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Characterization of volatiles *Tribolium castaneum* (H.) in flour using solid phase microextraction–gas chromatography mass spectrometry (SPME–GCMS)

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Abstract

The objective of this study was to identify volatile organic compounds (VOCs) from flour, *Tribolium castaneum* (Herbst) and flour infested by *T. castaneum* separately, to confirm the difference of healthy flour and flour infested by *T. castaneum* and to explore the new technique to diagnose stored flour's quality by its VOCs change. Headspace-solid phase microextraction (HS-SPME) coupled with gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) were used to detect the VOCs of three different samples. Totally, 71 different compounds were identified in flour, *T. castaneum* and *T. castaneum* infested flour. Therefore, 27 VOCs were identified from flour alone, 32 VOCs from *T. castaneum* and 39 VOCs from *T. castaneum* infested flour. The compound 2-ethyl-2,5-cyclohexadiene-1,4-dione is only found in *T. castaneum* infested flour. This suggests that 2-ethyl-2,5-cyclohexadiene-1,4-dione can be a useful VOC for detecting *T. castaneum* in flour.

Keywords: Flour; *Tribolium castaneum* (Herbst); Volatile organic compounds; Solid phase microextraction (SPME); Gas chromatography–mass spectrometry

1. Introduction

Flour is an important food ingredient for humans. It contains some basic nutrients such as fibre, protein and vitamins [1]. Although flour is a central component of human diet, there have been few reports concerning the volatile organic compounds (VOCs) in this staple food until recently [2]. Identification of the VOCs released by flour can help us know the components of its flavour and judge its quality over time during storage [3].

Tribolium castaneum (Herbst) is one of the most common insects affecting stored cereal grains, beans, nuts and other durable agricultural products all over the world [4,5]. The presence of this insect in flour, not only causes direct damage, but also results in the deterioration of grain quality, loss of feeding value for stock, and hygiene problems like off-odour damage [6].

Reliable and simple methods to detect the existence of pest infestations in stored products are critically important throughout the supply chain to ensure, for example, the maintenance of grain quality during domestic storage and compliance with international quarantine requirements [7]. The typical approaches for detecting insects in stored grain are based on collecting representative samples of grain from stacks, trucks and rail bogies, and manually inspecting these samples for adult insects by sieving, flotation and Berlese-funnels [8]. These techniques can easily trap or detect adult insects but are not suitable for immature insects. X-ray imaging and near infrared reflectance (NIR) spectroscopy have been studied for the detection of stored grain insects as they can detect hidden insects [9]. However, the operation of these technologies is relatively complicated and there has been no success with *in situ* detection.

A potential detection method is to analyze the air within a grain mass for specific volatile compounds (VOCs)

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released by insects. In recent years, headspace solid phase microextraction (HS-SPME) in conjunction with gas chromatography–mass spectrometry (GC–MS) has been employed to examine volatile compounds from stored product insects in grain [10]. It has been widely used to isolate and identify the VOCs of stored grain and has also been used to detect the aggregation pheromone and other volatile metabolites of the lesser grain borer *Rhyzopertha dominica* (F.) and *T. castaneum* [11]. SPME–GCMS has also been used to determine ongoing spoilage based on the production of unique VOCs by insects to communicate with their own species [12].

Based on previous studies [13], the goal of this study was to screen samples of healthy (non-insect-contaminated) flour, samples of *T. castaneum* adults and samples of flour infested with *T. castaneum* adults using a non-polar column at 25 °C to establish if there were clearly identifiable compounds unique to flour alone, infested flour and the contaminating insects, which would indicate that HS-SPME–GCMS could be used as a way of identifying an infestation of *T. castaneum* in flour. If this were the case, this technique might also be used to gauge the quality of stored products.

2. Materials and methods

2.1. Preparation of samples for analysis

The flour was made from harvested wheat (Australia Standard Wheat I) (2011–2012) with a moisture content of 11.5% (w/w). The wheat had initially been sealed in glass jars (4 L) and held first at −4 °C for one week to kill any pests, and then at 4 °C until milling. A coffee grinder was used for that purpose and the flour produced was also stored in jars at 4 °C.

