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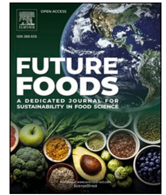

Characterization of all life stages of Tenebrio molitor: Envisioning innovative applications for this edible insect

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ABSTRACT

The human food security is unavoidably dependent on exploring novel nutritional sources, such as edible insects. However, insects farming needs to improve their rearing practices, such as extracting value from all growing stages, from larvae until adults. In this work, a full characterization of larvae, pupae and adults of *Tenebrio molitor* was carried out. Higher protein and ash contents were observed in pupae, while higher lipids (mainly polyunsaturated fatty acids, PUFA) were observed in larvae, decreasing until reaching the adult stage. Chitin content was 6-fold higher in adults than larvae and pupae. Also, it was possible to quantify the chitin resorting to computed 3D tomography, achieving similar values as the traditional and chemically based method. Lower mineral content was found in larvae, while high iron, zinc and copper levels were found in beetles. Phenolic compounds content was similar among larvae, pupae and beetles. FTIR analysis was a valuable, easy, and fast method to assess the nutritional composition of *T. molitor*, detecting vibrations characteristic of protein, lipids and chitin fractions. This study provides a full characterization of all life stages of *T. molitor*, highlighting the possible valorisation chains that can be adopted to obtain highly nutritive and/or functional compounds.

1. Introduction

In 2017, the protein demand of the 7.3 billion inhabitants was circa 2020 million tons worldwide (Henchion et al., 2017). Food demand is ever-increasing along with the growth of the world population, which is expected to reach nearly 10 billion people in 2050 (Shafique et al., 2021). Also, it is estimated that circa 600 million people worldwide will be chronically malnourished by 2030 (FAO, 2023). Therefore, the capacity of food production systems will continue to be surpassed beyond its limits, being also negatively affected by other societal factors, such as the increase in income and urbanization (Henchion et al., 2017; FAO, 2018). The most severe food insecurity is faced in Africa and Asia, housing 282 and 402 million undernourished people in 2022, respectively (FAO, 2023). Nonetheless, food insecurity is a result of intertwined causes of social, political, environmental and demographic origin, conditioning the access, availability and stability of food sources (Foini et al., 2023). For example, the rapid urbanization of regions in Africa and Asia increases the food demand and may trigger the consumption of highly processed foods due to higher cost of animal-based proteins, while food insecurity is undistinguishable between urban, peri-urban and rural areas (FAO, 2023). On the other hand, increasing income in low-income countries foresees the adoption of similar consumption patterns of high-income countries, such the increase of uptake of animal-based proteins such is observed in European and North American countries (Henchion et al., 2021). Nowadays, meat is still the principal supplier of protein, with poultry meat being the most produced due to its high affordability (FAO, 2018). Despite the high feed conversion efficiency and growth rates, poultry production is more prone to be negatively affected by market and availability fluctuations to feed the animals (Kleyn and Ciacciariello, 2021; Barbut and Leishman, 2022). Therefore, despite the fact that the main protein source consumed in the EU is of animal origin, the animal production sector is greatly dependent of importing protein-rich plants, increasing the volatility of food prices and the supply chain (Román, 2023). This scenario causes a major deficiency in the supply of plant protein in the EU, causing a high dependency of foreign imports of high-quality plant

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crops. For example, 97 % of soybean meal and 21 % of maize used as feeds is imported (Román, 2023). Therefore, novel and eco-conscious protein sources need to be explored to tackle the protein deficiency in the EU, improving the production systems efficiency, decreasing the pressure on ecosystems services and widening the food security prospects.

In the EU, "novel foods" are currently regulated by the Regulation (EU) 2015/2283 and comprehend items that were not consumed before 15 of May 1997, are safe to the consumers, properly labelled, may not be nutritionally disadvantageous when the novel food aims to replace an existent one, and not contain harmful levels of antinutritional compounds (European Commission 2015; Ververis et al., 2020). Nevertheless, novel proteins must also be highly available to the overall consumers, that is, have a steady supply and be economically attractive, and also be produced via green sources and/or methods (Henchion et al., 2017). Edible insects are alternative protein sources, and is estimated that circa 1600 species are consumed worldwide, mostly species of orders Coleoptera, Lepidoptera and Hymenoptera (Precup et al., 2022). The edible insects generally consumed present several advantages, such as high protein content, easiness of rearing, high feed utilization efficiency, and low dependency on water, energy and space (Hammer et al., 2023). In this sense, the EU (Regulation 2015/2283) approved the use of insect-derived products as novel foods for human consumption (European Commission 2015; Precup et al., 2022). Among edible insects, *Tenebrio molitor* L. is one of the most promising and the use of larvae (mealworms) are approved by the EU as novel food along with other six species (Regulation 2015/2283; (European Commission 2015)), and can be commercialized in frozen, dried or powder forms (Regulation 2022/169; (European Commission 2022)). Also, mealworms are authorized as a protein source in feeds for farmed animals, including fish, poultry and swine (Regulation 2017/893; European Commission 2017). Indeed, the larval stage of *T. molitor* is highly desirable since it contains high protein and low chitin contents, increasing the protein bioavailability and digestibility. Contrarily, the adult stage of *T. molitor* (beetles) presents high protein but also high chitin contents, which hinders its use as a nutritional source. Chitin is a polysaccharide formed by glucosamine and *N*-acetylglucosamine units, constituting the exoskeletons of insects and is tightly bound to proteins, decreasing the nutritional advantages of dietary utilization of insects in pupal and adult stages compared with larvae. However, chitin can be fractionated into chitosan, a highly water-soluble and biologically active molecule (Nafary et al., 2023), whose functional properties can be applied in a wide variety of industrial sectors, such as agriculture, medicine, pharmacy, biotechnology, animal feeds, among others (Khanal et al., 2023; Nafary et al., 2023). Therefore, further attention must be given to the characterization of all development stages of *T. molitor*, widening the valorisation and applicability of all biomasses produced within this industry. The adult insects should be considered to obtain protein-rich biomass and/or chitosan with bioactive properties, especially if this biomass is an inherent and no-value residue from insect farms.

Tenebrio molitor life cycle comprehends four stages: egg, larvae, pupae and adult (Khanal et al., 2023). Eggs of *T. molitor* hatch in 3–9 days after deposition, while larvae stage is the longest and may last between 57 days (under controlled conditions) and up to 2 years when individuals are submitted to low temperatures (Ribeiro et al., 2018; Hong et al., 2020; Gkinali et al., 2022a). During this time, larvae may experience between 9 and 23 instars (Ribeiro et al., 2018). After, larvae enter the pupa phase, which is the briefest of all development stages, lasting between 5 up to 48 days depending on the temperature (Gkinali et al., 2022b). During this time, the organisms do not eat and gradually evolve a rigid exoskeleton. Then, the adult organisms emerge, and this stage may last between 2 up to 3 months (Hong et al., 2020). Different factors influence the transition between each stage of development of *T. molitor*, such as temperature, diet type, moisture, photoperiod, oxygen and population density (Ribeiro et al., 2018).

