Quantification of the Rice Aroma Compound, 2-Acetyl-1-pyrroline, in Uncooked Khao Dawk Mali 105 Brown Rice

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Volatile components of uncooked Khao Dawk Mali 105 brown rice were extracted using indirect steam distillation under reduced pressure and controlled temperature in order to prevent cooking. Analysis of the fresh extract by capillary gas chromatography−mass spectrometry revealed that there were >140 volatile constituents. Among these, 70 volatiles were identified, including 2-acetyl-1-pyrroline (2AP), a key aroma compound of cooked rice. Further study concentrated on an improved method for the quantification of 2AP in uncooked brown rice. The method was simplified by utilizing a solvent extraction procedure. Quantitative analysis was performed using a capillary gas chromatographic system employing a flame ionization detector with the aid of a more selective column, CP-Wax 51, for amines. This improved chromatographic system had remarkable detection sensitivity for 2AP in the rice extracts so that 2AP in an extract of only 0.5 g of uncooked Khao Dawk Mali 105 brown rice could be detected.

Keywords: *2-Acetyl-1-pyrroline; gas chromatography−mass spectrometry (GC-MS); Khao Dawk Mali 105 rice*

INTRODUCTION

Khao Dawk Mali (KDML) 105 is the most popular rice variety in Thailand. This is due mostly to its pleasant aroma, which together with its white color and soft texture has resulted in its name “Khao Dawk Mali”, meaning “as white as jasmine flowers”. The name “jasmine rice” is, therefore, often used by foreign countries to refer to the KDML 105 Thai aromatic rice variety.

For the past two decades, KDML 105 has become increasingly popular in many other countries in Asia and Europe and more recently in the United States. Because it is in great demand in the rice market, Thai rice breeders have made a great effort to improve the effectiveness of aromatic rice breeding programs leading to higher productivity and yield but retaining aroma and texture.

A good understanding of the chemistry of KDML 105 rice, and especially of the compounds that contribute to its characteristic aroma, as well as an improved method for their quantification is considered a prerequisite for accurate detection and evaluation of aroma in rice. Since the key aroma compound of cooked rice, 2-acetyl-1-pyrroline (2AP), was first identified by Buttery et al. (*1*), there have been a number of studies involving the identification and determination of 2AP in various rice varieties (*2*−*7*). Quantitative analysis of the key aroma compound in rice has been performed with emphasis only on cooked rice. A few researchers (*8*, *9*) have studied volatile compounds in raw rice, but none have reported the presence of 2AP. Steam distillation and extraction (SDE) has accordingly been the method of choice for the isolation of aroma volatiles from cooked rice, although thermal decomposition of some volatiles has been experienced during cooking or steam distillation of rice (*10*, *11*).

A method developed by employing a microscale SDE device in association with improved chromatographic separation and detection has been found to decrease the amount of milled rice sample needed to only 1 g (*6*). However, a mass spectrometer using a sophisticated selected ion monitoring (SIM) mode, used as a detector for a gas chromatograph (GC), was required to improve detection sensitivity and specificity.

A study of volatile components isolated from uncooked brown rice was our primary objective in order to identify 2AP in raw KDML 105. The isolation procedure used was indirect steam distillation at low temperature under vacuum to prevent cooking. To fulfill rice breeders' needs, a secondary objective of our study was to develop a chromatographic method employing a conventional flame ionization detector (FID) allowing detection of 2AP isolated from as little as 1 g of rice. Isolation of 2AP from uncooked brown rice samples in our study employed a simple solvent extraction procedure to simplify the extraction process, the decomposition of 2AP by heat having been prevented.

MATERIALS AND METHODS

Materials. KDML 105 seeds were obtained from the Pathum Thani Rice Research Station, Pathum Thani, Thailand, harvested in 1997 in Surin, a province located in the eastern part of Thailand known for its consistent yield of high aroma quality KDML 105 rice crop. The rice seeds were collected, hulled by hand, and kept at 0−4 °C for no more than 48 h before being subjected to indirect steam distillation at low temperature under reduced pressure. For isolation of rice chemical components using a solvent extraction method, samples of KDML 105 rice harvested in 1998 at the Pathum Thani Rice Research Station were obtained. They were hulled by hand and kept at −20 °C before experiments. Milled samples of KDML 105 rice were purchased at a local supermarket. This processed rice was commercially available in different brands and packed in sealed plastic bags.

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A synthetic standard of 2AP was obtained using the method outlined by Buttery (*2*). Purification of the synthetic 2AP was done by utilizing gas chromatography (GC) with a packed column. The purified 2AP emerging from the detector outlet was collected in 3 mm o.d. Pyrex tubes, sealed under vacuum, and stored at −20 °C before spectral examination by capillary gas chromatography−mass spectrometry (GC-MS) and infrared spectroscopy (IR). A standard solution containing 30 ppm of 2AP was made by dissolving a known weight of the purified 2AP in a precisely measured volume of 0.1 M HCl.