T. castaneum (MUWTC-8) insects were supplied by School of Veterinary and Life Sciences, Murdoch University, Perth, Australia. Mixed age populations were produced by adding around 200 adults to 350 g wheat flour contained in 500 mL jars which were closed with a mesh lid. The cultures were then incubated for 4–5 weeks at 30 ± 1 °C and 65% relative humidity (RH) in constant darkness to produce adults. Newly emerged adults were then harvested as required.

The samples prepared for assay were 70 g flour, or 50 *T. castaneum* adults alone or 70 g flour plus 50 *T. castaneum* adults sealed in a 100 mL Erlenmeyer flask. Each sample was replicated 3 times and all samples were conditioned for 24 h at 25 °C in a constant temperature room prior to examination.

2.2. HS-SPME–GCMS equipments and methods

Samples were held in Erlenmeyer flasks (100 mL) with ground glass joints (Crow Scientific, NSW, Australia, Cat. No. FE100/3) fitted with half hole septa (Alltech Associates, P/N 6526), and obtain a sealed system suitable for sampling of the headspace.

The SPME fibre of 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (Sigma–Aldrich Australia, Cat. No. 57348-U) was used based on Niu et al. (2012), optimized HS-SPME–GC method for detection of stored grain insects. The fibre was conditioned before use as per manufacturer's recommendations. The sample's extraction time and desorption time was 4 h and 5 min respectively.

The profiles of the VOCs extracted by HS-SPME were analyzed with an Agilent 6890 GC manufactured by Agilent Technology (Palo Alto, CA, USA) with a Flame Ionization Detector (FID). Anon-polar Rxi®-5 ms column was used which was 30 m × 0.25 mm × 0.25 μm in size. The carrier gas used was hydrogen at a constant speed of 40 mL/min in the split-less mode. The GC inlet and FID temperature was 250 °C. To obtain the best possible GC conditions, the oven temperature was set at 45 °C for 5 min, then increased at 5 °C/min to 250 °C and held at each increment for 5 min. The total run was therefore 51 min and each flask was sampled three times.

An Agilent 6890 GC was used for GC–MS. This was combined with an Agilent 5793 Network mass selective detector (MSD). The GC inlet (at splitless mode), interface and MS source temperatures were 250 °C, 250 °C and 230 °C respectively. The carrier gas used was helium at a constant airflow of 1.0 mL/min. The column and oven temperatures were the same as those used above. The ionization potential was 70 eV and scanning was from 35 to 500 atomic mass units. The volatiles were identified by comparison of the mass spectrum with the NIST08 mass spectra library.

The GC-FID data including retention time, peak height and peak area were collected and integrated by the chromatography software, Agilent Chemistation, and then exported to Microsoft Excel for further analysis. The GC–MS data were collected by Agilent Chemistation and then exported to Analyzer Pro® and Pancho Data Analysis (Version 2.7.0.0 Manual) for further analysis.

2.3. Statistical analysis

The variations (standard deviations of mean) of VOCs concentrations, the replicate samples and injections in comparison with average readings were analyzed by Microsoft Excel 2007.

3. Results

3.1. GC-FID analysis

Fig. 1 shows representative chromatograms gained from the three test samples of flour, *T. castaneum* and *T. castaneum* infested flour under the same experiment conditions by GC-FID. The results showed that the chromatograms of flour alone and *T. castaneum* infested flour were almost the same. The compounds, 2-methyl-1,3-benzenediol, 4-ehtyl-1,3-benzenediol and 1-hexadecene were the main ones detected in the *T. castaneum* sample. However, these were not detected in the *T. castaneum* infested flour. Therefore, GC-FID alone cannot distinguish between healthy flour and *T. castaneum* infested flour under the experiment conditions that was used.

