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Edible insect processing pathways and implementation of emerging technologies

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REVIEW ARTICLE

Abstract

The processing of insects is paramount to deliver safe and high quality raw materials, ingredients and products for large-scale food and feed applications. Depending upon the nature of the initial material and the desired end product, the processing pathways vary and may include several unit operations currently already used in food and feed processing. Insect processing pathways can involve harvesting, pre-processing, decontamination, further processing, packaging and storage. Several traditional and industrial decontamination methods have been proposed for edible insects, which include smoking, drying, blanching/boiling, marination, cooking, steaming, toasting and their combinations. Further processing steps are employed to produce insect meal, insect flour or extracted insect fractions. Each operation will have a different impact on the chemical and microbiological properties of the final product. Novel food processing technologies (e.g. high pressure processing, pulsed electric field, ultrasound and cold plasma) have shown potential to modify, complement or replace the conventional processing steps in insect processing. These technologies have been tested for microbial decontamination, enzyme inactivation, drying and extraction. Further, these are considered to be environmentally friendly and may be implemented for versatile applications to improve the processing efficiency, safety and quality of insect based products. Future research focuses in insect processing are development of efficient, environmentally friendly and low-cost processes; waste minimisation and incorporation of by-products/co-products.

Keywords: pre-processing, nonthermal technologies, extraction, novel food, microbial decontamination

1. Introduction

Edible insects have been part of the human diet throughout history (Dobermann *et al.*, 2017). Their consumption is traditionally recognised in several countries, where they are predominantly collected from their natural habitat (Dobermann *et al.*, 2017); however, with increasing demand of insect based foods in western countries, many companies have started mass rearing systems (Wade, 2020).

In order to ensure food safety, insect processing and storage should follow the same health and hygiene standards as for any other traditional food or feed (Imathiu, 2020). Hazards which are commonly found in insects comprise pathogenic microorganisms and parasites (Gałęcki and Sokół, 2019; Schlüter *et al.*, 2017), allergens (De Gier and Verhoeckx, 2018), heavy metals, toxins and other chemical contaminants (Bosch *et al.*, 2017; De Gier and Verhoeckx, 2018; Poma *et al.*, 2017, 2019; Van der Fels-Klerx *et al.*, 2016). Prions and human pathogenic virus should be considered as well (EFSA, 2015), although they have not been detected in edible insects yet.

Processing technology depends on insect species, safety hazards and type of final product. Edible insects can be marketed in three different forms: (1) whole (dried, frozen, pre-cooked); (2) processed; and (3) extracts (EFSA, 2015). Whole insects are commercially available as dried, frozen or chilled products for direct use by the consumer or food manufacturer. Processing of insects results in powder or paste, which allow the insects to be incorporated into food products or directly into dishes prepared by the consumer.

In order to improve the acceptance of insects and insect products and extend their shelf life, several traditional cooking techniques such as steaming, roasting, smoking, frying, stewing, curing (Ebenebe and Okpoko, 2015; Grabowski and Klein, 2017b; Lautenschläger *et al.*, 2017; Manditsera *et al.*, 2019; Nonaka, 2009; Obopile and Seeletso, 2013; Ramos-Rostro *et al.*, 2016; Shockley *et al.*, 2018) have been proposed. Other techniques, such as traditional sun-drying (Manditsera *et al.*, 2018), microwave processing (Lenaerts *et al.*, 2018; Vandeweyer *et al.*, 2017b), freeze-drying, oven-drying (Azzollini *et al.*, 2016; Fombong *et al.*, 2017), dry heat treatment (Bußler *et al.*, 2015), dry fractionation (Purschke *et al.*, 2018a), freezing (Melis *et al.*, 2018), marination, fermentation (Borremans *et al.*, 2018, 2020b; Patrignani *et al.*, 2020) and new processing methods as ultrasound-assisted extraction (Mishyna *et al.*, 2019; Panja, 2018; Sun *et al.*, 2018), cold atmospheric pressure plasma (CAPP) (Bußler *et al.*, 2016a), supercritical CO_2 extraction (Purschke *et al.*, 2017), enzymatic hydrolysis (Purschke *et al.*, 2018a) for protein, fat, and/or chitin extraction, three-dimensional food printing technologies (Severini *et al.*, 2018; Soares and Forkes, 2014), and several modified atmosphere packaging methods (Flekna *et al.*, 2017; Stoops *et al.*, 2017) have also been tested for insects and insect products.

Main objective of processing is to ensure food safety; however, it is also important to satisfy quality standards and consumer expectations. In this context, an important aspect to consider is the microbiological safety. In countries where insects are already recognised as food or feed, specific microbiological criteria are present. However, in the other countries, lack of specific rules may be observed. For example, insects are classified as novel foods in European Union (EU) and therefore fall within the current novel food legislation (EU Regulation 2283/2015; EFSA, 2016). Whole edible insects and their derived ingredients can only be lawfully placed on the EU market after safety assessments and authorisations. When a novel food is accepted for consumption, it will be considered as any other food, therefore it should respect the current accepted limit for microbiological contamination, stated in the EU Regulation 2073/2005 (EC, 2005). However, in the aforementioned regulation, edible insects are not considered. They could be equated to traditional meat or fish, but since their particular nature, specific microbiological criteria should be formulated. In order to overcome this problem, some European countries, such as Belgium and the Netherlands, where insects consumption is already allowed, have fixed microbiological limits for edible insect (FASFC, 2014a,b; Bureau Risicobeoordeling en Onderzoeks-programmering, 2014).

In order to guarantee high safety standard for the final consumer, suitable processing pathways should be integrated in the insect value chain. In this context, the present review aims to illustrate the current scientific knowledge in edible insect processing, focusing on the traditional pathways and providing an overview on possible future processing routes.

2. Pre-processing

Pre-processing technologies represents the first step of each edible insects processing route and mainly consist of insect harvesting/separation from the substrate residuals, insect inactivation/killing, removal of wings/legs, and washing (Rumpold and Schlüter, 2013).

Once the insects have grown to the desired size or reach a certain age, they are harvested either manually or automatically. Larvae such as mealworms and lesser mealworms are usually separated from their substrate by sieving, while crickets and grasshoppers, which typically live apart from the feeding source, can be harvested by picking them from their rearing cage or by shaking them out of the crevices they hide in. Some farms apply a starvation period (Figure 1) before harvesting by separating the insects from their food (Garofalo *et al.*, 2019). This causes the insects to empty their intestines (Finke, 2002), which, according to the breeders, results in better taste and cleaner products with reduced microbial load (Fernandez-Cassi *et al.*, 2019). However, Wynants *et al.* (2017) reported that starvation of yellow mealworms did not reduce the total viable counts (TVC) or change the bacterial community composition consistently.

Following harvesting, the insects undergo several post-harvest treatments before being consumed. If not sold as living species, killing the insects is the first step. Freezing, drowning in hot or boiling water and steaming are some of the most common methods used for this purpose. Killing is a key step in insect processing since it can affect various final product quality parameters including microbial load, proximate composition, colour and taste (Adámková *et al.*, 2017; Farina, 2017; Larouche *et al.*, 2019). Larouche *et al.* (2019) compared blanching, desiccation, freezing, high hydrostatic pressure, grinding and asphyxiation (100% CO_2; 100% N_2; vacuum conditioning) as killing methods on black soldier fly larvae. They reported blanching (boiling water for 40 s) to be the most effective method in terms of time duration, final larval moisture, lipid oxidation, microbial load and colour alteration. However, due to the high TVC and presence of pathogenic microorganisms in the resulting product, a further stabilisation process was recommended (Larouche *et al.*, 2019). Variable results have been reported for effect of killing methods on nutritional composition of different insects. Adámková *et al.* (2017) observed lower fat content in mealworm killed by direct immersion in boiling water compared to freezing. However, Caligiani *et al.* (2019) and Singh *et al.* (2020) observed no difference in lipid concentration of *Hermetia illucens* and

