

## Differences in the Volatile Components and Their Odor Characteristics of Green and Ripe Fruits and Dried Pericarp of Japanese Pepper (*Xanthoxylum piperitum* DC.)

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The essential oils of green and ripe fruits and dried pericarp of Japanese pepper (*Xanthoxylum piperitum* DC.), which are commonly used in Japanese dishes as spices, were extracted with methanol, followed by adsorption to Porapak Q resin. Their aroma profiles were characterized by a sensory evaluation, and their chemical constituents were investigated by using gas chromatography (GC), gas chromatography–mass spectrometry, and aroma extract dilution analysis. Geraniol, citronellal, linalool, and methyl cinnamate were perceived to be important to the basic flavor of the three samples of Japanese pepper by GC–sniffing at high flavor dilution (FD) factors. Monoterpene hydrocarbons constituting almost 76% of the essential oil are the major flavor compounds in the green fruit, and the stronger green and pine leaf notes in the green fruit were considered to be imparted mainly by the large amount of *d*-limonene,  $\beta$ -phellandrene, and myrcene due to their high FD factors. On the other hand, the oxygenated terpenes including citronellal, geraniol, and geranial are predominant for the potent odorant in the ripe fruit. The marked citrus-like note in the ripe fruit was thought to be due to the amounts of geranial and citronellal, being 20% of the essential oil. In the dried pericarp, the ratio of oxygenated terpenoids was almost equal to that of monoterpene hydrocarbons. These seemed to induce the flavor character of the dried pericarp to be milder than that of the ripe fruit.

**KEYWORDS:** Japanese pepper; *Xanthoxylum piperitum* DC.; aroma, potent odorant

### INTRODUCTION

The flavor components of Japanese pepper (*Xanthoxylum piperitum* DC.) have already been investigated (1–7), because the fresh young leaves and green and dried fruits are commonly utilized in Japanese cuisine for their pleasant flavor note and pungent taste. The use of fresh herbs and spices is characteristic of Japanese dishes; for example, immature or mature ginger rhizomes grated or soaked in vinegar are used in preference to the dried type. These spices are utilized in quite different ways because of their different flavors, tastes, and textures. We are interested in this Japanese food culture and attempted to clarify the difference in the flavor of Japanese pepper and the formation mechanism.

Fresh young leaves of Japanese pepper are mainly used to impart a pleasant and fresh flavor to dishes after being slapped or crushed. We have previously investigated the flavor components from samples by the different mechanical stimuli and discussed the formation mechanism for the main aroma compounds (8, 9). The constituents and amounts of the C<sub>6</sub> compounds and oxygenated monoterpenes are different between slapped and crushed leaves, this difference being due to the

activity of lipoxygenase for unsaturated fatty acids and of glycosidase for the glycosides as alcoholic aroma precursors.

Green and dried fruits are differently utilized in Japanese cuisine. Green fruits, which are unripe and harvested from the end of May to the beginning of June, are widely used as a seasonal spice to impart a fresh flavor or to suppress any unpleasant fishy and meaty odor. After the fruits have become ripe from the end of July, the fresh ripe fruits are also utilized in boiled and seasoned Japanese dishes. The ripe fruits, after being dried in the sun or by heat treatment at 40–60 °C for 10 h, are separated from the seeds, and the resulting pericarps are ground. The ground pericarps are often used alone or mixed with hot pepper as a spice for cooking fish to mask the fishy odor. The dried pericarp is also commonly utilized in Chinese cooking, although the cultivar of the plant is different.

Efforts have been made in earlier work to investigate the constituents of the essential oil of the fruits. Sakai et al. have analyzed the volatile components in the essential oil of unripe and ripe fruits prepared by steam distillation (2). Wu et al. have reported the volatile aroma compounds in young leaves and green fruits by employing an adsorptive column method (5). One of the present authors has recently compared the compositions of the volatile compounds in various dried pericarps from Japan, China, and Korea and one unripe fruit by using a dynamic headspace gas analysis (7). These studies have identified

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limonene,  $\beta$ -phellandrene, citronellal, and geranyl acetate as the main compounds, although the quantities and constituents of the volatile components of the fresh fruit and dried pericarp from various regions or countries were significantly different. The suppressive effect of the volatile compounds on any unpleasant fishy odor in dishes has also been studied by some Japanese researchers (10–12), and it was reported that citronellal, geranyl acetate, limonene, and myrcene, in single or mixed form, played an important role in this odor-masking function.

