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RESEARCH ARTICLE

Influence of the waste substrate and extraction method on the lipid profile of black soldier fly (*Hermetia illucens*)

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Abstract

Hermetia illucens commonly known as black soldier fly (BSF), is a rich source of protein, lipids, vitamins, and minerals, fulfilling the nutritional needs of both humans and animals. The larvae (BSFL) are recognised for converting food waste into valuable organic fractions and is rich in several nutrients, thereby supporting a circular economy and addressing a significant global challenge such as organic waste management. The aim of this work was to evaluate the ability of attenuated total reflectance (ATR) mid infrared (MIR) spectroscopy combined with chemometrics to classify lipids extracted from BSFL fed with three different waste streams (supermarket, food waste mix and childcare centre waste). The results of this study indicated that most fatty acid (FA) levels, except for palmitic and stearic acids, vary across the different dietary groups, supporting that diet significantly influences fatty acid levels and the distribution of saturated (SFA) and unsaturated (UFA) in BSFL. The results also demonstrate the potential of ATR-MIR spectroscopy combined with principal component analysis (PCA) and Soft Independent Modelling of Class Analogy (SIMCA) to differentiate and classify *H. illucens* larvae lipids reared on various organic side streams. This method offers a promising alternative for the industry to link BSFL to their diet, based on their lipid profile, enabling rapid and accurate analysis with minimal sample amount and no need for pretreatment.

Keywords

black soldier fly – infrared – lipids – substrate – ultrasound

1 Introduction

Hermetia illucens, commonly known as the black soldier fly (BSF), is one of the most widely utilized insects used for converting by-products into valuable organic fractions, thereby supporting a circular economy and addressing a significant global challenge: organic waste management (Barroso *et al.*, 2014; Oonincx *et al.*, 2015). Several characteristics make *H. illucens* particularly noteworthy, including its short life cycle, high feed conversion ratios, and the fact that it only consumes food during its larval stage, not as an adult (Da Silva and Hesselberg, 2020; Oonincx *et al.*, 2015). Moreover, *H. illucens* can process a diverse range of waste types, including soy waste, cattle, pig, or chicken manure, sewage sludge, food waste, restaurant waste, and even faecal sludge,

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among others (Lalander *et al.*, 2013; Liu *et al.*, 2020; Rehman *et al.*, 2017). The different substrates used can impact the larvae (BSFL) growth rates, size, survival rate, fertility, and nutritional composition, including fat and protein contents (Kim *et al.*, 2021). Additionally, the diet can influence their fatty acid profile (Oonincx *et al.*, 2020; Riekkinen *et al.*, 2022). Nonetheless, *H. illucens* remains a rich source of protein, lipids, vitamins and minerals, fulfilling the nutritional needs of humans and animals (Barroso *et al.*, 2014). Despite the variation in feed substrates, BSFL consistently exhibit a high lipid content, storing up to 39% of their dry weight in lipids, which are used as an energy source during the non-feeding adult stages (Wang and Shelomi, 2017). *H. illucens* has a lipid fraction distinct from most other insects studied, characterized by a high percentage of saturated fatty acids (58-72%), particularly lauric, myristic, and palmitic acids, which contribute to its solid state at room temperature (Sosa and Fogliano, 2017). Additionally, it contains unsaturated fatty acids, mainly oleic and linoleic acids (Barragan-Fonseca *et al.*, 2017). This unique lipid profile makes *H. illucens* particularly appealing for some applications where other insect species may be less suitable. For instance, Smetana *et al.* (2020) obtained margarine with a lower environmental impact than butter, with the same spreading properties as plant-based margarine, by replacing vegetable fats with *H. illucens* fats, thanks to its similar physical state. Delicato *et al.* (2020) used *H. illucens* fat instead of butter in bakery products and found that substituting 25% of the butter with insect fat did not affect consumer preference. They could even replace up to 50% of waffle production without compromising taste or texture. Schiavone *et al.* (2018) partly and fully replaced soybean oil with *H. illucens* larva fat in broiler chicken diets and found no adverse effects on growth performance or blood parameters. Similarly, Cullere *et al.* (2019) studied the sensory characteristics of chicken meat under the same rearing conditions and concluded that substituting soybean oil with *H. illucens* fat is feasible, even up to 100%. In addition to its applications in food and feed production, *H. illucens* fat also has a range of industrial uses. It can serve as a non-food feedstock for biodiesel production or in the cosmetic industry, where it can be used in products like soaps and various personal care items (Franco *et al.*, 2021; Li *et al.*, 2011; Verheyen *et al.*, 2018).

