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Identification of the volatile components of the litchi unifloral honey by electronic nose

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Abstract
The study focused on studying the volatile components of Litchi Unifloral honey through the electronic nose. This electronic nose is ultrafast GC chromatography produced the chromatogram as the responses of the volatiles. Many flavour compounds especially monoterpenoids such as geraniol, linalool, nerol and p-cymene found in the litchi also founded in the volatile profile of the honey. The obtained volatile fingerprint also contained the aldehydes, ketones, carboxylic acids, esters, pyrazine and furanone compounds. The volatile components obtained includes both the honey and the litchi matched with each other. Thus, it is possible to determine the quality of honey through the e-nose.

Keywords: Volatile components, litchi unifloral honey, electronic nose

1. Introduction
Honey is the most valuable product and certification of its quality is done with its volatile components. Multiple small constituents from the nectar and the bees themselves give honey its unique properties (Malan and Marletto, 1974) [1]. The majority of unifloral honeys have distinctive fragrances and typically have higher commercial value (Bogdanov *et al.* 2008) [2]. Litchi has a particular flavoured and is frequently characterized as having a rose-floral and fruity-floral aroma as well as a pleasing, sweet taste. Litchi honey is a popular type of honey made from natural nectar that is collected from Litchi flowers in orchards (Linkon *et al.* 2015) [8]. A strong balsamic and woody aroma with floral undertones was said to characterize the lychee honey (Mahattanatawee *et al.* 2014) [5].
In order to measure a product's quality or recognise its aroma, electronic noses are indeed emerging as new technology. They function similarly and resemble the human nose greatly in that regard (Lozano *et al.* 2005) [6]. A wide range of possible uses in biomedical and agricultural applications are being explored for electronic nose (e-nose) devices, which are being created as adaptable, cost-effective replacements for GC-MS equipment. (Wilson *et al.* 2011) [11]. As a result, the current research focuses on studying and categorising the volatile compounds in litchi honey using ultrafast GC e-nose.

2. Materials and Methods
2.1 Sample collection
Litchi honey was collected from the tropical monsoon forest climatic regions of Morigaon, Mayong, Assam district. Until the analysis was completed, the samples were held at room temperature and out of direct sunlight in the PET bottle. The International Commission for Bee Botany (ICBB) proposed the melissopalynological technique for study of pollen frequency in the honey (*Louveaux et al.* 1978) [7]. Melissopalynology technique certifies the quality of honey based on the percentage of pollen frequency i.e. above 45 % presence of single pollen may represents the “unifloral” whereas below that categorized as “Multi-floral”.

2.2 Electronic nose
An ultra-fast GC electronic nose (Heracles, Alpha M.O.S., Toulouse, France) were utilised to identify volatile compounds. The two columns, a non-polar MXT-5 (5% diphenyl) and a moderately polar MXT-1701 (14% cyanopropylphenyl), have distinct polarities. Based on the in-built technique parameters for the honey provided in the Alpha M.O.S. software, the analytical procedure for analysing the volatile components of the honey was selected. Honey sample was analysed by the taking the 1 g of the sample in the vials and sealed to prevent the

escape of any volatiles. At 260 °C, the FID detectors were programmed to operate. Every 0.01 s during the 110 s of total integration time, the signal was digitalized. As the molecules eluted from each column, a flame ionisation detector detected them and displayed as a peak on a chromatogram.