Although the volatile components have been identified in many studies, little information has been presented about the potent flavor compounds in Japanese pepper. Citronellol and citronellal were demonstrated in our previous paper to be the characteristic aroma compounds in the young leaves, but the potent odorants in the fruits were not as obvious. The compounds contributing to the odor and the differences in the flavor characteristics of the unripe, ripe, and dried fruits from the same cultivar were therefore considered to be worth examining due to of their wide uses.

Our main aim in the present study is to clarify the aroma characteristics and their differences in the green and ripe fruits and dried pericarp from one cultivar of Japanese pepper. We extracted the essential oils by adsorption to Porapak Q resin and investigated their volatile components by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analyses. The potent odorants were additionally identified by an aroma extract dilution analysis (AEDA) method and from the results of a sensory evaluation.

## MATERIALS AND METHODS

**Materials.** The green and ripe fruits and dried pericarp of Japanese pepper (var. Budo sansho) were purchased from the same plantation in Wakayama prefecture of Japan in 2001. The fresh green fruit was harvested on May 28, and the ripe fruit was obtained on July 25. The dried fruit was purchased on October 2 after having been dried by a heat treatment at 40–60 °C for 10 h. The pericarp of the dried fruit was separated from the seeds and used for the analyses.

**Standard Compounds for Identification.** *d*-Limonene and linalool were obtained from Kanto Chemical Ltd. (Tokyo, Japan);  $\alpha$ -phellandrene, 2-carene,  $\beta$ -caryophyllene, isopulegol, (*E,E*)-2,4-hexadienal, neryl acetate, and cinnamyl acetate were purchased from Tokyo Kasei Kogyo Ltd. (Japan);  $\alpha$ -humulene was from Sigma-Aldrich (Tokyo, Japan); all of the terpenoids,  $\beta$ -phellandrene, sabinene, (*Z*)- and (*E*)- $\beta$ -ocimene, elemol, methyl benzoate, and piperitone were provided by T. Hasegawa Co. Ltd. (Tokyo, Japan); and other standard compounds came from Wako Co. Ltd. (Saitama, Japan).

**Extraction of the Volatile Compounds.** The green or ripe fruit (6.0 g) or the dried pericarp (2.5 g) was immersed in 50 mL of methanol to suppress the enzymatic activities of the fresh fruits, and ethyl decanoate was then added as an internal standard. After homogenization with a mixer, the suspension was filtered through a nylon cloth, and the remaining solids in the filtrate were separated by centrifugation for 15 min at 3000 rpm and 4 °C. The resulting supernatant, after being adjusted to a 10% methanol aqueous solution with purified water, was immediately submitted to chromatography in a column packed with 50 mL of Porapak Q resin, which had been washed with the same solvent and water just before using. The adsorbed compounds were eluted with 250 mL of pentane and diethyl ether (2:3), after the water-soluble compounds such as sugars and amino acids had been removed with 150 mL of purified water. The eluate was dried overnight on anhydrous sodium sulfate and concentrated at 39.5 °C under atmospheric pressure via a short-length Vigreux column to obtain the concentrate. The extract was additionally concentrated with nitrogen just before injection into the gas chromatograph.

The dried weight of each sample was measured after freezing with liquid nitrogen and subsequent lyophilization.

**Analysis by Gas Chromatography.** The GC analysis was carried out with a Hewlett-Packard (HP) 5890 series II gas chromatograph

coupled with a flame ionization detector (FID), in which a DB-Wax capillary column (J&W Scientific, 60 m  $\times$  0.25 mm i.d.) was connected to the GC instrument. The analytical conditions were as follows: helium carrier gas flow rate, 1.0 mL/min; respective injector and detector temperatures, 200 and 220 °C; oven temperature program, 60 °C (hold for 4 min) rising to 220 °C at a rate of 2 °C/min; and split ratio, 30:1.

**Analysis by Gas Chromatography–Mass Spectrometry.** The GC-MS analysis was also conducted with the Hewlett-Packard (HP) 5890 series II gas chromatograph coupled to an HP 5972 mass spectrometer. The GC analytical conditions were the same as those just stated. The MS instrument was operated in the electron impact (EI) mode and scanned at 70 eV in an *m/z* range of 30–400 mass units.

**Chiral Gas Chromatographic Analysis.** Linalool was analyzed by GC in a similar manner, a CP-cyclodextrin- $\beta$ -2, 3, 6-M-19 column (Chrompack, 50 m  $\times$  0.25 mm i.d.) being used in place of the DB-Wax column. The oven temperature was held isothermally at 95 °C, the other analytical conditions being the same as those described for the GC analysis.

