Packaging, storage and shelf life analysis

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This document is part of our literature review on nutritional quality, processing, functionality and shelf life and storage of insect-based products, and the associated analytical methods.

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1. Introduction

Insects, as any other biological materials utilized as food, are expected to preserve the raw material and the natural ingredients to the greatest extent possible. The procedures (the processing, cooking, storage, packaging and transporting techniques) have a relevant potential to affect characteristics such as aroma, appearance, taste, and texture appeal and spoilage including safety hazards to the final products, and cause a rejection/hazard by the market and potential consumers. As any food material, contaminants can be introduced into the insect-based product throughout the breeding, manufacturing, packaging and transporting process, resulting in undesirable changes which could greatly affect safety and market appeal. As such, defining preparation changes and determination of shelf-life standards for a given species will further enhance product safety as known ingredient bioactivity or potential toxicity-generating changes, therefore them can be addressed and mitigated (Marone 2016). There have been relatively few studies on insects as food in general and the evaluations and comparison of edible insects as food reported to date do not give conclusive results. Therefore, detailed and consistent studies targeting certain individual insects, preparation, storage and other aspects are needed to give a clearer picture of the use of insects as human food (Elhassan, Wendin et al. 2019).

Here we present a literature review based on the works and publications and protocols established by the International Platform of Insects for Food and Feed (IPIFF). Suggestions and protocols available in the literature have been written literally in order to present the available information with ease. However, it is highlighted the lack of studies related to the topic, especially for the specific case of each insect species.

2. Shelf life analysis

Shelf life has different definitions; to the general consumer, shelf life is the length of time before the food is considered unsuitable for human consumption. However, shelf life can equally be given as the length of time a food product can be stored and displayed maintaining an acceptable quality or specific functionality. Shelf life can be defined as: how long can the producer guarantee the stability of the quality characteristics. We differentiate between best before date and use by date. First is for products which may loose quality and second about products which are not safe after the date or if stored not correctly.

2.1. Shelf life of insects as components and ingredients

There is currently very little information available on the shelf-life properties of whole insects, insect components such as fats, oils, etc., and insect-based food ingredients. However, there are some known characteristics of insects, which suggest that insect-based food ingredients may have an advantage of longer shelf life, particularly for dry products or pastes with high levels of insect content. Several insects have been shown to contain antimicrobial substances such as secondary metabolites, fatty acids, and peptides, which may be helpful protecting the shelf life of insect-containing foods. Besides, the chitin from insects is known to have antimicrobial properties. However, there is a great need to conduct more studies about shelf life of insect-based food ingredients, particularly for whole insect, powders and insect oils (Dossey, Tatum et al. 2016).

2.2. Whole insect

The Netherlands is among the firsts countries to pioneer hygienic whole-animal processing for “ready-to-eat” human consumption, specifically for the mealworm beetle. Processing steps consisting of: (1) one-day fasting (to ensure an empty gut), followed by (2) heat treatment, and (3) whole-body freeze-drying for extended 52-week shelf life (Marone 2016). 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 with a sufficient heat treatment to ensure a microbiologically safe food product. Other simple preservation methods such as acidifying the insects with vinegar have been successful (Huis, Itterbeeck et al. 2013). 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 microorganisms (Klunder, Wolkers-Rooijackers et al. 2012).

For the species yellow mealworm larvae, lesser mealworm larvae and migratory locusts one-day fasting technique is applied to ensure that the insect has an empty gut (degutting), and the whole insect is then freeze-dried. This produces a safe product with a relatively long shelf life (one year), if stored appropriately in a cool, dry place (Huis, Itterbeeck et al. 2013). 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 the long-chained unsaturated fatty acids, decreasing the nutritional value of the product and resulting in “off” odours and tastes (Huis, Itterbeeck et al. 2013).

2.3. Powders

Processed and whole-animal dried insect-derived foods can be compact, easy to store, and transport. Their shelf life (powder) is considered advantageously longer than traditional meat/poultry/fish protein sources. This serves to heighten their practical safety appeal (Huis, Itterbeeck et al. 2013). A fine particle size dry powder will be the ideal insect-based ingredient format. The reasons are as follows: (1) powders have the longest shelf life, in general, more than a year, depending on production and processing method, packaging, and storage environment; (2) powders can be blended with other ingredients without compromising the texture or structural integrity of the product and; (3) depending on the processing method, a powder can have the mildest flavour and aroma as well as the lightest colour. The general usefulness and long shelf life of powders make them ideal for any product (even when the final product might not be a dry one) as the ingredient can be shipped, stored for long periods, and then used as needed. This can help a company purchase larger quantities of the insect-based ingredient at a time without worrying about having to use it quickly (Dossey, Tatum et al. 2016).

