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Stable isotope ratios and current-use pesticide levels in edible insects: Implications on chemical food safety

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ABSTRACT

In the past years, the European Union (EU) has added edible insects to the list of novel foods, allowing an increasing number of insect-based products into the European market. With insects gaining more popularity in the Western world, it is crucial to investigate their chemical food safety. This study aimed at investigating possible isotopic patterns in different edible insect species (n = 52) from Asia, Africa and Europe using stable isotope ratio analysis (SIRA) to provide a framework for future investigations on food authenticity and traceability. Additionally, complementary mass-spectrometric screening approaches were applied to gain a comprehensive overview of contamination levels of current-use pesticides (CUPs) in edible insects, to assess their chemical food safety. SIRA revealed significant differences between countries in $\delta^{13}C_{VPDB}$- ($p < 0.001$) and $\delta^{15}N_{air}$- ($p < 0.001$) values. While it was not possible to distinguish between individual countries using principal component analysis (PCA) and linear discriminative analysis (LDA), the latter could be used to distinguish between larger geographical areas (i.e. Africa, Europe and Asia). In general, African samples had a more distinct isotopic profile compared to European and Asian samples. When comparing the isotopic compositions of samples containing pesticides with samples with no detected pesticides, differences in sulphur compositions could be observed. Additionally, LDA was able to correctly classify the presence of pesticides in a sample with 76% correct classification based on the sulphur composition. These findings show that SIRA could be a useful tool to provide a framework for future investigations on food authenticity and traceability of edible insects. A total of 26 CUPs were detected using suspect screening and an additional 30 CUPS were quantified using target analysis, out of which 9 compounds had a detection frequency higher than 30%. Most detected pesticides were below the maximum residue levels (MRLs) for meat, suggesting low contamination levels. However, dichlorvos and fipronil could be detected in the same order of magnitude as the MRLs, even in samples purchased in Europe. These findings indicate a limited chemical risk for edible insects regarding pesticide contamination. Nevertheless, the study also highlights that further and more extensive investigations are needed to give a comprehensive assessment of the chemical risk of edible insects as a novel food source in Europe. With insects recently being potentially more incorporated into daily diets, more attention should be paid to possible chemical hazards to accurately assess their risk and to ensure food safety.

1. Introduction

With a continuously growing world population, alternative food sources with high nutritional value are becoming more and more important and will be a crucial factor in the future global food system (FAO, 2018a). With an estimated 10 billion people by 2050, it is believed that 50% more food will need to be produced to feed the world population (FAO, 2018a). To avoid irreversible changes in our environment and to ensure food security on a global scale, alternative and more sustainable food sources need to be introduced. While plant-based diets are gaining popularity in Western countries, another promising option that has already been consumed for centuries in many parts of the world is represented by edible insects (van Huis, 2020). Due to their high nutritional value, insects already play an important role in the diets of

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people from Africa, Asia and Latin America (FAO, 2013). While nutritional values depend on species and life stage, insects are generally considered a valuable food source of protein and fat (Belluco et al., 2013). In addition, they also provide important vitamins, minerals, and fibres, due to their high chitin content.

Compared to common livestock, insects are considered a more sustainable food source. They generally use less resources such as water, energy, and space, and produce less greenhouse gas emissions (Oonincx et al., 2010). Additionally, insects have a high feed-conversion efficiency, and they can be reared on organic residues, while simultaneously reducing waste (FAO, 2013). In recent years, more and more insect species have been introduced into the European market and are being regulated by the European Union (EU) under the novel food regulation (Regulation (EC) 258/97 of the European Parliament and the Council of 27 January 1997 Concerning Novel Food and Novel Food Ingredients, 1997). With insects gaining more popularity, it is crucial to investigate their chemical food safety to move forward with this novel protein source. While some European studies have been focusing on chemical food safety of edible insects in recent years (De Paepe et al., 2019; Poma et al., 2017; Poma et al., 2019; Truzzi et al., 2019), studies focusing on pesticides that might accumulate in the insects are still scarce and needed to ensure the safety of this novel food.

Pesticides are used in a variety of applications and purposes, such as conventional agriculture, gardening, forestry or railroad maintenance (Schleiffer & Speiser, 2022). While the highly toxic legacy pesticides (i.e. organochlorines) have been banned for agricultural use, there are more than 450 current use-pesticides (CUPs) approved in the European Union and each year more than 333,000 tonnes of CUPs are sold in the EU (Eurostat, 2023). This extensive use can lead to pesticide occurrence and accumulation far beyond the application sites, entering the environment and eventually the food chain. While CUPs are generally considered a less persistent and accumulative alternative to legacy pesticides, some of these compounds have been associated with adverse environmental and health effects (Veludo et al., 2022). To reduce the risk of human exposure to pesticides and the associated health impacts, it is crucial to gain a comprehensive understanding of contamination levels of different food sources to develop appropriate measures to guarantee food safety. However, due to the large variety of pesticide classes and structures, pesticide residue determination can be a challenging task. While targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) is commonly used to detect and quantify pesticide residues in food samples, it only focuses on a predefined list of compounds (Picó et al., 2018), while high-resolution (HR) MS allows for a broader screening of a large range of compounds without the need of any “a priori” defined list of standards (Masiá et al., 2016). By combining the two approaches, a more accurate and realistic estimation of pesticide residue contamination can be achieved.

Along with assessment of food safety, food authenticity and traceability have become important factors to protect consumer health and ensure food security. Food authenticity includes misidentification and extension of food using cheaper substitutes or adulterants, while traceability is the ability to follow a food product through all stages of the supply chain (Camin et al., 2016; Food Traceability, 2007). Misidentification and adulteration can affect overall food characteristics and quality and may even result in unsafe products. An objective and reliable assessment of food authenticity is therefore crucial to prevent food fraud and ensure food safety. One of the most important techniques used nowadays is stable isotope ratio analysis (SIRA). This technique is based on the fact that materials have an “isotopic fingerprint”, consisting of a specific combination of ratios of the stable isotopes of certain elements. While these abundances were fixed when the earth was formed, there are subtle variations introduced through physical, chemical or biological processes (Dunn & Carter, eds. 2018), which are characteristic for the origin and history of a substance and can be used to interpret them. To assess food authenticity or adulteration using SIRA, however, reference databases consisting of authentic samples with known characteristics (e.g. origin) need to be established, so unknown samples can be compared to this data (Camin et al., 2017; Donarski et al., 2019). For many food products, like wine (Dordevic et al., 2013), cheese (Pianezze et al., 2020) and honey (Bontempo et al., 2020), extensive datasets already exist and are widely used for authentication purposes. In 2021 Pianezze et al. (Pianezze et al., 2021) conducted a pilot investigation on stable isotopes in edible insects, providing a first reference background. However, extensive datasets for stable isotopes in edible insects have not yet been established and would be useful for future investigations on their authenticity, for origin testing, quality control and environmental impact assessment of edible insect products.

Therefore, in this study, SIRA was used to investigate possible isotopic patterns in different farmed and wild edible insect species from Africa, Asia and Europe to provide a framework for future investigations on food authenticity and traceability. Additionally, a suspect screening approach using HRMS was combined with quantitative targeted analysis to investigate the presence and occurrence of CUPs. These complementary screening approaches were applied to gain broader and more accurate information on contamination levels in edible insects, in order to assess their chemical food safety. In particular, this study aimed at (i) establishing a first reference database for isotopic values of different edible insect species and investigating possible patterns and (ii) using different mass-spectrometric screening approaches to gain a comprehensive overview of contamination levels of CUPs in edible insects.

