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Volatile Aroma Compounds of Khao Dawk Mali 105 Rice

Sugunya Mahatheeranont[1] , Somnuk Promdang[2], and Adisak Chiampiriyakul[3]

ABSTRACT

The volatile compounds of uncooked Khao Dawk Mali 105 brown rice were extracted using simple steam distillation method under reduced pressure. After solvent extraction in dichloromethane, the extract was concentrated and subjected to analysis twice using capillary GC-MS. The first analysis was performed immediately after distillation and extraction, while the second analysis was done after the extract was left at room temperature until its aromatic volatile compounds dissipated.

Comparison of these volatile compounds identified suggested that the compounds assumed to play an important role in aroma of Khao Dawk Mali 105 rice were 2-acetyl-1-pyrroline as the major component, butyl acetate, diethyl carbonate, butyl cyclopropane, 1,4-dimethylbenzene, isocyanato-methylbenzene, hexanal, nonanal, 7-octen-4-ol, 2-(2-propoxyethoxy)ethanol, and 2,6-bis(1,1-dimethyl-ethyl)-4-methylphenol.

Odor quality evaluation was performed among these compounds identified and came to a conclusio in that the oder of synthetic 2-acetyl-1-pyrroline was shown to be most match with the cooked pandan leaves aroma. However, this specific compound when added into cooked bland rice gave the odor which matched most closely with the aroma of Khao Dawk Mali 105.

Key words : volatile aroma, Khao Dawk Mali 105, 2-Acetyl-1-pyrroline, capillary gas chromatography-mass spectrometry (GC-MS)

INTRODUCTION

Khao Dawk Mali 105 is the most popular aromatic rice variety consumed in Thailand and some nearby countries. It has, for the last decade, gained increasing popularity in many other countries in Asia, Europe and the United State of America, due to its pleasant aromatic character and nice texture. Attempts have, thus, been made by many breeders and scientists to improve its productivity, yield and, especially, its aromatic quality. However, information on chemical basis of Khao Dawk Mali 105 is still limited. Only one research group has reported the present of 2-acetyl-1-pyrroline, an important aroma of cooked rice, in Khao Dawk Mali 105 quantitatively compared with those other aromatic and non-aromatic rice varieties (Buttery *et. al.*, 1983). The present work was carried out in order to study volatile flavor components of uncooked Khao Dawk Mali 105 rice. Further study was undertaken to evaluate the odor quality of particular volatile compounds which may be involved in contribution to the aromatic character of Khao Dawk Mali 105.

1 Department of Chemistry, Faculty of Science, Chiangmai University, Chiangmai 50200, Thailand

2 Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaengsean, Nakorn Pathom 73140, Thailand

3 Department of Plant Protection, Faculty of Agricultural Production, Majoe University, Chiangmai 50290, Thailand

MATERIALS AND METHODS

Khao Dawk Mali 105 rice harvested in Surin, a province in the Eastern part of Thailand, was hulled by hand and kept at 0°C within 24 hours before the experiment.

Collection of Volatile compounds by steam distillation

Under reduced pressure, fresh steam was directed to pass into a two-neck round bottom flask containing 200 g of uncooked brown rice. The vapor containing volatile compounds was then condensed while passing through a cooled condenser connected at another neck of the round bottom flask. The condensate was collected in a receiving flask until its volume reached 200 μL.

Extraction of volatile components

The above condensate was extracted twice, each time using 200 μL of dichloromethane. The extract was concentrated to 0.1 μL using rotary evaporator under reduced pressure and temperature set at 26°C. 1 μL of the concentrated extract was drawn to analyze using capillary gas chromatography-mass spectrometry. The remain was left at room temperature for six days until its smell of aroma was dissipated. By adding dichloromethane, the extract volume was brought up to 0.1 μL before subjecting to analysis again by capillary GC-MS using the same conditions as the previous analysis.