It is not surprising that, to the best of our knowledge, most works focus solely on the study and characterization of *T. molitor* larvae. The protein content of *T. molitor* may vary between 47.2 % dry weight (DW; larvae) and 65.3 % DW (adult beetles; Rumpold and Schlüter, 2013), having high amounts of essential amino acids (EAA; 124.9 mg/g DW), including leucine (22.19 mg/g DW), valine (18.99 mg/g DW), lysine (15.89 mg/g DW), and isoleucine (13.19 mg/g DW; (Wu et al., 2020)). However, the wide range of protein values reported in the literature is mainly obtained by using the conventional protein conversion factor of 6.25 (Kp), which often overestimates the protein content (Janssen et al., 2017).

The lipids content of *T. molitor* generally ranges between 14.9 % DW (beetles) and 43.1 % DW (larvae; Rumpold and Schlüter, 2013), mainly constituted by unsaturated fatty acids ranging between 12.1 % DW (adults) and 21.9 % DW (larvae; (Khanal et al., 2023)), followed by saturated and polyunsaturated fatty acids (Gkinali et al., 2022c). Therefore, the simultaneous richness of high-quality protein, the FA profile and the practical and economic advantages of *T. molitor* rearing, increases the interest of different industries in using this edible insect, such as for food and animal feeds production.

Since a great variety of compounds can be obtained from *T. molitor*, the present work centres in assessing the nutritional composition of all developmental stages of *T. molitor*, that is, larvae, pupae, and adult beetles. Also, the amino acids, fatty acids and mineral profiles were assessed, and Fourier-Transform Infrared Spectroscopy (FTIR) was also evaluated as an efficient method to assess the nutritional composition of *T. molitor*. Furthermore, an indirect measure of chitin content in *T. molitor* adults is proposed using computed 3D X-ray tomography (CT).

2. Materials and methods

2.1. *Tenebrio molitor*

Tenebrio molitor larvae, pupae, and beetles were acquired from the insect farm Galinsect S. L. (Ponteareas, Galicia, Spain). The organisms were fed a cereal-based diet mainly constituted of wheat bran, and occasionally adding vegetables as nutrient and water source. Larvae were collected aging between 180 and 210 days old, pupae were collected in the first day of pupation, and adults were sampled at the end of their life cycle, that is, between 80 and 90 days old.

2.2. *Proximate composition larvae, pupae and beetles of T. molitor*

Larvae, pupae and beetles' meal were fasted for 24 h, sacrificed by placing at −20 °C, dried at 50 °C for 48 h, finely ground and stored in a desiccator until analysis. The characterization was carried out in each sample in triplicate according to the standard procedures of the Association of Official Analytical Chemists (AOAC International 2016). Briefly, moisture was determined by drying samples in an oven at 105 °C until constant weight; total nitrogen and organic carbon were determined by a Thermo Finningan Flash Element Analyzer 1112 series (San Jose, CA, USA); the organic nitrogen was determined using the Kjeldahl method after digestion with concentrated sulphuric acid; the protein content was calculated using the kp factor of 6.25 to multiply the nitrogen content measured using the Kjeldahl method, discounting the nitrogen content of chitin fraction; lipids were assessed using petroleum ether as solvent in a Soxhlet extraction system; ash content by incineration in a muffle furnace at 450 °C, for 16 h. The minerals profile was analysed in the ashes using Flame Atomic Absorption and Atomic Emission Spectrometry (FFAS/FAES). Soluble protein was determined following the method of Bradford (1976).

Chitin was quantified in triplicate using the methods described by Kumari et al. (2015) and Nidheesh and Suresh (2015), with some modifications. First, the demineralization of samples was carried out using HCl 1 M and a 1:40 ratio (w/v), for 2 h, at room temperature (25 °C). After, the mixture was centrifuged (10,000 g, 10 min) and the recovered solid was washed with distilled water until neutral pH and

dried at 50 °C, for 24 h. The deproteinization was carried out using an alkaline solution of NaOH 5 N and a 1:20 ratio (w/v), during 18 h, at room temperature (25 °C). After, the mixture was centrifuged (10,000 g, 10 min) and the solid was washed with distilled water until neutral pH and treated with H_2O_2 solution (1 %, w/w) at a 1:10 ratio (w/v), for 10 min, at room temperature (25 °C). The mixture was centrifuged (10,000 g, 10 min) and the solid was washed with distilled water and dried at 50 °C, for 24 h, and chitin and the nitrogen content of chitin were quantified.

The total phenolic compounds were assessed using the Folin-Ciocalteu method described by Filipe et al. (2020).

Protein digestibility was assessed in triplicate using the method described by Morales et al. (2018). Briefly, each sample was weighed to contain 1 g of protein (DW), mixed with 7.5 mL of pepsin solution (2 mg mL^{-1} in HCl 0.1 M) and incubated at 37 °C for 3 h. After, 7.5 mL of NaOH 0.2 M was added to the samples, followed by adding 3.75 mL of pancreatin solution (2 mg mL^{-1} in phosphate buffer 0.1 M, pH 8) and incubated at 37 °C for 24 h. The reaction was stopped adding 5 mL of trichloroacetic acid (TCA) 30% w/v and adjusting the volume to 25 mL with the addition of TCA 5% w/v. After, the mixtures were centrifuged (10,000 rpm, 4 °C, 30 min), the precipitates (non-digested protein) were dried at 50 °C, and the protein content was determined by the Kjeldahl method. The digestibility of each sample was obtained calculating the difference between the initial protein content and the total protein measured in the precipitate.

2.3. Amino acids profile

To carried out the analysis of amino acids (AA), the samples were simply homogenized, hydrolysed at 110 °C with 6 N HCl, for 24 h (in sealed vials), neutralized and diluted in 0.1 % formic acid (extra pure, 98–100 %; Scharlau S.L., Madrid, Spain). The analysis of AA was carried out using a High-Pressure Liquid Chromatography (HPLC) 1290 (Agilent Technologies, Inc., Santa Clara, CA) coupled to a hybrid Triple Quadrupole mass spectrometer TRIPLE QUAD 3500 (AB Sciex, Framingham, MA). The AA extracts were placed in an autosampler kept at 15 °C and 10 μL of sample was injected into the column. The liquid chromatography analysis was carried out at 30 °C. AAs were separated using a C18 Symmetry column (4.6 × 150 mm; 3.5 μm) from Waters (Milford, MA). The gradient used to separate the AAs consisted of acetonitrile plus 0.1 % formic acid (solvent A) and 0.1 % formic acid in water (solvent B). The total LC–MS/MS run was 11 min with a flow rate of 300 μL min^{-1}. The gradient applied was as follows: $A = 0$–2 min 0 %, 2–6.5 min 20 %, 6.5–6.6 min 80 %, 6.6–8.5 min 80 %, 8.5–8.6 min 0 %, 8.6–11 min 0 %. The detection limits were obtained carrying out dilutions of amino acids mix solution and calculated considering a linearity with $R^2 > 0.999$.

The mass spectra were acquired using an electrospray ionization in a positive mode. The AAs were detected in the same LC–MS/MS run using multiple reaction monitoring (MRM). The source parameters such as curtain gas (30 psi), ionization (2500 V), temperature (650 °C), nebulizer gas (60 psi), heating gas (60 psi), and collision activated dissociation (Medium) were kept constant for MRM and scheduled MRM. The mass spectrometer was set to have a dwell time of 30 msec for the MRM scan survey whereas a 40 s scanning window was defined for each AA using scheduled MRM. LC–MS/MS data were acquired and processed using the Analyst 1.6.1 software (Sciex, Toronto, Canada). The AA profile was assessed in triplicate for each sample and was provided by SSADS-CACTI of University of Vigo.

