Miniaturized NIR and MIR spectroscopy for insect lipid characterization

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Abstract

The main objective of this research was to evaluate the performance of portable FT-MIR (4000 to 630 cm^{-1}) and handheld FT-NIR (7400 to 4000 cm^{-1}) spectrometers, combined with partial least squares regression (PLSR) multivariate analysis. This method was used to predict the percentage of insect lipids added to mixtures of vegetable and seed oils, as well as to profile the fatty acid composition in the lipid fractions of *Locusta migratoria* and *Acheta domesticus* mixed with these oils. Insect lipids from *L. migratoria* and *A. domesticus* were extracted from their powdered forms using organic solvent extraction and then mixed with five edible oils (olive, sunflower, avocado, almond, and linseed) at concentration ratios ranging from 0% to 100% (n = 140). The fatty acid (FA) profile of the samples was determined using the fatty acid methyl ester (FAME) method. PLSR was used to develop algorithms for predicting insect lipid content in mixtures and FA composition. The PLSR model for predicting the percentage of insect lipids in mixtures with vegetable and seed oils demonstrated good linearity, with high correlation (R_{CV} = 0.99), low standard errors of cross-validation ($SE_{CV} \leq 2.21\%$) and prediction ($SE_P \leq 2.32\%$), and high predictive performance as indicated by the ratio of prediction to deviation (RPD ≥ 9.23). The percentage of myristic, palmitic, stearic, oleic, linoleic, and α-linolenic fatty acid as well as the content of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) were accurately predicted with values of $SE_{CV} \leq 4.64\%$ and an $R_{CV} \geq 0.88$. However, the FT-MIR spectrometer tested showed superior performance in predicting fatty acids and SFA, MUFA, PUFA reaching values of 0.99 in coefficient of correlation (RP) and 0.66% in standard error in prediction (SE_P).

Key words: insect lipids, *L. migratoria*, *A. domesticus*, mid-infrared spectroscopy, near-infrared spectroscopy.

Conflicts of interest. The authors declare no conflict of interest.

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1. Introduction

Lipids, along with proteins, are primary components of insects. Recent research has characterized numerous insect species, revealing that lipid composition and content may be influenced by factors such as insect species, life stage, diet, and fat extraction method (Arrese and Soulages, 2010; Laroche *et al.*, 2019; Oonincx and Van Der Poel, 2011). Insects accumulate essential nutrients and energy as lipids to support survival through metamorphosis or other non-feeding activities (Dossey *et al.*, 2016). Insect lipids have been evaluated as alternative ingredients for various applications, including bakery products, spreads, frying oils, food-grade lubricants, and emulsions, with promising results (Tzompa-Sosa and Fogliano, 2017). Additionally, potential applications in the cosmetic, therapeutic, and industrial sectors have been proposed (Aguilar, 2021; Franco *et al.*, 2021). The quality and functionality of these lipids depend on their fatty acid (FA) profile. Notably, the primary FA in insects includes palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9) and linoleic acid (C18:2n6) (Rumpold and Schlüter, 2013). While palmitic acid can be produced endogenously, dietary intake may still be required (Carta *et al.*, 2017). Palmitic and stearic acids are of interest for biodiesel production due to their high calorific value and viscosity (Manzano-Agugliaro *et al.*, 2012). Oleic acid, a monounsaturated FA, is associated with health effects such as anti-inflammatory effects and is utilized in cosmetics for its skin penetration properties (Rabasco Alvarez and González Rodríguez, 2000; Sales-Campos *et al.*, 2013). Linoleic acid, an essential omega-6 FA, is linked to health benefits and participates in omega-3 metabolic pathways (Viola and Viola, 2009).

Several studies have demonstrated that insect lipids can effectively replace vegetable and seed oils in food formulations. For example, insect lipids from *Schistocerca gregaria* and *Ruspolia differens* were successfully used in cookies, with consumer preference favoring those made with *R. differens* and sesame oil (Cheseto *et al.*, 2020). Additionally, *Hermetia illucens* larvae fat was able to substitute up to 25% of butter in bakery products without altering taste (Delicato *et al.*, 2020), and *Tenebrio molitor* oil (crude or deodorized) was shown to replace partially replace peanut and rapeseed oils in crackers and hummus without negatively affecting consumer acceptance (Tzompa-Sosa and *et al*., 2021a, 2021b).

FA are commonly analyzed using gas chromatography (GC), a labor-intensive method, that requires complex sample pretreatment, and results in sample destruction. Infrared (IR) spectroscopy, when combined with pattern recognition analysis, has been proposed as an alternative technique for predicting FA percentages (Aykas *et al.*, 2020; Salas-Valerio *et al.*, 2022; Yao *et al.*, 2020). Benchtop Fourier-transform mid-infrared (FT-MIR) spectrometers have been also employed to study oxidative stability by quantifying peroxide and anisidine values (Guillén and Cabo, 2002), determine omega-6 and omega-3 amount and ratios (Olsen *et al.*, 2008), and authenticate extra virgin olive oil (Lerma-García *et al.*, 2010). Similarly, benchtop near-infrared (NIR) equipment has been used to assess quality parameters such as acidity, volatile matter, peroxide index, and insoluble impurities in olive oil (Garrido-Varo *et al.*, 2017) and other edible oils (Armenta *et al.*, 2007). Recent advancements in IR spectroscopy have enabled the miniaturization of equipment, allowing for in situ analysis (Cebi *et al.*, 2023; Rodriguez-Saona *et al.*, 2016). The integration of technologies such as Linear Variable Filters (LVF) and micro-electro-mechanical systems (MEMS) has resulted in the development of palm-sized NIR spectrometers (Yan and Siesler, 2021). Portable NIR equipment, such as the NeoSpectra Scanner, which operates in the 7400 to 4000 cm^{-1} range and incorporates MEMS advancements, has been used to predict trans-fat content in edible oils (Birkel and Rodriguez-Saona, 2011). The MicroNIR spectrometer,

utilizing an LVF wavelength selector and collecting spectra from 11000 to 6000 cm^{-1}, has been evaluated for quantifying olive oil in various vegetable oils (Borghi *et al.*, 2020). Portable MIR devices, such as those based on the Michelson interferometer, have also been applied to lipid analysis. For instance, spectral data obtained using a portable Agilent Cary 630 has been utilized to identify the type of vegetable oil used for frying chips (Aykas and Rodriguez-Saona, 2016) and determine fatty acid composition in frying oils and fish oil supplements (Aykas and Rodriguez-Saona, 2016; Plans *et al.*, 2015). The handheld TruDefender has been employed to create prediction models for quantifying major fatty acids in butter and margarine (Salas-Valerio *et al.*, 2022). Additionally, the Bruker-Alpha spectrometer has been tested for quantifying free fatty acids in olive oil (Tarhan *et al.*, 2017) and measuring refractive index, peroxide value, and total phenolic content in pomegranate kernel oil (Okere *et al.*, 2022).

The aim of this study was to assess the potential of miniaturized FT-MIR and FT-NIR devices, combined with PLSR, to predict the amount of *Locusta migratoria* and *Acheta domesticus* lipids mixed with sunflower, almond, olive, avocado, and linseed oils. Additionally, the lipid samples were analyzed to predict the percentages of saturated, monounsaturated, and polyunsaturated fatty acids, as well as the specific fatty acid profiles, including myristic, palmitic, stearic, oleic, linoleic, and α-linolenic acids.

2. Material and Methods

Lipid extraction procedure

Lipids from *A. domesticus* (AD) and *L. migratoria* (LM) powders supplied by Delibugs (Lelystad, Netherlands, two batches from the same lot) were extracted using the organic solvent extraction method described following the procedure of Wang *et al.* (2021). First, the insect powder was mixed with 2-methyltetrahydrofuran (2- MTHF) (Scharlab S.L., Barcelona, Spain) at 1/5 (w/w) ratio and stirred at 300 rpm for 1 h at room temperature. The mixture was allowed to fully separate in two phases. The upper phase, containing the lipid fraction, was collected, while the lower phase, with the partially defatted insect powder, underwent two additional defatting cycles using more 2-MTHF. The collected upper phases were combined in a round-bottom flask, and the 2-MTHF was evaporated (SBS-RV-2000, Steinberg, Germany) at 400 mbar and 65 °C and the lipid fraction was recovered. The insect lipids were stored at 4 °C under a nitrogen atmosphere and protected from ambient light until further use.

