Processing of insects as a whole or as fractions

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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. Processing insect as a whole and as fractions

Processing insects for the use in human diets requires some necessary steps. The process needs to make sure that sensory and nutritional qualities are preserved and the safety is guaranted. The processes reviewed in this research, start when the insect are employed as ingredient, not at the

breeding of insects. The process ends when there is raw insect material to use as ingredients to make different kinds of food products like snacks, cookies, hamburgers, protein bars and others.

This literature research focuses on three insects: *Tenebrio molitor, Locusta migratoria* and *Acheta domesticus*.

1.1. Processing technologies of insects in general

Within this research, many process technologies were discovered on how to make food products from insects. These technologies are not working alone, but they need to be connected with each other into a whole process flow, consisting of multiple steps from the insect to an ingredient for a food product. The technologies used for insect processing can be categorized into four subcategories: heat, cold, shred treatments and others. The following table shows an overview of all found processing technologies that are used for insects in general.

Table 1: Overview processing technologies of insects

Heat treatments	**Cold treatments**	**Shred treatments**	**Others**
Steaming	Freeze-drying	Crushing	Ultrasound-assisted extraction
Roasting	Freezing	Pulverizing	Fluidized bed drying
Smoking	Cold atmospheric pressure plasma	Grinding	Marinate
Frying		Milling	Fermentation
Stewing		Mashing	Microwave-drying
Drying (Sun-, Oven-)		Mincing	Spray drying
Toasting			Dry fractionation
			Curing
			Blanching