2. Materials and methods

2.1. Sampling

A total of 52 samples of edible insects were purchased between 2018 and 2021 from different shops and websites in Asia and Europe, while the samples from Africa, i.e. Nigeria and Uganda, were obtained from local farms or open markets in 2022 (McGrath et al., 2022; Poma et al., 2021; Poma et al., 2022; Poma et al., 2019). Samples were chosen according to availability in the different countries, aiming at acquiring a large selection of different species, while particularly focusing on the species that are currently regulated under the novel food regulation in Europe (i.e. house cricket, mealworm and locust). A detailed description of the collected samples can be found in Table S1. In brief, the insect samples belonged to seven orders, Blattodea (n = 1), Coleoptera (n = 10), Hemiptera (n = 4), Hymenoptera (n = 5), Lepitoptera (n = 6), Orthopetra (n = 25) and Trichoptera (n = 1). They were either purchased in their natural form (n = 41) or seasoned (n = 11) and were either farmed (n = 37) or harvested wild (n = 15). All samples were freeze-dried, except from the samples from Africa which were sundried. After purchasing, all samples that were not bought in a powdered form were homogenised using a mortar. Only the freeze-dried samples could be analysed by SIRA. Therefore, two fresh samples had to be excluded from the analysis (NGR-01 and NGR-02) and were not considered for SIRA but only for suspect screening. In their place, two other samples (SK-05, SK-08) were included to be investigated using SIRA.

2.2. Stable isotope ratio analysis (SIRA)

Dry samples were further homogenised into a fine powder using a ball mill (Spex Sample prep, Metuchen, USA). Samples were freeze-dried to remove non-tissue-bound water to avoid interference with hydrogen measurements and milled to ensure homogeneity. Lipids were removed from the samples to ensure an accurate isotopic profile of the protein fraction. To remove lipids, a Soxhlet extraction was performed using an automatic soxtherm unit (Velp Scientifica, Usmate, Italy). For that, the samples were washed with 40 mL of petroleum ether (Thermo Fisher, Waltham, USA). The work temperature was set to 110 °C, the immersion time was set to 30 min, the washing time to 60 min and the recovery time to 30 min. The washed samples were then dried at room temperature and subsequently sieved with a wire mesh (850 μm).

The individual defatted samples were loosely crimped in silver ($^{2}H/^{1}H$ analysis, 6 × 4 mm) or tin ($^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ analysis, 8 × 5 mm) capsules. For hydrogen and simultaneous carbon, nitrogen and sulphur (CNS) analysis, 1 mg and 5 mg, respectively, was weighted for each sample and placed in a 96-well plate using a microbalance. Samples were weighed and analysed in triplicate or quadruplicate for CNS and H analysis, respectively. Due to the exchange of hydrogen between ambient water vapour and the sample matrix, an equilibration step was performed before H analysis, where the weighted samples were stored in desiccators over silica beads at 40 °C for 48 h, before crimping the capsules. The reference materials NIST 1577c (LGC standards, Teddington, United Kingdom) and SC063 (SerCon, Crewe, United Kingdom) were used for the two-point normalisation of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ samples. For $^{2}H/^{1}H$ analysis, NIST 1577c and an in-house casein standard (the value of which was determined by inter-laboratory comparison using multiple reference materials) were used. NIST1577c was also run at intervals throughout the batches to be used for drift-correction (prior to two-point normalisation). Linearity was also tested for in each batch using a sample weighed to 0.5x, 1x, and 1.5x the masses listed above and a correction was also applied prior to two-point normalisation if appropriate. In-house matrix-matched QC materials were run with every batch to ensure validity of results.

The isotope measurement was conducted using a SerCon 20–22 Isotope Ratio Mass Spectrometer coupled to an elemental analyser (ISOEArth +) for simultaneous analysis of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios and a pyrolyzer (Vecstar HT furnace, high temperature pyrolysis furnace) for measurement of $^{2}H/^{1}H$ ratios. For CNS analysis, the tin capsules containing the samples were introduced to the instrument by a carousel. Afterwards, the samples were converted into gas by combustion at 1000 °C (metallic copper and two layers of quartz chips, SerCon) for CNS analysis. NO_x gases were reduced to N_2 by reduced copper wire. Excess water vapour was removed by a water trap containing magnesium perchlorate as a drying agent. The obtained gases were then purified and separated by a gas chromatography column at 60 °C with helium (grade 99.999% purity, BOC, Ipswich, United Kingdom) as carrier gas (53 mL/min) and subsequently sequentially admitted to the mass spectrometer by means of an interface (ISO Earth + software: Calisto 4011, SerCon).

For $^{2}H/^{1}H$ measurements, silver capsules containing the samples were also introduced to the reaction tube via carousel. The samples were converted to gas via pyrolysis at 1350 °C (molybdenum liner filled with glassy carbon chips, SerCon) via carousel and resultant gases separated by a GC held at 50 °C with a helium flow rate of 80 mL/min. Data was analysed using pyrolysis software Calisto 1050 V10.5.58.

Stable isotope ratios were expressed in delta (δ) notation in parts per thousand (‰). Delta values are used to represent the difference in isotope ratio of a sample relative to the international agreed zero points, according to the IUPAC protocol (Brand et al., 2014). The international standards are V-SMOW (Vienna Standard Mean Ocean Water) for $\delta^{2}H$, V-PDB (Vienna Pee Dee Belemite) for $\delta^{13}C$, V-CDT (Vienna Canyon Diablo Troilite) for $\delta^{34}S$ and air (atmospheric N_2) for $\delta^{15}N$. The maximum standard deviations of repeatability accepted were 0.3 ‰ for $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{air}$, 0.8 ‰ for $\delta^{34}S_{VCDT}$, and 3.0 ‰ for $\delta^{2}H_{VSMOW}$. The following equation was used:

$$\delta X = [(R_{sample}/R_{standard}) - 1]$$

where R_{sample} and $R_{standard}$ are the ratios of the sample and the standard, respectively. The ratios are always displayed as the heavier isotope divided by the lighter isotope. X represents the heavier isotope (e.g. ^{13}C). The δX value describes isotopic enrichment or depletion relative to the standard. A more positive δX value therefore indicates that a sample contains more of the heavier isotope.

Each analysis contained an in-house quality control for protein samples, two standards as well as linearity samples. Standards were run in triplicates (quadruplicates for hydrogen) at the beginning, middle and end of each batch. Drift correction was performed by the Calisto software between references. Quality control samples were run in triplicates (quadruplicates for hydrogen). A batch was accepted when the measured values of the quality control fell within two times the standard deviation of the established mean value. The estimated expanded uncertainty of measurement (coverage factor k = 2) was 0.57 ‰ for $\delta^{13}C_{VPDB}$, 1.24 ‰ for $\delta^{15}N_{air}$, 2.13 ‰ for $\delta^{34}S_{VCDT}$ and 7.73 ‰ for $\delta^{2}H_{VSMOW}$.

