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Chemical Composition of Agarwood Essential Oil (*Aquilaria malaccensis*) Upon Exposure Towards Heat Condition

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Agarwood (Gaharu) is highly valued due to extensive usage in industries including perfumery, pharmaceutical and traditional medicine. The strong and unique woody aroma of agarwood oil is dependent on the abundance of sesquiterpenoid or oxygenated sesquiterpene. Post-treatment of agarwood oil such as exposure towards heat, UV light and oxygen under certain period of time are practiced by the industry to induce the formation of sesquiterpenoid content through the oxidation process, thus enhancing the woody aroma of the oil. In this study, agarwood oil (*Aquilaria malaccensis*) from Pahang, Malaysia was subjected to exposure of heat at 40 ^{o}C for periods of 3, 7, 14, 20 and 30 days. Gas chromatography analysis identified the major compounds in the oils as 9-hydroxyselina-4,11-dien-14-oic acid (6.94-8.06 %), *epi*-α-cadinol (6.38-7.88 %), selina-3,11-dien-9-al (5.20-6.72 %), β-eudesmol (3.95-5.22 %), 10-*epi*-γ-eudesmol (3.95-3.87 %), selina-4,11-dien-14-oic acid (4.21-4.85 %), and α-eudesmol (3.25-3.79 %), kusunol (1.77-3.02 %) and kessane (2.42-2.90 %). The classification of compounds of treated oil (Day 3 to Day 30) showed that the sesquiterpenoid group dominated the aromatic compounds of agarwood oil in the range of 62.82 to 66.25 % with the highest content on Day 14. However, further investigation showed a decrease in total sesquiterpenoid content from 70.85 % (Day 0) to 66.25 % (Day 14) suggesting that the heating process did not increase the quantity of sesquiterpenoids as intended. Thus, further study with other parameters such as oxygen and UV light is recommended to be done together with headspace analysis of the samples.

Key words: Agarwood; *Aquilaria malaccensis;* post-treatment; heat; sesquiterpenoid

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Agarwood refers to the resinous heartwood tree of Thymelaeaceae, mainly from the *Aquilaria* species [1]. Other genera including *Gyrinops* and *Gonystylus* were also reported to produce agarwood, however, they are not commercialized due to their low market value [2]. Agarwood oil is a concentrate of volatile aromatic compounds produced from the distillation of low quality wood sawdust. Malaysian agarwood, known as gaharu, is mostly obtained from the native species *Aquilaria malaccensis* tree. Other species such as *A. crassna*, *A. sinensis* and *A. subintegra* that are cultivated across the country are originated from Indochina [3].

The grading of agarwood oil in the United Arab Emirates (UAE) is based on the country of origin and is ranked in the order of India, Cambodia, Malaysia and other countries [4]. The aroma is evaluated by the distinctive and spicier notes combined with duration of the fragrance that lingers on the skin or clothes. The colour of the oil is related to the age of the tree during harvest, while viscosity of the oil is dependent on the storage period. As the trade is conducted based on trust between a trader and a buyer, a case of fraud can damage the reputation of the agarwood oil industry. Other traditional method utilizes an experienced appraiser (nose or sniffer) with years of knowledge on agarwood oil. However, this method is prone to human error and inconsistence as it highly depends on one's capability [5].

With the advances in analytical chemistry, the composition of agarwood oils and extracts can be assessed by gas chromatography coupled a with flame ionization detector (GC-FID) or a mass spectrometry detector (GC-MS). The earliest study on this subject was conducted in 1959 where agarol and several sesquiterpenes were found in the petroleum ether extract of agarwood from India [6]. Research in this area was expanded and more chemical compounds were identified from agarwood, mainly in the dominant forms of sesquiterpenes and sesquiterpenoids (oxygenated sesquiterpene), followed by other groups such as carboxylic acids, aldehydes, ketones, and chromones [1, 7, 8]. Further investigations identified these two terpene groups as

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the main contributors to the unique and strong woody aroma of agarwood oil [1, 5, 9].

Most previous studies to enhance the quality of agarwood oil are focused on pre-treatment processes such as normal soaking of agarwood dust, enzymatic soaking and microwave irradiation [1, 3, 14]. In the industry, post-treatment process was long-practiced by some agarwood producer by exposing the oil to certain parameters such as heat, UV light and oxygen to induce the formation of sesquiterpenoid content through the oxidation process, thus enhancing the woody aroma of the oil. To date, no systematic tudies have been conducted on these practices. Thus, this study is aimed at investigating the effect of heat on the chemical composition of agarwood oil.

