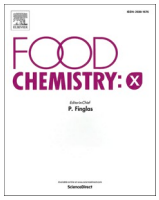
Food Chemistry: X 27 (2025) 102386

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



GC–MS/MS–based multiresidue pesticide analysis in mealworm (*Tenebrio molitor*) larvae: Optimization of standard QuEChERS-based method to minimize matrix effects

Hyun Ho Noh [a], Chang Jo Kim [b], So-Hee Kim [a], Hye-Ran Eun [a,c], Yongho Shin [c,*], Won Tae Jeong [a,*]

[a] *Residual Agrochemical Assessment Division, Department of Agro-Food Safety and Crop Protection, National Institute of Agricultural Sciences, 166 Nongsaengmyeong-ro, Wanju 55365, South Korea*
[b] *Safety Analysis Division, Experiment Research Institute, National Agricultural Products Quality Management Service, 141 Yongjeon-ro, Gimcheon 39660, South Korea*
[c] *Department of Applied Bioscience, Dong-A University, 225 Gudeok-ro, Busan 49315, South Korea*

A R T I C L E I N F O

Keywords:
Multiresidue pesticide analysis
Edible insect
QuEChERS
GC–MS/MS
Mealworm larvae
Fatty matrix
Matrix effect

A B S T R A C T

Given the unsuitability of the standard quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based method for multiresidue pesticide analysis in mealworm larvae due to their complex composition and high lipid content, we established a gas chromatography-tandem mass spectrometry–based procedure for the detection and quantitation of 247 pesticides in this matrix. The optimized extraction solvent (0.1 % formic acid in acetonitrile) was combined with the original QuEChERS salt packet to achieve high recovery rates, and matrix-derived interferents were effectively removed without analyte loss using primary secondary amine–based dispersive solid-phase extraction (cleanup step). Acceptable linearities, high recoveries, and low detection limits were achieved for most analytes. The detection of triadimenol in 24 field samples suggested that mealworms absorb pesticides from bran, their primary feed source. The developed method is suitable for monitoring pesticide residues in mealworm larvae and similar edible insects, providing a reliable tool for regulatory bodies and relevant industries.

1. Introduction

The consumption of animal products has increased with the increasing global population, and the demand for consumable crop–derived calories and proteins is expected to increase 2.1-fold by 2050 (Hunter et al., 2017). Given the need to promote sustainable food production, considerable attention has been drawn to decreasing the reliance of pest management on chemicals (Rockstrom et al., 2017). However, the fact that wheat, corn, and cotton losses due to pests reached 38.3 % in 1964–2003 (Poppe et al., 2003) indicates the importance of pesticides for preventing crop damage by fungi, insects, mites, weeds, and other pests and thus securing stable food production (Burger et al., 2008). Additionally, climate and weather changes directly and indirectly affect crop production and pest reproduction, distribution, and migration. Thus, food production should be increased while reducing the use of chemicals (Porter et al., 1991).

Insects are rich in proteins (40 %–70 %), minerals, and vitamins, thus holding promise as alternative protein sources for the food industry (Gravel & Doyen, 2020). Over 2000 insect species are consumed in various countries, including those in Asia and Europe (van Huis, 2020). The production of edible insects is more environmentally friendly and sustainable than livestock farming (FAO, 2013), and the growth of the domestic and international insect market is expected to continue. Edible insects include members of the orders Lepidoptera, Coleoptera, Orthoptera, and Hymenoptera (Yoo et al., 2013). In particular, the larvae of the mealworm beetle (*Tenebrio molitor*) have a protein content of 46.44 % and are nutritionally rich alternative food sources (Ravzanaadii et al., 2012). However, edible insects may contain harmful substances, such as microorganisms, chemicals, and parasites, with numerous studies reporting the detection of pesticides, including organophosphates and benzoquinones (Belluco et al., 2013).

Pesticides are toxic to humans and the environment and can harm

Abbreviation: MeCN, Acetonitrile; d-SPE, dispersive solid-phase extraction; EU, European Union; FA, formic acid; GC–MS/MS, gas chromatography-tandem mass spectrometry; LOQ, limit of quantitation; PSA, primary secondary amine; QuEChERS, quick, easy, cheap, effective, rugged, and safe; RSD, relative standard deviation.

* Corresponding authors.
E-mail addresses: yong6103@dau.ac.kr (Y. Shin), shewaspretty@korea.kr (W.T. Jeong).

https://doi.org/10.1016/j.fochx.2025.102386
Received 9 January 2025; Received in revised form 13 March 2025; Accepted 14 March 2025
Available online 18 March 2025

the immune, reproductive, and nervous systems. In response to the concerns related to pesticide residues in agricultural products and human exposure (Damalas & Eleftherohorinos, 2011; Lushchak et al., 2018), the European Union (EU) has established 464 maximum residue limits to regulate pesticides in terrestrial invertebrates, including insects (EU, 2024). In South Korea, the Rural Development Administration and Ministry of Food and Drug Safety set safety guidelines and residue limits for various crops, while the National Agricultural Products Quality Management Service and Ministry of Food and Drug Safety conduct safety investigations on harmful substances, including pesticide residues, in domestically produced and distributed agricultural products to ensure safe food production. However, no residue limits have been established for pesticides in edible insects in South Korea, which indicates the need to establish and monitor pesticide residue analysis methods, particularly for applying uniform PLS (positive list system) standards and future maximum residue limits.

Owing to the high protein and fat contents of mealworm larvae, the analysis of pesticide residues therein requires pretreatment to prevent contamination (Ravzanaadii et al., 2012), as exemplified by protein and fat removal based on the QuEChERS method and *n*-hexane treatment (Houbraken et al., 2016; Ramluckan et al., 2014; Shin et al., 2020). Acetonitrile/hexane partitioning is necessary to improve the recovery rates of high-distribution-coefficient pesticides during gas chromatography-tandem mass spectrometry (GC–MS/MS) analysis (Shin et al., 2018). Additionally, adsorbents commonly employed in the QuEChERS method, such as primary secondary amine (PSA), C18, *Z*-Sep, Z-Sep+, and HLB (hydrophilic lipophilic balance), should be used to remove potential interferents from the extract. Herein, we aimed to (i) develop a method for the simultaneous analysis of multiple pesticides and related metabolites in mealworm larvae using a GC–MS/MS–based modified QuEChERS approach and (ii) validate the effectiveness of this method by applying it to real mealworm larvae. The method improves routine residual pesticide analysis procedures to provide a breakthrough the analysis of high-protein and high-fat samples.