3.2. GC–MS analysis

Seventy-one VOCs were identified by GC–MS from one or more of the three samples. Twenty-seven VOCs were detected

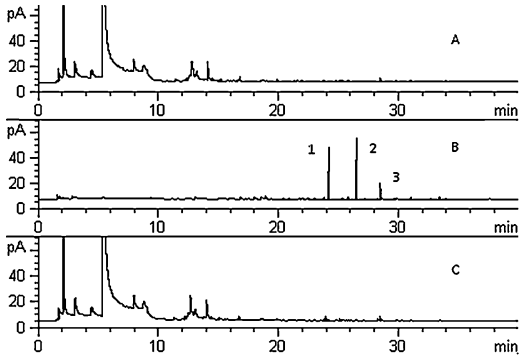

Fig. 1. Representative chromatograms of VOCs from the 3 samples investigated: (A) Wheat flour, (B) *T. castaneum* adults and (C) *T. castaneum* infested flour. The specific compounds identified in Chromatogram (B) are: (1) 2-methyl-1,3-benzenediol, (2) 4-ehtyl-1,3-benzenediol, (3) 1-hexadecene.

from healthy flour, 32 VOCs from *T. castaneum* and 39 VOCs from *T. castaneum* infested flour (Table 1).

3.3. VOCs from flour and flour infested with T. castaneum

Five special VOCs were detected only in flour alone and cannot be detected in flour infested by *T. castaneum*, these were dimethyl-diazene, dimethyl sulfide, monoisopropylcarbonotrihioate, nonanal and tridecane. 21 compounds were found in both of flour alone and *T. castaneum* infested flour (Fig. 2).

3.4. VOCs from T. castaneum and T. castaneum infested flour

The main compounds from *T. castaneum* were identified as 2-methyl-p-benzoquinone,4-ethyl-1,3-benzanediol and 2-ethyl-2,5-cyclohexadiene-1,4-dione (Fig. 3). These three compounds

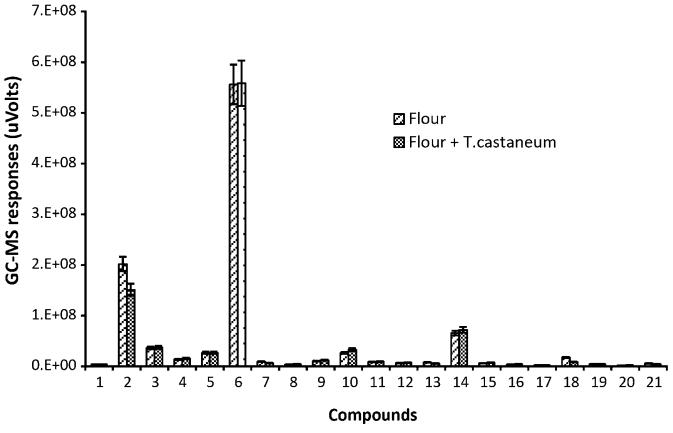

Fig. 2. GC–MS responses for VOCs common to both healthy wheat flour and *T. castaneum* infested flour. (1) Bromo-methane; (2) hexane; (3) pentanal; (4) heptane; (5) 1-chloro-hexane; (6) hexanal; (7) hexamethyl-cyclotrisiloxane; (8) (*Z*)-2-heptenal; (9) 1-octen-3-ol; (10) 2-pentyl-furan; (11) decane; (12) octanal; (13) octamethyl-cyclotetrasiloxane; (14) *D*-limonene; (15) (*E*)-3-octen-2-one; (16) (*E*)-2-octenal; (17) undecane; (18) decamethyl-cyclopentasiloxane; (19) dodecane; (20) decanal; (21) dodecamethyl-cyclohexasiloxane. (The data were alalysed with a variation less than 9% (SD) between three replicates and the duplicate injections, bars represent standard deviations of the mean.)