2.3. Pesticide screening

2.3.1. Chemicals and reagents

Detailed information on purchased chemicals and materials is reported in the supplementary data (Table S2). In brief, individual labelled pesticide standards were purchased from Cambridge Isotope Laboratories (Andover, USA) and Sigma-Aldrich (St. Louis, USA). Individual native compounds were purchased from Merck (Darmstadt, Germany) and Toronto Research Chemicals (Canada) and pesticide mixes (Canada mixture 1 and mixture 51) were purchased from LGC standards (Teddington, United Kingdom). Sodium chloride (NaCl) and magnesium sulphate ($MgSO_4$) were purchased from Sigma-Aldrich (St. Louis, USA). All solvents were chromatography grade, hexane was purchased from Acros Organics (Belgium), acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Biosolve Chimice (France) and the QuEChERS sorbents C_{18} and primary secondary amine (PSA) were purchased from Supelco (Bellefonte, USA).

2.3.2. Sample preparation and instrumental analysis

A previously described QuEChERS extraction method was used with minor modifications (Poma et al., 2022), including an additional extraction step with hexane to remove excess of lipids. In brief, 300–500 mg sample was weighed in polypropylene tubes, spiked with 50 μL internal standard mix (1 ng/ μL, Table S2) and extracted with 6 mL acetonitrile acidified with 0.1% formic acid. The sample was salted out by adding 1 g of $MgSO_4$ and 0.25 g of NaCl to the extract, afterwards it was vortexed for 1 min and centrifuged at 3000 rpm for 3 min. The supernatant was transferred to a glass tube and concentrated to 2 mL at 30 °C under a gentle nitrogen flow. Dispersed solid phase extraction was performed by adding 50 mg PSA and 100 mg C_{18}, followed by 1 min of vortexing and 3 min centrifugation at 3000 rpm. The supernatant was transferred to a new glass tube and 2 mL of hexane was added, followed by vortexing for 1 min and centrifugation for 3 min at 3000 rpm. To remove the excess of lipids, the hexane fraction was discarded. After adding 50 μL water to the acetonitrile fraction, the sample was concentrated to near dryness and added with 50 μL methanol. The extract was filtered through a 0.2 μm centrifugal filter and transferred to an autosampler vial for analysis.

Suspect screening was carried out using an Agilent Infinity 1290 liquid-chromatography system coupled to an Agilent 6530 Quadrupole Time-Of-Flight (QTOF) mass spectrometer equipped with an electrospray ionisation (ESI) source (Agilent Technologies, Santa Clara, USA). Chromatographic separation was achieved using a Kinetex XB-C_{18} column (3 mm × 50 mm, 1.7 μm, Phenomenex, Torrace, USA) equipped with a SecurityGuardTM ULTRA guard column (2.1 mm, Phenomenex). The mobile phase consisted of water (A) and methanol (B), both containing 0.01% formic acid (FA) for positive ionisation mode and 0.01% FA and 5 mM ammonium formate for negative ionisation mode. The QTOF was operated in both negative and positive ionisation mode. The final optimised chromatographic conditions and ionisation source parameters are summarised in the supplementary data (Section 1, Table S3). The instrument was operated in data-dependent (Auto MS/MS) acquisition mode, which allowed an automatic selection of a maximum of 3 precursor ions per cycle. The quadrupole was operated in narrow selection mode ($m/z \pm 1.3$), ranging from 50 to 1700 m/z with a scan rate of 5 spectra per second applying collision energies of 10, 20 and 40 eV. Based on the results from the data dependent acquisition

cycle, compounds with high probability but without fragmentation spectra were reanalysed using target MS2 acquisition mode and otherwise unchanged parameters. The quadrupole was operated in narrow selection mode, ranging from 100 to 950 *m/z* with a scan rate of 5 spectra per second applying collision energies of 10, 20 and 40 eV. Prioritised compounds were set as targeted *m/z*-values.

Targeted analysis was performed using a previously described method (Poma et al., 2022) with slight adaptations. The analysis was carried out using an Agilent Infinity 1290 liquid-chromatography system coupled to an Agilent 6460 Triple Quadrupole mass spectrometer equipped with an ESI source (Agilent Technologies) using both positive and negative ionisation mode. Chromatographic separation was achieved using a Kinetex XB-C_{18} column (4.6 mm × 100 mm, 2.6 μm, Phenomenex) with water (A) and methanol (B) as mobile phases, both containing 5 mM ammonium formate. The acquisition was carried out using dynamic multiple reaction monitoring (dMRM) mode. The final optimised chromatographic conditions and ionisation source parameters are summarised in the supplementary data (Section S1, Table S4).

2.3.3. Quality control and data analysis

To control potential background contamination, two procedural blanks (Na_2SO_4) were run in parallel with each batch of samples. Quality controls were included in each run spiked with a known mass of native compounds (Table S2).

For the suspect screening, a suspect list (n = 474) was developed based on the HBM4EU CECscreen database (Meijer et al., 2022), containing different pesticide classes. After deconvolution and alignment of the chromatograms, the suspect list was matched against the sample results using first the "targeted feature extraction" algorithm of the Profinder Software (version B.08.00, Agilent Technologies). 10 ppm was used as mass error limit and 0.5 min for retention time alignment. Afterwards, MassHunter Qualitative analysis (version 08.00, Agilent Technologies) was used to confirm results manually using the "Find by Formula" algorithm (Agilent Technologies). The mass error and retention time match tolerance were set to 10 ppm and 0.35 min, respectively. Expected variation was set at 2 mDa ± 5.6 ppm (7.5%) and a match score of above 70%. In positive ionisation mode, the data was screened for M + H, and M + Na and in negative ionisation mode the data was screened for M−H. The results were confirmed by matching against databases (MassBank, mzCloud) and by using *in silico* prediction tools (MS Fragmenter, CFM-ID). Confidence levels (CLs) were assigned according to Schymanski et al. (Schymanski et al., 2014). Details on the data analysis workflow and matching parameters can be found in the supplementary data (Section S2).

For the target analysis, the limit of quantification (LOQ) was ten times the signal-to-noise-ratio from the lowest calibration point + the concentration of each respective compound in the procedural blank (Gao et al., 2022). Individual LOQ values, together with additional information can be found in the supplementary data (Table S5, Section S2).

2.4. Statistical analysis

Statistical Analysis of SIRA was performed using XLSTAT (Addinsoft, New York, United States). Parametric tests were conducted after ensuring normal distribution (histograms and QQ-plots). Boxplots were generated for each element to visualise any differences between the isotope ratios. SIRA data was analysed further by grouping samples according to their origins and comparing multivariate statistics. One-Way Analysis of Variance (ANOVA) was used to see if statistically significant differences ($p < 0.05$) can be observed between groups. Principal component analysis (PCA) and linear discriminative analysis (LDA) were performed for dimensionality reduction. To simplify multidimensional data with multiple features that have a correlation with each other, dimensionality reduction is used to plot this data in just two or three dimensions. While PCA ignores class labels and aims at finding principal components that maximise variance, LDA aims at finding the discriminant that will represent the set class labels/axes which maximise separation between different classes. SIRA data was therefore grouped according to their origins and further analysed.

For descriptive statistics of CUP concentrations, values < LOQ were treated as LOQ × detection frequency (James et al., 2002) and performed with IBM SPSS 20 (Chicago, Illinois, USA).

