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Comparative Study on Aroma-Active Compounds in Thai, Black and White Glutinous Rice Varieties

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ABSTRACT

The aroma compounds of cooked, black and white glutinous rice (Kheow Ngu) that are commonly found on the Thai market were investigated. Volatile compounds were extracted from the steamed rice samples using diethyl ether and identified by gas chromatography-mass spectrometry (GC-MS). Twelve volatile compounds were identified in black glutinous rice and 14 compounds in white glutinous rice. In black glutinous rice, benzaldehyde was found in the highest concentration (1,203.7 ng/kg) but 4-vinyl-2-methoxyphenol (sweet, spicy and smoky odor) had the highest odor-active value (OAV = 133). However, the results from gas chromatography-olfactometry (GCO) indicated that there were four aroma-active compounds in black glutinous rice. The most prominent odorants were (*E,E*)-nona-2,4-dienal (sweet, fatty) and 2-acetyl-1-pyrroline (pandan) that had a flavor dilution (FD) factor of 3. Two other compounds that had an FD factor of 1 were 2,3-butanediol (sweet, balsamic) and 1-octen-3-ol that had straw and earthy-aroma characteristics. In white glutinous rice, the compound that had the highest OAV was butanoic acid (sweaty, rancid, OAV = 1,036). From GCO data, there were seven aroma active compounds that had sweet, cooked-flour and fatty-aroma characteristics in the white glutinous rice. These compounds were tentatively identified as nonanal, ethyl octanoate, (*E,E*)-nona-2,4-dienal, germacrene-D, methyl dodecanoate and two were unknown.

Key words: black glutinous rice, *Oryza sativa*, aroma active compound, 2-acetyl-1-pyrroline, (*E,E*)-nona-2,4-dienal

INTRODUCTION

Glutinous rice is cultivated in the north and the northeast regions of Thailand, as well as other Asian countries such as Laos, Vietnam, India and China. Both black and white glutinous rice cultivars produce grains that have different aroma characteristics to common long- and short-grain white rice.

Several research projects have been conducted to identify and quantify the aroma-active compounds of fragrant rice. It was concluded that 2-acetyl-1-pyrroline (2-AP) was the most prominent aroma compound in both cooked and uncooked fragrant rice (Mahatheeranont *et al.*, 2001). The compound 2-AP had an extremely low odor threshold of 0.1 μg/kg in water (Buttery *et al.*, 1983). The formation of 2-AP from proline and hydroxyproline through Strecker degradation was studied extensively by Hofmann and Schieberle (1998a; 1998b). However, 2-AP was not always the most prominent key odorant in all

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rice cultivars. Jezussek *et al*. (2002) studied aroma compounds in the cooked, brown rice cultivars of Malagkit Sungsong, Basmati 370, Khaskhani and Indica. They identified 41 odor-active compounds and reported that 2-AP was the key aroma constituent in all rice samples, but not in the Indica cultivar. Other key odorants in cooked, brown rice were; *bis*-(2-methy-3-furyl)-disulfide (meaty), 2-amino acetophenone (medicinal, phenolic), an unknown (spicy), vanillin, 4,5-epoxy-(*E*)-dec-2-enal (metallic), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (seasoning-like), 4-vinyl-2-methoxyphenol (spicy, clove-like), (*E,E*)-deca-2,4-dienal (fatty) and phenylacetic acid (honey-like).

Yang *et al*. (2008) studied volatile compounds of Korean, black rice and found 25 aroma-active compounds. Compounds that had a high aroma intensity in Korean black rice were 2-AP, (*E*)-2-nonanal, nonanal, hexanal and 3-octen-3-one. Besides these compounds, the same research also suggested that guaiacol, with an immediate aroma intensity, might have been one of the principal contributors to the unique, "smoky" character of Korean black rice. In white rice, volatile compounds had a higher relative proportion of alcohols, aldehydes, ketones and terpenoids. The aromas of cooked, black- and white- rice had large differences in the amounts of 2-AP, guaiacol, indole and *p*-xylene.

