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Difference in the Volatile Composition of Pine-Mushrooms (*Tricholoma matsutake* Sing.) According to Their Grades

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The differences in volatile components of pine-mushrooms (*Tricholoma matsutake* Sing.) according to their grades were observed by applying multivariate statistical methods to GC−MS data sets. A total of 35 and 37 volatile components were identified in raw and cooked pine-mushrooms, respectively. The volatile components in pine-mushrooms were primarily composed of C_8 species, such as 3-octanol, 1-octen-3-ol, 1-octanol, (*E*)-2-octen-1-ol, 3-octanone, 1-octen-3-one, (*E*)-2-octenal, and octanoic acid. The levels of ethyl octanoate, junipene, and 3-methyl-3-buten-2-one were much higher in raw pine-mushroom of higher grades, whereas the reverse was true for C_8 components. On the other hand, furfuryl alcohol, benzyl alcohol, phenylethyl alcohol, dihydro-5-methyl-2(3*H*)-furanone, 2(5*H*)-furanone, (*E*)-2-methyl-2-butenal, furfural, phenylacetaldehyde, benzoic acid methyl ester, camphene, and β-pinene were the major components of cooked mushrooms. These volatile components formed by various thermal reactions could be mainly responsible for the difference in volatile components of cooked pine-mushrooms according to their grades.

KEYWORDS: Pine-mushroom (*Tricholoma matsutake* Sing.); difference; grade; volatile; GC−MS; principal component analysis (PCA)

INTRODUCTION

Mushrooms have been widely used since ancient times not only as foods or food flavoring materials but also for medicinal or functional purposes. Pine-mushroom (*Tricholoma matsutake* Sing.) is the most valuable species throughout the world, exhibiting a characteristic and delicate flavor as well as several biological activities, such as cholesterol lowering, antioxidant, immunomodulating, and antitumor effects in humans (*1*−*3*). In particular, pine-mushrooms cultivated in the pine forests of South Korea are the most highly valued, mainly due to the unique environment and climate of South Korea.

Pine-mushrooms can be classified according to their appearance, which is affected mostly by their ripening stages and cultivating conditions. The standard for their classification was developed by the National Forestry Cooperatives Federation of South Korea (*4*). Pine-mushrooms of the first grade are of the highest quality and are more than 8 cm long with an unopened pileus. Pine-mushrooms of the second grade are generally 6−8 cm long, but their widths are irregular and their pilei unopened. Pine-mushrooms of the third grade are less than 6 cm long or have one-third opened pilei, and pine-mushrooms of the fourth grade have completely opened pilei (**Figure 1**). The quality of pine-mushrooms, such as aroma, taste, texture, and color, varies depending on their grades. In particular, pine-mushrooms of higher grades have distinctive aroma notes compared to those of lower grades.

(a) first (b) second (c) third (d) fourth

Figure 1. Pine-mushrooms of the first, second, third, and fourth grades.

The volatiles present in mushrooms have been investigated by many researchers (*5*−*15*). Nearly 150 different volatile components representing a variety of chemical classes have been identified in various mushroom species (*5*−*7*). In particular,

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several aliphatic C_8 components are the main contributors to mushroom volatiles (*7*, *11*, *12*). The profiles of volatile components vary with species and varieties and can also be influenced by the cultivating conditions (*6*, *7*, *13*). In addition, since raw mushrooms contain numerous reactive components, any processing (e.g., drying, canning, or other thermal treatments) normally results in significant changes to the compositions of diverse components, including volatiles (*5*, *8*, *11*). Some studies have investigated the effects of the origins (*16*), grades (*17*), and thermal processing (*18*) of pine-mushrooms on the volatiles that are present.

Multivariate statistical tools, including principal component analysis (PCA) (*19*, *20*), hierarchical clustering analysis (HCA) (*21*), and partial least-squares regression (PLSR) (*22*), have been specifically designed for the analysis and visualization of the complex data sets in different samples. Many studies have used GC−MS and multivariate analysis techniques to compare food samples, such as coffee (*19*), cheese (*23*), potato (*24*), apricot (*25*), maize (*26*), and cold-smoked salmon (*27*). In this study, volatile components in raw and cooked pine-mushrooms of different grades were investigated by applying multivariate statistical methods to GC−MS data sets, with the aim of comparing them according to their grades.

