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RESEARCH ARTICLE

Lipid extraction from *Galleria mellonella* larvae: introducing the use of eco-friendly solvents and emerging technologies

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Abstract

Edible insects are seen nowadays as important protein sources, thus holding a huge potential for becoming a food solution in the future. However, beyond their protein content, mainly at their larvae stage, they also offer significant potential as lipid sources for use as food ingredient. In this context, the present work aimed to investigate the lipid extraction of *G. mellonella* larvae through sustainable methods, employing green techniques, such as Supercritical fluid extraction (SFE) and ultrasound assisted extraction (UAE), using eco-friendly solvents like CO_2 and hydrophobic deep eutectic solvent (H-DES) composed by menthol and eucalyptol 1:1 molar ratio. Mass transfer kinetic modeling using the Two-site and Spline models revealed improved lipid extraction efficiency when SFE was combined with H-DES. Phase equilibrium results indicated homogeneous systems across all extraction conditions, likely enhancing mass transfer as demonstrated by the highest yield (58.8%). Palmitic and oleic were the main fatty acids in all extraction conditions applied. Alternative extraction methods improved linoleic acid recovery, although *G. mellonella* lipid extracts showed limited acetylcholinesterase inhibition (IC_{50} > 200 μg extract/ml) compared to plant-based extracts. Finally, *Path2Green* analysis identified SFE-H-DES as the method with the lowest environmental impact. These findings pose *G. mellonella* as a promising lipid source and underscore the importance of adopting sustainable extraction methods to efficiently recover lipid-soluble compounds from edible insects.

Keywords

eco-friendly solvent – edible insects – lipid extraction – mass transfer – phase equilibrium

1 Introduction

Worldwide population is continuously growing, with a projected 9.8 billion people in 2050 according to the United Nations Department of Economic and Social Affairs, Population Division (United Nations, 2022), thus encouraging governments to work through a main concern: feeding population. The use of insects as alternative protein is one promising way to mend the scenario, justifying the current focus of the scientists on this matter. At present, the consumption of edible insects is noticeable in Asia but still restrained in Europe. In fact, there are only four insects authorized to be used in the food industry in the European Union,

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including *Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus* and *Alphitobius diaperinus* (Regulation (EU) 2015/2283 on novel foods). Other insects were also approved to be used as animal feed: *Hermetia illucens*, *Musca domestica*, *Gryllodes sigillatus*, *Gryllus assimilis*, and, the most recent, *Bombyx mori*. Another promising specie is the *Galleria mellonella* (also known as "wax-moth"), and although not approved to the present date, it was introduced by the European Food Safety Authority (EFSA) in 2015 as having great potential to be used as food and feed (EFSA, 2015). However, their potential inclusion to the approved list as novel food is still speculative since its utilization remains unknown (Meijer *et al.*, 2025).

G. mellonella presents a poor protein content, suggested by their protein-to-lipid ratio of 0.64 (Ma *et al.*, 2024) in comparison to other larvae species current approved to be used as food, such as *Tenebrio molitor* and *Alphitobius diaperinus* that present 2.10 (Orkusz *et al.*, 2024) and 2.63 (Kurečka *et al.*, 2021), respectively. Therefore, special attention must be given to its lipid application, as it shows strong potential to become a valuable ingredient in the food sector.

Acetylcholinesterase (AChE) is an enzyme that catalyses the breakdown of the neurotransmitter acetylcholine, and its inhibition is one of the main therapeutic strategy for Alzheimer's disease (Marucci *et al.*, 2021). Lipid-based compounds, due to their ability to interact with neural pathways, offer a promising alternative for the development of neuroprotective agents. This potential is further supported by the fact that the brain contains the second highest concentration of lipids in the human body (Hamilton *et al.*, 2007). Thus, investigating the acetylcholinesterase inhibition potential of lipid extracts, such as those derived from *G. mellonella*, could open new opportunities for sustainable, natural sources of bioactive compounds targeting neurodegenerative diseases.

The extraction of lipophilic bioactive molecules, i.e., that exert various biological activities and confer potential health benefits (including fatty acids, sterols and terpenes), is usually obtained with conventional methods employing hexane as extractant. However, hexane presents a high toxicity and is forbidden in cosmetic and pharmaceutical formulations in the European Union, as described in the Regulation (EC) No 1223/2009 of the European Parliament. A healthy and environmentally safe alternative process is the supercritical fluid extraction (SFE) with CO_2, which provides several advantages over conventional extractions mainly due to: (a) the use of CO_2, a non-toxic (GRAS) solvent that can be recirculated (and recovered) into the system; and (b) the combination of high-diffusivity and high-density at supercritical conditions, conferring gas-like and liquid-like attributes (Brunner, 2005). Moreover, the final product is expected to be solvent-free, and without residues.

Another emerging extraction technique with potential application to recover lipid soluble compounds is ultrasound-assisted extraction (UAE), based on the promotion of the cavitation phenomena within the liquid solvent bubbles (Mason *et al.*, 1996), thus improving the release of the solutes into the solvent media. This method has been previously reported for lipid recovery from *Acheta domesticus* and *Tenebrio molitor* using pure ethanol or ethanol:water (1:1, v/v) as solvents (Otero *et al.*, 2020). Results confirmed the fatty acids profile variation due to the extraction method, finding a healthier profile than in the original insect lipid composition, suggesting a promising approach to obtain active extracts from insects. SFE kinetics of bioactive compounds are sometimes enhanced by cosolvents such as alcohols (Tirado *et al.*, 2018) and deep eutectic solvents (DES) (Bragagnolo *et al.*, 2022), although literature is still very scarce for the use of DES as entrainer. Abbott *et al.* (2004) firstly introduced the DES' properties of a mixture between carboxylic acids and choline chloride. Later on, van Osch *et al.* (2015) described hydrophobic DES (H-DES), represented by a mixture of a quaternary ammonium salt with decanoic acid to separated fatty acids from aqueous mixtures. Recently, Devi *et al.* (2023) revised several DES, confirming they can present high CO_2 affinity, and therefore a high potential to become a cosolvent in SFE processes. The use of SFE and DES for bioactive compounds recovery from insects is a novelty in the field. Recent studies have started to focus on the use of insects as a lipid source (Lorrette and Sanchez, 2022; Perez-Santaescolastica *et al.*, 2023) and the use of green solvents such as supercritical CO_2 is a potential sustainable alternative to obtain lipid extracts from insects (Hurtado-Ribeira *et al.*, 2023). However, the identification of liposoluble compounds presented in *Galleria mellonella* and their correlation with the extraction method used is unknown. Therefore, considering the high potential of using the lipid fraction of *G. mellonella* as an active extract or food ingredient, this work aims to compare the extraction effectiveness based in lipid-soluble composition of different emerging extraction techniques (SFE, SFE+H-DES and UAE+H-DES). In addition, the phase equilibrium between CO_2 and the selected H-DES was studied to understand the system's characteristics at the extraction conditions applied in this work.