Besides all the conditions before insect harvesting that impact their composition, the techniques employed during the killing process, manufacturing, fraction extraction, storage, and transport also play a significant role (Mendez-Sanchez *et al.*, 2024; Ojha *et al.*, 2021; Ravi *et al.*, 2020). Caligiani *et al.* (2018), for instance, followed three different protocols for fractioning *H. illucens*, revealing that these methods significantly impacted the purity, yield, and integrity of specific components. Numerous techniques have been described for lipid extraction from insects, with organic solvents being the most common. However, other methods, including aqueous extractions, three-phase partitioning, ultrasound, pressing, centrifugation, enzyme-assisted extraction, and supercritical fluid extraction, have also been explored (Laroche *et al.*, 2019; Li *et al.*, 2024; Tzompa-Sosa *et al.*, 2014). The choice of method significantly influences both the yield and composition of the final lipid extract.

The biochemical composition of the lipid fraction is important for its application, but traditional analysis methods are time-consuming, require chemicals, and demand significant investment in equipment. Furthermore, the use of harsh chemicals tends to negate the positive image of BSFL being a "green" answer to waste management within the circular economy. Infrared (IR) spectroscopy is emerging as an alternative analytical technique, enhanced by advances in miniaturized devices (Cebi *et al.*, 2023; Rodriguez-Saona *et al.*, 2020). These instruments enable real time analysis, with almost instant results and no destruction of the sample. IR spectroscopy is based on the interaction between the radiation and the chemical bonds in the sample, allowing for detailed structural identification (Cebi *et al.*, 2023; Rodriguez-Saona *et al.*, 2020). The fingerprint region, in the mid-infrared (MIR) spectrum, provides a unique pattern specific to each sample. This distinct spectral pattern makes it highly useful for identifying and comparing different samples (García-Gutiérrez *et al.*, 2021). Combined with multivariate analysis, MIR spectroscopy has been successfully used to classify and quantify components and variables in different samples, including lipids. There are already results of insect samples analysed using MIR spectroscopy. For instance, Hoffman *et al.* (2022) successfully classified *H. illucens* larvae powders reared on different waste stream diets. Similarly, Mellado-Carretero *et al.* (2020) differentiated insect powders by species and, within the same species, based on variations due to processing conditions while Alagappan *et al.* (2024) quantified insect powder mixed with chickpea and flaxseed flours. However, no reports were found in the literature that combines the use of IR spectroscopy to analyse lipids extracted via ultrasound from BSFL.

This work aimed to evaluate the ability of attenuated total reflectance (ATR) MIR spectroscopy combined with chemometrics in discriminating lipids extracted from BSFL fed with three different waste streams. Furthermore, the study assessed the impact of the ultrasound technique on the lipid extract profile during the extraction process based on organic solvents.

2 Materials and methods

Samples

Three waste streams, called supermarket (WL), food waste mix (WB) and childcare centre (CC) were used as feed for BSFL under commercial rearing conditions as reported previously (Alagappan *et al.*, 2024b). These commercial waste streams are supplied on a daily basis and were used by the commercial waste processing facility to feed the larvae. The waste streams were received from local suppliers, de-packaged, and ground using an industrial grinder in a commercial waste processing facility (particle size approximately between 10 and 15 mm) (Alagappan *et al.*, 2024b, 2025). Although the detailed rearing conditions were protected by industrial rights (e.g. temperature and humidity protocols) (Alagappan *et al.*, 2024b) and not disclosed in this paper. However, the BFSL were reared in a room where the temperature was maintained above 25 °C, and at approximately 80% ambient humidity. The harvested live 5th instar BSFL samples were blanched (100 °C for 5 mins) and freeze-dried (Labconco™ FreeZone™ 4.5 L −50 °C Benchtop Freeze Dryer, USA) (−50 °C, 0.054 bar for 96 h). The freeze-dried samples were finely ground into a fine powder before being stored at −20 °C in the freezer until analysis.