The digestible indispensable amino acid score (DIAAS) was calculated considering the digestibility values obtained for each life stage of *T. molitor* and using beef protein as reference protein, according to FAO (2013).

2.4. Fatty acids profile

The fatty acid methyl esters (FAMES) production was developed following Ferreira et al. (2020). Briefly, 0.4 g of sample was mixed with 1.6 mL distilled water, 4 mL chloroform and 2 mL methanol and stirred for 1 h to extract lipids. After, the mixture was passed through a Pasteur pipette filled with glass wool. The extraction flasks were washed with 2 mL chloroform and 2 mL methanol and the mixture passed again in the Pasteur pipette. After, the pipette was also washed with 2 mL chloroform and the final mixture were allowed to rest on a separating funnel. The chloroform-containing lipids was recovered (lower phase) and chloroform was evaporated using nitrogen injection. Weight differences between the empty flask and the flask after evaporation was used to assess lipids mass. The lipids were extracted in triplicate for each sample.

The extracted lipids were dissolved in 1 mL of KOH-MeOH solution (0.5 mL^{-1}), vortex-mixed and heated (40 °C, 10 min). The internal standard, pentadecanoic acid (1 g/L), was added to each sample (100 μL). The mixture was then cooled in water, and 2 mL of BF_3/CH_3OH (14 %) was added, vortex-mixed and heated (40 °C, 10 min). The mixture was cooled down and 1 mL of hexane and 2 mL of water were added, vortex-mixed (15 s), and centrifuged until reach the speed of 3000 rpm and stopped immediately. The hexane phase was collected, and FAMES were analysed by gas chromatography (GC) in a GC system (VARIAN 3800) equipped with a flame ionization detector (FID). The FAMES were separated using a TRB-WAX 30 m x 0.25 mm x 0.25 μm column (TR140232, Teknokroma, Tr-wax), with helium as the carrier gas (1 mL/min). The temperatures of the injection port and detector were 250 and 280 °C, respectively. The initial oven temperature was 40 °C for 2 min, with a 30 °C/min ramp to up 150 °C and a 3 °C/min final ramp up to 250 °C. The calibration curves were performed with commercial fatty acids following the same derivation process. The standards of fatty acids were palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), and linoleic (C18:2n6) acids.

2.5. Fourier-transform infrared spectroscopy (FTIR) spectroscopy

Triplicate samples of the three phases of *T. molitor* were dried and analysed by spectrometer Nicolet 6700 (Thermo Scientific, USA) with a detector DTGS KBr. The spectra were obtained in the wavelength range of 400–4000 with 4 cm^{-1} of resolution, and all the spectra resulted from the average of three measurements per sample.

2.6. Computed 3D tomography

The chitin content of larvae, pupae and adults was also determined using computed 3D tomography. The tomographic images were obtained using a YXLON FF20 (Comet-Yxlon GmbH) cone beam CT, with a 190 kV X-ray transmission tube and a flat panel detector, featuring a pixel matrix of 2880 by 2800 and a CsI scintillation screen (Varex Imaging Inc.). The reconstructed 3D images were processed for quantification and analysis using Avizo 3D version 2023.1 (FEI, ThermoFisher Scientific, Inc.). All individuals were immobilized using a commercial-grade 0.3 g/L alpha-cypermethrin spray. They were then affixed to a 5 mm carbon shaft with a nitrocellulose-ethyl acetate adhesive. This shaft was held by the precision chuck inside the tomography cabin. The scanning conditions were adjusted for each of the 3 stages of development. For all stages, the best combination of scanning parameters to achieve the maximum contrast and resolution of the images was microfocus at 45 kV, 50 μA intensity, 2048 projections 360° rotation and no filters. The resulting voxel size was 6.4 μm.

2.7. Statistical analysis

The data on chemical composition of *T. molitor* were analysed by one-way analysis of variance (ANOVA), using the stage of development as factor. The Tukey's multiple range test was used to detect significant differences among means ($p < 0.05$), using IBM SPSS Statistics 26 software (IBM, NY, United States).

3. Results

3.1. Protein content and amino acids profile of *T. molitor*

Insects are mainly constituted by protein, lipids, minerals and chitin, whose proportions vary among the developmental phases. In this sense, the three growth stages of *T. molitor* were characterized (Table 1). Protein content was determined after the extraction of chitin from the samples, with higher protein being observed in pupae, followed by beetles and larvae.

The amino acids profile was also analysed in the three phases of development of *T. molitor* (Table 2). The content in essential amino acids (EAA) was similar in larvae and pupae but it was lower in beetles. The main EAA varied between larvae (leucine, lysine, methionine), pupae (leucine, lysine, phenylalanine) and beetles (leucine, valine, lysine). On the other hand, glycine, alanine and proline were the most abundant non-essential amino acids (NEAA) in beetles, while in larvae and pupae were proline, glycine and glutamic acid. The limiting AAs in larvae meal were lysine and isoleucine (DIAAS of 42), and threonine in pupae (DIAAS of 37) and beetles' meal (DIAAS of 15).

Despite the high protein content observed in all three life stages of *T. molitor*, it is important to determine its digestibility. For this reason, a digestibility assay was conducted in larvae, pupae and beetle meals (Fig. 1). The digestibility of larvae and pupae meals was similarly high, ranging between 66 % and 67 %, while the digestibility of beetles' meal was substantially lower (17.8 %).

3.2. Lipids content and fatty acids profile of *T. molitor*

The lipids content in the larvae and pupae stages was twice as high as in the beetles (Table 1). Also, the polyunsaturated fatty acids represented between 69 % and 75 % of the total fatty acids in the three stages, with the linoleic acid present in higher amount (Table 3). On the other hand, the saturated fatty acids content was low in all life stages, comparatively with monounsaturated and polyunsaturated fatty acids. Oleic and palmitic acids content were higher in larvae and pupae stages, while beetles contained lower quantities of all fatty acids assessed.

3.3. Chitin determination using chemicals or computed 3D tomography in *T. molitor*

The chitin content was determined either using a conventional-chemically method and using computed 3D tomography. In this sense,

Table 1
Nutritional characterization of different growth stages of *Tenebrio molitor*.

g/100 g (dry weight, w/w)	Larvae	Pupae	Beetles[1]	*p*-value
Carbon	59.7 ± 1.17[b]	56.6 ± 0.26[ab]	53.5 ± 0.47[a]	0.0242
Total Nitrogen	10.0 ± 0.37[a]	11.0 ± 0.22[a]	10.1 ± 0.53[a]	0.2876
Organic nitrogen	8.13 ± 0.17[a]	9.2 ± 0.2[b]	10.3 ± 0.06[c]	0.0010
Ash	4.16 ± 0.03[a]	6.99 ± 0.11[c]	4.81 ± 0.2[b]	0.0011
Protein	48.5 ± 0.08[a]	55.8 ± 0.46[b]	50.0 ± 0.36[a]	0.0015
Soluble protein	0.82 ± 0.07	0.54 ± 0.06	0.55 ± 0.02	0.073
Lipids	30.49 ± 3.27[b]	27.73 ± 0.67[b]	13.01 ± 0.29[a]	0.0146
Chitin	3.61 ± 0.52[a]	4.11 ± 0.63[a]	22.7 ± 0.23[b]	0.0000
Total phenolic compounds	0.06 ± 0.01	0.113 ± 0.005	0.0899 ± 0.0002	0.097

[1] Values reported in (Muñoz-Seijas et al., 2024).
One-way ANOVA ($p < 0.05$) of 3 replicates. Means in the same row and different superscript letters are significantly different (Tukey's test, $p < 0.05$).