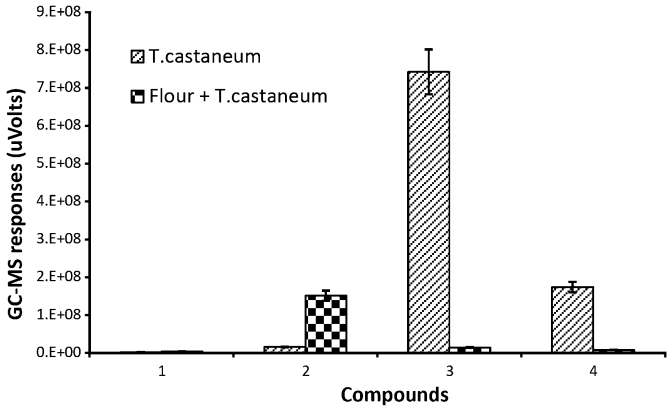

Fig. 3. GC–MS responses for VOCs common to both *T. castaneum* and *T. castaneum* infested flour. (1) Bromo-methane; (2) hexane; (3) 2-methyl-p-benzoquinone; (4) 2-ethyl-2,5-cyclohexadiene-1,4-dione. (The data were analyzed with a variation less than 9% (SD) between three replicates and the duplicate injections, bars represent standard deviations of the mean.)

can also be detected and identified in *T. castaneum* infested flour but at a very low trace level and often overlapped and dwarfed by other compounds.

3.5. VOCs from T. castaneum infested flour

There were 16 compounds identified only in *T. castaneum* infested flour (Fig. 4). Some compounds which come from *T. castaneum*, such as 1-pentadecene, have been known as common semi chemicals of flour beetles [11]. Other compounds, like (*E*,*E*)-3,5-octadien-2-one were previously detected from healthy flour [3]. However, there were some unique compounds detected in the current study which were just found in *T. castaneum* infested flour, such as, propylene oxide, 1-hexanol and 2-ethyl-2,5-cyclohexadiene-1,4-dione. The functions of these compounds are currently unknown.

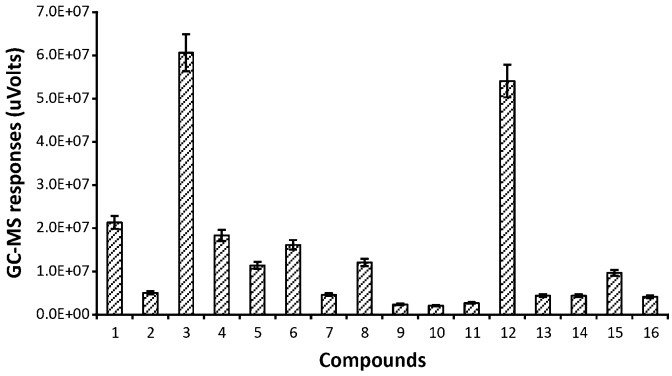

Fig. 4. GC–MS responses for VOCs from *T. castaneum* infested flour. (1) Propylene oxide; (2) 4-methyl-cyclohexanol; (3) 1-hexanol; (4) 2-heptanone; (5) 2-n-butyl furan; (6) heptanal; (7) pentyl-oxirane; (8) oxime-methoxy-phenyl-_; (9) 6-methyl-5-hepten-2-one; (10) (*E*,*E*)-3,5-octadien-2-one; (11) hexamethyl-cyclotrisiloxane; (12) 2-ethyl-2,5-cyclohexadiene-1,4-dione; (13) dodecanal; (14) 3,5-dihydroxytoluene; (15) 1-pentadecene; (16) pentadecane. (The data were analyzed with a variation less than 9% (SD) between three replicates and the duplicate injections, bars represent standard deviations of the mean.)

Table 1
Compounds from flour, *T. castaneum* and *T. castaneum* infested flour determined by GC–MS.

