



# Recent Advances in Biorefinery of *Tenebrio molitor* Adopting Green Technologies

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Received: 5 March 2024 / Accepted: 2 July 2024  
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## Abstract

Insects are promising alternatives to meet the world population's demand for high-quality foods and to overcome important issues in animal farming practices. Novel and green biorefinery processes must be applied to insects, overcoming chemically based techniques. Eco-friendly approaches increase the nutritional value of insects, widening the commercial applications. This review addresses the rearing practices and nutritional composition of *Tenebrio molitor*, highlighting the green methodologies that can be applied to obtain value-added compounds, replacing unsustainable practices. Also, useful applications of pre-treated *T. molitor* biomass are presented with a thoughtful insight into their advantages and limitations. The nutritional richness of *T. molitor* is being successfully explored by resorting to physical and biological procedures, resulting in valuable compounds for food, feeding, and biomedical and biotechnological industries. Novel ingredients and additives of insect origin may upgrade food and feed formulation, while chitosan of *T. molitor* origin may upgrade the packaging industries of food and feed.

**Keywords** *Tenebrio molitor* · New food · Green technologies · Biorefinery · Protein · Deep eutectic solvents

## Introduction

The ever-increasing demand for food is directly linked to the exponential growth of the world population, overwhelming the food production systems of high-quality and high-level protein sources. According to the United Nations, the global population is expected to increase by 2 billion people between 2020 and 2050, reaching 9.7 billion (Borges et al., 2022). Animal farming presents several environmental concerns since it originates large amounts of waste and greenhouse gas emissions. Therefore, it urges the need to increase the sustainability of food production systems to obtain high-quality protein without negatively impacting the environment, which may be attained by considering alternative food sources. Novel foods can be considered interesting replacements to conventional protein sources, highlighting the analog meat with microbial proteins, fungi

protein, plant protein, and microalgae such as *Chlorella* spp. (*Chlorella vulgaris*; Mazac et al., 2023). Among the novel foods, edible insects are gaining importance, since they are a rich source of protein, essential fatty acids, antioxidants, and micronutrients (Perez-Santaescolastica et al., 2022).

Edible insects are already produced and consumed mostly in South America y South Asia, while cultural barriers still limit the consumption in Europe and North America. The production of edible insects has many advantages, including low water, energy and space requirements, and fast growth rates. The principal advantage of insect farming is the low environmental impact, generating low greenhouse gas emissions and requiring less water since it is obtained from the feed (Madau et al., 2020). It is estimated that approximately 1 mg NH<sub>3</sub>/kg of body mass is produced during insects rearing, which is considerably low compared to the production of cattle (14 mg NH<sub>3</sub>/kg of body mass per day) or pigs (4.8 mg NH<sub>3</sub>/kg of body mass per day (Rocha et al., 2021).

To meet to increasing protein demands of world population, the production of edible insects must increase up to industrial scale and be based on sustainable practices, such as using inexpensive and nutritionally effective feeding substrates during insects farming. Agricultural by-products are abundantly generated and can be used as nutritional sources

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for edible insects, reducing costs and allowing the production of large amounts of insects. The nutritional source directly affects the chemical composition and growth of organisms, with protein-rich by-products resulting in high growth yields in insect farms (Mancini et al., 2019). Another central step to boost the production and utilization of edible insects relates to the existing legislation. In Europe Union (EU), the European Food Safety Authority (EFSA) carries out safety assessments that are used to adequately legislate food items. This committee considers “novel foods” (Regulation (EU) 2015/2283) all the items that have not been consumed in the EU before 15 May 1997, in which are also included the edible insects (European Commission, 2015). Novel foods must be safe for the consumers, properly labelled, and may not be nutritionally inferior compared to the existent traditional foods. In January 2021, *Tenebrio molitor* dried mealworms (larval stage) were one of the first edible insects authorized for human consumption in the EU (Regulation EU 2015/2283; European Commission, 2015). After, a novel EFSA report permitted approving the consumption of dried and frozen *Locusta migratoria* (Turck et al., 2022). In January 2023, *Acheta domesticus* (European Commission, 2023) and *Alphitobius diaperinus* larvae have also been deemed safe for human consumption (Turck et al., 2022). Edible insects not only have an important role in human nutrition but can also be used in the animal feeding industry, highlighting the research conducted in aquaculture fish and monogastric animals replacing the use of traditional feed ingredients. For example, the dietary inclusion of partially defatted mealworms meal to replace fishmeal did not have negatively affect the growth of rainbow trout (*Oncorhynchus mykiss*; Chemello et al., 2020) while improved growth and feed conversion ratio of poultry, chickens, and pork (Hong et al., 2020).

In addition to the high quality and content in protein, the chitin fraction of insects is considerably high in the adult stage, hindering nutrient utilization. In fact, insect meals are uniquely fabricated with larvae, given their low chitin and high protein contents, while adult insects are unutilized once their lifetime expires.

In recent years, the concept of biorefinery has been applied to insects aiming to fractionate insects into different products for both food/feed and other industrial applications (Kee et al., 2023). Furthermore, another green trait of insect rearing is the fact that they can be fed with agro-industrial by-products that otherwise would be disposed of. Currently, many of the industrial processes used to extract chitin or protein from insects involve chemical treatments using concentrated acid and alkaline solutions, generating highly pollutant wastes. Therefore, it is necessary to explore eco-conscious alternatives to fractionating insect biomass, aiming to achieve the yields that are attained with chemical treatments. This review focuses on environmentally friendly

technologies that have been recently applied to obtain various components from *T. molitor*, such as protein, chitin, and lipids.

## Production and Characterization of *Tenebrio molitor*

Among the edible insect species already used in the food industry, *Tenebrio molitor* is one of the most consumed and produced (Dobermann et al., 2017). This insect is commonly named mealworm, belongs to the Tenebrionidae family of the order Coleoptera, and has a medium size (12–20 mm) and black or brown coloration (Rumbos et al., 2020). *T. molitor* has three growth stages: larvae, pupa, and beetle. The larval phase can vary between 73 and 115 days (Bordiean et al., 2022), the pupal phase between 15 and 34 days, and the adult phase between 95 and 130 days (Riaz et al., 2023). The adult insects lay eggs, which hatch between the 7th and 10th day. The duration of the growth period and the final nutritional composition depend on the type of diet used, as well as the temperature and humidity. Riaz et al. (2023) compared feeding larvae, pupae, and beetles of *T. molitor* with wheat-based diets supplemented with *Bacillus clausii*, *Lactocaseibacillus rhamnosus*, *Calocybe indica*, or *Saccharomyces cerevisiae* and observed that *C. indica* supplementation promoted a faster growth and resulted in higher protein content.

The EFSA divides the production of *T. molitor* in three stages, such preharvest, harvest, and postharvest (Turck et al., 2022). The first stage, preharvest, includes the production of eggs and rearing of larvae, occurring when the eggs are separated from the adults and placed in a sterile high-density polyethylene container to reduce the ingestion of plastic material. The harvest stage includes the separation of larvae from the residual products accumulated during rearing, including a visual checking of the individuals until achieving the postharvest stage. In the postharvest stage, that larvae are subjected to a blanching process, which simultaneously kills the larvae and the microbiological pathogens that may affect the consumer. Finally, the blanching larva is dehydrated and grinded to produce the mealworms meal (Turck et al., 2022). Larvae have high protein and low chitin content, which potentiates the use for food and feeding purposes, while adults are discarded (Gkinali et al., 2022).

