LWT - Food Science and Technology 207 (2024) 116652

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Prediction of fat content in edible insect powders using handheld FT-IR spectroscopic devices

Carmen Mendez-Sanchez [a], Maria Carme Güell [a], Montserrat Ferrando [a], Luis Rodriguez-Saona [b], Rafael Jimenez-Flores [a], Joan Carles Domingo [c], Silvia de Lamo Castellvi [a,b,*]

[a] Universitat Rovira I Virgili, Departament D'Enginyeria Química, Av. Països Catalans 26, Campus Sescelades, 43007, Tarragona, Spain
[b] Department of Food Science and Technology, The Ohio State University Columbus, OH, 43210-1007, USA
[c] Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
Fat content
Tenebrio molitor
Alphitobius diaperinus
Mid-infrared spectroscopy
Near-infrared spectroscopy

ABSTRACT

The present study describes a new approach to predict the level of crude fat present in commercial edible *Tenebrio molitor* and *Alphitobius diaperinus* powders partially defatted using mid and near infrared spectral data combined with multivariate analysis. Insect powders were partially defatted by using three organic solvents and CO_2 in supercritical conditions obtaining samples with fat content ranging from 0 to 28.7% (n = 46). Lipid content and fatty acid profile were determined by using Soxhlet and fatty acid methyl esters (FAME) methods, respectively. Spectral data was acquired using a two handheld FT-NIR devices (1350–2550 nm) and two portable FT-MIR equipment (4000-630 cm^{-1}) equipped with ATR crystals. Partial least squares regression (PLSR) model was used to easily predict insect fat content. Ethanol had lipid extraction yields significantly lower, specially for *T. molitor*. FA composition was affected by the solvent used. PLSR results exhibited good linearity, predicting crude fat content with strong correlation ($R_{cv} \geq 0.9$) and low standard error of cross-validation (SE_{CV} = 1.06–3.22%). Nonetheless, the FT-NIR devices tested, showed higher performance for fat content prediction in insect powders, reaching values of 0.99 in coefficient of correlation (R_P) and 1.05% in standard error in prediction (SE_P).

1. Introduction

Insects are rich in proteins, lipids, vitamins, fiber, and minerals (Barker et al., 1998). Since 2021 to date, the European Food Safety Authority (EFSA) has recognized four different insect species as novel food, and a total of 9 species are currently accepted to breeding farmed livestock, farmed fish, or pets. Insect lipids can be used to replace vegetable oils and animal fats for feed and food formulation as well as other non-food applications such as such as biodiesel production or cosmetics (Franco et al., 2022; Manzano-Agugliaro et al., 2012). Insect lipid fraction has been mostly obtained by organic solvent (Soxhlet and Folch) (Tzompa-Sosa et al., 2014; Wang et al., 2021). Nonetheless, other techniques such as aqueous extraction, three-phase partitioning, pressing methods and supercritical fluids, had been also explored with promising results (Laroche et al., 2019; Tzompa-Sosa et al., 2014). The lipid fraction composition and its physicochemical properties depend on the extraction method, the solvent, and the extraction conditions used (Laroche et al., 2019; Purschke et al., 2018; Son et al., 2019). Controlling insects lipid content and its fatty acid profile is a key factor for insect rearing at farms. Nonetheless, the existing techniques depend on the solvent extraction-gravimetric method to quantify the crude fat content in insect powders and the need of using Gas Chromatography with Flame-Ionization Detection (GC-FID) or Gas Chromatography-Mass Spectroscopy (GC-MS) for profiling their fatty acids (Meullemiestre, 2015; Nielsen, 2010). These methods are expensive and time-consuming and are less feasible for integration into quality control processes at insect farms.

The developments in micro-electro-mechanical systems (MEMS) production have allowed the integration onto a chip, allowing the miniaturization of the components of the near-infrared (NIR) and mid-infrared (MIR) spectrophotometers (Pu et al., 2021; Rodriguez-Saona et al., 2020). These advances have led to the development of miniaturized and portable equipment, simplifying their use and integration into routine analyses. These innovative handheld NIR devices have been tested to identify insect powders mixed with wheat flour (Benes et al., 2022), adulteration of insect protein powders (Ni et al., 2024),

* Corresponding author. Universitat Rovira I Virgili, Departament D'Enginyeria Química, Av. Països Catalans 26, Campus Sescelades, 43007, Tarragona, Spain.
E-mail address: silvia.delamo@urv.cat (S. de Lamo Castellvi).

https://doi.org/10.1016/j.lwt.2024.116652
Received 6 May 2024; Received in revised form 7 August 2024; Accepted 16 August 2024
Available online 19 August 2024

determine protein content in a single larvae (Cruz-Tirado et al., 2022), discriminate protein content in complex matrices (Beć et al., 2021), and monitor the nutritional quality of black soldier fly larvae and frass (Alagappan et al., 2024). Portable MIR instruments are ideal for on-site analysis as the MIR region (4000–400 cm^{-1}) includes the fingerprint region characteristic of each sample analyzed (Guillén & Cabo, 1997a). In a previous research, spectral data obtained using a portable MIR equipment was used to develop multivariate classification models to successfully discriminate and classify edible insect powders by specie and origin (Mellado-Carretero et al., 2020). Moreover, MIR data combined with multivariate analysis, has also been used to distinguish black soldier fly larvae instar according to the diet (Hoffman et al., 2022).

The main objective of this project was to explore a spectroscopic approach for real-time screening of fat content in partially defatted insect powders by using portable Fourier transform mid infrared (FT-MIR) and handheld FT-NIR spectrometers. In this project four different equipment, two portable FT-MIR equipped with attenuated total reflection (ATR) crystals (Agilent Cary 630 and Agilent 4500) and two handheld FT-NIR spectrometers (NeoSpectra Micro Development Kit and NeoSpectra Scanner), were tested. Three organic solvents (ethanol, petroleum ether and 2-methyltetrahydrofuran) were tested to evaluate solvent polarity effect on lipid extraction yield and fatty acid composition.

2. Materials and methods

2.1. Materials and reactants

Tenebrio molitor (mealworm, *T. molitor*) and *Alphitobius diaperinus* (lesser mealworm, *A. diaperinus*) were purchased from Kreca Ento-Food BV (Ermelo, The Netherlands). The solvents used in this research, absolute ethanol, petroleum ether, 2-methyltetrahydrofuran (2-MTHF) and hexane (96% purity), were purchased from Scharlab S.L. (Barcelona, Spain). Food-grade liquid CO_2 was obtained from OSU Stores Gas Warehouse (Columbus, OH, USA).

2.2. Defatting procedure

2.2.1. Fat extraction with organic solvents

Edible insect powders were partially defatted using three organic solvents: ethanol, petroleum ether and 2-methyltetrahydrofuran following the protocol of (Wang et al., 2021). The insect powder was mixed with the organic solvent at a proportion 1:5 (w/w) at 300 rpm for 1 h. After that, a decantation step was used to separate the two phases. The organic phase containing the lipid extract, was collected and the partially defatted insect powder was defatted again one more time, following the sample protocol (two defatted cycles), or it was dried overnight under the hood to completely remove the organic solvent (one defatted cycle). The lipid fraction recovery was performed by rotary evaporator (Laborota 4000, Heidolph, Germany) using different conditions per each solvent. The recuperation of the petroleum ether was performed at 40 °C and 500 mbar; ethanol, 65 °C and 350 mbar; 2-MTHF, 65 °C and 400 mbar.

