Screening for 2-Acetyl-1-pyrroline in the Headspace of Rice Using SPME/GC-MS

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Solid phase microextraction (SPME) is used to collect and concentrate the compounds in the headspace of rice. This research describes optimization parameters of temperature, moisture, and sampling time. Optimization was based upon the recovered levels of 2-acetyl-1-pyrroline (2-AP), the popcorn aroma in aromatic rice. The method uses a sampling temperature of 80 °C and adds 100 μL of water to a 0.75 g sample of rice. The rice was preheated for 25 min, a carboxen/DVB/PDMS SPME fiber was exposed to the headspace for 15 min, and a subsequent GC-MS analysis took 35 min. Samples of rice can be analyzed as the flour, milled kernels, or brown rice. Twenty-one experimental rice varieties were analyzed by the SPME method and compared to a wet technique. Recoveries of several nanograms of 2-AP from 0.75 g samples of aromatic rice were observed, whereas only trace amounts of 2-AP were recovered from nonaromatic rice. Recovery from a single SPME headspace analysis is calculated to be 0.3% of the total 2-AP in the sample.

Keywords: *Solid phase microextraction (SPME); rice; volatiles; 2-acetyl-1-pyrroline; headspace; gas chromatography−mass spectrometry*

INTRODUCTION

By nature, compounds that elicit an aroma are volatile. Standard methods of analysis employ schemes to capture volatile compounds, concentrate them, separate them, and quantify them. Recent advances in analytical instrumentation and methodology have approached the threshold of selectivity and sensitivity demonstrated by the human nose. In this paper, new methodology is employed to measure some of the key odorants in rice.

Several hundred compounds can be observed in the headspace of cooked rice, and >100 have been identified (*1*−*3*). The chromatographic profile is dominated by lipid oxidation products. Only a few compounds have been found to have an impact on the flavor of cooked rice. Yajima et al. (*4*) identified α-pyrrolidone as a key odorant in Kaorimai (scented rice *O. sative japonica*) and noted the presence of indole. Buttery et al. (*2*) listed 64 volatile compounds known in rice and identified 7 compounds with low odor thresholds: (*E*,*E*)-2,4-decadienal (T = 0.07 ppb), (*E*)-2-nonenal (T = 0.08 ppb), (*E*)-2-decenal (T = 0.4 ppb), nonanal (T = 1.0 ppb), octanal (T) = 0.7 ppb), decanal (T = 2 ppb), and 2-acetyl-1-pyrroline (2-AP), the latter being the major odorant contributor of scented or popcorn rice. Widjaja et al. (*5*), in a comparative study of a nonfragment and fragrant rice, identified (*E*)-2-decenal, (*E*,*E*)-2,4-nonadienal, and (*E*,*E*)-2,4-decadienal as having a "waxy"-like aroma. Of the identified compounds and associated odors, 2-AP plays the greatest role in the sensory quality of rice, and measurement of its concentration can be used to differentiate between a scented and a nonscented rice.

The effective collection and analysis of these volatile compounds can now be accomplished using solid phase microextraction (SPME). SPME has proven to be a simple, rapid, and sensitive method for collecting the volatile compounds from the headspace of a sample (*6*). However, initial efforts to use SPME to measure rice volatiles proved to be less than satisfactory (*3*). The limitation was that the initial stationary phases used as coatings for SPME fibers were limited to temperatures slightly above ambient. Placing the fiber in the effluent (dynamic SPME) of a sample purged with nitrogen improved the recovery of volatile compounds. However, quantitation was problematic as the additional variables of purge flow rate, path, and time contributed to increased variance. The introduction of SPME fibers with the capability of operating at higher temperatures has resulted in improved collection of volatile compounds from the headspace of rice. Compounds not observed at room temperature can now be generated, collected, and concentrated in a single step. Additionally, heating the sample shifts the concentration equilibrium toward the gas phase, thus enhancing sensitivity.