2. Materials and methods

2.1. Reagents

Reference pesticides (>97 %) were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Wako pure chemical industries (Osaka, Japan), sigma-Aldrich (St. Louis, MO, USA), and ChemService (West Chester, PA, USA). Stock solutions (100–1000 μg/mL) were acquired from AccuStandard (New Haven, CT, USA). Methanol (LiChrosolv grade) and ammonium formate (liquid chromatography-mass spectrometry grade) were obtained from Merck (Darmstadt, Germany). Formic acid (98–100 %), acetonitrile (high-performance liquid chromatography grade), and *n*-hexane (analytical grade) were sourced from Thermo fisher scientific (Waltham, MA, USA). Deionized water was obtained using an automatic purification system (Wasserlab, Navarra, Spain), and the QuEChERS extraction packet was sourced from Agilent Technologies (Santa Clara, CA, USA). Dispersive solid-phase extraction (d-SPE) sorbents containing PSA and/or C18 were sourced from Agilent Technologies (Santa Clara, CA, USA). *Z*-Sep and Z-Sep + were obtained from Supelco (Bellefonte, PA, USA). The OASIS PRiME HLB cartridge (1 mL, 30 mg) was procured from waters (Milford, MA, USA)

2.2. Edible insects and pesticides

Mealworms were provided by the Industrial Insect Division of the National Institute of Agricultural Sciences in South Korea (for method optimization) or collected from domestic mealworm larva farms (for real sample analysis). The samples were homogenized with dry ice after lyophilization and stored at −20 °C until use. The test pesticides corresponded to 247 compounds used to inspect the safety of domestic and foreign agricultural products.

2.3. Working and matrix-matched standard solutions

Stock solutions were prepared using the reference pesticides, mixed with acetonitrile, and stored at −20 °C until use. The mixed standard solution (2.5 μg/mL) was serially diluted to prepare working solutions with concentrations of 1000, 500, 250, 80, 40, 20, 10, 4, 2, 1, 0.4, and 0.2 ng/mL. Matrix-matched standard solutions were prepared by mixing the final extract and standard solution in a ratio of 3:1 (*v*/v). The final extract was prepared following the protocol for blank mealworm sample preparation.

2.4. Instrumentation

Pesticide analysis was performed using an Agilent 7000C triple-quadrupole mass spectrometer coupled with an Agilent 7890B gas chromatograph (Santa Clara, CA, USA). The carrier gas (helium) was supplied at a flow rate of 1 mL/min. Samples were injected in the solvent vent mode, and the vent flow rate was held at 50 mL/min for the first 1.5 min, decreased to 25 mL/min, and maintained (5 psi) for 0.3 min. The target analytes were separated on an HP-5 ms column (30 m × 250 μm × 0.25 μm). The oven temperature was held at 50 °C for 3 min, increased to 185 °C at 30 °C/min, 250 °C at 5 °C/min, and 300 °C at 10 °C/min, and held for 7 min. The source and transfer line temperatures were set to 250 and 290 °C, respectively. Electron ionization was performed at 70 eV, and all pesticides were analyzed in the dynamic multiple reaction monitoring mode.

2.5. Optimization of sample preparation procedure

2.5.1. Extraction efficiency

Homogenized pesticide-free mealworms (5 g) were hydrated with water (7 mL) and spiked with pesticides to 25 μg/kg. Each sample was separately treated with acetonitrile (10 mL) and 0.1 % formic acid in acetonitrile (10 mL) and extracted/partitioned using the original QuEChERS (1 g NaCl and 4 g $MgSO_4$) or EN-15662 QuEChERS (1 g NaCl, 4 g $MgSO_4$, 1 g $Na_3Citrate{\cdot}2H_2O$, and 0.5 g $Na_2HCitrate{\cdot}1.5H_2O$) extraction packet. The extract (6 mL) was transferred to a 15 mL conical tube and partitioned with *n*-hexane (4 mL). The lower acetonitrile layer was transferred to a new tube, while the upper *n*-hexane layer was further partitioned with *n*-hexane-saturated acetonitrile (2 × 6 mL). Finally, the solution, acetonitrile, was purified using a d-SPE tube containing 25 mg PSA and 150 mg $MgSO_4$ and then mixed with acetonitrile in a 3:1 (*v*/v) ratio. Pesticide extraction efficiencies were expressed in terms of the corresponding recovery rates, which were calculated as follows.

$$\text{Recovery (\%)} = \frac{\text{Amount of Pesticide treated}}{\text{Recovered pesticide}} \times 100$$

2.5.2. Partitioning efficiency

Homogenized pesticide-free mealworms (5 g) were hydrated with water (7 mL) and spiked with pesticides to 25 μg/kg. Extraction and partitioning were performed using 0.1 % formic acid in acetonitrile (10 mL) and the original QuEChERS extraction packet. The extract (6 mL) was transferred to a 15 mL conical tube, mixed with *n*-hexane (4 mL), and centrifuged at 1300 rpm. The acetonitrile layer was transferred to a new tube, while the hexane layer was either discarded ($N = 1$) or subjected to up to three additional partitioning steps ($N = 2, 3, 4$) using *n*-hexane-saturated acetonitrile (6 mL). After each partitioning step, the acetonitrile layer was purified using d-SPE (25 mg PSA, 150 mg $MgSO_4$) and combined with acetonitrile in a 3:1 (*v*/v) ratio. Partitioning efficiencies were expressed in terms of the corresponding recovery rates, which were calculated as follows.

2.5.3. Cleanup efficiency

Homogenized pesticide-free mealworms (5 g) were hydrated with

Table 1
Recovery rates of 247 pesticides obtained for different extraction solution–QuEChERS salt combinations.

