Comparison of Aroma Compound (2-Acetyl-1-Pyrroline) in Leaves from Pandan (*Pandanus amaryllifolius*) and Thai Fragrant Rice (Khao Dawk Mali-105)

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ABSTRACT

A study was made to identify the aromatic compound 2-acetyl-1-pyrroline (AP) in fresh and aged (shelf-stored) Khao Dawk Mali (KDML) 105 (a well-accepted Thai aromatic rice variety), in nonaromatic rice, and in pandan (*Pandanus amaryllifolius*) leaves, using a steam distillation-extraction method. Gas chromatography-mass spectrometry identified a gas chromatographic peak for AP eluting at a retention time of 5.47 min. The compound from the volatile oil of pandan leaf was found to be similar to AP in molecular structure. Under the same gas chromatographic conditions used to identify the volatile oil of pandan leaf, the AP obtained from the volatile oil of fresh KDML-105 was found to have a clear separate peak that eluted at retention times between 5.43 and 5.59 min. The intensity of the AP was expressed as the ratio of the peak area of AP to the peak area of collidine (internal standard). The AP, however, was not found in nonaromatic rice and occurred in low concentrations in the aged KDML-105 rice. The study confirms the importance of AP as a key compound contributing to the pandanlike aroma in KDML-105 and indicates the need to determine measures to properly store the rice to ensure aroma stability.

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Aromatic rice has been very popular in Asia and has recently gained wider acceptance in Europe and the United States. Due to their special aroma and flavor, aromatic varieties are highly favored and command a higher price in the rice market than do nonaromatic rice varieties.

The volatile compounds of rice that provide this characteristic aroma and flavor, which are of commercial importance, have been studied during the last 15 years. Maga (1978) and Yajima et al (1978, 1979) identified 20–100 volatile compounds in cooked Japanese rice, but none of these individual compounds was found to cause the characteristic odor of cooked rice. The potent aromatic compounds in the aromatic rice were not confirmed until Buttery et al (1982) identified and determined the concentration of 2-acetyl-1-pyrroline (AP) as an important compound contributing to a popcornlike aroma in several Asian aromatic rice varieties. Paule and Powers (1989) reported AP in Basmati 370, an aromatic rice from Pakistan, and positively correlated the AP concentration with descriptive terms (*popcornlike aroma* as described by nonorientals and *pandanlike aroma* as described by orientals). Lin et al (1989) and Tanchotikul and Hsieh (1991) confirmed the findings of Buttery, and others, who indicated that AP was chiefly responsible for the characteristic odor of aromatic rice varieties.

The AP compound was later identified as a major component of the volatile oil of freeze-dried pandan (*Pandanus amaryllifolius*) leaves. However, the concentration of AP in the freeze-dried leaves was 10 times greater than that found in milled, scented rice varieties and 100 times greater than that found in common milled rice. Extensive panel evaluations showed that AP had a significant positive correlation with descriptive terms (pandanlike or popcornlike aroma). Therefore, some Asian people may traditionally use pandan leaves while cooking common nonaromatic rice to impart a resemblance of the aroma to the cooked rice.

Buttery et al (1982, 1983) indicated that AP was a key compound contributing to the characteristic popcornlike aroma in Khao Dawk Mali (KDML) 105, a well-accepted Thai aromatic rice variety. Although these authors provided the prerequisite knowledge on aromatic compounds in Thai rice varieties, limited studies have been done since then on these rice varieties in Thailand due to numerous constraints of equipment and facilities. More information is needed on the aromatic compounds of Thai fragrant rice as well as a comparison of these compounds with locally synthesizable aroma compounds. The comparison of aroma compounds in fresh KDML-105 with those in aged KDML-105 (stored in mills for a long duration) and with those in nonaromatic rice is important in determining the storability of the aromatic rice.

