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Volatile Flavor Components of Scent, Colored, and Common Rice Cultivars in Korea

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ABSTRACT

To compare the composition of volatile flavor components of three different cultivars of rice, Hyangnambyeo (aromatic cultivar), Heugjinjubyeo (pigmented cultivar) and Dongjinbyeo (normal cultivar), the volatile flavor components of brown rice were isolated by Likens-Nickerson simultaneous steam distillation and extraction apparatus. The flavor concentrates obtained were analyzed by gas chromatography and gas chromatography-mass spectrometry. A total of 65 components, including 14 aliphatic aldehydes and ketones, 7 aliphatic alcohols, 8 aromatic alcohols, 13 hydrocarbons, 9 esters, 7 aliphatic acids, and 7 miscellaneous components were identified. The aliphatic aldehydes, which are known as contributors to the overall flavor of cooked rice, were present in larger amounts in Hyangnambyeo than in Heugjinjubyeo and Dongjinbyeo, while the difference in quantity of these components between Heugjinjubyeo and Dongjinbyeo was not remarkable. Hyangnambyeo and Heugjinjubeyo contained 562 ng and 259 ng of 2-acetyl-1-pyrroline per gram of brown rice based on dry weight, respectively, which is a key compound contributing to the popcorn-like aroma in aromatic rice. Dongjinbeyo contained about 6 ng.

Keywords : aromatic rice cultivar, pigmented rice cultivar, volatile flavor components, 2-acetyl-1-pyrroline.

Rice is one of the most important edible crops for people in the world, especially in the Asian region. During the last decade in Korea, rice breeders and agronomy researchers have concentrated to develop new varieties with high yield and disease resistance and to reduce the cost of production. However, they have recently been interested in increasing the quality of rice according to the demands of consumers. Consequently, rice breeding programs now incorporate sensory evaluation of the product into their breeding objectives. For example, aromatic rice (scented rice) and pigmented rice cultivars were recently developed and cultivated commercially in Korea.

More than 100 volatile components have been identified in cooked rice (Yajima et al., 1978; Maga, 1984; Buttery et al., 1988). It has been the flavor characteristics of cooked rice that were different with the degrees of milling, duration after milling, and duration after cooking (Tsugita et al., 1980; Tsugita et al., 1983; Kato et al., 1983; Buttery et al., 1983; Lee et al., 1991). Yajima et al. (1979) reported that the volatile flavor components between cooked aromatic rice and nonaromatic rice (Koshihikari) were different. Buttery et al. (1983) reported that 2-acetyl-1-pyrroline, which has "popcorn-like" as an essential compound for cooked rice aroma, was more abundant in aromatic rice than in nonaromatic rice. Both growing conditions and genetic resources of cultivars might affect the volatile composition. However, the volatile flavor of domestic rice cultivars has not been studied a great deal in Korea.

Accordingly, the present work was carried out to compare the compositions of volatile flavor components in brown rice of three different types of rice cultivars developed in Korea.

MATERIALS AND METHODS

Materials

Three rice cultivars were used in this study. Hyangnambyeo (aromatic rice), Heugjinjubyeo(pigmented rice), and Dongjinbyeo(normal rice) were cultivated at the Research farm of Chungnam Rural Research and Extention Service in Taejon, Korea. After harvest in 1998, the unhulled rice was dried to 13% moisture content under sunlight, and then hulled to brown rice, and stored at 4℃ for 3 months until analysis. Authentic 2-acetyl-1-pyrroline(2-AP) was generously given by Dr. P.Schieberle(Deutsche Forschungsanstalt für Lebensmittelchemie, Germany). Chemical compounds were purchased from Sigma Chemical Co.(St. Louis, MO, USA), Aldrich Chemical Co.(Milwaukee, Wi, USA) or Tokyo Kasei Kogyo Co.(Tokyo, Japan). The other reagents used were guaranteed grades. A volatile-free antifoam agent was prepared by adding 100 ml of silicon antifoam emulsion(Fluka No. 85390) to 500 ml of distilled water in a 1,000 ml beaker and concentrating the mix-

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ture to 200 ml by boiling.

