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Characterization of Volatile Aroma Compounds in Cooked Black Rice

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Black rice (*Oryza sativa* L.), an aromatic specialty rice popular in Asia, has a unique flavor, the volatile chemistry of which has not been reported. The objectives of this research were to study volatile profiles of cooked black rice and to characterize the odor-active compounds. Thirty-five volatile compounds were identified by gas chromatography−mass spectrometry using a dynamic headspace system with Tenax trapping. Aldehydes and aromatics were quantitatively in the greatest abundance, accounting for 80.1% of total relative concentration of volatiles. The concentration of 2-acetyl-1-pyrroline (2-AP) was high, exceeded only by hexanal, nonanal, and 2-pentylfuran. A total of 25 odor-active compounds, determined by gas chromatography−olfactometry, were applied to principal component analysis, demonstrating significant differences between a black and a traditional white rice cultivar in terms of aroma and explaining 93.0% of the total variation. 2-AP, guaiacol, indole, and *p*-xylene largely influenced the difference between the aroma in cooked black and white rice. 2-AP and guaiacol were major contributors to the unique character of black rice based on odor thresholds, relative concentrations, and olfactometry.

KEYWORDS: Flavor chemistry; odor-active compound; *Oryza sativa*; dynamic headspace; Tenax trap; 2-acetyl-1-pyrroline; guaiacol; GC-O; principal component analysis

INTRODUCTION

Quantitatively, rice is the single most important food crop worldwide. It is consumed as a staple by over one-half of the world's population with approximately 95% of production in Asia (*1*). Most countries cultivate rice from the *Oryza* genus, which has more than twenty different species. *O. sativa* L. and *O. glaberrima* Steudel are the most widely grown and are cultivated primarily in Asia and Africa, respectively. In addition to traditional white or common rice, a diverse cross-section of specialty rice types have been developed that have unique flavor, nutritional, textural, esthetic, or other properties that often garner higher prices in the market place (*2*). Although there have been a number of studies on rice flavor chemistry, many of the uniquely flavored specialty rice types, such as black rice, have not been characterized.

Black rice is popular in Asian countries where it is often mixed with white rice prior to cooking to enhance the flavor, color, and nutritional value. It is intensely colored because of anthocyanins (e.g., cyanidin 3-glucoside and peonidin 3-glucoside) found in the surface cells of the grain (*3*). It has a number of nutritional advantages over common rice, such as higher protein, total essential amino acids, vitamin B_1 (*4*), and minerals (Fe, Zn, Mn, and P) (*5*), the latter of which varies with cultivar and production location (*6*).

Black rice has a relatively intense flavor that is distinctly different from other types of aromatic rice. Flavor is considered the single most critical quality trait in rice affecting consumer preference (*7*). Over 300 volatile compounds have been identified from various cultivars of aromatic and nonaromatic rice (*8*). The volatiles identified vary with the degree of milling (*9*), isolation technique (e.g., Tenax trapping, simultaneous distillation-extraction, solvents, steam-distillation, and solid phase microextraction) (*10–14*), cooking method (*10, 15*), and storage duration (*16*). Among the volatiles identified, there are a relatively small number of odor active compounds. For example, 2-acetyl-1-pyrroline (2-AP) has a very low odor threshold and is considered to be critical to the flavor of aromatic rice (*10*). Although aromatic types assessed to date contain 2-AP, they have very different aromas, indicating that other compounds contribute to their respective flavors. In that the aroma chemistry of black rice has yet to be characterized, the objectives were to identify the volatile compounds in a cooked black rice cultivar and to characterize the key odor active compounds versus a typical nonaromatic white rice cultivar.

MATERIALS AND METHODS

Materials. To investigate volatile compounds of cooked black rice, a dehulled black rice cultivar (Geomjeong-ssal Brand Premium Korean

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black rice, 1.84 kg package, Korean Farm Inc., Santa Fe Springs, CA) was purchased locally in Georgia. The black rice was not milled because the pigmentation is present in the surface cells. A milled common white rice (Jungilpum Brand Premium Korean nonaromatic rice, 9.07 kg, AOFW Co., Doraville, GA) was also purchased in a local supermarket to characterize the key odor active compounds that distinguish black rice from a typical nonaromatic white rice cultivar. Samples were sealed in glass and held at −20 °C until analysis.

