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Volatile Components of Pine Needles from *Pinus densiflora* S. using Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry

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Abstract

The volatile components of *Pinus densiflora* needles were studied by gas chromatography-mass spectrometry (GC-MS), using seven kinds of solid phase microextraction (SPME) fibers, seven in SPME fibers: 100 μm PDMS, 65 μm PDMS/DVB, 65 μm SF-PDMS/DVB, 85 μm PA, 75 μm CAR/PDMS, 65 μm CW/DVB and 50/30 μm DVB/CAR/PDMS fibers. A total of 40 components were identified by using the seven different SPME fibers. The identified components were classified, according to their functionalities, as follows: 26 hydrocarbons, 7 alcohols, 4 carbonyl compounds, and 3 esters. The major volatile components of *Pinus densiflora* needles identified by these SPME fibers were α-pinene (1.7~21.7 μg/g), β-myrcene (2.0~20.1 μg/g), β-phellandrene (4.6~22.8 μg/g), β-caryophyllene (6.7~26.0 μg/g) germacrene D (1.1~11.9 μg/g). In the comparison of the seven SPME fibers, PDMS appeared to be the most suitable fiber for the analysis of hydrocarbon compounds and CAR/PDMS, PDMS/DVB, CW/DVB and DVB/CAR/PDMS are shown to be optimal for analysis of the alcohols and carbonyl compounds.

Key words: *Pinus densiflora*, solid phase microextraction, volatile components, GC-MS

Introduction

Pinus densiflora S. is native to Korea, Japan, China and East Russia[1]. It is known to the west as Japanese red pine[2]. Pine needles have been valued for their medical effects and used in popular medicines for the treatment of hepatosis, various neurological disorders and arteriosclerosis[3]. Especially in Korea, pine needle have been widely used as food materials and folk medicines for a long time[4]. Various pine-based products, such as beverages, tea, wine and candy, have become available from the market[5]. Also, since the efficacy of the pine needles as an anti-microbial, anti-cancer, anti-oxidant, skin lightening and anti-agent in health has been recognized, studies of certain functional elements contained in pine needles have been conducted in medicines and cosmetics[6,7]. In recent years, there are large increases in the need for natural flavourings, following increases in the demand for natural products, as opposed to synthetic products. This trend seems to be continued for decades. In response to this situation, there have been some studies on the volatile ingredients of pine needles. Lee *et al.* analyzed the volatile components of *Pinus densiflora* needles grown in Korea by using purge and trap headspace apparatus[8]. Yu *et al.*[9] analyzed the differences in the extraction of volatile components of *Pinus densiflora* needles grown in Korea by using SDE and SPME. Hong *et al.*[10] have analyzed the volatile components of *Pinus rigida* needles by using steam distillation and solvent extraction. Woo *et al.*[11] reported the differences

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in the composition of volatile components of pine needles by using the supercritical fluid extraction and steam distillation. In recent years, Lee *et al.* established a new analysis method that analyzes the volatile component of pine needles by using a pyrolyzer device, which is used to analyze polymer substances.

Also, they reported that the pyrolyzer analysis presented several advantages in the analysis of the volatile components of pine needles compared to that of the SDE method, which is largely used as a conventional method[12]. Among various extraction methods for isolation of volatile compounds in plants, SDE is the most commonly used method[13]. Although SDE is often known as one of the best methods to obtain high recovery of various compounds, it has many drawbacks such as formation of artifacts, use of organic solvents, and undesirable reactions[13]. To avoid these obstacles in SDE, headspace sampling techniques have attracted special attention, because analytes can be obtained without the use of an organic solvent. Headspace solid phase microextraction (HS-SPME), in particular, has been widely applied to the analysis of volatile components in various food samples, because it is fast, inexpensive, and easy to use[14]. SPME has been used in various ranges of fields including studies of flavours and taints especially for quick screening of the volatile composition of a wide range of products[15]. Traditional fibres (PDMS and PA) have been used to determine terpenes and fermentation compounds in food samples, however these fibers present the poor sensitivity for polar compounds[16]. Mixed coating fibers containing divinylbenzene (DVB), PDMS, and carboxen (CAR) or polyethylene glycol (CW), increase the adsorption ability of the fiber by the synergic effect of adsorption and distribution within the stationary phase, producing higher sensitivity than PDMS and PA fibers[17]. In studies on SPME fibres up to the recent years, 3~5 different fibres were usually used to compare the characteristics between fibres. In the present study, 7 different SPME fibres that have differences in the stationary phase and film thickness in order to analyze the volatile component of pine needles were used.