MATERIALS AND METHODS

Sample Collection and Preparation

Agarwood oil (*Aquilaria malaccensis*) was procured from a trader in Rompin, Pahang, Malaysia. Five (5) clear 4 mL vials (sealed with aluminium foil) were filled with 0.1 g of this agarwood oil and the vials closed with the cap. The oil was heated at a constant temperature of 40 ºC for different periods of time, namely 3, 7, 14, 20 and 30 days.

Gas Chromatography Analyses

Gas chromatography (GC) analyses were performed using an Agilent 7890 GC equipped with a flame ionization detector (FID), and another Agilent 7890A GC coupled to a mass spectrometer (Agilent 5957C) with the detector operating in a full scan mode under an electron impact ionization (EI) of 70eV. Both instruments were fitted with a capillary column (DB1-ms, 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Ovens were programmed at 80 ºC to 230 ºC at a rate of 3 ºC/min. Data analysis of the GC-FID results were conducted using the calculation of Kovats index of homologous alkane C_7 to C_{20}, while GC-MS mass spectrum was identified by comparison with integrated NIST library.

RESULTS AND DISCUSSION

Identification of Compound through GC Analyses

A total of 47 compounds were identified through GC analyses and classified into four (4) groups, namely other compound, sesquiterpene, sesquiterpenoid and carboxylic acid. The chemical composition for agarwood oils heated at 40 ºC from Day 0 (untreated oil) to Day 30 are summarized in Table 1.

Table 1. Chemical composition of agarwood oil (*A. malaccensis*) heated at 40 ºC from Day 0 to Day 30

Compound	Molecular formula	Kovats DB1	Relative peak area (%)						Ident.
			D0	D3	D7	D14	D20	D30	
Other compounds									
4-Phenyl-2-butanone	$C_{10}H_{12}O$	1214	2.90	2.49	2.20	2.60	2.14	2.04	FID,MS
Vanillin	$C_8H_8O_3$	1364	0.83	1.29	1.20	1.31	1.39	1.02	FID,MS
nor-Ketoagarofuran	$C_{14}H_{22}O_2$	1563	0.30	0.26	0.44	0.27	0.24	0.32	FID
Sinenofuranol	$C_{14}H_{24}O_2$	1784	0.57	0.39	0.60	0.60	0.43	0.59	FID,MS
Sesquiterpene									
allo-Aromadendrene	$C_{15}H_{24}$	1462	0.84	0.75	0.72	0.88	0.78	0.87	FID,MS
α-Curcumene	$C_{15}H_{22}$	1469	0.21	0.19	0.17	0.19	0.18	0.18	FID,MS
γ-Gurjunene	$C_{15}H_{24}$	1473	0.43	0.38	0.35	0.38	0.37	0.37	FID
γ-Cadinene	$C_{15}H_{24}$	1496	0.44	0.37	0.31	0.35	0.27	0.21	FID,MS
cis-Calamenene	$C_{15}H_{22}$	1502	0.20	0.17	0.18	0.19	0.16	0.18	FID,MS
Dehydro-aromadendrene	$C_{15}H_{22}$	1534	2.24	2.01	1.84	2.44	2.19	2.46	FID
Sesquiterpenoid									
β-Agarofuran	$C_{15}H_{24}O$	1478	2.67	0.21	0.13	0.08	0.17	0.22	FID
Dihyro-β-agarofuran	$C_{15}H_{26}O$	1487	0.30	0.25	0.26	0.12	0.24	0.25	FID,MS
Kessane	$C_{15}H_{26}O$	1516	2.67	2.44	2.42	2.89	2.64	2.90	FID,MS
Elemol	$C_{15}H_{26}O$	1530	0.61	0.63	0.59	0.78	0.70	0.77	FID,MS
Epoxybulnesene	$C_{15}H_{24}O$	1573	0.21	0.18	0.22	0.19	0.17	0.17	FID
Caryophyllene oxide	$C_{15}H_{24}O$	1583	0.71	0.67	0.62	0.67	0.62	0.55	FID
Guaiol	$C_{15}H_{26}O$	1597	1.72	1.66	1.55	1.46	1.43	1.29	FID,MS
10-*epi*-γ-eudesmol	$C_{15}H_{26}O$	1602	3.87	3.75	3.42	3.63	3.71	3.57	FID,MS
γ-Eudesmol	$C_{15}H_{26}O$	1611	0.69	0.79	0.78	0.73	0.93	1.05	FID,MS
Agarospirol	$C_{15}H_{26}O$	1620	1.37	1.45	1.39	1.49	1.59	1.76	FID,MS
τ-Cadinol	$C_{15}H_{26}O$	1623	0.98	1.01	0.93	0.82	1.06	1.05	FID,MS
β-Eudesmol	$C_{15}H_{26}O$	1631	4.41	4.22	3.95	4.79	4.66	5.22	FID,MS
epi-α-Cadinol	$C_{15}H_{26}O$	1634	7.88	7.09	6.38	7.57	6.84	7.04	FID,MS