Chemicals. Analytical standards utilized were: benzaldehyde, decane, decanal, (*E*)-2-decenal, dodecane, guaiacol, heptanal, heptane, (*Z*)-linaloloxide, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, and undecane from Sigma-Aldrich Inc., St. Louis, MO; (*E*,*E*)-2,4-decadienal, (*E*)-2-hexenal, 1-hexanol, *d*-limonene, 2-nonanone, 2-methylnaphthalene, hexanal, (*E*)-2-octenal, and 1-octen-3-ol from Aldrich Chem. Co., Milwaukee, WI; octanal and *p*-xylene from Fluka Chem. Co., Milwaukee, WI; naphthalene and toluene from J. T. Baker Inc., Phillipsburg, NJ; indole, (*E*)-2-nonenal, 2-pentylfuran, and 4-vinylguaicol from TCI America, Portland, OR; and 3-octen-2-one from Alfa Aesar, Ward Hill, MA.

Sample Preparation. Because black rice is generally cooked with white rice, five ratios of black-to-white rice were tested: 0, 5, 20, 50, and 100%. The total amount of each sample was 60 g. Because of differences in water absorption between cultivars, the amounts of water added to the five ratios of black-to-white rice ranging from 0 to 100% were 60.0, 75.0, 84.0, 88.5, and 90.0 mL, with the cooking time (25 min) held constant, respectively. The appropriate amount, established in preliminary tests, was based on the cooked samples having similar textural properties.

Isolation of Volatiles Using Tenax Trap. Rice samples (60 g) were added to distilled water and cooked for 25 min at 100 °C in a specially constructed 1 L glass beaker that has the glass top part with entry and exit ports. The entry and exit ports were wrapped with aluminum foil to minimize diffusive losses during cooking. Immediately after cooking, the beaker containing the cooked rice was placed in a hot water bath (70 °C), and purified air was passed through the beaker (150 mL/min for 60 min), exiting into a Tenax trap where the volatiles were collected. The 10 cm long, 6 mm o.d., 4 mm i.d. stainless-steel sorbent trap (Scientific Instrument Services, Inc., Ringoes, NJ) held 150 mg of 60/80 mesh Tenax-TA (Alltech Assoc. Inc., Deerfield, IL) contained with silated glass wool. The Tenax trap was preconditioned at 280 °C for 2 h with purified He at 20 mL/min. A 50 mL glass flask was placed between the exit port and the trap to collect any condensation. All connections were made of glass material. Using a vacuum sampling pump (Aircheck Sampler, model 224-44XR, Eighty-Four, PA), the rice volatiles were swept from the exit port of the beaker into the Tenax trap using air purified by passing it through an activated charcoal filter (Alltech Assoc. Inc. Deerfield, IL) [Pyrex glass tube (10 × 1 cm i.d.) with 7 cm of 2.5 g of charcoal].

Short Path Thermal Desorption. The volatile samples were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min onto the gas chromatograph column (GC/MS, model 6890N/5973, Agilent, Palo Alto, CA) using an automated short-path thermal desorption system (model TD-5, Scientific Instrument Services, NJ). Analytes were collected on the first 4 cm of the GC column using a CO_2 cooled cryofocus trap (−40 °C) (SIS 2″ Cryo-Trap, Scientific Instrument Services, Ringoes, NJ). After desorption, the cryofocus trap was rapidly heated up to 200 °C, and the GC separation program started.

GC/MS Analysis. Identification and quantification of the volatile compounds were performed using a GC/MS equipped with a 30 m length, 0.25 mm i.d., 0.25 μm film thickness, fused silica capillary column (HP-5MS, Agilent, Palo Alto, CA). The injection port temperature was 225 °C, with a split ratio of 5:1. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The column temperature was held at 40 °C for 1 min and then programmed to increase at 1.5 °C/min to 65 °C, which was held for 1 min, increased at 2 °C/min to 120 °C, held for 1 min, and increased at 15 °C/min to 280 °C and held for 5 min. Mass spectrometry conditions were as follows: ion source, 230 °C; electron energy, 70 eV; multiplier voltage, 1247 V; GC/MS interface zone, 280 °C; and a scan range of 35−350 mass units.