3. Results and discussion

3.1. Isotopic ratios for determination of origin

The values of $\delta^{13}C_{VPDB}$ in edible insects ranged from −30.8 to −11.3 ‰. Carbon values are usually strongly influenced by the diet and give insights about whether an animal fed on C_3 or C_4 plants (Knobbe et al., 2006). Plants are categorised into 3 groups: C_3, C_4 and CAM plants. Due to isotopic discrimination capabilities of enzymes and different CO_2 concentrations in the photosynthesis cycle, C_3 plants (e.g. grass, hay or soybeans) and C_4 plants (e.g. maize) show different $\delta^{13}C_{VPDB}$-values (O'Leary, 1981). C_3 plants show $\delta^{13}C_{VPDB}$-values between –32 and –23 ‰, while C_4 plants show values between −10 and –19 ‰ (Knobbe et al., 2006). Most samples had $\delta^{13}C_{VPDB}$-values in the δ-range of C_3 plants (Fig. 1A). The African samples, however, showed $\delta^{13}C_{VPDB}$-values in the δ-range of C_4 plants, indicating that these plants have been a major component of the insects' diet. Since maize is the most widely grown crop in sub-Saharan Africa, this is in line with our findings (Badu-Apraku & Fakorede, 2017). Four other Asian insect samples also had $\delta^{13}C_{VPDB}$-values below −24 ‰, which is most likely due to the ingestion of C_4-plants.

$\delta^{15}N_{air}$-values ranged from −1.5 to 9.3 ‰ (Fig. 1B). Generally, nitrogen ratios are closely related to the trophic level of an organism as the ratios change considerably when they are processed by consumers (O'Brien, 2015). For carnivores, there is an enrichment of $\delta^{15}N_{air}$-values per trophic level, therefore, herbivores are expected to have lower $\delta^{15}N_{air}$-values (Camin et al., 2016). $\delta^{15}N_{air}$-values are also influenced by agricultural conditions, mainly fertiliser usage. However, no significant differences in $\delta^{15}N_{air}$-values between wild and farmed insects were observed in this study (Figure S1). It was expected to see differences between wild and farmed insects, due to the expected differences in agricultural conditions.

$\delta^{34}S_{VCDT}$-values ranged from 0.4 to 9.8 ‰. Sulphur ratios in animals are related to the food they consume. The values are influenced by geology, microbial processes in the soil (including aerobic and anaerobic growing conditions), fertilisation techniques and the distance from the sea ("sea-spray effect") (Krouse & Mayer, 2000; Rubenstein & Hobson, 2004). While most samples ranged between 0.4 and 7.0 ‰, the African samples showed again a distinct profile with values higher than 7.0 ‰ (Fig. 1C). It could be observed that wild samples contained heavier sulphur than farmed samples (Figure S1). This was the only isotope composition that showed differences between wild and farmed samples. It should also be mentioned that differences between natural and processed samples were also investigated, however, they achieved the same results as wild and farmed samples. This is probably because most wild samples were also natural (Table S1). It is therefore not possible to draw any clear conclusion whether the differences in sulphur isotopic composition in insects were due to the processing or the farming.

Values for $\delta^{2}H_{VSMOW}$ ranged from −107.6 to –32.2 ‰. $\delta^{2}H_{VSMOW}$-values are linked to drinking water and food (Bowen et al., 2007). They are generally more positive in low-latitude coastal regions and decrease in high-latitude inland regions. The $\delta^{2}H_{VSMOW}$-values of the samples from Asia and Europe were spread out over the whole range. The African samples were among the samples with the highest $\delta^{2}H_{VSMOW}$-values, ranging from −57.8 to − 32.2 ‰ (Fig. 1D). Both Nigeria and Uganda are Sub-Saharan countries and the climate there is considered tropical (Beck et al., 2018). This also corresponds to $\delta^{2}H_{VSMOW}$-values predicted by global isoscape prediction models simulated in literature (Terzer et al.,

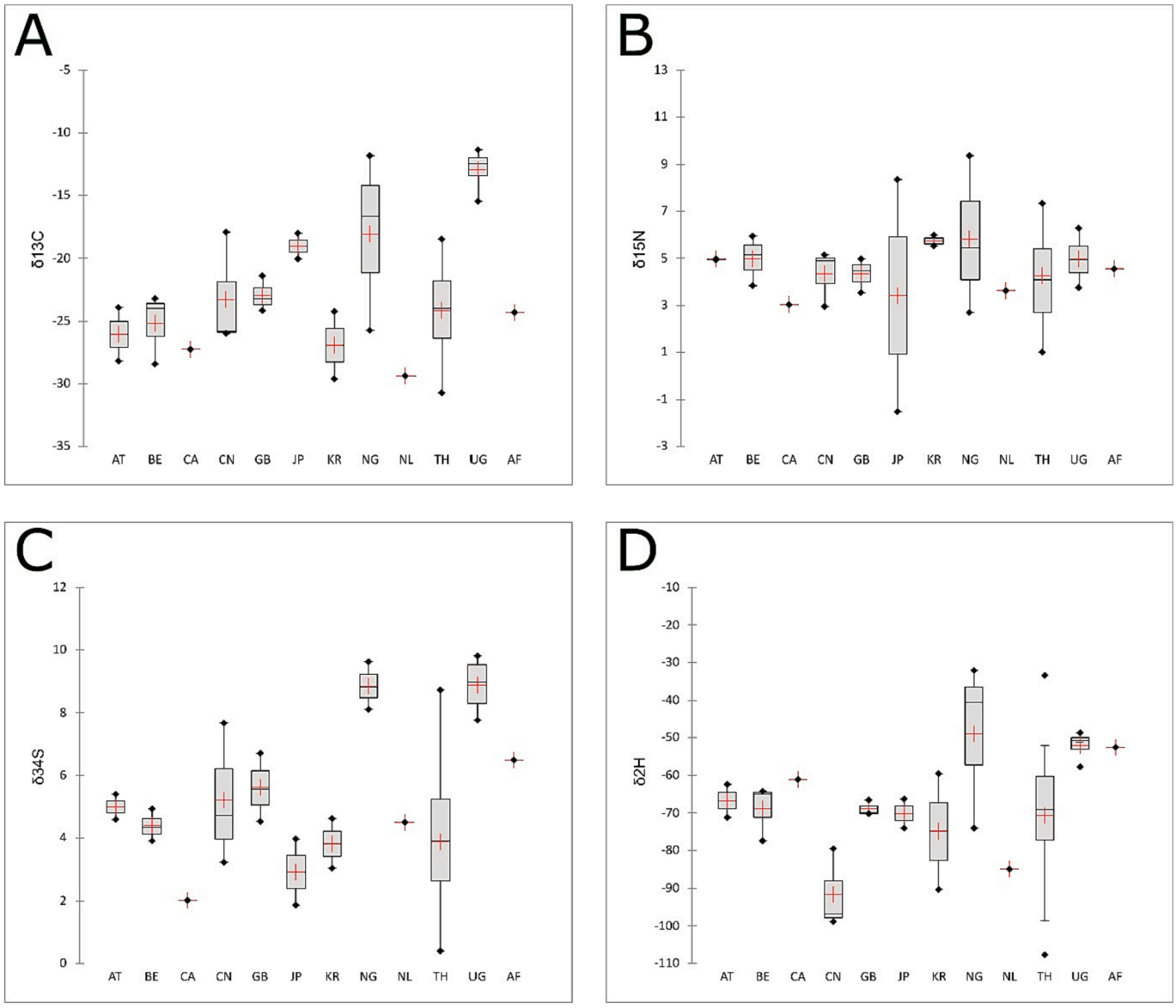

Fig. 1. (**A**) $\delta^{13}C_{VPDB}$, (**B**) $\delta^{15}N_{air}$, (**C**) $\delta^{34}S_{VCDT}$ and (**D**) $\delta^{2}H_{VSMOW}$-values of edible insect samples grouped by country. Country codes according to origin: AT = Austria (n = 2), BE = Belgium (n = 3), CA = Canada (n = 1), CN = China (n = 3), GB = Great Britain (n = 3), JP = Japan (n = 2), KR = South Korea (n = 2), NG = Nigeria (n = 2), NL = Netherlands (n = 2), TH = Thailand (n = 24), UG = Uganda (n = 5), AF = Africa (n = 1). $\delta^{13}C_{VPDB}$- ($p < 0.001$) and $\delta^{34}S_{VCDT}$- ($p < 0.001$) values showed significant differences between countries using ANOVA. Significance was labelled with * ($p < 0.05$) and ** ($p < 0.01$).

