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Analysis of Volatile Compound from Commercial Swiftlet Aroma Using Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (SPME-GC-MS)

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Abstract. This study was designed to investigate the compounds in the different types of commercial aroma used to attract swiftlets in the birdhouse. Analysis of the volatile compounds is done on two types of commercial aroma, which is categorized as floor aroma (F1, F2, F3) and top aroma (T1, T2, T3) using SPME-GC-MS. As a result, F1, F2 and F3 shows the presence of major constituents like 2-heptanone (13.53%, 3.22% and 0.37% respectively) and 2-nonanone (0.83%, 2.02%, and 0.82% respectively) using DB-1ms while 2-heptanone (5.87%, 0.78% and 0.45% respectively) and methoxy-phenyl-oxime (11.50%, 11.84% and 0.20% respectively) using DB-wax. Major constituents detected using DB-1ms for T1, T2 and T3 are 1-(2-methoxy-1-methylethoxy)-2-propanol (36.49%, 16.23% and 3.06% respectively). Meanwhile, no similar compounds detected by DB-wax for sample T1, T2 and T3. The overall findings concluded that most of the formulation used in the commercial aroma contained strong odor-producing chemicals to attract swiftlets. More studies should be done on investigating the effects of the commercial aroma towards swiftlet also on aroma made from natural substances instead of chemically produced commercial aroma.

Introduction

Swiftlets are from the family *Apodidae* and *Apodidae* refers to a mixed group of small sized swifts [1]. Three of the swiftlet species found in Malaysia, namely *A. fuciphagus*, *A. maximus* and *C. esculent* are commonly known as edible bird's nest (EBN) producer in Southeast Asia (SEA) [2]. In general, EBN is made using the saliva from a pair of swiftlet's salivary glands which bind the nest together during nest building [2].

Nowadays, the global demand for EBN has increased especially in Asia region with China as the main EBN consumers [3]. As an alternative for more EBN production, local community started to build more swiftlet birdhouses as the swiftlets also use vacant buildings as their nesting sites. These man-made buildings (birdhouses) are designed to imitate a cave-like environment in order to attract the swiftlet [4]. Aroma is also used in the birdhouse as it helps in increasing number of swiftlets however, it has not been scientifically proven on its effectiveness and safety towards the animal and environment [5].

Commercial aroma products may vary due to different manufacturers, may also contain ingredients which is toxic and harmful to swiftlet. Moreover, there is no documented scientific research on the chemical attraction on swiftlet. As there are lack of researches done on the use of swiftlet aroma, thus the aim of this study is to investigate the main compounds in the selected commercial aroma products.

Materials and Method

Extraction of Volatile Compounds. Three commercial swiftlet aroma products for top and floor aroma were purchased from various swiftlet farming suppliers. The volatile compounds in the products were identified by SPME-GC-MS. Each fresh liquid sample (1 mL) was transferred to a 4 mL SPME vial and the vial was allowed to rest at 30ºC for 30 min to reach the equilibriation. SPME sampling device equipped with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was used to absorb the analyte after conditioned at 250 ºC for 30 min. Headspace sampling was done by exposing the fiber in the upper space of the vial at 30 ºC for 30 min. The exposed fiber was withdrawn and transferred immediately to the GC injection port at 250 ºC for 10 minutes for thermal desorption. The GC analyses using DB-1ms and DB-Wax capillary column, (both are 30 m × 0.25 mm ID; 0.25 µm film thickness), were performed on an Agilent 7890B coupled with MS 5977A instrument. The oven was programmed at 60 ºC for 3 min, then ramped at 3 ºC/min to 240 ºC and held for 10 min. Identification of volatile compounds were conducted via comparison with National Institute Standard and Technology (NIST) library, with matching of above 80%.

Results and Discussion

Physical Observation and Aroma Assessment of Commercial Aroma. Different colors of liquid can be observed for floor and top commercial aroma ranging from goldish brown to colorless clear liquid (Figure 1). Table 1 showed the description of every sample in terms of appearance and its odor. Most of the samples expressed a strong smell with dark color of liquid except for top aroma sample T3, which is colorless.

