JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of Odor-Active Compounds from Six Distinctly Different Rice Flavor Types

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Using a dynamic headspace system with Tenax trap, GC-MS, GC−olfactometry (GC-O), and multivariate analysis, the aroma chemistry of six distinctly different rice flavor types (basmati, jasmine, two Korean japonica cultivars, black rice, and a nonaromatic rice) was analyzed. A total of 36 odorants from cooked samples were characterized by trained assessors. Twenty-five odorants had an intermediate or greater intensity (odor intensity ≥ 3) and were considered to be major odor-active compounds. Their odor thresholds in air were determined using GC-O. 2-Acetyl-1-pyrroline (2-AP) had the lowest odor threshold (0.02 ng/L) followed by 11 aldehydes (ranging from 0.09 to 3.1 ng/L), guaiacol (1.5 ng/L), and 1-octen-3-ol (2.7 ng/L). On the basis of odor thresholds and odor activity values (OAVs), the importance of each major odor-active compound was assessed. OAVs for 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal comprised >97% of the relative proportion of OAVs from each rice flavor type, even though the relative proportion varied among samples. Thirteen odor-active compounds [2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, 1-octen-3-ol, (*E*)-2-octenal, (*E*,*E*)-2,4-nonadienal, 2-heptanone, (*E*,*E*)-2,4-decadienal, decanal, and guaiacol] among the six flavor types were the primary compounds explaining the differences in aroma. Multivariate analysis demonstrated that the individual rice flavor types could be separated and characterized using these compounds, which may be of potential use in rice-breeding programs focusing on flavor.

KEYWORDS: Odor-active compound; aromatic rice; black pigmented rice; dynamic headspace; Tenax trap; GC-O; principal component analysis (PCA); odor activity value (OAV)

INTRODUCTION

Rice grain quality is a critical breeding objective in that quality has a significant impact on consumer preference. Of the various quality attributes of cooked rice, flavor is considered of primary importance in that superior flavor increases consumer satisfaction, overall acceptability, and the probability of repeated purchase (*1*). Indian consumers consider aroma and taste the most critical quality traits, whereas Asian consumers in the United States consider flavor one of the most important acceptance factors (*1, 2*). Flavor is composed of taste and aroma, the latter of which is conferred by volatile compounds emanating from cooked rice that interact with olfactory receptors. Although a relatively large number of compounds from cooked rice have been identified (*3–5*), a much more limited number appear to make up the characteristic aroma. Qualitative and quantitative differences in these critical odor-active compounds are thought to collectively create the aroma perceived and account for differences among flavor types.

Rice cultivars can be separated very generally into aromatic and nonaromatic types. Aromatic rice has a relatively diverse range of aromas that are much more dominant than in nonaromatic cultivars. Within aromatic rice there is a cross section of unique aromas. For example, jasmine rice was characterized as having buttery, corn, dairy, starchy, cooked grain, and nutty attributes, whereas basmati rice was characterized as having hay-like and earthy attributes (*6*).

The aroma of both aromatic and nonaromatic rice is composed of a complex mixture of odor-active compounds. Several odor-active compounds in cooked aromatic rice have been determined using odor units (*7*) and AEDA (*8*). In aromatic rice, 2-acetyl-1-pyrroline (2-AP), which is synthesized in aerial parts of aromatic rice during growth (*9*), is considered to be highly important, and although found in some nonaromatic types, its concentration is very low to negligible (*10*). 2-AP is described as having a "popcorn-like" odor by American and "pandan-like" odor by Asian consumers (*11*). However, the unique aromas of basmati, jasmine, black, and other rice flavor types cannot be accounted for simply by differences in 2-AP. Their

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10.1021/jf072685t CCC: $40.75
Published on Web 03/26/2008

aromas are due to qualitative and quantitative variations in a diverse cross section of odor-active compounds. Lipid-derived odor-active compounds in cooked rice were formed during the degradation of oleic, linoleic, and linolenic acid (*12*). For example, octanal, heptanal, nonanal, (*E*)-2-nonenal, decanal, and 2-heptanone are formed from oleic acid, whereas hexanal, pentanol, pentanal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal, and 2-pentylfuran are formed from linoleic acid (*13*). The lipid oxidation products can not only yield rancid odors but also induce various deteriorative reactions with proteins, amino acids, and other components (*14*). 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone and bis(2-methyl-3-furyl) disulfide, formed in rice during cooking, are good examples of thermally derived flavor compounds that have seasoning-like and meaty-like aromas, respectively (*8*).

Plant breeding is the primary avenue for altering the basic flavor of rice. The problem, however, is that sensory tests for flavor are expensive, time-consuming, and labor intensive such that the number of progeny that can be assessed in a breeding program is only a very small percentage of the total. The net effect is that flavor is assessed very late in the selection process after the majority of the progeny have already been discarded. If flavor could be measured analytically, integrating progeny flavor chemistry with the chemistry of preference of targeted consumer populations, assessment could be moved much earlier in the selection process, greatly increasing the chance of finding new cultivars with truly superior flavor.

As a preliminary step toward this objective, we have identified and quantified the volatile compounds emanating from six different rice flavor types using GC-MS, identified and characterized the odor-active compounds using GC−olfactometry (GC-O), and assessed the relative importance of each odor-active compound in the overall aroma using odor activity values (OAVs). The distinct aromas of the various flavor types were also compared through the presence or absence of single odorants and their OAVs. Our intent in this study was not to identify all possible volatiles emanating from the cooked rice samples but rather to focus on the critical odor-active compounds that would be useful in distinguishing differences among progeny in rice breeding programs.

