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# IDENTIFICATION AND QUANTITATIVE ANALYSIS OF RICE AROMA COMPOUNDS IN BLACK JASMINE AND SUNG-YOD KORAT RICE FROM NAKHON-RATCHASIMA PROVINCE, THAILAND

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**ABSTRACT**

***Black jasmine and Sung-yod Korat rice were two new types of rice promoted to cultivar in Nakhon Ratchasima province, Thailand. This research aimed to identify and quantitatively compare rice aroma compounds in black jasmine rice with aromatic compounds in Sung-yod Korat rice. Headspace was used to collect and concentrate the compounds in rice. The rice was preheated in headspace, and subsequent GC-MS analysis was performed. The identification was confirmed by comparing both mass spectra and retention values of 2-Acetyl-1-pyrroline (2AP) standards and samples. 2AP was reported as the key aromatic compound for aromatic rice. The results showed that the concentration of 2AP in black jasmine rice and Sung-yod Korat rice were 0.77±0.03, and 0.52±0.01 ppm, respectively.***

**Keywords:** *Rice aroma; 2-acetyl-1-pyrroline; Headspace; Black jasmine rice; Sung-yod Korat rice; GC-MS.*

## 1. INTRODUCTION

Rice (*Oryza Sativa* L.) is the main cereal crop in many countries around the world. Globally, Nakhonratchasima province in Thailand is one of the major rice producers (Phakdeewanich, 2015). There are two new rice cultivars promoted to grow in this area; black jasmine and Sung-yod Korat rice. Black jasmine, or 'Hom – nin' rice is a cultivar produced by hybridization between jasmine rice and black sticky rice. This dark purple long grain rice (Figure 1, a) has an anthocyanins, vitamin E and antioxidant activity. Normally, cooked rice is soft, sticky and fragrant (Singkong, 2015). Alternatively, a red brown long grain rice, Sung-yod rice (Figure 1, b) is a Geographically Indicated (GI) product from the Phatthalung province in South Thailand (Vorapai, 2015). As it is in high demand amongst consumers, it was also planted in Nakhonratchasima province to meet supply. These two rice cultivars have special characteristics, including color, high nutritional value, a rich, soft texture, and fragrance.

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Figure 1: images of rice grains; black jasmine (a) and Sung-yod Korat rice (b)

There are many kinds of scent found in rice, but very few scents possess an odor activity that can be detected by consumers (Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011). A five-membered N-heterocyclic compound with an acetyl group, 2-Acetyl-1-pyrroline (2AP), (described as a "popcorn" or "pandanus leaf" like flavor) has been reported as the main aroma of scented rice (Jezussek, Juliano, & Schieberle, 2002), (Buttery, Ling, Juliano, & Turnbaugh, 1983), (Paule & Powers, 1989). This compound is found in numerous processed food such as in sweet corn (Buttery, Stern, & Ling, 1994), blue crab craw meat (Chung & Cadwallader, 1994), toasted wheat bread (Rychlik & Grosch, 1996), roasted sesame (P. Schieberle, 1996) and cooked maize flour used for cooking (Bredie, Mottram, & Guy, 1998).

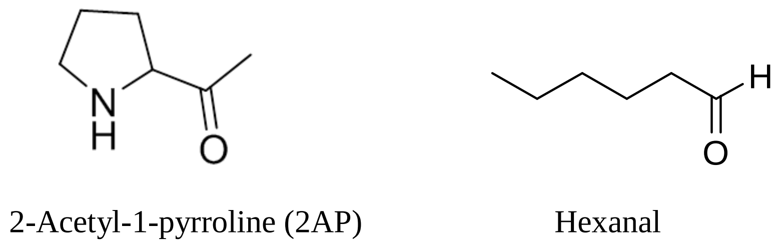


Figure 2: Structure of 2-Acetyl-1-pyrroline (2AP) and hexanal

Since the method for food processing involves heat, the presence of 2AP in food has been proposed to be generated from the Millard reaction between carbohydrates and amino acids during cooking at high temperatures (Weenen, 1998). Therefore, the quantitative studies of 2AP in food usually use a heat extraction, for instance steam distillation, solvent extraction (SDS), Headspace, Headspace Solid Phase Microextraction (HS-SPEME), followed by analysis of the extracts using Gas Chromatography-Mass Spectrometry (GC-MS).

