Analysis of 2-Acetyl-1-Pyrroline in Rice by HSSE/GC/MS

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ABSTRACT

An extremely sensitive method for the analysis of 2-acetyl-1-pyrroline (2AP) in rice, employing stir bar sorptive extraction (Twister) was studied. The Twister stir bar is placed in the headspace of a 20-mL vial containing 1 g of rice kernels, 7.5 mL of 0.1*M* KOH, and 2.2 g of NaCl, along with a second Teflon-coated stir bar for mixing. Analytes are adsorbed onto the Twister for 4 hr at 40°C and then desorbed at 270°C into a GC column while cryofocusing at −80°C. The headspace sorptive extraction (HSSE) method was able to detect <0.1 ppb of 2AP in rice. The precision of the HSSE method (>10%) was not as good as the GC/FID method (≈6%). Using HSSE, 2AP was observed in all samples generally considered to be aromatic and was not observed in any nonaromatic samples. Additionally, a modified method for the synthesis of 2-acetyl-1-pyrroline was studied and the presence of a tautomer of 2-acetyl-1-pyrroline was confirmed.

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Numerous compounds can be observed in headspace of cooked rice. Lipid oxidation products (aldehydes, alcohols, etc.) dominate the chromatographic profiles irrespective of the analytical method employed. However, only the volatile compound 2-acetyl-1-pyrroline (2AP) has been related to a characteristic rice aroma. Initial reports suggested 2AP concentrations in aromatic rice were <100 ppb (Buttery et al 1982). Subsequent reports have shown 2AP concentrations range from several hundred ppb to several thousand ppb. When present at concentrations of several hundred ppb, 2AP gives the rice a nutty or popcorn-like aroma, and the rice is referred to as aromatic. 2AP has been reported in nonaromatic rice at concentrations of only a few ppb (Buttery et al 1988; Grimm et al 2001). However, it is unclear whether 2AP is actually present in nonaromatic rice and, if so, does it contribute to the overall aroma of rice.

2AP is generated during the growing season in the plant and can be found in the leaves and stems as well as in the rice kernel (Yoshhashi 2002). The maximum 2AP concentration is variety-dependent (Guofo et al 2010) and occurs four or five weeks after heading (WAH) and decreases to 20% of maximum at seven or eight WAH (Itani et al 2004). Furthermore, 2AP concentration decreases with storage time and temperature (Tananuwong and Lertsiri 2010). Consequently, even rice of the same variety grown under the same conditions may show a significant difference in 2AP concentration due to differences in harvest date and postharvest handling.

Several methods have been developed for the isolation and concentration of 2AP in aromatic rice samples for subsequent analysis by gas chromatography with nitrogen-phosphorus, flame ionization, or mass spectrometry as a detector. These methods include concentration of 2AP in rice by purge and trap (Buttery et al 1988), steam distillation/solvent extraction (Lin et al 1990), solvent extraction followed by direct injection (Bergman et al 2000), solid-phase microextraction (SPME) (Grimm et al 2001) and headspace analysis (Sriseadka et al 2006). Recently, SPME has shown the presence of 2AP in a single kernel of aromatic rice (unpublished data).

Analytically, measuring the concentration of 2AP has proven challenging, not only becuase of its low concentration, but because the compound rapidly degrades, generally making high purity standards unavailable. Buttery et al (1986) used 2,4,6-trimethylpyrridine (TMP) as an internal standard because of similar physical properties (basicity, water solubility, volatility, and GC retention time). Employed as an internal standard, TMP with organic solvent extraction has provided a sufficiently reliable method for monitoring 2AP levels in aromatic rice samples using GC/FID (Bergman et al 2000). Lin et al (1991) expanded the use of TMP as an internal standard for 2AP by relating the relative ion abundances of the two compounds using quadrupole mass spectrometric analysis. Unfortunately, this method is instrument-specific and calibration must be made for each individual instrument.

With the advent of SPME, qualitative data is readily obtained for 2AP in rice (Grimm et al 2001). With SPME, a fiber coated with an adsorbent phase is deployed in the headspace of a sealed vial containing a sample. Volatiles and semivolatile compounds diffuse from the sample into the headspace and are adsorbed onto the fiber. The fiber is then thermally desorbed and qualitative data and semiquantitative data is readily obtained using SPME. Accurate quantitative data, however, remains elusive with this technique.

Headspace sorptive extraction (HSSE) is quite similar to SPME but has ≈50 times the adsorption capacity, allowing for more sensitive analyses. Like the SPME fiber, the Twister stir bar used in HSSE can be employed in either a liquid sample or in the headspace above it. When the stir bar is deployed in the headspace, similar to SPME, it concentrates volatile compounds under static headspace conditions. Both techniques rely upon the establishment of equilibrium between the sample and the headspace and between the headspace and the sorptive material on the HSSE or SPME fiber. Unlike liquid injections, where a known amount of standard is directly injected onto the column, the amount of standard absorbed by HSSE is unknown and may vary depending on such variables as placement, sample amount, headspace volume, temperature, and exposure time.

We explored the use of HSSE as a method for determining the qualitative and quantitative amounts of 2AP and other volatile and semivolatile compounds in rice.

MATERIALS AND METHODS

Rice Samples

A milled sample of Philippine Jasmine rice used for sample optimization was produced in 2007 in Los Banos, Philippines. The rice was kept at −10°C until analyzed. Specific cultivars (Jasmine 85, Dellrose, Drew, Giant Embryo, IAC600, and JES) were produced in a field trial conducted in 2008 at the Dale Bumpers National Rice Research Center, Stuttgart, AR, stored as rough rice at 4°C, and hulled to produce brown rice just before analysis. Commercial brands of rice of unknown purity and origin were pur-

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chased at a local retail store (Black Forbidden, a brown rice; Watermaid, a milled rice; Uncle Ben's parboiled rice; Goya aged Basmati rice; Bhutanese Red rice; and Himalayan Red rice).