All the lipid samples were storage at 4 °C, nitrogen atmosphere and protected from light until further analysis. All the experiments were performed in duplicate or triplicate.

2.2.2. Fat extraction with supercritical fluids

For this study, the Supercritical fluid extraction (SFE) System and ChromeScope IE Operating System (Waters Corporation, Milford, MA, USA) was also used to partially defatted insect powders. Approximately 40 g of *T. molitor* powder was packed in a staple-sealed milk-filter bag (DeLaval, Kansas City, MO, USA). Subsequently, the bag was placed inside a 1000 mL stainless-steel extraction vessel with a jacketed heating system (Venkatram et al., 2024). In this study, different extraction conditions were performed, treating pressure in the extraction vessel was set to 100, 120, 150, and 180 bar and treating temperature was controlled at 40, 45, 50, and 65 °C during 15, 30, 60, 75, and 90 min. Gas flow was set at 100 g/min and all samples were analyzed in duplicate.

2.2.3. Fat extraction with soxhlet

The total defatted insect powders were obtained by Soxhlet extraction. An aliquot of 3 g of insect powder was placed in the cellulose thimble (Filtros Anoia S.A., Barcelona, Spain) inside the Soxhlet extractor. The extraction was performed using 300 mL of hexane as solvent and ran for 6 h. Then the solvent was removed from the insect powder by drying overnight under the hood. Afterwards, hexane was recovered using a rotary evaporator ((Laborota 4000, Heidolph, Germany) at 400 mbar in a water bath at 50 °C.

2.2.4. Extraction yield

To calculate the performance of the extraction methods, the following equation (1) was used (Purschke et al., 2017):

$$\text{Lipid extraction yield (\%)} = (1 - (F_d/F_i)) \times 100 \quad (1)$$

The F_d represents the percentage in the partially defatted powder and F_i is the percentage of fat in the initial powder.

2.3. Reference analysis

2.3.1. Lipid content

The lipid level in the partially defatted insect powders was also determined by Soxhlet gravimetric method. The lipid content was calculated through the difference in weight between the thimble with the sample before and after the extraction.

2.3.2. Fatty acid profile

The fatty acid profile was determined by Gas Chromatography-Mass Spectroscopy (GC-MS) using the fatty acid methyl esters (FAME) method (Lepage & Roy, 1986).

The GC-MS analysis was performed using a Shimadzu GCMS-QP2010 Plus gas chromatograph/mass spectrometer (Shimadzu, Kyoto, Japan). The sample was injected with a split/splitless injector and a Shimadzu AOC-20i autoinjector and a Shimadzu AOC-20s autosampler. The column employed was a Suprawax-280 (Teknokroma), 15m length x 0.10 mm internal diameter x 0.10 μm film thickness. Helium was used as the carrier gas. A sample volume of 1 μL was injected and the injector was set at 250 °C. The oven conditions were 130 °C held for 0.25 min, then increased with a ramp of 35 °C/min until 200 °C, increased to 225 °C (8 °C/min) held for 3.2 min, and then 80 °C/min to 245 °C and held for 2.75 min.

The mass spectrometer interface temperature was 255 °C and ion surface, 200 °C. The mass range was 40–440 m/z and detector voltage of 1.0 kV.

The different fatty acids (FA) were identified by comparing the elution times of reference FAME mixture (GLC-744 Nu-Chek Prep. Inc., Elysian, Minnesota, USA) peaks. The values of the fatty acid profile indicate the percentage of each fatty acid on the total. All the samples were analyzed in duplicate.

From the individual FA values, the saturated FA (SFA) percentage was calculated as the sum of C12:0, C14:0, C16:0, C18:0, C20:0, and C22:0; the monounsaturated FA (MUFA) percentage as the sum of C16:1n7, C18:1n9, C18:1n7, and C20:1n9; the polyunsaturated FA (PUFA) percentage as the sum of C18:2n6, C18:3n3, and C20:2n6; and the unsaturated FA (UFA) percentage as the sum of MUFA and PUFA. The total amount of omega-6 (n6) is the addition of C18:2n6 and C20:2n6, and the total amount of omega-3 (n3) is composed by C18:3n3.

2.4. Spectral data acquisition

Four infrared (IR) equipments were used for this project, two

portable MIR spectrometers and two handheld NIR devices. These equipment are Agilent Cary 630 (Agilent Technologies Inc., Santa Clara, CA) equipped with a single bounce attenuated total reflectance (ATR) diamond crystal and a deuterated triglycine sulfate detector (DTGS); Agilent 4500 (Agilent Technologies Inc., Santa Clara, CA), equipped with an ATR diamond crystal, in this case it has 3 bounces, and a DTGS detector; NeoSpectra Micro development kit (NeoSpectra MDK) (Si-Ware Systems, Inc., Cairo, Egypt) is a single-chip Michelson interferometer with a monolithic opto-electro-mechanical structure combine with a single uncooled InGaAs photodetector (Aykas et al., 2020) and 3 Tungsten halogen lamps and reflectors surrounding the bulbs and NeoSpectra Scanner (Si-Ware Systems, Inc., Cairo, Egypt) that has 7 Tungsten halogen lamps and the collection window measure around 10 mm.

For the MIR analysis, around 4 mg of insect powder were placed on the crystal and pressed with a pressure clamp to be sure of contact between the sample and the crystal. The spectra were collected from 4000 to 800 cm^{-1} with a resolution of 8 cm^{-1} (for the 630 Agilent instrument) and from 4000 to 630 cm^{-1} and a resolution of 4 cm^{-1} (for the 4500 Agilent) and the spectra is the result of the average of 128 scans. Each sample was analyzed 3 times, and the background was also recorded between each sample scan with clean ATR crystal.

For the NIR devices, the background was collected using a 99% reflectance standard (Spectralon®, Labsphere, North Sutton, NH, USA) between each measurement. 1 g of sample at room temperature was placed in a Petri dish (Duroplan®, DWK Life Sciences GmbH, Mainz, Germany) and covered with a reflectance accessory. The spectra were collected from 1350 to 2500 nm with a resolution of 16 nm during 10 s. Each sample was analyzed 10 times, and the average was calculated to perform the data analysis.

2.5. Data analysis

Multivariate analysis and spectra processing were carried out using Piroutte 4.5 (Infometrix Inc., Washington, DC, USA). Partial least squares regression (PLSR) was employed to build up the quantitative prediction models by correlating IR spectra and the results from the reference analysis.

All the raw spectra were pre-treated with Normalization, Standard Normal Variate (SNV) and the derivative Savitsky-Golay (second derivative with a window from 7 to 15) algorithms, separated or combined. In all the cases, pre-treated spectra were mean-centered to create the PLSR models. All the models were validated by cross-validation to add robustness. To evaluate the models the parameters regression vector, coefficients of correlation in calibration (R_{CAL}) and validation (R_{CV}) and the standard error of cross-validation (SE_{CV}) were considered. The total number of samples was divided in two sets: the calibration set with the 75% of the total and the validation set that includes the rest, 25%, of the total samples (Table 1). All the created models include both species, they do not distinguish between *T. molitor* and *A. diaperinus*.

