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ARTICLE

Effect of milling degrees on volatile profiles of raw and cooked black rice (*Oryza sativa* L. cv. Sintoheugmi)

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Abstract Volatile compounds in raw and cooked black rice (cv. Sintoheugmi) samples with different degrees of milling (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w) were investigated by headspace solid-phase microextraction and gas chromatography–mass spectrometry. A total of 101 volatile compounds were found. Among them, 44 compounds found in raw black rice were absent in cooked black rice and 20 compounds were newly formed in cooked black rice. The 8 identified major odor-active volatile compounds in raw and cooked black rice included 3 phenols (guaiacol, 4-vinylphenol, and 2-methoxy-4-vinylphenol), 2 benzenes (benzaldehyde and *p*-xylene), 2 furans (2-butylfuran and 2-pentylfuran), and 1 terpene (calamenene). Additionally, fatty acid oxidation products such as hexanal, 2-nonenal, octanal, and 2-pentylfuran were found in raw and cooked black rice samples. The relative concentrations of these volatile compounds were significantly higher in step 0 than in step 2 of raw and cooked black rice ($p < 0.05$). Partially milled cooked black rice (i.e., step 1) contained ~ 80% guaiacol (a favorable unique black rice flavor) of unpolished rice (step 0), with similar levels of several lipid oxidation indicator volatile products (e.g., 2-nonenal and 2-pentyl furan) of fully milled rice (step 2). Thus, partially milled black rice should be consumed rather than fully milled black rice.

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Introduction

Black rice has received attention from the food industry and researchers because of its high levels of anthocyanins such as cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside [1]. Moreover, black rice has a relatively intense flavor that is distinctly different from that of white rice. 2-Acetyl-1-pyrroline, guaiacol, indole, and *p*-xylene are unique volatile compounds found in cooked black rice but not in cooked white rice [2]. Particularly, guaiacol is responsible for a smoky flavor, making cooked black rice acceptable to customers. Guaiacol and its derivatives have been used as flavor agents in the food and perfume industries. The smoky flavor imparts a roasted flavor to processed foods including bacon. Guaiacol can be also used as a starter compound for vanillin synthesis [3].

Black rice has been used as rice flour and kernel after milling. Rice bran is a by-product of rice milling and contains large amounts of nutritional components such as anthocyanins and phytic acids. The degree of milling is the weight ratio of removed rice bran to unpolished rice. The degree of milling alters rice quality characteristics such as nutritional composition and cooking quality. Polished rice, for which the bran has been completely removed from unpolished rice, shows not only improved taste but also low degeneration caused by lipid-associated rancidity during distribution [4]. However, when rice bran and germ are completely removed, favorable flavor components such as guaiacol are also removed.

The analysis of volatile compounds using headspace solid-phase microextraction fibers in conjunction with GC–MS has proven to be an effective method since its development in the 1990s [5]. Several different phases on fibers are available to choose [6]. The DVB/CAR/PDMS combination fiber has been used to analyze volatiles in rice [7, 8]. This combination has been found to trap a greater range of volatile compounds with different polarities, such as aldehydes, ketones, alcohols, esters, and terpenic hydrocarbons, than other fibers, which is important when analyzing complex mixtures of volatile components, such as rice volatiles [6, 9].

The volatile profiles of raw and cooked black rice milled to different degrees have not been characterized. Thus, the objectives of this study were to (1) identify and quantify the volatile compounds in raw black rice varying in degree of milling and (2) observe changes in volatile composition according to milling degrees by the cooking process by using headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS).

Materials and methods

Chemicals and reagents

Authentic standards for GC–MS analysis, C7–C40 saturated hydrocarbon standard, methyl alcohol, hexanal, octanal, nonanal (*E*)-2-octenal, benzaldehyde, 2-nonenal, acetone, 2-heptanone, 2-octanone, ethanol, 1-pentanol, 1-hexanol, 1-octanol, 1-nonanol, acetic acid, 3-methylbutanoic acid, hexanoic acid, methyl myristate, octanoic acid, nonanoic acid, methyl palmitate, methyl (9*Z*)-9-octadecenoate, benzoic acid, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heneicosane, chloroform, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Internal standards (octanal-d_{16}, 2-methylpyrazine-d_6, and *n*-hexyl-d_{13} alcohol) were purchased from C/D/N Isotope, Inc. (Quebec, Canada).

Rice sample preparation

Sintoheugmi was harvested in October 2016 at a local farm in Hoengseong-gun, Gangwon-do, Republic of Korea. Rough rice was dehulled at laboratory in the form of unpolished rice using a hand-operated rice huller. After dehulling, an FS-2000 rice polisher (Mosul, Seoul, Korea) was used to mill unpolished rice to 3 different milling degrees (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w). Step 0 and step 2 were milled to the typical brown rice level and typical white rice level.

Rice was cooked as described previously [10, 11]. The black rice samples (steps 0–2) were cooked using a consistent ratio of 1:1.8 (black rice/water, w/w). Black rice samples were presoaked in water for 1 h prior to cooking in a rice cooker (DWX-200K, Daewoong Co., Ltd., Seoul, Korea). Rice was cooked for 30 min in the cooker, and all cooked rice was allowed to steam-cool for 10 min after the heating was stopped [12]. Cooked samples were cooled for 2–3 min at 25 °C and then placed in a heat-insulating container and used for GC/MS analysis.

Color measurement of raw and cooked black rice

The color of raw and cooked black rice kernels varying in three different milling degrees was measured using an UltraScan PRO colorimeter (Hunterlab, Reston, VA, USA). Color characteristics were indicated as L^* (whiteness), a^* (redness), and b^* (yellowness) values. The colorimeter was calibrated using a standard white tile ($L^* = 100.03$, $a^* = 0.09$, $b^* = 0.15$). One hundred grams of each rice sample was added to a glass dish (40 Ø × 12 mm). The color was analyzed in the middle part of the glass dish. Samples were prepared in triplicate ($n = 3$). The total color difference (ΔE^*) between different milling degree levels of rice samples of step 0 (control) was calculated using the following equation.

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Black rice sampling for HS-SPME–GC/MS analysis

To prepare samples for analysis, a 60-g random raw rice sample was removed from each batch and ground for 60 s at low speed using a SFM-353NK food blender (Sinil, Ansung, Korea). After passing through an 18-mesh standard sieve, 2 g (± 1%) of the raw rice powder or cooked rice kernels was transferred into a 22.5 × 75 mm (20 mL) glass headspace vial (Gerstel, Baltimore, MD, USA). Samples were prepared in duplicate ($n = 2$). Internal standards (octanal-d_{16}, 2-methylpyrazine-d_6, n-hexyl-d_{13} alcohol, and 2,4,6-trimethylpyridine) were dissolved in methanol and then diluted with nano-pure water. The mixed internal standard solution was added to each headspace vial with a final internal standard concentration of 10 ng/g. The vials were immediately sealed with a magnetic crimp cap (Gerstel). An MPS 2L-XT SPME System (Gerstel) was used for all sequences for automated headspace extraction and analysis.

After a 5-min equilibration time and 18-min headspace extraction time at 80 °C, a 1-cm-long DVB/Car/PDMS fiber (Supelco, St. Louis, MO, USA) was injected into the GC and remained in the GC inlet for 25 s. This fiber is commonly used for HS-SPME analysis for complex mixtures of volatiles [13].

GC–MS analysis

Volatile analysis was conducted using GC–MS on a 7890A coupled to an Agilent 5975 mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Compounds were separated on a DB-Wax fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies) at 50 °C for 1 min, and then the temperature was increased by 5 °C/min to a final temperature of 260 °C, with a final holding time of 4 min. Injection was performed in splitless mode, and the injector temperature was 260 °C. Helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The MSD was fitted with an electron impact ionization source set at 250 °C at 70 eV. Total ion chromatograms were recorded by scanning from m/z 40–350 at a rate of 3.06 scans/s.

Identification and relative quantification of volatile compounds

Volatile compounds were identified by comparing their mass spectra and retention times with those of authentic standard compounds [13, 14]. Volatiles without authentic standard compounds were tentatively identified by comparison of mass spectrum with those reported in the Wiley 9 and NIST 08 with < 80% as a cutoff to match compounds and/or comparison of the Kovats' retention index (RI). Kovats' RIs were determined using a polar DB-Wax column and C7–C40 *n*-alkanes and compared with previously reported RIs at https://www.nist.gov/ or www.pherobase.com.