At the time the current study was undertaken, there had been no reports on the aroma-active compounds in Thai, black glutinous rice and "Kheow Ngu", white, glutinous rice. The purpose of this study was to identify the key odorants of the black glutinous rice and white, glutinous rice cultivars that were commonly grown in Thailand.

MATERIALS AND METHODS

Chemicals

2-Methyl-3-heptanone, *tert*-butylbenzene, 1,2-propanediol, 2,3-butanediol and *n*-alkane series (C5-C25) were purchased from Aldrich Chemical (Milwaukee, WI, USA). Diethyl ether and sodium chloride were obtained from Labscan (Dublin, Ireland). Sodium sulfate was sourced from Ajax Finechem (Sydney, Australia).

Sample preparation

Black glutinous rice and white glutinous rice (Kheow Ngu) were purchased from a local supermarket in Bangkok. Glutinous rice samples were soaked in odor-free water overnight, before they were steamed for 30 min.

Isolation of volatile compounds

Volatile compounds were extracted from 50 g of the cooked rice samples. The samples were suspended in 90 ml diethyl ether and stirred using a magnetic stirrer for 40 min at room temperature. The extraction was repeated twice. The three portions of the extracts were combined, dried over anhydrous Na_2SO_4 and concentrated to 30 ml using a 50 x 1-cm Vigreaux column at 40°C. The volatile fraction was further purified by high-vacuum distillation at 10^{-5} Torr. The volatile fraction was concentrated to 10 ml using a mild nitrogen stream and filtered through a Pasture pipette packed with anhydrous Na_2SO_4 and glass fiber. The sample was further concentrated to 100 µl under a mild nitrogen stream.

Analysis of volatile compounds by gas chromatography-mass spectrometry (GC-MS)

The volatile compounds were analyzed using an HP 6890 gas chromatograph equipped with an HP 5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA). High purity helium (99.999%) was used as a carrier gas at a flow rate of 1.6 ml/min. Samples were injected on-column to an HP-5 column and an FFAP column (30 m × 0.32 mm × 0.25 µm, Agilent Technologies). The oven temperature program used with HP-5 column started at 40°C, was held for 5 min, increased to 60°C at 2°C/min, increased to 90°C at 20°C/min and increased to 200°C at 10°C/min and then held for 10 min. The oven temperature program for the FFAP column started at 40°C was held for 5 min, increased to 60°C at 2°C/min, increased to 120°C at 5°C/min and increased to 200°C at 10°C/min. The ion source was by electron-impact ionization. The ionization energy level was 70 eV. The scan range was 30-300 amu and the scan rate was 2.74 scan/s.

The volatile compounds were identified by a comparison of mass spectrum data with the Wiley 275 mass spectrum library, a comparison of the retention index (RI) with the literature and by a comparison of the mass spectra and RI with authentic standards. RIs were determined using an HP-5 column and FFAP column and the calculation was based on the retention times of a series of *n*-alkanes (C5-C25). The internal standards were 2-methyl-3-heptanone and *tert*-butylbenzene. Information on odor descriptions and the thresholds of the aroma compounds were obtained from Flavor-Base 2004 (Leffingwell & Associates, 2004) and other literature.

Analysis of aroma-active compounds by gas chromatography-olfactometry (GCO)

The flavor dilution (FD) factors of the odor-active compounds were determined by GCO. The original extract was diluted stepwise with diethyl ether (1:3, 1:9, 1:27, 1:81 by volume, respectively). Determination of FD factors was done by two panelists who detected the greatest dilution that was odor-active. The GCO system consisted of an HP 5890 gas chromatograph (Agilent Technologies) equipped with a flame ionization detector, a sniffing port (SGE, Australia), and an on-column injector. The sample was concentrated to 50 μl under a mild nitrogen stream. The extract (1 μl) was on-column injected into an HP-5 and a FFAP capillary column (30 m × 0.32 mm × 0.25 μm film thickness, Agilent Technologies). The GC oven temperature used with an HP-5 column was programmed as follows; started at 40°C, held for 5 min, increased to 60°C at 2°C/min, increased to 90°C at 20°C/min, increased to 200 °C at 10 °C/min and then held for 10 min. The carrier gas, helium, flow was at 1.6 ml/min. The oven temperature program for the FFAP column started at 40°C, was held for 5 min, increased to 60°C at 2°C/min, increased to 120°C at 5°C/min and increased to 200°C at 10°C/min.