The results of composition of the different lipid extracts and lipid content were presented as mean ± standard error of the replicates. One-way Analysis of variance (ANOVA) was performed by using OriginPro Version 2024 (OriginLab Corporation, Northampton, MA, USA), to determine if the differences were significant and Tukey's and Fisher tests were used to identify the different groups ($p < 0.05$).

Table 1
Distribution of samples in calibration and validation set.

Sample Set	Agilent Cary 630	Agilent 4500	NeoSpectra MDK	NeoSpectra Scanner
Calibration	34	30	34	31
Validation	12	12	12	12
Total	46	42	46	43

3. Results and discussion

3.1. Lipid extraction yields and fatty acid composition

Lipid extraction yields for *T. molitor* and *A. diaperinus* powders after fat extraction using organic solvents are shown in Fig. 1a and b. The lipid extraction yields for *T. molitor* were significant higher ($p < 0.05$) compared to A. *diaperinus* regardless the organic solvent and the number of defatted cycles applied. This difference can be explained by the higher content of fat in *T. molitor* powders (29.4% w/w vs. 28.7%w/w). For *T. molitor*, the lipid extraction yield was enhanced by the less polar solvents tested, petroleum ether and 2-methyltetrahydrofuran, up to 57.0% and 82.9% after applying one and two deffating cycles, respectively. The lipid extraction rates were significant lower for ethanol after one (48.3%) and two (77.2%) deffating cycles. In the case of *A. diaperinus*, ethanol showed lower lipid extraction yields after one deffating cycle.

The lipid extraction yields using SFE with CO_2 were lower than those obtained with solvent extraction. The lipid extraction yield values between 0 and 30.3% (Fig. 1c). SFE with CO_2 technique was intentionally used under mild conditions to obtain partially defatted samples with higher lipid content than those obtained using the solvent to have partially defatted insect powder samples with a lipid content over 20%. Other researchers have reported that when 325 bar and 55 °C during 75 min are applied, lipid extraction yields for *T. molitor* can be higher than 90% (Purschke et al., 2017). The lipid yields using Soxhlet extraction were 100% for both insect species tested (Fig. 1d).

Six FAs were detected above 1% of the total content (Table 2 and Table S1), comprising myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9), linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3). Regardless the solvent used, the most common FAs were palmitic acid and oleic acid for *T. molitor* and palmitic acid, oleic acid and linoleic acid for *A. diaperinus*. These FAs were the 82.39–84.62 % of the total FAs content. Our results are in agreement with previous studies performed with *T. molitor* and *A. diaperinus* (Paul et al., 2017; Tzompa-Sosa et al., 2014). In *T. molitor* and *A. diaperinus*, FAs n3, linked to health benefits, were found in the form of α-linolenic acid. α-Linolenic acid is an essential fatty acid that humans cannot synthesized and needs to be consume through the diet (Simopoulos, 1999). The FAs n6 were present in higher content than FAs n3, especially for *A. diaperinus*. The ratio n6/n3 ranged from 3.68 to 4.93 for *T. molitor* and 12.58 to 13.90 for *A. diaperinus*, depending on the organic solvent used during the extraction procedure. The recommended value for human consumption is 4 and the levels found in the lipid fraction of both insect lipids were mostly higher than the recommendation, especially for *A. diaperinus* (Simopoulos, 2002). The total SFA and UFA were similar between the insects, but the distribution of mono and poly unsaturated FA differed. *A. diaperinus* had higher content in PUFA mainly for linoleic acid, while *T. molitor* had more MUFA percentage due to amount of oleic acid.

Ethanol is more polar than petroleum ether, this fact modifies the composition of the lipid extract. Ethanol produced a lipid fraction with higher proportion of PUFA (1.8% for *T. molitor* and 1.2% for *A. diaperinus*), lower proportion of MUFA (0.74% for *A. diaperinus* and 1.27% fot *T. molitor*) and changed the proportion of several UFA such as myristic, palmitic and arachidic (C20:0) acids (Laroche et al., 2019). Polar solvents are more efficient at extracting PUFA because are relatively more polar than MUFA and UFA and nonpolar solvents such are more efficient at extracting MUFA (Meullemiestre, 2015; Murali et al., 1993). Besides that, the green solvent 2-methyltetrahydrofuran hardly affected the FA composition, only in *A. diaperinus* a pair of trace (<1%) fatty acids, vaccenic acid (C18:1n7) and cetoleic acid (C20:1n7), presented proved differences in the percentage.

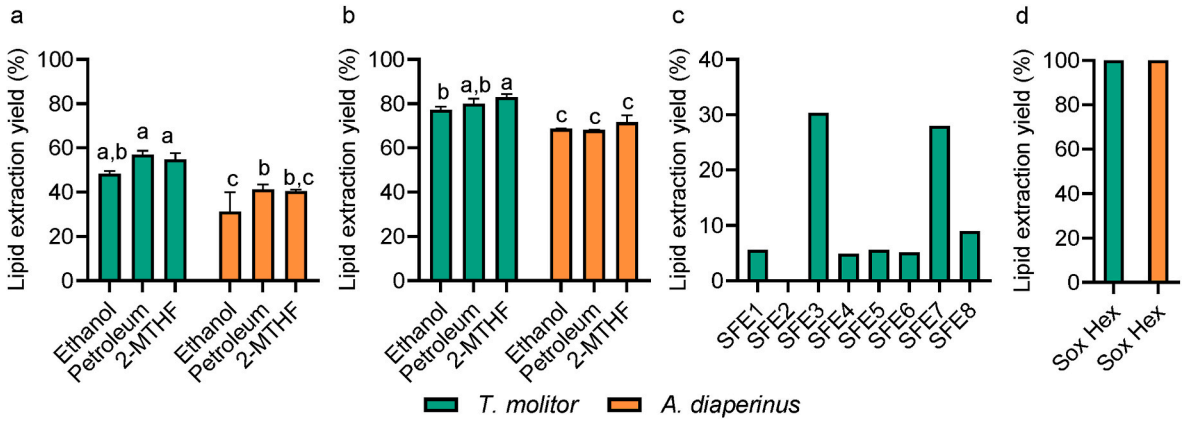

Fig. 1. Lipid extraction yield of *T. molitor* and *A. diaperinus* powders after fat extraction by (a) one cycle with organic solvents, (b) two cycles with organic solvents, (c) SFE with CO_2 under different conditions (°C, bar, min), and (d) Soxhlet. Different letters are significantly different (Fisher test, $p < 0.05$). Values are means of two replicates. Petr. Et.: petroleum ether; 2-MTHF: 2-methyltetrahydrofuran. SFE1: 40 °C, 100 bar, 30 min. SFE2: 45 °C, 120 bar, 30 min. SFE3: 45 °C, 165 bar, 15 min. SFE4: 50 °C, 100 bar, 90 min. SFE5: 50 °C, 150 bar, 30 min. SFE6: 50 °C, 150 bar, 60 min. SFE7: 50 °C, 150 bar, 75 min. SFE8: 65 °C, 180 bar, 30 min.

Table 2
Fatty acid profile of *T. molitor* and *A. diaperinus* extracted by organic solvents.