Kim, Kim et al. (2016), determined the shelf life of cricket powder and investigated the changes in its quality during storage at temperatures of 25, 35, and 40℃ for 6 months. The moisture content, acid value, oleic acid, and flavor were selected as the criteria for shelf-life establishment of cricket powder. They observed that the moisture content of the cricket powder increased during storage but did not show any significant difference at 6 months of storage. Furthermore, the composition of fatty acids of cricket powder (palmitic acid, oleic acid, and linoleic acid) was not changed at the various storage temperatures. No aerobic and coliform bacteria grew in the powder during the whole storage period. Cricket powder stored at 25℃ and 35℃ showed similar scores in sensory evaluation. The acidity value of cricket powder, monitored during storage, showed a negative relationship with storage temperature. This decrease in the acidity of cricket powder due to increased temperature can be attributed to the oxidation of fat. They concluded that based on these studied parameters, especially the moisture content, the shelf life of cricket powder was likely to be 18 months when stored at 25℃. Sensory attributes of cricket powder seem to be stable at 25 ◦C and 35 ◦C, while inferior sensory evaluation can occur at 40 ◦C.

2.4. Liquids, slurries and pastes

The shelf life of a liquid is shorter than that of a dry product (however that depends on the product treatment/processing). Liquid could be desirable over powder in limited cases, especially when the product manufacturing are done at a single location from raw insect to the finished product, for example, no shipping or long-term storage product. Using a liquid might be best in those situations since the insects are already mostly water and it is not necessary to dry. Aside from the shorter shelf life and more care needed in storage and handling to avoid microbial growth, care must also be taken with processing insect-based liquids. Since insects contain a large amount of protein and nonliquid tissue, insect material in liquid form can denature and turn into a paste or solid when heated or with other treatments (with protein denaturing salts, reagents, enzymes, etc.) (Dossey, Tatum et al. 2016). A very interesting approach to extend food products shelf life in the format of liquids or slurries is the application of high hydrostatic pressures (HHP); by means of this technique, it has been demonstrated that number of bacteria can be effectively decreased with no affecting the quality of the final product. Such technique has been successfully applied in product with high protein content as for example chicken (Rodríguez-Calleja, Cruz-Romero et al. 2012).

2.5. Oils

Oils, blended oil and oil formulations have different degrees of susceptibility to oxidation when stored alone or in food products. Therefore, different insect oils have different shelf lives, or impact in different ways depending on the products they are utilized in. Hence, it is necessary to obtaining shelf life studies in different insect oils and the products in which they are used (Dossey, Tatum et al. 2016). Lipid composition is one of the main factors affecting oils preservation. Oils with higher content of PUFA's tent to be more prone to oxidation. Therefore, strategies such as vacuum, inert atmospheres or encapsulations are of relevance. Due to the lack of information on shelf life in insects-food, here is a brief review on factors influencing shelf life of fish oils. Generally, the oxidative stability of fish oils depends on of factors, such as fatty acid composition, presence of impurities that in many cases may act as pro-oxidants, antioxidant protection, access to light and oxygen, and processing and storage temperatures (Mozuraityte et al 2016).

To process the raw material within a short time possible it is important to ensure high-quality oil, when producing crude oil. The raw material is exposed to high temperatures (90–95 °C) to cause protein coagulation and rupture of the fat depot, allowing the liberation of oil and water. It is common the use of a continuous steam cooker where the material is moved by a heated rotary screw conveyer, a processing step that takes around 20 min. Low-quality oil could result due to exposure to high temperature, oxidation can occur during processing. One way to reduce oxidation during crude oil production is by the addition of antioxidants to the raw material before production, by reducing the presence of oxygen in the production line (flushing with nitrogen) or by using equipment that requires less retention period for the raw material and oil at elevated temperatures (Mozuraityte, Kristinova et al. 2016).

3. Shelf life of preservation methods

3.1. Freezing

A feasible technique for initial temporary preservation of the insects in most cases is deep-freezing. When choosing a freezing method, it is important, to decrease the temperature and freeze the insects as quickly as possible (shock freezing) for shelf-life reasons as with any other food or raw biological material. Similarly, as any food or biological material insects contain bacteria, other microbes and fungi. The zone in which microbial growth occurs is in general from 4.5°C to 60°C. Therefore, products in the process of being frozen from warm or ambient temperatures are in this range until they are near frozen. Methods such as flash freezing or using small containers, such as plastic bags, etc. are ideal for freezing quickly and avoid microbial growth or degradation of the frozen product. For example, some farmers prefer to pack no more than 2.26 kg (5 lb) of crickets into a bag to be frozen at a time, and/or use large, flat bags instead of conventional bags that tend to accumulate into a thick pile. This maximizes the speed at which the cold can penetrate the core of the bagged insects. Insects also contain enzymes to degrade tissues when they have died, which are temporarily inactivated when they are frozen. Flash freezing is ideal because insects can be frozen rapidly then packaged in large bags, boxes, or containers in bulk (Dossey, Tatum et al. 2016).

