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REVIEW

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New lipid sources in the insect industry, regulatory aspects and applications☆

Bénédicte Lorrette* and Lorena Sanchez

Ynsect, 1 rue Pierre Fontaine, 91058 Evry, France

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Abstract – Edible insects constitute a sustainable and alternative source of nutrients: they have potential to become a valuable protein source for addressing animal and human markets addressing part of the global food demand. After protein, the second largest fraction of the insect is constituted of lipids. Lipids can represent 10 to 15% of the insect in dry matter, making this fraction one of the major co-products of insect industry. The composition of the insect fat may change in terms of quantity and composition of fatty acids profile. Insect species, stage of growth, extraction technologies are some parameters that can impact the fat quality. Many applications of insect fat are naturally focusing on animal nutrition to replace vegetal or fish oil in poultry or aqua feed. Health or human food applications are also envisaged to replace vegetal oil or butter in processed foods, and some technical and sensory tests are reported in this review. However, these last applications, concerning food are submitted to the regulation and especially to the Novel Food EU regulation. For these reasons, it is important to have more data about safety and innocuity of insect fats: a first study is dealing with this aspect, showing an absence of toxicity. Finally, some energy or surfactant applications can also be considered.

Keywords: insect / fat / oil / regulation / applications

Résumé – Nouvelles sources de lipides dans l'industrie des insectes, aspects réglementaires et applications. Les insectes constituent une source durable et alternative de nutriments : ils ont le potentiel de devenir une source de protéines précieuse pour répondre aux besoins des animaux, des humains et plus généralement de la demande alimentaire mondiale. Après les protéines, la deuxième plus grande partie de l'insecte est constituée de lipides. Ils peuvent représenter 10 à 15 % de l'insecte en matière sèche, ce qui en fait l'un des principaux coproduits de l'industrie des insectes. La composition de la matière grasse d'insecte peut changer en termes de quantité et de composition en acides gras. L'espèce d'insecte, le stade de croissance, les technologies d'extraction sont autant de paramètres qui peuvent avoir un impact sur la qualité de la matière grasse. De nombreuses applications des lipides issus d'insecte sont naturellement axées sur la nutrition animale pour remplacer les huiles végétales ou de poisson dans l'alimentation des volailles ou des animaux aquatiques. Des applications dans le domaine de la santé ou de l'alimentation humaine sont également envisagées pour remplacer l'huile ou le beurre dans certains produits alimentaires transformés, à travers des tests techniques et sensoriels. Cependant, ces dernières applications, concernant l'alimentation, sont soumises à la réglementation et notamment au règlement européen sur les nouveaux aliments « Novel Food ». Pour ces raisons, il est important d'avoir plus de données sur la sécurité et l'innocuité des graisses d'insectes : une première étude traite de cet aspect, montrant une absence de toxicité. Enfin, certaines applications énergétiques ou tensioactives peuvent être envisagées.

Mots clés : insecte / graisse / huile / réglementation / applications

☆ Contribution to the Topical Issue "Bioactive lipids and lipid droplets: green ressources for food and health / Lipides et gouttelettes lipidiques bioactifs : des ressources vertes pour l'alimentation et la santé".

*Correspondence: benedicte.lorrette@ynsect.com

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1 Introduction

The production of insects is promising from the perspective of novel and sustainable protein production. They are already well recognized as a very interesting alternative source of protein for addressing the global food demand, and more especially for animal and human nutrition. Most insect species have high levels of high-quality protein. The production forecast of IPIFF (International Platform of Insects for Food and Feed) indicates that insect sector may reach one million tons of insect meal by 2030 in Europe (IPIFF, 2021). Among edible insects, mealworms are the most commonly reared species in Europe. For example, the larvae of *Tenebrio molitor* contain 60% protein of dry matter. Their content of essential amino acids (isoleucine, leucine, lysine) (Ravzanaadii *et al.*, 2012) is high in comparison to proteins of plant origin, which also makes them a source of interest in the search for alternative protein to cope with the increasing world population. Insects can be used as ingredients in feed or food, such as protein powder to substitute other sources of protein for both markets.