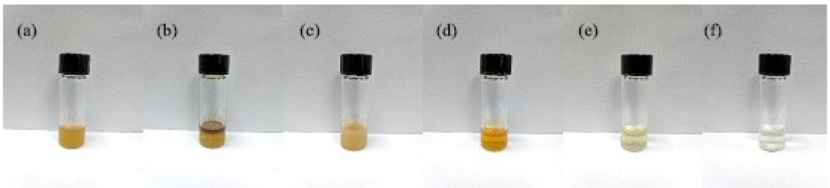

Fig. 1 Commercial aroma for swiftlet's birdhouse, (a) F1, (b) F2, (c) F3, (d) T1, (e) T2, (f) T3

Table 1 Physical observation and odor description of the commercial aroma

Sample	Physical appearance	Odor description	Odor strength
F1	Clear liquid, light brown, a thin white layer at the top	Ammonia-like	Very strong
F2	Clear liquid, a light brown, thick brown layer at the top	Ammonia like	Strong
F3	Cloudy liquid, whitish brown, a small brown layer at the top	Sweet	Medium
T1	Clear liquid, goldish brown	Pungent, sweet	Strong
T2	Clear liquid, light golden brown	Pungent, sweet	Strong
T3	Clear liquid, colorless	Ammonia-like, pungent, powdery	Strong

Identification and Quantification of Volatile Compounds. Volatile compounds in floor and top aroma (F1, F2, F3, T1, T2, T3) are listed in Table 2 and 3. Identified compounds belonged to different chemical classes, as follows: 3 alcohols, 3 aldehydes, 4 alkanes, 3 alkenes, 19 esters, 6 ketones, 1 oxime, 5 pyridines and 5 sesquiterpenes for floor aroma. Meanwhile, top aroma compounds are as follows: 5 alcohols, 3 aldehydes, 1 alkane, 3 alkenes, 6 esters, 1 oxime, and 1 sesquiterpene for top aroma. F1, F2 and F3 shows the presence of same compounds as follows: 2-heptanone (13.53%, 3.22% and 0.37% respectively) and 2-nonanone (0.83%, 2.02%, and 0.82% respectively) using DB-1ms while 2-heptanone (5.87%, 0.78% and 0.45% respectively) and methoxy-phenyl-oxime (11.50%, 11.84% and 0.20% respectively) using DB-wax. Major constituents detected using DB-1ms for T1, T2 and T3 are 1-(2-methoxy-1-methylethoxy)-2-

propanol (36.49%, 16.23% and 3.06% respectively). Meanwhile, no similar compounds detected by DB-wax for sample T1, T2 and T3. Different column is used because substance of different polarity in the sample will interact differently with each column, thus eluting different compound. However, the setting parameter for GC-MS is kept the same.

Compounds from floor sample: 2-heptanone and 2-nonanone belonged to ketone group, meanwhile methoxy-phenyl-oxime is from oxime group. Aliphatic ketones are widely found in nature, and for some species of animals such as mice, this class of volatile chemicals appears to be of particular behavioral relevance [7]. 2-heptanone functions differently depending on animal species. 2-heptanone has been identified as a pheromone in the urine of female mice [8], also as a urinary biomarker of male mice providing a chemosignal of stress level [9]. Meanwhile for honeybees, 2-heptanone is used to paralyse small invaders [10]. 2-nonanone is one of the compound identified in brown male rat bedding that have a pheromonal function and attract females [11]. 1-(2-methoxy-1-methylethoxy)-2-propanol from top aroma sample belongs to alcohol group.

F1 was predominated by alcohol (8.19 to 19.51%) and ketone (8.44 to 19.50%) while F2 contains high amount of pyridines (14.77%), alcohols (4.49 to 13.33%). Other than that, F3 contains highest amount of esters (90.32 to 93.17%). T1 and T2 are manufactured by the same company, however, using different formulation. T1 and T2 contains high amount of alcohols which are (17.25 to 36.49%) and (2.40 to 53.95%) respectively. T3 aroma, which is recommended by some farmers is mainly constituted by alkanes (10.26%). The variation in the predominant compound in each sample may be influenced by different formulation from the manufacturer of each commercial aroma. However, most of the aroma are made to give strong odour which is believed to be able to attract more swiftlets into the birdhouse.