GC/FID Analysis of 2AP

The method of Bergman et al (2000) was employed. In brief, 0.3 g of rice flour was placed in a 2-mL vial. A solution of 0.5 mL of methylene chloride containing 2,4,6-trimethylpyridine (Sigma-Aldrich, St Louis, MO) at a concentration of 485 ppb (ng/mL) was added to the vial and capped with a steel cap. The vials were heated at 85°C for 2.5 hr to maximize recovery of 2AP. A gas chromatograph (7890 GC, Agilent Technologies, Folsom, CA) equipped with a 30 m × 0.25 mm, DB-WAX capillary column (J&W Scientific, St. Joseph, MO) was used for analysis. Aliquots of 1 µL were injected into an injection port operated in splitless mode at 155°C. The GC oven was initially held for 1 min at 40°C then increased at a rate of 9°C/min to 120°C, then 25°C/min to 250°C and held for 5 min. Helium was the carrier gas at a constant flow of 1.1 mL/min. Samples were run in triplicate and the concentration of 2AP was calculated based upon the relative peak area of the TMP peak.

HSSE/GC/MS Analysis of 2AP

Twister stir bars coated with polydimethylsiloxane (PDMS, film 0.5 mm thickness × 10 mm length) were obtained from Gerstel (Baltimore, MD). Method development was performed using milled Philippine Jasmine rice, which was kept at –10°C until analyzed. Whole rice grains were analyzed as-is; flour samples were prepared by grinding rice in an analytical mill (model A-10, IKA-Labortechnik, West Germany) immediately before analyses. Sample preparation consisted of placing 0.5–2.5 g of brown rice, milled rice, or rice flour directly into a 20-mL vial containing a standard Teflon-coated magnetic stir bar and 2.2 g of NaCl. Deionized water (7.5 mL) (Milli-Q Gradient A-10, Millipore Billerica, MA) or 0.1*M* KOH was added to the vial. 2,4,6-Trimethylpyridine (TMP) (Sigma-Aldrich, St. Louis, MO) was employed as the internal standard. A 100 µL aliquot of a 0.1 ppm solution was added to each sample, thus effectively placing 10 ng of TMP in each vial. A Twister stir bar was suspended in the headspace from a steel wire inserted through a Teflon-lined screw cap (Sigma-Aldrich). Samples were heated to 40°C and volatile compounds were adsorbed onto the Twister for 0.5–24 hr. The Twisters were removed, rinsed with deionized water, blotted dry, and placed in sample tubes. Volatile compounds were thermally desorbed from the Twisters at 270°C with a 50 mL/min flow of He for 8 min and cryofocused into the programmed-temperature vaporizing injector held at –80°C. Following transfer of the analytes, the chromatographic run was started and the injection port was heated to 280°C at 12°C/sec and held for 10 min. The split valve was opened at 1 min. The GC oven was held at 40°C for 1 min then increased to 280°C at a rate of 8°C/min and held for 2 min. The scan range was m/z 33 to m/z 330 with a 150 count threshold and 8.69 scans were collected per second. The column interface was held at 280°C, ionization energy was 70 eV, and the electron multiplier was set at 1,750V. A standard calibration curve using synthesized 2AP was run in triplicate and yielded an r^2 of 0.976. Peak areas were determined for each compound by integrating a selected ion unique to that compound. The limit of detection data were gathered using the same conditions, except the MSD was operated in selected ion monitoring mode (m/z 68, 83, 106, 111, and 121 were monitored).

Synthesis of 2-Acetyl-1-Pyrroline

All starting materials and reagents were commercially available and used without further purification except as indicated. Diethyl ether was refluxed for 24 hr over pure sodium in the presence of benzophenone, distilled, and immediately used. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker 400 MHz spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard. Mass spectra were recorded using an Agilent 6520 Q-TOF mass spectrometer. A schematic representation of the synthesis of 2AP is given in Fig. 1.

The (S)-Tert-butyl-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate(4) was prepared following a procedure of Woo et al (2004). (S)-Tert-butyl-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate(4) *N*-(tert-Butoxycarbonyl)-L-proline (**2**) (5.381 g, 25.0 mmol) was dissolved in 240 mL of THF and cooled to 0°C, while stirring under Argon. To this solution was added triethylamine (10.45 mL, 75.0 mmol). Methanesulfonyl chloride (**1**) (2.14 mL, 27.5 mmol) was then added dropwise and the reaction was stirred for 10 min. Upon addition of methanesulfonyl chloride, white solids (triethylamine hydrochloride) appear in solution. Then freshly distilled *N,O*-dimethylhydroxyl-amine (**3**) (2.29 g, 37.5 mmol) was added by syringe, and the solution was stirred for 1 hr. Deionized water (120 mL) was then added to the reaction, dissolving all white solids, to form a clear solution. The solution was transferred to a separatory funnel and extracted with diethyl ether (120 mL × 3). The organic phase was washed with brine, dried over magnesium sulfate, and evaporated under reduced pressure to yield a light-yellow crude oil. The crude product was dried onto silica gel and chromatographed on a column of silica gel using a solvent system of 1:1 petroleum ether/diethyl ether. The collected fractions were concentrated under reduced pressure to provide the title compound as a light yellow oil in 64% yield. MS (EI) m/z (relative intensity) 129 (100), 57 (62), 70 (40), 43 (40), 112 (30), 147 (28), 101 (12), 241 (6), 259 (5); 1H-NMR (400 MHz, CDCl3): δ 4.71 (d, J=8.2 Hz, ½ H); 4.61 (d, J=8.2 Hz, ½ H); 3.79 (s, 3/2 H); 3.73 (s, 3/2 H); 3.34-3.66 (m, 2H); 3.20 (s, 3H); 1.77-2.29 (m, 4H); 1.46 (s, 9/2 H); 1.42 (s, 9/2 H).