Sample preparation

Samples were prepared by mixing lipid fractions from *A. domesticus* and *L. migratoria* with two batches from the same lot of sunflower oil (SF) (Borges, Tarragona, Spain), extra virgin olive oil (OL) (Aceites molisur, Málaga, Spain), avocado oil (AV) (Perseus Foods, Guecho, Spain), linseed oil (LI) (Ecoveritas S.A., Barcelona, Spain) and almond oil (AL) (Ecoveritas S.A., Barcelona, Spain) (supplementary Table S1 and Table S2). A total of 140 samples, each with 50 mL, were prepared by mixing one insect species with a vegetable or seed oil in increasing ratios of 10% by weight, ranging from 0% to 100% of the final weight.

Determination of fatty acids profile

The fatty acids profile from all the vegetables and seed oils and insect lipid extracts used in this study were obtained by Gas Chromatography-Mass Spectroscopy (GS-MS). Fatty acid methyl esters (FAME) were prepared following the method Lepage and Roy (1986) by transesterification of the fatty acids present in the samples by addition of methanol-hexane (4:1, v/v). Then, 200 µL of acetyl chloride was added and heated at 100 °C for 1h. After that, potassium carbonate was added into the tube and centrifuged. The upper phase, which contains the FAME, was collected and analyzed. 1 µL of the sample was analyzed to determine the fatty acid composition with Shimadzu GCMS- QP2010 Plus gas chromatograph/mass spectrometer (Shimadzu, Kyoto, Japan), equipped with a split/splitless injector, a Shimadzu AOC-20i autoinjector, a Shimadzu AOC-20s autosampler and a Suprawax-280 (Teknokroma, 15m length x 0.10mm internal diameter x 0.10 µm film thickness) column. The oven's settings were as follows: 130 °C for 0.25 min, followed by a ramp of 35 °C/min to 200 °C, 225 °C (8 °C/min) for 3.2 min, and 80 °C/min to 245 °C for 2.75 min. By comparing the elution times of the reference FAME mixture (GLC-744 Nu-Chek Prep. Inc., Elysian, Minnesota, USA), the fatty acids present in the samples were identified. The proportion of each fatty acid in the total is shown by the values of the fatty acid profile. Every sample was analyzed twice. The percentage of saturated fatty acids (SFA) was determined as the sum of C12:0, C14:0, C16:0, C18:0, and C20:0; the monounsaturated fatty acids (MUFA) as the sum of C16:1n7, C16:1n9, C18:1n9, C18:1n7, and C20:1n9; the polyunsaturated fatty acids (PUFA) percentage as the sum of C18:2n6, and C18:3n3; and the unsaturated fatty acids (UFA) percentage as the sum of MUFA and PUFA. The total amount of omega-6 (n6) is composed by C18:2n6, and the total amount of omega-3 (n3) is composed by C18:3n3.

Infrared analysis

The portable Agilent Cary 630 (Agilent Technologies Inc., Santa Clara, CA) equipped with a deuterated triglycine sulfate detector and single-bound attenuated total reflectance (ATR) diamond crystal accessory was used to acquire FT-MIR spectral data. The spectra were collected with an 8 cm^{-1} resolution, to enhance the signal-to-noise ratio 128 interferograms were coadded in the range of 4000 to 650 cm^{-1}. The samples were held at 45 °C and 0,8 µL of sample was placed on the ATR crystal to collect the spectrum. Each sample was measured five times.

The FT-NIR spectral data measurements were collected using Neospectra scanner (Si-Ware Systems, Cairo, Egypt) equipped with a single uncooled InGaAs photodetector and a monolithic MEMS-based Michelson interferometer chip. 350 µL of oil mixture at 45 °C were placed in the vials for analysis liquids (Si-Ware Systems, Inc., Cairo, Egypt) and covered with a reflectance accessory. Spectra were recorded using Neospectra Collect App (Si-Ware Systems) within the range of 7400 to 4000 cm^{-1} during 10 s. All the samples were analyzed in duplicate, collecting the background signal between each measurement.

Multivariate data analysis

Piroutte 4.5 (Infometrix Inc., Washington, DC, USA) was used for the multivariate analysis. The NIR spectra were treated by Standard Normal Variate (SNV) and a second Savitzky−Golay derivative (9-point window size). MIR data were transformed by normalization followed by the second derivative transformation (17-point window size). All the data were mean-centered before the analysis. Principal Component Analysis (PCA) is an unsupervised pattern recognition algorithm, it was performed to study the main differences between the samples included in this study. Partial least squares regression (PLSR) models were developed to quantify insect lipids content and the FA profile based on the reference values obtained by GC-MS. The dataset was

divided into a calibration set (n=107) for model training and a validation set (n=40) for performance assessment using the Kennard-Stone algorithm. PLSR models were generated based on results obtained by GC-MS. Internal cross-validation (leave-one-out) was performed during the calibration to evaluate model performance. The number of factors was determined by the minimum standard error of cross-validation (SE_{CV}) and the correlation coefficient of cross-validation (R_{CV}) values to avoid overfitting and underfitting. The best PLSR models were validated by predicting the insect lipid content and FA profile from the validation sample set. The standard error of prediction (SE_P), the correlation coefficient of prediction (R_P), and the ratio of performance to deviation (RPD) were calculated to determine regression model performance.

3. Results and Discussion

FTIR analysis and chemometric approach

Figure 1. shows a representative *A. domesticus* (AD), *L. migratoria* (LM), sunflower (SF), olive (OL), avocado (AV), linseed (LI), and almond (AL) oils acquired by FT-MIR. The spectra presented absorption bands at 3007 cm^{-1} linked to =C–H cis stretching and bands at 2955, 2922, and 2851 cm^{-1} corresponding to C–H symmetric and asymmetric stretching vibrations (Aykas and Rodriguez-Saona, 2016). The band at 1744 cm^{-1} was associated with the carboxyl group (C=O) of the esters stretching vibration, characteristic of the lipids (Salas-Valerio *et al.*, 2022). The signal at 1647 cm^{-1} was related to C=C stretching of cis double bonds; and the following bands at 1457 and 1379 cm^{-1}, to bending vibration of C-H from CH_2 and CH_3 group respectively (Aykas *et al.*, 2020), and the band at 723 cm^{-1} corresponds to cis C-H (Aykas and Rodriguez-Saona, 2016). The FT-NIR spectra of the insects and vegetable and seeds oil in Figure 2 showed substantial variations in five bands at 5800, 5678 4670, 4330, and 4234 cm^{-1}. The IR bands at 5800, 5678 cm^{-1} were linked to C-H from CH_2 stretching first overtone (Benes *et al.*, 2022), and the band at 4670 cm^{-1}, was associated with vibrations arising from C-H groups from double bonds (Garrido-Varo *et al.*, 2015). The bands ranged from 4384 to 4084 cm^{-1} were related to combination modes of C-H (Ozaki *et al.*, 2018). The spectra showed absorption bands at similar wavenumbers to those previously reported by the same device with other lipidic samples, such as butter or vegetable oils (Salas-Valerio *et al.*, 2022; Yao *et al.*, 2020).

Pattern recognition

In this study, Principal Component Analysis (PCA) was performed as an exploratory tool to detect patterns between the pure oils and mixed with *A. domesticus* and *L. migratoria* lipids. Figures 3 and 4 show the score plots of the resulting PCA obtained from the FT-MIR and FT-NIR input, respectively. The type of vegetable or seed oil used and its percentage in the mixture were indicated on the sample labels. The first principal component consistently discriminated the percentage of insect lipids from vegetable and seed oils, while the second principal component differentiated the various types of oils. The large spread of samples in the class projection plot showed the variability among the selected oils. The formation of clusters observed in this study was also noted by researchers combining insect powders with wheat flour (Benes *et al.*, 2022). In MIR analyses PC1 and PC2 explained 98.8 % and 97.7% of the variance for *A. domesticus* and *L. migratoria* samples, respectively. The loadings provided information about the variables that contributed most significantly to the discrimination. The bands of interest were centered in the lipid region. The most important bands identified by PCA were 2918, 2881, 2851, 1770, 1744, 1714, and 1155 cm^{-1}. The bands 2918, 2881 and 2851 cm^{-1} were related to the asymmetric stretching vibration of C-H from CH_2 groups; the bands at 1770, 1744, and 1714 cm^{-1} were associated with the stretching vibration

