Rapid Method for Quantitative Analysis of the Aroma Impact Compound, 2-Acetyl-1-pyrroline, in Fragrant Rice Using Automated Headspace Gas Chromatography

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A rapid method employing static headspace gas chromatography (HS-GC) has been developed and validated for quantitative analysis of the impact aroma compound, 2-acetyl-1-pyrroline (2AP), in grains of fragrant rice. This developed method excludes wet extraction, and the rice headspace volatiles are brought directly and automatically to GC analysis. The conditions of the static HS autosampler were optimized to achieve high recovery and sensitivity. The most effective amount of rice sample used was 1 g, which provided 51% recovery and a linear multiple headspace extraction (MHE) plot of the peak area of 2AP. The sensitivity of the method was enhanced by utilizing a megabore fused silica capillary column in conjunction with a nitrogen−phosphorus detector (NPD). Method validations performed for both static HS-GC-FID and HS-GC-NPD demonstrated linear calibration ranges of 20−10 000 (r^2 = 0.9997) and 5−8000 (r^2 = 0.9998) ng of 2AP/g of rice sample, respectively. The limits of detection for both systems were 20 and 5 ng of 2AP, and the limits of quantitation were 0.30 and 0.01 g of brown rice sample, respectively. Reproducibility calculated as intraday and interday coefficients of variation were 3.25% RSD (*n* = 15) and 3.92% RSD (*n* = 35), respectively, for SHS-GC-FID and 1.87% RSD (*n* = 15) and 2.85% RSD (*n* = 35), respectively, for SHS-GC-NPD. The method was found to be effective when applied to the evaluation of aroma quality, based on 2AP concentrations, of some fragrant rice samples.

KEYWORDS: 2-Acetyl-1-pyrroline; rice aroma; static headspace gas chromatography; SHS-GC; nitrogen−phosphorus detection; NPD

INTRODUCTION

Fragrant rice has been in great demand in the Asian rice market for decades. In recent years, this rice has earned a high reputation and wide popularity throughout Europe and North America. This bright prospect for fragrant rice in the world food market has led to attempts to develop new fragrant rice varieties having improved production yield and higher aroma quality. Attention has also been paid to the management of the grain after harvesting, resulting in many designs of particular post-harvest treatments that are able to maintain the aroma character of the fragrant rice product. Along with such research, effective analytical methods employing chemical analysis and instrumentation need further development to efficiently evaluate the aroma quality of a large number of fragrant rice samples.

The characteristic aroma of fragrant rice is derived mainly from a five-membered *N*-heterocyclic ring compound, 2-acetyl-1-pyrroline (2AP). This compound was originally identified as the key aroma compound of cooked rice (*1*), with an aroma threshold value as low as 0.1 ppb in water (*2*). The compound

was also reported to be present in the volatile moiety of various processed foods (*3−16*). Since the methods of food processing usually involve heating, the occurrence of 2AP in foods has been suggested to take place during cooking at elevated temperatures via a reaction between amino acids and carbohydrates, called the Maillard reaction (*17−19*). As a result, the early quantitative studies concerning the analysis of 2AP in foods frequently utilized a heat extraction, such as steam-distillation and solvent extraction (SDS), followed by analysis of the extracts using gas chromatography-mass spectrometry (GC-MS).

Many studies concerning the genetic and chemical basis of fragrant rice indicated that 2AP is formed naturally in the rice plant. Generation of this compound in rice is controlled by a single recessive gene and occurs in all aerial parts of the plant (*20*). A study on the quantification of 2AP in uncooked aromatic rice performed by solvent extraction at room temperature and followed by capillary GC analysis (*21*) has confirmed the natural occurrence of the compound. This nonheated method was also utilized to reveal the natural occurrence of 2AP in certain parts of two aromatic plants, pandanus leaves (*Pandanus amaryllifolius* Roxb) (*22, 23*) and bread flowers (*Vallaris glabra* Ktze)

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(*23*). However, the solvent extraction method is time-consuming, as it requires many steps for sample preparation and, thus, is not appropriate when a large number of limited amount of rice samples are to be analyzed.