After the protein fraction, the second largest part of the insect consists in lipids. Depending on the insect species, the content can vary from 10 to 15% in dry weight (*Chorthippus parallelus*, *Conocephalus discolor*, *Acheta Domesticus*) to more than 30% for *Tenebrio molitor* larvae (Aman *et al.*, 2017). Lipids represent the major co-product of insect protein meal industry (excluding frass, insect excrement). It may also be utilized as a substitution ingredient of other fat sources in feed or food applications. Despite this potential, the valorization of insect lipids at an industrial scale is today poorly documented and unlike insect meal, production volume forecasts of insect lipids are not available. The aim of this article is to review the current knowledge about the lipid composition of insects but also their potential applications regarding regulatory and safety aspects, with a special focus on *Tenebrio molitor* and *Hermetia illucens* (Black solider fly). Both of them are the most studied and documented insect species, as it is observed from the numbers of published articles in bibliography databases (Van Huis, 2020). Both of them are also the most widely reared insects for feed and/or food in the western world.

2 Composition of insect fat and sources of variations

Insects are the largest and most diverse group of organisms on Earth and the composition and proportion of fat is very different throughout species.

An extensive compilation of the nutritional content of edible insects, including their lipid content, was done by Rumpold and Schlüter (2013). In Table 1, lipid mean values based on dry matter were classified by insect order, the range of values for each order are presented as well. Beetles and termites are among the groups with the highest mean lipid values; while crickets, grasshoppers, locusts have the lowest mean values.

The lipid content of four insect species was investigated and variability in lipid content from 10.21% (expressed as dry matter) for *Chorhtippus parallelus* adults (Orthoptera) to 31.97% for *Tenebrio molitor* larvae (Coleoptera) was showed (Aman *et al.*, 2017). In the same way, a comparative study on the extraction of lipid fractions from four insect species, by different methods, underlined the variability of fat content between insect species (Tzompa-Sosa *et al.*, 2014). The authors concluded that *Tenebrio molitor* contained the highest amount of lipids among the four following insect species, *T. molitor*, *A. diaperinus*, *A. domesticus* and *B. dubia*.

As observed from the broad lipid value ranges, the lipid content of insects varies with individual species, but also with their stage of growth, their environmental conditions and their diets specifications. Regarding *Tenebrio molitor*, an increase of fat content is observed throughout its growth cycle. Starting from 6.6% of lipid (fresh matter) for young larvae (mass around 23 mg), it increases to 11.7% for older larvae (154 mg size) (Fig. 1, from internal data from Ynsect); while at adult stage, the fat content decreases to 6.2% (fresh matter).

Insect lipids are mainly composed of triacylglycerols. Other types of lipids present in minor amounts include cholesterol, partial glycerides, free fatty acids (FFA), phospholipids and wax esters (Tzompa-Sosa and Fogliano, 2017). Most insect lipids are liquid at room temperatures (20 °C); thus they are called "insect oils". That's the case for *Tenebrio molitor* where its fat consists in a light-yellow oil of very good quality after a mechanical extraction (Fig. 2). The peroxide value, first oxidation marker, and the oleic acidity (marker of an aqueous or enzymatic degradation of oil) of this crude oil are very low (2–3 meq O_2/kg and 0.25% oleic acid, respectively), underlining its very good quality. The liquid state is linked to the nature of the fatty acid profile of this oil, rich in unsaturated fatty acids. The fatty acid profile of *Tenebrio molitor* oil is represented in the Figure 3 (internal data from Ynsect). It presents a considerable concentration of unsaturated fatty acids, which confer high nutritional value to this ingredient. The main fatty acid is oleic acid (C18:1ω9, 44%) (monounsaturated fatty acid) followed by linoleic acid (C18:2ω6, 28%) (polyunsaturated fatty acid) and palmitic acid (C16:0, 18%) (saturated fatty acid). The composition of this oil is very similar to the one of some vegetal oils (especially rice bran and peanut oils) (Orsavova *et al.*, 2015). These internal data are in good accordance with a previous study (Aman *et al.*, 2017) in which the authors showed that oleic and linoleic acid were the main fatty acid components of *Tenebrio molitor* larvae but also of *Acheta domesticus* lipids. For *Chorthippus Parallelus*, α-linolenic acid (C18:ω3, 39%) formed the major lipid component, which was followed by oleic acid and linoleic acid.

