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RESEARCH ARTICLE

A Novel Method for the Analysis of Volatile Organic Compounds (VOCs) from Red Flour Beetle *Tribolium castaneum* (H.) Using Headspace-SPME Technology

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ARTICLE HISTORY

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Abstract: ***Background*:** The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the world's most serious stored grain insect pests. A method of early and rapid identification of red flour beetle in stored products is urgently required to improve control options. Specific chemical signals identified as volatile organic compounds (VOCs) that are released by the beetle can serve as biomarkers.

***Methods*:** The headspace solid phase microextraction (HS-SPME) technique and the analytical conditions with GC and GCMS were optimised and validated for the determination of VOCs released from *T. castaneum*.

***Results*:** The 50/30 µm DVB/CAR/PDMS SPME fibre was selected for extraction of VOCs from *T. castaneum*. The efficiency of extraction of VOCs was significantly affected by the extraction time, temperature, insect density and type of SPME fibre. Twenty-three VOCs were extracted from insects in 4 mL flask at 35 ± 1°C for four hours of extraction and separated and identified with gas chromatography-mass spectroscopy. The major VOCs or chemical signals from *T. castaneum* were 1-pentadecene, p-Benzoquinone, 2-methyl- and p-Benzoquinone, 2-ethyl.

***Conclusion*:** This study showed that HS-SPME GC technology is a robust and cost-effective method for extraction and identification of the unique VOCs produced by *T. castaneum*. Therefore, this technology could lead to a new approach in the timely detection of *T. castaneum* and its subsequent treatment.

Keywords: Headspace-SPME, stored grain insects, *Tribolium castaneum*, VOCs.

1. INTRODUCTION

Damage caused by insects decreases the quantity and quality of grain by consuming, contaminating and producing the ideal conditions for growing the microorganism in grain [1]. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a vital pest infecting a broad range of stored products including grains [2, 3]. The economic importance of this species derives from damaging and reducing the grain quality by contaminating the products by insect parts, ecdysis skins and individuals of each life stages in addition to off-odour [4, 5]. These insects can be very difficult to detect in large volumes of stored grains, especially when infestation rates are low. Many detection techniques used in stored products are not practical because of their high cost and low sensitivity [1].

Volatile organic compounds (VOCs) are carbon compounds that evaporate at room temperature [6]. In an organism, they are released products of metabolic activities or tissue damage, and the profile of VOCs may be unique to each species. Hence, analysing VOCs released into the airspace surrounding stored products is a potential method of diagnosis and species identification [7-11]. A range of commercial solid phase micro-extraction (SPME) fibres were used to extract VOCs from the headspace [12]. The VOCs were analysed and identified using GC and GC-MS methods [7]. However, this method requires further optimisation to enhance the efficacy of VOCs extraction [9].

This study aimed to optimise the parameters for the detection of *T. castaneum*-specific VOCs in the headspace of stored grains using the headspace SPME method, and various conditions like insect density, temperature, age, type of

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SPME fibre and time of equilibrium extraction were optimised to get a maximum number of VOC's to enhance the sensitivity of the detection.

2. MATERIALS AND METHODS

2.1. Insects

Adult insects of *T. castaneum* were obtained from the Department of Primary Industries and Regional Development (DPIRD), Australia. Insects of similar age were produced by incubating 3000 adult insects of *T. castaneum* with 500 g of wheat flour and yeast in a 12:1 ratio in 2000 mL jars sealed with meshed lids. Parent insects were removed after four days. Cultures were incubated at 27 ± 2 and 70% RH. As new insects emerged, the cultures were sieved and all adult insects removed and transferred to a new container with food to get adults of similar age. The insects used in the experiments were one month old. The flour was made from freshly harvested wheat (Australian Standard Wheat 1). Before using the wheat was sterilised by storing in a 60 L container at -20°C for seven days and then storing at 4°C until further use. The grain was milled using a Wonder Mill (Model WM2000, Korea), and the flour was also kept at 4°C until further use.

2.2. Analysis and Identification of VOCs

2.2.1. Glassware, reagents and SPME fibres

A screw top 4 mL amber vial coupled with a cap equipped with septa (SUPELCO, USA) was used as a chamber for collection of VOCs released from insects. All chemical reagents used were of analytical grade and supplied by Sigma-Aldrich (Castle Hill, NSW, Australia). The 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) and 85 µm polyacrylate SPME fibres (SUPELCO, Bellefonte, PA, USA USA) were used according to the manufacturer's recommendations.

2.2.2. Gas Chromatography-flame Ionisation Detector (GC-FID)

Desorption and separation of extracted VOCs were achieved with gas chromatography-flame ionisation detector (GC-FID); Hewlett Packard 5890 (series II USA) coupled with mid-polarity phase column (RESTIK Rxi-5ms 30 m x 0.25 mm x 0.25 µm) (Cat no. 13423; serial no. 978690). Hydrogen was used as a carrier gas at a constant flow of 40 mL/min. The temperature of the GC inlet (split-less mode) and the detector were 250 and 290°C respectively. The oven temperature was programmed at 40°C for 5 min increased at a rate of 5°C/min to 250°C and held for 5 min with a total running time of 52 min. The oven temperature program was designed to volatilize most of the VOCs released by the insects in addition to providing good separation among the compounds. High inlet temperature was used to ensure that all the VOCs were desorbed from the SPME fibre [9].

