Profiling of Volatile Components Using Gas Chromatography-Mass Spectrometry in Commercial Pine Needle (*Pinus densiflora* S. and Z.) Powder

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Abstract

Volatile components in commercial pine needle (*Pinus densiflora* S. and Z.) powder were extracted using simultaneous steam distillation and a solvent extraction (SDE) apparatus, and were analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 230 compounds divided into 13 groups were identified, which included alcohols (42), ketones (39), aldehydes (32), terpenes (30), alkenes (17), esters (14), furans (14), benzenes (10), alkanes (8), napthalenes (7), acids (6), miscellaneous compounds (6), and phenols (5). Among the 230 compounds identified, 96 compounds were positively confirmed and quantified, and the rest of the compounds were tentatively identified. The major volatile components identified at relatively high levels were dodecanoic acid, hexadecanoic acid, hexanal, benzaldehyde, (Z)-3-hexen-1-ol, 1-penten-3-one, limonene, and β-caryophyllene oxide. Among the groups, terpenes accounted for 60.18% of the total concentration of all the volatile components. Some volatile components might account for the unique aroma and the biological activity of the sample.

Key words: pine needle, gas chromatography-mass spectrometry (GC-MS), simultaneous steam distillation and extraction (SDE), volatile components

INTRODUCTION

Pinus densiflora S. and Z. belongs to the Pinaceae family, and is an evergreen tree with needle leaves indigenous to eastern Asia. It is a species most widely distributed in Korea, although it is known in the West as Japanese red pine (1,2). Its leaves have been widely used for centuries as a food ingredient, food additive and folk medicine throughout eastern Asia due to their unique flavor and health-promoting properties (3). Pine needle provides a characteristic bitter taste and aroma, which may contribute to its acceptability by consumers (4,5). Its leaves contain an essential oil (0.3~1.3%) that has been reported to possess biological activity (2,6). It has been found to have positive effects on liver sicknesses, gastroenteric problems, and skin diseases. It is also used as a nourishing tonic drug (6-8).

There is a growing public interest in incorporating naturally occurring functional ingredients into the diet due to their health-promoting effects. Along this trend, several pine needle based products are available, such as pine needle powder, pine needle tea, and pine needle wine (5,9). Currently, pine needle powder, prepared from natural sources is widely distributed in the market to satisfy the consumer demand for its unique flavor and

its nutritional and biological properties. Although the use of pine needle powder as a food ingredient is on the rise, there is limited qualitative and quantitative information on the composition of its volatile compounds.

Gas chromatography-mass spectrometry (GC-MS) profiles of volatile compounds in the twigs, sprouts, and needles of *Pinus densiflora* have been reported in several studies (10-12). However, the volatile compounds in pine needle powder have not yet been determined. One of the current industrial trends is to utilize more comprehensive information from composition analysis to search for the potent flavor chemicals of a food product, and make use of them to increase its sensory preference by the consumers. The objective of the study reported here was to determine the volatile components in pine needle powder in order to provide more basic information to the public for utilization.

MATERIALS AND METHODS

Materials

Pine needle (*Pinus densiflora* S. and Z.) powder, which was produced at Cheonan-Si located in the Chungchungnam-do, South Korea, was purchased three times with one month apart from a local supermarket in 2009. The

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powdery samples were kept at 20°C in a freezer until used. Chemical standard compounds for verification and quantification were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA), Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and Fluka (Buchs, Switzerland).

Simultaneous steam distillation and solvent extraction (SDE) of volatile components by boiling of samples

The pine needle powder was steam-distilled to simulate the food preparation method often used by the consumers. Fourteen grams of powder samples on a dry weight basis were mixed with boiled double-distilled water [1:35 (w/v)] in a 5-L round bottom sample flask. One and half milliliter of 2,4,6-trimethylpyridine (9.783 mg/mL), internal standard (IS), was added to the sample. Forty milliliters of distilled dichloromethane were used as extraction solvent. Extraction was carried out with a Likens and Nickerson (13) type of simultaneous distillation and extraction (SDE) apparatus (model 523010-0000, Kontes, Vineland, NJ, USA) for 2 hr as described by Kim and Chung (14). Three replicated extractions were carried out on the pine needle samples. Extracts were concentrated by a stream of ultra high purity (99.99%) nitrogen gas and dried by 2.8 g of anhydrous sodium sulfate. Concentrates were stored at -80°C in 15-mL conical tubes sealed with Teflon-lined screw cap. Before sample injection, extracts were further concentrated to 0.5 mL.

Gas chromatography-mass spectrometry (GC-MS)

A GC-MS system consisting of an Agilent Technologies Model 6890 GC and a 5975 Inert Mass Selective Detector (MSD) was used for both qualitative and quantitative analyses. Two microliters of extract was injected at splitless mode to a capillary column (Agilent, HP-5MS; 30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies Inc., Wilmington, DE, USA) through the hot injector (280°C) for the separation of volatile components. Helium gas (ultrahigh-purity grade, 99.999%) was used as a carrier gas at a constant linear velocity of 30 cm/s. GC oven temperature was programmed from 35 to 195°C at a ramp rate of 2°C/min. The initial and final hold times were 5 and 30 min, respectively. The MS interface temperature was set at 250°C, the ion source temperature was 230°C, the MS quadrupole temperature was 150°C, and the ionization voltage was 70 eV. The mass range of MS was set at 35~550 amu, the scan rate was 2.91 scans/s, and the electron multiplier voltage was 1400V.

Qualification and quantification of volatile compounds

Qualification and quantification of the volatile components in samples were carried out as described by Kim and Chung (14) and Chung et al. (15). Tentative identification of compounds was made by matching the mass spectra of unknowns with those found in the Wiley Chemical database (7th ed., Hewlett-Packard Co., Palo Alto, CA, USA). Positive identification of each compound was based on the comparison of the mass spectrum and the retention time or retention index (RI) of the unknown compound in the extracts with the corresponding authentic standard under the same experimental conditions. Calculations of retention indices were done according to the method of Van den Dool and Kratz (16). For quantification, a three point calibration curve was developed for each positively identified compound. In brief, the calibration curve was prepared by plotting the concentration ratio (i.e. between a standard compound and the internal standard) against its corresponding area ratio (i.e. from a specific mass/charge fragment of the standard compound to that of the internal standard). The concentration of a compound was calculated from the response factor derived from the calibration curve for the compound. Relative abundance of a tentatively identified compound was estimated from the ratio between the relative area of a specific fragment of the tentatively identified compound and that of the internal standard (2,4,6-trimethylpyridine; fragment chosen was m/z 121). The concentration of each tentatively identified compound was calculated assuming the response factor has a value of 1.

Moisture analysis

Moisture content of samples was determined by an oven-drying method (17).