Lipid extraction

The lipid extraction was based on the Bligh and Dyer method (Bligh and Dyer, 1959). Initially, 1 g of freeze-dried *H. illucens* larvae powder was mixed with 5 ml ethanol in a tube and vortexed. The suspension was then ultrasonicated for 15 min at room temperature. Methylene chloride (5 ml) and Milli-Q water (4 ml) were added to the tube, followed by vortexing and centrifugation. The upper phase was discarded, and the methylene chloride phase containing the lipids was collected. The insect powder underwent a second extraction by mixing it with a methylene chloride and methanol mixture 1:1 (v/v) and centrifuging again. The organic solvent portion was collected and combined with the previously collected phase. Finally, the solvent was removed to obtain the lipid fraction at 45 °C under a nitrogen flux. Each extraction was performed in triplicate.

Reference analysis

Fatty acids were derivatized and quantified using a Shimadzu QP-2010 Ultra gas chromatography-mass spectrometry (GC-MS) system (Shimadzu Scientific Instruments, Sydney, NSW, Australia) and FAME standards according to Srivarathan *et al.*, (2021). Fatty acids were derivatized according to and quantified using a Shimadzu QP-2010 Ultra GC-MS system (Shimadzu Scientific Instrumentsa) and an Agilent DB-23 fused silica capillary column (60 m × 0.25 mm diameter i.d, 0.15 µm film thickness; Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas, maintaining a constant linear velocity of 42.7 cm/s. The injection port temperature was set to 230 °C. The temperature gradient program started at 40 °C for 1 min, followed by an increase to 170 °C at a rate of 30 °C/ min, and then a slower rise to 230 °C at 3 °C/ min. The ion source and interface temperatures of the mass spectrometer were set to 200 and 230 C, respectively. Fatty acids were identified using a Supelco 37-component FAME mix standard (Sigma-Aldrich).

ATR-FTIR analysis

The infrared spectra of the lipids were collected using a Bruker Alpha II spectrometer (Bruker Optics, Ettlingen, Germany). This instrument was equipped with platinum diamond attenuated total reflectance (ATR) single reflection accessory. Each spectrum was an average of 24 scans with a resolution of 4 cm^{-1} and ranged from 4000 to 400 cm^{-1}. For lipid analysis, 0.8 µl of sample was placed on the sample stage, and measure twice. The ATR crystal was cleaned with 70% ethanol between measurements and dried with laboratory Kimwipes® before the measurement of each sample. The region between 2400 to 1900 cm^{-1} was removed from the analysis due to water vapour and CO_2 interferences. Air was used as the reference background spectra and was collected every 10 samples.

Data analysis

The multivariate analysis used Piroutte 4.5 (Infometrix, Bothell, WA, USA). The spectra were mean-centred, normalized using the Divide By function, and then processed with a second derivative Savitzky-Golay transformation (25-point window) (Savitzky and Golay, 1964; Rinnan *et al.*, 2009). Principal component analysis (PCA) was used to identify underlying patterns in the spectral data. For classification, Soft independent modelling of class analogy (SIMCA), a supervised algorithm based on individual PCA for each group, was employed to differentiate *H. illucens* larvae lipid samples by rearing waste stream (Bureau *et al.*, 2019).

One-way analysis of variance (ANOVA) was used to determine statistically significant differences in each fatty acid's amount between group means using R language. Following a significant ANOVA result, Tukey's post hoc test was conducted to identify which specific groups differed from each other, providing a detailed pairwise comparison ($p < 0.05$). Each of the three groups was evaluated individually to study the effect of sonication. The impact of the waste stream on the lipid profile was studied by comparing the three lipid extracts obtained through organic solvent extraction with ultrasound assistance.