(S)-Tert-butyl 2-acetylpyrrolidine-1-carboxylate was prepared following a modified procedure of Ferraris et al (2004). (S)-Tert-butyl-2-(methoxy(methyl)carbamoyl)-pyrrolidine-1-carboxylate (2.88 g, 11.2 mmol) was dissolved in dry diethyl ether (36 mL) to form a clear yellow solution and cooled to –78°C while stirring under Argon. Methylmagnesium bromide solution (3.0*M* in diethyl ether) (4.09 mL, 12.3 mmol) was added by syringe and formed white solids in a yellow solution. The dry ice/acetone bath was removed and the reaction vessel was allowed to warm to room temperature and stirred overnight. After confirming the reaction was complete using thin-layer chromatorgraphy, the reaction was cooled to 0°C and deionized water (50 mL) was added slowly. An additional 100 mL of ammonium acetate (1*M*) solution was added and the reaction was transferred to a separatory funnel. All white solids disappeared upon addition of the ammonium acetate solution. The aqueous phase was extracted with diethyl ether (75 mL × 4) and the organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield a dark yellow residue. The crude product was purified on a column of silica gel

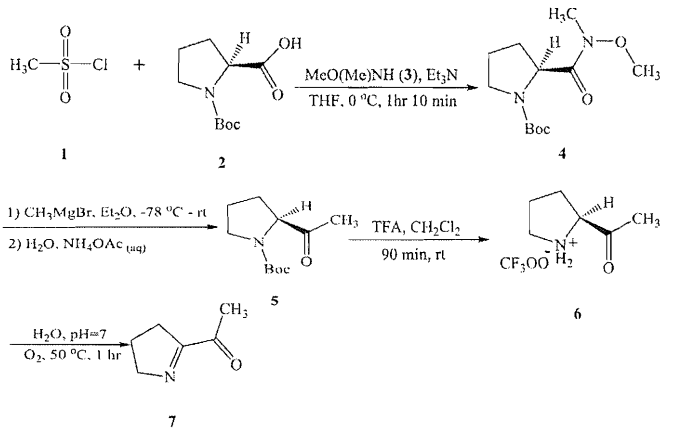

Fig. 1. Schematic representation of the synthesis of 2AP.

using a solvent system consisting of 1:9 diethyl ether/petroleum ether (250 mL), 1:5 diethyl ether/petroleum ether (250 mL), 2:3 diethyl ether/petroleum ether (250 mL), then 3:2 diethyl ether/petroleum ether (250 mL). The eluted product was collected and the solvent removed under reduced pressure to yield a white solid (1.080 g, 45%). MS (EI) m/z (relative intensity) 70 (100), 114 (74), 57 (70), 41 (20), 41 (20), 170 (18), 140 (10); 1H-NMR (400 MHz, CDCl3): δ 4.15-4.39 (m, 1H); 3.38-3.64 (m, 2H); 2.14 (dd, J=9.6, 7.8 Hz, 3H), 1.78-2.26 (m, 4H); 1.43 (dd, J=10.8, 9.0Hz, 9H).

2-Acetylpyrrolidine trifluoroacetate was prepared following a procedure of Hofmann and Schieberle (1998). (S)-Tert-butyl 2-acetylpyrrolidine-1-carboxylate (0.723 g, 3.39 mmol) was dissolved at 25°C in methylene chloride (2 mL) while stirring under Argon. Trifluoroacetic acid (6 mL) was slowly added by syringe and the solution stirred under Argon for 90 min. Silica thin-layer chromatography (1:6 hexane/diethyl ether), followed by exposure in an iodine chamber, confirmed the salt formation. Methylene dichloride was removed by purging with Argon, and the product (2-Acetylpyrrolidine trifluoroacetate) was carried to the next step.

2-Acetyl-1-pyrroline was prepared following a procedure of Hofman and Schieberle (1998). The pH of 2-acetylpyrrolidine trifluoroacetate was adjusted to neutral with the addition of 15 mL of saturated sodium bicarbonate solution. The orange solution was heated at 50°C for 1 hr under pure oxygen, during which time the solution darkened. The reaction was then cooled to room temperature and the aqueous phase was extracted with diethyl ether (10 mL × 3), dried over sodium sulfate, and filtered. The desired product was concentrated by distilling off (35°C) the organic solvent to yield an orange residue that was used without further purification. MS (EI) m/z (relative intensity) 43 (100), 41 (52), 42 (16), 83 (15), 68 (10), 69 (7), 111 (7).

RESULTS AND DISCUSSION

Synthesis of 2-Acetyl-1-Pyrroline

The total synthesis of 2-acetyl-1-pyrroline was achieved in four steps with an overall yield of 20%. The preparation of (S)-tert-butyl 2-acetylpyrrolidine-1-carboxylate required a modification of the published procedure of Ferraris et al (2004). Besides using diethyl ether instead of tetrahydrofuran as a solvent, our procedure allowed the reaction to stir at room temperature overnight, instead of 3 hr in earlier reports. This was due to the observation by thin-layer chromatography that the reaction had not gone to completion after 3 hr had elapsed. Furthermore, the procedure was altered by the addition of 100 mL of ammonium acetate (1*M*) solution. This additional step was necessary to clear the emulsion that formed after the addition of 50 mL of water to the cooled reaction. Upon addition of the ammonium acetate, the emulsion disappeared and the extraction proceeded without difficulty. All other procedures were followed.

