

Primary Volatile Aroma Compounds in Phka Rumduol, Cambodia

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Abstract: Phka Rumduol is a leading variety in Cambodian rice. Analysis and qualification of volatile aroma compounds of Cambodian rice were not reported yet, limiting a few papers about only 2-acetyl-1-pyrroline (2-AP). Although Cambodia has been very impressive and progressed for milling rice exports over the last 10 years, rice qualities in her value chain are still remained much more improvement, especially in domestic market. In this paper, volatile aroma compounds in Phka Rumduol (brown rice), cultivated by Cambodian Agricultural Research and Development Institute (CARDI) was analyzed by gas chromatograph mass spectrum (GCMS) and aroma extract dilution analysis (AEDA). As the results, 2-AP and other 21 aroma compounds were detected. Its flavor dilution (FD) factor of 2-AP was extremely high at 10,000. Since 2-AP was dominant aroma in Phka Rumduol, the analytical measure of 2-AP by solid phase micro extraction (SPME) and gas chromatograph (GC) in rice kernels were investigated. With the purpose to compare qualities between Phka Rumduol (CARDI) and Phka Rumduol purchased in a Cambodian market (commercial), 2-AP concentration of both samples, the changes of 2-AP by different milling degrees, using both samples and 2-AP concentration in red kernels in commercial were analyzed. The results showed that 2-AP of Phka Rumduol (CARDI) and Phka Rumduol (commercial) were 800 and 380 ppb respectively, that after 0, 1, 2, 3 and 4 minutes-milling, the 2-AP in CARDI ranged in 800 to 420 ppb, while commercial did in 380 to 250 ppb and that 2-AP of whole red kernels in commercial was 50 ppb. From the results changes of milling time, 2-AP was located more in out-layer than inside of rice kernel. Phka Rumduol (CARDI) was very good qualities, while Phka Rumduol (commercial) showed less 2-AP concentration with larger percentages of broken, red kernels and lower qualified kernels like immature, chalky, damaged ones etc. Aroma which is one of important factors for Cambodian consumers will be decreased by rice type and grade of processing in market. This paper also referred a little to decrease in qualities by excess milling and/or polishing.

Keywords: Cambodia, Phka Rumduol, 2-Acetyl-1-Pyrroline, Aromatic Rice, Milling Degrees

1. Introduction

Rice is the most important crop of farmers in Cambodia, with rice paddies occupying 75% of total cultivated land. The annual paddy production exceeds domestic consumption by around 5 million tons and this surplus paddy and milled rice are exported through formal and informal channels. Since 2010, milled rice exports by formal channels increased and

modernization of rice milling and processing have progressed. There are over 800 rice mills in Cambodia, 200 of which are medium or large-scale mills. About 40 of these are millers/exporters. However, others are small-scale or household mills. Meeting the international market, Cambodian standard of milled rice was required. In the standard prepared in 2012, the types are divided into Premium Aromatic Rice, Aromatic Rice, Premium White

Rice, Long Grain White Rice and Medium Grain White Rice. Milled rice with strong natural scent such as Phka Rumduol and Phka Rumdeng is included in Premium Aromatic Rice. According to glade of processing (5 classes) in each type, not exceeding percentages of grain length, grain composition (whole and broken), paddy rice and other composition are prescribed, moreover, not-exceeding percentages of damaged, red streaked, chalky and yellow kernels are also prescribed. For instance, in the case of Premium Aromatic Rice, not-exceeding percentage of red streaked kernel should be 0.10% in the highest class of glade of processing and this percentage increases until 4.8% in the lowest class. In the case of Medium Grain White Rice, not - exceeding percentage of red streaked kernel ranges 0.25 to 7.0%. Generally speaking, market values of Cambodian rice depend on grain length (long to medium), aroma (aromatic to no-aromatic) and grade of processing (high to low in whole rice and low to high in the impurities). The types above (not variety) are basic units in Cambodian marketing deals. Completely eliminating of red streaked kernels in milled rice (red kernels in brown rice) seems to be very difficult in the present farming, processing and seed manipulation systems in Cambodia. Therefore, milled rice in Cambodia is usually polished after normal milling to make it whiter.