MATERIALS AND METHODS

Materials. Six rice cultivars, each displaying distinctly different cooked aromas, were selected for analysis. Five were aromatic and one a traditional nonaromatic rice cultivar. The aromatic cultivars were Hyangmibyeo 1 (H1, a medium-grained Korean japonica), Hyangmibyeo 2 (H2, a medium-grained Korean japonica), Royal [basmati (BA), a long-grained Indian indica], Golden Elephant [jasmine (JA), a long-grained Thai indica], and Goemjeongssal (BP, a medium-grained japonica black rice from Korea). Jeongilpum (TM), a traditional medium-grained, nonaromatic japonica from Korea, was included for comparison. H1 and H2 were grown at the National Institute of Crop Science, Suwon, in 2006; the remainder, indicated as "New Crop 2007" on the packages, were purchased from a local supermarket in October 2006. All rice samples were milled to remove the bran layer from brown rice except for the black rice cultivar (BP). The samples were sealed in glass containers and kept at –20 °C until analysis.

Chemicals. Analytical standards utilized for identification were benzaldehyde, decane, decanal, (*E*)-2-decenal, furfural, guaiacol, heptanal, heptane, 2-heptanone, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, undecane (Sigma-Aldrich Inc., St. Louis, MO); (*E*,*E*)-2,4-decadienal, 2-decanone, hexanal, (*E*)-2-hexenal, 1-hexanol, 1-nonanol, 2-nonanone, 1-methylnaphthalene, 2-methylnaphthalene,1-octanol, (*E*)-2-octenal, 1-octen-3-ol (Aldrich Chemical Co., Milwaukee, WI); *p*-menthan-3-one, octanal (Fluka Chemical Co., Milwaukee, WI); naphthalene (J. T. Baker Inc., Phillipsburg, NJ); dodecane, indole, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, 2-pentylfuran, 4-vinylguaiacol (TCI America, Portland, OR); and 3-octen-2-one (Alfa Aesar, Ward Hill, MA). 2,4,6-Trimethylpyridine (Aldrich Chemical Co.) was used as an internal standard for 2-AP.

Dynamic Headspace Sampling. Dynamic headspace sampling of volatile compounds in cooked rice was performed using a method described previously (*15*). Rice samples (100 g) in a specially constructed 1 L glass beaker were cooked in distilled water (150 mL) for 30 min at 100 °C on a hot plate (Fisher Thermix Stirring Hot Plate, model 210T, Pittsburgh, PA). The beaker was sealed during cooking and sampling with a ground glass lid containing entry and exit ports. The entry and exit ports were wrapped with aluminum foil during cooking. Due to different water absorption characteristics, the black rice sample (100 g) was cooked in 100 mL of distilled water, which was established on the basis of the texture after cooking during preliminary tests; the volatiles were collected as with the other samples. The glass beaker with freshly cooked rice was immediately placed in a hot water bath and was maintained at 70 °C during sampling. Headspace volatiles emanating from the cooked rice samples were collected on a 10 cm long, 6 mm o.d., 4 mm i.d. stainless-steel Tenax trap (Scientific Instrument Services, Inc., Ringoes, NJ) with 150 mg 60/80 mesh Tenax-TA (Alltech Associates Inc., Deerfield, IL) using a vacuum sampling pump (Aircheck Sampler, model 224-44XR, Eighty-Four, PA). The Tenax trap was preconditioned at 280 °C for 2 h with purified He at 20 mL/min. Air purified using a charcoal filter (Alltech Associates Inc.; 1 cm i.d., 10 cm long Pyrex glass tube with a 7 cm bed of charcoal) connected to the entry port was passed through the beaker at 150 mL/min for 60 min. A 50 mL Erlenmeyer flask was placed between the exit port and the trap to collect any condensation. All connections were made of glass material. One milliliter of 18.34 mg/L 2,4,6-trimethylpyridine (TMP) solution in 0.1 M HCl, used as an internal standard for 2-AP (*16*), was injected into the Erlenmeyer flask at the beginning of volatile collection using a 1 mL syringe. The internal standard was chosen due to similar properties with 2-AP (e.g., basic, water solubility, volatility, stability, retention time) (*10*).

After sampling, the Tenax trap was connected to an automated short-path thermal desorption system (model TD-5, Scientific Instrument Services) on the injector port of the gas chromatograph−mass spectrometer (GC-MS, model 6890N/5973, Agilent, Palo Alto, CA). The collected samples were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min and the analytes collected on the first 4 cm of the GC column using a CO_2-cooled cryofocusing trap (−40 °C) (SIS 2 in. Cryo-Trap, Scientific Instrument Services). After desorption, the cryofocusing trap was rapidly heated to 200 °C, and the analytes were separated using temperature programming.

GC-MS and GC-O. The GC was equipped with a 30 m length, 0.25 mm i.d., 0.25 μm film thickness, fused silica capillary column (HP-5MS, Agilent). The injection port temperature was 225 °C with a split ratio of 0.5:1. He was used as the carrier gas with the flow rate of 2.0 mL/min. The column temperature was held at 40 °C for 1 min and then programmed at 1.5 °C/min to 65 °C, which was held for 1 min, at 2 °C/min to 120 °C for 1 min, and finally at 15 °C/min to 280 °C for 5 min. Volatiles exiting the column were split between the mass spectrometer for identification and quantification and an olfactory detector outlet for description and intensity assessment (ODO II, SGE International, Austin, TX). MS conditions were as follows: ion source, 230 °C; electron energy, 70 eV; multiplier voltage, 1247 V; GC-MS interface zone, 280 °C; scan range, 35−350 mass units. Three assessors were trained by describing 15 materials with different odors: popcorn-like (popcorn), starchy (rice starch), woody (toothpicks), cooked grain (cream of wheat), corn (cream-style corn), nutty (roasted peanut), floral (jasmine scent), dairy (2% milk), hay (hay), barn (white pepper), buttery (butter), green (alfalfa), rancid (rancid vegetable oil), waxy (candle), and earthy (mushroom). Each of the assessors had considerable prior experience in GC-O. An aroma extract of each rice flavor type was characterized by describing the aroma of the individual components and assessing their intensity via GC-O. Odor intensity was classified on a 1−5 scale: 1 = very weak; 2 = weak; 3 = intermediate; 4 = strong; 5 = very strong. Odor intensity values were averaged for the assessors, and odorants perceived by all assessors were accepted as odor-active compounds.

