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# Potential of Insect-Derived Ingredients for Food Applications

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Additional information is available at the end of the chapter

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*“The supreme irony is that billions are spent every year to save crops that contain no more than 14% of plant protein by killing another food source (insects) that may contain up to 75% of high quality animal protein”. [1]*

## Abstract

Insects are a sustainable and efficient protein and lipid source, compared with conventional livestock. Moreover, insect proteins and lipids are highly nutritional. Therefore, insect proteins and lipids can find its place as food ingredients. The use of insect proteins and lipids as food ingredients requires a deep understanding on the chemical and physical characteristics of these ingredients, as well as its functionality. Information on the chemical and physical characteristics of insect proteins and oils will help to assess the possibilities of its use on different food applications. In this chapter, we briefly review the nutritional aspects of insect proteins and lipids, insect processing, protein and lipid extraction as well as the perspectives of food applications of insect protein and lipids. Future studies should delve into extraction methods and into intrinsic properties of insect ingredients. This knowledge will be useful to introduce insect ingredients into various food preparations. Also valuable will be the study of other insect species with perspectives for its commercial rearing.

**Keywords:** fractionation, insect proteins, insect fats and oils, food ingredient, food applications

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## 1. Introduction

Sustainability is becoming increasingly important in the world. Alternative sources of proteins and oils have to be found to replace traditional less sustainable ingredients. One of

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the possible alternatives to replace traditional proteins and oils in food products is insects. About 1900 insect species are traditionally consumed as a whole or low processed by approximately two billion people worldwide [2]. In some cases, insects have been consumed as emergency food, in other circumstances as staple food, and in other cultures, they are even considered delicacies [3]. Insects are in general a healthy food source with a high content of protein, fat, vitamins, minerals and fibres. Furthermore, there is a wide range of edible insects which contributes to a high variation in terms of protein and fat profiles. Using insects as a source of protein and lipids can contribute to global food security through feed or as a direct food. Edible insects have been traditionally harvested from natural forests or in the fields, providing food in rural areas for self-consumption or local markets.

Despite the proven nutritional aspects of insects, in the western world, the acceptability of insects as food is low due to its association as pests and disease transmitters [4], which is often described as “dirty image.” In other countries with entomophagy tradition, the consumption of insects has decreased due to the growing urban society. FAO has pointed out the need to examine modern food science practices to increase insect trade, consumption and acceptance. Food scientists and food technologists have been innovating and have been looking for alternative solutions for postharvest handling, to improve processing and increase shelf life of insect products to increase availability and consumer acceptance. One of the proposed solutions to increase consumer acceptance is isolation of insect proteins and fats to be used as food ingredient. This requires a deep understanding on the chemical and physical characteristics of insect proteins and lipids, its functionality as well as an assessment on the consumer’s perception and motivations to accept this novel source. In this chapter we describe some nutritional aspects of insect proteins and lipids, insect processing, protein and lipid extraction as well as the perspectives of food applications of insect-derived ingredients. Since the use of insect-derived ingredients is still in its infancy, there are more questions than answers. In this chapter, we also point out the need of information on the chemical and physical characteristics of insect proteins and oils that will help to assess the possibilities of its use for different food applications.

## 2. Insect proteins

Food scientists need to tackle in the next future the challenge of food security: how to ensure enough protein production to the 2–3 billions of additional people that will populate the planet in the coming decades [5]. There are different possibilities that could be explored as alternative source to obtain a sustainable production of proteins in the future. In this vein, several studies have been done looking at the potentiality of protein from yeast [6], microscopic fungi [7] and microalgae [8].

Recently, insects have been proposed as one of the most promising alternative source of proteins to solve the global issue of protein shortage: the main advantage of insects over other protein sources is the low environmental costs of production, which becomes essential to satisfy the rise in the global protein demand [2, 9]. In **Table 1**, a short summary of the main

Organism	Pro	Cons	Reference
Insects	Thousands of species High conversion rate of feed into edible biomass Protein and lipid production	Low consumer acceptability Scale up of rearing facilities	[10]
Fungi (Quorn)	Easy to grow and harvest Good consumer acceptability	Lower growth rate and relatively low protein content	[7]
Microalgae	Metabolic versatility Environmental friendly Easy to grow and harvest High-quality proteins and lipids	Nondigestible cell wall Easily contaminated by heavy metals	[8]
Yeast	High consumer acceptability Production easy to scale up Low DNA amount	Slow growth rate Low protein content	[6]

**Table 1.** Intrinsic advantage and disadvantage related to the use of different organisms for feed and food production.

advantage and disadvantages related to the use of insect proteins in comparison with other alternative protein sources is provided.

**Table 1** focuses on the intrinsic advantage and disadvantage of the different organisms; however, it is clear that new technological solutions as well as changes in consumer awareness can probably solve many of the present disadvantages. It is very likely that in the next few years, the scenario will be significantly different.

