Effects of milling on aromatics, lipophilic phytonutrients, and fatty acids in unprocessed white rice of scented rice ‘Cheonjihyang-1-se’

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Received: 28 August 2017 / Revised: 27 October 2017 / Accepted: 10 November 2017 / Published online: 12 December 2017
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Abstract To understand effects of milling, scented rice ‘Cheonjihyang-1-se’ was milled from 10 to 140 s and changes in volatiles, phytonutrients, and fatty acids were evaluated. Among 43 identified odor-active compounds, four volatiles, including hexan-3-one, exhibited decreases of up to 78%, while four others including (*E*)-non-2-enal, increased following milling. Levels of 2-acetyl-1-pyrroline, the most distinctive popcorn-flavoring compound in scented rice, were not affected by the degree of milling (DM). Partial least squares discriminant analyses of volatiles were able to differentiate white rice according to the DM. Benzene and 2-pentylfuran showed the highest variable importance in projection scores, which could be applied in estimating the DM of rice. Milling significantly decreased tocopherols, tocotrienols, squalene, phytosterols contents and oleic acid composition, while palmitic acid composition was increased. These results suggest milling-dependent variations in phytonutrient levels and lipid composition, as well as changes in aroma and subsequent market quality, in ‘Cheonjihyang-1-se’ rice.

Keywords 2-acetyl-1-pyrroline · Flavor · Headspace · Solid phase microextraction · Volatile

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop, supporting more than half of the world’s population as a staple food, especially in Asian countries. Although high-yield rice varieties have been a major objective of farmers and breeders, recent customers’ preferences have shifted toward some of the qualitative traits of rice. Different cultures have different definitions of, and preferences in, rice quality. For example, Korean, Chinese, and Japanese customers prefer sticky rice with low amylose and protein contents, while aroma, grain size and shape, and non-sticky properties are important factors determining the quality of rice in countries with a prevalent Indian culture.

A typical means of enhancing the quality of white rice is milling, which removes the fiber-rich bran that is responsible for the low palatability of brown rice. Milling is a physical process that cuts off the exterior bran and embryo of brown rice and can cause physical damage resulting in structural changes, followed by changes in the physiochemical properties and perceived quality of white rice [1, 2].

Nutritional value has also become important to customers. Unfortunately, the rice bran that is removed by milling is a rich source of phytochemicals compared to the starch-rich endosperm. In addition to minerals and dietary fiber, major phytonutrients such as oryzanols, tocopherols, tocotrienols, squalene, phytosterols, phytic acid, and polyosanols are relatively concentrated in rice bran. The antioxidative, anticancer, hypolipidomic, blood glucose- and cholesterol-lowering effects of rice bran are well documented [3, 4]. The typical commercial degree of milling (DM) in Korea, termed ‘10% milling’, is equivalent to an 8% weight loss in brown rice. In some cases a higher DM is applied. For example, ‘12% milling’, refers to an

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equivalent 10% weight loss consisting of 2–3% germ and 5–8% bran. Milling processes may produce broken kernels and additional weight loss, which are important economic factors in the rice industry. Therefore, the DM needs to be carefully monitored and controlled [5].

The aroma or scent of rice also plays an important role in determining the rice quality, especially in South East Asian countries [6, 7]. Aromatic or scented rice varieties, such as Basmati and Jasmine rice, have unique flavor properties and are sold at higher prices in both local and global markets [8]. The flavor of scented rice is determined by a complex combination of volatile compounds, and more than 300 volatiles have been reported in unprocessed and cooked rice [6, 9, 10]. Among these, 2-acetyl-1-pyrroline (2AP) yields a popcorn-like aroma and is considered to be the most important aromatic compound in rice [11, 12]. Due to its importance in characterizing the flavor of scented rice, genes related to 2AP [13], the evolution [14] and biosynthetic pathway of 2AP production [15], and the effects of environmental conditions and cultivation practices on 2AP content [16] have been intensively studied. Additional odor-active compounds, including hexan-1-ol, benzaldehyde, hexanal, 2-pentylfuran, and octanal, have relatively low detectable odor thresholds and consequently high impacts on the overall flavor of rice [9].