2 Materials and methods

Galleria mellonella pretreatment and physicochemical characterization

The *G. mellonella* larvae were purchased alive from Zoopinto (Madrid, Spain). The larvae were in their adult stage and they were previously fed with honey and cereals (information provided by the suppliers). After reception, larvae were kept under refrigeration (–4 °C) for 24 h in order to eliminate excrements and avoid sample impurities. Then, fresh and health larvae (20 count) were evaluated with respect to their size (using a pachymeter) and weight, while non-health larvae (dark and dead ones) were discarded. Larvae were washed under tap water to eliminate solid residues, dried using paper towel, and sequentially frozen (–20 °C) for 24 h before undergo freeze-drying in a tray freeze-drying Lyobeta 15 (Telstar, Madrid, Spain) for 48 h. Samples were completely dried when no further changes in mass were observed. The lyophilized animals were rapidly frozen using liquid N_2 and subsequently milled, preventing lipid oxidation due the milling heat transfer. Samples were stored at –20 °C until further analysis.

Moisture, ash, lipid, and protein were obtained according to official methods described by the Association of Official Analytical Chemists (AOAC, 1997). Moisture was determined by drying 1 g of milled sample at 105 °C to constant weight, and ash were calculated after 2 h of oven-heating at 550 °C. Lipid content was calculated after weighing the total lipid recovered from 5 g of sample after 6 h of solvent reflux in a Soxhlet apparatus. Finally, total crude protein was determined according to the Kjeldahl method, and the residual N_2 was converted to equivalent protein using the appropriate conversion factor for insects of 4.76, as previously stated by Janssen *et al.* (2017).

Hydrophobic Deep Eutectic Solvent (*H-DES*)

The selected H-DES used in this work was menthol:eucalyptol (both purchased from TCI Chemicals, Japan) since it was previously reported as a potential H-DES for defatting samples (Bragagnolo *et al.*, 2022). The H-DES preparation was performed according to Rodrigues *et al.* (2020). In short, menthol (solid at room temperature) was mixed with eucalyptol (liquid at room temperature) at molar ratio 1:1. The mixture was heated at 55 °C with constant stirring until a clear liquid was formed and remained stable for over 24 h. The solvent was kept at room temperature and preventing from light until further use in the extraction.

Conventional extraction (*Soxhlet*)

Soxhlet was selected as the solid-liquid standard extraction to determine the reference yield when a conventional solvent is used. Briefly, 250 ml of hexane was refluxed into the 5 g of dried *G. mellonella* larvae during 6 h. The extract was evaporated under vacuum, at 40 °C in a rotary evaporator, and the yield was calculated according to equation (1). This procedure was performed in duplicate and the extracts were kept under freezing conditions until further analysis.

Green extraction methods

SFE and UAE together with hydrophobic deep eutectic solvent (H-DES) were employed to recover the target fractions from the *Galleria mellonella* biomass. The main studied aspects were related to mass transfer kinetics, extraction yield, fatty acid profile and AChE inhibitory activity.

Supercritical CO_2 extraction assisted (or not assisted) by H-DES: SFE was performed for *G. mellonella* samples in two different modes: (a) using pure supercritical CO_2 (SFE), and (b) using supercritical CO_2 assisted by the selected H-DES (SFE-H-DES). For both modes, the extraction equipment comprised a PU-2080-CO_2 Plus pump and a PU-2080 liquid pump (Jasco, Tokyo, Japan), that pumped the fluid into a 20 ml stainless steel cell placed inside an Finnigan MAT oven (Thermo-Finnigan, Somerset, NJ, USA). Approximately 0.5 g of milled larvae was placed inside the cell altogether with glass beads, while glass wool was put at the ends. In the first mode, only CO_2 was used as solvent at a flow rate of 4 ml/min, 200 bar, and 60 °C. In the second mode, same conditions were applied, except that SFE-H-DES (15% of flow rate) was also pumped and mixed to CO_2 before flowing into the cell, keeping the total flow at 4 ml/min. Then, the yield for extracts collected from 2 to 150 min (DES evaporation under N_2 continuous flow and 60 °C) was calculated according to equation (1), and a mass transfer kinetics for each mode was built. Finally, extractions were performed in the same conditions but at a defined time to compare pressure effects in SFE (200 and 400 bar) and H-DES concentration (15 and 50% of flow rate in SFE-H-DES). All extractions were performed in duplicate and extracts were kept at –20 °C until further analysis.

$$Y(\%, w/w) = 100 \times \left(\frac{E}{F}\right) \tag{1}$$

where,

Y is the yield of the extract (%, g extract/g dried larvae);

E is the extracted mass collected at a specific time (g);

F is the dried larvae mass used in the extraction (g).

Phase equilibria of the selected H-DES in supercritical CO_2 – synthetic visual method: Phase equilibrium tests for CO_2/H-DES systems were conducted in a phase equilibrium unit, which is described in detail in previous studies (Girardi *et al.*, 2023). The visual-synthetic method was employed for data acquisition. The apparatus consists of a variable-volume high-pressure equilibrium cell equipped with two sapphire windows (one for light intake and one for internal visualization), and connected to a syringe pump (ISCO 260D, Teledyne, Lincoln, NE, USA) and a CO_2 reservoir.

In each experiment, a precise amount of H-DES, corresponding to 15 and 50% (mass fraction), was measured and fed into the cell. The cell was then closed and mounted in the system. Liquid CO_2 (10 MPa, 7 °C) was pumped into the cell at a controlled flow rate until the desired CO_2 mass composition (50 or 85%) was reached. The mass fraction compositions were predefined based on the densities of H-DES (0.907 g/ml at 25 °C) and CO_2 in the feed (0.9374 g/ml at 10 MPa and 7 °C), according to Strieder *et al.* (2024) and NIST database, respectively. After reaching the desired temperature (60 °C), the system pressure was increased until complete homogenization occurred. The pressure was then reduced at a controlled rate of 0.3 MPa/min until a phase change occurred. A liquid-vapor transition with a bubble point (VLE-BP) is characterized by the formation of bubbles at the top of the cell during the depressurization of the system. For once, liquid-liquid equilibrium (LLE) is identified by the appearance of a new phase that extended across the top of the cell, followed by complete cloudiness of the system (cloud point). In this case, depressurization is continued until the appearance of a third vapor phase at the top of the cell (bubble point), characterizing vapor-liquid-liquid equilibrium (VLLE).

Ultrasound assisted extraction (UAE) using H-DES: UAE was employed for *G. mellonella* samples using a digital Branson 450 50/60 Hz sonifier (Branson Ultrasonics, Fremont, CA, USA) with a probe of 1.27 cm diameter that was immersed (35 mm) in vials containing 0.15 g of sample and 1.5 ml of H-DES. Previous to the extraction, a temperature profile was built, and a pulsed or continuous sonication process was tested, to confirm that overheating effects would not interfere in the extraction of the lipid-soluble compounds. Samples (immersed in an ice bath to avoid heating during the sonication process) were sonicated in different times intervals (0.17 to 5 min) at 30% amplitude. Then, amplitude effect (30 and 60%) was evaluated applying a fixed time of 1 min to avoid heating effects. Like other extraction processes, the yield was calculated according to equation (1) and the extracts were kept at –20 °C until further analysis.

Mass transfer kinetic modelling: In order to understand the effects of the selected solvent in the mass transfer kinetics, two different models (spline and two-site desorption model) were fitted to the experimental data (obtained according the extraction procedures described above).

