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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Tenebrio molitor as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects

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Abstract

Nowadays, it is urgent to produce in larger quantities and more sustainably to reduce the gap between food supply and demand. In a circular bioeconomy vision, insects receive great attention as a sustainable alternative to satisfy food and nutritional needs. Among all insects, *Tenebrio molitor* (TM) is the first insect approved by the European Food Safety Authority as a novel food in specific conditions and uses, testifying its growing relevance and potential. This review holistically presents the possible role of TM in the sustainable and circular solution to the growing needs for food and nutrients. We analyze all high value-added products obtained from TM (powders and extracts, oils and fatty acids, proteins and peptides, and chitin and chitosan), their recovery processes (evaluating the best ones in technical and environmental terms), their nutritional and economical values, and their biological effects. Safety aspects are also mentioned. TM potential is undoubted, but some aspects still need to be discussed, including the health effects of substances and microorganisms in its body, the optimal production conditions (that affect product quality and safety), and TM capacity to convert by-products into new products. Environmental, economic, social, and market feasibility studies are also required to analyze the new value chains. Finally, to unlock the enormous potential of edible insects as a source of nutritious and sustainable food, it will be necessary to overcome the cultural, psychological, and regulatory barriers still present in Western countries.

1 | INTRODUCTION

It is known by now that the world population in 2050 will reach 9.7 billion people (UN, 2019) together with billions of animals raised for food (cattle, fish, chickens, etc.) and recreational purposes (pets, zoos, sports, etc.). The expected global demographic increase, associated with the consequent urban and agro-industrial expansion, will inevitably produce a negative environmental and climatic impact that, if not well managed, could further aggravate global inequality. A further consequential aspect of this scenario is the increasing demand for food, especially animal-based protein sources. Currently, in the world, about 1 billion people are affected by food insecurity and malnutrition (inadequate intake of protein, vitamins, etc.) (FAO et al., 2020). It is necessary to resort to scientific research, innovation, and technological development to produce more food or alternative protein sources more sustainably and with a circular bioeconomy vision.

Many efforts and investigations have concerned vegetable proteins (legumes, nuts, etc.) due to greater interest, both in the feed sector (e.g., soya proteins in feed for aquaculture and intensive farming) and among consumers following plant-based diets (vegan and vegetarian) or among environmentally conscious people. However, diets conducted with exclusively plant-based proteins may be nutritionally inadequate, since they lack some essential amino acids (EAAs), are poor in vitamins and less digestible than proteins of animal origin. For example, cereals are typically deficient in lysine; legumes are low in methionine, cysteine, and tryptophan. However, recent studies have shown the adequacy of diets with vegetable proteins when the sources are diversified (Gorissen et al., 2018; Singh, 2017).

In the feed sector, protein sources like soybean meal and fishmeal are used in large amounts to feed farm animals. Soybean meal is debated for different reasons, including the ethical issues surrounding genetic modified organisms. Many countries, despite considerable investments in soybean production, are not self-sufficient. They must import large quantities of soybeans from abroad with severe repercussions for the environment and the economy. In addition, soybean meal prices are high and change according to the climatic and social conditions occurring in the producing countries (Selaledi et al., 2020). On the other hand, large-scale fishmeal production is also controversial. It can cause the depletion of resources and ecosystems, environmental damage, and the breakdown of local fisheries.

In this context, in recent years, insects have received particular attention as a sustainable alternative to meet future food demands and satisfy nutritional requirements, which overcomes most of the criticalities shown by conventional protein sources (Ordoner-Araque & Egas-Montenegro, 2021). Over one million insect species have been described so far, including more than 2000 edible species (Jongema, 2017). It has been estimated that 2 billion people of various ethnicities, representing approximately 30% of the world population, already consume insects (van Huis, 2020). Several studies report that edible insects have a higher protein content (on average 40%, and up to 70% on a dry weight basis) than traditional sources such as meat, dairy, and seeds. They have both a high content of EAAs and higher digestibility than vegetable proteins (peanuts and lentils) and conventional animal proteins (beef and egg white) (Gravel & Doyen, 2019; Orkusz, 2021; Payne et al., 2016). Furthermore, insects are also a good source of essential fatty acids, microelements (iron, calcium, potassium, selenium, copper, and zinc) and vitamins (in particular those of B-group), fiber (the chitin contained in the exoskeleton), and a large amount of bioactive substances (polyphenols, enzymes, peptides/proteins, terpenoids, and sulfur compounds) (Zielińska et al., 2018c).

Among these edible insects, *Tenebrio molitor* (TM), a beetle of the Tenebrionidae family, is one of the most studied insects in the scientific world. TM, like all insects, is a poikilothermic animal and has a high feed conversion ratio (describing the efficiency in transforming food into body weight) compared to meat livestock. Moreover, it reproduces and grows faster, and its edible fraction is almost 100%, compared to chicken and pigs (55%) and cattle (40%). TM production requires less land and water and emit fewer greenhouse gases and less ammonia (Oonincx & de Boer, 2012; van Huis & Oonincx, 2017). However, the comparison of the environmental impacts of 1 kg of trout fed with diets containing fishmeal and different levels of TM shows that increasing amounts of TM lead to an increase in global warming potential, energy demand, and land use. On the contrary, consumption of water and biotic resources are lower (Le Féon et al., 2019). Nevertheless, few LCA studies of TM production are in the literature.

TM larvae (hereafter TML) can grow on low-nutrient vegetables or agri-food industry by-products, several vegetable residues, various spent mushroom substrates, brewer's spent grain, crop residues rich in lignocellulose, hatchery waste, and milling by-products. The feed conversion ratio can compare that of traditional livestock and can reach values of 1.8 when the feeding substrates are rich in protein. (Harsányi et al., 2020; Riudavets et al., 2020; Stull et al., 2019; Zhang et al., 2019). All this makes TM a sustainable and circular solution for the growing needs of food and nutrients.

TM, as a whole larva or as a processed product (protein flours, protein extracts, and oils), is currently used on a large scale for feeding animals, such as birds, fish, and pets in general.

In the European Union, TM is in the list of insects with the highest potential as food and feed (EFSA, 2015). In the field of animal feed, at present, EU Regulations 2017/893 and 2021/1372, amending Regulation 999/2001, allow using insect-derived proteins for feeding fish, pets, poultry, and pigs, but not for ruminants (e.g., sheep and cattle). In the field of human nutrition, EFSA experts recently expressed a favorable opinion as a novel food under the Regulation EU 2015/2283, concluding that it is safe for the proposed uses in biscuits, snacks, bars, pasta (EFSA NDA, 2021).

After the EFSA opinion, the Commission Implementing Regulation 2021/882 authorized TM as the first insect as novel food, thus paving the way to new market opportunities.

TM production and marketing for human food is a little-explored field in Western countries due to legislative, cultural, and psychological barriers. However, it is expanding, showing very profitable economic prospects for its attractive natural compounds with multiple biological and industrial potentials.

In some countries, insects are already used as food, for example, in China, the Netherlands, and Korea. Nutritional profiles and harmful components of insects are quite well known. On the contrary, other characteristics are still untested, such as appropriate cooking methods for mealworms, physical and sensory evaluations after cooking, oxidative stability of their oils, manufacturing methods to obtain lyophilized powder and recipes using mealworms (Baek et al., 2019).

In many areas of Asia, Africa, and South America, people eat insects daily. The significant food risks derive from the ingestion of the insect gastrointestinal tract with microbiological load implications. These risks might be so relevant that several commercial insect farms are keeping the growth of edible insects under controlled hygiene conditions (Messina et al., 2019).

In Western countries, insects' consumption is limited: they are often consumed as flour added to some traditional food ingredients. Their functionality as sources of essential protein and fat for humans, pets, and farm animals is well known (Messina et al., 2019). However, in Western countries, people are still apprehensive about consuming insects also because, in these areas, the number of allergic or intolerants is high. Many studies aim to find food processing methods able to reduce the risk of cross-reactivity and allergenicity associated with edible insect proteins (Barre et al., 2019). Other than safety reasons, it is crucial to consider the acceptability of different insects as food for religious reasons, popular tradition, or sensory preference. In Korea, for example, the use of insects in traditional Korean medicine is an age-old practice, so it is easier for them to accept the consumption of some species and include them in traditional cuisine (Pyo et al., 2020).

This work aims to give a holistic view of TM. This insect shows multiple potentialities and numerous applications in the food, pharmaceutical, and nutraceutical sectors. In a single work, we analyze all the high value-added products obtained from TM, specifically: Powders and Extracts, Oils and Fatty Acids, Proteins and Peptides, and Chitin and Chitosan (Figure 1). For each TM-based product, we report the recovery processes (evaluating the best ones in technical and environmental terms), together with the nutritional and economic values and the biological effects on different organisms, including humans. For completeness, we make a safety overview of TM products, although it is not the main topic of this article. We particularly emphasize the recovery from TM of bioactive molecules such as peptides, together with the description of their beneficial effects on human health. To our knowledge, such relevance has never been given to this topic in previous reviews dedicated to *T. molitor*.

1.1 | Methodology for searching literature data

This review is the result of a literature search, conducted in Google Scholar, Web of Science, PubMed, and Scopus, using a wide range of research elements, including *T. molitor*, its uses, potential beneficial effects and safety aspects, chitin and chitosan, global market sizes and recovery processes of TM-based products.

The sources of EU legislation were EUR-Lex and the European Food Safety Authority (EFSA). After a careful study, the authors selected the papers based on the relevance of their content. Most of the publications are from the last decade. However, the authors chose older references to describe the succession of scientific discoveries or in the absence of more recent works.

2 | NUTRITIONAL AND ECONOMIC VALUE OF *TENEBRIO MOLITOR*

TML have an excellent nutritional profile (Table 1) expressed as content in proteins and EAAs (Table 2), in fats (Table 3), fibers, ashes, and dietary energy; therefore, TML represent an excellent alternative to meat, according to international nutritional guidelines. Many works describe the chemical composition of TM in detail (Laroche et al., 2019; Nowak et al., 2016; Ravzanaadii et al., 2012; van Broekhoven et al., 2015). Ravzanaadii et al. (2012) also analyzed TM exuviae and frass (i.e., excrement) and found proteins even in frass (18.51%), suggesting that this could also be used in some way as an additional supplement in the food recycling process. As an alternative to amino acid composition analysis, the crude protein content of insects is often calculated from the total nitrogen content using the N-protein conversion factor 6.25 (Maehre et al., 2018). This value leads to an overestimation of the protein content because it includes the non-protein nitrogen fraction (chitin, nucleic acids, phospholipids, residues of excretion products present in the intestinal tract) (Janssen et al., 2017). Some authors determined a new protein conversion factor: Boulos et al. (2020), for example, suggest using 5.33.

Although mainly used as a source of excellent protein, TM is also highly valued for its lipid component. TM fatty acids (FAs) profile and content can depend on multiple parameters, such as diet, environment, and life stage.

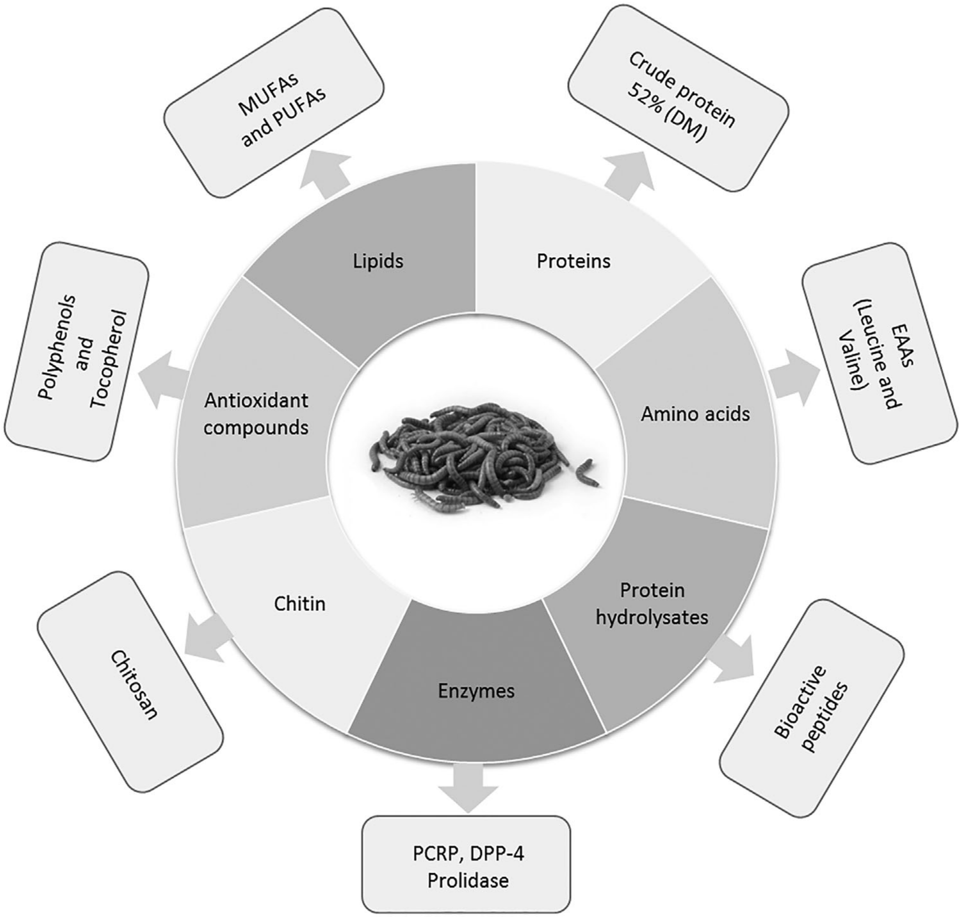


FIGURE 1 *Tenebrio molitor* as a source of high-value compounds. DPP-4, dipeptidyl peptidase 4; MUFA, monounsaturated fatty acid; PCRP, prolyl carboxypeptidase; PUFA, polyunsaturated fatty acid
Sources: Chae et al., 2012; Cho et al., 2020a; Chen et al., 2019a; Dai et al., 2013; Goptar et al., 2013; Jung et al., 1995; Moon et al., 1994; Morin-Crini et al., 2019; Rivero-Pino et al., 2020c; Roh et al., 2009; Son et al., 2020; Tereshchenkova et al., 2016; Tomchaney et al., 1982, 2017; Zielińska et al., 2017.

TABLE 1 Nutrition composition (% in dry matter) of *Tenebrio molitor* larvae (TML)

Protein	Fat	Carbohydrate	Fiber	Minerals (ash)	Energy (kcal/100 g)	Ref.
45.7	42.9[a]		7.2	4.3		Laroche et al., 2019
46.44	32.70	—	4.58	2.86	—	Ravzanaadii et al., 2012
47.18–49.43	35.17–43.08	—	5.00–14.96	2.36–3.08	539.63–577.44	Rumpold et al., 2013
(13.68–22.32)	(8.9–19.94)	(3.61)	(2.1)	(0.9–3.81)	(166–192)	Nowak et al., 2016
45.1	25.0	—	—	—	—	van Broekhoven et al., 2015
49.1	35.2	—	—	—	205.6	van Huis et al., 2013
44.72	42.48	—	—	3.69	—	Siemianowska et al., 2013
50.23–52.99	27.25–33.70	9.32–11.77	4.70–4.81	3.73–4.28	—	Yoo et al., 2013

[a] Value corresponding to the sum of the two components. Between brackets fresh weight values.

Either the body fat mass and the frass of these insects are rich in mono- and polyunsaturated FAs (MUFAs and PUFAs) (Moreno & Ganguly, 2016; Ravzanaadii et al., 2012). PUFAs, especially those of the omega-3 series, are crucial in terms of human health and, since humans cannot synthesize them, they must be acquired through the diet.

Although TM cannot meet the human requirements of total FAs, it can certainly provide a good amount of these macronutrients when consumed with other sources.

TABLE 2 Amino acid pattern of *Tenebrio molitor* larvae (TML) and FAO/WHO/UNU (2007) standards for adults essential amino acids (EAAs) requirements

	TML (mg/g protein)					FAO/WHO/UNU requirements
Essential amino acids						
Histidine	15.73	29	24.1	15.5	28.7–37.9	15
Isoleucine	22.26	43	50.7	24.7	43.5–50.3	30
Leucine	38.98	73	83.0	52.2	82.2–106.4	59
Lysine	29.16	54	59.0	26.8	44.3–64.9	45
Methionine + Cysteine	10.86	26	21.3	10.5	(12.7–19-5) + (6.8–10.9)	16 + 6
Phenylalanine + tyrosine	60.45	100	109.1	53.3	(26.2–43.7)[a]	30
Threonine	21.00	39	36.5	20.2	34.2–41.8	23
Tryptophan	ND	12	—	3.9	8.0–11.0	6
Valine	32.23	61	65.6	28.9	58.8–69.0	39
Sum of EAAs	230.67	437	449.3	236	—	269
Non-essential amino acids						
Alanine	39.38	70	69.8	40.4	—	
Arginine	27.89	54	55.5	25.5	—	
Aspartic acid	44.27	80	93.3	40.0	—	
Glutamic acid	62.36	109	128.8	55.4	—	
Glycine	29.51	50	52.9	27.3	—	
Proline	40.49	66	48.5	34.1	—	
Serine	24.97	44	40.2	25.2	—	
Hydrophobic amino acid	199.3	1.60	—	—	—	
Sum of total amino acid	499.52	910	940.2	483.9	—	
Ref.	Jiang et al., 2021	Yi et al., 2013	Zhao et al., 2016	van Huis et al., 2013	Zielińska et al., 2018c	

[a] Not available tyrosine data.

Two valuable antioxidants, tocopherol (vitamin E) and polyphenols, are also appreciably contained in TM oil (Son et al., 2020).

In Eastern countries, TML are widely consumed as whole insects in food, raw or cooked in different ways, such as steam cooking, roasting, smoking, frying, stewing and seasoning, salting, thus improving their sensory and nutritional qualities, as well as their shelf-life. In Western countries, despite the great food potential of this insect, its use, both as a food and as an ingredient for the food industry, encounters many cultural and psychological barriers that hinder its acceptance by consumers as food (van Huis et al., 2013). Those who have tasted the mealworms describe them with a savory taste similar to dried shrimps and a crunchy texture (Son et al., 2020). The color of the whole powder is too brown to be tempting. However, the degreasing process can significantly decrease the browning index and eliminate statistical color differences between powders, thus improving the product acceptability (Borremans et al., 2020).