The entire spectrum was scanned in total ion chromatogram mode. The relative concentration of each volatile compound in black rice was determined using a unique extracted ion peak area at its respective retention time and by comparison with the extracted ion peak area of one of 3 internal standards (i.e., octanal-d_{16}, 2-methylpyrazine-d_6, and *n*-hexyl-d_{13} alcohol for aldehydes, nitrogen-containing compounds, and alcohols, respectively). The remaining volatile compounds were quantified by comparison with the internal standard that eluted closest to each of these compounds [15]. Concentration was calculated as described by Baek and Cadwallader [16] and Lee et al. [15].

$$\text{Concentration}\left(\frac{\text{ng}}{\text{g}}\right) = \frac{\text{extracted ion peak area}}{\text{extracted ion peak area of I.S.}}\left[\text{I.S.}\left(\frac{10\ \text{ng}}{\text{g}}\right)\right]$$

Statistical analysis

To determine whether differences in volatile compound levels in black rice samples of varying milling degrees were significant, the results were tested by one-way analysis of variance followed by Duncan's multiple range test ($p = 0.05$). All statistical analysis was performed using SPSS statistical software version 23 (SPSS, Inc., Chicago, IL, USA).

Results and discussion

Color of raw and cooked black rice samples

Table 1 shows the L^*, a^*, and b^* values, which differed among the three milling degree levels. The L^* values of raw black rice kernels significantly increased with increased milling degree levels ($p < 0.05$), as reported previously [17]. In raw rice samples, the L^* values was the highest at 60.34 for step 2 and lowest at 39.68 for step 0 (i.e., unpolished rice) ($p < 0.05$). The a^* value was the highest at 4.53 for step 2 and lowest at 0.66 for step 0 (i.e., unpolished rice) ($p < 0.05$). The b^* value was significantly higher (1.94) in step 2 raw black rice than in other black rice samples ($p < 0.05$).

The changes in a^* and b^* of cooked black rice samples with different milling degrees were similar to those of raw black rice samples. The a^* and b^* values of cooked black rice increased as the milling degree increased from step 0 to step 2 ($p < 0.05$). Compared to the a^* and b^* values, the L^* value changed only minimally as the milling degree increased. There was a slight decrease in the L^* value of cooked black rice as the milling degree increased. For example, there was no significant difference in the L^* value in step 1 and step 2 of cooked black rice.

Additionally, ΔE^*, color differences between unpolished (step 0) and polished rice (steps 1–2) were determined. In raw rice samples, ΔE^* was 3.4 (obvious difference to an untrained eye) and 21 (obvious difference) at steps 1 and 2, respectively. In contrast, the cooked rice sample showed a value of 0.38 (normally invisible difference) and 1.07 (only obvious to a trained eye) at steps 1 and 2, respectively. Thus, the color difference was smaller in cooked black rice than in raw rice after cooking as the milling degree increased. This can be explained by the diffusion of black pigments, such as anthocyanin pigments, during cooking.

Volatile compound identification in raw and cooked black rice

A total of 101 volatile compounds were identified using Wiley 9, NIST 08 libraries, and Kovats' retention index in black rice (Table 2). The identities of 32 volatile compounds were confirmed using authentic standards and denoted as * after peak numbers in Table 2. Representative

Table 1 Hunter color of various milling degrees of black rice (cv. Sintoheugmi)

Sample	Milling degree	Color values			
		L^*	a^*	b^*	ΔE^*
Raw	Step 0	39.68 ± 0.00a*	0.66 ± 0.04a*	− 0.45 ± 0.03b*	
	Step 1	42.86 ± 0.01b*	1.71 ± 0.03b*	− 0.82 ± 0.06a*	3.4
	Step 2	60.34 ± 0.02c*	4.53 ± 0.01c*	1.94 ± 0.03c*	21
Cooked	Step 0	35.77 ± 0.19b	1.08 ± 0.07a	− 0.90 ± 0.04a	
	Step 1	35.04 ± 0.02a	1.43 ± 0.05b	− 0.56 ± 0.03b*	0.38
	Step 2	34.97 ± 0.20a	2.26 ± 0.16c	− 0.58 ± 0.08b*	1.07

Different letters within the same column denote significant difference, using Duncan's multiple test ($p < 0.05$). Values followed by * with each column in the same milling degree for raw and cooked black rice are significantly different ($p < 0.05$)

ΔE^* value 0–1: normally invisible difference, ΔE^* value 1–2: very small difference, only obvious to a trained eye, ΔE^* value 2–3.5: medium difference, also obvious to an untrained eye, ΔE^* value > 3.5: obvious difference, ΔE^* values were obtained from https://www.hunterlab.com/

chromatograms of raw and cooked black rice are shown in Fig. 1. Among the 101 volatile compounds, 80 and 56 compounds were identified in raw and cooked rice, respectively. These include 9 aldehydes, 10 ketones, 8 alcohols, 16 acids and esters, 13 alkanes, 5 olefins, and 19 additional compounds in raw samples (Fig. 1A), and 13 aldehydes, 13 ketones, 2 alcohols, 6 acids and esters, 5 alkanes, 2 olefins, and 15 additional compounds in cooked sample (Fig. 1B). As reported previously in a study using SPME fiber containing PDMS [18], siloxane derivatives affected the chromatogram peaks. These compounds were identified by comparing blank tests with the black rice samples. Other contaminants were also detected and are shown in Table 2. Some contaminants may be absorbed from the storage container (e.g., plastic or cloth bags) and/or pesticides used during cultivation [6]. Additionally, when other black rice samples purchased from local markets grown in different regions were analyzed, contaminants were detected in the samples. Five volatile compounds, not detected in previous studies, were identified as 4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one (ripe, apricot, woody odor), 1-methoxy-2-propanol (bitter taste), 1,4,7,10,13,16-hexaoxacyclooctadecane, methyl acetate (ethereal, sweet odor), and calamenene (herb odor), in raw black rice samples.

Of the volatile compounds identified in the black rice cultivar, 52 and 42 volatile compounds, excluding contaminants, were reported as odor-active compounds in raw and cooked black rice, respectively. The odor description and threshold of all aldehydes in black rice identified in this study were determined previously by GC–olfactometry (Table 2) [19, 20]. Some volatile compounds were unique to the raw and/or cooked black rice. Twenty volatile compounds were newly formed during cooking. Newly formed volatile compounds included aldehydes (2-furaldehyde, 2,6,6-trimethyl-1-cyclohexene-1-carbaldehyde, phenylacetaldehyde, 2-butyl-2-octenal, 4-hydroxy-3-methoxybenzaldehyde), ketones (2,5-octanedione, 2-nonanone, 3-octen-2-one, 6-methyl-3,5-heptadien-2-one, (5*E*)-6,10-dimethyl-5,9-undecadien-2-one, 2-pentadecanone, 5-pentyl-2(5H)-furanone, and 6,10,14-trimethyl-2-pentadecanone), acids and esters (dibutyl (2*Z*)-2-butenedioate), alkane (tricyclo[5.2.1.$0^{2,6}$]decane), olefins (3-ethyl-2-methyl-1,3-hexadiene), and additional compounds (propylbenzene, isopropenylbenzene, 2-sec-butylphenol, and 2-methoxy-4-vinylphenol). Additionally, 44 volatile compounds in raw black rice samples were absent in cooked black rice samples. This may be because of the low abundance of these volatile compounds or because they were removed during cooking.

Changes in volatile profiles of raw black rice samples by milling

Changes in volatile compounds in raw and cooked black rice (cv. Sintoheugmi) of three milling degree levels are shown in Table 3. Milling commonly refers to the process of removing rice bran and germ from unpolished rice. Thus, polished rice contains high endosperm contents. The milling degree levels of rice are commonly related to lower concentrations of surface lipids of rice [21]. Therefore, odor-active compounds in rice bran and germ, such as lipid-derived compounds, alcohols, and aldehydes, were effectively removed as the milling degree level increased.

In raw black rice, identified aldehydes included hexanal, octanal, (*E*)-2-heptenal, nonanal, (*E*)-2-octenal, decanal, benzaldehyde, 2-nonenal, and pentadecanal. The relative concentrations of these aldehydes generally decreased as the milling degrees increased ($p < 0.01$). For example, the relative concentration of hexanal (green tomato odor) showed a decrease of 52% after milling from step 0 to step 2. Odor-active aldehyde compounds have a low odor threshold (0.1–13 ppb except benzaldehydes) and octanal, (*E*)-2-heptenal, nonanal, (*E*)-2-octenal, decanal, 2-nonenal,

Table 2 Identified volatiles in raw and cooked black rice varying with milling degrees