Traditionally, in Korea, rice aroma has not been an important qualitative trait. This may be due to the lack of a gene source or model variety with aromatic properties in Korean landraces. Recently, however, customers' demands for scented rice have increased, stimulating the production of new scented rice varieties such as 'Hyangmibyeo-1-ho', 'Hyangmibyeo-2-ho', 'Hyangnambeo', 'Mihyangbyeo', 'Arangchalbyeo', 'Seolhyangchalbyeo', and 'Heukhyangbyeo' [17]. One of great concerns in these scented rice varieties is possible loss of aromatic property caused by milling process.

Although many reports describe the effects of milling on eating quality, physiochemical properties, and nutritional value of rice [18], only limited information is available on the spatial distribution of flavoring compounds in the bran, embryo, and endosperm. This study was conducted to understand milling-dependent flavor and phytonutrient-related properties of 'Cheonjihyang-1-se', a scented rice variety released in 2016.

Materials and methods

Plant materials and sample preparation

The rice variety 'Cheonjihyang-1-se' was cultivated in a paddy field in Suwon, Korea according to standard rice cultivation practices. Harvested rice samples, adjusted to a

16% water content, were dehusked into brown rice using a rice huller (BH-06, SY21 THC, Incheon, Korea), double-packed in Ziploc freezer bags, and stored at -50°C . Prior to analysis, frozen samples were thawed overnight to room temperature and abnormal rice grains were removed for sample uniformity. To obtain white rice at different DMs, 100-g samples of brown rice were milled for 10, 20, 40, 60, 80, 100, 120, or 140 s using a milling machine (TM-05, Satake, Japan). These DMs correspond to weight losses of 1.33, 2.49, 4.43, 5.69, 6.87, 7.99, 9.40, and 11.0%, respectively. Milled white rice samples were passed through a 1.4-mm sieve, to remove bran and broken kernels prior to analysis. Intact grains were used for analyses of volatile compounds, while grains used for phytonutrient analyses were pulverized into fine powders using a micro hammer mill (Culatti AG, Zürich Switzerland) immediately prior to extraction.

Analysis of volatile compounds by HS-SPME-GC/MS

For volatile compound analyses, 2.0 g of rice sample was placed in a 20-mL headspace (HS) vial and spiked with 100 μL of ultra-pure water containing 100 ng of 2-acetylpyrrole as an internal standard. The vial was then tightly sealed with a Teflon-lined magnetic headspace cap. Volatile compounds were released by pre-heating the sample at 80°C for 40 min and adsorbed onto a solid phase microextraction (SPME) fiber (divinylbenzene/carboxen/polydimethylsiloxane stableflex fiber, Supleco, Bellefonte, PA, USA) for 25 min at 80°C . Volatiles were desorbed by injecting the SPME fiber into the inlet port of a GC/MS (QP2010 Ultra, Shimadzu, Japan) for 10 min. After each injection, the SPME fiber was placed in an automatic thermal cleaning chamber for 5 min at 260°C prior to starting the next sample analysis. Volatile compounds were separated using an Rxi-5sil MS capillary column (30 m $\times$ 0.25 mm ID, 0.50 μm thickness, Restek, USA). The GC oven temperature was initially held at 40°C for 5 min, then increased to 160°C at $2.5^{\circ}\text{C}/\text{min}$, followed by an increase to 270°C at $20^{\circ}\text{C}/\text{min}$ and held for 5 min. The carrier gas consisted of helium (99.999%) at a constant flow rate of 1.04 mL/min in splitless mode. The inlet port was maintained at 250°C . The total GC cycle time consisted of a 63.5-min run and a 10-min re-stabilization step. The mass spectrometer (MS) conditions were as follows: ion source 200°C , interface temperature 270°C , electron energy 70 eV, solvent cut time 3 min, scan range 35–650 m/z. The consistent performance of the SPME fiber was evaluated by monitoring the peak area of the internal standard. The relative standard deviation (RSD) of the internal standards' peak area throughout the whole experiment was 7.2%.