Two-site desorption model: The experimental kinetics data obtained for *G. mellonella* using pure CO_2 (SFE) or CO_2+H-DES (SFE-H-DES) at high-pressure was fitted to the two-site desorption model, described previously in Hawthorne *et al.* (2001). In this model, two different processes are considered to occur simultaneously during the mass transfer kinetics: a washing period (in the very beginning of the extraction) where mass transfer is intensified from solid particle's surface to the solvent, and a diffusion period, representing the slow mass transfer rate from the inner part of the solid particles to the solvent. Both mechanisms are correlated to kinetic coefficients (K_1 and K_2) from the model (equation (2)). The fitting was performed using the nonlinear regression large-scale generalized reduced gradient (LSGRG) method available on Solver for Microsoft Excel® version 2402 (Microsoft, Redmond, WA, USA).

$$y_t = y_\infty \left(1 - f \cdot e^{-K_1 \cdot t} - (1 - f) \cdot e^{-K_2 \cdot t}\right) \tag{2}$$

where y_t is the yield (% g extract/g dried larvae); y_∞ is the yield at the saturation (i.e., when the extraction process has been completed); K_1 and K_2 are constants (per min); f is the fraction of extract easily desorbed from dried larvae (dimensionless).

Spline model: The spline model was also adjusted to the same lipid extraction curves. For that purpose, a spline model of three straight lines (equations (3)–(5)) as described in Meireles (2008), was used to estimate the kinetic parameters employing MATLAB R2018b software. Parameters of the model are associated to three relevant stages in mass transfer processes, corresponding to (from the fastest to the slowest): (1) the constant extraction rate period (CER); (2) falling extraction rate (FER) and (3) the diffusion-controlled period (DC). Each straight line in the spline model represents one of the described periods.

$$y = a_1 \cdot t + b_1 \quad \text{for } t \le t_{\text{CER}} \tag{3}$$

$$y = a_2 \cdot t + b_2 \quad \text{for } t_{CER} < t \leq t_{FER} \tag{4}$$

$$y = a_3 \cdot t + b_3 \quad \text{for } t_{FER} < t \tag{5}$$

where y is the yield (% g extract/g dried larvae); a_1, a_2, a_3, b_1, b_2 and b_3 are spline coefficients (g extract/g dried larvae ·min); t_{CER} is the constant extraction rate period (min) and t_{FER} is the falling extraction rate period (min).

The extract mass recovered in each extraction period was calculated according to equation (6).

$$\text{Recovery (\%)} = \frac{y \cdot 100}{y_t} \tag{6}$$

where y is the yield (g extract/g dried larvae) at either t_{CER} or t_{FER}, as applicable, and y_t is the yield at the end of the extraction.

Lipid extract characterization: fatty acid profile (GC-qToF-MS)

The fatty acids and lipid soluble compounds from *G. mellonella* extracts were assessed by gas chromatography (GC) using a 7890B equipment from Agilent (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent quadrupole time-of-flight mass spectrometry (qTOF-MS) 7200, accordingly to FIEHN GC/MS metabolomics method (Fiehn, 2017). Samples were prepared at 10 mg/ml ethanol and submitted to derivatization (steps described in detail in dos Santos *et al.*, 2021a). The final concentration of samples after derivatization was 1 mg/ml. The separation was performed using an Agilent DB5-MS capillary column (30 m × 250 μm × 0.25 μm). Injection volume was 1 μL at a split ratio of 10:1 at a total flow rate of 10 ml/min using Helium as the carrier gas (0.8 ml/min). The temperature ramped from 60 to 325 °C (at 10 °C/min), steadying at 325 °C for 10 min. Detection was performed under the following conditions: EI at 70 eV, 250 °C and, m/z scan range 50–600 amu (5 spectra/s). Data processing was completed in the software Agilent Mass Hunter Unknown Analysis tool, in which chromatograms were deconvoluted and the identification was performed with the help of updated mass spectrum data libraries (NIST and FIEHN).

Acetylcholinesterase (AChE) inhibition assay

The bioactivity of the extracts was evaluated concerning their possible neuroprotection effects by inhibitory activity of acetylcholinesterase (AChE). The inhibitory activity (IC_{50}, in μg/ml) of *G. mellonella* extracts was achieved following the method described in Sánchez-Martínez *et al.* (2021). Briefly, extracts at a concentration of 1.5 mg/ml (or positive control, galantamine, at 0.0125 mg/ml) were dissolved in aqueous ethanol solution (50%, v/v). Enzyme mean velocity (to attain K_m constant) was obtained using acetylthiocholine iodide (ACth) as substrate, and then a kinetic inhibition was determined under fluorescent parameters: 37 °C; λ excitation = 389 ± 20 nm and λ emission = 513 ± 20 nm. Finally, IC_{50} was calculated as the required concentration to achieve 50% inhibition (using the respective curve: % inhibition *vs.* extract concentration). All reagents used and calculation steps are described in details elsewhere (dos Santos *et al.*, 2021a).

Environmental impact of extraction methods

In order to assess the environmental impact of the process, an innovative metric tool (Path2Green; de Souza Mesquita *et al.*, 2024) was applied to compare scores among the different extraction methods used in this work. This greenness approach is based on 12 principles of green chemistry, individually evaluated in the application, attributing different principle weights, and scored from –1.00 to 1.00 according to biomass, transport, pre-treatment, solvent, scalability, purification, recovery yield, post-treatment, energy demand, application, repurposing, and waste management. Results are presented as process pictograms.

Statistical analysis

The analysis of variance (ANOVA) and Tukey's mean test (α = 0.05) were performed using OriginPro 2024b (OriginLab Corporation, Northampton, MA, USA) to evaluate the differences in recovery yield (%, w/w) of the *G. mellonella* extracts obtained from different extraction methods.

3 Results and discussion

Galleria mellonella biomass characterization

The characterization of the raw material can be useful not only to understand some process parameters effects (for instance, high temperature and use of water in high protein content samples could lead to tubing clog issues), but also to provide an estimation of its chemical composition that could dictate the efficiency of the selected extraction technique. In this sense, physicochemical characterization of *G. mellonella* (Figure 1) was performed and is presented in Table 1.

In the present work, lipid content of *G. mellonella* was around 50%, a value between those reported by Finke (2007) and Francardi *et al.* (2017), who found a

FIGURE 1 Freeze-dried (left) and milled (right) *Galleria mellonella* larvae.

TABLE 1 Physico-chemical characterization of *Galleria mellonella* larvae

	Galleria mellonella
Moisture (fresh sample) (wt.%)	57.2 ± 1.3
Moisture (freeze-dried sample) (wt.%)	1.3 ± 0.2
Protein (dwt.%)	33.05 ± 0.55
Lipids (dwt.%)	50.5 ± 6.4
Ash (dwt.%)	2.30 ± 0.03
Carbohydrates (dwt.%)	12.85*
L (m) (20 count)	0.023 ± 0.002
m (g) (20 count)	0.34 ± 0.09

wt.%, wet weight%; dwt.%, dry weight%; *L*, length; *m*, larvae weight.
* Estimated by difference.

lipid content of 58 and 42% (dwt.%) for *G. mellonella* in the larvae stage, respectively. In the latter, authors fed *G. mellonella* larvae with three different diets, in order to study its influence on the insects' fatty acid composition, mainly focusing on their role on polyunsaturated fatty acids synthesis. Thus, it was possible to attribute the lipid content to the type of reared (strict diet or industrial reared). In our work, no specific diet was applied to the larvae. As for protein and ash content, values are in accordance with those reported by Finke (2007), that is, 38 and 2% (dwt.%), respectively. On the other hand, *G. mellonella* has a low carbohydrate content (12.85% as estimated by difference), thus reinforcing the larvae's potential as a good lipid source.