No.	Volatile compound	t_R^a of unknown	Unknown RI[b]	Literature RI	Extracted ion[c]	Internal standard	Odor description[d]	Odor threshold[e]	After cooking
Aldehydes									
12*	Hexanal	4.47	1080	1080	82	Octanal-d_{16}	Green tomato, green, grassy	4.5	
29*	Octanal	8.78	1282	1284	84	Octanal-d_{16}	Citrus	0.7	
32	(*E*)-2-heptenal	9.65	1316	1318	83	Octanal-d_{16}	Pungent, green, fatty	13	Absent
37*	Nonanal	11.34	1383	1390	98	Hexyl-d_{13} alcohol	Aldehydic, waxy, citrus, tart, sweet	1	
42*	(*E*)-2-octenal	12.23	1418	1416	83	Octanal-d_{16}	Cucumber, fatty, fresh, green, waxy	3	
47	2-Furaldehyde	13.00	1449	1451	96	Octanal-d_{16}	Sweet, woody, almond, bread baked		New
49	Decanal	13.90	1484	1484	112	Octanal-d_{16}	Citrus	0.1	
50*	Benzaldehyde	14.45	1505	1508	106	Octanal-d_{16}	Almond	350	
51*	2-Nonenal	14.79	1519	1532	140	Octanal-d_{16}	Beany, cucumber	0.08	
56	2,6,6-Trimethyl-1-cyclohexene-1-carbaldehyde	16.75	1598	1600	152	Octanal-d_{16}	Tropical, saffron, herbal, clean, rose		New
57	Phenylacetaldehyde	17.16	1617	1613	120	Octanal-d_{16}	Sweet, floral, nutty, fruity		New
58	2-Butyl-2-octenal	17.84	1646	1653	55	Octanal-d_{16}	Fruity, pineapple		New
72	Pentadecanal	25.46	2012	2040	96	Octanal-d_{16}	Fresh		
96	4-Hydroxy-3-methoxybenzaldehyde (vanillin)	34.75		2566	151	Octanal-d_{16}	Vanilla, sweet, creamy	58	New
Ketone									
1*	Acetone	1.97	815	814	58	Octanal-d_{16}	Acetone, ethereal, fruity	500,000	
20*	2-Heptanone	6.39	1179	1180	58	Octanal-d_{16}	Cheesy	140	
28*	2-Octanone	8.72	1280	1283	58	Octanal-d_{16}	Parmesan cheese like	50	
31	2,5-Octanedione	9.60	1314	1319	99	Octanal-d_{16}			New
34	6-Methyl-5-hepten-2-one	9.99	1330	1339	108	Octanal-d_{16}			
36	2-Nonanone	11.22	1378	1378	142	Octanal-d_{16}	Fruity, cheesy		New
39	3-Octen-2-one	11.68	1395	1408	126	Octanal-d_{16}	Creamy, earthy, oily, mushroom		New
48	2-Decanone	13.81	1480	1480	113	Octanal-d_{16}	Fermented, generic cheese notes		
54	6-Methyl-3,5-heptadien-2-one	16.13	1574	1582	109	Octanal-d_{16}	Cinnamon, coconut, spicy		New
63	(5*E*)-6,10-dimethyl-5,9-undecadien-2-one	21.93	1832	1840	151	Octanal-d_{16}	Fresh, green, fruity, waxy	60	New
64	Geranyl acetone	21.97	1834	1843	136	Octanal-d_{16}	Floral, rose, fresh, slightly soapy	60	Absent
71	2-Pentadecanone	25.29	2002	1998	58	Octanal-d_{16}	Fresh, jasmine, celery		New
74	5-Pentyl-2(5H)-furanone	26.31	2059	2052	125	Octanal-d_{16}	Minty, fruity		New
77	6,10,14-Trimethyl-2-pentadecanone	27.29	2113	2110	250	Octanal-d_{16}	Oily, herbal, jasmine		New
78	Hexahydrofarnesyl acetone	27.33	2115	2110	43	Octanal-d_{16}	Fresh jasmine, celery		Absent

Table 2 continued

No.	Volatile compound	t_R^a of unknown	Unknown RI[b]	Literature RI	Extracted ion[c]	Internal standard	Odor description[d]	Odor threshold[e]	After cooking
85	3-Ethyl-4-methyl-1 h-pyrrole-2,5-dione	29.93	2269	2260	139	Octanal-d_{16}			Absent
89	4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one	31.16	2271	2291	180	Octanal-d_{16}	Ripe, apricot, woody		Absent
99	7,9-Bis(2-methyl-2-propanyl)-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	37.45			149	Octanal-d_{16}	Green, woody		Absent
Alcohols									
3*	Ethanol	2.71	927	926	46	Hexyl-d_{13} alcohol	Strong alcoholic, ethereal, medical	100,000	Absent
18	1-Methoxy-2-propanol	5.57	1140	1135	45	Hexyl-d_{13} alcohol			Absent
27*	1-Pentanol	8.06	1253	1246	55	Hexyl-d_{13} alcohol	Fusel, oily, sweet, balsamic, fatty	4000	Absent
35*	1-Hexanol	10.52	1352	1358	56	Hexyl-d_{13} alcohol	Green, fruity, apple-skin, oily	2500	
46	1-Octen-3-ol	12.86	1444	1442	57	Hexyl-d_{13} alcohol	Mushroom	1	
53*	1-Octanol	15.47	1548	1555	84	Hexyl-d_{13} alcohol	Waxy, green, citrus	110	Absent
59*	1-Nonanol	17.84	1647	1653	98	Hexyl-d_{13} alcohol	Floral, rosy, fatty, citrus-like	50	Absent
88$^+$	2,2′-[1,2-Ethanediylbis(oxy)]diethanol	31.03	2335		89	Hexyl-d_{13} alcohol			Absent
Acids and esters									
45*	Acetic acid	12.81	1441	1442	60	Hexyl-d_{13} alcohol	Pungent, vinegar		Absent
52	Ethyl nonanoate	14.84	1522	1516	88	Hexyl-d_{13} alcohol	Fatty, fruity brandy-like		Absent
60*	3-Methylbutanoic acid	18.05	1656	1684	60	Hexyl-d_{13} alcohol	Cheesy, dairy, creamy		Absent
62	Hexanoic acid	21.89	1830	1850	73	Hexyl-d_{13} alcohol	Cheesy, fatty	3000	
68	2-Ethylhexanoic acid	24.06	1939	1933	88	Hexyl-d_{13} alcohol			Absent
70*	Methyl myristate	25.12	1994	2008	143	Hexyl-d_{13} alcohol	Fatty, waxy, petal		Absent
73*	Octanoic acid	26.19	2053	2050	101	Hexyl-d_{13} alcohol	Oily, fatty, rancid	3000	Absent
75	Dibutyl (2Z)-2-butenedioate	26.75	2083		99	Hexyl-d_{13} alcohol			New
79*	Nonanoic acid	28.17	2164	2169	129	Hexyl-d_{13} alcohol	Cheese, dairy, fatty, waxy	3000	
82*	Methyl palmitate	29.02	2206	2206	143	Hexyl-d_{13} alcohol	Waxy, fat, candle		
83*	Butyl myristate	29.22	2213	2215	229	Hexyl-d_{13} alcohol	Oily, fatty		Absent
84	Ethyl palmitate	29.69	2227	2246	157	Hexyl-d_{13} alcohol	Mild, waxy	2000	Absent
86	Decanoic acid	30.13	2241	2250	172	Hexyl-d_{13} alcohol	Fatty, rancid	10,000	
92*	Methyl (9Z)-9-octadecenoate	32.94	2449	2452	264	Hexyl-d_{13} alcohol	Mild, fatty		
93*	Benzoic acid	33.20	2465	2457	105	Hexyl-d_{13} alcohol	Balsamic, urine		Absent
94	Methyl (9Z,12Z)-9,12-octadecadienoate	33.73	2497	2484	95	Hexyl-d_{13} alcohol	Oily, fatty		Absent
101	Palmitic acid	40.12		2910	213	Hexyl-d_{13} alcohol	Waxy, creamy fatty, soapy	10,000	Absent
Alkanes									
4*	Decane	3.31	998	1000	142	Hexyl-d_{13} alcohol			Absent
7	2-Methyldecane	4.08	1055	1053	141	Hexyl-d_{13} alcohol			Absent