The 2,4,6-trimethylpyridine (TMP), 99% purity, used as an internal standard was purchased from Aldrich Chemical Co., Milwaukee, WI. The exact weight of it was dissolved in a precisely measured volume of 0.1 M HCl to give an internal standard solution with 0.20 ppm concentration of TMP.

Indirect Steam Distillation of Uncooked Brown Rice. Under reduced pressure, fresh steam produced in a 1-L flask was directed to the bottom of a 500-mL two-neck round-bottom flask containing 200 g of whole uncooked brown rice. Vapor containing extracted volatiles from rice was then condensed by passing it through a cooled condenser connected at another neck of the round-bottom flask. Meanwhile, the vapor flow rate was adjusted by controlling the pressure in the system so that the vapor did not condense and accumulate at the bottom of the two-neck round-bottom flask. In this procedure, the brown rice sample remained uncooked. The condensate was collected in a receiving flask until its volume reached 200 mL. It was then transferred into a 500-mL separatory funnel and extracted twice, each time using 250 mL of dichloromethane. The organic layer was concentrated to ∼50 mL using a rotary evaporator under reduced pressure at a temperature of 26 °C. After drying with anhydrous sodium sulfate, the extract was further concentrated to a volume of 1 mL. The concentrated extract was left uncovered at room temperature and allowed to evaporate until its volume decreased to 0.2 mL before 1 μL was drawn for analysis by capillary GC-MS. The remainder was left at room temperature for 6 days until its aroma dissipated. By adding dichloromethane, the extract volume was brought up to 0.2 mL before it was subjected to analysis again by capillary GC-MS under the same conditions as the previous analysis.

Solvent Extraction of 2AP from Uncooked Brown Rice. Ground rice seed (10, 3, 1, or 0.5 g) of ground rice seed was added to a 125-mL flask containing 40.00 mL of internal standard solution. The mixture was stirred for 30 min before filtration. Twenty-five milliliters of the filtrate was transferred to a 125-mL pear-shaped separatory funnel. This was followed by the addition of 3 mL of 1.0 M NaOH to make the solution slightly basic. Then 50 mL of dichloromethane was immediately added as an organic solvent. The extraction was done twice, resulting in ∼100 mL of dichloromethane solution. After drying with anhydrous sodium sulfate, the extract was concentrated to 1 mL using a rotary evaporator under reduced pressure and a temperature of 26 °C. The concentrated extract was transferred to a V-shaped vial and left open to the air at room temperature until its volume decreased to 0.1 mL before it was subjected t to quantitative analysis by capillary GC with an FID. The whole experimental process was repeated for extraction and analysis of standard 2AP of exactly known amounts in a dilution series in order to obtain a standard calibration curve. For qualitative analysis of all the components in the extract by GC-MS, ground rice seed was extracted using the same solvent extraction procedure but without addition of the internal standard.

Capillary GC and GC-MS Conditions. To cover the wide range of compounds analyzed, two kinds of fused silica capillary columns were employed. One was a 30 m × 0.32 mm id. DB-5 column with a 0.25 μm film thickness (J&W Scientific, Folsom, CA). The other was a tailor-made column, CP-Wax 51 for amines (Varian Chrompack International B.V., Middelburg, The Netherlands) with the same dimensions. The temperature of the DB-5 column was programmed starting at 45 °C after injection, after which the temperature was increased at a rate of 2 °C/min from 45 to 200 °C and held there for 15 min. When an extract by solvent was to be analyzed, the initial temperature was set at 50 °C. It was ramped to 250 °C at a rate 4 °C/min and stayed at this upper limit for 10 min. The temperature of the CP-Wax 51 for amines column was programmed starting at 40 °C. After 4 min, it was ramped to 180 °C at a rate of 4 °C/min and held there for 5 min.

Purified helium gas with a linear velocity of 29 cm•s^{-1} was used as the GC carrier gas. The GC injector was in a split mode with a 1:10 split ratio. The injector temperature was set at 200 °C. The effluent from the capillary column went directly into the mass spectrometer, a double-focusing magnetic sector type (JMX-DX 505WA, JEOL Ltd. Japan), operated in the electron impact (EI) mode with an ionization voltage of 70 eV and an acceleration voltage of 3000 V. The ion source temperature was 200 °C, and the GC-MS transfer line was set to 200 °C.

Brown rice extract obtained by this solvent extraction procedure was analyzed by a capillary GC with an FID (HP 5890 Series II Plus, Hewlett-Packard Co., Wilmington, DE). The capillary column used was a CP-Wax 51 for amines. A programmed temperature elution was employed with an initial temperature of 50 °C held for 2 min. Then it was ramped to 200 °C at 4 °C/min. Purified helium gas was used as the carrier gas. The GC injector was operated in splitless mode at a temperature of 170 °C.