Insect feeding can also change the nutritive values of insect and especially of insect fats (Oonincx and Finke, 2021). The addition of flaxseed in *Galleria mellonella* feed increased the ω3 fatty acid content from 2 to 14.8% and the ω6 fatty acid value from 9.9 to 12.6%. Larvae fed with flaxseed showed a ω6/ω3 ratio optimal for human health and total PUFA incremented from 9.5 to 25.5% (Francardi *et al.*, 2017). The enrichment in specific fatty acid profile through feeding was also observed on *Tenebrio molitor* and *Hermetia illucens* larvae (Lawal *et al.* 2021). By supplementing a basal diet with specific seed meals, the authors also showed that it was possible to increase ω-3 fatty acid and to obtain a ω-6/ω-3 ratio closer to that recommended for healthy diet.

Table 1 . Lipid composition (%) of edible insects (based on dry matter).

Insect order	Common name	Lipid content % (value range)
Coleoptera	Beetles, grubs	33.4% (5.4–69.8)
Isoptera	Termites	32.7% (21.3–46.1)
Hemiptera	Tue bugs	30.3% (4–57.3)
Blattodea	Cockroaches	29.9% (27.3–34.2)
Lepidoptera	Butterflies, moths	27.7% (7–77.2)
Hymenoptera	Ants, bees	25.1% (5.8–62)
Diptera	Flies	22.8% (11.9–35.9)
Odonata	Dragonflies, damselflies	19.8% (16.7–22.9)
Orthoptera	Crickets, grasshoppers, locusts	13.4% (3.5–48.2)

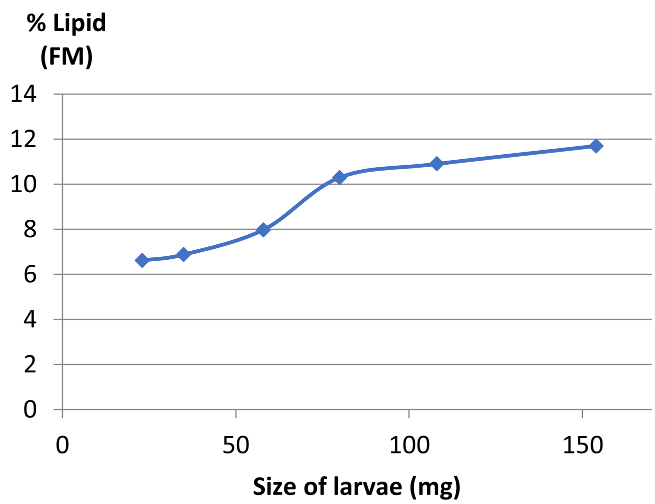

Fig. 1. Evolution of lipid content (expressed as Fresh matter) during the growth of *Tenebrio molitor larvae* (internal Ynsect R&D data).

Fig. 2. Picture of *Tenebrio molitor* oil.

There are a few known insect lipids that are solid at room temperature, such as the lipids extracted from black soldier fly larvae (*Hermetia illucens*). In this case, they are called insect "fat". The solid state of this insect fat reflects its high content in saturated fatty acids, which ranges from 57 to 75% of total fatty acids. The more abundant saturated fatty acids in this fat are lauric acid (C12:0) representing around 45%, myristic acid (C14:0), around 9% and palmitic acid C16:0 ($\approx$ 12 of total fatty acids) (Tzompa-Sosa and Fogliano, 2017).