It was also noted that some characteristics vary considerably with the cooking method used (Baek et al., 2019). A 2015 Korean study compared the effect of different cooking methods on the sensory characteristics of TML and showed that each process influences some specific sensory aspects making them more perceptible. For example, microwave cooking increases hardness and crushability; boiling enhances the adhesiveness, elasticity, and chewability. Hardness and crunchiness reached their highest values when the larvae were dried in the hot air or oven broiled while boiling and steaming preserves juiciness. The latter two cooking methods give the TML the aroma and flavor of cooked corn, canned pupa, and boiled mushroom. Finally, having found that oven-broiled TML had a higher aroma and flavor of worm oil, seafood, sweet and toasted sesame than those obtained with other cooking methods, the authors suggest this as the preferred method of providing TML with a more robust aroma and flavor (Baek et al., 2015).

TABLE 3 Fatty acid profile of *Tenebrio molitor* larvae (TML) oil (g/100 g oil)

Fatty acids	**Composition (g/100 g oil)**					
C4:0	—	—	—	—	—	—
C6:0	—	—	—	—	—	—
C8:0	—	—	—	0.01	0.19	—
C10:0	—	—	—	0.01	0.14	0.02
C11:0	—	—	—	—	—	—
C12:0	0.3	—	0.15 –0.32	0.29	—	0.58
C13:0	0.1	—	–	0.05	—	0.08
C14:0	4.0	1.58–1.72	2.73–4.86	1.98	2.13	5.89
C14:1$_{\mathrm{CIS}}$	—	—	—	0.01	—	—
C16:0	15.8	17.78–19.10	10.96–16.66	16.27	16.4	19.0
C16:1$_{\mathrm{CIS}}$	1.8	0.93–0.97	1.86–3.01	1.83	0.76	—
C17:0	0.1	0.40–0.44	0.06–0.14	0.22	—	0.07
C18:0	2.3	2.20–2.80	1.18–2.04	0.68	2.51	2.96
C18:1$_{\mathrm{CIS}}$	44.5	0.29–0.30	28.08–42.01	45.79	43.8	45.48
C18:1V	—	36.94–39.80	—	—	—	—
C18:2(n-6)	19.5	33.70–37. 0	35.56–51.51	27.99	30.3	20.55
C18:3(n-3)	0.4	1.21–1.30	—	2.98	1.59	0.33
C20:0	0.1	—	0.05–0.11	—	0.25	0.1
C20:1$_{\mathrm{CIS}}$	0.1	—	0.04–0.08	—	—	0.06
C20:2(n-6)	0.1	—	—	—	—	0.02
Total	88.6	—	—	100		
SFA	22.6	—	17.44–22.75	19.77	21.6	28.78
MUFA	46.2	—	31.04–44.01	31.48	46.6	49.50
PUFA	19.8	—	35.56–51.51	48.77	31.8	21.41
UFA	66.0	—	—	80.23	78.4	—
n-6:n-3 ratio	47.0	—	—	15.37	19.2	21.78
P:S ratio	0.9		—	—	—	—
Ref.	Son et al., 2020	Laroche et al., 2019	Otero et al., 2020	Wu et al., 2020	Benzertiha et al., 2019	Cito et al., 2017

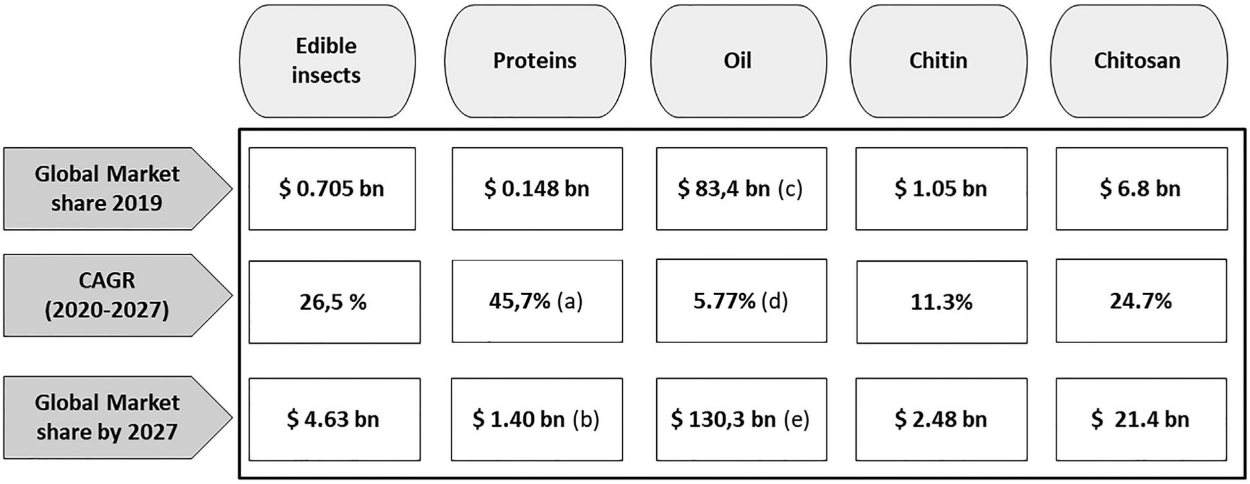
Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acids.

A recent Korean study analyzes the sensory differences between raw and cooked TML. The most perceived aroma in the raw TML was moist soil, while the oily ones, like shrimp and sweet corn, were less intense. On the contrary, the sweet corn aroma was strong in the steamed TML, while shrimp taste was more perceptible in the roasted and fried TML (Seo et al., 2020).

The characteristic aroma of roasted insects is influenced by pyrazines and carbonyl compounds that are formed (by Maillard reaction) during heat treatment, in particular from 11 odorous compounds that give the typical aroma of traditional European baked dishes, such as bread, potatoes, meat (Żołnierczyk & Szumny, 2021). Therefore, it is essential to choose the right cooking conditions. In their recent study, Żołnierczyk & Szumny (2021) found that mealworms roasted at 180°C had a pleasant and desirable smell of bread while cooking at a higher temperature (200°C) imparted an undesirable burning smell. Furthermore, according to this study, the sensory characteristics of roasted TML are not influenced by the type of feed.

A strategy adopted to reduce food neophobia toward edible insects is to make them "invisible" by incorporating them either as flour (powder) or as an extract (protein isolate) into "familiar" food products like bakery products, chips, biscuits, pasta, energy drinks, cookies, tortillas, chocolates, other snacks, etc. (Caparros Megido et al., 2016). A complementary strategy is to sensitize the Western consumer by emphasizing the ability of these foods to provide nutrients, other than proteins (also of excellent quality), not available in other foods, such as minor compounds that could have a functional interest (Navarro del Hierro et al., 2020).

Proteins and peptides extracted from TML are gaining great popularity and attention from the scientific world, especially as regards the study of the relationship



	Edible insects	Proteins	Oil	Chitin	Chitosan
Global Market share 2019	$ 0.705 bn	$ 0.148 bn	$ 83,4 bn (c)	$ 1.05 bn	$ 6.8 bn
CAGR (2020-2027)	26,5 %	45,7% (a)	5.77% (d)	11.3%	24.7%
Global Market share by 2027	$ 4.63 bn	$ 1.40 bn (b)	$ 130,3 bn (e)	$ 2.48 bn	$ 21.4 bn

FIGURE 2 Global market size of insects-based products. (bn = billions). Statistics referring to: (a) 2019–2025; (b) 2025; (c) 2017; (d) 2017–2024; (e) 2024. Data elaborated from: Cheseto et al., 2020; Meticulous Market Research, 2020; Research and Markets, 2020; Verified Market Research, 2019; Zion Market Research, 2020

between nutritional and healthy effects and their multiple applications both in the food and feed industry and in the pharmaceutical field (Hong et al., 2020; Okagu et al., 2020).

An interesting application can be the supply of TML proteins to astronauts in space through a bio-regenerative life support system, where the larvae are used as an efficient tool for the treatment of plant waste (Li et al., 2013).

The attention of the global market for edible insects and insect-based products has grown enormously in recent years (Figure 2); the increase in food needs due to the increasing prevalence of chronic diseases, together with the growing demand for high-protein and low-calorie products, is just one reason for this trend. The low environmental impact and nutritional benefits of edible insects are some of the other main factors that make their market growth. Currently, the most common edible insects are beetles, locusts, crickets, and ants. A further increase in the market can be expected due, on the one hand, to the greater availability of increasingly cheaper processing techniques and, on the other hand, to the increase in investments, especially in the field of innovative food and beverages for health and environmentally conscious consumers. Since they are a sustainable source of amino acids (AAs) and proteins, insect flours are compatible with a wide range of ingredients and natural flavors, have a low-calorie content, are easily soluble and have a neutral taste which makes them ideal for the preparation of various types of baked goods, protein bars, but also energy drinks.

As for the use of TM-based flours as feed, in 2016, the price of insect meal was 15 €/kg, higher than that of soy meal and fishmeal (0.33 and 1.22 €/kg, respectively). According to medium-term forecast analyses, insect meal cost is set to decrease, while the other two are likely to increase (Arru et al., 2019; Grau et al., 2017), especially if insects' rearing as a protein source will no longer be a niche sector. All this certainly requires economic investment and technological innovation (de Ruyter, 2016), but it can encourage the use of insect meal as feed.

Regarding chitin and chitosan, their growing application in the pharmaceutical, biomedical, cosmetic and food sectors, and water treatment is expected to drive market growth (Research & Markets, 2020). Currently, chitin and chitosan are produced commercially in countries such as Japan, United States, India, Poland, Australia, and Norway, and less so in Canada, Italy, Chile, and Brazil (FAO, 2020), and usually offered as flakes or powders with prices ranging from 13 to 84 €/kg, depending mainly on acetylation/deacetylation degree, purity parameters and products specifications (e.g., appearance, viscosity, ash content, and metal content lower than 10 mg/kg) (Morin-Crini et al., 2019).

3 | RECOVERY PROCESSES OF TM-BASED PRODUCTS

Figure 3 shows a scheme of the main production processes used to obtain TM-based products. The analysis of each step is given in detail in the following sections.

3.1 | Preliminary treatment

The quality of the larvae is a fundamental prerequisite for the quality of the final product. Both for industrial production and research tests, it is very important to have TML that maintain their characteristics unaltered over time, for this reason, some studies have been carried out

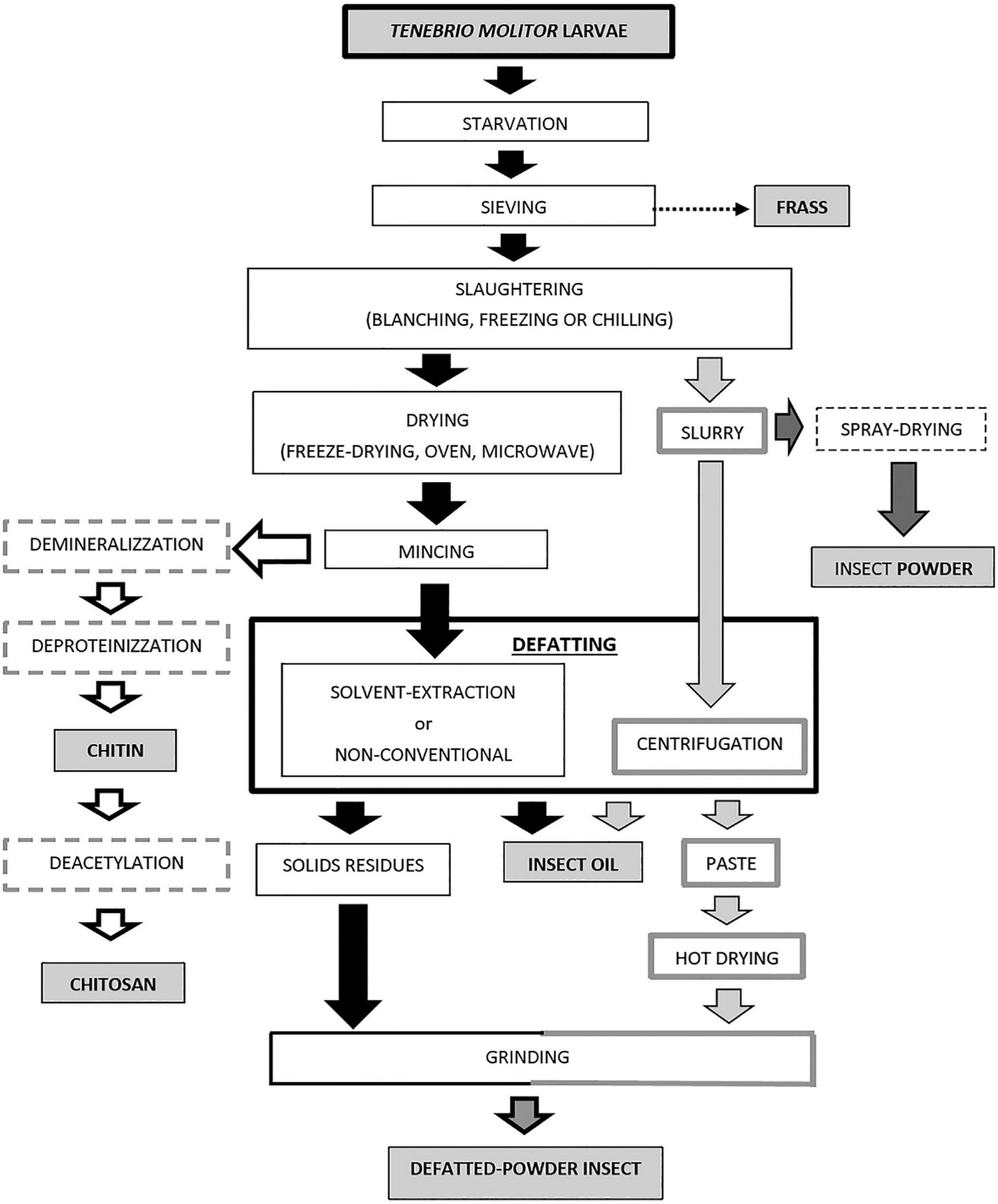


FIGURE 3 Production processes of TM-based products

to test the possibility of storing TML at low temperatures for short periods (48 h) (Koo et al., 2013) and long periods (30–120 days) (Errico et al., 2021).

In the production of insect meal, pre-treatment is highly dependent on the type of raw material used. It is a fundamental step needed to improve the efficiency of subsequent processing steps, ensure food safety, meet quality standards and consumer expectations. It is common in many insect farms to apply a starvation period before harvesting by separating insects from their food (Garofalo et al., 2019).

When associated with low temperatures (5°C) for a long period (8 days) it produces a reduction in the total microbial count of all microorganisms, especially yeasts and total anaerobic mesophilic spore-forming bacteria (Costa et al., 2020).

However, appropriate hygienic conditions should be applied, during the rearing period and processing of edible insects (Ojha et al., 2021).

The blanching and drying processes are the two main pre-treatments that are usually used in succession for the industrial and artisanal production of edible insect meals (Melgar-Lalanne et al., 2019).

The blanching process is a "wet-heat" pre-treatment in which the larvae are blanched for a short time and then cooled in ice. The main purpose of this thermal shock is to minimize the microbial load and to inactivate the degradative enzymes responsible for food spoilage (Mancini et al., 2019). The only drawback associated with blanching, so far encountered, is the variation of some color parameters, in particular the browning of the larvae and the reduction of luminosity. Azzollini et al. (2016) argue that this phenomenon is due to the probable change in the refractive index of the tissues following blanching and the increase in non-enzymatic browning reactions induced by the increase in the solubility of some nutrients. The drying process is a "heat-dry" treatment, generally used to increase food shelf-life. The reduction of the total water content consequently produces the inhibition of all the degradative reactions of enzymatic and microbial origin. Depending on the type and physical state of edible insects, different drying techniques are adopted. Sun-drying, freeze-drying, oven drying and microwave-dried are usually used to dry whole edible insects, while freeze-drying, oven drying, fluidized bed drying and non-conventional drying techniques (spray-dried, etc.) are mainly used for insect flours and powders (Melgar-Lalanne et al., 2019). Kroncke et al. (2018) compared various drying processes carried out on TML: from the comparison of the nutritional values obtained from freeze-drying, fluidized-bed, frying, microwave-drying, vacuum drying in a vacuum oven, and conventional hot drying on a rotating rack, only small changes emerged regarding the content of proteins, fats, and fibers. TML subjected to freeze-drying showed an increase in lipid oxidation and a decrease in the solubility of proteins, probably due to the high-fat content (40%) present in the larvae (Caparros Megido et al., 2017). Oven drying is the most widely used drying method at the industrial level, preferred because it is less expensive than others and provides a final product with quality comparable to those obtained with the freeze-drying method, in terms of extraction of proteins, fats, and chitin (Azzollini et al., 2016; Purschke et al., 2018). TML, freeze-dried or baked, are ground and sieved to produce a fine powder that promotes the contact between the matrix solvent, essential for the success of the subsequent phases (Buβler et al., 2016; Zhao et al., 2016; Zielińska et al., 2018b).

In a recent paper, the effects of nine different processing methods on the nutritional composition and antioxidant activity of TML were examined (Baek et al., 2019). Protein, some minerals, and vitamin contents increase by freeze-drying, hot air drying, baking, roasting, deep-frying, frying, boiling, steaming, and microwaving. Total mineral contents decrease after frying as well as vitamin B1 and B3 contents after microwave cooking. Tests with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) showed that microwaving, freeze-drying, frying, steaming, boiling, and cooking of TML produced scavenging activity similar to the level of activity shown by tocopherol. (Baek et al., 2019).

However, not all pre-treatments increase all the nutritional characteristics of TM flour: in the case reported by Borremans (2020), who evaluated some parameters (including crude protein, crude fat and dry matter content, water holding and oil binding, foaming capacity, emulsion capacity) on the blanched and degreased powder, the blanching and fermentation processes reduced the raw and soluble protein content of fat-filled powders and in general compromised their binding, foaming, and emulsifying properties of water and oil. However, the results they obtained confirmed that the properties of the unfermented powders are comparable to those of other sources of dietary proteins.

3.2 | Recovery

3.2.1 | Powders and extracts

Edible insects are mostly used as whole insects or as powder. The psychological hesitation of many consumers to eat whole insects makes it essential to process them into powder, paste, or ground meat-like products for use in the food industry. Breaking down the insect forms result in a more attractive, familiar, and desirable product for more consumers (Ojhia et al., 2021; Stoops et al., 2017). The powders, compared to the whole insect, have a milder taste, aroma, and color and are characterized by a longer shelf-life, as they consist of dried ingredients with a water activity of less than 0.5. The powders are also easy to transport and store and this makes them a very versatile product in the food industry (House, 2016). Considerable efforts are being made to optimize and secure the entire powder production process.

The flour obtained from insects' transformation retains all the benefits due to the physical and chemical qualities of the insects themselves and, at the same time, it takes on a form that can be easily mixed with many other ingredients and easily inserted into many recipes (snacks, crackers, pastries, biscuits, candies, chocolate, pasta, bread, sausages, hamburgers) (Dossey et al., 2016).