Table 1. Linearity, Sensitivity, and Precision of Major Odor-Active Compounds Detected in Cooked Rice Samples

compound	standard curve (r^a)	validation range (ng/L)	RSD (%)
1-pentanol	$y = 5.497 \times 10^{-5}x + 0.69333$ (1.0000)	4.1−406	1.97
hexanal	$y = 9.8 \times 10^{-5}x + 2.20510$ (0.9999)	4.0−401	2.94
(*E*)-2-hexenal	$y = 8.343 \times 10^{-5}x + 2.43582$ (1.0000)	4.1−414	2.39
2-heptanone	$y = 5.865 \times 10^{-5}x + 1.39308$ (0.9999)	4.0−404	1.82
heptanal	$y = 8.578 \times 10^{-5}x + 5.43402$ (0.9999)	4.0−404	2.26
benzaldehyde	$y = 3.996 \times 10^{-5}x + 3.91624$ (0.9999)	5.3−525	1.67
1-octen-3-ol	$y = 5.774 \times 10^{-5}x + 4.81201$ (0.9999)	4.2−417	1.81
2-pentylfuran	$y = 3.950 \times 10^{-5}x + 1.92809$ (0.9999)	4.5−447	1.97
octanal	$y = 6.120 \times 10^{-5}x + 3.85092$ (0.9999)	4.1−406	1.38
3-octen-2-one	$y = 5.687 \times 10^{-5}x + 4.75926$ (1.0000)	4.2−417	2.23
(*E*)-2-octenal	$y = 6.062 \times 10^{-5}x + 5.40639$ (0.9999)	4.2−416	0.82
1-octanol	$y = 3.717 \times 10^{-5}x + 5.61728$ (0.9991)	4.1−412	2.33
guaiacol	$y = 6.154 \times 10^{-5}x + 10.83110$ (0.9998)	5.6−555	2.57
2-nonanone	$y = 4.782 \times 10^{-5}x + 1.67076$ (0.9999)	4.1−408	1.75
nonanal	$y = 5.339 \times 10^{-5}x + 2.20882$ (0.9998)	4.1−408	2.97
p-menthan-3-one	$y = 3.759 \times 10^{-5}x + 1.71313$ (0.9999)	4.2−441	1.65
(*E*)-2-nonenal	$y = 6.372 \times 10^{-5}x + 8.30734$ (0.9997)	4.2−417	0.71
1-nonanol	$y = 4.293 \times 10^{-5}x + 7.53287$ (0.9998)	4.1−413	2.04
decanal	$y = 3.734 \times 10^{-5}x + 3.11112$ (0.9998)	4.1−409	2.49
(*E*,*E*)-2,4-nonadinenal	$y = 6.372 \times 10^{-5}x + 8.30734$ (0.9998)	8.6−428	0.76
(*E*)-2-decenal	$y = 4.986 \times 10^{-5}x + 6.91635$ (0.9999)	4.2−418	1.94
indole	$y = 4.500 \times 10^{-5}x + 19.50584$ (0.9994)	20.0−500	2.88
4-vinylguaiacol	$y = 4.686 \times 10^{-5}x + 23.70592$ (0.9997)	50.0−500	1.69
(*E*,*E*)-2,4-decadienal	$y = 5.497 \times 10^{-5}x + 16.78223$ (0.9998)	17.1−427	0.90

[a] *r*, linear correlation coefficient.

Identification and Quantification of Odorants. Each odorant was identified on the basis of its mass spectra using NIST 02 and Wiley 7 libraries. Identifications were confirmed by comparing Kovats retention indices (RI) and odor descriptions with those of authentic standards. Retention indices were calculated using a nonpolar HP-5MS column and a series of *n*-hydrocarbons (C_7−C_{15}) and compared with those reported previously (http://webbook.nist.gov/). The identification of 2-AP was confirmed by mass spectra, RI, and its distinct descriptor (popcorn odor) in that an authentic standard was not available. For quantification of each odorant, standard curves for each odorant were determined. Each standard solution was prepared with 5, 10, 20, 50, 100, 200, and 500 ppm in hexane, and three replications of 1 μL of each standard solution were injected directly into the GC-MS using a microsyringe. The data were tested for linearity, precision, and sensitivity using linear correlation coefficient (*r*), validation range, and relative standard deviation (RSD), respectively (**Table 1**). The quantification of 2-AP was expressed as TMP equivalents.

Odor Thresholds in Air and Odor Activity Values. Odor thresholds were determined using a modified Ulrich and Grosch GC-O method and required substantiation by at least two of the three assessors (*17*). A stock solution of each odorant (10 mg) dissolved in 10 mL of hexane was diluted stepwise (1:1 v/v), and 0.5 μL of each dilution was injected for GC-O. (*E*)-2-Decenal, which has an odor threshold in air of 2.7 ng/L, was used as an internal standard. The odor thresholds in air were calculated in relation to the odor threshold of (*E*)-2-decenal on the basis of the detectable minimum concentration of (*E*)-2-decenal and the other odorants. Odor activity values (OAVs) were calculated by dividing the absolute concentration of each odor-active compound by its odor threshold in air.

