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IDENTIFICATION OF ODOR COMPONENTS OF AGARWOOD

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Nor Azah Mohamad Ali[a*], Nurlaila Ismail[b], Mailina Jamil[a], Azrina Aziz[a], Sahrim Lias[a], Mohd Hezri Fazalul Rahiman[b], Mohd Nasir Taib[b]

[a]Herbal Product Development Programme, Natural Products Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia
[b]Faculty of Electrical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

*Corresponding author
norazah@frim.gov.my

Graphical abstract

Abstract

This article presents the use of Z-score in assessing the significant chemical compounds extracted by head space solid phase microextraction (HS-SPME) and gas chromatography – mass spectrometry (GC-MS) analysis of an agarwood oil obtained from Melaka, Malaysia. Two types of SPME fiber; polydimethylsiloxane (PDMS) and divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) were used. During the extraction analysis, the results showed that at least 27 and 29 compounds were identified using PDMS and DVB-CAR-PDMS fiber, respectively. DVB-CAR-PDMS fiber was found to be more efficient in terms of selectivity of compounds extraction. The application of Z-score showed that eight and eleven marker compounds were determined in PDMS and DVB-CAR-PDMS fibers, respectively. 4-Phenyl-2-butanone, α-guaiene, β-agarofuran, α-bulnesene, α-agarofuran and 10-epi-γ-eudesmol were some of the compounds selected and were often reported significantly in agarwood oils as key odor compounds. The information gathered will be used for compound selection towards grading of agarwood oils by sensor technology.

Keywords: Agarwood, chemical constituents, HS-SPME-GCMS, Z-score

Abstrak

Artikel ini membentangkan kegunaan skor-Z dalam menilai sebatian kimia utama yang diekstrak dengan menggunakanan alisis pengekstrakan mikro fasa pepejal (HS-SPME) dan gas kromatografi – spektrometri jisim (GC-MS) bagi minyak gaharu yang diperoleh dari Melaka, Malaysia. Dua jenis fiber SPME, *polydimethylsiloxane* (PDMS) dan *divinylbenzene-carboxen-polydimethylsiloxane* (DVB/CAR/PDMS) telah digunakan. Semasa analisis pengekstrakan, hasil analisis menujukkan sekurang-kurangnya 27 dan 29 sebatian telah dikenalpasti dengan menggunakan masing-masing fiber PDMS dan DVB/CAR/PDMS. Fiber DVB/CAR/PDMS dikenalpasti member hasil analisis yang lebih berkesan dalam pemilihan sebatian yang diekstrak. Aplikasi skor-Z menunjukkan lapan dan sebelas sebatian penanda yang telah dikenalpasti dalam setiap fiber PDMS dan DVB/CAR/PDMS. 4-Fenil-2-butanon, α-guaiena, β-agarofuran, α-bulnesena, α-agarofuran dan 10-epi-γ-eudesmol merupakan beberapa sebatian terpilih dan dilaporkan terdapat secara signifikan di dalam minyak gaharu sebagai kunci sebatian yang berbau. Maklumat yang dikumpulkan akan digunakan bagi pemilihan sebatian kearah pengredan minyak gaharu melalui teknologi sensor.

Kata kunci: Gaharu, sebatian kimia, HS-SPME-GCMS, skor-Z

1.0 INTRODUCTION

Agarwood (gaharu) is a fragrant and expensive resinous wood that is usually derived from the diseased timber of the genus *Aquilaria*.The wood is found to have many names such as jinkoh, kanankoh, oudh, eaglewood, gaharu and aloeswood [1-3]. The resinous heartwood has wide applications such as in traditional medicines preparation, religious ceremony as incense and especially in perfumery products preparation. The market demand for agarwood oil is popular due to its elegant, unique and long lasting odor. The long lasting aroma and very dark color is graded as high grade and is sold expensively at USD126 to USD631 per tola (12 mL) in year 2008 [4]. Agarwood is generally made up of a complex mixture of sesquiterpene, sesquiterpene alcohols and chromone derivatives. Some of the compounds which had been identified from agarwood included 4-phenyl-2-butanone, α-agarofuran, β-agarofuran, 10-epi-γ-eudesmol, agarospirol, jinkohol, jinkohol II and valerianol. These compounds had been reported to be responsible contributors to the agarwood unique scent [5-7].