Identification of volatile compounds

Volatile compounds were identified by comparing their mass spectra and relative abundances (similarity > 75%) with NIST08 (Shimadzu, Japan), and FFNSC 2 (Flavor and Fragrance Natural and Synthetic Compounds, ver 2.0, Shimadzu, Japan) spectral libraries. In addition to mass spectral similarities, retention indices (RI) were obtained under our experimental conditions by injecting a series of *n*-hydrocarbons (C₈–C₂₀), and were compared against those of the aforementioned spectral libraries (acceptance range: $-5 < \text{RI} < +5$). Additional confirmation of the presence of a particular compound in rice was performed by comparing the properties of a compound identified in our rice sample(s) with those given in previous reports and with the 335 rice-originated volatile compounds listed in the Volatile Compounds in Food (VCF) online library (<http://www.vcf-online.nl/VcfHome.cfm>) [19]. Among the 98 volatile compounds identified (data not shown), odor-inactive compounds that cannot be sensed by the human nasal system and, consequently, cannot contribute to the aromatic properties of rice, were excluded from this study.

Analysis of tocots, squalene, and phytosterols

Lipophilic phytonutrients such as tocopherols, tocotrienols, squalene, campesterol, stigmasterol, and sitosterol were analyzed in accordance with Bhandari et al. [20] with slight modifications. Rice powder (2.0 g) was placed in a 50-mL tube. After adding ascorbic acid (0.1 g) and ethanol (10 mL), phytonutrients were extracted in a water bath (80 °C) for 18 min. After adding 300 µL of saturated KOH, each sample was saponified for 18 min at 80 °C. After immediately cooling the tube in an ice bucket, 10 mL of distilled water and 10 mL of hexane were added and thoroughly mixed by vortexing and the hexane layer was collected. After repeating this process twice more for each sample, the combined hexane layers were washed three times with distilled water and dried by passing through anhydrous Na₂SO₄. The hexane was evaporated and the residue was resuspended in 1 mL of isooctane and injected into the GC/MS (QP2010 Ultra, Shimadzu, Japan). A BR-5 capillary column (25 m × 0.25 mm) was used for separation with a 1.2-mL/min flow rate of carrier gas (He, 99.99%) at a split ratio of 1:5. The oven temperature started at 240 °C for 1 min and was then increased to 290 °C at 3 °C/min and held for 15 min. The temperature of the ion source and interface were 200 and 280 °C, respectively. Quantification of each compound was performed by comparing the area of characteristic peaks against those of authentic standards (Sigma, USA). The following *m/z* values were used for compound quantification: squalene 69, δ -tocopherol 402, β - and γ -tocopherol 416, δ -

tocotrienol 346, α -tocopherol 430, γ -tocotrienol 410, campesterol 400, α -tocotrienol 424, stigmasterol 412, and β -sitosterol 414.

Analysis of fatty acid composition

A one-step extraction/methylation method [21] was applied with slight modifications for fatty acid analyses. Each powdered rice sample (0.2 g) was placed into a 1.5-mL amber vial. After adding 680 µL of reaction solution (MeOH:benzene:2,2-dimethoxypropane:H₂SO₄ = 39:20:5:2) and 400 µL of heptane, the vial was vortexed and kept at 80 °C for 2 h for extraction and methylation. After cooling the vial to room temperature, the heptane layer was collected, centrifuged at 12,000 rpm for 5 min, and transferred into another vial for injection into the GC (GC-2010 Plus, Shimadzu, Japan). Fatty acid methyl esters (FAME) were separated through a CP-SIL 88 CB FAME column (100 m × 0.25 mm) using the following temperature program: start at 100 °C and hold for 5 min, increase to 180 °C at 4 °C/min and hold for 5 min, increase to 210 °C at 5 °C/min. The injector and FID detector temperatures were 210 °C, and the helium carrier (99.99% purity) maintained a flow rate of 1.2 mL/min with a split ratio of 1:20. For identification, the retention time of each FAME peak was compared against those of 37 authentic FAME standards (Supelco, USA), and the relative composition of each fatty acid was expressed as a percentage of the total FAME peak area.

Statistical analysis

All measurements for volatile compounds, phytonutrients, and fatty acids were performed in 3 independent replications. For quantitative studies on volatiles, the peak area of each volatile compound in the total ion chromatogram (TIC) was normalized to the peak area of the internal standard in each sample. This relative amount was used in the following statistical analyses. For general statistics, the SPSS (ver. 22) software package [22] was used. MetaboAnalysis 3.0 (<http://www.metaboanalyst.ca/> [23]) was used for multivariate analyses, such as partial least squares discriminant analyses (PLS-DA), after mean-centered scaling of the data and dividing by the standard deviation of each volatile compound.