RESULTS AND DISCUSSION

In commercial pine needle (*Pinus densiflora* S. and Z.) powder, the average moisture content found was 4.34%, which was higher than that reported by Lee and Han (18) at 2.60% in the pine needle powder used as an ingredient in the preparation of Korean rice cake. A combined total of 230 volatile components were identified in the commercial pine needle powder (Table 1). The volatile components were classified to 13 chemical groups as shown in Table 1. Among them, alcohols, ketones, aldehydes, and terpenes were the four major groups in number, containing 42, 39, 32, and 30 compounds, respectively. The next groups were alkenes, esters, furans, and benzenes, comprised of 17, 14, 14, and 10 compounds, respectively. The rest of the chemical groups contained eight or less compounds. Each chemical group except the phenols had more than one volatile component at a relatively high concentration of more than 100

Table 1. Volatile components in commercial pine needle (*Pinus densiflora* S. and Z.) powder

No.[1]	Compound[2]	ID[3]	Ref[4]	CAS No.[5]	MW[6]	RT (min)[7]	RI[8]	m/z[9]	Conc. (μg/g)[10]
	Acids (6)								
1	Benzoic acid hydrazine*	MS	6	613-94-5	136.06	26.91	1062	105	5.1±0.9
2	Dodecanoic acid	RI, MS	3, 6, 8	143-07-7	200.18	61.59	1590	73	2518±492
3	Tetradecanoic acid	RI, MS		544-63-8	228.21	71.42	1772	73	678±130
4	Hexadecanoic acid	RI, MS		57-10-3	256.24	81.37	1973	73	1568±392
5	Dehydroabietic acid*	MS	9		314.23	104.21	2321	239	209±35
6	(+)Dehydroabietic acid*	MS	9	1740-19-8	300.21	115.66	2413	239	11.6±10.2
	Aldehydes (32)								
7	2-Butenal	RI, MS		4170-30-3	70.04	3.69	642	70	39.2±14.1
8	3-Methyl-butanal	RI, MS		590-86-3	86.07	3.75	646	44	21.4±8.5
9	Pentanal	RI, MS		110-62-3	86.07	4.67	697	44	387±122
10	(E)-2-Methyl-2-butenal	RI, MS		497-03-0	84.06	6.21	737	84	11.9±3.8
11	(E)-2-Pentenal	RI, MS	1	1576-87-0	84.06	6.67	748	55	235±56
12	Hexanal	RI, MS	1, 2, 3	66-25-1	100.09	8.75	800	56	2348±549
13	4-Methyl-2-pentenal*	MS		5362-56-1	98.07	9.65	815	55	0.4±0.1
14	(E)-2-Hexenal	RI, MS	1, 2	6728-26-3	98.07	11.81	849	69	329±66
15	4-Methylene-5-hexanal*	MS		17844-21-2	110.07	14.56	893	67	1.4±0.3
16	(Z)-4-Heptenal	RI, MS		6728-31-0	112.09	14.93	899	55	24.5±4.3
17	Heptanal	RI, MS		111-71-7	114.1	15.05	901	70	198±23
18	2,4-Hexadienal	RI, MS		142-83-6	96.06	15.61	909	81	28.8±4.0
19	Benzaldehyde	RI, MS	6	100-52-7	106.04	19.01	956	106	1694±283
20	Octanal	RI, MS		124-13-0	128.12	22.46	1003	57	54.9±5.3
21	(E,E)-2,4-Heptadienal*	MS	1	4313-03-5	110.07	22.95	1009	81	121±22
22	(E)-2-Octenal	RI, MS		2548-87-0	126.1	26.51	1057	70	228±35
23	2-Methylbenzaldehyde	RI, MS		529-20-4	120.06	27.02	1063	91	10.7±1.7
24	2,2,3-Trimethyl-3-cyclopentene-1-acetaldehyde*	MS		4501-58-0	152.12	31.35	1122	108	19.7±3.4
25	trans-2-cis-Nonadienal	RI, MS		557-48-2	138.1	33.47	1151	70	69.8±12.8
26	(E)-2-Nonenal	RI, MS		18829-56-6	140.12	33.96	1158	55	83.4±14.6
27	6,6-Dimethyl-bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde*	MS		564-94-3	150.1	36.33	1191	79	14.7±3.5
28	(E,E)-2,4-Nonadienal	RI, MS		5910-87-2	138.1	37.74	1211	81	25.2±4.8
29	4-(1-Methylethyl)-benzaldehyde	RI, MS		122-03-2	148.09	39.39	1235	133	23.7±4.7
30	(Z)-3,7-Dimethyl-2,6-octadienal	RI, MS		106-26-3	152.12	39.65	1239	69	4.4±0.9
31	4-Oxononanal	RI, MS			156.12	40.03	1245	85	6.7±1.1
32	4-Methoxybenzaldehyde	RI, MS		123-11-5	136.05	40.40	1250	135	6.9±1.1
33	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	RI, MS		472-66-2	166.14	40.60	1252	151	1.8±0.3
34	(E)-2-Decenal	RI, MS		3913-81-3	154.14	41.07	1259	41	22.7±4.8
35	3-Phenyl-2-propenal	RI, MS		104-55-2	132.06	41.50	1266	131	6.9±1.9
36	p-Menth-1-en-7-al	RI, MS		23963-70-4	152.12	41.75	1269	109	50.6±9.1
37	(E,E)-2,4-Decadienal	RI, MS		25152-84-5	152.12	44.72	1313	81	187±41
38	3-Dodecen-1-al*	MS		68083-57-8	182.17	47.80	1361	70	17.8±3.9
	Alkanes (8)								
39	Chloroform	RI, MS		67-66-3	117.91	3.15	612	83	21.4±22.0
40	2-Methylpentane*	MS		107-83-5	86.11	9.96	819	43	1.5±0.7
41	1-Chloropentane*	MS		543-59-9	106.06	10.37	826	70	0.2±0.1
42	7-Oxabicyclo[4.1.0]heptane	RI, MS		286-20-4	98.07	11.45	844	55	48.1±9.2
43	Tridecane	RI, MS		629-50-5	184.22	43.68	1297	57	2.8±0.2
44	(1-Methylethyl)-cyclohexane*	MS		696-29-7	126.14	45.33	1323	82	2.5±0.6
45	7-Acetyl-2-hydroxy-2-methyl-5-isopropyl-bicycle[4.3.0]nonane*	MS		96093-81-1	238.19	69.43	1734	43	143±16
46	19-Norabieta-4,8,11,13-tetraene+18-norabieta-3,8,11,13-tetraene(mixture)*	MS		23963-75-9	254.2	82.26	1991	197	46.8±7.7
	Alkenes (17)								
47	3-(Methylthio)-1-propene	RI, MS		10152-76-8	88.04	4.61	694	88	15.3±10.1
48	(Z)-3-Methyl-2-hexene*	MS		10574-36-4	98.11	9.09	806	69	1.4±0.4
49	5-(1,1-Ddimethylethyl)-1,3-cyclopentadiene*	MS		35059-40-6	122.11	11.14	839	107	5.4±1.9
50	1,3-cis,5-cis-Octatriene*	MS		40087-62-5	108.09	13.48	876	79	1.4±0.2
51	3,3-Dimethyl-6-methylenecyclohexene*	MS			122.11	16.57	922	79	0.5±0.0
52	5-Isopropyl-2-methyl-1,3-cyclohexadiene*	MS		99-83-2	136.13	16.68	923	93	13.1±4.1