Data Analysis. Principal component analysis (PCA) was carried out on the odor-active compounds using SAS for Windows v. 8 to visualize the differences in odor among rice samples. Duncan's multiple-range test was used to compare OAVs of the odor-active compounds from rice samples.

RESULTS AND DISCUSSION

Odor-Active Compounds from Rice Flavor Types. Odor-active compounds emanating from six different aroma types of cooked rice were identified and quantified and their relative intensities and descriptors determined using trained assessors (**Table 2**). The 30 odorants detected in the rice samples were identified by mass spectra (NIST 02 and Wiley 7 libraries), retention indexes (RI), and odor description using authentic standards. Six additional minor odorants were tentatively identified using GC-MS, RI, and odor descriptors from the literature.

A total of 30, 24, 17, 21, 27, and 24 odorants were detected in BA, JA, H1, H2, BP, and TM, respectively (**Table 2**). Aldehydes and aromatic compounds were the most abundant odor-active compounds in the aromatic rice types (BA, JA, H1, H2, and BP). In the nonaromatic rice (TM), aldehydes were the most abundant odor-active compounds. Of the total odorants detected across all samples, there were nine aldehydes [hexanal, (*E*)-2-hexenal, heptanal, octanal, nonanal, (*E*)-2-nonenal, decanal, (*E*)-2-decenal, and (*E*,*E*)-2,4-decadienal], two aromatics (benzaldehyde and 2-pentylfuran), two alcohols (1-pentanol and 1-octen-3-ol), and one nitrogen-containing compound (2-acetyl-1-pyrroline). Odor intensities of the 14 compounds varied with concentration among the flavor types (data not shown). Pentanal and benzothiazole were detected in only BA, 1-nonanol, 2-methylpyridine, guaiacol, and indole were detected in BP, and 1-hexanol was detected in TM. These compounds may contribute to the unique aroma of their respective cultivars; however, the average odor intensity was very low for all except 1-nonanol, guaiacol, and indole, which had an intermediate or higher intensity (odor intensity ≥ 3). Jezussek et al. (*8*) reported 2-AP, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, bis(2-methyl-3-furyl) disulfide, and 2-aminoacetophenone as key aroma compounds in cooked brown rice, which were not found in our research. The difference can be explained by differences in product chemistry (brown vs white rice) and method of isolation of the volatile compounds from the rice sample. We isolated the volatile compounds above freshly cooked rice samples using the dynamic headspace system with a Tenax trap, whereas Jezussek et al. (*8*) isolated the volatile compounds from a frozen cooked rice sample by high-vacuum distillation using the solvent-assisted flavor evaporation technique.

On the basis of odor intensity, 12, 11, 6, 6, 18, and 16 odorants were found to be at least intermediate in intensity (i.e., ≥3) from BA, JA, H1, H2, BP, and TM, respectively (**Table 2**). Among these, 4, 2, 1, 0, 6, and 8 odorants were classified

Table 2. Odor Intensity and Description of Odor-Active Compounds in Cooked Rice Flavor Types: Basmati (BA), Jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), Black Pigmented Rice (BP), and Traditional Nonaromatic Rice (TM)

		odor intensity[a]							
RI[b]	odorant	BA	JA	H1	H2	BP	TM	odor description[c]	identification[d]
732	pentanal	2.9	nd	nd	nd	nd	nd	nutty, sweet	MS, RI
766	1-pentanol	4.0	3.7	2.8	2.4	3.4	2.8	plastic	MS, RI, STD
803	hexanal	4.9	4.7	3.5	3.9	4.0	4.4	green tomato, green	MS, RI, STD
816	2-methylpyridine	nd	nd	nd	nd	1.0	nd	ash	MS, RI
842	chlorobenzene	2.6	2.8	2.0	2.3	nd	nd	nutty, burnt	MS, RI
857	(*E*)-2-hexenal	2.3	1.4	1.8	2.2	1.0	3.3	green, apple	MS, RI, STD
870	1-hexanol	nd	nd	nd	nd	nd	2.5	green	MS, RI, STD
895	2-heptanone	3.3	1.9	nd	2.2	nd	3.5	fruity, sweet	MS, RI, STD
903	heptanal	4.3	3.6	3.1	2.8	3.9	4.6	floral	MS, RI, STD
918	2-acetyl-1-pyrroline	4.3	4.0	4.0	3.5	4.4	3.6	popcorn	MS, RI
952	benzaldehyde	3.8	3.6	2.4	2.7	3.5	4.2	almond	MS, RI, STD
969	1-heptanol	1.0	nd	nd	nd	0.6	2.8	green	MS, RI, STD
984	1-octen-3-ol	2.6	1.9	1.8	2.2	3.7	3.5	mushroom	MS, RI, STD
992	2-pentylfuran	2.5	2.4	2.1	2.1	2.5	3.2	floral, fruit	MS, RI, STD
1005	octanal	2.1	3.2	3.1	3.0	3.8	4.0	citrus	MS, RI, STD
1026	3-ethyl-2-methyl-1,3-hexadiene	1.0	nd	nd	nd	nd	2.6	nutty	MS, RI
1036	3-octen-2-one	3.5	2.7	nd	2.8	4.0	3.5	rose	MS, RI, STD
1058	(*E*)-2-octenal	2.9	3.8	nd	3.2	2.9	4.3	nutty, cooked flour	MS, RI, STD
1075	1-octanol	2.8	nd	nd	nd	nd	2.9	citrus	MS, RI, STD
1086	guaiacol	nd	nd	nd	nd	3.2	nd	black rice-like, smoke	MS, RI, STD
1093	2-nonanone	2.1	3.3	nd	2.8	3.7	4.2	fruity, flora	MS, RI, STD
1106	nonanal	3.6	2.9	3.5	3.5	4.2	4.1	citrus, fatty	MS, RI, STD
1152	*p*-menthan-3-one	3.3	nd	2.6	nd	3.3	4.1	mint	MS, RI, STD
1160	(*E*)-2-nonenal	3.6	2.5	2.8	2.4	4.3	2.9	beany, cucumber	MS, RI, STD
1172	naphthalene	nd	1.8	2.1	1.9	2.5	nd	naphthalene	MS, RI, STD
1175	1-nonanol	nd	nd	nd	nd	3.0	nd	fatty	MS, RI, STD
1194	2-decanone	1.5	1.5	nd	nd	nd	nd	fruity, fatty	MS, RI, STD
1206	decanal	3.0	3.0	2.8	1.6	2.2	3.3	citrus	MS, RI, STD
1212	(*E*,*E*)-2,4-nonadienal	1.4	2.2	nd	nd	3.7	2.4	nutty, fatty	MS, RI, STD
1213	benzothiazole	1.7	nd	nd	nd	nd	nd	nutty, rubber	MS, RI
1262	(*E*)(*E*)-2-decenal	3.0	3.1	1.7	2.3	2.3	3.1	fatty	MS, RI, STD
1281	2-methylnaphthalene	2.5	nd	nd	0.7	2.2	nd	naphthalene	MS, RI, STD
1289	indole	nd	nd	nd	nd	3.5	nd	sour fruit	MS, RI, STD
1296	1-methylnaphthalene	1.5	1.9	nd	nd	nd	nd	naphthalene	MS, RI, STD
1311	4-vinylguaiacol	1.7	1.5	nd	nd	3.4	nd	nutty	MS, RI, STD
1315	(*E*,*E*)-2,4-decadienal	2.8	3.1	3.3	3.1	3.8	3.5	fatty	MS, RI, STD