2.2.3. Gas Chromatography-mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) was used for identification of the VOCs released by the insects. The instrument used was an Agilent technologies 7820A (serial# CN 14272038, USA) equipped with an Agilent 5977E Mass Spectrometry Detector (MSD) (serial# US1425R204, USA) fitted with an Agilent J&W mid-polarity column (HP-35 ms; 30 m x 0.25 mm x 0.25 µm). The GC inlet (split-less mode) operated under an SPME splitless mode injector. Helium was used as a carrier gas at a constant airflow of 1.1 mL/min. The oven temperature was programmed at 50°C for 5 min increased at 5°C/min to 250°C with a total operating time of 45 min. while the inlet and detector temperature were kept at (250 and 290°C respectively).

2.2.4. Optimization and Validation Studies

This study was conducted to determine the effect of some parameters on the extraction of VOCs from the red flour beetle T. castaneum. The extraction procedure involved cleaning the SPME fibre by heating it at 270°C for 15 min. The optimisation was started by evolution of two commercial fibre types different in polarity coating of 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) and 85 µm polyacrylate (PA) fibres. The aim of comparing the performance of 85 µm polyacrylate fibre with three phase fibre was to determine if there are specific compounds that can be extracted only by 85 µm polyacrylate, as it has only one polar phase. The experiment was implemented using 10 adult insects for 2 h extraction period at 27 ± 1°C and 70% RH with three biological replicates.

The screw top 4 mL amber vials containing 1, 5, 10, 20 and 30 insects respectively and sealed with cap equipped with septa for sampling were used to evaluate the effect of the insect density on the extraction of VOCs. The extraction was conducted at 27 ± 1°C and 70% RH for a 2 h.

For optimization of VOCs extraction, the clean fibre was inserted into the headspace of the vial containing the insects. Combinations of two extraction temperatures of 27 ± 1°C and 35 ± 1°C at 70% RH for four extraction times (2, 4, 6 and 8 h) was evaluated. After the extraction had finished, the SPME fibre was withdrawn and inserted directly into the GC inlet for 5 min to desorb the collected VOCs at 250°C.

The VOCs identification was implemented using GC-MS by applying optimal conditions that were obtained from the optimization studies. Each of the above test was replicated three times.

2.3. Evaluation of the Limit of Detection of the Analytical Method

The limit of detection (LOD) was determined by analysing two external standards including toluene and acetophenone. Diluted standards were prepared in a range of 0 to 100 ng/mL by adding a specific amount to extraction vials. The standard samples were extracted and analysed with optimized procedures and conditions. Each test was replicated for three times.

2.4. Statistical analysis and Compound Identification

The area that represents each peak was extracted using MassHunter Quantitative Analysis software for GC (Agilent

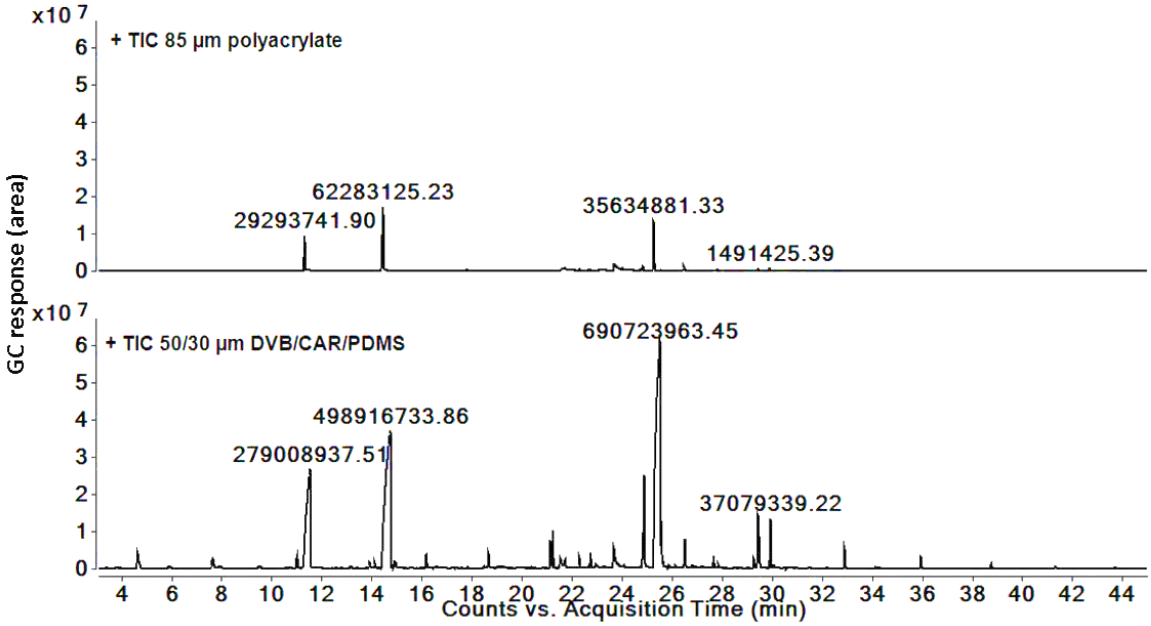

Fig. (1). Comparison between two types coating SPME fibres in the total number of GC signal chromatograms (TIC) and peak areas of volatile organic compounds from *T. castaneum*. The numbers on the chromatogram indicate the peak area of some identical peaks.

