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Determination of optimum conditions for the analysis of volatile components in pine needles by double-shot pyrolysis–gas chromatography–mass spectrometry

Jae-Gon Lee [a,*], Chang-Gook Lee [a], Jae-Jin Kwag [a], Alan J. Buglass [b], Gae-Ho Lee [c]

[a] *Department of Tobacco Research, KT&G Central Research Institute, Yuseong-gu, Daejeon 305-805, South Korea*
[b] *Department of Chemistry, Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 305-701, South Korea*
[c] *Department of Chemistry, Chungnam National University, Yuseong-gu, Daejeon 305-764, South Korea*

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Abstract

The optimum conditions for the analysis of the volatile organic components of pine needles from *Pinus densiflora* using double-shot pyrolysis–gas chromatography–mass spectrometry (DSP–GC–MS) were investigated with respect to thermal desorption temperature and duration of heating. A total of 41 compounds were identified using thermal desorption temperatures of 150 °C, 200 °C, 250 °C and 300 °C. Thermal decomposition products, which include acetol, acetic acid, furfurals and phenols, were observed only at thermal desorption temperatures exceeding 250 °C: they were not observed in the extract from a simultaneous distillation extraction (SDE) method. Heating times of 1 s, 6 s, 30 s, 150 s and 300 s were investigated at the thermal desorption temperature of 200 °C, whence it was found that thermal decomposition products were produced only at heating times over 30 s. The optimum pyrolyzer conditions for the analysis of pine needles using DSP–GC–MS is 200 °C for 6 s. Under these conditions, this method gives comparable results to the SDE method.

Keywords: Pine needle; Thermal desorption; Double-shot pyrolyzer; Volatile components

1. Introduction

Pine trees represent the most widely used species of tree in Korea. *Pinus densiflora* S. is common throughout Korea: it is known in the west as Japanese red pine [1]. Pine needles have long been valued for their medical effects and have been used in popular medicines for the treatment of hepatosis, various neurological disorders, and arteriosclerosis [2]. They are also valued for their flavouring properties: the essential oil of pine needles has found wide commercial use and is a constituent of certain beverages, cookies, detergents, cosmetics, amongst others [3,4]. In recent years, there has been an increase in the need for natural flavourings, following increases in the demand for natural products, as opposed to nature-identical or synthetic products. It is likely that this trend will continue for the time being. In response to this situation, there have been some studies on the volatile ingredients of pine needles. Hong et al. [5] have analyzed the volatile organic components of *Pinus rigida* needles using steam distillation and solvent extraction. Woo et al. [6] reported a difference in the composition of volatile ingredients of pine twigs from *P. densiflora* S. using of supercritical fluid extraction and steam distillation. In addition, Roussis et al. [7] analyzed the volatile components of five Greek varieties of pine needles and found large differences between the varieties. More recently, Yu et al. [8] have analyzed the volatile organic compounds in the pine needles of *P. densiflora* S. using SDE and headspace solid-phase microextraction (HS-SPME) and Stevanovic et al. [9] have analyzed the essential oil of the needles and twigs of the dwarf pine *Pinus mugo* Turra.

* Corresponding author. Tel.: +82 42 866 5591; fax: +82 42 866 5426.
E-mail address: jgolee@ktng.com (J.-G. Lee).

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Previously, steam distillation methods have been widely used to extract volatile ingredients from plant material. However, solid-phase microextraction (SPME) [10] and headspace [11] methods are more commonly used at present. Steam distillation has certain advantages, such as the use of small amounts of solvent, but it also has certain drawbacks, such as major changes in composition resulting from thermal decomposition due to the necessary maintenance of high temperatures during the extraction process [12]. SPME has been preferred to headspace methods since it is simple and can be used on a small sample without any organic solvents. However, the efficiency of this method has been found to be much lower than that of headspace methods [13,14].

Thermal desorption methods, which use a directly connected gas chromatograph–mass spectrometer (GC–MS), can analyze volatile compounds using small samples (lower than 0.01 g) and is economical, due to the ability to treat many samples in a short time. For example, Sanz et al. [15] analyzed volatile compounds by injecting them into the GC–MS after collecting them in a Tenax TA and desorbing *Lavandula luisier* L. at 320 °C. However, it has been shown that the composition of volatile components of a given sample varies with both the thermal desorption temperature and the heating time, and hence requires careful selection of optimum conditions. Moreover, thermal decompositions of certain components occur at higher temperatures or over prolonged heating times [16,17,18]. González-Vila et al. [18] reported that there were large differences in the volatile compound composition with regard to heating time, when Rye grass (*Lolium rigidum*) was heated at 350 °C using a curie-point pyrolyzer and GC–MS.

This study presents the results of an investigation to find the optimum conditions for the analysis of the volatile organic components of pine needles, using a double-shot pyrolyzer GC–MS set up, by varying the thermal desorption temperature and heating time. The double-shot pyrolyzer is a type of furnace where the range of temperature setting is wide compared with the more usual curie-point pyrolyzer.

2. Experimental

2.1. Plant material and reagents

P. densiflora pine needles were collected from mountains near Daejeon, South Korea in August 2004, stored in solvent-cleaned glass jars with aluminium foil-lined lids and were refrigerated at 3 °C in the laboratory until required for analysis. Pine needles were cut to 2 mm lengths immediately before use. All organic solvents were of analytical grade and were purchased from Sigma.