In particular, it is important to consider that the sustainability aspects are gaining more and more importance and for this reason, they are already shaping the production systems in many food chains. In this respect, insects and microalgae have now the competitive advantage that can be produced using nonsophisticated technological infrastructure. Moreover, the possibility to feed insect using by-products of food productions or organic municipality waste could represent the perfect solution to close the circle of food and feed production favouring the transition towards a zero waste system.

As for the sustainability and also food security standpoint, the best way to take advantage from the insect proteins is to use the whole insect eventually grinded to favour consumer acceptance. However, protein-rich ingredients, which can be used in various food preparations, are also very important, and their development would require a better knowledge of the intrinsic properties of these proteins and also to understand the behaviour of insect proteins during extraction and processing.

## 2.1. Insect processing and protein extraction

Protein extraction and fractionation are necessary steps to produce insect protein-rich ingredients. The protein concentration in the various insect species is usually very high (typically 50–70% of the dry matter), and this facilitates the isolation process.

Grinding of fresh insect is mechanically complicated, and it would result in the production of a slurry, which could be difficult to store and be further processed. Therefore, the first necessary processing step immediately after the insect harvesting is their drying. The conditions of the biomass preliminary treatment are critical: time/temperature can be adjusted according to the species and their initial water content. The damage of the tissue should be limited to avoid the contact of proteolytic and browning enzymes with their substrates. These enzymes are confined in specific organelles surrounded by membranes. The main objective of the drying step is to reduce the water content up to 5–10%. In this condition, the microbiological growth is ruled out, and the quality of the starting material can be better preserved. Fat oxidation is another main factor that could bring to quality decay during this step and during the following storage. In production, plants dedicated to insect protein production, drying and defatting conditions can be adjusted according to the need of the following steps.

In fact, for many purposes and especially to produce insect protein-rich ingredients, extraction of fats and grinding into small particles are essential prerequisite. Also when protein extraction is not the ultimate goal, it must be considered that the particle size of the final powder has a big impact on the technological properties of the insect-based ingredients. In particular grinding conditions will influence the:

- Dispersibility and solubility
- Water and lipid holding capacity
- Rheological performance when rehydrated

Therefore, a careful selection of the grinding conditions according to the species should be performed particularly when adult insect (such as crickets or grasshoppers) rich in chitins and with a structured exoskeleton must be processed.

Up to now mainly wet fractionation processes have been proposed in the literature, although in principle dry fractionation could potentially lead to a better quality, and it is also intrinsically more sustainable because you do not need to eliminate water after extractions.

Data on the amount of proteins that can be extracted by grinded insect powder in aqueous solutions showed an enormous variability [11]. Factors like solid/water ratio as well as the pH of the media and the temperature play an important role. Using alkaline pHs (i.e. far from the protein isoelectric point), higher extraction yield was obtained [12]. Also ionic strength, i.e. the presence of sodium chloride in the solution, can increase the solubility, and as above mentioned, the finer the granulometry, the higher the extraction yield. However, the most important factor affecting protein extractability is the thermal treatment insects undergo before extraction. When the biomass is heated before extraction, the yield of soluble protein extraction drops dramatically moving from about 50% of the total proteins below the 20%. **Figure 1** suggests that the denaturation of insect proteins decreases their water solubility in a similar way observed for meat proteins.

There are some good reasons to perform a thermal treatment before extraction. First of all, the higher the temperature, the faster is the drying step. Secondly, a treatment at high temperature can be used to prevent enzymatic browning. Enzymatic browning catalysed by polyphenol oxidases (PPOs) is a major phenomenon taking place during insect protein extraction on aqueous solutions [12]. The browning reaction has negative effects on proteins by affecting the solubility and other technological functionalities. Moreover, brown colour is generally not well accepted by consumers, and consequently brown ingredients are always more difficult to use in several food preparations. Browning can be prevented also with not thermal treatment adding chemical agent such as sodium bisulphite or ascorbic acid in the extraction buffer [12].

The design of an insect protein extraction and fractionation process could often result in a trade-off between yield and purity. A higher yield of the extraction always comes with a lower purity of the final product and *vice versa*. For instance, an aqueous extraction followed by an acidic precipitation step could bring to a fraction containing more than 85% of proteins, which is a remarkably high purity, but this comes at the expenses of a final yield below 20% [13].

In such a condition, it is useful to follow an approach driven by the ingredient final destination of use, i.e. when the final goal is to get the maximum nutrient value, it is logical to prioritise the protein yield. On the other hand, when specific techno-functionality of proteins is desired, such as gelling or foaming properties, it is better to elaborate strategies that allow to obtain more purified preparation also at the expenses of a low yield.

## 2.2. Insect protein techno-functional properties and their food applications

Unfortunately, due to lack of standardised fractionation procedure, studies about the technological functionality of insect proteins are still at their infancy. Looking at what happened first with the dairy proteins and later on with legumes proteins (soybean, pea, lupine), the capability to fractionate them in defined preparations opened many possibilities of use as ingredients. We can assume that also for insect proteins, their use in different foods will be mainly driven by the added value they can bring to specific food preparations.