A technique of gas extraction, called headspace sampling, has been reviewed as a rapid and efficient technique used with capillary GC for the analysis of volatile fractions in many food samples (*24*). With regard to rice, this technique has successfully been applied to analyses of volatiles in rice foliage (*25*) and in rice cake (*26*). With the introduction of a handy headspace solid-phase microextraction (SPME), the traditional techniques were then replaced. Despite its widespread application, SPME has not been reported as a successful analytical tool for the quantitation of 2AP in grains of fragrant rice. Its main limitation was derived from the difficulties in obtaining method validation due to the poor extraction reproducibility and recovery (*27*). Another approach to headspace sampling, static headspace (SH), has also shared popularity in food and flavor research (*28*–*30*). The technique has successfully been applied to both qualitative and quantitative approaches. With static headspace sampling, sample headspace volatiles are automatically brought directly to the GC, thus offering good validation as well as the possibility for a high number of samples to be processed.

In this study, methods employing static headspace sampling prior to GC for rapid quantitative analysis of the key aroma compound, 2AP, in grains of fragrant rice were developed and validated. Optimization of the sample headspace conditions was performed to make greater recovery of 2AP from the raw rice material. The chromatographic performance of GC was made more efficient by investigation through some selected capillary columns and two types of detectors, flamed ionization and nitrogen–phosphorus. The developed method was validated for each detection system used and was further applied to the evaluation of aroma quality, based on 2AP concentrations, of some fragrant rice samples.

MATERIALS AND METHODS

Rice Sample. Fragrant rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 (KDML 105) was cultivated in a paddy of a local farm in the Surin Province in northeastern Thailand during August and November 2004. After sun drying, the paddy was hulled and milled. In the experiment for optimization of chromatographic and headspace sampling conditions, milled rice samples of 5 kg were sealed in 0.2 mm polyethylene bags and kept under ambient conditions for 1 month before the experiment. A nonfragrant rice cv. Pijit was used as supporting material in the calibration procedure. It was grown in a paddy of the experimental farm of the Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand.

The grains used as rice samples to be analyzed by the developed SHS-GC methods for quantification of 2AP were divided into three groups: KDML 105 brown and milled rice from Surin Province cultivated during August and November 2005 and kept as whole grains for 1 month before the experiment, a set of Indian Basmati rice, and a set of Thai Hom Mali rice. The latter two groups of rice samples were obtained as shelf products from local markets and department stores in the Chiang Mai Province, Thailand.

Chemicals and Reagents. All solvents used were analytical-reagent grade and purchased from the following sources: benzene and methanol from Merck (Darmstadt, Germany) and benzyl alcohol from Fisher (Loughborough, UK). 2,6-Dimethylpyridine (2,6-DMP) and 2,4,6-trimethylpyridine (TMP) used as internal standards were 99% pure and were purchased form Merck (Schuchardt, Germany). 2-Acetylpyrole (98% purity), 5% rhodium on an activated alumina catalyst (99% purity), and celite were purchased from Fluka (Buchs, Switzerland). Silver carbonate was purchased from Aldrich (Milwaukee, WI).

2AP, used as the standard compound, was synthesized as outlined by Buttery and co-workers (*22*) with some modifications. The final product obtained in a solution of toluene was further purified by separation on a packed column of a Varian gas chromatograph, model 2000 (Walnut Creek, CA) and collected in a 3 mm o.d. Pyrex tube. This tube was then sealed under a N_2 atmosphere and kept at -20 °C. Purity and chemical structure of the synthetic 2AP were confirmed by capillary GC-MS and by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The exact weight of the purified 2AP was diluted in 1.0 mL of deuterated benzene (Aldirch, Steinheim, Germany) and spiked with a known quantity of tetramethylsilane (TMS) (Aldrich, Steinheim, Germany) used as an internal standard prior to analysis by ^{1}H NMR. The quantity of the synthetic 2AP was obtained by calculating the integrated proton signal of the methyl group of 2AP against those of the TMS.

GC Instrumentation and Conditions. Static HS-GC analyses were carried out using an Agilent Technologies (Wilmington, DE) model 6890N gas chromatograph equipped with an Agilent Technologies model G1888 headspace autosampler, a flame ionization detector (FID), and a nitrogen–phosphorus detector (NPD). A sample headspace was collected through a 3 mL sample loop and automatically transferred to the GC via a heated transfer line. A split/splitless GC injector equipped with a direct 0.2 mm i.d. glass liner was used. The system operation, as well as data acquisition, collection, and evaluation, were accomplished using an Agilent ChemStation software version A.01.04 and B.01.03 (Agilent Technologies, Waldbronn, Germany).

