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Volatile Compounds Detected by SPME-GC-MS in Fish Viscera Extracts with Attractant Activity against House Fly *Musca domestica* from Chemometric FTIR Fingerprints

(Sebatian Meruap Dikesan oleh SPME-GC-MS dalam Ekstrak Visera Ikan dengan Aktiviti Bahan Penarik terhadap Lalat Rumah *Musca domestica* daripada Cap Jari FTIR Kimometriks)

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ABSTRACT

Fish viscera has been locally applied as fly attractant in North Moluccas. The objective of this study was to evaluate the efficacy of attractant from fish viscera extracts and to determine the indispensable active extracts with chemometric FTIR fingerprints, as well as to characterize volatile compounds of the extracts. Attractant activity against house fly *Musca domesctica* and FTIR spectra were analyzed for the ethanol, acetone and hexane extracts of stomach, intestines, liver, and combination of the viscera from skipjack tuna stored for 1-2 days at room temperature. SPME-GC-MS analysis was applied for active extracts to determine volatile compounds. OPLS analysis result showed that FTIR spectra were correlated with the attractant activity (R^2 0.974 and Q^2 0.89), and compounds with functional groups S-H, N-H, O-H, and C-O have the strong correlation. Ethanol and acetone extracts of mixed viscera from 2 days storage are the active extracts. The volatile compounds in the extract through the SPME-GC-MS analysis belong to ketone, amine, phenol, heterocyclic, aromatic aldehyde, fatty acid, fatty acid ester and sulfur compounds are reported.

Keywords: Attractant activity; fish viscera extracts; *Musca domestica*; chemometric; FTIR fingerprint; SPME-GC-MS

ABSTRAK

Visera ikan telah digunakan secara tempatan sebagai bahan penarik lalat di Maluku Utara. Objektif kajian ini adalah untuk menilai keberkesanan bahan penarik daripada ekstrak visera ikan dan menentukan ekstrak aktif yang sangat diperlukan dengan cap jari FTIR kimometriks selain mencirikan sebatian meruap bagi ekstrak tersebut. Aktiviti bahan penarik terhadap lalat rumah *Musca domesctica* dan spektrum FTIR dianalisis untuk ekstrak etanol, aseton dan heksana perut, usus, hati dan gabungan visera daripada tuna cakalang yang disimpan selama 1-2 hari pada suhu bilik. Analisis SPME-GC-MS digunakan untuk ekstrak aktif dalam menentukan sebatian meruap. Keputusan analisis OPLS menunjukkan bahawa spektrum FTIR berkorelasi dengan aktiviti bahan penarik (R^2 0.974 dan Q^2 0.89) serta sebatian kumpulan berfungsi S-H, N-H, O-H dan C-O mempunyai korelasi yang kuat. Ekstrak etanol dan aseton daripada gabungan visera daripada simpanan 2 hari adalah merupakan ekstrak aktif. Sebatian meruap dalam ekstrak melalui analisis SPME-GC-MS dilaporkan tergolong kepada keton, amina, fenol, heterosiklik, aldehid aromatik, asid lemak, ester asid lemak dan sebatian sulfur.

Kata kunci: Aktiviti bahan penarik; cap jari FTIR; ekstrak visera ikan; kimometriks; *Musca domestica*; SPME-GC-MS

Introduction

Insect control against house fly (*M. domestica*) by using fish viscera has been found as a traditional method in North Moluccas. The viscera emits volatile signals which attract insects (Magalhães et al. 2018). The similar method is used in oil palm plantation of Malaysia, where pig carcasses are used to attract house fly, as reported by Chin et al. (2011). In addition, decayed rabbit meat is applied to attract *Chrysomya albiceps* in Kuwait, studied by Al-Mesbah et al. (2012). These fly attractant methods have similar mode of actions through volatile compounds produced from protein decomposition.

Several fishery products are identified to produce volatile compounds as the result of indigenous metabolism and microorganism-induced metabolism that occur immediately after the fish is caught, as found in carp (Pratama, Rostini & Rochima 2018a) and viscera of catfish and mackerel (Pratama, Rostini & Rochima 2018b). Generally, volatile compounds of fish consist of aldehydes, ketones, alcohols, esters, and hydrocarbons (Chang, Hou & Bafang 2016; Hou et al. 2013; Zhou et al. 2016). In this case, skipjack fish viscera is one of the main by-products of fishery industries in Indonesia; thus, it is potent material for house fly control agents. However, chemicals contributing to fly attracting activity are still rarely investigated, including the solvent for extraction and their characterization. To fill the gap, FTIR can be used. It is a rapid biochemical fingerprinting technique, widely used together with multivariate analysis for detecting functional groups or fingerprint related to food degradation (Castañeda-Perez et al. 2013; Saraiva, Vasconcelos & de Almeida 2017).

Volatile compounds of fishes have been studied using a solid-phase microextraction (SPME) linked to GC-MS (Erickson, Ma & Doyle 2015; Leduc et al. 2012; Xuan et al. 2023). Some volatile compounds (e.g., alcohol, acid, aldehyde, alkane, ketone, trimethylamine, and sulfur groups) are good indicator of fish freshness or spoilage (Leduc et al. 2012). Identification of volatiles in fish by SPME-GC-MS using headspace method is considerable because the compounds can be originally extracted and identified, without any changes during the extraction.

This present study aimed to determine the attractant activity of fresh and stored skipjack tuna fish viscera extracts against house fly, the active extracts as well as identify the functional groups of active compounds using chemometric method based on FTIR spectral data as fingerprints. Volatile compounds in the active extracts by SPME-GC-MS were identified. Multivariate analysis with orthogonal partial least squares (OPLS) of FTIR spectral data with corresponding attractant activities was used for the chemometric fingerprints. The use of viscera from skipjack tuna is important to promote a zero waste of tuna product industries, which give a benefit for pest management in the future.