With all these considerations, the present work focused on the lipid extraction of the larvae using supercritical CO_2, UAE, hydrophobic deep eutectic solvent or their combination. To facilitate lipid mass transfer and improve extraction efficiency, water was removed from the larvae by freeze-drying, reducing the initial moisture content by approximately 56%. This step was essential to eliminate water interference during the lipid extraction process and enhance solvent accessibility within the matrix.

High pressure extraction: the solvent composition effect in mass transfer kinetics

In order to elucidate the mass transfer kinetics of lipid extraction from *G. mellonella* for further efficiency enhancing, designing and scaling up of the process, the mathematical models (spline and two-site) were applied to SFE and SFE-H-DES. The experimental and the adjusted curves are presented in Figure 2a,b, while the fitting parameters are depicted in Table 2. The two models applied were overall well adjusted, revealed by their high coefficient of determination (R^2 ≈0.99) for both SFE and SFE-H-DES scenarios.

In Figure 2 it can be observed that the SFE kinetics was slower than SFE-H-DES, and achieved lower yield after 150 min, thus indicating an enhanced mass transfer kinetics when H-DES was used at 15% concentration.

Spline model with three straight lines describes the three different periods of extraction (CER, FER, and DC). The time to reach constant and falling extraction rate periods (t_{CER} and t_{FER}) were considerably smaller for SFE-H-DES, confirming the faster kinetic mass transfer for *G. mellonella* when 15% of H-DES is used (Table 2), which is also corroborated by the high values of $Recovery_{CER}$ and $Recovery_{FER}$ for SFE-H-DES. As noticed, the SFE-H-DES yield after CER (Y_{CER}) demonstrates a higher value even when com-

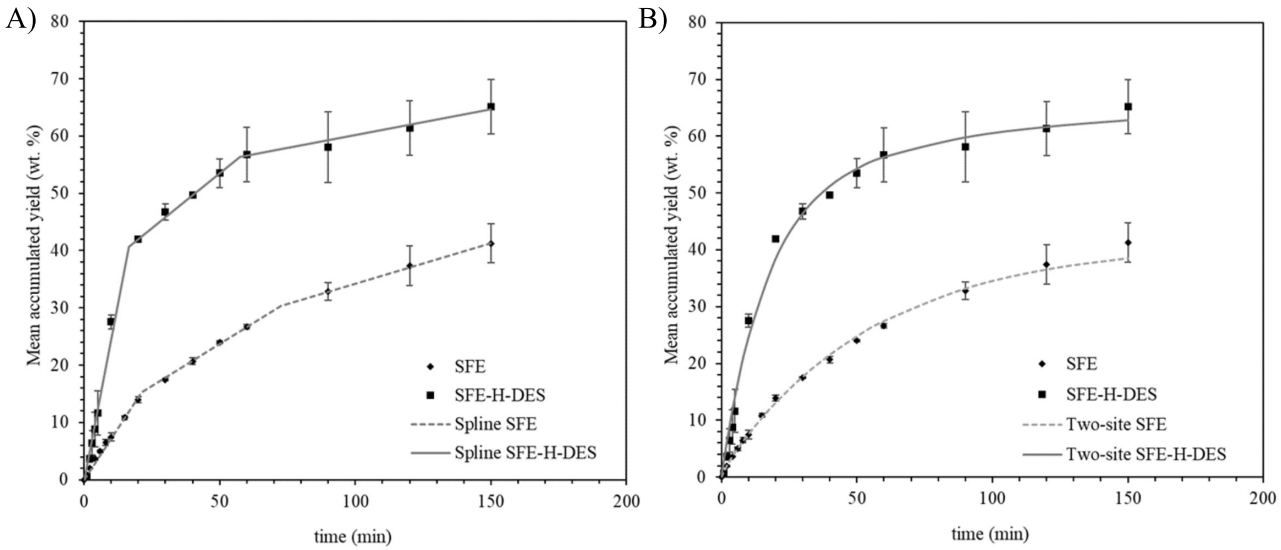

FIGURE 2 Lipid extraction kinetics of *G. mellonella* larvae fitted by Spline (a) and Two-site (b) models using 100% supercritical CO_2 (SFE) or using 85% supercritical CO_2 and 15% of menthol:eucalyptol (1:1; mol) as a hydrophobic deep eutectic solvent (H-DES).

TABLE 2 Fitting parameters and coefficient of determination (R^2) for kinetic models (Spline and Two-site) applied to yield (%, g extract/100 g dried larvae) of extraction curves from *G. mellonella* obtained by SFE and SFE-H-DES

	Kinetic yield (%, g extract/ 100 g dried larvae)		
	Kinetic parameter	SFE	SFE-H-DES
Spline	a_1	0.0072	0.0247
	a_2	0.0029	0.0038
	a_3	0.0014	0.0009
	b_1	0	0
	b_2	0.0904	0.3434
	b_3	0.2009	0.5118
	t_{CER} (min)	21.06	16.43
	t_{FER} (min)	72.49	57.45
	Y_{CER}	0.15	0.41
	Y_{FER}	0.30	0.56
	$Recovery_{CER}$ (%)	38.88	62.86
	$Recovery_{FER}$ (%)	73.46	87.16
	R^2	0.9954	0.9991
Two-site	K_1	0.43	0.06
	K_2	0.02	0.01
	f	0.025	0.737
	R^2	0.9959	0.9941

a_1, a_2, a_3, b_1, b_2 and b_3, spline linear coefficients (% g extract/g dried larvae per min); t_{CER}, constant extraction rate time (min); t_{FER}, constant falling rate time (min); Y_{CER} and Y_{FER}, g extract/g dried larvae at t_{CER} and t_{FER}, respectively; $Recovery_{CER}$ and $Recovery_{FER}$, recovered mass of extract at t_{CER} and t_{FER}, respectively (%); K_1 and K_2, two-site constants (per min); f, fraction of extract easily desorbed from dried larvae (dimensionless).

pared to Y_{FER} of SFE, indicating that the extraction kinetic was not only faster but also was able to reach considerable higher yields of lipid extract from *G. mellonella*. Although no other work was available for kinetic parameters comparison in larvae oil extraction, a recent defatting SFE process was applied to *Hermetia illucens* larvae by Fornari *et al.* (2023). The authors reported an approximate t_{cer} value of 30 min (graphically described) in a larger-scale SFE unit (1350 ml) at 130 g/min, 450 bar and 60 °C. The higher value in comparison to our work

(t_{CER} = 21 min) is expected considering the small scale applied (20 ml) for *G. mellonella* extraction. On the other hand, the increased pressure (450 bar against 200 bar applied in this work) did not seem to enhance the oil recovery from larvae. Still, the differences in the fatty acid composition of the different species might also have affected oil extraction kinetics due to their solubility differences in supercritical CO_2.