In theory, under ideal conditions, the volatile compounds in a liquid sample partition between the liquid and gas phases well as the gas phase and the SPME fiber. After sufficient time, equilibria are established between the sample and the headspace and between the headspace and the SPME fiber (*7*). In liquid samples, the equilibria can be shifted by adjusting the temper-

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Table 1. Target and Qualifier Ions Used for Peak Area Determinations for 2-AP and TMP

compound		target	Q1	Q2
2-AP	ion (*m*/*z*)	83	111	68
(111 Da)	abundance (%)	(100)	(43)	(45)
TMP	ion (*m*/*z*)	121	106	79
(121 Da)	abundance (%)	(100)	(16)	(26)

ature, using mechanical mixing, and the addition of salts, primarily NaCl. In a sample consisting of a complex matrix such as food, the ideal conditions are not readily achieved because the absolute concentration of a given compound is not fixed. Compounds are continually formed and broken down through a myriad of complex chemical reactions as a function of temperature, moisture, and pressure. However, relative quantitative information can be obtained by treating the samples under consistent conditions and measuring the resultant production/release of the volatile compounds.

This paper focuses on the rapid screening of rice cultivers for the relative amounts of 2-AP. Current sample preparation methods for determining the concentrations of 2-AP in rice include purge and trap (*2*), steam distillation−solvent extraction (*8*), and solvent extraction followed by direct injection (*9*).

MATERIALS AND METHODS

Method development was performed using milled Jasmine rice, purchased at a retail store and kept at −10 °C until analyzed. Whole rice grains were analyzed as is, whereas flour samples were ground immediately before analyses. Sample preparation consisted of placing 0.75 g of rice directly into a 2 mL vial. Water was added to the sample by spraying Milli-Q water onto the top of the rice kernels. 2,4,6-Trimethylpyridine (TMP; Sigma-Aldrich, St. Louis, MO) was employed as the internal standard. A 2 μL aliquot of a 1 ppm solution was added to each sample, thus effectively placing 2 ng of TMP in each vial. The standard was placed on the inside of the glass vial just below the neck. Following preparation, samples were placed in an autosampler tray and maintained at room temperature until analyzed. Samples were preheated for 25 min at 80 °C prior to sampling. Collection of volatile compounds was accomplished using a 15 min adsorption period at 80 °C during which the sample was shaken. The SPME fiber employed was a 1 cm 50/30 carboxen/DVB/PDMS fiber (Supelco, Bellefonte, PA). A CTC SPME autosampler equipped with a heated sample shaker and a needle heater for thermal cleaning of the SPME fiber was employed (Leap Technologies, Carrboro, NC).

Samples were desorbed for 5 min on an HP 5973 GC-MS system (Agilent Technologies, Palo Alto, CA), The injector temperature was held constant at 270 °C. The GC oven temperature was held for 1 min at 50 °C and then ramped to 250 °C at 10 °C/min. A 30 m, 0.25 μm, DB-5 capillary column was used with helium as the carrier gas under a constant flow of 40 cm/s. The total GC cycle time consisted of a 30 min run and a 5 min cooling period. Following the first GC-MS run, subsequent samples were prepared ahead so that one sample was run every 45 min. The mass spectrometer was operated in scan mode from *m*/*z* 50 to 350. Peak areas were determined for each compound by integrating a selected ion unique to that compound. Target and qualifying ions (Q1 and Q2) employed for the quantification of 2-AP and TMP are given in Table 1. The 2-AP standard was dissolved in $CHCl_3$ and not amenable to SPME analysis. Therefore, serial dilutions were made in $CHCl_3$, and triplicate injections at 0.1. 0.5, 1, 5, and 10 ng of 2-AP were made to generate a calibration curve. An r^2 of 0.9673 was obtained, and a 2 ng spike of TMP in each standard resulted in a relative standard deviation of 6.4% for all samples.

Solvent extraction was accomplished according to the method of Bergman et al. (*9*). Briefly, 0.3 g of ground rice was placed in a 2 mL vial, to which 500 μL of a 459 ng/μL solution of TMP in $MeCl_2$ was added. The TMP served as the internal standard. The sample vials were heated to 85 °C for an extraction period of 2.5 h. A 2 μL injection was then made, and the sample was analyzed by GC, equipped with a flame ionization detector.