Materials and Methods

Materials, reagents and instruments

Skipjack viscera samples (20 kg) were obtained from Pahala Bahari Nusantara Co., Bekasi, Indonesia. The viscera were sorted (stomach, intestines, liver and their mixture), cleaned and weighed. Each viscera (1000 g) was extracted by different solvents, i.e., ethanol, acetone, and hexane (analytical grade, Merck, USA). House fly *Musca domesctica* were reared and harvested after 22 days of rearing. Standards of 15 amino acids, i.e., phenylalanine, isoleucine, valine, alanine, glycine, leucine, tyrosine, proline, serine, glutamic acid, arginine, lysine, aspartic acid, threonine, histidine (Agilent amino acid analysis kits) were used for analysis by UHPLC-PDAD (WATERS UPLC Acquity PDA Det H Class, Waters Corp, Massachusetts, USA). DVB/CAR/PDMS fiber for SPME (Supelco, Bellefonte, PA, USA), GC-MS instrument (Agilent Technologies, Santa Clara, USA), KBr from analytical grade (Merck), and FTIR instrument (Tensor 37, Bruker Optic GmbH, Germany) were used to obtain FTIR spectra in this study.

Preparation of viscera samples

Fresh viscera (250 g) were transferred into four separated sterile containers (using code: stomach (L), intestine (U), liver (H) and mixture (J)). The samples were also stored for 1 - 2 days at room temperature (27 to 29 ºC), and observed in day 0 (L0, U0, H0, J0), day 1 (L1, U1, H1, J1), and 2 (L2, U2, H2, J2) for total amino acids. The samples were prepared in duplicate, for the chemical analysis and solvent extraction.

Total amino acids analyses of fresh and stored viscera

To determine the total amino acid composition (after acid hydrolysis), fresh and stored viscera samples were analyzed using UHPLC-PDAD method. A 10 µL sample was injected into a Waters reversed phase AccQ Tag Silica-bonded Amino Acid Column C18 (3.9 mm × 150 mm) using auto sampler (Waters 2707). The Waters AccQ Tag Eluent A Concentrate (WAT052890) was diluted to 10% in Milli-Q water and used as eluent A, and 60%

acetonitrile as eluent B in a separation gradient with a flow rate of 1.0 mL/min. The separation gradient used was 0-2 min (100% A), 2.0 min (98.0% A), 15.0 min (93.0% A), 19.0 min (90.0% A), 32.0 min (67.0% A), 38.0 min (0.0% A), and 56.0 min (100.0% A). The amino acids were detected using PDA at 254 nm with the column condition set at 37 ºC (Waters Corporation 2012).

VISCERA SAMPLE EXTRACTION

Fresh or stored viscera samples were extracted by a method of Harborne (1987). Samples were macerated for 72 h, initially by ethanol p.a. (polar) for 24 h, then extracted by acetone p.a. (semipolar) for 24 h, and finally by hexane p.a. (nonpolar) for 24 h with a ratio of sample weight to solvent volume 1:4. In each maceration, solvent extract was separated from the residue by filtration using vacuum filter unit and Whatman No. 1 filter paper. Subsequently, the residue was subjected to the next corresponding maceration step. The extracts obtained from the stepwise maceration were named as ethanol extracts (E), acetone extracts (A) and hexane extracts (H). Each extract was concentrated with a rotary evaporator at 40 °C, then evaporated with nitrogen gas (N2) to remove the solvent in the extract until it reached a constant weight. Totally, 36 extracts were obtained as follows: L0E, L1E, L2E, L0A, L1A, L2A, L0H, L1H, L2H (for stomach extracts); U0E, U1E, U2E, U0A, U1A, U2A, U0H, U1H, U2H (for intestine extracts); H0E, H1E, H2E, H0A, H1A, H2A, H0H, H1H, H2H (for liver extracts); and J0E, J1E, J2E, J0A, J1A, J2A, J0H, J1H and J2H (for mixed viscera extracts). The extracts were then tested for house fly (*M. domestica*) attractant activity and then analyzed by FTIR to obtain FTIR spectral data as described herewith.

ATTRACTANT ACTIVITY TEST

The test was designed to determine the attractant activity of skipjack viscera extract against house fly *M. domestica.* Each solvent extract of viscera was diluted with 25% dimethyl sulfoxide (DMSO) (1:4 w/v). Next, the extract solution (1 mL) was moved to a new vial, according to previous works (Kun et al. 2013; Sodiq et al. 2016). A wire with a cotton swab at the tip was dipped in 1 mL of the extract solution, then the cotton was put in a fly trap made of an empty mineral water bottle (600 mL in size) (Figure 1(A)). Each bottle was coded corresponding to extract (for all solvent extracts of stomach (L), intestine (U), liver (H) and mixtures (J)), subjected for attractant testing. The trap bottles (4 bottles) were put in a pet greedy (PG) room, followed by putting 100 *M. domestica* house flies using an aspirator into the PG, and the distance between trap bottles was 1 m to each other (Figure 1(B)). The four bottles with 4 different extracts were put in the same PG, noted as one replication. For the negative control, an empty trap bottle with water was used and these were placed in a separate PG room from the sample extracts. The test was run for 6 hours (09.00 am - 03.00 pm), which was known as the active time of the fly. Number of trapped flies were recorded using Abbot formula (number of dead flies divided by the number of flies applied multiplied by 100) (Abbott 1925). The testing was carried out in triplicate.

FTIR ANALYSIS FOR CHEMOMETRIC FINGERPRINTS

The fingerprints of skipjack viscera extracts were analyzed by a Fourier Transform Infrared (FTIR). Each extract (2 g) was mixed with 0.2 g of KBr, pressed to form pellet. The sample spectrum was analyzed for absorbance or transmission using FTIR at wavenumbers of 4000-400 cm^{-1}, producing data in Excel format (wavenumbers and absorbances) and FTIR spectra. The Excel data were used for chemometrics using a multivariate analysis. Functional groups observed from the FTIR spectral data were also interpreted to know the functional groups of active extracts.

VOLATILE COMPOUNDS IDENTIFICATION BY SPME-GC-MS

Volatiles in active fish viscera extracts were pre-concentrated using the solid-phase micro-extraction (SPME) method. The volatiles were absorbed using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber. Before use, the fiber was heated in a GC-MS injector at 250 ºC for 15 min to remove contaminants. For the volatile analysis, the condition of SPME sampling was conducted by taking 8 g of extract solution and added into a 22 mL glass vial with PTFE/Silicone septa (Agilent). The vial with SPME fiber was closed hermetically and put in a water bath for 60 min at 40 ºC to extract volatile compounds, while the extracting fiber was injected into GC-MS. Desorption of volatile compounds occurred in the injection port of GC-MS for 5 min.