The preliminary headspace autosampler conditions used for the SHS-GC-FID operation were as follows: oven temperature, 120 °C; vial equilibration time, 9 min with high-speed shaking; loop filling time, 0.10 min; pressurizing time, 0.50 min; and injection time, 0.50 min. The sample loop and transfer line temperatures were set at 10 and 20 °C higher than the oven temperature, respectively. When the SHS-GC-NPD was utilized, the loop filling time, pressurizing time, and injection time were set at 0.01, 0.10, and 0.40 min, respectively.

Three chromatographic columns of the fused silica capillary type were investigated. These were DB-1701 (15 m × 0.32 mm i.d. × 0.15 μm film thickness), DB-35MS (30 m × 0.53 mm i.d. × 1.0 μm film thickness), and HP-5 (30 m × 0.53 mm i.d. × 1.5 μm film thickness) columns (J&W Scientific, Folsom, CA). The optimum GC conditions were achieved using an HP-5 column with a splitless injection at 230 °C. For GC-FID, the column temperature program began at 50 °C. The temperature was increased at a rate of 1 °C/min to 70 °C. The FID temperature was 250 °C. When the NPD was utilized, its temperature was set at 275 °C. The column temperature was initially at 50 °C, and it was increased to 125 °C at a rate of 5 °C/min. The carrier gas flow rate was 7 and 5 mL/min for GC-FID and GC-NPD, respectively.

Sample Preparation. The KDML 105 rice samples were chilled at 4 °C for 24 h before they were ground and screened through an Endecotts test sieve (Endecotts Ltd., London) having an aperture size of 150 μm. The exact weight of rice powder was placed into a 20 mL headspace vial, followed by the addition of 1.00 μL of 0.50 mg/mL 2,6-DMP in benzyl alcohol using the open vial sample introduction technique (*31*). The headspace vial was then sealed immediately with a PTFE/silicone septum and aluminum crimp cap. It was shaken well at a room temperature of 27 °C for 10 min prior to analysis by SHS-GC.

Multiple Headspace Extraction (MHE) Procedure. KDML 105 brown rice and mill rice were prepared as described in the sample preparation step. The rice samples weighed 1.000 g each and were subjected to MHE, each of which was followed by GC-FID analysis. In this experiment, a set of consecutive analyses from the same rice sample vial was carried out. After each sampling, part of the remaining headspace was removed so that the pressure in the vial returned to atmospheric pressure or close to it, and then the rice sample vial was allowed to re-equilibrate. For each rice sample, MHE with six extraction steps was performed. The SHS-GC-FID conditions used were the optimized conditions obtained previously.

Calibration Procedure. A series of 2AP standard solutions having concentrations of 10.0, 8.00, 6.00, 4.00, 2.00, 1.00, 0.50, 0.20, and 0.10 mg/mL in benzyl alcohol was prepared. One microliter of each

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solution was added to a headspace vial containing 1.000 g of the nonscent rice (cv. Pijit) powder used as a supporting material. The internal standard, 1.00 μL of 0.50 mg/mL 2,6-DMP in benzyl alcohol, 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. Five replications were done 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.

RESULTS AND DISCUSSION

In some other methods of 2AP analysis, the compound is extracted from sample materials mainly by steam-distillation or solvent extraction. The extracts are then modified to follow the specific requirement of a particular analytical technique, resulting in many steps of sample preparation. This analytical complication, apart from being time-consuming, certainly affects recovery of the aroma compound and is followed by the lost of method sensitivity. As most aroma chemicals are volatile, procedures for their isolation from foods have been established that take advantage of this volatility. Being a low molecular weight volatile aroma substance, 2AP is readily transferred to a gas phase when subjected to an elevated temperature. Hence, direct introduction of 2AP in rice sample headspace for GC analysis, where extraction can be accomplished in one step, in addition to its simplicity and automated operation, can offer an appropriate solution to the problem. However, method development has to be performed to achieve the preferred recovery, sensitivity, and validation.

Optimization of Chromatographic Conditions. KDML 105 brown rice instead of the milled rice was used as the rice sample for this experiment because the rice bran is included with the grain. This maximizes the number of volatiles present in the headspace. For the analysis of 2AP in the rice headspace using GC-FID, separation of 2AP from the other rice headspace volatiles was optimized by using three types of fused silica capillary columns. First, a DB-1701 (14% cyanopropylphenyl, 85% dimethylpolysiloxane) column having an intermediate polarity phase was used. Then, 2AP was eluted at 9.19 min but overlapped with the other volatiles in the rice headspace. A more polar DB-35MS (35% phenyl, 65% methyl arylenesiloxane) capillary column was then utilized in an attempt to alter the elution order of 2AP. Still, the coelution was observed between 2AP and sample matrix volatile identified as hexanoic acid. The coexistence of these compounds in the same peak was revealed using the GC-MS single ion chromatogram technique. Finally, a column with a lower polarity stationary phase, HP-5 (5% phenyl, 95% dimethylpolysiloxane), and with a greater volume dimension was used. This megabore type column is generally preferred for headspace analysis because it can be operated at higher carrier gas flow rates so that peak broadening is reduced. Moreover, the thicker film stationary phase, which has a major advantage of higher solute capacities, is more compatible with volatile analysis. This necessitates a resolution for the highly volatile components as the retention time increase.