3 Results and discussion

Lipid profile

According to previous studies, the composition of *H. illucens* larvae can be significantly influenced by their diet, including variations in their lipid profile, which is also subject to variation based on dietary differences (Barragan-Fonseca *et al.*, 2017). It has been reported that diets rich in fruits (e.g. sugars, high carbohydrate content, protein to carbohydrate ratio) and meat can increase the lipid content and composition in the larvae (Danieli *et al.*, 2019; Tognocchi *et al.*, 2024). The fatty acid composition of the lipid fractions from larvae fed with different organic wastes in this study is presented in Table 1. Consistent with earlier research, *H. illucens* larvae are rich in saturated fatty acids (SFA) (Bessa *et al.*, 2020; Smets *et al.*, 2020), comprising between 58.58 and 65.48% of the total lipid content. The SFA group includes capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0), with notable levels of lauric (28.76–36.57%) and palmitic (16.84–17.63%). On the other hand, the total content of unsaturated fatty acids (UFA) is lower, ranging from 34.51 to 41.44%. The UFA fraction consists of palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), and α-linolenic (C18:3) acids. Most fatty acid levels, except for palmitic and stearic acids, vary across the different dietary groups, corroborating that diet significantly influences fatty acid levels and the distribution of SFA and UFA in BSFL. Among the BSFL, those reared on waste from the childcare center (CC) had the highest UFA content (41.44%), in contrast to those fed on supermarket (WL) and market (WB) waste. The CC waste comprises of salad, bread and other cooked dishes, with a high proportion of starch and non-structural carbohydrates (Alagappan *et al.*, 2025). Interestingly, there were also notable differences in the levels of omega-3 (α-linolenic acid) and omega-6 (linoleic acid) fatty acids, reflecting similar findings in other studies where *H. illucens* diets were supplemented with sources of these fatty acids to increase their content (Barroso *et al.*, 2017; Li *et al.*, 2022). These fatty acids are recognized for their health benefits, enhancing the nutritional value of the larvae's lipid fraction (Simopoulos, 2002). Despite these variations, the overall fatty acid profile remains similar to previous findings.

Infrared spectra

Using ultrasound-assisted extraction, Figure 1 displays the MIR spectra for lipid fractions extracted from larvae reared on different organic waste streams. These spectra exhibit the characteristic bands for lipids (Aykas *et al.*, 2020). The band at 3006 cm^{-1} is associated with the =C-H stretching found in cis double bonds present in UFA. The band at 2921 /cm and the shoulder band at 2958 cm^{-1} correspond to C–H asymmetric stretching (CH_2 and CH_3), while the absorbance at 2853 cm^{-1} is associated with the same bond but symmetric stretching. The main band, located at 1742 cm^{-1}, corresponds to the stretching of the ester carbonyl (C=O), a functional group characteristic of lipids. The IR spectra also show absorbances at 1463 cm^{-1}, and 1418 cm^{-1}, corresponding to C–H bending vibrations of the CH_2 group and cis double bonds, respectively. The band at 1377 cm^{-1} corresponds to the C–H bending (symmetrical) vibration of the CH_3 group. Additionally, bands in the range 1250–1110 cm^{-1} are related to stretching vibrations of the C-O ester groups and bending vibrations of CH_2. Another notable band is centered at 721 cm^{-1}, representing the rocking vibration of CH_2 and bending (out of plane) of HC=CH- (cis) group (Kaneko *et al.*, 2018; Lerma-García *et al.*, 2010; Salas-Valerio *et al.*, 2022). The lipids from larvae reared on the three waste streams display a similar spectrum.