3. Results and Discussion

3.1 Melissopalynology pollen analysis

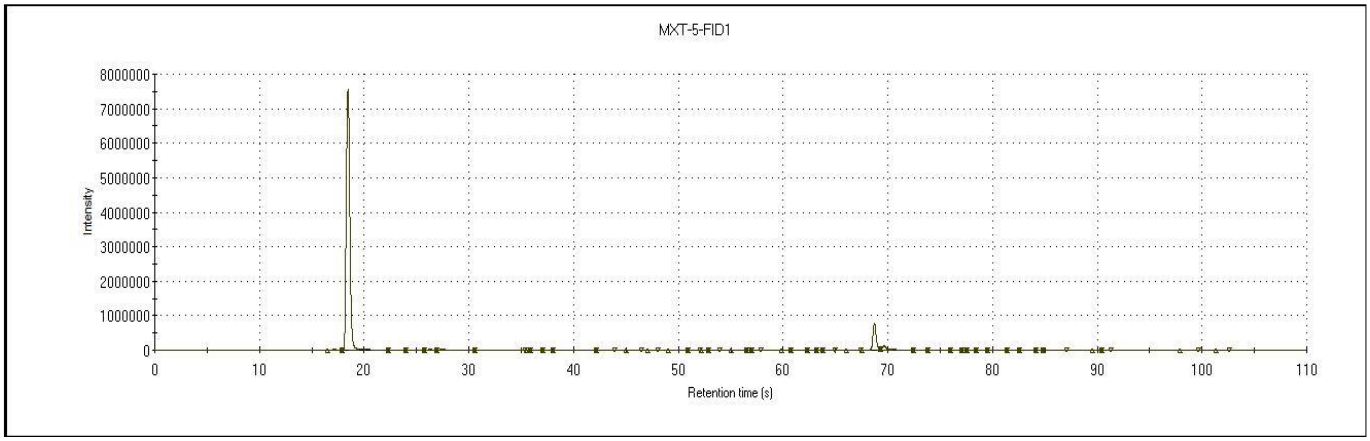
Melissopalynology report revealed that the collected honey contained the unifloral species of *Nephalium litchi* (57%), Ligustrum sp. etc. By the pollen test, the collected honey can be assured as the unifloral honey of litchi.

3.2. Electronic nose

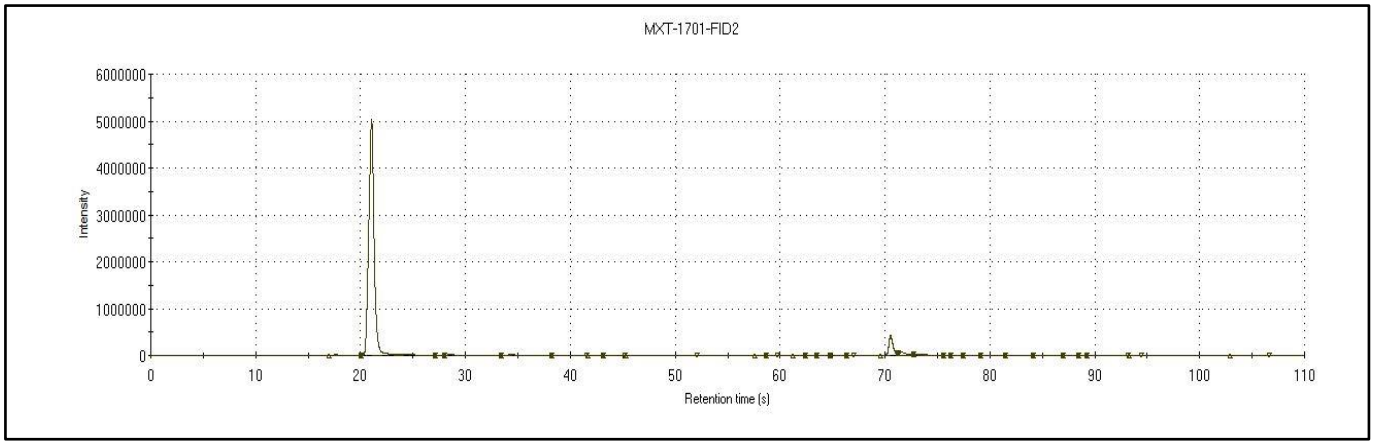
Two chromatograms (Figure-1 and Figure-2) representing the raw data from the Heracles ultra-fast GC E-nose is used to identify the compounds in the peak using the Arochembase database along with details on their retention indices, sensory characteristics, and detection thresholds. The volatile compounds of litchi honey detected by two flame ionization detectors (FID) in two separate columns, MXT-5 and MXT-17, listed in Table 1 and Table 2. Of the total compounds, 43 volatile compounds identified in the first column MXT-5-FID-1 and the remaining 28 volatile compounds identified in the second column MXT-FID-17-FID-2 respectively.