of C=O from the fatty acids functional group; and the signal from the stretching vibration of the C-O ester groups was observed at 1155 cm^{-1} (Aykas and Rodriguez-Saona, 2016; Lerma-García *et al.*, 2010). Variations in signal at these bands resulted from differences in the fatty acid chains of the oils particularly among the vegetable oils, as most of the bands were represented in PC1. These differences were attributed to variations in chain length and degree of unsaturation. Similar bands were reported by other authors in the discrimination of extra virgin olive, virgin olive, olive, and adulterated olive oils (Aykas *et al.*, 2020). A similar approach was considered in PCA analysis using FT-NIR spectra, the two main PC explained 98.3% of *A. domesticus* and 98.2% of *L. migratoria* of the variance. The NIR bands at 5923, 5868, 5827, 5787, 5759, 4411, 4343, 4221, and 4112 cm^{-1} explained most of the variance in the PCA. The bands between 5923 to 5759 cm^{-1} are associated with the first overtone of C-H stretching of CH_3 and carbons double bonds, the ones between 4411 and 4112 cm^{-1} are mainly related to C-H from CH_3 and stretching of CH_2 bonds, both from fatty acids chains (Christy *et al.*, 2004). The wavenumber absorptions described in the loadings were like those previously identified by other researchers in the authentication potato chip frying oil, they also found relevant the bands from 5900 to 5600 cm-1 authors (Yao *et al.*, 2020). Similar to our findings with FT-MIR spectral data, the primary differences were linked to the vegetable oils and their compositional variations.

PLSR models to predict the percentage of insect lipid content

PLSR calibration models created with FT-MIR and FT-NIR spectral data to predict the percentage of insect lipids added to mixtures of vegetable and seed oils are summarized in Table 1 and represented in Figure 5. To enhance prediction quality, specific wavenumbers identified through PCA loadings were selected rather than using the entire spectral range. This approach helps remove variables that might be irrelevant, noisy, or otherwise unreliable in the context of interest (Leardi and Nørgaard, 2004). For FT-MIR models, wavenumber regions between 3100–2600 cm^{-1} and 1850–650 cm^{-1} were used, while the 6100–4000 cm^{-1} range was chosen for FT-NIR PLSR models. PLSR models used 7 factors to explain most of the relevant variance in the training set while minimizing the standard error of cross-validation (SE_{CV}). A leave-one-out cross-validation approach was employed to prevent overfitting by mitigating the impact of random noise (Ayvaz *et al.*, 2016). The correlation coefficients of cross-validation were 0.99 and the SE_{CV} values ranged from 1.15 to 2.21% (w/w). An external validation set was used to validate the models, and the results followed the same trend of the cross-validation, obtaining SE_P values lower than 2.32% and good correlation (R_P>0.99). The predictive qualities of the models were reported based on the RPD values An RPD above 3 indicates suitability for routine analysis, while values above 5 reflect a reliable model for quality control and an RPD greater than 8 indicates excellent predictive capability. The best predictive ability was obtained for PLSR models with FT- MIR with a RPD value of 18.32.

Table 1. Performance statistics of calibration and external validation models developed to quantify insect lipid content based on MIR and NIR data.

Device	Range (cm^{-1})	N_{cv}	Factors	R_{CV}	SE_{CV}	N_P	R_P	SE_P	RPD
MIR (Agilent Cary 630)	3100-2600; 1850-650	106	7	0.99	1.15	40	0.99	1.21	18.32
NIR (NeoSpectra Scanner)	6100-4000	103	7	0.99	2.21	37	0.99	2.32	9.23

Abbreviations used: N_{CV}: number of samples in the calibration set; R_{CV}: correlation coefficient of cross-validation; SE_{CV}: standard error of cross-validation; N_P: number of samples in the validation set; R_P: correlation coefficient of validation; SE_P: standard error of validation; RPD: Ratio of performance to deviation.

Our results are in line with those of other authors who have quantified a specific oil in a blend. Mendes *et al.* (2015) quantified soybean oil in a mixture with olive oil by NIR and MIR spectroscopy in the same range as our study. They reported a good correlation to predict the content of soybean oil in the blend but had higher SEP = 4.89 % when using MIR spectral data. Du *et al.* (2021) also quantified by NIR the camellia oil mixed with corn, rapeseed, and sunflower oil, achieving SE_P from 2.17 to 3.19%.

Fatty acid profile

The fatty acid profile of *A domesticus, L. migratoria*, olive, linseed, avocado, almond, and sunflower oil are shown in Table 2. The most common FA were palmitic (C16:0, 37.86%), stearic (C18:0, 10.43%), oleic (C18:1n9, 20.50%) and linoleic (C18:2n6, 27.08%) for *A domesticus* and palmitic (35.19%), stearic (9.98%), oleic (25.94%), linoleic (17.84%) and α-linolenic (C18:3n3, 8.45%) for *L. migratoria*. These FA were the 95.87 and 97.4% % of the total FA content, respectively. Our results are in agreement with previous studies performed with *A domesticus* and *L. migratoria* (Egonyu *et al.*, 2021; Orkusz *et al.*, 2023; Osimani *et al.*, 2017). Olive and almond oils had high oleic acid content 69.29% and 66.06%, respectively, while avocado oil was notably rich in palmitic acid (22.84%). Sunflower oil contains a significant amount of linoleic acid (54.67%), an omega-6 (n6) fatty acid, whereas linseed has high α-linolenic acid content (45.37%), an omega-3 (n3) fatty acid. The individual fatty acids were classified into saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA) based on the structure of their lipid chains. Significant differences in the distribution of SFA and UFA were observed between vegetable and insect lipids. Vegetable and seed oils had a higher percentage of UFA (76.32- 87.49%). The FA of the insects was approximately equally divided between SFA (47.03-49.48%) and UFA (50.52-52.97%). In terms of UFA content, the

Table 2. Fatty acid composition (%) of vegetable, seed oils and insect lipids.

Fatty acids (%)	*A. domesticus*	*L. migratoria*	Linseed	Avocado	Olive	Almond	Sunflower
C12:0	0.10±0.02^{a}	0.26±0.03^{b}	0	0	0	0.06±0.01^{a}	0
C14:0	0.76±0.01^{a}	1.60±0.14^{b}	0.08±0.01^{c}	0.07±0.02^{c}	0.04±0.02^{d}	0.13±0.01^{c}	0.18±0.01^{c}
C16:0	37.86±0.15^{a}	35.19±1.07^{b}	8.97±0.23^{c}	22.84±0.23^{d}	15.24±0.04^{e}	9.79±0.61^{c}	9.26±0.07^{c}
C16:1 n9	0.27±0.02^{a}	0	0	0	0.08±0.03^{b}	0	0
C16:1 n7	0.55±0.07^{a,e}	0.73±0.13^{a}	0.07±0.01^{b}	9.17±0.04^{c}	0.72±0.06^{a}	0.46±0.00^{e}	0
C18:0	10.43±0.28^{a}	9.98±0.50^{a}	4.60±0.09^{b}	0.77±0.03^{c}	2.81±0.18^{d}	2.53±0.12^{d,e}	3.62±0.05^{d,f}
C18:1 n9	20.50±0.32^{a}	25.94±0.51^{b}	23.34±0.14^{c}	48.67±0.22^{d}	69.29±0.03^{e}	66.06±0.12^{f}	30.40±0.00^{g}
C18:1 n7	0.92±0.07^{a}	0	1.15±0.18^{a}	7.82±0.04^{b}	3.88±0.30^{c}	3.15±0.54^{c}	1.87±0.25^{a}
C18:2 n6	27.08±0.01^{a}	17.84±0.19^{b}	16.19±0.04^{c}	10.21±0.10^{d}	7.03±0.05^{e}	17.82±0.05^{b}	54.67±0.28^{f}
C18:3 n3	1.20±0.06^{a}	8.45±0.11^{b}	45.37±0.03^{c}	0.45±0.04^{d}	0.52±0.09^{d}	0	0
C20:0	0.33±0.01^{a}	0	0.13±0.05^{a}	0	0.26±0.12^{a}	0	0
C20:1 n9	0	0	0.09±0.00^{a}	0	0.13±0.02^{a}	0	0
SFA	49.48±0.43^{a}	47.03±0.46^{b}	13.78±0.39^{c}	23.68±0.28^{d}	18.35±0.11^{e}	12.51±0.71^{c}	13.06±0.03^{c}

UFA	50.52±0.43[a]	52.97±0.46[b]	86.22±0.39[c]	76.32±0.28[d]	81.65±0.11[e]	87.49±0.71[c]	86.94±0.03[c]
MUFA	22.24±0.48[a]	26.68±0.38[b]	24.66±0.32[c]	65.66±0.22[d]	74.11±0.16[e]	69.67±0.66[f]	32.27±0.25[g]
PUFA	28.29±0.05[a]	26.29±0.09[b]	61.56±0.07[c]	10.66±0.06[d]	7.54±0.04[e]	17.82±0.05[f]	54.67±0.28[g]
n-6	27.08±0.01[a]	17.84±0.19[b]	16.19±0.04[c]	10.21±0.10[d]	7.03±0.05[e]	17.82±0.05[b]	54.67±0.28[f]
n-3	1.20±0.06[a]	8.45±0.11[b]	45.37±0.03[c]	0.45±0.04[d]	0.52±0.09[d]	0	0

Results are expressed as mean ± standard deviation. Different letters are significantly different (Tukey test, $p < 0.05$) in each row.