Table 1. Continued

No.	Compound	ID	Ref	CAS No.	MW	RT (min)	RI	m/z	Conc. (μg/g)
53	(Z,Z)-3,5-Octadiene[*]	MS		7348-80-3	110.11	16.82	925	81	0.7±0.1
54	5-(1-Methylpropylidene)-1,3-cyclopentadiene[*]	MS		3141-02-4	120.09	24.46	1029	105	2.1±0.2
55	2,3-Dimethyl-bicyclo[2.2.1]hept-2-ene[*]	MS		529-16-8	122.11	37.88	1213	94	5.7±1.0
56	(E)-7,11-Dimethyl-3-methylene-1,6,10-dodecatriene[*]	MS		18794-84-8	204.19	53.65	1454	69	8.8±2.1
57	(+)-Epi-bicyclosesquiphellandrene[*]	MS		54324-03-7	204.19	53.79	1457	161	40.6±7.7
58	Cadina-1,4-diene[*]	MS	3, 8	16728-99-7	204.19	58.02	1527	119	37.1±6.0
59	Phenanthrene	RI, MS		85-01-8	178.08	70.90	1762	178	2.9±0.6
60	1,1,2,3,3,5-Hexamethyl-2,3-dihydroindene[*]	MS			202.17	77.10	1885	187	12.7±2.1
61	1,3,6,10-Cyclotetradecatetraene[*]	MS		1898-13-1	272.25	79.13	1926	93	418±85
62	9,10-Dehydrocycloisolongifolene[*]	MS			202.17	93.65	2203	187	18.1±3.1
63	Neophytadiene[*]	MS		504-96-1	278.3	94.78	2216	123	3.8±0.5
	Benzenes (10)								
64	Methylbenzene	RI, MS	8	108-88-3	92.06	7.09	759	91	2.4±0.9
65	1,3-Dimethylbenzene	RI, MS		108-38-3	106.08	12.69	863	91	0.9±0.2
66	Styrene[*]	MS		100-42-5	104.06	14.09	886	104	1.2±0.2
67	Methyl-(1-methylethyl)-benzene[*]	MS		25155-15-1	134.11	23.85	1021	119	122±8
68	Benzenemethanol	RI, MS		100-51-6	108.06	24.75	1033	79	35.1±4.2
69	Benzeneacetaldehyde	RI, MS		122-78-1	120.06	25.28	1040	91	69.2±12.4
70	Benzeneethanol	RI, MS		60-12-8	122.07	30.51	1110	91	32.8±3.7
71	Hexylbenzene[*]	MS		1077-16-3	162.14	31.56	1125	91	3.7±0.6
72	2-Methoxy-4-methyl-1-propan-2-ylbenzene[*]	MS		1076-56-8	164.12	39.22	1232	149	79.2±14.8
73	1,2-Dimethoxy-4-(2-propenyl)-benzene	RI, MS		93-15-2	178.1	50.52	1403	178	156±37
	Esters (14)								
74	Methyl benzoate	RI, MS		93-58-3	136.05	29.09	1091	105	4.9±0.9
75	L-Bornyl acetate	RI, MS	1, 2, 8	5655-61-8	196.15	42.67	1283	95	753±149
76	Ethyl-3-phenyl-2-propenoate	RI, MS		103-36-6	176.08	54.07	1461	131	8.2±1.9
77	1-Methylethyl-3-phenyl-2-propenoate[*]	MS		7780-06-5	190.1	56.56	1502	131	7.8±1.7
78	(Z)-3-Hexenyl benzoate[*]	MS		25152-85-6	204.12	60.51	1571	105	268±61
79	Benzyl benzoate[*]	MS	8	120-51-4	212.08	70.75	1759	105	89.2±17.2
80	Methyl-(E,Z)-2,6-nonadienoate[*]	MS		41654-18-6	168.12	73.25	1807	100	22.9±4.1
81	2-Phenylethyl benzoate[*]	MS	8	94-47-3	226.1	75.27	1848	104	84.6±15.3
82	2-Hydroxylphenylmethyl benzoate[*]	MS		118-58-1	228.08	75.95	1861	91	13.8±2.5
83	(Z)-3-Hexenyl cinnamate[*]	MS	3	68133-75-5	230.13	76.71	1877	131	189±36
84	Methyl isodextropimarate[*]	MS		1686-54-0	316.24	96.31	2234	121	60.2±10.1
85	Methyl communate[*]	MS		15798-13-7	316.24	99.67	2274	121	51.2±10.1
86	Methyl abietate[*]	MS		127-25-3	316.24	108.50	2357	256	2.4±0.5
87	Methyl (Z)-communate[*]	MS		10178-35-5	316.24	109.95	2370	79	25.2±20.9
	Furans (14)								
88	2-Methylfuran	RI, MS		534-22-5	82.04	3.10	609	82	67.6±60.6
89	2-Ethylfuran	RI, MS		3208-16-0	96.06	4.77	701	81	27.9±16.6
90	Dihydro-2-methyl-3(2H)-furanone	RI, MS		3188-00-9	100.05	9.20	808	72	0.5±0.3
91	3-Furaldehyde[*]	MS		498-60-2	96.02	9.51	813	95	9.2±2.2
92	2-Furancarboxaldehyde	RI, MS		98-01-1	96.02	10.56	829	96	130±45
93	1-(2-Furanyl)-ethanone	RI, MS		1192-62-7	110.04	15.68	910	95	13.1±2.3
94	Methylfuran-3-carboxylate[*]	MS		1334-76-5	126.03	16.52	921	95	1.9±0.3
95	2-Methyl-5-isopropenylfuran[*]	MS			122.07	17.37	933	122	9.7±1.6
96	4,6-Dihydrofuro[3,4-b]furan[*]	MS			110.04	18.45	948	81	1.5±0.3
97	5,5-Dimethyl-2(5H)-furanone[*]	MS		20019-64-1	112.05	18.76	952	97	1.5±0.3
98	5-Methyl-2-furancarboxaldehyde	RI, MS		620-02-0	110.04	19.47	962	110	11.4±4.7
99	2-Pentylfuran	RI, MS		3777-69-3	138.1	21.58	991	81	144±22
100	5-Pentyl-2(5H)-furanone[*]	MS			154.14	45.43	1324	84	1.4±0.3
101	Dihydro-5-pentyl-2(3H)-furanone	RI, MS		104-61-0	156.12	47.67	1359	85	7.5±1.6
	Miscellaneous (6)								
102	Pyridine	RI, MS		110-86-1	79.04	6.49	746	79	2.3±0.5
103	3-Octyne[*]	MS		15232-76-5	110.11	14.35	890	81	4.0±0.9
104	4-Nonyne[*]	MS		20184-91-2	124.13	24.35	1028	67	7.7±1.3
105	(Z)-4-Decen-6-yne[*]	MS		13343-76-5	136.13	41.63	1267	79	9.3±1.3
106	7,8-Dimethyl-benz[c]acridine[*]	MS		3518-01-2	257.12	77.91	1901	257	8.7±1.5
107	Simonellite[*]	MS		27530-79-6	252.19	85.05	2050	237	13.7±2.6