Rice Volatiles

A typical GC/MS chromatogram of the volatile compounds recovered from the headspace using HSSE is shown in Fig. 2. 2AP elutes at 7.05 min and is readily detected using the quadrupole mass spectrometer in scan mode. The large peak at 8.15 min is a siloxane contaminant from the stationary phase of the Twister stir bar. Of particular interest is the peak at 7.8 min. This unidentified compound was previously reported by Bergman et al (2000) and appears in aromatic rice samples. The mass spectrum of the unidentified compound along with that of 2AP and TMP is given in Fig. 3. The compound is an isomer of 2AP possessing a molecular ion at m/z 111. It readily loses CO to give the ion at m/z 83. Possible isomers include 3 acetyl-1-pyrroline, 4-acetyl-1-pyrroline, 2-acetyl-2-pyrroline, and 2-acetyl-3-pyrroline. The absence of the m/z 68 ion $[C_4H_6N]^+$ suggests the acetyl group does not fragment as an intact unit. Of particular interest, is that the isomer was observed in the synthesized 2AP as well as in rice samples containing natural 2AP.

Because of the large difference in fragmentation patterns of the mass spectra, the peak areas of TMP and 2AP are not readily comparable. The TMP molecular ion m/z 121 is relatively stable and is the most abundant ion in the TMP spectrum (Fig. 3C). 2AP, on the other hand, readily fragments with m/z 43 being the most abundant ion. Lin et al (1990) measured the relative response of a known amount of 2AP and TMP and developed a procedure to relate a known quantity of TMP to an unknown quantity of 2AP, based on the ratios of selected ions. However, this procedure is highly dependent on acquisition parameters and individual instrumental characteristics and is not readily transferred between different laboratories. Thus, use of TMP as a standard is widespread only when used in conjunction with an FID, where the response factor of the detector is similar for both compounds.

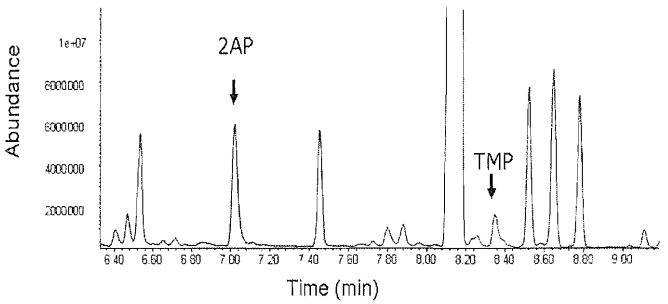

Fig. 2. Total ion chromatogram of Philippine Jasmine rice using HSSE/GC/MS.

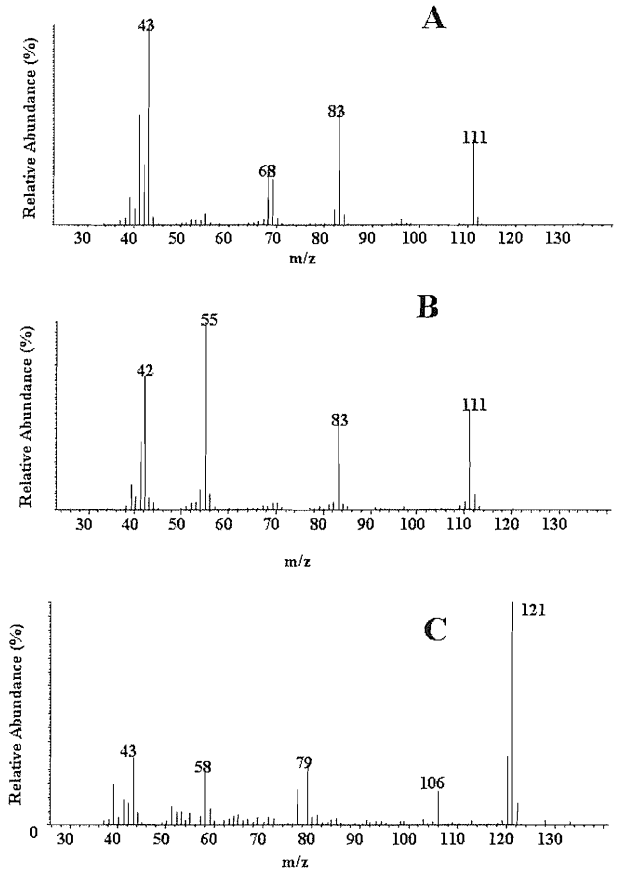

Fig. 3. Mass spectra of 2AP (**A**), isomer of 2AP observed in Philippine Jasmine rice and in synthesized 2AP (**B**), and TMP (**C**).