Table 2
Amino acids profile (g/100 g DI) in the different growth phases of *Tenebrio molitor*.

	Amino acids			DIAAS*		
	Larvae	Pupae	Beetles	Larvae	Pupae	Beetles
Essential AA						
Histidine	1.43	1.51	1.31	58	61	52
Isoleucine	1.23	1.52	1.39	42	52	48
Leucine	4.30	4.98	5.52	68	79	88
Lysine	2.82	2.65	1.45	42	39	22
Methionine	2.04	1.82	1.37	97	86	65
Phenylalanine	1.48	2.08	1.08	49	70	36
Threonine	1.63	1.29	0.53	46	37	15
Tryptophan	ND	ND	ND	–	–	–
Valine	1.30	1.50	1.53	41	48	49
Total EAA	16.24	17.34	14.18	–	–	–
Non-essential AA (NEAA)						
Alanine	4.32	4.68	6.42			
Arginine	2.07	2.09	1.19			
Asparagine	NA	NA	NA			
Aspartic acid	4.47	4.61	3.17			
Cysteine	0.19	0.13	0.11			
Glutamic acid	4.82	5.83	4.64			
Glutamine	NA	NA	NA			
Glycine	4.78	5.69	9.22			
Proline	5.05	4.81	5.75			
Serine	3.31	3.79	2.22			
Tyrosine	2.38	3.18	0.99			
Total NEAA	31.39	34.81	33.72			

AA: amino acids; DI: Dry insect; EAA: Essential amino acids; NEAA: non-essential amino acids; ND: not detected; NA: non analysed.
Values are present as mean of 3 replicates with standard deviation below 0.1.
* DIAAS: (mg of digestible EAA in 1 g of protein / mg of digestible EAA in 1 g of reference protein) x 100.

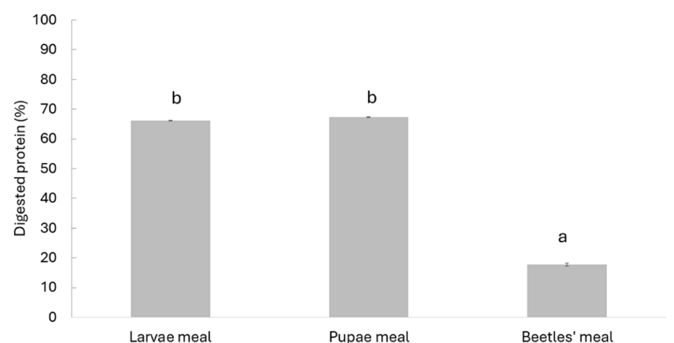

Fig. 1. Protein digestibility of *T. molitor* larvae, pupae and beetles' meals.

Table 3
Total fatty acids (g dry weight basis) profile of larvae, pupae and beetles of *Tenebrio molitor*.

Fatty acids	Larvae	Pupae	Beetles	*p*-value
Palmitic acid (C16:0)	1.06 ± 0.01[b]	1.26 ± 0.01[c]	0.76 ± 0.01[a]	0.000
Stearic acid (C18:0)	0.26 ± 0.02	0.27 ± 0.03	0.22 ± 0.03	0.169
Oleic acid (C18:1n9)	6.43 ± 0.11[b]	6.82 ± 0.07[c]	3.09 ± 0.04[a]	0.000
Linoleic acid (C18:2n6)	22.8 ± 0.11[c]	19.4 ± 0.09[b]	8.94 ± 0.02[a]	0.000
SFA	1.32 ± 0.01[b]	1.53 ± 0.03[c]	0.98 ± 0.03[a]	0.000
MUFA	6.43 ± 0.11	6.82 ± 0.07	3.09 ± 0.04	0.000
PUFA	22.8 ± 0.11	19.4 ± 0.09	8.94 ± 0.02	0.000

TFA: total fatty acids; SFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
One-way ANOVA ($p < 0.05$) of 3 replicates. Means in the same row and different superscript letters are significantly different (Tukey's test, $p < 0.05$).

lower chitin content was observed in larvae and pupae stages using both methods (Table 1). On the other hand, chitin of adult insects was 6-fold higher compared to the larvae and pupae.

During the 3D tomography method, the chitin of larvae presents a low level of polymerization, offering a very low X-ray absorption contrast. For pupae, the chitin contrast depends on the degree of sclerotization, observing significant variations between individuals in their degree of development. In the adults, the contrast between the chitinized organs and the remaining organs can be efficiently used to estimate the chitin quantity in dehydrated individuals (Fig. 2).

The image analysis of the dehydrated adults of *T. molitor* allowed us to estimate the distribution of chitin percentages along sections by measuring the volume of the sclerotised pieces regarding the total volume of the adult. The total volume is calculated by a procedure of digital filling of the internal gaps (Fig. 3). The longitudinal distribution of the chitin content expressed in volumetric relation is shown in (Fig. 3D). Higher concentrations of chitin are found in the mouthparts, the coxae and legs, specifically the third hind pair. The lower concentration of chitin is found in the central part of the abdomen. The results of the analysis show that the estimated mean value of chitin content in adult individuals is 21 ± 3 % (Fig. 4).

3.4. Minerals content and profile of T. molitor

Higher iron, zinc and copper contents were found in beetles, while lower quantities of minerals were generally found in larvae (Table 4). Higher potassium, magnesium and sodium contents were found in pupae compared to the larvae and beetles. Nonetheless, similar levels of manganese were found among the different stages.

3.5. FTIR analysis

The spectral obtained in the FTIR analysis of the different developmental stages of *T. molitor* is present in Fig. 5. The bands followed similar patterns in larvae, pupae and beetles. Different peaks were observed: peaks corresponding to lipids and chitin were observed between 2900 and 2800 cm^{-1}; related to lipids between 1700 and 1600 cm^{-1}; and between 1500 and 1200 cm^{-1} probably concerning to proteins. Another peak was observed at 3500 cm^{-1} that may also be due to the presence of water and other polysaccharides. Also, small bands were observed between 1000 and 900 cm^{-1} which may be indicative of the presence non-structural carbohydrates.

4. Discussion

4.1. Protein and amino acids profile in T. molitor

In this study, pupae exhibited 15 % and 5 % more protein content than the larvae and adult insects, respectively. Morales-Ramos et al. (2016) also observed a 13.4 % higher protein content in the pupae compared to the larval stage. On the other hand, Adámková et al. (2017) observed that protein of larvae and pupae were similar (51 % - 52 % DW). It is important to emphasize that most studies characterize the larval stage of *T. molitor* and that composition can be modulated with feeds provided during rearing. Indeed, *T. molitor* larvae fed with wheat bran exhibited 45.6 % DW (Costa et al., 2020) and 49.1 % DW (Liu et al., 2020) of protein when fed wheat bran. Likewise, the protein content of larvae varied between 14.4 % and 29.3% w/w when fed either a mixture of 50 % brewer's spent grain and 50 % of wheat bran, 100 % of distilled dried grains or 100 % of wheat bran (Kim et al., 2017).