RESULTS AND DISCUSSION

Separation by capillary GC-MS of >140 volatile components of the steam distillate extract at low temperature of uncooked KDML 105 brown rice as shown in Figure 1 shows the great number of volatile compounds contributing to the aroma of uncooked KDML 105 brown rice. Table 1 lists of the volatile compounds identified together with their major ions from mass spectra. Compounds were identified mainly by comparing their mass spectra with the mass spectral data of the standard compounds in the NIH library together with the comparison of their GC retention times with those of standard compounds and confirmed by the standard addition technique. Due to the limited availability of standard compounds, only one-third of the extracted components that gave proper matches with the reference data have been reported. Tentatively identified components based only on a comparison of their mass spectra with the reference spectra of the NIH library that yielded <90% matches were identified as unknowns and have not been included in Table 1. It was found that the utilization of a more specific CP-Wax 51 for amines column increased the detection sensitivity of 2AP remarkably compared to other components as shown by the reconstructed total ion chromatogram in Figure 2. However, more extracted components found were added, and some of them were identified using the same methods. It is believed that some of these components identified when the CP-Wax 51 for amines column was used as the chromatographic column were already present in the chromatogram obtained by using a nonpolar column, DB-5, but their mass spectra were of poor quality because of unresolved peaks. In accordance with the polar property of the CP-Wax 51 for amines column, higher detection sensitivities of more polar components were achieved. These resulted in identification of additional alcohols, aldehydes, ketones, and nitrogen-containing compounds.

Straight- and branched-chain hydrocarbons, including most of the unknowns, were found to be major constituents as expected. With the mild steam distillation method, oxidation of lipids, fatty acids, and certain amino acids was retarded, resulting in fewer aldehydes,

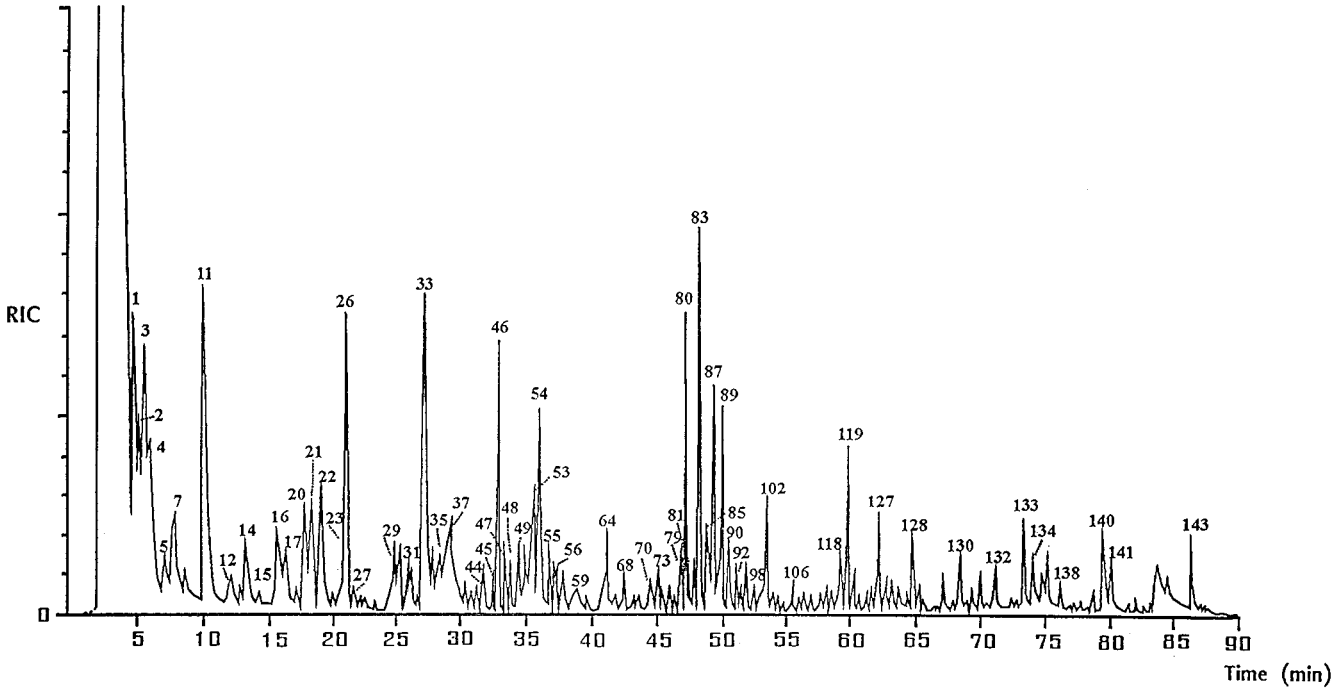

Figure 1. Reconstructed total ion chromatogram of a steam distillate extract from uncooked KDML 105 brown rice obtained by capillary GC-MS. A 30 m × 0.32 mm i.d., 0.25 μm film thickness DB-5 column was used.