As compared to the two capillary columns investigated before, greater sensitivity of 2AP was obtained with the HP-5 megabore column. The 2AP peak was clearly separated from the other matrix volatiles. However, an attempt to utilize TMP as the internal standard, as reported previously in many studies, failed in this experiment mainly because of peak overlapping between TMP and some other matrix volatiles. Therefore, only 2,6-DMP was used as an internal standard.

A good separation of the headspace volatiles of KDML 105 brown rice added with 1.00 μL of 0.50 mg/mL 2,6-DMP in

benzyl alcohol was obtained on the HP-5 megabore column. The 2,6-DMP and 2AP were eluted at 10.91 and 13.73 min, respectively. Peak purities of both compounds were confirmed by mass spectral data obtained by SHS-GC-MS analysis. The HP-5 column was, thus, chosen for further method development since it provided a good compromise between acceptable resolution, sensitivity, and analysis time.

To reduce problems with peak overlapping that is usually caused by the rather complex nature of rice headspace volatiles, the use of NPD, a more selective GC detector, was investigated. The GC chromatogram of the same rice headspace volatiles obtained by GC-NPD showed less complexity of the peak signals than that obtained by GC-FID. The 2,6 DMP and 2AP were eluted at 8.12 and 9.21 min, respectively. The use of NPD as a detector for GC not only provides a higher detection sensitivity for 2AP but also a shorter analysis time and higher chromatographic resolution.

MHE Plots. As rice samples to be analyzed are in solid form, quantitative analysis of the aroma compound, 2AP, by SHS-GC is possible if the rice samples can be treated as part of a partition system where the distribution coefficient of 2AP is constant and independent of its concentration. This can be investigated by performing a multiple headspace extraction (MHE) experiment in which a linear MHE plot should be resulted. Nonlinearity of the MHE plot, on the other hand, indicates that the distribution coefficient is dependent on concentration and that adsorption effects are present. A sequential analysis of a number of headspace samples taken from the same sample vial was performed. After each HS-GC analysis, the vial was opened, allowing the pressure to be reduced to atmospheric. Then, it was sealed again and left for the re-equilibration to take place before the next analysis by HS-GC. The areas under the 2AP peak obtained in consecutive measurements were plotted following the equation (*31*)

$$\ln A_i = -q(i - 1) + \ln A_1$$

where i represents the extraction step number and A is the peak area obtained. The exponent q describes the exponential decline of the peak areas during the MHE procedure.

In this experiment, MHE analysis was performed on both brown and milled rice samples, and peak areas of 2AP were calculated. The linear MHE plots of 2AP peak areas were obtained with regression coefficients of -0.9875 for brown rice and -0.9895 for milled rice (**Figure 1**). The direct analysis of 2AP in rice by SHS-GC was, consequently, practicable without any effects of adsorption by the nature of the rice sample and its matrix.

Optimization of Static Headspace Conditions: Effect of Sample Amount. Normally, sensitivity of the method is dependent on the sample amount and the extraction efficiency. In headspace analysis, the peak area obtained when analyzing an aliquot of the headspace is directly proportional to the concentration of the analyte in the headspace. This follows the expression (*31*)

$$\mathrm{A} \propto C_G = C_O/(K + \beta)$$

where C_G is concentration of the analyte in the headspace and C_O is its original concentration in the sample, K is the partition coefficient of the analyte between the sample and the headspace, and β is the phase ratio relating the volume of the gas phase in the vial to the volume of the condensed phase or rice sample.

