Natural Levels of Dimethyl Sulfide in Rough Rice and Its Products

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Natural levels of dimethyl sulfide (DMS) in rough rice and its products (polished rice, brown rice, and broken rice) were determined by a gas chromatograph equipped with a flame photometric detector and sulfur mode, after extraction with 25% KBr solution in a sealed system. DMS was found to occur naturally in nine newly harvested and stored Australian varieties of rough rice and its products and decreased during storage after harvesting. Natural levels of DMS in rough rice and its products varied with variety, fraction, and period of storage. The order of levels of DMS was rough rice = brown rice > polished rice = broken rice. The range of values was 0.002−30 mg kg^{-1} (ppm, w/w).

Keywords: *Rough rice; brown rice; polished rice; storage; dimethyl sulfide; analysis*

INTRODUCTION

Dimethyl sulfide (DMS) naturally occurs in many foodstuffs and vegetables and makes an important, and generally beneficial, contribution to the odor and flavor of many foodstuffs, including tea, cocoa, milk, wines, rum, beer, sweet corn, and numerous cooked vegetables (*1*−*6*). When present in quantities >35 ppm (v/v), DMS imparts a flavor that is usually described as "cooked sweet corn" or "blackcurrant-like" in these commodities. This flavor character is not present when DMS levels are <10 ppm (v/v) (*2*). DMS is the main volatile sulfur compound (VSC) emitted from wetlands, particularly from rice fields (*7*−*12*). However, there are few data on the natural occurrence of DMS or on the effect of variety, fraction, and period of storage on natural levels of DMS in rough rice and its products. Information about the natural occurrence of DMS in rough rice and its products is relevant to the establishment of maximum residue limits (MRL) and to the acceptance of DMS as a noncontaminating sulfur compound.

DMS is analyzed by headspace analysis (*3*, *13*) for solid commodities and cold trapping (*11*, *12*) for air or liquid samples. The traditional method of placing the solid sample in water and autoclaving forces the DMS from solution into its gaseous state, so that losses of analyte are inevitable (*13*). We therefore examined the possibility of using several different extraction solvents, including salt solutions such as 25% potassium bromide (KBr). Beyond this, like analysis of fumigants (*14*), headspace analysis relies on a number of assumptions about the concentration of the analyte in the gaseous and liquid phases. However, no published studies that describe headspace analysis of rough rice and its products have been located.

Here we report a method for the analysis of trace levels of DMS and the natural levels of DMS in rough rice and its products related to variety, products, and period of storage.

MATERIALS AND METHODS

Rough Rice and Their Fraction Samples. Six varieties of Australian rough rice (Koshhikarri, Millin, Waxy, Langi, Kyeema, and Amarro) and their products (polished, brokens, and brown rice) and five varieties of polished rice (Jasmine rice, SON 102275, Doogarra, Pelde, and Jarrah) were used. The samples were collected on stalk prior to harvesting to exclude any possible contamination. The rice products were obtained by milling rough rice at the laboratory. All commodities (0.5−1.0 kg) were placed into a sealed jar (1.5 L) at 25 ± 2 °C. The samples were removed and moisture content was checked before analysis of DMS. Moisture content (wet basis) was at 11.3−11.9% for rough rice and at 12.3−12.8% for polished rice, brokens, or brown rice. Moisture content was measured from loss of mass in ground samples after oven-drying at 130 °C for 2 h.

Reagents and Apparatus. DMS was supplied by Aldrich, Castle Hill, Australia, catalog no. 27,438-0. The other chemicals were purchased from BDH AnalaR, Poole, U.K. All reagents were of analytical grade, and water was glass distilled.

Glassware for extraction included 100-mL Erlenmeyer flasks with ground-glass joints (Crown Scientific, NSW, Australia, catalog no. FE100/3) that were fitted with half-hole septa (P/N 6526, Alltech Associates, Baulkham Hills, Australia).

DMS was determined by a Shimadzu GC6AM gas chromatograph (GC) (Shimadzu Seisakusho, Kyoto, Japan), equipped with a flame photometric detector (FPD). Separation was achieved on a 1 m × 3 mm i.d. glass column packed with HayeSep Q (Alltech Associates, catalog no. 2801) at 110 °C and carrier flow (N_2) of 40 mL/min at 0.8 psi.