**Sensory Evaluation. Sample Preparation.** The extraction of the volatile compounds for the sensory evaluation was done in a manner similar to that for the GC analysis, except that the internal standard was not added. After as much solvent as possible had been distilled off, each essential oil of the green and ripe fruits and dried pericarp, corresponding to 0.1 g of freeze-dried fruits, was dissolved in a small amount of propylene glycol and then diluted to 10 mL with water. A 50-mL glass bottle with a plastic screw cap containing each resulting solution was covered with white paper to unify the sample color and coded with a random three-digit number prior to the sensory evaluation.

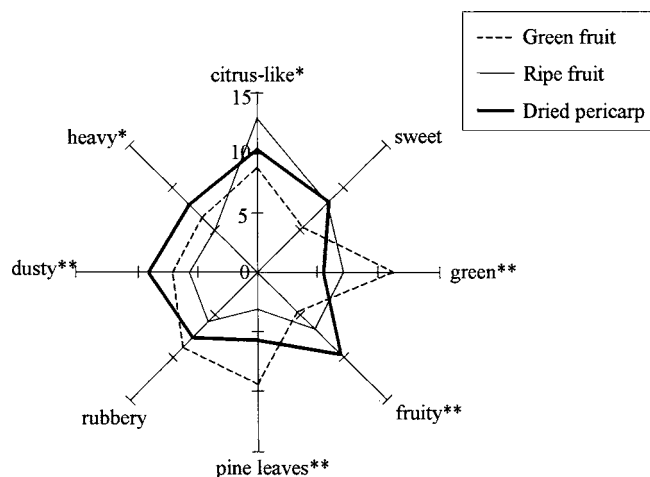
**Sensory Evaluation.** Sensory profiles of the flavor were evaluated by 10 well-trained females in the age range of 21–37 years. The terms for evaluating each essential oil were first determined. The panelists were requested to record the appropriate terms for evaluating the flavor of the three samples. Eight attributes were finally agreed upon after several consultations among the panelists: citrus-like, sweet, green, fruity, pine leaves, rubbery, dusty, and heavy. Each term was evaluated for the three samples by a 15-cm line scale (0, absent; 15, strong) as reported by Morita et al. (13). The samples prepared as already described were presented in a random sequence to the panelists, and they were asked to sniff and rate the score for the intensity of each flavor note. The resulting data were analyzed by Tukey's multiple-comparison test.

**Determination of the Potent Odorants. Sample Preparation.** The potent odorants were determined by an aroma extract dilution analysis (AEDA). The aroma concentrate was the same as used for GC analysis, corresponding to 1 g of a freeze-dried sample containing no internal standard, and was diluted with diethyl ether in a volume ratio of 4-fold and then subjected to the GC–olfactometry (GC-O) analysis. The flavor dilution factor of each odorant was determined, and the retention index of each odorant was calculated after being analyzed again by the GC analysis.

**Analyses by Gas Chromatography–Olfactometry.** The sniffing analysis was conducted by the GC apparatus already described. The GC instrument and temperature conditions were similar to those for the GC analysis. A column of 60 m  $\times$  0.53 mm i.d. (1  $\mu$ m film) coated with DB-Wax (J&W Scientific) was employed for GC-O analysis. The flow rate of helium as the carrier gas was set at 8.3 mL/min. After 1  $\mu$ L of the essential oil had been injected into the column in the splitless mode, the carrier gas was split 1:1 at the outlet of the column and passed through the FID detector and a glass sniffing port at 220 °C. Wet air was pumped into the sniffing port at 45 mL/min to quickly remove the odorant and keep the nose moist.

## RESULTS AND DISCUSSION

The classification of Japanese pepper is difficult due to the large number of varieties, so we employed the fruits of only the variety Budo sansho from Wakayama prefecture in Japan as our samples; this variety makes up ~70% of the market in Japan. The yields of the freeze-dried material used here were 18.5% for the green fruit, 33.5% for the ripe fruit, and 93.5%



**Figure 1.** Flavor profiles of the green and ripe fruits and dried pericarp of Japanese pepper. Each value represents the average score on a scale of 0 (absent) to 15 (strong). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

for the dried pericarp. There was a remarkable difference in the content of water between the green and ripe fruits, water being more abundant in the green fruit.