2013).

ANOVA was used to investigate differences in isotope ratios between countries. There was a significant difference in the $\delta^{13}C_{VPDB}$- ($p < 0.001$) and $\delta^{34}S_{VCDT}$- ($p < 0.001$) values when comparing the data of all countries. $\delta^{15}N_{air}$ and $\delta^{2}H_{VSMOW}$ did not show any significant differences. However, with a p-value of 0.055, $\delta^{2}H_{VSMOW}$ was close to being significant and might thus show differences between countries when considering a larger dataset.

Both LDA and PCA were used to further investigate whether it was possible to use isotopic data to classify samples according to their country of origin. After entering isotopic compositions and country of origin into the model, PCA reduced the multidimensional data to two principal components (PC1 and 2). PC1 was mostly composed of $\delta^{13}C_{VPDB}$, $\delta^{15}N_{air}$ and $\delta^{2}H_{VSMOW}$, while the largest contribution to PC2 was $\delta^{15}N_{air}$ (77%, Table S6). PCA could not identify any conclusive clusters for individual countries, and it was therefore not possible to distinguish between individual countries (Fig. 2A). However, the African samples clustered in PC1 (circled in black) showed a distinct profile compared to the other samples. The same procedure was applied for LDA and isotopic compositions and country of origin were fed to the model. LDA estimates the probability that a new set of inputs belongs to every class. The class with the highest probability is then considered the output class. The LDA model was therefore used to test whether it could correctly classify the origin of a sample based on the input information, i.e. the isotopic compositions. Similar to PCA, LDA could only correctly classify 66% of the samples according to the country of origin. Since both models were not able to distinguish the samples by country when considering all samples from different species, another investigation was performed, analysing whether it was possible to differentiate between samples belonging to a single species. Therefore, LDA and PCA were used to investigate whether it was possible to differentiate between countries if only considering one species.

House crickets (*Acheta domesticus*) were selected because this was the most abundant species (n = 8) in this sample set. The cricket samples were from Thailand (n = 3), Nigeria (n = 1), Belgium (n = 1), Canada (n = 1), Uganda (n = 1), and the United Kingdom (n = 1). The models were

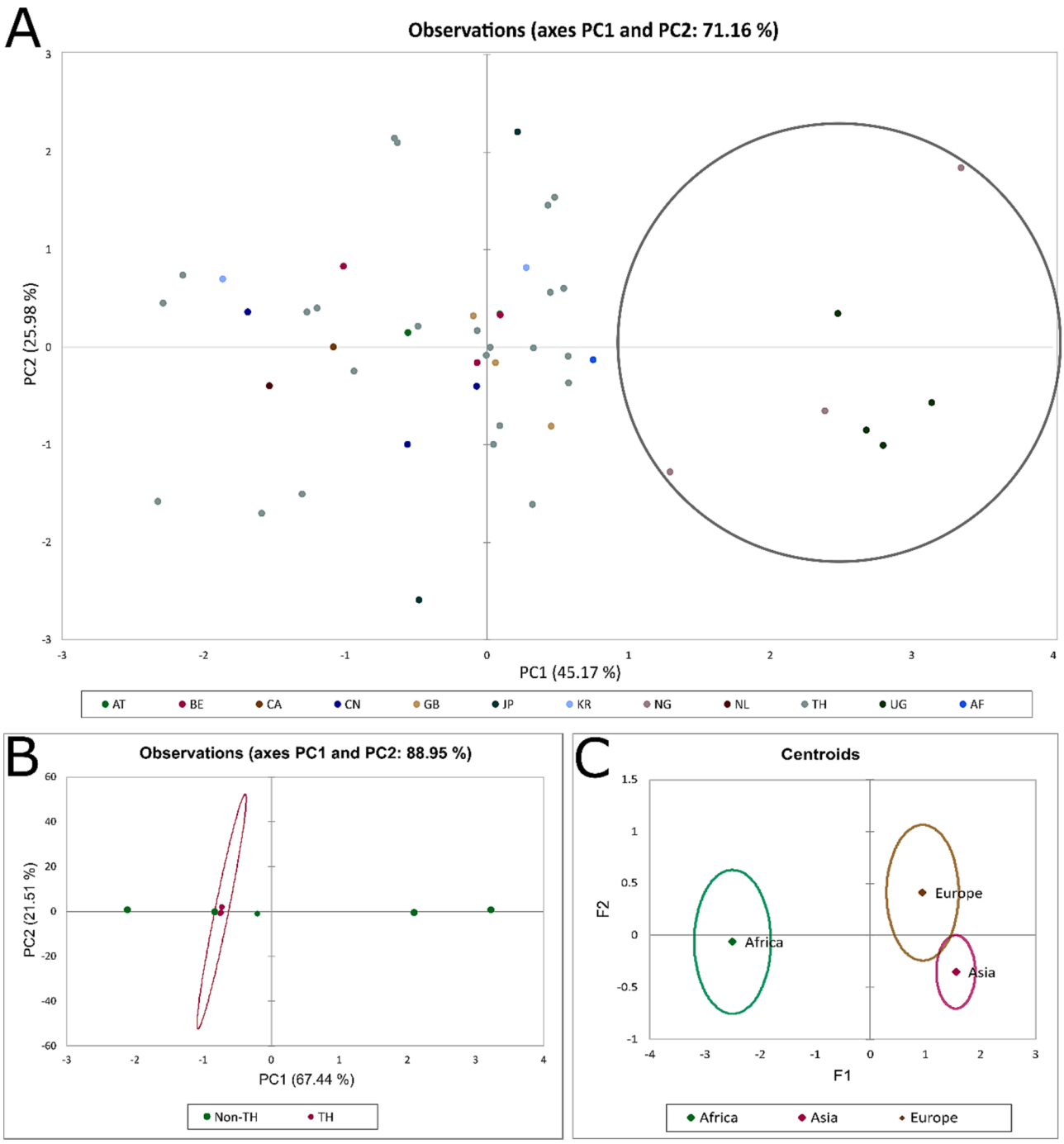

Fig. 2. Principal component analysis (PCA) and linear discriminative analysis (LDA) of isotopic patterns of edible insect samples. (**A**) PCA of all samples divided by country, samples from Africa (Nigeria and Uganda) are circled in black. (**B**) PCA of house crickets from Thailand (TH) and other countries (non-TH), confidence ellipse is circled in red, and (**C**) LDA of all insect samples divided by region.

therefore used to distinguish between samples from Thailand (TH) and samples that were not from Thailand (non-TH). Subsequently, PCA was able to separate the samples originated from Thailand vs the ones not from Thailand (Fig. 2B), with PC1 representing $\delta^{13}C_{VPDB}$, $\delta^{2}H_{VSMOW}$ and $\delta^{34}S_{VCDT}$ and the largest contribution of PC2 representing $\delta^{15}N_{air}$ (Table S6). Additionally, LDA was able to correctly classify 90% of the samples. Since food fraud might be one of the possible applications of SIRA in edible insects, the distinction between countries by SIRA can be an important tool to help discover any discrepancies regarding origin. By being able to distinguish between crickets from Thailand and other countries, it was shown that SIRA can be used to distinguish between edible insects from different areas when it comes to one species. This, however, needs to be confirmed with other species than crickets. With Thailand being the world's largest producer of edible insects, this distinction could be useful from a commercial perspective when investigating whether a sample originated from Thailand or not.