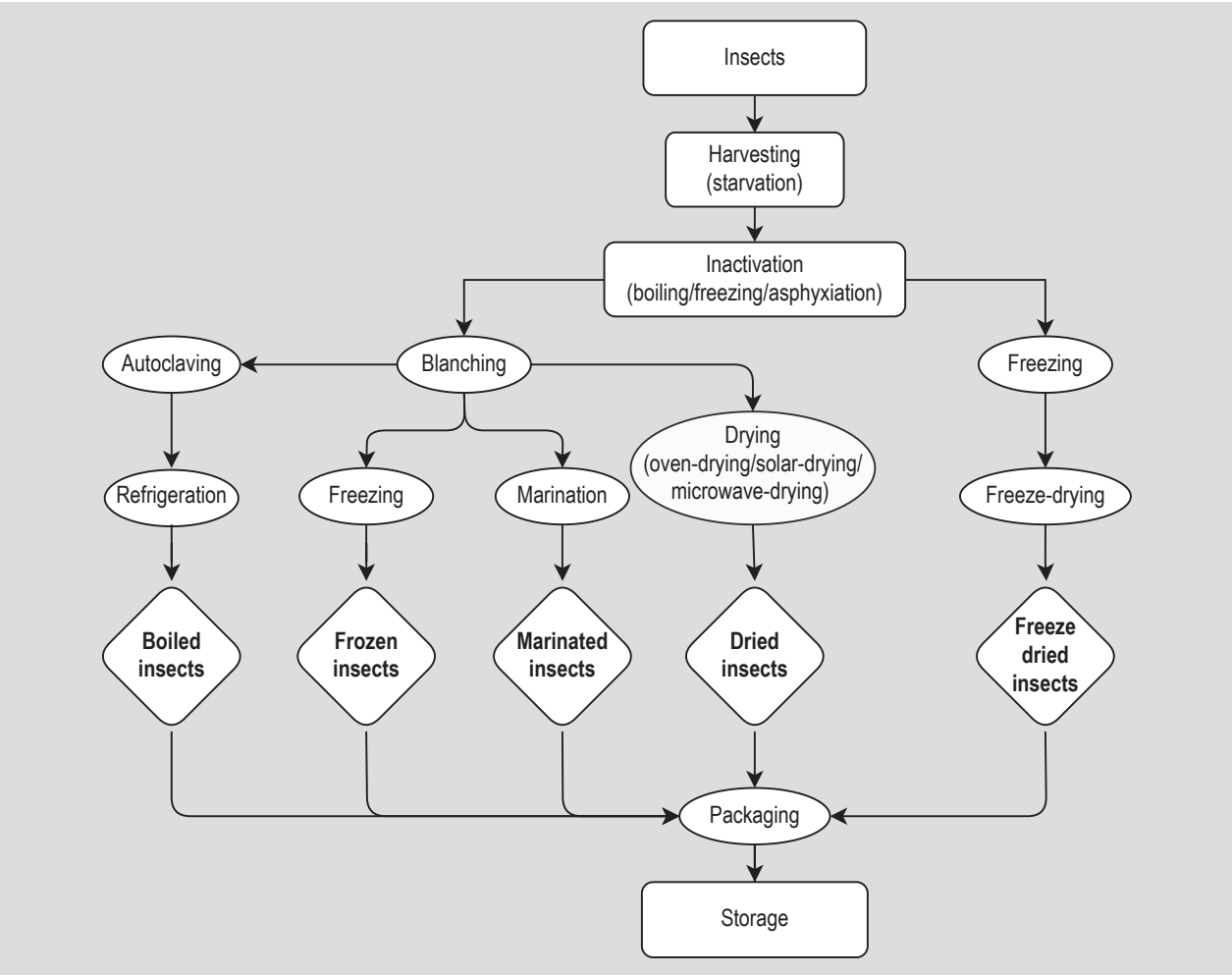

Figure 1. Processing pathways for the production of whole (boiled/frozen/oven-dried/freeze-dried/microwave-dried/marinated) edible insects.

Acheta domesticus killed by freezing (-20 °C) or blanching (100 °C, 40 s). Additionally, higher ether extract has been recorded when crickets were killed by asphyxiation (3 hour in plastic bag at room temperature).

The killing method can influence physiochemical properties as well. For instance, higher luminosity and lower browning have been detected in house crickets killed with CO_2 (40 min) followed by blanching (40 s), while reduction in taste and lower pH have been displayed when crickets were frozen before cooking. This was explained by the *ante-mortem* stress, which is responsible for an increasing glycolytic activity, with higher conversion of glycogen into lactic acid (Farina, 2017).

In order to reduce the microbiological load, killing process can be coupled with a rinsing/washing step, as it is already applied in traditional food products such as raw fruits and vegetables (Yoon and Lee, 2018) or carcasses and fresh meat (Huffman, 2002). However, experiments conducted by Wynants *et al.* (2016, 2017) and Ng'ang'a *et al.* (2019) on mealworm larvae and wild harvested *Ruspolia differens* showed no reduction in TVC and in mesophilic aerobic count, Enterobacteriace, bacterial endospores, yeast and moulds when 1 min of washing treatment with tap water or sterile water were applied (Ng'ang'a *et al.*, 2019; Wynants *et al.*, 2016, 2017). Better results have been obtained in lesser mealworms with sterile distilled water containing antimicrobial substances such as ethanol, hydrogen peroxide or sodium hypochlorite (Crippen and Sheffield, 2006). Although these concepts can be extended to dead insects, the washing experiments aforementioned were applied on living larvae. Use of washing procedures on dead insects has been described by Fröhling *et al.* (2020). In this study, three successive washing steps with tap water were applied on frozen/thawed crickets. Although TVC of crickets was not affected by washing procedure, a reduction of approximately one logarithmic cycle was detected for total aerobic mesophilic viable count, *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus* spp., *Staphylococcus* spp., yeasts and moulds (Fröhling *et al.*, 2020).

3. Conventional processing routes

Although pre-processing represents the first step to connect from rearing to consumption, it cannot be considered decisive for ensuring food safety. Further processing techniques are required to obtain a product suitable for consumption. In the following section, the common conventional processing routes applied in the edible insects sector are described. Current scientific results as well as positive aspects and challenges observed for each step are discussed.

Processing of whole insects

Whole edible insects are usually marketed in chilled (T≤5 °C), frozen (T≤-18 °C) or dried form. Dried insects have a low moisture and water activity, therefore can be stored stable for a longer time (up to months) at room temperature (Kamau *et al.*, 2018). When cold and freezing storage are used, the cold chain should be continuously maintained during the storage (James, 1996). Even though some reduction in TVC has been detected after freezing (Grabowski and Klein, 2017a; Ssepuuya *et al.*, 2016), both cold storage and freezing cannot be considered as decontamination steps as they can only decelerate or delay microbiological growth and chemical deterioration (Kamau *et al.*, 2020). A certain microbial inactivation can be reached with drying processes, despite several studies displayed high residual microbial count on dried insects (Vandeweyer *et al.*, 2017a, 2018; Wynants *et al.*, 2018). Therefore, in order to guarantee high food safety standards and extend the shelf-life, raw edible insects should be subjected to a series of steps to ensure overall safety. An overview of possible processing pathways for production of whole edible insects is presented in Figure 1.

Every processing route should be organised in 3 steps: (1) harvesting and pre-processing; (2) decontamination; and (3) packaging and storage. Several decontamination methods have been proposed on edible insects. These comprise blanching, cooking/boiling, steaming, marination, drying, smoking, toasting and their combinations. Each operation will have a different impact on the chemical and microbiological properties of the final product. For example, Nyangena *et al.* (2020) compared the effect of toasting (5 min, 150 °C), boiling (5 min, 96 °C), solar-drying (2 days, 50-60 °C, 15-25% RH) and oven drying (2 days, 60 °C) on *A. domesticus, H. illucens, Spodoptera littoralis* and *R. differensis*. They observed that in all the species, an important reduction could be detected for TVC and *Staphilococcus aureus* (5-6 log cycles), yeasts-moulds and *Salmonella* spp. (lower than detection limits) when toasting or boiling were used. On the contrary, solar-drying and oven-drying could not guarantee the same results, if not preceded by toasting or boiling (Nyangena *et al.*, 2020). The presence of pathogenic microorganisms on edible insects after drying process represent an important safety concern, whereby dried insects should be reheated before consumption (Grabowski and Klein, 2017b). However, drying at 100 °C for 4 hours was able to reduce about 5-8 log cycles of the microbial counts (TVC, total aerobic, *E. coli*, *Enterobacteriaceae*, *Enterococcus* spp., *B. cereus, C. perfringens, Stahilococcus* spp., yeasts and moulds) in *A. domesticus* (Fröhling *et al.*, 2020). Similar reduction levels have been obtained for TVC (from 7.5 to <1.7 log cfu/g), *Enterobacteriaceae* (from 6.8 to <1 log cfu/g) and bacterial endospores (from 2.1 to <1 log cfu/g) on *Tenebrio molitor, Brachytrupus* spp and *A. domesticus* after either boiling in water or roasting for 10 minutes (Klunder *et al.*, 2012).

A very common process in the food industry is blanching. Blanching allows the inactivation of vegetative microorganisms (but not bacterial spores). It refers to a process where a food is placed inside boiling water for short time (from few seconds to more than 10 minutes, depending by the product) and then cooled in cold water (Xiao *et al.*, 2014). It has been tested on edible insects by Vandeweyer *et al.* (2017b), who compared the effect of blanching for 10, 20 or 40 seconds, with or without microwave drying (16 microwave sources of 2 kW) on *T. molitor* larvae. They observed a significant reduction (from 8 log cfu/g to 1.3 log cfu/g) in total aerobic count when blanching for 40 seconds was followed by microwave treatment for at least 16 minutes (residual moisture 0.6%). Lower microbial reductions were detected when blanching was followed by shorter microwave treatments or no microwave process was applied. Blanching was able to reduce the number of *Enterobacteriaceae* and fungi (yeasts and moulds) below the detection limits (1 log cfu/g for *Enterobacteriaceae* and 2 log cfu/g for fungi), but an increase in bacterial endospores was observed (Vandeweyer *et al.*, 2017b). Similar results have been obtained by Vandeweyer *et al.* (2018) who studied the evolution in microbial counts during processing and storage of *Gryllus sigillatus.* They observed an initial reduction in TVC after smoking (80 °C, 40 min, residual moisture 5.1%) or smoking and oven-drying (80 °C, overnight, residual moisture 2.2%). However, an increase in TVC and endospores was displayed during the storage at room temperature (Vandeweyer *et al.*, 2018). Therefore, in order to obtain a final product safe for the consumer, alternative storage methods should be tested.

Still under-explored techniques in edible insects processing and storage are marination (treatment of raw meat with several ingredients to improve sensorial parameters and improve microbiological safety; Williams, 2012) and fermentation (subjecting food to the activity of microorganisms or enzymes, in order to obtain desirable sensorial and nutritional characteristics and extend the shelf life (Campbell-Platt, 1987)). While fermentation is usually conducted on insect powder or paste, marination can be

realised on whole insects. In a study from Borremans *et al.* (2018), effect of marination technology was analysed on blanched (40 s) *T. molitor* larvae. The authors concluded that 6 days of marination in red wine or soy sauce could inhibit the microbial growth, allowing shelf life extension of at least 7 days. However, marination might be responsible for germination of bacterial endospores, which might represent an issue for longer storage periods (Borremans *et al.*, 2018).