Table 1. Continued

No.	Compound	ID	Ref	CAS No.	MW	RT (min)	RI	m/z	Conc. (μg/g)
	Naphthalenes (7)								
108	Naphthalene	RI, MS		91-20-3	128.06	35.13	1174	128	5.3±1.1
109	1,2,3,4-Tetrahydro-1,1,6-trimethyl naphthalene*	MS		475-03-6	174.14	47.08	1350	159	2.0±0.3
110	(+)-Epi-bicyclosesquiphellandrene*	MS	8	54324-03-7	204.19	55.48	1485	161	77.7±15.0
111	1,2,4α,5,6,8α-Hexahydro-1-isopropyl -4,7-dimethylnaphthalene*	MS		31983-22-9	204.19	58.30	1533	105	33.8±6.2
112	1,2-Dihydro-1,1,6-trimethylnaphthalene*	MS		30364-38-6	172.13	59.77	1558	157	79.4±16.2
113	1,2,3,4,6,8α-Hexahydro-1-iso propyl- 4,7-dimethylnaphthalene*	MS		16728-99-7	204.19	63.52	1625	119	150±33
114	1,6-Dimethyl-4-(1-methylethyl)-naphthalene*	MS		483-78-3	198.14	66.07	1671	183	46.4±9.2
	Alcohols (42)								
115	2-Methyl-3-buten-2-ol	RI, MS	3	115-18-4	86.07	3.09	608	71	104±56
116	3-Methyl-3-buten-1-ol	RI, MS		763-32-6	86.07	5.79	727	56	8.5±2.1
117	3-Methyl-1-butanol	RI, MS		123-51-3	88.09	5.95	731	55	5.1±1.6
118	1-Cyclopenten-3-ol*	MS		3212-60-0	84.06	6.31	740	55	1.3±0.5
119	1-Pentanol	RI, MS	2	71-41-0	88.09	7.27	764	55	45.3±10.8
120	(Z)-2-Pentenol	RI, MS		1576-95-0	86.07	7.44	768	57	107±23
121	3-Methyl-2-buten-1-ol	RI, MS	7	556-82-1	86.07	7.67	774	71	6.5±2.0
122	2-Methyl-1,5-hexadiene-3-ol*	MS		17123-60-3	112.09	8.60	797	71	0.6±0.2
123	(Z)-3-Hexen-1-ol	RI, MS	3	928-96-1	100.09	12.11	854	67	844±150
124	(E)-2-Hexen-1-ol	RI, MS		928-95-0	100.09	12.86	866	57	28.9±2.4
125	1-Hexanol	RI, MS	5	111-27-3	102.1	13.02	869	56	51.8±7.0
126	1-(1-Propynyl)cyclopropanol*	MS		57951-62-9	96.06	14.13	887	67	3.3±1.7
127	1-Heptanol	RI, MS		111-70-6	116.12	20.28	973	56	19.7±3.0
128	1-Octen-3-ol	RI, MS	4	3391-86-4	128.12	20.91	982	57	17.5±3.3
129	2-Chlorocyclohexanol	RI, MS		1561-86-0	134.05	23.59	1018	57	12.6±0.5
130	1-Octanol	RI, MS		111-87-5	130.14	27.67	1072	56	67.4±12.9
131	cis-Linalyl oxide (linalool)*	MS	3, 5	5989-33-3	170.13	28.68	1085	59	38.1±6.9
132	3,7-Dimethyl-1,6-octadien-3-ol	RI, MS		78-70-6	154.14	29.66	1098	71	29.4±5.3
133	4-(1,1-Dimethylethyl)-2-cyclohexen-1-ol*	MS		35376-39-7	154.14	31.01	1117	93	6.0±1.6
134	trans-Pinocarveol*	MS		547-61-5	152.12	32.17	1133	92	38.5±7.4
135	p-Menth-2-en-1-ol*	MS	3, 4, 5	29803-81-4	154.14	32.36	1136	93	3.3±0.8
136	trans-Verbenol*	MS	8	1820-09-3	152.12	32.73	1141	94	9.2±1.1
137	1-Adamantanol*	MS		768-95-6	152.12	33.29	1149	95	6.2±1.2
138	Borneol*	MS	1, 2, 4	507-70-0	154.14	34.19	1161	95	265±51
139	p-Mentha-1,5-dien-8-ol*	MS	3	1686-20-0	152.12	34.39	1164	59	96±23
140	6-Ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol*	MS		14049-11-7	170.13	34.58	1167	68	8.9±1.4
141	4-Terpinenol	RI, MS	1	562-74-3	154.14	35.04	1173	71	78±15
142	para-Cymen-8-ol*	MS		1197-01-9	150.1	35.75	1183	135	91±18
143	α-Terpineol	RI, MS	3, 4, 7	98-55-5	154.14	36.08	1188	59	756±158
144	Myrtenol	RI, MS	3, 4, 7	515-00-4	152.12	36.47	1193	79	59.2±14.8
145	trans-(+)-Carveol*	MS	4	1197-07-5	152.12	38.08	1216	109	22.9±4.5
146	(E)-3,7-Dimethyl-2,6-octadien-1-ol	RI, MS		106-24-1	154.14	40.70	1254	69	33.8±7.2
147	4-Phenyl-2-butanol*	MS		2344-70-9	150.1	40.78	1255	117	19.4±3.8
148	4-(1-Methylethyl)benzene methanol*	MS		536-60-7	150.1	43.03	1288	135	22.0±3.6
149	4-(1-Methylethenyl)-1-cyclohexene-1-methanol*	MS		536-59-4	152.12	43.51	1295	67	3.2±0.9
150	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	RI, MS		7212-44-4	222.2	60.16	1566	69	75.7±16.8
151	(+)Spathulenol*	MS	1, 3, 4, 6	77171-55-2	220.18	60.96	1579	91	426±100
152	Caryophylladienol I*	MS		19431-80-2	220.18	63.94	1632	136	44.5±9.7
153	α-Cadinol*	MS	1, 5, 8	481-34-5	222.2	64.60	1645	161	153±32
154	Thunbergol*	MS	3, 8	25269-17-4	290.26	84.97	2049	81	1081±222
155	Manool*	MS	3	596-85-0	290.26	85.02	2050	137	19.3±7.3
156	Phytol*	MS	1, 3	150-86-7	296.31	87.89	2109	71	43.4±4.7
	Ketones (39)								
157	3-Butene-2-one*	MS		78-94-4	70.04	2.83	594	55	212±105
158	2-Butanone	RI, MS		78-93-3	72.06	2.94	600	43	63.3±27.8
159	3-Methyl-2-butanone	RI, MS		563-80-4	86.07	3.86	652	43	30.1±10.1
160	1-Penten-3-one	RI, MS	1	1629-58-9	84.06	4.41	683	55	955±322