According to the previous equation, sensitivity of SHS-GC method depends on the combined effect of K and β. For this
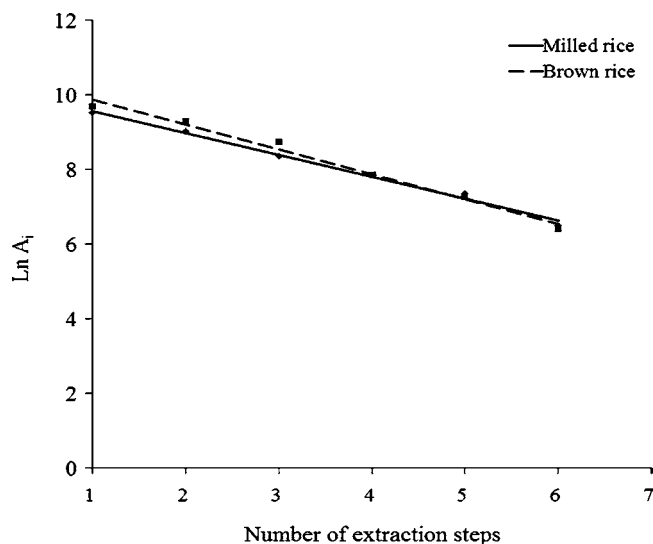
Figure 1. Linear regression plots for MHE measurements of 2AP in the brown and milled rice samples.

reason, the experiment was carried out by varying the rice sample amount. The influence of the phase ratio on the analytical result was investigated using a constant temperature. The size of the analyzed rice sample was limited to 8 g to prevent the needle of the headspace sampling system from protruding into the rice sample. **Figure 2** shows the relationship between peak areas of 2AP and phase ratios at different rice sample amounts up to 8 g. With the increase in sample amount, the peak areas of 2AP increased exponentially, while the phase ratio values decreased in the same manner. However, the most suitable amount of rice sample will be the amount that provides the highest percentage recovery of 2AP in the rice headspace as well as a linear MHE plot. Thus, percentage recovery of 2AP in the first headspace extraction step, or the peak area of 2AP relative to the sum of peak areas in the consecutive MHE measurements, was determined for each rice sample amount. Results show that the highest recovery was 51% when a rice sample of 1 g was used. A linear MHE plot with this sample amount was also obtained. Therefore, further experiment was performed using 1 g rice samples.

Effect of Equilibrium Temperature and Time. Apart from the phase ratio, there are many instrumental parameters of the headspace autosampler that can affect the sensitivity, precision, and accuracy of static headspace analysis. These include the temperature of the headspace oven, transfer line, and sample loop; the time of vial equilibration, pressurization, loop filling, and injection; and the sample shaking speed. Good chromatographic data, maximum recovery, sensitivity, and time saving were selected as criteria for optimization of these parameters. One gram of KDML 105 brown rice spiked with 1.00 μL of 0.50 mg/mL 2,6-DMP in benzyl alcohol was used as a model sample for these trials.

To manipulate the headspace concentration of 2AP, two parameters can easily be changed. These are the sample volume in a headspace vial and the equilibrium temperature. Since 1 g of the rice sample was previously determined as the optimum amount, the sample volume and phase ratio were constant. However, the time that a rice sample is subjected to a certain temperature has to be investigated to obtain an appropriate equilibrium time at each vial temperature. **Figure 3** shows peak areas of 2AP obtained by varying the temperature of a rice sample and the heating time. The equilibration temperature (headspace oven or vial temperature) and time had a consider-

able effect on the peak area of 2AP. The maximum responses of peak areas of 2AP at vial temperatures of 100, 120, 140, and 160 °C were obtained at equilibration times of 13.0, 7.0, 4.0, and 2.0 min, respectively. The higher the vial temperature, the shorter the equilibration time required for 2AP to reach equilibrium. However, the highest response of 2AP was obtained at an oven temperature 120 °C, and the equilibration time required for the maximum response at this temperature was 9.0 min. Although the easiest way to increase the peak intensity is to raise the oven temperature, heating the rice sample above 120 °C yielded increasing complexity of the rice headspace volatiles as compared to those at lower temperatures. This complexity may be caused by thermal degradation of some rice components resulting in consequent products eluted as interferences in the analysis. Considering this, a headspace oven temperature of 120 °C and a vial equilibration time of 9.0 min were used for the next steps of this study.