Monoterpenoids such as Linalool, citronellol Geraniol, p-cymene are present in the litchi honey, matched with the result of comparative analysis of litchi volatiles done by *Wu et al.* 2009 [12] and they quoted that terpenoids contributed significantly to the distinct flavour of fresh litchi fruits. Feng *et al.* 2017 [3] stated that the geraniol, nerol were the main components of the rose oil which contributes to the rose-like aroma to the lychee fruit. Linalool added the sweet, citric taste to the honey. Volatile compounds such as Butane 2, 3- diol, Nerol, Linalool, Beta damascenone, 2-phenylethanol, acetic acid, p-cymene, Linalool obtained through the e-nose also found in the Gas chromatography- Olfactometer analysis of unifloral litchi honeys done by Mahattanatawee *et al*. 2004 [5]. Alcohols such as 3-pentanol, 3-methyl-1-butanol, 1-hexanol, and 2-methyl-1-butanol presented in the volatiles of litchi honey. Aldehyde such as neroloxide, also presented in the litchi honey. Ketones such as the Propan-2-one and butane-2, 3-diol also presented in the litchi honey. Methane thiol and Dimethyl trisulphide are the organosulfur compounds found in the honey. Carboxylic acids such as formic acid, Acetic acid, Pentanoic acid also presented in the litchi honey. Other compounds such as Methyl propanaoate, methyl heptanoate, fufural, 2, 3-dimethyl pyrazine also presented in the collected honey.

The most commonly test melissopalynology is used for pollen analysis to certify the origin and type of honey. More than time-consuming Palynological conventional technique, volatile data of any food product will be specific and can be used as the fingerprint of the honey as it is directly correlated with its own aroma. GC-MS is usually employed for volatiles identification, but its tedious sample preparation before injecting, is the limitation of this method (soria *et al*. 2004) [9]. Electronic nose enable quick results, non-destructive sampling of odorants or analytes with enhanced sensitivity and specificity.



Chromatogram of Volatile components for Litchi honey obtained through the e-nose in the column MXT-5-FID-1



Chromatogram of Volatile components for Litchi honey obtained through the e-nose in the column MXT-17-FID-2

Table 1: Volatile components of Litchi honey obtained in the Column MXT-5-FID-1

S.no	Retention time	Retention index	Name	Sensory descriptors
1	17.12	447	Methanethiol	Cabbage, Cheese, Garlic
2	18.45	473	Propan-2-one	Apple, Characteristics, Fruity
3	22.70	560	Formic acid	Acidic, Pungent, Vinegar
4	24.32	592	Butane-2-one	Acetone, Butter, Cheese
5	26.28	617	1-propanethiol	Alliaceous, Cabbage, Sweet
6	27.42	629	Acetic acid	Acetic, Acidic, Pungent, Sharp, Sour, vinegar
7	31.02	667	Tricholoroethane	Etheral, Mild, Sweet
8	35.70	714	pyrazine	Bitter, Corn, Hazelnut
10	35.96	716	3-pentanol	Fruity, Green, Nutty
11	37.70	732	3-methyl-1-butanol	Alcoholic, Balsamic, Bitter, Burnt
12	38.90	742	2-methyl 1-butanol	Alcoholic, Balsamic, Banana, butter
13	42.74	776	1-octene	Gasoline
14	45.64	802	2,3 butane diol	Creamy, Fruity, Odourless, Onion
15	47.22	818	furfural	Almond, Baked, Benzaldehyde
16	49.50	842	1-hexanol	Alcoholic, Characteristics, Dry, Fatty
17	51.44	862	Z-2-Hexen-1-ol	Caramelized, Fruity, Green
18	52.48	873	3-heptanol	Green, Herbaceous
19	53.20	880	Cyclohexanone	Acetone, Minty, Peppermint
20	55.74	908	Pentanoic acid	Acidic, Sour, Sweaty, Sour
21	56.78	921	2,3-dimethylpyrazine	Baked, Bread, Burnt sugar
22	57.18	926	Methyl hexanoate	Acetone, Fresh, Fruity, Pineapple
23	60.38	968	3-methyl-3-sulfanylbutanol-1-ol	Broth, Chervil, Meat
24	61.24	979	Dimethyl trisulphide	Alliaceous, Cabbage, Cauliflower, Sulfrous
25	63.00	1002	Pis-cumene	Aromatic, Herbaceous
26	63.46	1009	Propyl pentanoate	Etheral, Fruity
27	64.44	1024	p-cymene	Aromatic, Balsamic,citrus,Fresh,Fruity
28	66.82	1061	4-hydroxy-5-methyl-3(2H)-furanone	Balsamic, Candy, Caramelized, Sweet
29	68.71	1091	2-phenylethanol	Floral, Flower, Honey
30	69.65	1107	Linalool	Anise, Bergamot, Citrus
31	73.24	1173	Neroloxide	Celery, Floral, Green
32	74.56	1197	(E,E)-2,4-Nonadienal	Cereal, Cucumber, Deep fried
33	76.50	1237	4-Octanolide	Caramelized, Coconut, Creamy
34	77.20	1251	Citronellal	Floral, Fresh, Rose
35	77.88	1265	Geraniol	Citrus, Floral, Fruity
36	78.74	1283	Geranial	Citrus, Floral, Fruity
37	79.82	1305	Cinnamaldehyde	Cinnamon, Clove, Pungent
38	81.82	1351	Gamma-Nona lactone	Coconut, Creamy, Fruity
39	82.90	1375	Beta-damascenone	Apple, Rose, Fruity
40	84.50	1413	Methyl Eugenol	Carnation, Cinnamon, Clove
41	85.24	1431	Bis(2-furylmethyl)sulfide	Coffee, Earthy,
42	89.92	1548	Methyl dodecanoate	Coconut, Creamy, Fatty
43	90.82	1571	Hexadecane	Alkane, Fruity,
44	98.02	1755	Alpha-hexyl cinnamaladehyde	Floral, Fresh, Green,
45	102.00	1857	1-hexanedecanol	Floral, Sweet