The main techno-functional properties that should be considered for insect proteins are:

- Water and lipid holding capacity
- Thickening capacity
- Emulsification capacity
- Foaming capacity
- Gelation capacity
- Structuring capacity

Interestingly, all these properties depend on the nature of the starting material (for instance, the species and maturity stage of the insects); however, they can be also modulated by the extraction and fractionation process especially by heating and the consequent protein denaturation.



Looking at the properties that can be influenced by the hydration of the proteins (i.e. the water holding and the thickening capacities), they can be particularly useful to improve the quality of meat and bakery products or even to modify the sensory properties of thick beverages such as the electrolyte-rich solutions for sportsman or the satiating products. As far as the emulsification and foaming capacities, they are particularly interesting for drinkable dairy products, desserts and salad dressing, while the gelation and structuring capacities are relevant for products such as cheese and tofu.

These potential applications of various insect protein fractions in real food product should also take into account the potential negative effects associated to their use. Although the increase of protein concentration is often desired parameters, insect proteins are often brown or even dark. The colour change in the final products caused by protein addition could be perceived as a very negative sensory attribute especially in dairy products. On the other hand, in many bakery products or when insect proteins will be used as meat replacers, this is not expected to be a significant problem.

### **2.3. Nutritional and healthy properties**

A large part of the literature focused on the nutritional quality and the benefit related to the whole insect consumption. The attention is focused on proteins and the most important parameter is their total concentration. When measured on fresh weight basis, the insect protein concentration is within the range of that of the other animals, i.e. between 10 and 30%. So the real nutritional advantage of using insect proteins can be only understood when looking at the bigger picture as it was done by the consortium of the SUSFAN project. What makes the insect proteins particularly interesting from the nutritional point of view is not only their high concentration and the favourable amino acid composition but also their potential to meet all the so-called sustainable, healthy, affordable, reliable, palatable (SHARP) principles [14]. To have a 360 degree evaluation of the potentiality, the protein sources of the future should confront with these five criteria. In this respect, insects will perform very well.

In the previous paragraph, the reasons highlighting the sustainability, affordability, reliability and palatability of insect proteins have been discussed. Regarding the healthiness which includes also the nutritional properties, it is essential to say that insect proteins are rich in all essential amino acids. A diet based on insect proteins can perfectly sustain the harmonious growth of laboratory and husbandry animals also indicated that there are no major limiting or anti-nutritional factors affecting the growth of mice, fish and poultry [15].

Interestingly, insect proteins can be a good vehicle to increase mineral bioavailability. Insect contain much more iron, zinc and calcium than beef, pork and chicken [16]. As observed for the casein-calcium system in milk, it can be hypothesised that thanks to the interaction with specific peptides formed during insect protein digestion, these minerals can be more bioavailable than in protein-free food matrix.

Recent papers also confirmed that insect proteins are well digested during gastroduodenal digestion: *in vitro* data showed that the extent of the digestion depends also on the fraction of the insect proteins considered. In particular, the water soluble proteins are digested more efficiently than those remaining in the insoluble moiety [13]. No consisting data have

been still published about the effect of browning and thermal treatment on the insect protein digestibility. Both phenomena can have opposite effects: on one hand they determine protein aggregation and crosslinking which decrease protein digestibility; on the other they favour protein denaturation, thus improving their degradation.

In addition to their use as nutrients, proteins from different sources have emerged as precursors of specific peptides, which are now known to be bioactive. A wide range of biological properties have been demonstrated with protein-derived bioactive peptides including anti-hypertensive, immunomodulatory, antimicrobial and antioxidant. These effects can be relevant in human and animal health promotion and can also be applied in food preservation for extended shelf life. The production of bioactive peptides has relied heavily on the use of food proteins, which further contributes to the depletion of their primary food sources. Edible insects can also be sustainable sources of proteins for bioactive peptide production. The availability of the gene sequence of major proteins in several insects such as *Tenebrio molitor* or *Hermetia illucens* allows the theoretical calculations of the linear structure, conformation and biological significance of peptide motifs released by specific proteinases used in industrial food processing. Open-access web-based tools can be used to verify the presence of these potential bioactive peptides, and enzymatic and microbiological methodology can be used to generate these peptides from the different insect biomasses.

We could imagine that the same variety of products and applications now available for dairy or soybean proteins will be soon available also for insects.

### 3. Insect lipids

Lipids in insects are either obtained from the diet or de novo synthesised. These lipids are stored in the insects in the fat body, where lipids are stored, degraded, transformed and further transported to the site of utilisation [17, 18]. Lipids are used by insects for several physiological functions, among them for reproduction, development, flight, buoyancy, integumental waterproofing, communication via pheromones, structure of cell membranes, etc. [17, 19]. Insects are rich in lipids; its content ranges from 10 to up to 50% in dry basis [20]. The fat content and the fatty acid (FA) composition of insect are related to its species, sex, life stage, diet, environmental temperature, diapause and migratory flight [17, 21, 22]. Therefore, it is not strange to find a wide variability of FA profiles among insects.