The powdering process is highly recommended for TM, since, given its high moisture level (60%), protein content (20%), microorganism, and enzymes, it is a food with a high decomposition rate (Costa et al., 2020; Rumpold et al., 2014). In a study conducted by Son et al. (2019), the optimal conditions for TML powder production were researched, analyzed, and verified. In particular, six different conditions of industrial powder productions were considered, giving great emphasis to the variations in physicochemical qualities of the final product obtained, the influence of the milling process on the morphological characteristics of the powders, and the consumer preferences. Only by using the spray-drying technique, it was possible to obtain a product of good quality, as TML high lipid content affects the powder production process; therefore, a previous defatting step was required. Powders were previously defatted using solvent (n-hexane) or pressure, and four different types of milling machines were used (pin, hammer, high-performance jet, or cutting). The solvent-degreased powder, due to its very low lipid content, showed brighter and more achromatic colors than the others, but it was tasteless and did not meet consumer preferences.

The use of powders as both feed and food shifts the focus of investigations to food safety items (see Safety section). In their study, Kooh et al. (2020) highlighted how all processes involving hot slaughter (5 min, 100°C) strongly reduce the risk of contamination only by vegetative bacteria; sporogenous bacteria require special attention to the quality of the raw materials and the performance of the subsequent heat treatment. All TML obtained powders have a long shelf-life, only if stored at room temperature in sealed packages without the possibility of re-wetting. These conditions are of paramount importance when it came to finished food products.

Chung et al. (2013) analyzed a process of indirect sterilization of TML before freeze-drying and processing into powder. For this purpose, larvae were fed on steam-sterilized bran for 3–5 days, starved without water or food for 3 days, and then freeze-dried and ground by a blender. They investigated the possible cytotoxicity of the powder used as food by assessing the viability of Raw 264.7 macrophages and concluded that raw powder is completely free of microorganisms (including bacteria and fungi), and its safety is high.

The "extracts" are another way to use insects for human and animal. They are very rich in compounds other than proteins, such as fibers, lipids, or minor compounds that exhibit strong biological properties. Their use often serves to enhance the quality of the final food product.

The recovery of this mixture of substances requires the use of specific extraction conditions in terms of the type of solvent and extraction method.

In the case of TML, the Soxhlet technique combined with low-polar solvents has been described to obtain predominantly lipid-rich extracts (Tzompa-Sosa et al., 2014). However, the use of conventional techniques seems to be disadvantageous for several reasons: low selectivity and extraction efficiency, demand for expensive solvents of high purity, long extraction times, degradation of thermolabile components. These disadvantages have been partly solved by new alternative techniques, such as ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE). Their use on matrices with high lipid content has led to higher extraction yields and the production of lipid-rich extracts with modified or improved fatty acid profiles compared to the raw material (Otero et al., 2020).

The extraction process can affect the biological effects of the resulting insect extracts. In the study conducted by Navarro del Hierro et al. (2020), all the extracts, regardless of the type of extraction, had antioxidant activity, but the most effective were those extracted in a mixture of ethanol and water. Similarly, all extracts showed lipase inhibitory activity, although those obtained by using PLE were the most effective.

The protein quality of the extracts is similar to or better than fish meat or soy powder. For this reason, they could be used as a source of bioactive peptides for functional foods or as natural alternatives to synthetic antioxidants (Messina et al., 2019). On the other hand, many proteins are allergenic, especially for people sensitized to shrimp and other shellfish (Barre et al., 2019).

3.2.2 | Oil, lipid, and fatty acids

As already mentioned, the high-fat content (30%) in TM makes the pulverization process very problematic as it produces a high degree of powder caking (agglomeration), reducing its flowability and making it difficult to handle and transport (Son et al., 2020). In addition, many works have shown that the hydrophobic nature of lipids and possible protein–lipid interactions severely limit the solubility of proteins in food, so directly removing the lipid part affects both the recovery and purity of the extracted proteins (Azagoh et al., 2016; Lam et al., 2018). The defatting process, the mainly used technique for the recovery of oils and fats from highly lipid-rich foods such as oily legumes (peanuts, soybeans, etc.) (Kang et al., 2017), is a crucial step in overcoming all the critical issues in the production process insect protein meal (Choi et al., 2017). Several studies have focused on lipid extraction methods applied to insects in general and TM in particular (Table 4). In most of these works, conventional methods, such as solvent or mechanical extraction, are used to search best conditions to produce good quality defatted flours and, at the same time, to obtain oils with optimal yields, good nutritional value and chemical characteristics suitable for versatile use as a food ingredient (Kroncke et al., 2018; Son et al., 2019, 2020; Zhao et al., 2016). Tzompa-Sosa et al. (2014) compared two conventional industrial methods (Soxhlet and aqueous extraction) and one laboratory

TABLE 4 Lipids extraction methods of *Tenebrio molitor* larvae (TML)

Matrix	Recovery methods	Main findings	Ref.
TML whole-fat powder	Solvent extraction (n-hexane)	Oil with a good nutritional value (oleic acid, rich in tocopherols and other minor nutrients) and characteristics for versatile use as a food ingredient. Lipid extraction yield: 29.5% ± 1.0%	Son et al., 2020
TML whole-fat powder	Soxhlet with petroleum ether (SOX) Aqueous extraction method (AEM) Folch extraction method (FEM)	Lipid extraction yield (%) (AEM/FEM): 60.3 (SOX/FEM): 98.4 Total lipid content % (g/100 g fresh insects) AEM: 7.8 SOX: 12.7 FEM: 12.9	Tzompa-Sosa et al., 2014
TML—differently dried whole-fat powder (freeze-drying, vacuum oven drying, and rack oven drying)	Folch-based methanol/chloroform extraction	Composition and fatty acid profile were comparable between the dried larvae. TML color impressions and profiles of volatile compounds depend on the processing.	Kroncke et al., 2019
TML coarse meal	Supercritical CO_2 extraction (in comparison to n-hexane extraction)	Solvent free. Lipid extraction yield: 21%–95% (depending on duration, temperature, and pressure applied). No substantial difference in the chemical composition of the oil extracted with the two methods.	Purschke et al., 2017
TML meals	Supercritical CO_2 extraction	Lipid extraction yield: 22.1% (75 min, 325 bar, 55°C)	Laroche et al., 2019
	Soxhlet method	Lipid extraction yield: Hexane 25.5% Petroleum ether 24.3% Ethyl acetate 25.7% Ethanol 28.8%	
	Three-phase partitioning method (TPP)	Lipid extraction Yield: 23.7% Significant differences of fatty acid profiles depending on solvent polarity.	

method (Folch method). They extracted various lipid fractions from four insect species (TM, *Alphitobius diaperinus*, *Acheta domesticus*, and *Blaptica dubia*). Aqueous lipid extraction produced the lowest total extracted lipid value for all insect types (1.6%–7.8%), while Soxhlet and Folch produced similar yields (6%–12.9%). These conventional extraction methods have considerable limitations and criticalities: long extraction times, use of expensive solvents for the high degree of purity required, inevitable degradation and decomposition of thermolabile compounds, denaturation and loss of the functional properties of proteins, and potential danger to human health and the environment (Russin et al., 2011). More eco-friendly alternatives to lipid extraction are the so-called unconventional methods, including extraction using supercritical CO_2. This lipid extraction method works in the absence of solvents and produces a marked reduction in the oxidation of lipid components and a higher protein yield. The application of this method on TML provides a degree of decreasing from 21% to 95% of the coarse meal, depending on the duration, temperature and pressure applied, and no substantial difference in the chemical composition of the oil extracted with n-hexane (Purschke et al., 2017). Laroche et al. (2019) described a comparison between conventional and nonconventional methods for oil extraction from TML. Specifically, this study, focusing on TM and domestic cricket (*A. domesticus*), examined the effects of six degreasing methods on lipid extraction yield, fatty acid profiles, protein extraction, and purification. These methods are Soxhlet (with n-hexane, petroleum ether, ethyl acetate, and ethanol), Three-phase partitioning (TPP) and supercritical CO_2 extraction. TM, due to its high lipid content, shows very high extraction yields (22.1%–28.8% w/w). The three most abundant FAs obtained are palmitic acid (C16:0)

(18%–19%), vaccenic acid (C18:1V) (37%–40%), and linoleic acid (C18:2) (33%–37%). The extraction yield values do not seem to be affected by the defatting method used ($p < 0.05$). On the contrary, there are some differences in the FA profiles of produced oils. Soxhlet extraction with ethanol and TPP are the two least efficient methods for extracting C16:0 (but most of C18:2 is extracted), while supercritical CO_2 extraction is the most efficient. These results agree with Otero et al. (2020) and confirm the direct correlation, in selectivity and yield terms, between the polarity of the lipid to be extracted and that of the extraction solvent. Indeed, PUFAs as C18:2 are relatively more polar than MUFAs as C18:1V and long-chain saturated FAs as C16:0 (Laroche et al., 2019; Otero et al., 2020). In many studies conducted to research and evaluate the biological properties of the lipid portion of TM, conventional extraction techniques have been preferentially adopted (Cito et al., 2017; Dabbou et al., 2020; Wu et al., 2020; Youn et al., 2014).

3.2.3 | Proteins, peptides, and bioactive molecules

Defatted insect meal, potentially useful as it is, is often the raw material on which various protein extraction processes are applied. Protein extraction is a process consisting of specific steps aimed at obtaining end-products enriched in high quality and purity proteins, which in turn can be used as substrates for the production of bio-peptides of interest.

The strategy used is to selectively eliminate all the other components of the mixture by exploiting both the specific physical–chemical characteristics of the protein pattern of the defatted meal (solubility, pH of solubilization, isoelectric point, molecular size, binding with other molecules, etc.) and the effect of physical and mechanical pre-treatments carried out on the insect or the meal. The main extraction methods deriving from the literature from 2013 onward, including some specific pre-treatments, are summarized in Table 5.

In the study by Azagoh et al. (2016), the influence of a thermomechanical pre-treatment, carried out directly on the larvae, on the extraction yield, and the physicochemical characteristics of the extracted proteins is evaluated. The data obtained show a doubling of its protein yield (59.9%), an increase in both protein level in the crude extracts (71.6%), and the soluble protein content (about 80%), as well as the presence of EAAs of excellent quality, except methionine.

An alternative method of protein extraction to other heat or pressure treatments (microwave-assisted, supercritical CO_2, high-pressure treatment), is UAE, known as sonication. Applied on TML flour after the degreasing process, it produces a protein extract with a low yield (35%), probably due to a suboptimal degree of homogenization of the sample, but with a high-quality and not altered AA profile that fully meets typical human food requirements (Choi et al., 2017).

In the study conducted by Laroche et al. (2019), the usefulness of the defatting method as a chemical pretreatment on the percentage protein yield and especially on the degree of purity obtained is investigated. All six defatting methods analyzed (consisting of four conventional solvents, TPP, and supercritical CO_2), produces a good extraction yield and an increase in the degree of purity of about 30% compared to that obtained without pretreatment.

Recently, a study conducted by Purschke et al. (2018) introduces the dry fractionation method to increase both the yield of protein-enriched fractions from TML and the quality of the flour produced. This method shows a strong dependence on the pre-treatments (blanching, freezing, etc.) and the type of drying treatment (oven drying, liquid bed drying, freeze-drying, etc.) used before fractionation. The highest protein recovery was obtained with the fraction with a particle size of 500 to 1000 μm. Dry fractionation is, therefore, a promising strategy to produce standardized insect-based intermediates given industrial applications and consumer acceptance.

Extraction techniques based on wet fractionation, on the other hand, exploit the chemical and physical characteristics of the proteins. Modulating the solubility of proteins in the extraction medium (water or solvents) is one of the strategies often used to selectively separate most proteins from the other components present in a defatted insect meal. pH and ionic strength of the extraction medium can modulate this parameter. The most commonly used method to solubilize and recover proteins from TML is the alkaline one, followed by isoelectric precipitation (IEP), in which the extraction yields obtained are generally higher than those obtained with aqueous ($\mathrm{pH} \sim 7$) or saline extraction (Bußler et al., 2016; Zhao et al., 2016; Zielińska et al., 2018b).

Aqueous extraction procedure has been found to show not only low protein yields but to cause browning of the water-soluble fraction, despite the use of ascorbic acid as a salt/anti-browning agent (Yi et al., 2013). In a subsequent study, Yi et al. investigated the use of sodium bisulfite as a salt/anti-browning agent and the introduction of an additional purification of crude proteins by exploiting their isoelectric point (pI). The authors concluded that sodium bisulfite in an alkaline environment partially prevents the browning of the TML water-soluble protein fraction. Moreover, they demonstrated that pH and salt concentrations can influence the yield of the water-soluble protein fraction: aqueous extraction at pH 11 in

TABLE 5 Purification processes of *Tenebrio molitor* larvae (TML) proteins

Matrix and pre-treatment	Defatting	Extraction	Main findings	Ref.
TML - Thermo-mechanical process: - Blanching with water at 90°C for 10 min - Pressing with a screw press to remove part of fat and water - Drying at 75°C for 6 h - Grinding in blender to have the larvae meal	n-hexane and isopropanol in an ASE 200	Alkaline extraction	Total protein: - Larvae 65.6% - Larvae meal 71.6% Good EAAs profiles	Azagoh et al., 2016
TML - Previously microwave drying - Ground into coarse meal using a traditional pestle and mortar	n-hexane	Ultrasound-assisted extraction with water and ascorbic acid	Total protein after defatting: 62%–74%. No significant changes in amino acids profiles (proline and tyrosine values were much higher)	Choi et al., 2017
TM meals	a. Soxhlet (n-hexane, petroleum ether, ethyl acetate and ethanol) b. TPP extraction ($(NH_4)_2SO_4$ and t-butanol) c. Supercritical CO_2	AEAP methods	No significant changes in protein extraction yield and purity among defatting processes	Laroche et al., 2018
TML - Blanched in boiling water - Shock-freezing - Thawing - Drying (oven drying, freeze-drying)	Only one of the five drying methods is followed by a partial defatting with supercritical CO_2	Dry fractionation: - Roller milling - TML grist - Sieve classification	Up to 5.4% protein enrichmentMaximum protein content : - Fluidized bed drying 58% (0–500 μm) - Defatted sample 66% (355–500 μm)	Purschke et al., 2018
TML Non-defatted insect flours production process: - Pureeing frozen larvae with water at 4°C - Freezing - Freeze-drying - Grinding	Two-step extraction with n-hexane	High-protein and low-protein fractions were obtained by sequential alkaline extraction (pH 10) and acid precipitation (pH 4 and 2)	Crude protein %: - TM flour 57.8% - Defatted TM flour 64.6% High-protein fraction 68.2%Low-protein fraction 11.2%	Bußler et al., 2016

(Continues)

TABLE 5 (Continued)

Matrix and pre-treatment	Defatting	Extraction	Main findings	Ref.
Freeze-dried TML	Ethanol (99.5%)	AEAP methods	Optimal extraction conditions: 0.25 M NaOH + ethanol defatted TML (40°C for 1 h) True protein content 75% Extraction rate 70% The protein extract was a good source of EAAs	Zhao et al., 2016
TML Frozen grinded TML were freeze-dried or N_2-frozen TML	n-hexane	Water extraction with ascorbic acid beforehand	Total protein 20%	Yi et al., 2013
TML Frozen grinded	The lipids removed after centrifugation like a upper layer	• TM grinded was blended in buffer with different amounts of salt/anti-browning agents [(NaCl)/(sodium bisulfite or ascorbic acid)] • Extraction (as in Yi et al., 2013)	Sodium bisulfite (0.5%–4%), unlike ascorbic acid (0.01–0.04%), prevents browning	Yi et al., 2017
Pulverized TML	n-hexane	Four different techniques: - AEAP methods - Salting-in-AEAP (adding 1% NaCl (w/v) to the NaOH solution) - AEAP-salting-out: 20% $(NH_4)_2SO_4$ (w/v) added to the supernatant - Salting-in-AEAP-out: extraction with 1% NaCl (w/v) added to the NaOH solution (salting-in) and then 20% $(NH_4)_2SO_4$ (w/v) added to the supernatant (salting-out)	The presence of 17 amino acids (including 7 EAAs except for methionine) Salting-in-AEAP-out extraction led to the highest protein yields. The treated sample showed the greatest emulsion activity index, foaming capacity and stability, and zeta potentials	Jiang et al., 2021

Black-filled bullets indicate the different steps in the pre-treatment processes.

Abbreviations: AEAP, alkaline extraction and acid precipitation; ASE, accelerated solvent extractor; EAAs, essential amino acids; TM, *Tenebrio molitor*; TPP, three-phase partitioning.

combination with NaCl produces a crude protein yield of 68.6% rich in heavy myosin chains, while precipitation performed at pH 4 increases the protein content by 42%, preferentially extracting the 12 kDa proteins present in the hemolymph (Yi et al., 2017).

Another factor that modulates protein solubility is the ionic strength of the extraction medium. As the surface charges of the various protein species are different, the salting-in/out method is a technique often used to isolate one or groups of proteins (Duong-Ly & Gabelli, 2014). In a recent work, Jiang et al. (2021) adopted this technique on TML powder. TML protein isolates were obtained by alkaline extraction and acid precipitation (AEAP) methods, assisted by NaCl (salting-in) and $(NH_4)_2SO_4$ (salting-out) procedures. The salt-assisted treatments are found to give a protein yield of 39.54%. The total AA content is not affected, but the specific AA compositions are altered (only 40% EAA). On the other hand, an increase in emulsifying activity, foaming capacity, stability, and overall protein solubility is found.

Proteins extracted from TM can be advantageously used as a source of bio-peptides, simulating what occurs naturally in the gastrointestinal tract, where activation or ex novo formation of bio-peptides is usually the result of enzymatic hydrolysis. Together with fermentation, this is the main technique used to produce protein hydrolysates (PHs) from edible insects. In particular, Nongonierma and FitzGerald (2017) reviewed the production of bioactive PHs (BAPs) from edible insects. Different preparations with various dietary proteases (Alcalase™, Flavourzyme™, Protamex™, papain, trypsin) were used, alone or in combination and with different hydrolysis times to obtain all bioactive peptides from TM proteins. Their bioactive power is extensively described in Section 4.