Furthermore, carbonyl compounds are another aromatic compound present in rice. They may cause the rancid, undesirable odor which is a great concern of the rice producer industry (Hofmann & Schieberle, 1998). These carbonyl compounds are generated from lipid oxidation and lipid deterioration during storage (Peter Schieberle, 1990). Among the carbonyl compounds found in rice, hexanal is most produced during rice storage. Therefore, it has been

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used as an indicator of rancidity (Yasumatsu, Moritaka, & Wada, 1966), (Shibuya, Iwasaki, Yanase, & Chikubu, 1974), (Tsugita, Ohta, & Kato, 1983), (Champagne & Hron, 1993).

The work described in this paper was carried out to compare the amount of 2AP and hexanal in black jasmine and Sung-yod rice. We also aimed to identify a variety of volatile compounds presented in both rice cultivars by using Headspace and GC-MS.

## 2. EXPERIMENTAL

### *2.1 Rice samples*

Black jasmine and Sungyod Korat rice seeds were obtained from the Huai Taleang rice organic group, Huai Taleang district, Nakhonratchasima province, Thailand (harvested in March 2016). The rice seeds were collected, sun-dried, dehulled, and packed in vacuumed-sealed polypropylene bags. Each package of rice was unpacked and randomly weighed about 5 cm from the top, the middle, and the bottom of the bag. All samples were chilled at 4 °C for 24 h before they were ground using a Cryomill (Retsch). The exact weight of rice powder was placed into a 20 mL Headspace vial (about 1.00xx g for 2AP quantitative study and 2.00xx g for hexanal and other volatile-component determination). The headspace vial was then sealed immediately with a PTFE/silicone septum and aluminum crimp cap. It was shaken well at room temperature of 27 °C for 10 min prior to analysis by SHS-GC (Sriseadka, Wongpornchai, & Kitsawatpaiboon, 2006).

### *2.2 Chemicals*

2AP was purchased from BOC Science. Cyclohexanol, 1000 ppm, was used as internal standard was purchased from CARLO ERBA. The exact volume of ethanol was added to dilute the stock standard of cyclohexanol to 0.5 ppm.

### *2.3 GC-MS Analysis*

GC-MS analysis was performed using an Agilent 7890A/7000 GC-MS instrument equipped with a Triple Quadrupole (Agilent Technologies Inc., Santa Clara, CA, USA). Samples were analyzed via a HP-5MS capillary column (30 m x 0.25 mm and 0.25 µm film thickness) from J&W Scientific, Folsom, CA, USA. The column temperature used in the analysis of volatile compounds was initially held at 40 °C for 1 min. The temperature was then increased to 65 °C at 2 °C/min and held for a further 1 min, and then finally increased to 240 °C at 10 °C/min and held for 5 min. Column temperature for 2AP and hexanal determination was initially held at 28 °C for 10 min. then the temperature was increased to 160 °C at 3 °C/min and then finally to 230 °C at 10 °C/min. The carrier gas was Helium (flow rate of 2 mL/min for detecting volatile compounds and 1 mL/min for 2AP and hexanal). Mass spectra were acquired in electron impact mode. MS was taken at 70 eV ionization energy in the 25–400 amu mass range, and the ion source temperature was 230 °C. The volatile compounds were tentatively identified by matching the mass spectra with the spectra of a reference compound library in both the Agilent Mass Hunter Qualitative Analysis B.04.00 and the NIST/EPA/NIH mass spectra library (version 2.0). The results from volatile analyses are provided in peak area counts of the compounds identified. These experiments were modified from Sriseadka (2006).

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Samples were run in triplicate and the concentration of hexanal was calculated based on the relative peak area of cyclohexanol.