MATERIALS AND METHODS

Solvent and Chemicals. Dichloromethane (≥99.9% pure) was obtained from Fisher Scientific Korea (Seoul, South Korea), and sodium sulfate, *n*-alkane standards (C_7−C_{22}), and an internal standard compound (dodecanoic acid methyl ester) were purchased from Sigma-Aldrich (St. Louis, MO). The stock solutions of 35 authentic standard compounds were prepared in dichloromethane. The authentic standards were obtained from various suppliers as follows: nos. 101−106, 108, 110−114, 116−119, 202−204, 208, 301−306, 402, 403, 405, 501, 502, 504, and 601 from Sigma-Aldrich, nos. 107 and 109 from Wako Pure Chemical Industries (Osaka, Japan), and nos. 109, 406, and 503 from Fluka (Buchs, Switzerland).

Materials. Pine-mushrooms of four grades cultivated in Inje-eup, Gangwon-do, South Korea, in 2004 were investigated in this study. Fresh pine-mushrooms were wrapped in LLD-PE (low-level-density-polyethylene) film and stored at −70 °C in a deep freezer until they were used, when they were thawed at 4 °C in a refrigerator for 3 h and then sliced using a cutter (model SFS-102, Shinomura, Sanjo, Niigata, Japan). A convection oven broiler (model 7091, Toastmaster, Boonville, MO) was preheated to 220 °C, and the pine-mushrooms were roasted at 187−193 °C (internal oven temperature) on each side for 1 min. The raw or cooked pine-mushrooms were placed in a stainless steel container, frozen in liquid nitrogen, and then ground in a blender (model HMC-400T, Hanil Electric, Seoul, South Korea).

Extract of Volatiles. The ground samples (100 g) were directly extracted with 200 mL of dichloromethane that was redistilled before being used. After 0.1 mL of 500 ppm dodecanoic acid methyl ester (v/v, in dichloromethane) was added as an internal standard, the ground sample suspended in dichloromethane was magnetically stirred at 400 rpm for 30 min and then filtered (paper no. 41, Whatman, Maidstone, U.K.) under vacuum. Volatiles were then separated from nonvolatiles using a high-vacuum pumping system (model VPC-250F, ULVAC KIKO, Yokohama, Japan) connected with custom-made glassware (Chang Young Scientific, Seoul, South Korea). The solvent extract was placed in an addition funnel and then added drop by drop into a 1 L round-bottomed flask when the operating vacuum level reached $<3 \times 10^{-5}$ Torr; the water bath temperature was 40 °C. Each sample droplet was dispersed in the flask and magnetically stirred at approximately 300 rpm. The distillate was collected in three cold traps immersed in liquid nitrogen. After all the extract was fed into the apparatus, the water bath temperature was increased to 45 °C and extraction continued for 2 h. The final operating vacuum was typically below 2×10^{-5} Torr. After the high-vacuum sublimation was complete, the cold traps in the apparatus were warmed to room temperature. The resulting extract collected from the three cold traps was dehydrated over anhydrous sodium sulfate (Na_2SO_4), concentrated on a Vigreux column (50 cm length × 3 cm inside diameter) in a 45 °C water bath, and then placed under a slow stream of nitrogen to obtain a final volume of 0.1 mL. All sample preparations were performed in triplicate.

Analysis by GC−MS. GC−MS analysis was performed using an HP 6890 gas chromatograph-5973 mass selective detector (GC-MSD) (Hewlett-Packard, Palo Alto, CA) equipped with a DB-wax column (60 m length × 0.25 mm inside diameter × 0.25 mm film thickness, J&W Scientific, Folsom, CA). The carrier gas was helium at a constant flow rate of 0.8 mL/min. One microliter of the extract was injected in the splitless mode. The oven temperature was held at 40 °C for 1 min, then increased to 200 °C at 4 °C/min, and held at 200 °C for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively. The mass detector was operated in the electron impact mode with an ionization energy of 70 eV and a scanning range of 33−550 amu.

Identification of Volatiles. Volatile components were positively identified by comparing mass spectra and RIs with those of the authentic compounds. When standards were not available, compounds were tentatively identified with the aid of Wiley 275 mass spectral database (Hewlett-Packard, 1995) or by manual interpretation. The RIs of the components were calculated using *n*-alkanes C_7−C_{22} as external references (*28*). The semiquantitative analysis of volatile components was performed by comparing their peak areas to that of the internal standard compound (0.1 mL of 500 ppm dodecanoic acid methyl ester in dichloromethane, v/v) on the GC−MS total ion chromatogram.

Statistical Analysis of GC−MS Data. Analysis of variance (ANOVA) was performed using the general line model (GLM) procedure in SPSS (version 10.1, SPSS, Chicago, IL) to evaluate significant differences in volatile components of pine-mushrooms according to their grades. Duncan's multirange test was used when the samples exhibited significantly different peak areas of volatiles, with the level of significance set at $P < 0.05$. Principal component analysis (PCA) was applied to the mean values of the relative peak area of volatiles to clarify the relationship between the pine-mushroom samples and the volatile components that are present. All the statistical analyses were performed using SPSS (version 10.0).