[a] Average intensity of compounds that were detected by all three assessors. nd = not detected. [b] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [c] Odorants were described by assessors during GC-O. [d] Method of identification: MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI, by comparison of RI with those from the literature; STD, by comparison of retention time, spectrum, and odor description of an identified compound with those of an authentic compound.

as at least high in intensity (i.e., ≥4) from BA, JA, H1, H2, BP, and TM, respectively. Hexanal (odor intensity = 4.9), 2-AP (4.3), and heptanal (4.3) in BA; hexanal (4.7), 2-AP (4.0), and (*E*)-2-octenal (3.8) in JA; 2-AP (4.0), nonanal (3.5), and hexanal (3.5) in H1; hexanal (3.9), 2-AP (3.5), and nonanal (3.5) in H2; 2-AP (4.4), (*E*)-2-nonenal (4.3), and nonanal (4.2) in BP; and (*E*)-2-octenal (4.6), and hexanal (4.4), and heptanal (4.3) in TM were found to be the most potent odorants in each of the flavor types. 2-AP, a central component in the aroma of aromatic rice (*10*), was the most potent odorant in the five aromatic rice cultivars (BA, JA, H1, H2, BP). Hexanal, which has been reported to be produced nonenzymatically or by an unknown pathway from linoleic acids via 9-D-hydroperoxy-10,12-(*E*,*Z*)-octadecadienoic acid (*18*), was a significant odorant in all rice samples except BP.

Assessment of the Relative Importance of Each Odor-Active Compound to the Overall Aroma. To assess the importance of individual odor-active compounds to the overall aroma, 25 compounds with an odor intensity score of equal to or greater than intermediate (i.e., ≥3) were considered to be potentially significant contributors and their odor threshold values in air determined (**Table 3**). The odor threshold of 2-AP was the lowest (0.02 ng/L) of the major odor-active compounds. Eleven aldehydes [(*E*)-2-nonenal, (*E*,*E*)-2,4-nonadinenal, octanal, heptanal, hexanal, (*E*,*E*)-2,4-decadienal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-decenal, and (*E*)-2-hexenal] had odor thresholds ranging from 0.09 to 3.1 ng/L. These compounds also have relatively low thresholds in water (*7*). The odor of guaiacol, described as "smoky", had an odor threshold of 1.5 ng/L and was detected only in the black rice sample (BP). Guaiacol has been reported as the key odorant in black rice due to its unique odor and low odor threshold (*15*); it is also responsible for the smoked odor in smoked salmon (*19*). In contrast, 1-octen-3-ol, described as "mushroom", had an odor threshold of 2.7 ng/L and was found in each of the rice cultivars, ranging in intensity from 1.8 to 3.5. 1-Octen-3-ol is a significant odorant in cheese due to its low odor threshold (*20*).