Besides the high protein content observed in pupae, the weight of pupas is lower (0.16 ± 0.02 g DW) than that of larvae (0.22 ± 0.03 g DW). Therefore, higher production of total protein is achieved in the larval phase of *T. molitor*. Yu et al. (2021) determined the maximum total protein content considering the weight of insects and observed that higher protein content was achieved at week 13 within the larvae stage (48.6 % DW), rightly before the transformation into pupae (45.2 % DW).

In protein assessment, a kp of 6.25 is the most commonly used for different food and feed matrices (Mariotti et al., 2008) unless when other kp value is scientifically certified to be used in a specific biomass (WHO, 2019). However, using specific factors for different protein sources seems to be the most accurate methodology since it may prevent an over- or underestimation of the real protein value of different biomasses when using the kp of 6.25 (Mariotti et al., 2008; Templeton and Laurens, 2015). Indeed, using the kp of 6.25 overestimates the protein content of insects since they also have chitin, a nitrogen-based polysaccharide that will account for the total nitrogen determined (Dobermann et al., 2017). Janssen et al. (2017) studied which conversion factor

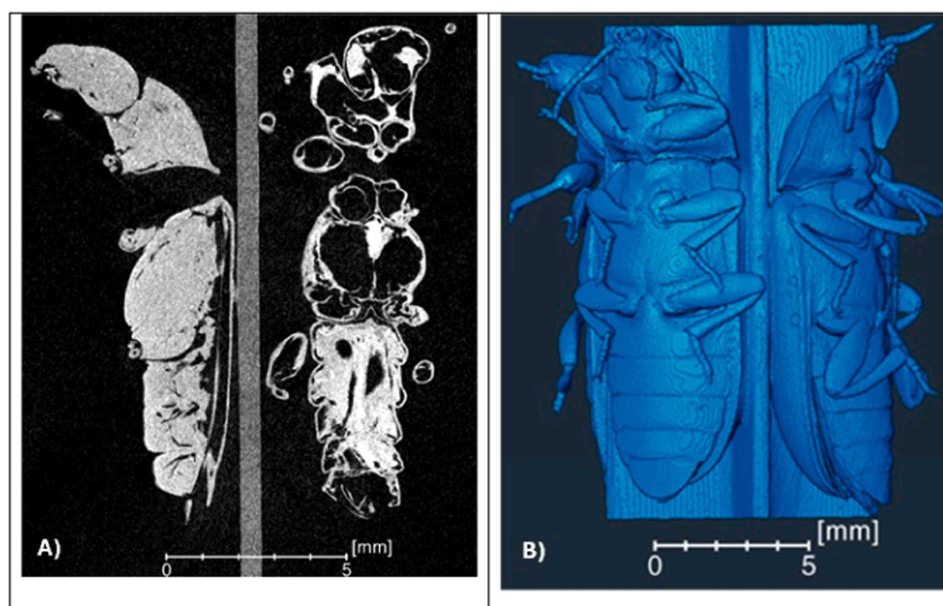

Fig. 2. Sagittal section of two imagoes with different degrees of fasting and dehydration. A) unaltered individual; B) individual in an advanced state of fasting and dehydration, preserving remains of soft tissues that occupy a smaller fraction of the skeleton. 3D rendered views of binarized 3D X-CT images of imagoes of *T. molitor*, where the effects of the degree of starvation and dehydration on the exoskeleton are negligible.

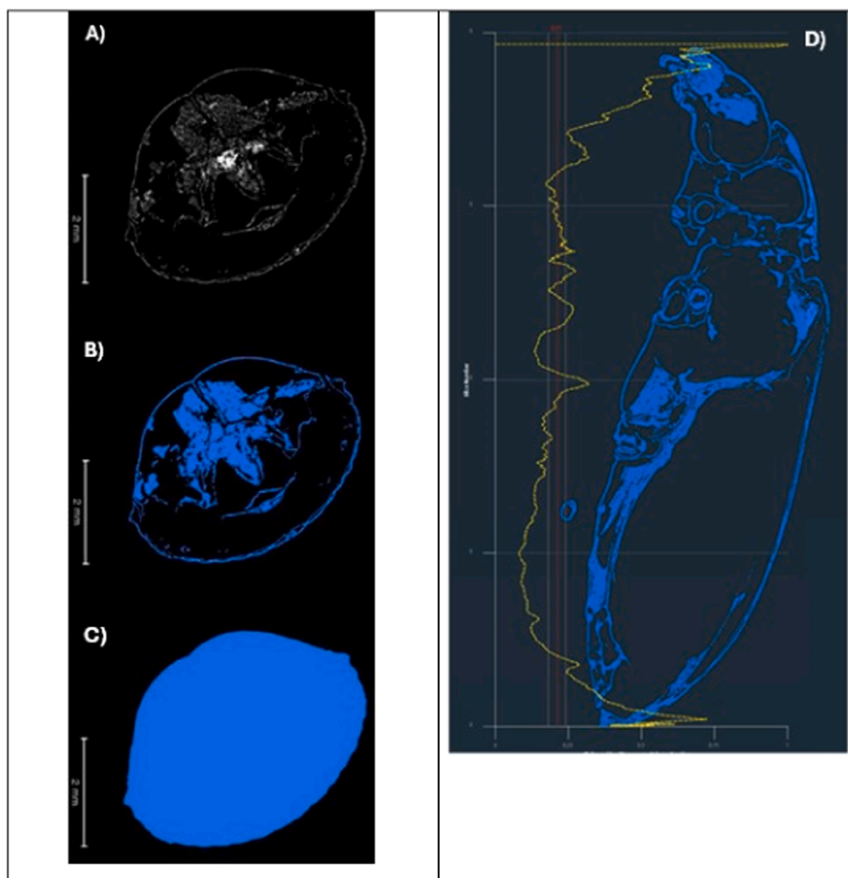

Fig. 3. Illustration of image processing for estimation of chitin content. Axial section of an imago of *T. molitor*, showing: A) the unprocessed image of a dehydrated individual, B) the binarized image highlighting the sclerotized organs, and C) the section filled in by image analysis. D) Longitudinal distribution of estimated volumetric chitin content in an imago of *T. molitor* along its sagittal plane (dashed yellow line). The vertical red line represents the mean value of the transect, and the grey lines flanking it represent its standard error.