Table 2 continued

No.	Volatile compound	t_R^a of unknown	Unknown RI[b]	Literature RI	Extracted ion[c]	Internal standard	Odor description[d]	Odor threshold[e]	After cooking
8	5-Ethyl-2,2,3-trimethylheptane	4.12	1058	1060	112	Hexyl-d_{13} alcohol			Absent
9	3,7-Dimethylnonane	4.27	1067	1060	127	Hexyl-d_{13} alcohol			Absent
10	3-Methyldecane	4.32	1070	1070	126	Hexyl-d_{13} alcohol			Absent
11	3,7-Dimethyldecane	4.41	1076	1079	141	Hexyl-d_{13} alcohol			Absent
13*	Undecane	4.57	1085	1100	71	Hexyl-d_{13} alcohol			
21*	Dodecane	6.47	1183	1200	170	Hexyl-d_{13} alcohol			
25	Tricyclo[5.2.1.$0^{2,6}$]decane	7.62	1234	1243	136	Hexyl-d_{13} alcohol			New
30*	Tridecane	8.91	1286	1300	184	Hexyl-d_{13} alcohol			
38*	Tetradecane	11.42	1386	1400	198	Hexyl-d_{13} alcohol			
55*	Hexadecane	16.30	1581	1600	99	Hexyl-d_{13} alcohol			Absent
76*	Heneicosane	26.81	2086	2100	85	Hexyl-d_{13} alcohol	Waxy		Absent
100	1,4,7,10,13,16-Hexaoxacyclooctadecane	39.46			207	Hexyl-d_{13} alcohol			Absent
Olefins									
24	1-Dodecene	7.54	1231	1233	97	Hexyl-d_{13} alcohol			Absent
40	3-Ethyl-2-methyl-1,3-hexadiene	11.87	1402	1404	124	Hexyl-d_{13} alcohol			New
43	(*Z*)-4-Tetradecene	12.34	1423	1427	111	Hexyl-d_{13} alcohol			Absent
44	1-Tetradecene	12.53	1430	1420	125	Hexyl-d_{13} alcohol			
66+	(4*Z*,8*Z*)-Tricyclo[10.2.1.02,11]pentadeca-4,8-diene	23.01	1885		79	Hexyl-d_{13} alcohol			Absent
95+	Decanamide	34.65			59	Hexyl-d_{13} alcohol			Absent
Additional compounds									
2	Methyl acetate	2.02	823	825	74	Octanal-d_{16}	Ethereal, sweet		Absent
5*+	Acetonitrile	3.38	1005	1003	40	Hexyl-d_{13} alcohol			Absent
6*+	Chloroform	3.59	1021	1021	83	Hexyl-d_{13} alcohol	Floral, rose, green		Absent
14	Ethylbenzene	5.15	1118	1116	91	Hexyl-d_{13} alcohol			
15	2-Butylfuran	5.23	1123	1126	81	Hexyl-d_{13} alcohol	Mild, fruity, wine, sweet, spicy		
16	*p*-Xylene	5.30	1126	1130	91	Hexyl-d_{13} alcohol	Medicinal	530	
17	*m*-Xylene	5.42	1133	1140	106	Hexyl-d_{13} alcohol			
19	*o*-Xylene	6.33	1177	1176	91	Hexyl-d_{13} alcohol	Peanut		
22	Propylbenzene	6.81	1197	1203	120	Hexyl-d_{13} alcohol	Solvent, sweet	6	New
23	2-Pentylfuran	7.36	1223	1226	138	Hexyl-d_{13} alcohol	Floral, fruit	6	
26	Styrene	8.00	1250	1254	104	Hexyl-d_{13} alcohol	Sweet, balsam, floral, plastic	730	
33	Isopropenylbenzene	9.75	1320	1321	118	Hexyl-d_{13} alcohol			New
41	2-Heptylfuran	12.10	1412	1420	81	Octanal-d_{16}	Green, fatty, lactonic, oily, roasted, nutty		Absent
61	Calamenene	21.47	1809	1808	159	Hexyl-d_{13} alcohol	Herb		Absent
65	Guaiacol	22.07	1839	1850	109	Hexyl-d_{13} alcohol	Smoky, black rice-like	3	
67+	4-Methyl-2,6-bis(2-methyl-2-propanyl)phenol	23.15	1892	1902	205	Hexyl-d_{13} alcohol	Smoky, meaty, phenolic		

Table 2 continued

No.	Volatile compound	t_R^a of unknown	Unknown RI[b]	Literature RI	Extracted ion[c]	Internal standard	Odor description[d]	Odor threshold[e]	After cooking
69	Diethylene glycol	24.51	1963	1968	45	Hexyl-d_{13} alcohol	Sweet		Absent
80	2-Sec-butylphenol	28.24	2168		150	Hexyl-d_{13} alcohol			New
81	2-Methoxy-4-vinylphenol	28.55	2185	2180	135	Hexyl-d_{13} alcohol	Amber, cedar, peanut	3	New
87[+]	2,4-Bis(2-methyl-2-propanyl)phenol	30.72	2258	2277	191	Hexyl-d_{13} alcohol			Absent
90	4-Vinylphenol	32.02	2392	2382	120	Octanal-d_{16}	Chemical, phenolic, medicinal	10	
91	1H-indole	32.87	2444	2441	117	2-Methylpyrazine-d6	Animal, fecal, naphthyl, with earthy	140	
97[+]	Dibutyl phthalate	36.90		2680	149	Hexyl-d_{13} alcohol	Faint odor		Absent

[a]t_R stands for retention time

[b]RI stands for retention index. RI values were obtained from https://www.nist.gov or flavornet.org

[c]Extracted ion from total ion scan used for quantitation

[d]Odor-active compounds and [e]odor threshold (ppb) were described in previous studies [19, 20]

*Verified using authentic standards

[+]Putative contaminants

and pentadecanal showed higher levels in the by-products than in the rice kernel [2].

The relative concentrations of alcohols (e.g., ethanol, 1-methoxy-2-propanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-octanol, 1-nonanol, 2,2′-[1,2-ethanediylbis(oxy)]diethanol) also significantly decreased as the milling degree increased in raw black rice samples ($p < 0.05$). Rice is typically contaminated by compounds present in water and/or pesticides used in the cultivation process [22]. The relative concentrations of the contaminants decreased as milling degree increased ($p < 0.05$). For example, 2,2′-[1,2-ethanediylbis(oxy)]diethanol was not detected in step 2, but was detected in step 0.

The 7 identified major odor-active compounds, including 2 phenols (guaiacol, 4-vinylphenol), 2 benzenes (benzaldehyde and *p*-xylene), 2 furans (2-butylfuran and 2-pentyl furan), 1 terpene (calamenene), and 1 nitrogen-containing compound (1H-indole), were detected in raw and/or cooked black rice (Fig. 2A, B) [2]. The relative concentration of the major odor-active compounds significantly decreased as the milling degree increased ($p < 0.05$). Guaiacol, a characteristic odor-active compound in black rice, is a phenolic compound with a low odor threshold (3 ppb) [19, 20]. According to previous studies, guaiacol is reported to impart a smoked odor such as those of roasted coffee, lightly roasted sesame seed, and smoked salmon [23]. As the milling degree increased from step 0 to step 2, the relative concentrations of guaiacol (smoky, black rice-like odor) decreased by 65% (Table 2). However, partially milling the rice such as step 1 retained 92% of the guaiacol in unpolished rice (step 0). As an off-flavor producing volatile, 1H-Indole is a nitrogen-containing compound conferring mothball and fecal odors. The relative concentration of 1H-indole in raw black rice decreased by 74% when milled from step 0 to step 2 (Table 2).

Additionally, 5 lipid oxidation products (i.e., hexanal, octanal, 2-nonenal, 1-pentanol, and 2-pentylfuran), representative lipid oxidation indicators of stored rice [24], were detected in the black rice samples. Among the lipid oxidation products, hexanal, 2-nonenal, octanal, 1-pentanol, and 2-pentylfuran were chosen based on their high relative concentrations and low odor threshold values. Lipid oxidation products may have been formed by the oxidation of unsaturated fatty acids in black rice. More than 60% of fatty acids are composed of unsaturated fatty acids in rice and unsaturated fatty acids act as precursors of lipid oxidation products [24]. Major unsaturated fatty acids in rice include oleic, linoleic, and linolenic acids [24]. The relative concentrations of hexanal, 1-pentanol, and 2-nonenal, which are indicators of secondary oxidation of linoleic acid [25], decreased significantly with increasing milling degrees of raw black rice samples ($p < 0.05$) (Fig. 2C).