Phka Rumduol is one of the Premium Aromatic Rice and is becoming a leading variety in world rice trade. Its high qualities in Cambodian markets and other qualities such as cooking qualities and sensory evaluation were reported in our previous papers [1-3], however, the aroma compounds in Phka Rumduol were reported a few yet.

CARDI (Cambodian Agricultural Research and Development Institute) examined the DNA fingerprints of Phka Rumduol and other Cambodian Premium rice varieties. The result showed that Phka Rumduol is each congruent with Thai Home Mali in 17 markers but differs in the position of their 18 markers. It means Phka Rumduol is indigenous as Cambodian variety.

Aromatic compounds in foods, especially those in rice, the eminent scientists were involved in research and reported in countless papers. Comprehensive and fundamental data were published in a book [4]. Although Basmati (India and Pakistan) and Khao Dawk Mari 105 (Thailand) with long grain length are dealt with high price in international market, other aromatic varieties in India, Thailand, Vietnam, Myanmar, Philippines, Cambodia, US, and China are also consumed in domestic and/or international markets. On the other hand, majority of indigenous aromatic rice are small and medium-grained, that are cultivating in much more wider areas such as Himalayan region, Afghanistan, Iran, China and Japan.

Buttery *et al.* [5] reported that 2-AP is a key aroma compound of aromatic rice. This compound is present not only in the all plant parts except root of aromatic rice [6] but also in pandan (*Pandanus amaryllifolius*) leaf and in other many crops and foods. Furthermore, many papers investigated on 2-AP in aromatic rice have made clear that the concentration of 2-AP changes by the growing period of rice

plant, cultivated places, storage time and milling degrees [7].

The present investigation was carried out using Phka Rumduol cultivated by CARDI and Phka Rumduol purchased at a market in Phnom Penh, Cambodia. The purpose is placed on characterization on volatile aroma compounds in Phka Rumduol and study on the changes of 2-AP concentration by different milling degrees and also 2-AP concentration of red rice kernel presented as impurities in commercial Phka Rumduol.

2. Materials and Methods

2.1. Materials

2.1.1. Reagents

Reagents were purchased from Tokyo Kasei Kogyo Co., Ltd. and FUJIFILM Wako Pure Chemical Corporation. 2-Acetyl-1-pyrroline (2-AP) was granted by San-Ei Gen F. F. I., Inc.

2.1.2. Rice Samples

Phka Rumduol (brown rice) cultivated by CARDI (Cambodian Agricultural Research and Development Institute) in 2018 and Phka Rumduol (brown rice) purchased in a market of Phnom Penh in 2018 were used. The rice samples were kept in a deep freezer until analysis.

These rice samples were milled (Testing Husker, Model THU 35B, Satake Corporation) for 0 (brown rice), 1, 2, 3 and 4 minutes. The ratio of whole (without any broken part), broken, red included broken red, husk, immature, chalky and damage were calculated by manual separation from 100g of each of the milled rice samples and the amount of each was weighed.

2.2. Methods

2.2.1. Extraction and Concentration of Aroma Compounds

Aroma compounds in the Phka Rumduol brown rice cultivated by CARDI were extracted using simultaneous steam distillation extraction [5]. Two flasks were loaded on a Likens-Nickerson apparatus. 100 g of brown rice in a 2 L flask was put in 300 mL of distilled water. On the other side, 100 mL of diethyl ether was put in a 200 mL flask. The 2 L flask was carefully heated for 30 minutes and the heating content of the 2 L flask was changed 10 times. In total, aroma substances were extracted from 1,000 g of brown rice samples. The ether extract obtained was dehydrated with anhydrous sodium sulfate for a night, and then it was filtered and concentrated under room temperature to about 1ml.

2.2.2. Identification of Aroma Compounds by GCMS

GCMS (a gas chromatograph mass spectrum) was conducted using a GCMS QP2010SE (Shimadzu Co.) equipped with a polar column (DB-WAX, Agilent, 0.25 mm ID, 30m long, 0.25µm film thickness). For this analysis the ether extract was further concentrated and then using the split injection method (split ratio of 1:50), 1 µL of the ether extract was injected into the column. GC conditions were as follows; initial temperature of 50°C for 1 minute, with a programmed

rate of 4°C per minute until the final temperature of 200°C was reached. Each peak was identified through comparison with mass spectrum and retention index (RI), using authentic standards.