Insect lipids are composed mainly of triacylglycerols. Other types of lipids present in minor amounts include cholesterol, partial glycerides, free fatty acids (FFA), phospholipids and wax esters [18, 23]. When insect lipids are extracted for its consumption as edible oils, the main types of lipids are triacylglycerols, which reflect the original composition of the lipids in the insect. The presence of other minor compounds in the lipid extraction greatly depends on the extraction process.

The extraction of insect lipids aiming its use as edible oils has been investigated using Soxhlet extraction, an aqueous method and supercritical CO<sub>2</sub> extraction [24, 25]. The lipid extraction process does not have a major impact in the FA composition of the extract,

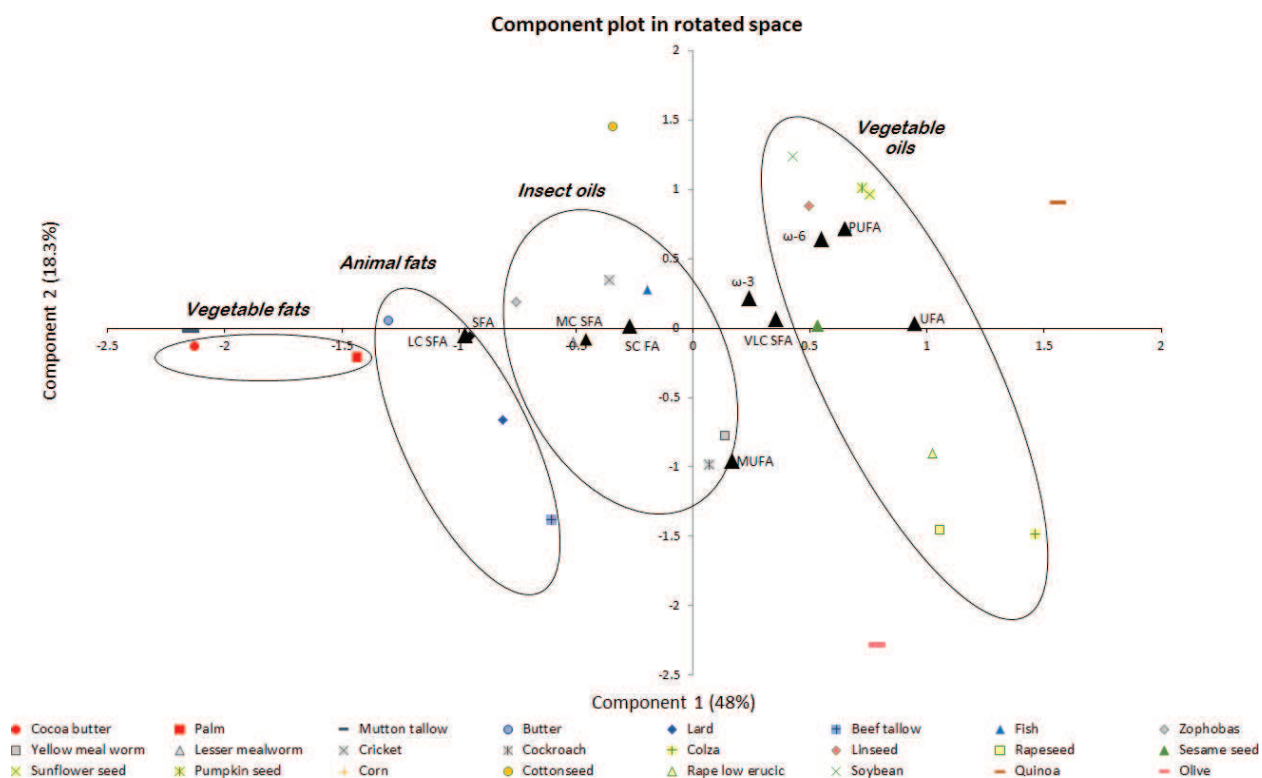


but it strongly influences the lipid extraction yield and the types of lipids extracted. For instance, when aqueous extraction is used, only triacylglycerols are extracted. In contrast, when organic solvents are used, phospholipids, partial glycerides and triacylglycerols are extracted [25]. Partial glycerides, FFA and phospholipids are undesired in edible oils and fats. To eliminate these undesired compounds, the fats and oils undergo refining processes, which increases the cost of the oils and fats. Insect lipid aqueous extraction provides a high oil quality similar to that of virgin oils. However the yield is lower than that obtained using organic solvents or supercritical CO<sub>2</sub> extraction in which more than 95% of the lipids are extracted [24, 25]. Therefore, the extraction process should be carefully selected according to desired application and the costs of each extraction process. Other method for insect lipid extraction with industrial potential is extrusion and expelling, which is a method commonly used in oil extraction of seeds. However, this method remains unexplored in the scientific literature.