Production systems on insect farms are carried out in rooms with temperature and humidity control. Insects are placed in plastic boxes, where a cereal-based food bed is provided, and pieces of vegetables or fruits are added to provide them with water during their growth. Once the larvae reach their maximum weight (150–200 mg), they are processed to obtain larvae meal. A portion of the production (10–20%) is allowed to continue until reaching the adult stage, with

the aim of laying eggs and continuing the production cycle. Adult insects are not used as food due to their high chitin content, so they are considered a by-product in insect farms. Another by-product generated in *T. molitor* production is the frass, accounting for up to 2 kg of frass produced per kilogram of larvae, and is often used as fertilizer (Chavez, 2021). Recent studies aim to find other applications for frass, such as a defense priming agent (Blakstad et al., 2023) or as substrate on solid-state fermentation to produce proteases (Muñoz-Seijas et al., 2024).

Larvae of *T. molitor* are usually named mealworms and are currently the only development stage that is commercialized for food and feeding purposes with many nutritional advantages, such as high energy (138 kcal/100 g FW) and protein (between 14 and 25% FW) contents (van Huis, 2020). In addition to mealworms content in macro and micronutrients, they also contain chitin but at much lower levels compared to the adults of *T. molitor* (Table 1; Adámková et al., 2017).

The proportion of protein, lipids, and chitin depends on the development stage, with the protein fraction being the most abundant nutrient on edible insects, including *T. molitor*, averagely reaching 50% of dry weight (DW) (Jajić et al., 2019; Boulos et al., 2020). Protein of insect origin presents high quality and high digestibility (Wu et al., 2020; Anusha & Negi, 2023), being considered a good supplementation for human food (Heidari-Parsa et al., 2018). Focusing on essential and not-essential amino acids profile (Table 2), leucine and valine are the most abundant essential amino acids, with important function on muscle mass on

protein production (Wang et al., 2023). Among the nonessential amino acids, alanine and glutamine + glutamic acid is present in higher quantities, being related to metabolic processes. Recently, Mihaly Cozmuta et al. (2023) observed a high value of isoleucine (essential amino acid) and tyrosine (nonessential amino acid) in mealworms.

However, the assessment of protein content in insects and insect-based biomass is challenging since this fraction is covalently bounded to chitin, which overestimated the nitrogen content of these biomasses (Khanal et al., 2023). Indeed, the common practice to assess the protein content of edible insects is determining the nitrogen content and multiplying it by a conversion factor (kp, protein/total nitrogen) of 6.25. However, considering the chitin fraction, it is important to rigorously guarantee that the nitrogen quantified is only of protein origin. Janssen et al. (2017) assessed that the most appropriate conversion factor to insects' protein should be 4.75 after determining the protein content of *T. molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. On the other hand, Boulos et al. (2020) studied two conversion factors, kp (protein/total nitrogen) and ka (protein/protein nitrogen), to determine the nitrogen-to-protein factors of *T. molitor*, *A. domesticus*, and *L. migratoria* and observed that a kp of 5.41 and a ka of 5.60 were the most accurate factors to assess insects protein, also confirming that the kp of 6.25 overestimates the protein content in about 17%. Mattioli et al. (2024) also tested kp factors of 4.76 and 6.25 and quantified a total protein content in mealworms meal of 48% DW and 62% DW, respectively. Therefore, since the chitin fraction of mealworms is considerably low, the nutrient digestibility is

**Table 1** Nutritional composition of larvae, pupae, and adults of *Tenebrio molitor* (% dry weight, DW)

Development stage	Protein	Lipids	Chitin	Minerals	Reference
Adult*	54.86	12.50	26.63	3.24	Flores et al. (2020)
Adult	55.3	28.3	ND	3.7	Hidalgo et al. (2022)
Adult	45.7	42.9	7.2	4.3	Laroche et al. (2019)
Pupae	60.18	23.01	ND	ND	Morales-Ramos et al. (2016)
Pupae	57.78	31.21	ND	3.63	Oliveira et al. (2024)
Pupae	51	32	12	5	Adámková et al. (2017)
Pupae	45	36	9.5	9.5	Yu et al. (2021)
Larvae	42.9	41.9	11.5	3.6	da Cruz et al. (2022)
Larvae*	57.9	10.9	11.2	4.3	Pasini et al. (2022)
Larvae	48.82	30.69	16.24	4.25	González et al. (2019)
Larvae	68.9	ND	6.97	3.21	Addeo et al. (2022)
Larvae	52.23	29.42	ND	4.30	Wu et al. (2020)
Larvae	55.83	32.3	7.15	4.84	Jajić et al. (2019)
Larvae	60.21	19.12	ND	4.20	Heidari-Parsa et al. (2018)
Larvae	52.63	29.47	6.32	6.36	H. W. Kim et al. (2016)
Larvae*	70.42	3.94	8.51	8.14	H. W. Kim et al. (2016)
Larvae	49.57	21.20	ND	4.22	Choi et al. (2017)
Larvae	55.88	34.05	ND	ND	S. Y. Cho and Ryu (2021)

\*Defatting; ND non-determined

**Table 2** Amino acids profile of *Tenebrio molitor* larvae

References	(Iaconisi et al., 2019)	(Yoo et al., 2019)	(Ghosh et al., 2017)	(Mihaly Cozmuta et al., 2023)	(Heidari-Parsa et al., 2018)	(Ao et al., 2020)	(Wu et al., 2020)
Essential amino acids (%)							
Arginine	3.03	5.46	2.23	ND	2.23	4.18	1.88
Histidine	5.55	2.93	2.80	ND	1.38	2.62	0.87
Isoleucine	4.95	3.76	1.98	8.83	1.83	<b>6.12*</b>	1.31
Leucine	<b>8.13*</b>	<b>6.88*</b>	<b>3.37*</b>	<b>18.53*</b>	<b>3.13*</b>	<b>5.87*</b>	<b>2.20*</b>
Lysine	4.59	5.42	2.01	0.21	2.50	5.02	1.58
Methionine	0.70	1.34	ND	0.45	0.52	1.15	0.60
Phenylalanine	2.99	3.72	1.76	6.19	1.55	3.02	1.31
Threonine	3.31	4.04	1.83	1.24	1.70	3.13	1.27
Valine	<b>7.72*</b>	<b>4.98*</b>	<b>2.94*</b>	<b>9.11*</b>	<b>2.57*</b>	4.21	<b>1.89*</b>
Non-essential amino acids (%)							
Alanine	<b>12.79*</b>	7.02	<b>3.96*</b>	<b>7.78*</b>	NA	<b>6.33*</b>	<b>2.48*</b>
Asparagine + Aspartic acid	8.68	8.01*	2.76	3.67	NA	6.16	1.54
Cystine	ND	0.86	3.16	NA	NA	0.88	NA
Glutamine + Glutamic acid	<b>11.90*</b>	<b>11.54*</b>	<b>5.78*</b>	ND	NA	<b>9.75*</b>	<b>3.92*</b>
Glycine	10.43	5.06	2.61	3.35	NA	4.14	1.71
Proline	7.67	6.73	1.66	5.01	NA	5.22	2.01
Serine	3.67	4.83	2.20	2.12	NA	3.61	1.36
Tyrosine	3.88	7.76	3.45	<b>11.92*</b>	NA	5.97	2.15