However, enzymes are not necessarily deactivated during deep-freezing.This is why many foods get heated up briefly before deep frozen, enzymes can cause off flavors due to oxidation - Floto-Stammen, personal communication). Hydrolytic enzyme activity in frozen foods can result in quality degradation such as, textural softening, and colour loss. Textural toughening and acceleration in lipid oxidation may be secondary consequences. Food-grade inhibitors are available to control excess proteolytic activity; however, no food-grade inhibitors exist to control lipolytic activity. The way to avoid the consequences of lipolysis in frozen foods is to minimize the release of the enzymes from the cellular organelles through processes involving fast freezing and minimization of ice recrystallization in the product (Sista, Erickson et al. 1997).

3.2. Drying whole insect

Drying is primarily a preservation step to extend shelf life. It makes a product more efficient and cost-effective to store because dry food products do not need to be refrigerated or kept cool. Also, it is ship due to dry products weigh less, due to water in a product adds additional weight and consequently adds to transport cost. Insects can be dried in their whole form or as pastes, slurries, or liquids. With drying, it is important to measure both the total moisture level as well as the water activity in the product. The water activity is a measurement of how much water is free versus bound in a product. Typically, the free water is what contributes to microbial growth. The guidance and recommendations provided by Food and Drug Administration (FDA) about ideal water activity and moisture levels in dry products to mitigate microbial growth during storage can be followed to insect-based foods (Dossey, Tatum et al. 2016).

Roasting whole insects is a common method. This is often done for insects, which will ultimately be eaten as a whole, or for production of dry ground insect meals or powders. Even though this is a very popular, widely available, and low-tech method, it is far from ideal. Roasting is typically more expensive at the industrial scale than other drying methods, utilizes more heat and energy, and causes a greater level of product degradation, due to the extended time exposed to higher temperatures, than other drying methods. Due to exoskeleton and cuticle are designed to keep moisture in, insects are aimed to resist desiccation in nature and conserve water efficiently. Therefore, removing water from them via heating and evaporation is very inefficient. In addition, the heat required to achieve this is in a reasonable amount of time tends to be high. High heat exposure over time degrades the quality of all foods, food ingredients, and biological materials. Fats and oils oxidize (leading to lower shelf life), proteins denature or agglomerate, nutrients such as vitamins degrade, undesirable flavors and aromas can become stronger, and so on. Roasted insects become darker in color, and have stronger aromas and flavors than insects dried in other ways. Additionally, proteins that adhere to the insect chitin exoskeletons are difficult to remove. This causes the overall functionality of products made from roasted insects to be much less desirable than insects dried in other ways. The proteins also separate from the fats and oils, which melt at high temperatures, rather than blend with them homogeneously, and thus become more exposed to oxidation and make the product generally more oily (Dossey, Tatum et al. 2016).

3.3. Drying pastes, slurries, and liquids

It is possible to drying insects whole however, it is not ideal for the production of powders (fine) or meals made from high moisture raw materials. Insects are indeed a high moisture material, with most species naturally containing around 50–70% moisture. Therefore, the particle size reduction is taken care of in the wet phase. Advantages can be described for this process. (1) Liquids, slurries, and pastes are typically easy to manipulate and move through pumps, hoses, and pipes. Thus, it makes them easier to process compared with the original whole materials. (2) Slurries, liquids, and pastes are a more homogeneous mixture, yielding a much more consistent dried product at the end. In the case of high protein/fat, or high fat/protein/starch food products (such as insect powders, milk powders, etc.), this allows the fats, oils, proteins, and other components to remain together throughout the drying process. When they dry together, the non-protein/starch components can become microencapsulated into the proteins and starches and somewhat protected from oxidization and other elements that affect shelf life. Homogeneous powders also tend to be more free-flowing, clump less, blend better with other ingredients, and be generally more functional than powders produced in ways that do not keep the components well homogenized (Dossey, Tatum et al. 2016).

There are various methods for drying liquids, pastes, and slurries into powders or meals. These include drum drying, spray drying, fluid bed drying, tray drying, freeze-drying, cooking, roasting, vacuum drying, and dehydrating. With spray drying fats, oils, vitamins, and other components do not heat, cook, oxidize, or degrade as much as with some other methods, such as roasting. This often results in lighter color and more neutral aroma and flavor of products, which are desirable traits of bulk food ingredients such as protein powders. In spray drying, the short time exposure to heat, as well as the microencapsulation of components such as fats and oils, can also impart a better shelf life and overall better functionality and usefulness in a wider range of food and beverage products, than products dried using other techniques (Dossey, Tatum et al. 2016).