The quality of insect fat also depends also on the processing method and extraction technology. Tzompa-Sosa and Fogliano underlined that the extraction process should be carefully selected according to desired application and the costs of each extraction process (Tzompa-Sosa and Fogliano, 2017). They reviewed and compared some Soxhlet extraction, an aqueous method and supercritical CO_2 extractions. These processes have not a major impact in the fatty acid composition but strongly influence the lipid extraction yield and the types of extracted lipids. In a case of aqueous extraction, only triacylglycerols were extracted in contrast with organic solvents extracting phospholipids, partial glycerides and triacylglyceride.

3 Regulatory aspects for feed and food applications

Insect, as a food or feed source, represent emerging protein and lipid sources. The growth of the insect industry is somewhat restricted due to outdated food and feed regulations covering insect use (Lahteenmaki-Uutela *et al.*, 2021). In this section, a focus is done on legal and specific regulatory rules for Europe. This part does not address the subject of the use of insects for technical uses (*e.g.*, biofuel production, cosmetic, biochemistry).

Seven insect species are authorized for the production of processed animal proteins intended as feed for farmed animals within the Europe, according to the Regulation (EU) No 2017/893. This act amended Regulations (EC) No 999/2001 and (EU) No 142/2011 defining the list of authorized species, including, *Grillodes sigillatus*, a tropical house cricket, *Gryllus assimilis*, a Jamaican field cricket, *Acheta domesticus*, a house cricket, *Tenebrio molitor*, common in Europe as a pest of the grain storages, *Alphitobius diaperinus*, also known as lesser mealworm, *Hermetia Illucens*, the black soldier fly, *Musca Domestica*, a house fly. It is important to add that an eighth specie, *Bombyx mori*, a silkworm was recently authorized. Moreover, the substrates on which insects may be reared (mainly of vegetal origin) are strictly defined in regulation (Lahteenmaki-Uutela *et al.*, 2021). This list of authorized species can also be applied to the

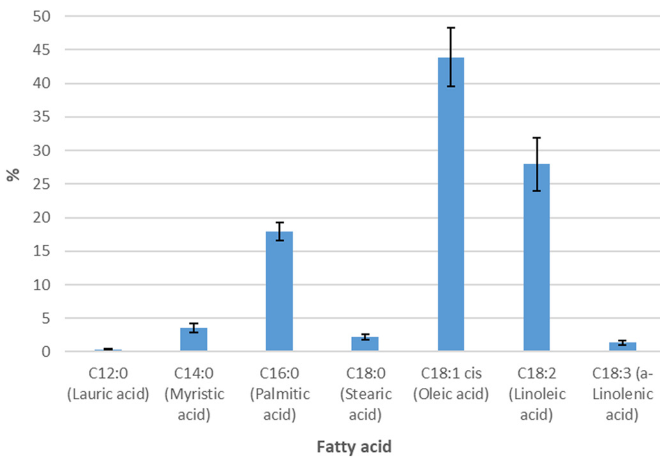

Fig. 3. Main fatty acids present in *Tenebrio molitor* Oil (internal Ynsect R&D data).

lipid fraction, since it is a side product of the protein production from insect processing.

Contrary to the use of protein from insects, which was first very restricted by legislation (Lahteenmaki-Uutela *et al.*, 2021), fat and oil from insects have always been very accessible on the feed market. They fall within the definition of animal fat as "product composed of fat from land animals, including invertebrates other than species pathogenic to humans and animals in all their life stages" (Regulation (EU) No 68/2013). By consequence, for the feed applications, they can fully address all the feed markets such as aquaculture, pet food, poultry, pigs, and other animals' nutrition, without any justification to EU authorities.