Edible insects, such as TM, represent not only a source of protein to cope with normal protein turnover but also an inexhaustible reservoir of biologically active proteins and peptides that may exert a beneficial effect on human health. Most of these peptides are present in mixtures in the total protein complex either in an active form, as insect components, or as inactive precursors (Karaś, 2019). Selective purification processes are the way for improving purity and increasing bioactivity. Several approaches can be adopted, based on different types of chromatography (concerning hydrophobic/hydrophilic, charge, molecular size, or affinity properties of peptides), electrophoresis techniques, and membrane separation. In particular, chromatographic methods are a useful tool used on TM to purify, isolate and characterize some specific proteins/peptides of non-food but mainly biological interest. For example, Francis et al. (2019) recently partially purified arginine kinase from TML by ion-exchange chromatography. Other authors, such as Strobl et al. (1997), isolated high-purity α-amylase from TM by successively combining chromatographic processes: anion exchange, affinity, and size exclusion.

Goptar et al. (2013) were the first to isolate digestive enzymes from insects, and in particular from TM, using chromatographic gel permeation purification. In their work, prolyl carboxypeptidase (PRCP)—a proline-specific lysosomal serine peptidase (PSP) involved in the hydrolysis of the main TM dietary protein, gliadins—was isolated and characterized from the anterior midgut. This enzyme is the first PRCP to be attributed to a digestive function. The same group of authors subsequently isolated and characterized another PSP from the TM anterior midgut, dipeptidyl peptidase 4 (DPP4), that can also hydrolyze gliadins efficiently. These data provide a comprehensive overview of the digestion of wheat prolamins in TML and when combined, indicate that DPP4 and PRCP participate in the digestion of gliadins after preliminary degradation by endopeptidases (Tereshchenkova et al., 2016). Finally, another PSP was purified and characterized by the soluble tissue fraction of the TML mid-posterior intestine, the enzyme Prolidase, a proline-specific metallopeptidase. This enzyme is crucial for the final stages of gliadin digestion as it can hydrolyze the intractable fragments formed: the imidodipeptides. (Tereshchenkova et al., 2017). These studies show that TM contains a broad spectrum of digestive proteinases that operate in the midgut. Two digestive peptidases (PRCP and DPP4) and one post-proline cleaving peptidase (Prolidase) readily hydrolyze the bonds formed by the major AAs of cereal gluten, Pro and Gln, and presumably have the potential for oral administration to treat coeliac disease (Elpidina & Goptar, 2007).

3.2.4 | Chitin and chitosan

Chitin, or poly-*N*-acetylglucosamine, is an insoluble aminopolysaccharide, a naturally abundant mucopolysaccharide, and the second most abundant natural biomass after cellulose, with an estimated annual production of approximately 10^{10}–10^{12} tons (Li et al., 2019; Zainol Abidin et al., 2020). This chemically inert macromolecule $(C_8H_{13}O_5)n$, similar to cellulose (Akakuru et al., 2018), consists mainly of repeating *N*-acetyl-D-glucosamine units linked by β-(1,4)-glycosidic bonds (GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose). It should have a degree of acetylation greater than 50% to be defined as chitin (Pighinelli et al., 2019). Although the chitin is widely distributed in arthropods, nematodes, and cell walls of fungi (Deringer et al., 2016), the insects could be the best choice for the chitin recovery and its derivatives, being the most common species in the world: out of 1.3 million species in the world, 900,000 are insects (Zainol Abidin et al., 2020).

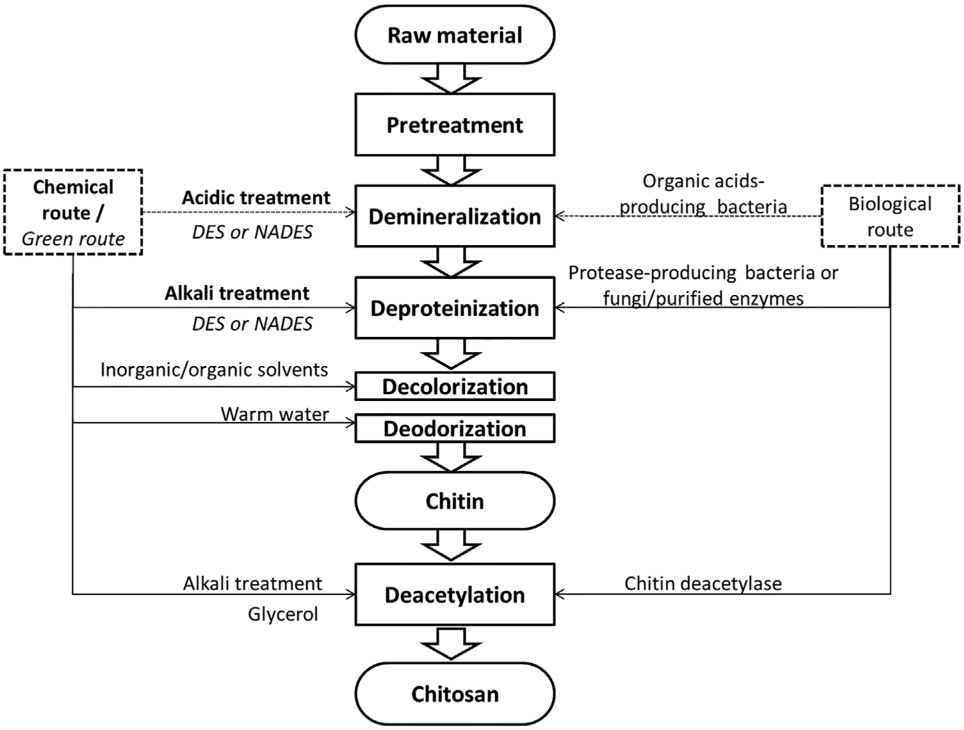


FIGURE 4 A general process for chitin and chitosan recovery. DES, deep eutectic solvent; NADES, natural deep eutectic solvent

In these species, the chitin acts as scaffold material, supporting the cuticles of the fibrous exoskeleton, head capsule, trachea, foregut, and hindgut, and the peritrophic membrane lining the midgut lumen. It also protects insects from external invasion and food abrasion (Yang et al., 2019b). Despite the source material, the purpose of the extraction process is to remove impurities and foreign organic matter, such as proteins, lipids, pigments, and inorganic filler, such as calcium carbonate and calcium phosphate, until only the material of interest is obtained (Pighinelli et al., 2019). At present, the main chitin sources are shells of crustaceans, such as lobsters, crabs, and shrimp, whereas alternative sources are still being investigated (Kumari & Kishor, 2020; Zainol Abidin et al., 2020). Since insects contain less inorganic material (less than 10%) compared to crustacean shells (20%–40%), chitin extraction can be easier, resulting in a more environmentally friendly process (Zainol Abidin et al., 2020). Compared to other insects, exuviae and exoskeletons of TM represent the best resource for chitin extraction due to the stable supply of raw materials and its low cost (El Knidri et al., 2019).

Through a process of chitin *N*-deacetylation, it is generally possible to produce chitosan, a copolymer with a degree of acetylation (the ratio between glucosamine and *N*-acetyl glucosamine) lower than 50%. Chitosan, discovered by Charles Rouget, physiologist, in 1859, consists of β-(1→4)-linked 2-acetamido-2-deoxy-d-glucopyranose and 2-amino-2-deoxy-d-glucopyranose units. It represents the most important chitin derivative (Maddaloni et al., 2020). A general process for chitin and chitosan recovery occurs in several steps, as shown in Figure 4.

The chitin extraction requires two main steps: demineralization (also known as decalcification) to remove calcium, phosphorus, and other minerals, and deproteinization, for the removal of proteins and other compounds. These steps can be carried out chemically, based on the use of acids and bases, or following a biological approach, by microorganisms and enzymes (Arbia et al., 2013; Pighinelli et al., 2019). It is also possible to make a combination of both methods to achieve an even higher process efficiency (Pighinelli et al., 2019). In addition to the two main steps, phases of decolourization (carried out using acetone, hydrogen peroxide, sodium hypochlorite, or potassium permanganate) and deodorization can be performed, if necessary (Maddaloni et al., 2020; Pighinelli et al., 2019). The chemical method for chitin extraction is currently the most widely used in both industrial and laboratory production, due to its purity, yield, and cost-effectiveness (Yang et al., 2019a). The parameters that can be modified are variation and ratio of acid and base, incubation time, and temperature. However, the chemical method requires strong acid and alkali solutions, large water amounts for neutralization steps between acid and alkali treatments, high temperatures, and a long process time. These conditions limit the sustainability of the whole process. In the last few years, scientific interest is focused on

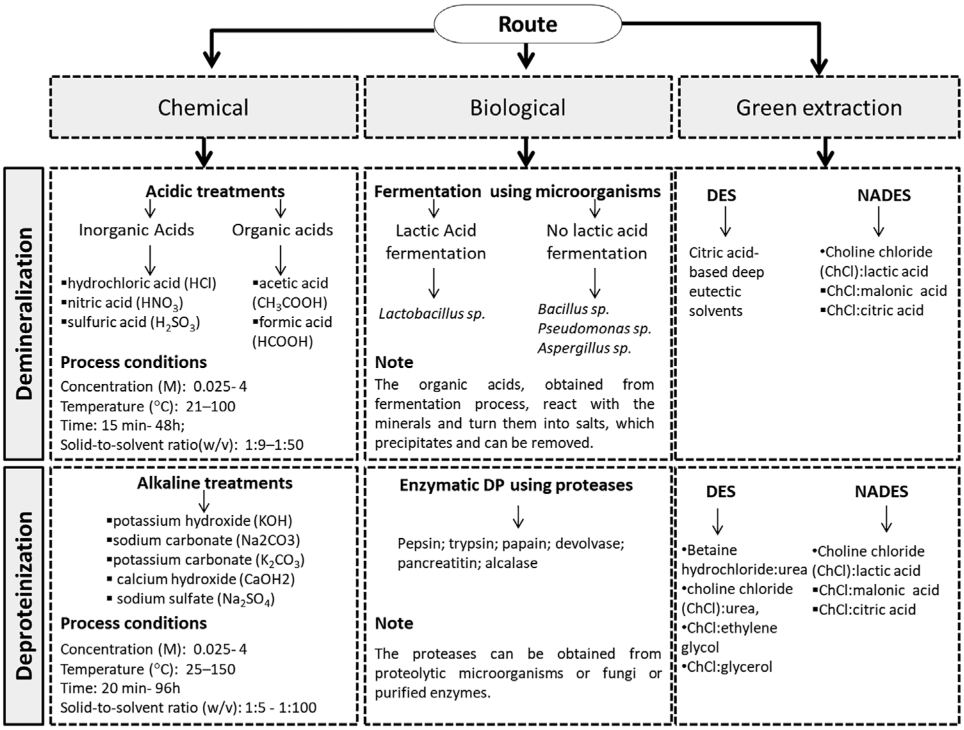


FIGURE 5 Main procedures for chitin extraction

eco-friendly alternative procedures for the recovery of several products. These procedures use green solvent mixtures, such as deep eutectic solvents (DESs)—formed by a hydrogen bond donor and a hydrogen bond acceptor with a certain molar ratio—and natural deep eutectic solvents (NADESs)—consisting of several compounds, including primary metabolites of plants, such as sugars, organic acids, AAs, alcohols, and amines (Maddaloni et al., 2020). DESs and NADESs are a new class of ionic liquids, widely used in extraction procedures thanks to their high ability to dissolve and extract materials from natural sources. In addition, these mixtures can be prepared easily, and their price is low (obtainable from low-cost components), nontoxic, low flammable, and biodegradable (Maddaloni et al., 2020; Saravana et al., 2018; Zhao et al., 2019; Zhu et al., 2017). However, the scientific literature on chitin extraction by green methods is still scarce, and additional specific studies shall be carried out. A summary of chitin extraction routes is reported in Figure 5.

The most recent research studies on chitin recovery from TM are conducted chemically in two steps. The first demineralization step is performed by using diluted acids, such as mineral acids (e.g., hydrochloric acid) or organic acids (e.g., acetic acid), to decompose minerals contained in the cuticle into their respective water-soluble salts. These salts are then separated from chitin by filtration and washing of the solid phase. The second deproteinization step allows proteins removal using alkaline treatments, mainly with a dilute solution of sodium hydroxide. Part of the lipids contained in the exoskeleton can be also extracted. The efficiency of demineralization and deproteinization depends on the reagent concentration, solid-to-solvent ratio, time, and treatment temperature. An additional step of insect chitin decolourization can be performed mainly using sodium hypochlorite, acetone, and hydrogen peroxide, to delete residual pigments involved in the insect cuticle coloration and to improve the purified chitin color (Hahn et al., 2018). Table 6 shows the newest methods for chitin extraction from TM, used as raw material.

The chitin quantification in insects is difficult, as this molecule is not soluble in water nor most solvents. The chitin content in insect materials can be indirectly measured by different methods. The gravimetric determination from acid-detergent fiber (ADF) measures the chitin as the insoluble residue obtained after acid extraction. The total amount of AAs from the ADF fraction is subtracted. This method is not specific for chitin but for all non-acid-labile compounds (Hahn et al., 2018).

Sipponen et al. (2018) have estimated the relative amount of chitin in insects by fluorescence microscopic observation of Calcofluor White stained chitin in air-classified insect fractions. The chitin amount was visually compared, but accurate levels of chitin were not studied. A more accurate and specific measure of the chitin content in TML and their isolated protein-rich fractions was recently performed by Han et al. (2021), using a UPLC/FLR method that requires a prior alkaline deproteinization, followed by acid hydrolysis of chitin. This method displayed good performance resulting in sensitive and specific quantification of insect chitin. The authors reported that the chitin levels in TML were approximately 5% dry matter, with chitin expressed as the amount of GlcNAc. However, this expression may have resulted in a slight overestimation, considering the polymerization degree of insect chitin.

Smets and Van Der Borght (2021) investigated and validated a procedure for determining chitin after its hydrolysis into glucosamine via UPLC/MS. This study shows chitin recoveries close to 100% and high specificity of the procedure, as no interference was observed from cellulose.

The insect chitin deacetylation for chitosan production commonly occurs using sodium hydroxide ranging from 40% and 60%, at 90–150°C for 1–9 h, with a few exceptions of longer times (up to 2 days). Occasionally, sodium borohydride, used as the protecting reagent, was combined with sodium hydroxide. As well as for demineralization and deproteinization, the efficiency of deacetylation can be optimized by adjusting alkali concentration, temperature, time, and solid-to-solvent ratio, other than particle size (Han et al., 2021).

The most recent protocols of TM chitin deacetylation and characteristics of respective chitosan are reported in Table 7, while the three routes for chitosan recovery are shown in Figure 6.

The chemical method for chitin deacetylation is the most commonly used, but it is characterized by high environmental impact and high cost, severely limiting its scale-up and industrial applications. The biological method for chitin deacetylation uses chitin deacetylase enzymes produced by fungi or bacteria. It is a valid alternative to chemical procedures and an eco-friendly process also due to its mild reaction conditions. However, the high cost of enzymes and the long reaction time limit the scale-up of this process (Maddaloni et al., 2020). An efficient and green process to obtain chitosan by chitin deacetylation is based on the use of glycerol, a by-product of biodiesel considered a recyclable, stable, and green solvent (Liu et al., 2017). However, this green chemical process needs high temperatures for a long time. Ultrasound or microwave technologies enhance the deacetylation of chitin, reducing extraction times with lower levels of sodium hydroxide. Ngo et al. (2017) evaluated the deacetylation process of chitin with and without low-frequency ultrasound. The results were similar for the two cases, but the ultrasound technology provides the same degree of deacetylation in less time, particularly in low concentrations of sodium hydroxide. Fiamingo et al. (2016) used high-intensity ultrasound irradiation in a multistep deacetylation process. They obtained high MW chitosan, randomly deacetylated. El Knidri et al. (2019) tested microwave heating to extract chitin and chi-

TABLE 6 Methods for chitin extraction from *Tenebrio molitor* (TM)

TM: Stage/body part	Demineralization step: Reagent and concentration	Demineralization step: Temperature (°C)	Demineralization step: Duration (h)	Deproteinization step: Reagent and concentration	Deproteinization step: Temperature (°C)	Deproteinization step: Duration (h)	Chitin Yield (%)	Ref.
Larvae	HCl, 1.25 M	20	6	NaOH, 1.25 M	80	24	4.72	Son et al., 2021
Larvae	HCl, 7% (v/v)	24	24	NaOH, 10% (v/v)	80	24	4.60	Shin et al., 2019
Superworm							8.40	
Adult							3.90	
Adult	HCl, 2 M	50	24	NaOH, 2 M	50	24	11.6	Sáenz-Mendoza et al., 2018
Larvae	HCl, 1 M	30	2	NaOH, 2 M	90	2	2.5	Luo et al., 2019
Larval exuvium	HCl, 2N	20	3	NaOH, 1.25N	95	3	18.01	Song et al., 2018
Whole body							4.92	

TABLE 7 *Tenebrio molitor* (TM) chitin deacetylation and characteristics of respective chitosan

	Deacetylation			Chitosan obtained		
TM:Stage/body part	**Reagent and concentration**	**Temperature (°C)**	**Duration (h)**	**Yield (%)**	**Deacetylation degree (%)**	**Ref.**
Larvae	NaOH, 50%	80	4	—	89.4	Son et al., 2021
Larvae	NaOH, 55%	90	9	80	75.59	Shin et al., 2019
Superworm				78.33	75.63	
Adult				83.33	75.67	
Adult	NaOH, 60%	120	2	—	88.55	Sáenz-Mendoza et al., 2018
Larvae	NaOH, 60%	100	8	2.5	—	Luo et al., 2019
Larvae	NaOH 40%,	105	3	3.65	91.90	Song et al., 2018
	NaOH 50%				96.19	

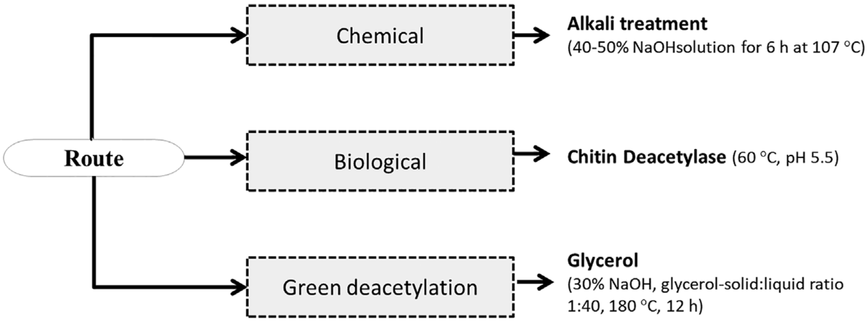


FIGURE 6 Chitosan extraction routes

tosan from shrimp shells. The authors report that chitin deacetylation leads to a high deacetylation degree (DD) in a few minutes.