Rice cultivers and breeding lines used to evaluate the SPME method were grown in the U.S. Uniform Regional Nursery, in Mississippi, during the 1998 crop year. Both brown rice and milled rice were analyzed in duplicate and reported on an as is moisture basis. The 2-AP standard was a generous gift from Dr. Ron Buttery (USDA-ARS-WRRC, Albany, CA).

RESULTS AND DISCUSSION

Unlike environmental samples, in which the target analytes are present in finite quantities, analyte concentrations in foods are variable and strongly dependent upon the analytical method employed to extract and concentrate them. This is particularly true of compounds found at trace levels. Consequently, reports of absolute concentrations of trace levels of endogenous analytes in foods must be presented with all of the experimental details, because meaningful comparisons can be made only between experiments that subject the sample to similar conditions. As reported below, slight changes in sample temperature, heating time, or variation of moisture can and do affect the measured amounts of analytes. Preliminary analyses were run on milled 1999 Jasmine rice samples. Parameters were optimized on the basis of the relative amounts of 2-AP collected.

The sample must reach a temperature sufficient either to liberate bound volatile compounds or to thermally generate the compounds, in order to successfully sample the headspace of rice with SPME. To determine the optimum sample temperature for the collection of volatile compounds from the headspace of the rice, samples of rice flour were run in triplicate from 60 to 85 °C at intervals of 5 °C (Figure 1). Recovered amounts of 2-AP from the headspace of rice doubled as the temperature of the sample was increased from 60 to 85 °C. Conversely, the internal standard, TMP, decreased with increasing temperature. As the recoveries of 2-AP at 80 and 85 °C were not significantly different, the 80 °C value was selected for use. The use of adsorption temperatures >85 °C resulted in the sporadic deformation and rupture of the Teflon seal of the sampling vials.

The SPME literature suggests that an equilibration time of 10−15 min is generally sufficient for most volatile compounds, whereas less volatile compounds such as organic acids and esters may take hours. After a comparison of 2-AP recoveries over adsorption times of 10, 15, 20, and 30 min, an adsorption period of 15 min was selected. Further exposure of the SPME fiber to the headspace resulted in no significant increase in the amount of 2-AP collected. The use of an incubation period to preheat the sample prior to adsorption was also investigated. The incubation period was varied from 0 to 35 min at 5 min intervals, resulting in a total heating time of 15−50 min for each sample. Samples were run in triplicate, and resultant recoveries of 2-AP from the headspace are displayed graphically in Figure 2. The level of 2-AP continues to increase up to 40 min (total heating time is 40 min consisting of a 25 min incubation period and a 15 min SPME adsorption period) and then plateaus. An incubation time of 25 min was determined to be the optimal incubation or preheating time for each sample.

In the United States, rice is generally dried to ~12% moisture to prevent microbial growth and/or germina-