Identification and Quantification of Volatile Compounds. The volatiles were identified based on comparison of their mass spectra and relative abundances with NIST 02 and Wiley 7 spectral libraries. The identity of a compound was confirmed by comparison of the Kovats retention index (RI) and mass spectra with authentic standards. Kovats RIs are determined using a nonpolar HP-5MS column and a series of *n*-hydrocarbons (C_7−C_{15}) and comparing with those reported previously in the literature and listed at http://webbook.nist.gov/. The levels of the volatile compounds were expressed as δ-carvone equivalents (assuming all of the response factors were 1). The concentrations are considered relative data because recovery after extraction and calibration factors related to the standard were not determined. The internal standard, δ-carvone, was introduced by placing 5 mL in a sealed 1 L Erlenmyer flask. After 24 h, 10 mL of air saturated with δ-carvone was removed and injected into the glass beaker containing cooked rice at the beginning of volatile collection. The concentration of the internal standard in the trapped sample was determined via direct GC injection of a range of concentrations in hexane. The results were expressed as the average of three replicates of five different ratios of black-to-white rice.

Gas Chromatography–Olfactometry (GC-O). Black rice was cooked (100 g rice/100 mL distilled water) for 30 min, and the volatiles were trapped, desorbed, and chromatographed as previously described. The desorbed odorants were assessed using an Olfactory Detector Outlet (ODO II, SGE Intl., Austin, TX) attached to the Agilent 6890N GC (30 m length, 0.25 mm i.d., 0.25 μm film thickness, fused silica capillary column). The temperature program was the same as described above. The split ratio was 0.5:1, and helium was used as the carrier gas at a flow rate of 2.0 mL/min. An aroma extract of the sample was characterized by describing the aroma of the individual components and assessing their intensity by three trained assessors. The 3 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 (vegetable oil), waxy (candle), and earthy (mushroom) odors. The trained assessors were familiar with GC-O from preliminary tests and previous research. Odorants perceived by all three assessors were classified as odor active compounds. The respective odor intensities were scored from 1 to 5: 1 = very weak; 2 = weak; 3 = intermediate; 4 = strong; 5 = very strong.

Data Analysis. Analysis of variance and principal component analysis (PCA) (*17*) were carried out using the SAS system for Windows v8 to assess differences in odor-active compounds between black and white rice.

RESULTS AND DISCUSSION

Black Rice Flavor Chemistry. Thirty-five volatile compounds emanating from cooked black rice, collected using a dynamic headspace system with a Tenax trap, were identified and quantified by GC-MS (**Table 1**). There were 10 aromatic, 4 nitrogen-containing, 6 alcohol, 10 aldehyde, 3 ketone, and 2 terpenoid compounds, the relative proportions of which are displayed in **Figure 1**. The relative proportion of the main classes of volatiles in 100% black rice was significantly different from that in 100% white rice. The relative proportion of aromatic and nitrogen-containing compounds in black rice was significantly greater than in white rice, whereas white rice had higher relative proportions of alcohols, aldehydes, ketones, and terpenoids. Aldehydes (51.7%) and aromatics (28.4%) quantitatively represented the highest percentage of the total volatiles emanating from black rice (**Figure 1**). Aldehydes, identified in decreasing order of their relative proportion, were hexanal, nonanal, octanal, heptanal, (*E*)-2-octenal, decanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, (*E*)-2-hexenal, and (*E*)-2-decenal. All aldehydes were detected through GC-O and were considered odor-active compounds in cooked black rice (**Table 2**). The aldehydes, products derived predominantly via lipid oxidation,

Table 1. Concentration of Volatiles Identified in Various Ratios of Cooked Black and White Rice