Effect of Time for Loop Filling and Injection. The amount of headspace sample to be analyzed by GC is related to both the volume of the sample loop used and the time needed for the gas sample to fill the sample loop (loop filling time). In this study, the sample loop size was fixed at 3 mL. The effect of loop filling time on the 2AP peak area was investigated for two pressurizing times, 0.50 and 1 min. For each of these pressuring times, loop filling was done at 0.01 min and 0.05−0.25 min at 0.05 min intervals. **Figure 4** shows the dependence of peak area of 2AP on a series of loop filling times at pressurizing times of 0.50 and 1 min. The magnitude of the 2AP peak area decreased as the loop filling time increased, and the maximum response of 2AP was obtained at a loop filling time of 0.01 min, regardless of the pressurizing time. This means that the distribution of masses occurs as the rice headspace is being forced to travel to the sample loop, and the lower masses, such as 2AP, reach the loop volume in a shorter time.

With a SHS-GC employing a pressure-loop sampling system as utilized in this study, the volume of headspace gas to be transferred to the GC is also controlled by the loop flushing time (injection time). The longer the injection time, the more headspace gas is allowed to reach the GC injection system. However, if the injection time is too long, some of headspace volume can be lost. To compensate for these effects, the optimum injection time was determined by varying the time in the range of 0.10−0.50 min at 0.10 min intervals. Results indicated that an injection time of 0.40 min yielded the highest count of peak area of 2AP. The optimum values of all headspace autosampler parameters are temperature of oven 120 °C; temperature of loop 140 °C; temperature of transfer line 160 °C; vial equilibration time 9 min with high-speed shaking; loop filling time 0.01 min; loop equilibration time 0.6 min; pressurizing time 0.1 min; and injection time 0.40 min.

Method Validation. Two analytical methods were developed for quantitative analysis of 2AP in fragrant rice using SHS-GC. These were SHS-GC-FID and SHS-GC-NPD, and they were validated with respect to linearity, range, sensitivity (limits of detection and quantitation), and precision. A nonfragrant rice (cv. Pijit) was subjected to analysis by SHS-GC using the optimum conditions obtained previously. It yielded a volatile component profile that closely matched that of the fragrant rice KDML 105 except for the absence of 2AP. This revealed that no additional 2AP was formed by heating the rice at the optimum conditions. One gram of the Pijit rice powder was added with an exact amount of standard 2AP and then spiked with 1.00 μL of 0.50 mg/mL 2,6-DMP in benzyl alcohol and then was used for this assay. The relationship between detector

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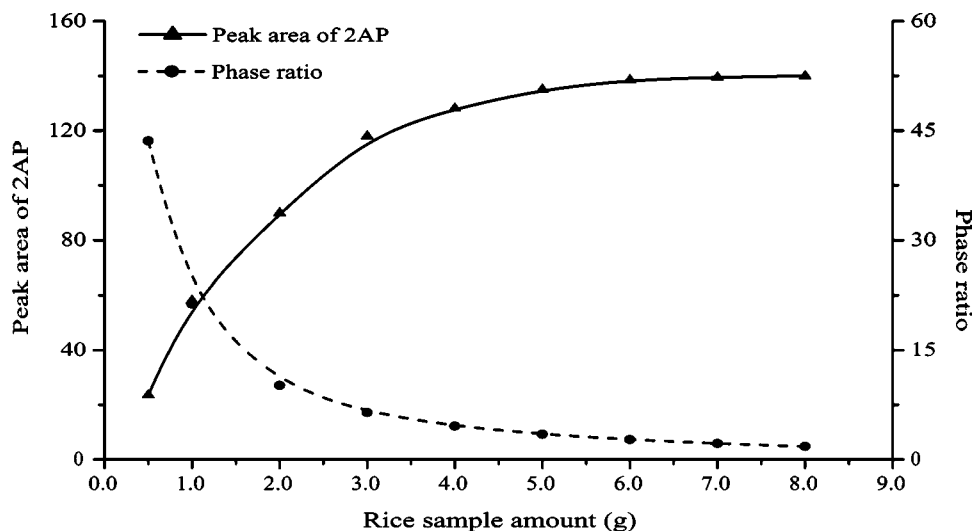*J. Agric. Food Chem.,* Vol. 54, No. 21, 2006 **8187**