α-Eudesmol	$C_{15}H_{26}O$	1637	3.70	3.60	3.25	3.55	3.70	3.79	FID,MS
Jinkoh-eremol	$C_{15}H_{26}O$	1644	0.62	0.63	0.64	0.71	0.81	0.92	FID
Kusunol	$C_{15}H_{26}O$	1651	2.14	2.39	1.99	1.77	2.19	3.02	FID
Bulnesol	$C_{15}H_{26}O$	1659	1.39	1.25	1.17	1.09	1.08	0.95	FID,MS
Dehydrojinkoh-eremol	$C_{15}H_{24}O$	1676	1.04	1.05	0.87	0.90	0.81	0.83	FID
epi-α-Bisabolol	$C_{15}H_{26}O$	1682	0.90	0.76	0.69	0.59	0.50	0.44	FID,MS
α-Bisabolol	$C_{15}H_{26}O$	1686	2.35	2.00	1.82	1.92	1.63	1.42	FID,MS
Selina-3,11-dien-9-one	$C_{15}H_{22}O$	1692	1.25	1.14	1.18	0.97	0.91	0.88	FID
Rotundone	$C_{15}H_{22}O$	1703	3.34	3.00	2.94	3.03	2.70	2.65	FID,MS
Selina-3,11-dien-9-ol	$C_{15}H_{24}O$	1716	0.89	0.89	0.92	0.84	0.74	0.58	FID
Selina-4,11-dien-14-oic acid	$C_{15}H_{22}O_2$	1725	4.63	4.21	4.55	4.81	4.50	4.85	FID
Selina-3,11-dien-9-al	$C_{15}H_{22}O$	1739	6.72	6.09	5.66	6.38	5.57	5.20	FID
9,11-Eremophiladien-8-one	$C_{15}H_{22}O$	1743	0.28	0.51	0.48	0.49	0.49	0.17	FID
Guaia-1(10),11-dien-9-one	$C_{15}H_{22}O$	1754	1.93	1.15	1.23	0.94	1.20	0.81	FID
Selina-4,11-dien-14-al	$C_{15}H_{22}O$	1759	1.02	0.89	1.10	0.46	0.65	0.41	FID
Guaia-1(10),11-dien-15-ol	$C_{15}H_{24}O$	1769	0.69	0.56	0.78	0.52	0.55	0.50	FID
Selina-3,11-dien-14-oic acid	$C_{15}H_{22}O_2$	1777	0.51	0.37	0.54	0.38	0.33	0.37	FID
Dihydrokaranone	$C_{15}H_{22}O$	1794	0.22	0.13	0.18	0.12	0.06	0.05	FID,MS
Guaia-1(10),11-dien-15-oic acid	$C_{15}H_{22}O_2$	1817	0.46	0.78	0.94	0.94	1.19	1.13	FID
Karanone	$C_{15}H_{20}O$	1825	0.55	0.86	1.09	1.01	1.07	1.00	FID
oxo-Agarospirol	$C_{15}H_{24}O_2$	1834	0.34	0.13	0.18	0.19	0.20	0.35	FID
2-hydroxyguaia-1(10),11-dien-15-oic acid	$C_{15}H_{22}O_3$	1913	1.18	1.06	1.04	1.36	1.39	1.03	FID
9-hydroxyselina-4,11-dien-14-oic acid	$C_{15}H_{22}O_3$	1957	6.61	7.18	6.94	8.06	7.07	7.92	FID
Carboxylic acid									
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1972	0.41	0.38	0.35	0.43	0.38	0.43	FID,MS
Total of other compounds (%)			**4.60**	**4.43**	**4.44**	**4.78**	**4.20**	**3.97**	
Total of sesquiterpene (%)			**4.36**	**3.87**	**3.57**	**4.43**	**3.95**	**4.27**	
Total of sesquiterpenoid (%)			**70.85**	**64.98**	**62.82**	**66.25**	**64.10**	**65.11**	
Total of carboxylic acid (%)			**0.41**	**0.38**	**0.35**	**0.43**	**0.38**	**0.43**	