Fatty acid	*A. diaperinus*			*T. molitor*		
	Petroleum Et.	Ethanol	2-MTHF	Petroleum Et.	Ethanol	2-MTHF
C12:0	0.13 ± 0.04 ^{a}	0.19 ± 0.05 ^{a}	0.14 ± 0.04 ^{a}	0.44 ± 0.06 ^{A}	0.40 ± 0.02 ^{A}	0.42 ± 0.04 ^{A}
C14:0	0.89 ± 0.04 ^{a}	1.01 ± 0.09 ^{b}	0.89 ± 0.07 ^{a}	4.53 ± 0.09 ^{A}	4.29 ± 0.05 ^{B}	4.47 ± 0.08 ^{A}
C16:0	26.73 ± 0.46 ^{a,b}	26.23 ± 0.47 ^{a}	26.83 ± 0.39 ^{b}	25.57 ± 0.23 ^{A}	24.90 ± 0.25 ^{B}	25.63 ± 0.24 ^{A}
C16:1 n7	0.36 ± 0.04 ^{a}	0.36 ± 0.03 ^{a}	0.32 ± 0.05 ^{a}	2.31 ± 0.14 ^{A}	2.19 ± 0.13 ^{A}	2.35 ± 0.13 ^{A}
C18:0	10.22 ± 0.91 ^{a}	9.72 ± 0.33 ^{a}	9.65 ± 0.14 ^{a}	7.17 ± 0.43 ^{A,B}	7.51 ± 0.57 ^{A}	6.77 ± 0.52 ^{B}
C18:1 n9	32.18 ± 0.36 ^{a}	31.40 ± 0.44 ^{b}	32.25 ± 0.41 ^{a}	54.38 ± 0.59 ^{A}	53 0.11 ± 1.20 ^{B}	54.54 ± 0.84 ^{A}
C18:1 n7	0.60 ± 0.04 ^{a}	0.67 ± 0.04 ^{a}	0.84 ± 0.19 ^{b}	0.79 ± 0.07 ^{A}	0.91 ± 0.26 ^{A}	0.84 ± 0.16 ^{A}
C18:2 n6	25.60 ± 0.37 ^{a}	26.52 ± 0.78 ^{b}	25.54 ± 0.78 ^{a}	2.77 ± 0.36 ^{A}	4.38 ± 0.50 ^{B}	2.97 ± 0.20 ^{A}
C18:3 n3	1.85 ± 0.08 ^{a}	2.14 ± 0.30 ^{b}	2.00 ± 0.12 ^{a,b}	0.78 ± 0.16 ^{A}	0.93 ± 0.26 ^{A}	0.68 ± 0.18 ^{A}
C20:0	0.36 ± 0.05 ^{a}	0.48 ± 0.07 ^{b}	0.37 ± 0.10 ^{a}	0.31 ± 0.04 ^{A}	0.41 ± 0.07 ^{B}	0.33 ± 0.05 ^{A}
C20:1 n9	0.16 ± 0.03 ^{a}	0.16 ± 0.03 ^{a}	0.21 ± 0.03 ^{b}	0.13 ± 0.02 ^{A}	0.14 ± 0.03 ^{A}	0.15 ± 0.03 ^{A}
C20:2 n6	0.06 ± 0.01 ^{a}	0.06 ± 0.02 ^{a}	0.08 ± 0.02 ^{a}	0.00	0.00	0.00
C22:0	0.14 ± 0.05 ^{a}	0.35 ± 0.08 ^{b}	0.20 ± 0.02 ^{a}	0.00	0.00	0.00
Total SFA	38.47 ± 0.58 ^{a}	37.98 ± 0.66 ^{a}	38.07 ± 0.59 ^{a}	38.00 ± 0.51 ^{A}	37.51 ± 0.70 ^{A}	37.62 ± 0.67 ^{A}
Total UFA	61.53 ± 0.58 ^{a}	62.02 ± 0.66 ^{a}	61.93 ± 0.60 ^{a}	62.00 ± 0.51 ^{A}	62.49 ± 0.70 ^{A}	62.38 ± 0.67 ^{A}
Total MUFA	34.02 ± 0.38 ^{a}	33.28 ± 0.43 ^{b}	34.31 ± 0.39 ^{a}	58.45 ± 0.75 ^{A,B}	57.18 ± 1.36 ^{A}	58.73 ± 0.85 ^{B}
Total PUFA	27.51 ± 0.30 ^{a}	28.73 ± 0.93 ^{b}	27.62 ± 0.78 ^{a}	3.55 ± 0.47 ^{A}	5.31 ± 0.73 ^{B}	3.65 ± 0.29 ^{A}
Total n6	25.66 ± 0.36 ^{a}	26.59 ± 0.78 ^{b}	25.62 ± 0.78 ^{a}	2.77 ± 0.36 ^{A}	4.38 ± 0.50 ^{B}	2.97 ± 0.20 ^{A}
Total n3	1.85 ± 0.08 ^{a}	2.14 ± 0.30 ^{b}	2.00 ± 0.12 ^{a,b}	0.78 ± 0.16 ^{A}	0.93 ± 0.26 ^{A}	0.68 ± 0.18 ^{A}
Ratio n6/n3	13.90 ± 0.76 ^{a}	12.58 ± 1.61 ^{a}	12.83 ± 0.92 ^{a}	3.68 ± 0.68 ^{A}	4.93 ± 1.06 ^{A}	4.73 ± 1.54 ^{A}

Results are expressed as mean ± standard deviation (values are means of eight replicates). Different letters are significantly different (Tukey test, $p < 0.05$) in each row. Uppercase letters for *A. diaperinus* and lowercase letters for *T. molitor*.
Petroleum Et: Petroleum ether; 2-MTHF: 2-methyltetrahydrofuran.

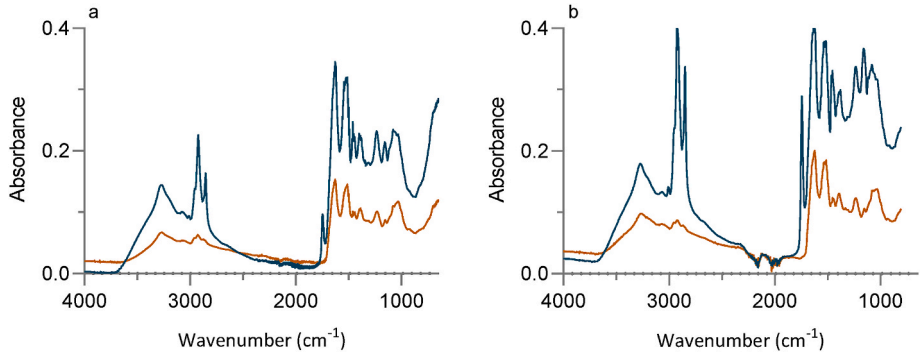

Fig. 2. ATR-FTMIR spectroscopy raw spectra. (a) Agilent Cary 630 and (b) Agilent 4500. Blue original *A. diaperinus* powder, brown *A. diaperinus* defatting powder. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Spectral characterization of insect powders