L. migratoria was divided in half for MUFA (26.68%) and PUFA (26.29%). While the *A. domesticus* had values more balanced toward the PUFA (28.29%), mainly due to the content of linoleic acid. *A. domesticus* and *L. migratoria* lipids were combined with all vegetable oils, resulting in samples with diverse fatty acid profiles, shown in Supplementary Table S3. The samples formulated with olive, almond, and avocado oils were richer in MUFA due to their high oleic acid content. In contrast, samples with higher amounts of sunflower and linseed oils had a higher percentage of PUFA, as they contribute linoleic and α-linolenic acid, respectively. Samples with a greater proportion of insect lipids had a more balanced composition, with insect lipids being the primary contributors to SFA.

PLSR models to predict SFA, MUFA, and PUFA

SFA, MUFA, and PUFA values were selected to create the prediction models, whereas UFA was discarded because of their roundness, and the n6 and n3 values were excluded because they did not differ from the linoleic and α-linolenic acid values, respectively. The statistical performance of the PLSR models developed is summarized in Table 3. The listed values include the data from both cross-validation and external validation, with the number of samples used varying between models due to the removal of samples with high leverage or residuals (Aykas *et al.*, 2020). To enhance the prediction quality, models were developed with the selected regions of the spectrum, focusing on characteristic lipid bands and removing infrared signals with irrelevant or noisy information. The number of factors selected was determined by minimizing SE_{CV} and R_{CV} values (factors<10), with 7 or 8 factors used during calibration to prevent under- or over-fitting the models. The PLSR models in this study showed a strong correlation ($0.987 \leq R_{CV} \leq 0.999$) and low error ($0.24\% \leq SE_{CV} \leq 2.21\%$) in the prediction of SFA, MUFA, and PUFA. Notably, models generated from spectra collected by the portable MIR spectrometer exhibited the best predictive performance, with lower error and higher correlation coefficients. The PLSR model performance was also evaluated using the external validation set, revealing similar performance across models in terms of error (SE_{CV} and SE_P) and correlation coefficient (R_{CV} and R_P). The SE_P values ranged from 0.30 to 0-54% for the three fatty acids groups in MIR validation models and ranged from 1.41 to 3.85% for NIR external validation models. Higher performance was observed with MIR compared to NIR, with RPD values based on MIR data exceeding 8, indicating excellent prediction, while NIR-based RPD values exceeded 3.1, making them suitable for screening or even quality control, particularly for SFA results. In both cases, SFA prediction showed better accuracy compared to MUFA and PUFA predictions. The predicted values from MIR and NIR for cross-validation and external validation are shown in Figure 6 and Figure 7, respectively. The regression line demonstrates how well the regression aligns with the best possible slope (slope=1) represented by the dotted line, with none of the predicted parameters deviating significantly from optimal. Additionally, it is possible to observe that the entire range included in the model is well-represented by the samples.

Table 3. Statistical performance of calibration and external validation models developed for predicting SFA, MUFA, and PUFA content based on MIR and NIR data.

		Range cal.	N_{CV}	F	R_{CV}	SE_{CV}	Range val.	N_P	R_P	SE_P	RPD
SFA	MIR	12.51-49.48	105	8	0.999	0.24	19.46-48.97	39	0.999	0.30	28.8
	NIR	12.51-49.48	88	7	0.993	1.26	19.46-48.97	40	0.989	1.41	6.2
MUFA	MIR	22.24-74.11	100	8	0.999	0.66	22.75-64.50	39	0.999	0.50	26.1
	NIR	22.24-74.11	84	7	0.987	2.21	22.75-64.50	40	0.965	3.40	3.7
PUFA	MIR	7.54-61.56	102	8	0.999	0.68	11.34-54.74	39	0.999	0.54	22.7
	NIR	7.54-61.56	80	8	0.992	1.49	11.34-54.74	40	0.955	3.85	3.1

Abbreviations used: N_{CV}: number of samples in the calibration set; F: number of factors; R_{CV}: correlation coefficient of cross-validation; SE_{CV}: standard error of cross-validation; N_P: number of samples in the validation set; R_P: correlation coefficient of validation; SE_P: standard error of validation; RPD: Ratio of performance to deviation.

PLSR models to predict fatty acid content

A total of 12 fatty acids were detected in the vegetable oils and insect lipids, with six selected to build PLSR prediction models. These fatty acids were chosen due to their high variability and prominence in insect lipids. The selected fatty acids were myristic (0.04–1.60%), palmitic (8.97–37.86%), stearic (0.77–10.43%), oleic (20.50–69.29%), linoleic (7.03–54.67%), and α-linolenic (0.00–45.37%). The remaining fatty acids almost overlap the minimum and the maximum values. The statistics of PLRS performance of each device for each parameter are summarized in Table 4 and represented in Figure 8 and Figure 9. The prediction models were developed following the same guidelines as the previous ones and using a limited spectral range focused on the lipid region. The plot of reference values versus those predicted by the PLSR models shows that the samples are well-distributed across the whole range, except in the case of α-linolenic acid. The highest contribution of α-linolenic acid was from linseed oil, which had a content 5.4 times higher than *L. migratoria* and 37.8 times higher than *A. domesticus*. Not in such a perfect way, this distribution can be also observed in the case of linoleic acid, with sunflower oil and *A. domesticus* being the two major contributors. Overall, the PLSR models based on the spectra collected by the portable MIR device showed greater predictive capabilities compared to the handheld NIR spectrometer, following the performance of both devices in predicting SFA, MUFA, and PUFA. The correlation coefficient achieved during the calibration (R_{CV}) was over 0.995 for all the variables and the errors (SE_{CV}) ranged from 0.04 to 0.87%. The models were applied to predict values from the external validation set and evaluate its predictive performance. The SE_P (0.04 - 0.66%) had values around the corresponding SE_{CV} and also for the coefficients of correlation (R_P>0.994). Based on RPD values obtained from the validation set prediction (RPD > 8.2), the models demonstrated excellent predictive performance, reaching a value of 23.9 for oleic acid quantification. These MIR models demonstrated better performance for the prediction of the main FA composition than the results obtained by Liu *et al.* (2021). These authors analyzed 50 insect oil samples with a benchtop MIR spectrometer and created PLSR models to quantify 7 fatty acids that achieved SE_P value under 4.87% but RPD values between 1.04 to 2.88. There are no other studies that analyze the lipid profile of insects based on mid-infrared spectra collected with a portable device with such variability, but

the results obtained can be compared with those obtained in oils of another origin. Plans *et al.* (2015) quantified levels of major FA in omega-3 oil dietary supplements of fish, cod liver, and flaxseed oil, samples rich in PUFA and n3, their sample set has higher variability in these compounds. The PLSR models based on the MIR spectra obtained with a 5-bounce ZnSe crystal ATR accessory also showed robustness with a high correlation coefficient ($R_P \geq 0.93$), and SE_P between 0.53 and 2.13%. Salas-Valerio *et al.* (2022) analyzed a smaller sample set of butter and margarine, validation with an independent sample set showed errors below 2.4% and a high correlation with a single bounce device as used in this work. Using an accessory ATR crystal with a higher number of bounces (five) the results improved to errors below 2.0%.

