EFFECTS OF SOAKING ON YIELD AND QUALITY OF AGARWOOD OIL

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NOR FAZILA K & KU HALIM KH. 2012. Effects of soaking on yield and quality of agarwood oil. The aims of this study were to investigate vaporisation temperature of agarwood oil, determine enlargement of wood pore size, analyse chemical components in soaking solvents and examine the chemical composition of agarwood oil extracted from soaked and unsoaked agarwood. Agarwood chips were soaked in two different acids, namely, sulphuric and lactic acids for 168 hours at room temperature (25 °C). Effects of soaking were determined using thermogravimetric analysis (TGA), scanning electron microscope (SEM) and gas chromatography-mass spectrum analysis. With regard to TGA curve, a small portion of weight loss was observed between 110 and 200 °C for agarwood soaked in lactic acid. SEM micrograph showed that the lactic acid-soaked agarwood demonstrated larger pore size. High quality agarwood oil was obtained from soaked agarwood. In conclusion, agarwood soaked in lactic acid with concentration of 0.1 M had the potential to reduce the vaporisation temperature of agarwood oil and enlarge the pore size of wood, hence, improving the yield and quality of agarwood oil.

Keywords: TGA analysis, SEM analysis, GC-MS analysis

NOR FAZILA K & KU HALIM KH. 2012. Kesan rendaman terhadap hasil dan kualiti minyak gaharu. Matlamat kajian ini adalah untuk menyiasat suhu pengewapan minyak gaharu, menentukan pembesaran saiz liang kayu, menganalisis komponen kimia yang larut dalam pelarut rendaman dan mengkaji komposisi kimia minyak gaharu yang diekstrak daripada kayu yang direndam dan yang tidak direndam dalam pelarut. Serpihan kayu gaharu direndam dalam dua jenis asid iaitu asid sulfurik dan asid laktik pada suhu bilik (25 °C) selama 168 jam. Kesan rendaman ditentukan menggunakan analisis termogravimetrik (TGA), mikroskop elektron pengimbas (SEM) dan analisis kromatografi gas spektrum jisim. Daripada graf TGA, didapati sebahagian kecil daripada kehilangan berat diperhatikan pada suhu antara 110 °C hingga 200 °C bagi gaharu yang direndam dalam asid laktik. Mikrograf SEM menunjukkan bahawa gaharu yang direndam dalam asid laktik menunjukkan saiz liang yang lebih besar. Minyak gaharu berkualiti tinggi dapat diperoleh daripada gaharu yang direndam. Kesimpulannya, gaharu yang direndam dalam asid laktik berkepekatan 0.1M mempunyai potensi untuk mengurangkan suhu pengewapan minyak gaharu dan membesarkan saiz liang kayu. Justeru, hasil dan kualiti minyak gaharu dapat dipertingkatkan.

INTRODUCTION

Agarwood or scientifically known as *Aquilaria* is the resinous hardwood native to South-East Asia. There are 15 *Aquilaria* species in tropical Asia. Natural distributions occur widely in South and South-East Asia. Agarwood or commonly called gaharu in Indonesia and Malaysia is a fragrant resin that is produced by the hardwood of *Aquilaria* species. Great demand for essential oil from this species resulted in the depletion of trees due to indiscriminate cutting. However, the genus is listed and protected as an endangered species internationally. Therefore, most countries in South-East Asia are cultivating *Aquilaria* trees to maintain agarwood products in a sustainable manner. Resins can be formed by notching, fungal infection, insect infestation or inoculation either by chemical or biological methods (Barden et al. 2002, Nor Azah et al. 2008, Rosli 2009). Agarwood is extensively used in incense, pharmaceutical and perfumery industries.

Even though agarwood is an angiosperm, the structure of agarwood is very unique whereby a bundle of phloem is observed in the xylem (Blanchette & Beek 2005). Parenchyma cells were reported to produce resin or identified as agarwood (Nobuchi & Siripatanadilok 1991). Botany (1976) and Prachakul (1997) stressed that resin did not only exist in parenchyma cells but also in included-phloem tissue and ray cells. The dark colour of agarwood indicates that the

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wood contains fragrant resin. Basically resins have numerous chemical compounds such as α-guaiene, α-bulnesene and γ-gurjunene (Nor Azah et al. 2008, Wetwitayaklung et al. 2009, Winarni & Waluyo 2009, Nizam & Mashitah 2010). A number of studies have found that the compounds not only give odour, taste and colour to wood but also act as a protector against insect invasion (Rowell et al. 2005, Horvath 2006).