### *2.4 Calibration Procedure*

A series of 2AP standard solutions having concentrations of 10.0, 5.00, 1.00, and 0.5 ppm in methanol was prepared. One microliter of each solution was added to a headspace vial containing 1.000 g of the nonscent rice (cv. Pijit) powder used as a supporting material [24]. The internal standard, 1.00 µL of 0.50 ppm cyclohexanol, was added to each vial using the open vial sample introduction technique. The headspace vial was sealed immediately with a PTFE/silicone septum and aluminum crimp cap. Three replications were carried out for each 2AP concentration. The vials were shaken constantly at a room temperature of 27 °C for 10 min prior to analysis by using the optimized SHS-GC conditions.

### *2.5 Data analysis*

All values are means of at least three replicates ± SD. Statistical analysis was performed using excel and Duncan's multiple range test (SAS Institute Inc., Cary, NC, USA) to determine significant differences among means ($p < 0.05$).

## 3. RESULTS AND DISCUSSION

### *3.1 Volatile components in black jasmine and Sung-yod Korat rice*

Table 1 : Relative contents of the 10 most potent volatiles in Black jasmine and Sung-yod Korat rice by Headspace GC-MS

| No. | RT (min)[d] | Volatile compounds[a] | Relative content (%)[b] | |
|---|---|---|---|---|
| | | | Black jasmine rice | Sung-yod Korat rice |
| 1 | 9.09 | Hexanal | 5.37 | 5.76 |
| 2 | 14.96 | 1-Hexanol | 11.40 | 7.97 |
| 3 | 22.88 | 2-Pentylfuran | 6.06 | 3.68 |
| 4 | 27.63 | 1-Octanol | 2.17 | 3.47 |
| 5 | 29.29 | Nonanal | 8.81 | 19.84 |
| 6 | 34.16 | Dodecane | 8.13 | 3.86 |
| 7 | 34.96 | Benzothiazole | 2.58 | 3.19 |
| 8 | 38.84 | Tridecane | 18.33 | 8.33 |
| 9 | 43.21 | Tetradecane | 7.21 | 4.17 |
| 10 | 59.20 | Methylhexa-decanoate | ND[c] | 7.81 |

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[a]Volatile compounds were identified mainly by comparing their mass spectra with the mass spectral data of the standard compounds in the NIST library together with the comparison of their GC retention times with those of standard compounds and confirmed by the standard addition technique.
[b]Relative content may vary from sample to sample.
[c]ND = Not determined.
[d]RT = Retention time (min)

Table 1 shows the relative contents of the 10 most potent volatiles in Black jasmine and Sungyod Korat rice by Headspace GC-MS. Volatile compounds were identified mainly by comparing their mass spectra with the MS data of standard compounds in the NIST library, together with the comparison of their GC retention times with those of standard compounds, and confirmed by the standard addition technique. Straight- and branched-chain hydrocarbons, including carbonyl compounds, were found to be major constituents as expected. Some of volatile compounds identified were hexanal, 1-hexanol, nonanal, dodecane, 2-pentylfuran, 1-octanol, tridecane, tetradecane, methylhexadecanoate, and benzothiazole. The results indicated that black jasmine and Sung-yod Korat rice have similar volatile compounds but they are present in different ratios. For example, there was 19.84% of nonanal present in Sung-yod Korat rice, but there was only 8.18% of nonanal in black jasmine rice. Alternatively, there was 18.33% of tridecane in black jasmine, but 8.33% of tridecane presented in Sung-yod Korat rice. Figure 3 depicts the chromatogram of various volatile components present in black jasmine and Sung-yod Korat rice.