Partitioning of DMS between Solvent and Air and Stability in the Sealed System. Acetone, toluene, water, methanol, 25% (w/v) potassium bromide (KBr), and 20% (v/v) H_2SO_4 (20 mL) were placed respectively in 100-mL Erlenmeyer flasks. DMS (2 μL) was injected into the flasks. DMS in both the gas and solvent phases was analyzed at timed intervals over a 78-h period at laboratory temperature (25 ± 2 °C). The headspace gas of 50 μL, or solvent of 2 μL, was taken using a gastight syringe or liquid syringe and injected into the GC. The concentrations in each phase were determined with reference to standards prepared daily. Concentrations recorded in the figures are the mean of duplicate samples.

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10.1021/jf000607z CCC: $20.00 © 2001 American Chemical Society
Published on Web 01/13/2001

Fortification Studies and Stability of DMS in Spiked Samples. Acetone, toluene, water, methanol, 25% KBr, and 20% H_2SO_4 (20 mL) were added respectively into 100-mL Erlenmeyer flasks containing rough rice or its fraction samples (25 g). DMS (2 μL) was injected into the flasks. DMS in the headspace was analyzed by GC over a 78-h period at laboratory temperature (25 ± 2 °C). The concentrations in the headspace were calculated by comparison with the standards prepared daily. Concentrations recorded in the figures are the mean of duplicate samples.

Studies on Time of Extraction. Waxy rough rice samples (50 g) were extracted in sealed flasks (100 mL) containing 50 mL of 25% KBr. Gas samples in the headspace over the solution (50 μL) were injected directly into the GC at timed intervals. The levels of DMS were calculated on the basis of peak areas. The peak areas were calibrated periodically using a gas standard and calculated as a percentage of maximum level of DMS versus time of extraction. The data recorded in the figures are the mean of duplicate samples.

Extraction and Determination of Natural Levels of DMS. Rough rice or its fraction samples (50 g) were extracted in sealed flasks (100 mL) containing 50 mL of 25% KBr for 20−25 h. An aliquot of the headspace over the extraction solution (50 μL) was injected directly into the GC. The levels of DMS were calculated on the basis of peak areas. The peak areas were calibrated periodically using a spiked standard, and the data recorded in the figures are the mean of duplicate samples.

Preparation of Gas Standard and Fortified Samples. The dilute gas was prepared by injecting a measured volume of liquid DMS into a bottle (250 mL) containing five glass beads (2−3 mm o.d.). After mixing, the diluted gas was used to prepare both fortified samples and gas standards. For partitioning and stability studies, reference standards were prepared by injecting measured volumes of gas into a sealed flask containing solvent. For extraction, spiked samples were prepared by injecting measured volumes of the diluted gas into a sealed flask containing commodity plus solution. Each spiked sample was duplicated. Analysis of DMS in the headspace over solvents or in the liquid phase required complete elution of solvent or solvent vapor before further injections, so a minimum interval of 10 min was kept between injections.

RESULTS

Partitioning of DMS between Air and Solvent and Stability in the Sealed System. Partitioning of DMS between air and six solvents (water, 20% H_2SO_4, 25% KBr, acetone, methanol, and toluene) is shown in Figure 1. Equilibrium partitioning between air and each solvent was achieved within 2 h. The mass of DMS in the headspace over each solvent and the solvents was stable. The mass (Ma) of DMS in the headspace was calculated from the concentration (Ca) of DMS in the air and the volume (Va), and the mass (Ms) in the solvent was calculated from the concentration (Cs) of DMS in liquid phase and the solvent volume (Vs). That is

$$Ma = Ca \times Va \quad (1)$$

$$Ms = Cs \times Vs \quad (2)$$

The mass distribution constant (K_d) is the ratio Ms/Ma. The equilibrium mass distribution ratios (K_d) were 6.0−30.0 for the organic solvents and 1.5−3.0 for water, 25% KBr, and 20% H_2SO_4. These studies show that these six selected solvents may be used for the extraction of DMS from commodities. However, using water, 25% KBr, or 20% H_2SO_4 gave higher sensitivity than the organic solvents for the determination of DMS by headspace sampling.