Choice of the most suitable processing methods is a key and should involve the consideration of the initial raw material and the final product that would be obtained. Besides microbial safety, choice of processing technique should also consider nutritional and sensorial parameters. For example, blanching has shown to be responsible for luminosity value and non-enzymatic browning (Azzollini *et al.*, 2016) although it is effective inactivating browning enzymes (Tonneijck-Srpova *et al.*, 2019). Another common effect connected with processing is the increase in protein solubility (Womeni *et al.*, 2012). However, these effects cannot be generalised for all insect species. For example, decrease in protein digestibility was observed on *R. differens* subjected to toasting (150 °C, 5 min) and solar drying (30 °C, 40% RH), while the same process applied on *Macrotermes subhylanus* has not shown the same results (Kinyuru *et al.*, 2010). Processing could also be responsible for nutrient loss. It has been shown for *Hemijana variegate* subjected to sun drying (Egan *et al.*, 2014), while reduction in vitamin B12 has been detected in mealworm exposed to microwave drying (14 sources of 2 kW for 24 min) (Lenaerts *et al.*, 2018). Whereas, no proximate composition alteration was observed when oven drying (60 °C, 24 h) was used on *R. differens* (Fombong *et al.*, 2017). Reduction in protein and *in vitro* true dry matter digestibility have been detected in *Imbrasia belina* (Madibela *et al.*, 2007), *Eulepida mashona* and *Henicus whellani* (Manditsera *et al.*, 2019) after 1 hour of boiling process. For the last two species, boiling without roasting operation, was also responsible for reduction in zinc and iron bio-accessibility (Manditsera *et al.*, 2019). Conversely, 5-7 minutes of roasting could improve the calcium availability in mopane caterpillar (Madibela *et al.*, 2007).

Insect meal production

Sometimes edible insects require to be formulated into homogenous forms, such as powder or paste (Figure 2). In such cases, milling or grinding processes are required. Edible insects can be milled when still fresh or after blanching (wet milling) or after a drying process (dry milling). Wet milling is usually applied at industrial level because it results into fluid material, whereby it will be easy to handle along the production line (Dossey *et al.*, 2016). However, due to the high moisture content, the resulting material is microbially unstable (De Smet *et al.*, 2019). It should be kept at low temperature (≤5 °C) and used within a short time (De Smet *et al.*, 2019). Conversely, dry milling results in a dry powder, which shows extended shelf-life and can easily be marketed and stored at room temperature (Klunder *et al.*, 2012).

In order to extend the shelf life and have a stable product, drying operations can also be performed after the wet milling process for obtaining a powder (Dossey, 2015). When insect paste has to be dried, spray drying is usually preferred. It is a drying method where the paste is propelled into hot air. In this way, every single drop of the fluid is dried singularly; therefore, the drying process happens in a short time and results in homogeneous products and with better nutritional properties (Bhandari *et al.*, 2008; Dossey *et al.*, 2016; Son *et al.*, 2019). However, since spray-drying cannot be used for products with a high fat level, drum drying is often adopted (Dossey *et al.*, 2016). Here, the insect paste is in contact with a warm surface until it is dry (Tang *et al.*, 2003). The result is a faster drying of the layer in direct contact with the drier surface and low drying of the remaining bulk. Therefore, the final powder can be inhomogeneous and characterised by low quality (Courtois, 2013; Dossey *et al.*, 2016). Table 1 shows an overview of studies where positive and negative effects connected to traditional drying technologies have been evaluated on edible insects.

Possible processing pathways resulting in several types of insect meal are shown in Figure 2 (whole fat insect meal) and 3 (defatted insect meal). Defatted insect meal is produced by extracting fat from the insect. This operation can be performed with chemical (Ravi *et al.*, 2019; Son *et al.*, 2019), physical (Arsiwalla and Aarts, 2015) or mechanical (Azagoh *et al.*, 2016; Tschirner and Simon, 2015) treatments, and results in final products with different physiochemical and sensorial characteristics (Son *et al.*, 2019) and longer shelf-life (Nadarajah *et al.*, 2015; Temba *et al.*, 2017). Defatting operation can be carried out before (Figure 3, pathways B and C) or after (Figure 3, pathway A) the milling. If milling foregoes the defatting process, enzymatic hydrolysis might occur. It is due to the action of lipases and proteases present inside the insect cells and released into the meal when the insects are pulverised. Further enzymatic hydrolysis can be reached by adding commercial enzymes to the insect paste, as suggested by Arsiwalla and Aarts (2015). In order to promote the physical separation and/or the enzymatic hydrolysis, water should be present; therefore, a drying process is conducted on the defatted paste (Figure 3, pathway A). When defatted insect meal is produced by a mechanical defatting process (Figure 3, pathway B and C), milling is usually conducted after oil extraction (Azagoh *et al.*, 2016; Son *et al.*, 2019), while drying can be performed on the final defatted paste (Figure 3, pathway B) or on the whole insect (Figure 3, pathway C). Finally, solvent defatting operation is usually performed on dry material

Table 1. Summary of studies exploring the effect of different drying processes on edible insects.

Insect species	Drying methods	Notable effect	Reference
Acheta domesticus, Hermetia illucens, Spodoptera littoralis, *Ruspolia differens*	Oven drying Freeze drying Solar drying	Oven drying decreased bacterial populations, yeasts and mould. Solar drying had no effect. All three drying processes combined with boiling eliminated bacterial populations, yeasts, moulds.	Nyangena *et al.* (2020)
Rhynchophorus phoenicis	Solar drying Oven drying Smoke drying	Smoke drying was suggested as the preferable method as it maintains the lipid profile, while solar drying increased peroxide value.	Tiencheu *et al.* (2013)
Sternocera orissa	Oven drying Freeze drying	Oven drying method led to a higher composition of the material in compare to freeze drying	Shadung *et al.* (2012)
Imbrasia epimethea	Oven drying Solar drying	The monosaturated fatty acid content was reduced.	Lautenschläger *et al.* (2017)
Polyrhachis vicina	Solar drying	Aldehydes, increase of free fatty acid content, decrease of ketone content and hydrocarbons were observed.	Li *et al.* (2009)
R. differens	Oven drying Freeze drying	Both processes led to no significant differences in composition and product quality.	Fombong *et al.* (2017)
Tenebrio molitor	Oven drying Microwave-assisted drying	Microwave-assisted drying, combined with 40 s of blanching for less than 16 min did not reduce water activity lower than 0.6.	Vandeweyer *et al.* (2017b)
T. molitor	Oven drying Freeze drying Fluidised bed drying	Higher temperatures of drying caused darkening and shrinking due to browning reactions and tissue disruption.	Purschke *et al.* (2018a)
T. molitor	Fluidised bed drying Microwave-assisted drying Freeze drying Vacuum drying Hot air drying	Freeze drying oxidised the lipid fraction. Protein solubility was higher with vacuum drying and freeze drying and decreased with microwave-assisted drying.	Kroncke *et al.* (2018)
H. illucens	Oven drying Microwave-assisted drying	Protein digestibility was higher with oven drying, since microwave treatment polymerised protein molecules.	Huang *et al.* (2019)
Musca domestica L.	Oven drying Solar drying	Oven dried samples contained more proteins and less fat	Aniebo and Owen (2010)
Macrotermes subhylanus, R. differens	Solar drying	Solar drying of termites and grasshoppers decreased vitamin content in both species and protein digestibility of the grasshoppers.	Kinyuru *et al.* (2010)
Locusta migratoria manilensis, Bombyx mori	Freeze drying Oven drying Microwave-assisted drying	Oven and microwave drying changed the sensory characteristics of the materials in a way that they were less acceptable.	Mishyna *et al.* (2020)

(Ravi *et al.*, 2019; Son *et al.*, 2019), which should be milled to ultrafine powder in order to increase the surface and to improve the solvent penetration (Son *et al.*, 2019). However, although defatting yield with solvent is higher than with other methods (Son *et al.*, 2019), solvent defatting operations are not preferred for both, environmental and sensorial aspects. Indeed, n-hexane defatted mealworm meal showed less flavour, lower colour preservation and consumer acceptability than pressure-defatted powder (Son *et al.*, 2019).

The type of blender used for meal production can also affect the final properties of the insect powder/paste. For example, Son *et al.* (2019) observed that mealworm defatted meal obtained by using a jet-mill showed lower humidity, higher luminosity, higher particle size uniformity and higher consumer acceptance than powder produced by pin, hammer or cutter mill (Son *et al.*, 2019).

Apart from the three pathways already described, several other processing routes have been described for insect meal production (Borremans *et al.*, 2020a,b; Bußler *et al.*,