Effect of ultrasound assisted extraction

The effect on the fatty acids extracted during the ultrasound-assisted extraction on the lipid profile is given in Table 2. It has been reported that ultrasound-assisted lipid extraction improves yield by enhancing cell rupture and increasing the amount of lipids released into the solution phase (Gharibzahedi and Altintas, 2022; Keris-Sen *et al.*, 2014). This study found no significant ($p > 0.05$) differences in lipid extraction using ultrasound across any of the three waste sources anal-

TABLE 1 Lipid profile of *Hermetia illucens* larvae fed with different organic wastes obtained by lipid extraction ultrasound assisted and measured using gas chromatography mass spectrometry

	CC	WL	WB
C10:0	1.27 ± 0.11 [a]	1.55 ± 0.13 [a]	3.04 ± 0.13 [b]
C12:0	28.76 ± 0.15 [a]	36.57 ± 1.17 [b]	33.94 ± 0.91 [b]
C14:0	8.76 ± 0.42 [a]	7.88 ± 0.80 [a]	5.98 ± 0.19 [b]
C16:0	16.84 ± 1.12 [a]	17.63 ± 0.30 [a]	16.96 ± 0.25 [a]
C16:1(n9)	3.49 ± 0.14 [a]	3.08 ± 0.09 [b]	4.12 ± 0.11 [c]
C18:0	2.94 ± 0.98 [a]	1.85 ± 0.43 [a]	2.00 ± 0.03 [a]
C18:1(n9)	25.38 ± 0.66 [a]	21.02 ± 0.81 [b]	23.26 ± 0.33 [c]
C18:2(n9,12)	10.68 ± 0.74 [a]	9.44 ± 0.31 [b]	9.29 ± 0.16 [b]
C18:3(n9,12,15)	1.89 ± 0.35 [a]	0.96 ± 0.12 [b]	1.41 ± 0.05 [a,b]
SFA	58.57 ± 1.19 [a]	65.48 ± 0.88 [b]	61.92 ± 0.52 [c]
UFA	41.44 ± 1.19 [a]	34.51 ± 0.88 [b]	38.08 ± 0.51 [c]

Values represent the percentage of the lipid fraction. Results are expressed as mean ± standard deviation (values are means of three replicates). Abbreviations: CC, childcare centre; WL, supermarket; WB, mixed.

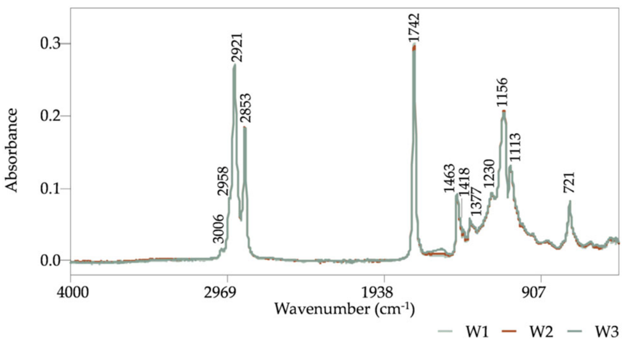

FIGURE 1 Mid-infrared (MIR) spectra of *Hermetia illucens* larvae lipids fractions extracted by organic solvents and ultrasound-assisted. Abbreviations: CC, childcare centre; WL, supermarket; WB, mixed.

ysed. Comparative pairwise analysis of the profiles consistently showed no notable variations. The only exception was capric acid, which was observed in larvae of *H. illucens* reared on supermarket waste (WL). However, since capric acid is present in low concentrations, its impact on the overall SFA was negligible. Therefore, the ultrasound-assisted extraction method did not produce meaningful changes in the lipid composition of the *H. illucens* larvae. Principal Component Analysis (PCA) applied to pre-treated MIR (second derivative) data was conducted to explore potential differences caused by using the ultrasound method during lipid extraction. To minimize the influence of variations in lipid composition due to diet, an independent analysis was carried out for each organic waste stream.