1.1.1. Processing *Tenebrio Molitor*

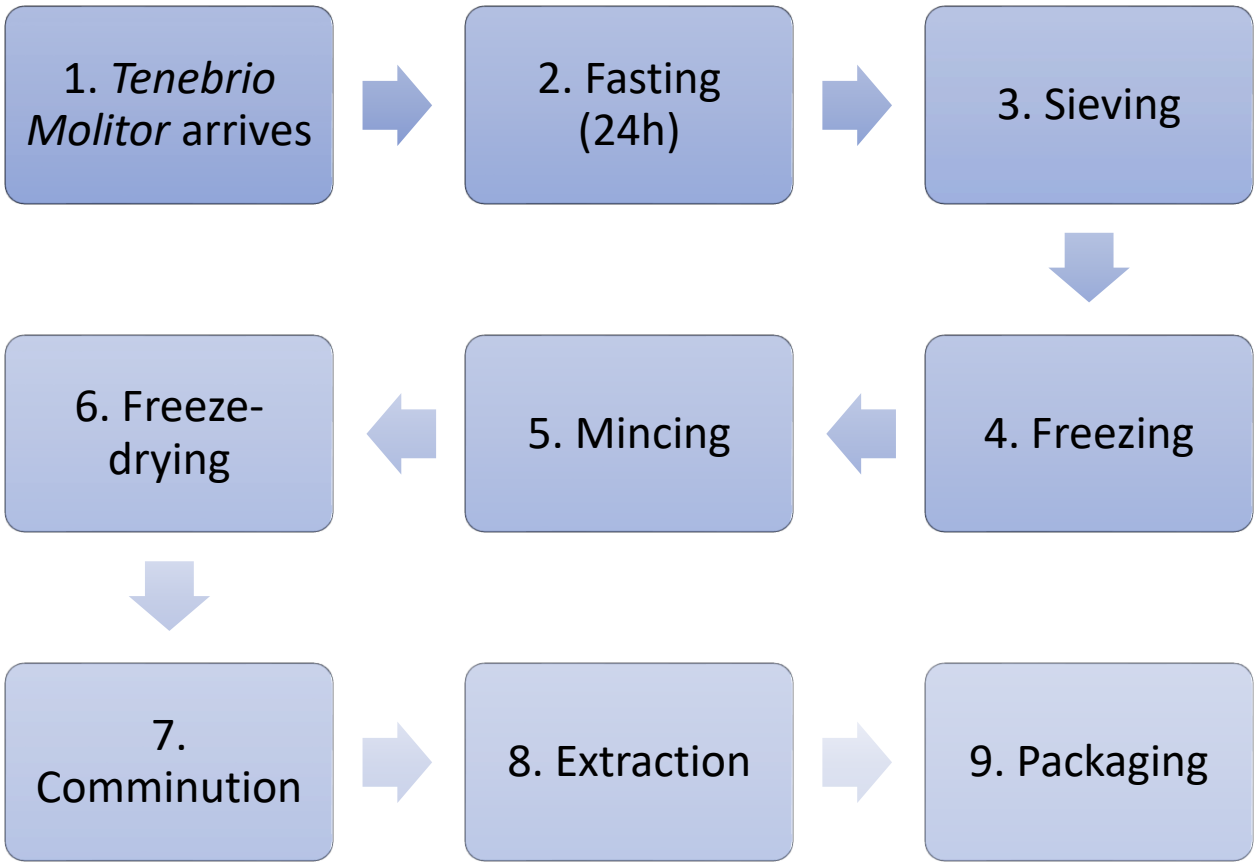

Figure 1: Flow chart for the processing of T. molitor as a whole

(2) When *Tenebrio molitor* arrives at the processing chain, it first gets on a diet for 24 hours. The fasting is done to reduce the gut content in the body of the insect.

(3) Before they are slaughtered they can potentially get sieved to eliminate frass.

(4) To slaughter the insects, they are frozen at -18 / 20 ° C for 24 hours (insects must be killed at temperatures below -10 ° C). The traditional way of processing insects is cooking (blanching) (black soldier fly: at least 80 ° C), although this method usually only involves pasteurizing them.

In the frozen state at minus 18 degrees, the reproduction of bacteria and the germination of spores is effectively suppressed. However, thawing carries an immediate risk of bacterial

regrowth. To avoid spoilage, deep freezing in sealed boxes / bags (to avoid enzyme activity and mass loss) is required.

(5) Once the insects have been killed, they are ground up.

(6) After chopping, the insects are freeze-dried at 0.2 millibars / 48 h in order to reduce the water content of the insects. Freeze-drying is a low temperature dehydration process, where the product gets freezed and the pressure is lowered. The ice is removed by sublimation. This is an alternative to the use of conventional heat drying by using an oven, because it results in less nutritional and sensory losses.

(7) The final processing steps within the crushing process are grinding the insects into fine particles or a homogeneous powder, sieving them and packaging them for further use to ensure that the products are not contaminated. The machines for grinding / milling must be cleaned regularly. The entire process is subject to strict hygiene regulations, and an HACCP plan is required.

(8) There is not yet much information available about the extraction of lipids, proteins and chitin from insects. Mechanical separation (pressing) and/or heat treatment is used for oil/fat extraction, lipids can also get extracted by the use of hexane or aqueous extraction. Chitin extraction requires chemical and/or enzymatic processing, e.g. fermentation with microorganisms or enzymes. Protein extraction can be done for example by alkaline protein extraction, first at alkaline then at acidic conditions or by dry-fractionation.

(9) Because of the high fat levels of many insects, the packaging should be made under modified atmosphere. Retard lipid oxidation can be avoided then. Protection from light and oxygen is necessary.

Some more specific strategies within the different process technologies of *Tenebrio molitor* can be found in the following table.

Table 2: Overview specific strategies of processing *Tenebrio molitor*

	Technology	**Strategy**
Drying technologies	Oven-Drying	• 60 °C/24 hr • Conventional hot air drying 60 °C/24 hr 80 °C/7 hr • with air circulation

		45 °C/48 hr
	Microwave-assisted drying	8, 10, 13, 16, 20 min / 2 kw
	Freeze-drying	0.2 mbars/48 hr
	Fluidized bed drying	Bed temperature: 60 °C Air outlet temperature: 55 °C Differential pressure bed: 15 bar Differential pressure filter: −1.3 bar Air flow: 500 m3/hr
	Oven-drying with aircirculation	45 °C/48 hr
Extracting technologies	Cold atmospheric pressure plasma (Microbiological charge reduction)	Surface dielectric-barrier air-discharge sinusoidal voltage: 8.9 kVpp frequency: 3.0 kHz using air as working air with 12 mm of distance below the plasma source continuous agitation. 350 rpm, 15 min. Thermal load <67 °C.
	Supercritical CO_2 Solvent extraction	400/250 bar, 45 °C, 105 min
	Dry-fractionation (Protein extraction)	Blanching 10 min 1/12 (w/w) and shock-freezing (−38 °C, 20 min); thawing 1 hr, room temperature and drying treatments. Roller milling and sieve classification (pore size 355, 500, 710, 1000, 1400 µm).

