Analysis of Different Quality Agarwood Oil (*Aquilaria Malaccensis*) and Sensory Study

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***Abstract*—Agarwood is increasing in demand in the global market due to the presence of resinous wood that is widely used as an ingredient in perfumes, aromatherapy and traditional medicines. The popularity comes with concern on its unique smell (woody) and quality adulteration. This paper presents the response between chemical compositions in agarwood oil, reflected on the reading of fabricated e-nose which is used for classification. Different quality of samples which were identified as pure and mixture agarwood oil were analyzed by using gas chromatography for chemical profiling. Reveals that common major compounds like 4-phenyl-2-butanone, β-agarofuran, 10-epi-γ-eudesmol and agarospirol were found in the pure sample. An electronic nose (E-nose) that consists of a sensor array is a device used to substitute human olfactory system. The sample was exposed to the sensor array for odor profile recognition. The collected dataset was normalized and analyzed using box plot for feature extraction. The result concludes that different chemical compound was able to produce a different pattern for electronic nose reading even though the odor profile is almost similar.**

***Index Terms*—Agarwood; Volatile Compounds; Odor-Profile Classification; E-Nose.**

I. INTRODUCTION

Agarwood (Gaharu) is commonly known as an aromatic product, obtained from an infected wood of Aquilaria species (Thymelaeaceae). This resinous wood of *Aquilaria sp* which is also recognized as gaharu, oud or eaglewood has been used for millennia as incense, an ingredient of perfumes and traditional medicines in many cultures [1]. Formation of dark aromatic resin that carries high complex mixture volatile organic compound gives the strong woody scent. The combination of these fragrant compounds give the special characteristic odor to the plant and often used by human in a form of agarwood essential oil and perfumery [2]. In perfumery industry, agarwood oil is highly valuable due to their limited availability as it is listed as endangered tree species that results in its skyrocket price. High demand of agarwood has led to the chaotic harvesting activity of the wild agarwood-producing trees in the forest; this situation has caused the agarwood market to suffer due to the shortage of resources leading to greater efforts to conserve and research into the formation of agarwood by natural and artificial means [3]. Consequently, some agarwood essential oils may be impure, adulterated or distilled with synthetic chemicals. Some agarwood chips were polluted with foreign chemicals to make it looks like high-quality agarwood chips. Due to the safety concern, it is important to ensure the purity and quality of agarwood chip and oil.

However, grading of agarwood oil and woodchip has become a big concern due to the fact that there is no standard available to be used as references for quality determination. Method to determine the quality of agarwood is not very accurate and inconsistent in this industry [4]–[6]. Currently, agarwood investors are depending on the skill of “the nose”, a person trained to identify the quality of agarwood oil and woodchip. However, there are a lot of restraints and limitations to standardize the results from the sniffers considering human nature.

One of the growing research subjects on relevant technologies is electronic noses (E-nose) that have the capability to detect odor molecule, comparable to human nose [7], [8]. As refer to Gardner and Barlett [9], the electronic nose is an instrument that contains an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odor. The artificial electronic model olfactory system has been constructed since 1982 ago [10]. It has been tested in many applications previously [11]–[13]. Generally, the concept of E-nose is similar to human nose function (refer figure). Sensor array that acts as a receptor will transform the featured extraction to the microprocessor for the software to classify the pattern and allow recognition.

The aim of this study is to evaluate the capability of the fabricate E-nose for agarwood oil classification. Therefore, reported in this paper the response of the E-nose sensor array towards volatile fragrant chemical compounds that present in the agarwood oil since the quality of oil was investigated by comparing their chemical composition [14].

II. MATERIAL AND METHOD

A. Samples Preparation

The data has been collected from six different samples of agarwood oil bases on different types. The samples are named as Pure 1, Pure 2, Mix 1, Mix 2, Mix 3 and Mix 4. Experts have classified Pure 1 and Pure 2 as a pure agarwood essential oil and the rest as a mixture agarwood. All of the samples are purchased from the trusted trader for quality confirmation.

B. Chemical Analysis

Chemical composition identification, 2µL agarwood oil was diluted in the dichloromethane (DCM) for gas chromatography analysis.