Results and discussion

Changes in the levels of odor-active compounds

The degree of milling is known to be related to the sensory properties of white rice [24]. A total of 43 odor-active

compounds were identified from unprocessed ‘Cheonjiyang-1-se’ rice (Table 1), consisting of 12 aldehydes, 12 alcohols, 1 base, 1 furan, 10 hydrocarbons, and 7 ketones. Nonanal, tetradecane, 2-acetyl-1-pyrroline, dodecane, and octan-1-ol were major components with average compositions of 19.8, 6.7, 5.8, 5.7, and 5.5%, respectively, of the total peak area of all compounds. The average peak area composition of other important odor-active compounds such as hexan-1-ol, benzaldehyde, hexanal, 2-pentylfuran, octanal were 3.4, 1.3, 3.6, 1.6, and 2.4%, respectively.

Out of the 43 compounds identified, eight odor-active compounds exhibited statistically significant ($p < 0.05$) changes in their relative amounts at different DMs (Table 1). The levels of hexan-3-one, benzene, 2-pentylfuran, and pentanal decreased continuously in proportion to increasing DM. For example, the amount of pentanal extracted after 10 s of milling decreased by 21, 47, and 78% after 40, 80, and 140 s of milling, respectively. The amounts of hexan-3-one (79%), benzene (70%), and 2-pentylfuran (54%) also decreased significantly between 10 and 140 s of milling. In contrast, other compounds increased with the DM. The most distinctive case was (*E*)-non-2-enal, which exhibited an increase of 252% between 10 and 140 s of milling. The sensory threshold of (*E*)-non-2-enal is reportedly as low as 0.08 ppb [9]. Therefore, even slight changes in its concentration may contribute to detectable changes in the odor properties of milled rice. Pentadecanal (185%), (*5E*)-6,10-dimethylundeca-5,9-dien-2-one (172%), and menthol (159%) also showed significant increases between 10 and 140 s of milling. Most of the remaining odor-active compounds, including 2-acetyl-1-pyrroline, (*E*)-2-decanal, octanal, and 1-octen-3-ol [9], showed no significant changes as a function of DM.

In scented rice, the popcorn-flavoring 2-acetyl-1-pyrroline (2AP) is considered to be the most important volatile compound characterizing aromatic properties. Thus, any milling-induced losses of 2AP will result in significant losses in market value. Under our experimental conditions, however, no significant differences in 2AP levels were observed as a function of DM (Fig. 1). These findings suggest that higher degrees of milling, to enhance eating quality, will not diminish the aromatic properties of ‘Cheonjiyang-1-se’ rice. These results are consistent with those of Grim et al. [25], who reported consistent 2AP levels across different milling fractions in 21 rice varieties. Contradictory reports, however, describe decreasing 2AP levels at high DM [26], which may be due, in part, to differences in rice variety.

Although no statistically significant differences among DM were observed, various compounds exhibited increasing or decreasing tendency as a response to milling, and consequently the DM also affected the relative contributions of chemical groups into which the individual

identified compounds are classified. Higher DMs resulted in an increase in aldehydes from 35 to 45% between 10 and 140 s of milling and a decrease in hydrocarbon content from 29 to 20%. These changes were more prominent between 100 and 120 s of milling, corresponding to 8.0 and 9.6% weight loss, which is the level of DM most popular in Korea. Levels of ketones and furans also decreased with extended milling. In contrast, alcohols maintained 21 to 24% regardless of the DM.

To optimize the differentiation of rice as a function of DM, a partial least squares discriminant analysis (PLS-DA) was applied to each identified odor-active compound and approximately 64% of the observed variations could be explained [Fig. 2(A)]. The overall classification of rice at different DMs was acceptable, although rice samples with close DMs could not be fully separated. Rice samples with DMs between 10 and 40 s exhibited similar odor-active compound profiles, resulting in poor classification by PLS-DA [Fig. 2(A)]. Similarly, rice samples with DMs between 80 and 100 s, or between 120 and 140 s, exhibited similar odor-active compound profiles. These results suggested possible 3 groups of white rice with distinctive volatile profiles corresponding to DM; low (10–40 s), medium (60–100 s), and high (120–140 s) degree of milling. Further interactive studies on cellular, physiochemical, and aromatic properties across the rice grain may elucidate customers’ palatability preference.