1.1.2. Processing *Locusta migratoria* and *Acheta domesticus*

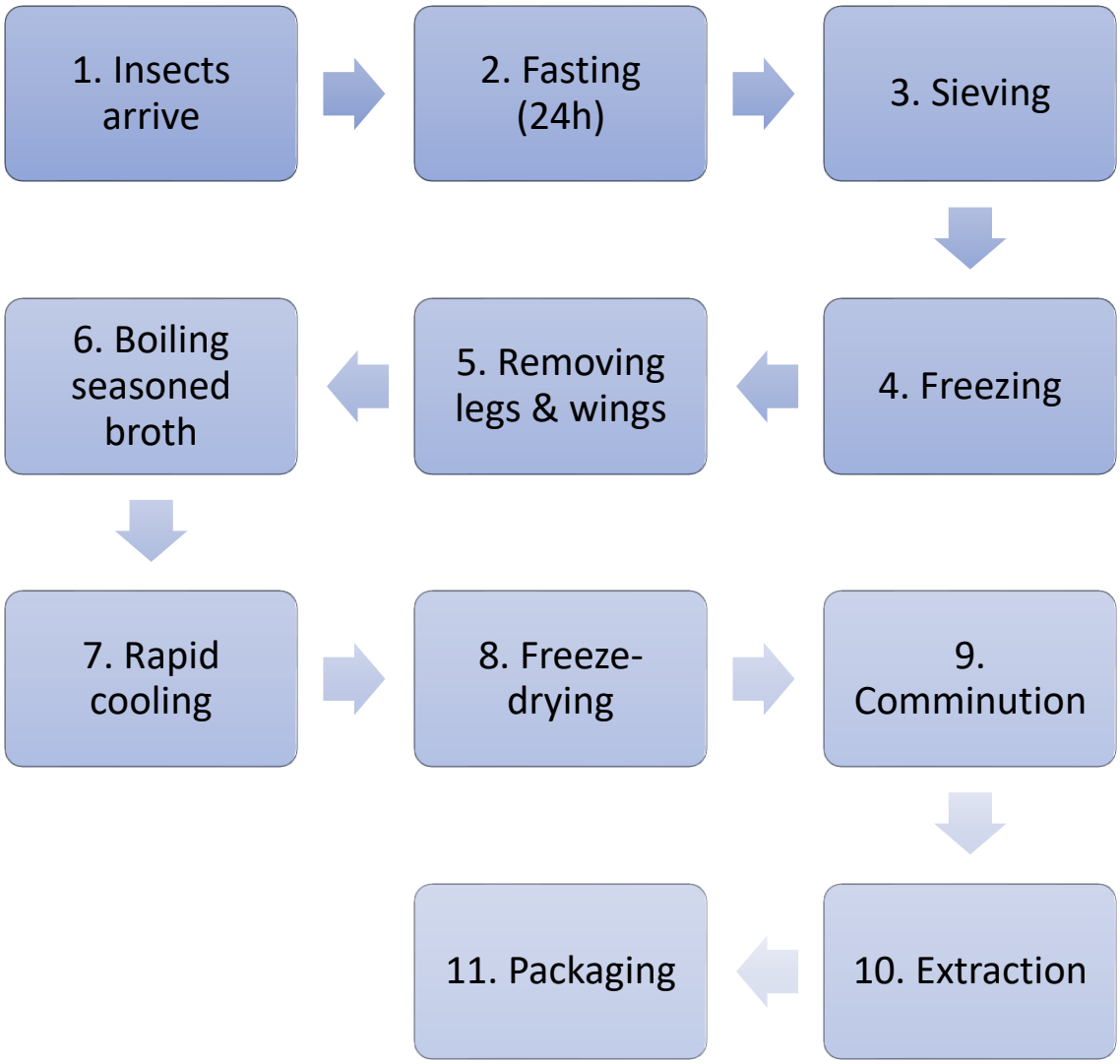

Figure 2. Flow chart for processing Locusta migratoria and Acheta domesticus

These processing steps are the same as with *Tenebrio molitor.* The new steps will be further explained.

(5) After slaughtering the insects by freezing, the legs and wings are removed.

(6) Cooking the insects in boiling water for 1 to 5 minutes ensures that all germs, at least all pathogens, are killed, but is also associated with nutritional losses. This cooking can also be done in seasoned broth to enhance the sensory properties of the end product.

(7) After cooking, the insects need to be cooled quickly so that no bacterial growth can take place in the event of a possible recontamination.