2.2. Thermal desorption using a double-shot pyrolyzer

Volatile fractionation was carried out by using a double-shot pyrolyzer 2020iD (Frontier Lab, Japan), which was connected directly to the injector of the GC. The Pyrolyzer was composed of a plunger for the sample, the sample cup, a deactivated needle (into the GC injector) and a furnace. Helium (high purity, 99.99%) was used both as the GC carrier gas and as the inert atmosphere for thermal desorption.

Pine needles (10 mg) and internal standard *n*-decanol (0.3 μl of a 0.45 mg ml^{-1} ethanol solution) were introduced into the sample cup, which was then placed in the furnace. In order to evaporate the solvent (ethanol) before commencing the thermal desorption, the system was purged for a short time (30 s) with the carrier gas. After purging, the sample cup was heated, whence the volatile organic components were transferred from the furnace to GC–MS without significant loss.

Experiments were carried out for 6 s (heating time) on separate samples at temperatures of 150 °C, 200 °C, 250 °C and 300 °C, respectively), in triplicate.

Also, to investigate the best heating time at the thermal desorption temperature of 200 °C, thermal desorptions were carried out for 1 s, 6 s, 30 s, 150 s, 300 s, respectively.

2.3. SDE

Pine needles (60 g), distilled water (500 ml), and *n*-decanol internal standard (1 g of a 0.45 mg ml^{-1} ethanol solution) were placed in a 2 l round-bottom flask. Diethyl ether (30 ml) and pentane (30 ml) were placed in a 100 ml round-bottom flask, and the two flasks were connected to the modified Likens-Nickerson micro SDE apparatus [19]. The extraction was performed for 2 h, during which time chilled water was circulated through the cold finger condenser. The fractions in the solvent flask were dried with anhydrous sodium sulfate, filtered and then concentrated by blowing with nitrogen. Three replications of the extraction and analysis procedure were performed for each of the samples.

2.4. GC–MS analysis

The GC–MS equipment consisted of an Agilent 6890 gas chromatograph equipped with an Innowax capillary column (50 m, 0.25 mm i.d., 0.25 μm film; polyethylene glycol as stationary phase). The double-shot pyrolyzer was directly connected to the GC injector, which was maintained at 230 °C, with a 1:100 split ratio at the initial time. The detector consisted of an Agilent 5973 mass selective detector operating in the scan modus. Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the *m*/*z* 30–500 range. Interface and source temperature were 250 °C and 230 °C, respectively. The carrier gas used was helium with a controlled flow of 1.0 ml min^{-1}. The GC oven temperature was programmed from 50 °C (3 min) to 220 °C (20 min) by increasing the temperature at the rate of 2 °C min^{-1}.

2.5. Qualitative and quantitative analysis

The identification of the separated volatile organic compounds was achieved through retention times (retention indices; RI) and mass spectrometry by the comparing mass

spectra of the unknown peaks with those stored in the Wiley mass spectrometry libraries. Retention indices, calculated by linear interpolation relative to the retention times of C_6–C_{26} *n*-alkanes, were compared with those reported in the literature [20]. Semi-quantitative values were obtained by using *n*-decanol as internal standard.

3. Results and discussion

Fig. 1 presents the total ion chromatograms (TIC), which were produced from the analysis of the volatile organic components of pine needles at four different thermal desorption temperatures using double-shot pyrolysis–GC–MS and a heating time of 6 s. Table 1 diplays the semi-quantitative results at these four thermal desorption temperatures, along with those from simultaneous distillation extraction (SDE)–GC–MS.

It can be seen in Table 1, that a total of 41 volatile ingredients were verified using four extracting thermal desorption temperatures. The identified compounds were classified, according to their functionalities, as follows: 23 hydrocarbons, 12 alcohols, 3 carbonyl compounds, 1 ester, and 2 carboxylic acids. The types of hydrocarbons, which are prominent, includes monoterpenes (10 carbon atoms), such as pinene, camphene, limonene, and β-phellandrene, and sesquiterpenes (with 15 carbon atoms), such as β-caryophyllene, germacrene D, and β-cubebene. It is already known that these compounds are odorous and present the flavouring properties described as woody, piney, and fruity [21]. In addition, Eakin has reported that compounds that give rise to flavouring properties described as herb, spicy, and citrus are not terpenoid hydrocarbons but are oxygenated terpenes, such as terpene alcohols or terpene esters [22]. This study verified the presence of the oxygenated terpene substrates 2-hexenal (22.2–56.9 $\mu g\,g^{-1}$), *cis*-2-penten-1-ol (2.9–7.2 $\mu g\,g^{-1}$) and *cis*-3-hexenol (44.9–95.6 $\mu g\,g^{-1}$).