RESULTS AND DISCUSSION

Compositions of Volatile Components in Raw and Cooked Pine-Mushrooms. The volatiles in raw and cooked pine-mushrooms of four grades cultivated in South Korea were isolated using high-vacuum sublimation and then analyzed by GC−MS. **Table 1** and **Table 2** list the volatile components identified in raw and cooked pine-mushrooms according to their grades, relative peak areas, and RIs on the DB-wax column, respectively. A total of 35 volatile components, including 16 alcohols, six ketones, three aldehydes, six acids and esters, three terpene hydrocarbons, and one miscellaneous, were identified in raw pine-mushrooms of four grades. In contrast, a total of 37 volatile components, comprising 16 alcohols, six ketones, five aldehydes, five acids and esters, four terpene hydrocarbons, and one miscellaneous, were found in the cooked samples. The volatile components in raw and cooked pine-mushrooms were primarily of the following C_8 components: 3-octanol, 1-octen-3-ol, 1-octanol, (*E*)-2-octen-1-ol, 3-octanone, 1-octen-3-one, (*E*)-2-octenal, and octanoic acid. These C_8 components are known to be formed in enzymatic reactions involving linoleic acid or linolenic acid (*6*, *10*). Among them, the levels of 1-octen-3-ol, 1-octen-3-one, and (*E*)-2-octen-1-ol were higher in raw pine-mushrooms than in cooked ones. The level of 1-octen-3-ol in raw and cooked pine-mushrooms varied with grade, being higher in the third and fourth grades than in the first and second grades. It is notable that 3-phenyl-2-propenoic acid methyl ester (methyl cinnamate) was dominant in both raw and cooked pine-mushrooms of all grades in this study, whereas 1-octen-3-ol

Table 1. Volatile Components Identified in Raw Pine-Mushrooms of Four Different Grades by GC–MS