Therefore, this study made a preliminary investigation to identify AP in KDML-105, using a method similar to that used by Buttery et al (1986). This study emphasizes the comparison of the major compounds in KDML-105 with that found in pandan leaves, aged aromatic rice (KDML-105), and nonaromatic rice varieties.

MATERIALS AND METHODS

Materials

Milled KDML-105 was obtained from the northeastern part of Thailand, an area well-known for the production of aromatic rice. Common milled nonaromatic rice and 200 g of fresh pandan leaves were purchased from a local market in Thailand to be used to compare the aromatic characteristics with those of the fragrant rice, KDML-105.

Collidine (2,4,6-trimethylpyridine, Aldrich Chemical Co., Milwaukee, WI), was used as an internal standard in gas chromatography (GC) analysis. The stock solution of collidine (300 ppm) was prepared and stored at room temperature. This stock solution was diluted to 30 ppm when used for steam distillation-extraction (SDE) to isolate AP from the rice samples and the pandan leaves.

Other reagents (diethyl ether, sodium bicarbonate, anhydrous sodium sulphate, and antifoam) used in the analysis were good-quality analytical-reagent grade.

Isolation of AP from Rice Samples

SDE was used to isolate AP from the rice sample. The procedure was similar to that used by Buttery et al (1986) and originated by Likens and Nickerson (1964). The SDE method began by adding 6.4 L of distilled water to a 12-L round-bottom flask containing 5 ml of antifoam. The water and antifoam were boiled together to obtain a volatile-free mixture. The boiling process was continued until the volume of the mixture was reduced to about 6 L. After reducing the volume of the mixture, a 200-g sample of milled rice was added gradually to the water-antifoam mixture in the flask. Collidine (5 ml) standard solution (30 ppm) was then added to the flask. The U-tube, located beneath the column of the distillation-extraction apparatus, was filled with water until water flowed over the water-return tube. The SDE column was cooled using a circulating water-cooling system, and the right arm of the SDE apparatus was then attached to the neck of the 12-L flask. The left arm of the apparatus was attached to a 250-ml round-bottom solvent flask containing 80 ml of distilled water with 2 ml of diluted sulfuric acid and 120 ml of diethyl ether. The 250-ml solvent flask was placed in a 500-ml beaker containing 100 ml of water. This served as a water bath to heat the dilute sulfuric acid-diethyl solvent mixture. The temperature of water in the 500-ml beaker was controlled by a 50°C setting on a hot plate; this was adequate to evaporate the diethyl ether at a boiling point of 39.5°C.

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When the rice sample in the 12-L flask began to boil, the hot plate temperature was lowered to prevent vigorous boiling. The solvent mixture was refluxed and stirred vigorously. After 2 hr of continuous distillation-extraction, the solvent flask was removed, and the sulfuric acid layer was separated into a 250-ml Erlenmeyer flask, using a 250-ml separatory funnel. The acid solution was neutralized by adding sodium bicarbonate. The neutral solution was poured into a clean 250-ml separatory funnel containing 120 ml of fresh diethyl ether. The separating funnel was shaken vigorously and left until the diethyl ether (upper layer) was clearly separated from the neutral solution (lower layer). The upper layer was poured into a clean 250-ml Erlenmeyer flask, and the lower layer was discarded. Anhydrous sodium sulphate was added to the flask to remove dissolved water.

The dry ether was filtered through a Whatman No. 1 filter into a 200-ml pear-shaped flask. The flask was attached to a Vigreux distillation column (Metha Group, Bangkok, Thailand), and the ether was concentrated to approximately 1 ml using a 60°C water bath. The concentrated solution was transferred to a micro tube and concentrated to 0.3 ml in a 50°C water bath. When not analyzed by GC on the same day, the micro tube was covered with a septum and parafilm and kept in a refrigerator.