Table 1
Chemical Composition of The Agarwood Oil Sample by Gas Chromatography FID and GC-MS

Compounds	Id. KI	Ref. KI	Pure Agarwood		Mixture Agarwood				ID
			Pure 1	Pure 2	Mix 1	Mix 2	Mix 3	Mix 4	
1,1'-oxybis-2-propanol	1017	1019			14.53	7.33			*MS*
2-(2-hydroxypropoxy)-1-Propanol	1041	1043			24.3	11.78			*MS*
4-phenyl-2-butanone	1213	1210	3.64	1.02	0.21	1.35	2.48	0.33	*FID,MS*
β-maaliene	1411	1414	0.15				0.19		*FID,MS*
aromadendrene	1445	1443	0.18						*FID,MS*
β-guaiane	1464	1466		0.33					*FID,MS*
γ-Gurjunene	1469	1472	0.36				0.34		*FID*
β-agarofuran	1474	1474	0.36	0.19					*FID*
α-selinene	1484	1486	0.17		5.17		0.37		*FID,MS*
α-muurolene	1494	1496	1.04				0.59		*FID*
α-bulnesene	1503	1503	0.10	0.27			0.23		*FID,MS*
α-elemol	1529	1530	1.48	0.72	0.20		0.52		*FID*
norketoagarofuran	1559	1555	0.88		0.28	0.95			*FID*
diethyl phthalate	1561	1561			1.30	42.72	2.22	7.91	*MS*
epoxybulnesene	1572	1572	0.55	0.58			0.45		*FID*
tetradecanal	1593	1593	1.95		0.15				*FID,MS*
caryophellene oxide	1601	1600						0.31	*FID*
guaiol	1602	1603	1.57	2.77	4.75	0.39	4.41		*FID,MS*
1,5-epoxy-nor-ketoguaiene	1614	1614	5.05	0.88	0.20			0.62	*FID,MS*
10-epi-γ-eudesmol	1616	1619	2.06	4.80			1.12	0.58	*FID,MS*
agarospirol	1629	1631	9.03	4.49			2.62	0.47	*FID,MS*
epi-α-cadinol	1636	1640	0.81	0.30	1.34				*FID*
jinkoh eremol	1643	1643	0.41		1.99				*FID*
tridecanoic acid	1648	1647	2.46		16.03	0.39			*FID*
kusunol	1650	1650		2.74	3.46		1.07		*FID*
α-Eudesmol	1652	1652	0.47	1.28					*FID,MS*
bulnesol	1665	1664	0.16	1.62	0.53		0.29		*FID,MS*
dehydrojinkol-eremol	1673	1673	0.57	1.45	2.81				*FID,MS*
epi-α-bisabolol	1678	1678	1.78	0.60	0.28		0.39		*FID,MS*
α-bisabolol	1682	1683	2.33						*FID,MS*
selina-3,11-dien-9-one	1687	1687	1.42						*FID,MS*
pentadecanal	1692	1695	4.98	0.46			0.83		*FID,MS*
rotundone	1701	1703	0.35	1.06			0.65		*FID*
selina-3,11-dien-9-ol	1720	1721	3.36	0.83					*FID*
selina-4,11-dien-14-oic acid	1730	1728	0.18						*FID,MS*
selina-3,11-dien-14-al	1735	1735	4.62	0.86		0.70			*FID,MS*
9,11-eremophiladien-8-one	1740	1740	0.85	1.45	3.73	0.84	1.28		*FID,MS*
selina-3,11-dien-14-ol	1745	1750	2.92						*FID*
selina-4,11-dien-14-al	1758	1758	0.88	1.46	0.57	1.08	0.20	0.68	*FID,MS*
guaia-1(10),11-dien-15-ol	1768	1770	0.79	0.88			0.12		*FID,MS*
selina-3,11-dien-14-oic acid	1774	1775	0.28	1.56			0.65		*FID,MS*
2-hexadecanone	1780	1782	1.10						*FID,MS*
dihydrokaranone	1796	1799	0.13		0.34	0.45			*FID*
isopropyl myristate	1805	1805			0.62	1.04			*FID*
karanone	1817	1812	0.64						*FID*
oxo-agarospirol	1823	1822	0.62	0.21					*FID*
pentadecanoic acid	1839	1842		0.38			0.17		*FID,MS*
n-hexadecanoic acid	1956	1950	6.03	8.85		0.36	1.96	5.76	*FID,MS*
methyl dehydroabietate						2.54	1.45	9.21	*MS*
methyl abietate						1.21		3.89	*MS*
diisooctyl phthalate							34.19	19.94	*MS*
Sesquiterpene hydrocarbon (%)			2.36	0.79	5.17	ND	1.72	ND	
Oxygenated sesquiterpene (%)			54.68	31.00	37.00	4.89	14.60	2.66	
Others (%)			9.67	10.25	40.34	67.29	42.47	47.04	
Total area (%)			66.71	42.04	82.51	72.18	58.79	49.70	