RT	VOC	RI	Samples			Rfs.
			1	2	3	
1.34	Bromo-methane	710	+	tr	+	–
1.43	Acetone	713	ND	tr	ND	–
1.43	Dimethyl-diazene	713	+++	ND	ND	–
1.43	Propylene oxide	713	ND	ND	+++	–
1.50	Dimethyl sulfide	715	+	ND	ND	–
1.56	Monoisopropylcarbonotrihioate	716	+	ND	ND	–
1.77	Hexane	722	++++++	+	++++++	–
2.46	Dimethyl-silanediol	741	++	+	ND	–
2.55	Pentanal	744	+++	ND	++++	–
2.58	Heptane	745	++	ND	+++	–
2.62	4-methyl-cyclohexanol	746	ND	ND	+	–
3.74	1-chloro-hexane	777	+++	ND	+++	–
4.75	Hexanal	805	+++++++	ND	+++++++	*
5.39	Hexamethyl-cyclotrisiloxane	822	+	ND	+	–
7.08	1-Hexanol	869	ND	ND	+++++	–
7.81	2-Heptanone	889	ND	ND	+++	*
7.87	2-n-Butyl furan	891	ND	ND	++	–
8.20	Heptanal	900	ND	ND	+++	*
8.34	Pentyl-oxirane	904	ND	ND	+	–
8.55	Oxime-methoxy-phenyl-_	909	ND	ND	++	–
10.23	(*Z*)-2-heptenal	955	+	ND	+	–
11.13	1-Octen-3-ol	979	++	ND	++	*
11.40	6-Methyl-5-hepten-2-one	986	ND	ND	+	–
11.55	2-Pentyl-furan	990	+++	ND	++++	*
11.87	Decane	999	+	ND	++	–
11.94	Octanal	1001	+	ND	+	*
12.00	Octamethyl-cyclotetrasiloxane	1003	+	ND	+	–
12.74	2-Methyl-p-benzoquinone	1025	ND	++++++	++	–
12.83	*D*-Limonene	1028	+++++	ND	+++++	–
13.20	(*E*)-3-Octen-2-one	1039	+	ND	+	–
13.83	(*E*)-2-Octenal	1059	+	ND	+	*
14.27	(*E*,*E*)-3,5-Octadien-2-one	1072	ND	ND	+	–
14.76	Hexamethyl-cyclotrisiloxane	1087	ND	ND	+	–
15.25	Undecane	1102	+	ND	+	*
16.15	2-Ethyl-2,5-cyclohexadiene-1,4-dione	1106	ND	++++	+++++	–
15.37	Nonanal	1106	+	ND	ND	*
16.22	2-Coumaranone	1132	ND	++	ND	–
17.12	Decamethyl-cyclopentasiloxane	1160	++	ND	++	–
17.60	3-Ehtyl-phenol	1174	ND	+++	ND	–
18.22	3,4-Dimethyl-phenol	1193	ND	+	ND	–
18.35	Dodecane	1197	+	ND	+	*
18.50	Decanal	1202	tr	ND	+	–
20.65	2-Ethyl-4-methyl-phenol	1277	ND	+	ND	–
21.21	Tridecane	1297	tr	ND	ND	–
21.66	2,2′-Bifuran	1315	ND	+	ND	–
22.06	Dodecamethyl-cyclohexasiloxane	1330	+	ND	+	–
22.14	Dodecanal	1334	ND	ND	+	–
22.34	3,5-Dihydroxytoluene	1342	ND	ND	+	–
22.54	2-Methyl-1,3-benzenediol	1350	ND	+++	ND	–
23.80	1-Dodecene	1400	ND	tr	ND	–
24.00	1-Tetradecene	1408	ND	++	ND	–
24.61	4-Ethyl-1,3-Benzenediol	1433	ND	++++	++	–
25.20	1-(2-Hydroxy-4-methoxyphenyl)-ethanone	1457	ND	+	ND	–
25.82	(*Z*)6-Pentadecen-1-ol	1481	ND	+++++	ND	–
26.20	1-Pentadecene	1497	ND	ND	++	–
26.39	Pentadecane	1504	ND	ND	+	–
26.49	n-Heptadecanol-1	1508	ND	+++++	ND	–
27.64	1-Tridecene	1554	ND	+++	ND	–
27.67	1-Pentadecene	1556	ND	+	ND	–
27.88	2′,4′-Dihydroxy-3′-methylpropiophenone	1564	ND	+	ND	–

Table 1 (*Continued*)