Unlike spline model, the two-site model described two extraction stages, where K_1 and K_2 are associated with washing and diffusion mechanisms, respectively. The washing mechanism is strictly linked to the mass transferred at the very beginning of the extraction, i.e., the solutes that are easily released from the outer surface of the particles to the solvent, while the diffusion mechanism is related to the solutes in the inner part of the particles (Hawthorne *et al.*, 2001). In this sense, the result of $K_1 \gg K_2$ in the SFE kinetics, suggests that washing period is somewhat relevant in the extraction yield, although the fraction of quickly desorbed extract (f) is only 0.025, which explains the slower mass transfer kinetic when using only CO_2 as solvent. In addition, the interpretation of the washing period is limited to the extraction equipment that under dynamic conditions will strictly depend on the contact time between the solvent and the feed, a parameter that is difficult to control in a lab-scale equipment. On the other hand, in the SFE-H-DES, the washing mechanism does not seem to control kinetics despite higher f (Table 2), which implies a significantly higher yield throughout the whole kinetics when using 15% H-DES. Although not applied to lipid extraction kinetics, a recent work described by Singh *et al.* (2024) aimed to compare the influence of an anthocyanin extraction kinetic from *Rhododendron arboreum* Sm., regarding variations in a deep eutectic solvent composition. Such as in this work, changes in the solvent composition highly affected the extraction kinetic, as demonstrated by two-site fitted kinetic coefficient parameters. In their findings, a f value of 0.83 was achieved when solvent was composed of 25% water, against only 0.20 when water was 20% of the extractant composition. In fact, the knowledge of other kinetic studies using supercritical CO_2 and H-DES would be helpful to understand the coefficient parameters variations but unfortunately, to date, there are no reports in literature. In this sense, is important to highlight that this is the first time (to the best of our knowledge) that mass transfer kinetic models are adjusted to lipid extraction from larvae. Although milled samples from some seeds and pulp with high lipid content (Chañi-Paucar *et al.*, 2023; dos Santos *et al.*, 2021b), presents a visual aspect very similar to *G. melonella* larvae (Figure 1b), comparisons here described were based on other works that mainly focused on plant or vegetable matrices extraction, and as a result of chemical composition differences among the matrices, the interpretation of the kinetic parameters might diverge.

Phase equilibrium between H-DES and supercritical CO_2: Figure 3 illustrates the photographs captured for the phase equilibrium between H-DES and CO_2 at 60 °C, with mass ratios of 15:85 and 50:50, at the point when the phase transition occurred. For a mass fraction of 15% of H-DES, homogeneity of the system was achieved at a pressure of 140 bar, and depressurization from this point was performed at a rate of 3 bar/min. A transition to liquid-liquid equilibrium (LLE) was noted at a pressure of 127 bar, which was subsequently followed by the complete clouding of the system (cloud point). This depressurization led to the appearance of a third phase, the vapour phase (bubble point equivalent) at a pressure of 122 bar, indicating the presence of vapour–liquid–liquid equilibrium (VLLE).

In the system containing 50% H-DES, homogeneity was maintained at a pressure of 125 bar. Upon pressure reduction, a transition to vapour–liquid equilibrium with bubble point (VLE-BP) was noted at a pressure of 112.9 bar. In both scenarios (VLE and LLE), the composition of the dominant phase is regarded as equivalent to the total composition of the mixture, assuming that the mass in the secondary phase is negligible. It is possible to assume, for both conditions applied in this work, the homogeneity of the system CO_2-H-DES is possibly responsible for a similar behaviour in the mass transfer between lipid soluble compounds in the *G. mellonella* and the solvent, which could explain the similar yield values for both conditions in SFE-H-DES (15 and 50% H-DES) (further described in Table 3).

Emerging extraction techniques are a great alternative for the sustainable exploitation of bioactive compounds from different sources. In this work, different extraction processes and conditions were tested to obtain lipidic extracts from *G. mellonella*; comparison among them can provide evidences allowing the selection of the most suitable technique and shedding some light on the behaviour of this material when submitted to different protocols.

Lipid extraction yield of G. mellonella: assessing extraction processes effects and environmental impact

Table 3 presents the extraction yields (%) obtained using different *G. mellonella* lipid extraction methods and conditions. As can be seen, the lowest yield (32.8%)

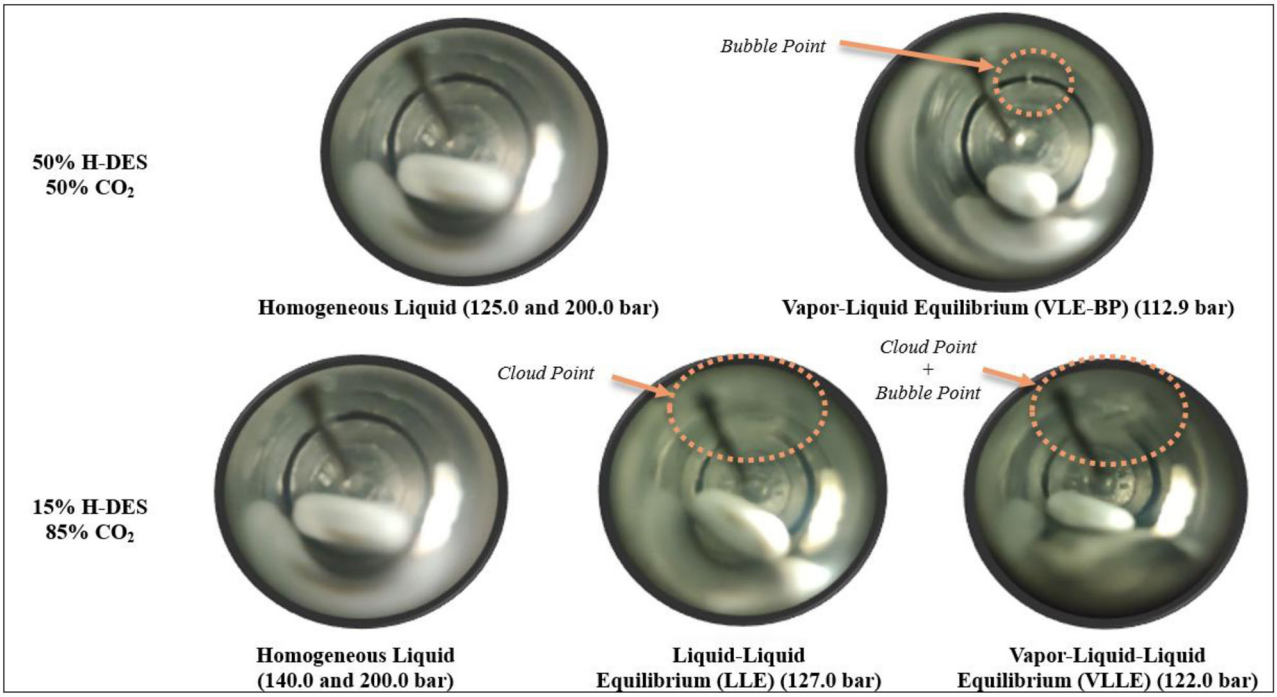

FIGURE 3 Effect of H-DES percentage (15 and 50%) on phase equilibrium at 60 °C between CO_2 and H-DES (menthol:eucalyptol 1:1 molar ratio).

TABLE 3 *Galleria mellonella* extraction yields (g extract/g dried larvae) achieved by using different extraction methods, solvent, and process conditions

Extraction method/condition		Yield (%, g extract/100 g dried larvae)
Soxhlet (8 h hexane reflux)		50.4 ± 6.1[a]
UAE-H-DES / probe, 1 min (T_{max} = 40 °C)	30% amplitude	44.4 ± 2.2[ab]
	60% amplitude	50.0 ± 3.4[a]
SFE-H-DES/200 bar, 60 °C, 4 ml/min	15% H-DES	58.1 ± 4.7[a]
	50% H-DES	58.8 ± 5.3[a]
SFE/60 °C, 4 ml/min	200 bar	32.8 ± 1.5[b]
	400 bar	31.8 ± 2.7[b]

Yield means that share the same letter are not significantly different at the 0.05 level.

was obtained for SFE at either 200 or 400 bar. Although SFE is recognized for its solvent-free advantage and noticeable reduced environmental impact, its efficiency can be highly dependent on the matrix composition and process parameters. The application of supercritical CO_2 extraction to *Tenebrio molitor* meals has been reported by Laroche *et al.* (2019). They performed SFE at 325 bar, 55 °C and 10 g/ml for 75 min and achieved a yield of 22.1% (with no significant differences in comparison to Soxhlet extraction using hexane). In comparison to our findings, these authors were more successful in recovering a higher percentage of larvae lipid when applying SFE. This could be due to the differences in the lipid composition (to be discussed in details below) between the different larvae species, which can influence solubility and mass transfer kinetics at the specific conditions of this work.