The two principal components account for between 58.6 and 67.3% of the variability in the data (Figure 2). Overlapping is observed between the ultrasound-assisted samples and those extracted using only organic solvents in the three groups. These results indicated that no clear differentiation existed between the samples obtained through extraction with organic solvents and those assisted with ultrasound, regardless of the waste stream utilised by the larvae. Furthermore, the PCA results obtained from both lipid analyses using the GC-MS suggested that the ultrasound method did not significantly impact the lipid composition extracted by solvents, highlighting the consistency of the extraction process proposed in this study. This finding aligns with Gharibzahedi and Altintas (2022), who concluded that ultrasound had no effect when using a mixture of ethanol-isopropanol to extract lipids from *Alphitobius diaperinus* larvae. While there is no prior research on the impact of ultrasound-assisted organic solvent extraction on *H. illucens* lipids, other studies have analysed various extraction techniques, including ultrasound combined with different solvents and microwaves (Almeida *et al.*, 2022). Although differences in fatty acid composition were noted (Almeida *et al.*, 2022), no specific reference was made to the potential impact of extraction.

Classification of H. illucens larvae lipids

The effect of the waste stream used to feed *H. illucens* larvae was evaluated using Soft Independent Modeling of Class Analogy (SIMCA) algorithm, aiming to classify lipid samples based on IR spectral data using the pre-treated spectra. Specific regions related to lipids were selected to construct the models, avoiding irrelevant information or noise. Each group represents lipids

TABLE 2 Lipid profile of *Hermetia illucens* larvae fed with different organic waste sources obtained with or without ultrasound during lipid extraction and measured using gas chromatography mass spectrometry

	CC		WL		WB	
	NO US.	US.	NO US.	US.	NO US.	US.
C10:0	1.20 ± 0.07	1.27 ± 0.11	1.31 ± 0.02	1.55 ± 0.13*	2.79 ± 0.11	3.04 ± 0.13
C12:0	29.19 ± 2.81	28.76 ± 0.15	36.75 ± 1.95	36.57 ± 1.17	35.07 ± 2.77	33.94 ± 0.91
C14:0	8.00 ± 0.58	8.76 ± 0.42	7.55 ± 0.73	7.88 ± 0.80	5.51 ± 0.35	5.98 ± 0.19
C16:0	16.86 ± 1.17	16.84 ± 1.12	17.27 ± 0.21	17.63 ± 0.30	16.43 ± 0.77	16.96 ± 0.25
C16:1	3.43 ± 0.51	3.49 ± 0.14	3.15 ± 0.40	3.08 ± 0.09	3.94 ± 0.09	4.12 ± 0.11
C18:0	2.79 ± 0.46	2.94 ± 0.98	1.72 ± 0.14	1.85 ± 0.43	2.74 ± 1.67	2.00 ± 0.03
C18:1	25.59 ± 1.01	25.38 ± 0.66	21.23 ± 1.14	21.02 ± 0.81	22.94 ± 0.83	23.26 ± 0.33
C18:2	10.92 ± 0.26	10.68 ± 0.74	9.94 ± 0.33	9.44 ± 0.31	8.97 ± 0.26	9.29 ± 0.16
C18:3	2.03 ± 0.12	1.89 ± 0.35	1.08 ± 0.18	0.96 ± 0.12	1.61 ± 0.34	1.41 ± 0.05

Values represent the percentage of the lipid fraction. Results are expressed as mean ± SD (values are means of three replicates). Abbreviations: CC, childcare centre; WL, supermarket; WB, mixed; US, ultrasound-assisted.