Gas chromatography mass spectrometer was employed in this study. Helium gas was used as a carrier at a constant flow rate of 1 mL/min. The injection port was equipped with a 0.75 mm i.d, Agilent liner suitable for SPME. GC-MS analysis was conducted by inserting the fiber previously exposed to the samples into the injection

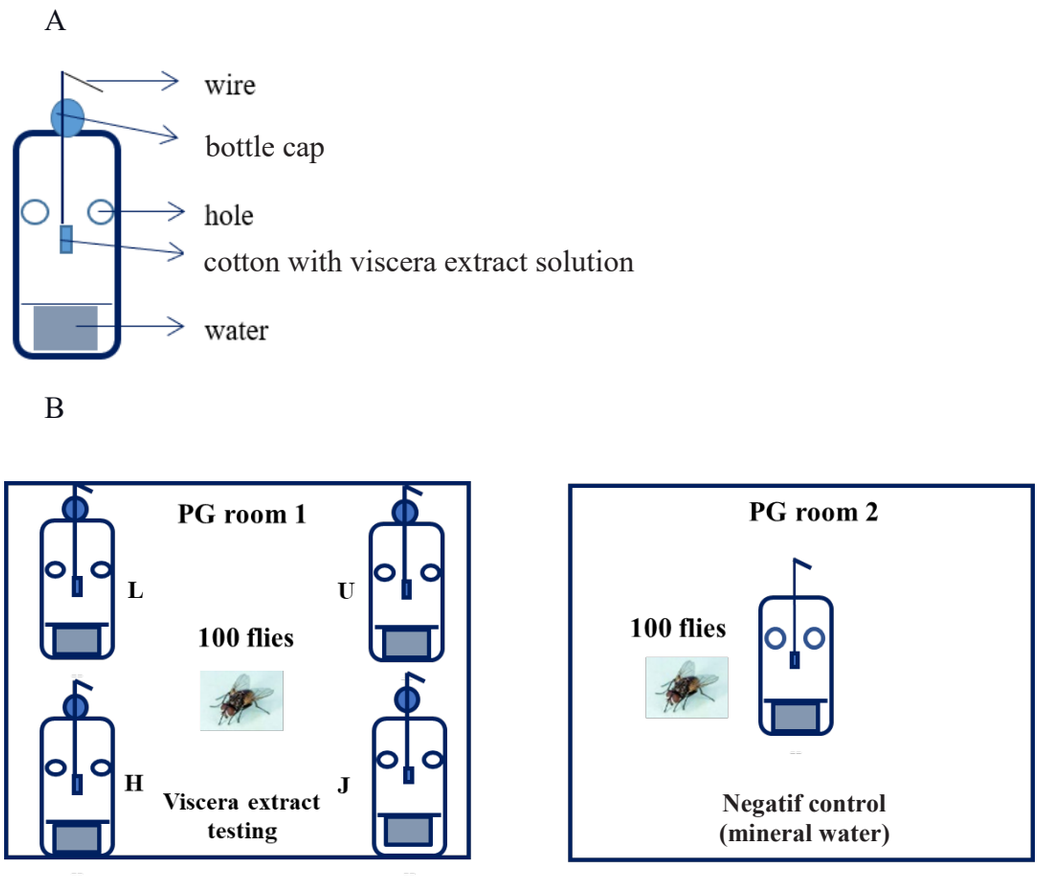


FIGURE 1. Bottle trap design for house fly trap (A) and separated pet greedy (PG) room (B) for attractant activity test for house fly *M. domestica*

port. The sample was injected in the splitless mode at 250 ºC. The compounds were separated in a capillary HP-Innowax column with 30 × 0.25 mm dimensions and a film thickness of 0.25 µm (Agilent Technologies). The temperature of oven was maintained at 40 ºC for 5 min and then increased at 4 ºC per min to 150 ºC. The temperature was further raised to 250 ºC at 30 ºC per min and held for 5 min, while the interface temperature was set at 280 ºC. The mass spectrometer was operated in the electron ionization mode with the electron energy set at 70 eV, a scanning range of 29-550 m/z, a speed of 4.37 scans/s, and a gain factor of 1. The ion source and quadrupole analyzer temperatures were set at 230 ºC and 150 ºC, respectively. Chromatographic peaks with different retention times were identified from GC-MS analysis of active extracts, and their mass spectra were compared to those reported in NIST147, NIST27, and WILEY7 standard MS libraries.

FATTY ACID ANALYSIS

The active extracts were also analyzed for fatty acid profiles using GC-FID method by BF_3-methanol methylation following AOAC Official Method 991.39 (AOAC 2012) to confirm the SPME-GC-MS result.

DATA ANALYSIS

Data of attractant activities were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence interval using SPSS software version 22. If there was a significant effect ($p<0.05$), then the Duncan post-hoc test was conducted. The FTIR spectral data indicated by wavenumbers and absorbance were then correlated with house fly attractant activity data using a multivariate data analysis by orthogonal projection to the least square (OPLS) method, using SIMCA-P software version 16 (Sartorius Umetric Umea, Sweden) after pre-processing

of the spectra data with MSC filtering. This chemometric fingerprint analysis could determine active extracts as well as active functional groups linked to house fly attractant activity.

Results and Discussion

Total amino acids of fresh and stored viscera

As depicted in Figure 2, the results showed that total amino acids (for 15 amino acids) of fresh viscera decreased after 1 and 2 days of storage at room temperature. The decrease clearly shows the degradation of proteins which was highly noted after 1 day of storage for all viscera. Our previous study reported the changes of amino acid composition in stomach, intestine, liver and mixture of skipjack viscera which concentrations were in the contrary of the observed volatile bases during viscera storage (Garwan et al. 2022). It can be inferred that abundant amounts of proteins transformed into nitrogenous volatiles, making them appropriate for fly attractants.

House fly attractant activity of viscera extracts

Figure 3 shows that three solvent extracts (ethanol, acetone and hexane extracts) of all viscera, derived from fresh and stored samples, demonstrated various levels of attractant activity against house flies. The activity from stomach extract was relatively higher than liver and intestine extracts. Interestingly, the combined viscera showed higher activity compared to other groups. The attractant activities of ethanol extracts of most viscera after 1-2 days storage tends to decrease. The contradictive results were found for acetone and hexane extracts, which shows the increase of attractant activities during storage. This links to the changes of polar compounds if stored at room temperature. Nonpolar compounds which are mostly volatile are more likely present in the acetone and hexane extracts. According to Duflos et al. (2010), Pratama et al. (2018a, 2018b), and Wang et al. (2022), volatile compounds detected in fisheries products were dominantly hydrocarbon, aldehyde, carboxylic acid, phenol, ketone, sulfide, amine and alcohol groups. Most of these compounds might be originated from enzymatic reactions, lipid oxidation and various environmental influences.