TABLE I
List of Compounds Observed in the Headspace of Rice Using HSSE Compound[a]

Compound	RI	MW	MF	Amt[b]	CAS	Compound	RI	MW	MF	Amt[b]	CAS
Ethanol	250	46	C2H6O	1,2	64-17-5	Nonane	900	128	C9H20		111-84-2
Acetone	259	58	C3H6O	1,2	67-64-1	Heptanal	902	114	C7H14O	1,2,3	111-71-7
Dimethyl sulfide	308	62	C2H6S	1	75-18-3	2-Butoxyethanol	905	118	C6H14O2	1,2	111-76-2
Hexane	600	86	C6H14		285-58-5	2,3,6-Trimethylheptane	913	142	C10H22	1	4032-93-3
Butanal	602	72	C4H8O	1,2	123-72-8	2-Acetyl-1-pyrroline	920	111	C6H9NO	1,2	85213-22-05
Acetic acid	622	60	C2H4O2	1,2,3	64-19-7	Methyl hexanoate	922	130	C7H14O2	1,2,3	106-70-7
3-Methyl-butanal	652	86	C5H10O	1,2	590-86-3	α-Pinene	932	136	C10H16	1	80-56-8
2-Methyl-butanal	660	86	C5H10O	1,2	96-17-3	1-Butoxy-2-propanol	938	132	C7H16O2	1,2	5131-66-8
Heptane	700	100	C7H16		142-82-5	(E)-2-Heptanal	956	112	C7H12O	1,2,3	57266-86-1
Pentanal	701	86	C5H10O	1,2,3	110-62-3	1-Ethyl, 4-methylbenzene	959	120	C9H12	1	611-14-3
Methyl butanoate	710	102	C5H10O2	1,2	623-42-7	Benzaldehyde	962	106	C7H6O	1	100-52-7
3-Methyl-butanol	730	88	C5H12O	1,2	123-51-3	1-Heptanol	969	116	C7H16O	1,2	111-70-6
2-Methyl-butanol	730	88	C5H12O	1,2	137-32-6	Dimethyl trisulfide	970	126	C2H6S3	1	3658-80-8
Dimethyl disulfide	741	96	C2H6S2	1,2	624-92-0	Hexanoic acid	983	116	C6H12O2	1,2	142-62-1
Pyridine	763	79	C5H5N	1	110-86-1	6-Methyl-5-hepten-2-one	983	126	C8H14O	1,2,3	110-93-0
3-Hexanone	744	100	C6H12O	1,2,3	589-38-8	2-Pentylfuran	990	138	C9H14O	1,2,3	3777-69-3
1-Pentanol	761	88	C5H12O	1,2,3	71-41-0	2,3,6-Trimethylpyridine	989	121	C8H11N	1	1462-84-6
Toluene	764	92	C7H8	1	108-88-3	1,2,4-Trimethylbenzene	994	120	C9H12	1	95-63-6
2,4-Pentandione	778	100	C5H8O2	1,2	123-54-6	Ethyl hexanoate	998	144	C8H16O2	1,	123-66-0
2,3-Butandiol	788	90	C4H10O2	1,2	513-85-9	Decane	1000	142	C10H22	1	124-18-5
1,3-Butandiol	791	90	C4H10O2	1,2	107-88-0	Octanal	1004	128	C8H16O	1,2,3	124-13-0
Octane	800	114	C8H18		111-65-9	2-Ethyl-1-hexanol	1029	130	C8H18O	1,2	104-76-7
Hexanal	800	100	C6H12O	1,2,3	66-25-1	Limonene	1030	136	C10H16	1	138-86-3
Methyl pentanoate	821	116	C6H12O2	1,2	624-24-8	Indane	1035	118	C9H10	1	496-11-7
Ethenyl cycohexane	832	108	C8H12	1	100-40-3	Benzyl alcohol	1036	108	C7H8O	1	100-51-6
Butanoic acid	838	88	C4H8O2	1,2	107-92-6	(E)-3-Octene-2-one	1038	126	C8H14O	1,2	18402-82-9
(E)-2-Hexenal	850	98	C6H10O	1,2,3	6728-26-3	Benzeneacetaldehyde	1045	120	C8H8O	1,2	122-78-1
(Z)-3-Hexenal	853	98	C6H10O	1,2	6789-80-6	5-Ethyldihydro-2(3H)-furanone	1049	114	C6H10O2	1	695-06-7
Ethyl benzene	858	106	C8H10	1	100-41-4	2-Octenal	1058	126	C8H18O	1,2,3	111-87-5
1-Hexanol	865	102	C6H14O	1,2	111-27-3	1-Octanol	1071	130	C8H18O	1,2	111-87-5
1,4-Dimethyl benzene	868	106	C8H10	1	106-42-3	2-Methyl-1-propenyl benzene	1082	132	C10H12	1,2	768-49-0
Trimethylheptane	874	142	C10H22	1	14720-74-2	1-Methyl, 2-(1-methylethyl)-benzene	1084	134	C10H14	1	527-84-4
Pentanoic acid	879	102	C5H10O2	1,2	109-52-4	Undecane	1100	156	C11H24	1	1120-21-4
2,2,4-Trimethylheptane	883	142	C10H22	1	14720-74-2	Nonanal	1106	142	C9H18O	1,2,3	124-19-6
2-Heptanone	888	114	C7H14O	1,2,3	110-43-0	1,2,3,4-Tetramethylbenzene	1123	134	C10H14	1	488-23-3
2-Butylfuran	890	124	C8H12O	1,2	4466-24-4	Methyl octanoate	1128	158	C9H18O2	1	111-11-5
Styrene	892	104	C8H8	1	100-42-5	5-Ethyl-6-methyl-(E)3-hepten-2-one	1147	154	C10H18O	1,2	57283-79-1
1,2-Dimethylbenzene	893	106	C8H10	1	94-47-6	3-Methyl-2-heptyl acetate	1151	172	C10H20O2	1	72218-58-7

[a] RI, retention index; MW, molecular weight; MF, molecular formula; Amt, amount; CAS, chemical registry number.
[b] Relative amount may vary from sample to sample, 1 denotes a trace amount, 2 a moderate amount, and 3 a major peak in the chromatogram.