OAVs, obtained by dividing each compound's concentration by its odor threshold in air, were used to assess the relative importance of individual odorants to the overall aroma (**Table 3**). To quantify the odor-active compounds, standard curves for 24 odor-active compounds were established using authentic standards (i.e., 7 concentrations for each compound). The accuracy of the standard curves was high as indicated by linear correlation coefficient (*r*), which ranged from 0.9991 to 1.0000, and relative standard deviations (RSDs) values, which ranged from 0.71 to 2.97% (**Table 1**). The most potent odor-active compound in BA was hexanal (OAV = 232, relative proportion

Table 3. Odor Activity Values (OAVs) and Odor Threshold Values of Major Odor-Active Compounds in Cooked Rice Flavor Types: Basmati (BA), Jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), Black Pigmented Rice (BP), and Traditional Nonaromatic Rice (TM)

		OAV[a] ($n = 3$)						
RI[b]	odorant	BA	JA	H1	H2	BP	TM	odor threshold in air (ng/L)
766	1-pentanol	0.002 c	0.01 a	0.004 bc	0.006 b	0.003 bc	0.004 bc	153
803	hexanal	232 a	117 c	31 d	44 d	16 d	167 b	1.1
857	(*E*)-2-hexenal	0.3 a	0.09 b	0.04 b	0.06 b	0.06 b	0.3 a	3.1
895	2-heptanone	0.9 a	0.5 b	nd	0.5 b	nd	0.7 ab	3.5
903	heptanal	12 a	3.2 d	4.0 cd	6.1 b	1.2 e	5.4 bc	0.9
918	2-acetyl-1-pyrroline	17 d	191 b	153 c	8.0 d	246 a	5.8 d	0.02[c]
952	benzaldehyde	0.1 a	0.05 b	0.01 c	0.02 c	0.007 c	0.06 b	85
984	1-octen-3-ol	1.8 a	0.8 c	0.3 d	0.4 d	0.3 d	1.3 b	2.7
992	2-pentylfuran	0.3 a	0.3 a	0.08 b	0.1 b	0.1 b	0.1 b	19
1005	octanal	19 a	6.2 bc	3.6 cd	4.9 cd	1.8 d	9.4 b	0.4
1036	3-octen-2-one	0.2 a	0.05 c	nd	nd	0.02 d	0.1 b	6.7
1058	(*E*)-2-octenal	1.7 a	0.9 c	nd	0.6 cd	0.2 d	1.3 b	2.7
1075	1-octanol	0.05 a	nd	nd	nd	nd	0.03 a	22
1086	guaiacol	nd	nd	nd	nd	0.3 a	nd	1.5
1093	2-nonanone	0.02 a	0.005 c	nd	0.007 c	0.004 c	0.01 b	31
1106	nonanal	5.1 a	1.4 c	1.7 bc	2.3 b	1.4 c	2.0 bc	2.6
1152	*p*-menthan-3-one	1.1 a	nd	0.3 b	nd	0.03 b	0.3 b	4.7
1160	(*E*)-2-nonenal	25 a	7.8 c	5.0 cd	5.7 cd	2.7 d	16 b	0.09
1175	1-nonanol	nd	nd	nd	nd	0.006 a	nd	18
1206	decanal	0.5 a	0.2 bc	0.2 bc	0.3 b	0.1 c	0.2 bc	2.6
1212	(*E*,*E*)-2,4-nonadinenal	1.3 a	0.9 b	nd	nd	0.5 c	1.1 ab	0.2
1262	(*E*)-2-decenal	0.4 a	0.3 bc	0.07 d	0.1 cd	0.04 d	0.3 ab	2.7
1289	indole	nd	nd	nd	nd	0.04 a	nd	8.1
1311	4-vinylguaiacol	0.1 a	0.09 b	nd	nd	0.08 b	nd	2.8
1315	(*E*,*E*)-2,4-decadienal	0.8 a	0.4 bc	0.2 cd	0.2 cd	0.1 d	0.4 b	2.3

[a] OAV is obtained by dividing the concentration of an odor-active compound by its odor threshold in air and means of three replicates per sample; means separation within rows by Duncan's multiple-range test at $P < 0.05$. nd = not detected. [b] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [c] Data from Schieberle (*33*).

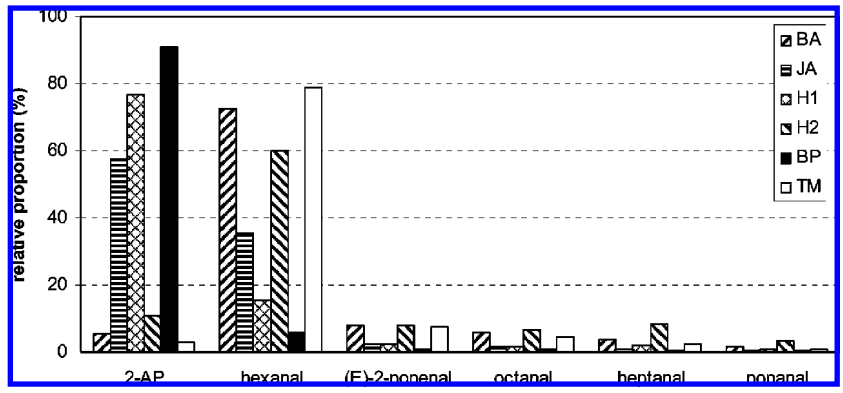

Figure 1. Relative proportion (percent) of OAVs of the primary odor-active compounds in cooked rice flavor types: basmati (BA), jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), black pigmented rice (BP), and traditional nonaromatic rice (TM).