3.4. Milling

The most technologically simple forms of grinding, milling, or particle reduction are typically done with dry products. However, milling of whole raw insects, which have not yet been dried is possible. For example, when conducting the heating kill-step (cooking, pasteurization, etc.) after grinding (ie, pasteurizing a liquid or slurry) or before (i.e., steaming or boiling insects before grinding), wet grinding and milling take a product containing a significant content of water or other liquid and reduce it into a liquid, paste, slurry or, a wet semisolid. One of the main differences between this and milling/grinding dry is that a wet product usually can more or less be treated like a liquid or semiliquid and thus, it can be pumped. This has several advantages in product handling, moving, pasteurization, manipulation, or movement for further processing within a processing plant. Being able to flow a product through pipes/hoses using pressure allows it to move into and out of various vessels and machines efficiently and consistently. Disadvantages of wet products over dry forms include: they have potentially lower shelf life, they need to be kept cool, they need to be more carefully handled or processed to mitigate microbial growth, and they weigh more and have greater volume, which negatively affects the economics of storing and shipping. Thus, shipping or storing a product in some dry format has several cost, quality, efficiency, and shelf life advantages. Cooking or pasteurization (even sterilization) can be done to insects used in wet form as a liquid or slurry and wet milling or grinding may still be useful in these situations if the material is to be utilized immediately. The wet milling process can be applied by batch (using things like rotor–stator apparatus, etc.) or continuously (using in-line machines). Additionally, for insects, removal of chitin may be desired. This will almost certainly require the product to be wet and the insects to have not been previously roasted or dried (Dossey, Tatum et al. 2016).

4. Microbiological aspects of shelf life

Levels of microorganisms in fresh insects were rather high and the sensory quality of insects deteriorated during the storage in refrigerator. Similar to other animal derived products, insects are rich in nutrients, moisture and contain their gut microflora providing a medium for growth of unwanted microorganisms in certain conditions. This makes insects susceptible for microbiological hazards if proper heat treatment or storage conditions are not applied. Klunder, Wolkers-Rooijackers et al. (2012) carried out an exploratory evaluation of the microbiological content of fresh, processed and stored edible insects, with focus on farmed mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*). In their work, authors recommend applying a heating step (boiling) before storing insects in the refrigerator; this would also preclude enzymatic spoilage such as manifested by black discoloration. The levels of bacteria on boiled (1 min) house crickets stored in the refrigerator remained stable during more than two weeks of storage. On the other hand, boiled insects (1 min) stored at room temperature spoiled rapidly. The high water content, high temperature and nutrient-rich environment creates a favorable environment for growth of surviving spoilage bacteria.

In the same study, it was observed that a short heating step was sufficient to eliminate Enterobacteriaceae; however, some spore forming bacteria will survive in cooked insects. Simple preservation methods such as drying/acidifying without use of a refrigerator were tested and considered promising. Lactic fermentation of composite flour/water mixtures containing 10, or 20% powdered roasted mealworm larvae resulted in successful acidification and was demonstrated effective in safeguarding shelf-life and safety by the control of Enterobacteria and bacterial spores.

It is possible to apply a fermentation process on composite flour containing 20% ground insects. It can also be concluded that Enterobacteriaceae are inactivated during the fermentation in all meals, likely because of the antimicrobial effect on Enterobacteriaceae during an acidic fermentation. The levels of spore forming bacteria remained stable at acceptable levels (Klunder, Wolkers-Rooijackers et al. 2012).

Non-heat or less-heat alternatives to reduce or eliminate microbial loads include acidification (addition of citric or other acids), preservatives, High-Pressure Processing (HPP), irradiation (currently not allowed), and others. Even dry products/powders can be pasteurized using longer time at moderately high temperatures (like egg powder), for products in which liquid pasteurization would damage the functionality or otherwise lower product quality. Technology even exists to pasteurize a raw egg without cooking it. Acidification also eliminates many types of microbes. Full microbial mitigation (i.e., complete sterilization) is not necessary for most food products, but the elimination of pathogenic species is critical. Non-pathogenic species may not be harmful to human health, but they may be spoilage microbes, which may cause degradation and reduction in quality of the product on the shelf. Insects sold raw or in need of cooking, steamed insects sold refrigerated or frozen, and so forth may be sold with higher acceptable microbial levels (with pathogens fully mitigated) than insect-based foods sold as ready-to-eat. While the microbiology of food as a topic is an entire field of study, and thus beyond the scope of this chapter, it is a critical matter to consider for anyone interested in food and agriculture (Dossey, Tatum et al. 2016).

Many foodborne pathogens and cases of microbial contamination come from within food processing and handling facilities, often from the environment, employees, or other products being processed at the same location. For other food commodities, the US Food and Drug

Administration (FDA) even has time and temperature regulations for various commodities such as milk, dairy products, meats, and other fools, but that does not yet exist for the insect-based food industry. It is evident the lack of information and standards need to be established (Dossey, Tatum et al. 2016).

In another experiment, chemical-physical and microbiological analyses of the following five insect species with rearing potential were carried out: super worm (*Zophobas morio*), yellow mealworm *(Tenebrio molitor*), wax moth (*Galleria melonella*), butterworm (*Chilecomadia moorei*) and house cricket (*Acheta domesticus*). Neither Salmonella nor *Listeria monocytogenes* were detected 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 trataments to render inactive or reduce their microbial content. This could involve cooking (e.g. boiling or roasting) or pasteurization (van Huis 2013).

5. Shelf-life in storage, packaging and transport

5.1. Legislative requirements

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, van Huis 2013). HACCP is mandatory for all food producing and food-handling facilities in the EU.