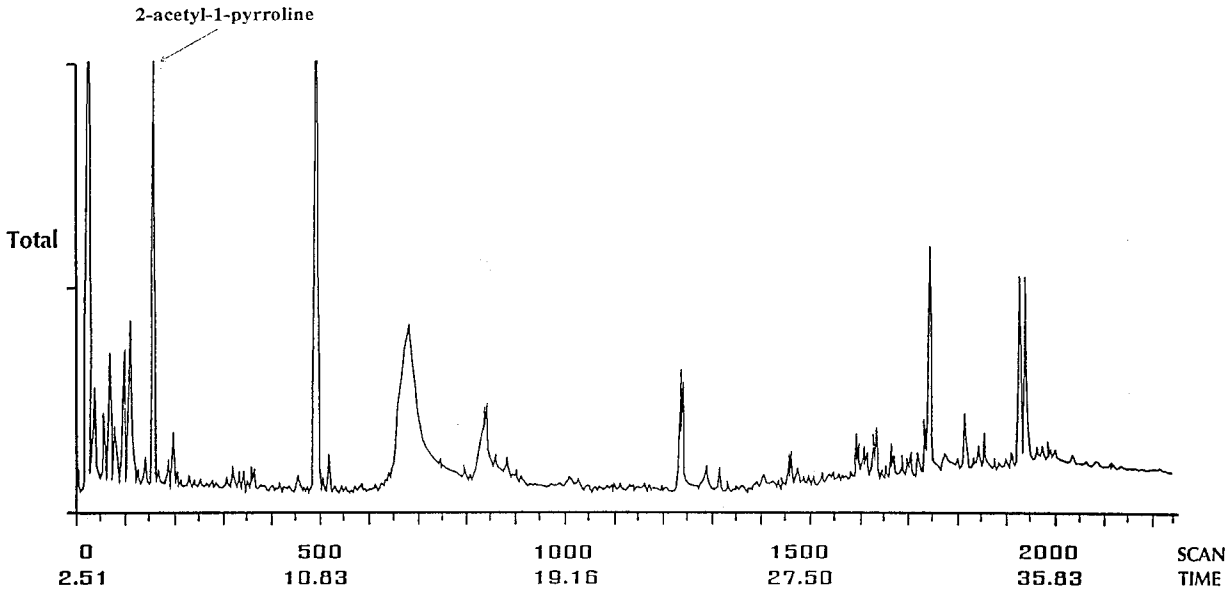

Figure 2. Reconstructed total ion chromatogram of a steam distillate extract from uncooked KDML 105 brown rice obtained by capillary GC-MS. A 30 m × 0.32 mm i.d., 0.25 μm film thickness CP-Wax 51 for amines column was used.

ketones, and other oxygenated compounds found compared with the volatile substances previously identified in cooked rice extracts of other rice varieties (*3*, *10*, *11*).

Fresh concentrated extracts of uncooked KDML 105 brown rice possessed a strong characteristic aroma, which differed greatly from the aroma of nonscented rice. These concentrated extracts when left at room temperature for a few hours had an odor similar to that of nonscented rice due to the dissipation of aromatic volatile compounds. Comparison of separate components of fresh concentrated extract and the same extract after its aromatic character had disappeared led to the identification of some volatile compounds assumed to play an important role in the characteristic aroma of KDML 105 brown rice. Some of the volatiles identified were hexanal, nonanal, butyl acetate, diethyl carbonate, butyl cyclopropane, 7-octen-4-ol, 2-(2-propoxyethoxy)-ethanol, 2AP, 1,4-dimethylbenzene, and 1-isocyanatomethylbenzene.

The presence of 2AP in the extract of brown rice not cooked during isolation has not been reported in previous studies. Accordingly, careful attention was paid to the identification method in order to identify the compound correctly. This included comparison of its mass spectral data and GC relative retention time with the synthetic 2AP under the same reproducible pressure and temperature conditions. The standard addition method of spiking the synthetic 2AP into extracts of brown rice and fresh pandan leaves was performed, and their chromatographic results were compared. Attempts

