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Analysis of Volatile Compound from Swiftlet Raw Material Using Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (SPME-GC-MS) and E-Nose Sensor

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Abstract. Aerodramus fuciphagus, or swiftlets are small insectivorous birds that breed throughout Southeast Asia. Edible birds' nest (EBN) from the swiftlets' saliva secretion are valuable in the market. EBN industry in Malaysia are large and recognized for its economic potential. Aroma is one of the factors believed to attract swiftlets to enter and build nests in the birdhouse. This study focused on the identification of volatile organic compounds (VOCs) from the aroma of EBN, feather, and guano as reference samples in a successful swiftlet birdhouse. E-nose technology was used to determine the VOC similarity index based on the reference samples collected. Headspace technique coupled with gas chromatography/mass spectrometry (HS-GC/MS) were performed for the chemical profile identification. The area favored by the population of swiftlet are shown through the similarity index of the e-nose results which are 87.06%, 67.15% and 60.23% obtained from feather, guano and EBN respectively. Next, HS-GC/MS using four types of solid-phase microextraction (SPME) fibers were used in the analysis. PDMS fiber showed the highest efficiency in extracting the VOCs from the reference samples. Pentadecane (15.5%) and 2,4-Di-tert-butylphenol (10.41%) were the main compounds from 16 compounds identified in EBN. Eleven compounds were identified in the feather sample with Heptadecane (7.26%) and Pentadecane (6.14%) as the main compounds, followed by ten compounds extracted from the guano sample, with 2-Bromo dodecane (11.9%) and Hexadecane (7.39%) as the main compounds. From this study, the volatile compounds of the possible natural aroma that attracts the swiftlets into the birdhouse can be identified.

INTRODUCTION

Swiftlets are well-known for their expensive nest known as edible birds' nest (EBN) built from the glutinous secretion of their two sublingual salivary glands. Among all swiftlet species in Malaysia, Aerodramus fuciphagus and Aerodramus maximus, are known to be producing marketable bird's nest. The A. fuciphagus white nest is expensive because it is made entirely of pure hardened saliva [1]. Birdhouses are built with cave-like characteristics to imitate the natural living habitat of swiftlets. Environmental factors affecting the population of swiftlets in swiftlet birdhouses are light intensity, humidity, air and building temperature, and air velocity are the key factors of successful swiftlet birdhouses [2]. The traditional use of aroma in attracting swiftlets is frequently applied in a swiftlet birdhouse to accelerate its occupancy. Two types of commonly used aroma are traditional or natural aroma (edible bird's nest, feather, and guano), and another one is commercial aroma, which is produced in the market for swiftlet ranchers. However, due to the risks associated with commercialized aroma that uses dangerous chemicals and can affect the health of swiftlets in long-term usage, a proper and less dangerous approach of producing aroma for swiftlets has been introduced using enzymatic hydrolysis and water extraction of the samples as the ingredients [3]. Therefore, in this research, the main compounds of EBN, feather, and guano are investigated to evaluate their usefulness as the ingredients to produce aroma for increasing swiftlets' EBN production. The aroma is used to increase the swiftlet

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population inside a swiftlet birdhouse. Nevertheless, the effectiveness and safety toward the animal and the environment has not been widely studied and scientifically proven. One of the commercial aroma samples tested contains chloroform, which is a hazardous chemical used as the solvent in the aroma. Chloroform is very dangerous and can potentially pose a threat to swiftlet population [4]. The smell of ammonia is strong in swiftlet caves as the smell accumulates from swiftlet droppings [5]. For this reason, many suppliers produced ammonia and commercialized the compound as swiftlet aroma. The inflammatory reaction can happen due to repeated exposure to ammonia [6]. In summary, this research addressed the volatile compound identification of swiftlet materials with the E-Nose similarity index used for comparison for identification of attractant aroma from the swiftlet materials to increase the population of swiftlets in the birdhouse. The success of this study is beneficial to the business of swiftlet farming as it helps to increase EBN production.