no.[a]	volatile component	RI[b]	relative peak area (mean ± SD)[c] first	second	third	fourth	ID[d]
	alcohols						
101	2-methyl-3-buten-2-ol	1037	0.039 ± 0.006a[e]	0.111 ± 0.007b	0.040 ± 0.004a	0.037 ± 0.002a	A
102	3-penten-2-ol	1170	0.180 ± 0.015a	0.479 ± 0.027b	0.191 ± 0.003a	0.167 ± 0.001a	A
103	3-methyl-1-butanol	1209	0.004 ± 0.001a	0.005 ± 0.001a	0.033 ± 0.003b	0.152 ± 0.008c	A
104	1-pentanol	1252	0.020 ± 0.007a	0.028 ± 0.007a	0.026 ± 0.001a	0.067 ± 0.008b	A
105	2-hexanol	1313	0.008 ± 0.002a	0.020 ± 0.001b	0.008 ± 0.001a	0.008 ± 0.001a	A
106	1-hexanol	1356	0.000 ± 0.000a	0.000 ± 0.000a	0.036 ± 0.001b	0.153 ± 0.001c	A
107	3-octanol	1396	0.275 ± 0.011c	0.095 ± 0.011a	0.223 ± 0.006b	0.627 ± 0.010d	A
108	1-octen-3-ol	1458	3.650 ± 0.433b	2.456 ± 0.395a	5.175 ± 0.112c	6.345 ± 0.247d	A
109	(*E*)-linalool oxide (furanoid)	1483	0.310 ± 0.011a	0.218 ± 0.014a	0.429 ± 0.019b	0.455 ± 0.019b	A
110	linalool	1547	0.056 ± 0.003b	0.039 ± 0.002a	0.184 ± 0.006d	0.085 ± 0.002c	A
111	1-octanol	1561	0.147 ± 0.005b	0.073 ± 0.007a	0.477 ± 0.011c	1.438 ± 0.011d	A
112	(*E*)-2-octen-1-ol	1620	0.421 ± 0.015b	0.207 ± 0.007a	0.954 ± 0.006c	1.623 ± 0.066d	A
114	(*Z*)-linalool oxide (pyranoid)	1768	0.152 ± 0.012b	0.107 ± 0.001a	0.214 ± 0.015c	0.263 ± 0.006d	A
115	α-methyl benzenemethanol	1820	0.086 ± 0.011b	0.049 ± 0.012a	0.166 ± 0.018c	0.219 ± 0.016d	A
118	nerolidol	2050	0.046 ± 0.004c	0.000 ± 0.000a	0.025 ± 0.003b	0.146 ± 0.001d	A
119	phenylpropyl alcohol	2061	0.066 ± 0.008a	0.060 ± 0.015a	0.395 ± 0.003c	0.374 ± 0.010b	A
	ketones						
201	3-methyl-3-buten-2-one	<1000	0.018 ± 0.001b	0.019 ± 0.001b	0.012 ± 0.001a	0.010 ± 0.001a	B
202	3-hydroxy-2-butanone	1292	0.073 ± 0.001a	0.606 ± 0.025b	0.093 ± 0.010a	0.937 ± 0.019a	A
203	3-octanone	1259	0.047 ± 0.004b	0.016 ± 0.002a	0.087 ± 0.004c	0.531 ± 0.020d	A
204	1-octen-3-one	1306	0.027 ± 0.001ab	0.018 ± 0.001a	0.031 ± 0.004bc	0.040 ± 0.010c	A
206	dihydro-2(3*H*)-furanone	1643	0.021 ± 0.002b	0.010 ± 0.000b	0.000 ± 0.000a	0.030 ± 0.010b	B
207	5-ethenyldihydro-5-methyl-2(3*H*)-furanone	1684	0.012 ± 0.001ab	0.015 ± 0.004b	0.009 ± 0.001a	0.015 ± 0.003b	B
	aldehydes						
302	hexanal	1085	0.011 ± 0.002ab	0.013 ± 0.002b	0.008 ± 0.001a	0.017 ± 0.001c	A
303	(*E*)-2-octenal	1437	0.029 ± 0.001a	0.068 ± 0.019b	0.027 ± 0.006a	0.084 ± 0.008b	A
305	benzaldehyde	1534	0.011 ± 0.003a	0.007 ± 0.001a	0.017 ± 0.003b	0.036 ± 0.003c	A
	acids and esters						
401	thiocyanic acid methyl ester	1276	0.009 ± 0.001c	0.000 ± 0.000a	0.008 ± 0.001c	0.004 ± 0.002b	B
402	octanoic acid ethyl ester (ethyl octanoate)	1440	0.053 ± 0.008d	0.034 ± 0.002c	0.005 ± 0.001b	0.000 ± 0.000a	A
404	benzenepropanoic acid methyl ester	1857	0.051 ± 0.003c	0.014 ± 0.004a	0.035 ± 0.003b	0.055 ± 0.004c	B
405	octanoic acid	2072	0.000 ± 0.000a	0.000 ± 0.000a	0.025 ± 0.003b	0.169 ± 0.007c	A
406	3-phenyl-2-propenoic acid methyl ester (methyl cinnamate)	2103	5.487 ± 0.177a	6.052 ± 0.511ab	6.452 ± 0.453b	7.552 ± 0.512c	A
407	2,4-furandicarboxylic acid dimethyl ester	2157	0.000 ± 0.000a	0.000 ± 0.000a	0.044 ± 0.001b	0.131 ± 0.005c	B
	terpene hydrocarbons						
501	α-pinene	1026	0.013 ± 0.002b	0.000 ± 0.000a	0.023 ± 0.001c	0.000 ± 0.000a	A
504	limonene	1205	0.002 ± 0.001b	0.000 ± 0.000a	0.003 ± 0.001b	0.000 ± 0.000a	A
505	junipene	1590	0.037 ± 0.001c	0.019 ± 0.001b	0.011 ± 0.003a	0.008 ± 0.001a	B
	miscellaneous						
601	dimethyl sulfone	1912	0.034 ± 0.001b	0.039 ± 0.009b	0.026 ± 0.001a	0.048 ± 0.001c	A

[a] Numbers correspond to those in **Table 2** and **Figures 2** and **3**. [b] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references. [c] Average of relative peak areas to that of the internal standard ($n = 3$) ± the standard deviation. [d] Volatiles were identified on the basis of the following criteria: A, mass spectrum and retention index consistent with those of an authentic standard; B, mass spectrum consistent with that of the Wiley 275 mass spectrum database or by manual interpretation (tentative identification). [e] There are significant differences ($P < 0.05$) among pine-mushrooms using Ducan's multiple comparison test between the samples having a different letter in a row.

(*5*, *13*), benzadehyde (*14*), and benzyl alcohol (*6*) were the most abundant in other mushroom species. Also, the level of carbonyls (3-hydroxy-2-butanone, hexanal, and benzaldehyde) was higher in cooked than in raw pine-mushrooms. In contrast, furfuryl alcohol, benzyl alcohol, phenylethyl alcohol, dihydro-5-methyl-2(3*H*)-furanone, 2(5*H*)-furanone, (*E*)-2-methyl-2-butenal, furfural, phenylacetaldehyde, benzoic acid methyl ester, camphene, and *β*-pinene were detected only in cooked pine-mushrooms. Dihydro-5-methyl-2(3*H*)-furanone, 2(5*H*)-furanone, and furfural could be formed by thermal degradation of carbohydrates, whereas phenylacetaldehyde could be produced by Strecker degradation of phenylalanine in the Maillard reaction (*30*). These volatiles were explained as the major components of cooked mushrooms (*11*).