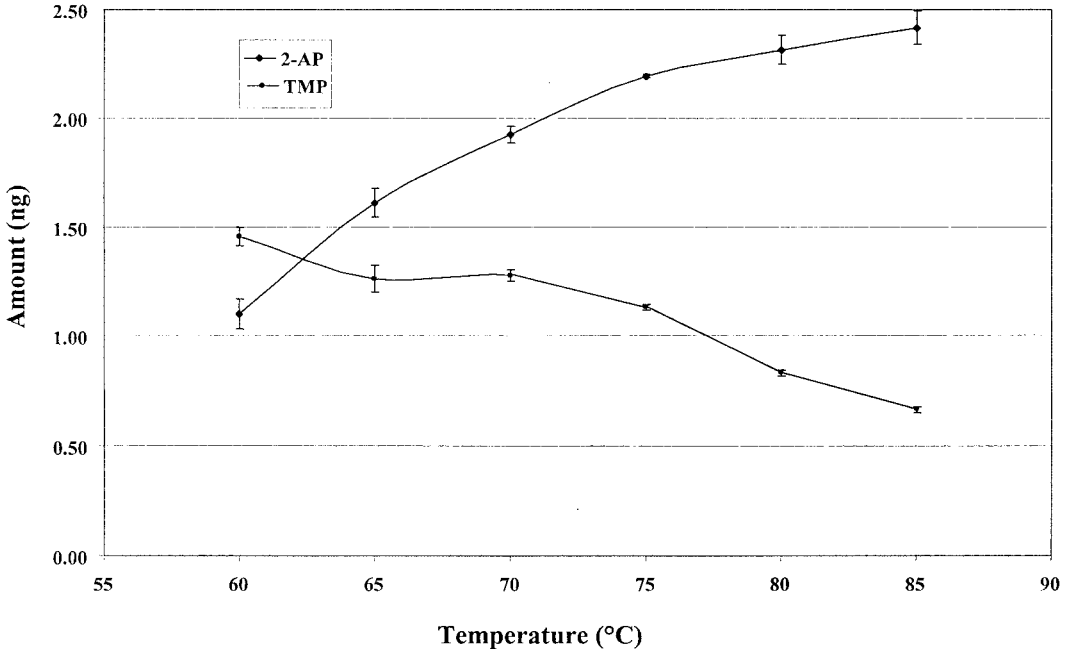

Figure 1. Amount of 2-AP recovered from Jasmine rice flour at sampling temperatures ranging from 60 to 85 °C. TMP is employed as the internal standard.

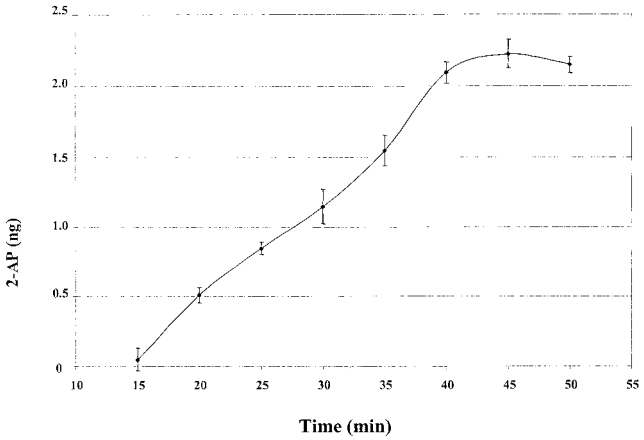

Figure 2. Determination of optimum heating time for the recovery of 2-AP from the headspace of Jasmine rice.

tion. It is then cooked in water at ratios ranging from 1:1 to 2:1 parts of water to rice. Initial experiments using normal amounts of water resulted in a large decrease in the amount of 2-AP recovered from the headspace. However, subsequent experiments in which only small amounts of water were added to the rice greatly increased the amounts of 2-AP observed in the headspace (Figure 3). With no water added, the amount of 2-AP recovered from the headspace of rice flour (Jasmine rice) is nearly 4 times that recovered from the headspace of milled kernels. As expected, the disruption of the tissue in the flour results in increased amounts of lipid oxidation products. There is nearly an 8-fold increase in the amount of hexanal recovered from the headspace of the flour and nearly a 6-fold increase in the amount of nonanal relative to the milled kernels (data not shown). With 50 μL of water added, the 2-AP recovered from the headspace increases slightly, whereas in the milled kernels a 5-fold increase was observed. The addition of 100 μL of water to the milled kernels produces a slight increase in 2-AP, whereas the amount recovered from the flour decreases. Subsequent addition of water results in a further decrease in the amount of 2-AP recovered from the flour and the milled kernels. Conversely, the amount of TMP collected by the SPME fiber drops by nearly half with the addition of 100 μL of water (Figure 3). Further addition of water tends to decrease the amount of 2-AP collected on the SPME fiber. The enhanced recovery of 2-AP from water added to milled rice eliminates the need to grind the sample to a flour, simplifying sample preparation.