Extraction of volatile components and isolation of basic fraction

The isolation procedures followed the methods described by Buttery et al.(1983). Two hundred grams of brown rice was put into a 6 ℓ round flask, and then 3,000 ml of distilled water, 2.0 ml of antifoam emulsion, 103.0 μg of 2-ethyl-1-hexanol in 5.0 ml of ethyl ether and 27.55 μg of 2,4,6-trimethyl pyridine (TMP) in 2.0 ml of distilled water as internal standards were added. After distillation at atmospheric pressure for 2 hours with vigorous magnetic stirring of the solvent flask, the solvent flask was detached from the simultaneous distillation & extraction (SDE) head. The ethyl ether layer and the aqueous layer were separated by using a 250 ml separatory funnel. The upper ethyl ether fraction was dried over anhydrous sodium sulfate and filtered. It was concentrated by heating in a water bath at 45℃ until the volume was reduced to about 1 ml using a short Vigreux column (20 cm long). This concentrate was then transferred to a GC autoinjector vial and the final volume was reduced to about 0.3 ml under nitrogen stream. The aqueous layer (basic fraction) was neutralized with excess sodium bicarbonate under ethyl ether(100 ml) with ice bath cooling. The ethyl ether fraction was separated and the aqueous layer extracted further (2 × 50 ml) with ethyl ether. The ethyl ether extracts were then combined, dried and concentrated to about 0.1 ml as described above.

Gas chromatography(GC) and gas chromatography-mass spectrometry(GC-MS)

GC analysis of volatile concentrate was carried out by Hewlett-Packard (HP) 5890A GC, flame ionization detector(FID) and Supelcowax 10 fused silica capillary column(30 m × 0.25 mm, film thickness; 0.25 μm). The column temperature was held at 40℃ for 10 minutes and programmed to 230℃ at 3℃/min. and maintained at 230℃ for 50 minutes. Injector and detector temperatures were 250℃, and column head pressure was 12 psi, which was maintained for about 1.0 ml/min. of nitrogen flow rate. The injection volume was 1.0 μl with split injection mode (split ratio = 28:1). GC-MS analysis was carried out with Hewlett-Packard (HP) 5890A GC and HP 5970 mass selective detector (MSD). Innowax fused silica capillary column (50 m × 0.20 mm, film thickness; 0.25 μm) was used. The column temperature was increased from 40℃ to 230℃ with the interval of 2℃/min. and then held at 230℃ for 60 minutes. Other details of the MSD condition, identification and quantification of components followed the method described by Lee et al.(1998). GC and GC-MSD conditions to identify and quantify 2-AP in the basic fraction was the same as described above, except for the modification of GC injector temperature; 170℃ and injection volume; 2 μl. The concentrations of 2-AP in the rice samples were estimated using the following equation:

$$\text{Concentration of 2-AP(ng /g, brown rice, dry weight)} = \frac{(A/B) \times 27{,}550 \text{ ng TMP} \times 3.57}{200 \text{ g rice} \times (1 - \text{moisture content } \%)}$$

Where A and B represent the peak area of 2-AP and an internal standard (TMP) in the basic fraction, respectively, and 3.57 represents the relative recovery factor of 2-AP and TMP (Buttery et al., 1986; Lin et al., 1990).

RESULTS AND DISCUSSION

Volatile components of cooked rice

Volatile flavor components of cooked rice are formed from raw rice during the cooking process. Therefore, the Likens-Nickerson type distillation and extraction apparatus were set up to mimic the cooking process of rice. During distillation, the basic components were extracted from the steam distillate and bound in the aqueous sulfuric acid layer when non-basic volatile components remained in the ether