On the other hand, a considerable higher yield was achieved when combining SFE and H-DES (≈58%), underscoring the potential of combining sustainable techniques with green solvents to enhance extraction performance. Also, for all tested methods, the extraction condition did not seem to significantly affect the yield. Regarding larvae extraction, Otero *et al.* (2020) have performed UAE (probe sonication at 60% amplitude, 15 min) and pressurized liquid extraction (PLE) to obtain extracts from *Tenebrio molitor* larvae using ethanol and ethanol:water (1:1, v/v) mixtures. Unlike

this work, the authors conclude that the extraction yield (%) was more dependent on the extraction method than on the solvent itself, since the yield for aqueous ethanol extraction were 17.14 and 33.87% for UAE and PLE, respectively. The results of this study demonstrate the viability of replacing hexane with H-DES, a greener solvent. Bragagnolo *et al.* (2022) previously used the same solvent to defat agro-soy by-products through pressurized liquid extraction, achieving results comparable to those obtained with n-heptane/hexane extraction.

The use of a natural deep eutectic solvent in this work (composed of menthol and eucalyptol) represents a promising alternative to conventional organic solvents, as demonstrated by the excellent yield results for lipid recovery from *G. mellonella*. H-DESs are derived from natural, biodegradable components and offer low toxicity, making them more environmentally benign, especially when combined with more sustainable techniques such as SFE. Moreover, this approach aligns with the principles of green chemistry by reducing the dependence on petrochemical-derived solvents and minimizing hazardous waste (Anastas and Warner, 1998).

Path2Green was selected as a metric tool to analyse the greenness of the UAE-H-DES, SFE, and SFE-H-DES processes. The relevant criteria applied for each extraction method are discussed following.

Principle 1 (weight 6.0), biomass/raw material selection: *G. mellonella* is used as a raw material in all selected extraction methods, which is a larvae easily found in nature (selected score = 0.75).

Principle 2 (weight 5.0), transportation: the source *G. mellonella* needs to reach its destination, respecting safety concerns and minimum environmental impact. Therefore, a reasonable criterion is the rearing near the extraction facilities, so effective transportation is defined as the maximum of 100 km in this case (score = 0.29).

Pre-treatment of *G. mellonella* (Principle 3, weight 2.5) involves physical methods as milling (to increase surface area) and freeze-drying (to reduce moisture for lipid extraction). Since the pre-treatments are only physical (no chemical, biological or combined pre-treatments were necessary) for both extraction methods, the score of –0.2 is selected.

The scores for solvents (Principle 4, weight 6.0) followed the CHEM21 guideline criteria (Prat *et al.*, 2016), which classified the solvents as recommended (score = 1.00), problematic (score = 0.00), or hazardous (score = –1.00). For solvent selection, the methods described in this work used a menthol:eucalyptol (H-DES), supercritical CO_2 or the combination of both. These solvents align with the principles of green chemistry, since they are non-toxic and replace hazardous solvents such as petroleum ether or hexane for lipid extraction. However, since supercritical CO_2 demands high energy inputs for its pressurization, a lower score is attributed to this solvent (score H-DES formulated using biobased materials = 1.00; score supercritical CO_2 = 0.00; score supercritical CO_2+H-DES (H-DES prevailed) = 1.00).

Principle 5 (weight 5) is related to the process scalability. Although scalability can be applied to all extraction operative modes, their effectiveness will be strictly dependent on time and the technology used. For SFE and SFE-H-DES processes, since they refer to a semi-continuous operation technique, this principle's score corresponds to the same (score = 0.50). On the other hand, a lower score is designated for UAE-H-DES which is a non-automated batch system (score = –1.00).

Principle 6 (weight 2.5) is scored under the purification steps needed to apply to the final extract. In this work, the lipid extract obtained from SFE is ready-to-use (score = 1.00). SFE-H-DES and UAE-H-DES generates lipids diluted in menthol:eucalyptol solvent that can be used straightforward depending on the application, or evaporated in case only the lipid is the goal for application. The latter is considered in this work (score = 0.50), since the aim is to recover the lipid fraction of the larvae.

Principle 7 (weight 4.0) aims to classify the environmental assessment through the extraction efficiency, often characterized by the yield of the processes. This parameter represents the feasibility and valorisation of the material. In this work, the lipid extract in the different extraction methods applied were compared to an exhaustive Soxhlet extraction (reference method, non-environmentally safe). In such way, the SFE-H-DES was the only exhaustive extraction (score = 0.00), while UAE-H-DES and SFE represent only semi-exhaustive extraction (score = –0.50).

Principle 8 (weight 2.5) focuses on post-treatment optimization to enhance the functionality of the extract. The bioactivity of *G. mellonella* extracts is still in the early stages of research, so it can currently only be considered a ready-to-use ingredient (score = 1.00). Further investigation into its bioactive compounds is expected, which may lead to additional assessments in the near future.

Principle 9 (weight 4.0) addresses the energy demand associated with the extraction process. Depending on the raw material, high energy input is often

FIGURE 4 Pictograms generated from Path2Green: environmental impact scores for lipid extraction of *G. mellonella* using SFE, SFE-H-DES and UAE-H-DES as extraction methods.

required to disrupt cells and release compounds into the solvent. In this study, two strategies were employed in order to overcome this limitation: two pressure-based (SFE and SFE-H-DES) and one cavitation-based extraction (UAE-H-DES). Since all scenarios relied on non-renewable energy sources, they received the lowest score (score = –1.00). However, integrating CO_2 recycling systems often used at industrial scale in SFE, can significantly reduce both CO_2 emissions and energy required for pressurization. Additionally, sourcing electricity from renewable energy (e.g., solar) could notably improve the sustainability score, emphasizing the critical role of energy source selection in lipid extraction methods.

Principle 10 (weight 4.5) highlights extract safety for several applications. A lipid extract from insect larvae can find applicability mainly in food, pharmaceutical, and cosmetic industries, thus including its versatility in three different domains (score = 0.50).

Principle 11 (weight 6.0) focuses on the repurposing of residual solid material and solvents, where applicable, after extraction. In this study, all three scenarios demonstrated that the remaining *G. mellonella* biomass is a valuable protein source, with potential for reuse as a new extraction matrix or as a ready-to-use ingredient in the food industry. This approach supports ecosystem health and reduces the environmental impact associated with waste disposal. Although DES are generally more challenging to recycle, the H-DES used in this work were demonstrated to be volatile at mild temperatures, and could be reused within the process. Therefore, repurposing strategies in this study present a viable opportunity and contribute positively to the environmental performance of the lipid extraction process (score = 0.00).