Several studies investigating the effect of solvents on wood cell expansion have been carried out. Soaking solvents play a role in reducing the strength of cell walls and breaking the oil glands. This eases extraction of chemical components. For this purpose, a variety of solvents were applied such as water (Rowell et al. 2005, Stokke & Groom 2006), acid and alkali (Rowell et al. 2005).

In commercial processing of agarwood oil, no standard operating procedures were applied. Distillers usually soak the wood in water for a few days up till two months. To date effects of the immersion technique on the vaporisation temperature and pore size enlargement of agarwood have not been studied extensively. Most publications focused only on the extraction of chemical compounds from agarwood (Nor Azah et al. 2008, Wetwitayaklung et al. 2009, Winarni & Waluyo 2009, Nizam & Mashitah 2010). Winarni and Waluyo (2009) extracted agarwood oil from unsoaked wood. Thus, only a small amount of extractive was obtained. Conversely, numerous chemical components were extracted from agarwood that was soaked in water for more than three days (Wetwitayaklung et al. 2009, Nizam & Mashitah 2010). Therefore, soaking is significant in improving chemical components extracted and yield of essential oil.

Hypothetically, the degradation of wood cell wall would enlarge the pore size. The objectives of this study were to determine effects of soaking method on vaporisation temperature of essential oil and the enlargement of agarwood pore size. These would increase the yield of essential oil and extract the active ingredients in agarwood. The study on soaking solvents was carried out to better understand the diffusion of chemical components through open wood pores as a result of soaking. Unsoaked agarwood acted as control. Chemical compositions of agarwood oil were analysed using gas chromatography-mass spectrum (GC-MS) and the yield was calculated in order to compare the quality of oils extracted from soaked and unsoaked agarwood.

MATERIALS AND METHODS

Plant materials and reagents

The heterogeneous ground agarwood species *Aquilaria malaccensis* used in this research was obtained from the forest of Temerloh, Pahang, West Malaysia. Chemicals such as sulphuric acid (95–98%), lactic acid (88%) and hexane (95%) used were of commercial grades.

Soaking with solvents

Ground agarwood samples were soaked in two solutions of 0.1 M lactic acid and 0.1 M sulphuric acid. Soaking process was performed at room temperature (25 °C) for 168 hours. The agarwood samples which were soaked in solvents were dried for 24 hours in an oven at 50 °C. For assessment using scanning electron microscope (SEM), agarwood was cut into 1 cm (length) × 1 cm (width) × 0.1 cm (height) before being soaked in solvents.

Thermogravimetry analysis (TGA)

Wood is essentially composed of hemicellulose, cellulose, lignin and extractives (Wiedenhoeft & Miller 2005, Shebani et al. 2009). Each component has its own temperature profile which can be interpreted from thermogravimetric and derivative thermogravimetric curves. However, the present research focused only on the vaporisation temperature of agarwood extractives. The temperature obtained is very practical as guidance to distillers in setting temperature for the extraction process. This practice was conducted with a Mettler Toledo analyser. The experiment was carried out with 20 mg of agarwood samples, heated from 30 to 900 °C at a rate of 10 °C min^{-1} under nitrogen gas flow of 60 mL min^{-1}.

Scanning electron microscopy analysis

Non-coated agarwood samples were introduced to SEM with magnification of 500 times. The changes in the structure of agarwood especially average pore size were measured and compared.

Hydrodistillation

Two hundred grams of each sample were subjected to hydrodistillation (Zulbadli et al.

2011). The agarwood samples were extracted with 1.5 L of water for 12 hours. Following this, mixtures of oils and water collected were separated using a separation funnel by adding hexane in a ratio of 1:2. Then, the hexane was separated using rotary evaporator at 60 °C. Evaporation of hexane under reduced pressure gave the agarwood oil, which was weighed to determine the yield of oil.

$$\text{Yield of oil (\%)} = \frac{\text{agarwood oil (g)}}{\text{agarwood chips (g)}} \times 100\%$$

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS was used to identify chemical compounds of agarwood which were dispersed in the solvents during the immersion process through open wood pores and chemical components extracted from soaked and unsoaked samples. Since acids could not be injected directly to GC-MS, the soaking solvents were mixed with hexane in a separation funnel in the ratio of 1:2. Two layers of liquid were formed, whereby hexane which was lighter would float on top of the acid. Hexane was collected and analysed via GC-MS.