Different chitin DD can be achieved using the different methods discussed in the text, based on the reaction parameters, such as temperature, time, and solvent concentration (He et al., 2016; da Silva Alves et al., 2021). Commercial chitosan preparations have DD degree within 70%–95%, and MW ranging from 104 to 106 g/mol (Kaczmarek et al., 2019). The large-scale production of the full chitosan (100% DD) is challenging due to the risks of side reactions and chain depolymerization (Morin-Crini et al., 2019; He et al., 2016).

4 | BIOLOGICAL EFFECTS OF TM-BASED PRODUCTS

4.1 | Powder and extracts

Much research has focused on the effects of an insect-based diet on animal and human health. Some authors have shown that TM feeds have no negative effects on animal health (Table 8); others have found that in some cases, they even have positive effects (Table 9). Many organisms have been studied, starting from the animals that usually feed on them (chickens, fishes) passing through monogastric land animals (chickens and pigs), up to mice. In vitro effects on murine and human cells were finally evaluated.

The possibility of replacing (at least partially) the diet with an insect-based meal is significant in aquaculture because in the last years, the availability and price of fishmeal, one of the main components in farmed fish diets, has decreased while its price has increased. The necessity to replace fishmeal with vegetable flours is ongoing, but it poses several issues as plant protein sources have shown adverse effects, such as highly variable protein content, EAA imbalances, and anti-nutritional factors, which limit their use in diet formulations (Egerton et al., 2020).

Insects use in aquafeeds has been approved by the European Commission (Annex II of Regulation 2017/893 of May 24, 2017), which authorized insect-derived proteins from seven insect species: two flies, two mealworms, and three crickets.

TM meal is also widely used for feeding chickens, as it is very rich in proteins, replacing fish and soy meal. Compared to these, however, it is poorer in calcium, thus calcium supplementation is required in poultry diets (Selaledi et al., 2020).

Given the promising results on other animals, many studies have recently also been conducted on mice to

TABLE 8 No negative effects of *Tenebrio molitor*-based diet on different animals

Organism tested	Feed	Parameters tested	Effects observed	Ref.
Chickens (female chickens farmed for 54 days)	TM meal as a replacement of corn gluten meal	Intestinal morphometric measurement (villus height and crypt depth in duodenum, jejunum, and ileum); blood and serum parameters	No effects on the welfare, productive performance or morphological features. Some histological changes and alteration in both the control and the diet with TM meal	Biasato et al., 2016
Weanling pigs (farmed for 28 days)	Dried mealworm as (partially) replacement of fishmeal (50% and 100%).	Feed intake, gain to feed ratio and average daily gain, growth performances, nutrient digestibility, immunological parameters (blood characteristics, cytokine secretion), gut morphometric parameters	No negative effects with the 50% replacement of fishmeal on the growth performance and blood parameters	Ko et al., 2020
Shrimps (Pacific white shrimps (*L. vannamei*) fed for 8 weeks)	Five tested diets designed to gradually reduce the proportion of fishmeal, while increasing the content of insect meal (0% as the control), 25%, 50%, 75%, and 100%)	Growth and feed conversion; Challenge test using *Vibrio parahaemolyticus*; immune parameters (total hemocyte, phenol oxidase activity, hemolymph protein, clearance ability)	No negative effects on shrimp survival or feed ingestion even with 100% replacement of fishmeal. Improvement of growth (when combined with fish meal) and of immunity and resistance to disease and to stress	Motte et al., 2019
Fishes (Gilthead sea bream (*Sparus aurata*) raised for 163 days)	Three tested diets: - A control diet (fishmeal) - Two tested diets with addition of TM meal (25% and 50%)	Growth performances; coefficients of total tract apparent digestibility; somatic indexes, slaughter traits, and marketable traits. These data were utilized to calculate the dressed yield, intestinal length/fish total length ratio and condition factor, as well as hepatosomatic and viscerosomatic index	25% TM meal diet: no negative effects on weight gain, crude protein and ether extract digestibility, and some post mortem traits; higher feed conversion ratio and protein efficiency ratio compared to the control 50% TM meal diet: no negative effects on growth performance, although nutrient digestibility was penalized and a lower dressed yield was observed	Piccolo et al., 2017
Fishes (juvenile mandarin fish (*Siniperca scherzeri*))	Three tested diets: 10%, 20%, and 30% TM meal added to the control diet. TM meal-rich diets were further supplemented with crystalline methionine and fish oil to avoid and/or compensate for any essential AA or fatty acid imbalances	Performance indices and survival rate (weight gain, specific growth rate, daily feed intake, feed efficiency, protein efficiency ratio, feed conversion ratio, protein retention, lipid retention, percentage of survival, condition factor); blood and immunological parameters; hepatosomatic and viscerosomatic indices	Positive effects: faster growth, more efficient use of feed, better humoral immune response and antioxidant defense status. Drawback: fish fillet has higher levels of saturated and monounsaturated fatty acids and lower levels of n-3 PUFA which is not ideal characteristic for human consumption	Sankian et al., 2018

TABLE 9 Positive effects of *Tenebrio molitor*-based diet on different animals

Organism tested	Feed	Parameters tested	Effects observed	Ref.
Fishes				
Rainbow trout farmed for 154 days (*Oncorhynchus mykiss*, Walbaum)	Four experimental diets, with increasing levels of a partially defatted TM meal to replace fishmeal (0%, 25%, 50% and 100%). All the diets were suitably added with wheat gluten, wheat meal, sardine oil and AAs	Growth performance (somatic indexes), diet digestibility, hepatic intermediary metabolism, hepatic AA catabolic and lipogenic enzymes	No negative effects on: fish growth, condition factor, and activity of hepatic AA catabolic and lipogenic enzymes. The apparent distestibility coefficients are high for all the TM meal diets (negative effects only for crude proteins)	Chemello et al., 2020
Rainbow trout farmed for 90 days. (*O. mykiss*) juveniles	A fishmeal-based control diet. Four different diets obtained by replacing fishmeal with 20%, 30%, 60%, and 100% TM protein meal. Squid and krill meal levels were kept constant across all diets to ensure palatability	Growth performance, whole-body composition, nutrient retention, apparent digestibility, nutrient utilization	100% defatted TM protein meal leads to the best growth performance and efficiency of feed conversion ratio	Rema et al., 2019
Monogastric land animals (chickens and pigs)				
Boiler chicks (male broiler chicks vaccinated against Newcastle disease and infectious bronchitis)	Five tested diets: four containing different percentages of dried insect meal (0%, 2%, 4%, and 8%) and one diet containing fresh insect meal (10.48%)	Growth performances; hematological characteristics; carcass and meat quality	The inclusion of 4% dried TM meal improves growth performance in the started phase. Similar effects are observed with both dried and fresh TM	Elahi et al., 2020
Growing pigs (surgically equipped with simple T-cannulas and farmed for 2 weeks)	Four tested diets with 10%: - defatted TM meal - hydrolysate of TML - fermented poultry by-product - hydrolyzed fish soluble	Contents of: nitrogen, crude protein (calculated), crude fat, fatty acids, total AA (methionine and cisteine analyzed independently); chromium concentrations	Higher digestibility compared to dietary supplementation with fermented poultry by-product and hydrolyzed fish soluble. High nitrogen solubility and in-vitro crude protein digestibility of TML hydrolysate	Cho et al., 2020b
Piglets farmed for 28 and 56 days	- Control diet (soybean-based diet + 5% plasma protein powder) - Tested diet in which 5% plasma protein powder was replaced by TM meal	Free AAs profiles in plasma and in the intestinal mucosa, AA transporter and sensing gene expression in intestinal mucosa	Improvement of amino acid transportation in the intestine (through regulating the sensing gene)	Liu et al., 2020

(Continues)

TABLE 9 (Continued)

Organism tested	Feed	Parameters tested	Effects observed	Ref.
Growing pigs (male, 5-week-old crossbred pigs farmed for 4 weeks)	- Control diet (soybean meal) - Two tested diets with 5% and 10% TM meal, respectively.	Growth performance and ileal digestibilities of AAs; hepatic and skeletal muscle's transcriptomes; plasma AAs, metabolites, carnitine species and bile acids; triglyceride, cholesterol, phospholipid and sphingolipid levels (in liver and plasma); hepatic mRNA levels of lipogenic and cholesterogenic genes; correlations between phospholipid parameters and mRNA levels in liver	Moderate differential regulation of many transcripts in liver and skeletal muscle; alterations in the plasma levels of several AAs and the AA metabolite methionine sulfoxide. No alterations in the plasma levels of major carnitine/acylcarnitine species and circulating bile acid species; No alterations in liver and plasma levels of main lipid classes	Meyer et al., 2020a
Growing pigs (male, five-week-old crossbred pigs farmed for 4 weeks)	- Control diet (soybean meal) - Two tested diets with 5% and 10% TM meal, respectively	Cecal microbiota composition and diversity, concentrations of SCFA in cecal digesta, bile acid concentration in the feces	Changes in the relative abundance of high-abundance and low-abundance bacterial taxa. TM meal is rich in crude fiber, so this effect cannot be ascribed to specific constituents, such as chitin	Meyer et al., 2020b
Mice				
Male ICR-based mice (male mice divided in 2 groups: one control and one with acute liver damage induced by intraperitoneally carbon tetrachloride injection)	Four tested diets: - The negative control - The positive control silymarin administered group (Sily, 200 mg/kg/day) - Fermented TM extract (500 mg/kg/day), - Non-fermented TM extract (500 mg/kg/day)	Serum biochemical parameters (total bilirubin and albumin, TNF-α and IL-6 levels triglycerides, total cholesterol, HDL-cholesterol and free fatty acids, AST and ALT levels); lipid content and antioxidant enzyme activity of liver tissue; morphological alterations of liver tissue	Serum free fatty acids and IL-6 as well as acute liver injury caused by carbon tetrachloride reduced in the fermented extract treated group compared with the non-fermented extract treated group and negative control	Choi et al., 2019
Hyperlipidemic obese Zucker rats (male homozygous (fa/fa), obese Zucker rats). Male heterozygous (fa/+), lean Zucker rats are used as the control	- Control diet (100% casein) - Two tested diets with 50% and 100% of TM meal	Daily weight gain and feed intake; hepatic transcript profiling (expression of 642 genes), bioinformatic analysis, gene expression and gene activities. Hepatic, plasma and tissue levels of lipids, carnitine and its precursor, methionine and its metabolites	Beneficial effects on metabolic health and pronounced lipid-lowering effects in liver and plasma	Gessner et al., 2019
Ovariectomized ddY mice	Four tested diets: - Control with vehicle (dH_2O) - Control with TM extract (10 ml/kg/day) - Ovariectomized mice with vehicle (dH_2O) - Ovariectomized mice with TM extract (10 ml/kg/day)	Body weights; estradiol and corticosterone levels; glucocorticoid receptor, cell proliferation and neuroblast expression levels and distribution in the hippocampus; bone histology	Increased cell proliferation and neurogenesis in the hippocampal dentate gyrus. Compensatory effects on attenuation of GR overexpression at the hippocampus. Anti-osteoporosis effects with ability to prevent decline of brain function	Kim et al., 2019

(Continues)

TABLE 9 (Continued)

Organism tested	Feed	Parameters tested	Effects observed	Ref.
Chronic alcohol-fed rats (male)	Six tested diets: - Control liquid diet - Alcohol liquid diet - Alcohol liquid diet + low-dosage fermented TM extract (50 mg/kg/day) - Alcohol liquid diet + medium-dosage fermented TM extract (100 mg/kg/day) - Alcohol liquid diet + high-dosage fermented TM extract (200 mg/kg/day) - Alcohol liquid diet + silymarin (200 mg/kg/day)	Food intake (daily) and body weight (weekly); serum parameters (AST, ALT, albumin, total bilirubin, g-GTP, TNF-α, IL-6 levels triglycerides, total cholesterol, HDL-cholesterol and free fatty acids); antioxidant enzyme activity; expression of lipogenesis-related genes	Ameliorated alcoholic hepatosteatosis, downregulated lipogenesis-related gene expression, inhibited alcohol-induced inflammatory response, reversed alcohol-induced antioxidant defense system dysfunction, altered the bacterial community composition	Choi et al., 2020
Spontaneously hypertensive rats. Wistar Kyoto rats as controls	Three tested diets: - Standard diet - Standard diet + TM - Standard diet + Captopril	Blood pressure and heart rate; plasma and brain ACE and inhibitory activity; red blood cells and plasma thiols; liver cytochrome P450 and b5 content, NADPH-cytochrome P450 reductase activity; brain IL-6, IL-1β, and TNF-α Ex-vivo functional studies (aorta ring mechanical activity, isolated heart preparation and perfusion, brain susceptibility to oxidative stress-mediated injury)	Antihypertensive, cardio- and neuro-protective effects, anti-obesity properties. Inhibition of the intrinsic pathways of blood clotting. Some effects of TM meal are not shared by captopril, so the former has added value in preventing hypertension and mitigating other severe cardiovascular risk factors	Pessina et al., 2020
Mice (male)	Five tested diets: - Negative control group (CMC Na 1%) - Positive control with glibenclamide (5 mg/kg body weight) - TM meal 9 mg/kg body weight - TM meal 45 mg/kg body weight - TM meal 90 mg/kg body weight	Oral glucose tolerance	TM meal can reduce blood glucose level (for 120 min) at doses of 45 and 90 mg/kg body weight, but has lower decreasing power than glibenclamide	Samsul et al., 2020

Abbreviations: AA, amino acid; ALT, aspartate transaminase; AST, alanine transaminase; CMC: carboxy methyl cellulose.; g-GTP, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein;IL-1β, interleukin-1β; IL-6, interleukin-6; NADPH, nicotinamide adenine dinucleotide phosphate; SCFA, short chain fatty acids; TM, *Tenebrio molitor*; TNF-α, tumor necrosis factor alpha.

TABLE 10 Possible positive effects of *Tenebrio molitor* larvae extracts on human health

Possible positive effects	Ref.
Protect against hepatocellular carcinoma	Lee et al., 2015
Inhibit BACE-1 enzyme activity related to accumulation of β-amyloid	Youn et al., 2014
Alleviate obesity (in vitro and in vivo)	Seo et al., 2017
Anti-thrombosis, anti-oxidant and hemolytic activities against human red blood cells	Pyo et al., 2020
Promote platelet aggregation	Pyo et al., 2020
Antioxidant capacity and anti-inflammation activity	Son et al., 2020
Antioxidant and tyrosinase inhibitions activities, perhaps also skin-whitening effects	Kim et al., 2018

Abbreviation: BACE-1, β-secretase 1.

study the positive effects of the TM-based diet that could also be helpful for the prevention or treatment of human diseases.

Some studies valued the ability of TML extracts to reduce adipogenesis and obesity (Seo et al., 2017). The drugs currently used to reduce obesity, in fact, despite being very effective, have many side effects, so research is now testing new natural solutions such as extracts of algae, plants, and recently also insects. The authors studied the effects of TML powder and ethanol extract on mouse 3T3-L1 preadipocytes and focused on some parameters as in vitro cytotoxicity, intracellular lipid accumulation, intracellular triglyceride content, gene expression, and protein production. In vivo studies investigated the effects of a TML diet on mice fed on different percentages of TML; both studies demonstrated the anti-obesity activity of TML extracts (Seo et al., 2017). In a more recent study, Lee et al. (2020) determined the effects of extracts from larvae of TM and *Allomyrina dichotoma* L. in a human hepatoblastoma (HepG2) cell line and animal models C57BL/6 male mice. In addition to the parameters considered in the study mentioned above, some serum parameter analyses (aspartate aminotransferase [AST], alanine aminotransferase [ALT], glucose, triglycerides, high-density lipoprotein (HDL) cholesterol and total cholesterol) and hepatic triglycerides assay were also considered. This study demonstrated that both larvae reduce intracellular lipid content and lipid accumulation in the liver tissues, by inhibiting lipogenesis-related genes based on antioxidant activity and can attenuate hepatic damage and symptoms of non-alcoholic fatty liver disease.

A very particular study is the one conducted by Hwang et al. (2019), in which the authors investigated the effects on ethanol-damaged HepG2 cells of koji, a fermented oriental food obtained by growing the filamentous fungus *Aspergillus oryzae* on a cereal (rice or barley) or soy prepared from TML instead from usual substrates.

TML paste and sauce have an inhibitory effect on AST and ALT enzyme activity, downregulating cytokines' expression, which are biomarkers of inflammation, and increase antioxidant effects in ethanol-damaged hepatocytes.

Some studies highlight the potential positive effects of TML-based products on humans (Table 10), but further experimental evidence is still needed to assess their influence on human health.

4.2 | Oil, lipids, and fatty acids

As already mentioned, TML oil is rich in MUFAs and PUFAs that show anti-inflammatory and healthy properties functionally against cardiovascular diseases and exhibit a significant impact on maternal lactation and the child's brain development during pregnancy (Koletzko et al., 2008).

TM oil can be used as an ingredient in nutrition, given its similar characteristics to vegetable oil, as Son et al. (2020) found in their study that compared two kinds of oil. TM oil is rich in bioactive nutrients, particularly γ-tocopherol, which accounted for 85.6% of the total tocopherol. Tocopherol is an antioxidant that prevents lipid oxidation in free radicals and hydroperoxides in food; plants and alga synthesize it in nature. TM oil has a polyphenol content equal to 10%–20% of olive and grape seed oil, while the cholesterol content (associated with atherosclerosis) is so low that it is impossible to quantify.

Mlček et al. (2019) analyzed the presence of sterols in edible insects, particularly in TM and in *Zophobas morio*. They found that the cholesterol content in TM is similar to that found in beef tallow or (northern) lobster. Cholesterol levels are lower in nutritionally stressed TML. In addition, TM contains phytosterols (stigmasterol and β-sitosterol), typical plant sterols that act as cholesterol antagonists, thus balancing the cholesterol levels. Mlček et al. observed that cholecalciferol (vitamin D3) content in TM is three to four times higher than that of animal products, except fish whose value is comparable. Therefore, TM can be a good source of cholecalciferol with a recommended daily intake of 25 g of dried mealworms.

In their study, Benzertiha et al. (2019) evaluated replacing palm oil and poultry fat with TM oil on growth performance, nutrient digestibility and pancreatic enzyme activity, some blood parameters, liver FA compositions, and breast muscle tissues in female Ross broilers. Birds fed on a diet supplemented with 5% palm oil, poultry fat, or TM oil were compared. With TM oil, reduced liver size, liver triglyceride concentration, and total cholesterol, the presence of n-3 and n-6 fatty acids in the breast muscle tissue of chickens also increased. TM oil, having the highest amount of PUFAs, may have resulted in the low concentration of hepatic triglycerides due to the PUFAs inhibition of hepatic lipogenesis, that is, the process through which acetyl-CoA is converted into triglycerides. These results agree with those obtained by Sosa and Fogliano (2017). In addition, Benzertiha et al. (2019) observed that TM oil diet leads to a decrease in amylase activity; no influence was observed on the activity of lipase and trypsin and in the size of the pancreas. The authors conclude that TM oil can be used as a source of fat in broilers' diets, as an alternative to palm oil. That has several adverse effects on the environment, related to deforestation and therefore loss of biodiversity, and from the nutritional point of view, associated with the high ratio of SFAs.