GC-MS conditions

In order to cover wide range of compounds analyzed, two kinds of fused silica capillary column were employed. One was a 30-m x 0.32-mm id. DB-5 column with a 0.25 μm film thickness (J&W Scientific). Another one was an Amine column (Chrompack) with the same dimension. The DB-5 column was temperature programmed starting at 45°C after injection and then the temperature was increased from 45°C to 200°C at the rate of 2°C/min and holding at this temperature for 15 min. When Amine column was used, the initial temperature was held at 40°C for 4 min. Then it was ramped up to 200°C at 2°C/min and stayed at this upper limit for 10 min. Purified helium gas with linear velocity 29 cm.s-1 was used as GC carrier gas. The GC injector was in splitless mode and its temperature was set at 170°C. The effluent from capillary column was directly introduced into the mass spectrometer, a double-focusing magnetic sector type (JMX-DX 505WA, JEOL Ltd.), operated in electron impact mode with ionization voltage of 70 eV and acceleration voltage of 3000 V. Ion source temperature was 200°C and GC-MS transferred line was set at 200°C.

Synthesis of 2-Acetyl-1-pyrroline and its sensory evaluation

Synthesis of 2-acetyl-1-pyrroline was performed by following the method outlined by Buttery (Buttery *et. al.*,1983). Authentic 2-acetyl-1-pyrroline yielded by the synthesis was examined by ^{1}H NMR and mass spectrometric techniques. Sensory evaluations on odor description of the synthetic 2-acetyl-1-pyrroline were carried out by means of panel judgments. Two groups of panelists were used. The first group was the trained judges with odor evaluation experience, consisted of 17 members. The second group consisted of 18 randomly selected untrained individuals. 0.1-ppm solution of the compound was prepared and contained in 250 mL glass bottle covered with opaque paper so that the judges could not see the contents. The judges were asked to describe the odor of the solution using as many descriptions as they could thing of another test was carried out in the same way but adding cooked bland rice variety into the solution containing approximately 0.1 ppm 2-acetyl-1-pyrroline.

RESULTS AND DISCUSSION

Separations of more than 140 compounds which constitute the concentrated extracts by capillary GC-MS method as shown in Figure 1., indicated the complexity of volatile compounds contributed to favor of uncooked Khao Dawk Mali 105 rice. Table 1. showed the list of all the volatile compounds identified together with their major ions. Additional volatile substances were found when Amine capillary column was employed and the separation was shown in Figure 3. Compounds were identified mainly by comparing their mass spectra with the mass spectral data of the standard compounds in the NIH library. Identification methods based on the comparison of GC retention time with those of authentic compounds were also used for confirmation.

Straight and branched chain hydrocarbons, including most of the unknowns, were found to be the major constituents as expected. With a mild steam distillation method, oxidation of lipids, fatty acids and certain amino acids were not facilitated, resulting in less aldehydes, ketones and other oxygenated compounds to be found compared with those volatile substances identified previously in cooked rice of some other varieties (Buttery *et. al.*, 1988). Fresh concentrated extracts of uncooked Khao Dawk Mali 105 rice, however, possessed strong characteristic scent which clearly isolate them from the flavor of non-scented rices. These concentrated extracts when left at room temperature for a few hours gave similar odor as those of the non-scented rices due to the dissipation of aromatic volatile compounds. Comparison between the separations of fresh concentrated extract as shown in Figure 1. and the same extract when its aromatic character disappeared, shown in Figure 2, led to the identification of some volatile compounds assumed to play significant role in characteristic scent of Khao Dawk Mali 105. These volatiles include 2-acetyl-1-pyrroline, a major aromatic compound