The storage and transport of insect for feed should be acording to the EU feed hygiene legislation (Regulation (EC) No 183/2005). The same legislation applies to them as to any other ‘feed business operator that is active in stages other than primary production'. Similar requirements are provided in EU food hygiene legislation for food business producers (Regulation (EC) No 852/2004). Besides, specifically for food business operators, Commission Regulation (EC) No 1935/200420 provides a legal EU framework on the general principles of safety and inertness for all Food Contact Materials (FCMs). The Regulation focuses on the core principles that require materials to not: release their constituents into food at levels harmful to human health, change food composition, taste and odour in an unacceptable way. FCMs Include direct or indirect contact of materials during the production process of insect-based food products (e.g. containers for transporting final insect-based food products, machinery to process food, packaging materials, etc.) (IPIFF 2019).

5.2. Storage facilities

Source (IPIFF 2019)

The facilities should ensure a safe environment and prevent contamination.Live insects to pet food or farmed food, should be stored in facilities that are designed to avoid any release of the insects into the environment. They should also be kept in a separate room, in the case that company produces different types of products. Storage areas should be arranged to allow segregation of raw materials, other ingredients (salt, oil, etc.), flavoring ingredients, packaging, chemicals (e.g. cleaning materials), waste and processed insects. A separate area for storing materials identified as potentially unsafe shall be provided. Where possible, employees should prevent non-dedicated vehicles from parking (e.g. fork-lift trucks, lorries) or circulating in storage areas. Dry storage should provide protection from dust, condensation, drains, waste and other sources of contamination. They shall be kept dry and well ventilated. The monitoring and control of temperature and humidity should be applied as required by the product or storage specifications. All materials and products shall be stored off the floor and with sufficient space between the material and the walls to allow inspection and pest control activities to be carried out (IPIFF, 2019). These rules and regulations are part of the GMP and more specifically covered in the HACCP plan.

5.3. Maintenance and cleaning of facilities

Source (IPIFF 2019)

Employees are required to indicate at what time the storage room or storage equipment was cleaned. The employee signs the document when finished with the cleaning operations. The storage handler keeps a book with the number of cleaning operations per day or per week, depending on the needs. It is important to establish a procedure including cleaning and disinfection activities, monitoring of hygiene and prevention of contamination by cleaning (agents). A documented cleaning and disinfection program is recommended to include the following elements: cleaning method, standards, frequency, chemicals used, equipment used, time and temperature specifications. Records of cleaning and disinfection procedures must be kept. The cleaning and disinfection agents used must be in accordance with the user instructions of the manufacturer and product safety data (information on the potential hazards: health, fire, reactivity and environmental). If cleaning and disinfection agents come into contact with feed/food material, then the responsible personnel must ensure that the control systems always ensure correct and effective levels of dilution of the agents. Cleaning and disinfection agents and other chemicals, which are used for hygienic purposes, must, if applicable, be stored separately in clearly identified areas. This is to avoid the risk of unintentional contamination or contamination due to negligence. Only permitted cleaning and disinfection agents must be in contact with food/feed materials. These activities should not form any risk under any circumstances to food/ feed safety. It is to be ensured that objects used for cleaning, and cleaning agents themselves, do not remain behind without need on/ in the cleaned equipment or areas. A proper ventilation system is required in the storage premises to make sure air-flow is constantly renewed in the premises. The storage premises must be ventilated to prevent excess humidity or heat (IPIFF 2019).

5.4. Storage conditions

Source: IPIFF 2019

Insects intended for food use, raw materials and ingredients shall be stored at the temperatures specified by the food processor responsible for their manufacture. Storage management of non-insect material should follow the principle of first expired – first out (FEFO). Material that requires cold storage shall be placed under chilled conditions without any unnecessary delay. Finished products must be clearly identified and appropriately handled and stored. Placement of finished products in the warehouse shall be managed according to the principle of first in – first out (FIFO). A thermometer must be fitted in a prominent position on a wall of the warehouse. Temperature readings must be taken regularly. Traffic arrangements shall be put in place to ensure the safety of the area. Pedestrianised areas should be clearly marked. Producers of insects intended for food should declare the proper storage conditions that are required depending upon the intended shelf life and use of the product on the label.

Some typical examples of the storage conditions are listed below:

1. Dry, cool and closed;
2. Avoid direct sunlight;
3. Frozen.

Producers of insects intended for food use should periodically check the stability of their product during the shelf life period. In case of deviations, there should be a strategic analysis to identify root causes and the appropriate actions to be taken. Any deviation during the shelf life period should be properly documented.

Summary recommendations of control measures in respect to storage operations for insect-based products

- (Locked) closed storage;
- (Locked) closed transport;
- Cleaning and disinfection procedures;
- Prevention accumulation of organic material;
- Visual check on objects foreign to the product;
- First in First Out (FIFO) strategy;
- Sampling plan for analysis of hazards for incoming raw materials and outgoing products;
- Adequate storage conditions with respect to product specifications;
- Documentation.