Recovery (%) at 25 μg/kg	RSD (%) $n = 4$	Number of pesticides (%)			
		Original salts		**EN 15662 salts**	
		MeCN	**0.1 % FA in MeCN**	**MeCN**	**0.1 % FA in MeCN**
<10	>0	0 (0)	0 (0)	0 (0)	0 (0)
10–30	≤20	4 (1.6)	2 (0.8)	2 (0.8)	2 (0.8)
	>20	1 (0.4)	0 (0)	1 (0.4)	1 (0.4)
30–70	≤20	43 (17.4)	29 (11.7)	39 (15.8)	28 (11.3)
	>20	7 (2.8)	3 (1.2)	6 (2.4)	6 (2.4)
70–120	≤20	182 (73.7)	201 (81.4)	190 (76.9)	194 (78.5)
	>20	4 (1.6)	5 (2.0)	1 (0.4)	9 (3.3)
>120	≤20	4 (1.6)	2 (0.8)	4 (1.4)	5 (2.0)
	>20	1 (0.4)	4 (1.6)	3 (1.2)	1 (0.4)
Not detected		1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)
Sum		247 (100)	247 (100)	247 (100)	247 (100)

FA = formic acid. MeCN = acetonitrile.

Table 2
Recovery rates of 247 pesticides obtained for different numbers of acetonitrile/*n*-hexane partitionings (*N*).

Recovery (%) at 25 μg/kg	RSD (%) $n = 4$	Number of pesticides (%)			
		***N* = 1**	***N* = 2**	***N* = 3**	***N* = 4**
<10	>0	2 (0.8)	0 (0)	0 (0)	0 (0)
10–30	≤20	7 (2.8)	4 (1.6)	2 (0.8)	3 (1.2)
	>20	1 (0.4)	0 (0)	0 (0)	0 (0)
30–70	≤20	72 (29.1)	58 (23.5)	29 (11.7)	23 (9.3)
	>20	3 (1.2)	7 (2.8)	3 (1.2)	4 (1.6)
70–120	≤20	155 (62.8)	172 (69.6)	201 (81.4)	209 (84.6)
	>20	2 (0.8)	2 (0.8)	5 (2.0)	2 (0.8)
>120	≤20	4 (1.6)	4 (1.6)	2 (0.8)	3 (1.2)
	>20	1 (0.4)	0 (0)	4 (1.6)	3 (1.2)
Not detected		0 (0)	0 (0)	1 (0.4)	0 (0)
Sum		247 (100)	247 (100)	247 (100)	247 (100)

water (7 mL) and spiked with pesticides to 25 μg/kg. Extraction and partitioning were performed using 0.1 % formic acid in acetonitrile (10 mL) and the original QuEChERS packet. The extract (6 mL) was transferred to a 15 mL conical tube, mixed with *n*-hexane (4 mL), and centrifuged at 1300 rpm. The acetonitrile layer was transferred to a new tube, while the *n*-hexane layer underwent additional partitioning using *n*-hexane-saturated acetonitrile (3 × 6 mL). The resulting solution was purified using different types of d-SPE sorbents (Type I (25 mg PSA, 150 mg $MgSO_4$), Type II (25 mg PSA, 25 mg C18, 150 mg $MgSO_4$), Type III (25 mg C18, 150 mg $MgSO_4$), Type IV (50 mg *Z*-Sep, 150 mg $MgSO_4$), Type V (50 mg Z-Sep+, 150 mg $MgSO_4$)) and an SPE sorbent (30 mg HLB). Each treated solution was mixed with acetonitrile in a 3:1 (*v*/v) ratio, and recovery efficiency was evaluated as follows.

2.5.4. Established sample preparation method

Homogenized pesticide-free mealworms (5 g) were hydrated with water (7 mL) for 15 min and then treated with 0.1 % formic acid in acetonitrile (10 mL) and a ceramic homogenizer. The mixture was shaken for 2 min at 1300 rpm (SPEX SamplePrep Geno/Grinder,

Table 3
Recovery rates of 247 pesticides obtained for different cleanup sorbents.

Recovery (%) at 25 μg/kg	RSD (%) $n = 4$	Number of pesticides (%)					
		PSA	**PSA + C18**	**C18**	***Z*-Sep**	**Z-Sep+**	**HLB**
<10	>0	0 (0)	0 (0)	1 (0.4)	0 (0)	1 (0.4)	0 (0)
10–30	≤20	3 (1.2)	3 (1.2)	3 (1.2)	4 (1.6)	3 (1.2)	2 (0.8)
	>20	0 (0)	0 (0)	1 (0.4)	0 (0)	0 (0)	0 (0)
30–70	≤20	23 (9.3)	33 (13.4)	38 (15.4)	56 (22.7)	25 (10.1)	40 (16.2)
	>20	4 (1.6)	3 (1.2)	2 (0.8)	8 (3.2)	5 (2.0)	1 (0.4)
70–120	≤20	209 (84.6)	194 (78.5)	174 (70.4)	163 (66.0)	191 (77.3)	183 (74.1)
	>20	2 (0.8)	6 (2.4)	18 (7.3)	9 (3.6)	8 (3.2)	9 (3.6)
>120	≤20	3 (1.2)	7 (2.8)	5 (2.0)	4 (1.6)	7 (2.8)	7 (2.8)
	>20	3 (1.2)	1 (0.4)	5 (2.0)	3 (1.2)	7 (2.8)	5 (2.0)
Not detected		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sum		247 (100)	247 (100)	247 (100)	247 (100)	247 (100)	247 (100)