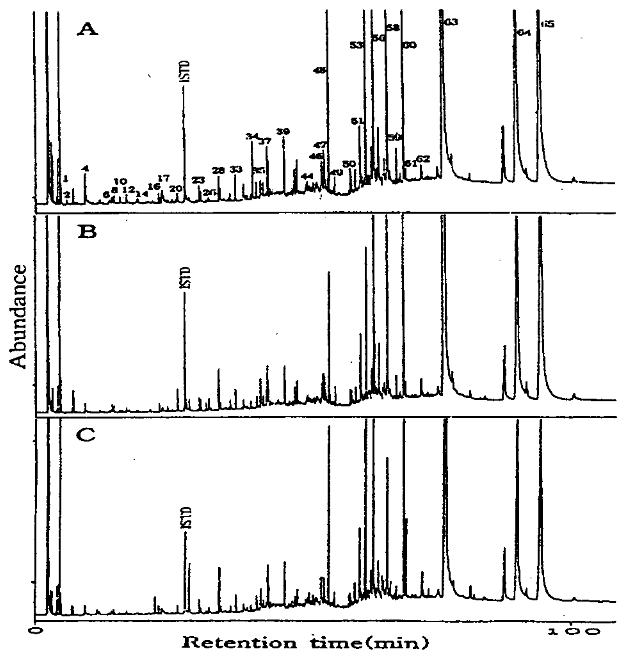

Fig. 1. Typical gas chromatograms of the nonbasic fraction isolated from brown rice of Hyangnambyeo(A), Heugjinjubyeo(B) and Dongjinbyeo(C). Peak numbers refer to Table 1.

Table 1. Components identified from the steam volatile flavor concentrates of the brown rice.

Peak no	Compounds	Concentration(μg/100g)†			Id**	Peak no	Compounds	Concentration(μg/100g)†			Id**
		Hy**	He**	Do**				Hy**	He**	Do**	
Aliphatic aldehydes and ketones(14)											
1	n-pentanal	7.73	12.83	13.51	1	4	n-hexanal	10.18	-*	9.14	1
7	2-heptanone	1.56	-	-	1	58	n-heptanal	4.22	1.93	-	1
10	trans-2-hexenal	2.39	-	-	1	13	2-octanone	2.00	-	-	2
15	trans-2-heptenal	1.75	-	-	2	17	n-nonanal	10.61	-	4.00	2
19	trans-2-octenal	2.38	-	-	1	21	n-decanal	1.72	-	-	1
25	trans-2-decenal	4.92	3.47	1.63	2	30	2,4-nonadiena	12.25	-	-	2
33	trans,cis-2,4-decadienal	9.72	11.56	8.7	1	35	trans,trans-2,4-decadienal	10.26	11.19	8.53	2
Aliphatic alcohols(7)											
6	n-butanol	4.31	-	-	1	12	n-pentanol	2.84	-	2.43	1
16	n-hexanol	3.19	3.52	2.67	1	18	trans-2-hexenol	4.26	-	-	1
20	1-octen-3-ol	11.30	7.57	5.92	1	24	n-octanol	10.26	5.24	1.68	1
9	n-dodecanol	20.27	30.97	15.97	1						
Aromatic alcohols(8)											
22	benzaldehyde	10.25	42.45	23.62	1	23	linalool	6.25	6.27	4.70	2
27	4-terpineol	2.68	-	-	2	28	menthol	18.56	10.26	14.54	1
29	α-terpineol	5.11	4.49	3.01	2	36	estragole	14.45	10.17	20.26	1
37	benzyl alcohol	13.94	15.03	11.10	1	46	patchouli alcohol	5.84	16.45	-	2
Hydrocarbons(13)											
2	n-decane	2.91	-	1.14	1	5	n-undecane	1.40	-	-	1
14	n-tridecane	3.80	-	-	1	26	n-hexadecane	0.44	2.52	-	1
31	n-heptadecane	3.03	4.43	2.59	1	34	n-octadecane	16.01	4.65	9.59	1
38	n-nonadecane	7.10	9.17	4.92	1	40	n-eicosane	3.50	4.35	2.31	1
44	n-heneicosane	7.70	10.25	5.04	1	47	n-docosane	24.99	36.41	22.32	1
50	n-tetracosane	2.99	4.23	2.76	1	55	n-pentacosane	48.03	13.90	6.17	1
58	n-hexacosane	79.05	119.57	65.28	1						
Esters(9)											
3	n-butyl acetate	1.81	-	-	2	32	benzyl acetate	3.30	2.67	-	2
48	methyl palmitate	238.71	106.71	53.38	2	49	ethyl palmitate	21.06	12.31	4.34	2
51	methyl oleate	35.71	23.90	11.82	2	53	methyl linoleate	133.70	6.09	2.80	2
56	ethyl oleate	179.30	12.35	6.16	2	57	ethyl linoleate	80.21	56.72	17.90	2
60	dibutyl phthalate	89.99	96.59	55.55	2						
Aliphatic acids(7)											
54	dodecanoic acid	14.64	12.47	6.8	1	59	tridecanoic acid	3.36	5.75	4.37	1
61	tetradecanoic acid	6.64	7.03	25.96	1	62	pentadecanoic acid	8.76	10.12	6.69	1
63	hexadecanoic acid	1501.6	1530.9	1186.5	1	64	stearic acid	788.3	302.5	442.8	1
65	oleic acid	960.4	935.7	547.2	1						
Miscellaneous compounds(7)											
9	limonene	2.07	2.38	2.09	1	11	2-pentyl furan	1.02	-	-	1
41	p-methoxy benzaldehyde	9.49	3.51	2.03	2	42	p-anisaldehyde	12.99	12.97	1.92	2
43	α-pyrrolidone	15.14	17.55	9.61	1	45	1-(4-methoxyphenyl) ethanone	12.65	19.72	8.49	2
52	indole	14.08	9.41	4.65	1						