RI[b]	compound	relative concentration (ng/100 g)[a] black 100%	black 50%	black 20%	black 5%	white 100%	method of identification[c]
			Aromatics				
760	toluene	47.7 ± 4.7	3.6 ± 0.9	ND[d]	ND	ND	MS, RI, STD
859	*p*-xylene	60.3 ± 0.5	45.2 ± 3.3	30.1 ± 0.9	18.4 ± 0.3	23.5 ± 0.3	MS, RI, STD
952	benzaldehyde	64.1 ± 3.8	135.5 ± 5.8	299.8 ± 10.3	355.3 ± 15.3	442.3 ± 8.6	MS, RI, STD
992	2-pentylfuran	180.8 ± 8.4	216.6 ± 11.0	273.3 ± 5.2	311.8 ± 4.8	341.6 ± 9.2	MS, RI, STD
1043	phenylacetaldehyde	13.4 ± 0.5	20.0 ± 2.1	ND	ND	ND	MS, RI
1086	guaiacol	68.9 ± 2.8	47.7 ± 1.7	9.1 ± 0.3	ND	ND	MS, RI, STD
1148	1,2-dimethoxybenzene	6.0 ± 0.3	3.1 ± 0.1	ND	ND	ND	MS, RI
1172	naphthalene	21.7 ± 0.4	22.0 ± 0.4	27.3 ± 2.2	11.7 ± 0.2	13.7 ± 0.2	MS, RI, STD
1281	2-methylnaphthalene	14.7 ± 0.2	14.8 ± 1.5	17.5 ± 0.4	26.9 ± 0.4	31.4 ± 0.2	MS, RI, STD
1311	4-vinylguaiacol	16.8 ± 0.5	20.3 ± 2.4	12.5 ± 0.3	9.3 ± 1.6	ND	MS, RI, STD
			N-Containing Compounds				
816	2-methylpyridine	2.9 ± 0.7	2.4 ± 0.0	ND	ND	ND	MS, RI
918	2-acetyl-1-pyrroline	169.3 ± 6.3	130.8 ± 13.1	98.4 ± 9.1	29.7 ± 1.5	ND	MS, RI
1213	benzothiazole	10.0 ± 0.5	8.1 ± 0.3	8.7 ± 0.3	19.3 ± 0.9	12.2 ± 3.3	MS, RI
1289	indole	41.9 ± 0.2	31.3 ± 1.9	18.9 ± 1.0	18.2 ± 1.5	12.4 ± 0.2	MS, RI, STD
			Aliphatic Alcohols				
771	3-methyl-1-butanol	5.5 ± 0.2	26.1 ± 1.9	85.1 ± 15.3	256.9 ± 13.9	304.5 ± 7.4	MS, RI
772	(*S*)-2-methyl-1-butanol	9.0 ± 1.3	41.2 ± 11.6	51.5 ± 4.0	58.6 ± 0.3	61.1 ± 0.8	MS, RI
787	1-pentanol	21.4 ± 1.6	105.7 ± 11.1	264.0 ± 0.5	243.0 ± 8.2	293.5 ± 4.4	MS, RI, STD
870	1-hexanol	20.3 ± 1.3	83.9 ± 9.7	222.0 ± 4.6	226.2 ± 11.4	267.8 ± 1.7	MS, RI, STD
969	1-heptanol	4.6 ± 0.2	21.0 ± 2.3	47.6 ± 4.1	57.1 ± 3.5	66.5 ± 0.7	MS, RI, STD
984	1-octen-3-ol	57.1 ± 4.2	96.0 ± 1.5	153.8 ± 5.9	165.7 ± 6.1	197.3 ± 2.7	MS, RI, STD
			Aliphatic Aldehydes				
803	hexanal	440.2 ± 13.8	837.5 ± 75.5	1507.3 ± 63.3	1790.0 ± 42.7	2327.9 ± 27.0	MS, RI, STD
857	(*E*)-2-hexenal	7.5 ± 0.6	14.5 ± 1.5	25.6 ± 3.4	28.6 ± 1.7	36.7 ± 0.6	MS, RI, STD
903	heptanal	41.0 ± 1.3	73.9 ± 7.7	117.3 ± 4.6	129.8 ± 3.1	153.0 ± 1.0	MS, RI, STD
1005	octanal	46.6 ± 1.5	78.1 ± 8.9	133.0 ± 4.7	151.9 ± 3.7	177.7 ± 4.6	MS, RI, STD
1058	(*E*)-2-octenal	35.0 ± 2.1	54.4 ± 4.5	134.0 ± 2.2	165.8 ± 6.5	214.0 ± 0.3	MS, RI, STD
1106	nonanal	258.3 ± 0.2	328.5 ± 6.5	384.5 ± 7.3	362.2 ± 11.4	381.5 ± 5.9	MS, RI, STD
1160	(*E*)-2-nonenal	16.1 ± 1.0	27.8 ± 2.3	60.1 ± 3.3	81.3 ± 3.7	100.2 ± 1.1	MS, RI, STD
1206	decanal	34.5 ± 1.1	48.7 ± 2.7	68.3 ± 0.6	68.0 ± 5.4	53.9 ± 6.0	MS, RI, STD
1262	(*E*)-2-decenal	6.0 ± 0.5	24.8 ± 1.3	46.0 ± 1.5	55.3 ± 4.4	63.8 ± 4.1	MS, RI, STD
1315	(*E*,*E*)-2,4-decadienal	15.2 ± 1.7	46.9 ± 0.5	65.7 ± 1.6	70.3 ± 3.8	72.4 ± 4.2	MS, RI, STD
			Aliphatic/Alicyclic Ketones				
1036	3-octen-2-one	8.7 ± 1.0	28.9 ± 1.7	57.7 ± 3.0	55.0 ± 0.7	57.9 ± 0.6	MS, RI, STD
1093	2-nonanone	6.8 ± 0.8	13.1 ± 1.3	26.9 ± 1.3	27.9 ± 1.3	31.3 ± 0.5	MS, RI, STD
1449	(*E*)-geranylacetone	17.8 ± 1.4	36.1 ± 0.4	59.9 ± 0.6	60.1 ± 2.3	69.7 ± 1.5	MS, RI
			Terpenoids				
1024	*d*-limonene	7.9 ± 0.7	7.9 ± 0.3	9.1 ± A0.5	52.6 ± 1.4	74.8 ± 0.4	MS, RI, STD
1069	(*Z*)-linaloloxide	7.9 ± 0.2	17.8 ± 0.4	12.0 ± 0.1	ND	ND	MS, RI, STD