6. Packaging

Source: IPIFF 2019

Sealing and packaging of insects is a crucial part of the manufacturing process, as it contributes to the condition the products will be when they reach the end-user. Good hygiene, environment, security and quality practices must, therefore, be respected throughout the packaging process to ensure safe food or feed is delivered. To this end, several measures are recommended.

1. The producer using packaging ensures it is clean before insects are placed inside. When necessary, it should be disinfected.
2. The producer ensures the packaging is closed immediately after filling.

3. Storage rooms and recipients/containers/vessels are kept clean to avoid development of non-desired
pests. Levels of humidity and lights are monitored and maintained in accordance with the end-product needs.

4. When sealing the final product, the operator ensures that no external source of contamination (e.g. pests, physical hazards, etc.) is included in the sealed bag.

5. Packaging is also labelled according to existing legislation. The operator labels the sealed package
on the basis of the intended use.

6. The packaging material used (i.e. FCMs) must come form a certified supplier in order to mitigate chemical, physical and microbiological hazards which could compromise the food/feed safety.

Summary recommendations of hygiene control measures in respect to packaging of insect-based products:

- Have certified suppliers for packaging materials receiving records of packaging materials to avoid packaging material contamination;
- Maintain staff hygiene and attire;
- Set up a cleaning and disinfection plan to avoid chemical residues and microbiological contaminations;
- Develop a quality control plan and preventive maintenance from physical hazards;
- Develop production planning and control to avoid labelling and packaging errors;
- Document the above measures.

Source: IPIFF 2019

7. Transport

Source: IPIFF 2019

Manufacturers or handlers, whose mission is to transport food and feed products derived from insects, should maintain the same hygiene standards applied throughout the production chain. These operators are, therefore, compliant with legal requirements set in Regulation (CE) No

852/2004 on the hygiene of foodstuffs and Regulation (EC) No 183/2005 laying down requirements on feedstuff.

A few examples of recommended practices are provided below:

• The selection of transports and their activities should be monitored through an appropriate vendor/supplier approval system, demonstrating their commitment to good hygiene practices, especially with their transportation units and workers;

• The transporter shall provide information of the previous shipment and proof of disinfection before insect ingredients are loaded.

Food and Feed business operators shall notify the appropriate competent authority of any establishments under their control, active in transport as laid down by Regulation (EC) No 183/2005 and Regulation (EC) No 852/2004 as amended.

A finished Product Specification document (often referred to as 'birth certificate') should be issued for each batch, specifying all the following requirements:

1. Product description and composition;
2. Ingredient list to be put on the label;
3. Chemical, physical and sensory requirements and characteristics;
4. Microbiological criteria applied to verify microbial controls;
5. Any allergenic contents;
6. Product packaging (including traceability data and information to be put on the pack);
7. Shelf life and corresponding storage and transport conditions (temperature, humidity and lighting conditions and all other applicable elements which can affect product quality and food safety);
8. Any conditions of use.

Any mode of transport used for transporting food and feed products derived from insects, should be designed appropriately to avoid cross-contamination due to simultaneous or consecutive transport. Transport means must be cleanable and must be made of appropriate material or should be appropriately coated. The design and manufacture of the transportation units should facilitate inspection, cleaning, disinfection, and when appropriate enable temperature and cooling control. Sanitary requirements are as important in the transport operations as they are in the manufacturing activities. Upon request by the competent authorities, evidence may be requested to demonstrate that the heating media or cooling system employed has been properly cleaned, evaluated and safely used. Before compartments are loaded, they must be inspected by staff authorised by the operator, the owner or the receiver of the goods (e.g. the truck driver in case of self-service truck stations). An inspection of the loading compartment will be carried out to establish that the loading compartment:

1. Is clean, dry, odorless and correctly maintained;
2. Is compatible with the loading and transport of the specific products;
3. Is suited to the transport needs and forms a closed whole;
4. Does not contain pests and rodents in the widest sense of the term;
5. Does not contain residues or remains from previous loads and/or from cleaning products.

Appropriate measures must be taken to prevent any harmful influence from other products that could arise during loading and transport. This must include consideration of other operations when transporting within a port complex.

Compartments that have been used to transport products regarded as 'high-risk' during the previous load must undergo a risk analysis and may be refused. Construction materials shall be selected to prevent contamination of reared insects and the stored products. Floors, walls and ceilings shall be washable or covered/painted with a washable protective layer. Technologies used for livestock building construction are recommended, including 'sandwich panel' etc. Raw timber and materials with rough surfaces should be generally avoided. The operator should prevent non-dedicated vehicles from parking
(e.g. fork lift trucks, lorries) or circulating in storage areas, where possible.

Food and feed storing temperatures:

1. Freeze-dried: unchilled
2. Fresh: 0-7°C
3. Frozen: -18°C or colder

Food and feed storing temperatures:
1. Freeze-dried: unchilled
2. Fresh: 0-7°C
3. Frozen: -15°C or colder

If the temperature is warmer than above-mentioned limits, the product must be rejected, or Q/A must prove that no damage occurred to the product due to the fact that was exceeding the above thresholds for a very short period only.