NA Not analysed; ND Not detected

\*Present in higher amount

not compromised, and it is not required to extract the chitin for an efficient use of protein (Toviho & Bársony, 2022). *T. molitor* has high lipid content (11–43% DW) compared to other edible insects accepted for consumption in Europe, such as *H. illucens* (32.6%) or *A. domesticus* (12.2%; Bbosa et al., 2019; Lawal et al., 2021). Also, the fatty acids profile

of mealworms is described in several studies which are included in Table 3. The monounsaturated fatty acids are present in higher proportion in *T. molitor*, highlighting the oleic, linoleic, and palmitic acids (Verheyen et al., 2023). Also, the fatty acids profile presents some variations due to the diet type (Mattioli et al., 2021) or the different methods

**Table 3** Fatty acids profile of *Tenebrio molitor* (%)

		References						
Fatty acid (%)		Adámková et al. (2017)	Paul et al. (2017)	Mattioli et al. (2021)	Otero et al. (2020)	Siow et al. (2021)	Wu et al. (2020)	Mancini et al. (2021)
C12:0	Lauric acid	0.1	0	0.19	0.32	0.74	0.24	0.46
C14:0	Myristic acid	2.5	4.45	4.05	2.12	4.94	3.53	4.61
C16:0	Palmitic acid	20.2	21.3	16.66	17.24	19.54	21.42	18.20
C16:1	Palmitoleic acid	0.4	1.97	3.01	1.94	4.73	1.68	1.69
C17:0	Margaric acid	0.3	ND	0.11	0.25	0.64	ND	ND
C18:0	Stearic acid	4.3	7.92	1.57	0.69	8.41	4.54	3.58
C18:1	Oleic acid	37.7	35.8	34.26	43.77	30.37	40.68	49.63
C18:2	Linoleic acid	31.9	22.8	39.85	29.39	25.07	14.49	19.72
C18:3	Alpha-linoleic acid	1.7	0.11	ND	2.27	2.80	ND	0.36
C20:0	Arachidonic acid	0.6	0	0.05	ND	0.33	0.65	ND

ND not detected

used to quantify the fatty acids content (Otero et al., 2020). Lawal et al. (2021) observed that *T. molitor* larvae accumulated 39.61% of lipids when fed a conventional diet (wheat meal based), and when replacing wheat meal with flaxseed, rapeseed, chia seed, or hempseed, the lipids content decreased. Nonetheless, since the mealworm meal is often defatted to improve protein accessibility and digestibility, the values presented in the literature may vary (Paul et al., 2017).

Another component of insects that can be extracted and valorized, including of *T. molitor*, is the chitin fraction. Chitin is an insoluble polysaccharide formed by linear chains of N-acetyl-D-glucosamine (GlcNAc), linked by  $\beta$  (1  $\rightarrow$  4) bonds, and is the main fiber found on *T. molitor*, ranging between 2% up to 21% (Mohan et al., 2022). Nevertheless, the chitin of natural sources is a heteropolymer of this GlcNAc and glucosamine in different proportions (Zhu et al., 2016). This fraction has an important structural function on the insect since it is one of the main substances present in the cuticle (Adámková et al., 2017). However, the high molecular weight of chitin makes it insoluble in water and several organic solvents, limiting its applications in several industries. To increase industrial applications, chitin must be transformed into chitosan by a deacetylation process, obtaining, which is water soluble (Mohan et al., 2022). Saenz-Mendoza et al. (2020) observed that chitosan from *T. molitor* had higher opacity and a brown coloration than the commercial chitosan films available (with different molecular weights), pointing out the protective function of this insect-based biofilm against UV radiation. Józwiak et al. (2023) proved that chitin obtained from *T. molitor* larvae was highly effective in removing anionic (RB5, RY84) and cationic (BV10, BV46) dyes from aqueous solutions, being more efficient for anionic dyes at pH between 2 and 3. The chitin fraction is lower in mealworms than in adults, increasing the direct use of mealworms meal on the food industry without previous extraction of chitin. Therefore, it is less common using the adults for food or feeding purposes since chitin extraction procedures must be carried out to improve protein accessibility to digestive enzymes, which is currently performed resorting to aggressive and chemically based procedures (Son et al., 2021).

Apart from protein, lipids, and chitin fractions, the presence of other compounds can be highlighted, such as minerals, phenolic compounds, and vitamins. Oliveira et al. (2024) observed that the most abundant minerals in *T. molitor* and *Gryllus assimilis* were potassium, phosphorus, and sodium, while *T. molitor* contained higher amounts of vitamins C and E and niacin compared to *G. assimilis*. Likewise, the vitamins profile observed in *T. molitor* also directly depends on the diet provided during the production phase (Kotsou et al., 2023).

## Biorefinery of Insects' Biomass Using Traditional Methods

The increasing interest in various value-added products of insect origin with many industrial applications boosted the development of biorefinery processes to fractionate this biomass, obtaining lipids, minerals, protein, and chitin.

### Extraction of Lipids

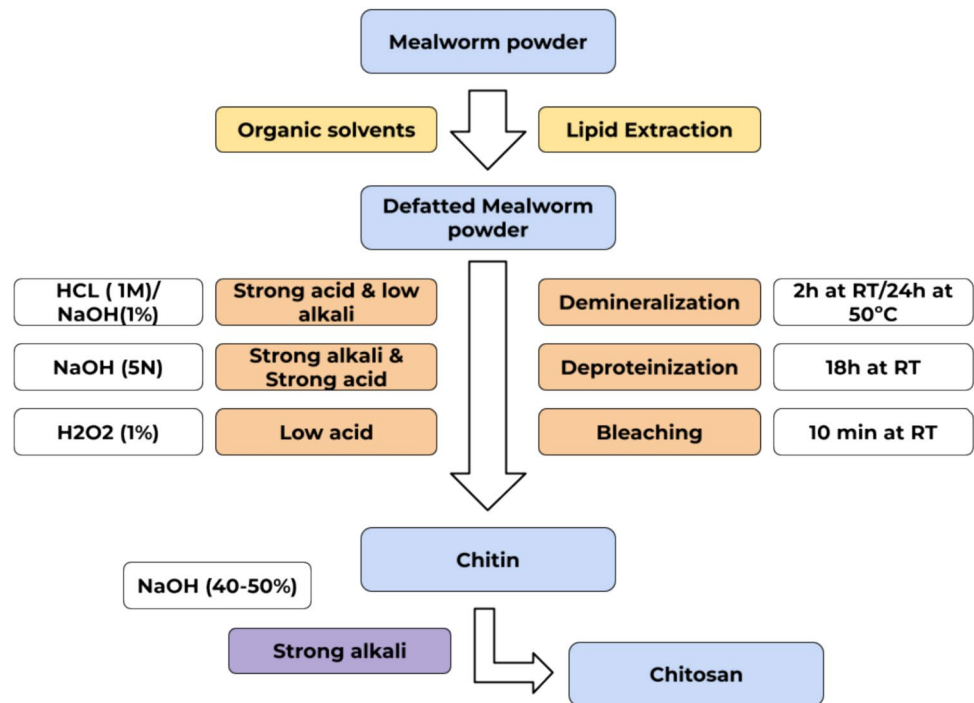
Lipids can be extracted to be used as feed supplements and to improve protein digestibility, being this procedure generally called defatting (Laroche et al., 2019). There are several defatting methods, with the most common resorting to organic solvents using a Soxhlet apparatus, such ethanol, petroleum ether, or hexane, in which the solids are in constant contact with the solvent. In the Soxhlet apparatus, the mixture is condensed in cool tubes, and the lipids are quantified after evaporation of the solvents (Zhang et al., 2018).