Isolation of AP from Pandan Leaves

Fresh pandan leaves (200 g) were blended and mixed with 800 ml of distilled water. The mixture was filtered to remove the blended leaf residue. The filtrate was made up to 1,000 ml with distilled water, and the resulting pandan solution was placed in the SDE apparatus. The AP compounds were extracted using the same procedures as for the rice sample. However, in this experiment, 1,000 ml of pandan solution was added to the 12-L flask (containing 5 L of volatile-free water and 5 ml of antifoam), instead of the 200-g rice sample.

GC-Mass Spectrometry Analysis

Using a flame-ionization GC detector, 0.6 μl each of the rice or pandan extract were injected into the GC apparatus, and the volatile compounds were separated on a silica capillary column coated with Carbowax 20-M. The column was 25 m in length and had an internal diameter of 0.25 mm. Helium was used as carrier gas at a flow rate of 60 ml/sec. The temperature of the injector and detector were set at 170°C. The capillary column temperature was maintained at 80°C for 30 min, gradually increased to 150°C at 1°C/min, and kept at 150°C for 15 min.

A JMS-DX-300 mass selective detector (Jeol, Tokyo, Japan) was used for determination of electron ionization mass spectra. Selective-ion monitoring was set up to monitor the peaks at a retention time (RT) of 5–7 min obtained for the AP.

RESULTS AND DISCUSSION

Buttery et al (1986) indicated that collidine was a suitable internal reference standard with properties related to AP. It was a stable compound and had a GC RT reasonably close to that of AP. Under the GC analysis conditions of this study, the RT for collidine was found to be about 7 min.

Attempts were made to synthesize the standard AP. However, the synthesis was not successful; a pure sample was not obtained, as indicated by the nuclear magnetic resonance spectrum (Fig. 1) that showed that the synthesized compound was a mixture of an intermediary compound (a), a starting compound (b), and other substances (c). The mixture had a dark-brown color and could not be identified by GC-mass spectrometry.

However, Buttery et al (1983) suggested that AP could be extracted from pandan leaves. Results from GC of volatile compounds extracted from both pandan leaves and authentic AP is shown in Figure 2. The mass spectrum of the peak at 5.47 min for pandan leaves was compared with that of the peak at 5.49 min attained by authentic AP. Both mass spectra were observed to be identical, as shown in Figure 3. These mass spectra were essentially similar to that of the authentic AP: molecular ion at *m/e* 111 (5), other major ions at 43 (100), 41 (50), 42 (24), 83 (11), 68 (8), 55 (2), 52 (0.9) 54 (0.2), and 67 (0.2), as reported by Buttery et al (1983). Therefore, it is possible that the volatile oil extracted from pandan leaves that peaked at 5.14 min (similar to AP) could be used as a reference mixture in GC analysis in studies related to Thai aromatic rice.

The basic fraction extracted by the SDE method from the KDML-105 and analyzed by GC indicated a major compound eluting at RTs similar to that of pandan leaves (5.43 min, Fig.