Comparison of Volatile Components in Raw Pine-Mushrooms of Different Grades. PCA is an unsupervised clustering method that does not require any knowledge of the data set, which reduces the dimensionality of multivariate data while preserving most of the variance therein (*29*). The covariance method for PCA was applied in this study. The raw pine-mushrooms of four grades could be clearly distinguished in the PCA plot (**Figure 2**). The different grades of raw pine-mushrooms could be easily separated in score plots by combining principal component 1 (PC1) (43.3%) with principal component 2 (PC2) (29.4%). The raw pine-mushrooms of the first and second grades (negative PC1 dimension) could be separated from those of the third and fourth grades (positive PC1 dimension) mainly in the score of PC1, while those of the first and third grades (negative PC2 dimension) were separated from those of the second and fourth grades (positive PC2 dimension) in PC2. The major components contributing to the PC1 dimension were ethyl octanoate (no. 402), phenylpropyl alcohol (no. 119), junipene (no. 505), 3-methyl-3-buten-2-one (no. 201), (*E*)-2-octen-1-ol (no. 112), α-methyl benzenemethanol

Table 2. Volatile Components Identified in Cooked Pine-Mushrooms of Four Different Grades by GC–MS

no.[a]	volatile component	RI[b]	relative peak area (mean ± SD)[c] first	second	third	fourth	ID[d]
	alcohols						
102	3-penten-2-ol	1172	0.006 ± 0.001b[e]	0.008 ± 0.001c	0.015 ± 0.002d	0.000 ± 0.000a	A
103	3-methyl-1-butanol	1212	0.021 ± 0.001b	0.137 ± 0.001d	0.002 ± 0.000a	0.084 ± 0.003c	A
104	1-pentanol	1255	0.012 ± 0.001a	0.011 ± 0.001a	0.016 ± 0.001b	0.017 ± 0.001c	A
106	1-hexanol	1359	0.034 ± 0.004b	0.029 ± 0.001b	0.014 ± 0.003a	0.059 ± 0.001c	A
107	3-octanol	1400	0.339 ± 0.003c	0.324 ± 0.001b	0.099 ± 0.001a	0.375 ± 0.004d	A
108	1-octen-3-ol	1461	2.837 ± 0.058a	3.455 ± 0.035b	3.522 ± 0.098c	3.569 ± 0.018c	A
109	(*E*)-linalool oxide (furanoid)	1484	0.310 ± 0.003a	0.529 ± 0.025c	0.431 ± 0.016b	0.674 ± 0.007d	A
110	linalool	1553	0.359 ± 0.022b	0.356 ± 0.008b	0.351 ± 0.040b	0.116 ± 0.001a	A
111	1-octanol	1566	0.221 ± 0.004b	0.359 ± 0.018c	0.126 ± 0.015a	0.845 ± 0.005d	A
112	(*E*)-2-octen-1-ol	1622	0.246 ± 0.002ab	0.232 ± 0.014a	0.269 ± 0.024b	0.378 ± 0.010c	A
113	furfuryl alcohol	1665	0.038 ± 0.003b	0.000 ± 0.000a	0.052 ± 0.004c	0.000 ± 0.000a	A
114	(*Z*)-linalool oxide (pyranoid)	1770	0.102 ± 0.007b	0.000 ± 0.000a	0.206 ± 0.007c	0.353 ± 0.004d	A
115	α-methyl benzenemethanol	1820	0.141 ± 0.006a	0.321 ± 0.013c	0.156 ± 0.006a	0.298 ± 0.002b	A
116	benzyl alcohol	1884	0.000 ± 0.000a	0.046 ± 0.002c	0.000 ± 0.000a	0.016 ± 0.001b	A
117	phenylethyl alcohol	1920	0.134 ± 0.001d	0.124 ± 0.005c	0.012 ± 0.000a	0.062 ± 0.001b	A
119	phenylpropyl alcohol	2058	0.157 ± 0.002b	0.129 ± 0.006a	0.195 ± 0.020c	0.315 ± 0.004d	A
	ketones						
202	3-hydroxy-2-butanone	1291	0.141 ± 0.001b	1.805 ± 0.019c	0.009 ± 0.001a	2.566 ± 0.071d	A
203	3-octanone	1261	0.125 ± 0.006b	0.386 ± 0.001c	0.000 ± 0.000a	0.000 ± 0.000a	A