Verheyen et al. (2023) extracted oil from mealworms using a Soxhlet-based and reactor-based methods, concluding that the Soxhlet (using hexane or ethyl acetate, overnight) resulted in higher lipids yield (32.04%) compared to when using the reactor (hexane or ethyl acetate, 2 h). Paul et al. (2017) also obtained a similar yield of lipids extraction but after washing three times with a chloroform and methanol solution. Nonetheless, using solvent-based techniques is linked to harmful effects in the environment and should, thereof, be avoided. Laroche et al. (2019) applied three methods to extract lipids from *A. domesticus* and *T. molitor* larvae, achieving higher lipids yield with Soxhlet ( $28.8 \pm 5.9\%$ ; w/w), followed by the supercritical CO<sub>2</sub> ( $22.1 \pm 0.6\%$ ; w/w) and the three-phase partitioning method ( $19.3 \pm 2\%$ ; w/w).

### Extraction of Protein

Wet fractionation is the most used method to obtain protein. This type of extraction is based on the physical and chemical properties of proteins, in which the separation is achieved by changing the ionic strength and the pH of the solution. The most common wet fractionation method used in mealworm meals resorts to alkaline conditions (Fig. 1) followed by an isoelectric precipitation of proteins. Zielinska et al. (2017) isolated protein from raw insects using 0.2% NaOH (1:10 ratio w/v) at room temperature. Azagoh et al. (2016) extract soluble protein from *T. molitor* larval stage, using solubilization at a pH 10 with water and NaOH. Aqueous- or salt-based extractions are less used than the alkaline conditions. Jiang et al. (2021) obtained higher protein yield from *T. molitor*

**Fig. 1** Schematic representation of procedures to fractionate mealworms powder into chitin (RT, room temperature)



(60%) using an alkaline extraction (1.5% NaOH) compared to using acid- (1% NaCl) or salt-assisted (20%  $(\text{NH}_4)_2\text{SO}_4$ ) treatments.

Recently, Laroche et al. (2022) used the protein fraction from defatted mealworms meal for an experimental design using one or two solubilization steps followed by one or two protein precipitation steps. Higher protein extraction rate was achieved when the process included one solubilization step, being maximum when a solubilization and a protein precipitation step were both used.

### Extraction of Chitin

There are several types of extraction and purification methods of chitin. Conventional extraction method of chitin entails three stages (Fig. 1): (1) the biomass is grounded; (2) a sequential demineralization (with a strong acid solution and low alkali solution) followed by deproteinization (with a strong alkaline solution and low acid solution) is carried out to eliminate minerals and protein, respectively; and (3) decolorization using  $\text{H}_2\text{O}_2$  to remove residual lipids and pigments (Kumari et al., 2015).

Chitosan can be obtained from chitin carrying out a deacetylation process using NaOH (da Silva Lucas et al., 2021). Contrarily to chitin, chitosan is highly soluble, disclosing varied utilizations within a wide range of industries.

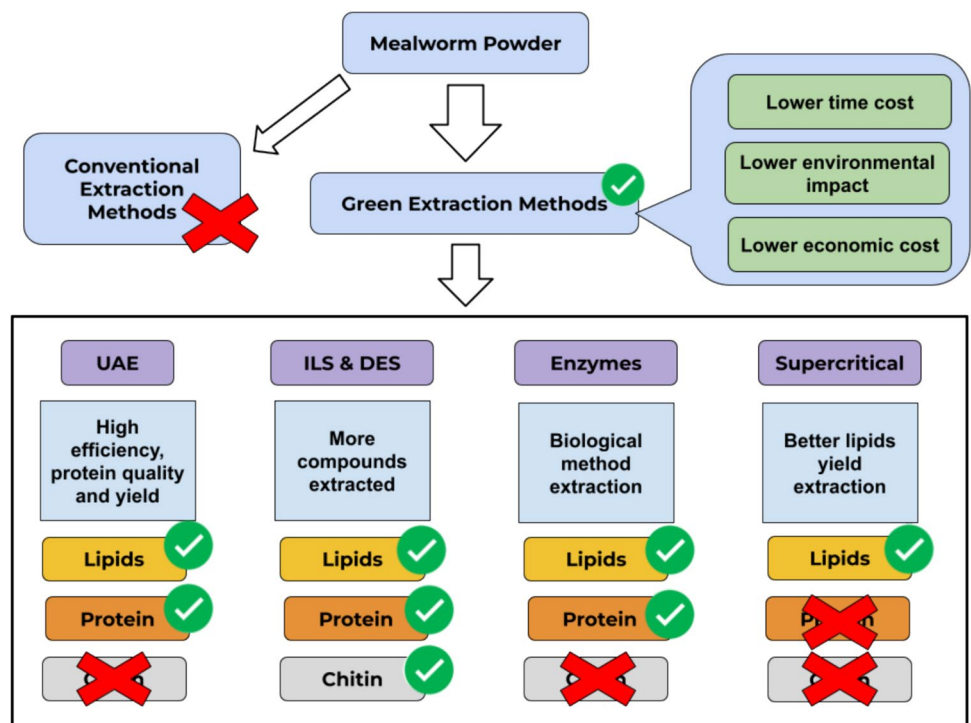
### Green Technologies Applied to Edible Insects Biorefinery

There are several nonconventional and green extraction methods to obtain biomolecules from edible insects, including *T. molitor* (Fig. 2). These methods include ultrasound-assisted extraction (UAE), ionic liquid solvents (ILs), deep eutectic solvents (DES), and enzymes-based extractions (EAE; Rocha et al., 2021). These methods have a lower environmental impact than the traditional methods using concentrated acid and basic solutions, are less time-consuming, and highly cost-effective.

#### Ultrasound-Assisted Extraction (UAE)

The ultrasound-assisted extraction (UAE) is based on the principle of acoustic cavitation, which consists in the creation, growth, and implosion of bubbles formed in the liquid (Queiroz et al., 2023). The shock between bubbles generates a thermochemical microreactor-like conditions, potentiating the separation of protein from the chitin fraction and increasing the protein solubility (Mohan et al., 2022). Usually, the frequency of waves used in the UAE is low but always higher than 20 kHz (Queiroz et al., 2023). The UAE has many advantages such as the easiness of the process, high efficiency, high yield, and high protein quality (Queiroz et al., 2023).

**Fig. 2** Green extraction methods used in mealworm powder to obtain different value-added fractions



Otero et al. (2020) carried out a UAE in *T. molitor* and *A. domesticus* meals using ethanol (E) and ethanol:water (E:W) as solvents, obtaining a lipids extraction yield of 15.48% (E) and 15.05% (E:W) and of 28.85% (E) and 17.14% (E:W) from *A. domesticus* and *T. molitor* meals, respectively. Navarro del Hierro et al. (2020) performed UAE using similar conditions as those used by Otero et al. (2020) obtaining different lipid fractions from *A. domesticus* and *T. molitor*, highlighting fatty acids and glycerides, and amino acids, carbohydrates, sterols, and hydrocarbons. Zhang et al. (2023) studied the effect of an alkaline-based UAE (UAAE) in *T. molitor* larvae, obtaining an extraction yield of protein of 60% after 30 min. The UAE have effects on the physicochemical properties of the extracts and may also alter the structure of proteins, improving the thermal stability, digestibility, and the digestion kinetics, for example. Wang et al. (2021) studied the effects of high-intensity ultrasounds on the physicochemical properties of *T. molitor* larvae protein and concluded that this treatment significantly reduced the particle size of proteins, affecting the secondary and tertiary structure, improving the hydrophobicity, the free sulfhydryl content, the stabilization effect, and the interface absorption, and exhibiting a tighter emulsion structure. These results were in accordance with Huang et al. (2023), who studied the effect of high intensity ultrasounds on physicochemical properties of *T. molitor* protein, observing that the treatment improved solubility, foam stability, emulsification, and foamability of the resulting protein.

