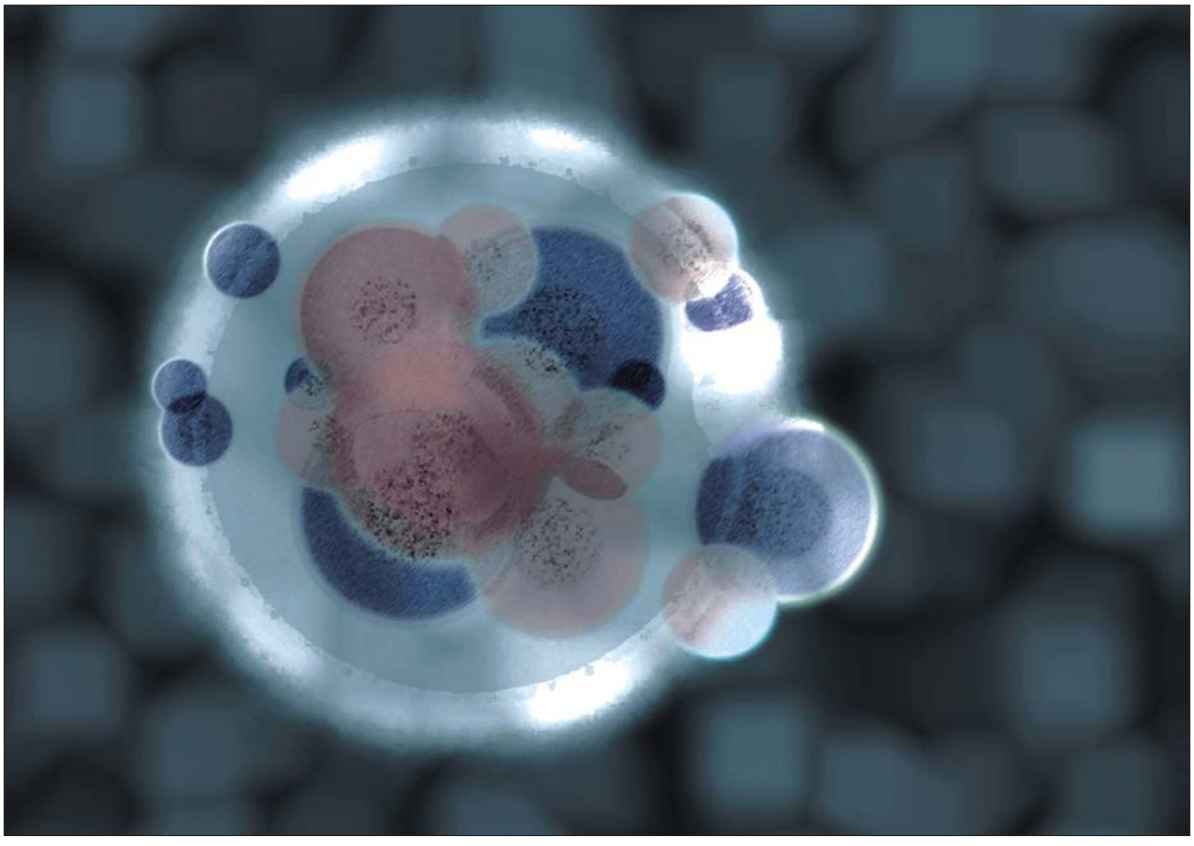

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.347.352

Research Article
Optimization and Validation for Determination of Volatile Organic Compounds from Mediterranean Fruit Fly (Medfly) *Ceratitis capitata* (Diptera: Tephritidae) by Using HS-SPME-GC-FID/MS