Variable importance in projection (VIP) values acquired by PLS-DA suggested benzene, 2-pentylfuran, and pentanal as characteristic compounds in low-DM rice, while (*E*)-non-2-enal, hexanal, and pentadecanal were indicative of high-DM rice [Fig. 2(B)]. Since variables with VIP scores higher than 1.0 have statistical meaning for group classifications, all of the compounds listed in Fig. 2(B) can be considered indicators for estimating the DM of ‘Cheonjiyang-1-se’ rice. These results suggest that profiles of volatile compounds, especially odor-active compounds, can be used as tools for discriminating rice as a function of DM. However, considering the complex interactions among aromatic compounds in determining the final flavor characteristics of rice [7, 27], and the distinctive differences in the sensory thresholds of each compound [9, 28, 29], it should be noted that further studies are required to assess not only quantitative changes in the levels of single compounds but also the interactive effects of multiple volatile compounds in a single sample. These studies would further our understanding of the effects of milling on the aromatic properties and subsequent market value of scented rice.

Table 1 Relative amount^a of odor-active compounds in unprocessed white rice of the scented 'Cheonjhyang-1-se' variety as a function of milling degree

Chemical name	Duration of milling (s) ^b							Odor description	RI ^c	m/z	Mw (g/mol)	CAS No.	
	10	20	40	60	80	100	120						140
Aldehydes													
(E)-Dec-2-enal	1832	1465	1866	2112	1974	2028	2219	2448	Fat, fish, hay, paint, tallow	1258	43,41,55	154	3913-81-3
(E)-Hept-2-enal	688	873	857	961	732	874	929	1143	Mushroom, soap	953	55,83,41	112	18,829-55-5
(E)-Non-2-enal [*]	729	544	511	1341	1187	1323	1803	2564	Cucumber, cut grass, fat	1156	55,83,41	140	18,829-56-6
Benzaldehyde	1954	1265	1354	1193	1691	1209	1781	2569	Bitter almond, burnt sugar	956	77,106	106	100-52-7
2-Phenylacetaldehyde	607	1806	1334	1224	720	667	680	761	Berry, geranium, honey	1038	91,120	120	122-78-1
Decanal	6046	5658	4523	5469	5456	5087	6144	6865	Foral, fried, orange peel	1202	57,41,82	156	112-31-2
Heptanal	518	647	924	757	883	644	745	914	Citrus, dry fish, fat, green	898	44,70	114	111-71-7
Hexanal	3188	3760	4039	4200	4098	4603	4725	7335	Fresh, fruit, grass, green	797	44,100	100	66-25-1
Nonanal	22,244	22,392	20,890	26,277	21,381	21,035	26,186	36,682	Citrus, fat, floral, green	1101	57,70,98	142	124-19-6
Octanal	3178	3363	3079	3222	2629	2642	2801	3076	Citrus, fat, floral, fruit, oil	999	43,57	128	124-13-0
Pentadecanal [*]	636	441	502	489	916	996	648	1177	Fresh	1709	57,73,82	226	2765-11-9
Pentanal [*]	139	139	109	81	74	67	33	31	Almond, bitter, malt, oil	703	44,86	86	110-62-3
Alcohols													
3-Methylbutan-1-ol	66	65	70	60	61	57	57	62	Fish oil, fusel oil, green	686	57,88	88	123-51-3
Decan-1-ol	1360	1322	1329	1528	1574	1411	1427	1843	Fat, oil, plastic	1268	57,43	158	112-30-1
2-Methyldecan-1-ol	2359	2991	2518	2983	3110	3035	3490	3606	Fat, wax	1298	43,57,69	172	18,675-24-6
Heptan-1-ol	1095	1019	973	974	635	839	831	1129	Chemical, green, putrid	967	70,56	116	111-70-6
Hexan-1-ol	4069	4447	4441	4019	3598	3954	3928	4728	Citrus, green, oil, rose	1025	57,70,98	130	111-27-3
Nonan-1-ol	2840	3093	2513	3041	2235	2163	2897	4276	Fat, floral, green, oil	1168	56,43	144	143-08-8
Octan-1-ol	6888	7073	7268	7494	6062	5802	6463	7068	Bitter almond, burnt matches	1067	56,69,70	130	111-87-5
2-Butyloctan-1-ol	2043	2026	1770	1760	2352	2170	2782	2704	Floral, pleasant	1407	57,69,70	186	3913-02-8
1-Octen-3-ol	1183	1078	1318	1385	1212	1296	1724	2073	Cucumber, earth, fat, floral	977	57,72	128	3391-86-4
Pentan-1-ol	463	512	561	578	546	661	638	755	Balsamic, fruit, green	771	42,70	88	71-41-0
(2E)-2-Octen-1-ol	2804	4894	5139	5876	4193	4683	3731	3608	Plastic, soap	1064	57,41,43	128	18,409-17-1
Menthol [*]	290	277	394	257	316	557	558	461	Lemon, mint	1175	71,81	156	89-78-1
Base													
2-Acetyl-1-pyrroline	5955	6143	7096	7569	6680	6994	7484	8876	Nut, oil, popcorn, roast	916	43,83,111	111	85,213-22-5
Furan													
2-Pentylfuran [*]	2627	2466	2367	2358	1697	1957	1230	1243	Butter, floral, green bean	987	81	138	3777-69-3
Hydrocarbons													
Pentadec-1-ene	817	762	908	794	654	779	772	592	Plastic	1488	41,57	210	13,360-61-7
Benzene [*]	7773	7429	6040	4951	2841	3738	2006	2347	Solvent	706	78	78	71-43-2