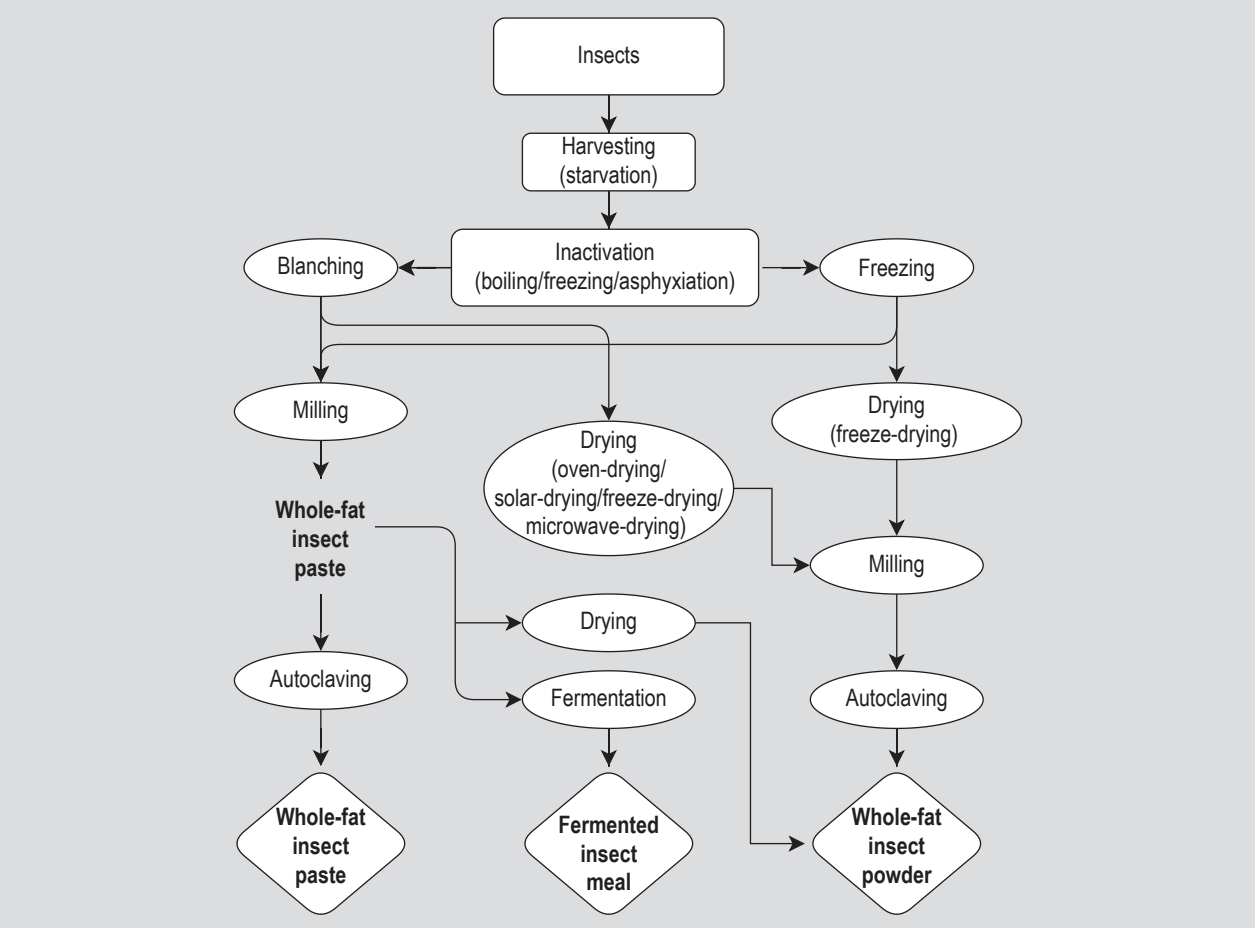

Figure 2. Processing pathways for the production of whole-fat and fermented insect meal.

2016b; Fröhling *et al.*, 2020; Purschke *et al.*, 2018a,b,c; Vandeweyer *et al.*, 2018). Depending on the process goal and the insect species, the order of several operations may vary (Stoops *et al.*, 2017).

The most critical step along each processing pathway is the thermal treatment, which is important to assure food safety. Microbial decontamination can be performed on whole insects (before milling operation) (Kamau *et al.*, 2020) or directly on the powder/paste resulting from the milling process (Bußler *et al.*, 2016a,b) (Figure 2). Applying the decontamination process on meal is preferred, since milling operations break down the insect body and a release of the insect gut microbiota is obtained increasing the total microbial count (Fröhling *et al.*, 2020; Klunder *et al.*, 2012; Rumpold *et al.*, 2014). Indeed, microorganisms at the surface of food materials will be more susceptible to high temperature (Rumpold *et al.*, 2014); therefore, acceptable results can be reached with lower temperature or shorter treatment time. In this way, less impact on physio-chemical and sensorial characteristics of the final product is realised (Fröhling *et al.*, 2020). However, every time high temperatures are applied on insect meal, it may result in burnings of the powder/paste (Arsiwalla and Aarts, 2015). Concerning the physicochemical stability, the presence of browning and lipolytic enzymes should be considered. They can be responsible for browning, lipid oxidation and rancidity of insect paste. Therefore, in order to prevent undesirable reactions, a blanching process can be realised on the whole insects (Figure 3, pathways B and C) as suggested by Azagoh *et al.* (2016). Nevertheless, when blanching is performed, the adding of water can result in loss of soluble nutrients and higher energy is required for drying (Fröhling *et al.*, 2020). However, blanching of whole insect can be responsible also for microbial inactivation, resulting in low number of *Enterobacteriaceae* (Stoops *et al.*, 2017), but possible reactivation of bacterial spores should also be considered (Stoops *et al.*, 2016). For this reason, further decontamination treatment should be carried out in the next steps. Besides drying and heat based operations, an interesting stabilisation method applied on insect-based food is fermentation (Figure 2). The ability of this technology to ensure quality and safety of insect-based food has been investigated in several studies. For example, Patrignani *et al.* (2020) used *Yarrowia lipolytica* and *Debaryomyces hansenii* strains to produce cricket flour hydrolysates with high food safety, functionality, sensory and technological properties. The hydrolysates obtained

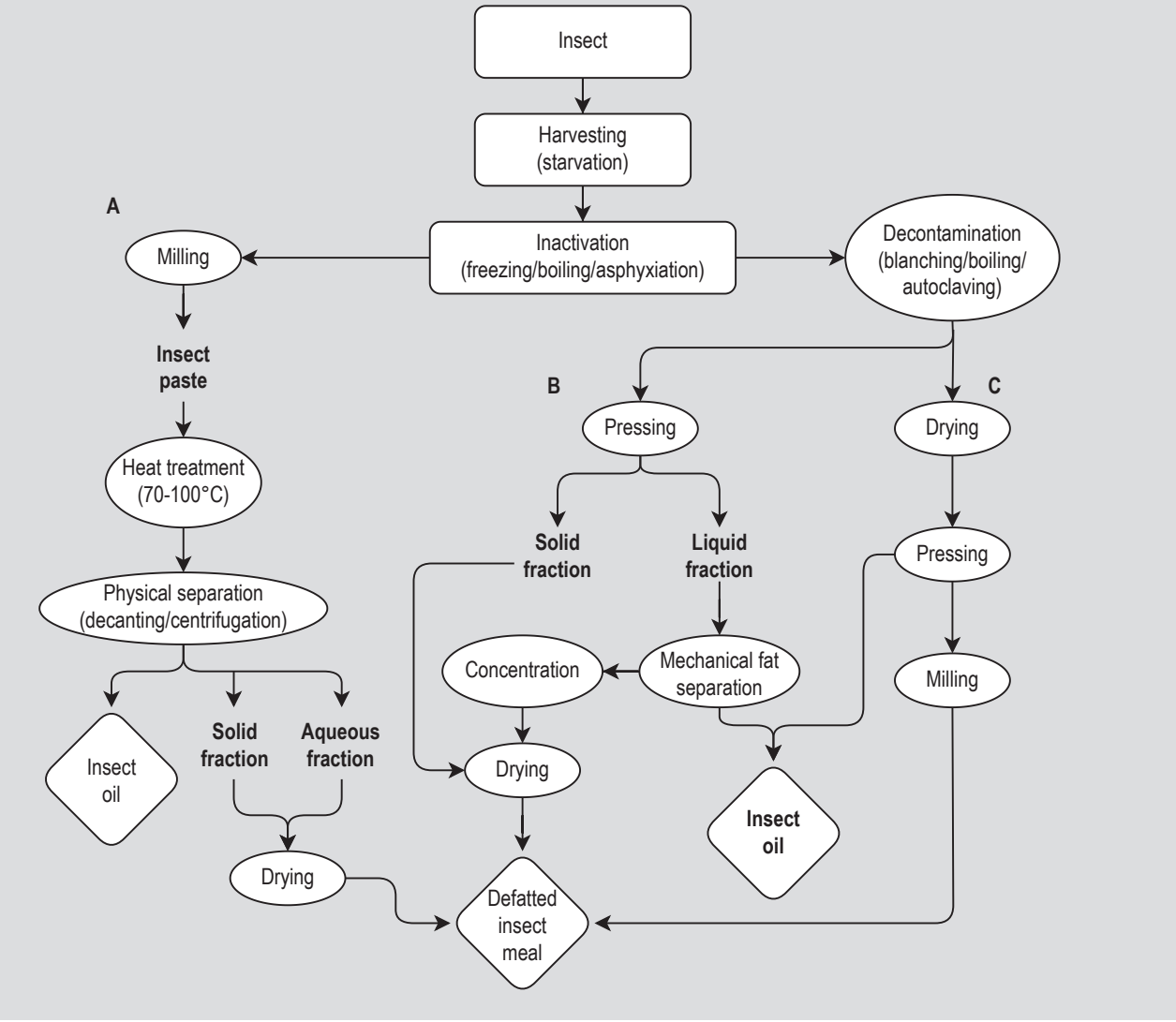

Figure 3. Processing pathways for production of defatted edible insect meal and insect oil: (A) as described by Arsiwalla and Aarts (2015), (B) analogous to fish meal production, (C) dry processing pathway.

by fermentation were characterised by a reduced chitin content and higher contents of antimicrobial substances (acetic acid, short chain fatty acids, chitosan, and GABA) and health-promoting molecules (arachidonic and linolenic acids, GABA, AABA, and BABA) when compared to the control. Borremans *et al.* (2018) observed that rapid acidification during fermentation, able to prevent microbial growth, could be reached within three days when blanched (40 s) paste of *T. molitor* was inoculated with a commercial starter containing *Pediococcus acidilactici, Lactobacillus curvatus* and *Staphylococcus xylosus* (Borremans *et al.*, 2018). However, the authors detected some lipid oxidation during refrigerated storage (4 °C) of the fermented paste. In order to prevent the oxidation, several additives have been tested. Borremans *et al.* (2020b) observed that sodium nitrite (150 mg/kg) and sodium lactate (60% w/w solution, 50 g/kg) are both able to reduce lipid oxidation. Different results were obtained by De Smet *et al.* (2019) who observed higher lipid oxidation of fermented insect paste stored for three weeks at 4 °C, when sodium nitrite or sodium lactate, rather than no additives, were used. Use of additive has displayed also a reduction in sulphite reducing bacteria in the fermenting paste (De Smet *et al.*, 2019). It is an important aspect because these bacteria are responsible for reduction of sulphite to hydrogen sulphite, compound associated with the unpleasant 'rotten egg' odour (Borremans *et al.*, 2018).