2. Processing insects to obtain fractions

Until now, it has been described how the insects can be processed as a whole, i.e., no specific components are being separated or purified. However, it has been well documented that several fractions can be obtained from the insect, and each one of them possesses different properties, and therefore, the range of applications and functions can be expanded. For instance, it has been described how protein, lipids, chitin or collagen can be obtained starting from insects as raw material. Table 17 shows different methods and technologies used to extract different compounds from edible insect.

***Table 3**: Methods and technologies used to extract different compounds from edible insect*

Specie	Method/ technology	Conditions
Ruspolia nitidula	Air convention dryer	80 °C/10 hr until moisture of 5% is reached.
Rhynchophorus phoenicis	Solar drying Oven-drying	5 days 50 °C/48 hr Smoke-drying Exposure to smoke heat for 6 hr
Sternocera orissa	Oven-drying Freeze-drying Frying pan	66 °C/24 hr −55 °C/24 hr/085 mtorr 130-cm diameter, 50-mL tap water Fried without cooking oil
Imbrasia epimethea	Oven-drying Solar drying	8 hr/80 °C 3 days
Macrotermes subhylanus	Solar drying	Approximately 30 °C RH 40% Time not indicated
Polyrhachis vicina	Solar drying	20–35 °C until dried, between 2 and 5 days
Ruspolia differens	Freeze-drying	Phase (1) −50 °C/0.40 bars /48 hr Phase (2) −55 °C/0.021 bars /48 hr
Cirina forda	Oven-drying	40 °C / 24 hr after boiling 2 hr
Rhynchophorus phoenicis	Oven-drying	60 °C to constant weight
Clanis bilineata	Ultrasound-assisted aqueous extraction (UAAE)	BILON-650CT multi-purpose constant-temperature ultrasonic extraction system equipped with one powerful ultrasonic transducer (20 kHz, 650 W)
Tenebrio molitor L.	Cold atmospheric pressure plasma Supercritical CO2 extraction Dry-fractionation	Surface dielectric-barrier air-discharge; sinusoidal voltage: 8.9 kVpp; frequency: 3.0 kHz using air as working air with 12 mm of distance below the plasma source; continuous agitation. 350 rpm, 15 min 400/250 bar, 45 °C, and 105 min Blanching 10 min 1/12 (w/w) and shock-freezing (−38 °C, 20 min); thawing 1 hr, room temperature and drying treatments (see Table 3). Roller milling and sieve classification (pore size 355, 500, 710, 1000, 1400 µm).

Tenebrio molitor, Alphitobius diaperinus, Acheta domesticus and Blaptica dubia	Soxhlet extraction	Soxhlet apparatus (6 hr, with petroleum ether as solvent); evaporation with rotary evaporator at 350 mbar, 40 °C, 30 min.
	Water extraction	Frozen insect mixed with water and blended 1 min, followed by 15 min of sonication; sieving (350 µm); centrifugation 15,000 × g, 30 min, 4 °C; after separation lipid fraction was centrifuged (15,000 × g, 15 min, 40 °C
	Folch extraction	Mixed ground powder with 200 mL dichloromethane/methanol (2:1) solution; shaking 20 s; sonication 10 min; shaking 2 hr; addition of 25 ml of water; centrifugation (1,006 × g, 20 °C, 20 min). Lipid fraction solubilized in organic solvents and filtered with dichloromethane. Evaporation of solvents 1.5 hr with rotary evaporator (800 mbar). Flushing with N2 in water bath at 40 °C
Holotrichia parallela Motschulsky	Chemical extraction	Dried powder milled and treated with 1M HCl, 100 °C, 30 min to remove minerals and catechol; deproteinization: 1 M NaOH, 80 °C, 24 hr. Washed until pH 7.0. Decolorized (KMnO4 (1%), 1 hr)
Locusta migratoria L.	Enzymatic hydrolisis	Hydrolysis conditions (50 °C, pH 8.0, enzyme substrate ratio: 0.05, 0.5 and 1.0 mL/100 mL); Single treatments with alcalase, Flavourzyme, neutrase, and papain or combined treatment: one-step (simultaneously added) or two steps (after 1 hr each); dispersion in water 5% (w/v) (11,000 rpm, 60 s and stirring 1 hr, 50 °C); pH 8.0 addition of enzymes (24 hr, 50 °C). Reaction stopped (90 °C, 20 min).

Source: adapted from Melgar-Lalanne. Comprehensive Reviews in Food Science and Food Safety Vol.18, 2019.