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Abstract

Background and Objective: The Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Diptera: Tephritidae), as most of the *Tephritidae* species, is a pest of great economic importance around the world. Volatile organic compounds (VOC) emitted by *Ceratitis capitata* (*C. capitata*) at different life stages (larvae, pupae and adults) can help us to understand the chemicals they produce when interacting. This study aimed to use GC-MS technique to determine the optimal method for accurate, rapid and cost-effective of extraction of VOC from different life stages of *C. capitata*. **Methodology:** This study used HS-SPME fibre coupled with flame ionization detection (FID) and gas chromatography with mass spectrometry (GC-MS) to determine the optimal method for accurate, rapid and cost-effective of extraction of VOC from different life stages of *C. capitata*. Qualitative Analysis software was used to analyse retention times and peak areas. Data were then analyzed by using SPSS. **Results:** Results indicated that a 4 h extraction time using 20 insects/ sample was optimal for the detection of VOC from all life stages of *C. capitata*. **Conclusion:** For saving time, 4 h as extraction time was selected. This study provide that different stage of Medfly has specific VOCs, which in turn explain the feasibility of this method as means of identifying stages of Medfly.

Key words: Mediterranean fruit fly, *Ceratitis capitata,* volatile organic compounds (VOC), head-space solid phase microextraction (HS-SPME), SPME fibre

Citation: Hasan AL-Khshemawee, Manjree Agarwal and YongLin Ren, 2017. Optimization and validation for determination of volatile organic compounds from mediterranean fruit fly (Medfly) *Ceratitis capitata* (Diptera: Tephritidae) by using HS-SPME-GC-FID/MS. J. Biol. Sci., 17: 347-352.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is an invasive pest species that impacts agronomic production and exports worldwide. Damage caused by the fly in the field and laboratory has been described by several researches[1]. It can infest over 200 fruit and vegetable varieties and is particularly damaging to stone fruits such as peaches, apricots and nectarines[2]. The United States spends around 57 million USD each year on *Ceratitis capitata* control[3]. Current management techniques rely almost exclusively on chemicals that are harmful to both human health and to the environment[4].

Volatile organic compounds (VOC) emitted by *C. capitata* at different life stages (larvae, pupae and adults) can help us to understand the chemicals they produce when interacting. VOC can be profiled and quantified using many different extraction methods including microwave-assisted hydro distillation, solvents and Head-Space Solid Phase Microextraction (HS-SPME)[5-6]. The latter, HS-SPME, is considered the best technique for extracting volatiles from food products, plants, fruits and insects[7].

There were several factors that affect the optimization of VOC extraction including temperature, jar size, number of insects/jar, duration of extraction and gas chromatography (GC) conditions[8]. Other studies have used HS-SPME fibre coupled with flame ionization detection (FID) and gas chromatography with mass spectrometry (GC-MS) to optimize these factors[9]. This study aimed to use this technique to determine the optimal method for accurate, rapid and cost-effective of extraction of VOC from different life stages of *C. capitata*.

MATERIALS AND METHODS

Culture of insects: *C. capitata* was obtained from the Department of Agriculture and Food (Perth, Western Australia), in February, 2016 and reared in the Murdoch University Laboratory (Perth, Western Australia). Insects were reared in a controlled temperature cabinet at 23±2°C, 75±5% relative humidity (RH), with 12 h of light and 12 h of dark (12:12 LD)[2]. Eggs were raised on carrot media and then transferred to screen cages (40 cm^3 in size) at pupae stage where it took 12-14 days for adults to emerge. Adults were fed with a paste made from crystalline sugar (Bidvest, Australia) and yeast hydrolysate (Australian Biosearch) at a ratio of 4:1, mixed with water. *C. capitata* eggs deposited onto the sides of the screen cages and fallen into the water tray adjacent to the cage were collected each day.

Apparatus and equipment: VOC samples were collected with 'Sigma-Aldrich' Solid Phase Microextraction (SPME) fibre (50/30 μm) with divinylbenzene/carboxen/polydimethylsiloxane coating (2 cm).

The profile of chemicals was analyzed with an 'Agilent Technologies' gas chromatograph (GC) 7829A (serial number CN14272038), fitted with a 'Restek' non-polar HP-5MS column (30 m×0.25 mm, 0.25 μm film thickness, catalogue number 13423) and flame ionization detector (FID). Helium (99.999% He from BOC Australia) was used as the carrier gas, injected at 250°C in a constant flow of 1.1 mL min^{-1}. FID temperature was set at 290°C. The GC-FID instrument was operated in a splitless mode. Total run time was 45 min and each flask was sampled 3 times.