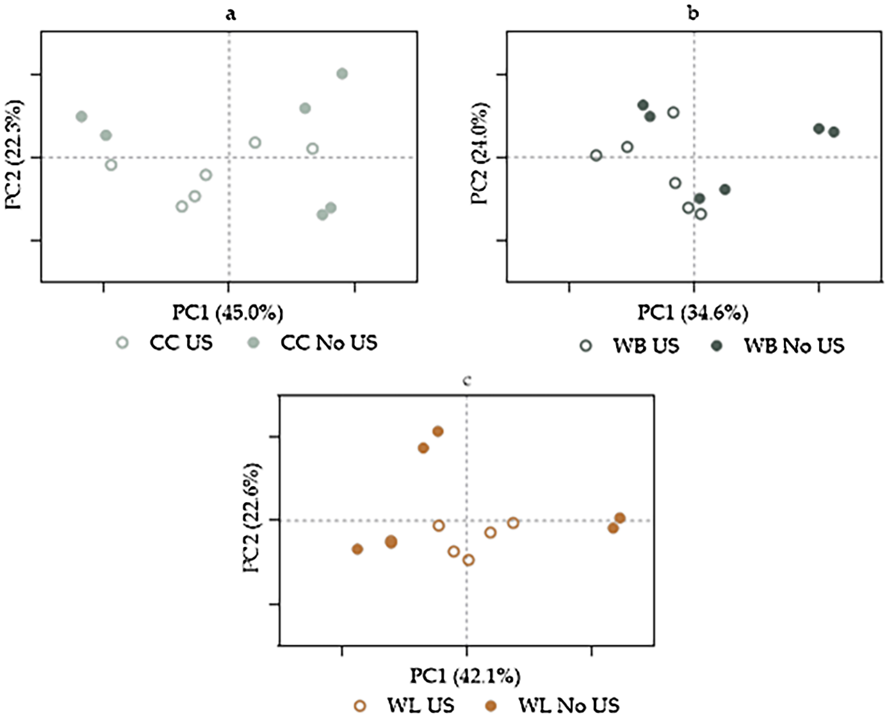

FIGURE 2 Principal component (PCA) score plots of *Hermetia illucens* larvae lipids extracted by organic solvent with or without ultrasound-assisted: (a) childcare centre, (b) market, and (c) supermarket waste. Each waste stream was analysed using three independent samples, and each sample was measured in duplicate Abbreviations: CC, childcare centre; WL, supermarket; WB, mixed; US, ultrasound-assisted.

obtained from a different organic waste source, including samples with and without ultrasound assistance during fractioning. Figure 3 shows the distribution of these classes based on the two main principal components. Four factors were selected for each class to create the model, explaining more than 90% of the variance in the dataset. The analysis shows clear separation between the three groups. The interclass distance (ICD) value represents the distance between clusters. An ICD value greater than 1.0 indicates that there are differences between the groups, while a value larger than 3.0 means that the groups are statistically different from each other (Mellado-Carretero *et al.*, 2020). All ICD values were greater than 3.0, indicating that the groups are statistically different from each other, demonstrating SIMCA's effectiveness in classifying all

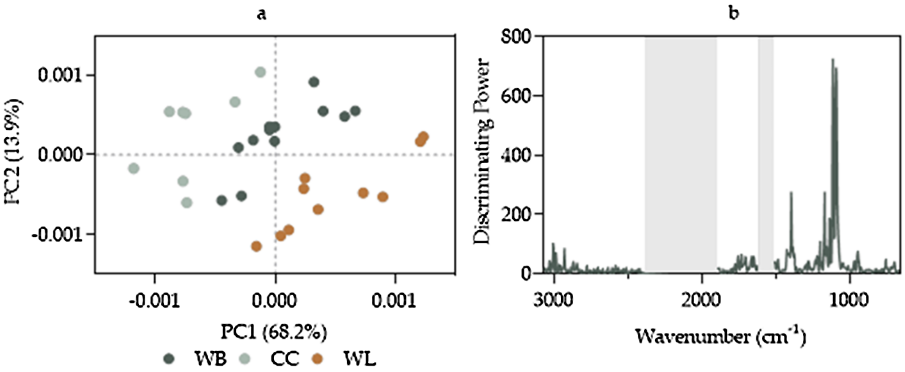

FIGURE 3 Soft Independent Modelling of Class Analogy (SIMCA) model constructed using spectral data in the wavelength ranges 3070–2400 cm^{-1}, 1880–1620 cm^{-1}, and 1500–655 cm^{-1}, showing (a) class projection plot and (b) discriminating power. Abbreviations: CC, childcare centre; WL, supermarket; WB, mixed.