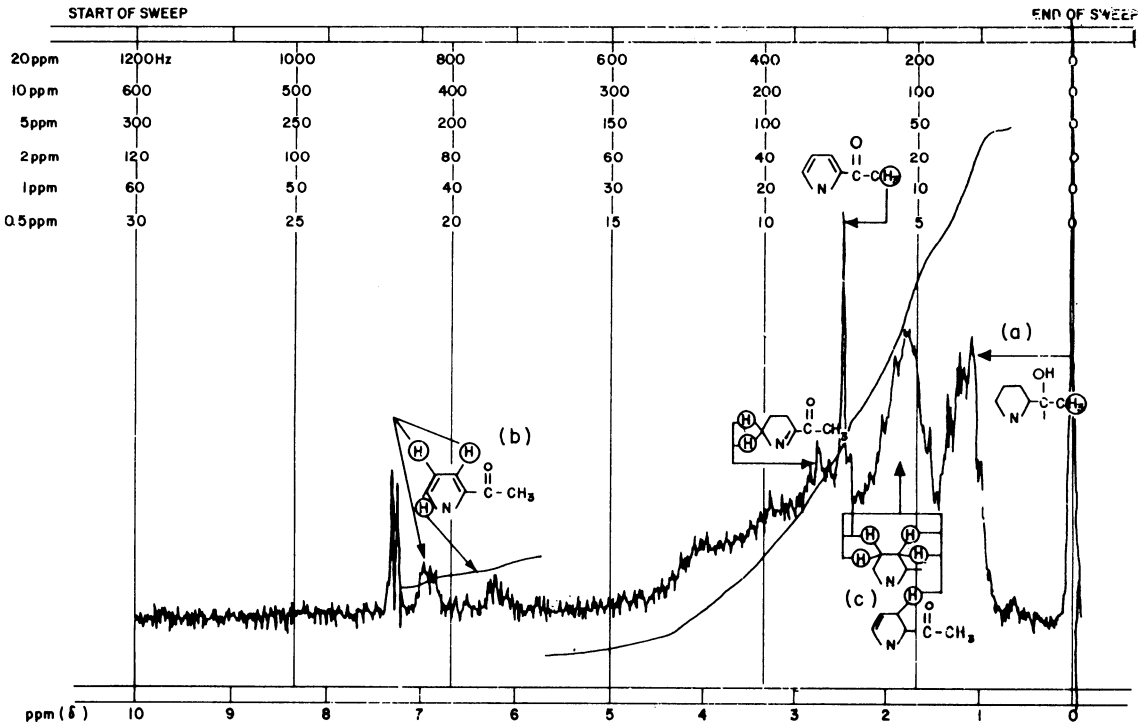


Fig. 1. Nuclear magnetic resonance spectrum of the synthesized 2-acetyl-1-pyrroline compound showing a mixture of an intermediary compound (a), starting compound (b), and other substances (c).

4A). However, mass spectrometry could not identify whether the peak at that RT was similar to AP because of the limited ability of mass spectrometry to identify AP intensities as low as those found in rice. One possible way to identify the peak is to mix the extracted solutions of both KDML-105 and pandan leaves at a suitable ratio of 2:1 just before injection into the GC. The additive concentration of AP obtained from the rice extract, as well as that from pandan leaves, could provide a higher peak area ratio (the AP peak area at 5 min divided by the collidine peak area at 7 min) than the peak area ratio from rice extracts only (Fig. 4B). Without analyzing the mass spectrum, the observed increase in peak area ratio may confirm that the major compound of aromatic rice eluted at about 5 min is similar to that of pandan leaves and, therefore, similar to that of the AP compound.

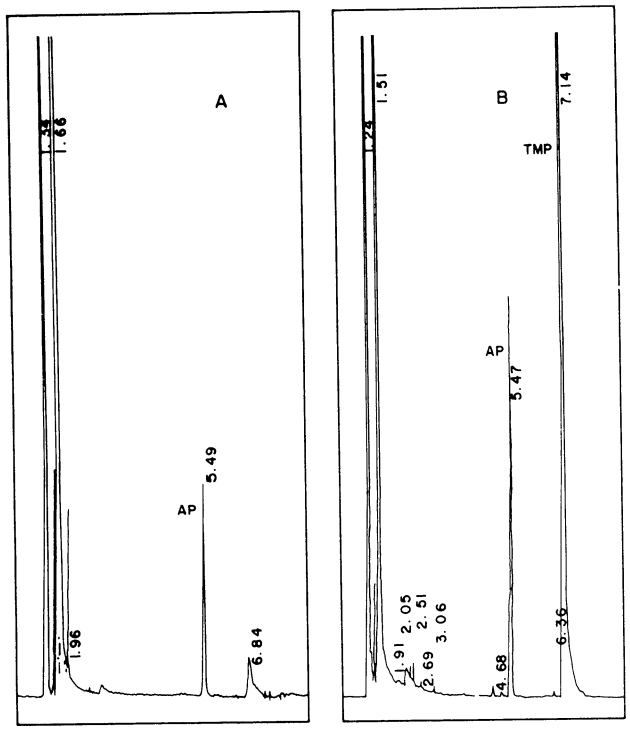


Fig. 2. Gas chromatograms of the authentic 2-acetyl-1-pyrroline (**A**) and volatile oils extracted from pandan leaves (**B**). AP = 2-acetyl-1-pyrroline, TMP = collidine (2,4,6-trimethylpyridine).