Technologies). The treatment groups were loaded to the software as one patch, and same parameters were applied to the whole patch. Reports of a compound of peak areas were generated to Excel. Each peak area was given a metabolic identification indicating the GC flame ionisation detector and the retention time of each peak. The averages of compound areas were statistically analysed by Metaboanalyst 3.0 using Partial Least Squares - Discriminant Analysis (PLS-DA) [13]. The qualitative identification of VOCs was made by Automatic Mass Spectral Deconvolution and Identification System (AMDIS-32) software coupled with NIST 2.2 mass spectra library. Three criteria were taken into consideration when identifying compounds: similar mass spectra, high match factor and the comparison of retention Kovat's index with retention index obtained from NIST (Table **S1**).

3. RESULTS AND DISCUSSION

3.1. Effect of SPME Fibre Type on the Efficacy of Extraction VOCs

Four factors were optimised and investigated including SPME fibre type, extraction time, extraction temperature and sample size. Fig. (**1**) shows the different performance between two types of SPME fibres (50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) and 85 µm polyacrylate (PA)) on the extraction of volatile organic compounds (VOCs) from the adults of red flour beetle insects. The three phase DVB/CAR/PDMS SPME fibre extracted more broad VOCs than the PA SPME fibre.

However, the PA SPME fibre could extract some compounds from *T. castaneum* like compounds at RT=14.45 and 25.27 min, but no new compounds different from the peak numbers and intensities compared to DVB/CAR/PDMS SPME that produced higher peak abundances for most of the VOCs chromatogram. Therefore, the DVB/CAR/PDMS SPME fibre was selected as the most appropriate fibre to extract VOCs for evaluation of the other optimisation parameters. In this context, it was reported that fibre coating is a vital factor in development appropriate SPME method, confirming that DVB/CAR/PDMS SPME is the best fibre for high efficient extraction VOCs and the further increasing the method sensitivity. It was also indicated that using mid polarity fibre coating like DVB/CAR/PDMS is more efficient to extract a number of the volatiles [12]. Similar results to our finding were obtained in a study which confirmed that the DVB/CAR/PDMS SPME fibre was the most efficient fibre among six commercial fibres including PA SPME fibre to extract VOCs from *Phytophthora cinnamomi* [11]. The superior performance of DVB/CAR/PDMS SPME was also reported in many studies in comparison with other fibre coatings [14-19]. The polarity of the SPME strongly influences the extraction capacity, as well as the polarity of volatile compounds also affects the SPME ability [12]. PA SPME was characterized for attracting polar semi-volatiles [15, 20, 21], whereas DVB/CAR/PDMS fibre can absorb more extensive range of VOCs that have different physical and chemical properties [15, 22]. Since that the PA fibre was reported to have a little capacity to extract VOCs from a waste gas [23].

3.2. Effect of Insect Densities on the Efficacy of Extraction VOCs

Once the DVB/CAR/PDMS was selected as the optimal fibre, different insect densities (1, 5, 10, 20 and 30) per vial were tested to investigate the optimal density of insects that should be used to improve the recovery of volatile organic compounds from *T. castaneum* adult insects. The average of the peak areas was analysed using Partial Least Squares -