Dabbou et al. (2020) evaluated in vitro antimicrobial activity of two insect fats, *Hermetia illucens* and TM, to assess their effectiveness as a total replacement to soybean oil in the cecal fermentation and intestinal microbiota of growing rabbits. The use of fats extracted from HI and TM retards the growth of both Gram-positive and Gram-negative pathogenic bacteria. Moreover, these fats stimulate volatile FA production at the cecum and positively affect the cecal and fecal microbiota of rabbits. The authors also found that, compared to other diets, TM supplementation reduces the relative abundance of *Klebsiella*, *Lachnospira*, *Parabacteroides*, and *Odoribacter*, while enriching the presence of *Clostridiales*, *Desulfovibrionaceae*, *Ruminococcus*, and *Akkermansia*, which are the major taxa in the gut microbiota of rabbits. *Akkermansia* is important because it can be considered as a new generation probiotic that degrades mucin in the intestine with the production of beneficial molecules such as short-chain FAs (SCFAs), thus exerting a significant improvement in the intestinal barrier and maintenance of intestinal health.

Dabbou et al. stated that insect fat in feed could control the growth of microbial pathogens, such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Pasteurella multocida*, thus leading to improved animal welfare. Insect fat is also beneficial for food safety in the slaughtering phase, as it reduces the gut flora that can contaminate rabbit meat during slaughtering operations. Moreover, insect fat in feed could limit classical antibiotics in meat rabbit production, thanks to its antimicrobial molecules.

Gasco et al. (2019) also evaluated the effect of fat from *H. Illucens* and TM supplementation to the rabbits' diet, in place of soybean oil, on growth performance, nutrient digestibility, blood parameters, intestinal morphology, and health of growing rabbits. The different diets fed to rabbits were similar in major crude protein and gross energy content, while their fiber contents differed. Substituting insect fat in place of soy changed the FA profile of lipids in the diets; total SFA in *H. Illucens* and MUFA in TM were higher. No changes were found in other parameters, precisely no negative effect: this concludes that insect fat can replace soy fat in rabbit diets.

Insect lipids as an alternative ingredient is an emergent topic in animal nutrition for their many beneficial effects, as shown in Table 11.

4.3 | Proteins, peptides, and bioactive molecules

Many TML proteins and peptides show various biological and regulatory functions in humans (Figure 7). To date, there is no direct scientific evidence of their effects on humans, but only studies conducted in vitro and on experimental animal models. These studies show the wide range of biological activities of some TML molecules and their potential uses. However, TML are far from being considered a source of drugs. The following sections report the main TML activities found in the literature.

4.3.1 | Antimicrobial activity

Insect hemolymph possesses several antimicrobial peptides (AMPs), small cationic peptides that exhibit a broad spectrum of antimicrobial activity ranging from Gram-positive and Gram-negative bacteria to protozoa, viruses, and fungi (Browne et al., 2020; Wu et al., 2018). These AMPs are involved in the humoral response in insect defense (Sheehan et al., 2018) and are divided into three groups, based on their biological targets: defensins, which act against Gram-positive bacteria; diptericins, attacins, drosocins, and cecropins, which act against Gram-negative bacteria; drosomycins and metchnikowin, which act against fungi (Lemaitre & Hoffmann, 2007).

A Korean research team has studied various molecular aspects of TM immune response, and due to its large size, they were able to easily collect enough hemolymph needed to purify some AMPs, serine proteases, and serine protease inhibitors (serpins). Using a purely biochemical approach, they also determined the molecular activation of the TM Toll signaling pathway (Kim et al., 2008; Roh et al., 2009; Yu et al., 2010) and identified and

TABLE 11 Biological effects of the *Tenebrio molitor* larvae (TML) oil

Organism tested or in vitro test	Biological effects of TML oil	Uses and shelf-life	Ref.
Rabbit	Feed ingredient. Positive effects on the gut microbiota and cecal fermentation. MCFAs (caproic acid and caprylic acid): antimicrobial activity against *Salmonella typhimurium*; beneficial effects on intestinal health and microbial growth inhibition; positive influence on the digestive health. SCFAs (propionic acid and butyric acid): antimicrobial activity against *Salmonella typhimurium*.	Substituted for dietary soya oil, produces a positive influence on cecal fermentation and the intestinal microbiota of growing rabbits. Sustainable lipid alternative with possible interesting applications in the feed industry.	Dabbou et al., 2020
Molecular docking studies	Inhibitory activity of the BACE 1 enzyme by oleic and linoleic acid.	For treatment of Alzheimer's disease.	Youn et al., 2014
Broiler chicken	Decreasing the fat content in the liver tissue (hepatic triglycerides and total cholesterol). Very good performance on chicken: nutrient digestibility, pancreatic enzyme activity, various blood parameters and lipid fatty acid composition of liver and breast muscle tissues.	Total replacement for palm oil in chicken diets. Alternative feed ingredients in broiler chicken with good performance.	Benzertiha et al., 2019
Human 14 hepatocellular carcinoma (HepG2) and colorectal adenocarcinoma (Caco-2)	Antiproliferational effects against hepato-human cell carcinoma and colorectal adenocarcinoma due to the presence of omega-3 fatty acids, oleic acid and palmitic acid in TML oil.	Treatment of liver diseases including cancer (in Asia). TML oil might be used in the development of natural cancer therapeutics.	Wu et al., 2020
Rabbit	Dietary replacement of soybeans oil with TML fats is favorable in growth performance, apparent digestibility, intestinal mucosa traits and health, and therefore is a suitable source of lipids in rabbit diets to replace soybeans oil.	Alternative to soybean oil in rabbit diet.	Gasco et al., 2019

Abbreviations: APP, amyloid precursor protein human; BACE-1, β-secretase 1; MCFAs, medium chain fatty acids; SCFAs, short-chain fatty acids; TML, *Tenebrio molitor* larvae.

purified four AMPs from TM larval hemolymph (Table 12). The first antibacterial protein, called Tenecin 1, was purified to homogeneity, characterized, and fully sequenced; it is a defensin-like molecule that targets Gram-positive bacteria and consists of 43 AA residues, including 6 cysteine residues (Moon et al., 1994). Tenecin 2, instead, is a molecule similar to coleoptericin and diptericin that targets Gram-negative bacteria (Roh et al., 2009). Tenecin 3 is a thermostable peptide made up of 78 AAs, particularly rich in glycine (43.6% as a molar percentage) that shows marked antifungal activity (vs. *Candida albicans*) and very little antibacterial activity (vs. *Escherichia coli* and *Staphylococcus aureus*). Comparative and in-depth studies on its mechanism of action have demonstrated the existence and membership of a family of antifungal proteins whose production represents a kind of evolution by the insect in the fight against fungal pathogens widespread in natural ecosystems (Jung et al., 1995; Maistrou et al., 2018). Tenecin 4, the last AMP purified so far, is a protein of 14 kDa which contains 14% glycine residues. Data obtained show that Tenecin 4 is a defense protein only against Gram-negative pathogens and is induced by multiple ligands in TML (toll cascade activating ligands and monomeric meso-diaminopimelic peptidoglycan) (Chae et al., 2012).

AMPs now draw much attention to studying their mechanisms of action in bacterial infections (Vigneron et al., 2019) to exploit this potential as novel therapeutic agents (Zasloff, 2019).

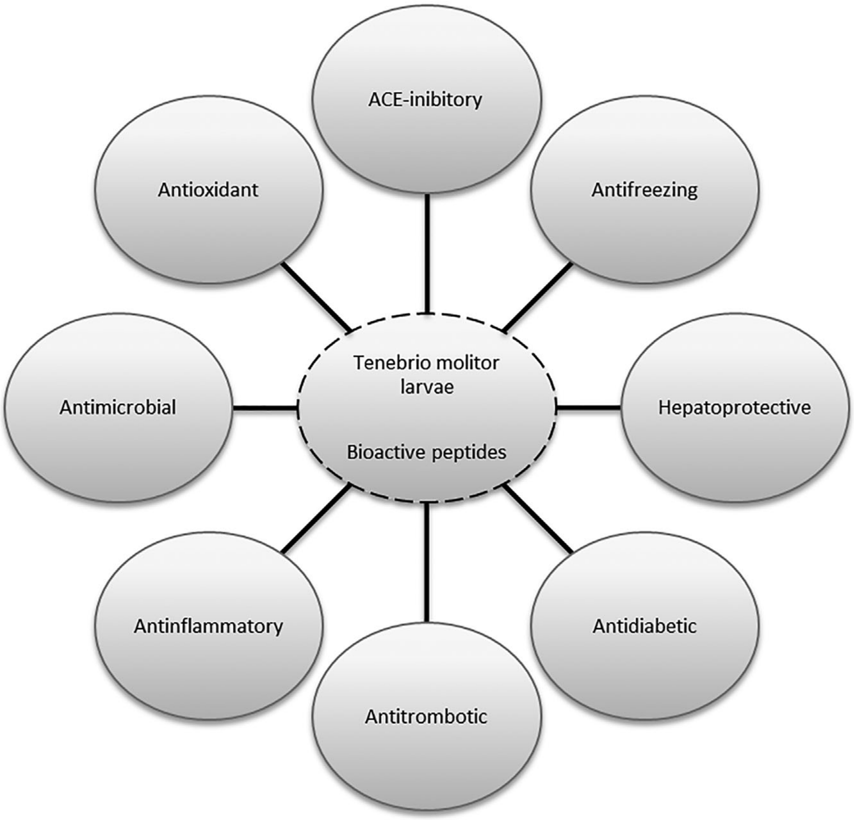


FIGURE 7 Properties of bioactive peptides from *Tenebrio molitor* larvae (in vitro studies)

4.3.2 | Antithrombotic activity

Thrombosis is a prevalent pathology of the circulatory system and is often associated with cardiovascular diseases. The current objective of scientific research is to identify antithrombotic drugs with different mechanisms of action, which are safer, more effective, and of lower toxicity than conventional antithrombotic drugs. In this context, many efforts have been made to identify bioactive components from natural sources, in particular from TML (Table 13).

Chen et al. (2019a), using Plackett–Burman design and response surface methodology, optimized conditions and parameters of the enzymatic hydrolysis process of TML with pepsin and trypsin, obtaining proteolytic peptides with an antithrombotic activity of 82.8%. To improve its application as a new antithrombotic drug, subsequent studies by the same authors focused on the purification and sequencing process of these antithrombotic peptides by using in silico screening and simulation by molecular docking at the thrombin active site. These peptides were shown to interact with the thrombin 1 exosite. This significant result would explain why these peptides show such strong antithrombotic activity (Chen et al., 2019b).

4.3.3 | Anti-ACE activity

Hypertension is a chronic disease that affects more than 30% of the adult population worldwide (Chockalingam, 2008). The main enzyme involved in this disease is angiotensin-converting enzyme (ACE), which converts angiotensin I into the powerful vasopressor octapeptide, angiotensin II.

In the last 20 years, many peptides or enzyme hydrolysates with ACE inhibitory activity have been identified and characterized from natural foods of both animal and plant origin (Gu & Wu, 2013; Neves et al., 2017). Given their high protein content (60%–70%), these peptides with antihypertensive activity have also been studied and identified from edible insects (Table 14).

Dai et al. (2013) is the first study to identify and isolate small peptides with ACE inhibitory activity from TML, using enzymatic hydrolysis with Alcalase™. The antihypertensive activity, note as inhibitory capacity (IC), was studied in vivo in spontaneously hypertensive rats, obtaining an IC50 value of 0.39 mg/ml. At the same time, the peptide identified, Tyr–Ala–Asn, showed ACE inhibitory activity with an IC50 value of 0.17 mg/ml.

In a recent study on pupae and TML, the in vitro ACE IC50 (0.097 mg/ml) of PHs obtained using a mix

TABLE 12 Extraction processes and bioactivity of the antimicrobial peptides (AMPs), extracted from *Tenebrio molitor* larvae (TML)

Matrix	Protein purification process	Peptide name sequence or molecular mass (kDa)	Antimicrobialactivities	Ref.
Hemolymph TML	• Reverse-phase (C18) open column chromatography • Reverse-phase HPLC	Tenecin 1 43 Amino acid residues (partial AA sequence) Val-Glu-Ala-Lys-Gly-Val-Lys-Leu, Asn-Asp-Ala	Antibacterial activity against Gram-positive bacteria	Moon et al., 1994
	• HPLC-CAPCELL PAK C18 column • Lyophilization of active fraction • HPLC-Synchropak RP C_{18} column • Concentration of active fraction • TSK gel G2000SW$_{XL}$ size exclusion column (TOSOH)	Tenecin 2	Antibacterial activity against Gram-negative bacteria	Roh et al., 2009
	• Heat treatment (15 min at 100°C) • C_{18} reverse-phase open column chromatography • C_{18} reverse-phase HPLC	Tenecin 3 78 amino acid residues 11 times repeated motif Gly-X-X-Gly (X = Glu, His, Leu)	Antifungal activity against *C. albicans* No antibacterial activities against *E. coli* and *S. aureus*	Jung et al., 1995
	• HPLC-CAPCELL PAK C18 column • YMC ODS Pack C_{18} column • CAPCELL PAK C_{18} column (MG-type) • Symmetry C_{18} column (Waters)	Tenecin 4 14-kDa 120 amino acid	Antibacterial activity against Gram-negative bacteria (*E. coli*) No antibacterial activity against Gram-positive bacteria (*Bacillus subtilis*) and antifungal activity against C. *albicans*	Chae et al., 2012

Black-filled bullets indicate the different steps in the purification processes.
Abbreviations: Ala, alanine; Asn, asparagine; Asp, aspartic acid.; Glu, glutamic acid; Gly, glycine; His, histidine; HPLC, high performance liquid chromatography; Leu, leucine; Lys, lysine; TML, *Tenebrio molitor* larvae; Val, valine.

of gastrointestinal proteases (pepsin, α-chymotrypsin, and trypsin) was much lower than that obtained in the work of Dai et al. in 2013, possibly due to the different types of analytical assay used (Cito et al., 2017).

Recently, TM hydrolysates obtained by enzymatic treatment using commercial food-grade proteases (e.g., subtilisin, trypsin, ficin, and Flavourzyme™) have been investigated, both alone and in combination. The authors assessed the influence of enzyme treatment and degree of hydrolysis (DH) on ACE inhibitory activity in vitro: both of them had a significant impact on the bioactivity of the hydrolysates obtained (trypsin at 10% DH IC50 0.27 mg/ml; subtilisin at 20% DH IC50 0.35 mg/ml) (Rivero-Pino et al., 2020c). These natural compounds, obtained by enzymatic hydrolysis, have recently been considered proper biological regulators that exercise physiological functions in the body and could therefore be used as components of functional foods to prevent certain diseases (Perez-Gregorio & Simal-Gandara, 2017).

4.3.4 | Anti-diabetic activity

Many bioactive peptides extracted and isolated from plants, animals, and microorganisms can be considered natural alternatives for managing the non-insulin-dependent form of diabetes mellitus (type 2). These peptides can inhibit the activity of many enzymes involved in the carbohydrate metabolism their action results in a modulation of blood glucose levels and a reduction in insulin resistance (Yan et al., 2019c).

TABLE 13 Extraction, purification processes and bioactivity of the antithrombotic peptides, extracted from *Tenebrio molitor* larvae

Matrix	Protein hydrolysis and purification processes	Peptide sequence or molecular mass (kDa)	Activities (%)	Ref.
Defatted TM powder	Hydrolysis with tripsine + pepsine (the significant variables were selected according to the Plackett–Burman design and optimized by the response surface methodology)	nd	Antithrombotic activity 82.8%	Chen et al., 2019a
	Hydrolysis with tripsine + pepsine 3 different purification processes:	SLVDAIGMGP AGFAGDDAPR	Antithrombotic activities of the 3 processes:	Chen et al., 2019b
	a. Ion exchange chromatography		a) 40.87% (8.0 mg/ml)	
	b. Gel filtration chromatography		b) 65.61% (8.0 mg/ml)	
	c. Reverse-phase liquid chromatography		c) 28.66% (0.2 mg/ml)	

Abbreviation: TM, *Tenebrio molitor*.

TABLE 14 Enzymatic extraction processes and bioactivity of the ACE-inhibitory peptides, extracted from *Tenebrio molitor* larvae (TML) and pupae

Matrix	Protein extraction processes	Enzymatic hydrolysis process (enzyme (s) and/or post-process)	DH (%)	Peptide sequence or molecular mass (kDa)	IC 50 (ug/ml)	Ref.
TML	Defatted TML powders (defatting with petroleum ether)	• Hydrolysis : Alcalase™	20	nd	390	Dai et al., 2013
		• Separation: Sephadex G–15 gel column (Peck 2)		0.18–0.50	230	
		• Purification: RP–HPLC (P2–F6 fraction)		0.366	nd	
		• Identification: UPLC–MS/MS +		Tyr–Ala–Asn	17	
	TM Meal 46.8% w/w of protein	Food-grade protease -Subtilisin -Pancreatic trypsine -Ficin -Flavourzyme 1000L™	5–20	Small-chain peptide (0.5–2)	260–280	Rivero Pino et al., 2020c
TML and pupae	Crude Protein Extract (Buffer Tris/HCl 50 mM pH 7.4)	Hydrolysis: gastrointestinal enzymes (Pepsin. Trypsin and α–Chymotrypsin)	nd	nd	97 (larvae) 77 (pupae)	Cito et al., 2017

Black-filled bullets indicate the different steps in the purification processes.

Abbreviations: IC, Inhibitory concentration.; RP-HPLC, reversed-phase high performance liquid chromatography; TM, *Tenebrio molitor*; TML, *Tenebrio molitor* larvae; UPLC-MS/MS, ultraperformance liquid chromatography/mass spectrometry.