Since both models were, however, not able to distinguish the samples across all species combined according to the country of origin, it was investigated whether the models could classify the samples according to region/continent – Africa, Europe, and Asia. LDA was able to separate the samples according to region (Fig. 2C). Moreover, the model was able to correctly classify 96% of the samples according to the region. This

could become a useful tool for food authenticity and traceability of edible insects. The model could be used in the future to investigate whether a sample originated from Europe or Asia, preventing fraud. Many companies in Europe state that their edible insect products are reared in Europe, however, at this point, that is difficult to prove. These findings could help to provide a framework for future investigations of origin in edible insects and for food authenticity and traceability.

3.2. Suspect screening of pesticides

In total, 26 compounds were identified in the samples by suspect screening using HRMS, 18 of these with CL 2 and 8 with CL 3 (summary Table 1, extended version Table S7). The identification was performed according to Schymanski et al. (Schymanski et al., 2014). Most of the identified compounds were insecticides (42%), followed by herbicides (27%) (Table S8). However, the most abundant CUP in the samples was trifloxystrobin (16%), a systemic broad-spectrum fungicide used to protect cereals, fruits, vegetables and ornamental plants (PubChem). In

Table 1
Summary of the CUP suspect screening results of edible insect samples (extended version Table S7). Confidence levels (CL) were assigned according to Schymanski et al.

Compound	EU-AT-02	EU-BE-03	EU-NL-03	EU-UK-04	JPN-06	JPN-09	JPN-10	JPN-11	JPN-12	JPN-13	JPN-15	JPN-17	JPN-21	JPN-26	NGR-01	NGR-04
2,6-Xylidine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4-Nitrophenol	2A	–	–	–	–	–	–	–	2A	–	–	–	–	–	–	–
Aldicarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Atrazine mercapturate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bendiocarb	–	–	–	–	–	–	–	–	3	–	–	3	–	–	–	–
Butocarboxim	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cinerin I	–	–	–	–	–	2B	–	2B	–	–	–	–	–	–	–	–
Diethofencarb	–	–	2B	–	–	–	–	–	–	–	–	–	–	–	–	–
Diethyltoluamide	–	–	–	–	–	–	–	–	–	2A	–	–	–	–	–	2A
Dinotefuran	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dodemorph	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethofumesate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–
Fenpropidin	–	2B	–	–	–	–	–	–	–	2B	–	–	–	2B	–	–
Fenuron	–	–	–	–	2B	–	–	–	–	–	–	–	2A	–	–	–
Hexazinone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2B
Icaridin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Imazapyr	–	–	–	–	–	–	2A	–	–	–	–	–	–	–	–	–
Isoproturon	–	–	–	3	–	–	–	–	–	–	–	–	–	–	2A	–
Jasmolin II	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Metamitron	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–
Oxadixyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Phenothrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Promecarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2A
Propargite	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pymetrozine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trifloxystrobin	–	–	–	–	3	–	2B	–	2B	–	–	–	–	–	–	3
Total number	*1*	*1*	*1*	*1*	*2*	*1*	*2*	*1*	*3*	*2*	*1*	*1*	*1*	*1*	*2*	*4*

Compound	PRC-03	SK-03	SK-11	TH-02	TH-03	TH-04	TH-06	TH-07	TH-08	TH-09	UGD-01	UGD-02	UGD-03	UGD-04	UGD-05
2,6-Xylidine	–	–	–	–	–	–	–	–	–	–	2A	–	2A	2A	–
4-Nitrophenol	2A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aldicarb	–	–	–	–	–	–	–	2B	–	–	–	–	–	–	–
Atrazine mercapturate	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–
Bendiocarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Butocarboxim	–	–	–	–	–	–	–	2B	–	–	–	–	–	–	–
Cinerin I	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
Diethofencarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diethyltoluamide	–	–	–	–	2B	–	–	–	–	–	–	–	–	–	–
Dinotefuran	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–
Dodemorph	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethofumesate	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–
Fenpropidin	–	2B	–	–	–	–	–	–	–	–	–	–	–	–	–
Fenuron	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–
Hexazinone	2B	–	–	–	–	–	–	–	–	–	–	–	3	–	–
Icaridin	–	–	–	2B	–	–	–	–	–	–	–	–	–	–	–
Imazapyr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Isoproturon	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Jasmolin II	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–
Metamitron	–	2B	–	–	–	–	–	–	–	–	–	–	–	–	–
Oxadixyl	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–
Phenothrin	–	–	–	–	–	–	–	–	–	–	–	3	2A	–	2A
Promecarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Propargite	–	–	–	–	–	–	–	–	–	–	–	–	3	3	3
Pymetrozine	–	–	–	–	2B	–	–	–	2B	–	–	–	–	–	–
Trifloxystrobin	–	–	–	–	–	–	–	–	–	3	–	3	3	–	3
Total number	*2*	*3*	*1*	*1*	*2*	*2*	*1*	*3*	*2*	*1*	*2*	*2*	*5*	*2*	*3*

14 samples, no CUPs were identified. In samples originating from Europe, fewer CUPs could be identified compared to samples from Asia and Africa. Most CUPs were found in the samples from Uganda. It should be mentioned, however, that suspect screening is a qualitative approach and can only give information about the presence of listed compounds and the corresponding level of confidence. De Paepe et al. conducted a first multi-residue screening of different contaminants in edible insects from Belgium (De Paepe et al., 2019). They investigated 77 compounds, including 25 pesticides. Only a few compounds were identified and isoproturon was the only pesticide that was detected in the analysed insect samples using suspect screening. Similar to our study, this suggests that the chemical safety of edible insects in regard to pesticide contamination can be considered high. Nevertheless, quantification is needed to give more comprehensive insights into the actual contamination levels.

Stable isotopes have previously been used to investigate contamination levels and elucidate factors that play a role in the occurrence of contamination, for example, the pollution source (Da Souza et al., 2018; IAEA, 2018). To investigate correlations between pesticide occurrence and isotopic patterns in edible insects, LDA was used as a model. The aim was to test whether the model could classify correctly if a sample contained a certain pesticide (CL 2) based on the input isotopic composition. Therefore, isotopic compositions and pesticide occurrence were entered into the model. The percentages with which the model was able to correctly classify whether a sample contained a certain pesticide ranged from 58% (Cinerin I) to 98% (Aldicarb) (Table S9). Interestingly, the model was able to classify the occurrence of most pesticides correctly, based on the isotopic composition. However, it did not successfully classify the occurrence of every individual pesticide. It appears that the model's ability to correctly classify whether or not a sample contained a pesticide depended on the individual pesticide. Evidently, there are many factors that play a role in stable isotope compositions, and while these findings need to be validated to ensure their significance, they can still give an indication and be an interesting starting point for future investigations on pesticide occurrence in edible insects using SIRA.