For human consumption, insects must be approved as a Novel Food under regulation (EU) No 2015/2283. Under this new regulation, insect food products may only be marketed when authorized after a safety assessment by the European Food Safety Authority (EFSA). At the time of the present document's writing, "only" dried *Tenebrio molitor* larvae can be legally placed on the EU market, following its authorization as "novel food" pursuant to Commission Implementing Regulation (EU) 2021/882. The conditions under which the product may be commercialized (*e.g.*, forms under which dried *Tenebrio molitor* larvae may be marketed, food categories in which it may be incorporated as an ingredient, applicable maximum limits, labelling requirements, maximum thresholds for chemical and microbial contaminants) are being defined in the above regulation. Two other positive safety assessments have been recently given by EFSA to crickets and frozen mealworm and many others applications for insect proteins have been submitted and are waiting for the EFSA agreement. The same requirements are expected for insect lipids as a Novel Food product even if the composition of the insect oil and fat are very similar to the one of some plant oils. To orient the use of insect fat towards human consumption, the deposit of a novel food file is also mandatory to justify its safety, innocuity and the harmlessness of the fat. Thanks to their composition, their oxidative stability, and safety aspects (discussed in the following section), this authorization should be reached, in the near future, for insect fat.

4 Safety aspects

Like products from other biological sources, insect derived food and feed ingredients, including oil, may contain microbiological or chemical hazards. The presence of these microbiological (*e.g.*, bacteria, fungi) and chemical hazards (*e.g.*, heavy metals, mycotoxins) have to be quantified and evaluated for safety concerns to be able to obtain regulatory approval and commercialize the product (EFSA, 2015).

Toxicity studies might also be required, in which a tiered approach has been suggested by EFSA. It integrates the core areas of kinetics, genotoxicity, repeated dose toxicity testing (subchronic, chronic toxicity and carcinogenicity) and reproductive and developmental toxicity (EFSA, 2016).

Consumption studies in the targeted species are another approach to evaluating the safety of the ingredients.

Freel, *et al.* studied the safety of black soldier fly larvae (BSFL) oil in dogs in a 28-day study. Three insect oil inclusions were tested: 1, 2.5 and 5%. Blood parameters, indicative of canine health, were maintained in normal ranges for the duration of the trial. There were no significant differences either in body weight, food consumption and stool quality between treatment groups ($p<0.05$) and the control. The ensemble of these results indicates that the general health of the dogs was maintained throughout the study without any safety concerns up to an inclusion of 5% of BSFL oil.

In the perspective of human consumption of insect lipids, studies to determine the safe doses to consume these edible insects are necessary. This was the purpose of a study dealing with the *Tenebrio molitor* (TM) and *Pachymerus nucleorum* (PN) oil toxicity (Alves *et al.*, 2019). Using rats' models through a single oral administration (acute toxicity) and 28 consecutive daily administrations (subacute toxicity), this first work on lipid toxicity demonstrated a considerable reduction in cholesterol and glucose levels of the treated rats. Acute and subacute toxicology experiments suggested that the TM and PN oils had low toxicity since it did not cause any lethality as well as no changes in hematological parameters.

For the moment, both articles are the only studies found in the literature focused on assessing safety consumption in animals.

5 Perspectives and applications

Due to their composition, different applications can be foreseen for oil and fat extracted from insects. Some promising applications naturally concern feed and food markets. Energy, surfactants and therapeutic applications may also be considered as technical uses.

5.1 Feed applications

The quality of insect's lipids is comparable to already commonly used energy sources in animal production. Soybean oil is one of the most used energy source ingredients in poultry diets. The effects of replacing soybean oil with selected insect lipids, *Tenebrio molitor* and *Zophobas morio* oil were investigated on broiler performance (Kieronczyk *et al.*, 2018). Researchers showed that a complete replacement of soybean oil was possible, with no impact on growth performance. Insect oil positively affects broiler digestibility, and improved meat quality by fatty acid profile. A positive effect of insect oil addition to the basal diet was also observed on selected gene expression in the liver. Other studies have come to the same conclusions, regarding the total replacement of palm oil and poultry fat by *Tenebrio molitor* oil in broiler chicken diets (Benzertiha *et al.*, 2019). *Tenebrio molitor* oil did not show any adverse impacts on performance and improved the fatty acid profiles of liver and breast muscle tissues with an increase in ω3 and ω6 fatty acids. The effects of partial or total replacement of soybean oil with *Hermetia illucens* (HI) larvae fat were also assessed on the growth performance, carcass traits, blood parameters, intestinal morphology and histological features of broiler chicken (Schiavone *et al.*, 2018). Half or total replacement of soybean oil with HI larva fat has showed no adverse effects on the different parameters and no beneficial effect was observed on gut health.