Note : The rice contained 13% moisture.

† Concentration by GC-FID(μg/100g dry wt. moisture content at 13%), Mean of two replications,

† 1 : GC-MS, GC:Identification by comparing the mass spectra and GC retention time to those of authentic samples.

2 : GC-MS:Identified by comparing the mass spectra only.

* - : Not detected, ** Hy : Hyangnambyeo, He : Heugjinjubyeo, Do : Dongjinbyeo

layer. Fig. 1 shows gas chromatograms of volatile concentrates of non-basic fraction. It can be seen that the volatile concentrate of cooked rice is composted with a large number of volatile components. Among them, a total of 65 components, including 14 aliphatic aldehydes and ketones, 7 aliphatic alcohols, 8 aromatic alcohols, 13 hydrocarbons, 9 esters, 7 aliphatic acids and 7 miscellaneous components were identified by GC and GC-MS. The concentrations of volatile compounds identified in the three samples are shown in Table 1. Of the components identified, the interesting groups are aliphatic alkanals and alkenals, due mainly to their low odor thresholds and characteristic odor profiles, and the importance of aliphatic aldehydes such as hexanal, octanal, nonanal, trans-2-nonenal, decanal and trans, trans-2, 4-decadienal as major contributors to the cooked rice aroma have been emphasized by Tsugita et al.(1983) and Buttery et al.(1986). In this work, these components were present in relatively larger amounts in Hyangnambyeo than in Heugjinjubyeo and Dongjinbyeo, while the differences in quantity of these components between Heugjinjubyeo and Dongjinbyeo were not remarkable. The presence of aliphatic aldehydes and ketones in rice volatiles is mainly considered to be caused by enzymatic reactions from lipids or Streaker degradation of sugars during the soaking or the cooking process (Kato et al., 1983). Of aliphatic alcohols in Table 1, n-hexanol and trans-2-hexenol have green, grassy-like aroma while 1-octen-3-ol and n-octanol have mushroom-like aroma (Arctander, 1969). The concentration of n-hexanol was not different in the three samples, while those of 1-octen-3-ol and n-octanol were higher in Hyang nambyeo than in Heugjinjubyeo or Dongjinbyeo. Paule and Powers (1989) reported that n-hexanol is negatively correlated with the desirable aroma of cooked rice. Therefore, they insisted that the rice variety with low n-hexanol and high 2-AP should be selected in breeding programs of aromatic rice. Of the groups listed in Table 1, the contribution of hydrocarbons, aromatic alcohols and aliphatic acids in the aroma of the cooked rice are not well known. Although these components are present in large amounts in a relative comparison with aliphatic aldehydes and alcohols, they may not significantly contribute to the characteristic aroma of the cooked rice, due mainly to their high odor thresholds (Buttery et al., 1988).