### Ionic Liquids (ILS) and Deep Eutectic Solvents (DES) Extraction

Ionic liquid solvents (ILs) are formed by large organic cations with several anions or by a mixture of acids and bases (Sulthan et al., 2023). Among ILs, deep eutectic solvents (DES) are the most remarkable. DES are a novel class of green solvents, formed by binary or ternary mixtures of different compounds which are linked via strong hydrogen bonds. These solvents are formed by at least one compound that acts as a hydrogen bond donor (HBDs) and another compound that functions as a hydrogen bond acceptor (HBAs), lowering the melting points of both substances compared to when they are individually used. DES presents several advantages since they are cost-effective, are environmentally respectful, are easy to prepare, and can be recovered and reused as much times as other extraction solvents, reducing the total production costs of the target products (Li et al., 2022). The most common method to prepare DES is heating and/or stirring the compounds at temperatures between 50 and 100 °C, without needing further purification steps. There are five types of DES, but the most referenced and used are those of type III, which are formed by a quaternary ammonium salt acting as HBA and an HBD that is often an amine, a carboxylic acid, or a polyol. However, the definition of DES is still very controversial and is in constant open debate, still being mostly associated with the ILs. Another category of DES comprehends those formed by metabolites naturally occurring in living cells, being named natural deep

eutectic solvents (NADES). These solvents have several potential activities on different reactions, such as catalysis reactions, isolation of polysaccharides, or synthesis of different nanoparticles (Sulthan et al., 2023).

The recent application of DES to extract chitin is present in Table 4. Zhou et al. (2019) used NADES formed for choline chloride or betaine as HBD and lactic acid, urea, n-butyric acid, glycerol, urea, and oxalic acid as HBA to extract chitin of *H. illucens*. Huet et al. (2021) studied the application of DES to purify the chitin of two insects, *H. illucens* and *Bombyx eri*, obtaining a purity close to 100% and yielding between 65 and 68%, at 110 °C, for 2 h (Table 4).

The treatment with DES and/or NADES can be used to deacetylate chitin into chitosan. To the best of our knowledge, there is no literature focusing on the application of DES or NADES using edible insects as raw material. Nonetheless, Vicente et al. (2021) studied the utilization of DES to obtain chitosan, comparing commercial or shrimp shells chitin as raw materials (Table 4). These authors observed that using DES formed by chlorine chloride and acid malic resulted in deacetylation degrees of 40% and 80% with commercial and shrimp shells chitin, respectively.

Apart from chitin, other fractions can be extracted with DES, such as phenolic compounds, proteins, lipids, and different carbohydrates from chitin (Boateng, 2023). However, the application of DES to obtain these fractions is underexplored in insect biomasses. Nonetheless, other authors used DES to extract protein from plant biomasses with promising results. For example, Chen et al. (2021) used a mixture of choline chloride and glycerol (1:1) and extracted up to more 10% of protein from soy compared using traditional extraction methods. Likewise, bamboo shoots treated with a mixture of levulinic acid and choline chloride (1:6) yield

up to more 60% of protein than when conventional methods were used (Lin et al., 2021). Therefore, it is fair to affirm that further work must be carried out to assess the feasibility of using DES to extract valuable compounds from insects in addition to chitin, including of *T. molitor*.

## Enzymes-Based Extractions

Enzymatic hydrolysis is an eco-friendly method that uses a single or a combination of specific enzymes to disrupt the recalcitrant structure of different biomasses, upgrading the access and extraction of value-added compounds. The enzymatic hydrolysis is more specific for the compound of interest, usually requires low volumes of water, does not involve hazardous chemicals, and results in high product yield (Nadar et al., 2018). This extraction process can improve the nutritional, functional, and sensorial properties of insect proteins, also highlighting the increase of free amino acids content (Chewaka et al., 2023). The final yield of these type of extractions depends on the solvent, the enzyme specificity, temperature, pH, and the ratio of enzyme/substrate. These parameters have a great influence on the hydrolysis efficiency, highlighting the pH, extraction time, concentration of the enzyme, and temperature (Gligor et al., 2019). The pH required for enzymes efficiency is usually acidic in the range of the isoelectric point of proteins, that is, around 4.5. However, proteins may not be efficiently extracted at this exact pH; thereof, pH values may oscillate from 4.5 depending on the protein source (Nadar et al., 2018). The extraction time and enzyme concentration are deeply related parameters since long periods of extraction may negatively affect the compounds' yield, leading to enzyme denaturation (Muniglia et al., 2014). The temperature is another important

**Table 4** DES used in extraction of chitin from insect biomass

Raw material	HBD	HBA	Purity (%)	Yield (%)	DD (%)	Reference
<i>H. illucens</i> powder	Choline Chloride	Lactic acid	91.34 ± 1.39	23.33 ± 0.21	84.75	Zhou et al. (2019)
	Choline Chloride	n-Butyric acid	86.41 ± 2.34	26.26 ± 0.35	82.49	
	Choline Chloride	Glycerol	87.62 ± 3.46	22.85 ± 0.62	86.56	
	Choline Chloride	Urea	88.14 ± 2.48	26.02 ± 0.65	80.19	
	Choline Chloride	Oxalic acid	87.89 ± 2.76	23.83 ± 0.68	90.70	
	Betaine	Lactic acid	85.13 ± 2.98	25.70 ± 0.52	89.29	
	Betaine	n-Butyric acid	83.75 ± 2.59	24.53 ± 0.34	83.92	
	Betaine	Glycerol	86.22 ± 3.81	25.47 ± 0.57	88.91	
	Betaine	Oxalic acid	87.19 ± 2.45	22.85 ± 0.81	84.17	
	Betaine	Urea	90.52 ± 2.91	26.71 ± 0.29	88.01	
<i>H. illucens</i> chitin purificate	Choline chloride	Lactic acid	100 ± 0.9	68	ND	Huet et al. (2021)
<i>Bombyx eri</i> chitin purificate	Choline chloride	Lactic acid	96.8 ± 2.2	65	ND	
Commercial chitin	Choline chloride	Malic acid	ND	ND	40	Vicente et al. (2021)
Chitin from shrimp shell	Choline chloride	Malic acid	ND	ND	80	

HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; DD, deacetylation degree; ND, non-determined



parameter, with high temperatures often decreasing the activity of the enzyme. Nevertheless, low temperatures may not be adequate to activate the enzymes, compromising the process efficiency (Nadar et al., 2018).