MATERIALS AND METHOD

Extraction of Volatile Compounds

EBN, feather and guano samples were collected from the Aspa Cottage swiftlet birdhouse in Kuantan, Pahang. The samples were kept dried in sealed bottles and stored at room temperature prior to extraction. SPME-GC-MS with PDMS/DVB/CAR, PDMS/DVB, PDMS/CAR and PDMS were fibers used for volatile compounds detection in the samples. Agilent 7890B coupled with MS 5977A instrument equipped with DB-1ms capillary column, (0.25 µm film thickness; 30 m × 0.25 mm ID) were used to conduct GC analyses. The ionization voltage of 70 Ev for was chosen for the electron ionization (EI) mode in the instrument detector. Carrier gas helium with 1.5 mL/min constant flow rate were used. The instrument was set in a splitless mode with the temperature of 260 °C for the front inlet. Conditioning was set for 30 min at 250 ºC before SPME analysis with different fiber was performed. 2 mL of the sample was transferred to a 4 mL SPME vial, then let to reach the equilibrium at 70ºC for 30 min. The heated space inside the vial was exposed with the fiber for 30 min at 70 ºC. For thermal desorption, the exposed fiber was transferred immediately to the GC injection port at 250 ºC for 10 minutes. For the GC/MS parameters of EBN and feather sample, the initial temperature was set at 30 °C with a 3-min hold time, ramped to 70 °C at 2.5 °C/min, and then increased to 150 °C at 8 °C/min. Lastly, the temperature was increased by a ramp of 20 °C/min to 260 °C and held for 5 min. Volatile compounds with matching of above 80%, were identified by comparing the results with National Institute Standard and Technology (NIST) library.

Tailoring E-Nose library into Sensor Body

Each natural source material (EBN, feather, and guano) was inserted in different 150 mL Schott bottles. A 5-min reading time was taken using an e-nose odour sensor at 30 °C for each sample. The electronic data were saved as a reference database for odour assessment later. The e-nose reading was taken for 5 min at each point (seven points) outside and inside of the studied birdhouse in Aspa Cottage, Pahang. The sensor was pre-conditioned for 5 min using ethanol prior to every data collection to avoid cross-contamination. During data collection, the surrounding temperature inside the birdhouse was estimated to be at most 30 °C. The data were saved and compared to the reference database of natural source materials. The similarity percentage generated was used to determine the odour type in the selected area. The database behavior or the pattern of the signal abstract was studied for the first step of tailoring process. Using several analysis methods such as normalization technique, mean formulation, and clustering technique, pattern similarity study and the normalized extracted features were analyzed. The study of case library abstraction and E-CBR similarity function were performed in the next step. The optimization of voting procedure was done after the analytical and correlation analysis. The E-Nose sensor employed with E-CBR was the main focus of the third step, involving the comparison of the performance measurement and simulation of the result for the optimization of the sensor reaction towards odour samples. Lastly, the data in the system being stored and stabilized using Arduino library.