The major volatile organic compounds of *P. densiflora* S. needles verified by using DSP–GC–MS were α-pinene (323.1–1434.1 $\mu g\,g^{-1}$, 7.5–22.9%), limonene

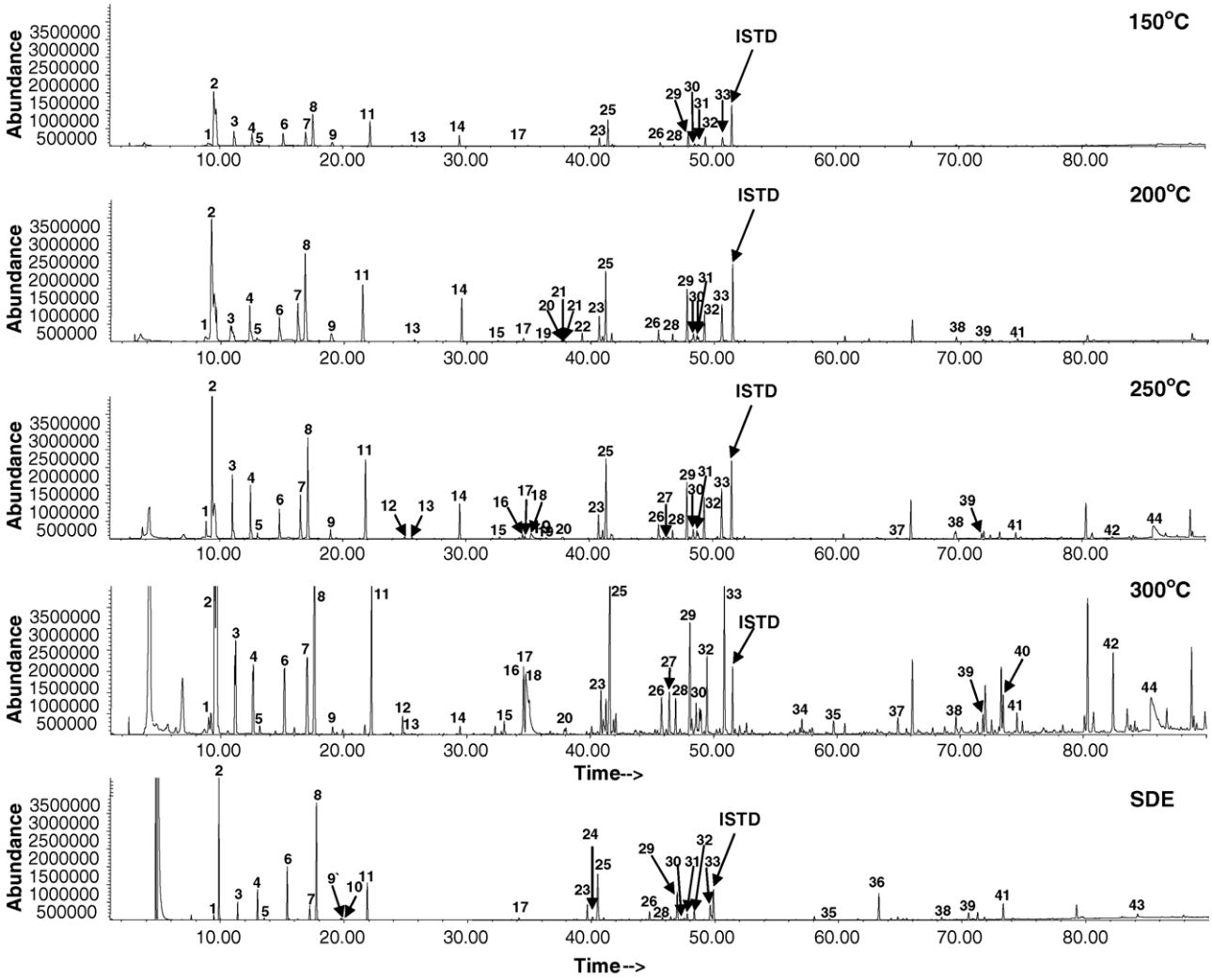

Fig. 1. TIC profiles of pine needle volatiles obtained at different thermal desorption temperatures, for 6 s heating time.

Table 1
Semi-quantitative results for major organic volatile components of *P. densiflora* S. needles obtained by DSP–GC–MS at different thermal desorption temperatures (heating time 6 s) and by SDE–GC–MS