Profiled chemicals were then identified using an 'Agilent Technologies' gas chromatograph (serial number GCMS 7820A), equipped with a 'Agilent Technologies' mass spectrometer detector (5977E) and non-polar HP-5MS column (30 m×0.25 mm, 0.25 μm film thickness, catalogue number 95051).

Optimization of extraction time and number of insects: The optimal number of insects required for VOC extraction at each life stage was tested using samples of 6, 10, 20, 30 and 40 insects. Larvae and pupae were placed in 4 mL flasks, adults in 36 mL jars. Optimal extraction time for VOC was analysed for each life stage by comparing the peak area of the main compounds after 2, 4 and 6 h of extraction. All samples were replicated 3 times.

Limit of detection for HS-SPME coupled to GC-FID method: The limit of detection was evaluated with n-alkanes standard C7-C30 (Supelco, USA) at ppm and ppb levels. After 1 h of extraction at room temperature, the SPME fibre was injected into the gas chromatograph mass spectrometer (GC-MS) at 270°C. Each limit was tested twice.

Statistical analysis: Qualitative Analysis B.07.00 software was used to analyze retention times and peak areas of the main compounds from chromatography to determine optimal insect number, extraction time and temperature. Data were then exported to Microsoft Excel 2010 for further analysis using IBM SPSS statistic 24.

RESULTS

Extraction time: Results showed that the best extraction time for the larval stage was 4 h. There was no significant difference between 4 and 6 h extractions; the lowest peak area was 2 h (Fig. 1). For the pupae stage, the best extraction time was also 4 h; the 2 h extraction had the lowest peak area (Fig. 2). For adults, there was no significant difference between 4 and 6 h extractions, the 2 h extraction had the lowest peak area (Fig. 2).

Number of insects: Results showed that the optimal number of larvae for VOC extraction was 20 (Fig. 1). There were no significant differences between 20 and 30 pupae (Fig. 2). For the adult stage, 20 adults (10 males and 10 females) was optimal (Fig. 2).

Limits of detection and number of peaks: Five out of the 23 chemicals in the n-alkanes standard C7-C30 were detected using the SPME fibre. The limits of detection of the five diluted samples were evaluated under optimal experimental conditions (Table 1). Octane, decane, undecane, pentadecane and heptadecane could be detected at less than ppb level.

The number of peaks was identified using the GC-FID and GC-MS. The highest number of peaks (chemical compounds) emitted by larvae was 27, pupae were 23 and 29 from adults (Table 2). There were no significant differences between 2 and 6 h extractions for all life stages.

VOC isolated from adult *C. capitata:* The GC-MS was used identify chemicals in samples taken from adult *C. capitata*. There were differences in the amounts of each chemical detected. Compounds detected include: 2,3-Hexanedione, o-dimethylbenzene, nonane, 2,3,4-Trithiapentane, octanal, acetophenone, 2,4,6-Octatriene, 2,6-Dimethyl-(E,Z), 1h-Pyrrole-2-carboxylic acid, 2,6,10-Trimethyltridecane, dimethyl phthalate, farnesene, (E)-Y-bisabolene, undecane, 5-phenyl, carboric acid, 2-Ethylhexyl octyl ester, 2(3H)-furanone and 5-Dodecyldihydro (Table 2).