ND-Non Detected; Id KI - Identified Kovat Index; Ref. KI - Reference Kovat Index (Tajuddin and Yusoff, 2010)

1) Gas Chromatography-Flame Ionization Detector (GC-FID)

Gas chromatography analysis was carried out by Agilent 7890 gas chromatography equipped with flame ionization detector (GC-FID). The carrier gas was helium with a linear velocity of 30 cm/s and using DB-1 column (30 m x 0.25 mm i.e., film thickness 0.25µm). By using autosampler, the injection was undergoing the conditioned volume 1.0 µL in the split mode (ratio 1:5). The oven temperature was programmed from 80°C to 230°C at 3°C/min. Analysis of the compound will analysis as refer to kovat index in the previous study [15].

2) Gas Chromatography-Mass Spectroscopy (GC-MS)

The gas chromatography-mass spectroscopy (GC-MS) was performed using an Agilent 7890A Network System coupled to a mass spectrometer (Agilent 5975C) with the detector in full scan mode under electron impact ionization (EI, 70eV) and fitted with a capillary column (DB-1 30m × 0.25mm, i.e.; 0.25µm film thickness). Oven temperature was programmed at 80°C to 230°C at the rate 3°C/min. The chemical compounds were identified by matching to the mass spectral library NIST.

C. E-nose Data Measurement

For E-nose data measurement, odor profile method has been applied. The E-nose was fabricated by using four sensor arrays that sensitive to volatile compounds attached to the chamber as refer to the Figure 1. The samples were exposed in the chamber while cooling fan roles as air circulating. Total 800 data were collected (200 column x 4 row) within 2 minutes and 5 minutes interval measurement without samples and repeat for five times resulting total off dataset with 8000 data [13], [16]. Four columns represent the reading of each sensor odor profile measurement while the row is the set of numerical data. The data were pre-processed using normalization technique. Then, the odor profile data were extracted by plotting the graphical representation to visualize.

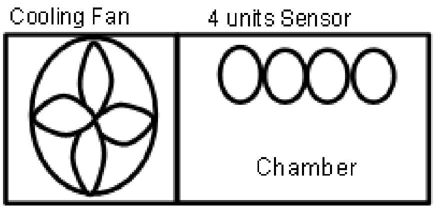

Figure 1: Illustration of fabricated E-nose

III. RESULT AND DISCUSSION

Table 1 lists the identified constituents, representing the chemical composition, percentage of compounds and retention indices of the samples. There were considerable qualitative variation and quantitative differences between the samples. In this analysis, forty compounds accounted for 66.71% of the oil were identified as Pure 1 while twenty-seven compounds, representing 42.04% of the oil were identified as Pure 2. For samples Mix 1, Mix 2, Mix 3 and Mix 4, the number of compounds identified was twenty-one, fifteen, twenty-five and eleven respectively and it represented 82.51%, 72.18%, 58.79% and 49.70% of the total oil respectively.

The main composition of agarwood oil has been revealed to be oxygenated sesquiterpene through this analysis, identified as a major constituent in luxury perfume [4], [14], [17], [18]. Only 4-phenyl-2-butanone and selina-4,11-dien-14-al were found present in all selected samples. The previous study reported 4-phenyl-2-butanone as one of the major compounds in agarwood oil extracted in the lab by hydrodistillation method [15]. Other constituents that were found present in both pure samples are β-agarofuron, α-Eudesmol and oxo-agarospirol, while 1,1'-oxybis-2-propanol and 2-(2-hydroxypropoxy)-1-Propanol was only identified in sample Mix 1 and Mix 2. These compounds are not naturally present in pure agarwood but it is recognized as a common fragrant compound in commercial perfume and cosmetic application due to the low skin irritation potential. Based on this study, all the samples are having the different chemical. However, the untrained human nose is difficult to differentiate may due to present of woody compounds like β-agarofuran, α-bulnesene, agarospirol, norketoagarofuran, epoxybulnesene and jinkoh-eremol [19].