The calibration results obtained by NIR were lower than those achieved by MIR analysis. However, NIR spectra showed SE_{CV} ranged from 0.04 and 2.74% and good linearity (R_{CV}>0.979), except for linoleic acid, whose prediction was not as robust (R_{CV}=0.88, SE_P=4.64%), it could be a problem of sampling as mentioned above. The results of the external validation show values similar to those obtained in the internal validation carried out using cross-validation. The RPD value was also calculated, and the results were above 3 for the prediction of the individual unsaturated fatty acid (myristic, palmitic, and stearic acids), and α-linolenic acid suggesting that the generated models could be successfully used for screening. In the case of myristic acid, the prediction by NIR is comparable to MIR, the errors and correlations are equal in both calibration and validation, and even the RPD value was higher for the PLSR algorithm-created NIR spectral data. These results indicate that the spectral information in the IR region can provide enough information to obtain the fatty acid profile. The PLSR NIR models built up in this study were comparable to those reported by Yao *et al.* (2020) and Salas-Valerio *et al.* (2022) for the prediction of individual FA using handheld NIR devices with the same technology, but with three tungsten lamps, specifically the NeoSpectra Micro device (Si-Ware Systems, Inc., Cairo, Egypt). Yao *et al.* (2020) reported SE_P ranging from 1.60 to 3.55% for the main FA present in chip frying oil, while Salas-Valerio *et al.* (2022) predicted the main FA in butter and margarine with RPD values ranging from 2.5 to 6. These findings are in line with the RPD values obtained in this study for the quantification of FA in insect lipid samples, which ranged from 2.3 to 9.0.

Table 4. Statistical performance of calibration and external validation models developed for fatty acids content based on MIR and NIR data.

		Range cal.	N_{CV}	F	R_{CV}	SE_{CV}	Range val.	N_P	R_P	SE_P	RPD
Myristic acid C14:0	MIR	0.04-1.60	102	6	0.995	0.04	0.20-1.43	39	0.994	0.04	8.2
	NIR	0.04-1.60	102	6	0.994	0.04	0.20-1.43	40	0.994	0.04	9.0
Palmitic acid C16:0	MIR	8.97-37.86	100	8	0.999	0.34	14.23-37.31	39	0.999	0.40	16.8
	NIR	8.97-37.86	88	7	0.993	0.97	14.23-37.31	40	0.976	1.68	4.1
Stearic acid C18:0	MIR	0.77-10.43	105	8	0.999	0.13	2.64-10.34	39	0.999	0.11	18.1
	NIR	0.77-10.43	87	6	0.996	0.19	2.64-10.34	40	0.971	0.41	4.5
Oleic acid C18:1 n9	MIR	20.50-69.29	102	8	0.999	0.67	21.10-60.51	39	0.999	0.49	23.9
	NIR	20.50-69.29	84	8	0.979	2.74	21.10-60.51	40	0.945	4.15	2.8
Linoleic acid	MIR	7.03-54.67	102	8	0.996	0.87	9.22-49.00	39	0.998	0.58	16.2

C18:2 n6	NIR	7.03-54.67	84	8	0.880	4.64	9.22-49.00	40	0.902	4.07	2.3
α-linolenic acid C18:3 n3	MIR	0.00-45.37	99	7	0.997	0.73	0.25-37.96	39	0.998	0.66	14.8
	NIR	0.00-45.37	82	9	0.984	1.97	0.25-37.96	40	0.980	2.07	4.8

Abbreviations used: N_{CV}: number of samples in the calibration set; F: number of factors; R_{CV}: correlation coefficient of cross-validation; SE_{CV}: standard error of cross-validation; N_P: number of samples in the validation set; R_P: correlation coefficient of validation; SE_P: standard error of validation; RPD: Ratio of performance to deviation.

4. Conclusion

Portable MIR and handheld NIR spectroscopic spectrometers represent a reliable, cost-effective, and rapid tool for quantifying insect lipids and determining their fatty acid profile *in situ*. Both miniaturized devices successfully quantified the insect lipid content in oil mixtures, with a SE_P below 2.21%, R_P higher than 0.99, and RPD values greater than 9.23. PLSR models developed using both MIR and NIR spectral data demonstrated strong performance in predicting the SFA, MUFA, and PUFA content, as well as individual fatty acids, in insect lipids and related samples. Overall, the models based on MIR spectra exhibited superior predictive accuracy compared to those from the portable NIR spectrometer, obtaining SE_P values ranging from 0.04% to 0.66% and RPD values over 8.2. This method offers a promising alternative for farmers and the insect industry, enabling rapid and accurate analysis of lipids with minimal sample amounts and no need for sample pretreatment.

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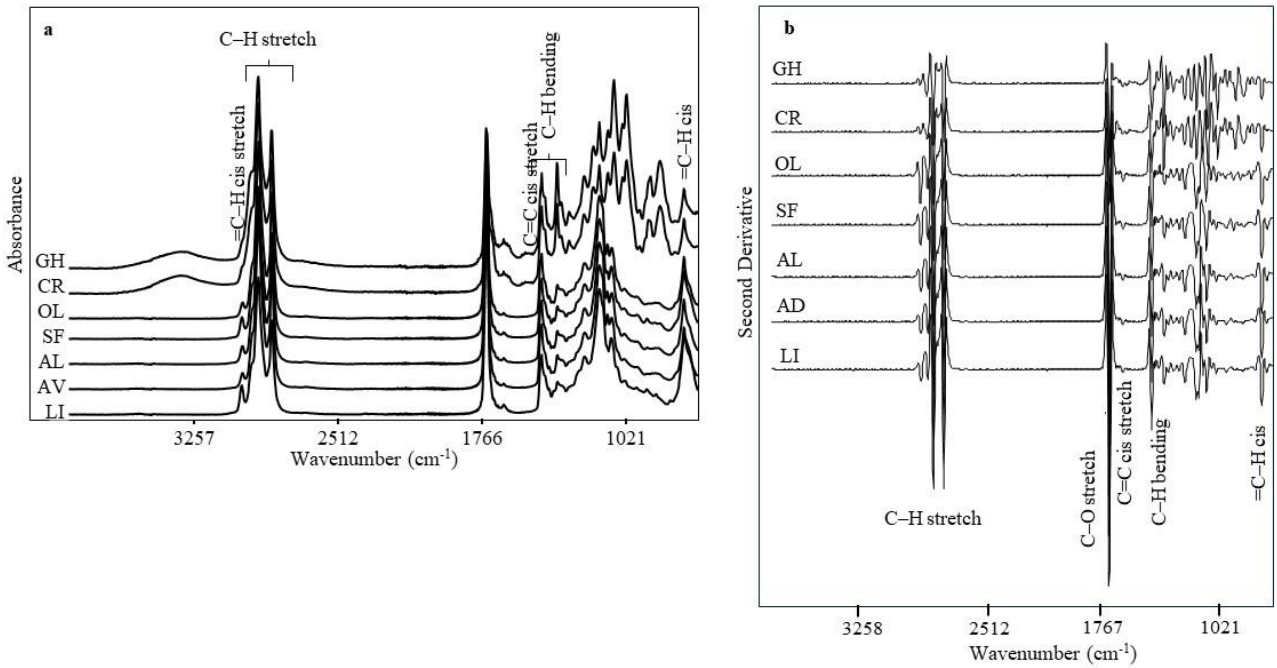

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571 **Figure 1.** Raw MIR spectral data (a) and (b) second derivative (17-point window size) of no mixed
572 insect lipids and vegetable and seeds oils.