The aim of this study is to compare the results of the composition of the volatile components obtained from the 7 different SPME fibres.

Materials and Methods

1. Plant Material and Reagents

Pinus densiflora needles were collected from mountains near Daejeon, South Korea in August 2005, stored in solvent-cleaned glass jars with aluminium foil-lined lids and were refrigerated at 3℃ in the laboratory until the required time for analysis.

All organic solvents were analytical grade and were purchased from Sigma.

2. Solid Phase Microextraction (SPME)

After placing pine needles (0.2 g) and internal standard *n*-decanol (0.5 μL of a 0.45 mg/mL ethanol solution) into the 20 mL vial, the vial was sealed using a teflon-faced septum cap. the SPME fiber was fitted into the sealed vial, whence it was exposed and its height was adjusted in the headspace above the pine needles. Then, the vial was placed in a heating block and the SPME fiber was exposed to the headspace above the sample for 20 min at 35℃. The SPME fibers containing the extracted volatile components were fitted in the GC injector at 250 ℃ and the desorption process was applied for 2 minutes in order to transfer the volatile components into the GC-MS.

In these experiments, a 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PD MS/DVB), 65 μm stableflex polydimethylsiloxane/divinylbenzene(SFPDMS/DVB), 85 μm polyacrylate(PA), 75 μm carboxen/polydimethylsiloxane(CAR/PDMS), 65 μm carbowax/divinylbenzene(CW/DVB), and 50/30 μm stableflex divinylbenzene/carboxen/polidimethylsiloxane (DVB/ CAR/PDMS) were used as SPME fibers, and they were conditioned by fitting them into the GC injector at 260℃ for 10 min prior to the extraction experiments.

3. GC-MS Analysis

The GC-MS equipment consisted of an Agilent 6890 gas chromatograph, is equipped with an Innowax capillary column (50 m, 0.25 mm i.d., 0.25 μm film; polyethylene glycol as stationary phase). Injector temperature was maintained at 250℃ , with splitless type at the initial time. The detector is 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 ranges. Interface and source temperature were 250℃ and 230℃, respectively.

The carrier gas used was helium with a controlled flow of 1.0 mL/min. The GC oven temperature was programmed from 50℃ (3 min) to 220℃ (20 min) by increasing the temperature at a rate of 2℃/min.

The identification of the separated volatile 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_{29} n-alkanes, were compared with those reported in the literature[18]. Semi-quantitative values were obtained by using n-decanol as an internal standard.

Results and Discussions

Several fiber coatings are commercially available for the extraction of volatile compounds. The affinity of the fiber for an analyte depends on the principle "like dissolves like", and coating fibers that have different properties or thickness are selected in accordance with different compounds. In order to analyze the adsorption efficiency, 7 SPME fibers, such as 100 μm PDMS, 65 μm PDMS/DVB, 65 μm SFPDMS/DVB, 85 μm PA, 75 μm CAR/PDMS, 65 μm CW/DVB, and 50/30 μm DVB/ CAR/PDMS fibers, were used.