The GC-MS analyses were performed via Varian 450 gas chromatograph with attached Varian 240 mass spectrometer. This apparatus was equipped with DB-1 capillary column (30 m × 0.25 mm × film thickness 0.25 µm). Injector temperature was set at 230 °C and oven temperature was programmed between 50 and 230 °C at a rate of 3 °C min^{-1}. Injection volume was 1 µL with split ratio 20:1. Helium was used as carrier gas and the flow rate was set at 1 mL min^{-1} (Nizam & Mashitah 2010).

RESULTS AND DISCUSSION

Effect of soaking on the vaporisation temperature of agarwood oil

Figure 1a depicts the weight loss (thermogravimetric) curves of unsoaked agarwood samples and agarwood soaked in lactic acid and sulphuric acid. Figure 1b represents the derivative thermogravimetric curves.

All agarwood samples revealed a small amount of weight loss at temperatures ranging from 40 to 100 °C due to evaporation of moisture (Figure 1a). Agarwood soaked in sulphuric acid showed the highest moisture loss which was 6.5% as visibly shown in Figure 1b. It occurred due to the characteristic of sulphuric acid as an excellent dehydrating agent. Agarwood which was soaked in sulphuric acid was volatile at about 100 °C, followed by agarwood soaked in lactic acid and unsoaked sample at temperatures of 110 and 170 °C respectively. A possible explanation for this might be due to the break up of parenchyma cells as a result of immersion, thus easing wood components to volatilise. Apparently, all samples except for agarwood soaked in sulphuric acid showed rapid weight loss between 50 and 60% at temperatures 200 to 400 °C. Conversely, agarwood soaked in sulphuric acid displayed a large vaporisation temperature range starting from 110 to 500 °C with weight loss of 55%.

Derivative thermogravimetric curve gave a clear picture of the weight loss comparison for all agarwood samples. One unanticipated finding was that there was weight loss of 4.14% at temperatures 110 to 200 °C for agarwood soaked in lactic acid. It could be presumed as vaporisation of agarwood oil. As mentioned in previous studies, the yield of essential oil obtained was between 0.03 and 4.43% (Wetwitayaklung et al. 2009, Winarni & Waluyo 2009, Nizam & Mashitah 2010). However, no weight loss was displayed by unsoaked samples within that range of temperature. With regard to agarwood soaked in sulphuric acid, there was abrupt decline at 100 to 300 °C. Obviously, agarwood soaked in sulphuric acid exhibited the highest weight loss of 32.6%. The weight loss could be referred to as the degradation of salts that were formed from the reaction between sulphuric acid and agarwood components.

Effect of soaking on pore size

Figure 2 depicts the structure of agarwood samples examined by SEM at magnification of 500 times. Figure 2a shows the image of unsoaked agarwood. The average pore size of this sample was 9.09 µm. Apparently, the pore sizes were small and cell walls were not deformed. Presumably, chemical components were difficult to escape. Therefore, soaking agarwood prior to extraction of essential oil is crucial.

Figures 2b and c show considerable changes in morphology for soaked samples. Figure 2b clearly displays that the pore size is expanded to 18 µm when agarwood is soaked in lactic acid.