RESULTS AND DISCUSSION

Identification and Quantification of Volatile Compounds of EBN

The peak of volatile organic compounds with quality of 80% and above were obtained from the raw data analysis of HS-GC-MS. The raw materials collected from the birdhouse contain their own volatile organic compounds (VOCs) related to odour production (Figure 1). Therefore, the analysis of the volatile compounds obtained from the swiftlets' feather, EBN, and guano samples using SPME-GC/MS with four different types of fibers, namely divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/ CAR/ PDMS), polydimethylsiloxane/ divinylbenzene (PDMS/ DVB), carboxen/ polydimethylsiloxane (CAR/ PDMS), and polydimethylsiloxane (PDMS) was performed. The best fiber among the four different types of fibers was chosen for the compounds extraction in swiftlet raw materials. The methods were performed on the samples in dry conditions. Table 1 lists different volatile compounds obtained from the EBN sample and the PDMS fiber extracted the most compounds compared to the other three fibers. PDMS fiber detected sixteen compounds while fourteen compounds by the PDMS/DVB fiber. PDMS/DVB/CAR fiber identified six compounds, and only one compound was detected using the CAR/PDMS fiber. Major compounds in the dry EBN sample were tentatively identified as 2,4-di-tert-butylphenol (10.41%), 1-iodo-octadecane (8.14%), heptacosane (5.87%), 2-ethylhexyl salicylate (4.92%), and dibutyl phthalate (4.92%). Meanwhile, the main compounds found for the PDMS/DVB fiber were 2,4-di-tert-butylphenol (4.15%), tetracosane (3.42%), hexadecane (2.89%), and trans-1,10-dimethyl-trans-9-decalinol (2.26%). For the PDMS/DVB/CAR fiber, the compounds identified were 1-iodo-octadecane (2.21%) and 4TMS derivative dihydroxyphenylglycol (2.17%), whereas 5-hydroxy-pentanoic acid, 2,4-di-t-butylphenyl esters (1.42%) were found using the CAR/PDMS fiber. CAR/PDMS fiber identified more acid and alcohol compounds. 2,4-di-tert-butylphenol was detected by three of the fibers with 10.41%, 4.15%, and 1.92%, respectively [7].

(a) (b) (c)

FIGURE 1. Swiftlets' a) EBN, b) feathers, and c) guano

TABLE 1a. Volatile compounds and its relative peak of the EBN sample using 4 types of fibers

	EBN			
Group Compound	**PDMS/DVB/ CAR**	**PDMS/DVB**	**CAR/PDMS**	**PDMS**
Alkane				
Triacontane	-	-	-	0.43

TABLE 1b. Volatile compounds and its relative peak of the EBN sample using 4 types of fibers

Group Compound	PDMS/DVB/CAR	PDMS/DVB	CAR/PDMS	PDMS
2-methyloctacosane	-	-	-	1.46
3-methyl-pentadecane	-	-	-	1.35
1-iodo-tridecane	-	-	-	0.41
2,6,11-trimethyl-dodecane	-	-	-	0.63
1-iodo-eicosane	-	0.6	-	-
Hexadecane	-	2.89	-	0.6
Hentriacontane	-	0.3	-	0.62
Heptacosane	-	0.42	-	5.97
Heneicosane	-	1.99	-	2.86
Octacosane	-	1.51	-	3.69
Tetracosane	-	3.42	-	-
1-iodo-Octadecaneoctadecane	2.21	-	-	8.14
Pentadecane	-	-	-	15.5
Phenol	-	-	-	-
4'-(1-methylethylidene) bis-Phenolphenol	-	-	-	0.95
2,4-Didi-tert-butylphenol	1.92	4.15	-	10.41
2,6-Bisbis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	-	0.48	-	-
2-Ethylhexyl ethylhexyl salicylate	-	1.36	-	4.92
4,4'-(1-methylethylidene) bis-Pphenol	-	0.62	-	-
Ester	-	-	-	-
Dibutyl phthalate	-	-	-	4.92
2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester Propanoic propanoic acid	-	0.62	-	-
Propanoic acid 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	0.33	-	-	-
Pentanoic acid 5-hydroxy-, 2,4-di-t-butylphenyl esters	-	-	1.42	-
Sesquiterpene				

TABLE 1c. Volatile compounds and its relative peak of the EBN sample using 4 types of fibers