Table 2 Total relative peak area of non-polar and polar volatiles from commercial floor aroma using Agilent DB-1ms and, DB-wax GC capillary column, respectively

		Relative Peak Area					
		Floor Aroma					
		DB-1ms			DB-wax		
Group	**Volatile Compound**	**F1**	**F2**	**F3**	**F1**	**F2**	**F3**
Alcohol	2-ethylhexanol	8.35	3.74	-	7.81	2.49	-
	Dimethyl-silanediol	11.16	9.59	-	0.38	-	-
	1-penten-3-ol	-	-	-	-	2.00	0.14
Aldehyde	2-methyl-butanal	-	0.90	-	-	-	-
	Pyran aldehyde	0.71	-	-	-	-	-
	2-methyl-2-butenal	-	-	-	-	-	0.76
Alkane	Hexane	-	1.70	-	-	-	-
	Hexanenitrile	7.68	-	-	4.34	-	-
	Dichloromethane	-	0.72	-	0.86	0.59	-
	Trichloromethane	-	4.67	-	-	-	-
Alkene	1-Tetradecene	-	0.76	-	-	-	-
	1,4-dichloro-benzene	-	-	-	0.48	-	-
	1,7-octadiene	-	-	-	-	0.69	-
Ester	3-hydroxy-2,2,4-trimethylpentyl isobutyrate	1.33	-	-	-	-	-
	Ethyl benzoate	-	-	0.21	-	-	0.17
	Ethyl decanoate	-	-	10.83	-	-	7.13
	Ethyl dodecanoate	-	-	12.21	-	0.37	8.64
	Ethyl heptanoate	-	-	0.26	-	-	0.26
	Ethyl hexanoate	-	-	2.49	-	-	3.51
	Ethyl octanoate	-	4.00	36.14	-	1.52	40.49

	Ethyl nonanoate	-	-	0.14	-	-	-
	Ethyl tetradecanoate	-	-	0.56	-	-	0.38
	Methyl decanoate	-	0.33	5.11	-	-	4.37
	Methyl dodecanoate	-	-	7.34	-	-	5.28
	Methyl hexanoate	-	-	0.73	-	-	1.09
	Methyl octanoate	-	1.79	16.77	-	-	18.31
	Methyl tetradecanoate	-	-	0.38	-	-	0.27
	Methyl benzoate	-	-	-	-	-	0.11
	Methyl butanoate	-	-	-	-	-	0.14
	Methyl heptanoate	-	-	-	-	-	0.17
	Methyl nonanoate	-	-	-	-	0.39	-
Ketone	2-Heptanone	13.53	3.22	0.37	5.87	0.78	0.45
	2-Hexanone	5.14	-	-	1.11	-	-
	2-Nonanone	0.83	2.02	0.82	0.50	1.52	-
	2-Undecanone	-	-	0.60	0.96	-	-
	2-Tridecanone	-	0.31	-	-	-	-
	6-methyl-5-hepten-2-one	-	-	0.11	-	-	-
Oxime	Methoxy-phenyl-oxime	-	-	-	11.50	11.84	0.20
Pyridine	1,3-Diazine	-	1.89	-	-	1.65	-
	2-ethyl-6-methylpyridine	0.54	9.39	-	0.92	3.91	-
	2-ethyl-pyridine	-	3.49	-	-	1.57	-
	Methyl pyrazine	-	-	-	-	1.05	-
	Pyridine	-	-	-	-	1.70	-
Sesquiterpene	β-Caryophyllene	-	3.13	0.19	-	2.26	-
	α-Copaene	-	0.31	1.63	-	-	1.44
	γ-Muurolene	-	-	0.14	-	-	0.11
	β-Bourbonene	-	-	-	-	-	0.46
	β-Elemene	-	-	-	-	-	1.25
Total of alcohols		**19.51**	**13.33**	**-**	**8.19**	**4.49**	**0.14**
Total of aldehydes		**0.71**	**0.90**	**-**	**-**	**-**	**0.76**
Total of alkanes		**7.68**	**7.09**	**-**	**5.20**	**0.59**	**-**
Total of alkenes		**-**	**0.76**	**-**	**0.48**	**0.69**	**-**
Total of esters		**1.33**	**6.12**	**93.17**	**-**	**2.28**	**90.32**
Total of ketones		**19.50**	**5.55**	**1.90**	**8.44**	**-**	**0.45**
Total of oximes		**-**	**-**	**-**	**11.50**	**11.84**	**0.20**
Total of pyridines		**0.54**	**14.77**	**-**	**0.92**	**-**	**-**
Total of sesquiterpenes		**-**	**3.44**	**1.96**	**-**	**2.26**	**3.26**

Table 3 Total relative peak area of non-polar and polar volatiles from commercial top aroma using Agilent DB-1ms and, DB-wax GC capillary column, respectively