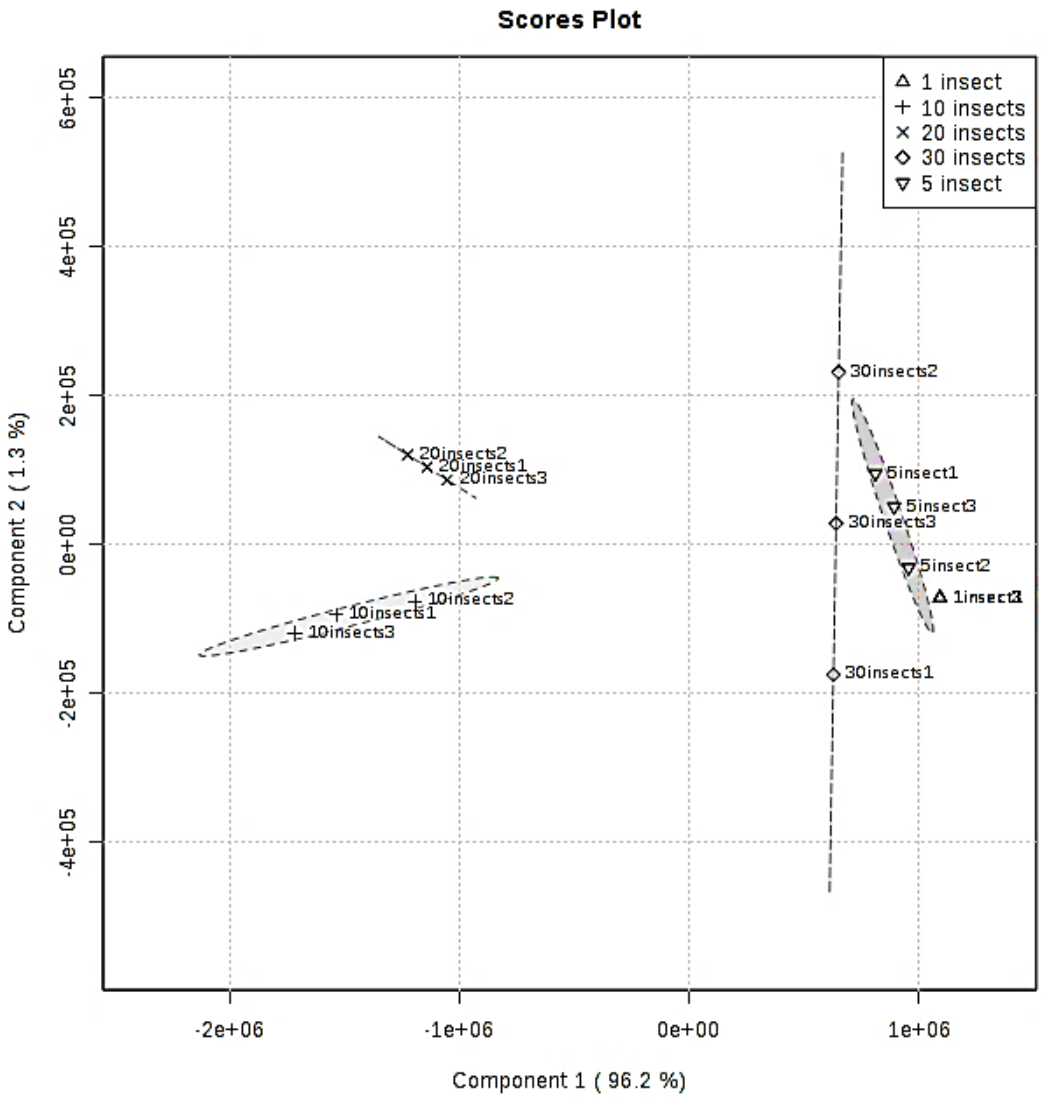

Fig. (2). PLS-DA score plot shows the data obtained from *T. castaneum* in different insect densities based on VOCs with three biological replicates.

Discriminant Analysis (PLS-DA). The result shows the scores plot between the selected PCs (Fig. **2**). The model showed a good separation among the tested treatment groups in this experiment, demonstrating the impact of the insect population density on the ability to emit the VOCs.

The density of 10 insects had the highest abundance of most metabolic products, including those which had high intensity in the GC-FID chromatogram like FID-25.01, FID-24.78, FID-20.74, FID-29.44 and FID-25.54 (Fig. **3**). In all cases, most of the VOCs increased steadily from one insect to 10 insects and then decreased from 20 and 30 insects. This decreasing can be attributed to the overcrowding in the small vial (4 mL). The overcrowding might have caused a reduction in the metabolism of insects due to an increase in the CO2 quantity which has a critical effect on the biological and physiological processes of insects [24, 25]. This is because the amount of sample strongly affects the amount of the extracted analyte [15]. The higher concentration of VOCs extracted from ten insects proved that this number of insects has an optimal density to produce an abundant amount of VOCs that can be collected by SPME. Ten insects in 4 ml bottle (2.5 insects for 1 cm3) were chosen as the optimum insect density to implement further optimization.

3.3. Effect of fibre Exposure Period and Temperature on the Efficacy of Extraction VOCs

Four extraction periods (2, 4, 6 and 8 h) were applied combined with two different extraction temperatures (27 and 35°C). The areas of the identical main peaks have been used to determine the optimal extraction period. The segregation of the results in the score plot model of the PLS-DA proves that extraction period and temperature have a significant effect on the VOCs emission from the adult of *T castaneum* (Fig. **4**). Variable Importance in Projection (VIP) scores (Fig. **5**) show the importance of the metabolites that contributed in the PLS-DA model.

At 27°C, no change was observed in the number of compounds peaks over the four extraction periods. However, the

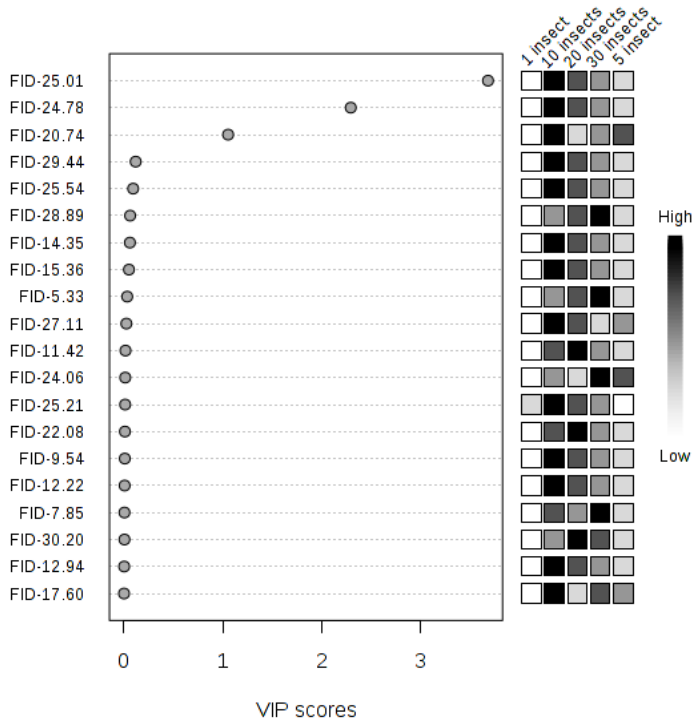

Fig. (3). Variable Importance in Projection (VIP) shows important features identified by PLS-DA from data of different insect densities based on VOCs with three biological replicates. The greyest boxes on the right indicate the high relative abundance of the corresponding metabolites in each group under study. Codes in left side are the metabolic ID (FID indicates GC-FID detector and numbers indicate the retention times min).