Storage of insect-based food

Although the aforementioned techniques can guarantee microbial safety, they cannot prevent the re-contamination of the product in the subsequent working steps. In order to guarantee microbiological safety for the consumer, appropriate storage methods with respect to the specific product properties should be applied. Refrigeration and

freezing, as well as air containing packaging, vacuum packaging and modified atmosphere packaging (MAP) are common preservation methods used for edible insects products. Klunder *et al.* (2012) observed that crickets boiled for 1 minutes could maintain low TVC (4.1 log cfu/g) over 16 days when stored at 5-7 °C, while TVC higher than 10 log cfu/g was reached after 2 days in room temperature storage (28-30 °C) (Klunder *et al.*, 2012). Similar results have been obtained on blanched *T. molitor* larvae stored at 3 °C for 6 days (Vandeweyer *et al.*, 2017b), while stable TVC over 180 days has been found on refrigerated (about 5 °C) black soldier fly larvae (Kamau *et al.*, 2020). Refrigeration has also shown promising results for storage of fermented or marinated insects, guaranteeing microbiological and chemical stability for up to two and four weeks respectively (Borremans *et al.*, 2018, 2020b; De Smet *et al.*, 2019). Cold storage can provide good lipid stability and thus the sensorial quality of insect products (Kamau *et al.*, 2020; Lee *et al.*, 2020; Tiencheu *et al.*, 2013). However, since refrigeration temperatures are not able to inactivate lipases, enzymes responsible for lipid oxidation, freezing can be applied to inhibit enzyme activities (Tiencheu *et al.*, 2013).

Important differences in terms of lipid oxidation and microbial growth were also observed when different packaging was used: insect meal packaged in polypropylene bags and stored at room temperature showed higher lipid oxidation than insect meal packaged in polyethylene bags (Kamau *et al.*, 2020). It was attributed to the permeability of polypropylene to water, which was considered responsible for propagation of moulds able to produce lipases (Kamau *et al.*, 2020). Combined effects of packaging and storage conditions have been tested also by Ssepuuya *et al.* (2016, 2019) who observed that storage at room temperature (20 °C) could guarantee microbiological, chemical and sensorial stabilities only if opaque vacuum packaging was applied (Ssepuuya *et al.*, 2016, 2019). An alternative to vacuum packaging is MAP. It has been investigated by Stoops *et al.* (2017), who observed that MAP (60% CO_2 40% N_2) reduced microbial growth guaranteeing at least 21 days of microbiological stability for insect paste stored at 4 °C (Stoops *et al.*, 2017). However, they did not observe any reduction in bacterial spore counts.

Use of MAP may also be helpful to reduce the moulds contamination of dried whole insects or insect powder. At the best of our knowledge, no study has been conducted in this area, but this technology can be extended to insects from other dried food (Rodriguez *et al.*, 2000). MAP can represent an important step in edible insect shelf-life extension because moulds are responsible for mycotoxin production, which can be a fundamental safety issue in edible insects when the water activity is higher than 0.6 (Kamau *et al.*, 2018). In a study conducted by Kachapulula *et al.* (2018), high moulds proliferation has been observed on dried caterpillars and termites after 7 days of storage at room temperature (31 °C). It was accompanied with high aflatoxin contamination, which made the insects not safe for human or animal consumption (Kachapulula *et al.*, 2018).

Extraction of valuable compounds from insects

Despite a large number of studies aiming to use whole insects or insect meal as food or food ingredient, many consumers, especially from the western world, may still not accept them (Chen *et al.*, 2009). In order to overcome these limitations and promote their consumption, isolation of protein and fat-rich fractions can be performed. The resulting products may then be used as ingredients for both industrial processing and culinary home-made preparation.

Isolation of protein has been performed from insect meal obtained from several species such as migratory locust (Clarkson *et al.*, 2018; Purschke *et al.*, 2018c), desert locust (Mishyna *et al.*, 2019) house cricket (Laroche *et al.*, 2019; Ndiritu *et al.*, 2017), mealworm (Bußler *et al.*, 2016b; Kim *et al.*, 2019; Laroche *et al.*, 2019; Yi *et al.*, 2013, 2017; Zhao *et al.*, 2016) black soldier fly (Bußler *et al.*, 2016b; Caligiani *et al.*, 2018; Soetemans *et al.*, 2019). Extraction conditions, as well as solvent and insect species can strongly influence extraction yield and properties of the isolated proteins. Satisfactory protein yields have been obtained by using alkaline solutions (Laroche *et al.*, 2019; Mintah *et al.*, 2020; Mishyna *et al.*, 2019; Zhao *et al.*, 2016) since pH values between 10 and 12 have displayed to lead to higher insect protein solubility (Bußler *et al.*, 2016b; Purschke *et al.*, 2018b,c; Udomsil *et al.*, 2019; Yi *et al.*, 2017; Zhao *et al.*, 2016). However, use of chemical solvents has shown negative effects on protein functionality, impacting several parameters such as emulsion capacity, foaming capacity and foaming stability (Ndiritu *et al.*, 2017). In order to prevent it, aqueous extraction may be performed. However, lower extraction yields have been shown when aqueous solutions of ascorbic acid have been used (Amarender *et al.*, 2020; Ndiritu *et al.*, 2017; Yi *et al.*, 2013). Improved results have been reached by using NaCl, as displayed on *T. molitor* (Yi *et al.*, 2017), or increasing pH (Purschke *et al.*, 2018b), temperature, solid/liquid ratio and/or extraction time (Bußler *et al.*, 2016b; Mintah *et al.*, 2020). Defatting can also increase the protein extract yields, as shown for several insect species (Amarender *et al.*, 2020; Kim *et al.*, 2019).

Several extraction systems have been implemented for recovery of fat from edible insects. Common fat extraction methods comprises Folch, Soxhelet, Supercritical CO_2 and aqueous extraction (Laroche *et al.*, 2019; Ramos-Bueno *et al.*, 2016; Ravi *et al.*, 2019; Tzompa-Sosa *et al.*, 2014). Choice of the method should consider the insect species because the same method applied on several species can results in different yields, as shown by Laroche *et al.* (2019), Pan *et al.* (2012), Ramos-Bueno *et al.* (2016) and Tzompa-Sosa *et al.* (2019, 2014). Moreover, different extraction systems, as

well as different solvents, lead to various extraction yield when applied on the same insect species (Amarender *et al.*, 2020; Feng *et al.*, 2020; Laroche *et al.*, 2019; Mariod *et al.*, 2010). Fatty acid profiles of the extract can change with the extraction system as well (Laroche *et al.*, 2019; Purschke *et al.*, 2017; Ramos-Bueno *et al.*, 2016; Ravi *et al.*, 2019; Tzompa-Sosa *et al.*, 2014).

An interesting bioactive compound that can be extracted from insects is chitin. It is the precursor of chitosan, an amino-polysaccharide characterised by important biological activities (Mohan *et al.*, 2020). The process for chitin extraction consists in four sequential steps: delipidation, deproteinisation, demineralisation, decolorisation (Mohan *et al.*, 2020). The aim of this processing pathway is to remove all the extractible compounds from the insect powder in order to obtain the insoluble chitin fraction. This method has been implemented on a wide range of insect species, such as *Bombyx mori* (Luo *et al.*, 2019; Simionato *et al.*, 2014), *T. molitor* (Luo *et al.*, 2019; Song *et al.*, 2018), *Zophobas morio* (Shin *et al.*, 2019; Soon *et al.*, 2018), *H. illucens* (Caligiani *et al.*, 2018; Purkayastha and Sarkar, 2020), *Musca domestica* (Jing *et al.*, 2007; Kim *et al.*, 2016), *Apis mellifera* (Marei *et al.*, 2016, 2019), *Gryllus bimaculatus* (Kim *et al.*, 2017), *Schistocerca gregaria* (Marei *et al.*, 2016) and others. In order to reduce the use of chemical compounds, new eco-friendly chitin extraction systems have been developed. For example, the aforementioned method has been enhanced by using a sustainable deep eutectic solvent, which has shown attractive results on chitin extraction from black soldier fly (Zhou *et al.*, 2019). Furthermore, biological methods, based on fermentation with bacterial strains isolated from mealworm surface, have shown to be strong candidates for replacement of chemical demineralisation (Da Silva *et al.*, 2017).

4. Emerging technologies in insect processing

Recent advances in agri-food sector have shown that the emerging technologies (e.g. high hydrostatic pressures (HHP), pulsed electric fields (PEF), ultrasound (US) and CAPP) are promising alternatives for sustainable eco-friendly processing with negligible environmental impacts (Pojic *et al.*, 2018). These technologies aim to improve the safety and the quality of final products and enhance processing efficiency. However, requirement of highly trained operators and presence of remarkable initial investments may represent important hurdles for their spreading (Priyadarshini *et al.*, 2019). Legislative aspects need to be considered as well. For example, in Europe the novel food regulation (EU Regulation 2283/2015) considers products obtained by using emerging technologies as novel food if the applied technology was not used within the EU before 15 May 1997 and shows an impact on nutritional composition, structure or safety of the obtained food, which cannot be compared with the same food produced using traditional technologies. Since HHP, PEF and US are already applied in industrial food processing, the relation between emerging technologies and novel food legislation is not always clear and should be evaluated case by case every time one of these technologies is used.

In the present section, the most applied innovative technologies are described. A general overview of each technology, its fundamental effects and main applications in the food industry are given. Afterwards, possible applications of emerging technologies in the edible insects sector are discussed.