Table 1. Continued

No.	Compound	ID	Ref	CAS No.	MW	RT (min)	RI	m/z	Conc. (μg/g)
161	3-Hydroxy-2-butanone	RI, MS		513-86-0	88.05	5.03	708	45	38.5±13.7
162	3-Penten-2-one	RI, MS		625-33-2	84.06	6.10	735	69	22.6±7.3
163	2,4-Pentanedione	RI, MS		123-54-6	100.05	7.84	778	85	20.4±1.5
164	2-Hexanone	RI, MS		591-78-6	100.09	8.33	790	58	5.0±1.0
165	3-Cycloheptenone*	MS		1121-64-8	110.07	10.13	822	54	1.2±0.4
166	3-Hexen-2-one*	MS		763-93-9	98.07	11.25	841	83	1.4±0.2
167	2-Heptanone	RI, MS		110-43-0	114.1	14.37	891	43	28.6±4.3
168	(1S)-(-)-Verbenone*	MS	8	80-57-9	134.11	18.51	949	91	14.6±2.2
169	1-Octen-3-one*	MS		4312-99-6	126.1	20.65	978	55	3.7±0.4
170	3-Methyl-3-cyclohexen-1-one*	MS		31883-98-4	110.07	20.98	983	67	4.6±1.5
171	2,2,6-Trimethylcyclohexanone*	MS		2408-37-9	140.12	24.56	1030	82	5.5±0.8
172	2-Methyl-3-octanone*	MS		923-28-4	142.14	26.71	1059	99	15.7±2.0
173	3,5-Octadien-2-one*	MS		30086-02-3	124.09	29.16	1092	95	3.1±0.7
174	1-Cyclopentylethanone*	MS		6004-60-0	112.09	30.37	1108	69	4.9±0.6
175	(1R)-6,6-Dimethyl-bicyclo[3.1.1]heptan-2one*	MS		38651-65-9	138.1	32.04	1131	83	3.6±0.8
176	4-Isopropylcyclohexanone*	MS	3	5432-85-9	140.12	33.64	1153	55	0.9±0.1
177	2,6,6-Trimethyl-bicyclo[3.1.1]heptan-3-one*	MS		18358-53-7	152.12	33.75	1155	83	4.8±0.8
178	1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone*	MS		70987-81-4	152.12	34.52	1166	109	12.2±1.7
179	cis-3-Pinanone*	MS		15358-88-0	152.12	34.72	1169	55	3.8±0.9
180	1-(3-Methylphenyl)-ethanone	RI, MS		585-74-0	134.07	35.53	1180	119	8.3±1.7
181	4-Isopropyl-2-cyclohexenone*	MS		500-02-7	138.1	35.65	1181	96	69.2±13.8
182	(-)-Verbenone*	MS	8	1196-01-6	150.1	37.29	1204	107	45.3±9.1
183	L-Carvone	RI, MS	3	6485-40-1	150.1	39.71	1240	82	15.4±3.1
184	4-Phenyl-2-butanone	RI, MS		2550-26-7	148.09	39.74	1240	148	8.4±1.7
185	3-Methyl-6-(1-methylethyl)-2-cyclohexen-1-one*	MS		89-81-6	152.12	40.40	1250	82	8.1±2.0
186	4-Butoxy-3-penten-2-one*	MS		3431-87-6	156.12	47.02	1349	85	2.8±0.3
187	1-Ethyl-3-piperidinone*	MS		43152-93-8	127.1	48.72	1375	84	10.8±1.0
188	1-(6,6-Dimethyl-2-methylene-3-cyclohexenyl)-buten-3-one*	MS			190.14	52.01	1428	43	11.3±2.4
189	(E)-6,10-Dimethyl-5,9-undecadien-2-one	RI, MS		3796-70-1	194.17	53.41	1450	43	96±22
190	β-Ionone	RI, MS		14901-07-6	192.15	55.34	1482	177	71.2±14.6
191	Salvial-4(14)-en-1-one*	MS			220.18	61.58	1590	123	183±36
192	(E)-1-(2-Hydroxy-5-methylphenyl)-2-hexen-1-one*	MS		41873-82-9	204.12	64.30	1639	161	702±151
193	6,10,14-Trimethyl-2-pentadecanone*	MS		502-69-2	268.28	75.03	1843	43	47.5±7.3
194	(E,E)-6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one*	MS		1117-52-8	262.23	78.63	1915	69	40.2±7.2
195	3-Ethyl-2-methyl-2H-naphtho[2,3-b]pyran-5,10-dione*	MS		105438-05-9	254.09	83.16	2011	239	18.1±3.2
	Phenols (5)								
196	4-Methylphenol	RI, MS	8	106-44-5	108.06	28.20	1079	107	3.9±0.5
197	2-Methoxy-4-vinyl-phenol*	MS		7786-61-0	150.07	44.48	1310	150	17.4±3.3
198	2-Methoxy-4-(2-propenyl)-phenol	RI, MS		97-53-0	164.08	47.34	1354	164	10.9±2.3
199	2-Methoxy-4-(1-propenyl)-phenol*	MS		97-54-1	164.08	48.16	1367	164	2.7±0.6
200	4-(1,1-Dimethylpropyl)-phenol*	MS		80-46-6	164.12	68.89	1724	135	51.8±9.8
	Terpenes (30)								
201	Tricyclene*	MS	3, 4, 5	508-32-7	136.13	16.14	916	93	7.9±2.2
202	α-Pinene	RI, MS	1, 3	80-56-8	136.13	17.05	928	93	230±64
203	Camphene*	MS	1, 2, 3, 4	79-92-5	136.13	18.04	942	93	19.3±4.0
204	(-)-β-Pinene	RI, MS	1, 2, 3	18172-67-3	136.13	20.11	970	93	100±18
205	α-Terpinene	RI, MS	1, 3, 4	99-86-5	136.13	23.22	1013	121	40.3±8.7
206	Limonene	RI, MS	2, 4, 9	138-86-3	136.13	24.18	1026	93	25806±3073
207	γ-Terpinene	RI, MS	5	99-85-4	136.13	26.43	1055	93	66.9±9.9
208	α-Terpinolene	RI, MS	1, 2, 4	586-62-9	136.13	28.58	1084	121	45.8±5.6
209	Camphor	RI, MS	3, 4	76-22-2	152.12	32.53	1138	95	44.1±8.2
210	α-Cubebene*	MS	1, 2, 3, 6	17699-14-8	204.19	46.76	1345	105	28.6±4.4
211	α-Ylangene*	MS		14912-44-8	204.19	48.10	1366	119	2.4±0.5
212	α-Copaene*	MS	1, 2, 3, 4	3856-25-5	204.19	48.38	1371	119	30.0±4.6