		Relative Peak Area					
		Top Aroma					
		DB-1ms			DB-wax		
Group	**Volatile Compound**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
Alcohol	1-(2-methoxypropoxy)-2-propanol	-	36.26	-	-	1.50	-
	1-(2-methoxy-1-methylethoxy)-2-propanol	36.49	16.23	3.06	16.15	0.90	-
	2-(2-methoxypropoxy)-1-propanol	-	1.46	-	1.10	-	-
	Diisobutylcarbinol	-	-	1.41	-	-	-
	5-Nonanol	-	-	-	-	-	0.57
Aldehyde	Benzaldehyde	-	-	6.68	-	-	0.55
	Furfural	-	5.92	-	-	-	-
	Piperonal	1.49	-	-	0.40	-	-
Alkane	2-methyl-butane	-	-	10.62	-	-	-
Alkene	Benzene	-	3.33	0.34	-	3.82	1.23
	Diisobutene	-	-	1.34	-	0.94	-
	Toluene	-	0.89	-	-	-	-
Ester	Cylohexyl isocyanate	-	-	0.19	-	-	-
	Ethyl butanoate	1.90	-	-	-	-	-
	Methyl benzeneacetate	0.36	-	-	0.14	-	-
	Methyl butanoate	12.40	-	-	11.11	-	-
	Methyl valerate	2.28	-	-	0.80	-	-
	Ethyl dodecanoate	-	-	-	-	-	-
Oxime	Methoxy-pheny-oxime	-	-	-	-	0.33	2.06
Phenolic	Butylated hydroxytoluene	-	-	-	-	-	1.69
	Phenol	-	-	3.69	-	-	0.33
Sesquiterpene	α-Santalene	0.10	-	-	-	-	-
Total of alcohols		**36.49**	**53.95**	**4.47**	**17.25**	**2.40**	**0.57**
Total of aldehydes		**1.49**	**5.92**	**6.68**	**0.40**	**-**	**0.55**
Total of alkanes		**-**	**-**	**10.26**	**-**	**-**	**-**
Total of alkenes		**-**	**4.22**	**1.68**	**-**	**4.76**	**1.23**
Total of esters		**16.94**	**-**	**0.19**	**12.16**	**-**	**-**
Total of ketones		**-**	**-**	**-**	**-**	**-**	**-**
Total of oximes		**-**	**-**	**-**		**0.33**	**2.06**
Total of pyridines		**-**	**-**	**-**	**-**	**-**	**-**
Total of sesquiterpenes		**0.10**	**-**	**-**	**-**	**-**	**-**

Aroma Concept and Its General Contents. Traditionally, floor and top aromas are produced by the fermentation process of swiftlet's guano and leftover of EBN. Both aromas are applied inside birdhouse for the same purpose to imitate cave-like odor inhabited by the swiftlet colony. A total of 69 from 235 (29.36 %) respondents (farmers) used the aroma to attract and encourage swiftlets to nest and roost inside their birdhouse [6]. In general, floor aroma is used by farmers to remove cement odor, especially for newly built birdhouses by direct application to the floor and lower parts of the building. Floor aroma is applied on the wall with a minimum distance of 3 ft. from the nesting plank. As formulated from swiftlet's guano, floor aroma contains dirty components that may deter swiftlet from building the nest on nesting plank if misapplied. Swiftlet tends to mark the wall of the birdhouse with their guano, but no traces of guano were found in the upper area of a birdhouse. This top area is instead filled with nest and saliva marking by swiftlet, thus sparked the idea of using the leftover from EBN cleaning water or crushed nest for aroma formulation. The simple formulation of top aroma using leftover nest also shown a good response from swiftlet as the population started to increase, and nest markings were found on the previously unoccupied nesting plank. The content of both floor and top commercial aroma is a mixture of alcohols, aldehydes, alkanes, alkenes, esters, ketone, oximes, pyridines, and also sesquiterpenes that may also comes from the fermentation process of swiftlet's guano and leftover of EBN.

Conclusion

In conclusion, based on GC-MS analysis, swiftlet's birdhouse aroma that sold in the market are formulated based on guesswork of manufacturers. There are variation of predominant volatile compounds in each sample, however in floor commercial aroma, ketone compound is observed, in which in previous study it is identified as volatile compound in animal urine which conclude that most commercial aroma may contain swiftlet's guano as the ingredient. Although some aromas are said to be effective based on testimonial by farmers, the chemicals content in those aromas need to be identified to avoid harmful effect to swiftlet and farmer.

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