Depending upon the compound and the fiber employed, specific analyte recoveries can be as much as 90%. However, when a solid sample is sampled, agitation and salting techniques are not possible and analyte recoveries are typically much lower. By employing the solvent extraction method of Bergman et al. (*9*), the concentration of 2-AP in the Jasmine rice was determined to be 810 ppb or 810 ng of 2-AP/g of rice. To determine the absolute amounts of 2-AP recovered from the headspace of milled rice, integrated peak areas were converted to mass by employing a calibration curve developed from a 2-AP standard injected as a $CHCl_3$ solution. Attempts to develop a calibration curve employing SPME failed as the 2-AP standard was available only in $CHCl_3$, which drastically affected the recovery of 2-AP. Recoveries of 2-AP from the headspace resulted in an average of 2.2 ng of 2-AP from 0.75 g of Jasmine rice, equivalent to a concentration of 2.9 ppb. A concentration of 810 ppb was determined by using the wet method for 2-AP in the same rice. This equates to a $<$0.3% recovery by the SPME analysis. Because of this low recovery there will be a large error associated with absolute concentrations of 2-AP in rice determined by SPME analysis. However, the data suggest that the SPME method may be suitable for the relative comparison of 2-AP concentrations between rice varieties.

To evaluate the ability of the SPME method to screen for relative concentrations of 2-AP, 21 cultivers and breeding lines were evaluated. Columns two and three in Table 2 give the amount of 2-AP recovered from the headspace of 0.75 g sample of milled and brown rice (samples were weighed and values adjusted for weight). The next two columns give the concentration and

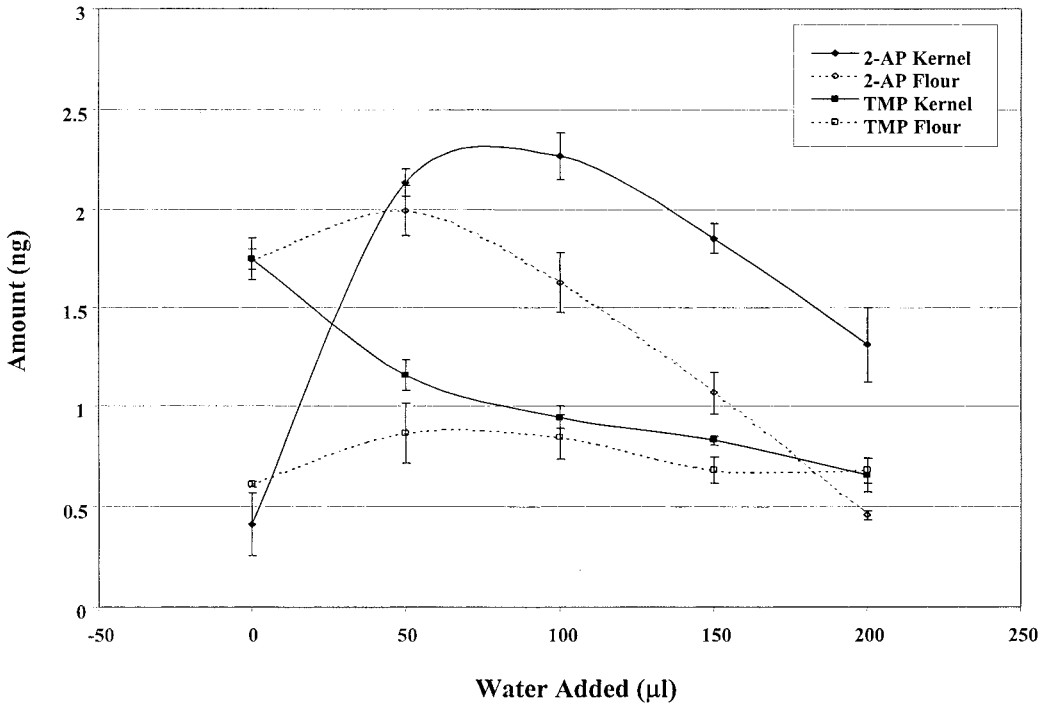

Figure 3. Amounts of 2-AP and TMP recovered, based on the addition of 0, 50, 100, 150, and 200 μL of water to 0.75 g of rice kernels and flour.