Table 1. Continued

No.	Compound	ID	Ref	CAS No.	MW	RT (min)	RI	*m/z*	Conc. (μg/g)
213	β-Bourbonene*	MS	3	5208-59-3	204.19	48.94	1379	81	8.1±1.6
214	(+)-Sativene*	MS	3	22338-87-0	204.19	49.22	1383	108	3.2±0.5
215	β-Cubebene*	MS	3	13744-15-5	204.19	49.36	1386	161	12.8±1.9
216	β-Elemene*	MS	2	515-13-9	204.19	49.50	1387	93	9.9±1.4
217	Junipene	RI, MS	1, 2	475-20-7	204.19	50.15	1398	91	216±33
218	trans-Caryophyllene*	MS	2, 7	87-44-5	204.19	51.13	1414	93	132±24
219	(+)-Aromadendrene*	MS	1, 2, 3	489-39-4	204.19	52.28	1432	91	7.9±1.1
220	Germacrene-D*	MS	2, 3, 4	23986-74-5	204.19	52.80	1441	161	10.1±1.9
221	α-Humulene*	MS	1, 2, 3, 4	6753-98-6	204.19	53.19	1447	93	93.8±18.2
222	γ-Cadinene*	MS	1, 3, 5	39029-41-9	204.19	54.50	1468	161	62.8±11.4
223	γ-Muurolene*	MS	3	30021-74-0	204.19	54.72	1472	161	108±18
224	α-Guaiene*	MS		3691-12-1	204.19	55.21	1480	105	35.1±5.5
225	α-Muurolene*	MS	2, 3, 5	31983-22-9	204.19	56.16	1496	105	144±27
226	α-Amorphene*	MS	1, 5	23515-88-0	204.19	56.97	1509	161	361±65
227	δ-Cadinene*	MS	1, 2, 7, 8	483-76-1	204.19	57.62	1520	161	659±125
228	β-Caryophyllene oxide	RI, MS	1, 3, 4, 8	1139-30-6	220.18	61.12	1582	79	5977±2233
229	Manoyl oxide*	MS	1, 3	596-84-9	290.26	81.79	1982	275	39.9±6.9
230	β-Selinene*	MS	1, 3, 8	17066-67-0	204.19	86.27	2076	204	8.3±1.2

[1] Compound number.
[2] Compounds in order of their retention indices in a chemical class.
[3] Positive identification of a compound was based on the comparison of the mass spectrum (MS) and retention index (RI) between the tentative compound with that of the authentic standard under the same experimental conditions; tentative identification was based on mass spectrum only.
[4] Articles in which the compounds were reported: 1. Yu et al., 2004a (5); 2. Kim and Shin, 2005 (2); 3. Kurose et al., 2007 (27); 4. Yatagai and Sato, 1986 (38); 5. Yu et al., 2004b (24); 6. Lee et al., 2005 (3); 7. Kim and Chung, 2000 (11); 8. Ka et al., 2005 (33); 9. Sultan et al., 2008 (39).
[5] Chemical Abstracts Service Registry number.
[6] Molecular weight.
[7] Retention time (min).
[8] Linear retention indices calculated with reference to the method of Van den Dool and Kratz (17).
[9] Ion fragment selected used to calculate the concentration of a particular compound.
[10] Mean concentration and standard deviation (μg/g) calculated from three replicate samples on a dry weight basis.
* Tentatively identified compound by MS database (7th ed., Wiley Interscience Co., New York, NY, USA); tentatively identified compounds were calculated in the basis of the response factor assumed by 1.

μg/g in the sample (Table 1). Among all of the compounds detected, 96 compounds were positively confirmed and quantified, and the rest of them were tentatively identified. From the GC-MS analysis, the group of volatile compounds with the highest quantity in the pine needle powder was the terpenes (60.18%), followed by aldehydes (11.01%). The other compound groups had concentrations below 10%, but higher than 1%, included acids (8.75%), alcohols (8.34%), ketones (4.90%), esters (2.77%), and alkenes (1.03%). The remaining groups showed concentrations below 1%. Based on the micromolar value per gram dry sample of each volatile compound, 33 compounds had values higher than 1 micromole/g on a dry weight basis. Eight compounds with molar concentrations higher than 6 μmoles/g included dodecanoic acid (12.58 μmoles/g), hexadecanoic acid (6.12 μmoles/g), hexanal (23.46 μmoles/g), benzaldehyde (15.97 μmoles/g), (Z)-3-hexen-1-ol (8.43 μmoles/g), 1-penten-3-one (11.37 μmoles/g), limonene (189.57 μmoles/g), and β-caryophyllene oxide (27.15 μmoles/g).

These eight compounds accounted for 73.16% of the total concentration of all the components identified, which comprised 4.42, 2.75, 4.12, 2.97, 1.48, 1.68, 45.26, and 10.48%, of the sample respectively.

Kang et al. (7) reported that the main components in a hexane extract of *P. densiflora* were α-pinene, β-thugene, *trans*-caryophyllene, β-myrcene, and β-cubebene, while their SDE extract contained β-cubebene, *trans*-caryophyllene, 2-hexenal, τ-mururolol, and δ-cadinene. Later, Woo et al. (19) reported that the main components in pine twig extracts obtained using the SDE method were limonene, β-pinene, β-myrcene, and α-pinene. Similarly, Lee et al. (3) using solid phase microextraction technique, reported that the main components in pine needles were β-pinene, camphene, germacrene D, α-terpinene, and β-caryophyllene. Components in the *P. densiflora* leaf extract identified by Kim and Shin (2) were α-ocimene, sabinene, β-myrcene, β-caryophyllene, β-pinene, and 2-hexenal, which accounted for 78.00~81.70% of the total peak area observed. Based on these reports, the

observed differences in the main components identified might be due to variations in extraction conditions, methods, collection times, and sample collection locations.

In Table 1, three saturated fatty acids including dodecanoic acid (or lauric acid (C12:0), 12.58 μmoles/g), tetradecanoic acid (or myristic acid (C14:0), 2.97 μmoles/g), and hexadecanoic acid (or palmitic acid (C16:0), 6.12 μmoles/g) were identified at relatively high molar value. Dodecanoic acid was described as fatty, and waxy, while was, and oily (20). The fatty products might be by-products degraded from ferulic acid esters or its derivatives present in pine needle powder. Hogan et al. (21) reported that C12 and C14 saturated fatty acids had strong antibacterial activity in inhibiting the growth of mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium bovis*. In addition, Feng et al. (22) reported that pine needle extract containing a high concentration of hexadecanoic acid (C16:0) had an effective growth inhibition effect on common spoilages and pathogenic bacteria even at low concentrations. Therefore, the medium to long chain saturated fatty acids found in our pine needle powder might possess similar antibacterial activity. Hexanal was described as having green and grassy notes, and (E)-2-hexenal was described as sweet, green, and fruity (20). Both components were reported as major C6-aldehydes contributing to the green aroma (23). Yu et al. (24) also reported that the compounds in the conifer plant possessed a characteristic green/apple aroma. Later, the same group found the same compounds in an SDE extract of red pine needle (*Pinus densiflora*) and considered them as aroma-active compounds of pine needles (5). Hexanal was described as grassy, while (E)-2-hexenal was described as sweet and fruity. In our study, these two volatile compounds have been identified at relatively high levels (Table 1), and are expected to have a similar flavor impact on the powder.