Fig. 1 presents the total ion chromatogram (TIC) produce by the results of the analysis of the volatile component of *Pinus densiflora* needles using 7 SMPE fibers. Table 1 presents the results of the analysis of the peak, which was separated using the GC, using the GC retention time and GC/MS. As noted in Table 1, a total number of 40 components were verified using 7 SPME fibers. The identified compounds were classified, according to their functionalities, as follows: 26 hydrocarbons, 7 alcohols, 4 carbonyl compounds, and 3 esters. The major volatile compounds of *Pinus densiflora* needles verified by using SPME were α-pinene (1.7~21.7 μg/g), β-myrcene (2.0~20.1 μg/g), β-phellandrene (4.6~22.8 μg/g), β-caryophyllene (6.7~26.0 μg/g), germacrene D (1.1~11.9 μg/g). These results are different from those of Lee *et al.*[12], who used a double-shot pyrolysis method for the analysis of *Pinus densiflora* needles, grown in Korea. The main components were α-pinene (323.1 μg/ g), β-myrcene (87.1 μg/g), β-phellandrene (193.4 μg/ g), β-caryophyllene (91.3 μg/g), germacrene D (68.0 μg/ g). However, these results have a similar tendency from the results of the study reported by Lee *et al.* and Yu *et al.* in which they reported that the major component of *Pinus densiflora* needles are α-pinene, β-myrcene, β-phellandrene, β-caryophyllene, and germacrene D[8,9].

Thus, We can see that these differences are due to the differences in extraction methods. Eakin *et al.* have reported that compounds give rise to flavouring properties described as herb, spicy, and citrus are not terpenoid hydrocarbons but are oxygenated terpenes, such as terpene

Table 1. Comparison on volatile components obtained from the seven kinds of SPME fibers

Concentrate (μg/g)

Peak No.	RI[a]	Components	PDMS[b]	PDMS-DVB[c]	SF-PDMS-DVB[d]	PA[e]	CAR-PDMS[f]	CW-DVB[g]	DVB-CAR-PDMS[h]
1	990	Tricyclene	0.69	0.29	0.36	–	0.20	0.03	0.42
2	1003	α-Pinene	21.70	10.08	12.92	1.71	7.19	1.98	14.19
3	1064	Camphene	2.89	1.32	1.73	0.18	0.89	0.31	1.89
4	1116	β-Pinene	4.49	2.10	2.82	0.43	1.88	0.60	2.86
5	1128	Sabinene	0.73	0.35	0.53	0.08	0.53	0.16	0.53
6	1171	β-Myrcene	9.58	8.18	9.09	1.97	20.55	3.19	10.12
7	1206	Limonene	3.26	2.28	3.02	0.68	5.27	1.10	3.28
8	1218	β-Phellandrene	22.83	12.78	18.77	4.58	20.09	6.29	19.32
9	1230	2-Hexenal	–[i]	0.05	0.17	tr[j]	Tr	tr	0.12