Major Compounds in Agarwood Essential Oil upon Heating

The major sesquiterpenoid compounds present in agarwood oils are primarily from the sesquiterpene groups namely agarofuran, agarospirane/vetispirane, cadinane, eremophilane/valencane, eudesmane/ selinane, guaiane, prezizane and nootkatane [5]. Thus, high quality agarwood oil should contain a high amount of sesquiterpenoids, as the desired woody aroma is mostly produced by this group. This statement is in accordance with gas chromatography data that identified the major compounds in the oils as 9-hydroxyselina-4,11-dien-14-oic acid (6.94-8.06 %), epi-α-cadinol (6.38-7.88 %), selina-3,11-dien-9-al (5.20-6.72 %), β-eudesmol (3.95-5.22 %), 10-epi-γ-eudesmol (3.95-3.87 %), selina-4,11-dien-14-oic acid (4.21-4.85 %), α-eudesmol (3.25-3.79 %), kusunol (1.77-3.02 %) and kessane (2.42-2.90 %) (Figure 1).

In this study, the aromatic compounds of agarwood oil from Malaysia (*A. malaccensis*) is dominated by compounds from the sesquiterpenoid group (62.82-70.85 %) (Table 1). Other studies found different amounts of sesquiterpenoids ranging from 63.87 % (India, *A. malaccensis*), 67.40 % (Malaysia, *A. malaccensis*) and 49.91 % (Thailand, *A. crassna*) indicating that the differences in composition of the sesquiterpenoids depends on the species, origin and treatment process prior to and after extraction of the oil [5]. The classification of compounds on the treated agarwood oil in the present study (Day 3 to Day30) showed that aromatic compounds of the sesquiterpenoid group is dominant in the agarwood oil

by 62.82 to 66.25 % with the highest content observed in Day 14 after exposure to heat. The percentages of sesquiterpenoid major compounds observed from Day 0 (untreated oil) to Day 30 are summarized in Figure 2.