Since the western consumer is not used to consuming whole insects, the processing into powders will ease the introduction to new markets. This processing has been referred to as "de-animalizing" and will lead to increased acceptance (Henchion et al., 2016; according to this authors processing

raw materials (such as offal) into ingredients will reduce rejection, although it can be seen as processed food, which has negative connotations.

According to Tzompa-Sosa and Fogliano (2017), for many purposes and specifically for those focused on producing high protein content extracts, it is an essential prerequisite to remove the fat and then grind the material into small particles. This facilitates the following steps of purification. Special attention has to be put on the final particle size, since it will have an impact on several properties of the ingredient as for example solubility, dispersability, water and oil holding capacity or rheological performance. It has been also suggested that thermal treatments, taken place before protein extraction, can minimize the process yield since the solubility of the proteins is negatively affected, and therefore affecting the subsequent processes. Such a thermal process can alter the lipid profile, leading to higher oxidation degrees, therefore, reducing lipid quality and sensory characteristics.

In the next figure, from Ravi, Degrou et al. (2020), the extraction protocols have been classified into wet and dry fractioning. The main difference between processes is when the drying is performed. In the first case the insects are not dried, and the moisture content is employed during processing; once the different fractions have been obtained, the drying process is carried out. On the second case, insect are dried as a whole, and then the dried powder is used for further fractionation steps. Nevertheless, by means of either process it can be obtained a lipid and a protein fraction. It has been suggested that dry processing will be more economical since only one drying process is required. Although wet processing would be more selective and fractions of better quality could be obtained.