Several types of enzymes are used in these extractions, being the most used cellulases, hemicellulases, pectinases, proteases, lipases, and chitin (Gligor et al., 2019; H. Kim et al., 2023). The proteases reduce the size of proteins and increase their solubility. Hall et al. (2017) carried out enzymatic hydrolysis of *Gryllodes sigillatus* meal with protease from *Bacillus licheniformis* and observed that an enzyme/solvent ratio between 0.5 and 3 during 30–90 min yielded between 55% up to 71% of protein and between 2.6% up to 11% of lipids. Likewise, Rivero Pino et al. (2020) applied an enzymatic hydrolysis in mealworm meal with four commercial proteases (subtilisin, pancreatic trypsin, ficin, flavourzyme) obtaining bioactive peptides with angiotensin-converting enzyme inhibition capacity, antioxidant activity, and dipeptidyl peptidase IV activity. An enzymatic hydrolysis carried out in mealworm meal using alcalase decreased the protein but increased the presence of branched-chain amino acids and antioxidant activity (Yoon et al., 2023). Furthermore, proteases can be used to extract other compounds in addition to protein, such as chitin and chitosan. Da Silva Lucas et al. (2021) used a bacterial protease (from *Bacillus licheniformis*, 2%) to extract chitin from *T. molitor* exuvias, obtaining a deproteinization efficiency of 85% and producing 70.9% of chitin and 45.1% of chitosan, with the total procedure yielding 31.9%. Chewaka et al. (2023) hydrolyzed mealworm meal using three different enzymes (commercial alcalase and flavourzyme, and concentrate nuruk extract), observing higher hydrolysis degree using the nuruk extract. Recently, H. Kim et al. (2023) extracted chitin from mealworm meal using alcalase and observed that the derived chitosan had similar properties to chitosan of commercial chitin origin.

### Supercritical Extraction Methods

The supercritical extraction methods are considered green-saving procedures in which the solvents are heated and compressed beyond their critical point, disrupting several biocomponents (Tzima et al., 2023). The supercritical extractions require specific equipment, in which the solid material is placed in direct contact with gaseous solvents, dissolving the biomass and extracting the biocomponents of interest. A cosolvent can also be added to the mixture at the end of the process to increase the compound yield, such as ethanol, and separate the dissolved biocomponent from the liquid stage (Uwineza & Waśkiewicz, 2020).

Among the supercritical methods, CO<sub>2</sub> is one of the most used gaseous solvents since it is suitable to extract nonpolar biocomponents, such as lipids, presenting many

advantages, such as high selectivity, low cost, and non-toxicity, and favors the extraction of thermally labile compounds (Zhang et al., 2018). Nonetheless, supercritical extraction can also be carried out without using cosolvents, therefore not degrading lipids as occurs when applying other methods using strong solvents (Uwineza & Waśkiewicz, 2020). The CO<sub>2</sub>-based extraction can extract up to 95% of lipids, which is in close range to values obtained using Soxhlet or hexane-based extractions (Purschke et al., 2017). Therefore, using supercritical extractions reduces the use of harmful chemicals that are used in traditional methods, and the extraction yields can be increased by applying different conditions during the supercritical extractions (Purschke et al., 2017). Nonetheless, some authors have been focusing on the application of supercritical methods to extract valuable compounds from insects' biomass. Purschke et al. (2017) carried out a supercritical CO<sub>2</sub>-based extraction of *T. molitor* mealworms at a pilot scale and observed that using 400 bar, 45 °C, and 105 min yielded up to 95% of lipids. Likewise, Laroche et al. (2019) extracted 22.1 ± 0.6% (w/w) of lipids from *T. molitor* meal in a pilot scale extraction using CO<sub>2</sub> as a solvent, at a flow rate of 10 g/mL, 325 bar, and 55 °C, for 75 min. More recently, Laurent et al. (2022) used a supercritical CO<sub>2</sub>-based extraction in *H. illucens* and *T. molitor* mealworm meals, yielding up to 85.7% and 92.9% of lipids, respectively.

Other compounds can be used as solvents in supercritical extractions, such as *n*-propane. Da Cruz et al. (2022) carried out a pilot scale extraction in *Zophobas morio* and *T. molitor* with *n*-propane using between 40 and 60 bar, at 40 °C, and obtained up to 94% of lipids, which was comparatively higher than the lipids obtained using the conventional Soxhlet extraction with *n*-hexane (32.19%; Verheyen et al., 2023) and a bench-scale extraction previously conducted, concluding that this extraction is feasible to upscale.

## Applications of Biorefined Insect-Based Products

### Food and Feeding Industries

*Tenebrio molitor* mealworm meal has many applications in the food and feeding industries given its high-quality nutritional profile, with some examples in the bakery and meat sectors. Important commercial brands of insect-based products already exist in Belgium, such as the “Goffard sisters” and “Nimavert,” using protein isolates or raw mealworm meal in bakery products, such as bread (Borges et al., 2022). González et al. (2019) compared the inclusion of 5% of defatted *H. illucens*, *A. domesticus*, and *T. molitor* meals in bread and observed a protein increase of 2.59%, 1.43%, and 1.13%, respectively. Similarly, Roncolini et al. (2019)

also observed that the inclusion up to 10% of TMM in bread increased the amino acids, protein, and lipids contents, also improving several organoleptic properties of this food, such as the volume and softness. Indeed, an ultrasound-based extraction of mealworm protein showed higher in vitro digestibility and digestion kinetics than when the proteins were extracted using an alkaline method (Zhang et al., 2023). Pasini et al. (2022) used defatted mealworm and *A. domesticus* meals to produce pasta, observing an increase in protein digestibility probably related to the high content of lysine and valine present in the insects' meals. Additionally, the texture of the insects' protein-made pasta also presents great firmness and adhesiveness, and color changes to brown tones (Table 5). Therefore, the use of insects to manufacture common foods can effectively substitute the use of costly ingredients while also improving the nutritional value of these foods. Azzollini et al. (2018) observed that cereal snacks containing between 10 and 20% of *T. molitor* mealworms meal presented higher protein digestibility; increased protein and lipids contents almost 2- and fivefold, respectively; and reduced the starch content up to 19%. Replacing 15% of wheat meal with mealworms or *L. migratoria* meals in muffins increased the protein and lipids fractions, decreased the carbohydrate content, and resulted in a softer texture (Çabuk, 2021). However, the same authors observed that the sensory acceptance varied according to the insect meal used, that is, decreasing when *L. migratoria* meal was added due to a dark coloration and annoying odor, while mealworms meal was well accepted by the consumers. Cho and Ryu (2021) observed that adding mealworm meal to a meat-like product, constituted by defatted soy protein and corn starch, decreased the hardness, cohesiveness, springiness, and chewiness while also increasing the nitrogen solubility index of the final product. Kim et al. (2016) replaced up to 10% of lean pork with defatted or acid-hydrolyzed mealworms meal, which increased the protein solubility and cooking yield and decreased the pH. Therefore, different studies point out the high potential of using *T. molitor* mealworms meal to replace traditional ingredients, maintaining the organoleptic properties of foods.

Nonetheless, instead of using insect meals for direct human consumption, they may also be applied in animal feeds. Vasilopoulos et al. (2023) observed that the dietary inclusion of 5 or 10% of mealworm meal in diets for chickens improved the nutritional profile of feeds, increasing protein, fiber, and lipid contents while also improving the growth and wellness of the chicken, increasing the protein level, and decreasing the lipids content of chicken breast and thigh meats. Addeo et al. (2022) included 5%, 10%, and 20% mealworms meal in diets for Japanese quail, *Coturnix japonica*, and observed that the inclusion up to 5% did not negatively affect the growth and improved the number of acids

mucopolysaccharide-secreting cells (AB+) and other intestinal cells. Dong et al. (2021) studied the production of selenium-enriched food products using *T. molitor* mealworms protein, attaining an antioxidant activity between 55% ( $0.75 \text{ mg mL}^{-1}$ ) and 70% ( $0.5 \text{ mg mL}^{-1}$ ).