Table 1. Structural Assignments and EI-MS Data Obtained by Capillary GC-MS of a Fresh Steam Distillate Extract of Uncooked KDML 105 Brown Rice

compound	*m/e* (% relative abundance)	peak	compound	*m/e* (% relative abundance)	peak
		Hydrocarbons			
pentylcyclopropane	112, 84, 70, 69, 56, 55, 43 (100)	22	tetradecane	199, 141, 99, 85, 71, 57 (100), 43	*a*
2,4-dimethylheptane	128, 85, 71, 57, 43 (100)	*a*	2-methyltetradecane	169, 113, 99, 85, 71, 57 (100), 43	45
6-ethyl-2-methyloctane	127, 85, 71, 57, 43 (100)	*a*	4-methyltetradecane	212, 197, 169, 155, 113, 99, 85, 71 (100), 57, 43	54
2,4,6-trimethyloctane	127, 113, 99, 85, 71, 57 (100), 43, 41	47			
2,6-dimethylnonane	156, 113, 85, 71, 57, 43 (100)	*a*	5-methyltetradecane	183, 155, 141, 127, 113, 99, 85, 71, 57, 43 (100)	44
2,9-dimethyldecane	170, 155, 127, 113, 99, 85, 71, 57, 43 (100)	20	pentadecane	212, 127, 90, 85, 71, 57 (100)	55
5-ethyl-5-methyldecane	169, 155, 127, 113, 99, 85, 71, 57 (100), 43	48	2-methylpentadecane	226, 183, 99, 85, 71, 57, 43 (100)	*a*
undecane	156, 113, 85, 57 (100), 43	*a*	hexadecane	226, 169, 127, 140, 122, 99, 71, 57 (100), 43	102
5-methylundecane	170, 155, 141, 127, 113, 99, 85, 71, 57 (100), 43	49	7,9-dimethylhexadecane	254, 169, 155, 127, 99, 71, 57 (100), 43	80
2,5-dimethylundecane	184, 113, 85, 71, 57 (100), 43	*a*	heptadecane	211, 197, 169, 155, 127, 113, 99, 85, 71 (100)	119
3,8-dimethylundecane	155, 141, 127, 113, 99, 85, 71, 57 (100), 43	73	tricosane	324, 127, 113, 85, 71, 57 (100)	133
			tetracosane	338, 309, 267, 225, 169, 141, 99, 85, 71, 57 (100)	143
5,5-dimethylundecane	169, 140, 127, 113, 99, 85, 71, 57 (100), 43	68			
			2-methoxy-1-pentene	100, 85, 72, 59, 55, 43 (100)	7
2,10-dimethylundecane	184, 113, 99, 85, 71, 57 (100), 43	*a*	1,3,5-cycloheptatriene	92, 91 (100), 65, 63, 51, 39	1
4,6-dimethyldodecane	169, 155, 127, 133, 99, 85, 71, 57 (100), 43	46	1-octadecene	252, 224, 182, 139, 111, 97, 83, 69, 55, 43 (100)	140
2,5-dimethyldodecane	198, 141, 85, 71, 57 (100)	64			
		Alcohols			
1,3-butanediol	90, 75, 57, 45 (100)	*a*	7-octen-4-ol	111, 83, 55 (100), 42, 41	14
1-pentanol	70, 57, 55, 42 (100)	*a*	1-nonanol	139, 97, 83, 69, 56, 43, 40 (100)	31
1-hexanol	84, 69, 56 (100), 43	*a*	2-butoxyethanol	100, 87, 57 (100), 45	*a*
2-cyclohexen-1-ol	98, 97, 83, 70 (100)	*a*	2-(2-propoxyethoxy)ethanol	132, 89, 75, 57 (100), 45	*a*
1-heptanol	98, 70, 55 (100), 43	*a*	1-(2-butoxyethoxy)ethanol	132, 100, 87, 75, 57, 45 (100)	33
1-hepten-4-ol	96, 73, 71, 43 (100)	*a*	benzyl carbinol	122, 92, 91 (100), 65	*a*
(*E*)-2-octen-1-ol	110, 95, 82, 68, 57 (100), 41	*a*			
		Aldehydes and Ketones			
hexanal	100, 82, 72, 56, 44 (100)	3	6,10-dimethyl-5,9-undecadien-2-one	194, 151, 69, 57, 43 (100)	*a*
nonanal	127, 114, 98, 82, 70, 57 (100), 43	26			
(*E*)-2-nonenal	122, 96, 83, 70, 55, 41 (100)	*a*	1,7,7-trimethylbicyclo-[2.2.1]heptan-2-one	152, 108, 95 (100), 81, 69, 55, 41	*a*
decanal	138, 112, 82, 70, 68, 57 (100), 41	*a*			
3-hydroxy-2-butanone	88, 73, 45 (100), 43	*a*			
		Aromatics			
methylbenzene	93, 91 (100), 65, 39	1	1,3-dimethylbenzene	106, 105, 91 (100), 77, 65	*a*
ethylbenzene	106, 91 (100), 78, 65, 51	*a*	1,4-dimethylbenzene	106, 105, 91 (100), 77, 52	6
1,2-dimethylbenzene	106, 91 (100), 77, 65	*a*	naphthalene	128 (100), 102, 87, 64, 51	32
		Acids and Esters			
hexanoic acid	101, 87, 73, 60 (100), 41	17	1,2-benzenedicarboxylic acid bis(1-methyl-ethyl)ester	209, 192, 167, 149 (100), 83, 57, 41	130
2-ethylheptanoic acid	101, 88 (100), 73, 57, 55, 43, 41	29			
octanoic acid	144, 95, 87, 73, 60 (100), 41	36			
diethyl carbonate	91, 75, 63, 45 (100)	2	1,2-benzenedicarboxylic acid butyl 2-methyl-propyl ester	278, 223, 205, 167, 160, 149 (100), 93, 77, 56	134
butyl acetate	116, 87, 73, 56, 43 (100)	*a*			
		Phenolic Compounds			
phenol	94 (100), 66, 65, 39	*a*	2,4-bis(1,1-dimethyl-ethyl)phenol	206, 192, 191 (100), 175, 163, 147, 74, 57	85
2,6-bis(1,1-dimethyl-ethyl)-4-methyl-phenol	220, 205 (100), 177, 161, 145, 105, 81, 57, 83	83	2,6-bis(1,1-dimethyl-ethyl)-4-ethylphenol	234, 219 (100), 203, 159, 131, 107, 88, 57, 41	93
		Nitrogen-Containing Compounds			
2-acetyl-1-pyrroline	111, 83, 69, 68, 43 (100), 41	11	isocyanatomethylbenzene	133 (100), 105, 104, 91, 89, 78, 77, 63, 51	21
N,*N*-dimethylformamide	73 (100), 44, 42, 30, 28	*a*			
1-(1*H*-pyrrol-2-yl)ethanone	109, 94 (100), 66, 43, 39	*a*	benzothiazole	135 (100), 108, 91, 82, 63	*a*