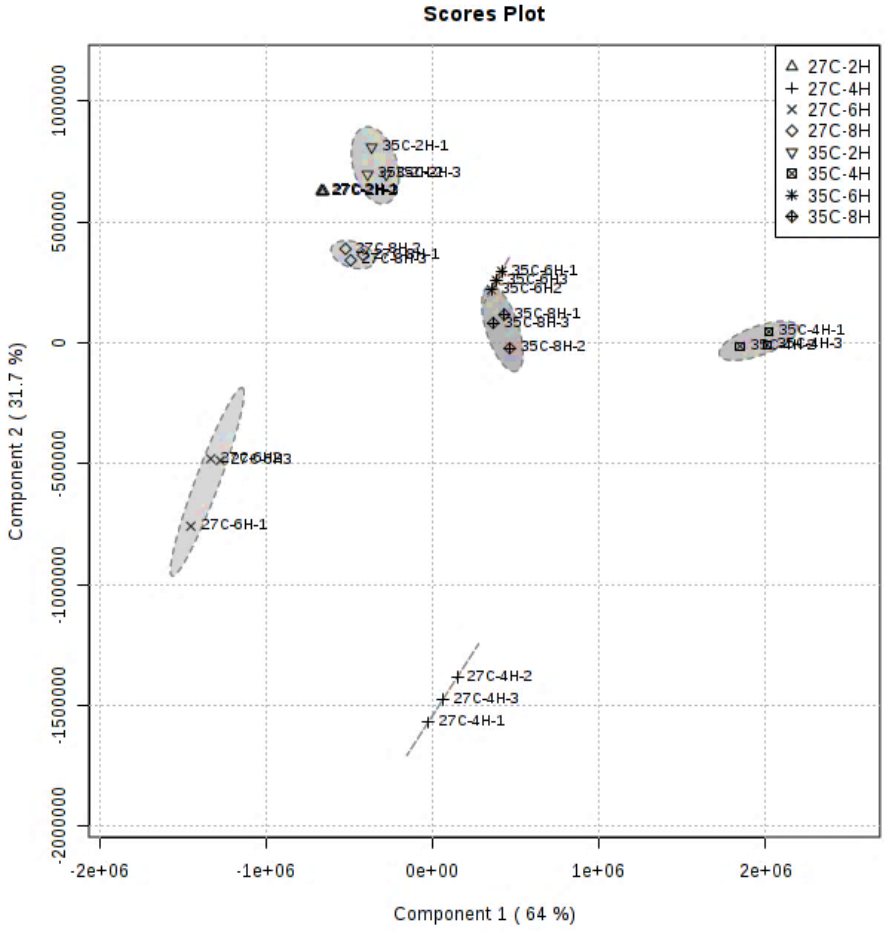

Fig. (4). PLS-DA score plot shows the data obtained from *T. castaneum* in different extraction time and temperature based on VOCs with three biological replicates.

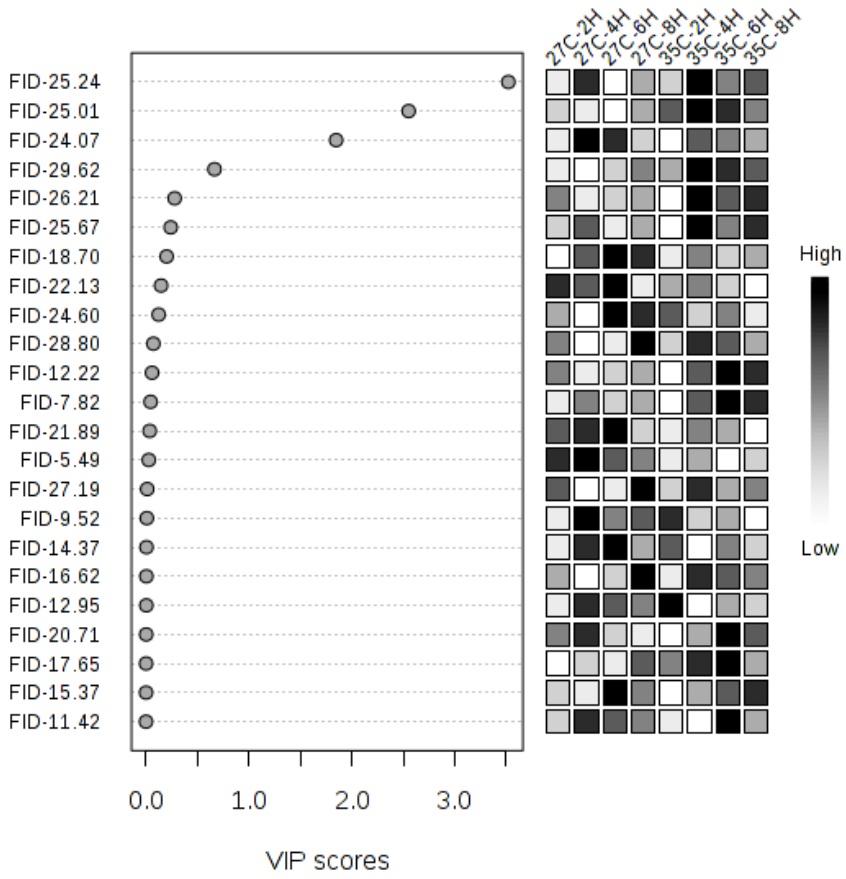

Fig. (5). Variable Importance in Projection (VIP) shows important features identified by PLS-DA from data of different extraction time and temperature based on VOCs with three biological replicates. The greyest boxes on the right indicate the higher relative abundance of the corresponding metabolites in each group under study. Codes in left side are the metabolic ID (FID indicates GC-FID detector and numbers indicate the retention times min).

intensities of chromatogram peaks increased significantly after increasing the extraction time from 2 to 4, 6 and 8 h (Fig. **5**). However, at 2 h, some of the early compounds had higher abundance than other extraction periods like a compound at FID-5.49 and FID-12.22. While at longer extraction time, some of the later compounds like FID-27.19, FID-28.80 and FID-24.60 had higher peak abundance. That is probably because at shorter extraction times, the highly volatile compounds were absorbed faster in comparison with the intermediate and low volatility compounds that need more time to be collected. This explains the large area of the later peaks in longer extraction time [7]. In this regard, other studies focused on the importance of extraction time, finding it as a crucial factor in recovering VOCs from a range of sample types [7, 9].