Another study on the total replacement of soybean oil by *Tenebrio molitor* and black soldier fly fats was performed on rabbits (Dabbou *et al.*, 2020). The authors showed the potential of these insect lipids as an antibacterial feed ingredient with a positive influence on the rabbit cecal microbiota.

It is already known that insects are an alternative protein source for fish diets with improvement of growth and immunity functions (Henry *et al.*, 2015; Henry *et al.*, 2018; Motte *et al.*, 2019). Insect fats and oils also demonstrated potential for applications in the aquaculture field. It was the case for the black soldier fly fat to substitute fish oil (Hender *et al.*, 2021). The authors showed that the substitution was possible in the diets of barramundi with no deleterious effects on the biological performance and flesh quality. In another review, the use of Lepidoptera such as domesticated silkworm as fish oil substitute was reported (Henry *et al.*, 2015). Silkworm pupa oil showed benefits in attracting, stimulating the appetite, growth, and organoleptic parameters for common carp.

All these studies demonstrate the potential of insect-derived fat and oil for numerous feed applications in order to substitute traditional lipid sources. Some human consumption of insect lipid may also be considered.

5.2 Food applications

As previously indicated, insect oils are highly nutritional, close to vegetable oils and they can easily find their place as food ingredients, suitable for the replacement of either olive, peanut or canola oils (Berezina, 2017). Applications such as mayonnaises, vinaigrettes and frying oils may be considered. The total replacement of vegetable oil by *Tenebrio molitor* lipidic fraction was investigated on sensory perception of crackers and houmous formulations (Tzompa-Sosa *et al.*, 2021). In this study, the impact of deodorization of oil was investigated since the main issue with insect oil is its strong odor. Results indicated that deodorized *Tenebrio molitor* oil could replace vegetable oils in both products, without changing the overall food experience, liking and visual appearance in the products. In contrast, using crude *Tenebrio molitor* oil impacted the overall liking and certain sensory attributes, mostly related to flavor. The potential of substitute plant lipids by insect oils (*Hermetia illucens* and *Tenebrio molitor*) to process margarines was also investigated (Smetana *et al.*, 2020). They showed that it was possible to substitute up to 75% of lipids with a mixture of the two insect species fats without negative effects on spreading abilities and with improvement of product coloring (natural yellowish coloration).

For solid fat, the potential in bakery applications is ideal. This was envisaged by some scientists and the potential of bakery products containing crude Black Soldier Fly larvae fat as a substitute to butter was investigated (Delicato *et al.*, 2020). Black Soldier Fly larvae fat was used for replacement of butter to cook cookies, cakes and waffles at different percentages of incorporation. A 25% replacement of butter in bakery products had no impact on liking and preference and the texture was conform to expectations. A 50% substitution of butter was possible only for bakery products in direct contact with heat, such as waffles, without influencing consumer's acceptance. However, in other products, a 50% substitution led to a lower preference because of a rancid aroma, off flavor, highlighting the necessity of refining fat for higher inclusion percentage.

To conclude, human food studies indicate that insects' lipids have good functionalities in foods. However, the crude fats and oils have after tastes, and it will be necessary to refine them to improve their organoleptic properties.