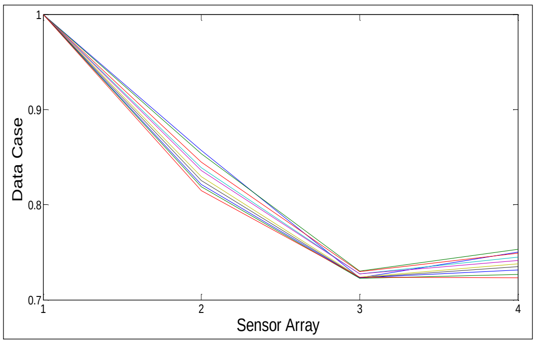

Figure 2: Graph Normalized Data for Pure 1

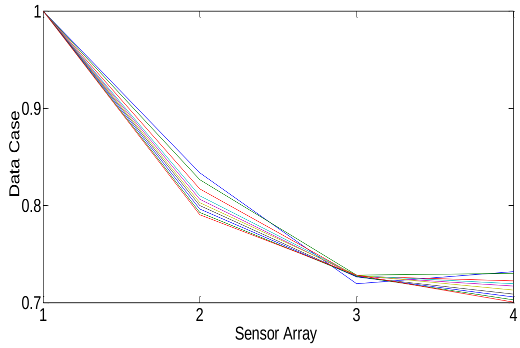

Figure 3: Graph Normalized Data for Pure 2

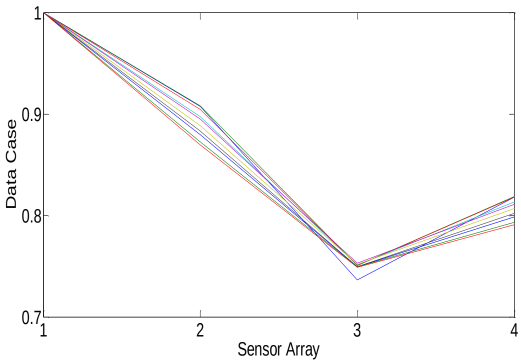

Figure 4: Graph Normalized Data for Mix 1

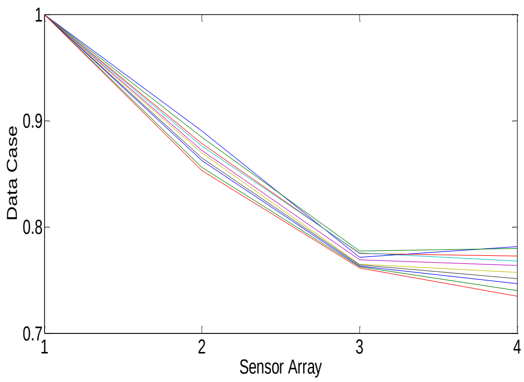

Figure 5: Graph Normalized Data for Mix 2

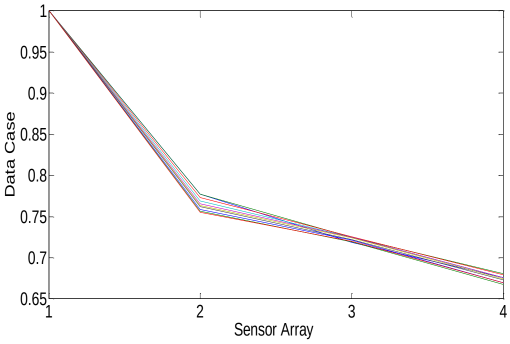

Figure 6: Graph Normalized Data for Mix 3

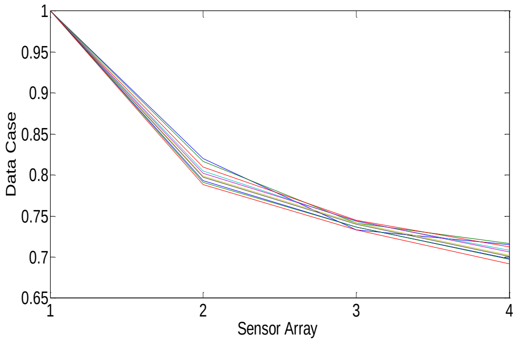

Figure 7: Graph Normalized Data for Mix 4

Classification based on pattern recognition graphical visualization technique have been reported previously [16], [20], [21]. The same technique was applied in this study by which the classification is based on the significant difference in feature extraction.

For odor pattern profile analysis of the agarwood samples, all measured data were pre-processed by using normalization technique to get the standard value (0-1). Graphical representation of the pattern profile for samples (Pure 1), (Pure 2), (Mix 1), (Mix 2), (Mix 3) and (Mix 4) were shown in Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7 respectively.

The data was divided into ten cases whereby each case represented 20 measurements of data set. These visualization patterns showed slight variances in odor profile of agarwood samples on Sensor 2 (S2), Sensor 3 (S3) and Sensor 4 (S4). The maximum peak of normalization graph recorded on Sensor 1 (S1) was similar to that of other samples. Therefore, the result indicated that S2, S3 and S4 were less sensitive as compared to S1 but the formers were more significant for pattern recognition and classification of agarwood oil.