= 72.6%) followed by (*E*)-2-nonenal (25, 7.8%), octanal (19, 5.9%), 2-AP (17, 5.3%), heptanal (12, 3.8%), and nonanal (5.1, 1.6%) (**Table 3**; **Figure 1**). To date, 2-AP has generally been considered to be the most critical odorant in aromatic rice cultivars; however, this is clearly not always the case. For example, hexanal (167, 78.9%) had the highest OAV in TM followed by (*E*)-2-nonenal (16, 7.6%), octanal (9.4, 4.4%), 2-AP (5.8, 2.7%), heptanal (5.4, 2.6%), and nonanal (2.0, 0.9%). The OAVs of these five odor-active compounds, with the exception of 2-AP in BA, were significantly higher than those in TM. The most potent compound in H2 also was hexanal (44, 60.0%); however, 2-AP (8.0, 10.9%), heptanal (6.1, 8.3%), (*E*)-2-nonenal (5.7, 7.8%), octanal (4.9, 6.7%), and nonanal (2.3, 3.1%) followed. The most potent compound in JA, H1, and BP was 2-AP (191, 57.7%; 153, 76.7%; 246, 90.8%) followed by hexanal (117, 35.3%; 31, 15.5%; 16, 5.9%) and (*E*)-2-nonenal (7.8, 2.4%; 5.0, 2.5%; 2.7, 1.0%), respectively. The OAV of guaiacol (0.3) coupled with its unique odor made it a significant contributor to the distinct aroma of BP (*15*). OAVs and the relative proportions of 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal were different among the rice flavor types; however, they had the first to sixth highest OAVs in all rice samples (**Table 3**; **Figure 1**). With the exception of 2-AP, aldehydes were thought not to be particularly important odorants in rice (*7*); however, we found they made up >97% of the OVAs in the rice cultivars studied, indicating a critical role in the overall aroma. It has also been reported that hexanal, (*E*)-2-nonenal, and octanal contribute to off-flavors in rice that develop during storage (*21*). Changes in the concentration of these compounds may have a pronounced effect on flavor.

Comparison of Flavor Types. The concentration of 2-AP has been used as an indicator of aroma in the selection of aromatic lines (*10, 22, 23*). Due to its significant importance in aromatic rice, several techniques have been developed for the identification and quantification of 2-AP (*24–26*). However, aromatic rice cultivars with distinctively different flavors cannot

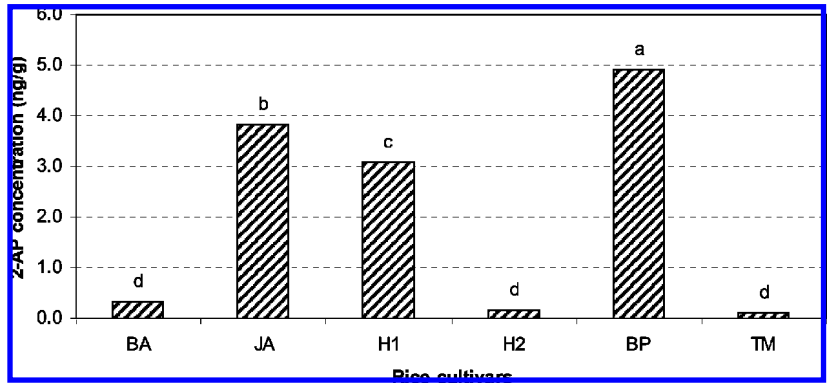

Figure 2. Comparison of concentration of 2-acetyl-1-pyrroline (2-AP) in cooked rice flavor types: basmati (BA), jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), black pigmented rice (BP), and traditional nonaromatic rice (TM). Value was calculated in ng/g equivalent of 2,4,6-trimethylpryridine (TMP) as relative data. Vertical bars with different letters are significantly different ($p < 0.05$).

be adequately characterized just by 2-AP concentration (popcorn-like odor) because other distinct odors (e.g., earthy, nutty, roasty, and green) are present, indicating that the overall aroma is made up of a cross section of compounds (*11, 27*). For example, whereas three of the aromatic cultivars had high levels of 2-AP [i.e., BP (4.9 ng/g), JA (3.8 ng/g), H1 (3.1 ng/g)], two [BA (0.3 ng/g), H2 (0.2 ng/g)] had concentrations on par with the nonaromatic cultivar [TM (0.1 ng/g)] (**Figure 2**). Variation in 2-AP concentration among aromatic rice cultivars confers the relative intensity in their popcorn-like odor (*28*), not the unique aromas found across cultivars. Therefore, a cross section of critical odor-active compounds, including 2-AP, would be required to distinguish differences among progeny in rice-breeding programs.

PCA of the OAVs for the 25 major odor-active compounds from the six flavor types in **Table 3** is presented in **Figure 3**. The five aromatic rice cultivars (BA, JA, H1, H2, BP) segregated distinctly from the nonaromatic cultivar (TM) with 68.4 and 13.9% of the total variance accounted for by the first (PC 1) and second (PC 2) principal components, respectively (**Figure 3A**). BA was positioned on the positive side of JA, H1, and H2 and BP on the negative side of PC 1. JA was separated from H1 and H2 in PC 1 and PC 2. BP distinctly segregated from JA, H1, and H2 in PC 2, and BA in PC 1 and PC 2. The indica aromatic cultivars (BA and JA) were segregated from each other as well as from the japonica aromatic cultivars (H1 and H2). The results indicate that the indica/japonica classification is not an adequate indication of flavor.

It is evident that PCA using all 25 odor-active compounds gives a distinct separation of the various rice flavor types. However, in the assessment of the flavor of large numbers of progeny, it would be advantageous if the number of odor-active compounds that need to be quantified could be reduced while maintaining adequate discrimination potential. A PCA plot using the OAV values for the 13 most important odorants [i.e., 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, 1-octen-3-ol, (*E*)-2-octenal, (*E*,*E*)-2,4-nonadienal, 2-heptanone, (*E*,*E*)-2,4-decadienal, decanal, and guaiacol] is presented in **Figure 3B**. Guaiacol was included in that it is unique to black rice. Reducing the number of compounds from 25 to 13 gave a good separation accounting for 88.3% of the total variance [PC 1 (78.3%) + PC2 (10.0%)] and was adequate for segregating the representative flavor types tested. Although the nonaromatic TM was placed in the middle of the distribution of the aromatic cultivars (BA, JA, H1, H2, BP), they were distinctly separated from TM, indicating the aromatic rice cultivars could be readily separated from nonaromatic (**Figure 3B**). The primary