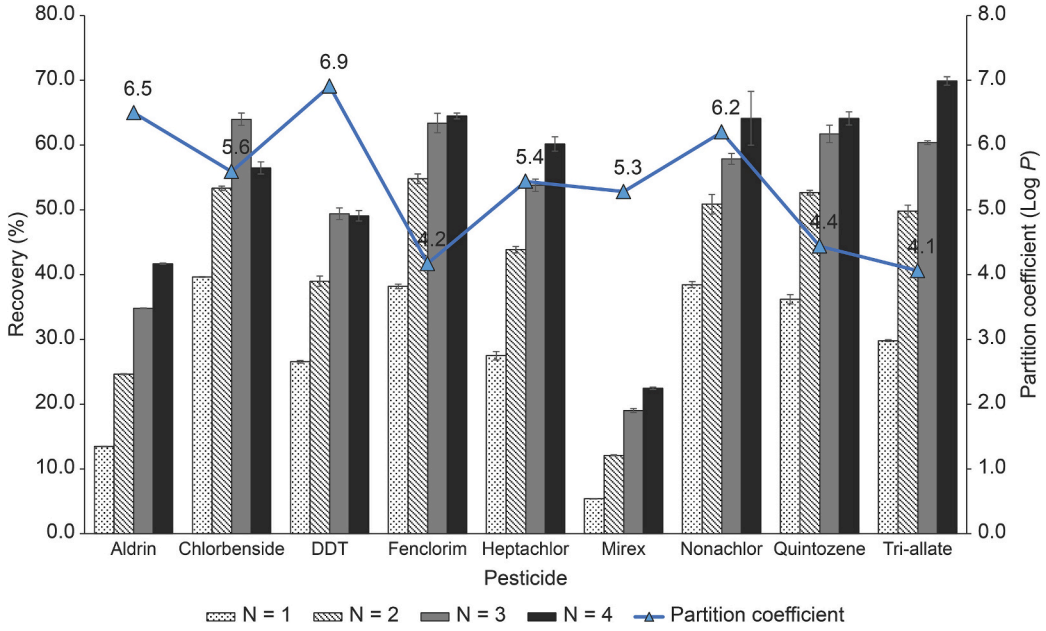


Fig. 1. Recovery rates (bars) and partition coefficients (log *P*, triangles) of 10 representative pesticides showing a strong dependence of recovery (>25 %) on the number of partitionings ($N = 1, 2, 3$, and 4). Error bars are the standard deviations of the recovery rates ($n = 4$).

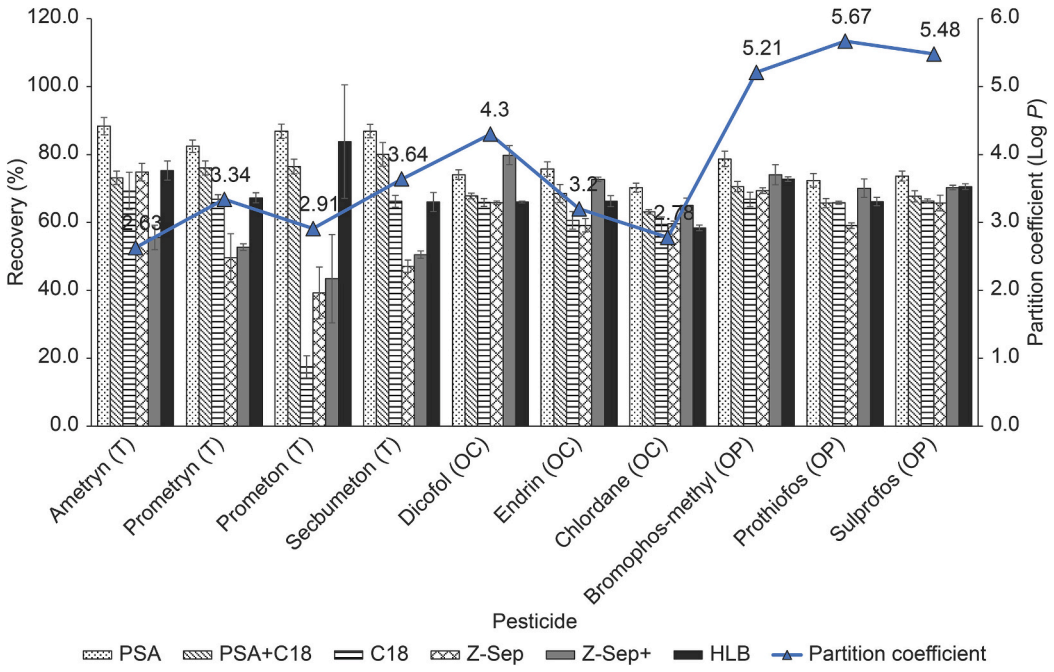


Fig. 2. Recovery rates (bars) and partition coefficients (log *P*, triangles) of 10 representative pesticides showing a strong dependence of recovery on the employed absorbent (primary secondary amine (PSA), C18 + PSA, C18). T = triazine, OC = organochlorine, OP = organophosphate. Error bars are the standard deviations of the recovery rates ($n = 4$).