Headspace-solid phase microextraction (HS-SPME) is relatively a new sampling method for the extraction of essential oils from leafy and flowery materials and when combined with GCMS, the chemical profiles of the oils could be easily determined. In previous studies, this technique had been developed for the analysis of essential oils [8-9]. In this study, the solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GCMS) analysis are chosen because this technique are one of the preferred methods in odor compounds analysis [8]. In Malaysia, the grading system of agarwood essential oil using chemical profiles of the oil is still developing. Therefore as a part of our research towards a grading system of agarwood oil in the country, this study is carried out to analyse the marker odor compounds contributing to the agarwood essential oil characteristics using chemometric analytical methods, Z-score.

2.0 EXPERIMENTAL

2.1 Plant Materials

Agarwood samples (MA2) were purchased from a supplier in Melaka. The samples were ground and soaked with water for several days prior to hydrodistillation in a clavenger type apparatus for three consecutive days. The oils (MAO2) obtained were dried using anhydrous sodium sulphate and stored in amber vial for the analysis purposes.

2.2 Headspace-Solid Phase Microextraction-Gas Chromatography Mass Spectrometry (HS-SPME-GCMS)

HS-SPME-GCMS analyses were performed by using GCMS from Agilent Technologies (7890A/5975C MSD). Two types of SPME Stableflex^{TM} fibers were purchased from Supelco (Bellefonte, PA, USA) which are 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) and 100 μm polydimethylsiloxane (PDMS) were used to extract the odor volatiles of agarwood oil (MAO2). The MAO2 samples were placed into three repetitions of 20 mL tightly capped and screw-top clear vials. The fiber type PDMS was placed in an agitator for 10 min at incubation temperature of 40 ºC. In order to trap the volatile compounds, the extraction time was set to 10 min. After that, the PDMS fiber was injected immediately into the GC port for 10 min. Similar procedure was followed for the fiber type DVB/CAR/PDMS. Three repetitions of PDMS fiber and three repetitions of DVB/CAR/PDMS fiber were recorded and their average was calculated and analysed.

The GCMS analysis was done using *Agilent Technologies 7890A/5975C* Series MSD with HP-5MS column (30 m x 0.2 mm ID x 0.25 μm film thickness). Initially, the apparatus was set at 60 ˚C for 10 min time duration, followed by 180 ˚C for 1 min at 3 ˚C/min. The gas carrier helium was introduced at a flow rate of 1 mL/min and the ion-source temperature was programmed at 280 ˚C. The chemical compounds were identified by matching them to the mass spectral library (HPCH2205.L; NIST05a.L). The chemical compositions were presented in terms of percentage of relative peak area (%).

3.0 RESULTS AND DISCUSSION

3.1 Chemical Compositions

The summary of chemical compositions of MA2 agarwood oil (MAO2) using two SPME fibers; PDMS and DVB-CAR-PDMS are tabulated in Table 1. In average, at least 27 and 29 aroma compounds were extracted by PDMS and DVB-CAR-PDMS, respectively. The major compounds identified by PDMS fiber are 4-phenyl-2-butanone (relative peak area average of 14.18%), α-copaene (relative peak area average of 10.2%) and α-bulnesene (relative peak area average of 10.76%). The 4-phenyl-2-butanone (relative peak area average of 14.17%) was found to be dominant as recognized by DVB-CAR-PDMS fiber. It is noticeable that 4-phenyl-2-butanone is a major compound detected by both fibers: PDMS and DVB-CAR-PDMS.