Statistical analysis

The experiments were performed using a complete randomized design. All experiments were done in triplicate. Mean separation was analyzed by Duncan's multiple range test using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Volatile compositions

The volatile compounds of black, glutinous rice and white, glutinous rice (Kheow Ngu) were studied. Steamed, black glutinous rice had a mild pandan and straw-like aroma. White glutinous rice, on the other hand, had a milder aroma with fatty and rancid notes.

The volatile compounds of cooked, black glutinous rice are listed in Table 1. The major volatile compounds were benzaldehyde (1,203.7 ng/kg) and 1,2-propanediol (459.0 ng/kg). In addition to 1,2-propanediol, also detected were 2,3-butanediol, 2-butanol and 1-propanol. It should be noted that the black glutinous rice had a higher aliphatic alcohol content compared to the white, glutinous rice. These alcohols are usually generated from lipid oxidation (Zeng *et al.*, 2007). It has been found that the level of 2,3-butanediol increased during the storage of rice (Champagne *et al.*, 2004).

Black glutinous rice contained phenol and 4-vinyl-2-methoxyphenol that could contribute to its smoky note. White glutinous rice did not have these compounds. Phenol and 4-vinyl-2-methoxy-phenol have been identified as the products of oxidation-reduction in plants (Tresol and Albrecht, 1986). In addition, they can be generated by decarboxylation of *p*-coumaric acid and ferulic acid during heating (Maga, 1984). The precursors are *p*-coumaric acid and ferulic acid that collapsed to phenol compounds (Maga, 1978).

Black glutinous rice also contained terpenoids such as *trans*-β-farnesene and β-bisabolene. It has been reported that some rice cultivars have sesquiterpene synthase. Cheng *et al*. (2007) revealed that rice had β-caryophyllene synthase and (*E,E*)-farnesol synthase that were responsible for sesquiterpene synthesis.

White, glutinous rice had a higher concentration of total volatile compounds, but its overall aroma intensity was weak. This could be because most of the volatile compounds in white glutinous rice had high aroma thresholds. The volatile components of white, glutinous rice were dominated by alkanes and alkenes such as pentadecane, nonadecane and 1-butene. These hydrocarbons were not prominent in the aroma profiles of black glutinous rice. The hydrocarbons were probably from grain wax (Zeng *et al*., 2007).

Table 1 Concentrations of volatile compounds in black glutinous rice (BG) and white glutinous rice (WG).

Compound	RI	RI	Relative concentration (ng/kg)	
	HP-5	FFAP	BG	WG
Benzaldehyde	975	-	1206.4 ± 15.7	-
1,2-Propanediol	984	1413	457.0 ± 5.9	659.7 ± 6.1
1-Butene	987	-	-	1569.0 ± 21.5
2,3-Butanediol	-	1489	169.0 ± 4.3	876.4 ± 7.3
2-Butanol	1037	-	163.1 ± 2.5	-
Phenol	1089	-	157.7 ± 7.8	-
1-Dodecene	1186	-	209.5 ± 13.8	-
1-Propanol	-	1356	57.2 ± 2.4	-
Benzeneethanol	-	1769	87.6 ± 3.3	-
Pentadecane	1251	-	-	1785.3 ± 21.5
Butanoic acid	-	1501	-	250.8 ± 8.4
Indole	1287	-	-	705.9 ± 5.2
4-Vinyl-2-methoxyphenol (*p*-vinylguaiacol)	1309	-	101.3 ± 9.2	-
Heneicosane	1323	-	-	624.2 ± 12.3
2-Tetradecene	1364	-	-	318.0 ± 2.4
7-Tetradecene	1378	-	-	66.8 ± 7.1
1-Tetradecene	1382	-	131.1 ± 10.6	-
(E)-β-Farnesene	1446	1562	65.6 ± 4.5	-
β-Bisabolene	1498	1628	49.5 ± 3.1	-
Benzoic acid	1500	-	-	307.2 ± 13.0
5-Octadecene	1555	-	-	324.1 ± 5.6
Octadecane	1677	-	-	159.6 ± 8.7
1-Octadecane	1754	-	-	656.9 ± 10.8
Nonadecane	1850	-	-	1152.5 ± 17.9