Typical attenuated total reflection-Fourier transform mid infrared (ATR-FTMIR) spectra obtained from crude *A. diaperinus* and completely deffated powders are shown in Fig. 2. The ATR triple-bounce accessory used on the portable spectrometer Agilent 4500 provided increased absorbance intensity sample. The number of bounces of the ATR accessory improves the signal resolution and the bands are more defined (Wilkerson et al., 2013). Differences mainly in three spectral regions linked to the presence/absence of lipids were identified. An IR band at 1740 cm^{-1} associated with C=O ester, the spectral region IR bands from 2800 to 3000 cm^{-1} linked to C–H symmetric and asymmetric stretching bonds (Mellado-Carretero et al., 2020; Smets et al., 2020) and the region from 1500 to 1350 cm^{-1} related to C–H bending vibrations. The FT-NIR spectral profiles of crude *A. diaperinus* and completely deffated powders showed vibrational transitions between the fundamental energy level and energy levels (Fig. 3). The differences in the NIR spectral region were mainly observed at 1700–1800 nm and associated with C–H overtone stretching of lipids (Alagappan et al., 2024; Osborne, 2000). The overall spectra showed spectral band definition for NeoSpectra MDK. These differences can be mainly explained by the fact than NeoSpectra Scanner has higher number of light sources than NeoSpectra MDK, seven tungsten halogen lamps versus three. Other researchers have reported similar differences when working with these NeoSpectra devices. Gorla et al. (2023) detected lower values of reflectance (up to 17%) were registered with the NeoSpectra development kit compared to the NeoSpectra Scanner.

3.3. Calibration and validation models

For each ATR-FTMIR and FT-NIR equipment, a PLRS model to predict fat content was created by optimizing data preprocessing and selecting the best model performance and validation error. The statistical performance of the calibration and validation models for predicting fat in *T. molitor* and *A. diaperinus* powders were summarized in Table 3. Samples with high leverage, and/or studentized residuals were excluded from calibration models and the optimum number of factors were selected specifically to eliminate under or overfitting (Yao et al., 2022). Table 3 showed that the regressions developed from using the spectral data obtained from the handheld FT-NIR devices had a lower SE_{CV} (1.06–1.26%) in predicting fat than had the portable ATR-FTMIR (SE_{CV} 2.01–3.22). PLSR calibration and validation plots generated with spectral data from handheld FT-NIR devices (Fig. 4c and d) exhibited a strong correlation between the measured reference data and predicted, specially NeoSpectra Scanner. To analyze powdered samples by ATR-FTMIR, a pressure clamp needs to be used to ensure full contact of the sample against the ATR crystal. This pressure seems to be contributing to extract the lipid fraction from the powders and the spectral signal obtained is mainly a contribution of the lipid fraction not the whole insect powder. This effect increased with the lipid content of the insect powders. PLSR plots generated for ATR-FTMIR Agilent Cary 630 (Fig. 4a) and Agilent 4500 (Fig. 4b) showed higher deviation between the reference values and the predicted values specially at concentrations higher than 20%. Moreover, the PLSR models using diamond ATR had higher number of outliers and needed double amount of PLS-factors than those in the NIR region. Several researchers have reported better performance of PLSR models created with NIR spectral data to predict quality traits in ground soybean and beans (Brás et al., 2005; Plans et al., 2013). Predictive ability of PLSR models can be evaluated by RPD values. RPD between 2.5 and 3.0 suggest a good model for prediction and RPD >3 indicates an excellent predictive ability (Saeys et al., 2005). Overall, PLSR models gave RPD values ranging between 2.31 and 10.09 for predicting fat using NIR and MIR systems. The best predictive ability was obtained for PLSR models with NeoSpectra Scanner with a RPD value of 10.09. The regression vector plot (Fig. 5) demonstrated that the discrimination was dominated mainly by lipids as expected. PLSR model for MIR showed positive correlation with 1718 and 1709 cm^{-1} linked to C=O stretching ester carbonyl group, 1640 and 1613 cm^{-1} assigned to doble bond C=C, 1462 cm^{-1} to C–H bending vibrations and 1181 and 1017 cm^{-1} to C–O stretching (Guillén & Cabo, 1997b; Lerma-García et al., 2010; Mellado-Carretero et al., 2020). The IR bands at 2914, 2846, 2831, and 2918 cm^{-1} related to C–H stretching of chitin and fats, were also relevant in the regression vector. PLSR model for NIR showed, as well, positive correlation with spectral bands related to lipids. NIR bands at 2247, 2266, 2297, 2309 and 2391 nm linked to bonds C–H, 1765, 1744, 1719 and 1683 nm associated to bonds of C=O were identified (Benes et al., 2020, 2022).

Calibration models were externally validated using an independent sample set that has not been introduced before. The green dots in Fig. 4a–d represent the samples in the external validation set and distribute within the range of the calibration set (red dots). The slopes of regression line for handheld FT-NIR devices were close to 1, specially for NeoSpectra Scanner. The comparison between the statistical performance (R_p and SE_P) of the external validation set (Table 4) demonstrated once more, better performance for handheld FT-NIR devices than portable ATR-FTMIR instruments. SE_P values ranged from 1.05 to 4.94% being more favorable for handheld devices, specially NeoSpectra Scanner. Our PLSR calibration and external validation models for fat content in *T. molitor* and *A. diaperinus* powders using the same handheld FT-NIR devices showed similar or superior performance compared to previous studies using the same instrument highlighting the potential of handheld sensor technology to provide real time screening for fat in insect powders. Cruz-Tirado et al., 2023 analyzed *Hermentia illucens* powders with NeoSpectra Scanner to predict fat content and reported SE_P and RPD values of 5.73% and 2.65, respectively. In this research the number of samples analyzed was higher as well as the fat content range. Riu et al., 2022 used NeoSpectra MDK to predict fat content in five commercially available insect powders, *Acheta domesticus* (2 farms), *T. molitor*, *A. diaperinus* and *Locusta migratoria*, and reported SEP and RPD values of 1% and 3.80, respectively.

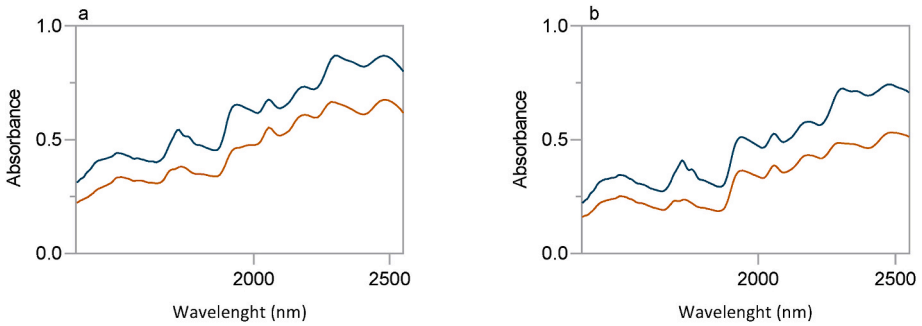

Fig. 3. ATR-FT-NIR spectroscopy raw spectra. (a) NeoSpectra MDK and (b) NeoSpectra Scanner. Blue original *A. diaperinus* powder, brown *A. diaperinus* defatting powder. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Statistical performance of the PLSR models to predict lipid content.