Within animal feeds, the nutritional composition of insect meals increases its suitability to be used in fish feeds, although different results have been observed (Barroso et al., 2014). Basto et al. (2022) studied the partial and total replacement of dietary fishmeal by defatted mealworms meal in diets for European seabass, *Dicentrarchus labrax*, observing a similar feed consumption when fish were fed diets with full replacement of fishmeal and the control diet, but also detrimental changes in hepatic and plasmatic metabolites, which may lead to long-term growth and health problems. In another study, mealworm meal was used to replace 15%, 30%, and 40% of soybean meal in diets for Nile tilapia, *Oreochromis niloticus*, observing that dietary inclusion levels above 30% increased the hardness and gumminess of fillets and decreased muscle antioxidant capacity (Zhang et al., 2022). *Tenebrio molitor* mealworm meal was included up to 30% in feeds for tench, *Tinca tinca*, and did not negatively affect fish growth, increased the specific growth rate and decreased lipid peroxidation, but the inclusion of 15% of *H. illucens* decreased growth (Hidalgo et al., 2022).

In addition to protein content of *T. molitor*, the chitin fraction also unveils new utilities in the food and feeding industries. Saenz-Mendoza et al. (2020) characterized a chitosan film obtained from *T. molitor* and observed a more intense brown tonality and higher opacity than a commercial packaging product, envisioning using this natural chitosan in food packages with protective properties against the UV radiation. Furthermore, chitosan obtained from *T. molitor* exhibited high antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Escherichia coli* (Shin et al., 2019). Therefore, the high opacity and antimicrobial properties of products obtained from *T. molitor* processing disclose novel utilizations in food and feed packaging, simultaneously preserving the nutritional quality and preventing microbial contamination during long storage periods (Ma et al., 2022).

## Biomedical Industry

Several products extracted from both mealworms (larvae) and adults of *T. molitor* can be potentially used in the biomedical sector (Table 5). Indeed, the high protein content of mealworm meal can be used to produce protein-dense and therapeutic foods to combat serious malnutrition problems, replacing the conventional and less healthy ingredients (such as vegetable oils and sugars, among others) and decreasing the overall costs (Feng, 2018).

**Table 5** Applications of derivate products of *Tenebrio molitor* processing

<i>Food industry</i>				
Product	Insect fraction	Process	Results	Reference
Bread	TMM	5% and 10% levels	↑ Protein content	González et al. (2019)
		5% and 10% levels	↑ Protein: free and essential AA	Roncolini et al. (2019)
		10%, 20%, and 30% levels	↑ Protein content ↓ Springiness and cohesiveness	Kowalski et al. (2022)
Pasta	TM protein fraction	14% level	↑ Protein texture	Pasini et al. (2022)
Snacks	Grinded TMM	10% level	↑ Digestible protein content	Azzollini et al. (2018)
Muffins	TMM	2%, 5%, 6%, and 10% levels	↓ Texture and lightness	Zielińska et al. (2021)
		15% level	↑ Protein and lipid contents ↓ Carbohydrate's content	Çabuk (2021)
Meat-analog product	TMM	Inclusion of 0%, 15%, & 30%	↑ Protein digestibility ↑ Water holding	Cho and Ryu (2021)
Emulsion sausages	Defatted TMM	10% level	↑ Protein solubility	H. W. Kim et al. (2016)
Frankfurt sausages	TMM	5% and 60% level	↑ Tensile strength	Choi et al. (2017)
Burgers	TMM	Burger beef: TMM (50:50%) Lentil: TMM (50:50%)	Good acceptance	Caparros Megido et al. (2016)
<i>Animal feeding industry</i>				
Animal feed	Insect fraction	Process	Results	Reference
Chicken	TMM	5% & 10% level	↑ TPC	Vasilopoulos et al. (2023)
			↑ Growth parameters ↑ Protein	
Japanese quail ( <i>Coturnix japonica</i> )	TMM	5%, 10%, and 20% levels	↑ AB +	Addeo et al. (2022)
European seabass ( <i>Dicentrarchus labrax</i> )	Defatted TMM	Inclusion of 20% and 40% levels	↑ Glucose in the liver	Basto et al. (2022)
Nile tilapia ( <i>Oreochromis niloticus</i> )	TMM	Replace at 15%, 30% & 45% levels	↑ Hardness ↑ Gumminess	Zhang et al. (2022)
Tench ( <i>Tinca tinca</i> )	TML powder	Replace 15 & 30%	↑ Growth performance; improved oxidative status	Hidalgo et al. (2022)
<i>Biotechnological industry</i>				
Function	Insect part	Process	Results	Reference
Antioxidant	TMM	Selenium enrichment	Hydroxyl radical 70% (0.5 mg mL <sup>-1</sup> )	Dong et al. (2021)
		Enzymatic hydrolysis with alcalase, trypsin, neutrase, and flavourzyme	DPPH 55% (0.75 mg mL <sup>-1</sup> ) ↓ ROS production	
	TMM protein	Enzymatic hydrolysis with subtilisin, trypsin, ficin, and flavourzyme	DPPH IC50 1.03–2.31 mg mL <sup>-1</sup> FRAP IC50 4–7 mg mL <sup>-1</sup> Fe21 chelating activity: IC50: 0.53–2.12 mg mL <sup>-1</sup>	Rivero Pino et al. (2020)
	TMM	Insects breed with, bread and brewer's spent grain	DPPH: 0.28–34 mmol TE kg <sup>-1</sup> ABTS: 1.70–2.45 mmol TE kg <sup>-1</sup> FRAP: 0.75–1.04 mmol TE kg <sup>-1</sup>	Mancini et al. (2021)
	TMM	Extraction with UAE and PLE using ethanol and ethanol:water (1:1)	DPPH inhibition of 11–87% at 10 mg mL <sup>-1</sup>	Navarro del Hierro et al. (2020)
	Defatted TMM	Defatting with <i>n</i> -hexane	DPPH: 21 mg TE g <sup>-1</sup> dry basis ABTS: 1.26 mg TE g <sup>-1</sup> dry basis	Song et al. (2018)
	TMM protein hydrolysate	Enzymatic hydrolysis with alcalase for 12 h	↓ ROS production ↑ Antioxidant genes expression	Cho and Lee (2020)
	TM protein	Extraction NaOH (0.2%) and precipitation at isoelectric point	↑ Pancreatic lipase inhibition	Zielińska et al. (2021)