[a] Additional compounds identified when CP-Wax 51 for amines column was used.

to confirm the presence of 2AP in brown rice extract resulted in a further study of the isolation of 2AP from uncooked KDML 105 brown rice by a simple solvent extraction procedure. This extraction technique subsequently proved to be not only convenient and inexpensive but also an efficient way to extract and quantify rice aroma.

The number of extracted components obtained by the solvent extraction method, as shown in the reconstructed total ion chromatogram in Figure 3, was less than those obtained by steam distillation when they were subjected to analysis by GC-MS using the same nonpolar capillary column, DB-5. This reduction of component complexity undoubtedly makes quantitative analysis based on peak area determination more efficient. To achieve the highest detection sensitivity of 2AP, a CP-Wax 51 for amines column was employed so that detection sensitivity of the chromatographic system was optimized. At the highest FID response, low amounts of 2AP present in the solvent extracts of only 0.5 g of brown rice were correctly detected as shown in Figure 4. However, when subjected to analysis by GC-MS using the same capillary column, the results revealed that the minimum amount of brown rice sample needed was 3 g for chromatographic detection with a proper mass spectrum of 2AP, which are shown in Figure 5.

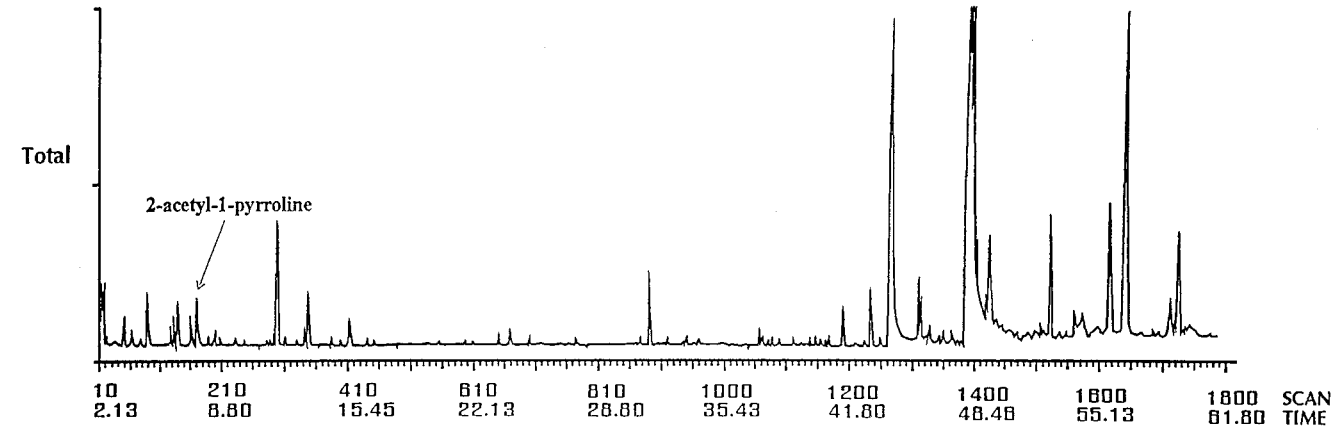

Figure 3. Reconstructed total ion chromatogram of an extract by solvent of uncooked KDML 105 brown rice obtained by capillary GC-MS using a DB-5 column.