To investigate which isotopes played a factor in this distinction, it was analysed which individual isotopic compositions show differences between pesticide-containing samples (Yes) and samples with no detected pesticides (No). No differences could be observed for $\delta^{13}C_{VPDB}$, $\delta^{2}H_{VSMOW}$, and $\delta^{15}N_{air}$ (Figure S2). However, sulphur compositions did show differences; samples that contained pesticides had more positive $\delta^{34}S_{VCDT}$-values than the samples with no detected pesticides. To exclude geographical dependence and further validate the significance of the results, only samples from Thailand (as they were the most abundant in the sample set) were investigated using the same approach. This led to the same result of pesticide-containing samples showing heavier $\delta^{34}S_{VCDT}$. As mentioned before, sulphur compositions can depend on different factors, particularly involving soil (Krouse & Mayer, 2000; Rubenstein & Hobson, 2004). Sulphur has also been used as an indication for environmental pollution stress in different matrices. Generally, sulphur is one of the elements that can be found in a surplus in more anthropogenically influenced areas (Kosior et al., 2015). It has been demonstrated that industrial areas are also more enriched in heavy sulphur compared to more natural areas (CARITAT, 1997; Kosior et al., 2015). While it was not possible to prove that the heavier $\delta^{34}S_{VCDT}$ in the insect samples containing pesticides was in fact correlated with the pesticide occurrence, the fact that more polluted areas also entailed heavier $\delta^{34}S_{VCDT}$ compares well with our findings. It could therefore be possible that the samples containing more pesticides also originated from a generally more polluted or contaminated area, leading to the more positive $\delta^{34}S_{VCDT}$-values. It should also be stated that the sulphur composition did not depend on whether or not the pesticide itself contained sulphur. Sulphur-free pesticides showed the same results as pesticides containing a sulphur atom. This indicates that the $\delta^{34}S_{VCDT}$-values are not dependent on sulphur atoms present in the compound, which supports the hypothesis that these samples could originate from generally more polluted areas.

Due to the previous findings indicating a correlation between pesticide occurrence and isotopic patterns, LDA was performed to investigate if the model could distinguish whether or not a sample generally contained pesticides, as opposed to testing for specific pesticides as detailed above. When including the ratios of all isotopes ($\delta^{2}H_{VSMOW}$, $\delta^{13}C_{VPDB}$, $\delta^{15}N_{air}$ and $\delta^{34}S_{VCDT}$) in the model, it was able to correctly classify 72%. However, when only including $\delta^{34}S_{VCDT}$ compositions – which proved to be the main isotope of interest for insects – it could correctly classify 76%. This suggests that $\delta^{34}S_{VCDT}$ is the main isotope of interest when investigating pesticide occurrence in insects. To exclude again any geographical dependence, subsequently only samples from Thailand were included, as they were the most abundant. The model could also correctly classify with this geographically limited dataset whether a sample contained pesticides or not (75%). This leads to the hypothesis that pesticide occurrence could indeed be correlated to the isotopic composition of sulphur and might even be used as an indication if a sample contains pesticides. Compared to the individual pesticide analysis – where the model could not be used for the correct classification of all the individual pesticides – the model investigating the combined pesticide occurrence showed more robust results. Naturally, this was a relatively small sample size and needs to be confirmed by a larger dataset to further investigate the importance of sulphur compositions in pesticide occurrence of edible insects. Additionally, it was not possible to link the $\delta^{34}S_{VCDT}$ compositions to pesticide contamination in particular, as other factors might also play a role. However, heavier sulphur could generally indicate more contaminated samples. Considering industrial pollution as an important aspect for food safety of edible insects (Charlton et al., 2015), these results therefore hold promise and could be beneficial for indicating any contamination, without undergoing a full analysis, for example for pesticides. Conspicuous isotopic compositions could give an indication of whether there is a need to perform a full pesticide analysis or not. These options should be further explored in the future.Target analysis of CUPs in edible insects.

As a complementary approach to the qualitative suspect screening, a quantitative target analysis was performed on the insect samples to gain additional information on the pesticide contamination levels and to further compare them with maximum residue levels (MRLs). MRLs define the highest legally tolerated amount of pesticide residues in food and are meant to protect consumer health (Carrasco Cabrera & Medina Pastor, 2022). While there are MRLs available for various types of food, there are none available for edible insects yet. Since there are no MRLs available yet for insects and they are considered a protein-alternative, the pesticide residues were compared with MRL values of meat from mammals or poultry.

Since the samples from Africa were analysed for CUPs in a previous study (Poma et al., 2022), they were therefore not included in this target analysis. The remaining samples were tested for 47 CUPs, consisting of commonly used compounds belonging to the groups of neonicotinoids, organophosphates, carbamates and triazines (Table S5). Out of these, bifenazate, novaluron, spiromesifen, oxamyl, phosmet, boscalid, chlorantraniliprole, aldicarb and triazines were below LOQ in all samples. A total of 30 compounds were quantified in the insect samples (Table S10, Fig. 3). Out of these compounds, 10 CUPs had a detection frequency (DF) higher than 30% (Table S11). The neonicotinoid imidacloprid had the highest DF (70%), followed by fipronil (48%). In 2018, the use of imidacloprid was banned in the EU, due to its untargeted toxicity against insects, which can also affect bees and other pollinators (European Food Safety Authority [EFSA], 2016). The insect samples investigated in this study were collected over several years and the high detection frequency of this compound could therefore partly be explained by the year of collection. Particularly the European samples were collected in the year 2018, so before imidacloprid was banned in Europe. Generally, the long collection period of the samples might influence pesticide occurrence, since some pesticide regulations have changed throughout the years.