Table 1. Continued

Peak No.	RI[a]	Components	PDMS[b]	PDMS-DVB[c]	SF-PDMS-DVB[d]	PA[e]	CAR-PDMS[f]	CW-DVB[g]	DVB-CAR-PDMS[h]
10	1253	r-Terpinene	0.22	0.21	0.21	tr	0.27	0.08	0.25
11	1277	p-Cymene	0.03	0.06	0.06	-	7.86	-	0.11
12	1289	α-Terpinolene	9.79	7.38	9.58	2.69	6.81	4.76	10.40
13	1306	Ethyl-3-hexenoate	0.02	0.09	0.03	-	0.05	0.03	0.06
14	1357	Hexanol	0.06	0.18	0.08	0.38	0.15	0.12	0.35
15	1390	cis-3-Hexenol	1.22	2.15	2.74	6.03	5.10	2.24	4.73
16	1463	α-Cubebene	0.18	0.13	0.09	0.03	0.28	0.05	0.20
17	1473	α-Longipinene	0.31	0.13	0.14	0.02	0.15	0.08	0.36
18	1486	Bicycloelemene	0.68	0.29	0.29	0.20	0.32	0.26	0.64
19	1505	Decanal	0.06	0.06	0.04	-	0.11	0.03	0.10
20	1524	β-Bourbonene	0.11	0.06	0.06	tr	0.06	0.04	0.13
21	1539	Benzaldehyde	-	0.02	-	-	0.03	-	0.11
22	1577	Isolongifolene	4.05	1.66	1.73	0.46	1.38	1.07	3.09
23	1590	Bornyl acetate	4.98	2.41	2.70	1.37	3.32	1.45	2.93
24	1600	β -Elemene	1.84	0.84	0.73	0.72	0.84	0.62	1.59
25	1608	β -Caryophyllene	26.00	14.54	11.82	6.69	13.66	7.82	20.78
26	1648	Butyrolactone	-	-	-	0.92	-	0.34	-
27	1677	α-Humulene	3.59	1.94	1.50	1.05	2.07	1.08	2.91
28	1694	α-Amorphene	0.85	0.61	0.35	0.28	0.61	0.28	0.84
29	1709	Borneol	0.08	0.06	0.03	0.04	0.09	0.05	0.07
30	1717	Germacrene D	11.88	4.87	3.99	4.44	1.12	3.66	8.75
31	1727	β -Selinene	0.77	0.38	0.28	0.23	0.34	0.22	0.60
32	1731	α-Selinene	0.87	0.52	0.33	0.36	0.41	0.31	0.78
33	1742	Bicyclogermacrene	2.83	1.15	1.13	1.04	0.90	0.95	2.19
34	1766	δ-Cardinene	1.64	1.03	0.64	0.86	0.81	5.83	1.48
35	1799	α-Cardinene	0.10	0.11	0.05	-	0.11	0.06	0.13
36	1842	Calamenene	-	0.02	tr	-	0.19	0.01	0.04
37	1850	Ethyl laurate	0.07	0.03	0.01	0.11	0.02	0.02	0.04
38	2028	Methyl eugenol	0.40	0.35	0.26	1.07	0.07	1.74	0.79
39	2181	T-Cadinol	-	-	-	-	tr	-	-
40	2243	T-Muurol	-	-	-	-	tr	-	-

[a] Retention indices on the Innowax column relative to C_6~C_{26} n-alkanes, [b] 100 μm polydimethylsiloxane(PDMS). [c] 65 μm polydimethylsiloxane/divinylbenzene(PDMS/DVB), [d] 65 μm stableflex polydimethylsiloxane/divinylbenzene(SF/PDMS/DVB), [e] 85 μm polyacrylate(PA), [f] 75 μm carboxen/polydimethylsiloxane(CAR/PDMS), [g] 65 μm carbowax/divinylbenzene (CW/DVB), [h] 50/30 μm stableflex divinylbenzene/carboxen/polydimethylsiloxane(DVB/CAR/PDMS), [i] Not detected, [j] Trace.