**Table 5** (continued)

Food industry				
Product	Insect fraction	Process	Results	Reference
Anti-inflammatory	TMM oil	Supercritical extraction	↓ TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 expression ↓ MPO	Park et al. (2022)
	Defatted TMM	Unsaponifiable lipids extracted with methanol	↓ NO production of LPS-induced RAW 264.7 cells	Son et al. (2020)
	TMM protein fraction	Fungal fermentation of soybean enriched with TMM protein	↓ TNF- $\alpha$ and IL-6 expression	Hwang et al. (2019)
	Defatted TMM	Extraction of lipids with ethanol 99.5%	↓ TNF- $\alpha$ and IL-6 expression	Pessina et al. (2020)
Anti-hypertensive	Hydrolysate protein	Enzymatic hydrolysis using subtilisin, trypsin, ficin, and flavourzyme	ACE IC50: 0.26–1.28 mg mL <sup>-1</sup>	Rivero Pino et al. (2020)
	Hydrolysate protein	Enzymatic hydrolysis using alcalase, neutrase, flavourzyme, Protamex	Inhibition ACE: 60–80%	Yoon et al. (2019)
	Defatted TMM	Extraction of lipids with ethanol 99.5%	↑ ACE plasma inhibitory activity ↓ Heart rate, coronary perfusion pressure, and systolic blood pressure	Pessina et al. (2020)
	Boiled TMM	Boiling at 10 min at 100 °C in water	↑ ACE plasma inhibitory activity	Zielińska et al. (2021)
Anti-lipidemic	TMM	Extraction with UAE and PLE with ethanol and ethanol:water (1:1)	↓ Lipids Pancreatic lipase IC50: 0.1–0.7 mg mL <sup>-1</sup>	Navarro del Hierro et al. (2020)
	TM protein	Extraction of NaOH (0.2%) and precipitation at isoelectric point	↑ Pancreatic lipase inhibition	Zielińska et al. (2021)

*TM*, *T. molitor*; *TMM*, *T. molitor* mealworm meal; *TPC*, total phenolic compounds; *AB+*, mucous cells blue; *DPPH*, 2,2-diphenyl-1-picrylhydrazyl; *FRAP*, ferric-reducing antioxidant power assay; *ABTS*, 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) assay; *ROS*, reactive oxygen species; *TE*, Trolox equivalents; *ACE*, angiotensin I-converting enzyme; *TNF- $\alpha$* , tumor necrosis factor; *IL-1 $\beta$* , interleukin-1 beta; *IL-6*, interleukin 6; *MPO*, myeloperoxidase; *NO*, nitric oxide; *LPS*, lipopolysaccharide; *UAE*, ultrasound-assisted extraction; *PLE*, pressured-liquid extraction

Furthermore, the antioxidant properties of *T. molitor* mealworm meal also envision great biomedical utilities. Cho and Lee (2020) obtained a protein hydrolysate with a high scavenging activity of reactive oxygen species (ROS). *Tenebrio molitor* mealworms protein is shown to be a suitable source of bioactive peptides with beneficial modulating effects, since the high antioxidant activity leads to the activation of antioxidant genes, lowers the ROS production, and modulates physiological processes in mouse hepatocyte cells during in vitro trials, with promising applications in nutraceutical products (Rivero Pino et al., 2020). Oh et al. (2022) obtained a protein hydrolysate from mealworms during an enzymatic hydrolysis with alcalase and used it to produce tofu, improving the protein digestibility, antioxidant and anti-inflammatory capacities, and cell proliferation compared to mixtures of tofu produced with the unhydrolyzed mealworm meal or the protein isolate.

Nonetheless, attention has also been given to the anti-inflammatory properties of mealworm meal. Park

et al. (2022) observed that adult mice with ulcerative colitis administered with mealworm oil showed less symptomatology associated with the disease and decreased the disease activity index (DAI), as well as the expression of colitis-related genes, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, and MPO peroxidase, suggesting that mealworms oil decreases the degree of inflammation. Zielińska et al. (2020) studied the inhibitory activities on angiotensin-converting enzyme (ACE), pancreatic lipase, and  $\alpha$ -glucosidase of *T. molitor* meal and observed that the ACE inhibition increases after applying a boiling procedure, while lipase and  $\alpha$ -glucosidase increased after isolation of protein. However, baking or boiling the protein decreased the inhibition activity of ACE and  $\alpha$ -glucosidase, while lipase inhibition activity slightly increased after the baking treatment. Chitin and chitosan have several utilities on biomedical industry highlighting the antimicrobial activity against pathogenic microorganisms. Nafary et al. (2023) studied the antimicrobial effects of chitin and chitosan of mealworm

meal origin, observing that an acidic solution containing 8% of mealworm meal chitosan exhibited inhibited the growth of *Pseudomonas aeruginosa*.

## Challenges and Future Work

The use of insects for food and feeding purposes still faces several challenges, such as the acceptance by consumers and the establishment of a cost-effective and eco-friendly production and utilization of all insect fractions, especially the chitin of adult insects. This is especially important considering that the use of larvae-based meals in the food and feeding industries will keep increasing, highlighting the application in bakery products and food analogs to improve the nutritional profile of the final products. Insect-based products are exclusively elaborated with larvae which foresees the generation of different by-products, such as adults and frass, with low or no economic value. Therefore, in the current scenario of increasing use of larvae-based meals, it is mandatory to develop efficient, green, and safe processes to extract the “hidden” value of insect by-products, solving the accumulation of these materials and enlarging the applicability and functionality of varied insect species, including *T. molitor*.

Considering an eco-based and future-oriented approach in fractioning insects’ biomass, establishing a biorefinery concept is the most suitable solution to extract value-added compounds from the insect by-products, which can then be applied in different industries, including agriculture, food and feeds production, resources management, biomedicine, among others. Green extraction methods can be applied to obtain protein, lipids, and chitin from insects, including of *T. molitor* biomass, resulting in higher extraction yields and improving the quality of the final products in comparison to those obtained using traditional methodologies. Furthermore, green methods will enable the use of insect-based products in different industries and will enlighten the general consumers regarding their potential uses, especially since the use of these methods is still underexplored in insect by-products, including the adults. Nonetheless, the future of insect biorefinery passes through the implementation of efficient exploitation methods of adults and frass, largely depending on addressing regulatory and technical challenges, such as the development of methodologies adapted to these specific biomasses. Fostering a more sustainable insect industry will positively impact the circular economy, promoting a highly efficient use of resources and generating low or no wastes, at the expense of minimal greenhouse gas emissions. Therefore, a synergetic approach between regulatory agencies, the academia, and the industries will boost the insect industry towards tackling the emergent societal problems.

## Conclusions

*Tenebrio molitor* multiple applications rely on its top-quality nutritional composition and richness in bioactive compounds. This review provides a broad insight about the different methods that can be applied to obtain diverse insect-derived products, which can then be used in animal feed or food to enhance the nutritional value and promote healthier livestock. Insects produce a wide range of compounds, including chitin, which can be used in the production of bioplastics, biodegradable materials, and biochemicals, contributing to the development of more sustainable packaging materials and chemical feedstocks. Since the insect biorefinery industry continues to evolve, addressing regulatory challenges and ensuring consumer acceptance are essential as well as developing clear guidelines for production and marketing. Insect biorefineries have the potential to expand globally, providing sustainable solutions to various rural regions facing different environmental and economic challenges.

**Author Contributions** Nuno Muñoz-Seijas: wrote the main manuscript Helena Fernandes: revision of the manuscript José Manuel Domínguez: revision of the manuscript José Manuel Salgado: structure of the original draft and revision of the manuscript

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research was financed by the Spanish Ministry of Science and Innovation (Project PID2021-122176OA-I00 and CNS2023-145453). This work forms part of the activities of the Group with Potential for Growth (GPC-ED431B 2021/23) funded by the Xunta de Galicia (Spain). José Manuel Salgado is contracted by the “Beatriz Galindo” program of the Ministry of Education from Spain (BG20–00156). Nuno Muñoz Seijas is contracted by the industrial doctoral program of Xunta de Galicia (04\_IN606D\_2023\_2542150). Funding for open access charge: Universidade de Vigo/ CRUE-CISUG.

**Data Availability** Data will be made available on request.

## Declarations

**Competing Interests** The authors declare no competing interests.

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