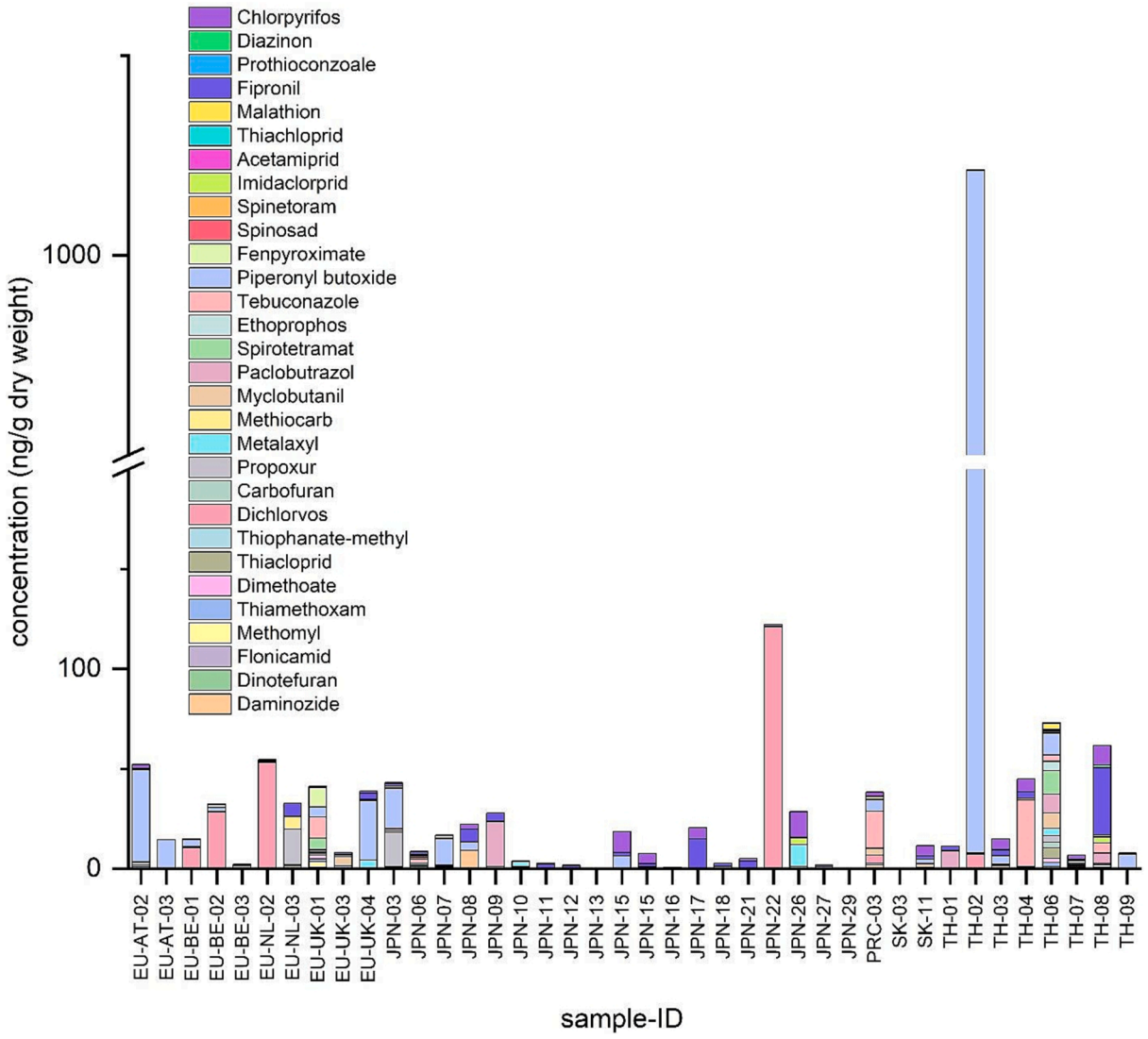

Fig. 3. Individual pesticide concentrations in ng/g dry weight in edible insects and their individual contribution to overall contamination.

Future work should investigate whether the DF of imidacloprid in more recent insect samples is still noticeably high. Compared to previously conducted experiments with African insects from Uganda and Nigeria (Poma et al., 2022), our experiments showed fairly different results regarding the compounds with the highest DF, as in African insects the organophosphates dichlorvos (89%) and chlorpyrifos (100%) proved to be the most abundant.

In the African insects (Poma et al., 2022), the highest measured concentration was observed for propoxur with 325 ng/g dry weight (dw), while in this study piperonyl butoxide had the highest individual concentration of 1034 ng/g dw (Table S10, Fig. 3) in a giant water bug (*Lethocerus americanus*) from Thailand. A possible explanation for this high concentration could be that this was one of the few aquatic insects in the sample set. The aquatic environment might be a possible source for this contamination and accumulation (Berenzen et al., 2005). Piperonyl butoxide is used as a synergist with different insecticides (e.g. pyrethroids) and its applications include pest control for hygiene and health purposes in public places (Regulation No 528/2012, 2017).

To better understand CUP contamination levels in edible insects, the obtained results were compared to MRLs (Table S12). Generally, the concentrations of most of the individual CUPs were below the corresponding MRLs for meat (expressed in ng/g wet weight (ww)) (Codex Alimentarius Commission). However, the organophosphate dichlorvos was in the same order of magnitude as the MRL (10 ng/g ww) in three samples: two locusts (*Locusta migratoria*) from the Netherlands (sample EU-NL-02) and Belgium (sample EU-BE-02) and a superworm (*Zophobas morio*) from Thailand (sample JPN-22). Fipronil could also be detected in concentrations close to the MRL (10 ng/g ww) in sample TH-08, a cicada (*Cryptotympana atrata)* from Thailand. Interestingly, two of the samples with concentrations close to the MRL were not only from Europe, but they were also the species (*Locusta migratoria*) that are now regulated under the novel food law and are therefore allowed in the EU. While these findings do not necessarily indicate a reason for concern, they might have implications on the chemical food safety of edible insects in Europe. MRLs are not toxicological parameters but rather a legislative standard set to ensure that residues are controlled nationally and internationally (Codex Alimentarius Commission). Exceeding those limits does therefore not necessarily mean that they pose any health risk for the consumer. However, since the MRLs in this study have been exceeded – also in European insects – the extension of the legislative framework is advisable for edible insects. Improving and increasing legislative guidelines and ensuring chemical food safety are likely to have a positive effect on the acceptancy of insects as a viable protein source in developed countries, whereas a lack thereof might only increase food neophobia and scepticism in regard to edible insects in Western Countries, including Europe (Mlcek et al., 2014).

Only limited compounds were detected using target analysis, most of them far below internationally defined MRLs for meat, suggesting low contamination levels for edible insects. However, the study highlights that further and more extensive investigations are needed to give a comprehensive assessment of the chemical risk of edible insects as a novel food source in Europe. Additionally, with increasing consumption of insects and to be able to properly estimate their risk, the availability of specific MRL values for edible insects is advisable, since consumption patterns of meat and insects vary and MRLs for meat are thus not fully representative.

4. Conclusions

This study showed that SIRA could be a useful tool to provide a framework for future investigations on food authenticity and traceability of edible insects. However, prior to that, extensive databases consisting of authentic samples with known characteristics (e.g. origin) need to be established as reference values. Particularly sulphur proved to be an interesting isotope in edible insects, for both origin and pesticide occurrence, and might be used as an indicator for chemical contamination. These findings can be useful in the future for the continuously growing edible insect market and help support fair trade practices, ensure food quality and safety, and protect consumer health.

Complementary screening approaches identified a limited number of CUPs, most of them below the internationally defined MRLs (for meat), suggesting low contamination levels of pesticides in edible insects. While this study offers novel and useful insights into the occurrence of different CUPs and their contamination levels in diverse edible insect species, it also highlights that more studies should focus on quantification of pesticides and generally chemical contaminants in edible insects. Now that insects have been legislated in Europe, allowing them to be more incorporated into daily diets, more attention should be paid to possible chemical hazards to accurately assess their risk and to ensure food safety.

CRediT authorship contribution statement

Alicia Macan Schönleben: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Shanshan Yin:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Ethan Strak:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Alison Johnson:** Writing – review & editing, Visualization, Formal analysis. **Lidia Belova:** Writing – review & editing, Formal analysis. **Yu Ait Bamai:** Writing – review & editing, Visualization, Formal analysis. **Alexander L.N. van Nuijs:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Giulia Poma:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Adrian Covaci:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114020.

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