Peak number	RI[a]	Component number	Concentration ($\mu g\,g^{-1}$) ± SD[b] (RSD[c] (%); $n = 3$)				
			150 °C	200 °C	250 °C	300 °C	SDE
1	1047	Tricyclene	27.81 ± 3.89 (13.98)	20.77 ± 11.09 (53.38)	31.19 ± 8.38 (26.88)	94.68 ± 29.92 (31.61)	22.21 ± 2.95 (13.26)
2	1056	α-Pinene	323.05 ± 32.62 (10.10)	621.66 ± 28.79 (4.63)	636.89 ± 68.72 (10.79)	1434.07 ± 667.54 (46.55)	567.46 ± 71.44 (12.59)
3	1083	Camphene	50.93 ± 17.79 (34.93)	85.99 ± 25.20 (29.31)	87.20 ± 25.41 (29.13)	199.55 ± 101.70 (50.96)	72.72 ± 9.80 (13.47)
4	1107	β-Pinene	44.04 ± 7.40 (16.80)	77.07 ± 1.39 (1.81)	97.67 ± 40.08 (41.03)	151.23 ± 57.08 (37.74)	178.36 ± 41.53 (23.28)
5	1119	Sabinene	5.91 ± 0.84 (14.17)	10.87 ± 6.56 (60.39)	12.44 ± 5.12 (41.12)	19.86 ± 4.99 (25.11)	12.81 ± 2.38 (18.59)
6	1154	β-Myrcene	87.08 ± 56.19 (64.53)	102.25 ± 53.58 (52.40)	102.79 ± 62.38 (60.69)	158.02 ± 33.52 (21.21)	212.38 ± 42.46 (19.99)
7	1180	Limonene	56.77 ± 10.03 (17.67)	101.59 ± 12.99 (12.79)	124.63 ± 46.21 (37.08)	168.53 ± 82.29 (48.83)	56.55 ± 10.07 (17.80)
8	1189	β-Phellandrene	193.41 ± 82.44 (42.62)	350.60 ± 170.33 (48.58)	402.98 ± 199.46(49.50)	457.38 ± 123.03 (26.90)	595.89 ± 104.10(17.47)
9	1225	2-Hexenal	43.69 ± 22.28 (50.99)	25.84 ± 3.34 (12.93)	22.21 ± 6.59 (29.67)	56.91 ± 51.26 (90.08)	5.88 ± 1.21 (20.59)
10	1248	1-Pentenol	–[d]	–	–	–	4.90 ± 1.12 (22.85)
11	1267	α-Terpinolene	98.55 ± 5.73 (5.81)	148.82 ± 110.85 (74.49)	164.60 ± 125.83(76.44)	554.36 ± 149.61 (26.99)	123.35 ± 20.24 (16.41)
12	1318	Acetol	–	–	1.38 ± 1.15 (83.82)	65.73 ± 36.87 (56.09)	–
13	1334	*cis*-2-Penten-1-ol	3.90 ± 1.37 (35.25)	2.87 ± 1.02 (35.61)	3.02 ± 0.45 (14.78)	7.19 ± 7.01 (97.56)	–
14	1394	*cis*-3-Hexenol	44.93 ± 27.43 (61.04)	55.30 ± 26.81 (48.48)	46.26 ± 25.02 (54.09)	95.62 ± 109.16 (114.16)	1.93 ± 0.91 (46.99)
15	1442	α-Cubebene	1.26 ± 0.55 (43.45	2.11 ± 0.59 (27.68)	4.87 ± 2.73 (56.06)	27.09 ± 7.76 (28.66)	3.26 ± 0.96 (29.42)
16	1471	Furfural	–	–	6.71 ± 3.88 (57.90)	208.18 ± 49.25 (23.66)	–
17	1473	α-Copaene	2.71 ± 1.34 (49.51)	5.32 ± 0.76 (14.24)	7.94 ± 2.99 (37.73)	*t*	15.01 ± 5.75 (38.32)
18	1486	Acetic acid	–	–	13.84 ± 10.12 (73.12)	662.66 ± 196.92 (29.72)	–
19	1498	β-Bourbonene	*t*[e]	2.04 ± 0.64 (31.35)	2.54 ± 1.61 (63.33)	6.01 ± 3.00 (49.98)	–
20	1525	β-Cubebene	–	1.77 ± 0.45 (25.51)	4.23 ± 1.88 (44.37)	11.17 ± 4.11 (36.81)	*t*
21	1529	Benzaldehyde	0.82 ± 0.46 (56.89)	1.27 ± 1.24 (98.08)	0.55 ± 0.07 (12.86)	–	*t*
22	1551	Isolongifolene	7.49 ± 7.17 (95.72)	10.96 ± 5.64 (51.47)	7.65 ± 6.26 (81.89)	3.63 ± 4.34 (119.60)	10.99 ± 1.95 (17.79)
23	1575	Bornyl acetate	33.20 ± 24.19 (72.86)	76.62 ± 35.33 (46.11)	79.93 ± 46.31 (57.94)	206.56 ± 112.25 (54.34)	60.80 ± 10.67 (17.55)
24	1579	β-Elemene	4.67 ± 1.07 (22.89)	14.56 ± 4.73 (32.50)	27.07 ± 9.43 (34.82)	76.11 ± 5.52 (7.25)	20.58 ± 7.36 (35.78)
25	1584	β-Caryophyllene	91.34 ± 3.38 (3.70)	196.21 ± 49.88 (25.42)	256.72 ± 104.69(40.78)	485.56 ± 181.71 (37.42)	321.11 ± 37.00 (11.52)
26	1656	α-Humullene	13.46 ± 0.07 (0.51	32.29 ± 9.44 (29.22)	41.85 ± 15.83 (37.83)	89.46 ± 27.98 (31.28)	51.10 ± 6.00 (11.75)
27	1669	Furfuryl alcohol	–	–	19.27 ± 24.30 (126.15)	62.86 ± 42.68 (67.89)	–
28	1674	α-Amorphene	4.98 ± 0.73 (14.73)	11.03 ± 7.54 (68.37)	24.26 ± 10.02 (41.30)	70.95 ± 34.51 (48.65)	17.19 ± 5.25 (30.53)
29	1694	Germacrene D	68.02 ± 43.00 (63.23)	222.48 ± 210.40 (94.57)	305.67 ± 226.68(74.16)	354.18 ± 108.34 (30.59)	201.27 ± 24.02 (11.93)
30	1708	β-Selinene	6.64 ± 2.52 (37.98	16.31 ± 7.07 (43.34)	26.92 ± 14.48 (53.79)	55.69 ± 43.69 (78.46)	13.84 ± 2.27 (16.41)
31	1713	α-Selinene	4.91 ± 0.85 (17.29)	13.57 ± 2.24 (16.49)	23.08 ± 8.20 (35.53)	50.31 ± 12.34 (24.52)	38.87 ± 9.36 (24.08)
32	1720	Bicyclogermacrene	26.63 ± 4.62 (17.33)	66.95 ± 22.04 (32.93)	110.32 ± 45.11 (40.89)	182.19 ± 48.35 (26.54)	67.87 ± 6.98 (10.29)
33	1747	δ-Cardinene	26.42 ± 15.28 (57.84)	73.06 ± 30.01 (41.07)	146.03 ± 72.24 (49.47)	346.51 ± 152.41 (43.98)	92.99 ± 14.35 (15.43)
34	1871	2-Methoxy phenol	–	–	–	32.23 ± 18.78 (58.27)	–
35	1921	1-Phenylethyl alcohol	–	–	–	39.81 ± 19.50 (48.98)	8.85 ± 1.95 (22.03)
36	2013	Methyl eugenol	–	–	–	–	64.09 ± 29.08 (45.38)
37	2028	Phenol	–	–	1.15 ± 0.83 (72.56)	128.88 ± 132.37 (102.70)	–
38	2127	Spathulenol	–	5.84 ± 3.94 (67.37)	7.98 ± 4.74 (59.38)	29.26 ± 22.48 (76.85)	9.21 ± 1.01 (10.98)
39	2189	T-Cadinol	–	3.99 ± 1.56 (39.21)	9.42 ± 4.83 (51.25)	21.60 ± 9.99 (46.22)	24.96 ± 3.22 (12.90)
40	2211	4-Vinyl-2-methoxy phenol	–	–	–	108.70 ± 24.96 (22.96)	–
41	2236	T-Muurolol	–	6.20 ± 2.69 (43.39)	12.49 ± 4.04 (32.38)	37.45 ± 19.04 (50.83)	54.80 ± 1.21 (2.21)
42	2417	4-Vinyl phenol	–	–	1.60 ± 1.59 (94.42)	146.94 ± 122.53(83.39)	–
43	2486	Lauric acid	–	–	–	–	9.69 ± 2.14 (22.13)
44	2497	Benzoic acid	–	–	66.68 ± 67.07 (100.59)	270.84 ± 127.14 (46.94)	