**Sensory Evaluation of the Flavor.** The sensory odor profiles of the green and ripe fruits and dried pericarp were examined by sensory evaluation. The results are summarized with a radar chart in **Figure 1**. It can be seen that the three samples had their own respective flavor characteristics. The green fruit, which was unripe, was characterized most strongly for its green note at a score of 11.2 by the panelists, this being followed by pine leaves (9.3) and rubbery (8.9) notes. The scores for fruity and sweet notes were the weakest among the samples, being only 4.7 and 5.3. This evaluation indicates that the green fruit had a very fresh and pleasant flavor. On the other hand, the citrus-like note (12.8) was strongest in the ripe fruit, whereas other odors were very weak. It is likely that the citrus-like note was so strong as to overpower other notes. The fruity note was significantly strongest in the dried pericarp. The heavy and dusty notes (8.0 and 9.1, respectively) were also stronger than in the fresh green and ripe fruits, which was considered to be due to some fresh flavor compounds dispersed during the heat treatment process.

**Identification of the Volatile Compounds.** The volatile components in the essential oil from the green and ripe fruits and dried pericarp were investigated by using GC and GC-MS analyses. The compounds were confirmed by comparing the mass spectra from GC-MS and the Kovats indices (KI) with those of authentic chemicals. The contents, calculated as the peak area percent and the ratio of peak area to that of the internal standard, are summarized in **Table 1**. The respective numbers of compounds detected were 57, 79, and 83 in the green and ripe fruits and dried pericarp. Most of the compounds reported previously (2–8) were identified as common components in the three samples.

It was generally apparent that the quantities of the main compounds in the three samples were different. The most abundant compounds in the green fruit were *d*-limonene,  $\beta$ -phellandrene, and myrcene. Their relative contents (RCs) in comparison with the internal standard of **1** were 12.79, 7.96, and 2.20, respectively, constituting almost 75% of all the volatile constituents, with *d*-limonene in particular being above 40%. This is similar to the results reported previously (5, 7), in which the monoterpene hydrocarbons were quantitatively predominant.

Geranyl acetate was the main compound among the oxygenated terpenoids, with an RC value of 3.14 (10.11%) in the green fruit. Methyl cinnamate and linalyl acetate were also detected as 0.19 (0.61%) and 0.11 (0.36%), these levels being higher than those of the other esters. Other oxygenated terpenoids such as citronellal and geraniol were relatively low in their contents, being 0.57 (1.82%) and 0.23 (0.73%).

On the other hand, the monoterpene hydrocarbons were markedly less in the ripe fruit at 10.77 (20.45%), whereas the contents of geraniol, citronellal, and geranyl acetate were greatly increased to 5.81 (11.02%), 8.55 (16.22%), and 21.10 (40.04%), respectively. The increase of geranyl acetate was especially predominant. It is well-known that esters increase with esterification during the development of many fruits, for example, strawberry (14), and that esters often have a “fruity” odor. It seemed that a similar reaction was promoted in the ripe fruit of Japanese pepper. In addition, geraniol was first detected at 1.79 (3.40%) in the ripe fruit.

The contents of oxygenated terpenoids in the dried pericarp were almost equal to that of monoterpene hydrocarbons. Many flavor compounds seemed to be scattered or changed to other compounds during the heat treatment process. The amount of isopulegol as the cyclization product of citronellal maintained almost the same level as that in the ripe fruit, indicating the change in citronellal for the heat drying process. This heat change was also reported in our previous study (8). Moreover, most of the oxygenated terpenoids were less than those in the ripe fruit; geraniol especially decreased to 0.34% due to its relative instability to heat, oxygen, and light (15). The ratios of aldehydes and esters decreased from 20.23 and 42.52% in the ripe of fruit to 7.81 and 21.60% in the dried pericarp, respectively. This quantitative balance among the oxygenated terpenoids resulted in a gentler flavor of the dried pericarp than of the ripe fruit.

In addition, the volatile components from the seeds contained in the green and ripe fruits were also analyzed in the same manner. The yields were only ~1% of essential oil on a whole-fruit basis, and few aroma compounds were detected (data not shown). Therefore, the amount of oil from the seeds was ignored in our experiment.

**Identification of the Potent Odorants.** To clarify the potent odorants contributing to the characteristic flavor of the green and ripe fruits and dried pericarp, the AEDA method (16) was carried out.

The aroma-active compounds recognized and their odor properties are given in **Table 2**. Thirty, 40, and 55 compounds were detected as odor-active compounds in the green fruit, ripe fruit, and dried pericarp, respectively. In the green fruit, geraniol and linalool were detected to have high  $\log_4$  FD factor (6 and 5), followed by citronellal (4), myrcene (4), *d*-limonene (4),  $\beta$ -phellandrene (4), and methyl cinnamyl acetate (4). It seemed that geraniol and linalool, in the small amounts reported in **Table 1**, presented a basic pleasant flavor of the fruits of Japanese pepper for their characteristic notes and their low aroma threshold value (17). Myrcene, *d*-limonene, and  $\beta$ -phellandrene, showing high FD factors, were thought to be responsible for the strong pine leaf and green odors of the green fruit, because they were contained in large amount as shown in **Table 1** regardless of their higher aroma threshold value. Citronellal, having a green and sweet note, was also considered to play an important role in intensifying the green note in the green fruit. Methyl cinnamate, which was perceived as having the fatty, sweet, and sweat note, was speculated to also contribute to the sweet note.