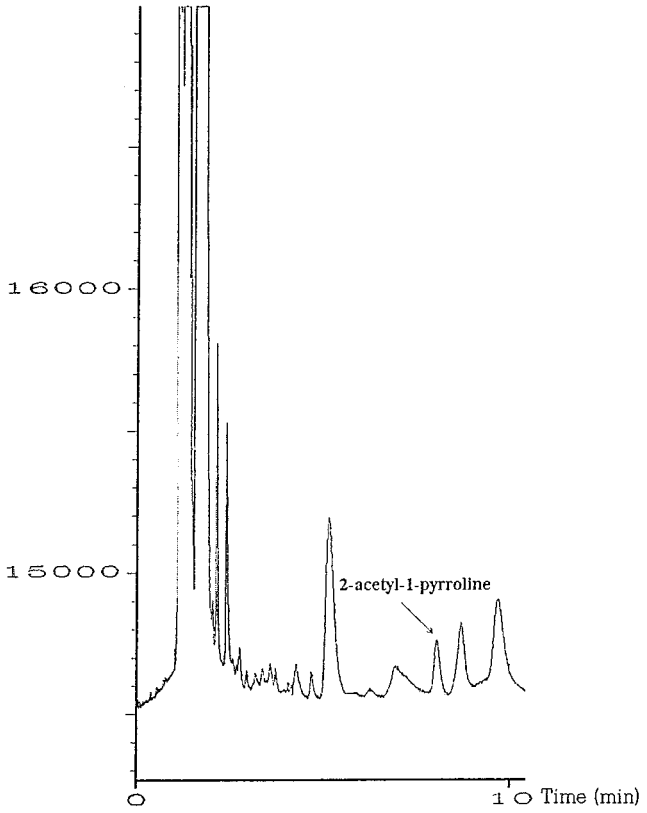

Figure 4. GC-FID chromatogram of an extract from 0.5 g of uncooked KDML 105 brown rice utilizing a solvent extraction method. A 30 m × 0.32 mm i.d., 0.25 μm film thickness CP-Wax 51 for amines column was used.

Quantitative Analysis of 2AP in the Extracts of Brown Rice. Using the basic property of 2AP, the compound was readily partitioned in a weak acid solution when ground rice seed was immersed in an appropriate quantity of 0.1 M HCl. Repeating this process one more time allowed recovery of the maximum amount of 2AP from 10 g of ground rice sample because the GC chromatogram of the extract of the same rice sample that had been extracted twice had an undetectable signal of 2AP. Removal of almost the entire amount of 2AP from the aqueous to the organic dichloromethane phase in the secondary step of the extraction procedure was done after the aqueous phase was made slightly basic by adding 1.0 M NaOH. Although a second extraction of the aqueous phase with fresh dichloromethane yielded an undetectable signal of 2AP, the reextraction process was performed in order to recover all of the 2AP from the aqueous layer. Consequently, the amounts of 2AP extracted by this solvent extraction method were assumed to be equal to the actual amounts of 2AP present in the rice samples, regardless of the minute amount lost during the solvent evaporation process.

Quantitative determination of 2AP in brown rice extracts was generally performed by using measurements of peak area with the aid of the instrument's digital integrator. Correlating peak areas with concentrations was performed by means of a standard calibration curve. To minimize errors occurring when accurate weights or volumes of samples were required, an internal standard method as reported in previous studies by other researchers was used with TMP as the internal standard in quantitative analysis of 2AP. Appropriate properties of TMP used as an internal standard were reported by Buttery et al. (*12*). As concentrations of 2AP in rice have been reported in the parts per billion range, the concentration of TMP used should be at the same level. Thus, low precision is presumably encountered when exact amounts of TMP are to be spiked into the extracts of rice. This error can be minimized in an isolation method employing solvent extraction because an internal standard is readily dissolved in the primary solvent used for extraction. For quantitative determination of 2AP in rice utilizing a solvent extraction method, the TMP solution was prepared in a large quantity as a stock solution. Thus, concentrations of TMP added into each replica of rice samples were constant, providing that volumes of extracting solvent were equally measured.