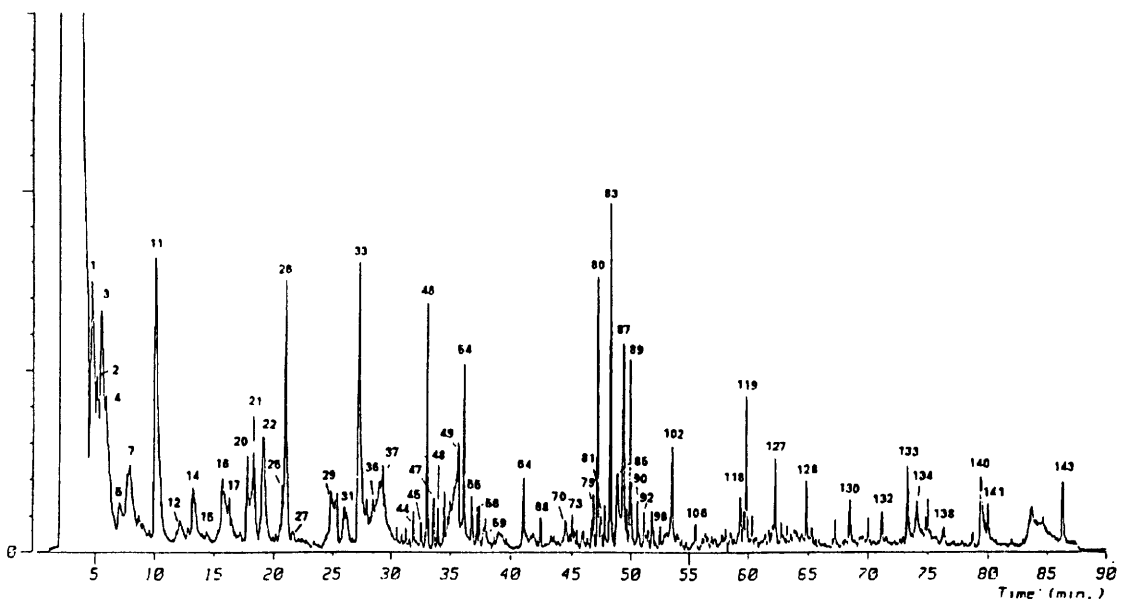

Figure 1 Reconstructed total ion chromatogram obtained by capillary GC/MS using DB-5 column of steam distillate extract from uncooked brown rice of Khao Dawk Mali 105.

Table 1 Mass spectral data obtained for volatile components identified in Khao Dawk Mali 105.

Compounds	Major MS ions	Peak No.
Hydrocarbons		
pentylcyclopropane	112,84,70,69,56,55,43(100)	a
2,4-dimethylheptane	128,85,71,57,43(100)	a
6-ethyl-2-methyloctane	127,85,71,57,43(100)	a
2,6-dimethylnonane	156,113,85,71,57,43(100)	a
undecane	156,113,85,57(100),43	73
2,5-dimethylundecane	184,113,85,71,57(100),43	a
2,10-dimethylundecane	184,113,99,85,71,57(100),43	a
dodecane	170,113,99,85,71,57(100),43	20
2,5-dimethyl dodecane	198,141,85,71,57(100)	64
tetradecane	199,141,99,85,71,57(100),43	a
pentadecane	212,127,90,85,71,57(100)	55
2-methylpentadecane	226,183,99,85,71,57,43(100)	a
hexadecane	226,140,122,99,71,57(100)	102
7,9-dimethyl hexadecane	254,155,127,99,71,57(100)	80
octadecane	252,224,139,97,69,57,43(100)	140
tricosane	324,127,113,85,71,57(100)	133
tetracosane	338,141,99,85,71,57(100)	143
1-octadecene	252,224,139,111,97,83,69,55	140
Alcohols		
1,3-butanediol	90,75,57,45(100)	a
1-pentanol	70,57,55,42(100)	a
1-hexanol	84,69,56(100),43	a
2-cyclohexen-1-ol	98,97,83,70(100)	a
1-heptanol	98,70,55(100),43	a
1-hepten-4-ol	96,73,71,43(100)	a
(E)-2-octen-1-ol	110,95,82,68,57(100),41	a
7-octen-4-ol	111,83,55(100),42,41	14
1-nonanol	139,97,83,69,56,43,40(100)	31
benzene ethanol	122,92,91(100),65	a
2-butoxy ethanol	100,87,57(100),45	a
2-(2-propoxyethoxy) ethanol	132,89,75,57(100),45	33
1-(2-butoxyethoxy) ethanol	132,100,87,75,57,45(100)	a
Aldehydes & ketones		
hexanal	100,82,72,56,44(100)	3
nonanal	127,114,98,82,70,57(100),43	26
(E)-2-nonenal	122,96,83,70,55,41(100)	a
decanal	138,112,82,70,68,57(100),41	a

Table 1 (Continued).