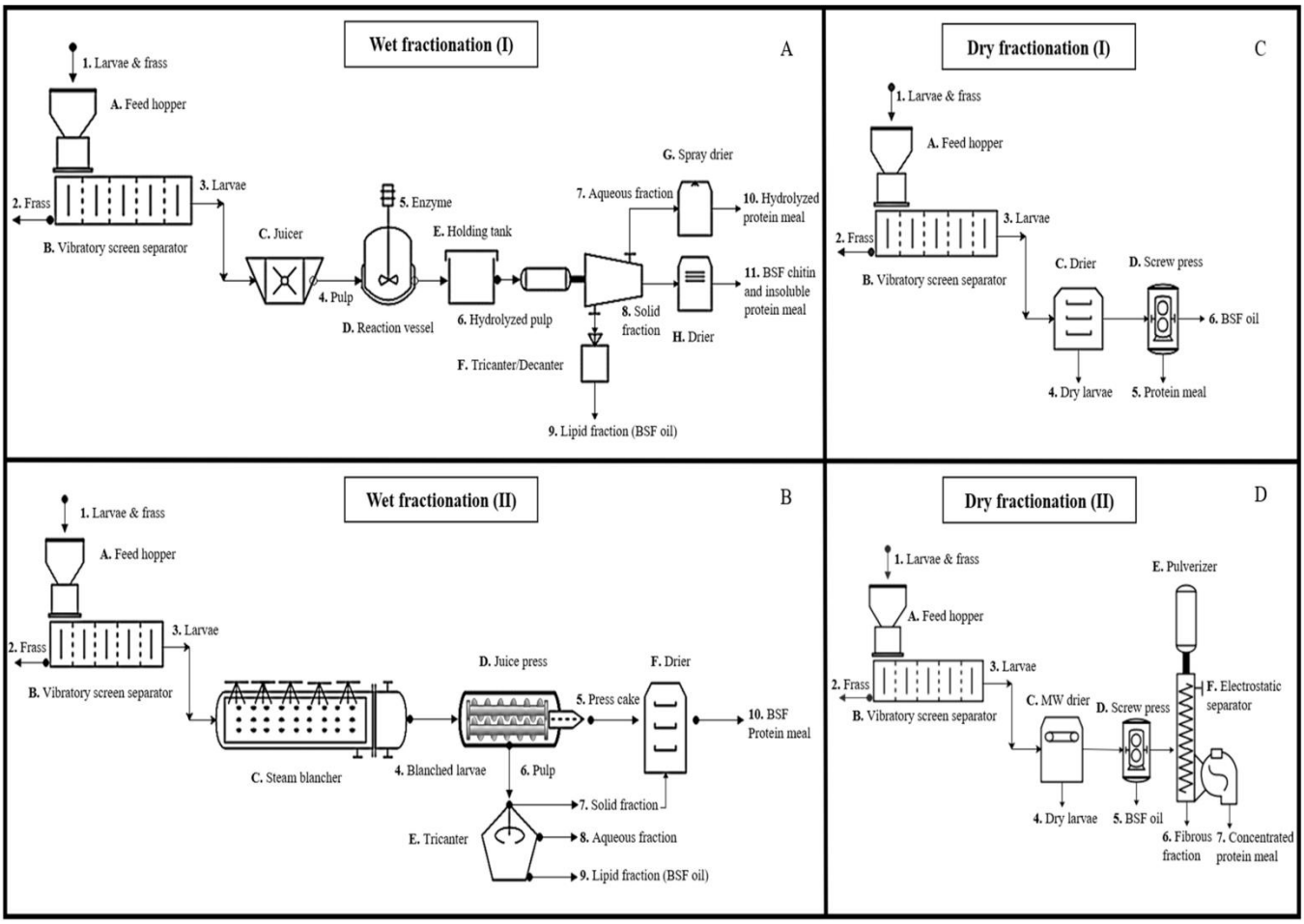

Figure 3: Schematic diagram of wet and dry fractionation

Table 4: Main fractions from insects, potential applications and extracting technologies

Fraction	Application/use	Extraction method
Protein	Protein supplement Functional ingredient	Aqueous extraction: -ionic strength -pH shift Organic solvents Enzymatic hydrolysis Dry fractionation (sieve) Deffating Sonication Microwave
Lipid	Edible oils Source of essential FA	Aqueous extraction Deffating (organic solvent) Supercritical CO_2 Ultrasounds
Chitin	Biomaterial Antimicrobial Oil-binder	Chemical de-proteinization (Weak acid and alkaline extraction)

2.1 Chitin extraction

It has been reported that chitin from insect can perform as good as chitin extracted from shrimp shells in terms of thermal stability, acetylation degree or crystallographic structure (Huet, Hadad et al. 2020). Same authors reported that because of the lower ash content in insects compared to crustaceans, chitin extraction and purification processes are simpler.

In the next figure, it can be seen the regular steps followed to obtain chitin from insects, in this case, the insect employed was silky worm (*Bombyx eri*), although similar approach was employed for house crickets (*Brachytrupes portentosus)* (Ibitoye, Lokman et al. 2018).

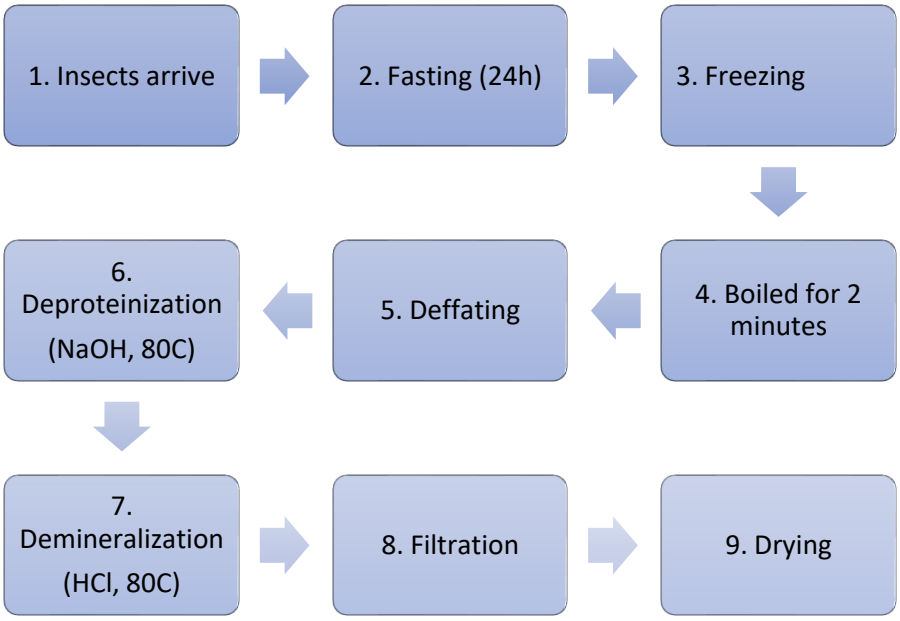

Figure 4: Flow chart for chitin extraction from insects

2.2 Lipid extraction

So far, the most common techniques employed for insect lipid extraction are those focused on using cold press filter, aqueous extraction, organic solvent extraction and supercritical CO_2 systems. According to Tzompa-Sosa and Fogliano (2017), the processing method has not a main impact on the final fatty acid profile; however, the yield and the types of lipids extracted are remarkably affected by processing conditions. In addition, the quality of the final product is affected by extraction technology; it has been found that water extraction provides the higher quality. Since the fractioning of insects is an emerging practice, there is still a lack of understanding

and the data available vary a lot depending on the species, biological phase (larva, pupae, adult), processing conditions and sample pre-treatments. The next table has been summarized based on results obtained for lipids extracted from *Tenebrio molitor, Alphitobius diaperinus, Acheta domesticus and Blaptica dubia* (Tzompa-Sosa, Yi et al. 2014, Purschke, Stegmann et al. 2017).