Emerging technologies

High hydrostatic pressure is a discontinuous or semi-continuous process that involves a fluid, submitted at a pressure in a range of 100-1000 MPa (Koutchma, 2014), surrounding the product to be treated at 0 to 120 °C (Aganovic *et al.*, 2021). However, pressure in the range of 200 up to 600 MPa at ambient or chilled temperature is usually applied in commercial food applications, with holding times rarely longer than 5 min (Aganovic *et al.*, 2021). The pressure can damage the microbial cells, leading to the inactivation of microorganisms at significantly lower temperature than conventional thermal processing and resulting in a better final product quality (Kashiri *et al.*, 2018). In food processing applications, HHP alone or in combination with heat can cause various physical, chemical, or biological changes in food, responsible for formulation of modified foods and ingredients (Bolat *et al.*, 2021; Hugas *et al.*, 2002; Rumpold *et al.*, 2014; Ugur *et al.*, 2020). Further, HHP can also be used to support the nutrient extraction from food (Preece *et al.*, 2017), to maximise the extraction yield of oils (Andreou *et al.*, 2020) and to improve product acceptability through the degradation of allergenic compounds which are naturally present in some food products (Boukil *et al.*, 2020; Li *et al.*, 2012; Penas *et al.*, 2011). However, HHP may have a negative impact on protein stability, resulting in loss of important nutritional components and changing the food texture (Boukil *et al.*, 2020; Tonneijck-Srpova *et al.*, 2019; Wang *et al.*, 2016). Moreover, when food is treated with HHP at moderate temperature, residual contamination with bacterial spores can still be detected (Campbell *et al.*, 2020; Kashiri *et al.*, 2018; Stoica *et al.*, 2013; Wang *et al.*, 2016). Therefore, the requirement of further heat operations and the high cost of HHP generating devices (Wang *et al.*, 2016), represent important challenges for using this technology for edible insect processing in food industry.

Pulsed electric field application is a relatively recent and widely employed technology in the food sector for several purposes. PEF consists of processing the food product with short high voltage electric pulses (Nowosad *et al.*, 2021). Depending on the purpose, several pulse frequencies

and exposition times can be applied. The mechanism of the technology is related to triggering a change in the cell membrane integrity with consequent enhancement of its permeability (Nowosad *et al.*, 2021). This phenomenon can be exploited in several ways, including improvement of extraction efficiency of nutritional and bioactive compounds from raw materials (Alles *et al.*, 2020; Andreou *et al.*, 2020; Kaferbock *et al.*, 2020; Rahaman *et al.*, 2020) and food waste (Franco *et al.*, 2020). Further, PEF coupled with some conventional processes, like freezing, drying or mechanical extraction of juices or oils, can lead to increase in yields, efficiency optimisation (Alles *et al.*, 2020; Andreou *et al.*, 2019; Shorstkii *et al.*, in press), and milder stabilisation treatments which allow obtaining fresh-like products with texture, flavour, colour and odour closer at their natural state (Liu *et al.*, 2020; Tao *et al.*, 2019). Hurdles for PEF application in food industry include the high initial costs (Stoica *et al.*, 2013) and its lack of activity on bacterial endospores inactivation, which requires a further treatment (Reineke *et al.*, 2015; Siemer *et al.*, 2014). However, unlike HHP, no negative impact on bioactive compounds has been detected with PEF treatment (Nowosad *et al.*, 2021; Shorstkii *et al.*, in press).

Ultrasound technology consists of mechanical acoustic waves propagating in an elastic (solid or fluid) medium (Lempriere, 2003). These waves can generate compression and decompression on the matrix particles, producing a huge amount of energy (Gallo *et al.*, 2018). High intensity ultrasound operates at frequencies between 20 and 100 kHz and the intensity ranged between 10 and 1000 W/cm^2. This high intensity allows them to be disruptive by inducing cavitation inside the treated medium, with consequent alterations on physical, chemical and mechanical properties (Bhargava *et al.*, 2021). In the food industry, US is usually used to improve the efficiency of several unit operations. US can be used to enhance the extraction yield by creating cavities inside the tissues and favouring the breakdown of cell walls with a reduction of time and energy used for compounds' extraction (Choi *et al.*, 2017; Mishyna *et al.*, 2019; Ojha *et al.*, 2020; Sun *et al.*, 2018). With a similar mechanism, US can also be coupled with drying process. The micro-channels generated inside the bulk because the molecular cavitation, can favour the water elimination, reducing time and temperature of the drying processes. This can lead to a higher quality of the final product (Huang *et al.*, 2020). Besides the mass transfer, US can also be used for heat transfer. For example, US can facilitate the freezing process through improved initial nucleation (Chow *et al.*, 2005). Further, US can be also used during thawing with the goal to reduce the defrosting time in order to avoid product degradation (Li *et al.*, 2020). US coupled with heat treatments (thermosonication) or high pressure (manosonication) has found application also in sterilisation and enzyme inactivation, allowing to obtain interesting results in terms of shelf-life extension, without altering the sensorial characteristics of the food (Cameron *et al.*, 2009; Mintah *et al.*, 2019b; O'Donnell *et al.*, 2010). However, when US is not applied properly, it can be responsible for increase of temperature, with negative effects on nutritional and organoleptic characteristics (Farkas and Mohacsi-Farkas, 2011; Mintah *et al.*, 2020). Despite these drawbacks, US is the mostly used novel technology in the food sector so far (Priyadarshini *et al.*, 2019).

CAPP is a new technology with high potential, but to the authors best knowledge not applied in food industry yet. Plasma constitutes a mixture of several active chemical species, i.e. electrons, free radicals, ions, protons, excited atoms, reactive oxygen species, reactive nitrogen species, and UV radiation (Moreau *et al.*, 2008). These reactive compounds are created by applying electric or electromagnetic fields, generated at several voltages, to a gas (Ramazzina *et al.*, 2015). CAPP indicates a specific kind of plasma, generated at low temperatures (usually between 30 and 60 °C) and atmospheric pressure (Moreau *et al.*, 2008). The lower operating temperature provides interesting opportunities for shelf-life extension of several food matrices, without altering the quality parameters of temperature-sensitive products (Bußler *et al.*, 2016a; Pan *et al.*, 2019). CAPP has also shown its potential to be active not only on the viable microbial cells but also on spores, thus allowing low-temperature sterilisation of the food product (Bußler *et al.*, 2016a; Liao *et al.*, 2018; Rumpold *et al.*, 2014). The high reactivity and instability of the compounds produced during plasma treatment can also be used for inactivation of certain enzymes due to the alteration of their protein structures (Bußler *et al.*, 2016a, 2017; Han *et al.*, 2019). With the similar mechanism, CAPP can facilitate the decontamination of foods from allergens, pesticides, mycotoxins and other toxic contaminants (Gavahian and Cullen, 2020; Gavahian and Khaneghah, 2020). Another application of CAPP in the food industry is related to the modification of some food components and their physical characteristics, with the purpose to improve their quality and increase the processing efficiency (Bußler *et al.*, 2016a; Ekezie *et al.*, 2017). However, reactive oxygen species present in CAPP have shown negative activities on lipid stability, promoting lipid oxidation, which can result in off-flavour (Varilla *et al.*, 2020). CAPP can also find application in food fortification to obtain a product with specific healthy properties (Akasapu *et al.*, 2020). Furthermore, CAPP has also been applied on some by-products to extract interesting secondary nutrients as well as phenolic compounds (Bao *et al.*, 2020). Main drawbacks for CAPP application in food industry are connected with the uncertainty of find toxic residuals in food. Despite no evidences of potentially dangerous compounds in plasma treated food have been found so far (Kromm *et al.*, unpublished data; Priyadarshini *et al.*, 2019), it is recommended that each product should be assessed individually (Schlüter *et al.*, 2013).

Innovative processing routes

Novel technologies have shown their potential for different processes in the food industry; however, their use in edible insect processing is still scarce. Traditional methods, which are well known and easy to operate, are still preferred, due to the high variability of the insect-products and the high difficulty of process optimisation. However, novel technologies can lead to improved quality and safety of insect based products, assuring a larger consumer acceptance. Several studies have been performed in this regard. Table 2 offers an overview of these studies. Further, Figure 4 and 5 present possible innovative processing pathways, for the production of whole edible insects and insect products respectively, where novel processing technologies can modify, complement, or replace the conventional processing routes.

Microbial decontamination

CAPP and HHP have been studied to reach an acceptable microbial reduction grade and guarantee safety for the final consumer. Rumpold *et al.* (2014) observed that indirect plasma treatment for 10 min is very effective for surface decontamination of mealworm larvae, allowing microbial reduction of 7 log cycles. However, the same treatment has not shown any effect on gut microbial load. On the contrary, HHP is more active on gut microbial count reduction than on the surface (Kashiri *et al.*, 2018). Therefore, combination of CAPP and HHP can be a suitable alternative to traditional heat treatments in order to reach adequate microbial reduction on whole edible insects (Figure 4, pathway A). Another possibilities is to couple HHP or CAPP with traditional heat treatments realised at lower temperature and shorter time (Rumpold *et al.*, 2014). Similar reasoning can be done for insect meal. Bußler *et al.* (2016a) investigated the impact of CAPP on the microbial load of *T. molitor* flour showing reduction of the total microbial load from 7.7 to 4.3 log cfu/g. However, reduction from 6.8 log cfu/g to below detection limit has been observed when a thermal treatment for 15 min in an oven at temperatures of 120 °C was applied (Bußler *et al.*, 2016a,b). The microbial species to be inactivated should be considered as well. For example, Campbell *et al.* (2020) observed that a HHP treatment at 400 MPa for 10 min on *H. illucens* larvae is able to reduce yeasts and moulds below the detection limits, while reductions of *Enterobacteriaceae* (from 7.65 to 3.32 log cfu/g) and lactic acid bacteria (from 6.50 to 4.73 log cfu/g) were remarkable but not satisfactory. These results have confirmed the finding of Kashiri *et al.* (2018), who observed that HHP at 400 MPa for 2.5 min was responsible for a total inactivation of yeasts and moulds, but no significant effect was observed for total aerobic mesophilic microbial count, where the reduction was only 0.35 log cycles (Kashiri *et al.*, 2018).

Enzyme inactivation

In order to promote shelf life extension of food products, undesirable chemical reactions, such as enzymatic browning, should be prevented. Interesting results in terms of enzyme inactivation have been obtained when HHP treatments were used on *T. molitor* flour (Tonneijck-Srpova *et al.*, 2019). In this study, HHP has shown a potential to prevent enzymatic browning, which was comparable with traditional blanching ($L^*90 \geq 60$ after 10-11 days for HHP and $L^*90 \approx 60$ after 3-4 days for blanching). However, HHP treatment had a negative impact on the product texture, with a high amount of serum released from the mealworm powder. On the contrary, treatment at 500 MPa was able to prevent the enzymatic browning and at the same time to reduce the amount of released serum, obtaining a product with a weaker texture, therefore more desirable by the consumer (Tonneijck-Srpova *et al.*, 2019).