Table 1. Volatile Compounds Identified from the Essential Oil of Fruits of Japanese Pepper

KI <sup>a</sup>	compound	green fruit		ripe fruit		dried fruit	
		RC <sup>b</sup>	PA <sup>c</sup> (%)	RC	PA (%)	RC	PA (%)
	hydrocarbons						
998	decane	0.47	1.52	0.01	0.02	tr <sup>f</sup>	0.01
1005	4-methyldecane <sup>g</sup>	0.07	0.24				
1025	$\alpha$ -pinene	0.13	0.42	0.02	0.03	0.01	0.05
1041	toluene	1.21	3.88	0.01	0.01		
1053	2-methyldecane	0.11	0.35				
1082	butylcyclohexane	0.06	0.20				
1097	undecane	0.34	1.11	0.08	0.15	0.01	0.06
1110	$\beta$ -pinene <sup>g</sup>	0.02	0.08	0.01	0.01	0.01	0.04
1122	sabinene <sup>g</sup>	0.09	0.29	0.03	0.05	0.03	0.15
1163	myrcene <sup>g</sup>	2.20	7.08	0.92	1.75	0.83	4.40
1167	$\alpha$ -phellandrene <sup>g</sup>	0.05	0.15				
1170	$\alpha$ -terpinene <sup>g</sup>	0.01	0.02	tr	0.01	tr	0.02
1202	<i>d</i> -limonene <sup>g</sup>	12.79	41.14	6.04	11.47	5.55	29.54
1211	$\beta$ -phellandrene <sup>g</sup>	7.96	25.60	3.64	6.91	3.35	17.79
1244	( <i>Z</i> )- $\beta$ -ocimene <sup>g</sup>	0.08	0.24	0.02	0.04	tr	0.03
1248	$\gamma$ -terpinene <sup>g</sup>					tr	0.01
1250	( <i>E</i> )- $\beta$ -ocimene <sup>g</sup>	0.04	0.12	0.01	0.03	0.01	0.05
1261	<i>p</i> -ethyltoluene <sup>g</sup>	0.05	0.16	0.01	0.02	tr	0.02
1271	<i>p</i> -cymene <sup>g</sup>	0.01	0.02	tr	0.01	tr	0.01
1282	terpinolene <sup>g</sup>	0.17	0.55	0.05	0.09	0.02	0.12
1285	2-carene <sup>g</sup>	0.01	0.02	0.01	0.01	tr	0.01
1469	$\alpha$ -cubebene <sup>d</sup>	0.01	0.02			tr	0.02
1492	$\alpha$ -copaene <sup>d</sup>	0.01	0.04	0.03	0.05	0.01	0.05
1586	$\beta$ -elemene <sup>d</sup>			0.02	0.04	0.01	0.04
1592	$\beta$ -caryophyllene	0.20	0.65	0.23	0.44	0.08	0.43
1610	aromadendrene <sup>d</sup>			0.01	0.01	tr	0.01
1665	$\alpha$ -humulene	0.03	0.11	0.06	0.12	0.01	0.05
1681	<i>p</i> -mentha-1,4,8-triene <sup>o,g</sup>			0.02	0.04	0.01	0.04
1703	germacrene D	0.09	0.30	0.23	0.43	0.12	0.65
1709	$\beta$ -cubebene <sup>d</sup>	tr	0.01	0.02	0.04	0.01	0.05
1714	$\beta$ -selinene <sup>d</sup>	0.01	0.03	0.01	0.03		
1719	$\alpha$ -selinene <sup>d</sup>			0.02	0.04		
1730	( <i>E,E</i> )- $\alpha$ -farnesene					0.03	0.17
1763	$\beta$ -bisabolene <sup>d</sup>	0.03	0.11				
1892	isomer of farnesene <sup>e</sup>					0.01	0.07
	total	26.26	84.46	11.52	21.87	10.10	53.85
	alcohols						
1091	2-methylpropanol					tr	0.01
1385	( <i>Z</i> )-3-hexenol					tr	0.01
1528	1,2-propanediol <sup>e</sup>					0.34	1.80
1546	linalool	0.04	0.14	0.44	0.84	0.15	0.78
1563	1-methyl-4-(1-methylethyl) 2-cyclohexen-1-ol <sup>e</sup>	0.03	0.09	0.12	0.24	0.05	0.29
1571	isopulegol			0.05	0.10	0.05	0.27
1599	terpinen-4-ol			0.03	0.05	0.01	0.04
1628	1-terpineol	0.01	0.05	0.06	0.11	0.03	0.14
1670	<i>p</i> -mentha-( <i>E</i> )-2,8(9)-dienol					0.01	0.05
1684	$\delta$ -terpineol			0.03	0.05	0.01	0.07
1698	$\alpha$ -terpineol	0.03	0.10	0.01	0.01	0.05	0.26
1744	piperitol <sup>d</sup>			0.04	0.07	0.01	
1765	citronellol	0.02	0.08	0.28	0.53	0.05	0.28
1797	myrtenol			0.02	0.03	0.01	0.05
1832	( <i>Z</i> )-carveol			0.01	0.03	0.01	0.05
1834	( <i>E</i> )-carveol			0.01	0.03	0.01	0.07
1849	geraniol	0.23	0.73	5.81	11.02	1.67	8.90
1873	benzyl alcohol					tr	0.01
1945	3,7-dimethyl-1,5-octadiene-3,7-diol	0.02	0.06	0.01	0.03	0.01	0.03
2028	ledol <sup>d</sup>			0.01	0.02	tr	0.02
2048	<i>endo</i> -1-bourbananol <sup>d</sup>			0.05	0.09	0.03	0.14
2078	elemol	0.01	0.03	0.11	0.21	0.03	0.15
2101	4-(1-methylethyl)benzenemethanol <sup>e</sup>			0.04	0.08	0.01	0.06
2121	spathulenol <sup>d</sup>	tr	0.02	0.03	0.05	0.01	0.06
2189	$\delta$ -cadinol <sup>e</sup>			tr	0.01	0.11	0.60
2224	bisabolol <sup>d</sup>	tr	0.01	0.10	0.20	0.12	0.66
2242	$\beta$ -eudesmol <sup>d</sup>			0.02	0.04	0.01	0.05
2273	1-hydroxylinalool <sup>e</sup>	0.07	0.24	0.01	0.03	0.03	0.16
2362	8-acetoxylinool <sup>e</sup>			0.06	0.11	0.06	0.29
	total	0.47	1.54	7.35	13.96	2.88	15.37