573

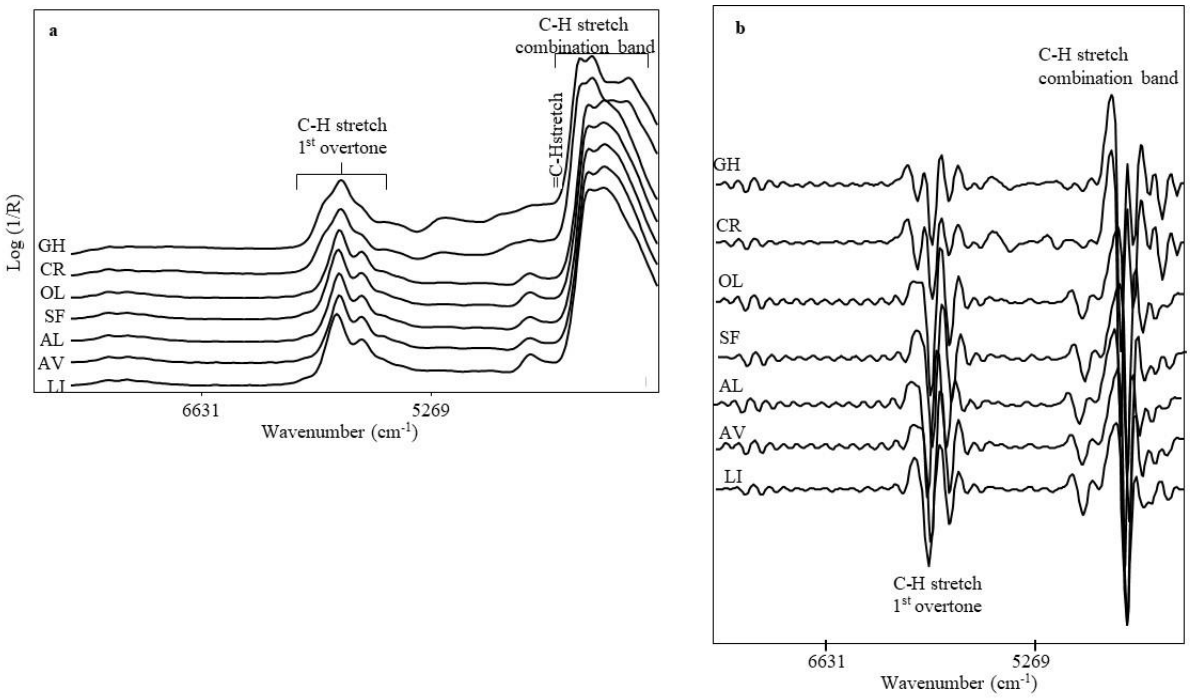


Figure 2. Raw NIR spectral data (a) and (b) second derivative (9-point window size) of no mixed insect lipids and vegetable and seeds oils

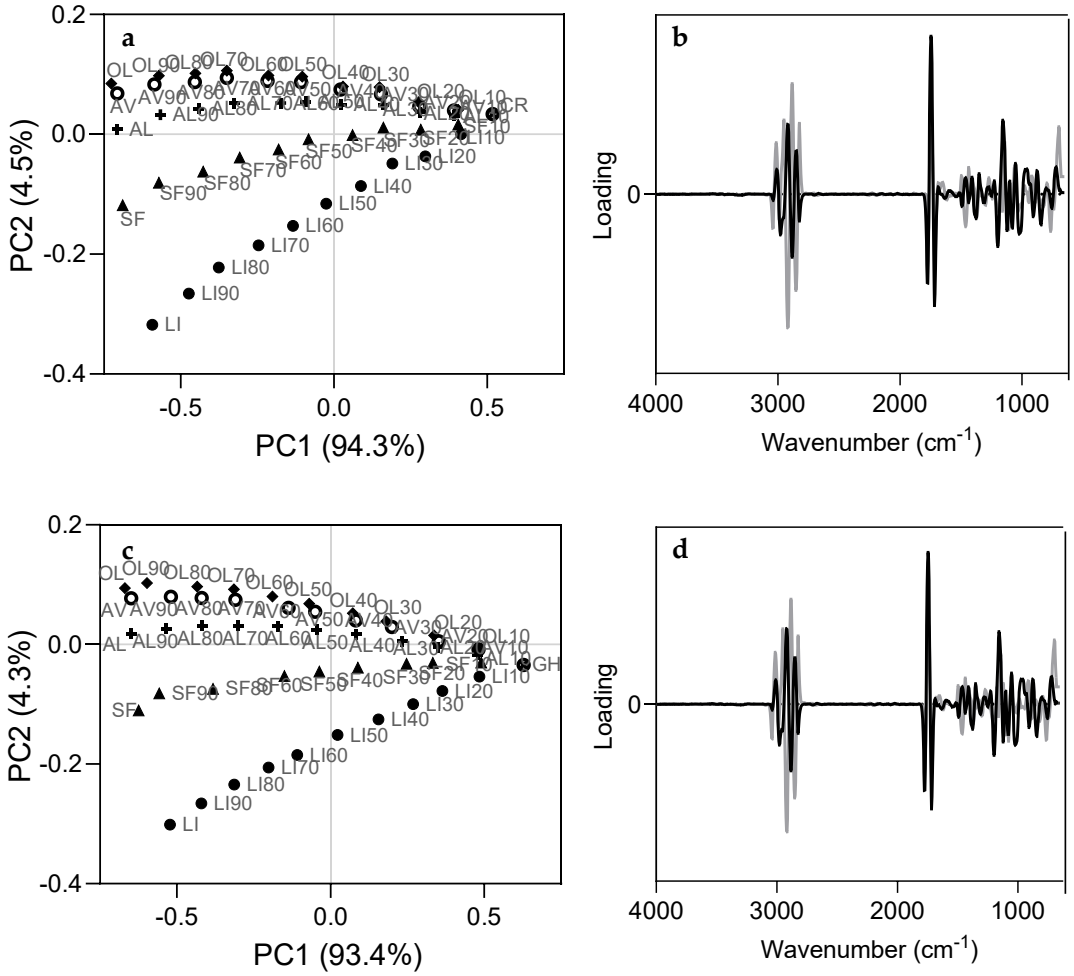


Figure 3. PCA MIR plot of (a) *A. domesticus* and (c) *L. migratoria* and loadings of (b) *A. domesticus* and (d) *L. migratoria*. PC1: black; PC2: grey.
Abbreviations used: LM: *L. migratoria*, AD: *A. domesticus*, OL: olive oil, SF: sunflower oil, AL: almond oil, AV: avocado oil, LI: linseed oil.

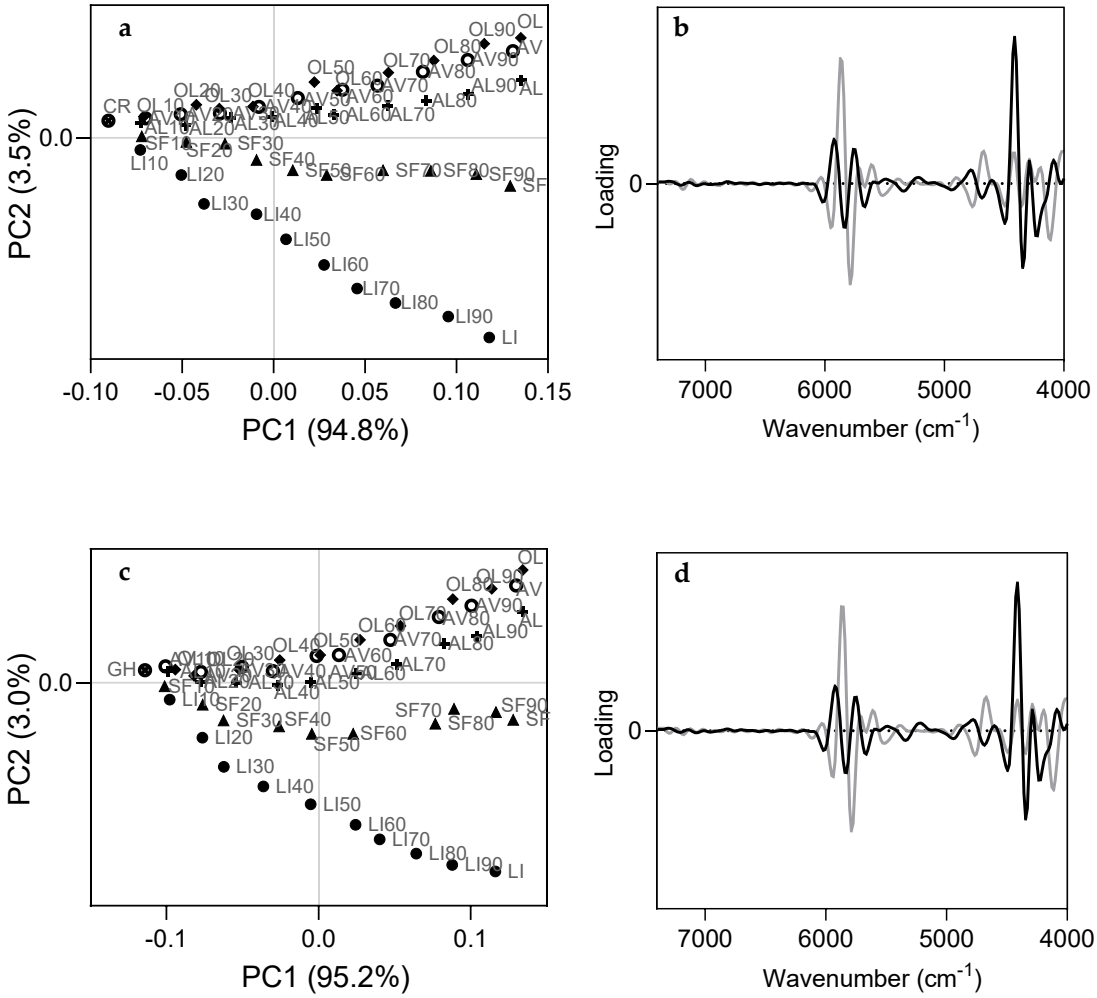
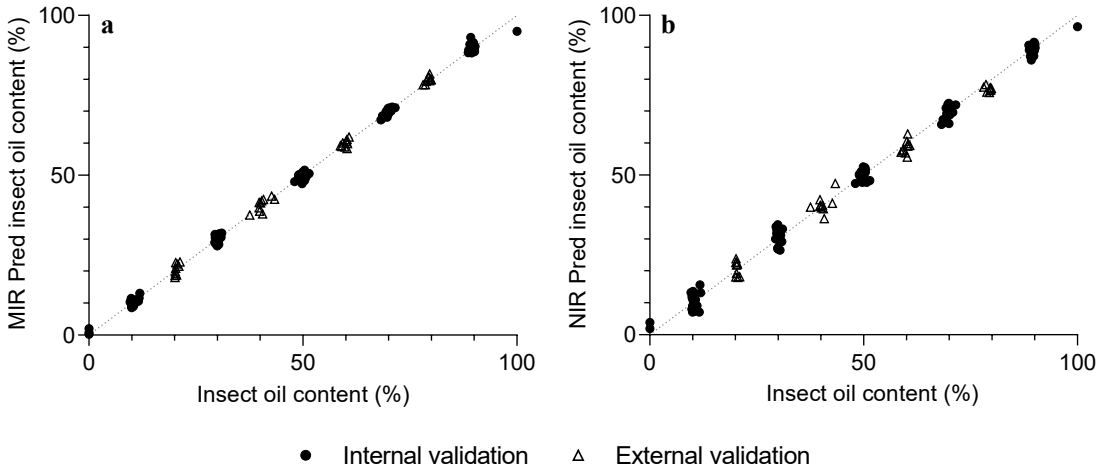