[a] Retention indices on an innowax column relative to C_6—C_{26} *n*-alkanes.
[b] Standard deviation.
[c] Relative standard deviation.
[d] Not detected.
[e] Trace.

(56.8–168.5 μg g^{-1}, 1.6–4.2%), β-phellandrene (193.4–457.4 μg g^{-1}, 3.2–9.6%), α-terpinolene (98.6–554.4 μg g^{-1}, 2.8–8.0%), β-caryophyllene (91.3–485.6 μg g^{-1}, 2.9–6.4%), and germacrene D (68.0–354.2 μg g^{-1}, 1.6–4.6%). The 100% value in this sense corresponds to all detected compounds.

The above results are similar to those of Yu et al. [8], who used SDE and HS-SPME method for the analysis of *P. densiflora* pine needles, from trees growing in South Korea. Here, the main components were α-pinene (21.8–34.2%), β-phellandrene (17.1–21.4%), β-caryophyllene (7.7–10.0%), and germacrene D (5.5–11.0%). Also, the analysis of Woo et al. [6] using a supercritical fluid extraction device on Korean pine twigs (*P. densiflora* S.), found that the main ingredients were α-pinene (5.3–11.7%), β-pinene (10.8–18.5%), β-myrcene (11.5–17.3%), limonene (32.6–43.4%), and germacrene D (5.6–11.3%). In addition, using an SDE device, the same workers found the main components to be α-pinene (12.0%), β-pinene (19.1%), β-myrcene (12.0%), and limonene (37.1%). Thus, it can be seen that these variations are due to differences not only in extraction methods, but also to differences in the organic compound composition of pine needles and twigs of the same species.

The data in Table 1 show that increases in thermal desorption temperatures generally leads to increases in both the number and quantity of volatile constituents extracted from the pine needles. In addition, compounds that are assumed to be the results of thermal decomposition (acetol, furfural, acetic acid, furfuryl alcohol, phenol, and 4-vinylphenol) were not identified at desorption temperatures below 250 °C. Also, benzaldehyde was identified at 250 °C but was absent at 300 °C, and 2-methoxyphenol, 1-phenethyl alcohol and 4-vinyl-2-methoxyphenol were not detected until the 300 °C thermal desorption temperature. All of the above-mentioned compounds, except 1-phenethyl alcohol and benzaldehyde, were not identified in the pine needles using the SDE method, thus supporting the presumption that they are the products of thermal decompositions of certain natural components. Consequently, it appears that the analysis of the volatile components of pine needles using a double-shot pyrolyzer is best carried out at thermal desorption temperatures below 250 °C: in particular, at 200 °C.

A comparison of the DSP results at 200 °C with the SDE results (Table 1) shows that 31 compounds were identified by the former method, as opposed to 33 identified by the latter method, otherwise the TIC profiles (Fig. 1, second and fifth chromatograms) for the two methods are very similar.

As presented in Table 1, the components spathulenol, T-cadinol and T-muurolol, which are sesquiterpenoid alcohols, were not identified at 150 °C, and were increasingly desorbed at higher temperatures. These results are in accord with the known relatively low volatility of sesquiterpenoid alcohols.