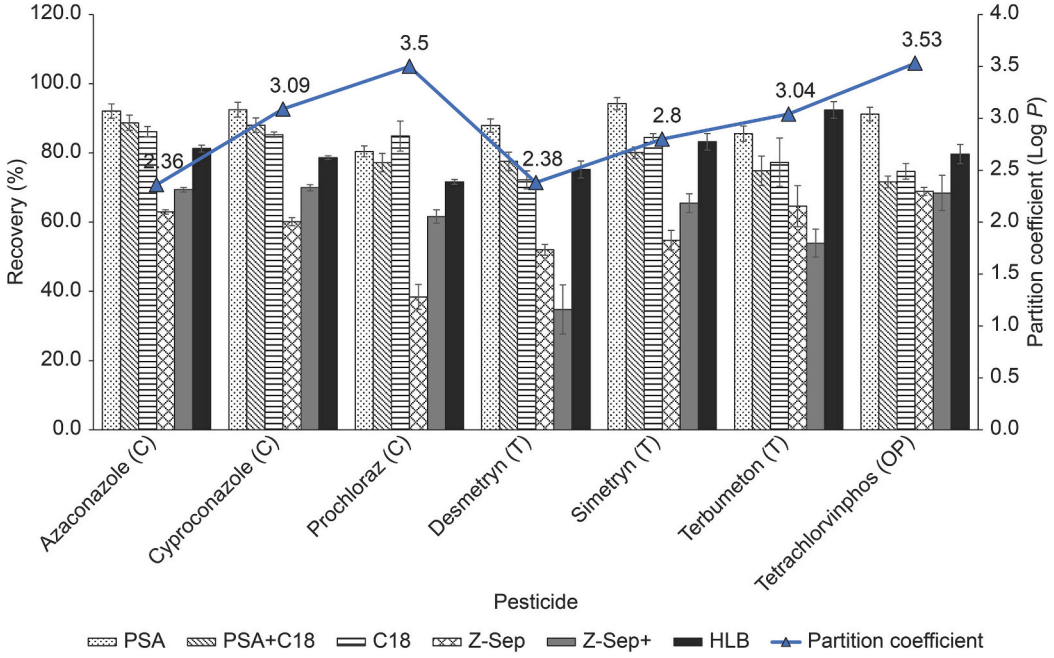


Fig. 3. Recovery rates (bars) and partition coefficients (log *P*, triangles) of seven representative pesticides showing a strong dependence of recovery on the employed absorbent (Z-Sep, Z-Sep+, HLB). C = conazole, T = triazine, OP = organophosphate. Error bars are the standard deviations of the recovery rates ($n = 4$).

Metuchen, NJ, USA). The contents of the original QuEChERS packet were added, and the mixture was shaken again at 1300 rpm for 1 min. The sample was centrifuged at 3500 rpm for 5 min (Combi-514R, Hanil Science Co., Ltd., Incheon, South Korea). The supernatant (6 mL) was transferred to a 15 mL conical tube, mixed with *n*-hexane (4 mL), and centrifuged at 3500 rpm for 5 min. The lower acetonitrile layer was transferred to a new tube, while the upper *n*-hexane layer underwent additional partitioning with *n*-hexane-saturated acetonitrile (3 × 6 mL). The extract was purified using a d-SPE tube containing PSA (25 mg) and $MgSO_4$ (150 mg), and the purified solution was mixed with acetonitrile in a 3:1 (*v*/v) ratio and analyzed using GC–MS/MS.

2.5.5. Method validation and matrix effect evaluation

Limits of quantitation (LOQs) were determined based on a signal-to-noise ratio of ≥10. Calibration curves were constructed using a matrix-matched standard, and linearity was evaluated using the correlation coefficient (r^2) with a weighting factor of $1/x$. Recovery tests were conducted at two spiking levels (LOQ and high concentration), and

Table 4
Validation test results obtained for 247 pesticides in mealworm larvae.

Validation factor	Range	Number of pesticides (%)
Limit of quantitation (LOQ)	1 μg/kg	26 (10.5 %)
	2 μg/kg	43 (17.4 %)
	5 μg/kg	102 (41.3 %)
	10 μg/kg	75 (30.4 %)
	50 μg/kg	1 (0.4 %)
	Sum	247 (100 %)
Linearity (r^2)	> 0.990 (LOQ to 200 μg/kg)	247 (100 %)
Recovery (%) (RSD, 0 %–19.9 %)	Low and high (70 %–120 %)	197 (79.8 %)
	Low and high, <30 % or > 120 %)	41 (16.6 %)
	Low or high (70 %–120 %)	9 (3.6 %)
	Sum	247 (100 %)
Matrix effect	Less than −50 % (strong)	8 (3.2 %)
	−50 % to −20 % (medium)	9 (3.6 %)
	−20 % to 0 % (soft)	42 (17.0 %)
	0 %–20 % (soft)	52 (21.1 %)
	20 %–50 % (medium)	52 (21.1 %)
	>50 % (strong)	84 (34.0 %)
	Sum	247 (100 %)

accuracy and precision were assessed using the average recovery rates and relative standard deviations (RSDs) of six replicate analyses. The matrix effect was calculated from the slopes of the calibration curves for matrix-matched (a) and pure (b) standards as.

$$\text{Matrix effect (\%)} = 100 \times (a/b - 1). \quad (1)$$

3. Result and discussion

3.1. Optimization of sample preparation procedures

The multiple reaction monitoring parameters used for the 247 pesticides, along with the corresponding GC–MS/MS conditions, are listed Table S1. After determining the optimal number of acetonitrile and *n*-hexane partitionings, we identified the optimal extraction solvent–QuEChERS salt combination and then compared the effects of d-SPE and SPE sorbents as cleanup agents.

3.1.1. Extraction efficiency

The extraction efficiencies obtained using different extraction solvents and QuEChERS salts are presented in Table 1. The replacement of pure acetonitrile with acetonitrile containing 0.1 % formic acid increased the pesticide extraction efficiencies by 1.6–7.7 percentage points, regardless of the QuEChERS salts used, which was ascribed to the stabilizing effect of formic acid on pesticides sensitive to basic conditions (Walorczyk & Gnusowski, 2009). Additionally, the citrate buffer in the QuEChERS EN-15662 packet maintained the extraction solution pH at ~5, preventing pesticide ionization and degradation (González-Curbelo et al., 2015). Shin et al. (2020) maximized the efficiency of pesticide extraction from mealworm larvae using acidic extraction solvents combined with the EN-15662 packet. However, herein, the highest extraction efficiency was achieved by combining 0.1 % formic acid in acetonitrile with the original QuEChERS packet, and 201 pesticides were recovered (81.4 %). Considering that different extraction methods have been used depending on the physicochemical properties of the pesticides, sample types, and analytical instruments (Perestrelo et al., 2019), we concluded that the combination of an acidic extraction solvent with the original packet was most suitable for extracting 247 of 254pesticides from mealworm larvae and analyzing them using GC–MS/MS.

3.1.2. Partitioning efficiency

Mealworm larvae have a fat content of 21.9 wt% (Imathiu, 2020) and should therefore be defatted prior to analysis to remove interferents and prevent the contamination of the GC–MS/MS instrument. Therefore, *n*-hexane, a nonpolar solvent effective for fat removal, was employed (Ramluckan et al., 2014). Additionally, *n*-hexane (partition coefficient = 3.90) has a higher affinity than acetonitrile (partition coefficient = −0.34) and can therefore extract nonpolar pesticides that may remain in the extraction solvent. Using *n*-hexane-saturated acetonitrile, we examined the effect of partitioning frequency on recovery rates (Table 2). Each additional partitioning led to a higher pesticide recovery, with four partitions yielding a 22 percentage point increase in recovery (84.6 %) compared with a single partition. At this level, 209 pesticides demonstrated acceptable recovery rates (70 %–120 %) and precisions (RSD ≤ 20 %). Thiocarbamate and organochlorine pesticides exhibited low recovery rates, probably because of their high partition coefficients (log P = 4.1–6.9) due to their low polarities, and were therefore retained in the *n*-hexane phase (Mathieu et al., 2015; Mdeni et al., 2022). However, the recovery rates of these pesticides increased with the increasing partitioning frequency (Fig. 1). Therefore, partitioning with *n*-hexane-saturated acetonitrile was concluded to be essential for improving the recovery of pesticides with high partition coefficients, and the optimal number of partitions was identified as four.