Table 2: Volatile Components of Litchi honey obtained on the column MXT-1701-FID-2

S.no	Retention time	Retention index	Name	Sensory Descriptors
1	17.63	505	Methanethiol	Cabbage, cooked potato
2	21.04	583	Propan-2-one	Apple, Characteristics, Fruity
3	27.75	679	Trichoroethane	Chloroform, Etheral, Mild, Sweet
4	28.59	690	Methyl propanaoate	Apple, Etheral, Fresh, Fruity
5	34.39	746	Acetic acid	Acetic, Odourless, Pungent
6	38.69	785	n-butanol	Alcoholic, Amyl alcohol, Banana
7	41.91	815	3-pentanol	Fruity, Green, Nutty
8	43.65	833	3-methyl-1-butanol	Alcoholic, Balsamic, Bitter, Burnt
10	46.27	858	2-methyl-1-butanol	Alcoholic, Balsamic, Banana, butter
11	58.21	993	1-hexanol	Alcoholic, Characteristics, Dry, Fatty
12	58.97	1003	2,3-Butanediol	Creamy, Fruity, Odourless, Onion
13	61.75	1044	Dimethyl trisulfide	Alliaceous, Cabbage, Cauliflower, Sulfrous
14	62.93	1061	Propyl pentanoate	Etheral, Fruity
15	64.09	1078	1,8-cineole	Camphor, Herbaceous, Licorice
16	65.23	1095	Methyl heptanoate	Berry, Floral, Fruity
17	66.67	1119	3-methyl-3-sulfanylbutanol-1-ol	Broth, Chervil, Meat

18	70.54	1187	Nonan-2-one	Baked, Cheese, Earthy
19	71.59	1207	Linalool	Anise, Bergamot, Citrus
20	72.99	1235	citronellol	Floral, Fresh, Rose
21	75.87	1292	4-hydroxy-5-methyl-3(2H)-furanone	Balsamic, Candy, Caramelized, Sweet
22	76.65	1309	2-phenylethanol	Floral, Flower, Honey
23	78.15	1342	Nerol	Citrus,Floral,Rose
24	79.43	1370	Geraniol	Citrus, Floral, Fruity
25	81.73		Geranial	Citrus, Floral, Fruity
26	84.63	1493	p-cymene	Aromatic, Balsamic,Citrus
27	87.61	1570	Beta-damascenone	Apple, Rose, Fruity
28	88.93	1604	Bis(2-furylmethyl)sulfide	Coffee, Earthy,
29	89.63	1622	Methyl Eugenol	Carnation, Cinnamaon
30	93.71	1726	E-Cinnammic acid	Floral, Honey,Sweet
31	103.51	1978	1-hexadecanol	Faint, Floral, Sweet

Conclusion

The obtained chromatogram for the litchi honey in the electronic nose composed of the compounds such as aldehydes, Alcohols, ketones, fatty acid esters, carboxylic acid ester, pyrazine, furanones and Monoterpenoids. Butane 2, 3- diol, Nerol, geraniol Linalool, Beta damascenone, 2-phenylethanol, acetic acid, p-cymene, and neroloxide were commonly identified as the litchi flavour. Thus, the collected volatile profile clearly characterized the litchi flavour in the honey and acted as the fingerprint for the litchi honey. The findings of this study show that using e-nose fingerprints to specify the floral origin and quality components of honey can prevent deception. In addition, the electronic nose analysis of volatile compounds does not require the tedious sample preparation, this method is easy to employ for analyzing the quality of the honey.

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