Most insect lipids are liquid at room temperature (20°C); thus, they are called “insect oils.” Insect oils are rich in unsaturated fatty acids (UFA > 60% of total FA), being the most abundant C18:1 cis 9 (>30% of total FA) and C18:2 cis 9,12 (>20% of total FA). The most abundant saturated fatty acid (SFA) found in these oils is C16:0 (20–30% of total FA) [10, 26]. In general, insect oils are rich in essential fatty acids such as C18:2 cis 9,12 (LA) and contain other FA associated to health benefits such as C18:3 cis 9,12,15 (ALA) and  $\omega$ -3 FA, which further points its nutritional value. The concentration of these FA varies among species [21], and it is strongly influenced by the insect diet. Studies showed that the insects caught in the wild are richer in  $\omega$ -3 than those commercially reared.

There are a few known insect lipids that are solid at room temperature, such as the lipids extracted from black soldier fly larvae (*H. illucens*). In this case, they are called “insect fats.” The solid state of this insect fat reflects its high content in saturated FA, which ranges from 57 to 75% of total FA. The most abundant SFA in this fat are C12:0 ( $\approx$ 45% of total FA), C14:0 ( $\approx$ 9% of total FA) and C16:0 ( $\approx$ 12% of total FA) [22, 27]. This insect fat is especially interesting for food and feed applications since it is rich in C12:0, with a concentration similar to that of coconut oil. C12:0 is more water soluble and is readily digestible than long-chain FAs. Moreover, after digestion C12:0 is transported to the liver and is immediately converted to energy; thus, it is not stored as fat [28]. Other properties related to lauric acid and monolaurin are antimicrobial activity against gram-positive bacteria and a number of fungi and viruses [28, 29]. Therefore, the fat from black soldier fly larvae could be used as a functional ingredient in food applications.

In **Figure 1**, we show the spatial arrangement of vegetable oils and fats, animal fats and insect oils. This plot was obtained using the FA composition of different fats and oils and was analysed using principal component analysis. When the FA of insect oils are compared with that of vegetable oils, vegetable fats and animal fats, it appears that insect oils have a FA profile similar to that of vegetable oils and animal fats. This is due to the increase content of SFA, namely, C16:0 and C18:0, and at the same time a high content of mono- and polyunsaturated FA, namely, unsaturated C18 FA. Saturated C18 and C16 FA are increased in most animal fats, whereas unsaturated C18 FA are typical of vegetable oils.



**Figure 1.** The first and second principal components of the principal component analysis. The amount of explained variance is provided in parenthesis in each axis. Fatty acids per group; LC SFA, long-chain saturated fatty acids; SFA, saturated fatty acids; MC SFA, middle-chain saturated fatty acids; SC FA, short-chain fatty acids; MUFA monounsaturated fatty acids; VLC SFA, very-long-chain saturated fatty acids; PUFA, polyunsaturated fatty acids; and UFA, unsaturated fatty acids. Source: Refs. [25, 30, 31] and the authors.

### 3.1. Potential food uses of insect oils

The liquid nature of insect oils makes them ideal for its use in mayonnaise, in vinaigrettes, as frying oils and as food grade lubricants, among others. Applications such as bakery, spreads, or confectionery are ideal for insect fats, such as the fat extracted from black soldier fly larvae, because these applications require a certain percentage of solid fat. In order to evaluate the specific applications of these oils, a detailed chemical and physical characterisation of the oils is necessary. Ekpo et al. [32] performed detailed chemical analysis in the extracted oil of four insect species consumed in Nigeria. They concluded that these insect oils had potential used in the pharmaceutical industry due to their low melting point, specific gravity (<0.89) and refractive index (<1.3) [32]. Other insect application oils remain unexplored in the scientific literature.

Traditionally, insect oils and fats have only being characterised in terms of its FA composition, mainly because this determines the nutritional value of fats and oils. However, other chemical and physical analyses are required in order to evaluate its potential applications as food ingredient. For instance, crystallisation, texture analysis and solid fat content at different temperatures are required to evaluate the use of a fat as a spread, in confectionery or its use as an ingredient in bakery. Rheological properties help to determine spreadability, ease of cutting and stand-up in margarines. Volatile compound analysis and sensory evaluations are required to assess its taste, mouth feel and aroma. Detailed triacylglycerol profiling and