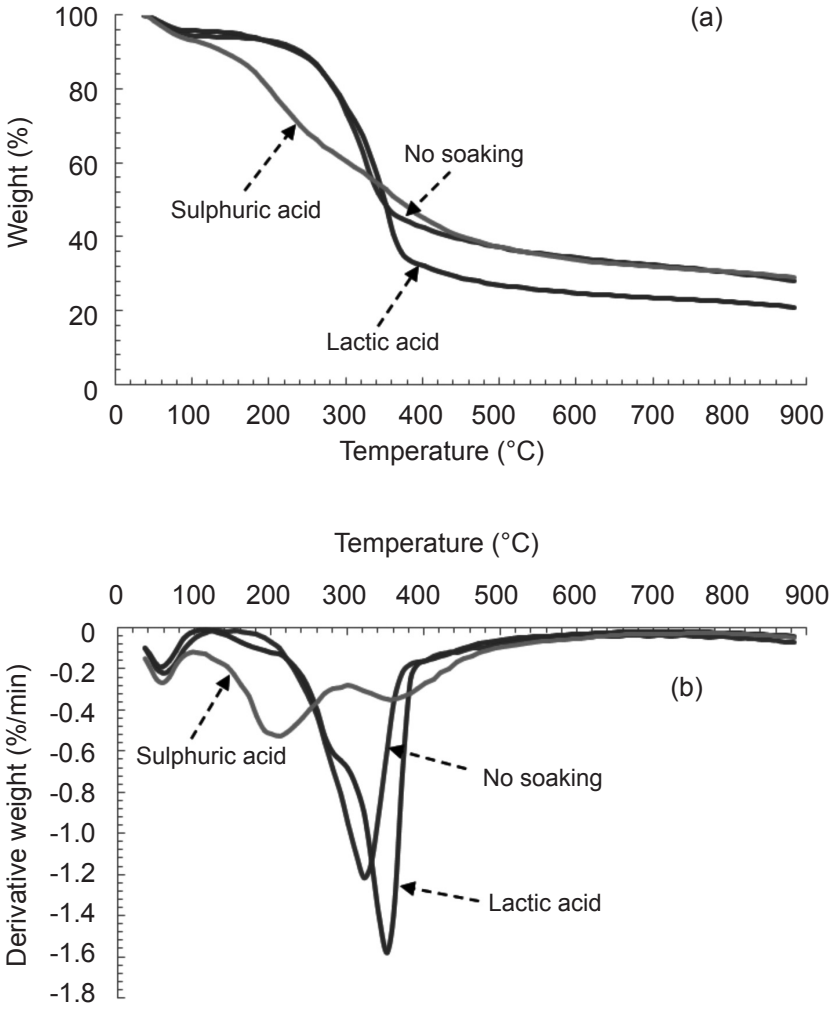
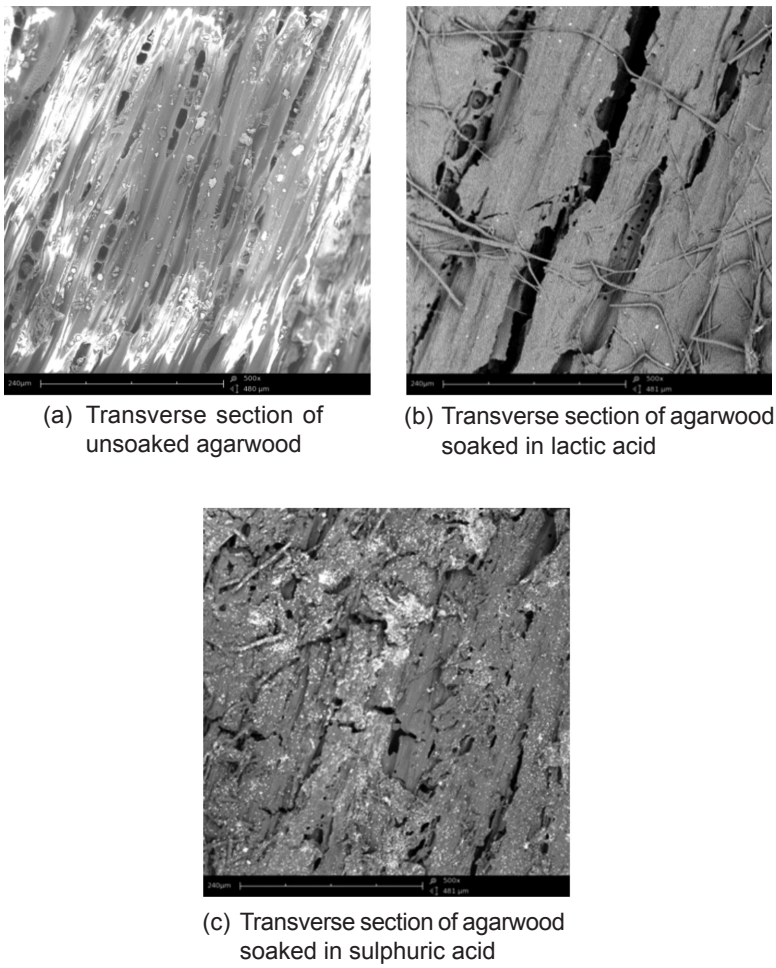


