of most micro-organisms. In humid areas, however, even sun-dried caterpillars are susceptible to moisture, which can stimulate the growth of microbes. Insects can also be re-contaminated during the drying process through air or soil; for this reason, hygienic practices during processing are of great importance and an additional heating/cooling step is recommended before consumption (Amadi *et al.*, 2005; Giaccone, 2005).

In many parts of the world, "ready-to-eat" insects are often sold in local markets after frying or roasting. In such cases, hygienic handling is equally important to prevent the potential risk of re-contamination and cross-contamination. At a household level, fresh insects should be prepared hygienically and sufficient heat treatment applied to ensure a microbiologically safe food product. Other simple preservation methods such as acidifying the insects with vinegar have been successful. Another example is the use of insects for protein enrichment in fermented food products. This is a viable processing option with mutual benefits, since the decreased pH in lactic acid-fermented products prevents the growth of potentially harmful micro-organisms (Klunder *et al.*, 2012).

There has been some success in processing and commercializing insects in the Netherlands. Three insect species (yellow mealworm larvae, lesser mealworm larvae and migratory locusts) can be found in specialized shops in the country that are produced and processed specifically for human consumption. One-day fasting is applied to ensure that the insect has an empty gut (degutting), and the insect is then freeze-dried whole. This produces a safe product with a relatively long shelf life (one year), if stored appropriately in a cool, dry place. Additional advantages of freeze-drying are the maintenance of the insect's nutritional value and the capacity of the product to re-absorb water. Nevertheless, obstacles remain: freeze-drying is expensive and often results in undesirable oxidation of

BOX 10.1

Processing the mopane caterpillar for human consumption

Care should be taken to avoid contamination throughout the various processing stages to ensure a safe product.

Degutting

- Ensure that holes have been dug for disposing of the gut contents.
- Holes must be covered immediately after degutting.

Drying

- Boil the bags (made of hessian or polypropylene) for at least 30 minutes and sun-dry for at least two hours before using them in the field.

Storage

- Ensure that bags are clean and disinfected before placing the caterpillars inside them.
- Ensure that bags are tied immediately with rope and the seams sewn up. They should then be covered with polythene material and placed on a raised platform to prevent cross-infestation from the surrounding environment and moisture from the ground from penetrating the bags.

Source: taken from Allotey and Mpuchane, 2003.

the long-chained unsaturated fatty acids, decreasing the nutritional value of the product and resulting in "off" odours and tastes.

A host of other contemporary preservation methods should be explored, such as the application of ultraviolet light and high-pressure technologies, as well as adequate packaging methods. Other important considerations need to be made in selecting the preservation method: the capacity to prolong shelf life (and in turn, contain costs), particularly if large amounts of insects need to be processed simultaneously; the extent to which the process preserves the nutritional value of the insects; and the cultural acceptability of the chosen preservation/processing method.

10.2 INSECT FEATURES, FOOD SAFETY AND ANTIMICROBIAL COMPOUNDS

Several issues related to food safety are distinct to insects because of their biological makeup: microbial safety; toxicity; impalatability; inorganic compounds; and the use of waste as insect feed.

Insect animal feed developed from manure and related organic waste streams raises bacteriological, mycological and toxicological concerns. Although some of these have been mentioned in the literature (Téguia, Mpoame and Okourou, 2002; Awoniyi, Adetuyi and Akinyosoye, 2004), they still have not been adequately researched (see section 5.2). The question is whether and to what extent insects sequester pathogenic organisms and toxic substances from manure and organic waste products.

10.2.1 Microbial safety

Insects may have associated micro-organisms that can influence their safety as food. Both insects collected in nature and insects raised on farms may be infected with pathogenic micro-organisms, including bacteria, virus, fungi, protozoa and others (Vega and Kaya, 2012). Such infections can be common. In general, insect pathogens are taxonomically separate from vertebrate pathogens and can be regarded as harmless to humans. Even within the genus *Bacillus* the insect pathogenic species *B. thuringiensis* and the vertebrate pathogen *B. anthracis* seem to have differing, non-overlapping life cycles (Jensen *et al.*, 1977). Also, insects have a high diversity of associated micro-organisms in their gut flora. Again, these organisms should, in general, not be seen as potential human pathogens. Finally, spores of various micro-organisms may be present on insect cuticles, including micro-organisms that grow saprotrophically on edible insect products and may even contribute to the degradation of the edible product. The above-mentioned micro-organism–insect association should, from the perspective of food consumption, be seen as microbial contamination and be treated as such.