[a] Values expressed as δ-carvone equivalent (ng/100 g) and given as average ± standard deviation ($n = 3$). [b] Retention index based on a series of *n*-hydrocarbons. [c] 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; and STD, by comparison of retention time and spectrum of an identified compound with those of an authentic compound. [d] ND = not detected.

had low odor thresholds and were considered to be important contributors to the overall aroma of black rice. Aromatics in black rice included two phenols, one furan, five benzenes, and two naphthalene derivatives. Among them, 2-pentylfuran and 4-vinylguaiacol were reported as odor-active compounds in California long-grain rice and brown rice cultivars, respectively (*10, 18*). Although nitrogen-containing compounds (10.3%), aliphatic alcohols (6.8%), aliphatic/alicyclic ketones (1.9%), and terpenoids (0.9%) were quantitatively substantially lower in abundance, several represented significant contributors to the aroma. For example, 2-acetyl-1-pyrroline (2-AP) is considered the single most important volatile in aromatic rice (*10*). In black rice it represented 9.7% of total volatiles and had the fourth highest relative concentration, following hexanal (25.3%), nonanal (14.8%), and 2-pentylfuran (10.4%); its concentration increased dramatically with increasing proportion of black rice in the mixture and was not present in the 100% white rice sample (**Figure 2A**). 2-AP has a very low odor threshold in water (0.1 ppb) (**Table 2**) and confers a popcorn-like odor (*19*) that is considered a critical component in the overall aroma of black rice. 2-AP is often reported as a key aroma compound in many food products (e.g., aromatic rice, bread, green tea, and popcorn) (*20–23*), so its presence in black rice is not surprising. Although hexanal, nonanal, and 2-pentylfuran were abundant

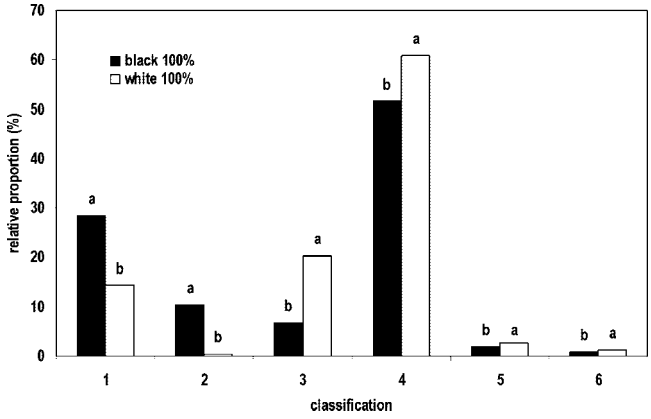

Figure 1. Relative proportion of the primary classes of volatile compounds emanating from a black (Geomjeong-ssal) and white (Jungilpum) cultivar. Vertical bars with different letters are significantly different ($p < 0.05$): (1) aromatics, (2) nitrogen-containing compounds, (3) aliphatic alcohols, (4) aliphatic aldehydes, (5) aliphatic/alicyclic ketones, and (6) terpenoids.