Drying

Novel technologies are also known for increasing efficiency and yield of basic unit operations such as drying and freezing (Bassey, 2021; Wu, 2017) (Figure 4, pathway B). Concerning edible insects, impact of PEF pre-treatment on drying time has been evaluated by Alles *et al.* (2020). They observed that PEF at 2 kV/cm and 20 KJ/kg was able to reduce the drying time of *H. illucens* pre-pupae. However, these benefits were not observed at higher electric field strength (3 kV/cm), highlighting the importance of process optimisation (Alles *et al.*, 2020; Shorstkii *et al.*, in press).

Interesting results in terms of increase in mass transfer rate and time reduction during drying process may be obtained by applying US on insects as a pretreatment and/or in combination (Figure 4, pathway B). At the best of our knowledge, no study has investigated this potentiality on edible insects. However, basic knowledge can be transferred from other food materials (Huang *et al.*, 2020).

Extraction

Traditional extraction procedures are often time consuming, inefficient and non-selective (Otero *et al.*, 2020). Ultrasound and PEF have shown important solutions for these problems. For example, in a recent study, Otero *et al.* (2020) compared two different methods (i.e. ultrasound assisted extraction and pressurised liquid extraction) and two solvents (pure ethanol and a mixture of ethanol and water 1:1 v/v) to extract oil from *A. domesticus* adults and *T. molitor* larvae. They observed that ultrasound can increase the content of polyunsaturated fatty acids and decrease the content of saturated and monounsaturated fatty acids of the extract. Despite these positive results, every extraction method was found to increase the cholesterol level of the final extract. Besides, the effect of ultrasound is strictly related to the

Table 2. Innovative technology in insect processing.[1]

Process goal	Technology and processing condition	Insect species and form	Main findings	Reference
Microbial decontamination	HHP treatment (35 L vessel, at room temperature) at 400 MPa and 600 MPa; 1.5 min and 10 min	*Hermetia illucens* larvae	HHP resulted in a lower inactivation for TVC and 600 MPa was needed to achieve similar reductions against Enterobacteria, LAB, and YM.	Campbell *et al.* (2020)
	HHP treatment (250 to 400 MPa, for 1.5 to 15 min)	*H. illucens* larvae	HHP was effective against natural contaminating YM, resulting more than 5 log cycle reductions at 400 MPa for all treatments; however, a low reduction of total microbial load was achieved.	Kashiri *et al.* (2018)
	CAPP generated at sinusoidal voltage of 8.8 kV_{PP} at a frequency of 3.0 kHz using air as working gas; treatments up to 15 min	*Tenebrio molitor* flour	Total microbial load reduced from 7.72 to 4.73 log_{10} cfu/g in 15 min CAPP treatment.	Bußler *et al.* (2016a)
	HHP treatment (400, 500, and 600 MPa; up to 15 min)	*T. molitor* larvae	A HHP treatment at 600 MPa for 10 min resulted in an inactivation of 3 log cycles.	Rumpold *et al.* (2014)
	Indirect plasma treatment at frequency of 2.45 GHz and a power consumption in the range of 1.2 kW	*T. molitor* larvae	Plasma treatment (10 min) resulted in up to 7 log cycles reduction.	Rumpold *et al.* (2014)
Drying	PEF at 2 and 3 kV/cm; 5, 10 and 20 kJ/kg wet basis	Live *H. illucens* larvae	PEF pre-treatment can significantly reduce (up to 30%) drying time of insect biomass.	Alles *et al.* (2020)
Enzymatic hydrolysis	HHP treatment at 380 MPa for 1 min applied before or during the enzymatic hydrolysis by pepsin or Alcalase®	*T. molitor* larvae powder	HHP applied before the enzymatic hydrolysis improved the enzymatic hydrolysis by Alcalase® (but not by pepsin) at the very beginning of the process. Partial reduction of allergenic properties was observed. No alterations on the protein profile were observed.	Boukil *et al.* (2020)
	Ultrasound pretreatment at 600 W and 40±2 kHz for 30 min. Pulse interval of 15 s on and 5 s off. Temperature of 50 °C	Defatted *H. illucens* meal	US pretreatment enhance the enzymolysis reaction, by altering the molecular structure and arrangement and improving the enzyme-substrate interactions.	Mintah *et al.* (2019a)
	Ultrasound pretreatment at 600 W and 40±2 kHz for 10-20-30 min. Pulse interval of 15 s on and 5 s off	Dried defatted *H. illucens* meal	US assisted hydrolysis leads to hydrolysates with higher antioxidant activity.	Mintah *et al.* (2019b)
	Ultrasound pretreatment at 600 W and 40±2 kHz with a pulse interval of 15 s on and 5 s off	Dried defatted *H. illucens* larvae	Ultrasound pretreatment increase the sample lightness by reducing the protein hydrolysate molecular weight. Sonic treatments alter the secondary protein structure, reducing the hydrolysate turbidity and increasing the protein dispersibility.	Mintah *et al.* (2020)
Enzyme inactivation	HHP treatment (4 l vessel) at 250, 300, 400 and 500 MPa for 3 min	*T. molitor* larvae	Pressure of 400 MPa prevented the enzymatic browning.	Tonneijck-Srpova *et al.* (2019)

Table 2. Continued.

Extraction	Ultrasound assisted extraction by direct sonication at 20 kHz, amplitude of 89.9 µm in continuous pulse; 15 min	Lyophilised and grounded *Acheta domesticus* and *T. molitor*	The extraction yield was dependent on the nature insect species and the solvent of extraction. Ultrasound can be used to obtain insects extracts with improved fatty acid profile.	Otero *et al.* (2020)
	Ultrasonic assisted aqueous extraction by sonication at 20 kHz (several parameters combinations tested)	Lyophilised and grounded *Clanis bilineata* larvae	Ultrasound treatment allowed increasing oil extraction yield and oil quality. The higher yield was reached with an ultrasound treatment of 50 min at 40 °C and 400 W, with a pulsation interval of 2 s. Ultrasound treatment can increase the thermos-stability and antioxidant activity of the oil.	Sun *et al.* (2018)
	Ultrasound assisted extraction by sonication at 20 kHz, 75% of amplitude, 3 seconds of pulsation interval; 1, 15 and 20 min	Lyophilised and grounded *Bombyx mori* Microwave dried and grounded *T. molitor* and *Gryllus bimaculatus*	Ultrasound sonication significantly affects the protein extraction yield without changing the amino acid profile. Sonication time had a different impact on protein extraction yield among several insect species (maximum yield reaches after 15 min for *T. molitor* and *G. bimaculatus* and 5 min for *B. mori*) The protein extraction yield was dependent on the insect species and their life stage.	Choi *et al.* (2017)
	Ultrasound assisted protein extraction by sonication with amplitude 70% for 6 min. pulsation interval of 30 s.	Defatted powder of *Apis mellifera* larvae and pupae Defatted powder of *Schistocerca gregaria* adults	Ultrasound sonication improve the protein extraction yield Method used for protein extraction alter the molecular characteristics of protein, determining a changing on its hydrophobicity. But a specie-specific effect has to be addressed.	Mishyna *et al.* (2019)
	PEF at 2 and 3 kV/cm; 5, 10 and 20 kJ/kg wet basis	Live *H. illucens* larvae	Oil extraction yield was not affected significantly by PEF treatment. Higher amount of amino acids was recovered in oil after PEF treatment	Alles *et al.* (2020)

[1] CAPP = cold atmospheric pressure plasma; HHP = high hydrostatic pressure; LAB = lactic acid bacteria; PEF = pulsed eclectic field; TVC = total viable count; US = ultrasound; YM = yeasts and moulds.

insect species and the solvent used, with better results found for *T. molitor* oil extracted with ethanol-water mixture. Important parameters that determine the extraction yield are also temperature, treatment time, pulse interval and ultrasound power. Optimisation of ultrasound treatment in order to increase the oil extracted from *Clanis bilineata* was conducted by Sun *et al.* (2018). In this study, they applied Response Surface Methodology to find out the best parameters combination in order to increase the extraction yield without altering the antioxidant properties of the oil. A possible pathway where integration of ultrasound is used for insect oil extraction is shown in figure 5 (pathway A).

PEF has also been tested for oil extraction from insects (Figure 5, pathway B). In a recent study conducted on *H. illucens* larvae, Alles *et al.* (2020) observed that the PEF pre-treatment resulted in a size reduction and a more homogeneous distribution of fat droplets in insect cells; however, no significant increase in the extraction yield was observed. Further, the fatty acid profile of the oil extracted from PEF pre-treated sample was observed to be similar to the oil extracted without any pre-treatment.

Ultrasound, PEF, HHP and CAPP can also have an impact on protein extraction from defatted insect meal (Figure 5, pathway C). For example Choi *et al.* (2017) observed an enhanced protein extraction yield on *B. mori*, *T. molitor* and *G. bimaculatus* treated when ultrasound was used, while Mishyna *et al.* (2019) observed that ultrasound assisted extraction can enhance solubility, coagulability and foaming stability of *S. gregaria* protein extracts, while the same treatment has been responsible for reduction in solubility and coagulability in *A. mellifera* extracts.