2.2.3. Analysis of Flavor Dilution Factor by AEDA

AEDA (Aroma Extract Dilution Analysis) was performed as follows: the aroma concentrate dissolved in diethyl ether was diluted exponentially to 2 or 10 with diethyl ether into different concentrations. Then, 3 µL of each solution was injected into a gas chromatograph equipped with a polar column (DB-WAX, Agilent, 0.53mm ID, 30m long, 1 µm film thickness) using the direct injection method. The GC conditions were the same as for GCMS. The column end was divided into two ports. One port led to the FID and another port led out of the GC oven so that the peak aroma could be monitored nasally. In this way, the maximum dilution ratio at which the aroma could be smelt using AEDA was obtained. The maximum dilution ratio at which the aroma was recognized is the flavor dilution factor (FD factor).

2.2.4. Analysis of 2-AP by SPME

By examining the following points, the abstraction parameters for the SPME (solid phase micro extraction) were established. The 2-AP was added to 0.50 g of crushed Niigata Koshihikari rice (approx. 50 meshes) and 0, 0.025, 0.050, 0.10 and 0.20 mL of distilled water, at 20 ppb, 100 ppb and 500 ppb. These were put into a 4 mL vial. Then the vial was capped with an open top closure with PTEE / silicone septum and installed with SPME fiber (divinylbenzen / carboxen / polydimethylsiloxane 50 / 30 µm DVB / CAR / PDMS 2 cm, Supelco). The 2-AP in the vial was extracted into SPME fiber by using a heating block, heated at 70, 80, 90, and 95°C for 30, 60 and 90 minutes. Then SPME fiber was inserted into GC-14A equipped DB-WAX (0.25 mm ID, 30 m long, df = 0.25 µm). The GC conditions were the same as for GCMS. The signal of detecting 2-AP was found by FTD (Flame Thermionic Detector, other name NPD: Nitrogen Phosphorus Detector). The concentration of 2-AP in whole kernels of Phka Rumduol (CARDI and commercial) and the ones after 1, 2, 3 and 4 minutes-milling were analyzed based on extraction parameters of SPME. Whole, red kernels of Phka Rumduol (commercial, brown) were also analyzed as same as the above.

Rice samples above were ground by mortar and pestle to approximately 50 meshes. Each sample (0.50 g) was put into a 4 mL vial with 0.05 mL of distilled water and the internal standard (3, 4- dimethylpyridine at 50 ppb). The vial was capped and installed with SPME fiber (divinylbenzene / carboxen / polydimethylsiloxane 50 / 30µm DVB / CAR / PDMS 2 cm, Supelco). The contents were heated at 95°C for 30 minutes by using a heating block. The SPME fiber was inserted into GC-14A equipped DB-WAX (0.25 mm ID, 30 m long, df = 0.25 µm). The GC conditions were the same as for GCMS. The column end was separated two ports, one port led to FID to detect RI, and the other port led to FTD to detect 2-AP. Each analysis was performed three times [8, 9].

3. Results and Discussion

3.1. Rice Qualities of Phka Rumduol (CARDI) and (Commercial)

Table 1 shows rice qualities of Phka Rumduol samples and Figure 1 shows images of rice kernels of Phka Rumduol (commercial, brown). As shown in Table 1, Phka Rumduol (brown rice) cultivated in CARDI did not contain husk, red, immature, chalky and damage kernels but had only 7% broken kernels, including head kernels in this paper. On the other hand, 23.7% of the commercial (brown rice) contained husk, red, damage, immature, chalky and damage kernels, 8.2% of which were red and broken red kernels. With milling time increased in which the seed coat and bran layer were removed, the appearance of commercial Phka Rumduol milled was almost similar (white) to CARDI Phka Rumduol milled. However until 2min-milling time, red and red broken kernels were presented and the percentages of broken kernels increased in commercial Phka Rumduol than those in CARDI Phka Rumduol. CARDI Phka Rumduol was not easily damaged by milling, therefore, the yields of its milled rice was higher than those of commercial Phka Rumduol. The reason was suggestible from larger contents of red and red broken kernels in commercial Phka Rumduol. In the case of commercial Phka Rumduol, red kernels (red streaked kernels) were disappeared after 3 min-milling as shown in Table 1, when the milling degree was 89%; It seemed to be too much milling.