Group Compound	PDMS/DVB/CAR	PDMS/DVB	CAR/PDMS	PDMS
Trans-1,10-Dimethyldimethyl-trans-9-decalinol trans-1	-	2.26	-	-
transTrans-1, 10-Dimethyldimethyl-trans-9-decalol	1.69	-	-	-
2,2,4-Trimethyltrimethyl-1,3-pentanedioldiisobutyrate	1.04	-	-	-
Other				
4TMS derivative Dihydroxyphenylglycoldihydroxyphenylglycol	2.17	-	-	-
Total of alkanes	**2.21**	**11.13**	**-**	**41.66**
Total of phenol	**1.92**	**6.61**	**-**	**16.28**
Total of esters	**0.33**	**0.62**	**1.42**	**4.92**
Total of sesquiterpene	**2.73**	**2.26**	**-**	**-**
Others	**2.17**	**-**	**-**	**-**

Identification and Quantification of Volatile Compounds of Feathers

Table 2 shows different volatile compounds obtained from the feather sample and the PDMS fiber extracted the most compounds compared to the other three fibers. Eleven compounds were detected by the PDMS fiber. Meanwhile, ten compounds were identified by PDMS/DVB fiber, two compounds by PDMS/DVB/CAR fiber, and CAR/PDMS fiber detected no compound. The major compounds extracted in the dry feather sample by the PDMS fiber were tentatively identified as heptadecane (7.26%), pentadecane (6.14%), 2,6,10,14-tetramethyl pentadecane (4.09%), and hentriacontane (2.46%). In contrast, the main compounds found for the PDMS/DVB fiber were 1,2-dimethoxy-4-propenyl-(Z)-benzene (3.51%), 1,2- dimethoxy-4-(1-propenyl)-benzene (22.82%), and pentadecane (0.91%). The compounds observed were 2,4-di-tert-butylphenol (0.93%) and heptadecane (0.45%) for the PDMS/DVB/CAR.

Table 2a. Volatile compounds and its relative peak of the feather sample using four types of fibers

	Feather			
Group Compound	**PDMS/DVB/CAR**	**PDMS/DVB**	**CAR/PDMS**	**PDMS**
Alkane				
Heptadecane	0.45	0.87	-	7.26
Hexacosane	-	-	-	1.26
Hentriacontane	-	-	-	2.46

Table 2b. Volatile compounds and its relative peak of the feather sample using four types of fibers

Group Compound	PDMS/DVB/CAR	PDMS/DVB	CAR/PDMS	PDMS
Heptacosane	-	-	-	1.50
2,6,10-trimethyltridecane	-	0.39	-	-
pentadecane	-	0.91	-	6.14
Pentacosane	-	0.88	-	1.75
Dodecane	-	0.83	-	-
2,6,10,14-tetramethyl pentadecane	-	0.67	-	4.09
Eicosane	-	0.29	-	-
2-methyltetracosane	-	0.43	-	-
Phenol				
2,4-di-tert-butylphenol	0.93	-	-	-
4,4'-(1-methylethylidene) bisphenol	-	-	-	2.45
2,5-bis(1,1-dimethylethyl)phenol	-	-	-	1.76
Ester				
Sulfurous acid butyl tridecyl ester	-	-	-	1.73
Phenyl				
(Z)-1,2-dimethoxy-4-propenyl-benzene	-	-	-	3.01
1,2-dimethoxy-4-propenyl-, (Z)-benzene	-	3.51	-	-
1,2-dimethoxy-4-(1-propenyl)-benzene	-	22.82	-	-
Total of alkanes	**0.45**	**5.27**	**-**	**24.46**
Total of phenol	**0.93**	**-**	**-**	**4.21**
Total of esters	**-**	**-**	**-**	**1.73**
Total of phenyls	**-**	**26.33**	**-**	**3.01**