In most tropical countries, insects are consumed whole, including their gut microflora. An exception is the mopane caterpillar, which is degutted (emptying the stomach by putting pressure on the body with two fingers), or fasted for one or two days before consumption. This process can affect the microbiological composition of an insect food product. Yet existing studies on the microbial safety of edible insects focus mostly on traditional practices of insect harvesting and consumption, making it difficult to decipher causal sources of infestations. Insect farming can allow greater control over hygienic practices and safe feed sources for insects, mitigating potential microbiological hazards.

The sanitary quality of the mopane caterpillar has been studied extensively given its frequent consumption in many African countries (Mpuchane, Taligoola and Gashe, 1996; Allotey and Mpuchane, 2003). One study carried out in Botswana demonstrated deterioration in sun-dried phane (the mopane caterpillar; *Imbrasia belina*) quality (i.e. disintegration of the inner flesh and change in colour due to mouldy growth and cavities in the chitinous exoskeleton). The most frequent fungal isolates found were species of *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium* and *Phycomycetes*. Species or strains of *Aspergillus*, *Penicillium* and *Fusarium* are associated with mycotoxin production. Mpuchane, Taligoola and Gashe (1996) found levels of aflatoxins varying from 0–50 µg per kg of product; the maximum safe level set by FAO is 20 µg per kg. Frequent consumption of infected foods over longer periods is likely to pose health risks. Although the caterpillars in this particular study were degutted, boiled for 15–30 minutes and spread out on sheets or on the ground to sun-dry for 1–3 days, it was hypothesized that contamination was caused by one of the following sources: water of poor quality, insect vectors (such as flies and dipterans) and soil. To maintain the best sanitary quality, the study recommended drying the caterpillars quickly and evenly after harvesting and processing and storing them in a cool, dry place.

In West Africa, three rhinoceros beetle species of the genus *Oryctus* are commonly consumed: *O. monoceros* and *O. owariensis*, which breed in dead-standing coconut and oil palms, and *O. boas*, which occurs in rotting vegetation and manure heaps. Of the three beetle species, pathogenic bacteria were found in *O. monocerus*, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus*, which may pose a risk to consumers (Banjo, Lawal and Adeyemi, 2006a). This contamination may have been due to improper processing, handling during retail and purchase, or exposure to air. As the consumption of the beetles contaminated with the pathogenic bacteria could pose risks to consumers, it was recommended that retailers of the partially dried and fried grubs ensure proper heating of the products to eliminate the pathogens.

The importance of hygienic handling and correct storage was highlighted by Klunder *et al.* (2012) in a laboratory experiment looking at the microbiological content of farmed yellow mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*). Boiling the insects in water for a few minutes eliminated Enterobacteriacae, but spores were found to survive this process, with the potential that the spores could germinate and the bacteria grow given favourable conditions, such as temperatures around 30 °C and a moist environment, causing food spoilage. The spore-forming bacteria were found in the insect gut and on the skin and are likely to have been soil-borne. Alternative preservation techniques that do not involve the use of a refrigerator are drying and acidifying. Lactic fermentation of composite flour/water mixtures containing 10–20 percent powdered roasted mealworm larvae resulted in successful acidification and was demonstrated to be effective in safeguarding shelf life and safety by the control of enterobacteria and bacterial spores.

In another experiment, chemical–physical and microbiological analyses of the following five insect species with rearing potential were carried out: superworm (*Zophobas morio*), yellow mealworm (*Tenebrio molitor*), wax moth (*Galleria melonella*), butterworm (*Chilecomadia mooreï*) and house cricket (*Acheta domesticus*). Neither *Salmonella* nor *Listeria monocytogenes* were identified in the analysed samples and it was concluded that it is unlikely that these insects attract microbial flora that pose risks to humans. However, it is still recommended that insects undergo a transformation to render inactive or reduce their microbial content. This could involve cooking (e.g. boiling or roasting) or pasteurization (Giaccone, 2005).