Table 1 The chemical compositions of SPME using PDMS and DVB-CAR-PDMS fibers

No	Chemical compounds	RI	Relative peak area, %							
			PDMS				DVB-CAR-PDMS			
			1	2	3	Average for PDMS	1	2	3	Average for DVB-CAR-PDMS
1	Benzaldehyde	952	0.2	0.01	-	0.07	0.2	0.01	-	0.07
2	4-Phenyl-2-butanone	1241	16.62	11.90	14.02	14.18	15.60	12.93	13.99	14.17
3	α-Copaene	1374	3.92	23.49	3.18	10.20	3.44	2.88	3.03	3.12
4	β-Elemene	1389	2.95	2.22	2.71	2.63	2.66	2.37	2.38	2.47
5	α-Funebrene	1402	-	0.80	1.01	0.91	0.85	0.84	0.79	0.83
6	α-Gurjunene	1403	1.28	1.30	1.36	1.31	1.26	1.17	1.13	1.19
7	α-Cedrene	1411	2.96	2.57	0.22	1.92	3.16	0.28	2.82	2.09
8	α-Guaiene	1433	5.16	4.09	8.68	5.98	4.21	3.73	3.98	3.97
9	Aromadendrane	1443	1.37	0.19	1.67	1.08	1.35	-	1.28	0.88
10	α-Humulene	1452	-	2.47	-	0.82	-	3.73	0.00	1.24
11	γ-Gurjunene	1472	-	0.58	0.39	0.32	0.38	0.65	0.51	0.51
12	β-Agarofuran	1474	8.64	7.20	-	5.28	7.70	-	6.90	4.87
13	γ-Muurolene	1478	0.17	1.82	3.19	1.73	1.96	1.71	1.82	1.83
14	*Cis*-β-guaiene	1492	0.60	1.58	0.71	0.96	0.64	1.00	0.97	0.87
15	α-Muurolene	1496	-	-	-	0.00	2.38	2.08	2.22	2.23
16	α-Selinene	1498	-	2.64	3.06	1.90	-	-	-	0.00
17	β-Dihydroagarofuran	1502	-	2.90	3.62	2.17	3.12	2.98	3.03	3.04
18	α-Bulnesene	1509	11.17	9.92	11.18	10.76	6.23	5.57	5.86	5.89
19	γ-Cadinene	1513	2.71	-	2.92	1.88	2.52	2.42	2.46	2.47
20	δ-Cadinene	1522	-	-	-	0.00	2.13	2.09	2.10	2.11
21	α-Agarofuran	1547	2.70	3.44	2.73	2.96	2.81	2.96	2.97	2.91
22	Elemol	1550	-	1.09	0.14	0.41	0.41	1.22	1.12	0.92
23	β-Vetivenene	1554	-	-	1.25	0.42	1.19	-	1.30	0.83
24	Viridiflorol	1592	3.33	0.00	0.93	1.42	1.85	-	-	0.62
25	β-Gurjunene	1600	4.02	3.95	5.09	4.35	-	0.33	-	0.11
26	10-Epi-γ-eudesmol	1621	1.84	5.64	1.89	3.12	2.85	3.83	3.79	3.49
27	γ-eudesmol	1635	0.67	2.94	0.59	1.40	1.30	2.73	2.76	2.26
28	Alloaromadendrene epoxide	1639	-	0.30	0.20	0.17	-	0.15	0.11	0.09
29	β-Eudesmol	1649	-	-	-	0.00	-	3.96	4.17	2.71
30	α-Eudesmol	1652	-	4.43	-	1.48	0.2	0.78	0.5	0.49

This compound stands as a marker compound to the aroma of MAO2 agarwood essential oil. The result is in agreement with Pripdeevech *et al.* 2011 where 4-phenyl-2-butanone was recognized as one of the major compounds contributing to the odor of *Aquilaria crassna* and *A. subintegra* oils [6].

3.2 The Data Processing

The data processing such as Principle Component Analysis (PCA) and Z-score are among the techniques used by researchers to separate the data belongs to the different group according to the mean value of the respective group [10-11].