Table 5: comparison of most common techniques employed for lipids extraction from insects

Extraction technique	Yield	Lipids extracted
Organic solvents (Soxhlet and Folch)	>95 %	Phospholipids, partial glycerides and triacylglycerols
Aqueous extraction	Between 40-60%	Triacylglycerol
Supercritical CO_2	> 95%	Depending on extraction conditions
Cold press filter	Between 50-70%	Mostly triacylglycerols

When supercritical CO_2 was employed, the highest yields were obtained at 400 bar, 45 °C for 105 minutes; although temperature had a minimal effect, so lower ones are recommended to avoid lipid deterioration. Processing conditions employed for Soxhlet extraction are as follows. Total lipid content was determined in freeze-dried insect powder. A Soxhlet apparatus was employed for 6 hours using petroleum ether as a solvent. Solvent was removed by means of a rotary evaporator until no solvent remained. Folch extraction was performed using a dichloromethane/methanol (2:1) solution, in ratio 1:40 regarding the sample mass. This solution was sonicated for ten minutes and then stirred for two hours, distil water was added and then centrifuged. The lower layer rich in lipids was recovered and then dried in a rotatory evaporator. The last method frequently used is the one based on aqueous extraction; samples and water are mixed in a ratio of 1:3 and homogenized. After this, the mixture is sonicated for 15 minutes and the resulting product is filtered. The filtrate is then centrifuged and the lipids are collected in the upper layer. All these extraction technologies have been mostly tried at lab scale, or pilot scale. However, such processes can be easily transferred to industrial scale level, with maybe the exception of applying ultrasounds, which is still not really implemented at industrial scale.

Final method for lipid extraction is that one based on press forces. For this purpose, dried insects are milled and the processed using an oil press heated at temperatures above the oil melting point, to facilitate the separation (Mätthaus et al., 2019). After that, a refining process can be carried out to obtain different lipid fractions.

2.3 Protein extraction

Protein extraction can be performed either after oil has been extracted in a previous step (see previous section), or at the same time that lipids are fractionated. Most common methods for protein extraction are those based on enzymatic extraction, pH shift or ISP (isoelectrical solubilization precipitation), ionic strength (i.e. adding sodium chloride to an aqueous extraction buffer) or as previously discussed filter press.

It has to be considered that the yield and the quality of the proteins extracted are directly related to any previous thermal treatment. High temperatures pre-treatment will lead to protein modifications, which can alter negatively their solubility patterns and therefore reducing the extractability. However, high temperatures might have additional advantages as for example shorter drying times and deactivation of browning enzymes. It has been suggested that browning or discoloration can be prevented by adding sodium bisulphite (0.5 to 4%) or ascorbic acid (0.01 to 0.04%), when alkaline solutions are employed for protein extraction. The first one, performed much better than the second (Yi, Van Boekel et al. 2017). As a general rule, with the exception of enzymatic process, higher yields of extraction are also generating the lowest purity (i.e. total protein content) on the final product. So, it has to be decided, depending on the final application and properties required the best approach for protein extraction. IN the case of enzymatic extraction, the higher yields are obtained at high degree of hydrolysis, which means that proteins are degraded into very low molecular weight peptides (LMWP). When this happens, the most of the techno functional properties are lose and only solubility is usually improved. But also, LMWP are a source of bioactive peptides, which can be used to prevent oxidative damage, as antibacterial compounds, or as preservatives.

Establishing general rules of yield and performance is complicated, since the final results will depend on several factors such as species, state of life cycle, pretreatments and extraction conditions. In this sense, it is likely that each process needs to be optimized for particular raw materials and focused on the final application.