Table 2. Odor Intensity, Description, and Threshold of Odor-Active Compounds in Cooked Black Rice

no.	RI[a]	odorant	identification[b]	intensity[c]	odor description[d]	reported odor threshold (ppb)
1	760	toluene	MS, RI, STD	2.4	paint	1000[e]
2	787	1-pentanol	MS, RI, STD	3.4	fruity	4000[f]
3	803	hexanal	MS, RI, STD	4.0	green tomato, green	5[f]
4	857	(*E*)-2-hexenal	MS, RI, STD	1.0	green, apple	17[f]
5	859	*p*-xylene	MS, RI, STD	3.6	medicinal, solvent	530[e]
6	903	heptanal	MS, RI, STD	3.9	fatty, rancid	3[f]
7	918	2-acetyl-1-pyrroline	MS, RI	4.4	popcorn	0.1[f]
8	952	benzaldehyde	MS, RI, STD	2.2	almond	350[f]
9	969	1-heptanol	MS, RI, STD	0.6	green	3[g]
10	984	1-octen-3-ol	MS, RI, STD	3.7	mushroom	1[f]
11	992	2-pentylfuran	MS, RI, STD	2.5	floral, fruit	6[f]
12	1005	octanal	MS, RI, STD	3.8	citrus	3[f]
13	1036	3-octen-2-one	MS, RI, STD	4.0	rose	
14	1058	(*E*)-2-octenal	MS, RI, STD	2.9	nutty	3[f]
15	1086	guaiacol	MS, RI, STD	3.2	smoky, black rice-like	3[f]
16	1093	2-nonanone	MS, RI, STD	3.7	fruity, floral	200[f]
17	1106	nonanal	MS, RI, STD	4.2	citrus	1[f]
18	1160	(*E*)-2-nonenal	MS, RI, STD	4.3	beany, cucumber	0.08[f]
19	1172	naphthalene	MS, RI, STD	2.5	naphthalene	5[e]
20	1206	decanal	MS, RI, STD	2.2	citrus	2[f]
21	1262	(*E*)-2-decenal	MS, RI, STD	2.3	fatty	0.4[f]
22	1281	2-methylnaphthalene	MS, RI, STD	2.2	naphthalene	20[e]
23	1289	indole	MS, RI, STD	3.5	mothball	140[f]
24	1311	4-vinylguaiacol	MS, RI, STD	3.4	clove	3[f]
25	1315	(*E*,*E*)-2,4-decadienal	MS, RI, STD	3.8	fatty	0.07[f]

[a] Retention index based on a series of *n*-hydrocarbons. [b] 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; and STD, by comparison of retention time, spectrum, and odor description of an identified compound with those of an authentic compound. [c] Average intensity of compounds that were detected by all three assessors. [d] Odorants were described by assessors during GC-O. [e] Odor threshold in water by Van Gernert and Nettenbreijer (*29*). [f] Odor threshold in water by Buttery et al. (*10*). [g] Odor threshold in water by Fazzalari (*30*).

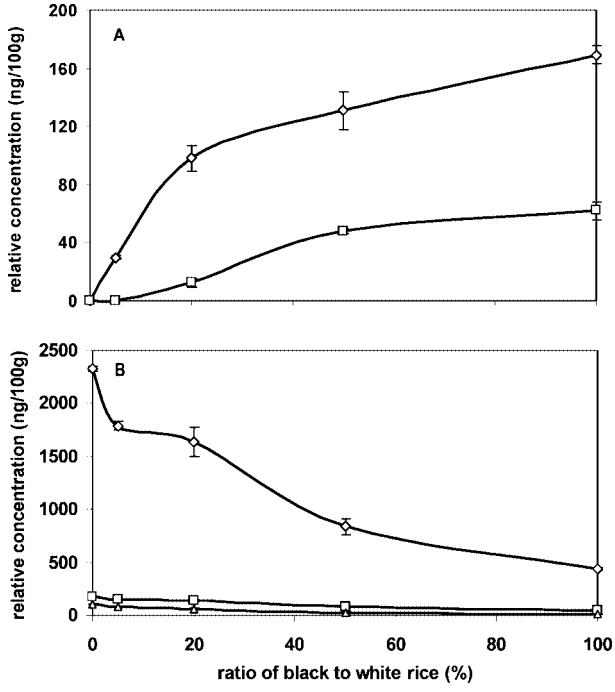

Figure 2. (**A**) Changes in relative concentration (δ-carvone equivalent ng/100 g) of 2-acetyl-1-pyrroline (◇) and guaiacol (□) and (**B**) changes in concentration of hexanal (◇), octanal (□), and (*E*)-2-nonenal (Δ) in varying ratios of black-to-white rice (0, 5, 20, 50, and 100%). Vertical bars represent the standard deviation.

in black rice, their relative concentrations were 5.3, 1.5, and 1.9 times lower, respectively, than in white rice. They were not considered significant contributors to the unique character of the aroma of black rice in that their concentrations increased as the percentage of white rice increased (**Table 2**). The relative proportion of aldehydes in cooked white rice was 60.9%, followed by alcohols (20.3%), aromatics (14.5%), ketones (2.7%), terpenoids (1.3%), and nitrogen-containing compound (0.3%) (**Figure 1**). Hexanal (39.6%) was the most abundant compound, followed by benzaldehyde (7.5%), nonanal (6.5%), and 2-pentylfuran (5.8%) (**Table 1**).