Table 2. Comparison of the Recovered Amounts and the Calculated Concentration of 2-AP by SPME and by Solvent Extraction

	SPME (ng)		SPME* (ppb)		wet method (ppb)	
rice	milled	brown	milled	brown	milled	brown
ALAN	0.0 ± 0.0	0.0 ± 0.0	1 ± 1	26 ± 7	3 ± 1	2 ± 0
MBLE	0.0 ± 0.0	0.0 ± 0.0	2 ± 2	9 ± 9	4 ± 0	4 ± 1
CCDR	0.0 ± 0.0	0.0 ± 0.0	0 ± 1	6 ± 4	4 ± 2	2 ± 0
RU970203	1.6 ± 0.1	1.4 ± 0.1	602 ± 59	516 ± 51	618 ± 2	703 ± 33
LFTE	0.0 ± 0.0	0.0 ± 0.0	0 ± 1	12 ± 13	1 ± 0	2 ± 1
BNGL	0.0 ± 0.0	0.0 ± 0.0	2 ± 2	9 ± 9	1 ± 1	2 ± 1
MARS	0.0 ± 0.0	0.0 ± 0.0	2 ± 2	14 ± 13	1 ± 0	2 ± 1
GFMT	0.0 ± 0.0	0.0 ± 0.0	3 ± 3	11 ± 8	3 ± 1	7 ± 2
PSCL	0.0 ± 0.0	0.0 ± 0.0	1 ± 1	12 ± 10	3 ± 0	4 ± 1
RU970302	2.4 ± 0.1	1.6 ± 0.3	913 ± 49	622 ± 136	751 ± 41	724 ± 51
RU980313	3.1 ± 0.1	3.1 ± 0.1	1150 ± 39	1169 ± 7	1256 ± 31	1171 ± 55
RU960214	0.9 ± 0.0	1.0 ± 0.1	361 ± 15	399 ± 39	305 ± 4	365 ± 8
RU980214	N/A	1.1 ± 0.2	N/A	440 ± 74	419 ± 4	432 ± 6
DLRS	1.7 ± 0.1	1.2 ± 0.2	645 ± 63	467 ± 100	624 ± 9	529 ± 4
DLLA	1.9 ± 0.3	1.3 ± 0.1	736 ± 135	499 ± 51	694 ± 7	620 ± 45
RU970316	0.8 ± 0.1	0.6 ± 0.0	313 ± 40	246 ± 27	304 ± 21	386 ± 42
RU980316	2.5 ± 0.0	1.7 ± 0.0	930 ± 22	642 ± 13	819 ± 8	696 ± 3
RU950217	0.8 ± 0.0	1.6 ± 0.2	296 ± 10	619 ± 74	426 ± 9	442 ± 14
RU980217	2.3 ± 0.2	2.2 ± 0.3	871 ± 86	819 ± 111	886 ± 2	761 ± 12
RICO1	0.0 ± 0.0	0.0 ± 0.0	4 ± 5	6 ± 6	2 ± 1	9 ± 4
AB647	0.0 ± 0.0	0.0 ± 0.0	4 ± 4	9 ± 9	1 ± 0	4 ± 0

standard deviation for brown and milled rice adjusted using the recovery factor (0.3%) obtained from the Jasmine rice standard. The final two columns give the concentration and standard deviations of 2-AP determined according to the wet method (*9*) for both the milled and brown rice samples. There was an insufficient amount of milled rice to analyze rice sample RU9800214. With the exception of the milled rice sample RU970302, the results from the SPME method are within 20% of the results of the wet method for the amount of 2-AP. The average standard deviation for all aromatic rice (2-AP > 100 ppb) samples is 11%. Comparing the amount of 2-AP in the brown rice gave similar results with a 20% error, with the exceptions of RU970316 and RU950217. Although the error is greater with the brown rice, the trade-off is a simpler sample preparation method. In all cases the SPME method is able to distinguish between aromatic and nonaromatic rices.

Employing full-scan mode gave sufficient sensitivity for the analysis presented here as 2-AP was detected at trace levels even in the nonaromatic rice. Operating the mass spectrometer in selected ion mode would most likely increase the lower limit of detection by at least an order of magnitude.