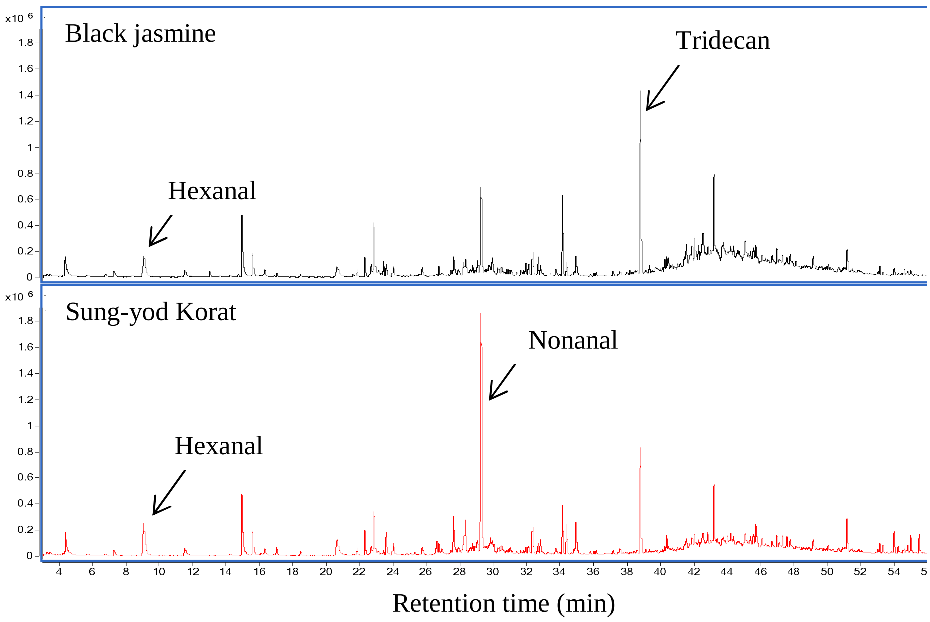


Figure 3: GC-FID Chromatogram of various volatile components presented in black jasmine and Sung-yod Korat roce.

### *3.2 Quantitative analysis of 2AP and hexanal in rice samples*

The presence of 2AP and hexanal in both rice samples is shown in Table 2. Like the method described by Sriseadka (2006), the concentration of 2AP in rice samples were determined using a standard calibration curve by using 2AP at 0.5, 1.0, 5.0, and 10.0 ppm. The results

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showed that the concentration of 2AP in black jasmine rice is higher than Sung-yod Korat rice. When comparing the concentration of 2AP in these two cultivars with the previous report (Sriseadka et al., 2006), the concentration of 2AP in both rice samples were shown to be higher than Indian Basmati rice, but lower than Khao Dawk Mali 105 (or jasmine rice 105). However, the concentration of 2AP in black jasmine and Sung-yod Korat rice was significantly different from Thai Hom Mali shelf rice. Of note, the concentration of 2AP in aromatic rice was reported at 500–600 ppb (Yoshihashi, Nguyen, & Kabaki, 2004).

Since cyclohexanol was used as an internal standard, the relative concentration of hexanal was determined from the ratio of the peak area of hexanal and cyclohexanol. As mentioned earlier, hexanal is considered an indicator for rancidity (Yasumatsu et al., 1966), (Shibuya et al., 1974). The results demonstrated that the relative concentration of hexanal in black jasmine rice was slightly lower than Sung-yod Korat rice. However, future studies will be performed to determine the efficacy of packaging materials and method of using the concentration of hexanal as an indicator.

Table 2 : Quantitative analysis of 2AP and hexanal in black jasmine and Sung-yod Korat rice

| Rice samples | Concentration of 2AP[a] (ppm) | Relative Concentration of Hexanal[b] (ppm) |
|---|---|---|
| Black jasmine | $0.77 \pm 0.03$ | $0.75 \pm 0.06$ |
| Sung-yod Korat | $0.52 \pm 0.01$ | $0.87 \pm 0.05$ |

[a]Concentration of 2AP in rice samples were determined by using a standard calibration curve with $r^2 = 0.9993$.
[b]The relative concentration of hexanal were derived from the ratio of the peak area of hexanal and cyclohexanol.

## 4. CONCLUSION AND FUTURE DIRECTION

This paper describes a comparative study of volatile components in black jasmine and Sung-yod Korat rice cultivars by Headspace and GC-MS. The results showed that there were similar volatile compounds presented in both black jasmine and Sung-yod Korat rice. Most of volatile compounds were straight-chain, branched-chain hydrocarbon and carbonyl compounds. There were moderate levels of 2AP present in both rice samples. However, the amount of hexanal in both samples was quite high, considering that they were fresh rice from packages. Thus, the packaging methods and materials may affect the rice quality. Further studies will be performed to find the optimal method to pack rice products for both local and international markets.

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