In contrast to being a potential microbial hazard, some edible insects are known to contain antibacterial peptides. A novel peptide (Hf-1) from the larvae of the common housefly (*Musca domestica*), for example, has been found to inhibit strains of food pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Bacillus subtilis*. The presence of Hf-1, also found in orange juice, suggests that the insect has potential as a food preservative (Hou *et al.*, 2007).

10.2.2 Toxicity

Some insect species considered toxic are eaten after precautionary measures are taken (Box 10.2). In Cameroon and Nigeria, *Zonocerus variegatus* has to be prepared in a specific way (Barreteau, 1999) by heating the insects in tepid water and then changing the water before cooking (Morris, 2004). Similarly, the tessaratomid *Encosternum* (=*Natalicola*) *delegorguei* in Zimbabwe and South Africa excretes a pungent fluid (Faure, 1944; Bodenheimer, 1951) that can cause severe pain and even temporary blindness if it comes into contact with the eyes (Scholtz, 1984). Therefore, the insect is consumed after removal of the fluid by squeezing the thorax and placing the bug in tepid water.

BOX 10.2

The stink bug *Nezara robusta* in southern Africa

Pentatomid bugs are widely consumed in southern Africa. Among these is the stink bug *Nezara robusta*, commonly found in *Brachystegia* woodland and blue gum (*Eucalyptus globulus*) plantations. The shield bug, another type of pentomid, is found at around 1 200 m above sea level in shady conditions and is associated with trees such as *Uapaca kirkiana*, *U. nitida*, *Brachystegia spiciformis* and *B. floribunda*. As its name suggests, the bug releases a powerful smell.

Women typically collect the bugs in the early morning because in cold conditions the cold-blooded insects are immobile and easy to collect. Collectors hit tree trunks with a log or long bamboo pole with a pouch fastened to one end to shake the bugs from the trees. The captured bugs are transported in bamboo baskets. They are prepared for consumption in tepid water, although care must be taken because the bug's bitter juice stains fingers brown and can be painful if it comes in contact with the eyes. After soaking in water, the bug ejects its bitter juices and loses its powerful smell. The bug is never placed in boiling water, as this would kill it immediately and cause the poison to be retained. After washing, the bug loses its green coloration and becomes a pale golden yellow. It is then cooked with a little water and salt. It is commonly eaten as a snack or side dish and the surplus sold in local markets. The water used to wash the bugs is said to be a useful pesticide against termites.

Source: Bodenheimer, 1951; Morris, 2004.

In the Carnia region of northeast Italy it is customary for children to eat the sweet ingluvies of a brightly coloured moth from the genus *Zygaena* (Zagrobelny *et al.*, 2009) (the ingluvies is the crop-widened portion of the oesophagus in many mollusks, insects and birds, which serves to accumulate, store and sometimes also begin the chemical processing of food). The moths contain cyanogenic glucosides, which release toxic hydrogen cyanide on degradation. They contain very low amounts of the toxic substance but high quantities of various sugars. Children collect the moths in early summer when they are plentiful and dissect them themselves, eating only the ingluvies.

There are limited reports, however, of adverse reactions caused by insect consumption. Cases of ataxia syndrome, characterized by tremors, ataxia and varying levels of impaired consciousness, were reported after consumption of the seasonal silkworm *Anaphe venata* in southwest Nigeria (Adamolekun, 1993; Adamolekun, McCandless and Butterworth, 1997). Further studies indicated that the reaction was most probably related to a structural undernourishment in the consumers who, being marginally thiamine deficient as a result of a largely carbohydrate-based diet containing thiamine-binding cyanogenetic glycosides, experience seasonal exacerbation of their thiamine deficiency from thiaminases in seasonal foods. In turn, they experience an adverse reaction to *A. venata*, which contains such thiaminases.

Edible insects can sometimes contain certain features that may be hazardous. For example, the consumption of caterpillars with hairs containing toxic substances can be very dangerous. These hairs have to be burned off (Muyay, 1981).

10.2.3 Unpalatability

In the Democratic Republic of the Congo, Bouvier (1945) observed that consuming grasshoppers and locusts without removing the legs caused intestinal constipation because the large spines on the tibia (shinbone) would catch in the gut. The only remedy in humans after consumption is often surgery to remove the legs from the gut. Similarly, in eastern Java, Indonesia, patients found to have eaten large quantities of roasted scarab beetles (*Lepidiota* spp.), whose indigestible chitinous remains can accumulate in several places inside the gut and cause total constipation, had to undergo surgery (Kuyten, 1960). Autopsies of dead monkeys following locust invasions revealed that the consumption of locusts proved to be fatal for the same reason. The product label of the migratory locust, Bugs Locusta, currently sold on the Dutch market, clearly states that the insect's legs and wings should be removed prior to consumption.