205	dihydro-5-methyl-2(3*H*)-furanone	1619	0.041 ± 0.001b	0.000 ± 0.000a	0.000 ± 0.000a	0.000 ± 0.000a	B
206	dihydro-2(3*H*)-furanone	1640	0.106 ± 0.006a	0.207 ± 0.003d	0.130 ± 0.016b	0.165 ± 0.003c	B
207	5-ethenyldihydro-5-methyl-2(3*H*)-furanone	1679	0.015 ± 0.002b	0.149 ± 0.001d	0.000 ± 0.000a	0.023 ± 0.001c	B
208	2(5*H*)-furanone	1767	0.375 ± 0.013b	0.855 ± 0.005c	0.148 ± 0.006a	0.157 ± 0.002a	A
	aldehydes						
301	(*E*)-2-methyl-2-butenal	<1100	0.000 ± 0.000a	0.218 ± 0.005c	0.171 ± 0.005b	0.218 ± 0.005c	A
302	hexanal	<1100	0.022 ± 0.002d	0.014 ± 0.001b	0.011 ± 0.001a	0.022 ± 0.001d	A
304	furfural	1465	0.000 ± 0.000a	0.000 ± 0.000a	0.009 ± 0.001c	0.003 ± 0.001b	A
305	benzaldehyde	1530	0.021 ± 0.001b	0.036 ± 0.001c	0.013 ± 0.001a	0.037 ± 0.001c	A
306	phenylacetaldehyde	1648	0.016 ± 0.002b	0.016 ± 0.001b	0.007 ± 0.001a	0.025 ± 0.001c	A
	acids and esters						
401	thiocyanic acid methyl ester	1274	0.013 ± 0.001c	0.011 ± 0.001b	0.003 ± 0.000a	0.003 ± 0.000a	B
403	benzoic acid methyl ester	1631	0.019 ± 0.001b	0.441 ± 0.014d	0.000 ± 0.000a	0.053 ± 0.001c	A
404	benzenepropanoic acid methyl ester	1854	0.030 ± 0.001b	0.044 ± 0.001c	0.023 ± 0.002a	0.044 ± 0.001c	B
406	3-phenyl-2-propenoic acid methyl ester (methyl cinnamate)	2095	7.074 ± 0.577a	7.172 ± 0.124a	7.279 ± 0.112a	7.293 ± 0.067a	A
407	2,4-furandicarboxylic acid dimethyl ester	2153	0.000 ± 0.000a	0.037 ± 0.001b	0.111 ± 0.004c	0.145 ± 0.002d	B
	terpene hydrocarbons						
502	camphene	<1100	0.007 ± 0.001c	0.005 ± 0.001b	0.000 ± 0.000a	0.000 ± 0.000a	A
503	β-pinene	1114	0.002 ± 0.007b	0.002 ± 0.001b	0.001 ± 0.000a	0.002 ± 0.000ab	A
504	limonene	1204	0.025 ± 0.001c	0.004 ± 0.001b	0.002 ± 0.000a	0.002 ± 0.000a	A
505	junipene	1583	0.036 ± 0.002b	0.000 ± 0.000a	0.064 ± 0.000a	0.000 ± 0.000a	B
	miscellaneous						
601	dimethyl sulfone	1908	0.075 ± 0.012ab	0.098 ± 0.002c	0.065 ± 0.001a	0.079 ± 0.001b	A

[a] Numbers correspond to those in **Table 1** and **Figures 2** and **3**. [b] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references. [c] Average of relative peak areas to that of the internal standard ($n = 3$) ± the standard deviation. [d] Volatiles were identified on the basis of the following criteria: A, mass spectrum and retention index consistent with those of an authentic standard; B, mass spectrum consistent with that of the Wiley 275 mass spectrum database or by manual interpretation (tentative identification). [e] There are significant differences ($P < 0.05$) among pine-mushrooms using Ducan's multiple comparison test between the samples having a different letter in a row.

(no. 115), 2,4-furandicarboxylic acid dimethyl ester (no. 407), linalool oxide (pyranoid) (no. 114), 1-octen-3-ol (no. 108), methyl cinnamante (no. 406), (*E*)-linalool oxide (no. 109), 1-octanol (no. 111), and 1-hexanol (no. 106). In contrast, the important components of the PC2 dimension were α-pinene (no. 501), dimethyl sulfone (no. 601), dihydro-2(3*H*)-furanone (no. 206), limonene (no. 504), hexanal (no. 302), (*E*)-2-octenal (no. 303), and 3-hydroxy-2-butanone (no. 202).