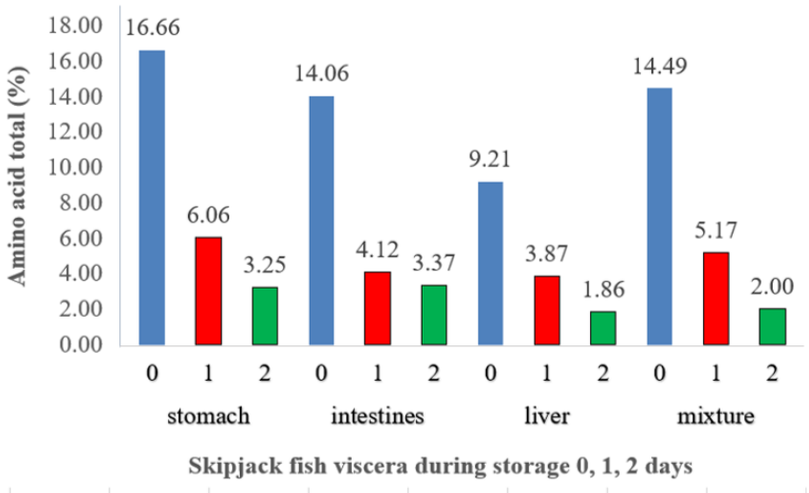
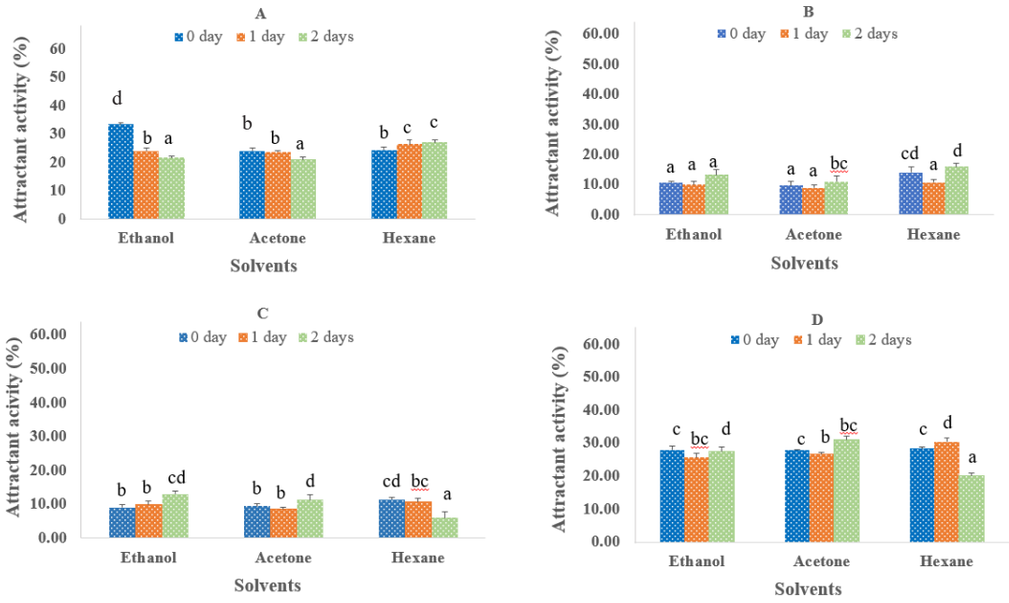


FIGURE 2. Changes of total amino acids of fresh skipjack fish viscera (0 day) and after storage (1 and 2 days)



- value are shown as mean ± stdev of replicates; n=3
- value followed by different letters as superscript are significantly different, Duncan test ($p<0.05$)

FIGURE 3. Attractant activities of *M. domestica* from viscera extracts using different Solvents: stomach (A), intestine (B), liver (C), mixture (D)

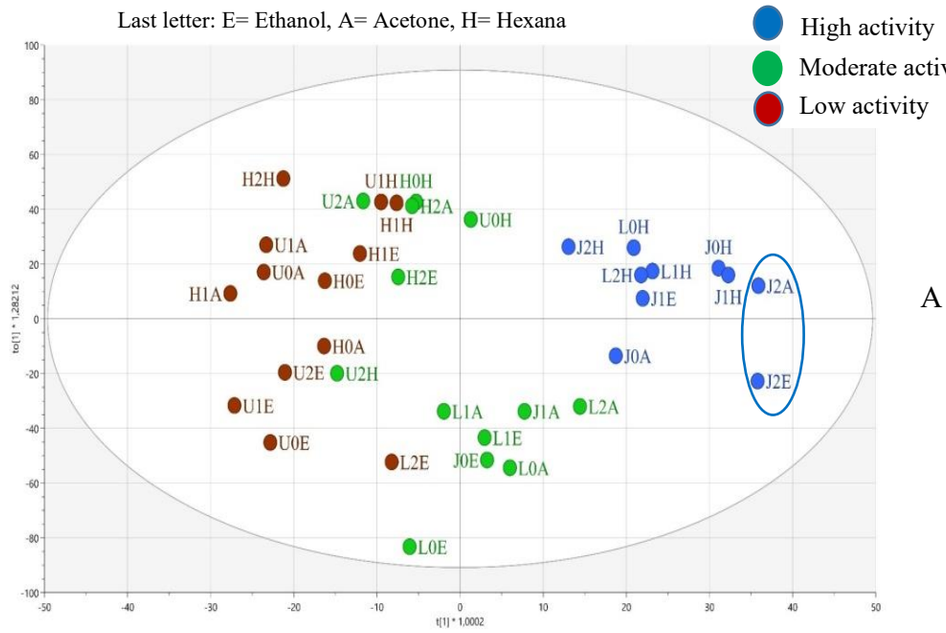
OPLS ANALYSIS FOR THE ATTRACTANT OF HOUSE FLY *M. domestica* BASED ON FTIR RESULTS

The OPLS analysis (Figure 4(A), 4(B) and 4(C)) for FTIR spectra in the form of wavenumbers with absorbances (as X-matrix) and attractant activities to the house flies *M. domestica* (as Y matrix) showed a good correlation, where the values of R^2Y at 0.974 and Q^2Y at 0.89 (Figure 4(A)) were obtained. This clearly divides the attractant activities into three groups: low, moderate and high activities. Based on the score plot (Figure 4(A)), it can be seen that mixed viscera extract was the most active extract followed by stomach extract. From this chemometric method, it is shown that extracts of mixed viscera from 2 days storage, from ethanol (J2E) and acetone (J2A) were highly correlated to the high attractant activities (Figure 4(A) to Figure 4(C)). Thus, these polar to semipolar extracts are the active extracts of fish viscera after 2 days of storage for attractant activity. Based on the average activity, the viscera extracted with acetone (J2A) had a higher activity (31%) compared to the viscera extracted with ethanol (J2E), reaching up to 27.33%. Acetone is a semi-polar solvent, which is almost similar to ethanol that can attract polar and semi-polar compounds (Ilza & Diharmi 2022).