Identification and Quantification of Volatile Compounds of Guano

Adult birds may have memorized some specific local odours along with their migratory routes. Birds may depend on the olfactory information and physical attributes, such as aerosols, which are used to ensure that olfactory chemicals are partially water-soluble [8]. In this analysis, volatile compound detection of dry swiftlet guano was performed. The main group of compounds found in dry guano is alkane. The profiles of volatile compounds in dry guano obtained using SPME are listed in Table 3 based on the retention time on a DB-1 column. From the table, different volatile compounds were obtained and the PDMS fiber extracted the most compounds compared to the other three fibers. PDMS fiber detected ten compounds, while three compounds obtained using PDMS/DVB fiber, PDMS/DVB/CAR fiber detected one compound, and CAR/PDMS fiber detected no compound. Major compounds in guano were tentatively identified as 2-bromo dodecane (11.9%), hexadecane (7.39%), octacosane (5.05%), 4,4'-(1-methylethylidene) bisphenol (4.83%), and heptadecane (3.72%). Meanwhile, the main compounds found for the PDMS/DVB fiber were hexadecane (2.03%), 2,4-di-tert-butylphenol (1.33%), and phenyl (0.56%). 3,5-bis(1,1-dimethylethyl)phenol (0.89%) was the main compound found for the PDMS/DVB/CAR fiber, while no compound was obtained by the CAR/PDMS fiber. The similar compounds obtained by two SPME fibers were 2,4-di-tert-butylphenol and hexadecane.

Table 3. Volatile compounds and its relative peak of the guano sample using four types of fibers

	GUANO			
Group Compound	**PDMS/DVB/CAR**	**PDMS/DVB**	**CAR/PDMS**	**PDMS**
Alkane				
3,9-dimethyl-undecane	-	-	-	2.86
2-bromo dodecane	-	-	-	11.9
Hexadecane	-	2.03	-	7.39
Heneicosane	-	-	-	2.82
Octacosane	-	-	-	5.05
Heptadecane	-	-	-	3.72
Phenol				
2,4-di-tert-butylphenol	-	1.33	-	3.35
4,4'-(1-methylethylidene) bisphenol	-	-	-	4.83
3,5-bis(1,1-dimethylethyl)phenol	0.89	-	-	-
Ester				
Benzoic acid 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester	-	-	-	0.88
Carboxyl				
Methyl 3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane)carboxylate	-	-	-	2.62
Phenyl	-	0.56	-	-
Total of alkanes	**-**	**2.03**	**-**	**33.74**
Total of phenols	**0.89**	**1.33**	**-**	**8.18**
Total of esters	**-**	**-**	**-**	**0.88**
Total of carboxyl	**-**	**0.56**	**-**	**2.62**

Odour profiles at different test point

All points of reference produced different values of similarity index. Based on the similarity index, the main aroma at each point of reference inside the birdhouse was identified. An e-nose sensor and GC/MS were used to assess the comprehensive aroma characterization as well as to identify volatile compounds of swiftlet waste products, respectively. The odour assessment results using the e-nose odour sensor were compared with the EBN, feather, and guano from the reference database, which is a library developed based on the swiftlet materials from the birdhouse (Table 4). Swiftlet populations were concentrated in VIP room 1 and 2 (Figure 2). Starting from P1, a swiftlet hole was used as the birdhouse entrance for the swiftlet to enter and leave the birdhouse. The main hall, P3, referred to the buffer area for the swiftlets from the roving area (P2) toward the VIP room (P4 and P5). Swiftlets rarely made the nests inside the main hall as they mostly occupied the VIP room (P4 and P5). P6 and P7 are the last areas occupied by the swiftlets for making nests.

Therefore, the odour sensor data also aligned with the observation as the odour of feather and guano showed high similarity to those areas with the amount of 65.98%–87.06%. These findings showed that swiftlets tend to live in areas covered with their smell. Farmers used aroma or hormone spray for the purpose of attracting swiftlets to nest and roost inside their birdhouses. The commercial aroma used by the swiftlet farmers inside the swiftlet birdhouses was categorized by the top and floor aroma based on the placement for the application of the aroma. Floor aroma is usually formulated using guano and feathers to create a comfort area for swiftlet breeding [9]. Meanwhile, the lower similarity for the odour of nest (27.41%–60.23%) may be because the odour was taken in an open area of each place, whereas nest-based aroma is commonly applied at the nesting plank. Although some top aroma was dispersed via a humidifier, the air particles tended to fall to the floor, thus enhancing the smell of guano. Other than that, the odour in the roving area and the main hall also showed low similarity (15.17%–56.69%). Therefore, farmers should avoid the application of aroma in this area as the room acts as a buffer room for light and swiftlets may not build their nest in this hot area.