The application of ANOVA to the GC−MS data set demonstrated that the mean values of the relative peak areas of all 35 volatile components identified in raw pine-mushrooms differed significantly with grade ($P < 0.05$) (**Table 1**). It was possible to determine the major volatile components contributing to the difference of raw pine-mushrooms according to their grades by analyzing the correlation of each variable (**Table 1**) with PC1 and PC2 scores (**Figure 2**). In particular, raw pine-mushrooms of the first grade contained much higher levels of ethyl octanoate (no. 402), junipene (no. 505), and 3-methyl-3-buten-2-one (no. 201), of which ethyl octanoate was previously described as a fruity odorant (*30*). In contrast, 2-methyl-3-buten-2-ol (no. 101), 3-penten-2-ol (no. 102), 2-hexanol (no. 105), dihydro-2(3*H*)-furanone (no. 206), 5-ethenyldihydro-5-methyl-2(3*H*)-furanone (no. 207), and 3-methyl-3-buten-2-one (no. 201) were the main contributors to raw pine-mushrooms of the second grade. The level of these volatiles, which could be mainly formed by the degradation of unsaturated lipids or carbohydrates (*30*), was higher in raw pine-mushrooms of the second grade than in those of the first grade. In raw pine-mushrooms of the third grade, linalool (no. 110), phenylpropyl alcohol (no. 119), furanoid (no. 109), and α-pinene (no. 501) were the dominant components (on positive PC1 and negative PC2). Among many volatile components loaded on both positive PC1 and PC2,

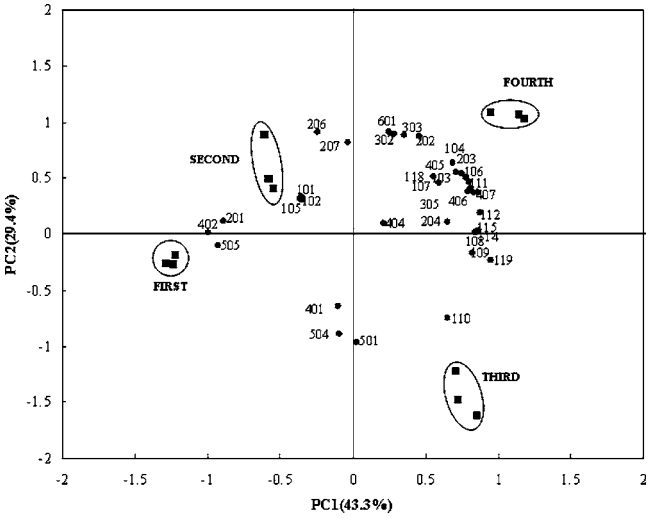

Figure 2. PCA score plots from raw pine-mushrooms of four different grades by the combination of PC1 and PC2.

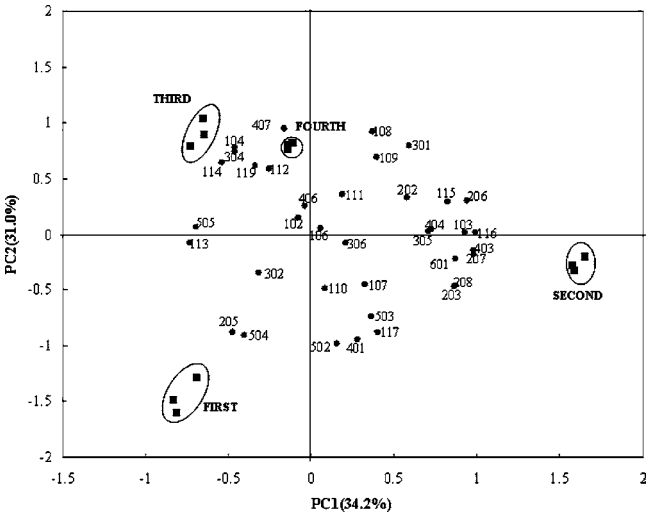

Figure 3. PCA score plots from cooked pine-mushrooms of four different grades by the combination of PC1 and PC2.

1-pentanol (no. 104), 3-octanone (no. 203), 3-methyl-1-butanol (no. 103), 1-hexanol (no. 106), octanoic acid (no. 405), 1-octanol (no. 111), benzaldehyde (no. 305), methyl cinnamate (no. 406), 3-hydroxy-2-butanone (no. 202), nerolidol (no. 118), 3-octanol (no. 107), (*E*)-2-octen-1-ol (no. 112), α-methyl benzenemethanol (no. 115), pyranoid (no. 114), and 1-octen-3-ol (no. 108) were associated with raw pine-mushrooms of the fourth grade. It has been thought that ethyl octanoate, junipene, and 3-methyl-3-buten-2-one were found in larger amounts in higher-quality pine-mushrooms. In contrast, the levels of the C_8 components of 3-octanone, 1-octanol, 3-octanol, (*E*)-2-octen-1-ol, and 1-octen-3-ol which had been reported to be important volatiles in mushrooms (*7*−*12*) were higher in lower-quality pine-mushrooms.