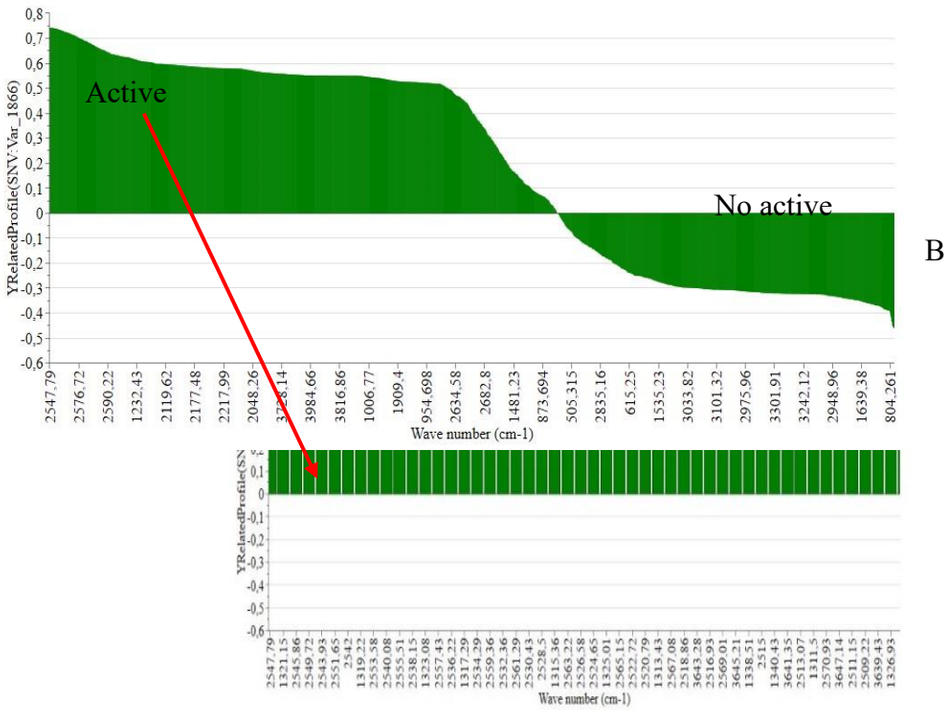
Some wavenumbers of the chemical components in viscera extracts have a positive correlation with attractant activities, shown by *Y-related coefficient plot* (Figure 4(B)). These wavenumbers corresponding to functional groups represented active compounds for the attractant activity by looking at the positive values of *Y-related coefficient* and high VIP (*Variable Influence on the Projection*) values, VIP higher than 1.0 as presented in Table 1. FTIR spectra obtained from active viscera extracts J2E and J2A, is presented in Figure 5.

Different FTIR profiles for the viscera extracts were found. In J2E extract, wavenumber 3500 cm^{-1} which indicates a stretching vibration from N-H and strong absorption up to a wavenumber of 3100 cm^{-1} indicating vibrations of O-H and followed by vibrations of C-H at 3000 cm^{-1} as well as vibrations of C=O at 1750 cm^{-1} were observed in the extract, which are different from those of J2A extract. The C=O band in the FTIR spectra is not positively correlated with the attractant activity. However, the absorption bands in J2E supported the indication of hydrogen bonds from amine, carboxylic acid and aromatic groups (Pavia et al. 2014). Figure 5 shows J2E and J2A bands occur with weak intensity absorption at wavenumbers of 2208 cm^{-1} and 2146 cm^{-1} for the

respective extracts, which are thought to originate from the S-H stretching vibrations. This S-H functional group has a positive correlation to attractant activity (Table 1 & Figure 4). The S-H group was known in some studies to be present as a result of the degradation of methionine, cysteine and their derivatives by microorganism or indigenous enzymes (Orabi & English 2016). The positive correlated-functional groups in Table 1 can be further confirmed by the determination of volatile compounds by SPME-GC-MS.



A



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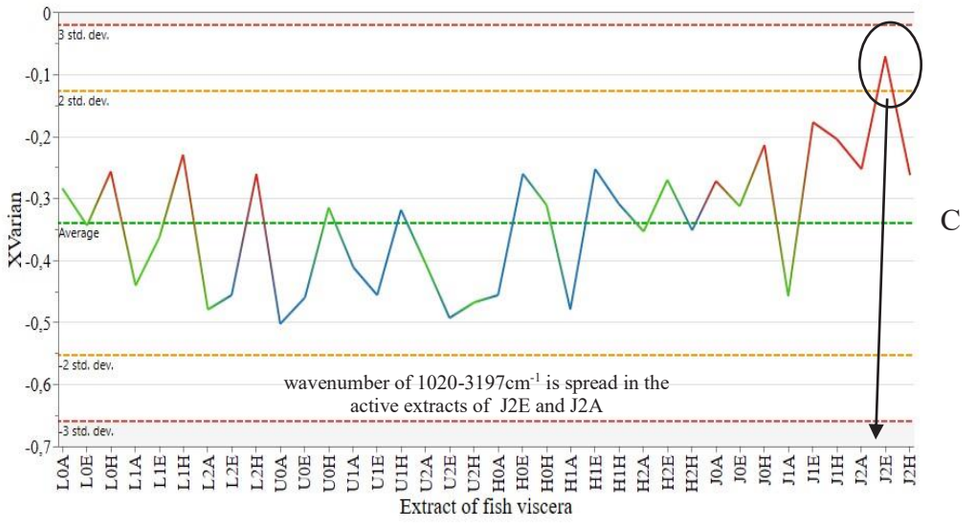