Compounds	Major MS ions	Peak No.
3-hydroxy-2-butanone	88,73,45(100),43	a
6,10-dimethyl-5,9-undecadien-2-one	194,151,69,57,43(100)	a
1,7,7-trimethyl bicyclo--2,2,1-heptan-2-one	152,108,95(100),81,69,55,41	a
Aromatics		
methyl benzene	93,91(100),65,39	1
ethyl benzene	106,91(100),78,65,51	a
1,2-dimethylbenzene	106,91(100),77,65	a
1,3-dimethylbenzene	106,105,91(100),77,65	a
1,4-dimethyl benzene	106,105,91(100),77,52	6
Acids & esters		
octanoic acid	144,95,87,73,60(100),41	36
butyl acetate	116,87,73,56,43(100)	a
diethyl carbonate	91,75,63,45(100)	2
1,2-benzenedicarboxylic acid, bis(1-methylethyl) ester	192,167,149(100),83,57,41	130
1,2-benzenedicarboxylic acid, butyl-2-methylpropylester	278,160,149(100),93,77,56	134
Phenols		
phenol	94(100),66,65,39	a
2,6-bis(1,1-dimethylethyl)-4-methyl phenol	220,205(100),145,105,81,57	83
2,4-bis(1,1-dimethylethyl) phenol	206,191(100),111,85,71,57	8
2,6-bis(1,1-dimethylethyl)-4-ethylphenol	234,219(100),159,107,88	93
Nitrogeneous compounds		
2-acetyl-1-pyrroline	111,83,69,68,43(100)41	11
N,N-dimethyl formamide	73(100),44,42	a
1(1H-prrole-2-yl) ethanone	109,94(100),66,39	a
Miscillaneous		
isocyanato methylbenzene	133(100),105,104,91,89,78	21
benzothiazole	135(100),108,91,82,63	a

a = additional compounds identified by using Amine column

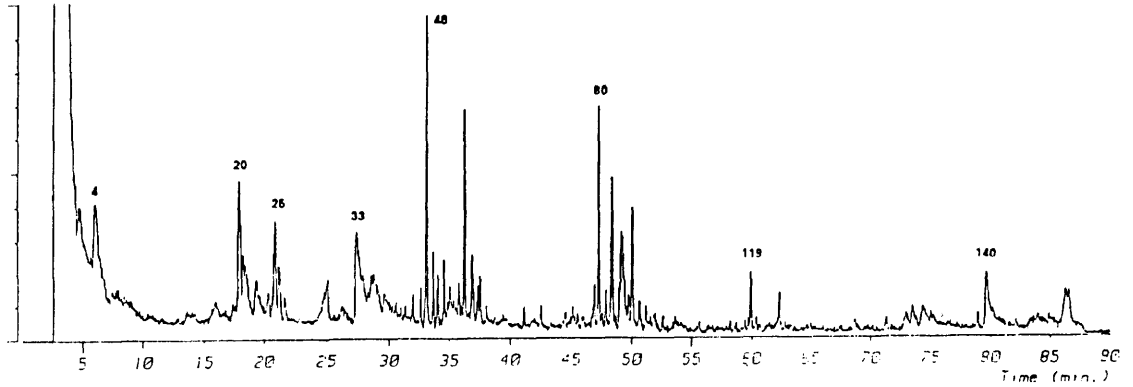

Figure 2 Reconstructed total ion chromatogram obtained by capillary GC/MS using DB-5 column of steam distillate extract from uncooked brown rice of Khao Dawk Mali 105 after left at room temperature for 6 days.