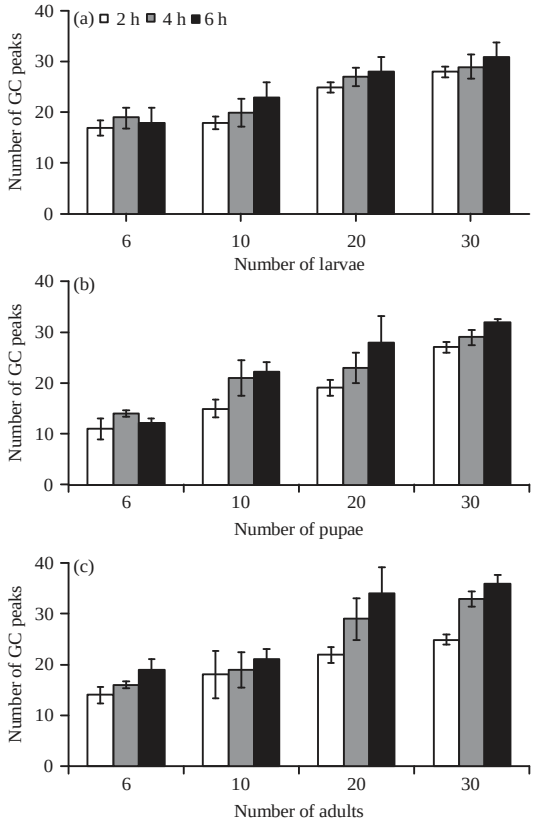

Fig. 1(a-c): Number of GC peaks emitted from different number of insect at different stages (a) Larvae (b) Pupae and (c) Adult and extraction times
Mean±SD

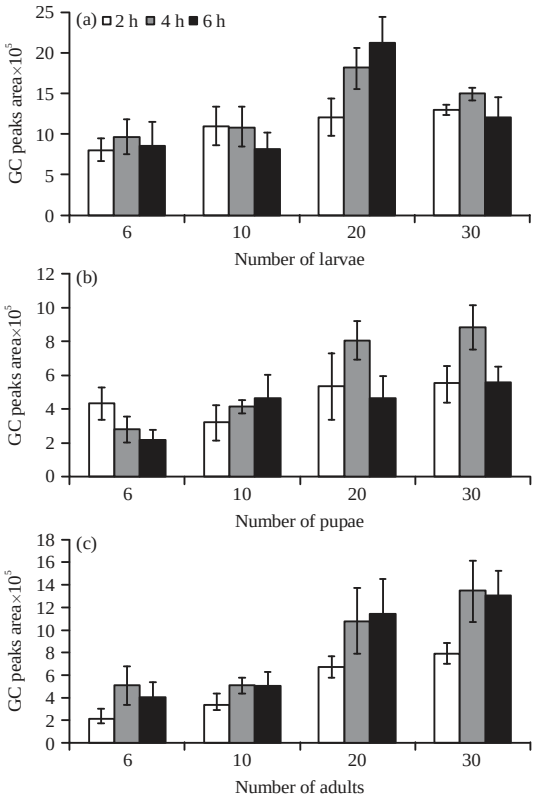

Fig. 2(a-c): Peaks area produced from different number of insect at different stages (a) Larvae (b) Pupae and (c) Adult and extraction times
Mean±SD

Table 1: Compounds were identify from adult stage in optimize extraction time and number of insect (one unit corresponds to a 10^5 area) determined by GC-MS

RT	Compounds	RI	Peak area
3.61	Acetoin	717	97.830
4.21	Toluene	755	20.493
5.54	Hexaldehyde	769	9.270
7.87	o-dimethylbenzene	862	5.312
8.29	Nonane	900	4.095
9.67	Butanoic acid,4-hydroxy	933	8.433
11.29	2,3,4-Trithiapentane	943	1.765
12.19	Octane, 2,7-dimethyl-	964	46.140
12.79	Octanal	982	2.035
13.75	4-Methyl-5-hexen-4-olide	996	3.624
14.57	Acetophenone	1049	0.851
15.52	3,3-Dimethylstyrene	1099	2.474
16.06	Cosmene	1134	5.422
16.52	2,4,6-Octatriene,2,6-dimethyl-(E,Z)-	1292	4.970
19.08	Undecane, 2,6-dimethyl-	1214	1.554
19.26	1H-Pyrrole-2-carboxylic acid	1276	2.032
21.89	Tridecane	1300	2.965
22.66	2,6,10-Trimethyltridecane	1467	1.025
25.89	Dimethyl phthalate	1440	1.275
26.54	Cuparene	1496	1.677
27.01	Farnesene	1499	0.871
28.25	(E)-Y-Bisabolene	1523	1.849
30.27	Undecane,5-phenyl-	1626	0.862
32.81	Tetradecanoic acid	1748	1.287
34.27	Carboric acid, 2-ethylhexyl octyl ester	1857	0.422
36.82	n-hexadecanoic acid	1968	2.129
38.56	2(3H)-Furanone, 5-dodecyldihydro-	2120	0.382
40.07	Octadecanoic acid	2187	1.743