3.1.3. Cleanup efficiency

Table 3 lists the purification efficiencies of different sorbents. PSA demonstrated the highest purification efficiency, yielding an acceptable recovery range (70 %–120 %) with RSD ≤ 20 % for 209 pesticides (84.6 %). PSA is typically used to remove sugars, fatty acids, and organic acids but may engage in polar interactions with low-partition-coefficient pesticides and thus reduce their recovery rates (Sobhanzadeh and Nemati, 2013; Belarbi et al., 2021). Herein, this effect appeared to be mitigated by the use of an acidic extraction solvent. Triazine herbicides, organochlorines, and organophosphate insecticides showed a 6.1–14.2 percentage point decrease in recovery when C18 was used instead of PSA (Fig. 2). Given that C18 is used to remove fats and lipids and can therefore adsorb nonpolar pesticides (Tuzimski & Rejczak, 2016; Walorczyk & Gnusowski, 2009), the high partition coefficients of these pesticides could have led to their reduced recovery through adsorption onto C18 (Fig. 2). *Z*-Sep and Z-Sep+, which feature ZrO_2-coated silica surfaces, effectively remove lipids and lipid-associated carboxylic acids through Lewis acid–base interactions (Belarbi et al., 2021; Walorczyk & Gnusowski, 2009). However, Z-Sep + has been reported to adsorb azole fungicides, while Z-Sep strongly binds phosphates (Rajski et al., 2013; Tuzimski & Rejczak, 2016). Herein, the recovery rates of azole and organophosphate pesticides obtained for Z-Sep and Z-Sep + were lower than those obtained for the other adsorbents (Fig. 3), which was attributed to the presence of ZrO_2 in Z-Sep and Z-Sep+. HLB, characterized by a hydrophilic–lipophilic balance, contains divinylbenzene and *N*-vinylpyrrolidone moieties that can adsorb both nonpolar and polar compounds (Lucci & Nunez, 2014). The recovery rates of the nonpolar triazine pesticides obtained for HLB exceeded those obtained for Z-Sep or Z-Sep+. However, PSA was determined to be the most suitable sorbent for the GC–MS/MS–based analysis of 247 pesticides in mealworm larvae.

3.2. Method validation

The developed method was validated by assessing its LOQ, linearity, recovery, and matrix effect according to the SANTE/12682/2019 guidelines (Tables 4 and S2). The analysis of 247 matrix-matched standards was used to establish LOQs as concentrations at which the signal-to-noise ratio exceeded 10. For 246 compounds, the LOQ was equal to or less than the default limit of 10 μg/kg; however, because of low sensitivity, the LOQ for endosulfan-alpha was 50 μg/kg. The matrix-matched standards of the 247 pesticides showed high linearity with correlation coefficients above 0.99. Among the tested compounds, 197 (79.8 %) showed acceptable recovery rates at both levels and were therefore suitable for quantitative analysis. However, 41 compounds