Table 1. Rice qualities of Phka Rumduol samples.

Rice Sample of Phka Rumduol	Milling time (min)	Yield after milling (%)	Grain Composition		Husk, immature, chalky and damaged (%)	Red (Whole and broken)(%)
			Whole (%)	Broken (%)		
CARDI	0	100	93	7	0	0
	1	96	91.4	8.6	0	0
	2	94	90.9	9.1	0	0
	3	92	88.4	11.7	0	0
	4	91	87.3	12.8	0	0
Commercial	0	100	76.3	12.4	3.1	8.2
	1	93	79.6	13.4	2.2	4.8
	2	92	81.4	15.5	1.2	2
	3	89	84	16	0	0
	4	87	82.6	17.4	0	0



Figure 1. Images of rice kernels shown in Table 1 of Phka Rumduol (commercial, brown).

Concerning to in Table 1 and Figure 1, whole kernels were kernels without any broken parts, while broken kernels included most of head kernels and small broken. This classification resulted in larger percentages of broken kernels. The percentages of broken kernels in Phka Rumduol (brown, CARDI) were 2-3% by counting followed to usual standard. In this paper, the analysis of 2-AP was done, using whole kernels of brown rice, whole kernels of milled-rice after 1, 2, 3 and 4 minutes-milling and also whole red- kernels.

3.2. Aroma Compounds of Phka Rumduol and Their Flavor Dilution Factors

Table 2. Volatile aroma compounds.

Retention Index		Identification	Compound name
Exper.*1)	Auth.*2)		
1110	1100	MS&RI*3)	hexanal
1178	1179	MS&RI	2-heptanone
1320	1318	MS&RI	4-methyl-1-pentanol
1324	1324	MS&RI	2-penten-1-ol
1323	1326	MS&RI	2-heptanol
1352	1352	MS&RI	1-hexanol
1360	1360	MS&RI	2-acetyl-1-pyrroline
1445	1442	MS&RI	acetic acid
1454	1454	MS&RI	1-heptanol
1455	1455	MS&RI	1-octen-3-ol
1494	1493	MS&RI	decanal
1495	1495	MS&RI	2-ethyl-1-hexanol
1531	1530	MS&RI	2-nonenal
1557	1557	MS&RI	1-octanol
1615	1617	MS&RI	2-octen-1-ol
1657	1657	MS&RI	2-decenal
1666	1668	MS&RI	1-nonanol
1700	1700	MS&RI	2,4-nonadienal
1706	1707	MS&RI	dodecanal
1752	1752	MS&RI	2-undecenal
1765	1762	MS&RI	1-decanol
1863	-	GCMS*4)	2-dodecenal

*1); Experimental. *2); Authentic. *3); Mass spectrum and retention index. *4); Mass spectrum only. GCMS; QP2010SE (Shimadzu Co.) Column; DBWax (0.25mm ID, 30m long, 0.25 μ m df. Agilent), Column initial temperature; 50°C (1min hold), temperature program; 4°C /min, final temperature; 200°C.

Volatile aroma compounds in the concentrated solution of Phka Rumduol (brown rice, CARDI 2018) were analyzed by GCMS and for identification and qualification; they were compared to the retention index of Reagents Authentic. This analysis confirmed the 22 compounds as those shown in Table 2. Table 3 shows the Flavor Dilution factor (FD factor) and aroma character by AEDA (Aroma Extract Dilution Analysis) analysis of the same ether extract concentrate.

Table 3. Analysis of flavor Dilution factor (FD factor) by AEDA.