Odor-Active Compounds of Black Rice Using GC-O. Twenty-five compounds emanating from black rice were characterized as odor-active based upon GC-O (e.g., 8 aromatics, 10 aldehydes, 3 alcohols, 2 ketones, and 2 nitrogen-containing compounds) (**Table 2**). Of these, 15 compounds were of intermediate intensity (≥3), and 2-AP, (*E*)-2-nonenal, nonanal, hexanal, and 3-octen-2-one were classified as strong (≥4) (**Table 2**).

The eight detected aromatic compounds included three benzenes, two phenols, one furan, and two naphthalenes. The benzene derivatives had low odor intensities except for *p*-xylene, apparently due to their high odor thresholds (**Table 2**). The phenolic compounds, guaiacol and 4-vinylguaiacol, were greater than intermediate in intensity and had low odor thresholds. Guaiacol, in particular, was not detected in the 0 and 5% black rice samples, but was found in the 20, 50, and 100% samples (**Figure 2A**). Guaiacol was described as having a "smoky" or "black rice-like" aroma by assessors. On the basis of its characteristic odor and low odor threshold, it was considered to be a principal contributor to the unique aroma in cooked black rice. Guaiacol has previously been reported as a key aroma compound responsible for the smoked odor in smoked salmon (*24*).

Many of the aldehydes were found to be equal to or greater than intermediate in intensity, with the exception of (*E*)-2-hexenal, decanal, and (*E*)-2-decenal. The aldehydes are thought to mainly be produced via lipid oxidation and decomposition. Lipid oxidation in rice occurs by (1) oxidative reaction of unsaturated fatty acids mediated via enzymatic, thermal, or light reactions and by (2) thermal mediated oxidative reactions of saturated fatty acids (*25*). Lipase and lipoxygenase activity increases with storage duration in rice, enhancing the production

of lipid oxidative products (*26*). Several studies have reported increased rancidity during rice storage, concurrent with elevated levels of hexanal (green tomato odor), octanal (citrus odor), and 2-nonenal (cucumber odor) (*27*). The concentrations of hexanal, octanal, and (*E*)-2-nonenal (18.9%, 26.2%, and 16.1%, respectively) in black rice were significantly lower than in white rice (**Figure 2B**).

2-AP and indole are nitrogen-containing compounds conferring popcorn and mothball odors, respectively. The protein content of black rice is higher than in brown rice (*4*) and may serve as a nitrogen source in the biosynthesis of these volatiles (*28*). 2-AP, which was not present in the white rice sample, was readily detected when only 5% black rice was present (**Figure 2A**) and contributed significantly to the unique aroma.

Several odor-active, lipid-derived alcohol and ketone compounds were found: 1-pentanol (plastic odor), 1-heptanol (green odor), 1-octen-3-ol (mushroom odor), 3-octen-2-one (rose odor), and 2-nonanone (fruity odor). The concentrations of 1-pentanol, 1-octen-3-ol, 3-octen-2-one, and 2-nonanone, which were found to be greater than intermediate in intensity, were lower than in white rice (**Table 1**) and did not appear to be significant contributors to the distinctive different aroma of black rice.