Identification and quantification of 2-AP in basic fraction

2-acetyl-1-pyrroline is considered the key component contributing to the popcorn-like aroma or cracker-like aroma in cooked aromatic rice (Buttery et al., 1983; De Kimpe et al., 1993). However, it is

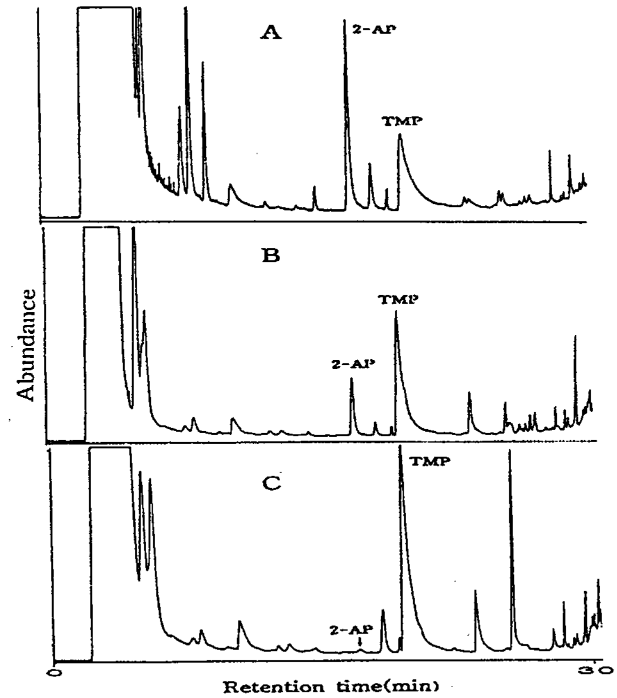

Fig. 2. Typical gas chromatograms of the basic fraction isolated from brown rice of Hyangnambyeo(A), Heugjinjubyeo(B) and Dongjinbyeo(C). Marked peaks are 2-AP (2-acetyl-1-pyrroline) and TMP(2,4,6-trime-thylpyridine, internal standard).

known that the determination of an accurate concentration of this component from cooked rice is difficult because of either the presence of other interfering compounds or a considerably low concentration (ppb units). A method using dilute acid to extract only the basic components can lessen the effect of interfering components. The basic components were bound in the aqueous sulfuric acid layer during steam distillation. The basic components are regenerated from the aqueous acid layer by neutralization with sodium bicarbonate and re-extracted with ethyl ether. Gas chromatograms are shown in Figure 2 for the basic fraction from Hyangnambyeo(A), Heugjinjubyeo(B) and Dongjinbyeo(C), and the result shows that the peaks of 2-AP were well resolved from those of other components. The mass spectral data for the peak labeled 2-AP in Figure 2 were identical to those reported for 2-AP from aromatic rice previously(Lin et al., 1990; Seitz et al., 1993) and GC retention times were matched with that of authentic 2-AP. The concentrations of 2-AP in the rice samples were quantified using TMP as an internal standard and the result was shown in Table 2. The aromatic type rice, Hyangnambyeo and the pigmented type rice, Heugjinjubeyo, contained 562 ng and 259 ng of 2-AP per gram of brown rice, res-

Table 2. Concentration of 2-acetyl-1-pyrroline in the cooked brown rice.

Cultivar	Concentration (ng/g±SD)†
Hyangnambeyo(aromatic rice cultivor)	562 ±29
Heugjinjubyeo(pigmented rice cultivor)	259 ±72
Dongjinbeyo(nonaromatic rice cultivor	6.3± 1.5

† Mean of three replications ± standard deviation.
Moisture contents of brown rice used were 13%.