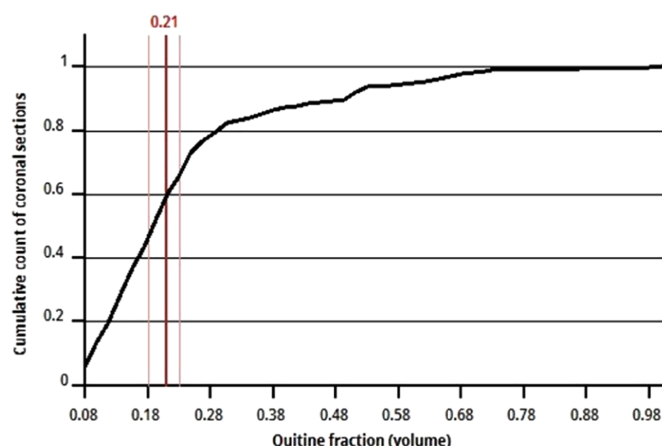

Fig. 4. Cumulative distribution of estimated chitin volumetric fraction values along the sagittal section of a *T. molitor* imago.

should be more appropriate to determine the protein fraction based on the nitrogen content of larvae of three insect species, including *T. molitor*, concluding that the kp should be 4.76. This conversion factor depends on the chitin content, with the authors suggesting that the kp of 4.76 should not be used in insects at stages with high chitin content, such as the adults. In our study, the content of chitin was assessed in the three phases of *T. molitor*, analysing the nitrogen content of the chitin fraction, which averaged 10.11 % ± 0.06 %. Then, the nitrogen of chitin origin was subtracted from the organic nitrogen present in the insect meals, which was assessed using a conversion factor of 6.25. Indeed, the protein content determined in beetles following the present analysis (separate quantification of nitrogen of protein and chitin origins) was 50.0 % ± 0.36 %, which is very similar to the content that would be obtained (49.5 % DW) if the conversion factor (kp 4.76) proposed by Janssen et al. (2017) was adopted. With this approach, it is ensured that the protein content of insects is not overestimated due to nitrogen of chitin origin. Also, if the kp of 6.25 already used in other published articles (Adámková et al., 2017; Mancini et al., 2019) was followed in

Table 4
Minerals profile of the different growth stages of *Tenebrio molitor*.

g/kg dry weight, w/w	Larvae	Pupae	Beetles	*p*-value
Potassium	9.92 ± 0.01^{a}	13.22 ± 0.45^{b}	10.33 ± 0.02^{a}	0.0052
Magnesium	2.49 ± 0.06^{a}	2.93 ± 0.06^{b}	2.38 ± 0.03^{a}	0.0059
Sodium	0.78 ± 0.01^{a}	1.52 ± 0.08^{c}	1.30 ± 0.03^{b}	0.0007
Iron	0.081 ± 0.001^{a}	0.076 ± 0.001^{a}	0.10 ± 0.0003^{b}	0.0000
Zinc	0.116 ± 0.002^{a}	0.15 ± 0.002^{b}	0.15 ± 0.002^{b}	0.0023
Manganese	0.0110 ± 0.0004^{a}	0.020 ± 0.003^{a}	0.020 ± 0.004^{a}	0.1916
Copper	0.0210 ± 0.0003^{a}	0.023 ± 0.001^{a}	0.03 ± 0.001^{b}	0.0086

One-way ANOVA ($p < 0.05$) of 3 replicates. Means in the same row and different superscript letters are significantly different (Tukey's test, $p < 0.05$).

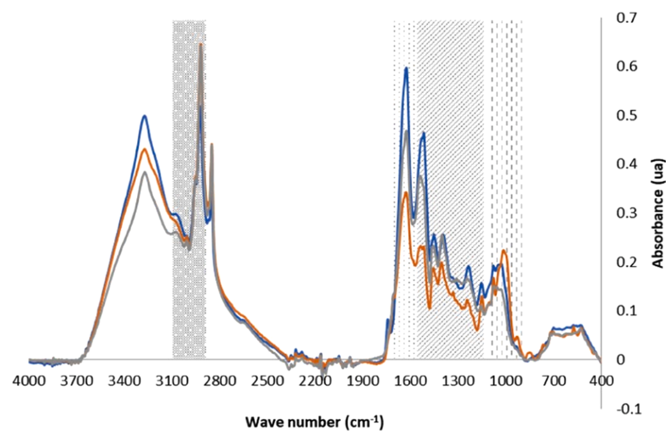

Fig. 5. FTIR spectra analysis of larvae (blue line), pupae (orange line) and beetles (grey line) of *T. molitor.* Identification of bands in each wave number range is based on the work of (García-Gutiérrez et al., 2021).

this study, the protein content of beetles would reach 63.12 % DW. In fact, Mancini et al. (2019) evaluated both kp values in protein quantification of *T. molitor* larvae and observed differences in protein content when larvae were fed different diets, ranging between 37.3 % - 51.3 % DW and 28.4 % - 39.1 % DW if a kp value of 6.25 or 4.76 was used, respectively.

In this study, the EAA content was lower in beetles compared to larvae and pupae, while NEAA content was similar among all stages. Ravzanaadii et al. (2012) observed that *T. molitor* adults were mainly constituted by glycine (5.44 g/100 g protein), glutamic acid (5.24 g/100 g protein) and leucine (5.17 g/100 g protein), while the larvae were mainly formed by glutamic acid (5.68 g/100 g protein), aspartic acid (3.59 g/100 g protein) and isoleucine (3.56 g/100 g protein). However, other authors also observed a similar AAs profile as the present study, but mostly in *T. molitor* larvae meals. Indeed, depending on the pre-extraction treatment used, larvae of *T. molitor* contained 26.2 g/100 g DW (microwave-dried) and 35.6 g/100 g DW (freeze-dried) of EAA, while the non-dried larvae contained 36.5 g/100 g DW of EAA (Zhang et al., 2022), which are higher levels comparatively to those observed in this study. Similarly to this study, Jajic et al. (2020) also observed that *T. molitor* larvae were mainly constituted by leucine (2.96 g/100 g DW), lysine (2.67 g/100 g DW) and methionine (1.76 g/100 g DW), although these values are lower than those assessed in this study. Furthermore, Yu et al. (2021) observed that the higher EAAs present in *T. molitor* larvae were valine (4.07 g/100 g DW), lysine (up to 3.69 g/100 g DW) and leucine (up to 3.66 g/100 g DW), which decreased when the larvae grew into pupae. Contrarily, in this study, the content of valine and leucine increased when *T. molitor* larvae grew into pupae. The main EAAs of *T. molitor* larvae were valine, leucine and lysine, ranging between 7.2–7.6, 9.2–10.0 and 6.7–8.1 g/100 g DW, respectively (Belghit et al., 2019). Contrarily, glutamic acid (5.78 g/100 g DW), tyrosine (3.45 g/100 g DW) and alanine (3.96 g/100 g DW) were the main EAAs found in *T. molitor* larvae (Ghosh et al., 2017). Differences in the amino acids content of *T. molitor* is related to stage of development and the of type diet provided during rearing. Indeed, valine, lysine and leucine are involved in the energy supply and development of muscles, which justifies that (Yu et al., 2021) observed higher content in these amino acids in the early development of larvae (3–12 weeks), which decreased afterwards and until reaching the pupal stage. Since the larvae used in this study were collected with 26–30 weeks-old, it is reasonable to expect that lower values of valine, lysine and leucine are found.

Protein digestibility was assessed in the three developmental stages of *T. molitor*, observing higher digestibility in larvae and pupae, while digestibility of beetles was lower than 20 %. The main reason for the poor digestibility of beetles' meal is the high chitin content, which can be improved by pre-treating the biomass to separate the chitin from the protein. Also, the degree of protein digestibility in the larvae and pupae observed in this study is comparatively similar to other high-quality proteins, such as soy protein (Pettersson and Pontoppidan, 2013). In this sense, the DIAAS values were calculated for each EAA in all samples, observing that the lysine was the most limiting AA in larvae and threonine in pupae and beetles' meal. Accordingly, *T. molitor* larvae meet the threonine requirements of infants, children and adults, while lysine and threonine were limiting AA in pupae and adult meals (FAO, 2013). A high quality protein must meet the nutritional requirements of essential amino acids of a certain age group, whose protein needs may vary with age, health status and physiological conditions (FAO, 2013). Nonetheless, despite the pupae and beetles' meal are less advantageous in terms of EAA and thereof of DIASS scores, different valorisation processes may be applied to potentiate their use as protein source. For example, enzymatic hydrolysis may be applied to fractionate the insects' protein into small-chain peptides, which may improve the nutritional, functional and sensory traits of the final products (Grossmann et al., 2021).