FIGURE 4. OPLS score plot (R^2Y =0.974; Q^2= 0.893) (A), Y-related coefficient (B) and X-variant plot (C) of viscera extracts based on attractant activity and FTIR spectral data: ethanol extracts (with final code E), acetone extracts (final code A) and hexane extracts (final code H) of liver (H), intestine (U), stomach (L) and mixed viscera (J) after 0, 1 and 2 days of storage

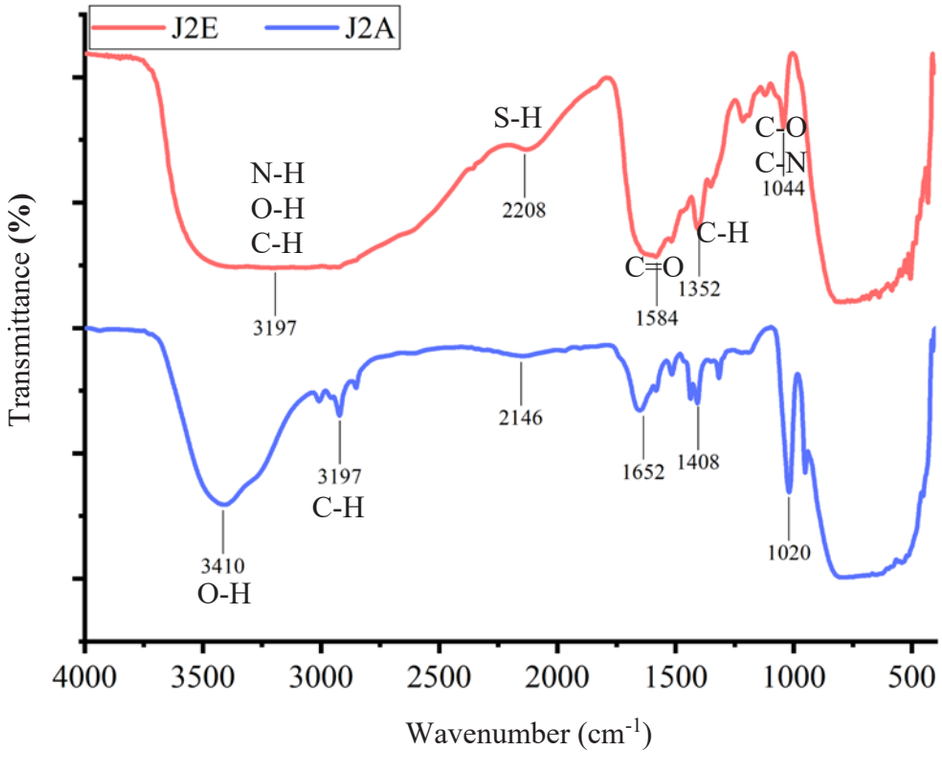


FIGURE 5. Spectral of FTIR from J2E and J2A of fish viscera extracts

TABLE 1. Active functional groups from FTIR spectral data based on wavenumbers correlated to house fly attractant activity from skipjack fish viscera extracts (spread on J2E and J2A extracts), analyzed by OPLS method (SIMCA v.16.0)

Vibration type	Functional group	Extracts	Wavenumber (cm^{-1})	Y-related coefficient	VIP	References*
Strech	N-H	J2E	3197	0.38	0.96	3500-3200
		J2A	3197	0.38	0.96	
Strech	O-H C-H	J2E	3197	0.38	0.96	3500-3200
		J2A	3410	0.38	0.96	
		J2E	3917	0.38	0.96	3000-2850
		J2A	2922	0.37	0.73	
Strech	S-H	J2E	2208.34	0.58	1.15	2550
		J2A	2146.62	0.59	1.17	
Bending	C-H C-O-H	J2E	1352.01	0.57	1.13	1440-1220
Strech	C-O C-N	J2A	1317.29	0.74	1.33	
		J2E	1043.42	0.65	1.19	1250-1000
		J2A	1020.27	0.53	1.03	

*Pavia et al. 2014

IDENTIFICATION OF VOLATILE COMPOUNDS

Based on OPLS result, J2A and J2E were subjected to SPME-GC-MS analysis, aiming to identify volatile compounds in the extracts. Chromatographic peaks of compounds identified in J2E and J2A are presented in Figure 6. A total of 26 volatile compounds were identified in J2E extract (Table 2), meanwhile 15 volatile compounds were identified in J2A (Table 3). It can be seen that polar solvent produced more extracted compounds than semi polar solvent. The identified volatile compounds in J2E included 2 amines, 1 phenol, 1 furan, 1 aromatic aldehyde, 7 ketones, 1 furane, 6 heterocyclics, 1 fatty acid and 7 fatty acid esters (Table 2). The volatiles in J2A consisted of 3 ketones, 3 heterocyclics, 2 amines, 2 alcohols, 1 sulfur, 1 phenol, 1 fatty acid and 2 fatty acid esters compounds (Table 3). The similar compounds were reported in fish from the other studies (Balino-zuazo & Barranco 2016; Bourigua et al. 2011; Toniolo et al. 2014).

Sulfur compound was identified in J2A, including methylsulfide, dimethylsulfide and other alkyl and dialkyl sulfides as the resultant of fish protein decomposition. The sulfur compounds such as dimethyl trisulfide in fish and pork waste (He et al. 2018) and dimethyl sulfide in stored nila fish (Cheng, Mei & Xie 2023) and in fillet nila (Sae-Leaw & Benjakul 2015) were detected. They may be derived from amino acids (cysteine, cystine and methionine) or from thiamine (Francioso et al. 2020).

J2E extract which has a strong C=O signal (Figure 5) is more dominated by ketone, esters and fatty acids compounds. Generally, ketones with lower aroma thresholds result in greater contributions to overall fresh fish-like odors (Ma et al. 2020). Ketones may be produced by oxidation of polyunsaturated fatty acids (PUFA), degradation of amino acids and microbial oxidation (Sun et al. 2022).

Trimethylamine (TMA) is formed in fish during bacterial spoilage (Wang et al. 2022). Several studies successfully identified volatile amines, including TMA, dimethylamine (DMA), and isobutylamine, as critical markers of fish freshness due to their gradual accumulation during the spoilage process and the contribution of characteristic fishy odor (Dehaut et al. 2016; Leduc et al. 2012).