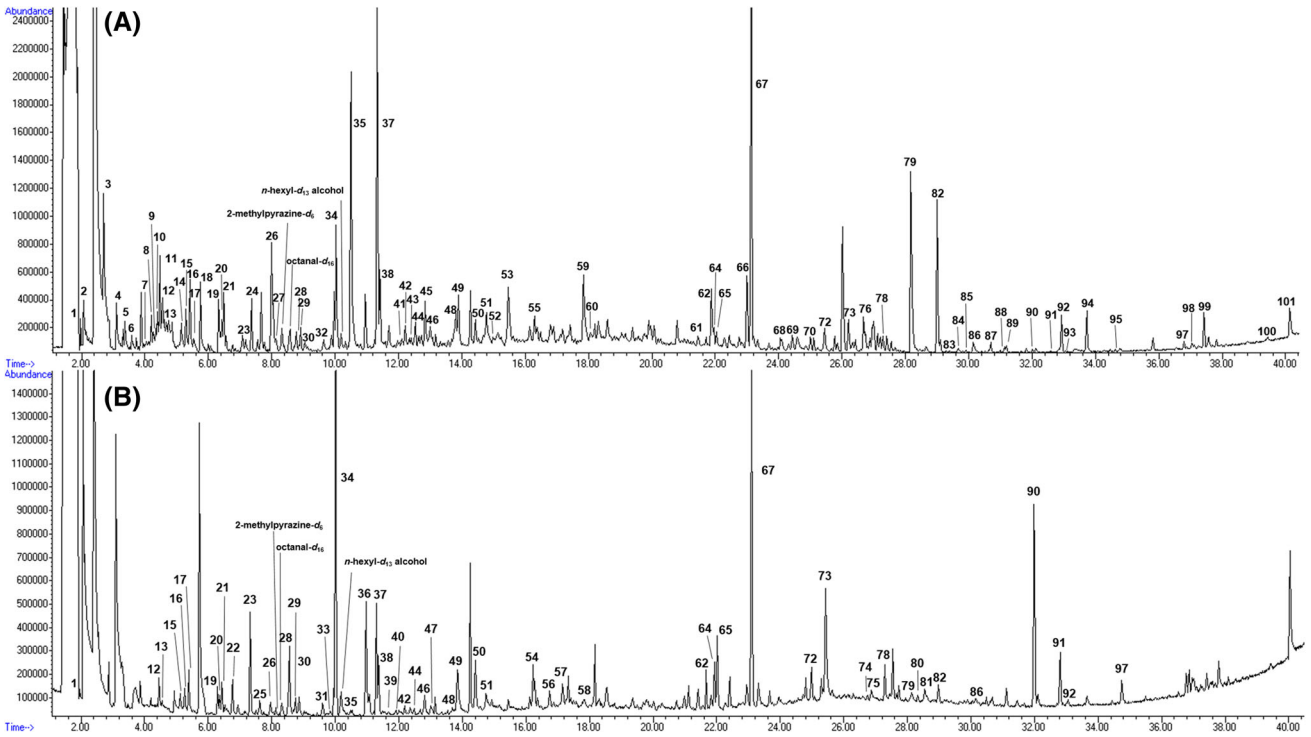

Fig. 1 Typical chromatogram of (**A**) raw and (**B**) cooked unpolished black rice using SPME/GC–MS showing the peak of volatile compounds and internal standards

Step 1 black rice contained hexanal concentrations by 60% of unpolished rice (step 0). The relative concentration of octanal, a representative secondary oxidation product of oleic and linoleic acids [24], decreased significantly with increasing milling degree ($p < 0.01$). The concentration of 2-pentylfuran, a secondary oxidation product of linolenic and linoleic acid, also decreased significantly with increasing milling degrees ($p < 0.01$). Additionally, the 1-pentanol concentration decreased significantly with increasing milling degree levels ($p < 0.001$). Thus, milling can also remove lipid oxidation products.

Changes in volatile profiles of cooked black rice samples by milling

In cooked black rice samples, (*E*)-2-heptanal (pungent odor), an odor-active compound, was not detected, regardless of the milling degree. In contrast, favorable volatile compounds (e.g., 2-furaldehyde (sweet, woody, almond, bread baked odor), 2,6,6-trimethyl-1-cyclohexene-1-carbaldehyde (saffron, herbal odor), phenylacetaldehyde (sweet, floral, nutty odor), 2-butyl-2-octenal (fruity, pineapple), and 4-hydroxy-3-methoxybenzaldehyde (vanilla odor)) were newly detected in cooked black rice.

The relative concentrations of the newly detected volatile compounds decreased significantly as milling degree increased ($p < 0.05$). For example, vanillin (4-hydroxy-3-methoxybenzaldehyde), a unique odor-active compound in cooked black rice, levels in step 0 (78.4 ng/10 g), decreased significantly in step 1 (37.4 ng/10 g) and step 2 (20.9 ng/10 g) ($p < 0.05$).

In cooked black rice samples, 7 volatile ketone compounds (2-nonanone (fruity, cheesy odor), 3-octen-2-one (creamy, earthy, oily odor), 6-methyl-3,5-heptadien-2-one (cinnamon, coconut odor), (5*E*)-6,10-dimethyl-5,9-undecadien-2-one (green, fruity, waxy odor), 2-pentadecanone (jasmine, celery odor), 5-pentyl-2(5H)-furanone (minty odor), and 6,10,14-trimethyl-2-pentadecanone (fresh jasmine, celery odor)), which were absent in raw black rice samples, were newly found after cooking. The concentrations of the ketones decreased significantly in polished black rice (i.e., steps 1–2) compared to that in unpolished black rice (step 0) ($p < 0.05$). For example, 3-octen-2-one was not detected in polished black rice.

Among the odor-active alcohols found in raw black rice samples, only 1-hexanol and 1-octen-3-ol were detected in cooked black rice samples, while the other odor-active alcohols were not detected in cooked black rice samples. Cooking appeared to greatly influence the alcohol concentration.

The major odor-active compounds of cooked black rice included 3 phenols (guaiacol, 4-vinylphenol, and

Table 3 Concentration of volatile compounds identified in raw and cooked black rice varying with milling degrees

No.	Volatile compound	ANOVA[a]	Raw black rice (ng/g)			ANOVA[a]	Cooked black rice (ng/10 g)		
			Unpolished	Step 1	Step 2		Unpolished	Step 1	Step 2
Aldehydes									
12	Hexanal	***	20.9 ± 0.0c	12.6 ± 0.2b	10.1 ± 0.0a	**	8.8 ± 0.0b	8.3 ± 0.3b	5.9 ± 0.2a
29	Octanal	**	15.1 ± 0.1c	12.0 ± 0.8b	9.9 ± 0.5a	**	19.9 ± 1.1c	13.4 ± 0.4b	9.0 ± 0.4a
32	(*E*)-2-heptenal	***	8.8 ± 0.6c	7.2 ± 0.2b	5.2 ± 0.0a		n.d.	n.d.	n.d.
37	Nonanal	***	299.7 ± 6.7c	271.4 ± 3.5bc	215.4 ± 0.2a	**	117.3 ± 2.2c	97.3 ± 4.1b	69.6 ± 4.6a
42	(*E*)-2-octenal	**	32.8 ± 0.1c	31.8 ± 0.9b	26.7 ± 1.9a	***	9.9 ± 0.1c	7.6 ± 0.2b	4.9 ± 0.2a
47	2-Furaldehyde		n.d.	n.d.	n.d.	**	10.2 ± 0.1b	5.9 ± 0.6a	5.3 ± 0.2a
49	Decanal		11.7 ± 0.3	11.4 ± 0.2	10.9 ± 0.0	*	14.8 ± 1.4c	9.6 ± 0.3b	8.0 ± 0.6a
50	Benzaldehyde	***	82.5 ± 0.0c	56.5 ± 0.1b	41.7 ± 0.4a	*	157.1 ± 3.0b	145.0 ± 12.3ab	121.1 ± 3.9a
51	2-Nonenal	***	1.6 ± 0.0c	1.5 ± 0.0b	1.3 ± 0.1a	*	1.7 ± 0.1b	1.2 ± 0.1a	1.0 ± 0.0a
56	2,6,6-Trimethyl-1-cyclohexene-1-carbaldehyde		n.d.	n.d.	n.d.	***	10.9 ± 0.4b	10.1 ± 0.2b	4.7 ± 0.2a
57	Phenylacetaldehyde		n.d.	n.d.	n.d.	**	23.5 ± 0.6c	15.9 ± 2.8b	6.5 ± 0.1a
58	2-Butyl-2-octenal		n.d.	n.d.	n.d.	*	5.5 ± 1.0b	3.0 ± 0.0a	1.3 ± 0.1a
72	Pentadecanal	**	15.0 ± 0.1c	12.1 ± 0.7b	9.5 ± 0.4a	***	88.3 ± 3.7c	76.6 ± 0.8b	34.6 ± 0.5a
96	4-Hydroxy-3-methoxybenzaldehyde(vanillin)		n.d.	n.d.	n.d.	***	78.4 ± 2.5c	37.4 ± 2.0b	20.9 ± 0.2a
Ketone									
1	Acetone	**	33.7 ± 1.3c	28.2 ± 0.5b	21.3 ± 0.3a	***	13.4 ± 0.4c	6.3 ± 0.0b	3.5 ± 0.2a
20	2-Heptanone	***	23.6 ± 1.7c	16.7 ± 1.1b	10.6 ± 0.1a	*	22.9 ± 1.9c	18.1 ± 0.9b	13.5 ± 0.1a
28	2-Octanone	***	8.8 ± 0.6c	6.3 ± 0.1b	4.4 ± 0.2a	**	18.5 ± 0.9c	11.2 ± 0.3b	6.3 ± 0.3a
31	2,5-Octanedione		n.d.	n.d.	n.d.	**	14.9 ± 0.3c	11.2 ± 0.6b	8.3 ± 0.2a
34	6-Methyl-5-hepten-2-one	***	53.8 ± 0.4c	41.9 ± 2.5b	28.9 ± 1.2a	*	26.2 ± 4.6b	16.5 ± 1.6a	12.7 ± 0.7a
36	2-Nonanone		n.d.	n.d.	n.d.	*	1.1 ± 0.0c	0.9 ± 0.0b	0.8 ± 0.1a
39	3-Octen-2-one		n.d.	n.d.	n.d.	***	0.9 ± 0.0b	0.7 ± 0.1a	
48	2-Decanone	**	7.8 ± 0.5b	7.3 ± 0.1b	5.5 ± 0.0a	**	4.3 ± 0.2b	4.2 ± 0.1b	2.7 ± 0.2a
54	6-Methyl-3,5-heptadien-2-one		n.d.	n.d.	n.d.	**	26.4 ± 1.3b	27.0 ± 2.3b	10.0 ± 0.3a
63	(5*E*)-6,10-dimethyl-5,9-undecadien-2-one		n.d.	n.d.	n.d.	**	29.1 ± 0.1b	19.3 ± 1.0a	20.7 ± 1.8a
64	Geranyl acetone	***	9.4 ± 0.1c	7.5 ± 0.0b	4.7 ± 0.2a		n.d.	n.d.	n.d.
71	2-Pentadecanone		n.d.	n.d.	n.d.	**	136.8 ± 3.9c	98.2 ± 17.2b	49.4 ± 1.0a
74	5-Pentyl-2(5H)-furanone		n.d.	n.d.	n.d.	**	5.6 ± 0.3c	4.5 ± 0.1b	3.4 ± 0.3a
77	6,10,14-Trimethyl-2-pentadecanone		n.d.	n.d.	n.d.	**	3.7 ± 0.4c	1.4 ± 0.0b	0.5 ± 0.0a
78	Hexahydrofarnesyl acetone	***	9.6 ± 0.0c	7.6 ± 0.0b	6.6 ± 0.1a		n.d.	n.d.	n.d.
85	3-Ethyl-4-methyl-1 h-pyrrole-2,5-dione	***	3.6 ± 0.1c	2.5 ± 0.0b	1.3 ± 0.0a		n.d.	n.d.	n.d.
89	4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one	***	2.9 ± 0.1c	2.2 ± 0.0b	1.3 ± 0.0a		n.d.	n.d.	n.d.