Benzaldehyde was described as bitter, almond-like, and sweet (20) in the tea volatile extracts (25), and was identified as the component the second highest in molar concentration (15.97 μmoles/g) among the aldehydes (Table 1). In fresh tea leaves, benzaldehyde could be produced by enzymatic hydrolysis of the purunasin present in fresh tea leaves. Purunasin is distributed widely in edible plants and is known as an aroma precursor of benzaldehyde (25). Similarly, benzaldehyde found in pine needle powder might be formed by the same mechanism in fresh pine tissue. (Z)-3-Hexen-1-ol, known as an aroma-active compound, was reported to have combined green and cucumber notes in the Korean perilla leaf (26). The compound was also identified at a high

concentration (8.43 μmoles/g) in the alcohol group. Kurose et al. (27) reported that this volatile compound was identified in the GC-MS profile in the leaves of the *Pinus* species such as *P. koraiensis*, *P. parviflora*, *P. petula*, and *P. rigida*. According to Yu et al. (5), 1-penten-3-one was an aroma-active compound in *Pinus densiflora* possessing a pungent aroma note. This compound had the highest concentration (11.36 μmoles/g) among the ketones in pine needle powder. The 1-penten-3-one exhibited a rancid and fatty aroma in linseed oil, and a solvent-like aroma in peanuts (5,28,29). It is considered to be one of the secondary lipid oxidation products generated from hydroperoxide (29). In this study, 1-penten-3-one could be generated by either non-enzymatic or enzymatic oxidation of the essential oil components present in the tissue of pine needle leaves.

Terpenes, a type of hydrocarbon, were found to have relatively high concentrations as shown in Table 1. The group included monoterpenes such as α-pinene, (-)-β-pinene, and limonene and sequiterpenes such as junipene, trans-caryophyllene, α-amorphene, and δ-cadinene is known to contain 10 or 15 carbon atoms in its parent chemical structure, respectively (3). It was known that these terpenes were odorous and provided flavoring properties described as woody, piney, and fruity (30). According to Eakin's report (31), compounds that gave rise to flavor properties described as herbal, spicy, and citrus-like were not due to the terpenoid hydrocarbons, but due to the oxygenated terpenoid hydrocarbons such as terpene alcohols or terpene esters. This study confirmed the presence of oxygenated terpenoid hydrocarbons such as L-bonyl acetate, (Z)-3-hexenyl benzoate, (Z)-3-hexenyl cinnamate, borneol, α-terpineol, (+)-spathulenol and thunbergol in the esters and alcohols groups. Among the oxygenated terpenoid, borneol and L-bornyl acetate are known as naturally-occurring volatile components in the oils of various conifers belonging to the family Pinaceaeas (24,32). Their aroma was described as pine/camphoraceous and pine/herbaceous-like, respectively, and these were found to be key aroma-active compounds in fresh pine needles (*Pinus densiflora*) (24). α-Terpineol (4.90 μmoles/g) and thunbergol (3.72 μmoles/g) were considered as major oxygenated terpene alcohols in terms of molar concentration in this study. They were also identified in the pine needle volatile SDE extracts reported by Yu et al. (5) and Ka et al. (33). As a monoterpene, limonene occupied 45.26% of the total concentration of all volatile components, and the highest concentration among all volatile components identified. β-Caryophyllene oxide is an oxygenated ses-

quiterpene occupying 10.48%. Limonene has a citrus note, and was reported as a major flavor constituent in citrus fruit (34). It was identified in the essential oils from the leaves of *Pinus* species such as *P. merkusii*, *P. koraiensis*, and *P. pumila* at high quantity (27). According to Petrakis et al. (35), *P. heldreichii* growing in Greece contains limonene as a major volatile compound at 34.30%. That result agrees well with our results that limonene is a major component in the powder. β-Caryophyllene oxide was reported to have a woody odor note (20), and this component was also found at high levels in the leaf oil of the *Pinus* species such as *P. rudis*, *P. petula*, and *P. merkusii*. Beside limonene and β-caryophyllene oxide, the other major terpenes identified in this study include δ-cardinene, α-pinene, α- amorphene, junipene, etc. The terpenes and their oxygenated hydrocarbons described above were reported to have antifungal, antibacterial, and antioxidant effects (2,33,36, 37). The SDE extracts of *P. pondesora* with high levels of monoterpenes such as α-pinene and β-pinene were very active against fungi like *Fusarium culmorum*, *F. solani* and *F. poae*. They fully inhibited the growth of fungi at 2 and 5% concentration levels of the extract towards *F. solani* and *F. poae*, respectively (36). According to Kim and Shin (2), the SDE extracts of *P. densiflora* with high levels of monoterpenes effectively inhibit the growth of food-borne bacteria such as *Escherichia coli* O157:H7, *Bacillus cereus* and *Staphylococcus aureus*. In addition, the SDE volatile extracts of pine needles containing high levels of borneol, bornyl acetate, thunbergol, caryophyllene oxide, spathulenol, and dodecanoic acid compounds showed strong biological activity in increasing the human neuroblastoma cell (SK-N-SH) viabilities in an H_2O_2-induced cytotoxicity test, but decreased the malonaldehyde level in the H_2O_2-induced lipid peroxidation test (33). Overall, volatile components, particularly those belonging to the terpenes and oxygenated terpenes, contributed to antimicrobial and other biological activities in SK-N-SH cells described previously; this was corroborated in this investigation, suggesting that pine needle powder might have similar properties.

In conclusion, the SDE volatile profile of commercial pine needle (*Pinus densiflora*) powder was investigated both qualitatively and quantitatively. Commercial pine needle powder had 230 components which were divided into 13 different chemical groups. Eight compounds dominated the composition of pine needle power based on their quantity, which included dodecanoic acid, hexadecanoic acid, hexanal, benzaldehyde, (Z)-3-hexen-1-ol, 1-penten-3-one, limonene, and β-caryophyllene oxide. Among them, limonene had the highest concentration

(25.81 mg/g) level. The terpene group had 60.18% of the total concentration of all volatiles covering both positively and tentatively identified compounds. Some terpenoid compounds and their oxygenated forms identified and found at high levels in pine needle powder might contribute to its characteristic aroma as well as its biological activities. In future investigations, an optimized product development scheme and extraction methods will be explored to maximize the recovery of functional volatile compounds from pine needles. In the long run, it is hoped that a desirable powder ingredient prepared from pine needles will be more readily available and acceptable to consumers.

ABBREVIATIONS

GC-MS, gas chromatography-mass spectrometry; IS, internal standard; SDE, simultaneous steam distillation and extraction.