Similar compounds were also found to attract *M. domestica* flies and other insects from other attractant sources such as fermented wheat bran (phenol, amine, and aldehyde groups respectively 2-ethyl-phenol, trimethylamine, hexanal, heptanal, tricodecanal, octanal, pentanal, nonanal, decanal, 2,4-nonadienal-1-al and 2-undecanal, as well as alcohols such as 2-penten-1-ol, 2-octanol) (Kun et al. 2013), and egg powder with sugar

(carbohydrates cyclic such as pyrazine compounds, fatty acids and esters such as isovaleric acid, butyric acid, methyl 4-acetylbenzoate, as well as sulfur compound (naphthalene, thiirane) (Kun et al. 2013; Mloston, Shermolovich & Heimgartner 2022).

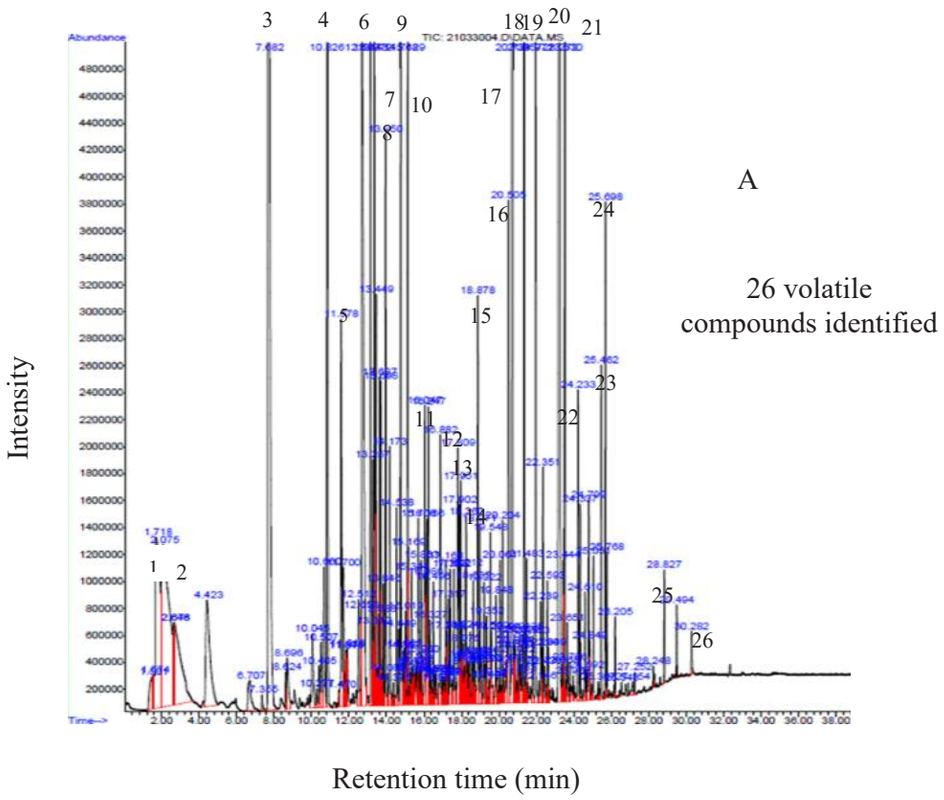
The identification of compounds with GC-MS were confirmed by analyzing the fatty acid profiles of the two extracts which are shown in Figure 7. The analysis of fatty acid profiles of both extracts showed the dominant fatty acids, consisting of DHA, EPA, palmitic, oleic, stearic, arachidonic, and pentadecanoic acids which are also found in the GC-MS analysis of the extracts (J2E and J2A). Therefore, this present study successfully uncovers the science behind the art of a local wisdom in controlling house flies using fish viscera for days. The extract of acetone could be mixed with extract of ethanol for the effective house fly control, and this may benefit to the pest control in future.

TABLE 2. Volatile compounds identified in J2E fish viscera extract (from ethanol extract of mixed viscera after 2 days storage) using SPME-GC-MS

RT (min)	Compounds	CAS	Group	RT (min)	Compounds	CAS	Group
1.718	*Trimethylamine*	75-50-3	amine	17.961 18.877	*Methyl laurate* *9-methyl-4-decanone*	111-82-0 29379-11-1	fatty acid ester ketone
2.075	*Betaine*	107-43-7	amine	20.448	*1-methyl-2-aminobenzimidazole*	1622-57-7	heterocyclic
8.364	*2-ethyl phenol*	90-00-6	phenol	20.884 21.435	*2-pentadecanone* *1,3-Cyclodecadiene, (E,Z)-*	2345-28-0 1129-92-6	ketone heterocyclic
10.659	*6-methyl-5-hepten-2-one*	110-93-0	ketone	21.971	*Methyl pentadecanoate*	7132-64-1	fatty acid ester
11.780	*2-nonanone*	821-55-6	ketone	22.351	*Acridine 10-oxide*	10399-73-2	heterocyclic
13.036	*2-methyl-5-propyl pyrazine*	29461-03-8	heterocyclic	23.272	*Methyl palmitate*	112-39-0	fatty acid ester
13.312	*2-methyl-6-vinyl 2-pyrazine*	13925-09-2	heterocyclic	24.232	*Ethyl 9-heptadecanoate*	1000336-4	fatty acid ester
13.846	*Benzaldehyde*	100-52-7	aromatic aldehyde	25.462	*Methyl stearate*	112-61-8	fatty acid ester
15.018	*2-undecanone*	112-12-9	ketone	25.697	*Methyl elaidate*	1937-62-8	fatty acid ester
15.169	*2-acetylpyridine*	1122-62-9	heterocyclic	25.826	*Methyl arachidonate*	2566-89-4	fatty acid ester
16.047 16.247	*2-furanmethanol* *4-9(1,3-benzodioxol-5-yl)-2- butanone*	98-00-0 55418-52-4	furane ketone	29.494	*Eicosapentaenoic acid*	10417-94-4	fatty acid
16.882	*3-decen-2-one*	10519-33-2	ketone				

TABLE 3. Volatile compounds identified in J2A fish viscera extract (from acetone extract of mixed viscera after 2 days storage) using SPME-GC-MS