To improve the features extraction for visualization comparison, the data were further subjected to analysis by using box plot. Box plots are used to evaluate the individual sensor and data distributions performance as well as identify the outlier [22]. The box plot results for this experiment are shown in Figure 8 - Figure 13. The pattern was generally similar with slight difference observed in Sensor 3 and Sensor 4. However, all data distribution is close to inter-quartile range (IQR) value. Out of 8000 data collected, fewer outliers were recorded and therefore made this data reliable for statistical analysis.

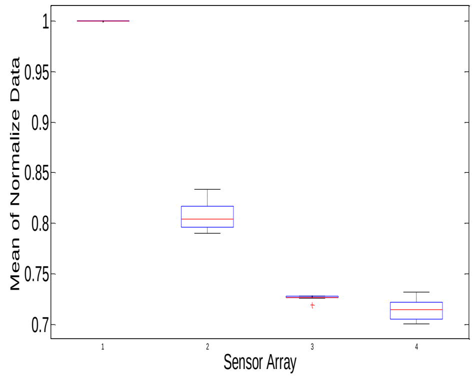

Figure 8: Box Plot for Pure 1

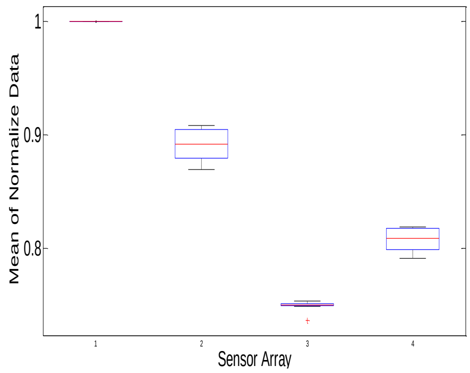

Figure 9: Box Plot for Pure 2

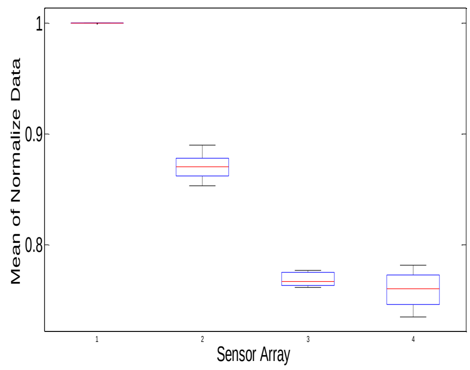

Figure 10: Box Plot for Mix 1

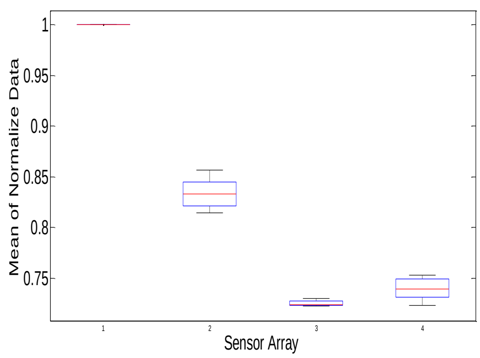

Figure 11: Box Plot for Mix 2

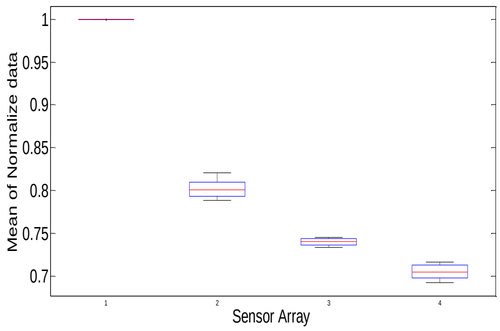

Figure 12: Box Plot for Mix 3

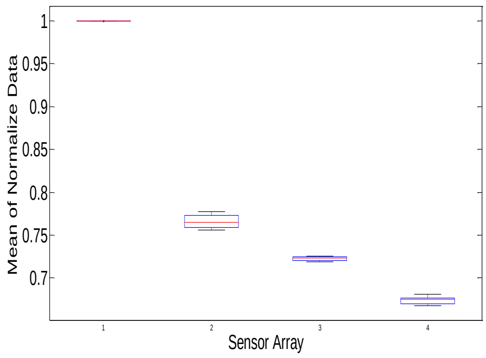

Figure 13: Box Plot for Mix 4

IV. CONCLUSION

This paper has successfully demonstrated that the fabricated E-nose attached to sensor arrays that react to different volatile compounds are capable to differentiate between pure and mixture agarwood oil. The resulting different feature extractions based on normalization and boxplot graph produced by E-nose measurement are a reflection of the different chemical composition. It is suggested that improvements be made to this research by adding further statistical analysis for intelligent classification.

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