Table 1 continued

Chemical name	Duration of milling (s) ^b								Odor description	RI ^c	m/z	Mw (g/mol)	CAS No.
	10	20	40	60	80	100	120	140					
4-Isopropyltoluene	4557	4235	5157	4598	4003	4165	3656	3496	Citrus, fresh, gasoline	1022	119, 134	134	99-87-6
Dodecane	6365	6896	7216	7352	6545	7014	7121	6886	Alkane, undesirable	1198	57,43	170	112-40-3
Ethylbenzene	638	709	690	507	421	392	365	372	Strong	856	91,106	106	100-41-4
Hexadecane	1842	2063	1276	2273	2072	1445	1583	2193	Alkane, root	1597	57,71	226	544-76-3
D-Limonene	2901	2426	2982	2669	2476	2518	2092	2364	Citrus, mint	1027	68,93	136	5989-27-5
Pentadecane	1066	747	769	744	871	685	633	1045	Alkane	1496	57,71	212	629-62-9
Tetradecane	8124	7698	6440	7626	8283	7566	9731	10,314	Alkane, hydrocarbon	1397	57,71	198	629-59-4
Toluene	711	883	533	573	498	478	479	448	Glue, paint, solvent	770	91,92	92	108-88-3
<i>Ketones</i>													
Heptan-2-one	182	263	280	216	220	230	200	300	Bell pepper, blue cheese	885	43,58,114	114	110-43-0
Nonan-2-one	228	265	306	266	174	227	170	134	Fragrant, fruit, green, soap	1042	43,58	142	821-55-6
Octan-2-one	205	181	201	252	223	187	150	180	Fat, fragrant, gasoline	950	43,128	128	111-13-7
6,10,14-Trimethylpentadecan-2-one	1096	1085	809	1030	729	711	848	922	Fat	1838	43,58,71	268	502-69-2
Hexan-3-one [*]	3244	4194	3796	2236	1058	1572	608	687	Ether, grape	725	43,57,71	100	589-38-8
6-Methylhept-5-en-2-one	1862	2243	1360	1637	1165	865	1450	1456	Citrus, mushroom, pepper	981	43,108,126	126	110-93-0
(5E)-6,10-Dimethylundeca-5,9-dien-2-one [*]	3380	3243	2132	3211	3338	2605	4277	5823	Green, hay, magnolia	1442	43,69	194	3796-70-1

^{*} Compounds which showed significant ($p < 0.05$) difference among degree of millings by analysis of variance; see details in text