Equipment	Preprocessing	Spectra range	Range (%)	N	F	SE_{cal}	R_{cal}	SE_{CV}	R_{cv}	RPD
Agilent Cary 630	Normalize	Full 4000–630 cm^{-1}	0–28.7	34	4	2.64	0.94	3.22	0.89	2.31
Agilent 4500	Normalize + 2nd deriv (15)	Full 4000–800 cm^{-1}	0–28.7	32	2	1.80	0.98	2.01	0.98	4.88
NeoSpectra MDK	SNV + 2^{nd}deriv (9)	Full 1350–2500 nm	0–28.7	34	2	1.11	0.98	1.26	0.98	7.42
NeoSpectra Scanner	SNV + 2nd deriv (9)	Full 1350–2500 nm	0–28.7	31	2	0.96	0.99	1.06	0.99	10.09

N: number of samples in the calibration set; F: number of factors; SE_{cal}: standard error of calibration; R_{cal}: correlation coefficient of calibration; SE_{CV}: standard error of cross validation; R_{cv}: correlation coefficient of cross validation; RPD: Ratio of performance to deviation; 2nd deriv (9,15): second Savitsky-Golay derivative (window points); SNV: Standard Normal Variate.

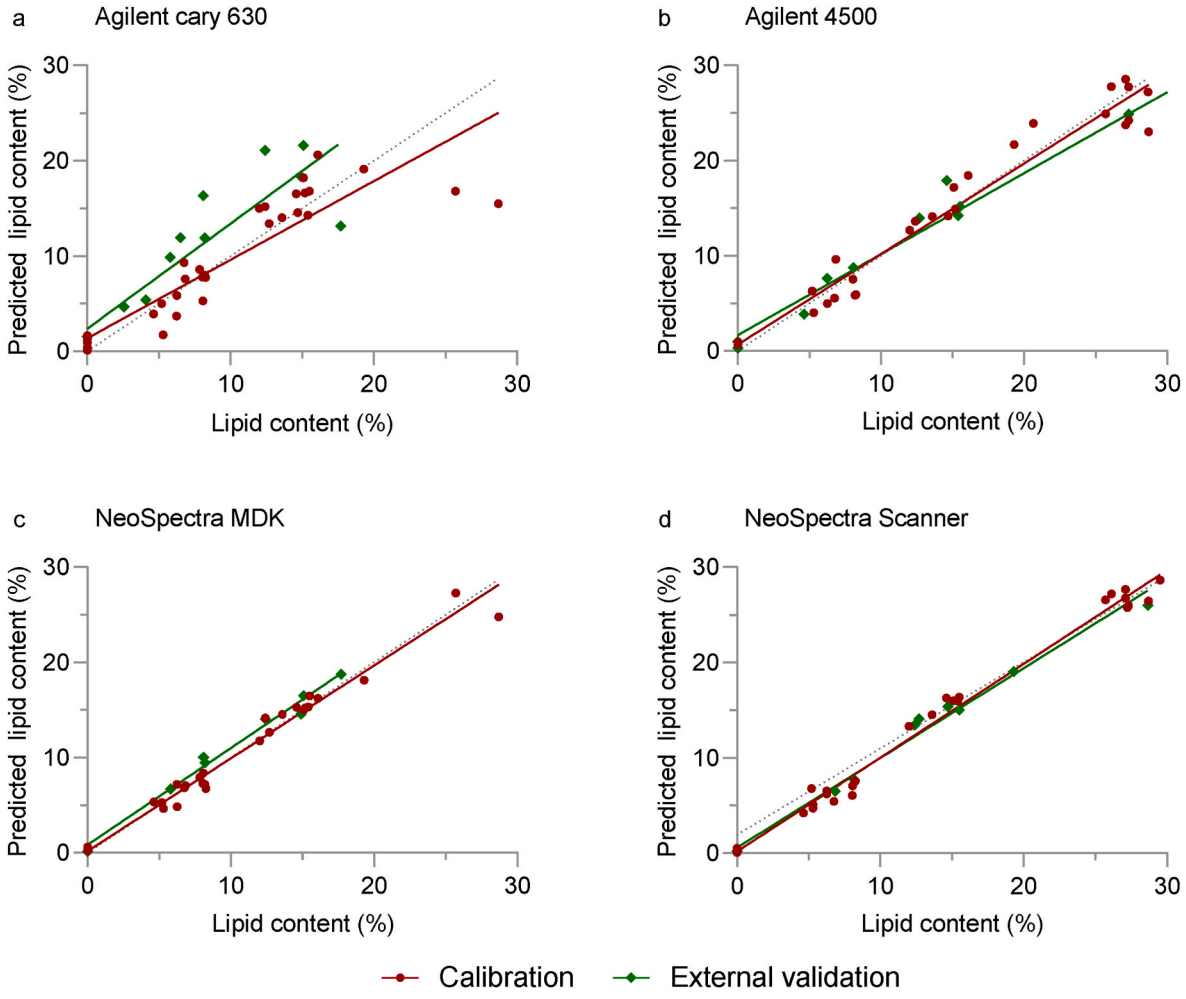

Fig. 4. Partial least squares Y fit for predicting lipid content on insect powder. (a) Agilent Cary 630 (b) Agilent 4500 (c) NeoSpectra MDK (d) NeoSpectra Scanner.

4. Conclusion

This research showed the potential of using handheld FT-NIR devices in conjunction with multivariate analysis to rapidly quantify the fat content in insect powders without the need of using reference methods. PLSR models developed using spectral data from FT-NIR devices, specially Neospectra Scanner, provided much stronger correlation as seen in the tighter predictions and lower SE_{CV} (1.06–1.26%) and SE_P (1.05–1.23%) making these models suitable for screening applications, Handheld FT-NIR devices allow fat quantification thought spectral signature profiles enabling for real time and field-based measurements for controlling insect processing. Moreover, the importance of polarity the organic solvent selected to perform the fat extraction in insect powders was also highlighted. Ethanol had lipid extraction yields significantly lower than petroleum ether and 2-methyltetrahydrofuran specially for *T. molitor* but favored the extraction of PUFAs.

Funding

This project was financially supported by the fundings from Ministerio de Economia y Competitividad PGC 2018-097095-B-I00. C. Mendez reports support Diputació de Tarragona and the Universitat Rovira i Virgili (2020PMF-PIPF-30).