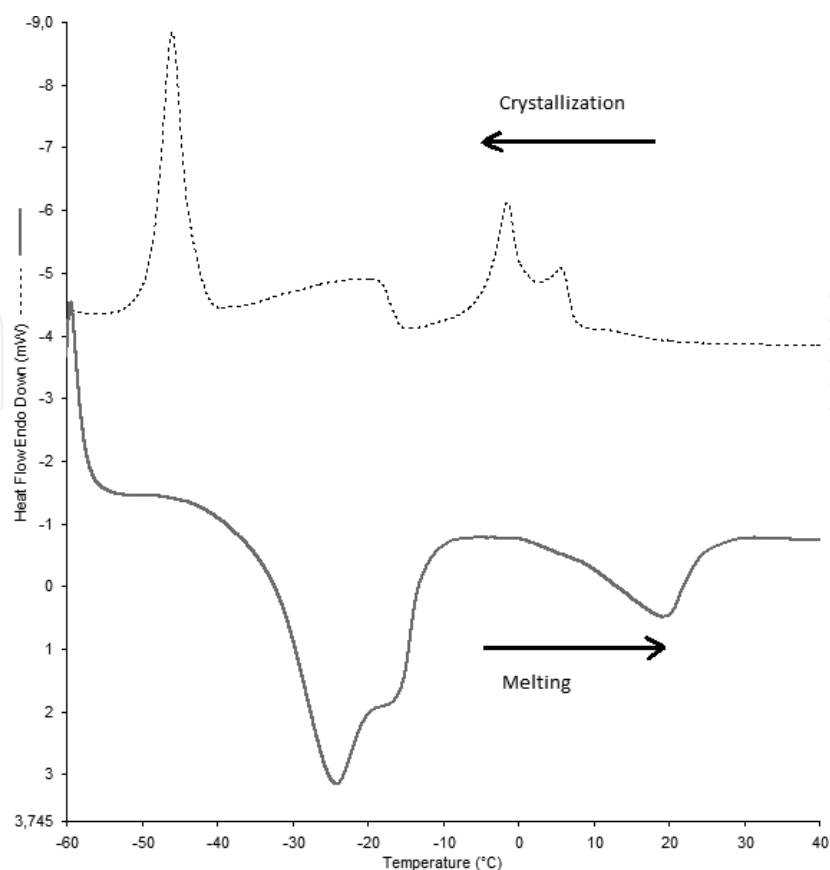
thermal behaviour analysis are required to evaluate the possibilities of fractionation of an insect fat or oil. Profiling of minor lipid compounds such as phospholipids and sterols help to fully assess its nutritional value. Currently, none of these information is available. However, information on the physical and chemical characteristics of insect fats and oils will become available as the attention of the consumers and the food industry for insect lipids increases.

### 3.2. Fractionation of an insect oil

Dry fractionation is a thermal procedure use to separate oils and fats into two or more components with different melting points. The aim of this separation is to extend the range of applications of the original oil or fat as well as to increase its commercial value. Fractionation is applied mainly to palm oil but also to coconut, palm kernel, butter oil and beef tallow, among others. Dry fractionation can be applied to insect oils showing different melting fractions. To show the possibilities of this process in insect oils, we applied dry fractionation to oil extracted from yellow mealworm (*T. molitor*). Then we performed physical and chemical analysis to the original oil and its fractions.

The first step of this study included a thermal analysis of the original oil. This analysis was performed to study the possibilities for fractionation of the yellow mealworm oil. In this analysis, the oil was heated to 70°C to eliminate all the crystals present in the oil; then the oil was cooled at a rate of 5°C/min from 70 to -60°C. In this step, the oil is crystallised and the crystallisation points of the fat are obtained. Then, the fat was melted again at 5°C/min from -60 to 70°C. In this step, all the formed crystals are melted and the melting points are obtained. The results of yellow mealworm oil showed four crystallisation points and three melting points (**Figure 2**). Each peak indicates the crystallisation or melting of crystals formed by triacylglycerols with similar structure. Therefore, when more than one crystallisation/melting point is obtained, it indicates that the triacylglycerols in the oil are crystallising/melting independently. This is because the structure and/or FA within the triacylglycerols are different and they cannot co-crystallise into one single crystal lattice. The results from this first thermal analysis showed that the yellow mealworm oil can be fractionated because several crystallisation and melting peaks were obtained and these peaks were clearly separated from each other.

In the second step of this study, we aimed to fractionate yellow mealworm oil into a high melting fraction (or stearin) and a low melting fraction (or olein). We applied two cooling temperatures, namely, 2 and 4°C, and then the solid and the liquid fractions were separated. These temperatures were selected because in the previous analysis, it was shown that the highest crystallisation point was 3.6°C. The first step of dry fractionation was to melt the fat at 60°C for 30 min to eliminate the crystals present. Then, the oil was placed in a water bath, at the crystallisation temperature (2, or 4°C) for 24 h. During this crystallisation process, only triacylglycerols capable of crystallising at this temperature nucleate and grow into crystals (solid fat); the rest of the triacylglycerols having lower crystallisation temperatures will remain liquid (liquid fat). Finally, the liquid fraction was separated from the solid fraction by centrifugation at 4800 g for 20 min at the cooling temperature. Both fractions were placed in a separated tube and weighted to obtain the fractionation yield. The physical (thermal analysis and colour) and chemical characteristics (FA composition) of the original oil and the obtained solid and liquid fractions were performed to assess the effect of fractionation in the oil.



**Figure 2.** Non-isothermal crystallisation and melting of unfractionated yellow mealworm oil. Cooled from 70 to  $-60^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$  and heated from  $-60$  to  $70^{\circ}\text{C}$  at the same rate.