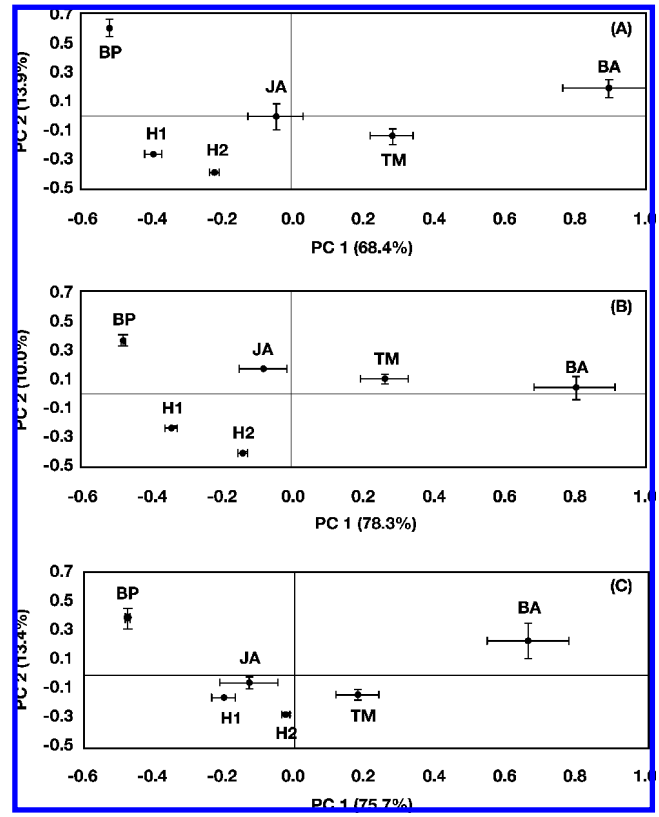

Figure 3. PCA plot using OAVs of (**A**) 25 odor-active compounds, (**B**) 13 odor-active compounds, and (**C**) 7 odor-active compounds in cooked rice flavor types: basmati (BA), jasmine (JA), Hyangmibyeo 1 (H1), Hyangmibyeo 2 (H2), black pigemented rice (BP), and traditional nonaromatic rice (TM). Vertical and horizontal bars represent the standard deviation.

compounds contributing to PC 1 were hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, 1-octen-3-ol, (*E*)-2-octenal, (*E*,*E*)-2,4-nonadienal, 2-heptanone, (*E*,*E*)-2,4-decadienal, and decanal. OAVs for these compounds were significantly different in each rice sample. In contrast, the major compounds contributing to PC 2 were 2-AP and guaiacol, which are critical to the overall aroma of BP. Reducing the number further to the seven most important odorants [i.e., 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, guaiacol] resulted in a PCA plot with less than adequate separation (**Figure 3C**). PCA using 25 odor-active compounds required three components to account for 89.4% of the total variance,

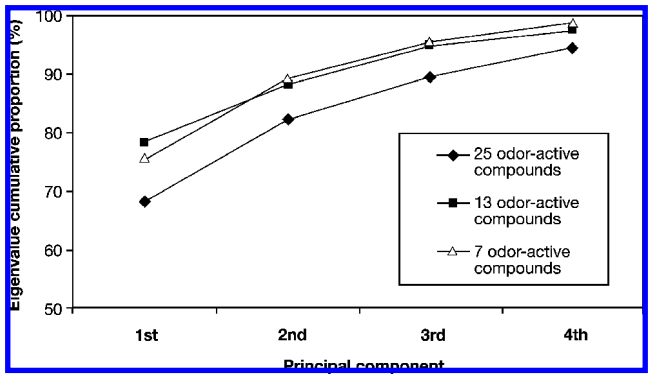

Figure 4. Eigenvalue cumulative proportion of first four principal components from PCA using 25 odor-active compounds, 13 odor-active compounds, and 7 odor-active compounds in cooked rice flavor types.

whereas 13 odor-active compounds accounted for 88.3% using only the first two components (**Figure 4**). It would appear that 13 odor-active compounds can potentially be used to segregate flavor types, although this needs to be substantiated with additional cultivars within each flavor type.

Initial rice flavor selection decisions are currently made in some breeding programs by chewing individual grains or sniffing the aroma of leaf tissue or grains after either heating in water or reacting with KOH or I_2−KI (*29*). The technique is not quantitative and allows classification of progeny only as scented, moderately scented, and nonscented. Decisions are made primarily on the amount of a single volatile compound (2-AP), even though 25 odor-active compounds contribute to the aroma, especially 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal. Likewise, use of molecular markers associated with 2-AP can indicate the presence of the gene but do not indicate the level of expression (*30, 31*). Coupled with this is the apparent absence of concurrent changes in other odorants with changes in 2-AP. Due to numerical, accuracy, and other limitations, these current sensory and chemical tests do not appear to be adequate for making selection decisions.

An analytical method for flavor assessment has the potential to facilitate the selection of lines with superior flavor attributes. Wang and Kays (*32*) used a principal component reference standard, which was developed using multivariate analysis of sweetness and the 17 most important odor-active compounds, as a marker for selecting sweetpotatoes for flavor. If applied to rice, this approach would allow the accurate classification of progeny for aroma, the simultaneous selection of multiple flavor types, and the development of superior new cultivars for a wide cross section of flavors without using sensory tests. Our data indicate that a reference standard with 13 odor-active compounds should allow accurate characterization of rice aroma.

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Received for review September 10, 2007. Revised manuscript received February 6, 2008. Accepted February 9, 2008.

JF072685T