Finally, the potential residuals from the extraction (when no process integration is applied) accounts in the waste management that must fulfil the environmental requirements (Principle 12, weight 6.0). Environmental factors (E-factor) calculated for the highest yields in SFE, SFE-H-DES and UAE-H-DES, were 67, 40 and 50%, respectively, resulting in scores of –0.34, 0.20 and 0.00, respectively.

The overall scores for the three extraction methods are shown in Figure 4. As illustrated, the use of H-DES as a solvent in SFE reduced the environmental impact associated with lipid extraction from *G. mellonella*. It is important to notice that, despite lower extraction time for UAE-H-DES, other process limitations contribute significantly to the environmental impact of this type of extraction, given the lowest score reported for UAE-H-DES. Furthermore, the need for H-DES removal after extraction depends on the intended application, which could significantly alter the final scores if solvent elimination steps are not required. For instance, a recent work reported by Strieder *et al.* (2024) states that eucalyptol-based solvents (including the menthol:eucalyptol mixture of this present work) are safe to use with the extract under permitted concentrations. These solvents demonstrated a general score of 0.155 against –0.023 obtained for hexane/CPME extraction processes, reinforcing the sustainable potential of the eucalyptol mixtures as a novel hydrophobic solvent in lipid extraction systems.

Figure 5 shows the results obtained by using H-DES in the ultrasound-assisted extraction (UAE) kinetics of *G. mellonella* larvae, as well as the temperature profile during the probe sonication process (fixed at 30% amplitude). In order to keep the temperature below 50 °C and to avoid a negative influence on the extraction of the lipophilic compounds, the extraction time was limited to 5 min. Results demonstrated that UAE-H-DES was considerably faster than the other tested methods, since 0.17 min was enough to achieve a maxi-

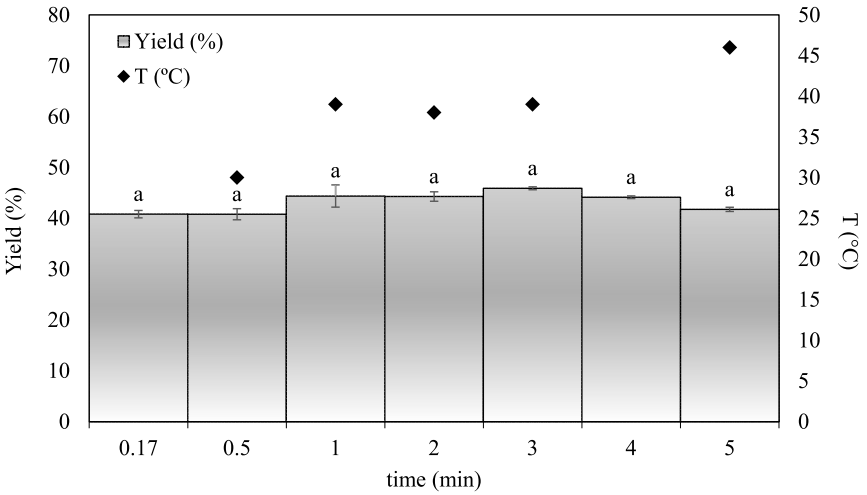

FIGURE 5 Ultrasound-assisted extraction (UAE) of *G. mellonella* using H-DES as extraction solvent. Amplitude = 30%. Initial probe temperature (t = 0 min) was approximately 14 °C. Yield means that share the same letter are not significantly different at the 0.05 level.

mum yield ≈ 42% (with no statistical differences to the yield% after 5 min). Still, this value is far below the SFE-H-DES yield of 58.8% (Table 3).

Fatty acid profile

The fatty acid profiles of *G. mellonella* lipid extracts are summarized in Table 4. Using the FIEHN method (threshold >2%), a total of five fatty acids were consistently identified in relatively high abundance across all extracts: oleic acid, palmitic acid, linoleic acid, stearic acid, and gondoic acid, in decreasing order of abundance: oleic>palmitic>linoleic>stearic>gondoic.

The lipid composition of *G. mellonella* has previously been characterized by Perez-Santaescolastica *et al.* (2023) who employed solid-liquid extraction with a chloroform:methanol mixture (1:1) to assess the fatty acid profiles of seven edible insect species. Their results showed that *G. mellonella* extracts were particularly rich in oleic acid and contained the lowest levels of stearic acid among the evaluated insects. Notably, gondoic acid was only detected in *G. mellonella* lipid extracts, highlighting a distinctive feature of this species. Recent findings by Fan *et al.* (2022) have also linked gondoic acid to anti-inflammatory effects in liver cells, suggesting additional potential health benefits.

With respect to the polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) ratio, it is well established that a higher intake of linoleic acid (n-6 PUFA) is associated with lower SFA consumption and contributes to a reduced risk of cardiovascular diseases (Czernichow *et al.*, 2010). Among the extracts analyzed, the SFE *G. mellonella* extract exhibited the highest relative content of linoleic acid, positioning it as the most promising candidate for health-promoting applications. However, it is also important to highlight that linolenic acid was not detected in any of the *G. mellonella* lipid extracts. This absence suggests that the larvae used in this study possibly did not obtain this polyunsaturated fatty acid from their diet, considering dietary intake as their primary source of this essential fatty acid. This is consistent with the fact that their typical diet is naturally low in n-3 fatty acids.

From a technological standpoint, the lipid profile of *G. mellonella* is particularly interesting. The combination of high SFA and oleic acid content is similar to high-oleic oils such as soybean and canola oils, while simultaneously preserving characteristics typical of SFA-rich oils like palm oil (which contains approximately 51% SFA). This unique fatty acid composition may confer greater oxidative stability at elevated temperatures while offering improved health benefits compared to traditional SFA-rich oils. Nevertheless, it is important to emphasize that the specific health impacts of each oil should be evaluated individually, as suggested by Desjardins and Rudkowska (2023).

In addition to fatty acids, *G. mellonella* extracts untargeted lipid soluble compounds also demonstrated the presence of cholesterol, plasticizers (e.g. triethylene glycol di(2-ethylhexoate), aldehydes (e.g. 4-methyloctadecanal), and acids (e.g. phtalic acid and L-glutamic acid).

Acetylcholinesterase inhibition

The acetylcholinesterase (AChE) inhibition by lipid extracts (mostly obtained from plant matrices) has demonstrated great potential, since the lipophilic compounds are more prone to cross the blood brain bar-

TABLE 4 Fatty acid profile (relative area%) of *G. mellonella* obtained by GC-qTOF-MS using different extraction methods