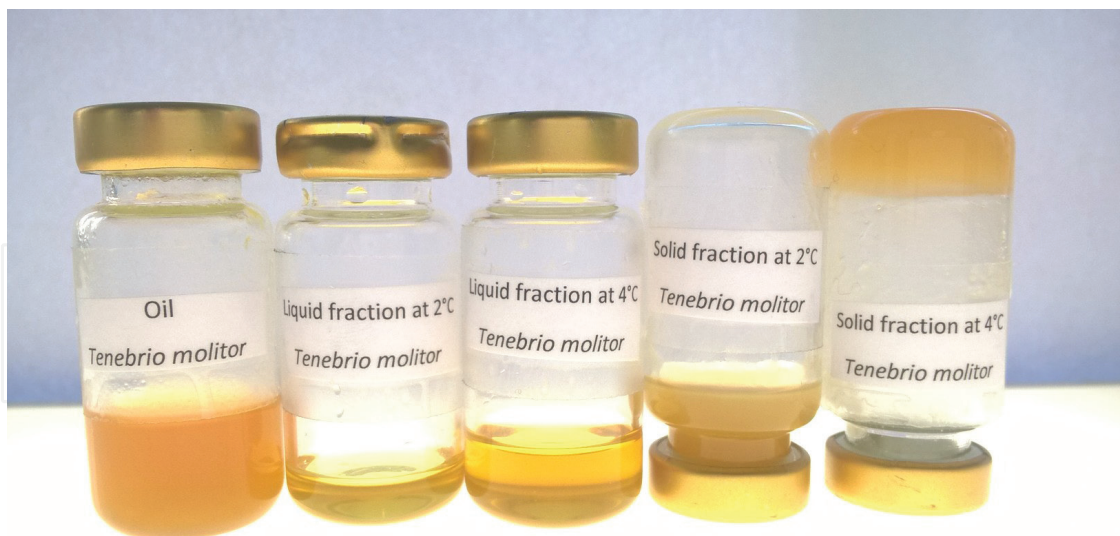
### 3.2.1. Physical characteristics of the yellow mealworm oil and its fractions

Separation into solid and liquid fat was possible at 2 and  $4^{\circ}\text{C}$ . Fractionation changed the colour of the liquid and solid fractions as compared with the unfractionated oil as analysed by Hunter Lab colorimeter in  $L^*a^*b^*$  scale (**Table 2**). In general, the solid fractions had a more red-yellow colour when compared with the liquid fractions. The liquid fraction had a bright appearance, and the red and yellow tones were lower in the liquid oil fractionated at  $2^{\circ}\text{C}$  than oil separated at  $4^{\circ}\text{C}$  (**Figure 3**). As expected, the amount of liquid fat fraction increased as the crystallisation temperature increased (**Table 3**).

Sample	$L^*$	$a^*$	$b^*$
Unfractionated oil	15.72	1.35	12.86
Solid fraction $4^{\circ}\text{C}$	34.27	3.26	25.33
Solid fraction $2^{\circ}\text{C}$	21.01	3.14	18.97
Liquid fraction $4^{\circ}\text{C}$	5.12	0.15	2.81
Liquid fraction $2^{\circ}\text{C}$	4.40	-0.56	1.74

$L^*a^*b^*$  scale was used. The data shown are the average of two repetitions. Taken from [33]

**Table 2.** Colour of unfractionated and fractionated yellow mealworm oil measured at  $20^{\circ}\text{C}$ .



**Figure 3.** Unfractionated and fractionated yellow meal worm oil (*Tenebrio molitor*). This picture was taken after stabilising the oil for 2 h at 24°C.

The unfractionated oil of yellow mealworm showed four crystallisation points at  $-45.4$ ,  $-19.9$ ,  $-5$  and  $3.6^{\circ}\text{C}$  and three melting points at  $-24.7$ ,  $-17.4$  and  $16.7^{\circ}\text{C}$  (Table 3). The liquid fractions lack the two highest crystallisation points at  $-5$  and at  $3.6^{\circ}\text{C}$ , and the last melting point was reduced from  $16.7$  to  $1.7$  and  $3.2^{\circ}\text{C}$  (L4 and L2, respectively). The solid fractions lack the crystallisation point at  $-5^{\circ}\text{C}$  and showed an increase in the highest crystallisation points from  $3.6$  to up to  $10.7^{\circ}\text{C}$  (S4 and S2, respectively). The final melting point of the solid fractions was increased from  $16.7$  to  $21.5$  and  $27.1^{\circ}\text{C}$  (S2 and S4, respectively).

Sample	Yield (%)	Crystallisation points ( $^{\circ}\text{C}$ )				Melting points ( $^{\circ}\text{C}$ )		
YMW		$-45.4$	$-19.9$	$-5.0$	$3.6$	$-24.7$	$-17.4$	$16.7$
S4	54	$-46.3$	$-19.3$	$10.7$	–	$-24.4$	$-18.0$	$27.1$
S2	81	$-46.3$	$-19.8$	$2.7$	–	$-24.4$	$-16.9$	$21.5$
L4	46	$-49.4$	$-19.7$	–	–	$-25.5$	$-18.3$	$1.7$
L2	19	$-44.7$	$-19.5$	–	–	$-24.3$	$-17.4$	$3.2$

Non-isothermal analysis cooled from  $70$  to  $-60^{\circ}\text{C}$  and then heated from  $-60$  to  $70^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$ .