At each of the four thermal desorption temperatures, the relative standard deviation (%RSD) for each component was calculated from three experiments in order to determine the reproducibility of the double-shot pyrolysis method (Table 1, columns 4–7). It can be seen that, for those components that are not thermal decomposition products, most of the RSD values are less than 60%, with a few components showing a larger deviation. In general, the reproducibility of the DSP method is somewhat lower than that of the SDE method, where most of the RSD values are less than 40% (Table 1, column 8). However, the present DSP results are similar to the ATD 400 thermal desorber results of Sanz et al. [15] where most of the RSD values for the analysis of the volatile constituents of lavender leaves were reported to be less than 60%.

As mentioned above, this pyrolytic technique gives a larger deviation and lower reproducibility than the SDE method. This is because the pyrolysis device requires very small samples (∼ 0.02 g, as opposed to 10–200 g for SDE) and because desorption of volatile matter in the device occurs over a short period of time.

As presented in Fig. 1, the peaks of the SDE-chromatogram below the retention time of 20 min are sharp, whereas the corresponding peaks of the DSP chromatograms are broader. In addition, there are small differences in retention times between the same components in the SDE and DSP chromatograms. These differences are explained as follows. Direct injection of an SDE sample into the GC injector, using a syringe, delivers the volatile organic components to the column in about 1 s or less. However, in the case of the DSP method, the same components are delivered to the column over 6 s, since volatilization occurs in the desorption device, which is installed on top of the GC injector, and this takes 6 s.

Fig. 2 presents the total ion chromatograms obtained from the analysis of pine needles by heating the sample for 1 s, 6 s, 30 s, 150 s, and 300 s in order to determine the optimal heating time at the thermal desorption temperature of 200 °C in the double-shot pyrolyzer. Table 2 summarizes the semi-quantitative data from these experiments, where it can be seen that the quantities of volatile organic components mostly increase as the heating time is increased. In the case of the major constituent α-pinene, the desorbed quantities were 206.4 μg g^{-1} after heating for 1 s, 621.7 μg g^{-1} after heating for 6 s, 1283.9 μg g^{-1} after heating for 30 s, 1549.8 μg g^{-1} after heating for 150 s, and 2039.9 μg g^{-1} after heating for 300 s. Also, numbers of desorbed volatile compounds significantly increased with increase in heating time. Thus, there were only 16 such components identified when the volatized ingredients were directly injected into the GC–MS (i.e. 1 s), whereas 31 volatile compounds were identified when the sample was desorbed at 200 °C in the double-shot pyrolyzer for 6 s.

The identified numbers of volatile organic compounds were 33 for a heating time of 30 s and 32 for the heating times of 150 s and 300 s. This demonstrates that there are no large differences in the numbers of identified components for heating times over 6 s.

The constituents 2-hexenal and *cis*-2-penten-1-ol were identified only at the shorter heating times of 6 s and 30 s.

Table 2
Semi-quantitative results for major volatile organic components of *P. densiflora* S. needles obtained by DSP–GC–MS at the thermal desorption temperature of 200 °C, using different heating times