TABLE 15 Enzymatic extraction processes and bioactivity of the anti-diabetic peptides, extracted from *Tenebrio molitor* larvae (TML)

Matrix	Pre-treatment and extraction	Enzymatic hydrolysis process (enzyme (s) and/or post-process)	Peptide sequence or molecular mass (kDa)	Antidiabetic activity IC 50 (mg/ml) or inhibition %	Ref.
TML	• Defatting (n-hexane) • Sonication (Sonics® Vibra-Cell™ VCX 750) • Filtration • Freeze-dried	Flavourzyme 12% and Alcalase 3% (55°C for 8 h)	nd	α-glucosidase inibithion 33.5%–35.0%	Yoon et al., 2019
TM meal	• Grounding • Sonication (500 W and 20 KHz of frequency for 15 s)	Sequential enzymatic treatment using subtilisin and trypsin	nd	High levels of α-glucosidase inhibition (after pre-treatment with ultrasound for 15 min and 1 h of subtilisin. and 1.5 h of trypsin)	Rivero-Pino et al., 2020b
TML	Heat treated (boiled and baked) Freeze-dried Alkaline extraction Isoelectric point precipitation	• Simulated digestion processes: • Simulated saliva solution (7 mM NaHCO3 and 0.35 mM NaCl. pH 6.75) • Simulated gastric digestion (the supernatants were adjusted to pH 2.5 with 1 M HCl +pepsin) • Simulated intestinal digestion (the solution was neutralized with 1 M NaOH and added a mixture containing 0.7% pancreatin and 2.5% bile extract); • Simulated absorption process (the hydrolysates were dialyzed with a membrane tube) • Gel filtration chromatography on Sephadex G10	Raw—NYVADGLG Boiled—AAAPVAVAK Baked—YDDGSYKPH Protein—AGDDAPR	α-glucosidase inhibition: 0.0203 (mg/ml) α-glucosidase inhibition: 0.0109 (mg/ml) α-glucosidase inhibition: nd α-Glucosidase inhibition: 0.0195 (mg/ml)	Zelińska et al., 2020b
384 sequences of yellow mealworm proteins	**Silico analysis of mealworm proteins and peptides Signal peptides were deleted and mature proteins were virtually digested with two proteases: pepsin and papain**		nd	DPP-IV inhibition same trend as the vitro experiment	Terán et al., 2020

(Continues)

Depending on the enzyme involved, they can be classified as α-amylase and α-glucosidase inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and dipeptidyl peptidase-IV (DPP-IV) inhibitors (Rivero-Pino et al., 2020a).

Several TML peptides with antidiabetic activity have been studied and identified, showing marked inhibitory activity against α-glucosidase and DPP-IV enzymes (Table 15).

Yoon et al. (2019) produced PHs with antidiabetic activity from TML by using commercial enzymes as Flavourzyme™, an exopeptidase, and Alcalase™, an endopeptidase. These enzymes are chosen both for their different mechanism of action and for their greater efficiency in hydrolyzing insect proteins. The obtained

TABLE 15 (Continued)

Matrix	Pre-treatment and extraction	Enzymatic hydrolysis process (enzyme (s) and/or post-process)	Peptide sequence or molecular mass (kDa)	Antidiabetic activity IC 50 (mg/ml) or inhibition %	Ref.
TML	• Defatting (n-hexane) • Alkaline extraction of non-cuticular proteins (1% potassium tetraborate decahydrate. pH 9.1) • Acid extraction of cuticular proteins (6 M urea in 0.02 M ammonium acetate at 1:4 (w/v). pH 5.0) • Freeze-dried • Milling to obtain powders containing cuticular proteins and non-cuticular proteins	Cuticular proteins and non-cuticular proteins + pepsin and papain	nd	DPP-IV inhibition Non-cuticular proteins 32.3% Cuticular proteins 43.2%	Terán et al., 2020
TM meal	TM meal 46.8% w/w of protein	Set of food-grade proteases (subtilisin–flavourzyme–DH 20%)	nd	DPP-IV inhibition 2.62 mg/ml	Rivero-Pino et al., 2020b
TM meal	Grounding Powdered	• Set of food-grade proteases (subtilisin, trypsin, and Flavourzyme 1000L^{TM}) • Size exclusion chromatography (Superdex Peptide 10/300GL column coupled to a Frac-902 collector)	500–1600 kDa (APVAH e AAGAPP) <500 Da (AR. CSR)	DPP-IV inhibition IC50 0.91 mg/ml 2.58 mg/ml α-Glucosidase inibithion	Rivero-Pino et al., 2021

Black-filled bullets indicate the different steps in the pre-treatment and enzymatic hydrolysis processes.
Abbreviations: DPP-IV, dipeptidyl peptidase IV; IC, Inhibitory concentration.

hydrolysates showed an in vitro α-glucosidase inhibitory activity of 33.5%–35.0%, which is considered effective but relatively low compared to the positive control with acarbose.

The enzymatic hydrolysis process was optimized in further studies by including an ultrasonic pre-treatment of the reaction mixture in the production protocol. The ultrasound-induced modification of the native structure of TML proteins resulted in increased efficiency of the hydrolysis process, especially of the enzyme subtilisin, with an increase in the yield of small bioactive peptides and an increase in their efficacy on inhibiting α-glucosidase activity (Rivero-Pino et al., 2020b). Zielińska et al. (2020b) evaluated the effect of heat treatment (raw, boiled, cooked) induced in TML on the formation of bioactive peptides. All peptides obtained from the in vitro simulation of gastric digestion and intestinal absorption showed improved efficacy as α-glucosidase inhibitors.

Bioinformatics was also used to study the best strategy for producing bioactive peptides from TM proteins. In silico analysis of 384 TM proteins revealed that specific structural proteins, in particular cuticular ones, are the best precursors of DDP-IV inhibitory peptides. These data were confirmed using the classic approach (extraction, hydrolysis with pepsin and papain in vitro) in parallel for this study (Terán et al., 2020). In another study, however, it is shown that PHs (di- and tripeptides), obtained by combined treatment with food-grade proteases (subtilisin—Flavourzyme™) and 20% DH, showed the highest inhibition of DPP IV (IC50 2.62 mg/ml) (Rivero-Pino et al., 2020c).

From these hydrolysates, several peptides with α-glucosidase inhibitory activity (AR and CSR; IC50 2.58 mg/ml) and DPP-IV (APVAH and AAGAPP; IC50 0.91 mg/ml) have recently been fractionated and identified. The results obtained elect these PHs and their purified fractions as potential functional ingredients to prepare functional foods that regulate the glycemic index (Rivero-Pino et al., 2021).

4.3.5 | Antifreeze activity

Psychrophilic organisms have developed the ability to synthesize particular peptides and proteins that modulate the growth of ice crystals. One category of these proteins is called antifreeze proteins (AFPs). AFPs protect the body from possible damage or lethal effects due to freezing through thermal hysteresis (TH) (Białkowska et al., 2020).

This phenomenon was first highlighted by studies conducted on the mechanism of water reabsorption in the TM rectal complex and the subsequent identification of these AFPs in his hemolymph (Ramsay, 1964; 1971).

In 1979, for the first time, it was possible to isolate and purify from an insect (TML), both certain AFPs and glycoproteins with TH activity (Table 16), in particular six AFPs and three glycoproteins. In numerous studies carried out on these AFPs, some interesting peculiarities have been highlighted, exclusively concerning TM:

(i) The co-presence, in an insect, of chemically different molecules (proteins and glycoproteins) that show TH activity (Patterson & Duman, 1979);
(ii) The display of a more significant TH activity than the AFPs present in Antarctic marine fish (*Trematomous borchgrevink*, *Hernitripterus americanus*, etc.). The ability of insect AFPs to lower freezing points more than fish AFPs has led to the definition of "hyperactive proteins" (Graham et al., 1997);
(iii) The presence of qualitative and quantitative differences in the primary structure of these proteins compared to those purified so far from other animal species (fish and molluscs). AFPs purified from TM were less rich in alanine and richer in cysteine and hydrophilic AAs such as asparagine, threonine, serine (Schneppenheim & Theede, 1980);
(iv) The dependence of the TH activity of these proteins on the integrity of the S—S bond present in the contained cysteines and on the cooperative activity between the individual peptides (Patterson & Duman, 1982; Schneppenheim & Theede, 1980).

The use of more selective purification techniques allowed the purification from TM of another AFP (with a molecular mass of 17 kDa) with peculiar variations in the primary structure, that are the absence of cysteine and the presence of methionine (Tomchaney et al., 1982). Graham et al. (1997) isolated another small protein (84 kDa) from TM hemolymph: it showed hyperactivation of TH, consisting of tandem repeats of 12 residues (CTxSxxCxxAxT).

From the isolation of four AFPs cDNA clones, it was possible to obtain the first complete characterization of a large family of AFPs and the corresponding insect cDNA sequences. A three-dimensional β-helix model is proposed, placing most of the Thr in a regular matrix on one side of the protein to form a putative ice-binding surface (Liou et al., 1999). The discovery of hyperactive AFPs in insects has led to growing interest and stimulated ideas for their applications in the fields of food technology, agriculture, cryobiology and materials technology, and manufacturing (Białkowska et al., 2020). Regarding the latter, an alternative and highly reproducible method has recently been developed on a laboratory scale compared to those used so far (Bar et al., 2006; Liou et al., 2000) in which it is possible to obtain simultaneously and rapidly from TML, both the natural AFPs and all its naturally produced isoforms. This purification method is based on the affinity of AFPs for ice, as highlighted by Kuiper et al. (2003). This innovative technique can be readily upscaled, completed in 1 day, and does not require expensive equipment (Tomalty et al., 2019).

4.3.6 | Antioxidant and anti-inflammatory activities

The imbalance between the production and removal of reactive oxygen species (ROS) and reactive nitrogen species, commonly referred to as "oxidative stress," generates pro-inflammatory molecules that play a crucial role as promoters of cellular and tissue damage, giving rise to various diseases:

- Cardiovascular diseases (hypertension, atherosclerosis, etc.) (de Almeida et al., 2020),
- Metabolic syndrome (cluster of interconnected metabolic abnormalities involving glucose metabolism, lipid metabolism, central obesity) (Grandl et al., 2018),
- Cellular ageing (Davalli et al., 2016),
- Neurodegenerative diseases (Alzheimer's, Parkinson's, etc.) (Singh et al., 2019),
- Respiratory diseases (asthma, acute lung injury, etc.) (Zuo et al., 2013).

Therefore, due to this close correlation, the continuous search for new molecules from natural sources with antioxidant activity and their use (Sangiorgio et al., 2020) seems to be one of the crucial steps to control the progress of these diseases or prevent their complications (Castellani et al., 2018; Jain et al., 2018; Ma et al., 2018; Magrone et al., 2017, 2019). In addition to the already known natural antioxidants—such as vitamin C, polyphenols, flavonoids,

TABLE 16 Extraction processes and bioactivity of the antifreezing proteins extracted from *Tenebrio molitor* (TML)

Matrix	Extraction	Protein purification process	Peptide sequence or molecular mass (kDa)	Thermal hysteresis (°C)	Ref.
TML acclimated for 3 weeks	Crude extract	• Ethanol fractionation • Ion exchange chromatography • Gel filtration chromatography	Six proteins Three glycoproteins	1.46	Patterson et al., 1979
TML placed at −1°C for 28 days in darkness	• Homogenization in 50% ethanol • Centrifugation	• Series of cation exchange chromatography : Elution Buffer: (0.05 M Tris/HCI buffer (pH8) + 1 M Na CI) • DEAE Sephadex pH 8/<3 mS • CM Sephadex pH 5/<3 mS • SP Sephadex pH 3/<3 mS • Concentration by ultrafiltration (Amicon UM 50) • Gel filtration on Sephadex G5O • Desalting by gelfiltration on Sephadex G 1O • Series ion-exchange chromatography: • SP Sephadex C 25, using salt gradient elution (0.05 M Na acetate, pH 4.2 adding NaCI) • SP Sephadex C 25 under equilibrium conditions (0.05 M Na acetate, pH 4.2 + NaCI; 13.7 mS) • Gelfiltration on Sephadex G 50 superfine (eluant: 0.05 M Tris-HCl; 1 M NaCl, pH 8) • Preparative isoelectric focusing in a fiat gel	Three peptides (I; II; III.) 9 kDa (III)	Nd 0.38	Schneppenheim et al., 1980
TML placed at 10°C for 3 weeks	• Homogenization in of cold (4°C) 50% ethanol • Centrifugation • Dialysis • Freeze-drying of the dialyzed	• DEAE-Sephadex (A-25) Chromatography with a NaCl gradient • QAE-Sephadex (A-25) Chromatography with a NaCl gradient • Freeze-drying • Dialysis • Sephadex G-100 Chromatography with 0.2 M NaCl	Two Proteins with high level of Cys	nd	Patterson et al., 1982
TML placed at 5° in darkness for 2–3 weeks	• Homogenization in of cold (4°C) 50% etanol	• DEAE-Sephadex Chromatography. 0.1–0.3 M NaCl gradient (twice) • Sephadex G-100 Gel Filtration • HPLC (twice)	17 kDa	nd	Tomchaney et al., 1982
TML	Dilute hemolymph	• Gel exclusion chromatography • Reversed phase (HPLC) C18 column	8.4 kDa	5.5	Graham et al., 1997

(Continues)

TABLE 16 (Continued)

Matrix	Extraction	Protein purification process	Peptide sequence or molecular mass (kDa)	Thermal hysteresis (°C)	Ref.
TML at 4°C for 4 weeks	• Homogenization • Skimming	• Rotary ice-affinity purification	nd	3.22–3.73	Tomalty et al., 2019

Black-filled bullets indicate the different steps in the extraction and protein purification processes.
Abbreviations: HPLC, high performance liquid chromatography; TM, *Tenebrio molitor*; TML, *Tenebrio molitor* larvae.

and carotenoids—proteins and peptides of animal, plant, and insect origin, especially TML (Table 17), have also long been the focus of numerous studies demonstrating their multiple antioxidant properties (Jakubczuk et al., 2020; Zielińska et al., 2018a).

Recently, it has been reported that the antioxidant activity of individual peptides and PHs derived from edible insects is relatively higher than that of other PHs derived from other food sources (Nongonierma & FitzGerald, 2017).

Zielińska et al. (2017) studied the influence of the heat treatment process on the antioxidant activity of TML hydrolysates obtained by gastrointestinal digestion and absorption in vitro. Heat treatment makes proteins more accessible to the action of the digestive enzymes (Alcalase™, thermolysin, or other proteases). Peptides with increased antioxidant activity are obtained.

These hydrolysates also showed significant anti-inflammatory activity by inhibiting the activity of lipoxygenase (5-LOX) and cyclooxygenase-2 (COX-2). These dual 5-LOX/COX inhibitors induce an enhanced anti-inflammatory effect by blocking prostaglandins and leukotrienes without affecting the formation of lipoxins and preventing damage to the gastrointestinal mucosa (Martel-Pelletier et al., 2003).

Given the extraordinary biological activity demonstrated by these types of new hydrolysates, Zielińska et al. (2018a) worked on separating and identifying these peptides, obtaining four new and unique ones (NYVADGLG, AAAPVAVAK, YDDGSYKPH, AGDDAPR) responsible for anti-inflammatory activity.

The relationship between the bioactivity of a given peptide and its length and AA composition was investigated on TML hydrolysates by Tang et al. (2018). Distilled water (DW) extract has the worst performance in terms of protein extraction efficiency, a balance between quality and amount of free AA, and antioxidant capacity. Hydrolysates obtained by treatment with Alcalase™ are the richest in total free AA, most of which are hydrophobic (Ala, Val, Leu, Iso, Pro, Phe, Try, Cys, and Met), while hydrolysis performed with Flavourzyme™ produce hydrolysates less rich in total free AA but more affluent in hydrophilic AA (Ser, Thr, Asn, Glu, His, and Tyr). On the other hand, antioxidant activity occurred in all extracts in a dose-dependent manner, but the hydrolysates provide the best results from combining the two enzymes.

The impact induced by the enzymatic treatment (subtilisin, trypsin, ficin, and Flavourzyme™) and the degree of adopted hydrolysis (between 5% and 20%) on the hydrolysates antioxidant capacity obtained from TML has also been recently studied. The hydrolysates obtained with subtilisin and trypsin at 10% DH showed an excellent level of DPPH scavenging and ferric reducing activity. In contrast, the iron-chelating activity was strongly favored by increasing DH, obtaining a minimum IC50 of 0.8 mg/ml at 20% DH, regardless of the enzyme treatment adopted (Rivero-Pino et al., 2020c).

Bioactive peptides extracted from TM are gaining increasing popularity and attention from the scientific world, especially regarding the relationship between the nutritional and health effects of these biomolecules added to specific food matrices. A recent study showed an increase in the nutritional and antioxidant properties of some shortbread biscuits prepared with added TM flour. This addition led to the rise in the antioxidant capacity of the biscuits and an increase in slow-digesting starch, with a decrease in fast-digesting starch (Zielińska et al., 2020a). Many studies are in progress to estimate the effects of adding TM flour to other food matrices.

4.3.7 | Hepatoprotective activity

Acute and chronic liver failure (ACLF) is a common syndrome worldwide, characterized by acute decompensation of chronic liver disease due to a precipitating event (alcohol-related liver damage, drug-induced liver damage, bacterial infection, etc.), often accompanied by high mortality (Shah et al., 2020). Many studies on the pathophysiology of ACLF have demonstrated the critical role of excessive hepatic decompensated oxidative stress and an excessive immune response in the liver (Clària et al., 2016; Li et al., 2015). Over the past decade, the search for peptides with antioxidant properties from TML has intensified. Cho et al. (2020a) produced PHs of TML rich in low

TABLE 17 Antioxidant and antinflammatory activities of the peptides extracts from *Tenebrio molitor* larvae by different extraction processes

Protein extraction methods	Enzymatic hydrolysis process (enzyme (s) and/or post-process)	Degree of hydrolysis (%)	Peptide sequence or molecular mass (kDa)	Bioactivity IC 50 (ug/ml) or (ABS_{700})*		Ref.
• Insect(s) fasting 48 h • Raw and Heat treatment (boiling: 100°C 10 min or baking: 150°C 10 min) • Freezing • Lyophilization • Grinding • Alkaline solubilization (pH not show) • Isoelectric precipitation (pH not show) • Lyophilization.	• Vitro digestion (VD) (α-amylase, pepsin, pancreatin and bile extract solution) • Absorption process (AP) (dialysis whit a membrane tube)	nd	29.0–66.4	<u>Antioxidant activities</u>		Zelińska et al., 2017
				-ABTS scavenging	5.3—28.9 (VD) 18.1—24.31 (AP)	
				-DPPH scavenging	40.3—109.4 (VD) 18.4–85.55 (AP)	
				-Fe^{2+} chelating	52.14–69.71 (VD) 9.93–19.5 (AP)	
				-Fe^{2+} reducing	0.373–0.566 (VD)* 0.203–0.280 (AP)*	
				<u>Anti-inflammatory activities</u>		
				-LOX Inhibitory	890–2290 (VD) 3.82–94.68 (AP)	
				-COX Inhibitory Activity	58.09–108.51 (VD) 9.53–11.46 (AP)	
• Insect(s) fasting 48 h • Raw and heat treatment (boiling: 100°C, 10 min or baking: 150°C, 10 min) • Freezing • Lyophilization • Grinding • Alkaline solubilization (pH not shown) • Isoelectric precipitation (pH not shown) • Lyophilization	• Vitro digestion (VD) (α-amylase, pepsin, pancreatin and bile extract solution) • Absorption process (AP) (dialysis with a membrane tube) • Gel-filtration chromatography (Sephadex G—10)	nd	NYVADGLG AAAPVAVAK YDDGSYKPH AGDDAPR	<u>Antioxidant activities</u>:		Zelińska et al., 2018a

(Continues)

TABLE 17 (Continued)

Protein extraction methods	Enzymatic hydrolysis process (enzyme (s) and/or post-process)	Degree of hydrolysis (%)	Peptide sequence or molecular mass (kDa)	Bioactivity IC 50 (ug/ml) or (ABS_{700})*		Ref.
				-ABTS scavenging	3.09—93.4	
				-DPPH scavenging	9.51—52.7	
				-Fe^{2+} chelating	2.21—53.3	
				-Fe^{2+} reducing	0.069—0.198*	
				Anti-inflammatory activities		
				-LOX inhibitory	0.17–29.8	
				-COX inhibitory activity	0.35—3.11	
TM meal (46.8% w/w protein)	Food-grade protease	5–20	Small-chain peptide (0.5–2)	Antioxidant activity:		
	-Subtilisin -Pancreatic trypsine -Ficin -Flavourzyme 1000L ™			-DPPH scavenging	1030—2310	Rivero Pino et al., 2020c
				-Fe^{2+} chelating	530—2120	

Black-filled bullets indicate the different steps in the protein extraction methods and enzymatic hydrolysis processes.
Asterisks indicate absorbance value at 700 nm.
Abbreviations: ABTS, azinobis ethyl benzthiazoline sulfonic acid; AP, absorption process; COX, cyclooxygenase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC, inhibitory concentration.; LOX, lipoxygenases; VD, vitro digestion.