Table 1 (Continued)

KI <sup>a</sup>	compound	green fruit		ripe fruit		dried fruit	
		RC <sup>b</sup>	PA <sup>c</sup> (%)	RC	PA (%)	RC	PA (%)
	aldehydes						
1392	( <i>E,E</i> )-2,4-hexadienal	0.01	0.02	0.01	0.02	tr	0.01
1479	citronellal	0.57	1.82	8.55	16.22	1.36	7.26
1676	neral			0.31	0.59	0.04	0.20
1727	geranial	0.02		1.79	3.40	0.06	0.34
1747	4-ethylbenzaldehyde <sup>e</sup>	tr	0.01				
	total	0.60	1.86	10.66	20.23	1.47	7.81
	esters						
1185	methyl hexanoate	0.01	0.02	0.01	0.02		
1233	ethyl hexanoate	0.01	0.03	0.01	0.02		
1353	isobutyl hexanoate	0.04	0.13	0.09	0.17	0.02	0.12
1553	linalyl acetate	0.11	0.36	0.33	0.62	0.33	1.75
1615	methyl benzoate			0.01	0.02		
1658	citronellyl acetate	0.03	0.10	0.11	0.20	0.07	0.39
1691	$\alpha$ -terpinenyl acetate	0.05	0.15	0.12	0.22	0.12	0.63
1760	geranyl acetate	3.14	10.11	21.10	40.04	3.33	17.71
1779	neryl acetate			0.02	0.04		
1814	geranyl butyrate	0.01	0.02	0.03	0.07	0.02	0.13
2071	methyl cinnamate	0.19	0.61	0.56	1.07	0.16	0.83
2145	cinnamyl acetate			0.02	0.04	0.01	0.03
	total	3.59	11.53	22.41	42.52	4.06	21.60
	ketones						
1668	cryptone <sup>d</sup>					0.06	0.30
1722	piperitone	0.06	0.21	0.31	0.60	0.08	0.43
1823	1-(3,4-dimethylphenyl)ethanone <sup>e</sup>	0.01	0.03	0.03	0.06	tr	0.02
1826	isomer of ethylacetophenone <sup>e</sup>			0.03	0.06	0.01	0.05
1860	isomer of ethylacetophenone <sup>e</sup>	tr	0.01	0.03	0.06	0.01	0.05
2107	valeranone <sup>e</sup>			0.04	0.08	0.03	0.13
	total	0.07	0.25	0.45	0.85	0.19	0.99
	acids						
1407	3-hexenoic acid <sup>e</sup>	0.01	0.02	0.04	0.07	0.01	0.05
1448	acetic acid			0.02	0.04	tr	0.02
1842	hexanoic acid					0.02	0.09
1952	heptanoic acid			0.01	0.02	0.01	0.04
2060	octanoic acid			0.01	0.03	0.01	0.06
	total	0.01	0.02	0.08	0.16	0.05	0.26
	others						
1213	1,8-cineole			0.15	0.29		
1403	2,5-dihydro-3-methyl furan <sup>e</sup>	0.01	0.03	0.02	0.03	tr	0.02
1978	caryophyllene oxide	0.01	0.02	0.02	0.03	0.01	0.05
	total	0.01	0.05	0.19	0.35	0.01	0.07