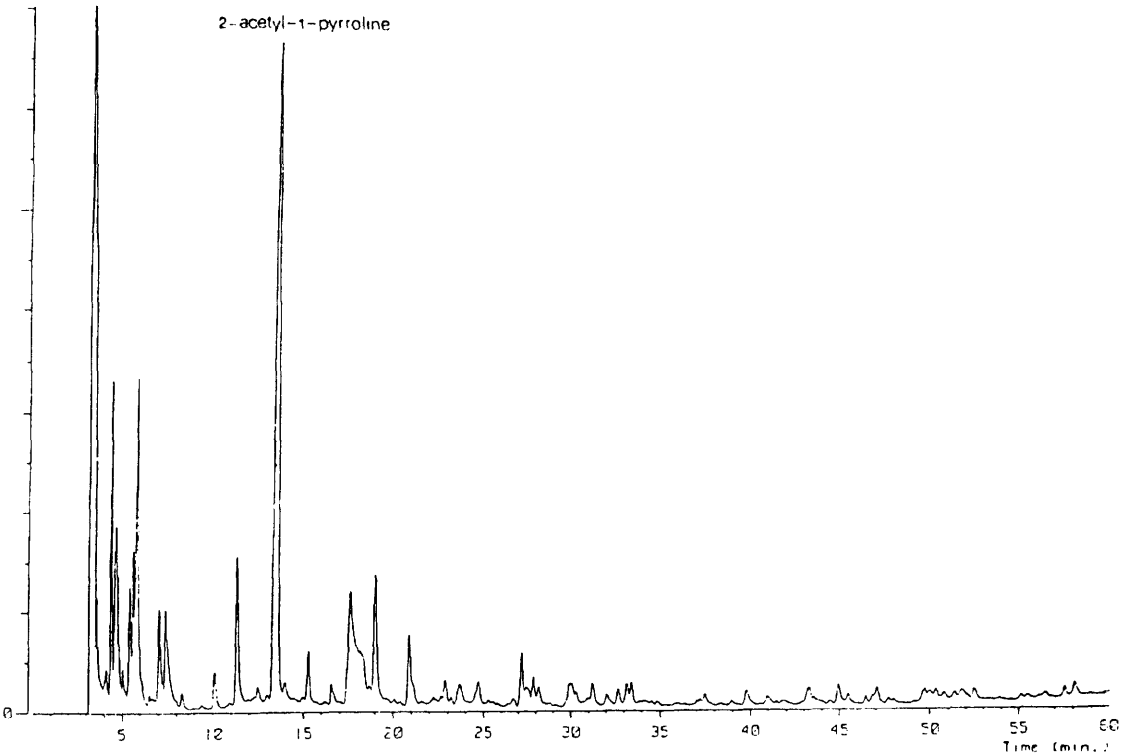

Figure 3 Reconstructed total ion chromatogram obtained by capillary GC/MS using Amine column of steam distillate extract from uncooked brown rice of Khao Dawk Mali 105

Table 2 Most often used panel odor descriptions of a 0.1 ppm solution of 2-acetyl-1-pyrroline and of 0.1 ppm solution of 2-acetyl-1-pyrroline added with cooked bland rice.

Odor description	% of judges using description	
	0.1 ppm solution of 2-acetyl-1-pyrroline in water	0.1 ppm solution of 2-acetyl-1-pyrroline added with cooked bland rice
Cooked pandan leaves	68	16
Cooked Khao Dawk Mali 105 rice	20	74
Scented coconut milk	12	8
Jasmine flower	12	-

found in scented rice varieties. Additional volatiles contributed to aroma of Khao Dawk Mali 105 are butyl acetate, diethyl carbonate, butyl cyclopropane, 1,4-dimethylbenzene, isocyanato methylbenzene, hexanal, nonanal, 7-octen-4-ol, 2-(2-propoxyethoxy) ethanol, and 2,6-bis(1,1-dimethyethyl)-4-methylphenol. Some of these volatiles including 2-acetyl-1-pyrroline was also found in concentrated extract of some non-scented rice varieties (Yajima *et. al.*, 1979; Bullard and Holguin, 1977) Except 2-acetyl-1-pyrroline, all the volatiles did not possess similar odor as that of Khao Dawk Mali 105 aroma.

Table 2 showed descriptions given by the 25 panelists for a solution of approximately 0.1 ppm authentic 2-acetyl-1-pyrroline. The most description chosen by the panelists to match odor of this compound was "cooked pandan leaves" which implied the present of such compound in pandan leaves in relatively high quantity. The odor description "cooked Khao Dawk Mali 105 rice" for cooked bland rice added in the solution of 2-acetyl-1-pyrroline suggested that this compound was the major volatile components mainly imparts the characteristic scent of Khao Dawk Mali 105 rice.

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