Indeed, the dietary incorporation of *T. molitor* larvae meal in diets for pigs (Yoo et al., 2019), fattening rabbits (Volek et al., 2021) and fish (European seabass; Gasco et al., 2016) resulted in higher protein digestibility than when the animals were fed, respectively, with diets mainly formulated with fishmeal/poultry meal, soybean meal and fishmeal. Furthermore, besides evaluating the protein digestibility of a single ingredient, is essential to predict and/or determine the true digestibility of the whole food/feed since interactions may occur between dietary ingredients and between the food/feed and the intestinal tract of the organism, affecting the overall bioavailability of the protein sources.

4.2. *Lipids and fatty acids profile of T. molitor*

Higher lipids content was found in larvae and pupae compared to the beetles, which is common in holometabolous insects such as *T. molitor* and may be attributed to their need for a readily available source of energy to grow, which decreases when the organism grows into pupae and later into adults (Oonincx and Finke, 2021; Kröncke et al., 2023). Ghosh et al. (2017) and Costa et al. (2020) assessed similar lipids content in *T. molitor* larvae (34.5 % DW) as observed in the present study. Also, Dreassi et al. (2017) assessed 34.4 % and 48.3 % DW of total lipids in *T. molitor* larvae fed with beer yeast or a mixture of oatmeal and wheat meal (1:1 w/w), respectively. The high protein and lipids combined with low chitin content of *T. molitor* larvae increases their utilization for food and feeding purposes. Indeed, a paste produced using non-defatted *T. molitor* larvae showed higher protein and lipids contents than beef, pork, turkey and salmon products (De Smet et al., 2019).

Concomitantly, higher linoleic acid content was observed in larvae followed by pupae and beetles, while higher SFA was observed in beetles. Ravzanaadii et al. (2012) observed higher linoleic (9.89 g), stearic (0.81 g) and palmitic (5.37 g) acids in *T. molitor* larvae than in beetles (2.46 g, 0.47 g and 1.42 g, respectively). High PUFA content was also detected in larvae at different weeks of growth, ranging between 38.1 % - 40.8 % of TFA, and being mainly constituted by linoleic and oleic acids (Kröncke et al., 2023). Likewise, high unsaturated fatty acids were detected in *T. molitor* larvae, achieving 0.33 g TFA of linoleic acid, while the most abundant SFA was the palmitic acid (0.15 g TFA; Costa et al., 2020). In another study, *T. molitor* larvae presented higher oleic (5.37 g TFA) and palmitic (0.25 g TFA) acids compared to the other fatty acids (Ghosh et al., 2017).

The linoleic acid was the most common among the different stages of *T. molitor*, while oleic and palmitic acids decreased when *T. molitor* grew from larvae into pupae and beetle. Nonetheless, besides the influence of the life stage in lipids and fatty acids profile, other factors may affect these parameters. For instance, the diet type may strongly influence the fatty acids profile of insects. Boukid et al. (2021) evaluated the effect of three different diet types (apricot, brewer's spent grain and yeast, and feed mill) in three insect species (larvae of *Ephestia kuehniella, T. molitor* and *Hermetia illucens*) observing that sterols varied according to diet type

and insect species, while *T. molitor* larvae exhibited high palmitic, oleic and linoleic acids. Also, the supplementation of a basal diet (wheat meal and wheat bran for *T. molitor* and *H. illucens* larvae, respectively) with whole-seed meals (flax, chia, hemp, and rapeseed) revealed to be a promising strategy to improve the fatty acids profile of both species, increasing the content in n-3 and lowering the n-6 fatty acids (Lawal et al., 2021). The modulation of fatty acids in edible insects is advantageous since this biomass must be enriched in highly unsaturated fatty acids, specially of the n-3 group, with varied associated benefits, such as boosting health status and preventing the development of cardiovascular and inflammatory diseases (Ververis et al., 2022). However, despite that beetles showed lower PUFA content than larvae and pupae, they are mainly constituted with oleic acid, a monounsaturated fatty acid with bioactive properties and which is among the healthiest lipids in human nutrition (Choi et al., 2010).

Safety assessments are extremely important when considering the use of *T. molitor* or other insect species for consumption. For that, the European Food Safety Authority (EFSA) is the agency responsible to provide independent reports that are used to establish regulations that ensure food safety. Different EFSA reports extensively analyse and report no safety concerns regarding the composition of the different mealworms products, such as dried (Turck et al., 2021ab), powder or frozen (Turck et al., 2021a), and UV-treated (Turck et al., 2023) mealworms under the proposed use levels. The same approach is adopted for other edible insects, such stating the safety of commercializing frozen and freeze-dried *Alphitobius diaperinus* larvae (Turck et al., 2022). Nonetheless, although mealworms consumption is considered safe under proper utilization and levels, allergens were identified and may trigger allergic reactions, especially in persons allergic to crustaceans and dust mites due to the risk of cross-reactivity (Turck et al., 2021a; Turck et al., 2021b). Also, mealworms allergenicity may me potentiated with additional allergens depending on the type of feed used in the rearing *T. molitor*, such as gluten-containing cereals (Turck et al., 2021a). Nonetheless, it is fair to conclude that besides the possible allergic events that may occur in allergic individuals, the safety of using *T. molitor* larvae is vastly covered, including proving the absence of benzoquinones.

However, when considering using *T. molitor* adults, attention must be given not only to the nutritional profile but also to the presence of allergens and other undesirable substances, such as benzoquinones (Turck et al., 2021a). These molecules are secreted in the abdominal glands of Tenebrionidae organisms, defending them against predators and functioning as aggregation pheromones (Hassemer et al., 2015). Benzoquinones may have harmful effects, such as skin irritation, decrease cellular respiration, vision disturbances, among others (Lis et al., 2011). The extensive safety assessments carried out in mealworms proved the absence of benzoquinones, guaranteeing the safety of this product for the consumers (Turck et al., 2021b). Nonetheless, although the legislation and safety reports are related to the larvae of *T. molitor*, the presence of allergens and benzoquinones needs to be addressed when considering the adults for food or feeding purposes. Therefore, when considering the valorisation of adult insects to obtain protein and chitin, it is essential to assess the presence of these substances, ensuring the safety of the derived products.

4.3. Chitin determination by chemical or computed 3D tomography in T. molitor

In this study, two methods were used to assess chitin content, and higher chitin content found in adult beetles compared to larvae and pupae. The formation of a rigid and dense exoskeleton in adult insects justifies the presence of more chitin comparatively with larvae (Kim et al., 2023). Adámková et al. (2017) observed similar chitin content in larvae (12 % DW) and pupae (13 % DW) stages, which were higher than those observed in the present study. The chitin content observed in beetles in this study was 63.4 % higher than that quantified by Shin et al. (2019), while Nafary et al. (2023) isolated up to 17.7 % of chitin from *T. molitor* beetles, yielding up to 78.3 % of chitosan. Nonetheless, is important to emphasize that, besides the effect of life stage, the variations of chitin content may also be attributed to the methodology used in the quantification of this fraction among the different studies.