Figure 4. PCA NIR plot of (a) *A. domesticus* and (c) *L. migratoria* and loadings of (b) *A. domesticus* and (d) *L. migratoria*. PC1: black; PC2: grey.

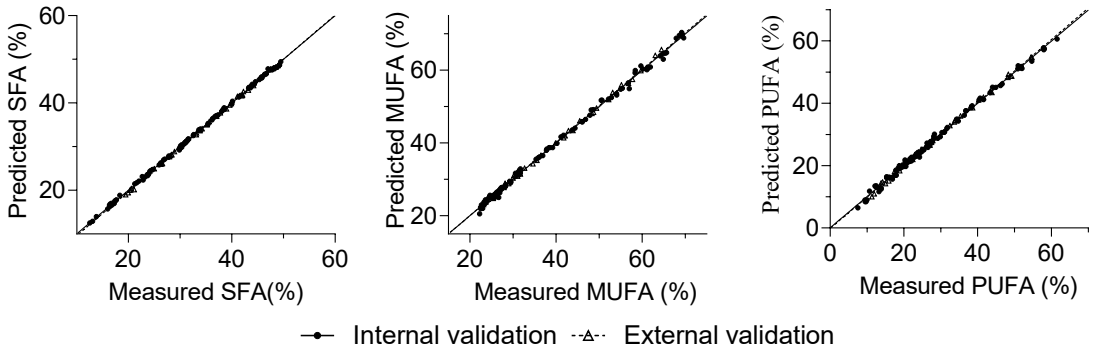
Abbreviations used: LM: *L. migratoria*, AD: *A. domesticus*, OL: olive oil, SF: sunflower oil, AL: almond oil, AV: avocado oil, LI: linseed oil.



596

597 **Figure 5.** PLSR models calibration and validation plots for insect content prediction using (a) MIR
598 and (b) NIR spectra.
599

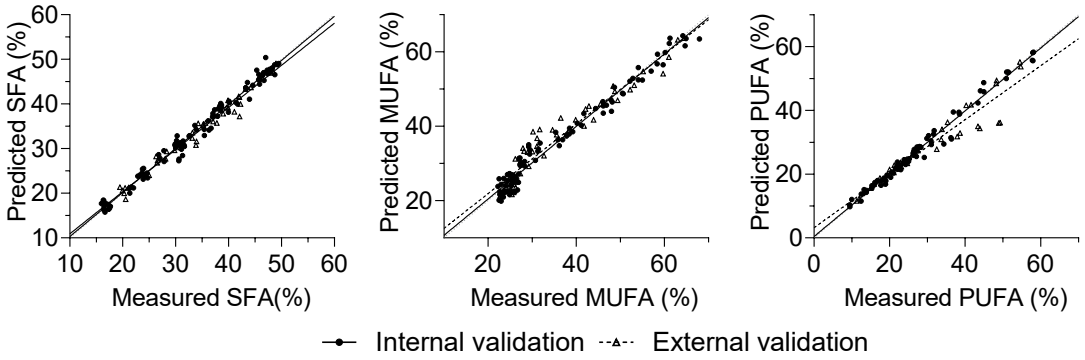
600



601

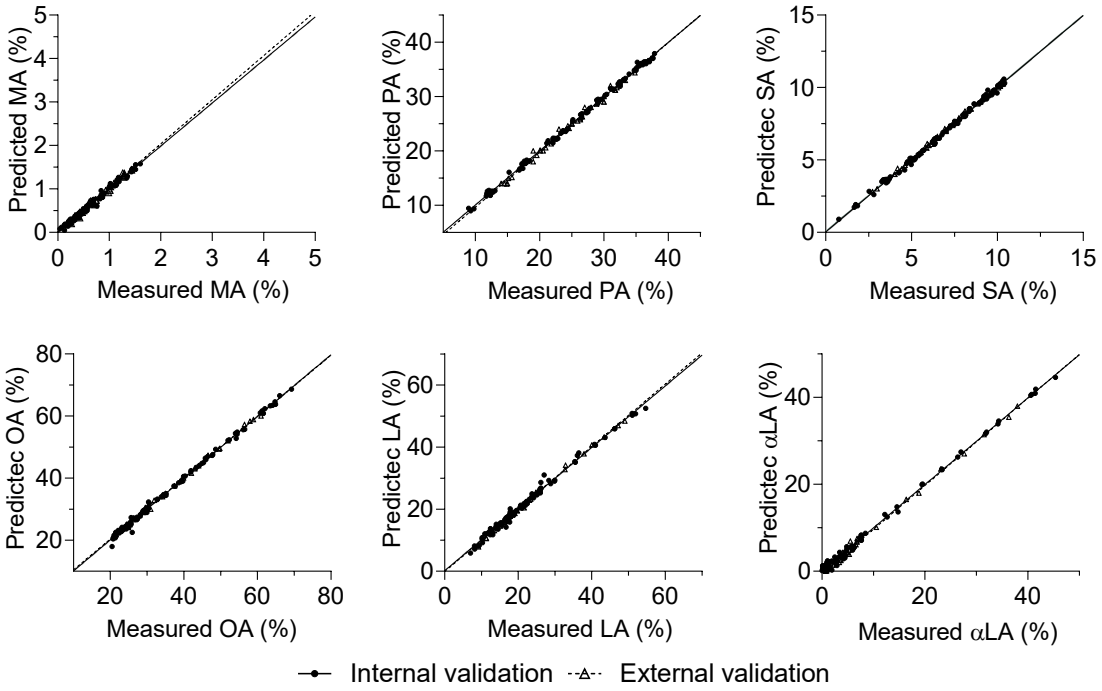
602 **Figure 6.** PLSR MIR models calibration and validation plots for SFA, MUFA, and PUFA
603 quantification.

604



605
606 **Figure 7.** PLSR NIR models calibration and validation plots for SFA, MUFA, and PUFA
607 quantification.

608



609

610 **Figure 8.** PLSR MIR models calibration and validation plots for fatty acid prediction.
611 Abbreviations used: MA: myristic acid; PA: palmitic acid; SA: stearic acid; OA: oleic acid; LA:
612 linoleic acid; αLA: α-linolenic acid.