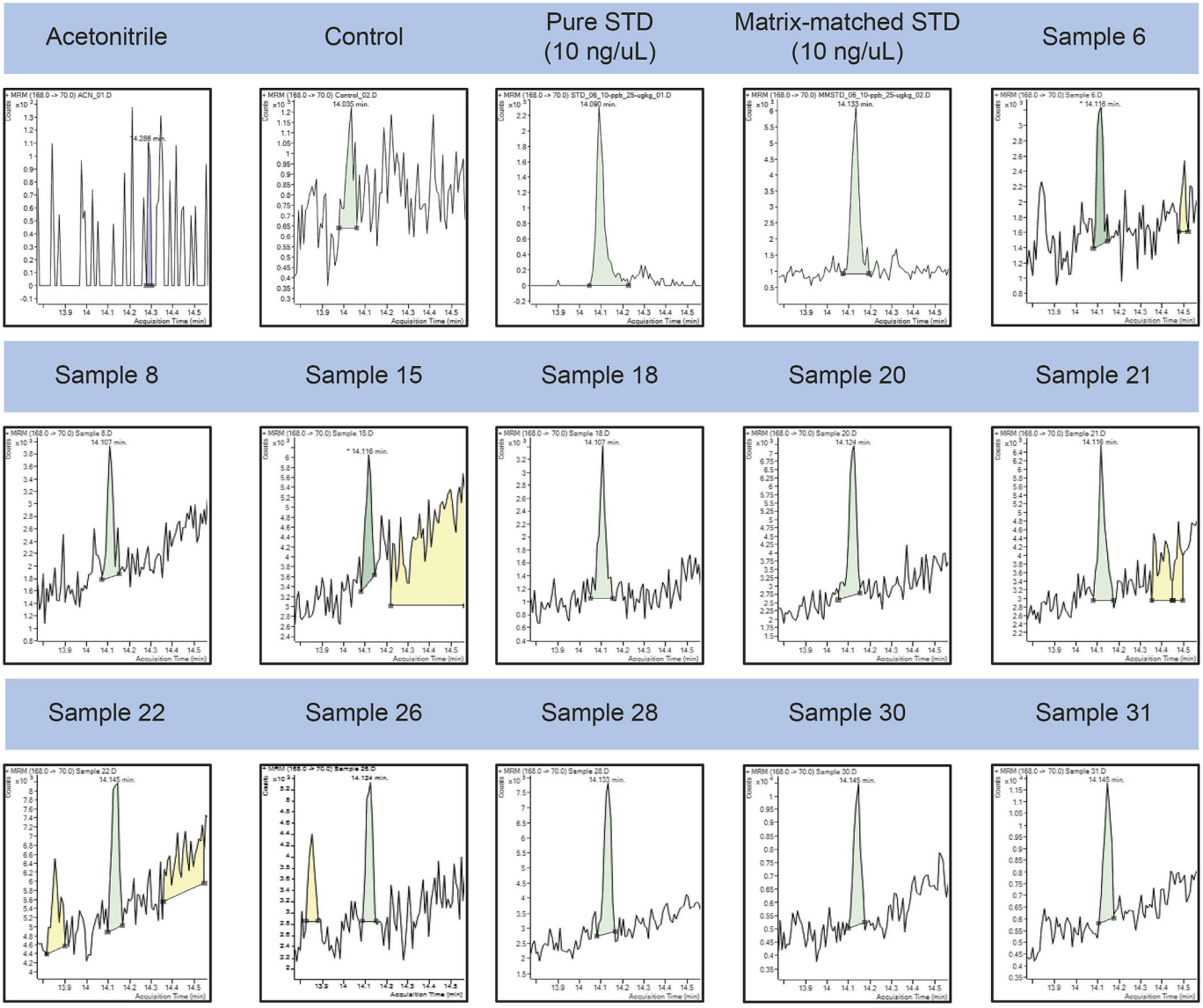


Fig. 4. Chromatograms of triadimenol obtained for acetonitrile, control, pure standard (10 μg/kg), matrix-matched standard (10 μg/kg), and mealworm samples (6, 8, 15, 18, 20, 21, 22, 26, 28, 30, and 31).

met the recovery criteria at only one level, and 9 compounds failed to meet the criteria at both levels, which suggested that these compounds were more suitable for qualitative analysis. According to the matrix effect, 94 compounds fell within the "soft" range (|matrix effect| < 20 %), i.e., could be quantified without matrix-matched standards. However, 153 compounds fell within the "medium" (|matrix effect| = 20 %–50 %) or "strong" (|matrix effect| > 50 %) ranges, which indicated the need for matrix-matched standards to offset matrix effects during inlet and ionization processes for accurate quantitation.

3.3. Real sample analysis

Among the 24 samples collected from domestic mealworm larva farms, 11 contained the triazole fungicide triadimenol at concentrations of 14–996 μg/kg (Fig. 4) and were therefore noncompliant with the EU residue limit for terrestrial invertebrates (10 μg/kg). Considering that the triadimenol residue limit for wheat, the primary ingredient of bran fed to mealworms, is 100 μg/kg, pesticide absorption probably occurred through bran consumption. Thus, the established method for the multiresidue analysis of 247 pesticides in mealworm larvae was concluded to be applicable in field settings and enable both quantitative and qualitative analyses.

4. Conclusions

A GC–MS/MS–based multiresidue method for the detection and quantitation of 247 pesticides in mealworm larvae was developed, optimizing the standard QuEChERS procedure to account for matrix complexity and high fat content, and validated. Pesticide recovery rates were maximized by combining 0.1 % formic acid in acetonitrile (extraction solvent) with the original QuEChERS packet. Further cleanup (PSA d-SPE) effectively removed matrix interferences without significant analyte loss, enhancing detection accuracy. According to the results of validation tests, the developed method exhibited high linearities, acceptable recovery rates, and low detection limits for most analytes and was therefore concluded to be robust and suitable for both quantitative and qualitative analyses. The analysis of 24 field samples revealed detectable levels of triadimenol, suggesting its potential uptake from bran feed. Thus, this validated approach was concluded to be suitable for application in regulatory and industrial settings, offering a reliable tool for monitoring pesticide residues in mealworm larvae and similar edible insect matrices.

CRediT authorship contribution statement

Hyun Ho Noh: Writing – review & editing, Writing – original draft, Resources, Project administration. **Chang Jo Kim:** Writing – original draft, Formal analysis. **So-Hee Kim:** Formal analysis. **Hye-Ran Eun:** Formal analysis. **Yongho Shin:** Formal analysis, Conceptualization. **Won Tae Jeong:** Writing – review & editing.

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.

Acknowledgements

Funding This study was supported by the Research Program for Agriculture Science & Technology Development (Project No. PJ015920), National Institute of Agricultural Sciences, Rural Development Administration, South Korea.

Declaration of interests

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102386.

Data availability

Data will be made available on request.

References

Belarbi, S., Vivier, M., Zaghouani, W., De Sloovere, A., Agasse, V., & Cardinael, P. (2021). Comparison of different d-SPE sorbent performances based on quick, easy, cheap, effective, rugged, and safe (QuEChERS) methodology for multiresidue pesticide analyses in rapeseeds. *Molecules, 26*, Article 6727. https://doi.org/10.3390/molecules26216727

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety, 12*, 296–313. https://doi.org/10.1111/1541-4337.12014

Burger, J., de Mol, F., & Gerowitt, B. (2008). The "necessary extent" of pesticide use – Thoughts about a key term in German pesticide policy. *Crop Protection, 27*, 343–351. https://doi.org/10.1016/j.cropro.2007.06.006

Damalas, C. A., & Eleftherohorinos, I. (2011). Pesticide exposure, safety issues, and risk assessment indicators. *International Journal of Environmental Research and Public Health, 8*, 1402–1419. https://doi.org/10.3390/ijerph8051402

European Commission. (2024). *EU pesticides database: 10600000 terrestrial invertebrate animals*. Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/products/details/377 Accessed October 15, 2024.

Food and Agriculture Organization (FAO). (2013). *Edible insects: future prospects for food and feed security*. Retrieved from https://www.fao.org/4/i3253e/i3253e.pdf Accessed December 15, 2024.