2.4 Biorefinery approach

As it was described in the previous sections, as by now three main fractions of economic interest can be obtained from insects: proteins, lipids and chitin. However, an effort was done on optimizing the different processes to obtain just one of these fractions. However, with the current research for more sustainable processes and a better use of available resources, a new approach called “bio-refinery” is focused. By means of bio-refinery, it would be possible to fractionate the

insect into its three main components, while optimizing the yield and the purity without compromising quality and functionality. A very simple and tentative diagram of this process is shown in the next figure.

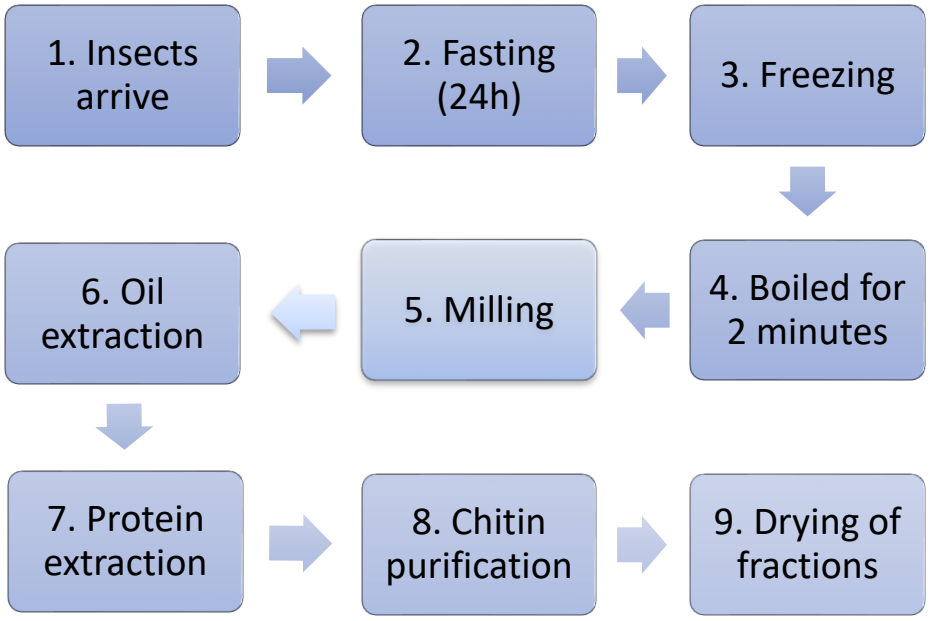

Figure 5: Schematic diagram of insect fractioning into its main fractions.

The main idea is to extract the different fractions in a consecutive way. In the proposed example, after the insects have been milled and homogenized in a fine particle powder, the next step will be to remove the fat by means of one of the methods already described. It has been recently reported that hexane has the highest capacity to extract lipids from insects; while keeping protein functionality (solubility, foaming and emulsifying properties) (Kim, Yong et al. 2021). Once the sample has been defatted, the remaining material is rich in chitin and proteins, and therefore these two fractions can be easily separated based on the low chitin solubility in aqueous and alkaline solutions; or by using enzymatic extraction.

3 Challenges in insect processing

Processing insects to ingredients of food products brings some kind of challenges with it. Many process technologies are expensive and scaling them up to an industrial level would be too costly. For example, freeze-drying is very expensive on a per unit basis, unless the final products have a

very high benefit. This process step needs a long time and is not very efficient. It also requires access to energy-intensive and specialized equipment that is not always available. In addition, heat drying methods bring problems because the longer it takes to dry, the higher the risk of microbial growth gets. It also degrades the quality of the food because fats, oils and proteins agglomerate or denature. Nutrients degrade and undesirable flavors can become stronger. In addition, drying takes a lot of time and is inefficient. In general, hygiene during all processes of production, packaging and storing must be in line with an approved HACCP plan.

Beneath some issues with processing insects, there is also a problem with integrating insects in human food because people are scared/disgusted by the idea of eating insects (see Figure 8 as an example of a meal from whole insects). In some cultures, eating insects is normal but in the western cultures, it is not. So it should be targeted through the development of food technologies and innovations that new food products made from insects as ingredients use them in an unrecognizable form to gain more acceptance from the public (see Figure 9 as an example of the use of insects as ingredients in an unrecognizable form). If the insects are not recognized by the eye as insects the feeling of disgusting can better get avoided.

Figure 6: Hole insects as meal, source: https://pixabay.com/de/photos/thail%C3%A4ndisch-essen-insekten-teller-1222261/

Figure 7: Protein bar made with insects as ingedrients, source: https://www.flickr.com/photos/160866001@N07/48848032292

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