Fatty acid*	Molecular formula	Match factor (%)	RT (min)	Relative area (%)						
				Soxhlet	UAE-H-DES (amplitude,%)		SFE-H-DES (H-DES (%, v/v))		SFE (*P*, bar)	
					30	60	15	50	200	400
Pelargonic acid	$C_9H_{18}O_2$	72	10.23	n.d.	n.d.	n.d.	trace†	n.d.	n.d.	n.d.
Azelaic acid	$C_9H_{16}O_4$	80	15.42	n.d.	n.d.	trace	trace	trace	n.d.	trace
Palmitelaidic acid	$C_{16}H_{30}O_2$	80	17.73	trace	trace	trace	trace	trace	trace	trace
Palmitic acid	$C_{16}H_{32}O_2$	88	17.93	40 ± 6^{a}	39 ± 3^{a}	39 ± 2^{a}	40.8 ± 0.5^{a}	39 ± 3^{a}	35 ± 4^{a}	38 ± 3^{a}
Linoleic acid n-6	$C_{18}H_{32}O_2$	94	19.44	8.2 ± 0.8^{a}	12 ± 1^{ab}	12 ± 2^{ab}	12 ± 2^{ab}	11.7 ± 0.8^{ab}	17 ± 3^{b}	16 ± 2^{ab}
Oleic acid n-9	$C_{18}H_{34}O_2$	93	19.50	48 ± 8^{a}	43 ± 5^{a}	43 ± 5^{a}	39.3 ± 0.8^{a}	43 ± 2^{a}	40.31 ± 0.13^{a}	39.4 ± 0.7^{a}
Stearic acid	$C_{18}H_{36}O_2$	86	19.73	3.2 ± 0.8^{a}	4.01 ± 0.01^{a}	3.76 ± 0.09^{a}	5 ± 1^{a}	4.2 ± 0.7^{a}	6 ± 1^{a}	4.5 ± 0.7^{a}
Petroselinic acid	$C_{18}H_{34}O_2$	86	20.22	trace	n.d.	trace	trace	n.d.	trace	trace
Gondoic acid n-9	$C_{20}H_{38}O_2$	89	21.15	trace	2.2 ± 0.6^{a}	2.7 ± 0.6^{a}	2.7 ± 0.5^{a}	2.2 ± 0.5^{a}	2.4 ± 0.2^{a}	2.7 ± 0.4^{a}
Paullinic acid	$C_{20}H_{38}O_2$	88	21.85	n.d.	n.d.	n.d.	trace	trace	trace	trace
MUFA				48 ± 8^{a}	45 ± 4^{a}	45 ± 4^{a}	42.0 ± 0.2^{a}	45 ± 2^{a}	42.68 ± 0.03^{a}	42.1 ± 0.2^{a}
PUFA				8.2 ± 0.8^{a}	12 ± 1^{ab}	12 ± 2^{ab}	12 ± 2^{ab}	11.7 ± 0.7^{ab}	17 ± 3^{b}	16 ± 2^{ab}
SFA				43 ± 7^{a}	43 ± 3^{a}	43 ± 2^{a}	46 ± 2^{a}	43 ± 3^{a}	41 ± 3^{a}	42 ± 3^{a}
PUFA/SFA				0.19	0.27	0.27	0.26	0.27	0.41	0.38

Fatty acids as TMS derivative; Relative area% means (for a single fatty acid) that share the same letter are not significantly different at the 0.05 level. trace, relative area ≤0.3%; n.d., not detected; UAE-H-DES, ultrasound-assisted extraction using hydrophobic deep eutectic solvent; SFE-H-DES, supercritical fluid extraction using hydrophobic deep eutectic solvent as cosolvent; SFE, supercritical fluid extraction using CO_2; MUFA, PUFA, SFA, monounsaturated, polyunsaturated and saturated fatty acids, respectively.

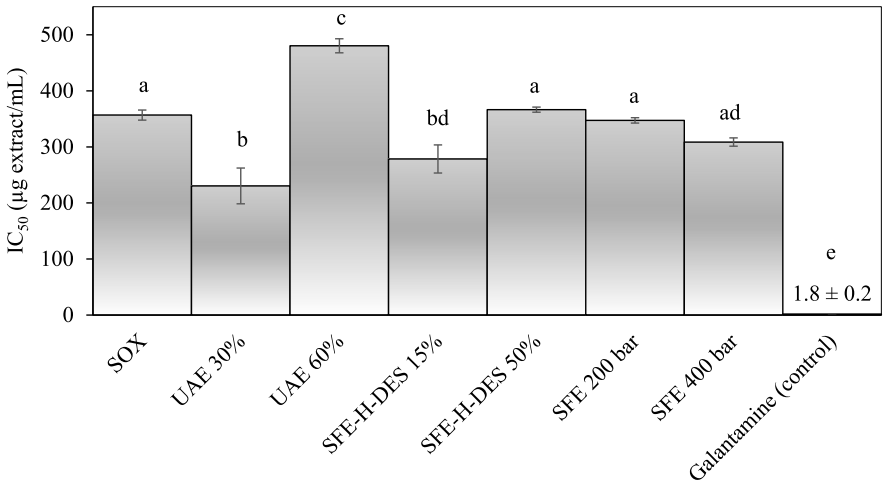

FIGURE 6 Acetylcholinesterase IC_{50} values for *G. mellonella* extracts obtained using different extraction methods and galantamine (control).

rier (Amiri *et al.*, 2021; Sánchez-Martínez *et al.*, 2022). Therefore, extracts obtained at different conditions were tested in terms of AChE inhibition. To our knowledge, this is the first time a larvae extract is tested against a neurodegenerative associated enzyme activity. The inhibitory concentration required to reduce AChE activity by 50% is presented in Figure 6.

As seen in Figure 6, IC_{50} values were in the range of approximately 200–500 µg extract/ml, and the highest and lowest values were found for UAE using 60 and 30% amplitude, respectively. Therefore, extracts can be considered as poor inhibitors of the enzyme. An extensive review on potential AChE inhibitors was reported by Santos *et al.* (2018). According to their detailed study, it was possible to categorize good inhibitors when the extracts present IC_{50} < 20 µg extract/ml, which were found mostly in plant bulb, aerial and root parts. However, higher IC_{50} results obtained in the present work do not exclude the potential of the existing compounds in the *G. mellonella* lipid extracts on other enzymes and/or mechanisms associated with Alzheimer's and other diseases. For instance, Francardi *et al.* (2017) significantly enhanced the n-3 fatty acid production in *G. mellonella* by supplementing larvae diet with minced leen seeds, demonstrating their health-benefits for human consumption. In this sense, other lipid compounds could also be achieved through *G. mellonella* rearing.

4 Conclusion

The characterization of *G. mellonella* larvae biomass and extracts highlighted their potential as a valuable lipid source, which could offer advantages for several applications, particularly for food industry. The study of sustainable lipid extraction methods using CO_2 and a novel hydrophobic deep eutectic solvent (H-DES, menthol:eucalyptol) revealed their combination significantly enhanced mass transfer kinetics compared to a conventional extraction method, achieving yields up to 58.8%. Phase equilibrium measurements indicated system homogeneity under all conditions tested, which may explain why varying the H-DES concentration did not further improve extraction efficiency. For the first time, the mass transfer kinetics of lipid extraction from *G. mellonella* were described using two mathematical models, with fitted kinetic parameters demonstrating that the use of H-DES not only improved extraction rates but also promoted more sustainable techniques by reducing dependence on traditional organic solvents and presenting the lowest environmental impact according to *Path2Green* scores. Ultrasound assisted extraction using H-DES showed limited yield efficiency with extraction time, and fatty acid profile remained stable across different ultrasound amplitudes. Moreover, the fatty acid profile (with oleic and palmitic as the main ones, and saturated fatty acids fraction accounting with around 43% of their total fatty acid composition) in *G. mellonella*, places their extracts in a position of a promising functional ingredient, where linoleic acid percentage was significantly higher in extracts other than conventional Soxhlet. Despite limited inhibition of acetylcholinesterase activity, this study encourages further research in exploring other health benefits properties of *G. mellonella* extracts. In summary, the extraction method and solvent choice played a crucial role in the lipid recovery efficiency from *G. mellonella*. These findings suggest that *G. mellonella* larvae represent a

promising, sustainable bioresource for the production of novel ingredients with industrial relevance.

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