5.3 Energy applications

In a review on insect lipid applications, the potential utilization of oils coming from insects for biofuels was considered (Berezina, 2017). The advantages and drawbacks of different sources of biodiesel were compared (algal, plant such as canola, palm, and insect...). Especially different indicators such as the lipids' content, the needed surfaces and the rapidity of growth were considered for each potential biofuel source. It was noticed that the "insect" source was much less time consuming (2–3 months of growth before being processing) comparing to the plant source and much less space consuming comparing to the algal source. Moreover, the quality of the obtained biodiesel was studied (Zheng *et al.*, 2013). It was found that the cetane number, the main intrinsic characteristic of the biofuel, was compliant with the requirements of the European norm EN 14214. The cetane number was 53 for *Hermetia illucens* and even as high as 58 for *Tenebrio molitor*. These values are much higher than what was observed for canola oil, 45. More recently, it was also demonstrated that the transesterification process to convert the triglycerides in the *Tenebrio molitor* oil into biodiesel was effective using a conventional base catalyst and resulting in 96.98% of (Fatty acid methyl esters (FAME) (Siow *et al.*, 2021). The quality of fuel met the requirement of ASTM D6751 biodiesel specification. *Tenebrio molitor* oil is feasible to be an alternative feedstock of third-generation biodiesel. Other scientists suggest that new technical routes are possible, and demonstrated that the oil extracted from *Tenebrio molitor* larvae was easily converted to biodiesel by a thermal non-catalytic reaction, achieving a 87.75% yield (Lee *et al.*, 2022).

5.4 Soap and surfactant applications

Soap and surfactants are other options that insect fats may address because of their chemical composition. A surfactant is a specific molecule possessing lipophilic "tail" and hydrophilic "head". The potential of such applications was already well described in the following review (Berezina, 2017). Surfactant may easily be obtained from triacylglycerol by a saponification step. Taking into account the poor properties for body care obtained with soap produced from animal fat (tallowates, mainly composed from saturated fatty acids), insect' oil may be rather interesting because of its better quality. However, the production of soaps and other surfactants was originally based on the plant fatty acids. Today the cosmetic market is fully focused on vegetal sources (plant oils), avoiding animal origin in the product and the acceptance of consumers for this market should first be improved.

5.5 Therapeutic applications

It has already been described, in the "Feed applications" part of this review, that oil and fat from insects may have positive effects on immune functions and cecal microbiota in animals such as fish or rabbits (Hender *et al.*, 2021; Dabbou *et al.*, 2020).

Another recent article suggested that insect oil, and especially *Tenebrio molitor* oil, could be considered as a therapeutic agent for the treatment of skin wounds. *Tenebrio molitor* oil significantly increased the re-epithelization after skin injury (Kim *et al.*, 2021). The authors suggest that *Tenebrio molitor* oil positively affects the role of interactions among keratinocytes, fibroblasts and myofibroblasts.

5.6 Plant applications

In a completely different field, it has recently been shown that certain components (fatty acids) of the fat of *Hermetia illucens* larvae have antimicrobial properties against phytopathogens (Marusich *et al.*, 2020). This work has highlighted the antibacterial potential against plant pathogens and opened the way to research for natural solution for crop protection.

6 Conclusions

Today, edible insects are an attractive alternative to conventional protein sources. They become increasingly more popular and can be one of the solutions to face the increased population growth and the necessity of increased livestock-rearing. They are suitable to address human nutrition as well as animal nutrition markets. Insect lipids are side product of insect protein production, and although less documented than the protein fraction, this review showed that insect lipids (oil or fat) have high interest from the nutritional and technological point of view. Lipids from insects can be included in feed and food for animal and human nutrition. Studies showed no restrictions in terms of safety and toxicity, and they showed similar or better performance on animal growth. Some beneficial health effects (immunity, colonic bacteria) have also been observed in some studies. For the human market, insect oil will have to be authorized by Europe through a Novel Food dossier. Several promising applications, substituting plant oil or animal fat, have been technically validated. Finally, some applications in the energy and crop protection area may also be considered.

Disclosure

Bénédicte Lorrette and Lorena Sanchez are employed by Ynsect.

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