Comparison of Volatile Components in Cooked Pine-Mushrooms of Different Grades. As shown in **Figure 3**, PCA allowed cooked pine-mushrooms of different grades to be easily separated in score plots by combining PC1 (34.2%) with PC2 (31.0%). The cooked pine-mushrooms of the first, second, and third grades (negative PC1 dimension) were separated from those of the second grade (positive PC1 dimension) predominantly in the score of PC1, while cooked pine-mushrooms of the first and second grades (negative PC2 dimension) were separated from those of the third and fourth grades (positive PC2 dimension) in PC2. Benzyl alcohol (no. 116), benzoic acid methyl ester (no. 403), 5-ethenyldihydro-5-methyl-2(3*H*)-furanone (no. 207), dihydro-2(3*H*)-furanone (no. 206), 3-methyl-1-butanol (no. 103), dimethyl sulfone (no. 601), 2(5*H*)-furanone (no. 208), 3-octanone (no. 203), and α-methyl benzenemethanol (no. 115) contributed to the PC1 dimension of cooked pine-mushrooms. In contrast, camphene (no. 502), thiocyanic acid methyl ester (no. 401), 2,4-furandicarboxylic acid dimethyl ester (no. 407), 1-octen-3-ol (no. 108), limonene (no. 504), phenylethyl alcohol (no. 117), and dihydro-5-methyl-2(3*H*)-furanone (no. 205) were the major components of the PC2 dimension.

The application of ANOVA to the GC−MS data set demonstrated that the mean values of the relative peak areas of 36 volatile components identified in cooked pine-mushrooms (i.e., all except methyl cinnamate) differed significantly with grade ($P < 0.05$) (**Table 2**). It was possible to determine the major volatile components contributing to the difference in cooked pine-mushrooms according to their grades by analyzing the ANOVA data (**Table 2)** and PCA score plots (**Figure 3**). In cooked pine-mushrooms of the first grade, dihydro-5-methyl-2(3*H*)-furanone (no. 205) and limonene (no. 504) were the dominant components. In contrast, benzoic acid methyl ester (no. 403), 5-ethenyldehydro-5-methyl-2(3*H*)-furanone (no. 207), 2(5*H*)-furanone (no. 208), 3-octanone (203), dimethyl sulfone (no. 601), benzyl alcohol (no. 116), 3-methyl-1-butanol (no. 103), benzenepropanoic acid methyl ester (no. 404), and benzaldehyde (no. 305) were the main contributors to cooked pine-mushrooms of the second grade. Junipene (no. 505) and furfuryl alcohol (no. 113) were the dominant components in cooked pine-mushrooms of the third grade, and the levels of (*E*)-2-octen-1-ol (no. 112), 2,4-furandicarboxylic acid dimethyl ester (no. 407), phenylpropyl alcohol (no. 119), 1-pentanol (no. 104), furfural (no. 304), pyranoid (no. 114), 1-octanol (no. 111), 1-octen-3-ol (no. 108), and furanoid (no. 109) were higher in cooked pine-mushrooms of the fourth grade. Dihydro-5-methyl-2(3*H*)-furanone, benzyl alcohol, 2(5H)-furanone, furfuryl alcohol, and furfural were identified only in cooked pine-mushrooms, which contributed to their difference in cooked pine-mushrooms. These results suggest that volatile components formed by various thermal reactions during cooking were mainly responsible for the difference in cooked pine-mushrooms according to their grades.

In conclusion, differences in the composition of volatile components from pine-mushrooms of each grade were observed, and those data could be used for the characterization of each grade. The difference in the volatile components of raw pin-mushrooms according to their grades was mainly due to phenylpropyl alcohol, (*E*)-2-octen-1-ol, dimethyl sulfone, dihydro-2(3*H*)-furanone, and others. In contrast, benzyl alcohol, benzoic acid methyl ester, 2,4-furandicarboxylic acid dimethyl ester, 1-octen-3-ol, and others were the main components contributing to the difference in the volatile components of cooked pine-mushrooms.

ABBREVIATIONS USED

GC−MS, gas chromatography−mass spectrometry; PCA, principal component analysis; RI, linear retention indices; ANOVA, analysis of variance.

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