A list of compounds identified in the headspace of rice samples is given in Table I. Relative abundance of the compounds lists 1 for a trace amount, 2 for a moderate amount, and a 3 for a major peak. Depending on the sample, concentration for a given compound may range from a trace amount to a major peak. GC of a fresh, carefully handled rice will contain only trace levels of some of these compounds. As the rice ages, concentration of lipid oxidation products becomes larger, as in aged basmati rice, and will dominate the volatile profile. The terpenoids and aromatic compounds are typically found only in specialty rice. Compounds such as BHT, ionol, and naphthalene are contaminants and are introduced during the handling of the rice.

Bounphanousay et al (2008) noted that 2AP is found in higher concentration in brown rice than in milled rice when overall 2AP concentration is high; there is little difference between brown and milled rice when 2AP concentration is low. This suggests that 2AP distribution is initially heterogeneous and, upon storage, 2AP concentration not only decreases but becomes more homogenous. Therefore, to perform a quantitative measurement, 2AP must be liberated from the rice interior by grinding the rice to flour and extracting with an organic solvent or by heating the rice in excess water and treating with KOH to break up the rice kernels. Due to the nature of the PDMS coating, organic solvents are not conducive to analysis using the Twister stir bars.

Optimization

The Twister stir bar was initially placed in the sample solution, but after limited recoveries of 2AP were observed, the stir bar was subsequently suspended in the headspace (HSSE). Equilibriums can be shifted from a liquid to the headspace by the addition of ionic compounds such as NaCl to the sample solution. Use of NaCl and stirring gave a fourfold increase of 2AP integrated peak area counts relative to samples where NaCl was not added (data not shown). Integrated peaks areas of 2AP from raw and cooked milled rice kernels show no significant difference (Fig. 4). The addition of 0.1*M* KOH doubled the recovery of 2AP relative to uncooked rice. Unlike SPME, in which adsorption times in the headspace rarely require 1 hr to achieve acceptable results, the adsorption period for HSSE takes several hours. The relative amount of 2AP, as a function of adsorption time, was determined at intervals of 1–24 hr (Fig. 5). Average 2AP peak area increased with adsorption time ≤3 hr and then decreased. TMP peak area was optimal at 16 hr. The different optimal adsorption periods demonstrate why TMP cannot be employed as a standard for extraction methods such as SPME and HSSE.

The rates of adsorption onto the fiber are not equal. An adsorption period of 4 hr was chosen to maximize the recovery of 2AP and TMP. A desorption temperature of 270°C was employed while comparing cryofocusing temperatures of 0, –40, –80, and –120°C. A cryofocusing temperature of –120°C resulted in frequent failures of the auto sampling system. The best recovery and precision of the 2AP was observed with a cryofocusing desorption temperature of –80°C. The optimal HSSE parameters for 1 g of rice in a 20-mL vial were 2.2 g of NaCL, 7.5 mL of 0.1*M* KOH, adsorption at 40°C for 4 hr, desorption at 280°C with a cryofocusing temperature of –80°C.

TABLE I *(continued)*
List of Compounds Observed in the Headspace of Rice Using HSSE Compound[a]

Compound	RI	MW	MF	Amt[b]	CAS	Compound	RI	MW	MF	Amt[b]	CAS
5-Propyldihydro-2(3H)-furanone	1156	128	C7H12O2	1	105-21-5	(E) 6,10-dimethyl-5,9 undecadien-2-one	1450	194	C13H22O	1	3879-26-3
β-Terpineol	1157	154	C10H18O	1	138-87-4	2,6-Bis(t-butyl)-2,5-cyclohexadien-1,4-dione	1471	220	C14H20O2	1	719-22-2
E-2-nonenal	1166	142	C9H16O	1,2	18829-56-6	1-Hexadecene	1492	224	C16H32	1	629-73-2
Nonanol	1176	144	C9H20O	1,2	143-08-8	Pentadecane	1500	212	C15H32	1	629-62-9
Naphthalene	1190	128	C10H8	1	91-20-3	BHT	1510	220	C15H24O	1,2	128-37-0
Ethyl octanoate	1196	172	C10H20O2	1	106-32-1	Methyl dodecanoate	1523	214	C13H26O2	1	111-82-0
α-Terpineol	1198	154	C10H18O	1	12/2/2438	1-S, *cis*-calamene	1534	202	C15H22	1	483-77-2
Dodecane	1200	170	C12H26	1	112-40-3	Ionol 2	1561	234	C16H26O	1,2	4130-42-1
γ-Terpineol	1203	154	C10H18O	1,2	586-81-2	Ethyl dodecanoate	1592	228	C14H28O2	1	106-33-2
Decanal	1207	142	C10H22	1	112-31-2	Hexadecane 2,6-bis-(*t*-butyl)-2,5	1600	226	C16H34		544-76-3
E,E-2,4-nonadienal	1218	138	C9H14O	1,2	5910-87-2	2,6-Bis-(*t*-butyl)-2,5-cyclohexadien-1-one	1637	232	C16H24O	1	6378-27-8
Benzothiazole	1234	135	C7H5NS	1	85-16-9	2,5,10-Trimethylpentadecane	1646	254	C18H38	1	3892-00-0
2-Hexyl-1-octanol	1254	214	C14H30O	1	19780-79-1	Heptadecane	1700	240	C17H36		629-78-7
5-Butyldihydro-2(3H)-furanone	1259	142	C8H14O2	1,2	104-50-7	2,5,10,14-Tetramethylpentadecane	1703	268	C19H40	1	1921-70-6
E-2-Decenal	1267	154	C10H18O	1,2	3913-81-3	Methyl tetradecanoate	1724	242	C15H30O2	1	124-10-7
1-Tridecene	1293	182	C13H26	1	2437-56-1	Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl	1731	236	C18H20	1	3910-35-8
3-(*t*-Butyl)-phenol	1295	150	C10H14O	1	585-34-2	2,6-Diisopropylnaphthalene	1744	212	C16H20	1	24157-81-1
Ethyl nonanoate	1296	188	C11H22O2	1	123-29-5	2-Methyl-2,4-diphenylpentane	1762	238	C18H22	1	31516-55-9
2-Undecanone	1300	170	C11H22O	1,2	112-12-9	Ethyl tetradecanoate	1793	256	C16H32O2	1	124-06-1
Indole	1300	117	C8H7N	1,2	120-72-9	Octadecane	1800	254	C18H38		593-45-3
Tridecane	1300	184	C13H28	1	629-50-5	2,6,10,14-Tetramethylhexadecane	1807	282	C20H42	1	638-36-8
Undecanal	1310	170	C11H22O	1,2	112-44-7	6,10,14-Trimethyl-2-pentadecanone	1844	268	C18H36O	1	502-69-2
Methyl decanoate	1325	186	C11H22O2	1,2	110-42-9	Nonadecane	1900	268	C19H40		629-92-5
5-Pentyldihydro-2(3H)-furanone	1368	156	C9H16O	1	104-61-0	Methyl hexadecanoate	1925	258	C17H34O2	1	112-39-0
2-Butyl-2-octenal	1375	182	C12H22O	1,2,3	13019-16-4	Ethyl hexadecanoate	1993	284	C18H36O2	1	628-97-7
Tetradec-1-ene	1392	200	C14H28	1	1120-36-1	Hexadecanoic acid	1995	256	C16H32O2	1,2	57-10-3
Ethyldecanoate	1394	200	C12H24O2	1	110-38-3	Eicosane	2000	282	C20H42		112-95-8
Tetradecane	1400	198	C14H30	1	629-59-4	Methyl linolate	2051	294	C19H34O2	1	2566-97-4
Isolongifolene	1408	204	C15H24	1	1135-66-6	Methyl oleate	2052	296	C19H36O2	1	112-62-9
trans-Caryophylene	1433	204	C15H24	1	87-44-5	Ethyl linoleate	2081	308	C20H36O2	1	544-35-4
Bis-(1-methylethyl) hexadecanoate	1448	230	C12H22O2	1	6938-94-9	Ethyl oleate	2086	310	C20H38O2	1	111-62-6