REFERENCES

1. Hotta M, Ogata K, Nitta A, Hosikawa K, Yanagi K, Yamazaki K. 1989. *Useful plants of the world.* Heibonsha, Tokyo, Japan. p 816.
2. Kim YS, Shin DH. 2005. Volatile components and antibacterial effects of pine needle (*Pinus densiflora* S. and Z.) extracts. *Food Microbiol* 22: 37-42.
3. Lee JG, Lee CG, Kang JJ, Buglass AJ, Lee GH. 2005. Determination of optimum conditions for the analysis of volatile components in pine needles by double-shot pyrolysis-gas chromatography-mass spectrometry. *J Chromatogr A* 1089: 227-234.
4. Korea Food Drug & Administration. 1997. *An illustrated guide to medicinal plants.* Woojin Publishing Co., Seoul, Korea. p 95.
5. Yu EJ, Kim TH, Kim KH, Lee HJ. 2004a. Aroma-active compounds of *Pinus densiflora* (red pine) needles. *Flavour Fragr J* 19: 532-537.
6. Jung MJ, Jung HA, Kang SS, Hwang GS, Choi JS. 2009. A new abietic acid-type diterpene glucoside from the needles of *Pinus densiflora*. *Arch Pharm Res* 32: 1699-1704.
7. Kang SK, Kang KH, Choi OJ, Kim YW, Kim YD. 1996. Volatile flavor compounds of *Pinus densiflora* Sieb and Zucc according to extracting solvents and steam distillation method. *Korean J Diet Cult* 11: 403-408.
8. Lee E. 2003. Effects of powdered pine needle (*Pinus densiflora* Seib. et Zucc.) on serum and liver lipid composition and antioxidative capacity in rats fed high oxidized fat. *J Korean Soc Food Sci Nutr* 32: 926-930.
9. Hong TK, Lim MH, Lee JH. 2001. The functional effects of pine needles and their application to foods. *Kor Food Sci Ind* 34: 48-52.
10. Jo JE, Lee MJ, Lee YB, Yun JR. 1999. Comparison of volatile compounds of *Pinus densiflora* on kinds of extraction solvents and parts of Pinus. *J Korean Soc Food Nutr* 28: 973-979.
11. Kim KY, Chung HJ. 2000. Flavor compounds of pine sprout tea and pine needle tea. *J Agric Food Chem* 48:

1269-1272.

12. Tsitsimpikou C, Petrakis PV, Ortiz A, Harvala C, Roussis V. 2001. Volatile needle terpenoids of six *Pinus* Species. *J Essent Oil Res* 13: 174-178.

13. Likens ST, Nickerson GB. 1964. Detection of certain hop oil constituents in brewing products. *Am Soc Brew Chem Proc* 2: 5-13.

14. Kim JS, Chung HY. 2009. GC-MS analysis of the volatile components in dried boxthorn (*Lycium chinensis*) fruit. *J Korean Soc Appl Biol Chem* 52: 516-524.

15. Chung HY, Fung PK, Kim JS. 2005. Aroma impact components in commercial plain sufu. *J Agric Food Chem* 53: 1684-1691.

16. Van den Dool H, Kratz PD. 1963. A generation of the retention index system including linear temperature programmed gas liquid partition chromatography. *J Chromatogr* 2: 463-471.

17. AOAC. 1980. *Official methods of analysis*. 13th ed. Association of Official Analytical Chemists, Washinton, DC, USA.

18. Lee HG, Han JY. 2002. Sensory and textural characteristics of Solsulgi using varied levels of pine leaves powders and different types of sweetners. *Korean J Soc Food Cookery Sci* 18: 165-172.

19. Woo GY, Kim KH, Lee MJ, Lee YB, Yoon JA. 1999. A comparison of volatile compounds in pine extracts obtained by supercritical fluid extraction with those by simultaneous steam distillation solvent extraction. *Korean J Food Sci Technol* 31: 1268-1274.

20. Aldrich. 2007. *Flavors and fragrances*. International edition, Sigma-Aldrich Co., Milwaukee, WI, USA.

21. Hogan JS, Pankey JW, Duthie AH. 1987. Growth inhibition of mastitis pathogens by long-chain fatty acids. *J Dairy Sci* 70: 927-934.

22. Feng S, Zeng W, Luo F, Zhao J, Yang Z, Sun Q. 2010. Antibacterial activity of organic acids in aqueous extracts from pine needles (*Pinus massoniana* Lamb.). *Food Sci Biotechnol* 19: 35-41.

23. Hatanaka A. 1993. The biogeneration of green odor by green leaves. *Phytochemistry* 34: 1201-1218.

24. Yu EJ, Kim TH, Kim KH, Lee HJ. 2004b. Characterization of aroma-active compounds of *Abies nephrolepis* (Khingan fir) needles using aroma extract dilution analysis. *Flavour Fragr J* 19: 74-79.

25. Guo W, Sasaki N, Fukuda M, Yagi M, Watanabe N, Sakata K. 1998. Isolation of an aroma precursor of benzaldehyde from tea leaves (*Camellia sinensis* var. sinensis cv.

Yabukita). *Biosci Biotechnol Biochem* 62: 2052-2054.

26. Seo WH, Baek HH. 2009. Characteristics aroma-active compounds of Korean perilla (*Perilla frutescens* Britton) leaf. *J Agric Food Chem* 57: 11537-11542.

27. Kurose K, Okamura D, Yatagai M. 2007. Composition of the essential oils from the leaves of nine *Pinus* species and cones of three of *Pinus* species. *Flavour Fragr J* 22: 10-20.

28. Matsui T, Guth H, Grosch W. 1998. A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution of headspace samples (GCOH). *Lipid-Fett* 100: 51-56.

29. van Ruth SM, SS Emad SS, Morrissey PA. 2001. Influence of methanolic extracts of soybean seeds and soybean oil on lipid oxidation in linseed oil. *Food Chem* 75: 177-185.

30. Furia TE, Bellanca N. 1975. *Fenaroli's handbook of flavor ingredients*. 2nd ed. CRC Press, Cleveland, OH, USA. p 45.

31. Eakin NAM. 1979. *Terpenoids and flavonoids*. Academic Press, New York, NY, USA. p 65.

32. Burdock GA. 1994. *Fenaroli's handbook of flavor ingredients*. 3rd ed. CRC Press, Boca Raton, FL, USA. p 68.

33. Ka MH, Choi EH, Chun HS, Lee KG. 2005. Antioxidative activity of volatile extracts isolated from *Angelica tenuissimae* roots, peppermint leaves, pine needles, and sweet flag leaves. *J Agric Food Chem* 53: 4124-4129.

34. Pakdel H, Sarron S, Roy C. 2001. α-Terpineol from hydration of crude sulfate turpentine oil. *J Agric Food Chem* 49: 4337-4341.

35. Petrakis PV, Tsitsimpikou C, Tzakou O, Vagias C, Roussis V. 2001. Needle volatiles from five *Pinus* species growing in Greece. *Flavour Fragr J* 16: 249-252.

36. Krauze-Baranowaka M, Mardarowicz M, Wiwart M, Poblock L, Dynowska M. 2002. Antifungal activity of the essential oils from some species of the genus *Pinus*. *Z Naturforsch* 57c: 478-482.

37. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem* 91: 621-632.

38. Yatagai M, Sato T. 1986. Terpenes of leaf oils from conifers. *Biochem Syst Ecol* 14: 469-478.

39. Sultan Z, Jeon YM, Moon SS. 2008. Labdane-type diterpenes active against acne from pine cones (*Pinus densiflora*). *Planta Med* 74: 449-452.