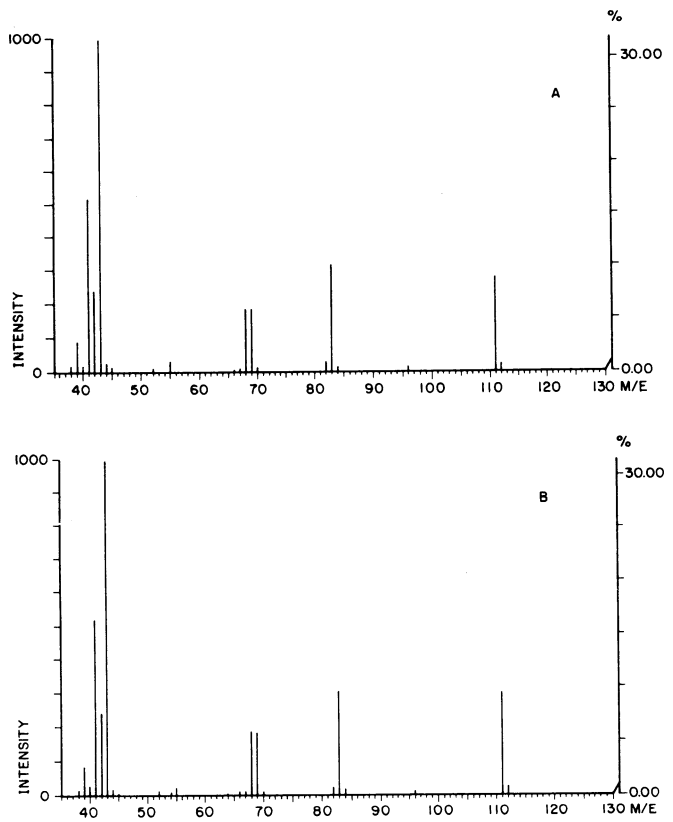


Fig. 3. Mass spectrum of the peak eluted at 5.49 min for authentic 2-acetyl-1-pyrroline (**A**) compared with that of the peak eluted at 5.47 min for the basic fraction from pandan leaves (**B**).

Buttery et al (1986) estimated the concentration of AP in the rice sample as follows:

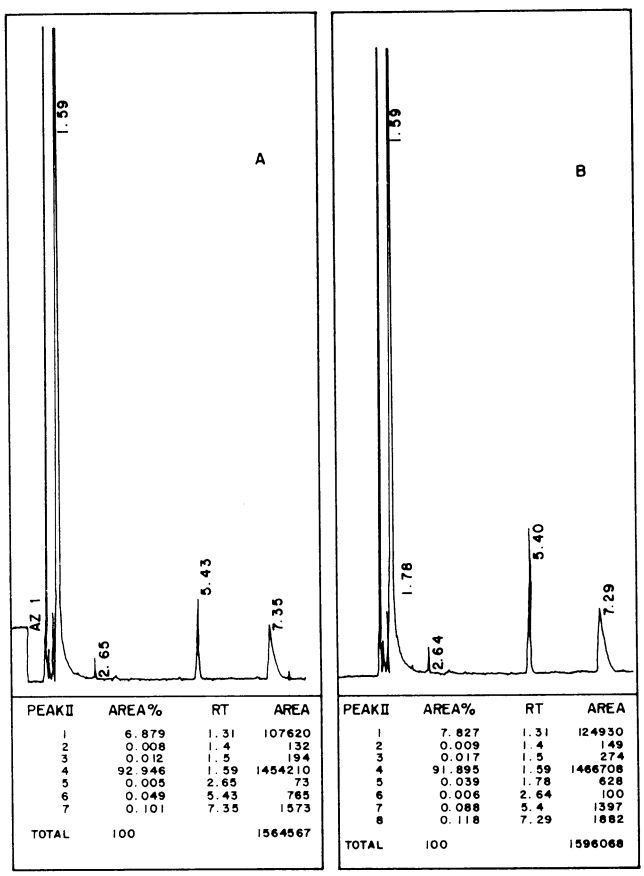
$$\text{AP concentration} = \frac{\text{area of AP peak}}{\text{area of collidine peak}} \times \text{collidine concentration} \times \text{RRF}$$