Summary recommendations of hygiene control measures in respect to transport of insect-based products:

- Specifications carrier loads to ensure carrier cleanliness and avoid hazard contaminations;
- Specifications carrier loads to ensure adequate product conditions to avoid hazard contaminations;
- Shipping control to ensure package integrity to avoid hazard contaminations;
- Shipping control to ensure presence of harmful pests;
- Document the above measures.

Source: IPIFF 2019

8. Specific case studies

Ssepuuya et al. (2015) showed that for a grasshopper *Ruspolianitidula*, vacuum packaging can maintain sensory acceptability, and thus enhance storage stability at ambient temperature and that the quality can be further improved by low-temperature storage. After 12 weeks of storage at ambient temperature, processed and vacuum packed, ready-to-eat grasshoppers maintained their edible quality with an acid value of 3.2 mg KOH/g and an overall acceptability of 6.7–7.2 on a 9-point hedonic scale.

Oonincx and De Boer performed a study in 2012 and they concluded that, at this time, there was insufficient evidence available for the standardization of storage conditions for mealworms.

Stoops et al. (2017) examined the effect of different storage conditions on microbial growth in mealworms and concluded that replacing air in the storage environment with carbon dioxide and nitrogen reduced microbial growth during storage.

Musundire et al. (2014) found aflatoxin in stinkbug packed in recycled grain containers, which are traditionally used to package these insects, and attributed this to cross-contamination from the packaging. However, there is still a lack of published data on the effect of packaging type on the shelf-life of edible insects prepared as human food.

9. Methodologies to determine shelf-life in insect based products

As previously revised in this document, shelf life is one of the most relevant properties of a product when in the market. In order to determine the shelf life of a specific product, several tests can be conducted to ensure both quality and safety. While many different analysis can be carried out, the most of them are focused on microbiological status of the food product, to determine its safety for the consumer (the type of analysis has to be in agreement with legislation and product nature); and also those related to the inherent quality of the product and how this quality is retained over a period of time, as for example lipid oxidation, protein aggregation or moisture content.

9.1. Microbiological analysis

When analyzing insect based products two considerations need to be done, the first one is the format of the products; i.e., the whole insect as such, it can be a dry powder with a very low water activity, or it can be as part of a product where the water activity is much higher or as a paste (Klunder, Wolkers-Rooijackers et al. 2012) and the second one the storing conditions.

Some microorganisms affecting the safety of insect as food may be associated to them; regardless if they are harvested in nature or in farms. However, in general, insect pathogens are taxonomically different from vertebrate pathogens and can be regarded as harmless to humans. Also, spores from some microorganisms can be found in the insect cuticles, these can even growth on the final edible product and contribute to of its degradation and spoilage. Although according to Jensen, Newsom et al. (1977) the species predominant in insects is different to that affecting humans, *Bacillus thuringiensis* and *B. anthracis* respectively. Also, important is to look at anaerobic sporogenic bacteria described as *Clostridium* or *Bacillus cereus.* Nevertheless, the above mentioned microorganism should be considered as microbial contamination and be treated accordingly. However, growing and harvesting insect in strictly controlled farms will reduce the risk of potential microbiological hazards (Huis, Itterbeeck et al. 2013).

The most relevant analysis for dried products are those related to aerobic mesophilic microorganisms, counted as TVC (total viable count); enterobacteriaceae; lactic acid bacteria (LAB), aerobic spore forming bacteria (ASFB), including specifically *B. cereus;* anaerobic bacteria; bacteria from the phylum Pseudomonas, and finally (depending on the water activity of the final powder, if it falls between 0,95 to 0.60) the presence of xerotolerants moulds and yeast.

Table 1: Conditions employed to perform microbiological analysis of dry samples

Analysis	Media	Temp (°C)	Time	Others	ISO ref
TVC	PCA	30	48 h		ISO 4833
Enterobacteria	Violet red bile glucose	37	24 h	2 g/L Delvocid (Natamycin)	ISO 21528-1
LAB	MRS broth + agar	30	48 h	Microaerophilic conditions	ISO 15214
ASFB	PCA + Agar	30	48 h	Sample heated for 5 min at 80 °C	
Bacillus cereus	Mannitol egg yolk polymyxin agar (MEYP)	30	24 h		ISO7932
Total anaerobic	PCA	30	48 h	Under anaerobic atmosphere (CO_2)	
Pseudomonas	Cetrimide fusidin cephaloridine agar (CFC; Oxoid)	30	48 h		ISO 13720:2010
Xerotolerant yeast and moulds	Dichloran (18%) Glycerol Agar	25	5-7 days		ISO 21527 part 2

In addition, common food-borne pathogens should be analyzed as for example *Listeria monocytogenes, Escherichia coli* and *Salmonella.* Up to date, there is not specific criteria for insects used as human food; however, the same process hygiene followed by minced meat should be contemplated (European Union Regulation (EC) No. 1441/2007) (Nganga, Imathiu et al. 2018). According to this legislation the number of TVC range from 5 x 10^5 to 5 x 10^6 cfu/g; *Salmonella* has to be absent in 25 g; *E. coli* has to be lower than 500 cfu/g, and L. monocytogenes is of 100 cfu/g once in the market and absence in 25 g before the food has left the immediate control of the food business operator who has produce it.