Aroma active compounds

The odor-activity values (OAV) of the aroma compounds were calculated and are listed in Table 2. OAVs were calculated from the relative concentrations of the volatile compounds divided by their odor-threshold values in water. The volatile compounds are selectively listed based on the availability of their odor-threshold data in the literature. The results indicated that 4-vinyl-2-methoxyphenol (OAV = 133) and 1-tetradecene (OAV = 2) contributed to the aroma of black, glutinous rice. Butanoic acid (OAV = 1,036) and indole (OAV = 5) could be key odorants in the white glutinous rice. Both butanoic acid and indole were also found in cooked, brown rice (Jezussuk *et al*., 2002).

Aroma-active compounds were studied using GCO and aroma extraction dilution analysis (AEDA). There were four aroma-active compounds in black glutinous rice (Table 3). The descriptors used for these aroma-active compounds were sweet, balsamic, pandan, straw, earthy, fatty and cooked rice, which agreed with the overall aroma description of black glutinous rice. White glutinous rice had seven aroma compounds with the characteristics of cooked rice, fatty and waxy, which fitted the overall "fatty" aroma description of white glutinous rice.

The aroma of black glutinous rice was dominated by the pandan-like characteristic of 2-AP and the fatty odor from (*E,E*)-nona-2,4-dienal. Both compounds had a flavor dilution (FD) factor of 3. This was similar to the result reported by Yang *et al*. (2008) that indicated that 2-AP was the most prominent odorant in Korean black rice. The other compounds that had an FD factor of 1 were 2,3 butanediol and 1-octen-3-ol. 2-AP has been reported as a key aroma compound in fragrant rice, bread and popcorn (Buttery *et al*., 1983; Wongpornchai *et al*., 2003). 2,3-Butanediol contributed to the faint, sweet and balsamic characteristics whereas 1-octen-3-ol contributed to the straw and earthy characteristics of black, glutinous rice. 1-Octen-3-ol has been described as a "mushroom odor" in several reports on rice aroma (Jezussek *et al*., 2002; Zeng *et al*., 2007). It was not detected in the white glutinous rice in this experiment.

Table 2 Thresholds and odor-active values (OAV) of selected aroma compounds in black glutinous rice (BG) and white glutinous rice (WG).

Compound	Odor description[1/]	Threshold[2/] (ppb)	OAV	
			BG	WG
1,2-Propanediol	Sweet	340,000[a]	<1	<1
2,3-Butanediol	Butter, creamy	>100,000[b]	<1	<1
2-Butanol	Fermented, yeasty	40,000[c]	<1	-
Phenol	Phenolic medicinal	5,000[d]	<1	-
1-Propanol	Floral balsamic	306[e]	<1	-
Butanoic acid	Sweaty, rancid	0.24[f]	-	1036
Indole	Sweet, burn, floral	140[g]	-	5
Benzaldehyde	Bitter almond-like	350[f]	<1	-
4-Vinyl-2-methoxyphenol (*p*-vinylguaiacol)	Sweet, spicy, clove-like, smoky	0.75[h]	133	-
1-Tetradecene	-	60[i]	2	-

[1/] Odor description from Flavor-Base (Leffingwell & Associates, 2004).
[2/] Thresholds in water are from [a] Alexander *et al*. (1982) [b] Buttery *et al*. (1999) [c] Schnable *et al*. (1988)
[d] Dietrich *et al*. (1999) [e] Leffingwell & Associates (2004) [f] Buttery and Ling (1998) [g] Buttery *et al*. (1988)
[h] Pyysalo *et al*. (1977) [i] Tamura *et al*. (1995).