where RRF is percent recoveries of collidine and AP from the rice extract.

RRF was determined by adding a known quantity of the standard AP and collidine to 6 L of water in the 12-L flask and performing the SDE as previously described.

However, in this study, the amount of the standard AP was not sufficient to carry through the whole process. The concentration of AP was, therefore, estimated based on the peak area ratio of AP over the peak area ratio of collidine.

Figures 5 and 6 compare the chromatograms of aged KDML-105 and nonaromatic rice samples with that of a fresh KDML-105 sample. Results indicated that the peak area ratio of AP to collidine in an aged KDML-105 and a nonaromatic rice sample was low or nearly zero, respectively, as compared with the peak area ratio in fresh KDML-105 (Table I). A higher peak area



PEAK#	AREA%	RT	AREA
1	6.879	1.31	107620
2	0.008	1.4	132
3	0.012	1.5	194
4	92.946	1.59	1454210
5	0.005	2.65	73
6	0.049	5.43	765
7	0.101	7.35	1573
TOTAL	100		1564567

PEAK#	AREA%	RT	AREA
1	7.827	1.31	124930
2	0.009	1.4	149
3	0.017	1.5	274
4	91.895	1.59	1466708
5	0.039	1.78	628
6	0.006	2.64	100
7	0.088	5.4	1397
8	0.118	7.29	1882
TOTAL	100		1596068

Fig. 4. Gas chromatograms of the basic fraction from Khao Dawk Mali-105 rice (**A**) compared with that of a mixture of basic fractions from Khao Dawk Mali-105 rice and pandan leaves (**B**). The peak area ratio between 6 and 7 of **A** = 0.5. The peak area ratio between 7 and 8 of **B** = 0.7.

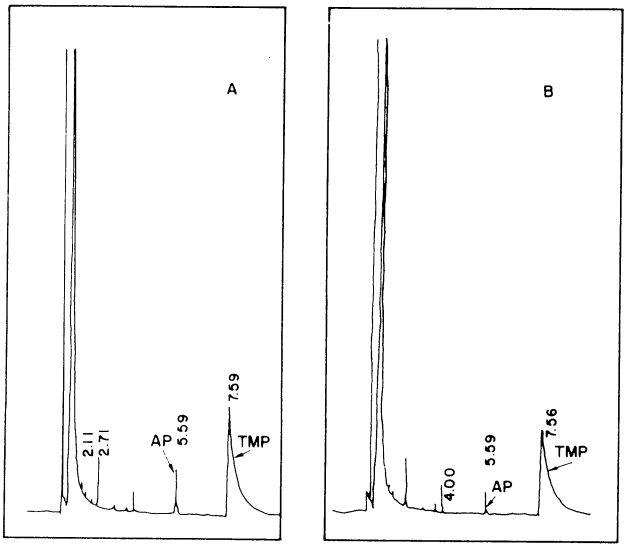


Fig. 5. Gas chromatograms of the basic fraction from fresh Khao Dawk Mali-105 rice (**A**) compared with that of the basic fraction from aged Khao Dawk Mali-105 rice (**B**). AP = 2-acetyl-1-pyrroline, TMP = collidine (2,4,6-trimethylpyridine).