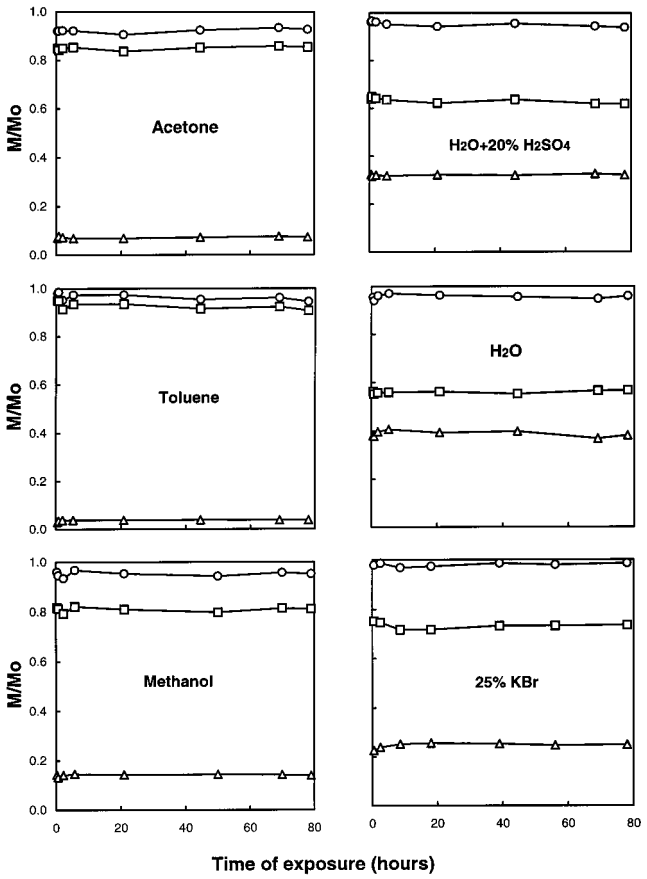

Figure 1. Distribution of DMS between gaseous and liquid phases in a closed system of air and solvent, where M/Mo is the ratio of mass of DMS in each phase to total calculated applied mass of DMS: ○, total recovery; △, gas phase; □, liquid phase. The coefficient of variation in replicates is <8%.

Fortification and Stability of DMS in Spiked Samples. The concentration of DMS in the headspace over the solvents plus rice samples is shown in Figure 2. DMS was stable in the headspace over methanol, acetone, and toluene plus rice. Equilibrium partitioning between air and methanol, acetone, and toluene plus rice was obtained after 5 h, but <1% of DMS was present in the headspace. DMS was not stable in the headspace over water and 20% H_2SO_4 plus rice. However, DMS was stable in the headspace over 25% KBr solution plus rough rice sample. The level of DMS in the headspace was 6 mg/L from an application of 24 mg/L, which is much greater than that with the organic solvents.

Time of Extraction. The effect of time of extraction of DMS from rough rice is shown in Figure 3. The amount of DMS in the headspace increased over a period of 20 h and then declined slightly. This result is consistent with results from fortified studies; that is, the amount of DMS in the headspace reached a plateau after ~25 h (Figures 2 and 3). However, the time for DMS to attain an equilibrium distribution between solvent and air (Figure 1) is much less than the time required to extract DMS from the rough rice sample (Figure 3).

Natural Levels of DMS in Rough Rice and Its Products. DMS was found to be naturally present in all tested newly harvested and stored Australian rough rice and its products, such as polished rice, brokens, and brown rice (Figures 4−6). The levels of DMS in rough

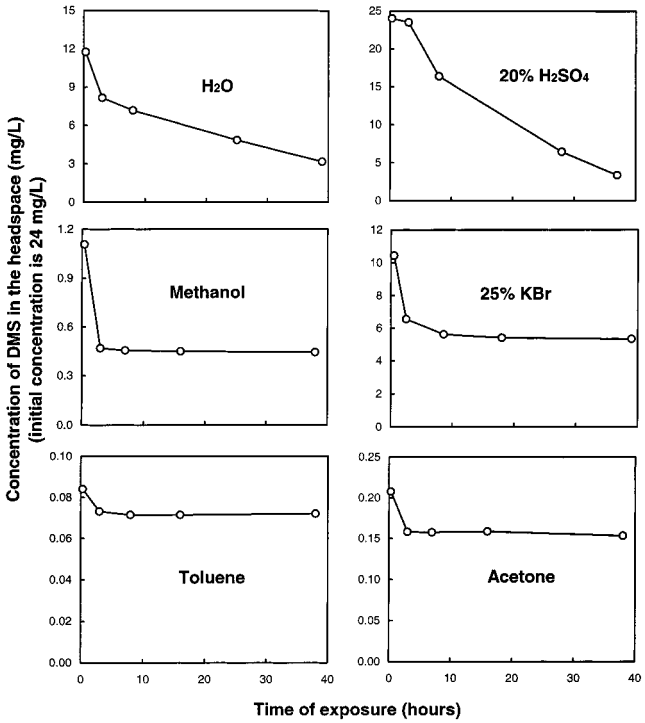

Figure 2. Stability and time to extraction of DMS from solvent (water, 20% H_2SO_4, methanol, 25% KBr, acetone, and toluene) plus paddy rice (Waxy 1998).