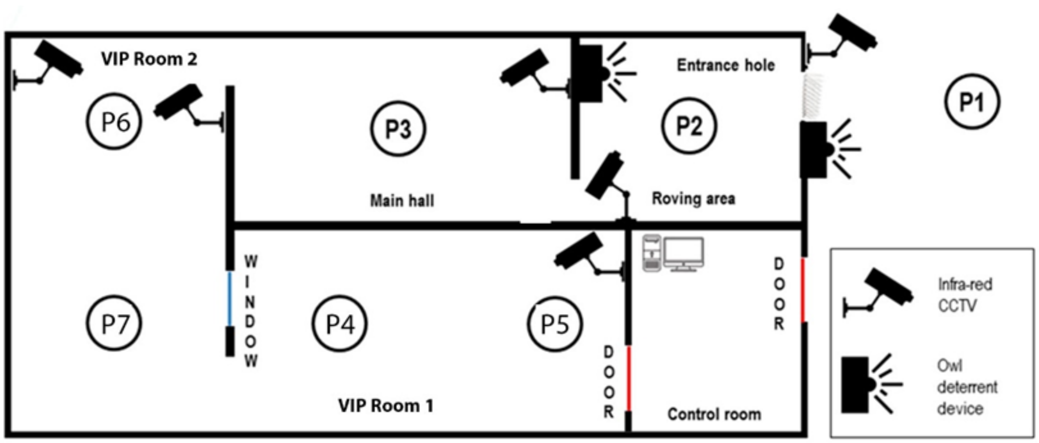

FIGURE 2. An overview of each point inside the birdhouse

TABLE 4. Results for odour assessment in the birdhouse of Aspa Cottage, Pahang

		Similarity index (%)		
Location	**Point/Sample**	**Feather**	**Guano**	**Nest**
Outdoor	P1	41.14	41.53	1.00
Roving area	P2	35.09	15.78	56.21
Main hall	P3	34.48	15.17	56.69
VIP room 1	P4	68.42	69.41	31.98
VIP room 1	P5	65.98	66.97	27.41
VIP room 2	P6	83.66	81.35	43.52
VIP room 2	P7	87.06	67.15	60.23
Reference	Feather	100.00	80.09	58.41
Database	Guano	80.09	100.00	39.10
Continued				
Reference Database	Nest	58.41	39.10	100.00

CONCLUSION

In conclusion, the analysis of swiftlet attractant factors using gas chromatography (GC), found that the chemical composition also varied for each sample, which contained several groups of compounds, namely alkane, phenol, carbonyl, ester and phenyl. In this research, the extraction of volatile organic compounds (VOCs) from swiftlet aroma samples was done using solid-phase microextraction. Extraction efficiency was enhanced using SPME fiber with four different types of coatings. Polydimethylsiloxane (PDMS) extracted the most abundant swiftlet aroma samples VOCs, hence chosen as the most preferred SPME fiber. The main compound detected was 2,4-di-tert-butylphenol for both raw EBN and guano, while Heptadecane was obtained from the feather sample. Next is the successful identification and extraction of volatile organic compounds (aroma) using the technology of e-nose. Based on the e-nose analysis from the reference samples, the odor intensity in a successful birdhouse originated from the feather, followed by guano and EBN. This research has successfully achieved the objective, which is to identify and extract volatile organic compounds (aroma) in a successful swiftlet birdhouse using gas chromatography (GC) and e-nose technology.

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