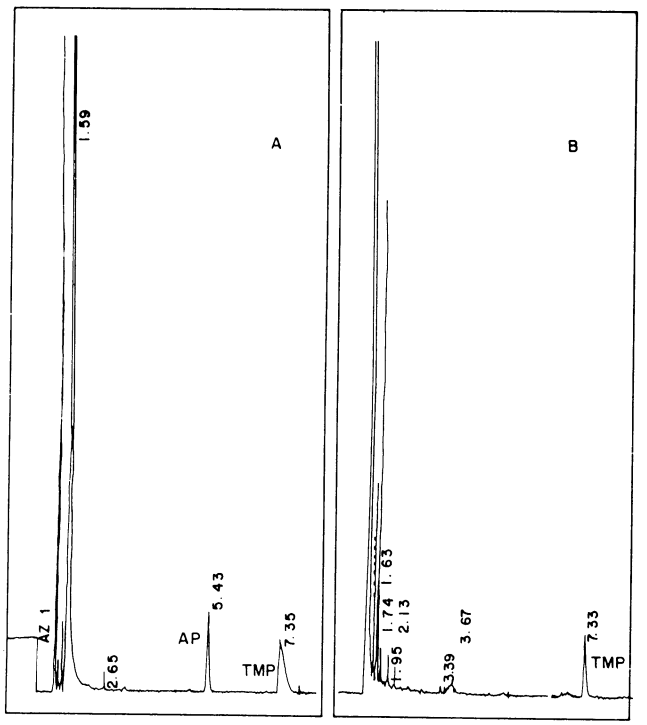


Fig. 6. Gas chromatograms of the basic fraction from fresh Khao Dawk Mali-105 rice (**A**) compared with that of the basic fraction from non-aromatic rice (**B**). AP = 2-acetyl-1-pyrroline, TMP = collidine (2,4,6-trimethylpyridine).

in the aromatic rice could confirm the important contribution of AP to the aroma of KDML-105. The low intensities in the stored rice indicate that the intensity of AP decreased as the rice was stored for longer periods. The zero peak area for non-aromatic rice indicates the absence of AP in this rice variety.

TABLE I
Estimated Intensity of 2-Acetyl-1-Pyrroline (AP) in Cooked Rice Samples Based on Peak Area Ratio of AP and Collidine in Gas Chromatography Analysis

Rice Samples	Peak Area Ratio[a]
Fresh aromatic	0.10
Aged aromatic	0.05
Nonaromatic	0.00

[a] Peak area of AP divided by peak area of collidine.

CONCLUSIONS

AP was secured from a natural pandan leaves. The pandan volatile compound obtained from the extracted oil could be useful as a reference mixture in GC analysis, where the AP eluted at a 5-min RT. Therefore, under the same GC conditions used for the pandan oil extract, the 5-min RT was used as a standard elution time to analyze the AP in fresh KDML-105, in aged KDML-105, and in nonaromatic rice.

Because the recovery factor (the correction term to convert the amount of AP in the extract to the actual amount of AP in the rice) was not obtained, the AP was estimated by the peak area ratio (i.e., the peak area of AP divided by the peak area of collidine). Based on the peak area ratio, the intensity of AP was greater in the fresh KDML-105 than it was in aged KDML-105. However, in nonaromatic rice, there were no traces of AP. The storability of the aromatic compound seems to decrease with duration, indicating the need to study the effects of storage conditions on AP degradability.

The study also provided information on a method to analyze aroma characteristics in Thai fragrant rice varieties using the RT of AP extracted from pandan leaves. The AP acted as a major compound contributing to the pandanlike aroma in fragrant rice. However, further study must be done to determine the recovery factor to benefit the estimation of actual AP concentrations in aromatic rice.

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