On the other hand, 3D tomography was effective to determine chitin content in adult beetles, while larvae and pupae showed a low level of polymerization and a highly variable degree of sclerotization, respectively, impairing the determination of chitin content. The best chitin assessment was carried out using starved beetles followed by air dehydration at ambient temperature, achieving a similar value to that observed using the chemical method. Indeed, the contraction of the internal soft organs during starvation and dehydration produces a dramatic reduction of their volume, while the completely sclerotised organs maintain their original volume intact. In this way, it is possible to use starved and dehydrated individuals to estimate the chitin content with an acceptable error. However, in the case of larvae and pupae, this operation is more complicated because two CT scans need to be performed: one with the individual intact and another with the individual dehydrated, with sclerotized cuticles, and then carry out a comparative study of the volumes of both CT scans, which is tedious and time-consuming.

Therefore, it is possible to conclude that the CT can be used as an alternative physical and non-destructive method to quantify the chitin content of adult insects since a similar value was attained compared to the chemical method. Indeed, despite larvae are the unique insect fractions currently used, other life stages of insects must be valorised so a self-sustainable and profit-oriented industry can be developed. Specifically, adult beetles are often discarded when their reproductive cycle expires due to chitin content, constituting a biological waste of insect farming. Chitin impairs nutrients digestibility since is tightly bounded to proteins, precluding the use of adult meals without any further preprocessing step. However, chitin is also a promising compound with biological functions and can be fractionated into chitosan, a deacetylated and cationic polymer that can form biofilms, with health benefits and antibacterial activities (Shin et al., 2019; Muñoz-Tebar et al., 2023). Therefore, separating the chitin fraction of *T. molitor* beetles will not only improve the digestibility of the other dietary components but can also be used to obtain biologically active products with advantageous industrial applications. Furthermore, the different compounds that can be obtained from *T. molitor* beetles can also be synergistically used. One example is shown by Vargas et al. (2009), which observed that the addition of oleic acid in chitosan-based biofilms increased their gloss and translucent appearances, improved the resistance to break and increased the water absorption capacity. Therefore, the chitin extracted from beetles can be conjugated with the high oleic acid content also found in these insects and originate industrially target compounds with great utilities.

4.4. Minerals content and profile of T. molitor

Insects are not often considered important sources of minerals but, as observed in this study, minerals content of *T. molitor* does not differ from that found in traditional protein sources (Gere et al., 2019). In other studies, *T. molitor* was reported as having high amounts of potassium, iron and magnesium (Gkinali et al., 2022b). Ghosh et al. (2017) also determined similar minerals content in *T. molitor* larvae, such as 7.37 g/kg of potassium, 3.15 g/kg magnesium, 1.09 g/kg sodium, 0.1 g/kg iron and 0.12 g/kg of zinc. Fresh or powdered *T. molitor* larvae had high phosphorous and potassium contents, reaching between 3.19 g/kg (fresh) and 7.0 g/kg (powder) and between 3.73 g/kg (fresh) and 7.26 g/kg (powder), respectively (Siemianowska et al., 2013).

Minerals have important metabolic functions, being involved in molecules formation, boosting the immune system and influencing the function of muscles and nervous system (Weyh et al., 2022). The higher minerals content found in beetles in the present study indicates their

high suitability for human consumption. Indeed, 100 g of *T. molitor* beetles contribute to fulfil 21 %, 59.5 %, 8.67 % and 87 % of the daily needs of potassium, magnesium, sodium and manganese, respectively, while even lower amounts of are needed to meet iron (80 g DW), zinc (73.3 g DW), and cooper (30 g DW) requirements (van Huis et al., 2013).

4.5. FTIR analysis

The larvae, pupae and beetles exhibited peaks correlated to lipids, chitin, protein and non-structural carbohydrates. Indeed, the peaks observed between 2900 and 2800 cm^{-1} are probably due to the stretching of C—H bonds of methyl groups from lipids and chitin fractions; the peaks between 1700 and 1600 cm^{-1} are identified as stretching of C = O bonds from ester groups of lipids; the spectra found between 1500 and 1200 cm^{-1} may be related to proteins, such as the C—N stretching, the C = O vibrations of N-acetyl groups, and the N—H bending from amide II groups; and the smaller bands observed between 1000 and 900 cm^{-1} may be due to C—O stretching vibrations, which most certainly correspond to the presence non-structural carbohydrates (Mellado-Carretero et al., 2020; García-Gutiérrez et al., 2021).

Comparing the structural changes among the development stages of *T. molitor*, it is possible to observe that beetles have a higher peak in the 2900 – 2800 cm^{-1} spectra, which is in accordance with the higher lipids and chitin contents found in the adults (35.71 % DW) compared to the larvae (34.1 % DW) and pupae (30.34 % DW). Regarding solely lipids, larvae exhibited a higher peak in the 1700 – 1600 cm^{-1} band (30.5 g/100 g DW) compared to pupae and adults, which is in accordance with the higher lipids content present in Table 1. However, adults exhibited a higher peak than pupae in the 1700 – 1600 cm^{-1} band even that pupae have higher lipids (27.7 g/100 g) content than beetles (13.0 g/100 g DW). That may be explained by the presence of body surface lipids, which are present in the cuticle and protect the organisms against harsh environmental conditions, water loss and pathogenic infections (Durak et al., 2022).

Despite the FTIR analysis does not provide an exact quantification of nutrients and other compounds present in edible insects, it is still a readily available, fast, and cost-effective method to identify the specific infrared spectrum of each material, since a single identification pattern is associated to the structure of each material when correlating the intensity of absorption with the wavenumber (Shin et al., 2019). In this way, the FTIR analysis can be a helpful tool to identify, in a first approach, which components are present in insect biomass and later proceed with more labour-intensive methods to quantify the different fractions.

5. Conclusions

The present study provides an exhaustive characterization of all developmental stages of *T. molitor*, enlightening the promising fractions that can be valorised within different industrial purposes. Despite the larvae of *T. molitor* are currently the most nutritionally and economically advantageous, this study highlights the advantages of valorising the adult insects to obtain chitin and protein, thereof widening the alternative uses of *T. molitor*. However, it is important to assess the presence of benzoquinones and allergenic substances in adult insects, since their presence may compromise the safety and nutritional advantages of using this biomass for food and feeding purposes. Since nowadays only the larvae are commercialized, this work evidence that it is possible to valorise the different nutritional fractions of adult insects, extracting the hidden value of this direct by-product of insect industry. Furthermore, this study provides evidence that three-dimensional computed tomography is highly efficient to estimate the chitin content of *T. molitor* adults, improving the sustainability of this type of characterization. Similarly, the possibility of also using FTIR analysis to firstly assess the proximate composition of *T. molitor* favours and fastens the applicability of insects-based biomass.

Ethical statement

The authors confirm that this study did not involve the experimentation on human or animal subjects.

CRediT authorship contribution statement

Nuno Muñoz-Seijas: Writing – original draft, Investigation, Formal analysis. **Helena Fernandes:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **José Eugenio López-Periago:** Writing – review & editing, Methodology, Formal analysis. **David Outeiriño:** Formal analysis. **María Guadalupe Morán-Aguilar:** Investigation, Formal analysis. **José Manuel Domínguez:** Writing – review & editing, Supervision. **José Manuel Salgado:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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