Figure 2. Variation of phase ratio and peak area of 2AP vs the amount of solid rice sample. Headspace parameters: static mode; equilibration temperature 120 °C; 9 min equilibration time; 0.5 min pressurization time; 0.1 min loop filling time; and 0.5 min injection time.
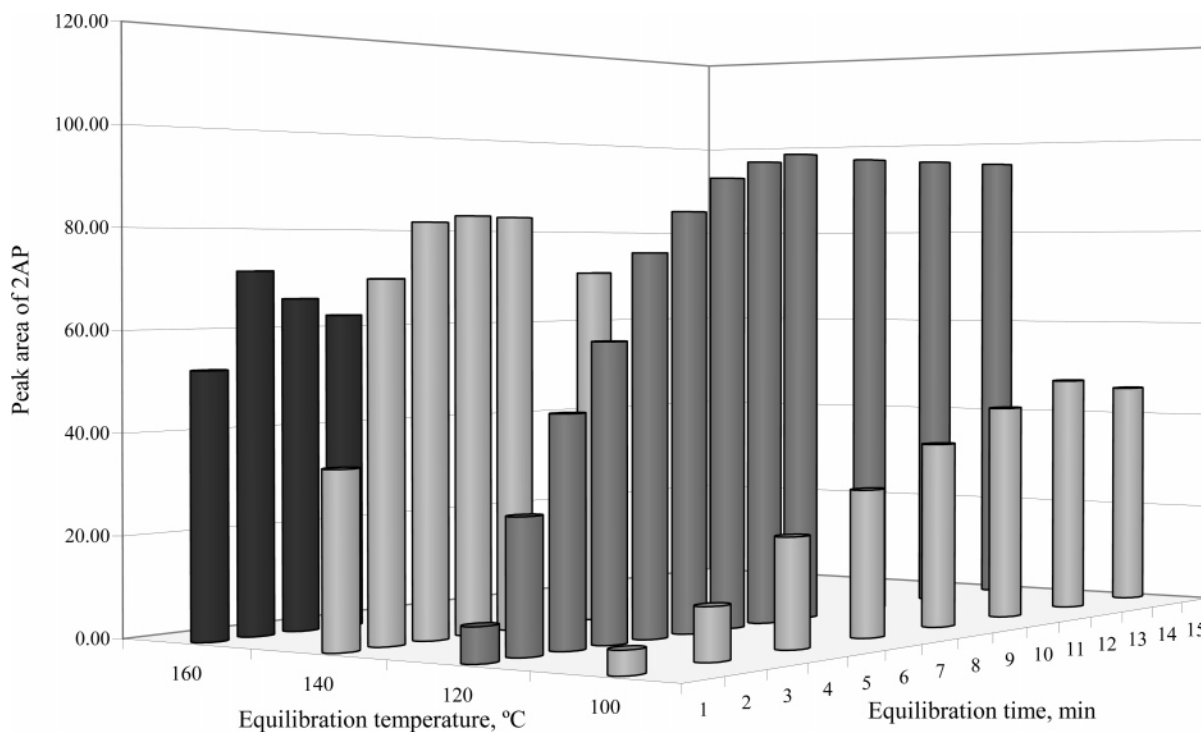

Figure 3. Effect of equilibration temperature and time on the concentration of 2AP in the rice headspace measured in terms of peak area of 2AP. Headspace autosampler conditions: static mode; equilibration temperature varied from 100 to 160 °C; equilibration time varied from 1 to 25 min; 0.5 min pressurization time; 0.1 min loop filling time; 0.5 min injection time; and 1 g of rice sample amount.

responses measured in terms of peak area ratios between 2AP and 2,6-DMP and the amount of standard 2AP was linear and reproducible for both FID and NPD with a correlation coefficient (r^2) of 0.9997 and 0.9998, respectively. The effective linear concentration ranges of the method were from 20 to 10 000 ng of 2AP/g of rice sample for SHS-GC-FID and from 5 to 8000 ng of 2AP/g of rice sample for SHS-GC-NPD.

Sensitivity is reflected by the limit of detection (LOD) and limit of quantitation (LOQ) defined as the concentration at which $S/N \geq 3$ in terms of the least amount of 2AP and the concentration at which $S/N \geq 10$ in terms of the least amount of rice sample, respectively. Analysis of a series of 2AP standard solutions by SHS-GC-FID and NPD had a LOD at 20 ng with a relative standard deviation (RSD) of 3.51% and 5 ng with a RSD of 2.93%, respectively. The LOQ determined was 0.30

and 0.01 g of brown rice with RSD of 3.73 and 2.09%, for SHS-GC-FID and NPD, respectively.

The intraday and interday coefficients of variation determined using brown rice samples were 3.25% RSD ($n = 15$) and 3.92% RSD ($n = 35$), respectively, for SHS-GC-FID and 1.87% RSD ($n = 15$) and 2.85% RSD ($n = 35$), respectively, for SHS-GC-NPD. Overall, the validation data confirm higher sensitivity and usefulness of the GC-NPD system over the GC-FID system.