RI*1)	Compound name	Aroma character	FD-factor
1320	4-methyl-1-pentanol	nutty	1
1324	2-penten-1-ol	mushroom like, green	1
1352	1-hexanol	cut grass like	1
1360	2-acetyl-1-pyrroline	nutty, popcorn like	10000
1495	2-ethyl-1-hexanol	sweet, green like	1
1666	1-nonanol	citronella oil like	1
1700	2,4-nonadienal	nutty, fatty	1
1752	2-undecenal	waxy, citrus peel like	1

*1) retention index

Table 3 shows that the FD factor of 2-acetyl-1-pyrroline (2-AP) of Phka Rumduol brown rice was extremely high at 10,000, followed by 4-methyl-1-pentanol, 2-penten-1-ol, 1-hexanol, 2-ethyl-1-hexanol, 1-nonanol, 2,4-nonadienal, and 2-undecenal aroma compounds which had high FD factors but these FD factors were 1 / 10000 in the strength of 2-AP. However, 4-methyl-1-pentanol, 2-penten-1-ol, 1-hexanol, 2-ethyl-1-hexanol, 1-nonanol, 2, 4-nonadienal and 2-undecenal aromas were not detected in the twice diluted ether extract concentrate AEDA. As shown in Table 2, several other compounds such as aldehydes, alcohols, 2-heptanone and acetic acid were detected. The contribution of these compounds to aroma of Phka Rumduol, however, was even less than the compounds shown in Table 3 with the FD factor 1 and were not considered as main aroma compounds of Phka Rumduol.

The aroma components of aromatic rice were previously reported [10-12]. The main aroma components of aromatic rice is 2-acetyl-1-pyrroline, which has a threshold value as small and is known to have poor storage stability [13]. In addition, 2-AP is regarded as a preferable aroma component of aromatic rice and is considered an indicator of rice variety, production area, cultivation method, and storage method [13-15]. Preservation of aromatic rice was reported to reduce the content of 2-AP and significantly to increase the lipid oxides of hexanal, 2-pentylfuran, 1-octanol and 4-vinylguaiacol [13]. In this experiment, 2-pentylfuran and 4-vinylguaiacol were not detected.

3.3. Analysis of 2-AP in Phka Rumduol by SPME

In a study to obtain the optimal method for the analysis of 2-AP by SPME and GC, the optimum addition of distilled water to 0.50 g rice was 0.05 mL (Figure 2). A suitable extraction temperature and time was 95°C for 30 minutes (Figures 3 and 4). The extraction conditions of SPME were that the addition amount of distilled water was 0.05 mL, and

the extraction temperature and time were 95°C for 30 minutes. The concentrations of 2-AP in whole kernels of Phka Rumduol (brown, CARDI) and in commercial Phka Rumduol (whole, white kernels) were analyzed based on the extraction parameters of SPME. The 2-AP concentration in CARDI Phka Rumduol and commercial Phka Rumduol were 800 ppb and 380 ppb respectively. On the other site, whole red - kernels in Phka Rumduol (commercial, brown, that is, 0 minutes -milling) were collected and analyzed by the same analytical condition by SPME. The concentration of 2-AP of the red - kernels above was 50 ppb.

Table 4. Analysis of 2-AP in Phka Rumduol by SPME.

Rice sample (Phka Rumduol)	Milling time (min)	Average of 2-AP concentration (ppb)	SD of 2-AP concentration
CARDI	0	800	89
	1	520	119
	2	510	101
	3	510	35
	4	420	80
Commercial	0	380	88
	1	420	102
	2	300	45
	3	360	24
	4	250	41

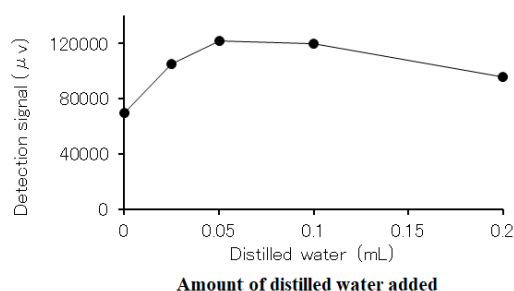


Figure 2. Effect of water addition.