At 35°C, the extraction of the VOCs was mostly enhanced in comparison with 27°C. The abundant metabolic compounds (main compounds) in VIP score like FID-25.24, FID-25.01, FID-29.63, FID-26.21 and FID-25.67 were found to be higher at the 4 h extraction period than other the extraction periods (Fig. **5**). The decrease of these peak areas at 6 h and 8 h might be because of accumulation of CO2 in the extraction container, which would adversely affect the role on the insects bioactivities [24, 25], although this was not measured. The results of the extraction time are consistent with a result confirmed that 4 hours are required to extract the VOCs from *T. castaneum* [9].

3.4. Validation Study on *T. castaneum*

As a validation to the method developed in this study, VOCs compounds released from *T. castaneum* were extracted using a three-phase DVB/CAR/PDMS fibre at 35ºC for 4 h extraction of 10 insects in 4 ml bottle and identified using gas chromatograph mass spectrometry. The identification was according to specific criteria, including a high match factor, similar mass spectra and similar retention index; Twenty-three VOCs were identified from the beetles (Fig. **6**). The most abundant compound identified in this study was 1-Pentadecene (area= 34.99%). This compound was also reported as a common compound released by *Tribolium SPP.* in many previous studies [26-29]. This pheromone can be secreted by both males and females and can stimulus both gender [27]. However, it can also act as a spacing pheromone for *Tribolium spp.* and a defensive secretion against their enemies [26]. Another compound that was detected in this study was 1,3-benzenediol, 4-ethyl-, this compound was also reported for the first time by [29].

Other main compounds identified were p-Benzoquinone, 2-methyl- (area= 14.8%) and p-Benzoquinone, 2-ethyl- (area

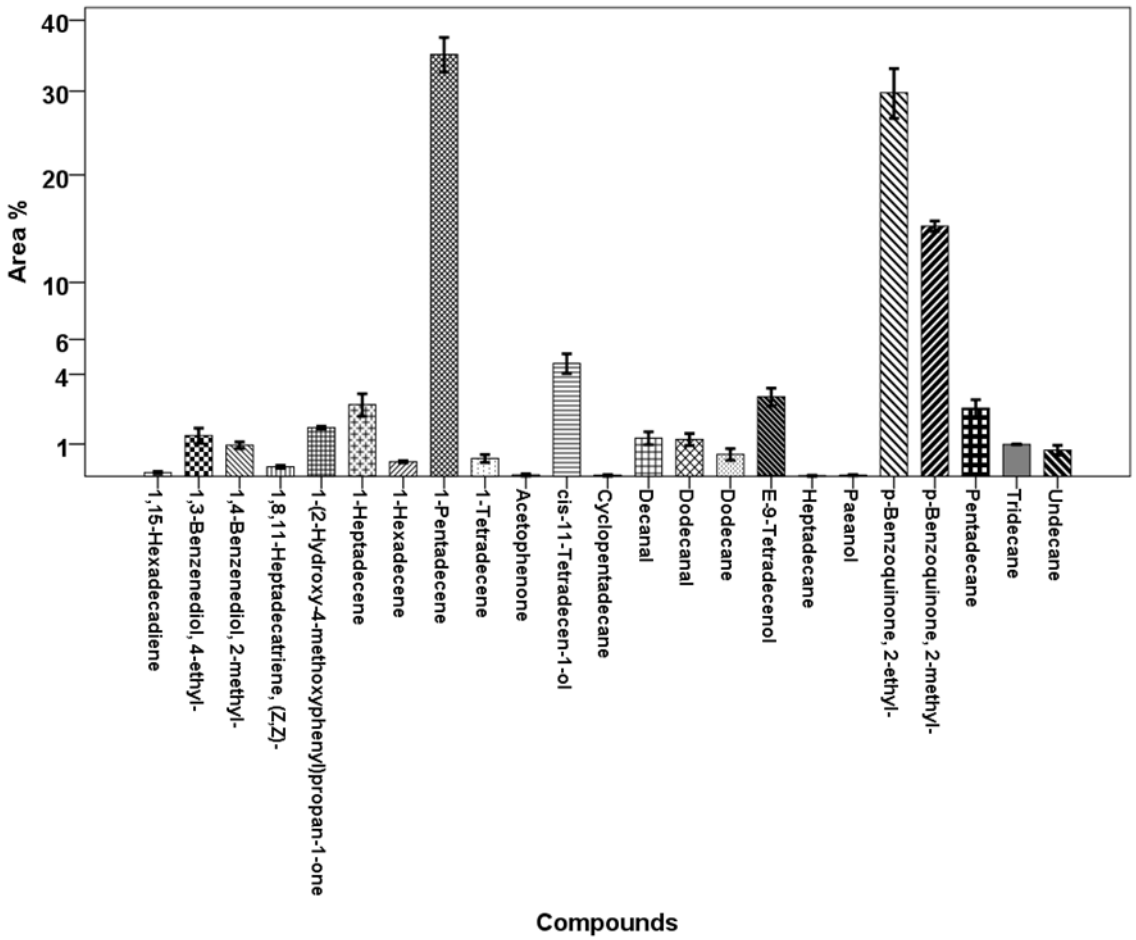

Fig. (6). Area percentages of VOCs that were identified from *T. castaneum* using GCMS.