The chromatograms from the previous experiment provide an opportunity to compare the volatile compounds found in the headspace of brown rice and milled white rice. The overlapping total ion chromatograms (TIC) of the DLRS rice samples are shown in Figure 4. The brown rice TIC is offset by 0.5×10^7 for easier comparison. The bran, present on the brown rice and

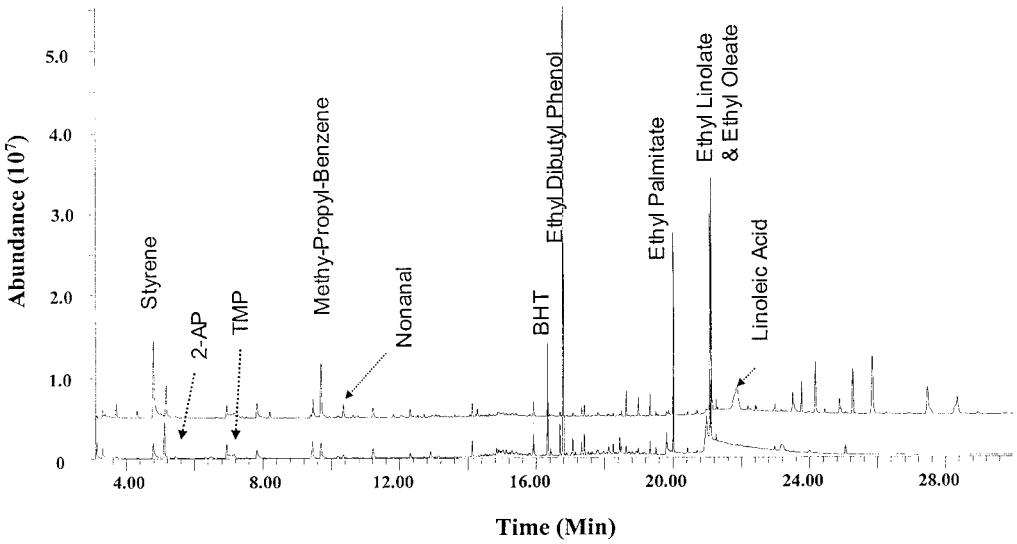

Figure 4. GC-MS TIC profiles of a brown rice (top) and milled white rice (bottom).

but milled away from the white rice, is rich in lipids relative to the starchy endosperm of the milled rice. As expected, lipid oxidation products such as nonanal, free fatty acids, and esters of palmitic, oleic, and linoleic acid are found in greater abundance in the brown rice. The ubiquitous antioxidants butylated hydroxytoluene (BHT) and 4-ethyl-2,6-bis(*tert*-butyl)phenol are found in greater amounts in the white rice. The presence of these compounds most likely occurs from contamination from the packaging and during the milling process. The 2-AP elutes at 5.42 min and constitutes only a small fraction of the total headspace, yet this amount is sufficient to dominate the aroma of the cooked rice. There appears to be only a slight difference between the levels of 2-AP recovered from milled and brown rice samples. This is in agreement with a study by Bergman et al. (*9*), which showed little difference in the 2-AP contents of brown and milled rice. The internal standard TMP elutes at 7.15 min. The TMP coelutes with 2-pentylfuran, a lipid oxidation product. The coelution does not hinder the quantitation of TMP because the target ion, at *m*/*z* 121, is not found in the fragmentation pattern of 2-pentylfuran.

CONCLUSIONS

This paper describes a rapid, sensitive analytical technique for the screening of 2-AP in the headspace of rice. The method is readily amenable for the analysis of additional compounds once they have been identified as having an impact on the sensory quality of rice. A key requirement of this methodology is the heating of the sample to produce sufficient amounts of analytes to be successfully analyzed. The method uses <1 g quantities of rice, which is important when rice varieties are screened as only a few grams of a particular variety may be available.

ABBREVIATIONS USED

2-AP, 2-acetyl-1-pyrroline; BHT, butylated hydroxytoluene; $CHCl_3$, chloroform; *m*/*z*, mass-to-charge ratio; ppb, parts per billion; Q1, qualifier ion 1; Q2, qualifier ion 2; SPME, solid phase microextraction; TIC, total ion chromatogram; TMP, 2,4,6-trimethylpyridine.

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