RT (min)	Compounds	CAS	Group
7.841	*4-methyl-3-penten-2-one*	107-43-7	ketone
8.827	*2-heptanone*	110-43-0	ketone
11.902	*3,4-dimethyl pyridine*	583-584-	heterocyclic
12.384	*4-hydroxy-4-methyl-2-pentanone*	123-42-4	ketone
13.893	*1-heptanol*	111-90-6	alcohol
14.787	*2-methyl-tetrahydropyran*	1000386-40	heterocyclic
14.988	*2-Acetylhydroquinone*	490-78-8	heterocyclic
16.076	*dimethyl sulfoxide*	67-68-5	sulfur
16.457	*2,6-dimethyl cyclohexanol*	5337-72-4	alcohol
18.495	*5-methoxytryptamine*	608-07-1	amine
22.082	*methyl myristate*	124-10-7	fatty acid ester
22.725	*5,6-dimethyl-2-benzothiazolamine*	29927-08-0	amine
24.632	*methyl palmitate*	112-39-0	fatty acid ester
25.732	*2,5-di-tert-butyl- phenol*	5875-45-6	phenol
32.340	*palmitic acid*	57-10-3	fatty acid



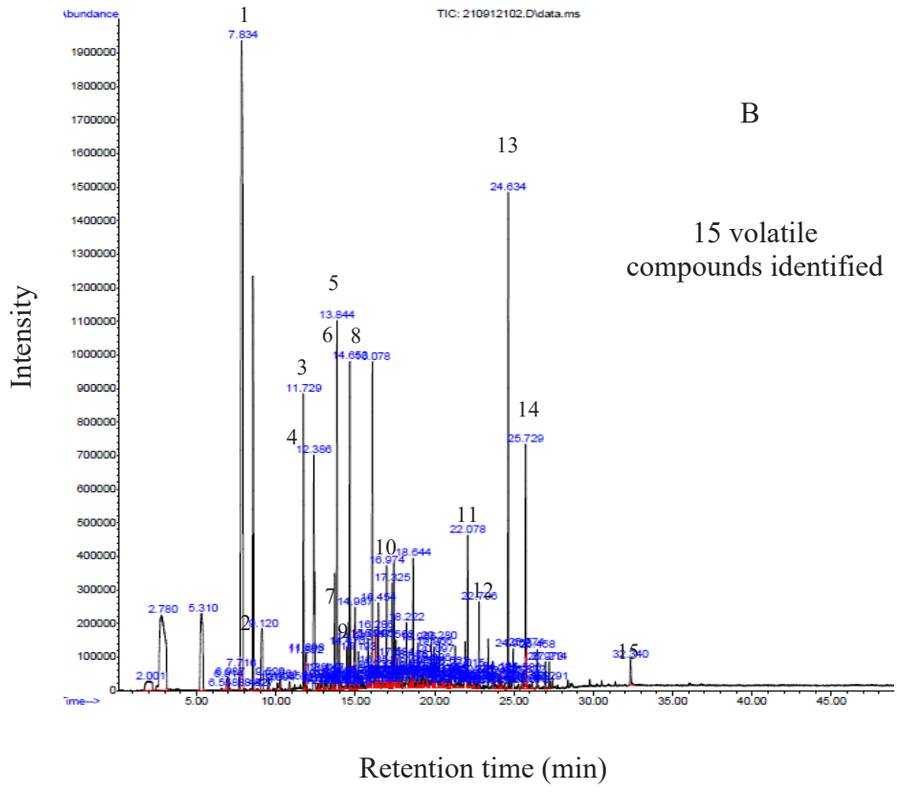


FIGURE 6. Cromatogram of GC-MS from J2E extract (A) and J2A extract (B) suspected as house fly attractant

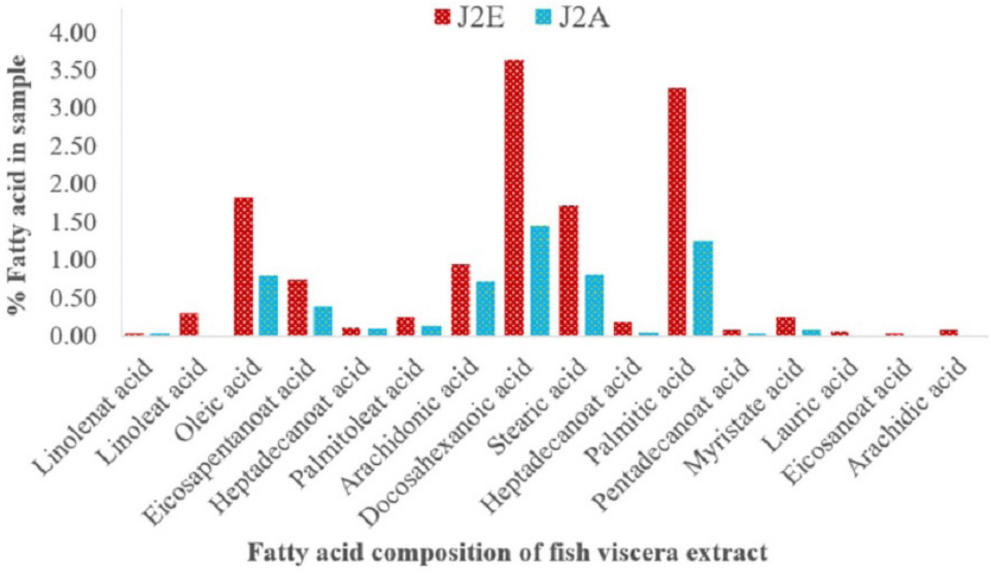


FIGURE 7. Fatty acid profile of J2E and J2A viscera extracts

Conclusions

Solvent extracts of skipjack fish viscera from 0 until 2 days storage, either from stomach, intestine, liver or mixture of the three, have attractant activities to house fly *M. domestica*. Chemometric by OPLS analysis on FTIR spectral data and attractant activities of 36 extracts showed that ethanol and acetone extracts of mixed viscera from 2 days storage had the significant house fly attractant activity. Compounds having functional groups S-H, N-H, O-H, and C-O were correlated to the activity. A total of 26 types of volatile compounds identified in the ethanol extract, meanwhile 15 types identified in the acetone extract. Both of extracts (J2E and J2A) showed the dominant by fatty acids consisting of DHA, EPA, palmitic, oleic, stearic, arachidonic and pentadecanoic acids which are also found in the GC-MS analysis. These compounds are commonly found in fishes as decomposition products and have been found in other extracts with house fly *M. domestica* attractants. This finding gives an insight about the application of fish viscera extract for pest management in the future.

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