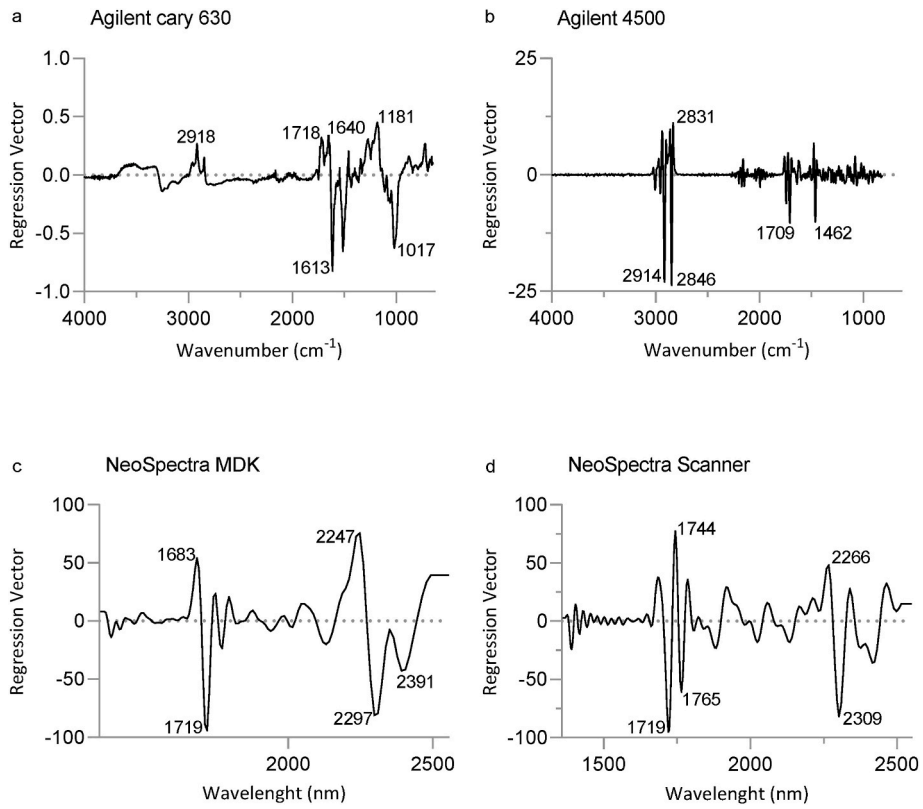

Fig. 5. Partial least squares discriminating power for predicting lipid content on insect powder. (a) Agilent Cary 630 (b) Agilent 4500 (c) NeoSpectra MDK (d) NeoSpectra Scanner.

Table 4
Statistical performance of the PLSR models prediction.

Equipment	SE_P	R_P
Agilent Cary 630	4.94	0.86
Agilent 4500	2.15	0.98
NeoSpectra MDK	1.23	0.98
NeoSpectra Scanner	1.05	0.99

SE_P: standard error of prediction; R_P: correlation coefficient of prediction.

CRediT authorship contribution statement

Carmen Mendez-Sanchez: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Carme Güell:** Supervision, Resources, Funding acquisition. **Montserrat Ferrando:** Supervision, Resources, Funding acquisition. **Luis Rodriguez-Saona:** Writing – review & editing, Resources. **Rafael Jimenez-Flores:** Writing – review & editing, Resources. **Joan Carles Domingo:** Resources, Investigation. **Silvia de Lamo Castellvi:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116652.

References

Alagappan, S., Hoffman, L., Mikkelsen, D., Mantilla, S. O., James, P., Yarger, O., & Cozzolino, D. (2024). Near-infrared spectroscopy (NIRS) for monitoring the nutritional composition of black soldier fly larvae (BSFL) and frass. *Journal of the Science of Food and Agriculture, 104*(3), 1487–1496. https://doi.org/10.1002/jsfa.13044

Aykas, D. P., Ball, C., Menevseoglu, A., & Rodriguez-Saona, L. E. (2020). In situ monitoring of sugar content in breakfast cereals using a novel ft-nir spectrometer. *Applied Sciences, 10*(24), 1–11. https://doi.org/10.3390/app10248774

Barker, D., Fitzpatrick, M. P., & Dierenfeld, E. S. (1998). Nutrient composition of selected whole invertebrates. *Zoo Biology, 17*(2), 123–134. https://doi.org/10.1002/(SICI)1098-2361(1998)17:2<123::AID-ZOO7>3.0.CO;2-B

Beć, K. B., Grabska, J., Plewka, N., & Huck, C. W. (2021). Insect protein content analysis in handcrafted fitness bars by nir spectroscopy. Gaussian process regression and data fusion for performance enhancement of miniaturized cost-effective consumer-grade sensors. *Molecules, 26*(21). https://doi.org/10.3390/molecules26216390

Benes, E., Biró, B., Fodor, M., & Gere, A. (2022). Analysis of wheat flour-insect powder mixtures based on their near infrared spectra. *Food Chemistry X, 13*. https://doi.org/10.1016/j.fochx.2022.100266

Benes, E., Gere, A., & Fodor, M. (2020). Predicting macronutrients and energy content of snack products using FT-NIR analysis and chemometric techniques. *Journal of Food Engineering, 280*. https://doi.org/10.1016/j.jfoodeng.2020.109954

Brás, L. P., Bernardino, S. A., Lopes, J. A., & Menezes, J. C. (2005). Multiblock PLS as an approach to compare and combine NIR and MIR spectra in calibrations of soybean flour. *Chemometrics and Intelligent Laboratory Systems, 75*(1), 91–99. https://doi.org/10.1016/j.chemolab.2004.05.007

Cruz-Tirado, J. P., Amigo, J. M., & Barbin, D. F. (2022). Determination of protein content in single black fly soldier (Hermetia illucens L.) larvae by near infrared hyperspectral imaging (NIR-HSI) and chemometrics. *Food Control, 143*. https://doi.org/10.1016/j.foodcont.2022.109266

Cruz-Tirado, J. P., Vieira, M. S., dos, S., Amigo, J. M., Siche, R., & Barbin, D. F. (2023). Prediction of protein and lipid content in black soldier fly (Hermetia illucens L.) larvae flour using portable NIR spectrometers and chemometrics. *Food Control, 153*. https://doi.org/10.1016/j.foodcont.2023.109969

Franco, A., Salvia, R., Scieuzo, C., Schmitt, E., Russo, A., & Falabella, P. (2022). Lipids from insects in cosmetics and for personal care products. *MD, 13*(1). https://doi.org/10.3390/insects13010041. In Insects.

Gorla, G., Taborelli, P., Ahmed, H. J., Alamprese, C., Grassi, S., Boqué, R., … Giussani, B. (2023). Miniaturized NIR spectrometers in a nutshell: Shining light over sources of

variance. *Chemosensors, 11*(3), Article 182. https://doi.org/10.3390/chemosensors11030182

Guillén, M. D., & Cabo, N. (1997a). Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. *Journal of the American Oil Chemists' Society, 74*(10), 1281–1286. https://doi.org/10.1007/s11746-997-0058-4

Guillén, M. D., & Cabo, N. (1997b). Infrared spectroscopy in the study of edible oils and fats. *Journal of the Science of Food and Agriculture, 75*(1), 1–11. https://doi.org/10.1002/(SICI)1097-0010(199709)75:1<1::AID-JSFA842>3.0.CO;2-R

Hoffman, L. C., Zhang, S., Alagappan, S., Wills, V., Yarger, O., & Cozzolino, D. (2022). Monitoring compositional changes in black soldier fly larvae (BSFL) sourced from different waste stream diets using attenuated total reflectance mid infrared spectroscopy and chemometrics. *Molecules, 27*(21). https://doi.org/10.3390/molecules27217500

Laroche, M., Perreault, V., Marciniak, A., Gravel, A., Chamberland, J., & Doyen, A. (2019). Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods, 8*(11). https://doi.org/10.3390/foods8110572

Lepage, G., & Roy, C. C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research, 27*(1), 114–120. https://doi.org/10.1016/S0022-2275(20)38861-1

Lerma-García, M. J., Ramis-Ramos, G., Herrero-Martínez, J. M., & Simó-Alfonso, E. F. (2010). Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. *Food Chemistry, 118*(1), 78–83. https://doi.org/10.1016/j.foodchem.2009.04.092

Manzano-Agugliaro, F., Sanchez-Muros, M. J., Barroso, F. G., Martínez-Sánchez, A., Rojo, S., & Pérez-Bañón, C. (2012). Insects for biodiesel production. *Renewable and Sustainable Energy Reviews, 16*(6), 3744–3753. https://doi.org/10.1016/j.rser.2012.03.017

Mellado-Carretero, J., García-Gutiérrez, N., Ferrando, M., Güell, C., García-Gonzalo, D., & De Lamo-Castellví, S. (2020). Rapid discrimination and classification of edible insect powders using ATR-FTIR spectroscopy combined with multivariate analysis. *Journal of Insects as Food and Feed, 6*(2), 141–148. https://doi.org/10.3920/jiff2019.0032

Meullemiestre, A. (2015). *Modern techniques and solvents for the extraction of microbial oils.* Springer. B. C. and A.-V. M. and C. F.