RT	VOC	RI	Samples			Rfs.
			1	2	3	
28.11	4-Ethoxy-3-anisaldehyde	1573	ND	+++	ND	–
28.47	*cis*-9-Tetradecen-1-ol	1588	ND	+++	ND	–
28.65	11-Hexadecen-1-ol	1595	ND	+	ND	–
28.89	1-Hexadecene	1605	ND	++++++	ND	–
28.97	Hexadecane	1609	ND	tr	ND	–
30.41	1,*E*-11,*Z*-13-Octadecatriene	1673	ND	++++	ND	–
30.83	*E*,*Z*-2,13-Octadecadien-1-ol	1692	ND	++++++	ND	–
30.91	*E*-2-Octadecadecen-1-ol	1695	ND	+	ND	–
31.12	8-Heptadecene	1705	ND	++++	ND	–
31.22	*E*-14-Hexadecenal	1710	ND	++++++	ND	–
33.44	Sulfuric acid, 5,8,11-heptadecatrienyl methyl ester	1815	ND	tr	ND	–

RT, retention time (min); RI, retention indices; Rfs, * means the VOC also detected by Maeda et al. [3].
Samples: 1, flour; 2, *T. castaneum*; 3, *T. castaneum* infested flour.
Trace level: tr, <1%; +, 1–5%; ++, 5–10%; +++, 10–20%; ++++, 20–30%; +++++, 30–50%; ++++++, 50–100%; +++++++, >100%; ND means the compound was not detected.
For flour and flour + TC, the percentages were calculated using the total ion current (TIC) for each compound relative to the Tic of Hexanal that recorded Hexanal = 100%.
For TC, the percentages were calculated using the total ion current (TIC) for each compound relative to the Tic 2-methyl-p-benzoquinone that recorded 2-methyl-p-benzoquinone = 100%.

4. Discussion

Senthilkumar et al. [14] were the first to detect 1-tridecene from *T. castaneum*. They speculated that this compound was a sex pheromone of the species in accordance with a study of male *Parastizopus transgariepinus* (Koch) by Geiselhardt et al. [15]. While1-tridecene was detected in the current study, two other previously reported volatiles, methyl-1,4-benzoquinone and ethyl-1,4-benzoquinone were not. These compounds were considered to be defensive secretions [12]. However, 2-methyl-p-benzoquinone and 4-ethyl-1,3-benzanediolwere detected for the first time under the current experiment conditions.

Nonanal has also been identified in healthy flour by Maeda et al. [3]. The major VOCs in descending order of response level were hexanal, hexane, *D*-limonene, pentanal and 2-pentyl-furan. Again, 2-heptanone and heptanal have previously been detected in *T. castaneum* infested flour and hexanal has been detected in both flour and *T. castaneum* infested flour [3]. However, some compounds like heptane, pentanal and decamethyl-cyclopentasiloxane were identified for the first time in the present study. The quantity of the 21 compounds found in the samples of flour alone and *T. castaneum* infested flour were almost same. This suggests that the presence of *T. castaneum* has little impact on the VOCs of flour.

Most compounds from flour and *T. castaneum* were different. Hexane was the only compound that could be detected from all of three samples, but it was found at a very low level in *T. castaneum*. Most compounds found in healthy flour were detected in *T. castaneum* infested flour. On the other hand, just a few compounds found from *T. castaneum* alone could be detected in *T. castaneum* infested flour. There were some specific compounds that were only detected in *T. castaneum* infested flour. The common peak in the spectra of healthy flour and *T. castaneum* infested flour was hexane, so the data for them in Table 1 were scaled relative to hexane for comparison. The equivalent levels of 2-ethyl-2,5-cyclohexadiene-1,4-dione in *T. castaneum* and Flour with *T. castaneum* could be a compound to determine *T. castaneum* infestations in flour (Table 1). For *T. castaneum*, 2-methyl-p-benzoquinonewas used as a scale for comparison.

5. Conclusion

Totally, 71 different compounds were identified in flour, *T. castaneum* and *T. castaneum* infested flour use HS-SPME coupled with GC–MS technique. However, most of compounds from *T. castaneum* infested flour are same as healthy flour. The compound 2-ethyl-2,5-cyclohexadiene-1,4-dione was only found in *T. castaneum* infested flour (e.g. it is listed in Fig. 4 and Table 1). This suggests that 2-ethyl-2,5-cyclohexadiene-1,4-dione is considered to be a very useful VOC for detecting *T. castaneum* in flour.

Ethical approval

All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of interest

The authors declare that they have no conflict of interest.

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