[a] RI, retention index; MW, molecular weight; MF, molecular formula; Amt, amount; CAS, chemical registry number.
[b] Relative amount may vary from sample to sample, 1 denotes a trace amount, 2 a moderate amount, and 3 a major peak in the chromatogram.

TABLE II
Recovery Efficiency of HSSE/GC/MS Method

	GC/FID 2AP (ng)[a]	HSSE/GC/MS 2AP (ng)[b]	Recovered (%)[c]
Aromatics			
Philippine Jasmine	1,234 ± 49	15.0 ± 3.0	1.2
Dellrose	1,555 ± 87	27.7 ± 4.2	1.8
Jasmine 85	1,171 ± 80	16.3 ± 5.1	1.4
Black Forbidden	1,408 ± 79	9.6 ± 2.3	0.7
Sierra	1,648 ± 41	35.8 ± 8.5	2.2
JES	881 ± 34	30.7 ± 7.3	3.5
IAC600	680 ± 24	15.8 ± 3.7	2.3
Goya Aged Basmati	nd	2.0 ± 0.2	–
Nonaromatics			
Drew	nd	nd	0
Giant Embryo	nd	nd	0
Watermaid	nd	nd	0
Uncle Ben's	nd	nd	0
Bhutanese Red	nd	nd	0
Himalayan Red	nd	nd	0

[a] Calculated concentration of 2AP using GC/FID method.
[b] Amount of 2AP measured using HSSE/GC/MS method.
[c] Amount of 2AP recovered by HSSE/GC/MS method.

Recovery

Initially, depletion experiments were attempted where the same sample was repeatedly subjected to HSSE. The 2AP peak area decreased an average of 30% after each sampling with substantial material still present after the sixth sampling. This approach was abandoned when no clear decrease in the integrated peak area of 2AP was observed, suggesting a very low extraction efficiency of 2AP. A second approach consisted of running 2AP standards by HSSE/GC/MS, measuring the 2AP present, and comparing with the expected amount of 2AP as determined by the liquid extraction method. The results listed in Table II are 0.7–3.5% for seven aromatic rice samples. The HSSE/GC/MS method yielded 15 ng of 2AP from 1 g of Philippine Jasmine rice. The rice contained 1,233 ± 49 ppb (ng/g) according to the liquid extraction method. This resulted in a recovery of ≈1.2% which is ≈4× that observed for SPME (0.3%) (Grimm et al 2001) and far less than the order of magnitude one might expect due solely to the increased capacity of the stationary phase. This lower than expected recovery may be due in part to the difference in stationary phase. At this time, the Twister stir bar is only available with a PDMS phase, which is not as efficient in extracting slightly polar compounds as the carboxen/DVB/PDMS phase used for SPME fibers.

Limit of Detection

The limit of detection was determined by spiking a 0.1*M* KOH/nonaromatic rice solution with various amounts of synthesized 2AP. Figure 6 shows representative reconstructed ion chromatograms resulting from the addition of 0.1, 1, and 10 pg, along with the associated S/N ratio, from five replicates. No signal was observed for samples spiked with <0.1 pg of 2AP. If the extraction resulted in100% recovery, this would indicate that a concentration of 0.1 ppt of 2AP could be detected by this method using 1 g of rice. Because the average recovery observed is only 2%, the lowest concentration of 2AP in rice that would be observable with the HSSE/GC/MS method would be 50 ppt (<0.1 ppb). 2AP has not been observed thus far in any of the nonaromatic rice samples examined using the HSSE/GC/MS method.