^a Peak area of each compound normalized to the peak area of internal standard in each sample; mean value of 3 independent measurements

^b Weight loss of brown rice at each duration of milling was as follows: 1.3% (10 s), 2.5% (20 s), 4.4% (40 s), 5.7% (60 s), 6.9% (80 s), 8.0% (100 s), 9.4% (120 s), and 11.0% (140 s)

^c Retention indices obtained under our experimental conditions by using n-hydrocarbons (C8 to C20)

Changes in squalene, tocals, and phytosterols content

Several reports have detailed the higher phytonutrient content of rice bran compared to white rice. High degrees of milling may decrease the nutritional value of white rice [3]. In the current study, levels of all of the evaluated

Fig. 1 Changes in the relative amount of 2-acetyl-1-pyrroline in unprocessed white rice of the scented 'Cheonjiyang-1-se' variety as a function of milling degree. Vertical bars represent mean $\pm$ standard deviation of 3 independent measurements. Mean values of the same letters on top of each bar are not significantly different at $p < 0.05$ by Duncan's multiple range test. Weight loss of brown rice at each duration of milling was as follows: 1.3% (10 s), 2.5% (20 s), 4.4% (40 s), 5.7% (60 s), 6.9% (80 s), 8.0% (100 s), 9.4% (120 s), and 11.0% (140 s)

Fig. 2 Partial least squares discriminant analysis (PLS-DA) score plot (A) and variable importance in projection (VIP) plot (B) of odor-active compounds in white rice of the scented 'Cheonjiyang-1-se' variety as a function of milling degree. Weight loss of brown rice at

phytonutrients, including tocals, squalene, and phytosterols, decreased significantly as a function of increasing milling time (Table 2). The total content of vitamin E, consisting α -, β -, γ -tocopherols and α -, γ -tocotrienols, decreased from 16.31 $\mu\text{g}/100\text{ g}$ at 10 s of DM to 5.72 $\mu\text{g}/100\text{ g}$ after 140 s of milling, representing a 70% reduction. All five tocol isomers exhibited similar patterns, although the tocopherols showed slightly greater reductions compared to tocotrienols. Squalene content decreased continuously by 13, 37, 54, and 65% between 10 s and 20, 60, 100, and 140 s of milling, respectively. All of the tested phytosterols experienced decreasing levels as a function of increasing DM. The total phytosterol content of 179 $\mu\text{g}/100\text{ g}$ at 10 s DM decreased to 71.22 $\mu\text{g}/100\text{ g}$ after 140 s of milling. Among the phytosterols, campesterol and stigmasterol were affected less than sitosterol. The observed decreases in phytonutrient content with increasing DM are as expected. The phytonutrients evaluated herein are lipophilic and the rice bran that is removed by milling contains a higher lipid content than white rice [18, 30]. Considering the degree of phytonutrient loss as a function of increasing milling time, the milling of scented white rice needs to be carefully optimized to preserve its aromatic properties and nutritional value.

Changes in fatty acid composition

Regardless of DM, the major fatty acids in white 'Cheonjiyang-1-se' rice were linoleic, oleic, and palmitic acids, representing 38, 30, and 27% of all 10 fatty acids,

each duration of milling was as follows: 1.3% (10 s), 2.5% (20 s), 4.4% (40 s), 5.7% (60 s), 6.9% (80 s), 8.0% (100 s), 9.4% (120 s), and 11.0% (140 s)

Table 2 Changes in the levels (in $\mu\text{g}/100\text{ g}$) of tocopherols, tocotrienols, squalene, and phytosterols in unprocessed, scented white rice of the 'Cheonjhyang-l-se' variety as a function of milling degree