The use of an FID for a capillary GC system is known to have an advantage over other detectors in that a wide linear range useful for quantitative analysis of compounds of interest can be obtained. Figure 6 shows that a plot of concentrations of standard 2AP against peak area of 2AP divided by peak area of TMP yielded a linear calibration curve. For a solvent extraction method utilizing an acidic solvent to extract basic components, it is assumed that almost all of each basic component is extracted from the sample when an appropriate volume of solvent is used. Accordingly, the amounts of 2AP present in the rice extracts were presumed to be close to those present in the rice samples. Hence, the

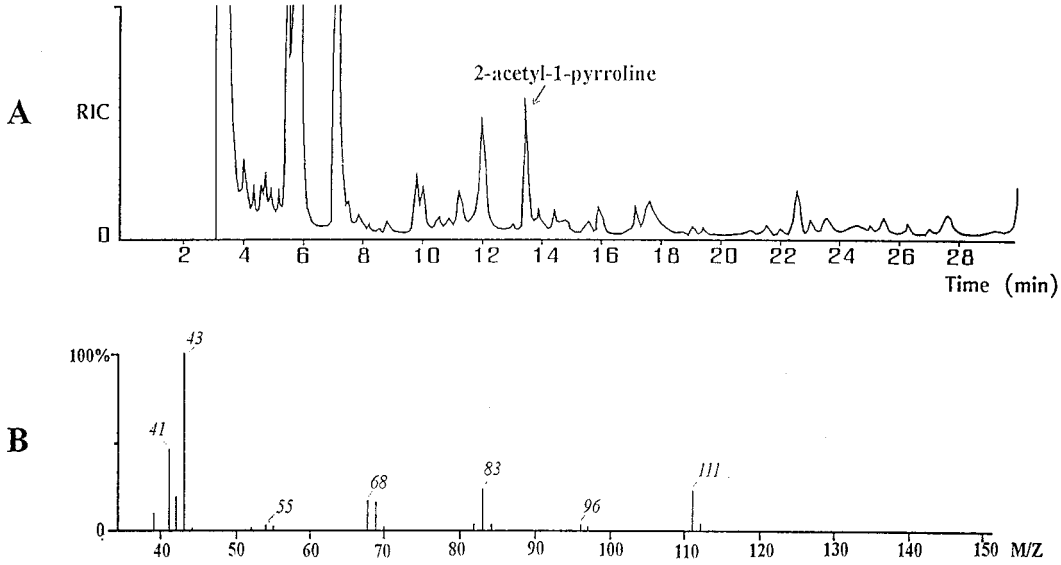

Figure 5. (A) Reconstructed total ion chromatogram of an extract by solvent from 3 g of uncooked KDML 105 brown rice obtained by capillary GC-MS using a CP-Wax 51 for amines column. (B) EI mass spectrum of 2AP present in the extract.

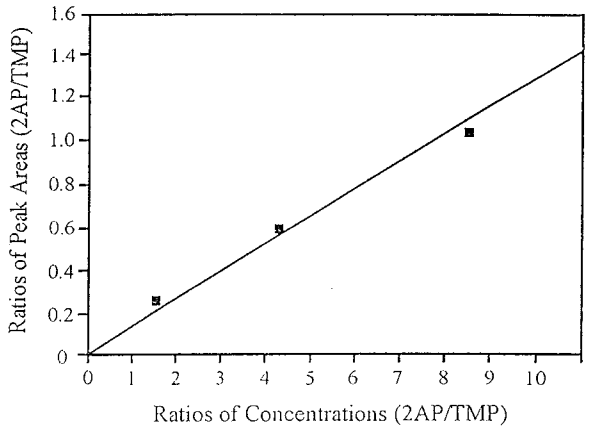

Figure 6. Relationship between the ratios of concentrations and ratios of peak areas of 2AP and TMP shows linear response of the FID used.

Table 2. Quantitative Results Obtained by Capillary GC for the Aroma Compound, 2AP, in Some KDML 105 Rice Samples

sample of KDML 105	concentration of 2AP (ppm)
fresh brown rice	0.34
brown rice stored for 12 months	0.12
brown rice from local market (no brand)	0.32
milled rice (Surintip brand)	0.25
milled rice (Matusorn brand)	0.12
milled rice (Changchuroungkhao brand)	0.05

amounts of 2AP in rice samples were obtained by using the area ratios of 2AP and TMP and plotting them on the calibration curve. Because the amount of TMP added earlier was known, the amounts of 2AP in rice samples could be readily calculated. This method was applied to the quantification of 2AP in different types of uncooked KDML 105 rice, both brown and milled; results are shown in Table 2. 2AP concentrations in fresh brown rice were found to be the highest of all the rice samples selected. This agrees with the earlier findings of Buttery et al. (*2*). The slightly higher 2AP concentrations found in this study compared with the earlier studies is believed to be due to the effectiveness of the low-temperature solvent extraction method in which the degradation of 2AP by heat was minimized. As expected, it was found that samples of rice stored for some time had decreased 2AP concentrations. The differences in aroma quality according to concentrations of 2AP found among milled rice samples were probably due to the freshness of rice as well as their harvest locations.

In comparison with the traditional steam distillation method, an inexpensive solvent extraction has advantages in its simplicity, which makes it suitable for aroma quantification of several rice samples as requested by rice breeders.

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