YMW: original yellow meal worm oil extracted by Soxhlet using petroleum ether

S4, solid fraction at  $4^{\circ}\text{C}$ ; S2, solid fraction at  $2^{\circ}\text{C}$ ; L4, liquid fraction at  $4^{\circ}\text{C}$ ; and L2, Liquid fraction at  $2^{\circ}\text{C}$

Adapted from Ref. [33]

**Table 3.** Yield, crystallisation and melting points of unfractionated and fractionated yellow meal worm oil (N = 2).



After fractionation the solid and the liquid fraction did show differences in their physical properties, as shown by the change in colour and by the changes in crystallisation and melting points. The liquid fractions obtained after fractionation had bright and transparent appearance and were liquid even at refrigeration temperatures (4°C). Therefore, these liquid fractions can be used for sauces, dressings, vinaigrettes, mayonnaise, etc. Regarding the solid fractions obtained, the solid fraction obtained after fractionation at 4°C was solid when it was kept at room temperature (24°C). This solid fraction can be potentially used as margarine. Further studies on crystal form ( $\alpha$ ,  $\beta'$  and  $\beta$ ) are necessary to fully assess the use of this solid fraction as a margarine. To produce high-quality spreads, such as margarines, the crystals should be in the  $\beta'$  form because these crystals are relatively small and can incorporate large volumes of oil; moreover,  $\beta'$  crystals give the product a glossy surface and a smooth lustre [34, 35]. In contrast,  $\beta$  crystals are less desirable in spreads because they grow into large needle-like crystals producing a sandy mouthfeel and are less able to incorporate liquid [36].

### 3.2.2. Chemical characteristics of the yellow mealworm oil and its fractions

The FA composition of the unfractionated oil and its fraction was determined by GC-FID. Similar FA composition of the unfractionated oil was found in previous studies done in our laboratory [25]. It is interesting that the FA composition between the unfractionated oil and its fractions did not change (**Table 4**). The solid fat separated at 4°C was highly unsaturated (74% of FA). In theory, this fraction should have remained liquid. However the experimental results showed a different behaviour. Several possibilities exist to explain this behaviour. It is likely that the arrangement of FA within the triacylglycerol molecule is different in the liquid than in the solid fat, if the arrangement differs, so will the physical properties of the triacylglycerol. The second possibility is that the solid fraction contains a compound that is structuring the oil and turning it into a solid-like material. It is known that natural waxes and partial glycerides are used to structure highly unsaturated oils, forming oleogels [37]. Waxes and partial glycerides are present in insect tissues and in the oil fraction [18, 25] and could remain in the solid fraction after fractionation. Therefore, it can be suggested that these two components can be structuring the solid fraction of the yellow mealworm oil and turning it into a solid-like material or oleogel.

The differences seen in physical properties of the fractions cannot be explained by its FA composition. To understand the differences in physical properties seen in this study, it will be necessary to analyse the profile and structure of the triacylglycerols as well as to analyse the concentration of other minor compounds such as waxes.

The fractionation process presented in the present study succeeded in changing the physical properties of yellow mealworm oil. This fractionation can broaden the applications of this oil as a food ingredient. However, further physical, chemical and sensory analyses are necessary to fully assess the potential use of yellow mealworm oil as a food ingredient.



FA	Unfractionated oil	Fractionated			
		Solid 2 °C	Solid 4 °C	Liquid 2 °C	Liquid 4 °C
Total CLA	<0.10	<0.10	0.13	0.14	<0.10
Total $\omega$ -3	1.68	1.66	1.65	1.69	1.71
Total $\omega$ -6	30.96	30.63	30.75	31.19	31.18
$\omega$ -6/ $\omega$ -3 Ratio	18.43	18.45	18.64	18.46	18.23
Total SFA	21.35	21.05	21.80	21.85	21.00
Total UFA	73.98	73.36	73.28	74.72	74.70
Total MUFA	41.23	40.87	40.70	41.73	41.70
Total PUFA	32.71	32.45	32.54	32.94	32.96

Adapted from Ref. [33]

**Table 4.** Fatty acid composition (g/100 g of fat) of unfractionated and fractionated yellow meal worm oil (N = 2).

## 4. Concluding remarks

Insects represent a viable source of proteins and fats since they have a low environmental cost of production. However, its effective inclusion in food preparations or as a food ingredient relies on several factors including consumer's acceptance, rearing facilities and technological functionalities. A lot of work is needed regarding protein and fat extraction methods as well as on technological functionality of insect proteins and fats. Understanding the behaviour of insect proteins and fats during extraction and processing as well as better knowledge of the intrinsic properties of insect ingredients is required for the development of various food preparations. It is very likely that in the next few years, the scenario will be significantly different as there is an increase attention of the consumers and the food industry for the use of insects as food.

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