Table 1. Limits of detection (LOD) and limits of quatification (LOQ) obtained from analyse external standards using the optimised method.

Compounds	RT (min)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Toluene	3.34	0.982	0.215	0.653
Acetophenone	13.18	0.970	0.347	1.051

RT = Retention time
R^2 = Regression coefficient

= 29.7%), consistent with previous studies that quinones are the major group of VOCs from *Tribolium spp* such as red flour beetle [28, 30, 31], in addition to a variety of traces of different chemicals some of them to our knowledge detected for the first time like E-9-Tetradecenol. Methyl-1, 4-benzoquinone and ethyl-1, 4-benzoquinone were detected in this study; these compounds were previously identified for their function as defensive secretions in *Tribolium spp* [28].

3.5. The Limit of Detection and Quantification

The LOD was used to determine the efficiency of extraction and analysis method along with the method of sensitivity. The low detection and quantification value to ng level (Table **1**) obtained for the external standards after applying the optimal conditions of the extraction and analysis demonstrate a high efficient and sensitive method which can be applied in early detection of infestation in sealed storage.

CONCLUSION

Optimal parameters to extract the VOCs from *T. castaneum* adult insects were the use of 50/30 µm DVB/CAR/PDMS at 35 ± 1°C with 10 insects in 4 mL vial for 4 h extraction. The method was validated on the identification of many volatile organic compounds from the insects using GCMS. The unique VOCs specific to *Tribolium* species (*e.g.* p-Benzoquinone, 2-methyl-; p-Benzoquinone, 2-ethyl- and 1-Pentadecene) can further be explored to develop a sensi-

tive method for early and timely detection of infestation or development of lures.

AUTHOR CONTRIBUTIONS

Ihab Alnajim: Conducted part of research and statistical analysis, interpreted data and wrote the manuscript.

Manjree Agarwal: Conducted part of bench research, interpreted data and wrote the manuscript.

Liu Tao: Contributed to the experimental design and help in the in manuscript writing.

YongLin Ren: provided the initial idea, supervised the research progress and provided the work instruments.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The authors approve that this manuscript is from their own work and they have no conflict of interest.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors approve that this manuscript is from their own work and they have no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Neethirajan S, Karunakaran C, Jayas D, White N. Detection techniques for stored-product insects in grain. *Food Cont*., 2007; 18, 157-62. https://doi.org/10.1016/j.foodcont.2005.09.008.

[2] Arbogast R. Beetles: coleoptera. Ecology and management of food-industry pests FDA. *Tech. Bull.*, 1991, 4, 131-76.

[3] Lorini I, Ferreira Filho A. Integrated pest management strategies used in stored grain in Brazil to manage phosphine resistance. Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia, 2004. pp. 293-300. https://www.alice.cnptia.embrapa.br/bitstream/doc/841164/1/SP15442.pdf.

[4] Hameed A, Freed S, Hussain A, Iqbal M, Hussain M, Naeem M, *et al.* Toxicological effects of neem (*Azadirachta indica*), Kanair (*Nerium oleander*) and spinosad (Tracer 240 SC) on the red flour beetle (*Tribolium castaneum*)(Herbst.). *Afr. J. Agric. Res*., 2012, 7, 555-60. https://doi.org/10.1146/annurev.ento.53.103106.093402.

[5] Trematerra P, Sciarreta A, Tamasi E. Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. *Entom. Experi. et Appli.*, 2000, 94, 195-200. https://doi.org/10.1046/j.1570-7458.2000.00619.x.

[6] Bennett JW, Inamdar AA. Are some fungal volatile organic compounds (VOCs) mycotoxins?. *Toxins*, 2015, 7, 3785-804. doi:10.3390/toxins7093785

[7] Laopongsit W, Srzednicki G, Craske J. Preliminary study of solid phase micro-extraction (SPME) as a method for detecting insect infestation in wheat grain. *J. stored Prod. Res*., 2014, 59, 88-95. https://doi.org/10.1016/j.jspr.2014.06.002.

[8] Laothawornkitkul J, Jansen R, Smid H, Bouwmeester H, Muller J, Van Bruggen A. Volatile organic compounds as a diagnostic marker of late blight infected potato plants: A pilot study. *Crop Protect.*, 2010, 29, 872-8. https://doi.org/10.1016/j.cropro.2010.03.003.

[9] Niu Y, Hardy G, Hua L, Trengove R, Agarwal M, Cheng H, *et al.* Optimization of HS-SPME-GC method for detection of stored grain insects. Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, 2012. pp. 15-9. http://researchrepository.murdoch.edu.au/id/eprint/38564/

[10] Niu Y, Hua L, Hardy G, Agarwal M, Ren Y. Analysis of volatiles from stored wheat and *Rhyzopertha dominica* (F.) with solid phase microextraction–gas chromatography mass spectrometry. *J. Sci. Food Agric*., 2015, 96, 1697-703. https://doi.org/10.1002/jsfa.7274.

[11] Qiu R, Qu D, Trengove R, Agarwal M, Hardy GESJ, Ren Y. Headspace solid-phase microextraction and gas chromatography-mass spectrometry for analysis of VOCs produced by *Phytophthora cinnamomi*. *Plant Dis*., 2014, 98, 1099-105. https://doi.org/10.1094/PDIS-01-14-0049-RE.