González-Curbelo, M.Á., Socas-Rodríguez, B., Herrera-Herrera, A. V., González-Sálamo, J., Hernández-Borges, J., & Rodríguez-Delgado, M.Á. (2015). Evolution and applications of the QuEChERS method. *TrAC –Trends in Analytical Chemistry, 71*, 169–185. https://doi.org/10.1016/j.trac.2015.04.012

Gravel, A., & Doyen, A. (2020). The use of edible insect proteins in food: Challenges and issues related to their functional properties. *Innovative Food Science & Emerging Technologies, 59*, Article 102272. https://doi.org/10.1016/j.ifset.2019.102272

Houbraken, M., Spranghers, T., De Clercq, P., Cooreman-Algoed, M., Couchement, T., De Clercq, D., Verbeke, S., & Spanoghe, P. (2016). Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. *Food Chemistry, 201*, 264–269. https://doi.org/10.1016/j.foodchem.2016.01.097

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., & Mortensen, D. A. (2017). Agriculture in 2050: Recalibrating targets for sustainable intensification. *Bioscience, 67*, 386–391. https://doi.org/10.1093/biosci/bix010

Imathiu, S. (2020). Benefits and food safety concerns associated with consumption of edible insects. *NFS Journal, 18*, 1–11. https://doi.org/10.1016/j.nfs.2019.11.002

Lucci, P., & Nunez, O. (2014). On-line solid-phase extraction for liquid chromatography mass spectrometry analysis of pesticides. *Journal of Separation Science, 37*, 2929–2939. https://doi.org/10.1002/jssc.201400531

Lushchak, V. I., Matviishyn, M., Husak, V. V., Storey, J. M., & Storey, K. B. (2018). Pesticide toxicity: A mechanistic approach. *EXCLI Journal, 17*, 1101–1136. https://doi.org/10.17179/excli2018-1710

Mathieu, C., Duval, R., Xu, X., Rodrigues-Lima, F., & Dupret, J. M. (2015). Effects of pesticide chemicals on the activity of metabolic enzymes: Focus on thiocarbamates. *Expert Opinion on Drug Metabolism & Toxicology, 11*, 81–94. https://doi.org/10.1517/17425255.2015.975691

Mdeni, N. L., Adeniji, A. O., Okoh, A. I., & Okoh, O. O. (2022). Analytical evaluation of carbamate and organophosphate pesticides in human and environmental matrices: A review. *Molecules, 27*. https://doi.org/10.3390/molecules27030618. Article 618.

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J. A. M., Silva, C., Medina, S., & Camara, J. S. (2019). QuEChERS—Fundamentals, relevant improvements, applications and future trends. *Analytica Chimica Acta, 1070*, 1–28. https://doi.org/10.1016/j.aca.2019.02.036

Poppe, L., Vanhoutte, S., & Hofte, M. (2003). Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. *European Journal of Plant Pathology, 109*, 963–973. https://doi.org/10.1023/B:EJPP.0000003747.41051.9f

Porter, J., Parry, M., & Carter, T. (1991). The potential effects of climatic change on agricultural insect pests. *Agricultural and Forest Meteorology, 57*, 221–240. https://doi.org/10.1016/0168-1923(91)90088-8

Rajski, R., Lozano, A., Ucles, A., Ferrer, C., & Fernandez-Alba, A. R. (2013). Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A, 1304*, 109–120. https://doi.org/10.1016/j.chroma.2013.06.070

Ramluckan, K., Moodley, K. G., & Bux, F. (2014). An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the Soxhlet extraction method. *Fuel, 116*, 103–108. https://doi.org/10.1016/j.fuel.2013.07.118

Ravzanaadii, N., Kim, S. H., Choi, W. H., Hong, S. J., & Kim, N. J. (2012). Nutritional value of mealworm, *Tenebrio molitor* as food source. *International Journal of Industrial Entomology, 25*, 93–98. https://doi.org/10.7852/ijie.2012.25.1.093

Rockstrom, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., Wetterstrand, H., Declerck, F., Shah, M., Steduto, P., de Fraiture, C., Hatibu, N., Unver, O., Bird, J., Sibanda, L., & Smith, J. (2017). Sustainable intensification of agriculture for human prosperity and global sustainability. *Ambio, 46*, 4–17. https://doi.org/10.1007/s13280-016-0793-6

Shin, Y. H., Kim, C. J., Baek, S. J., Kim, L. S., Son, K. A., Lee, H. D., … Noh, H. H. (2020). Liquid chromatography-tandem mass spectrometry for the simultaneous analysis of 353 pesticides in the edible insect *Tenebrio molitor* larvae (mealworms). *Molecules, 25*, Article 5866. https://doi.org/10.3390/molecules25245866

Shin, Y. H., Lee, J. H., & Kim, J.-H. (2018). A simultaneous multiresidue analysis for 203 pesticides in soybean using florisil solid-phase extraction and gas chromatography–tandem mass spectrometry. *Applied Biological Chemistry, 61*, 543–548. https://doi.org/10.1007/s13765-018-0388-y

Sobhanzadeh, E., & Nemati, K. (2013). Liquid–liquid extraction/low-temperature purification (LLE/LTP) followed by dispersive solid-phase extraction (d-SPE) cleanup for multiresidue analysis in palm oil by LC-QTOF-MS. Journal of Chemistry, 2013, Article 9. doi:https://doi.org/10.1155/2013/915048.

Tuzimski, T., & Rejczak, T. (2016). Application of HPLC-DAD after SPE/QuEChERS with ZrO_2-based sorbent in d-SPE clean-up step for pesticide analysis in edible oils. *Food Chemistry, 190*, 71–79. https://doi.org/10.1016/j.foodchem.2015.05.072

Van Huis, A. (2020). Edible insects. In H. L. Meiselman (Ed.), *Handbook of eating and drinking: Interdisciplinary perspectives* (pp. 965–980). Springer International Publishing.

Walorczyk, S., & Gnusowski, B. (2009). Development and validation of a multi-residue method for the determination of pesticides in honeybees using acetonitrile-based extraction and gas chromatography-tandem quadrupole mass spectrometry. *Journal of Chromatography A, 1216*, 6522–6531. https://doi.org/10.1016/j.chroma.2009.07.045

Yoo, J., Hwang, J. S., Goo, T. W., & Yun, E. Y. (2013). Comparative analysis of nutritional and harmful components in Korean and Chinese mealworms (*Tenebrio molitor*). *Journal of the Korean Society of Food Science and Nutrition, 42*, 249–254. https://doi.org/10.3746/jkfn.2013.42.2.249