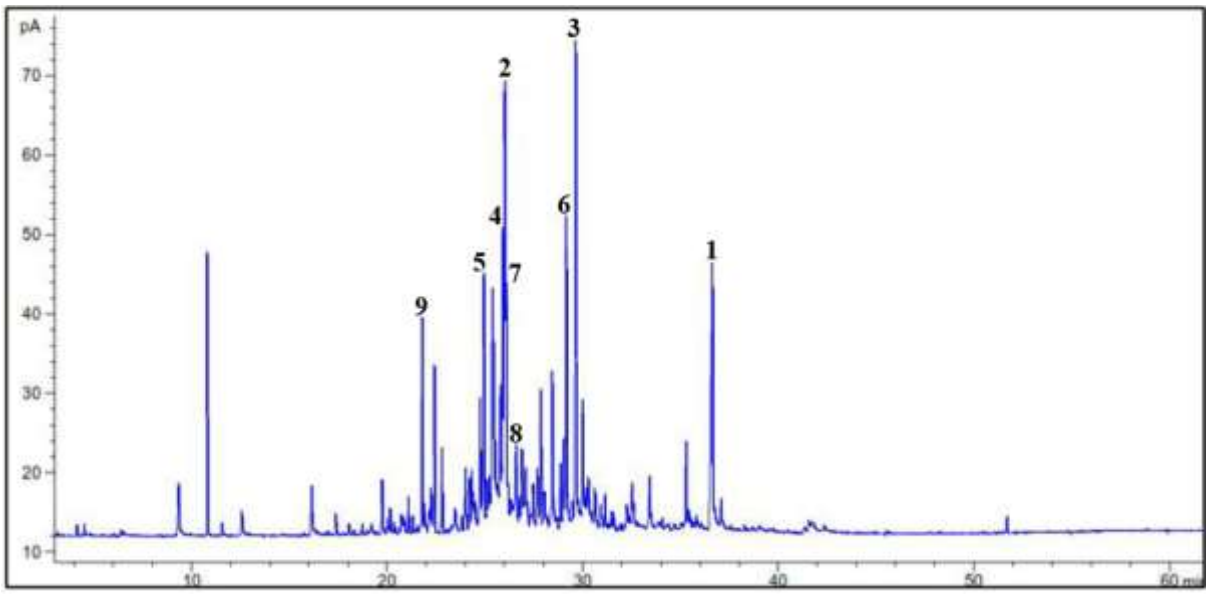

Figure 1. Chromatogram of agarwood oil (Day 0 – untreated oil) with positions of main sesquiterpenoid compounds; (1) 9-hydroxyselina-4,11-dien-14-oic acid, (2) epi-α-cadinol, (3) selina-3,11-dien-9-al, (4) β-eudesmol, (5) 10-epi-γ-eudesmol, (6) selina-4,11-dien-14-oic acid, (7) α-eudesmol, (8) kusunol and (9) kessane.

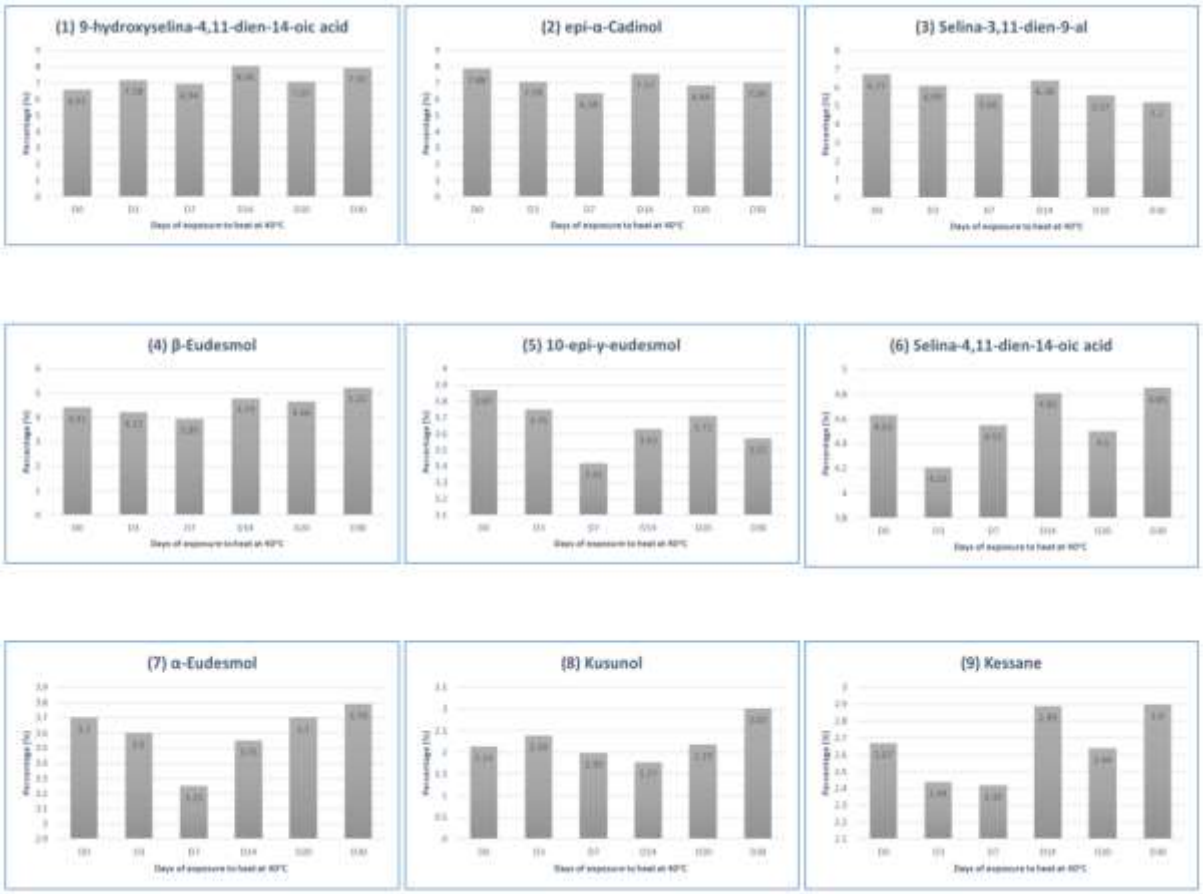

Figure 2. Effect of heat on the composition of major sesquiterpenoid compounds in agarwood oil.