pectively, while the non-aromatic type rice, Dongjinbeyo, contained about 6 ng of 2-AP per gram. The results indicate Hyangnambeyo contained almost 120 times more 2-AP than Dongjinbyeo. It is an interesting result that the pigmented type rice, Heugjinjubyeo, contained about 56 times more 2-AP than the nonaromatic rice, Dongjinbyeo. These results are consistent with the results of Buttery et al.(1986) that the aromatic rice, including Malagkit Sungsong, Basmati 370 and IR- 840-76-1 from the Philippines, contained 560-760 ng of 2-AP per gram of brown rice, whereas Texas long grain rice(nonaromatic type) contained almost 6 ng 2-AP per gram, and also Lin at al.(1990) reported that Louisiana aromatic Della rice and nonaromatic Lemont rice contained 330 ng and 4 ng of 2-AP per gram, respectively. According to previous reports (Tsugita et al., 1980; Buttery et al.,1983; Fushimi and Ishitani, 1990), the numbers and concentrations of volatile flavor components in cooked rice were known to be different with the degrees of milling. Accordingly, further work should be done to compare the compositions of volatile flavor components between brown rice and white rice or in relation to the degrees of milling.

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REFERENCES

Arctander, S. 1969. Perfume and Flavor Chemicals, Det Hoffensbergske Etablissement. Copenhagen, Denmark.

Buttery, R. G., L. C. Ling, B. O. Juliano, and J. G. Turnbaugh. 1983. Cooked rice aroma and 2-acetyl-1-pyrroline. J. Agri. Food Chem. 31: 823-826.

Buttery, R. G., L. C. Ling, B. O. Juliano, and T. R. Mon. 1986. Quantitative analysis of 2-acetyl-1-pyrroline in rice. J. Agric. Food Chem. 34: 112-114.

Buttery, R. G., Turnbaugh, J. G. and and T. R. Mon. 1988. Contribution of volatiles to rice aroma. J. Agric. Food Chem. 36: 1006-1009.

De Kimpe, N. G., C. V. Stevens, and M. A. Keppens. 1993. Synthesis of 2-acety-1-pyrroline, the principal rice flavor component. J. Agric. Food Chem. 41: 1458-1461.

Kato, H., T. Ohta, T. Tsugita, and Y. Hosaka. 1983. Effect of parboiling on texture and flavor components of cooked rice. J. Agric. Food Chem. 31: 818-823.

Lee, B. Y., J. R. Son, U. Matuskura, K. Kaimura, and A. Maekawa. 1991. Changes of volatile components of cooked rice during storage at 70℃. Korean J. Food Sci. Technol. 23: 610-61

Lee, J. C., and Y. H. Kim. 1999. Comparison of volatile flavor components of Korean aromatic rice and nonaromatic rice, J. Korean Soc. Food. Sci. Nutr., 28(2): 299-304.

Lin, C. F., T. C.-Y. Hsieh, and B. J. Hoff. 1990. Identification and quantification of the "popcorn-like" aroma in Louisiana aromatic Della rice(*Oryza sativa* L.). J. Food. Sci. 55: 1466-1469.

Maga, J. A. 1984. Rice product volatiles: A review. J. Agric. Food Chem. 32: 924-970. Paule, C. M. and J. J. Powers. 1989. Sensory and chemical examination of aromatic and nonaromatic rice. J. Food Sci. 54: 343-346.

Seitz, L. M., R. L. Wright, R. D. Waniska, and L. W. Rooney. 1993. Contribution of 2-acetyl-1-pyrroline to odors from wetted ground pearl millet. J. Agric. Food Chem. 41: 955-958.

Tsugita, T., T. Kurata, and H. Kato. 1980. Volatile components after cooking rice milled to different degrees. Agric. Biol. Chem. 44: 835-840.

Tsugita, T., T. Ohta, and H. Kato. 1983. Cooking flavor and texture of rice stored under different conditions. Agric. Biol. Chem. 47: 543-549.

Yajima, I., T. Yanai, M. Nakamura, H. Sakakibara, and T. Habu. 1978. Volatile flavor components of cooked Rice. Agric. Biol. Chem. 42: 1229-1233.

Yajima, I., T. Yanai, M. Nakamura, H. Sakakibara, and K. Hayashi. 1979. Volatile flavor components of cooked Kaorimai-(Scented rice, O. sativa japonica). Agric. Biol. Chem. 43: 2425-2429.