<sup>a</sup> KI, Kovats index on DB-Wax column. <sup>b</sup> RC (relative content), average values are calculated by comparing the peak area of each compound with that of the internal standard, which is assigned the numerical value of 1,  $n = 3$ . <sup>c</sup> PA (peak area), average of relative percentage of total peak area,  $n = 3$ . <sup>d</sup> Identified by the mass spectrum, with comparison of KI on DB-Wax column with reported data. <sup>e</sup> Tentatively identified only by the mass spectrum. <sup>f</sup> tr, trace. <sup>g</sup> monoterpene hydrocarbon.

In the ripe fruit, geraniol, citronellal, and linalool also showed the highest FD factors (7, 6, and 5), followed by geranial (4) and geranyl acetate (4). On the other hand, the influence of monoterpene hydrocarbons was much less than that in the green fruit. These alcohols and aldehydes, with contents being almost 10 times more than those in the green fruit as described in **Table 1**, were represented to be the most common constituents in the ripe fruit. They were considered to be the key factor for the flavor character of the ripe fruit. Especially, geranial, known to be important to the citrus-like odor of lemon oil (18), was thought to strengthen the citrus-like odor as shown in the sensory evaluation section due to its strong flavor note.

However, in the dried pericarp, the FD factor of geranial fell to 2, although geraniol, citronellal, and linalool were still shown to have high FD factors (6, 5, and 5). Geranyl acetate (4) and

methyl cinnamate (4) were also perceived as main odorants. In addition, piperitol (3), linalyl acetate (3), and many other odorants were recognized as relatively important odorants. These compounds seemed to be detected when the citrus-like note was weaker, whereas the fruity note became remarkable in the dried pericarp. Moreover, a few unknown compounds (A–I), not being detected as GC peaks, were detected as having stronger odors such as sweet, unpleasant rubber, and sweaty notes. To clarify the heavy and dusty notes of the dried pericarp, additional experiments including the identification of the unknown compounds should be continued.

On the other hand, citronellol, which has previously been determined as one of the most important contributors to the aroma characteristics of young leaves of Japanese pepper (6), was perceived as only a minor odor-active compound in the fruits of Japanese pepper.