Comparison of Odor-Active Compounds in Black and White Rice Using Multivariate Analysis. Because black rice is generally prepared as a mixture with white rice, the volatiles emanating from various ratios (e.g., 0, 5, 20, 50, and 100% black rice) were compared. Principal component analysis (PCA) was used with odor-active compounds of cooked black rice to establish differences among the five rice samples and to determine which volatiles contributed most to the differences. **Figure 3** is a PCA biplot of the five ratios of black-to-white rice based upon the concentration of the odor-active compounds. The first principal component (PC 1) explained approximately 85.9% of the total variation. As the percentage of black rice increased, the plotted position, based on the PC 1, moved sequentially toward the 100% black rice sample. The second principal component (PC 2) explained 7.1% of the total variation, indicating the difference between pure rice samples and mixed rice samples; collectively, PC 1 and 2 accounted for 93.0% of the variation. The level of precision of the analytical method is indicated by the precise separation of the individual samples and by the distinct separation of samples containing only 5% black rice (95% white) from those of 100% white (**Figure 3A**). The compounds with negative values on the PC 1 were in decreasing order of their contribution (**Figure 3B**): guaiacol (15), indole (23), *p*-xylene (5), 2-AP (7), toluene (1), 4-vinylguaiacol (24), and naphthalene (19) (numbers correspond to **Table 2**). They indicate that the main odor-active compounds are derived exclusively or predominately from black rice. Guaiacol, indole, *p*-xylene, and 2-AP were greater than intermediate in intensity. In contrast, the compounds with a positive value on the PC 1 were, in decreasing order of their contribution, 1-octen-3-ol (10), benzaldehyde (8), heptanal (6), 1-heptanol (9), octanal (12), 2-nonanone (16), hexanal (3), (*E*)-2-octenal (14), (*E*)-2-nonenal (18), 1-pentanol (2), (*E*)-2-decenal (21), 2-pentylfuran (11), (*E*)-2-hexenal (4), 3-octen-2-one (13), (*E*,*E*)-2,4-decadienal (25), nonanal (17), 2-methylnaphthalene (22), and decanal (20). They are indicative of a white rice origin. Therefore, the main odor-active compounds with negative and positive values characterized the aroma difference between cooked black rice and white rice. 2-AP, guaiacol, indole, and *p*-xylene are responsible for the unique aroma in cooked black rice (**Figure 3B**).

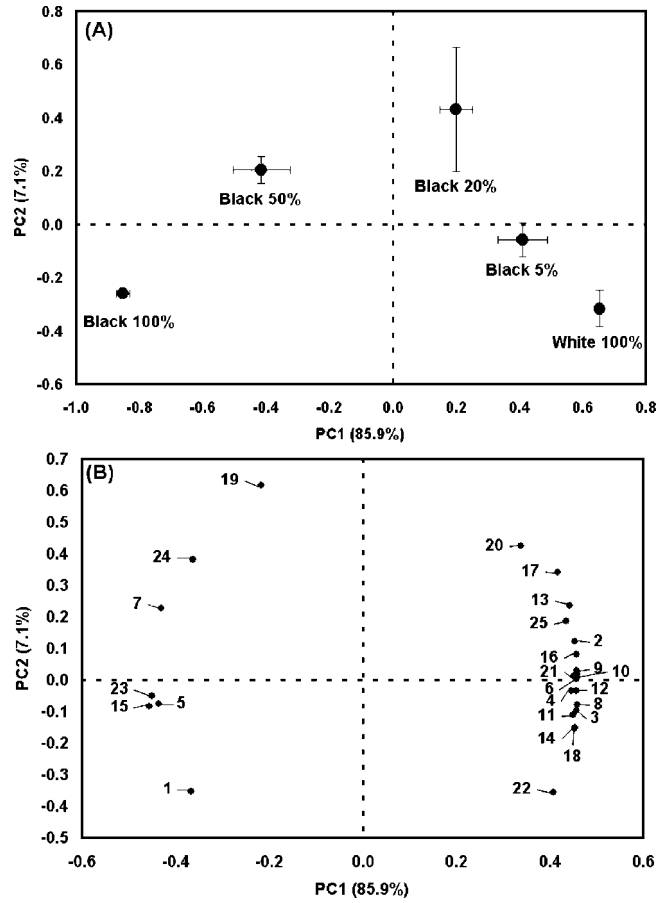

Figure 3. PCA plots from five cooked rice samples. (**A**) The separation of different ratios of black pigmented and traditional white rice based on odor-active compounds, and (**B**) the distribution of 27 odor-active compounds (loadings) in relation to ratios of black-to-white rice. Vertical and horizontal bars represent the standard deviation. Numbers correspond to those in **Table 2**.

A broad cross-section of volatiles, of which aromatics and aldehydes predominate, emanate from cooked black rice. Of these, 25 were considered odor active; 21 were found in white rice, and 4 were unique to black rice (2-AP, guaiacol, toluene, and 4-vinylguaiacol). Critical odorants were 2-AP and guaiacol, which appear to be major contributors to the characteristic aroma of black rice due to their high intensity and the unique description, respectively. The uniqueness of the aroma and the level of precision of the analytical technique described allowed detecting even a small number of black rice grains in white rice.

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