Figure 1 (a) Thermogravimetric curve and (b) derivative thermogravimetric curve for agarwood at heating rate of 10 °C min^{-1}

It is interesting to note that the pore size for agarwood soaked in lactic acid was augmented greatly. This could be due to the effect of microbial activities in lactic acid, significantly degrading wood components. Furthermore, extensive soaking period allowed the bacteria to acclimatise aggressively in the soaking mixture. These findings further supported the findings of Rowell et al. (2005) who found that cell wall structure degraded when the wood was exposed to acids for a long time. Conversely, the pore size of agarwood became smaller than that of unsoaked wood when the wood was soaked in sulphuric acid. The phenomenon was perceived in Figure 2c with an average pore size of 8.61 µm. This could be due to elimination of hydrogen and oxygen atoms from carbohydrates contained in agarwood, leaving behind carbon atoms (Shakhashiri 1989). Thus, carbon atoms might obstruct the pores of agarwood and distract the extraction of agarwood oil.

Effect of soaking on the diffusion of chemical components in immersion solvents

Table 1 shows the chemical components that are extracted from the agarwood during immersion process. As expected, there were more chemical components found in lactic acid compared with sulphuric acid. However, the percentage of components extracted were not as high as components extracted with sulphuric acid. Most of the compounds that diffused in lactic acid such as α-guaiene, α-selinene and α-gurjunene could influence the unique odour of agarwood oil. The major components extracted in lactic acid and sulphuric acid



(a) Transverse section of unsoaked agarwood

(b) Transverse section of agarwood soaked in lactic acid

(c) Transverse section of agarwood soaked in sulphuric acid

Figure 2 Scanning electron micrographs of unsoaked and soaked agarwood

were α-gurjunene (0.818%) and benzaldehyde (0.856%) respectively.

Effect of soaking on the yield of extract

Comparison between soaked and unsoaked samples based on yield of agarwood oil extracted using hydrodistillation is shown in Table 2. Higher yield was obtained from soaked agarwood compared with unsoaked samples. The findings of this study were consistent with those of Rashid and Zuhaidi (2011) and Pornpunyapat et al. (2011). They found that soaking could rupture the parenchyma cells, hence facilitating diffusion of oil from the fractured oil glands. Agarwood soaked in acids gave significantly greater yield of oil than the control. Our findings seemed to contrast with other researchers who found that the yield of essential oil derived from agarwood soaked in water was between 0.03 and 4.43% (Wetwitayaklung et al. 2009, Winarni & Waluyo 2009, Nizam & Mashitah 2010). This could be due to the effect of acid on the agarwood structure. Furthermore, distillation of acid-soaked agarwood samples within the shorter period gave extraordinary oil yield. It could be proven by comparing the current study with research done by Wetwitayaklung et al. (2009) who obtained only 0.2% oil yield from 168 hours distillation of 15 kg of agarwood soaked in water. On the other hand, in the present study, oil yield obtained was higher, i.e. 4.74 to 6.78%, extracted from 0.2 kg soaked agarwood samples for 12 hours.

Isolation of agarwood oil from unsoaked and soaked agarwood by hydrodistillation

The volatile components obtained from the extraction of various agarwood samples are shown in Table 3. The greatest numbers of volatile components were obtained from soaked samples. Agarwood soaked in lactic acid isolated the highest number of compounds. This accorded with earlier observations using SEM, which showed that the pore size of agarwood soaked

Table 1 Chemical components of agarwood diffused in immersion solvents

Compound	Retention time (min)	Immersion solvent	
		Lactic acid (%)	Sulphuric acid (%)
4-phenyl-2-butanone	17.40	0.258	0.609
Benzaldehyde	5.80	–	0.856
Caryophellene oxide	35.50	0.401	0.728
α-Guaiene	37.50	0.175	–
α-Selinene	35.60	0.401	–
α-Gurjunene	44.50	0.818	–

– denotes data not available

Table 2 Comparison of yield percentage of agarwood oil extracted via hydrodistillation

Sample	Yield (%)
Unsoaked	1.72
Soaked in lactic acid	4.74
Soaked in sulphuric acid	6.78

in lactic acid was much bigger than the rest, thus facilitating compounds escaping from the oil glands.