Milling time (s) ¹	α -Tocopherol	β -Tocopherol	γ -Tocopherol	α -Tocotrienol	γ -Tocotrienol	Total tocopherol	Total tocotrienol	Total tocols	Squalene	Campesterol	Stigmasterol	β -Sitosterol	Total phytosterols
10	6.66a*	0.38a	0.78a	6.43a	9.88a	7.82a	16.31a	24.13a	29.04a	25.12a	34.75a	119.89a	179.75a
20	5.53a	0.34a	0.65b	5.11b	8.21b	6.52a	13.32b	19.84b	25.30b	22.97a	31.55a	107.46a	161.99a
40	4.07b	0.19bc	0.48c	3.88c	5.90cd	4.74b	9.77c	14.51c	16.89c	16.59b	21.22bc	66.41bc	104.22bc
60	3.90bc	0.20bc	0.45c	3.60cd	6.12d	4.55b	9.72c	14.27cd	18.38c	17.21b	22.20bc	75.18bc	114.59bc
80	2.73cd	0.08cd	0.28d	2.87cde	4.85de	3.09c	7.73cd	10.81de	13.27d	13.69c	18.22cd	53.95cd	85.86cd
100	2.70cd	0.11bcd	0.30d	2.67de	4.61e	3.11c	7.28d	10.38e	13.39d	13.29c	17.69d	52.44cd	83.42cd
120	1.78de	0.06d	0.22de	2.10e	3.88e	2.06cd	5.97d	8.03e	10.80de	11.96c	15.94d	45.25d	73.15d
140	1.41e	0.04d	0.14e	1.98e	3.74e	1.59d	5.72d	7.31e	9.66e	11.53c	15.97d	43.73d	71.22d

* Mean values of the same letters within a column are not significantly different at $p < 0.05$ by Duncan's multiple range test¹Weight loss of brown rice at each duration of milling was as follows: 1.3% (10 s), 2.5% (20 s), 4.4% (40 s), 5.7% (60 s), 6.9% (80 s), 8.0% (100 s), 9.4% (120 s), and 11.0% (140 s)

Fig. 3 Changes in the fatty acid composition of unprocessed white rice of the scented 'Cheonjiyang-1-se' variety as a function of milling degree. Data represent mean $\pm$ standard deviation of 3 independent measurements. Weight loss of brown rice at each duration of milling was as follows: 1.3% (10 s), 2.5% (20 s), 4.4% (40 s), 5.7% (60 s), 6.9% (80 s), 8.0% (100 s), 9.4% (120 s), and 11.0% (140 s)

respectively, which is consistent with previous reports [21]. These three major fatty acids, however, exhibited different patterns as a function of DM. The amount of oleic acid decreased with increasing DM, dropping from 34.7% at 10 s to 32.4, 29.2, and 25.3% at 40, 100, and 140 s of milling, respectively (Fig. 3). In contrast, palmitic acid, which presented at 23.3% after 10 s of milling, increased to 24.7, 28.1, and 31.2% after 40, 100, and 140 s of milling, respectively. Although its composition was as low as 1%, myristic acid also exhibited continuously increasing pattern under higher milling degree conditions. Another major fatty acid, linoleic acid, maintained a constant composition between 37.3 and 38.7% regardless of DM, corresponding to a relative standard deviation as low as 1.4%. These results suggest a varying fatty acid composition through the outer layers of the rice grain. If this is indeed the case, then different degrees of milling would result in white rice kernels covered by outer layers with different lipid compositions, which may in turn produce unique profiles of odor-active compounds, as observed in Table 1. It has been reported that degradation of fatty acids can alter the flavor properties of rice by producing lipid-derived odor-active compounds [31]. Various aldehydes, such as octanal, heptanal, nonanal, are produced by the degradation of oleic acid, while hexanol, pentanol, and 2-pentylfuran can be produced from the degradation of linoleic acid [31, 32]. In our experiments, however, no distinctive correlation between the aforementioned fatty acid composition and the concentrations of volatile compounds was observed. This may be due to our use of uncooked white rice immediately after milling allowing no time for the thermal degradation of lipids and subsequent conversion of fatty acids into their corresponding volatile compounds. In this research only

relative composition of fatty acids were evaluated, and additional quantitative researches on changes in amount of each individual fatty acid will be helpful to understand relationship between fatty acid alterations and flavor property in scented rice as affected by milling (Fig. 3).

Our experiments utilized only unprocessed white rice for characterizing the effects of milling on aromatics and phytonutrients. Further research is required on the relationships between those in unprocessed white rice and resultant properties in cooked rice as a function of milling degree, including sensory evaluations and customer preference tests.

Acknowledgements This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the High Value-added Food Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (115011-03-1-HD030) and a Soonchunhyang University Research Grant.

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