Method Comparison

Table II gives a list of rice samples analyzed by solvent extraction with the GC/FID and the HSSE/GC/MS method. The first column gives the concentration of 2AP measured in each sample using the GC/FID method. An extraction efficiency of 80% recovery (Bergman et al 2000) is included in the calculation of the 2AP concentration. The second column gives the amount of 2AP measured using the HSSE/GC/MS method. Because the extraction efficiencies are so low in the third column, any calculations of absolute concentrations of 2AP could be inaccurate by as much as an order of magnitude using the HSSE/GC/MS method. However, comparison of relative concentrations can be valid and the sensitivity of the HSSE/GC/MS method provides a means for investigating rice samples with 2AP concentrations as low as 0.1 ppb. 2AP was detected in all aromatic rice samples using the HSSE/ GC/MS method, and in all but one case (Goya Aged Basmati), using the GC/FID method. The concentration of 2AP in Goya Aged Basmati is just at the GC/FID method limit of detection. If one assumes an average extraction rate of ≈2% using the HSSE method, this would suggest a 2AP concentration of ≈50 ppb. This amount is just below the theoretical limit of detection of 0.1 ng detection limit using the GC/FID liquid extraction method (0.3 g rice, 80% recovery). 2AP was not observed in any nonaromatic rice samples using either the GC/FID or HSSE/GC/MS method.

Relative standard deviations of the 2AP peak area for five replicates ranged from 9.3% for the Philippine Jasmine rice to >30% for Jasmine 85 rice using the HSSE/GC/MS method. The relative standard deviations using the GC/FID method were 3–8%. Thus the HSSE/GC/MS method is not as precise as with the liquid injections but may be acceptable depending on the end use of the data.

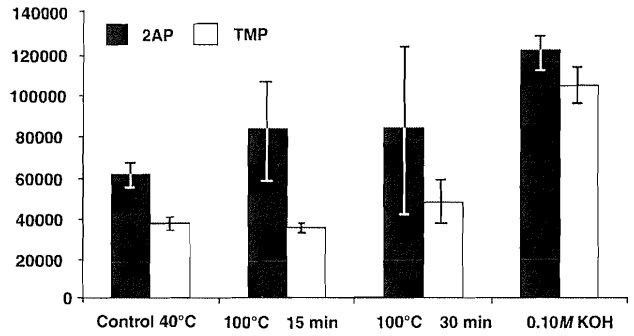

Fig. 4. Relative abundance of 2AP and TMP under different extraction conditions.

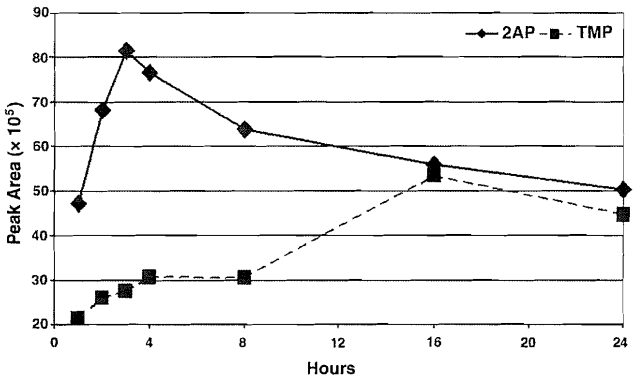

Fig. 5. Variation of 2AP and TMP with adsorption time.

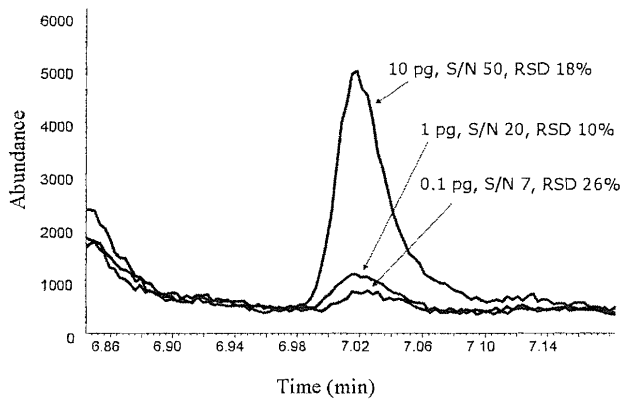

Fig. 6. Extracted ion chromatograms (m/z = 83) of 2AP at three concentrations.

CONCLUSIONS

This work reports on the use of HSSE with a sorptive coated stir bar suspended in the headspace of a sealed vial to collect volatile compounds found in rice, with a focus on the aromatic compound 2-acetyl-1-pyrroline (2AP). The method was extremely sensitive with a detection limit for 2AP of 0.1 ppb in rice and can provide semiquantitative data. Using this method, 2AP was readily detected in all rice samples deemed aromatic and was not detected in any nonaromatic rice. Although, the method was more sensitive than methylene chloride extraction with GC/FID method, it was less precise. Extraction of 2AP from rice was highly inefficient using HSSE (avg ≈2%), as is the case with SPME (0.3%). Unless a 2AP standard is available, HSSE data is semi-quantitative, because the TMP standard was adsorbed at a different rate relative to the 2AP. Relative comparisons are valid assuming experimental parameters are held constant, but determinations of absolute concentrations are not legitimate. The advantage of HSSE is the ability to detect 2AP in rice at sub-ppb concentrations.

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