As an extra measurement, since some of the powders can be vaccum packed, analysis fro chlostridium should be performed, since is prone to growth in anaerobic conditions and it has been previlsuy found in fresh farmed insects (T. *molitor, A. domesticus and Brachytrupes sp.*).

However, the analysis of of *C. botulinum* implies a veryhigh risk, due to the risk associated, instead, it is recommened to perform the analysis for sulphite reducing anaerobes (Clostridia); since its absence will be a good indicator of *C. botulinum* absence.

According to EFSA (2015), ther are two main types of microbiota to be considered as a hazard, the intrinsic ones related to the nature of the insect itself, and those that could have been introduced during farming and processing and carried over. Main conclusion in the EFSA report in 2015 was that:

"Pathogenic bacteria (such as *Salmonella, Campylobacter* and verotoxigenic *E. coli*) may be present in non-processed insects depending on the substrate used and the rearing conditions. Most likely, the prevalence of some of these pathogens for example campylobacter, will be lower compared to other non-processed sources of animal protein, since active replication of the pathogens in the intestinal tract does not seem to happen in insects. Furthermore, the risk of transmission of these bacteria could be mitigated through effective processing".

"The substrate used and the farming environment strongly influences insects microbiota, and therefore the foodborne risk is influenced by the nature and the hygienic conditions of the substrate and the farming environment. In general, food and feed-grade substrates (substrates groups A, B and C[1]), if maintained in good hygienic conditions, should not pose any additional risk when fed to insects as compared with other approved foods or feeds."

Table 2: Analysis performed for pathogenic bacteria in food products

Analysis	Others
Salmonella	EN/ISO 6579
E. coli	ISO 16649-1 or 2
L. monocytogenes	EN/ISO 11290-1
Clostridia	ISO 153213-1

[1] A. Animal feed materials according to EU catalogue of feed materials (EU 68/2013) and authorised as feed for food producing animals. B. Food produced for human consumption, but which is no longer intended for human consumption for reasons such as expiored use-by date or due to problems of manufacturing or packaging defects. Meat and fish may be included in this category. C. By-products from slaughterhouse that do not enter the food chain but originated from animals fit for human consumption.

9.2. Oxidation analysis

One of the main effects of storing is the oxidation of the compounds that may be present in the food product, as previously discussed; and that can be accelerated or reduced based on the processing and storing conditions.

Most relevant analysis in order to determine oxidation stability are those based on Thiobarbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation (i.e. as degradation products of fats) which can be detected by the TBARS assay. In this assay it is determined the presence of malondialdehyde (MDA), which is generated after the decomposition of primary and secondary lipid peroxidation products. Usually, a maximum limit of 2 mg MDA/Kg of sample is considered, since values higher than that are related to rancidity and overcomes the flavor of the product.

Oxidative stability is another way to determine how susceptible is the material to be oxidized over time. In this case it has been reported that peroxide and acid values are good indicators when analyzing insect samples (Jeon, Son et al. 2016).

Another analysis that can be performed is a detailed fatty acid profile, by means of GC couple the MS/MS or FID detectors. By means of this techniques, it may be possible to determine the fate of the polyunsaturated fatty acids over time, which are the most susceptible to be oxidized.

Oil can be also analyzed in terms of quality parameters, in which case the next test can be conducted: Iodine value [g of iodine/100 g] (DGF-C 11d (14)); saponification number [mg KOH/g] (DGF-C-V 3 (02)); peroxide value [meq O2/kg] (DGF-C-VI 6a Part 1 (05)); or free fatty acids [%] (DGF C-V 2 (06))(DGF, 2013).

9.3. Color analysis

Colour analysis can be a good indicator of changes in the quality of the product due to process of oxidation, entrapping moisture from the environment or even microbial spoilage. Also, colour plays a main role in consumer acceptability. Most common technique is the use of a calibrated colorimeter by which it is possible to determine the values of the three parameters used to describe any single colour (L, a and b). These values represent the lightness, redness and yellowness of any sample, respectively.

In order to determine whether the colour has changed over time; a factor comparing two different readings can be calculated. Such factor is known as ΔE and is calculated as follows:

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

Equation 1: Determination of ΔE for color changes

According to this equation, values of ΔE lower than 2 units means that colour differences can't be noticed by visual inspection, while values above this limit can be easily perceived. n the other hand, values higher than 5 units can be considered as different colors.

Specifically for oils, using a similar approach, it can be determined the browning index. This index is a good indication of molecules generated after a thermal treatment (drying, roasting or grilling) usually coming from Maillard reactions; but also because of phospholipid degradation (Jeon et al, 2016). This analysis is performed by carrying out an hexane extraction, and then reading the solution absorbance at 420 nm of wave length.

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