Table 3 continued

No.	Volatile compound	ANOVA[a]	Raw black rice (ng/g)			ANOVA[a]	Cooked black rice (ng/10 g)		
			Unpolished	Step 1	Step 2		Unpolished	Step 1	Step 2
99	7,9-Bis(2-methyl-2-propanyl)-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	***	5.0 ± 0.2c	4.5 ± 0.2b	2.7 ± 0.1a		n.d.	n.d.	n.d.
Alcohols									
3	Ethanol	***	83.7 ± 1.4c	70.3 ± 0.4b	22.4 ± 0.3a		n.d.	n.d.	n.d.
18	1-Methoxy-2-propanol	*	35.6 ± 0.6b	43.2 ± 3.1c	15.9 ± 1.5a		n.d.	n.d.	n.d.
27	1-Pentanol	***	30.3 ± 2.1c	19.3 ± 1.3b	6.6 ± 0.8a		n.d.	n.d.	n.d.
35	1-Hexanol	***	286.7 ± 15.6c	190.5 ± 6.5b	56.8 ± 0.5a	***	56.4 ± 0.6c	29.9 ± 0.1b	22.4 ± 0.5a
46	1-Octen-3-ol	***	73.9 ± 3.4c	46.8 ± 2.1b	26.4 ± 0.1a	**	115.2 ± 6.2c	90.1 ± 5.1b	45.4 ± 0.1a
53	1-Octanol	***	21.7 ± 0.4c	16.4 ± 1.0b	9.1 ± 0.1a		n.d.	n.d.	n.d.
59	1-Nonanol	***	12.5 ± 0.2c	8.0 ± 0.1b	4.4 ± 0.2a		n.d.	n.d.	n.d.
88[+]	2,2′-[1,2-Ethanediylbis(oxy)]diethanol	***	0.5 ± 0.0a	0.8 ± 0.1b	n.d.		n.d.	n.d.	n.d.
Acids and esters									
45	Acetic acid	***	18.4 ± 1.3c	12.3 ± 0.4b	3.4 ± 0.3a		n.d.	n.d.	n.d.
52	Ethyl nonanoate	***	31.2 ± 1.5c	27.2 ± 0.2b	21.9 ± 0.7a		n.d.	n.d.	n.d.
60	3-Methylbutanoic acid	***	7.3 ± 0.5c	4.5 ± 0.4b	0.8 ± 0.0a		n.d.	n.d.	n.d.
62	Hexanoic acid	***	42.8 ± 0.4c	22.7 ± 2.5b	7.4 ± 0.9a	**	69.3 ± 0.1c	57.0 ± 2.3b	40.5 ± 1.2a
68	2-Ethylhexanoic acid	***	27.7 ± 0.3c	23.5 ± 0.7b	3.8 ± 0.4a		n.d.	n.d.	n.d.
70	Methyl myristate	***	5.7 ± 0.3c	3.1 ± 0.2b	0.7 ± 0.0a		n.d.	n.d.	n.d.
73	Octanoic acid	**	39.8 ± 0.3c	21.7 ± 3.4b	5.2 ± 1.1a		n.d.	n.d.	n.d.
75	Dibutyl (2Z)-2-butenedioate		n.d.	n.d.	n.d.	**	13.4 ± 0.1c	11.3 ± 0.4b	9.4 ± 0.4a
79	Nonanoic acid	*	100.5 ± 7.4c	61.0 ± 4.1b	20.6 ± 5.9a	**	6.6 ± 0.1c	5.6 ± 0.1b	5.2 ± 0.1a
82	Methyl palmitate	***	53.5 ± 0.2c	28.3 ± 1.5b	7.1 ± 0.2a	**	4.8 ± 0.4b	2.0 ± 0.1a	1.8 ± 0.2a
83	Butyl myristate	**	6.3 ± 0.3b	6.1 ± 0.5b	2.5 ± 0.6a		n.d.	n.d.	n.d.
84	Ethyl palmitate	***	3.5 ± 0.2c	1.0 ± 0.0b	0.8 ± 0.0a		n.d.	n.d.	n.d.
86	Decanoic acid	***	3.0 ± 0.1c	0.8 ± 0.1b	0.2 ± 0.0a	***	5.7 ± 0.2c	3.2 ± 0.1b	2.2 ± 0.1a
92	Methyl (9Z)-9-octadecenoate	***	7.0 ± 0.5c	2.9 ± 0.0b	0.9 ± 0.1a	**	0.1 ± 0.0b	0.1 ± 0.0b	0.0 ± 0.0a
93	Benzoic acid	***	27.0 ± 1.5c	12.6 ± 0.3b	3.0 ± 0.5a		n.d.	n.d.	n.d.
94	Methyl (9Z,12Z)-9,12-octadecadienoate	***	23.0 ± 1.5c	13.0 ± 0.4b	2.3 ± 0.2a		n.d.	n.d.	n.d.
101	Palmitic acid	***	3.4 ± 0.2c	1.6 ± 0.1b	0.5 ± 0.1a		n.d.	n.d.	n.d.
Alkanes									
4	Decane	***	0.8 ± 0.0c	0.7 ± 0.0b	0.2 ± 0.0a		n.d.	n.d.	n.d.
7	2-Methyldecane	***	9.5 ± 0.1c	4.5 ± 0.0b	2.0 ± 0.3a		n.d.	n.d.	n.d.
8	5-Ethyl-2,2,3-trimethylheptane	***	6.3 ± 0.1c	3.3 ± 0.0b	1.5 ± 0.2a		n.d.	n.d.	n.d.
9	3,7-Dimethylnonane	***	4.1 ± 0.0c	2.3 ± 0.0b	1.0 ± 0.2a		n.d.	n.d.	n.d.