Analysis of Rice Samples. The developed SHS-GC method was applied for quantitative analysis of 2AP in three groups of fragrant rice samples: KDML 105 brown and milled rice, a set of Indian Basmati shelf rice, and a set of Thai Hom Mali shelf rice. Triplicate determinations were performed for each rice sample. **Table 1** shows concentrations of 2AP found in all types of rice samples studied. The RSDs of three replicates of each
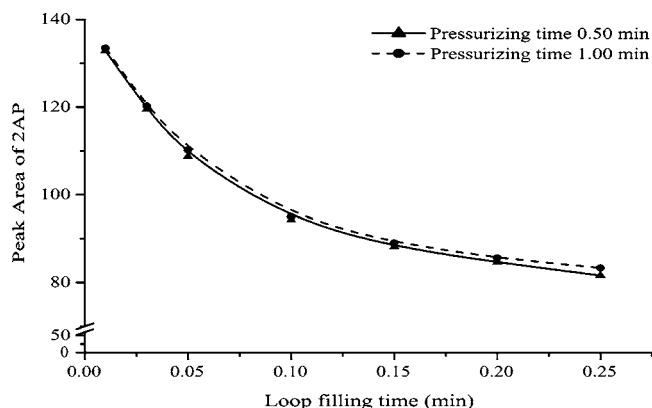
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Figure 4. Effect of loop filling time at pressurizing times of 0.50 and 1 min on the concentration of 2AP in the rice headspace measured in terms of peak area of 2AP. Headspace autosampler conditions: static mode; 120 °C equilibration temperature; 9 min equilibration time; 0.5 and 1 min pressurization times; loop filling time varied from 0.01 to 0.25 min; 0.5 min injection time; and 1 g of rice sample amount.

Table 1. Quantitative Analysis of 2AP in Fragrant Rice Samples

	concentration of 2AP, μg/g (mean $\pm$ SD)	
rice samples	*GC-FID*	*GC-NPD*
	KDML 105	
brown rice	3.83 $\pm$ 0.04	3.72 $\pm$ 0.07
milled rice	2.37 $\pm$ 0.05	2.32 $\pm$ 0.04
	Shelf Rice	
Indian Basmati, no. 1	0.26 $\pm$ 0.01	0.28 $\pm$ 0.01
Indian Basmati, no. 2	0.27 $\pm$ 0.01	0.29 $\pm$ 0.01
Indian Basmati, no. 3	0.38 $\pm$ 0.01	0.41 $\pm$ 0.01
Thai Hom Mali, no. 1	0.76 $\pm$ 0.01	0.78 $\pm$ 0.01
Thai Hom Mali, no. 2	0.91 $\pm$ 0.02	0.90 $\pm$ 0.01
Thai Hom Mali, no. 3	0.68 $\pm$ 0.02	0.69 $\pm$ 0.01

rice sample were acceptable, being in the range of 0.29−3.92%. The excellent agreement was obtained for the concentrations of 2AP in the fragrant rice samples determined by SHS-GC-FID and SHS-GC-NPD. The concentrations of 2AP in the fragrant rice KDML 105 were higher than those of Indian Basmati and Thai Hom Mali shelf rice. These results confirm the earlier report (*32*) that the storage time plays an important role on the aroma quality of fragrant rice. Also, the brown rice samples had higher concentrations of 2AP than did samples of the milled rice.

The method employing static headspace GC has been developed and has proven to provide an effective means for simple and rapid quantitative analysis of 2AP, the impact aroma compound of rice. The accuracy obtained by using a similar type of compound (2,6-DMP) as an internal standard for quantitation of 2AP is not comparable to those methods employing stable isotope-labeled analog and mass spectrometric detection (*33−35*), where differential recoveries of the analyte and internal standard compound can be avoided. This method has advantages over other methods that employ steam-distillation or solvent extraction in that the sample preparation step and the total analysis time are much shorter and it is fully automated and solvent-free. This newly developed method offers satisfactory recovery and sensitivity of 2AP for the utilization of both FID and NPD as a GC detector. Additionally, SHS-GC-NPD provided higher selectivity and sensitivity than previously reported methods and made the analysis of 2AP as low as 5 ng possible. The optimized headspace autosampler parameters enable the use of an internal standardization method for quantitation without any effects of matrix adsorption. The

superiority of this method in terms of simplicity, sensitivity, and precision makes it a suitable laboratory tool for routine analysis of the rice aroma compound and, thus, the aroma quality of fragrant rice products.

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