Murali, H. S., Mohan, M. S., Manja, K. S., & Sankaran, R. (1993). Polar and nonpolar lipids and their fatty acid composition of a few *Fusarium* species. *Journal of the American Oil Chemists' Society, 70*(10), 1039–1041. https://doi.org/10.1007/BF02543034

Ni, D., Nelis, J. L. D., Dawson, A. L., Bourne, N., Juliano, P., Colgrave, M. L., Juhász, A., & Bose, U. (2024). Application of near-infrared spectroscopy and chemometrics for the rapid detection of insect protein adulteration from a simulated matrix. *Food Control, 159*. https://doi.org/10.1016/j.foodcont.2023.110268

Nielsen, S. S. (Ed.). (2010). *Food analysis*. Springer US. https://doi.org/10.1007/978-1-4419-1478-1.

Osborne, B. G. (2000). Near-infrared spectroscopy in food analysis. In *Encyclopedia of analytical chemistry*. Wiley. https://doi.org/10.1002/9780470027318.a1018.

Paul, A., Frederich, M., Megido, R. C., Alabi, T., Malik, P., Uyttenbroeck, R., Francis, F., Blecker, C., Haubruge, E., Lognay, G., & Danthine, S. (2017). Insect fatty acids: A comparison of lipids from three orthopterans and Tenebrio molitor L. larvae. *Journal of Asia-Pacific Entomology, 20*(2), 337–340. https://doi.org/10.1016/j.aspen.2017.02.001

Plans, M., Simó, J., Casañas, F., Sabaté, J., & Rodriguez-Saona, L. (2013). Characterization of common beans (Phaseolus vulgaris L.) by infrared spectroscopy: Comparison of MIR, FT-NIR and dispersive NIR using portable and benchtop instruments. *Food Research International, 54*(2), 1643–1651. https://doi.org/10.1016/j.foodres.2013.09.003

Pu, Y., Pérez-Marín, D., O'shea, N., & Garrido-Varo, A. (2021). Recent advances in portable and handheld NIR spectrometers and applications in milk, cheese and dairy powders. *Foods, 10*(Issue 10). https://doi.org/10.3390/foods10102377. MDPI.

Purschke, B., Brüggen, H., Scheibelberger, R., & Jäger, H. (2018). Effect of pre-treatment and drying method on physico-chemical properties and dry fractionation behaviour of mealworm larvae (Tenebrio molitor L.). *European Food Research and Technology, 244*(2), 269–280. https://doi.org/10.1007/s00217-017-2953-8

Purschke, B., Stegmann, T., Schreiner, M., & Jäger, H. (2017). Pilot-scale supercritical CO2 extraction of edible insect oil from Tenebrio molitor L. larvae – influence of extraction conditions on kinetics, defatting performance and compositional properties. *European Journal of Lipid Science and Technology, 119*(2). https://doi.org/10.1002/ejlt.201600134

Riu, J., Vega, A., Boqué, R., & Giussani, B. (2022). Exploring the analytical complexities in insect powder analysis using miniaturized NIR spectroscopy. *Foods, 11*(21). https://doi.org/10.3390/foods11213524

Rodriguez-Saona, L., Aykas, D. P., Borba, K. R., & Urtubia, A. (2020). Miniaturization of optical sensors and their potential for high-throughput screening of foods. *Current Opinion in Food Science, 31*, 136–150. https://doi.org/10.1016/j.cofs.2020.04.008. Elsevier Ltd.

Saeys, W., Mouazen, A. M., & Ramon, H. (2005). Potential for onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. *Biosystems Engineering, 91*(4), 393–402. https://doi.org/10.1016/j.biosystemseng.2005.05.001

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition, 70*(3), 560S–569S. https://doi.org/10.1093/ajcn/70.3.560s

Simopoulos, A. P. (2002). *The importance of the ratio of omega-6/omega-3 essential fatty acids*. www.elsevier.com/locate/biopha.

Smets, R., Verbinnen, B., Van De Voorde, I., Aerts, G., Claes, J., & Van Der Borght, M. (2020). Sequential extraction and characterisation of lipids, proteins, and chitin from black soldier fly (hermetia illucens) larvae, prepupae, and pupae. *Waste and Biomass Valorization, 11*(12), 6455–6466. https://doi.org/10.1007/s12649-019-00924-2

Son, Y. J., Lee, J. C., Hwang, I. K., Nho, C. W., & Kim, S. H. (2019). Physicochemical properties of mealworm (Tenebrio molitor) powders manufactured by different industrial processes. *Lebensmittel-Wissenschaft und -Technologie, 116*. https://doi.org/10.1016/j.lwt.2019.108514

Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. S., & Lakemond, C. M. M. (2014). Insect lipid profile: Aqueous versus organic solvent-based extraction methods. *Food Research International, 62*, 1087–1094. https://doi.org/10.1016/j.foodres.2014.05.052

Venkatram, R., García-Cano, I., & Jiménez-Flores, R. (2024). Reduction in the antigenicity of beta-lactoglobulin in whole milk powder via supercritical CO2 treatment. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2023-24565

Wang, J., Ballon, A., Schroën, K., de Lamo-Castellví, S., Ferrando, M., & Güell, C. (2021). Polyphenol loaded w1/o/w2 emulsions stabilized with lesser mealworm (Alphitobius diaperinus) protein concentrate produced by membrane emulsification: Stability under simulated storage, process, and digestion conditions. *Foods, 10*(12). https://doi.org/10.3390/foods10122997

Wilkerson, E. D., Anthon, G. E., Barrett, D. M., Sayajon, G. F. G., Santos, A. M., & Rodriguez-Saona, L. E. (2013). Rapid assessment of quality parameters in processing tomatoes using hand-held and benchtop infrared spectrometers and multivariate analysis. *Journal of Agricultural and Food Chemistry, 61*(9), 2088–2095. https://doi.org/10.1021/jf304968f

Yao, S., Ball, C., Miyagusuku-Cruzado, G., Giusti, M. M., Aykas, D. P., & Rodriguez-Saona, L. E. (2022). A novel handheld FT-NIR spectroscopic approach for real-time screening of major cannabinoids content in hemp. *Talanta, 247*, Article 123559. https://doi.org/10.1016/j.talanta.2022.123559