MW peptides (MW < 3 kDa) that showed protective activity against H_2O_2-induced cytotoxicity in AML12 mouse hepatocytes. In particular, PHs obtained with Alcalase™ showed a reduction in ROS production and increased in the expression of antioxidant genes in the same AML12 cells treated with H_2O_2 by activating factor Nrf2. Thus, these PHs represent potential sources of natural hepatoprotective agents.

4.4 | Chitin and chitosan

As revealed from the analysis of scientific literature, insect chitin and chitosan exhibit functional characteristics, like low toxicity, biocompatibility, and biodegradability, and multiple biological effects, such as antioxidant, antimicrobial, and antitumoral effects (Table 18), that make them particularly suitable for applications in numerous fields: from the chemical and agrochemical industry to the medical, pharmaceutical and cosmetic industry, from the food industry and nutrition to textile and paper industry, etc. (Figure 8) (Kaczmarek et al., 2019; Li et al., 2019; El Knidri et al., 2019).

Based on the research carried out by Shin et al., a solution of 8% chitosan obtained by deacetylation of chitin from TML showed a clear inhibitory zone ranging from 1 to 2 mm, indicating antimicrobial activity against four pathogenic bacteria strains, including three Gram-positive bacteria (*Bacillus cereus*, *L. monocytogenes*, and *S. aureus*), and one Gram-negative bacterium, *E. coli* (Shin et al., 2019). According to the literature, there are a few main mechanisms of action of chitosan against microorganisms, as reported in Figure 9 (Matica et al., 2019).

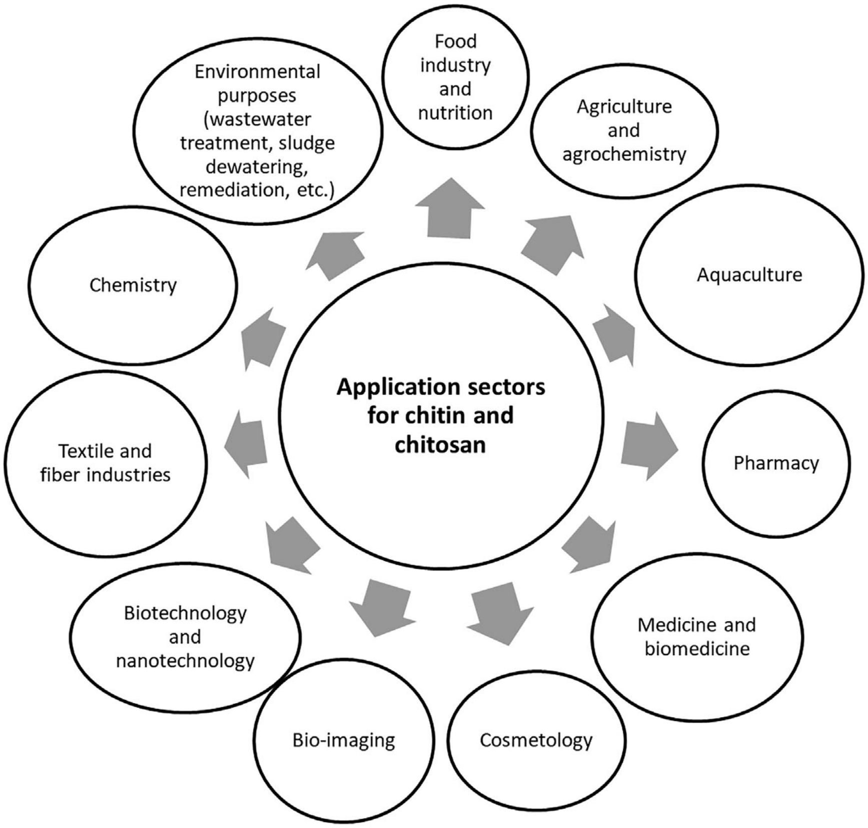
Therefore, TM chitosan could be exploited as a new antibacterial material in many areas, such as medicine, cosmetics, pharmaceuticals, food, environment, textiles, and others.

In addition, Son et al. highlighted the excellent anti-inflammatory effects of chitin and chitosan obtained from TML, tested in the lipopolysaccharide (LPS)-induced murine macrophage cells. In particular, TM chitosan showed notable nitric oxide reduction activity on the macrophage cells, with an efficacy relatively higher than that achieved by using chitosan obtained from other animal sources (Son et al., 2021). Nitric oxide is a soluble endogenous gas produced by immune myeloid cells,

TABLE 18 Main characteristics and biological effects of insect chitin and chitosan

Matrix/sources		**Characteristics**	**Ref.**
Order	**Species**		
Coleoptera	*Tenebrio molitor* *Zophobas morio* *Allomyrina dichotoma*	Low toxicity	Shin et al., 2019,
Coleoptera	*Holotrichia parallela*	Biocompatibility Biodegradability Non-antigenicity	Liu et al., 2012
Matrix/sources			
Order	**Species**	**Biological effects**	**Ref.**
Coleoptera	*Melolontha sp.*	Adsorbable	Kaya et al., 2014
Coleoptera	*Tenebrio molitor*	Anti-inflammatory	Son et al, 2021
Coleoptera	*Tenebrio molitor* *Zophobas morio* *Allomyrina dichotoma*	Antimicrobial (antibacterial, antifungal)	Shin et al., 2019
Dictyoptera	*Blattella germanica*		Basseri et al., 2019
Coleoptera Orthoptera Diptera	*Zophobas morio* *Pterophylla beltrani* *Chrysomya megacephala*	Antioxidant	Soon et al., 2018 Torres-Castillo et al., 2015 Song et al., 2013
Diptera	*Musca domestica*, *Lucilia sericata* *Chrysomya albiceps*	Antitumoral	Hasaballah 2019
Orthoptera	*Schistocerca gregaria*	Wound healing	Marei et al., 2016
Lepidoptera	*Clanis bilineata*	Antiageing	Wu et al., 2017
	Clanis bilineata	Hypolipidemic	Xia et al., 2013

FIGURE 8 Application sectors for chitin and chitosan



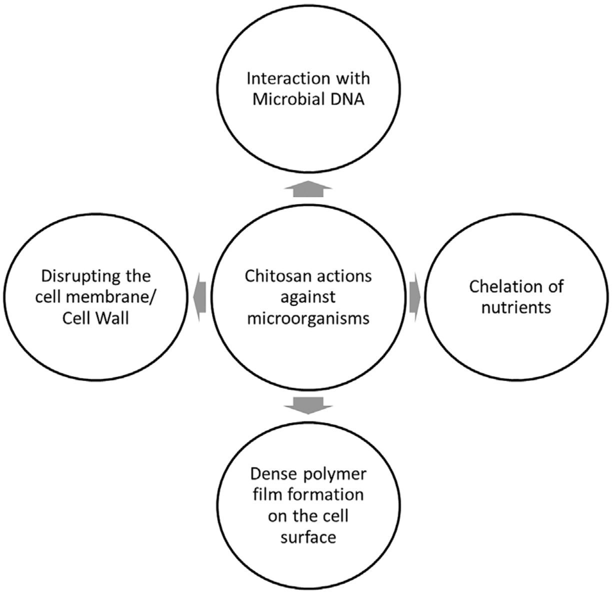


FIGURE 9 Main action mechanisms of chitosan against microorganisms

like macrophages, in response to inflammatory signals for pathogen killing (Palmieri et al., 2020). Subsequently, TM chitosan can potentially be used in inflammatory disorders for therapeutic applications (Son et al., 2021).

The most decisive factors affecting the biological activities of chitin and chitosan are molecular weight (MW) and deacetylation degree (DD) (He et al., 2016; Li et al., 2019; Morin-Crini et al., 2019). MW determines the solubility and viscosity of these polymers. Chitin and chitosan with high MW have poor solubility in water and high solution viscosity, which limit their use in different sectors, such as cosmetics, agriculture, and food industries. MW reduction is a common way to improve their solubility/viscosity and biological properties (Journot et al., 2020; Mohan et al., 2020). Insect chitin and chitosan with a low MW have higher antimicrobial, antioxidant, and wound healing activities (Sahariah et al., 2019) and exhibit excellent antiseptic and anticancer properties useful for drug development (Mohan et al., 2020). However, according to Davydova et al. (2016), the main contribution to the anti-inflammatory activity of chitosan was driven by structural elements that make up its molecule but not depending on MW.

The MW of chitin and chitosan is associated with the monomer units' number per polymer molecule. It can be measured in insects by high-performance liquid chromatography (HPLC), light scattering, and viscosimetric methods (Dotto, 2017; Mohan et al., 2020).

In chitin and chitosan from insects, the DD can be determined by several analytical methods, including UV spectrophotometry, Fourier-transform infrared (FTIR) spectroscopy, 1H-NMR spectroscopy, Raman spectroscopy, X-ray diffraction, circular dichroism, potentiometry, conductimetry, hydrolytic and enzymatic technique, and titration methods (da Silva Alves et al., 2021; Morin-Crini et al., 2019).

Chitin and chitosan with a higher DD have improved biological activities. In the case of chitosan, for example, higher DDs increase the solubility of chitosan and the number of reactive amino groups that can interact with other molecules (Kim et al., 2018). Therefore, comprehensive knowledge of MW and DD of chitin, chitosan, and derivatives is fundamental for their applications.

5 | SAFETY OF *TENEBRIO MOLITOR* LARVAE AS FEED AND FOOD

Edible insects are a promising food and feed source that can replace or supplement "classic" protein sources. As seen in previous sections, TM, in whole or powdered (mostly defatted) larvae, has a high nutritional profile that can meet animal and human needs. In addition to the nutritional aspects, however, the food safety of TM products must be considered and proved. The food safety risks of TM can arise from the insect itself or the substrate it feeds on, as well as from the conditions of rearing, harvest and processing (EFSA, 2015; EFSA NDA, 2021). Below is a quick overview of the main risks associated with the consumption of insects, especially TM; many of them are common to many other foods and are not characteristic of insects alone.

As described by Grau et al. (2017), TML were consumed or used without removing their gut, so if some pathogens are present, they are transmitted to livestock and humans. According to the same authors, their cuticle is also covered with microbes because they feed and defecate in the same place. In the last years, the practice of inserting starvation before transforming treatments has reduced the risk of microbial contamination (Garofalo et al., 2019). On the other hand, although it is known that the microbial load can be killed by thermic treatment, not all microorganisms have to be eliminated because a group of these, especially some bacteria, is found to be responsible for beneficial effects (Grau et al., 2017).

Foodborne pathogens can be found in fresh larvae, together with sporulated pathogens that are resistant to the food industrial treatments and can grow during the storage period (Messina et al., 2019). Wynants et al. (2019) studied the transmission of *Salmonella* sp. to TML when mealworms feed on contaminated bran. They found that the presence of TML reduces the *Salmonella* sp. level in the bran. They also demonstrated that bacterial retention in the larvae depends on the bran contamination level. Thus, the authors recommend frequent testing of the bran used for breeding.

As foodborne pathogens, Vandeweyer et al. (2020) evaluated the presence of foodborne viruses in edible insects and did not detect hepatitis A virus, hepatitis E virus, and genogroup II norovirus. Limited to these viruses, therefore, they can declare a low food risk. However, the authors recommend extending the field of research to other species, especially considering the speed with which the production process of insect-based products is being automated and upscaled.

As reported by Garofalo et al. (2019), many studies about the TM microbiota have shown how different microbial taxa can colonize the microbiota of edible insects. The numerous variables concerning insects' species, stage of development, rearing conditions, applied treatments, geographic zone considered, and sampling method could explain this wide range of microorganisms found. Even the most innovative analysis methods could lead to results that are not always comparable with those obtained using older microbial identification methods (Garofalo et al., 2019). The same authors note scarcity of information regarding fungal communities.

Contamination, both biological and chemical, can occur throughout the supply chain, from TM rearing to the consumer. The biological implications of microbial load and parasites can be so relevant; therefore it is necessary to maintain edible insects' growth in commercial insect farms under controlled hygienic conditions. Further down the supply chain, an HACCP protocol has recently been developed to assess the biological risks associated with the different production processes of TM powders destined to several flour-based foods (hamburger, protein shake, porridge, and biscuits) (Kooh et al., 2020). With a view to the circular economy, agro-industrial by-products of plant origin can be used to feed insects: it is essential to ensure the safety of these substrates to avoid the introduction of contamination into the supply chain. In any case, the production and storage of feed are crucial for the final product's microbiological and chemical safety (FAO, 2021).

Many risks, especially those related to parasites in insects, can be reduced by following strict hygiene rules both in the production and cooking phases (Bellucco et al., 2013).

Insects can also contain other toxic substances, sequestered directly from the plants they feed or produced as a defense in response to danger; these substances can be of various kinds; the most relevant for human nutrition are antinutritional factors, mycotoxins, heavy metals, alkalis, and toxins. In particular, in TM, some authors found the possibility of accumulating selenium over the acceptable levels for food consumption (van der Spiegel et al., 2013) and arsenic (van der Fels-Klerx et al., 2018). In contrast, there is currently no evidence that insects can accumulate mycotoxins or host pathogenic viruses and prions, although they can act as vectors (van der Fels-Klerx et al., 2018). However, the risk cannot be excluded entirely because similar situations occur in other insects and because prion diseases (transmissible spongiform encephalopathies) are fatal neurodegenerative diseases that affect both humans and livestock (Grau et al., 2017).

People allergic to shellfish can also react to TM due to tropomyosin, a pan-allergen also present in these crustaceans. For this reason, the risk of a potential cross-reaction in people sensitized to shellfish should be labeled in insect products. Arginine kinase, α-amylase, and other proteins may also be responsible for cross-reactivity. Several studies aimed to test which are appropriate food processing methods (e.g., thermal processing or enzymatic proteolysis) to reduce the risk of cross-reactivity and allergenicity associated with edible insect proteins; results show that heat treatment can reduce but not eliminate the allergenicity (van der Fels-Klerx et al., 2018). In addition, allergic reactions can occur due to mold contamination. For this reason, it is also essential to evaluate the effects, especially the indirect ones, not only on consumers but also on workers in production systems (Grau et al., 2017).

A separate discussion is that about chitin, for which allergenicity via inhalation seems to be related to the size of the particles; however, no data are available related to the allergenic effects of chitin through consumption (van der Fels-Klerx et al., 2018).

In January 2021, the EFSA published its opinion on the safety of *T. molitor*, both as whole larvae and as flour derived from them. The European Commission requested the opinion regarding regulation (EU) 2015/2283 on novel foods. EFSA experts believed that the levels of contaminants depend on the amount present in the feed of TM. Furthermore, they did not detect safety problems regarding stability or toxicity. However, they found that some allergens in the feed can pass into the larvae and that consumption of these can induce primary sensitization, allergic reactions to proteins, and allergic reactions in subjects with allergy to shellfish and dust mites. EFSA, therefore, gave a favorable opinion on the use proposed by the applicant (whole dried insect in the form of snack and food ingredient) for the entire population, underlining that, in the proposed conditions of use, consumption "is not disadvantageous from a nutritional point of view" (EFSA NDA, 2021).

As soon as EFSA gave its favorable opinion, the European players creating insect-based food for human consumption (in the Netherlands, France, Switzerland, and Spain) have taken steps to increase production. In particular, in France, a company has invested heavily in the production of TM to supply wet food for pets, protein products for humans, and specific products for athletes (Ledsom, 2021).

Maybe, before we can see TM-based food products on the shelves of our markets, it will need to investigate the still unclear aspects regarding food safety and overcome some regulatory obstacles in individual EU countries.

6 | CONCLUSIONS

In the scenario of a growing need for sustainable food, alternative sources of protein, in a circular economy vision, have become urgent and vital for the health of the Earth.

The recent approval by the European Commission (2021) to use TM as a novel food (the first insect in Europe) will give a new boost to its production, consumption, and the related market, promoting investments in this sector. It is also desirable to stimulate further research to clarify the still little-explored aspects of TM, especially regarding the effects of a TM-based diet on human health and issues related to safety. In Western countries, it will be necessary to remove cultural, psychological, and regulatory barriers that still harness the production and consumption of TM in a "niche" sector. It is indispensable to make every effort to communicate knowledge on the nutritional and environmental beneficial effects of insects since only good information can determine a change in the mentality and behavior of consumers.

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AUTHOR CONTRIBUTIONS

Simona Errico: conceptualization; investigation; visualization; writing—original draft, review, and editing. Anna Spagnoletta: conceptualization; investigation; visualization; writing—original draft, review, and editing. Alessandra Verardi: conceptualization; investigation; visualization; writing—original draft. Stefania Moliterni: conceptualization; investigation; visualization; writing—original draft. Salvatore Dimatteo: conceptualization; writing—review and editing. Paola Sangiorgio: conceptualization; investigation; supervision; visualization; writing—review and editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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