It should be noted that most aroma-active compounds of both glutinous rice samples had very low odor-threshold values. These compounds were not detected when the samples were analyzed with GC-MS but they were detectable with GCO. The human nose is more sensitive to volatile compounds. 2-AP, (*E*,*E*)-nona-2,4-dienal, and 1-octen-3-ol were found in the black rice to have thresholds of 0.1, 0.01, and 0.005 ng/kg in water, respectively (Buttery and Ling, 1998). The threshold values were much lower than those of the compounds detected by MS as reported in Table 2.

It should also be noted that the panelists could not detect 4-vinyl-2-methoxyphenol that had a high OAV of 133 (Table 2). This could be because there was a smoky background note detected by the panelists throughout the sniffing period so that the smoky aroma of 4-vinyl-2-methoxyphenol could have been masked. In Korean, black rice, it was guiacol that contributed to the smoky and black rice-like aroma characteristic (Yang *et al*., 2008).

White glutinous rice had (*E*,*E*)-nona-2,4-dienal and methyl dodecanoate that had an FD factor of 3 (Table 3). These compounds were described by the panelists as having a fatty, waxy and cooked flour odor. Other aroma-active compounds that had an FD factor of 1 were; nonanal, ethyl octanoate, germacrene-D, unknown A and unknown B (Table 3).

CONCLUSIONS

The aroma-active compounds in the cooked black glutinous rice were; 2-AP, (*E,E*)-nona-2,4-dienal, 2,3-butanediol, 1-octen-3-ol and possibly 4-vinyl-2-methoxyphenol. White glutinous rice was rich in alkanes and alkenes, but these compounds did not contribute much to the aroma characteristics of the rice. Aroma- active compounds detected in the cooked white, glutinous rice were: (*E,E*)-nona-2,4-dienal, methyl dodecanoate, nonanal, ethyl octanoate,

Table 3 Average FD factor of aroma-impact compounds in black, glutinous rice (BG) and white, glutinous rice (WG).

Compounds	Odor description [1/]	RI HP-5	RI FFAP	Average FD factor[2/] BG	WG
2,3-Butanediol [a]	Sweet, balsamic	865	1413	1	-
2-Acetyl-1-pyrroline [b,A]	Pandan, cooked rice	922	1321	3	-
1-Octen-3-ol [b,B]	Straw, earthy	978	1289	1	-
Nonanal [b,C]	Flour, sticky rice, fatty	1108	1406	-	1
Unknown A	Sticky rice	1116	na	-	1
Ethyl octanoate [b,C]	Sweet, cooked flour	1192	1430	-	1
(*E*,*E*)-Nona-2,4-dienal [b,D]	Sweet, fatty, cooked flour	1218	1684	3	3
Germacrene-D [b,D]	Cooked flour, woody	1478	na	-	1
Unknown B	Fatty, cooked flour	1534	na	-	1
Methyl dodecanoate [b,D]	Waxy, sticky rice	1525	na	-	3

1/ Odor description at the GC-sniffing port during GCO.

2/ Average of FD factor (n=2) on HP-5 and FFAP columns; FD factor = 1 means detected odor before diluted aroma extract for AEDA; - means no detected odor.

a Compound positively identified by comparing with retention index (RI) on HP-5 and FFAP columns, mass spectra obtained by MS and odor quality at the sniffing port on the same column.

b Compound tentatively identified with the reference compound on the basis of a comparison of RI on HP-5 and FFAP columns and odor description at the sniffing port with the data from [A] Karagul-Yuceer *et al*., (2003),

B Jezussek *et al*., (2002), [C] Yang *et al*., (2008), [D] Boelens Aroma Chemical Information Service (1999).

germacrene-D, two unknown compounds and tentatively butanoic acid.

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