10.2.4 Inorganic contamination

Harmful metals from the environment have been found in the cells of several insect body parts – such as the fat, integument (exoskeleton), reproductive organs and digestive tracts – where they bio-accumulate. A study on the yellow mealworm larvae (*Tenebrio molitor*), for example, showed that the insects accumulate cadmium and lead in their bodies when they feed on organic matter in soils that contain these metals (Vijver *et al.*, 2003). However, Lindqvist and Block (1995) showed that after each moult, larvae lose some cadmium, and even larger amounts of the metal are lost after metamorphosis. Further research into the consequences this might have for human consumption is necessary.

Another issue of concern is the uptake of pesticides by edible insects such as locusts and grasshoppers, which can cause problems when they are consumed in large quantities. These risks are of major concern in the traditional practices of harvesting and consuming insects in the wild, where the control of chemical applications is difficult (Box 10.3). This is yet another potential benefit of insect rearing, where chemical hazards can be controlled to a larger extent.

BOX 10.3

Bogong moths in Australia

Each spring, the cutworms of the bogong moth (*Agrotis infusa*) leave the lowland breeding grounds of eastern Australia to escape the harsh summer environment. They migrate up to 1 000 km to the Snowy Mountains and the Victorian Alps in southeastern Australia, spending the summer in large congregations in caves and crevices between rocks at about 1 200 m above sea level. In autumn, they make the return journey home. During this migration, they are often considered a nuisance because they are attracted to lights on houses and other buildings at night.

Indigenous Australians are documented to have once harvested the moths from the Alps using the heat and smoke of torches, a custom no longer practised. They feasted on this tasty, high-protein and high-fat resource. Yet a study by Green *et al.* (2001) showed that the insects transport sub-lethal quantities of arsenic taken up in the lowland breeding grounds from plants in areas where herbicides have been used. Arsenic was concentrated in damaging levels on the aestivation (similar to hibernation) sites on the Alps, as a result of the millions of moths congregating in these areas. The arsenic was also detected in soil inside the caves and on grass from outwash areas.

Source: Green *et al.*, 2001.

10.3 ALLERGIES

10.3.1 Allergic reactions to edible insects

Like most protein-containing foods, arthropods can induce allergic reactions in sensitive humans (Immunoglobulin E (IgE) mediated). These allergens may cause eczema, dermatitis, rhinitis, conjunctivitis, congestion, angioedema and bronchial asthma. While some people have a history of atopy (allergic hypersensitivity), it is also possible to develop allergic sensitivity through long-term exposure. The majority of cases are inhalant or contactant in nature (Phillips and Burkholder, 1995; Barletta and Pini, 2003). Allergic reactions to bee and wasp venom (injectant allergens) are well known.

Individuals in constant contact with insects, such as entomologists, laboratory workers (working mostly with beetles, cockroaches, locusts, blowflies, crickets, moths or flies) and agricultural and industrial workers (working mostly with bean weevils, grain weevils, mushroom flies, sewer flies, houseflies, silkworms or fish bait, such as the larvae of flies and moths) are most vulnerable to such allergies. Ways of developing allergic reactions include inhalation of dust containing cockroach fecal matter, and skin contact with caterpillar hairs. Studies suggest that people frequently in contact with larvae of *T. molitor*, for example, run the risk of developing certain allergic reactions (Senti, Lundberg and Wüthrich, 2000; Siracusa *et al.*, 2003). The same was found for the closely related species *Alphitobius diaperinus*. The symptoms of the allergic reactions include inflammation of the eyes and nose (*T. molitor*) and itching, mild swelling, inflammation of the nose, asthma and skin rash (*A. diaperinus*) (Schroeckenstein *et al.*, 1988; Schroeckenstein, Meier-Davis and Bush, 1990). Cross reactivity can also occur between the two species, meaning that the antibodies for a specific allergen in one insect species is capable of identifying allergens in another and may thus induce an allergic reaction to that insect as well. Cross-reactivity is not absolute, however; some people develop allergic reactions to specific insects with little cross-reactivity to other insects due to long-term exposure to high amounts of allergens from that specific insect. In household settings where several insects and other arthropods can co-occur, it is difficult to assess whether an allergic person has multiple sensitivities caused by all arthropods or a general allergic sensitivity to invertebrates (cross-reactivity) (Barletta and Pini, 2003). Tropomyosins (actin-binding proteins that regulate muscle contractions) from cockroaches, mites and shrimps have been reported to be allergenic. Some patients allergic to dust mites that were increasingly exposed to mite antigen became sensitive to seafood tropomyosins, for example (Reese, Ayuso and Lehrer, 1999). These findings suggest that people with seafood allergy, for example, could experience allergic reactions to the consumption of edible insects.