lipid samples according to the waste stream due to differences in their composition. No samples were misclassified during the construction of the model. These results demonstrate how small differences in the lipid fractions of insects, such as *H. illucens*, can be quickly and affordably detected using portable devices combined with multivariate data analysis. These results can be contrasted with other research done in the same IR spectrum range, where insect samples with varying compositions have been successfully classified. For instance, Hoffman *et al.* (2022) successfully classified *H. illucens* larvae at different development stages based on the waste stream (soy or bread-vegetable mix waste) using Partial Least Squares Regression Analysis (PLS-DA). They tested diverse wavelength combinations within the spectrum, which focused exclusively on the lipid specific region between 3000 and 2500 cm^{-1}, they were able to correctly classify 100% of the samples with a determination coefficient of 0.9. Additionally, Mellado-Carretero *et al.* (2020) used a portable MIR spectrometer to classify insect powders using SIMCA models. They separately analysed *Acheta domesticus* and *Alphitobius diaperinus* powders, successfully highlighting differences between the samples. In both cases, many of the bands identified in the discriminating power analysis were associated with lipids.

The results of these studies further support the idea that the composition of the lipid fraction significantly contributes to distinguishing insect samples. Although there is limited research on insect lipids analysed by MIR spectroscopy, the classification results are comparable to those obtained for other fatty samples, such as vegetable oils. For example, highly similar samples of olive oil were classified by their purity with sensitivity and specificity values of 100% (Aykas *et al.*, 2020). Beyond food samples, even crude petroleum oils have been 100% correctly classified by SIMCA and PLS-DA models based on their geographical origin (Galtier *et al.*, 2011). This supports the broad applicability and effectiveness of MIR spectroscopy in distinguishing and classifying insect lipids.

The discriminating power identifies the wavenumbers that provide relevant information for class differentiation. The main bands identified were 3006, 1393, 1197, 1167, 1134, 1111 and 1088 cm^{-1}. The bands at 3006 and 1393 cm^{-1} are associated with different types of C–H related to double bonds in lipid chains, which correspond to differences in the proportion of UFA and SFA between the groups. The other bands correspond to C–H and C–O bond vibrations present in the fatty acids (Kaneko *et al.*, 2018; Lerma-García *et al.*, 2010; Salas-Valerio *et al.*, 2022).

Overall, knowledge about efficient and green lipid extraction methods will provide the insect industry with better analytical methods. These methods can provide improved methods to search for alternate lipid sources for animal feed, food, biodiesel, cosmetic, and pharmaceutical industries (Franco *et al.*, 2021; Siddiqui *et al.*, 2025). Solid Safety Regulation would bring innovative food businesses to the EU and the worldwide market, guaranteeing at the same time their safety. Consequently, the knowledge about health benefits of insect oils as an alternative food source could help to improve the global market of insects and their farming practices in communities with limited access to food sources. To conclude, based on a good FA profile with PUFAs, edible insects make a valuable alternative for feed, and food if it is safe, legal, and accepted by the consumers. For this reason, more research is required to evaluate their optimal inclusion level and their safety as a source of fats and FAs in diets (Franco *et al.*, 2021; Siddiqui *et al.*, 2025)

4 Conclusions

The results demonstrate the potential of MIR spectroscopy combined with multivariate analysis tools to differentiate and classify *H. illucens* larvae lipids based on changes in lipid profile composition, specifically due to rearing on different organic side streams. This method offers a promising alternative for the industry to link BSFL to their diet, enabling rapid and accurate analysis with minimal sample amount and no need for pre-treatment. Additionally, our findings indicate that ultrasound/sonification during the lipid extraction phase does not significantly impact/improve the fatty acid composition obtained through lipid fractionation using organic solvents. Although these results are encouraging, some limitations need to be highlighted such as the relatively small number of waste streams and samples used and no analysis of other lipid functional properties (e.g. melting point, oxidative stability).

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Author contributions

C. Mendez Sanchez, S. De Lamo Castelvi, S. Alagappan, L. Hoffman, O. Yarger, D. Cozzolino: investigation, methodology, writing (original draft). L. Hoffman, O. Yarger, D. Cozzolino: methodology, writing (review and editing the final version). D. Cozzolino: conceptualization, investigation, methodology, project administration, writing (original draft). All authors: writing (review and editing the final version).

Conflict of 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.

Ethical approval

Ethics approval was not required for this work.

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