As one of the scaling methods, the Z-score was applied to index the Intelligent Quotient (IQ) level into several groups using correlation between IQ scores and Electroencephalogram (EEG) power spectrum density (PSD). This technique has been found to be successful in classifying, clustering and standardizing the data and the information. The formula for Z-score technique as in Eqn. (1) is given below:

$$Z = (x\_i - \bar{X})/SD \ldots.. (1)$$

where:

x_i is the individual value of *i*,
$\bar{X}$ is the mean of population
SD standard deviation of population

The significant compounds identified by Z-score technique are tabulated in Table 2 and Table 3 for PDMS and DVB-CAR-PDMS fiber, respectively. There are eight and eleven compounds found by Z-score from the total compounds extracted by PDMS and DVB-CAR-PDMS fibers, respectively. In average, 4-phenyl-2-butanone (14.18%), α-copaene (10.20%), α-guaiene (5.98%), β-agarofuran (5.28%), α-bulnesene (10.76%), α-agarofuran (2.96%), β-gurjunene (4.35%) and 10-epi-γ-eudesmol (3.12%) are recognized from the total compounds extracted by PDMS fiber. 4-Phenyl-2-butanone (14.17%), α-copaene (3.12%), β-elemene (2.47%), α-guaiene (3.97%), β-agarofuran (4.87%), β-dihydroagarofuran (3.04%), α-bulnesene (5.89%), γ-cadinene (2.47%), α-agarofuran (2.91%), 10-epi-γ-eudesmol (3.49%) and β-eudesmol (2.71%) are identified by DVB-CAR-PDMS fiber. These compounds especially 4-phenyl-2-butanone, α-copaene, α-guaiene, β-agarofuran, α-bulnesene, α-agarofuran and 10-epi-γ-eudesmol represent the major compounds contributed to the elegant aroma of high quality agarwood oil in Malaysia since they are common compounds identified in both fibers.

4.0 CONCLUSION

The use of Z-score in this study has shown its effectiveness in assessing the odor compounds of agarwood essential oil from Malaysia. The extraction methods using SPME and GCMS have proven their capability in analyzing the compounds from the oil. Several odor compounds i.e. 4-phenyl-2-butanone, α-copaene, α-guaiene, β-agarofuran, α-bulnesene, α-agarofuran and 10-epi-γ-eudesmol have being identified significant by Z-score, thus beneficial as marker compounds in grading the quality of agarwood oil in future.

Table 2 The significant compounds based on PDMS fiber data using Z-score

No	Chemical compounds	Relative peak area, %			Mean	Z-score value
		1	2	3		
1	4-Phenyl-2-butanone	16.62	11.90	14.02	14.18	3.31
2	α-Copaene	3.92	23.49	3.18	10.20	2.16
3	α-Guaiene	5.16	4.09	8.68	5.98	0.93
4	β-Agarofuran	8.64	7.20	0.00	5.28	0.73
5	α-Bulnesene	11.17	9.92	11.18	10.76	2.32
6	α-Agarofuran	2.70	3.44	2.73	2.96	0.06
7	β-Gurjunene	4.02	3.95	5.09	4.35	0.46
8	10-Epi-γ-eudesmol	1.84	5.64	1.89	3.12	0.11

Table 3 The significant compounds based on DVB-CAR-PDMS fiber data using Z-score

No	Chemical compounds	Relative peak area, %			Mean	Z-score value
		1	2	3		
1	4-Phenyl-2-butanone	15.60	12.93	13.99	14.17	4.41
2	α-Copaene	3.44	2.88	3.03	3.12	0.28
3	β-Elemene	2.66	2.37	2.38	2.47	0.04
4	α-Guaiene	4.21	3.73	3.98	3.97	0.60
5	β-Agarofuran	7.70	0.00	6.90	4.87	0.94
6	β-Dihydroagarofuran	3.12	2.98	3.03	3.04	0.26
7	α-Bulnesene	6.23	5.57	5.86	5.89	1.32
8	γ-Cadinene	2.52	2.42	2.46	2.47	0.04
9	α-Agarofuran	2.81	2.96	2.97	2.91	0.21
10	10-Epi-γ-eudesmol	2.85	3.83	3.79	3.49	0.42
11	β-Eudesmol	0.00	3.96	4.17	2.71	0.13

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