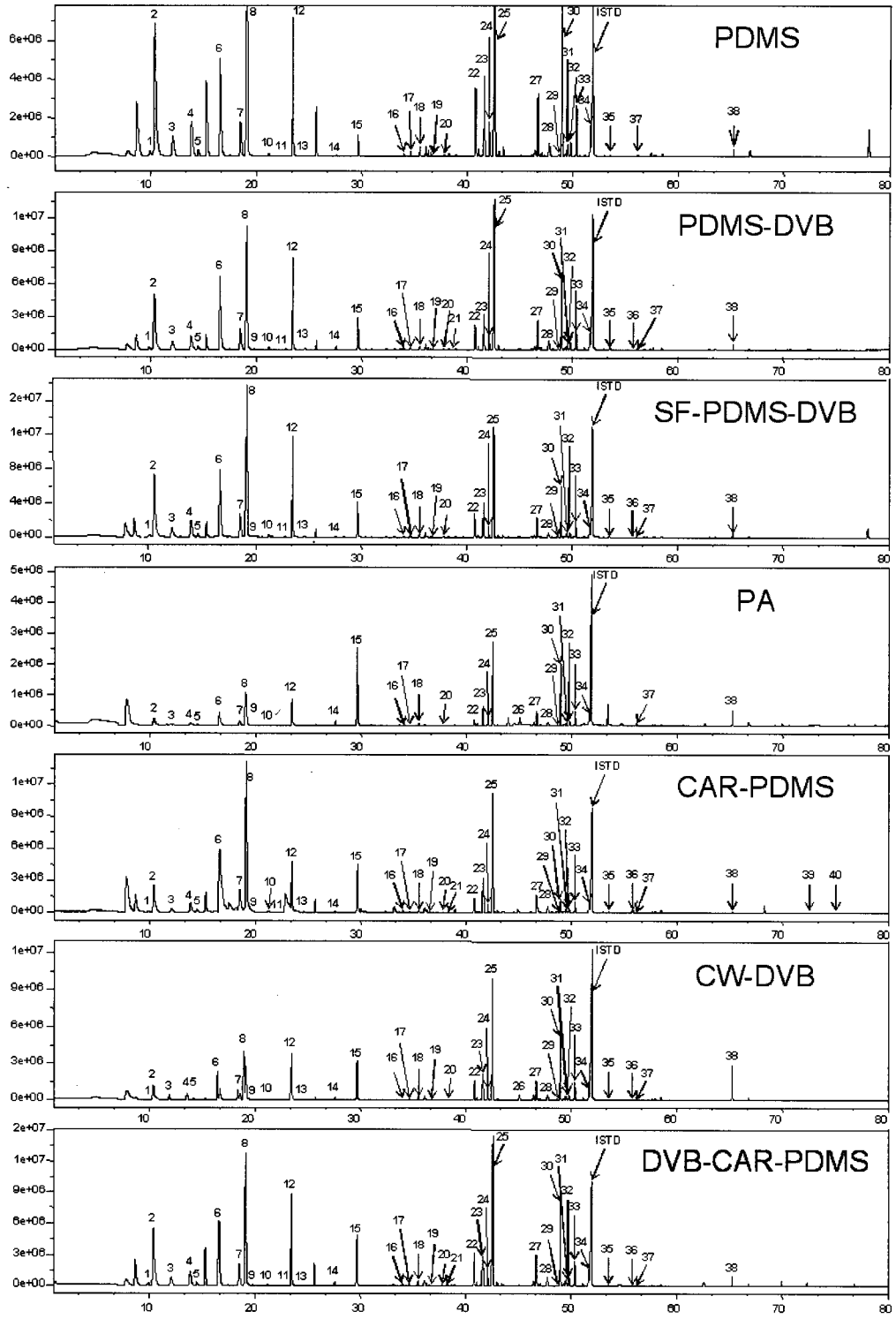

Fig. 1. TIC profiles of pine needle volatiles obtained from the seven kinds of SPME fiber.

alcohols or terpene esters[19]. These studies verified the presence of the oxygenated terpene substrates, such as 2-hexenal and *cis*-3-hexenol. From the comparison of the volatile component of 7 SPME fibers, the largest number of 39 components was observed in the 75 μm CAR/PDMS, 37 components were identified in the 65 μm PDMS/DVB, 36 components were identified in the 65 μm CW/DVB and 50/30 μm DVB/CAR/PDMS, respectively, 34

components were identified in the 100 μm PDMS, and the smallest number of 32 components was identified in the 85 μm PA. These results presented a similar result compared to the report of Marco *et al.*[20] where 82 components were identified in the 75 μm CAR/PDMS fiber, and 66 components were identified in the 50/30 μm DVB/CAR/PDMS fiber from the results of the analysis of the volatile component of fermented sausages using CAR/PDMS and DVB/CAR/PDMS fibers.