The major compounds (chromatogram area > 1%) for unsoaked agarwood were dibutyl phthalate, eudesma-5,11(13)-dien-8,12-olide and dodecanoic acid. Furfural, 4-phenyl-2-butanone, vanillin, dibutyl phthalate, eudesma-5,11(13)-dien-8,12-olide and dodecanoic acid were detected as the major compounds of oil extracted from agarwood soaked in lactic acid. The findings for agarwood soaked in sulphuric acid were slightly different, whereby furfural, 5-methyl-2-furancarboxaldehyde, 4-phenyl-2-butanone, vanillin, dibutyl phthalate and dodecanoic acid were identified as major compounds.

Nevertheless, dibutyl phthalate and eudesma-5,11(13)-dien-8,12-olide were not the fragrant agents. It is interesting to note that furfural, 4-phenyl-2-butanone, 5-methyl-2-furancarboxaldehyde, vanillin and dodecanoic acid contribute to the unique odour of agarwood oil. The pherobase database website El-Sayed (2011) and the Good Scents Company website (Company TGS 2012) have described the odour characteristics of some chemical components: vanillin (sweet), furfural (woody, almond, sweet, fruity, flowery), 4-phenyl-2-butanone (floral jasmine, herbal fruity, balsam) and 5-methyl-2-furancarboxaldehyde (caramel, burnt sugar, spicy, acid, coffee). However, the findings of the current study did not support those of previous studies (Nor Azah et al. 2008, Nizam & Mashitah 2010). Previous researchers had reported that α-bulnesene, kusunol, α-guaiene, β-agarofuran, jinkoh eremol, oxo-agarospirol, selina-3,11-dien-9-one and guaia-1(10),11-dien-15,2-olide were marker compounds of *A. malaccensis* oil. A possible explanation for this might be that some of the compounds such as α-guaiene, α-selinene and α-gurjunene were diffused into immersion solvents during soaking. Meanwhile the pore size of unsoaked agarwood was too small as analysed using SEM. Perhaps, the 12-hour extraction was not enough to isolate the marker compounds.

CONCLUSIONS

Agarwood oil that existed in samples which were soaked in lactic acid was volatile at low temperature of 110 °C. The results indicated that agarwood soaked in lactic acid gave a remarkable impact in reducing energy consumption in the agarwood oil processing.

Agarwood soaked in lactic acid caused extraordinary enlargement of pore size with average of 18 µm. This could provide better condition for the extraction process by facilitating the diffusion of either solvent or chemical component through the enlarged pores. High yield and greatest numbers of chemical compounds were obtained from agarwood soaked in lactic acid.

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Table 3 Comparison of volatile oil components extracted from unsoaked and soaked agarwood

Compound	Retention time (min)	(% Area)		
		Unsoaked	Lactic acid	Sulphuric acid
3-Hexanone	3.56	–	0.09	0.13
Methyl isobutyl ketone	3.60	–	0.12	–
2-Hexanone	3.61	–	–	0.16
Furfural	4.19	–	5.69	19.16
5-Methyl-2-furancarboxaldehyde	7.55	–	0.70	2.45
Benzaldehyde	7.55	–	–	–
4-Phenyl-2-butanone	19.04	0.49	3.79	2.51
3,4-Dimethoxyphenol	22.08	–	–	0.89
Vanillin	23.68	–	1.33	2.02
2-Methyl-4-chromone	28.67	0.29	0.20	–
4-(4-Methoxyphenyl)-2-butanone	29.64	0.37	0.42	0.20
γ-Gurjunene	35.58	–	0.46	–
Aromadendrene	38.29	–	–	0.24
α-Selinene	40.99	–	0.32	–
β-Guaiene	41.15	0.18	0.40	0.49
Velleral	41.30	0.37	0.75	0.22
Caryophelene oxide	42.20	–	0.63	–
β-Gurjunene	43.35	–	0.16	0.53
Valencene	44.62	0.28	0.49	–
Dibutyl phthalate	44.94	26.76	40.98	30.91
Aromadendrene oxide (2)	49.36	0.54	0.51	–
Eudesma-5,11(13)-dien-8,12-olide	51.29	5.69	5.38	0.33
Dodecanoic acid	54.32	4.64	10.97	2.98

Compounds listed here are based on the relative peak area > 0.1%; – denotes data not available

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