Table 3 continued

No.	Volatile compound	ANOVA[a]	Raw black rice (ng/g)			ANOVA[a]	Cooked black rice (ng/10 g)		
			Unpolished	Step 1	Step 2		Unpolished	Step 1	Step 2
10	3-Methyldecane	***	7.1 ± 0.4c	3.9 ± 0.0b	1.8 ± 0.3a		n.d.	n.d.	n.d.
11	3,7-Dimethyldecane	***	7.0 ± 0.5c	4.3 ± 0.0b	2.0 ± 0.4a		n.d.	n.d.	n.d.
13	Undecane	**	4.4 ± 0.3b	4.8 ± 0.7b	1.9 ± 0.2a	**	5.1 ± 0.1b	3.5 ± 0.1a	3.8 ± 0.4a
21	Dodecane	***	4.1 ± 0.1c	4.3 ± 0.0b	2.5 ± 0.0a	**	7.9 ± 0.4c	6.3 ± 0.5b	4.2 ± 0.1a
25	Tricyclo[5.2.1.$0^{2,6}$]decane		n.d.	n.d.	n.d.		23.8 ± 1.2	15.6 ± 0.5	10.1 ± 0.8
30	Tridecane	***	1.5 ± 0.1b	1.9 ± 0.0c	1.0 ± 0.0a	*	4.1 ± 0.2c	3.3 ± 0.3b	2.6 ± 0.1a
38	Tetradecane	***	4.6 ± 0.3b	5.7 ± 0.0c	2.8 ± 0.0a	**	10.6 ± 0.1c	8.2 ± 0.4b	6.7 ± 0.6a
55	Hexadecane	***	5.3 ± 0.4c	3.8 ± 0.2b	2.2 ± 0.2a		n.d.	n.d.	n.d.
76	Heneicosane	***	1.1 ± 0.0c	0.5 ± 0.0b	0.2 ± 0.0a		n.d.	n.d.	n.d.
100	1,4,7,10,13,16-Hexaoxacyclooctadecane	***	8.1 ± 0.1b	12.6 ± 0.0c	6.5 ± 0.1a		n.d.	n.d.	n.d.
Olefins									
24	1-Dodecene	***	9.5 ± 0.0c	8.7 ± 0.3b	5.0 ± 0.3a		n.d.	n.d.	n.d.
40	3-Ethyl-2-methyl-1,3-hexadiene		n.d.	n.d.	n.d.	**	3.3 ± 0.3b	2.1 ± 0.1a	1.7 ± 0.1a
43	(*Z*)-4-Tetradecene	***	9.2 ± 0.1c	7.1 ± 0.1b	5.1 ± 0.0a		n.d.	n.d.	n.d.
44	1-Tetradecene	***	1.3 ± 0.0a	2.7 ± 0.2b	1.2 ± 0.1a	**	8.0 ± 0.7c	5.7 ± 0.3b	3.3 ± 0.0a
66^{+}	(4Z,8Z)-Tricyclo[10.2.1.02,11]pentadeca-4,8-diene	***	24.5 ± 1.2a	67.2 ± 4.6b	28.4 ± 4.2a		n.d.	n.d.	n.d.
95^{+}	Decanamide		63.2 ± 1.0c	27.7 ± 2.9b	15.4 ± 1.9a		n.d.	n.d.	n.d.
Additional compounds									
2	Methyl acetate	***	7.1 ± 0.0c	4.0 ± 0.2b	1.9 ± 0.3a		n.d.	n.d.	n.d.
5^{+}	Acetonitrile	*	21.0 ± 0.9c	26.2 ± 2.1b	7.9 ± 1.0a		n.d.	n.d.	n.d.
6^{+}	Chloroform	***	48.2 ± 0.9c	36.8 ± 1.4b	15.9 ± 0.6a		n.d.	n.d.	n.d.
14	Ethylbenzene	***	33.2 ± 0.4c	30.1 ± 2.5b	13.8 ± 0.6a	*	33.6 ± 1.7b	31.0 ± 0.2ab	27.7 ± 1.6a
15	2-Butylfuran	***	3.1 ± 0.0c	1.9 ± 0.2b	0.5 ± 0.1a	***	16.5 ± 0.1c	10.8 ± 0.3b	5.8 ± 0.0a
16	*p*-Xylene	***	24.0 ± 0.3b	23.1 ± 0.6b	5.5 ± 0.3a	*	176.5 ± 8.9c	144.1 ± 12.2b	113.9 ± 3.7a
17	*m*-Xylene	***	20.8 ± 1.0c	25.4 ± 2.2b	6.3 ± 0.3a	**	201.6 ± 5.8c	142.3 ± 7.1b	102.6 ± 8.7a
19	*o*-Xylene	***	54.5 ± 0.2b	50.3 ± 3.7b	12.8 ± 0.5a	**	214.1 ± 3.3c	191.5 ± 3.8b	168.4 ± 8.4a
22	Propylbenzene		n.d.	n.d.	n.d.		1.7 ± 0.0	1.5 ± 0.2	1.3 ± 0.0
23	2-Pentylfuran	***	20.6 ± 0.4c	14.4 ± 1.0b	4.2 ± 0.1a	**	145.0 ± 3.3b	90.6 ± 1.2a	69.5 ± 12.2a
26	Styrene	***	487.2 ± 11.7c	363.1 ± 29.9b	208.0 ± 12.1a	***	90.4 ± 0.2c	76.7 ± 0.2b	70.9 ± 0.5a
33	Isopropenylbenzene		n.d.	n.d.	n.d.	**	6.7 ± 0.4c	4.8 ± 0.3b	3.5 ± 0.0a
41	2-Heptylfuran	***	4.8 ± 0.0c	4.2 ± 0.5b	3.4 ± 0.8a		n.d.	n.d.	n.d.
61	Calamenene	***	35.4 ± 2.5c	23.8 ± 2.1b	8.3 ± 0.2a		n.d.	n.d.	n.d.
65	Guaiacol	***	33.8 ± 2.5c	31.1 ± 0.2b	11.8 ± 0.2a	**	397.4 ± 33.8c	311.2 ± 15.7b	185.3 ± 0.9a
67^{+}	4-Methyl-2,6-bis(2-methyl-2-propanyl)phenol	***	6291.6 ± 190.9c	5872.3 ± 153.9b	2878.5 ± 1.0a	*	19.4 ± 1.0b	15.4 ± 0.4a	15.0 ± 0.8a

Table 3 continued

No.	Volatile compound	ANOVA[a]	Raw black rice (ng/g)			ANOVA[a]	Cooked black rice (ng/10 g)		
			Unpolished	Step 1	Step 2		Unpolished	Step 1	Step 2
69	Diethylene glycol	***	178.1 ± 0.9c	11.7 ± 0.9b	6.0 ± 1.5a		n.d.	n.d.	n.d.
80	2-Sec-butylphenol		n.d.	n.d.	n.d.		t.r.	t.r.	t.r.
81	2-Methoxy-4-vinylphenol		n.d.	n.d.	n.d.	**	42.4 ± 1.7c	32.5 ± 0.8b	26.2 ± 0.3a
87[+]	2,4-Bis(2-methyl-2-propanyl)phenol	***	33.1 ± 0.2b	68.7 ± 0.5c	30.2 ± 1.2a		n.d.	n.d.	n.d.
90	4-Vinylphenol	**	12.5 ± 0.6c	10.4 ± 0.7b	8.1 ± 0.0a	**	920.8 ± 49.4c	572.9 ± 18.5b	447.0 ± 35.7a
91	1H-indole	***	15.3 ± 0.4c	12.1 ± 0.4b	4.0 ± 0.0a	**	500.7 ± 25.2c	402.4 ± 34.2b	212.8 ± 6.9a
97[+]	Dibutyl phthalate	**	2349.2 ± 25.6c	1790.7 ± 201.7b	1087.6 ± 54.4a		n.d.	n.d.	n.d.

n.d. stands for not detected; [+], putative contaminants

[a]Different letters within the same column for raw or cooked black rice denote significant difference at $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$, using Duncan's multiple test ($p < 0.05$)

2-methoxy-4-vinylphenol), 2 benzenes (benzaldehyde and *p*-xylene), 2 furans (2-butylfuran and 2-pentyl furan), 1 terpene (calamenene), and 1 nitrogen-containing compound (1H-indole) (Fig. 2D, E) [2]. The relative concentration of odor-active compounds in cooked black rice samples significantly decreased as milling degree increased ($p < 0.05$). By milling unpolished black rice (step 0) to polished black rice (step 2), the relative concentration of guaiacol (smoky, black rice-like odor) decreased by ~ 50% from 397.4 ng/10 g (step 0) to 185.3 ng/10 g (step 2) (Table 2). However, partially polished rice (step 1) maintained guaiacol levels (311.2 ng/10 g) of 78% in unpolished rice (step 0). As an off-flavor producing volatile, 1H-Indole is responsible for mothball and fecal odors. In step 2, the relative concentration of 1H-indole decreased by 42%, compared to that in step 0.

Among the 5 lipid oxidation products (i.e., hexanal, octanal, 2-nonenal, 1-pentanol, and 2-pentylfuran) found in raw black rice, hexanal, octanal, 2-nonenal, and 2-pentylfuran were found in cooked black rice, and 1-pentanol was not detected in cooked black rice (Fig. 2F). As the milling degree increased, the sum of the relative concentrations of hexanal, octanal, 2-nonenal, and 2-pentylfuran decreased to 65% (step 1) and 49% (step 2) of step 0. Hexanal concentrations of step 0 cooked black rice were significantly higher than those of polished rice (steps 1–2) ($p < 0.05$). In contrast, step 1 cooked rice showed no significant difference from step 2 ($p > 0.05$). The relative concentrations of octanal decreased significantly (step 0 > step 1 > step 2) as milling degrees increased ($p < 0.05$). The relative concentrations of 2-nonenal and 2-pentylfuran in step 0 were significantly higher than those of steps 1–2; however, those of step 1 and step 2 showed no significant difference ($p > 0.05$). Thus, by partially milling (step 1), 2-nonenal and 2-pentylfuran concentrations decreased significantly as did the concentrations of these compounds in fully milled rice (step 2).

In summary, various volatile compounds differed significantly among black rice samples. As milling degrees increased, unique aromatic compounds (e.g., guaiacol and vanillin) of black rice as well as off-flavor (e.g., lipid oxidation products) compound levels decreased in raw and cooked black rice. Partially milled rice (step 1) maintained guaiacol levels of unpolished rice (step 0) by up to 90 and 80% for raw and cooked black rice, respectively. The relative concentrations of several lipid oxidation products such as 2-nonenal and 2-pentylfuran were not significantly different in partially milled black rice (step 1) and fully milled black rice (step 2) ($p > 0.05$). Thus, partially milled rice can be used to retain favorable unique volatile compounds in black rice with low lipid oxidation levels.