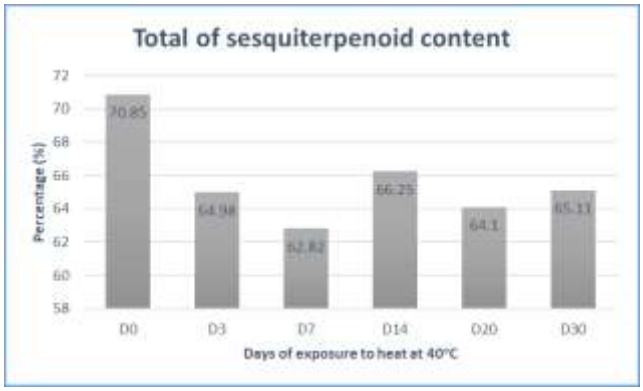

Figure 3. Trend of percentage of total sesquiterpenoid content in agarwood oils (Day 0 to Day 30)

Generally, chemical reactions tend to accelerate with increasing heat due to the temperature-dependence of the reaction rate as expressed by the Arrhenius equation. Oxidation of essential oil depends on the temperature and atmospheric condition which will influence the stability of the essential oil [13]. At low temperature, peroxyl radicals and hydroperoxides are involved in the oxidation process, followed by alkyl or hydroxyl radicals as the temperature continues to increase. Thus, thermal degradation of compounds occurs in four different oxidative reactions including (i) cleavage of double bond, (ii) epoxidation, (iii) dehydrogenation into aromatic system and (iv) allylic oxidation into alcohol, ketone and aldehyde [14]. The heating effect applied in the present study should be able to affect the amounts of individual sesquiterpenoid compounds during the experiment. However, the graphs showed inconsistent trends in the variations of the compounds of interest.

Although this process may degrade the oil quality of other essential oil, agarwood oil should benefit from the process, as more sesquiterpenoids should be produced to enhance its woody aroma. This maturation process is analogous to that of different 'vintages' associated with wine. In addition, the process should result in thicker oil that is claimed to be high quality [4]. However, the results from this study indicated reductions in sesquiterpenoid contents form the concentration of Day 0 (untreated oil) which is 70.85 %. Agarwood oil that was exposed to heat for 14 days, showed the highest percentage of sesquiterpenoids content of about 66.25% (Figure 3) amongst the five exposed samples in the study. This would suggest that the heating process did not increase the quantity of sesquiterpenoids of the treated samples as expected. However, it must be noted that in the present study the headspace above the oil in the vials were not analysed prior to the analysis of the liquid oils. This is because part of the aromatic compounds of interest may have volatilized with the applied heating treatment.

CONCLUSION

In conclusion, agarwood oil aromatics in this study is dominated by a group of sesquiterpenoids consisting of 9-hydroxyselina-4,11-dien-14-oic acid (6.94-8.06 %), epi-α-cadinol (6.38-7.88 %), selina-3,11-dien-9-al (5.20-6.72 %), β-eudesmol (3.95-5.22 %), 10-epi-γ-eudesmol (3.95-3.87 %), selina-4,11-dien-14-oic acid (4.21-4.85 %), and α-eudesmol (3.25-3.79 %), kusunol (1.77-3.02%) and kessane (2.42-2.90 %). This investigation found that the heating process applied reduced the percentage of sesquiterpenoids contents in the liquid oil compared to the concentration of Day 0 untreated oil. The highest percentage of sesquiterpenoids content in this series of agarwood oil exposed to heat was observed in the sample of oil exposed to 14 days of heating at a concentration of 6.25%. Therefore, we recommended that further study with other parameters such as oxygen and UV light need to be done accordingly together with headspace analysis of the samples.

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