[12] Balasubramanian S, Panigrahi S. Solid-phase microextraction (SPME) techniques for quality characterization of food products: a review. *Food Biop. Tech*., 2011, 4, 1-26. https://doi.org/10.1007/s11947-009-0299-3.

[13] Xia J, Wishart DS. Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. *curr. protoc. bioinforma*., 2016, 14.0.1-.0. 91. https://doi.org/10.1002/cpbi.11.

[14] Davoli E, Gangai M, Morselli L, Tonelli D. Characterisation of odorants emissions from landfills by SPME and GC/MS. *Chemosphere*, 2003, 51, 357-68. https://doi.org/10.1016/S0045-6535(02)00845-7.

[15] Jeleń HH, Obuchowska M, Zawirska-Wojtasiak R, Wasowicz E. Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J. Agric. Food Chem*., 2000, 48, 2360-7. DOI: 10.1021/jf991095v.

[16] Tuduri L, Desauziers V, Fanlo JL. Potential of solid-phase microextraction fibers for the analysis of volatile organic compounds in air. *J. Chromatogr. Sci.*, 2001, 39, 521-9. https://doi.org/10.1093/chromsci/39.12.521.

[17] Garcia-Esteban M, Ansorena D, Astiasarán I, Ruiz J. Study of the effect of different fiber coatings and extraction conditions on dry cured ham volatile compounds extracted by solid-phase microextraction (SPME). *Talanta,* 2004, 64, 458-66. https://doi.org/10.1016/j.talanta.2004.03.007.

[18] Gianelli MP, Flores M, Toldra F. Optimisation of solid phase microextraction (SPME) for the analysis of volatile compounds in dry-cured ham. *J. Sci. Food Agric*., 2002, 82, 1703-9. https://doi.org/10.1002/jsfa.1249.

[19] Marco A, Navarro J, Flores M. Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chem*., 2004, 84, 633-41. https://doi.org/10.1016/S0308-8146(03)00288-7.

[20] Matisová E, Sedláková J, Slezáčková M, Welsch T. Solid-phase Microextraction of Volatile Polar Compounds in Water. *j. high resolut. chromatogr*., 1999, 22, 109-15. https://doi.org/10.1002/(SICI)1521-4168(19990201)22:2<109::AID-JHRC109>3.0.CO;2-Z.

[21] Sostaric T, Boyce MC, Spickett EE. Analysis of the volatile components in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. *J. Agric. Food Chem*., 2000, 48, 5802-7. DOI: 10.1021/jf000515+.

[22] Risticevic S, Lord H, Górecki T, Arthur CL, Pawliszyn J. Protocol for solid-phase microextraction method development. *Nature protocols*, 2010, 5, 122. https://www.nature.com/articles/nprot.2009.179.

[23] Kleeberg K, Liu Y, Jans M, Schlegelmilch M, Streese J, Stegmann R. Development of a simple and sensitive method for the characterization of odorous waste gas emissions by means of solid-phase microextraction (SPME) and GC–MS/olfactometry. *Waste Manag.*, 2005, 25, 872-9. https://doi.org/10.1016/j.wasman.2005.07.003.

[24] Guerenstein PG, Hildebrand JG. Roles and effects of environmental carbon dioxide in insect life. *Annu Rev Entomol*., 2008, 53, 161-78. https://doi.org/10.1146/annurev.ento.53.103106.093402.

[25] Nicolas G, Sillans D. Immediate and latent effects of carbon dioxide on insects. *Annu Rev Entomol.,* 1989, 34, 97-116. https://www.annualreviews.org/doi/pdf/10.1146/annurev.en.34.010189.000525?casa_token=7f058_oSSY4AAAAA:04ImiRD2RuGq85-.

[26] Arnaud L, Lognay G, Verscheure M, Leenaers L, Gaspar C, Haubruge E. Is dimethyldecanal a common aggregation pheromone of Tribolium flour beetles? *J. chem. Ecol*., 2002, 28, 523-32. https://doi.org/10.1023/A:1014587927784.

[27] Keville R, Kannowski PB. Sexual excitation by pheromones of the confused flour beetle. *j insect physiol*., 1975, 21, 81-4. https://doi.org/10.1016/0022-1910(75)90070-0.

[28] Villaverde ML, Juárez MP, Mijailovsky S. Detection of *Tribolium castaneum* (Herbst) volatile defensive secretions by solid phase microextraction–capillary gas chromatography (SPME-CGC). *J. stored Prod. Res*., 2007, 43, 540-5. https://doi.org/10.1016/j.jspr.2007.03.003.

[29] Niu Y, Hardy G, Agarwal M, Hua L, Ren Y. Characterization of volatiles *Tribolium castaneum* (H.) in flour using solid phase microextraction–gas chromatography mass spectrometry (SPME–GCMS). *Food Sci. Human Wellness*, 2016, 5, 24-9. https://doi.org/10.1016/j.fshw.2015.11.002.

[30] Hodges R, Robinson R, Hall D. Quinone contamination of dehusked rice by *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae). *J. stored Prod. Res*., 1996, 32, 31-7. https://doi.org/10.1016/0022-474X(95)00036-7.

[31] Unruh LM, Xu R, Kramer KJ. Benzoquinone levels as a function of age and gender of the red flour beetle, *Tribolium castaneum*. *Insect Bioch. Molec. Biol*. 1998;28:969-77. https://doi.org/10.1016/S0965-1748(98)00085-X.