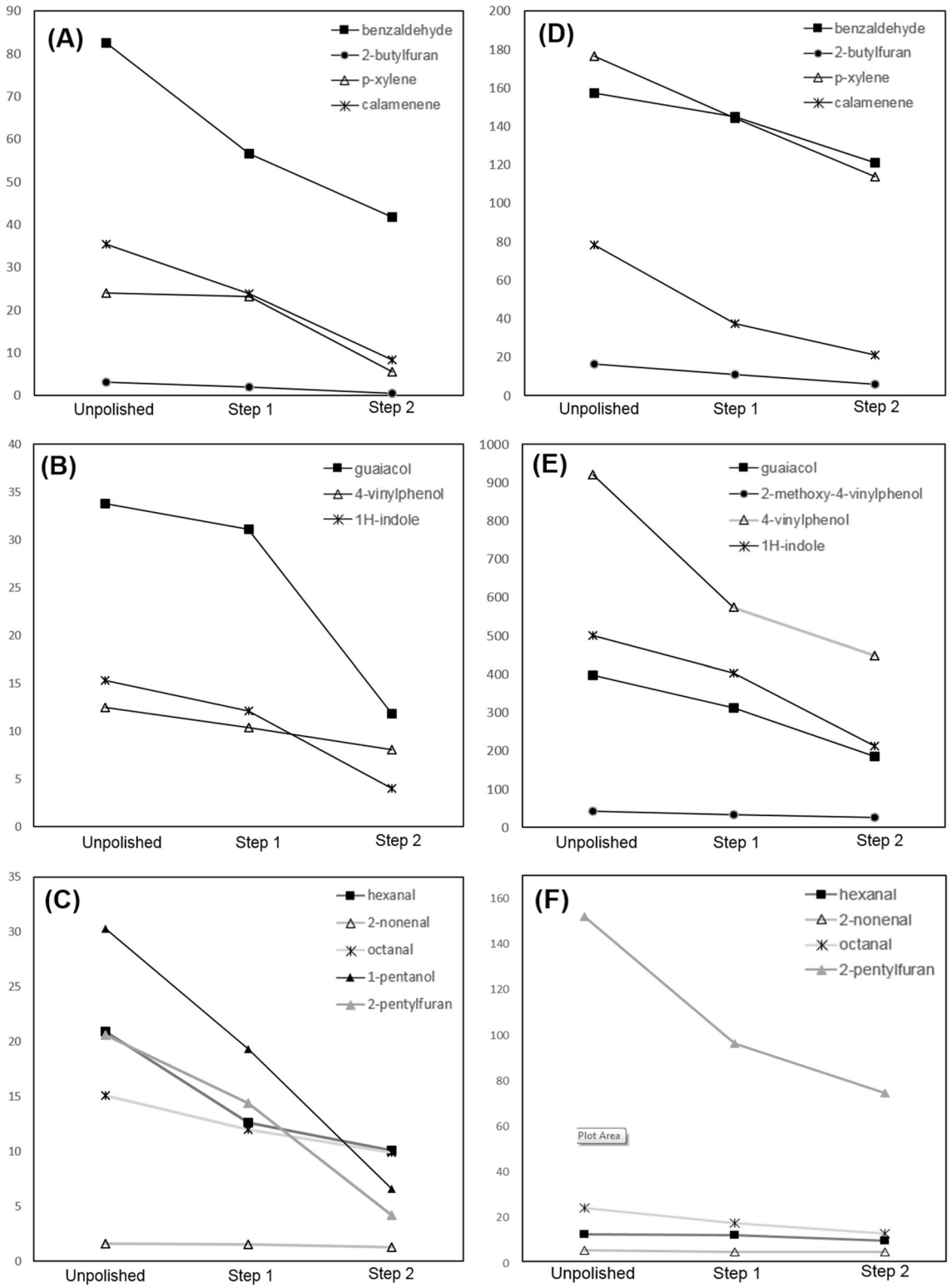

Fig. 2 Change in relative concentrations of volatile compounds in varying milling degrees of black rice: (**A**, **B**) aromatic compounds in raw black rice, (**C**) lipid oxidation products in raw black rice, (**D**, **E**) aromatic compounds in cooked black rice, and (**F**) lipid oxidation products in cooked black rice. Error bars are standard deviations ($n = 2$)

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References

1. Hu C, Zawistowski J, Ling W, Kitts DD (2003) Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J Agric Food Chem 51:5271–5277
2. Yang DS, Lee KS, Jeong OY, Kim KJ, Kays SJ (2007) Characterization of volatile aroma compounds in cooked black rice. J Agric Food Chem 56:235–240
3. Schranz M, Lorber K, Klos K, Kerschbaumer J, Buettner A (2017) Influence of the chemical structure on the odor qualities and odor thresholds of guaiacol-derived odorants, part 1: alkylated, alkenylated and methoxylated derivatives. Food Chem 232:808–819
4. Shitanda D, Nishiyama Y, Koide S (2001) Performance analysis of an impeller husker considering the physical and mechanical properties of paddy rice. J Agric Eng Res 79:195–203
5. Arthur CL, Pawliszyn J (1990) Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 62:2145–2148
6. Bryant R, McClung A (2011) Volatile profiles of aromatic and non-aromatic rice cultivars using SPME/GC–MS. Food Chem 124:501–513
7. Grimm CC, Bergman C, Delgado JT, Bryant R (2001) Screening for 2-acetyl-1-pyrroline in the headspace of rice using SPME/GC–MS. J Agric Food Chem 49:245–249
8. Laguerre M, Mestres C, Davrieux F, Ringuet J, Boulanger R (2007) Rapid discrimination of scented rice by solid-phase microextraction, mass spectrometry, and multivariate analysis used as a mass sensor. J Agric Food Chem 55:1077–1083
9. Ceva-Antunes PMN, Bizzo HR, Silva AS, Carvalho C, Antunes O (2006) Analysis of volatile composition of siriguela (*Spondias purpurea* L.) by solid phase microextraction (SPME). LWT Food Sci Technol 39:437–443
10. Samyor D, Deka SC, Das AB (2016) Evaluation of physical, thermal, pasting characteristics and mineral profile of pigmented and nonpigmented rice cultivars. J Food Process Preserv 40:174–182
11. Bhat FM, Riar CS (2017) Physicochemical, cooking, and textural characteristics of grains of different rice (*Oryza sativa* L.) cultivars of temperate region of India and their interrelationships. J Texture Stud 48:160–170
12. Hiemori M, Koh E, Mitchell AE (2009) Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. japonica var. SBR). J Agric Food Chem 57:1908–1914
13. Kim HY, Hwang SH, Lee JH (2017) Effect of fermented vinegar on the reduction in trimethylamine in konjac glucomannan gel. Appl Biol Chem 60:281–285
14. Kim MS, Baek SH, Park SU, Im KH, Kim JK (2017) Targeted metabolite profiling to evaluate unintended metabolic changes of genetic modification in resveratrol-enriched rice (*Oryza sativa* L.) Appl. Biol Chem 60:205–214
15. Lee J, Xiao L, Zhang G, Ebeler SE, Mitchell AE (2014) Influence of storage on volatile profiles in roasted almonds (*Prunus dulcis*). J Agric Food Chem 62:11236–11245
16. Baek H, Cadwallader K (1996) Volatile compounds in flavor concentrates produced from crayfish-processing byproducts with and without protease treatment. J Agric Food Chem 44:3262–3267
17. Lamberts L, De Bie E, Vandeputte GE, Veraverbeke WS, Derycke V, De Man W, Delcour JA (2007) Effect of milling on colour and nutritional properties of rice. Food Chem 100:1496–1503
18. Alizadeh R, Najafi NM, Kharrazi S (2011) A new solid phase micro extraction for simultaneous head space extraction of ultra traces of polar and non-polar compounds. Anal Chim Acta 689:117–121
19. Buttery RG, Turnbaugh JG, Ling LC (1988) Contribution of volatiles to rice aroma. J Agric Food Chem 36:1006–1009
20. Guadagni DG, Buttery RG, Okano S (1963) Odour thresholds of some organic compounds associated with food flavours. J Sci Food Agric 14:761–765
21. Siebenmorgen T, Sun H (1994) Relationship between milled rice surface fat concentration and degree of milling as measured with a commercial milling meter. Cereal Chem 71:327–329
22. Signes A, Mitra K, Burlo F, Carbonell-Barrachina AA (2008) Contribution of water and cooked rice to an estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. Food Addit Contam 25:41–50
23. Varlet V, Knockaert C, Prost C, Serot T (2006) Comparison of odor-active volatile compounds of fresh and smoked salmon. J Agric Food Chem 54:3391–3401
24. Monsoor M, Proctor A (2004) Volatile component analysis of commercially milled head and broken rice. J Food Sci 69:632–636
25. Ullrich F, Grosch W (1987) Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. Z Lebensm Unters Forsch 184:277–282