Table 2. Aroma-Active Compounds in Fruits of Japanese Pepper<sup>a</sup>

KI	odor description	log <sub>4</sub> FD factor			compound
		green <sup>b</sup>	ripe <sup>b</sup>	dried <sup>b</sup>	
1025	weak pine	1			α-pinene
1130	green, sweet				unknown
1163	strong pine	4	3	3	myrcene
1185	green	3	3	1	unknown A
1202	sour and peel	4	3	3	α-limonene
1211	mint and fresh/pungent	4	4	3	β-phellandrene
1213	weak sweet, citrus	0	3	1	1,8-cineole
1250	pleasant	1			(E)-β-ocimene
1282	mild sweet		1	0	terpinolene
1353	watermelon	0	2	2	isobutyl hexanoate
1385	green			1	(Z)-3-hexenol
1392	vegetable			1	(E,E)-2,4-hexadienal
1397	unpleasant vegetable		2		unknown
1412	paint			2	unknown
1418	strong unpleasant			2	unknown
1428	vegetable			1	unknown
1446	pleasant rubber			1	unknown
1448	vinegar, acid			0	acetic acid
1461	milk candy			1	unknown
1469	pleasant	2			α-cubebene
1479	green, sweet	4	6	5	citronellal
1513	pleasant floral			3	unknown
1543	astringent pine		2		unknown
1546	pleasant floral	5	5	5	linalool
1553	astringent pine, ester	2	3	3	linalyl acetate
1563	pleasant, fresh	2	3	1	1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol
1571	fresh, mint		2	2	isopulegol
1585	citrus			2	unknown
1586	green, fresh, cool			1	β-elemene
1592	mild, fresh	2	2	3	β-caryophyllene
1599	fresh		1		terpinen-4-ol
1628	pleasant plum/sweat	0	2	0	1-terpineol
1658	pleasant, ester, rubber	1	2	1	citronellyl acetate
1665	mint, green	1	0		α-humulene
1668	pleasant			2	cryptone
1676	pleasant fresh		2	2	neral
1691	strong tree, pungent	1	2	2	α-terpinenyl acetate
1703	pleasant, mild	1	2	1	germacrene D
1709	floral green, dried grass	0	1		β-cubebene
1714	dried grass		1		β-selinene
1722	strong rose, sweet rubber, fresh	2	3	2	piperitone
1727	pleasant citrus		4	2	geranial
1744	mint, pleasant			3	piperitol
1760	strong pine, sweet, pungent	3	4	4	geranyl acetate
1765	green	0	0	0	citronellol
1814	unpleasant rubber, sweet	2	3	3	unknown B
1826	mild sweet	3			unknown C
1832	pleasant Chinese medicine/unpleasant rubber		3	2	unknown D
1849	rose, strong sweet	6	7	6	geraniol
1857	strong sweet, fresh			1	isomer of 4-ethylacetophenone
1868	cucumber	1	1	0	unknown
1874	strong sweet	2	2		unknown E
1892	Chinese medicine			2	isomer of farnesene
1911	fresh		1		unknown
1939	sweet-scented osmanthus	2	3	3	unknown F
1945	watermelon	1			3,7-dimethyl-1,5-octadiene-3,7-diol
1962	unpleasant rubber		2		unknown
1978	Chinese medicine			0	caryophyllene oxide
2028	sugar sweet			2	ledol
2035	mould, sweat			3	unknown G
2057	mild	3			unknown H
2071	fatty, sweet, sweat	4	3	4	methyl cinnamate
2078	sweet/sweet vegetable	2		0	elemol
2084	vegetable, green leaf			3	unknown I
2145	unpleasant rubber/citrus		1	2	cinnamyl acetate
2173	sweet, rose			1	unknown
2189	strong Chinese medicine			1	unknown
2212	strong Chinese medicine			1	unknown
2246	pungent Chinese medicine			1	unknown

<sup>a</sup> KI, Kovats index on DB-Wax. <sup>b</sup> Green, green fruit; ripe, ripe fruit; dried, dried pericarp.

**Table 3.** Enantiomeric Ratios of Linalool in Fruits of Japanese Pepper

sample	peak area <sup>a</sup> (%)		% ee (for <i>R</i> )
	<i>R</i>	<i>S</i>	
green fruit	72	28	44
ripe fruit	87	13	74
dried pericarp	60	40	20

<sup>a</sup> Peak area was calculated from the FID response.

**Chiral Gas Chromatographic Analysis of Linalool.** The absolute configuration of linalool was investigated because it is known that its flavor character is different between enantiomers (19), and it was identified as one of the main potent odorants as already stated. The chiral GC analysis of linalool was conducted by using modified  $\beta$ -cyclodextrin as the chiral stationary phase as reported previously (20). The elution order was determined from the reported results (20), and the enantiomeric ratios (ee) deduced by comparison with the authentic racemic standard are summarized in **Table 3**. (*R*)-Linalool was 44, 74, and 20% ee in the green fruit, ripe fruit, and dried pericarp, respectively. The absolute configuration of linalool has often been investigated in lavender, jasmine flower, and tea leaves (20–22), and it has also been clarified that the absolute configuration greatly affected the overall odor of jasmine tea (23). The odor threshold value of (*R*)-linalool (0.035–0.040 ppm), with a strong, green floral note, is 3–4 times lower than that of (*S*)-linalool, with an oily, heavy, green note (24). Our results indicate that the optical ratios of linalool were different in the green fruit, ripe fruit, and dried pericarp and that the odor of (*R*)-linalool had a tendency to have a greater effect on the flavor of the three samples than the (*S*)-isomer. However, due to its low content, it was considered that the effect of enantiomeric ratios of linalool was not strong in the fruits of Japanese pepper.

The data obtained from this study indicate that the flavor differences between the green and ripe fruits were induced mainly by the decrease of monoterpene hydrocarbons and the increase of oxygenated terpenoids during the maturation. The formation mechanism of the potent odorants including geranial and geranyl acetate will be studied continually.

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