Peak number	RI[a]	Component number	Concentration ($\mu g\,g^{-1}$) ± SD[b] (RSD[c] (%); $n = 3$)				
			1 s	6 s	30 s	150 s	300 s
1	1047	Tricyclene	5.36 ± 2.52 (47.01)	20.77 ± 11.09 (53.38)	101.25 ± 16.48 (16.28)	102.21 ± 68.47 (66.99)	116.10 ± 31.06 (26.75)
2	1056	α-Pinene	206.42 ± 19.56 (9.47)	621.66 ± 28.79 (4.63)	1283.85 ± 200.20 (15.59)	1549.80 ± 566.86 (36.58)	2039.85 ± 260.50(12.77)
3	1083	Camphene	17.95 ± 6.65 (37.05)	85.99 ± 25.20 (29.31)	226.80 ± 50.34 (22.19)	270.00 ± 82.87 (30.69)	360.45 ± 95.76 (26.57)
4	1107	β-Pinene	25.38 ± 7.97 (31.39)	77.07 ± 1.39 (1.81)	167.40 ± 68.35 (40.83)	244.35 ± 63.76 (26.09)	272.70 ± 71.51 (26.22)
5	1119	Sabinene	3.71 ± 1.00 (26.93)	10.87 ± 6.56 (60.39)	17.55 ± 6.82 (38.85)	36.45 ± 14.58 (40.01)	35.10 ± 7.11 (20.26)
6	1154	β-Myrcene	49.95 ± 15.11 (30.24)	102.25 ± 53.58 (52.40)	168.75 ± 58.11 (34.44)	167.40 ± 39.87 (23.82)	221.40 ± 46.10 (20.82)
7	1180	Limonene	10.73 ± 4.41 (41.09)	101.59 ± 12.99 (12.79)	221.05 ± 73.61 (33.30)	220.05 ± 58.15 (26.43)	249.75 ± 97.49 (39.04)
8	1189	β-Phellandrene	143.66 ± 46.16 (32.13)	350.60 ± 170.33 (48.58)	450.90 ± 159.41 (35.35)	496.80 ± 110.60 (22.69)	765.45 ± 177.96 (23.25)
9	1225	2-Hexenal	–[d]	25.84 ± 3.34 (12.93)	8.10 ± 2.24 (27.69)	–	–
11	1248	α-Terpinolene	17.54 ± 8.68 (49.51)	148.82 ± 110.85 (74.49)	399.60 ± 134.62 (33.69)	306.45 ± 103.43 (33.75)	352.35 ± 130.27 (36.97)
13	1334	*cis*-2-Penten-1-ol	–	2.87 ± 1.02 (35.61)	*t*[e]	–	–
14	1394	*cis*-3-Hexenol	4.95 ± 2.54 (51.35)	55.30 ± 26.81 (48.48)	6.75 ± 5.03 (74.58)	9,45 ± 5.21 (55.13)	2.70 ± 0.89 (32.83)
15	1442	α-Cubebene	–	2.11 ± 0.59 (27.68)	9.45 ± 3.06 (32.43)	17.55 ± 2.94 (16.77)	13.50 ± 1.19 (8.85)
16	1471	Furfural	–	–	–	13.50 ± 18.56 (139.68)	12.50 ± 16.79 (134.32)
17	1473	α-Copaene	–	5.32 ± 0.76 (14.24)	12.15 ± 3.06 (25.16)	36.45 ± 31.83 (87.33)	40.50 ± 37.72 (93.14)
18	1486	Acetic acid	–	–	1.35 ± 0.44 (32.81)	32.40 ± 9.84 (30.37)	36.40 ± 5.76 (15.83)
19	1498	β-Bourbonene	–	2.04 ± 0.64 (31.35)	4.05 ± 3.22 (79.59)	6.75 ± 2.34 (34.71)	2.70 ± 0.70 (25.80)
20	1525	β-Cubebene	–	1.77 ± 0.45 (25.51)	6.75 ± 1.79 (26.50)	13.50 ± 4.91 (36.33)	9.45 ± 1.00 (10.59)
21	1529	Benzaldehyde	–	1.27 ± 1.24 (98.08)	*t*	2.90 ± 4.54 (156.44)	*t*
22	1551	Isolongifolene	–	10.96 ± 5.64 (51.47)	4.05 ± 3.59 (88.71)	*t*	20.25 ± 24.95 (123.23)
23	1575	Bornyl acetate	4.75 ± 3.14 (66.17)	76.62 ± 35.33 (46.11)	112.05 ± 74.36 (66.36)	155.25 ± 17.62 (11.35)	182.25 ± 129.98 (71.32)
24	1579	β-Elemene	–	14.56 ± 4.73 (32.50)	32.40 ± 8.84 (27.30)	56.70 ± 16.24 (28.64)	59.40 ± 7.29 (12.27)
25	1584	β-Caryophyllene	88.76 ± 10.62 (11.96)	196.21 ± 49.88 (25.42)	379.35 ± 66.24 (17.46)	514.35 ± 82.40 (16.02)	672.30 ± 41.42 (6.16)
26	1656	α-Humulene	12.79 ± 0.55 (4.28)	32.29 ± 9.44 (29.22)	75.60 ± 15.66 (20.71)	95.85 ± 19.56 (20.41)	126.90 ± 10.26 (8.08)
28	1674	α-Amorphene	–	11.03 ± 7.54 (68.37)	32.40 ± 6.77 20.89)	52.65 ± 10.13 (19.23)	62.10 ± 0.32 (0.52)
29	1694	Germacrene D	44.58 ± 3.62 (8.11)	222.48 ± 210.40 (94.57)	214.65 ± 26.48 (12.34)	276.75 ± 85.37 (30.85)	314.55 ± 85.62 (27.22)
30	1708	β-Selinene	–	16.31 ± 7.07 (43.34)	44.55 ± 11.68 (26.12)	70.20 ± 10.68 (15.22)	85.05 ± 5.97 (7.02)
31	1713	α-Selinene	–	13.57 ± 2.24 (16.49)	32.40 ± 9.03 (27.87)	51.30 ± 12.78 (24.91)	103.95 ± 9.74 (9.37)
32	1720	Bicyclogermacrene	16.30 ± 3.18 (19.53)	66.95 ± 22.04 (32.93)	178.20 ± 43.64 (24.49)	202.50 ± 40.92 (20.21)	247.05 ± 36.24 (14.67)
33	1747	δ-Cardinene	21.67 ± 9.12 (42.07)	73.06 ± 30.01 (41.07)	271.35 ± 72.75 (26.81)	365.85 ± 70.16 (19.18)	465.75 ± 23.41 (5.03)
38	2127	Spathulenol	–	5.84 ± 3.94 (67.37)	17.24 ± 2.88 (16.70)	53.86 ± 10.48 (19.45)	26.27 ± 9.09 (34.59)
39	2189	T-Cadinol	–	3.99 ± 1.56 (39.21)	22.63 ± 0.78 (3.45)	33.75 ± 12.74 (37.74)	26.01 ± 6.75 (25.94)
41	2236	T-Muurol	–	6.20 ± 2.69 (43.39)	20.93 ± 6.90 (32.98)	40.23 ± 10.13 (25.19)	45.27 ± 9.49 (20.97)
44	2497	Benzoic acid	–	–	122.99 ± 43.47 (35.35)	439.19 ± 317.12 (72.21)	133.66 ± 24.93 (18.65)

[a] Retention indices on an Innowax column relative to C_6—C_{26} *n*-alkanes.
[b] Standard deviation.
[c] Relative standard deviation.
[d] Not detected.
[e] Trace.