In addition, the results of these studies were similar to the report of Oscar *et al.*[21] where the largest number of 9 components were identified in the 75 μm CAR/PDMS fiber and 50/30 μm DVB/CAR/PDMS fiber, respectively, 8 components were identified in the 65 μm PDMS/DVB fiber, 6 components identified in the 85 μm PA fiber, and the smallest number of 4 components were identified in the 100 μm PDMS fiber from the results of the analysis of the volatile component of packaging materials using 5 different SPME fibers. It is evident that these two results presented similarities, even though they also presented slight differences. In the case of the 100 μm PDMS fiber, the largest amount of hydrocarbon components, which are included in the group of monoterpenes and sesquiterpenes, were extracted, but carbonyl compounds, such as 2-hexenal, benzaldehyde, and butyrolactone, were not extracted. These were due to the fact that the adsorbed volatile component can be varied according to the material of SPME fibers. Thus, it is very important to select the most optimal fiber for achieving the objective substance in the analysis of the volatile component using a SPME device.

In the case of the 85 μm PA fiber, where the smallest number of 32 components were identified among 7 fibers, it is known that the smallest amount of hydrocarbons, which present the characteristics of woody, piney, and fruity aroma[22] was extracted.

However, the largest amount of hexanol, which presents a wine-like and fatty-fruity odor, and *cis*-3-hexenol, which presents green odor, were extracted in the 85 μm PA fiber. Butyrolactone, which presents a coconut-butter aroma, was only observed in the 85 μm PA and 65 μm CW/DVB fibers, respectively, and was not observed in other fibers. This means that the lactone compound is not easily adsorbed by the fiber with PDMS materials.

Benzaldehyde, which presents bitter and sweet-cherry odors, was only verified in the PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS, respectively, and was not found in other fibers. These results are similar to the report of Eva *et al.* where benzaldehyde was not extracted in the CW/DVB fiber from the results of the analysis of the volatile component of grapes using the SPME fiber, such as PDMS/DVB, DVB/CAR/PDMS, and CW/DVB even though it was extracted in the PDMS/DVB and DVB/CAR/PDMS fiber, respectively.

The results of these studies were similar to the report of Song *et al.*[16] where PDMS and PA fibres well adsorbed non-polar components, and CAR-PDMA and CW-DVB fibres well adsorbed polar components [16,17].

From these results, it is obvious that the PDMS fiber is the most appropriate for analyzing the component of non-polar monoterpenes or non-polar sesquiterpenes using a SPME device, the CAR/PDMS, PDMS/DVB, CW/DVB, and DVB/CAR/PDMS fibers are optimal for analysis of alcohols and carbonyl compounds, and the PA fiber is the most appropriate for analyzing the component of low molecular alcohol components.

Conclusions

Volatile components of pine needles (*Pinus densiflora*) were isolated by seven solid phase microextraction (SPME) fibers and analyzed by gas chromatography-mass spectrometry (GC-MS). Seven in SPME fibers: 100 μm PDMS, 65 μm PDMS/DVB, 65 μm SFPDMS/DVB, 85 μm PA, 75 μm CAR/PDMS, 65 μm CW/DVB, and 50/30 μm DVB/CAR/PDMS fibers.

A total of 40 compounds were identified by using the seven different SPME fibers. The identified compounds were classified, according to their functionalities, as follows: 26 hydrocarbons, 7 alcohols, 4 carbonyl compounds, and 3 esters. The major compounds were α-pinene (1.7~21.7 μg/g), β-myrcene (2.0~20.1 μg/g), β-phellandrene (4.6~22.8 μg/g), β-caryophyllene (6.7~26.0 μg/g), germacrene D (1.1~11.9 μg/g).

The PDMS fiber appeared to be the most suitable fiber for the analysis of hydrocarbon compounds, and the CAR/PDMS, PDMS/DVB, CW/DVB and DVB/CAR/PDMS fibers have shown to be optimal for analysis of alcohols and carbonyl compounds.

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