RT: Retention time (min), Name of compounds detected by GC-MS, RI: Retention indices

Table 2: Limits of detection (LOD) and linearity data in gas chromatography coupled to a flame ionization detector GC-FID

Compounds	RT (min)[a]	Linearity (r^2)[b]	LOD (ppm)[c]	LOD (ppb)[d]
Octane	5.868	0.948	100.061	33.394
Decane	12.789	0.957	224.999	74.246
Undecane	16.083	0.997	164.794	77.475
Pentadecane	26.997	0.994	17.307	10.262
Heptadecane	31.540	0.946	6.499	2.521

[a]RT: Retention time, [b]Regression coefficient, [c]ppm: Part per million, [d]ppb: Part per billion

DISCUSSION

Four-hour extractions had the highest VOC peak areas using HS-SPME for all life stages of *C. capitata.* There were no significant differences between 4 and 6 h extractions in any stage but differences between 2 and 4 h extractions for all stages. Optimal extraction time was therefore identified as 4 h, a relatively short time period compared with other studies[10-11].

Determining extraction time is an important step in the development of the SPME method[12]. However, its accuracy is very much dependent on equilibrium during extraction of VOCs[13]. Barbieri *et al.*[14] and Alfaro *et al.*[15], showed that the equilibrium between wheat and its volatiles within a flask had an impact on the final VOC extraction made with SPME fibre. In this study, the number of VOC detected increased with the number of insects up to 30 insects/sample. Insects started to die, possibly due to overcrowding, in samples with 30 or more and there were no significant differences between samples with 20 and 30 insects. The optimal number of insects for this study was therefore identified as 20. In addition to insect numbers, equilibrium can also be influenced by other factors including relative humidity, temperature and light; these will need to be considered in future studies.

SPME fibre technique has been used in previous studies for the qualitative analyses of *C. capitata* emanations[16-18]. Some of these studies worked with headspace, solvent extraction and analytical techniques, which explains the differences seen in VOC profiles[19]. Alfaro *et al.*[15] identified 31 compounds emitted by *C. capitata* in study of fly sex, age and mating status. Baker *et al.*[20], identified nine compounds emitted by males of *C. capitata*. Techniques used in this study, headspace collection of VOC using GC-FID and GC-MS, identified 28 VOC in the adult stage of *C. capitata*. Some of

these compounds have been described previously as being part of volatile constituents of *C. capitata* and others were not, including acetophenone and eicoseN[17,21-25]. The main constituents of male VOC identified in this study included geranyl acetate, (E,E)-R-farnesene and ethyl (3E)-3-octenoate, consistent with the results of similar studies[19]. The acetophenone group has also been identified previously in female *Dendroctonus* species[26-28].

CONCLUSION

HS-SPME fibre has been validated as a fast, easy and solvent-free technique for the analysis of VOC from different life stages of *C. capitata.* It also allows the separation of a variety of chemicals that can be identified using GC-MS. The technique was optimized using a 50/30 µm Divinylbenzene/carboxen/polydimethylsiloxane fibre for extraction. Analysis using this tool showed that the optimal extraction time for all stages is 4 h and the optimal number (sample size) of insects for all life stages (larvae, pupae and adults) is 20.

SIGNIFICANCE STATEMENTS

This research has validated the optimized HS-SPME fibre method and has identified the optimal extraction time and insect numbers for the collection of VOC from different life stages of *C. capitata.* This provides the researcher with an excellent tool to collect VOC and will save time in laboratory analysis.

ACKNOWLEDGMENTS

Authors would like to acknowledge the Iraqi government for their financial support (HCED, D11-1473 Scholarship) and Murdoch University for their technical support. The authors also thank the staff of the biosecurity and food safety laboratory at Murdoch University for technical assistance.

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