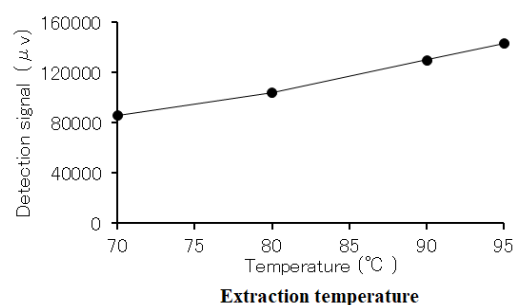


Figure 3. Effect of temperature.

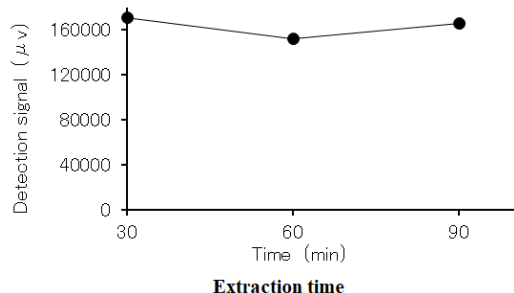


Figure 4. Effect of heating time.

Table 4 showed the 2-AP concentrations in whole kernels after 0, 1, 2, 3 and 4 minute-milling of CARDI and commercial Phka Rumduol. The 2-AP in whole kernels of CARDI decreased from 800 to 420 ppb and commercial decreased from 380 to 250 ppb. The concentration of 2-AP in CARDI Phka Rumduol decreased from 800 ppb to 520 ppb with a milling time of 1 minute. It was considered because high concentration of 2-AP was contained in the outlayer of aromatic rice grain. As milling time increased, the concentrations of 2-AP decreased more.

In Table 4, the 2-AP concentration in commercial Phka Rumduol decreased from 380 to 250 ppb, which were much lower than those of CARDI Phka Rumduol, however, the changes after milling seemed to be less. In the 2-AP analysis, rice kernels taken in a vial were whole kernels so that the concentration of 2-AP in commercial Phka Rumduol might have different tendency. That was because higher percentages of red - kernels (which were lower in 2- AP) in commercial brown rice.

The analytical results showed that the 2-AP concentration in Phka Rumduol (CARDI) was higher than that in commercial Phka Rumduol. Furthermore, the 2 - AP concentration in whole red - kernels were markedly lower than white kernels. One of the Cambodian varieties sited in the previous paper [14], showed 254.4 ppb in the 2-AP concentration. It was a little bit lower but could be almost equivalent.

The results above showed that 2-AP, the principle aromatic compound in Phka Rumduol decreased by increasing red kernels contaminated, even when the color became almost white by milling and polishing.

4. Conclusion

Volatile aroma compounds in Phka Rumduol (brown rice, cultivated in CARDI) showed that it's principal aromatic component was 2-acetyl-1-pyrroline (2-AP). Following to 2-AP, 4-methyl-1-pentanol, 2-penten-1-ol, 1-hexanol, 2-ethyl-1-hexanol, 1-nonanol, 2, 4-nonadienal, and 2-undecenal aroma compounds were detected. Its FD factor of 2-AP was extremely high.

Phka Rumduol (CARDI) and Phka Rumduol purchased in a market (commercial) were compared to study rice qualities in Cambodian market. First of all, an optimal method for the analysis of 2-AP was investigated. By the optimal method, changes in 2-AP as affected by 0, 1, 2, 3 and 4 min-milling

were analyzed, using Phka Rumduol (CARDI and commercial). The 2-AP in CARDI ranged in 800 to 420 ppb, while commercial did in 380 to 250 ppb. The 2-AP of whole kernels was decreased depending upon milling degrees. It means out-layers of rice kernel contained higher in 2-AP than the inside. Cambodian commercial milled rice is often polished after normal milling to make it appear whiter. The process might eliminate more the out-layers in where aromatic compounds and also protein. Protein may also be important for Cambodian people who take at least 65% of the energy intake in the diet from rice.

Moreover, 2-AP in red kernels was remarkably lower than white kernels. It means that the aroma which is one of the important factors by Cambodian consumers decreased.

Although Cambodian rice industry has been very impressive over the last 10 years, Cambodia will be required much more due to improve her value chain from seed to commercial products, relating to better quality.

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