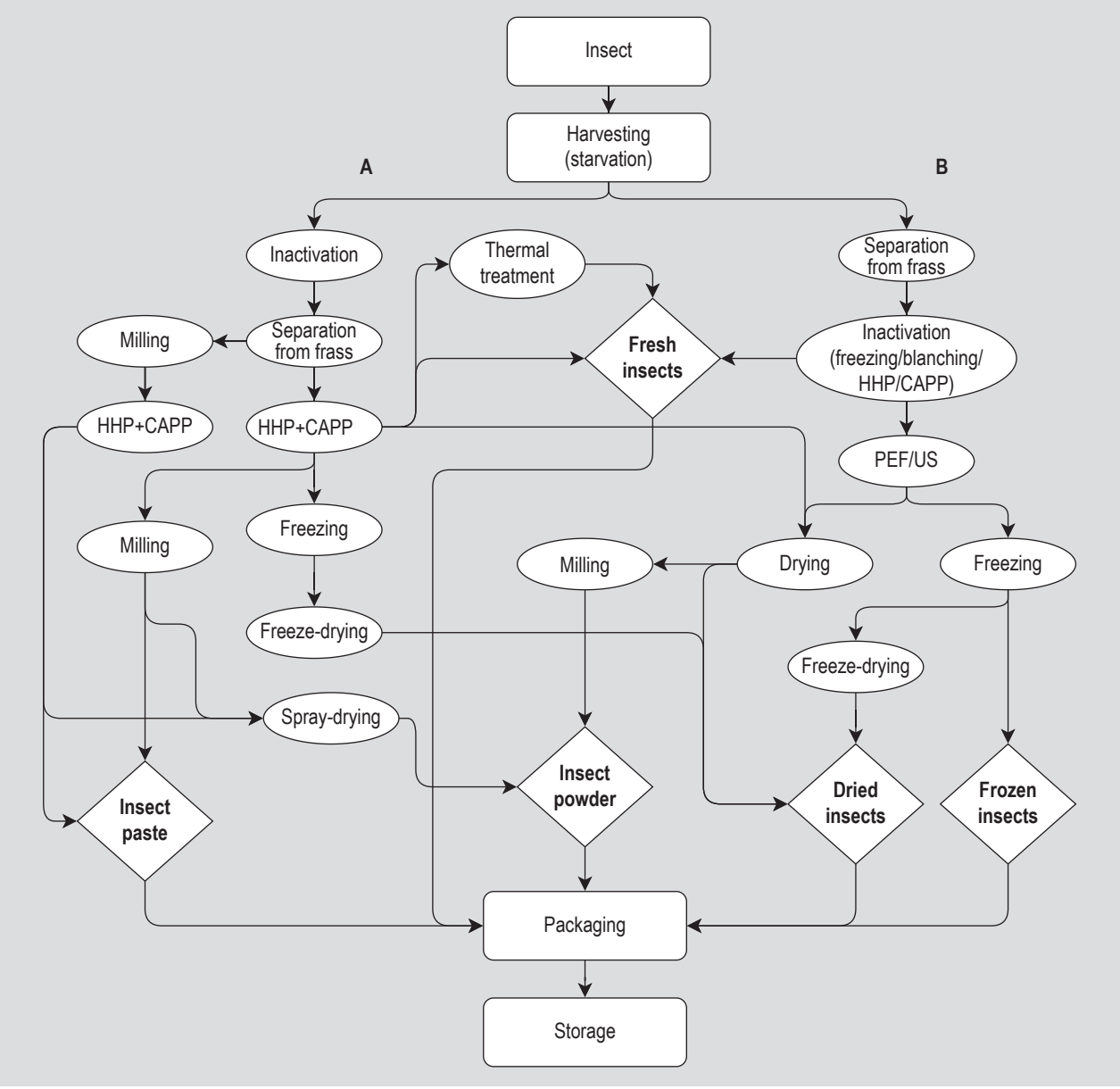

Figure 4. Possible innovative processing pathways for the production of whole (fresh/frozen/oven-dried/freeze-dried/microwave-dried) edible insects and full-fat insect meal

Enzymatic hydrolysis

Innovative food processing methods, such as microwave, pulsed-electric field, ultrasound and HHP alone or coupled with enzymatic hydrolysis can reduce protein allergenicity of food proteins including edible insect products (Dong et al., 2021). In particular to insects, HHP and ultrasound have been studied to enhance the effect of hydrolysis of these allergenic compounds in order to increase the product quality. In a recent study, Boukil *et al.* (2020) reported that HHP at 380 MPa for 1 min applied in combination with Alcalase® enzyme could improve the protein hydrolysis degree (by up to 24%), allowing a partial reduction in allergenic activity. Ultrasonic treatment can also enhance the hydrolysis degree of insect powders (Mintah *et al.*, 2019a). For example, defatted *H. illucens* meal treated with ultrasound (600 W, frequency of 40 kHz) at 50 °C for 30 min, presented higher enzymatic activity (Mintah *et al.*, 2019a). Moreover, the damaged cells could lead to increased enzyme releases/enzyme concentrations, enhancing the hydrolysis of allergens (Mintah *et al.*, 2019a). Nevertheless, ultrasound treatment can also affect the antioxidant properties of the powder. Optimisation of the process depending on some antioxidant parameters has been conducted and validated on defatted *H. illucens* meal, concluding that sonication before the enzymatic hydrolysis allows obtaining a product with higher antioxidant power (Mintah *et al.*, 2019b). Furthermore, ultrasound can cause a change in the

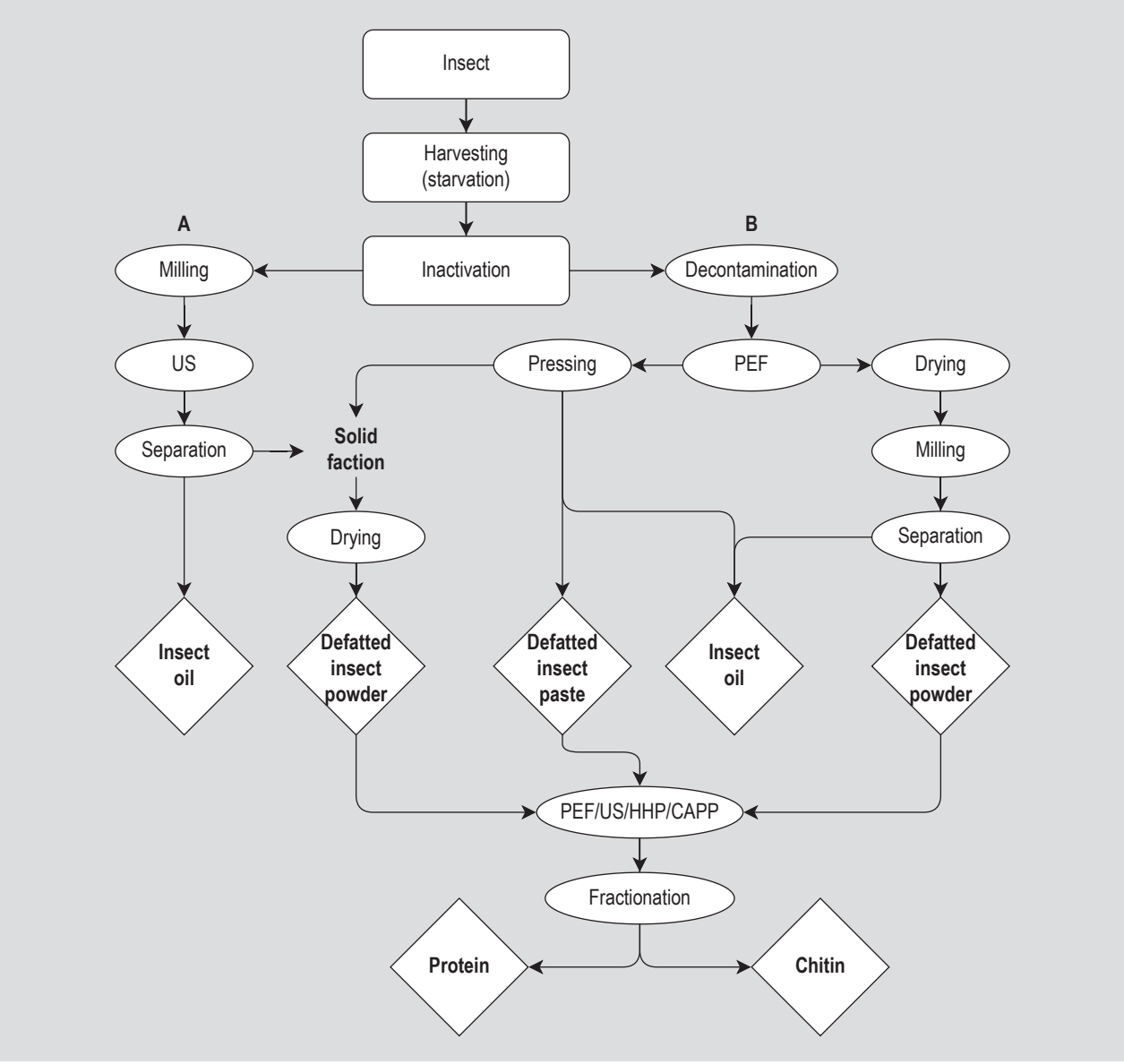

Figure 5. Possible innovative processing pathways for the production of defatted insect meal, insect oil, insect protein and chitin.

protein structure. Mintah *et al.* (2020) observed a reduction of β-sheet and a correspondent increase of α-helix and β-turn conformations. However, the proportion of each structure depends on the treatment parameters applied (Mintah *et al.*, 2020).

5. Conclusion and future perspectives

With the growing production of edible insects at an industrial scale, it is crucial to implement appropriate post-harvest and processing technologies for the safety, preservation, quality improvement, fractionation and storage of insects and insect products. Conventional processing pathways may include several unit operations used in traditional food and feed processing. The insect processing pathways can vary depending upon the nature of the initial material and the desired end product. In recent years, novel food processing technologies (e.g. high pressure processing, PEF, ultrasound and CAPP) have shown potential as an alternative or synergistic to the conventional technologies. The main challenges ahead of insect processing are the development of efficient, environmentally friendly and low-cost processing technologies, waste minimisation, recovery, and incorporation of by-products/co-products. Novel food processing technologies are considered to be environmentally friendly and may improve the processing efficiency, safety, quality, and sustainability of insect based products. However, the high knowledge level requirement, regulations and initial plant costs represent important hurdles to overcome.

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Conflict of interest

The authors declare no conflict of interest.

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