There is a certain amount of evidence of allergies induced through the ingestion of insects. Because honeybee larvae contain pollen, for instance, people allergic to pollen are advised not to eat them (Chen *et al.*, 1998). Asthmatic symptoms were recorded on ingestion of *Orthoptera* (Auerswald and Lopata, 2005). In one study conducted in the Lao People's Democratic Republic, one respondent with a history of consuming insects is said to have developed an allergy to giant water bugs, while another is said to have developed an allergy to all edible insects, as well as shrimps (J. Van Itterbeeck, personal communication, 2012). This raises the question of the potential of developing sensitivity caused by ingesting edible insects and by handling while cooking and eating. It is doubtful whether processing measures such as boiling will destroy allergenic components (Phillips and Burkholder, 1995). For the great majority of people, however, eating and/or exposure to insects do not pose significant risk of causing allergenic reactions, especially if the individuals have no history of arthropod or insect allergen sensitivity acquired through long-term exposure to an allergen in sufficient quantities.

BOX 10.4

The allergy–hygiene hypothesis[13]

Allergies are an increasing problem in Western populations, contrary to developing countries where their prevalence is far lower. The hygiene hypothesis states that the high prevalence of allergies in Western populations is induced by a lack of exposure to pathogens, including intestinal parasites, and to increased vaccination practices during childhood.

Most parasites contain chitin. It is hypothesized that the variation in exposure to chitin and to intestinal parasites may be a key to explaining the asymmetric prevalence of allergies in populations. The presence of chitinases in human gastric juice has been associated with responses to parasitic infections and linked to allergic conditions. A review of the immunological response to chitin and its possible role in inducing asthma and allergies revealed that the responses appeared to depend on the particle size of the chitin substance; in other words, medium-sized chitin particles induce allergic inflammation, while small-sized chitin particles may have the reverse effect of reducing the inflammatory response (Brinchmann *et al.*, 2011). The consequences for the pathogenesis of asthma and allergies following increased consumption of chitin through the promotion of insects as food are unpredictable. However, if allergies are catalysed by a lack of exposure to chitinous substances in childhood, as suggested, increasing the consumption of insects in early childhood could, by extension, support better protection against allergies later in life.

[13] N. Roos contributed this box.

Source: FAO/WUR, 2012.

10.3.2 Immunological effects of chitin, a major component in insect cuticle

Chitin, the second most abundant polysaccharide in nature, contains nitrogen and is commonly found in lower organisms such as fungi, crustaceans (e.g. crabs, lobsters and shrimps) and insects, but not mammals. Although the anti-viral and anti-tumour activities of chitin/derivatives have been known for some time, the immunological effects of chitin have only recently been recognized (Lee, Simpson and Wilson, 2008). Recent studies have demonstrated that chitin has complex and size-dependent effects on innate and adaptive immune responses (see Lee, Simpson and Wilson, 2008). In several studies, it was suggested that chitin is an allergen (Muzzarelli, 2010). However, chitin and its derivative, chitosan (produced commercially by the de-acetylation of chitin), rather than acting as allergens have been found to have properties that could improve the immune response of specific groups of people (Goodman, 1989; Muzzarelli, 2010; H. Wichers, personal communication, 2012) (Box 10.4). By inducing non-specific host resistance against infections by pathogenic bacteria and viruses, there are indications that chitin reduces allergic responses in individuals. Moreover, chitin has shown potential for boosting immune system functioning, making it a promising alternative to antibiotics currently used in livestock (H. Wichers, personal communication, 2012). The use of chitin for medical and industrial purposes needs to be explored further.