613

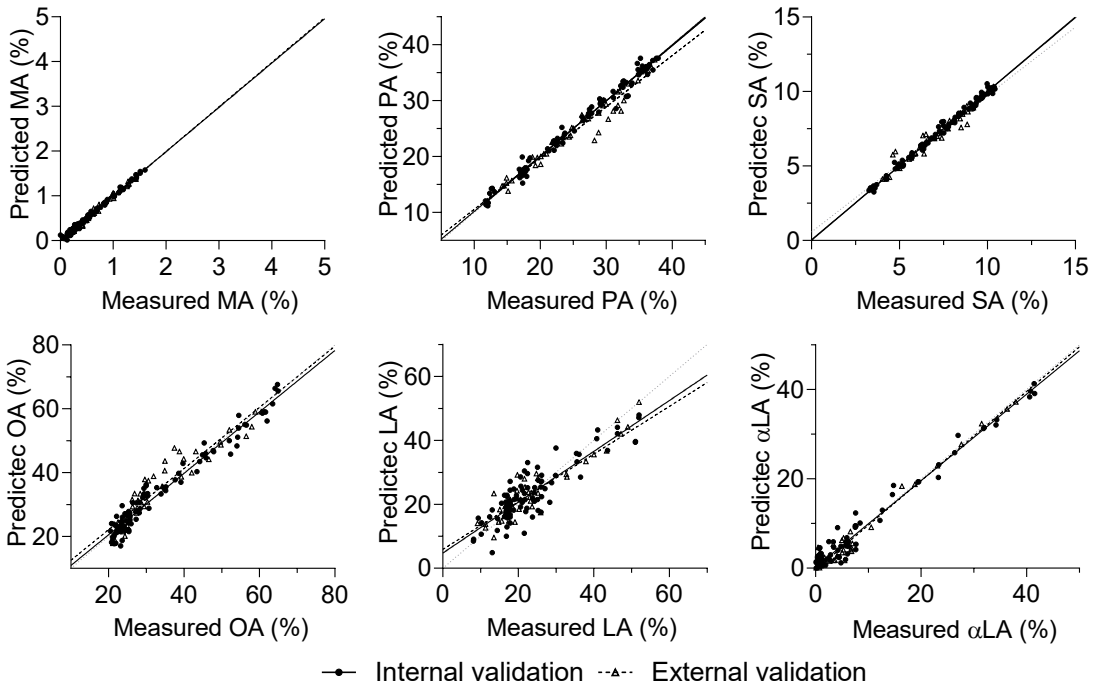


Figure 9. PLSR NIR models calibration and validation plots for fatty acid prediction. Abbreviations used: MA: myristic acid; PA: palmitic acid; SA: stearic acid; OA: oleic acid; LA: linoleic acid; αLA: α-linolenic acid.

Table S1. The average oil content in blends of *A. domesticus* lipids with vegetable and seed oils is expressed as the percentage of insect lipids relative to the total weight of the sample.

***A. domesticus* (%)**	**Olive oil (%)**	**Sunflower oil (%)**	**Almond oil (%)**	**Avocado oil (%)**	**Linseed oil (%)**
0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
10.6 ± 0.7	88.8 ± 1.0	90.1 ± 0.2	89.0 ± 0.8	90.0 ± 0.1	89.3 ± 0.3
20.7 ± 0.0	78.7 ± 0.0	79.4 ± 0.0	79.0 ± 0.0	79.7 ± 0.0	79.5 ± 0.0
30.4 ± 0.4	69.7 ± 0.1	69.5 ± 0.1	69.5 ± 0.5	69.7 ± 1.1	69.5 ± 0.2
40.8 ± 0.0	59.6 ± 0.0	59.2 ± 0.0	57.3 ± 0.0	60.2 ± 0.0	59.9 ± 0.0
49.6 ± 0.7	50.5 ± 0.7	50.1 ± 0.6	51.1 ± 1.1	50.3 ± 1.0	50.0 ± 0.1
59.8 ± 0.0	39.8 ± 0.0	39.2 ± 0.0	41.2 ± 0.0	40.7 ± 0.0	39.9 ± 0.0
69.6 ± 0.6	30.1 ± 0.1	30.4 ± 0.3	30.2 ± 1.1	30.8 ± 0.8	30.5 ± 0.3
79.6 ± 0.0	20.1 ± 0.0	20.4 ± 0.0	20.0 ± 0.0	20.3 ± 0.0	21.2 ± 0.0
89.6 ± 0.5	10.0 ± 0.1	10.3 ± 0.3	10.6 ± 1.0	10.9 ± 0.2	10.4 ± 0.3
100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table S2. The average oil content in blends of *L. migratoria* lipids with vegetable and seed oils is expressed as the percentage of insect lipids relative to the total weight of the sample.

L. migratoria (%)	Olive oil (%)	Sunflower oil (%)	Almond oil (%)	Avocado oil (%)	Linseed oil (%)
0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
10.3 ± 0.6	89.7 ± 0.2	90.0 ± 0.1	89.5 ± 0.7	89.3 ± 1.5	89.5 ± 0.2
20.3 ± 0.0	79.7 ± 0.0	79.7 ± 0.0	79.9 ± 0.0	79.8 ± 0.0	79.9 ± 0.0
28.5 ± 0.4	70.1 ± 0.7	78.9 ± 0.2	70.3 ± 0.4	70.3 ± 0.1	69.9 ± 0.2
40.2 ± 0.0	60.1 ± 0.0	60.2 ± 0.0	59.4 ± 0.0	56.6 ± 0.0	62.4 ± 0.0
50.1 ± 0.6	50.4 ± 0.3	50.1 ± 0.7	49.4 ± 0.2	49.8 ± 0.2	49.4 ± 1.2
59.9 ± 0.0	39.7 ± 0.0	40.9 ± 0.0	40.2 ± 0.0	39.6 ± 0.0	39.7 ± 0.0
69.8 ± 0.9	31.1 ± 0.9	29.1 ± 0.9	30.3 ± 1.7	30.2 ± 0.2	29.9 ± 0.1
79.2 ± 0.0	20.7 ± 0.0	21.9 ± 0.0	20.6 ± 0.0	20.4 ± 0.0	21.4 ± 0.0
89.3 ± 0.5	10.8 ± 0.1	10.0 ± 0.3	10.9 ± 0.2	10.9 ± 0.7	10.8 ± 0.9
100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

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Table S3. The average SFA, MUFA, and PUFA content in blends of *A. domesticus* and *L. migratoria* lipids with vegetable and seed oils included in the calibration set. The values are expressed as a percentage of the total sample weight. The label number indicate the vegetable oil percentage.

	A. domesticus			*L. migratoria*		
	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Sunflower 90%	16.64	31.28	52.08	16.45	31.71	51.84
Sunflower 70%	24.16	29.21	46.63	23.33	30.58	46.09
Sunflower 50%	31.09	27.30	41.61	30.19	29.45	40.36
Sunflower 30%	38.34	25.31	36.36	36.90	28.34	34.75
Sunflower 10%	45.67	23.29	31.05	43.70	27.23	29.08
Olive 90%	22.05	67.93	10.01	21.24	69.32	9.44
Olive 70%	27.76	58.42	13.82	26.78	60.17	13.05
Olive 50%	33.91	48.17	17.91	32.64	50.48	16.88
Olive 30%	40.14	37.79	22.06	38.28	41.15	20.57
Olive 10%	46.38	27.39	26.22	43.90	31.85	24.25
Avocado 90%	26.24	61.36	12.41	25.93	61.90	12.17
Avocado 70%	31.29	52.85	15.86	30.61	54.09	15.30
Avocado 50%	36.32	44.39	19.29	35.38	46.13	18.49
Avocado 30%	41.68	35.36	22.96	40.01	38.39	21.60
Avocado 10%	46.64	27.01	26.35	44.38	31.09	24.52
Linseed 90%	17.51	24.40	58.08	17.33	24.87	57.80
Linseed 70%	24.60	23.92	51.48	23.85	25.27	50.88
Linseed 50%	31.64	23.45	44.91	30.89	25.70	43.41
Linseed 30%	38.52	22.98	38.50	37.06	26.07	36.87
Linseed 10%	45.85	22.48	31.67	43.65	26.47	29.87
Almond 90%	16.79	64.18	19.03	16.32	64.92	18.76
Almond 70%	23.92	55.03	21.05	22.68	57.00	20.32
Almond 50%	30.86	46.12	23.02	30.01	47.87	22.12
Almond 30%	38.62	36.17	25.21	36.16	40.21	23.63
Almond 10%	45.31	27.58	27.11	43.32	31.29	25.38