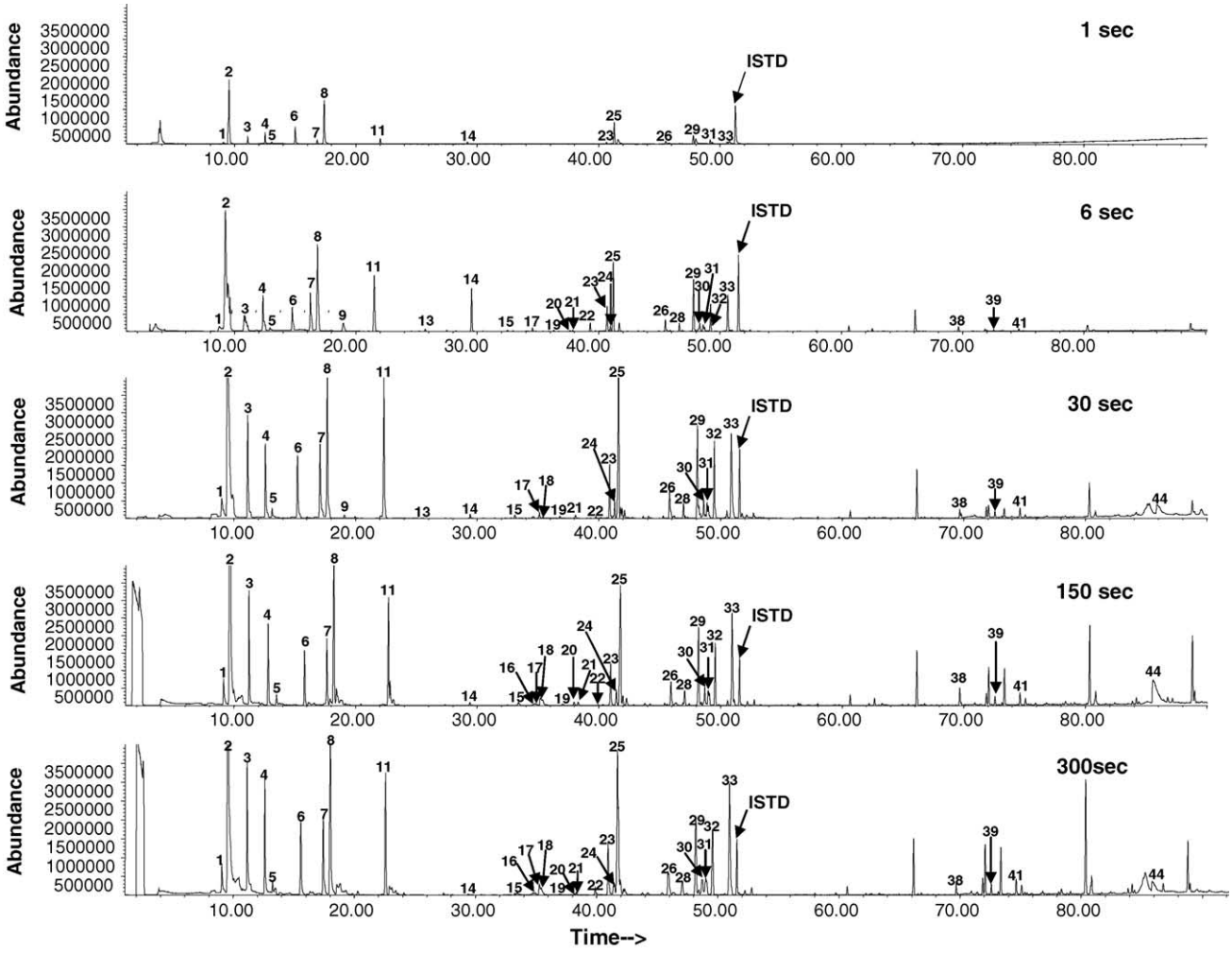

Fig. 2. TIC profiles of pine needle volatiles obtained at different heating times, at a thermal desorption temperature of 200 °C.

On the other hand, furfural, which has already been assumed to be a thermal decomposition product, was only identified at heating times of 150 s and above, and acetic acid was first identified at the heating time of 30 s. These results suggest that 2-hexenal and *cis*-2-penten-1-ol are thermally less stable than many other components at heating times of 150 s and above.

4. Conclusions

A thermal desorption temperature of 200 °C and a heating time of 6 s were found to be the optimum conditions for the analysis of the volatile organic constituents of pine needles using a double-shot pyrolyzer and GC–MS. A total of 41 volatile compounds were verified for thermal desorption temperatures of 150 °C, 200 °C, 250 °C, and 300 °C. However, at temperatures of 250 °C and above, significant amounts of thermal decomposition products were observed, including acetol, acetic acid, furfural compounds and phenols. Similarly, thermal decomposition products were detected at the thermal desorption temperature of 200 °C when heating times exceeded 6 s. The reproducibility of the DSP–GC–MS method was found to be rather lower than the SDE–GC–MS method that was used for comparison. However, total ion chromatographic profiles of the volatile components of pine needles presented by the two methods under optimum conditions are very similar. Moreover, use of the double-shot pyrolyzer was found to have certain advantages: it requires no sample preparation, it gives a rapid extraction from small amounts (under 10 mg), it is not labour-intensive and thus the method may be applied to quality control and other related fields.

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