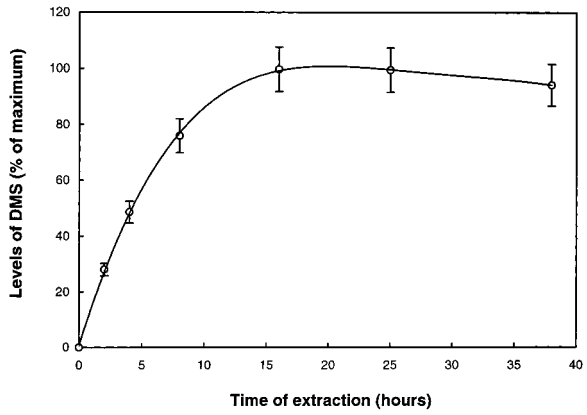

Figure 3. Extraction of natural levels of DMS in paddy sample (Waxy 1998), plotted as the percentage of maximum levels of DMS versus time of extraction (error bars indicate the SD, $n = 2$).

rice and its products varied with varieties, period of storage, and products. The values ranged from 0.03 to 30.0 mg/kg (ppm, w/w) of DMS in nine varieties (Jarrah, Doogarra, Kyeema, Langi, Koshhikarri, Pelde, Amarro, SON 102275, and Jasmine rice) of newly harvested (1999) polished rice (Figure 4). DMS decreased during storage; for example, the yearly loss (percent) of DMS from four tested varieties (Koshhikarri, Kyeema, Pelde, and Jarrah) of polished rice was ~50% (Figure 5). The effect of rice products on natural levels of DMS is shown in Figure 6. DMS was presented at the same level in polished rice and broken rice. Rough rice or brown rice also contained the same mass of DMS, if calculated on the basis of 85% milling production from rough rice to brown rice. However, levels of DMS in brown rice and rough rice were much higher than those in polished rice and brokens.

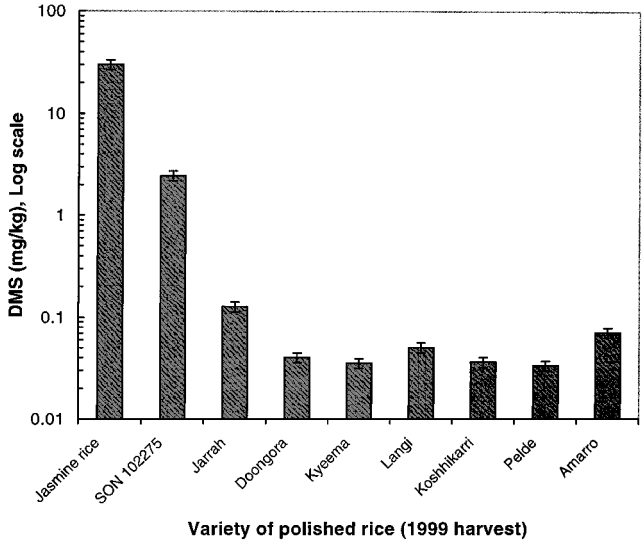

Figure 4. Natural occurrence of DMS in different varieties of newly harvested polished rice (error bars indicate the SD, $n = 2$).

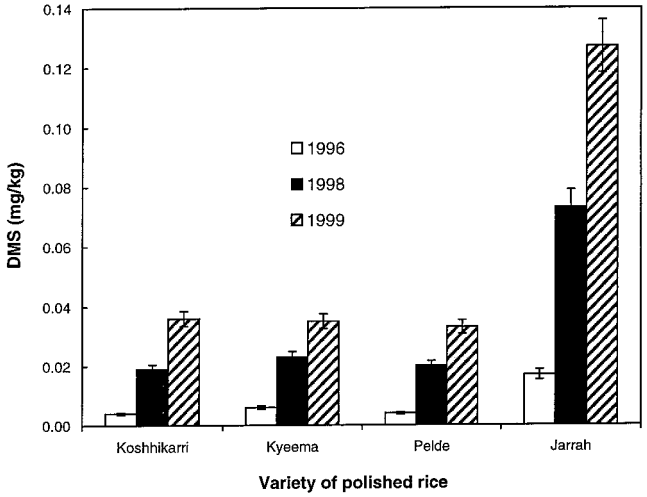

Figure 5. Natural occurrence of DMS in different varieties of polished rice after different periods of storage (error bars indicate the SD, $n = 2$).

DISCUSSION

The solvents can extract DMS from rough rice, but the limit of detection by headspace sampling might be much higher than the levels of naturally occurring DMS. DMS degraded in the headspace over water and 20% H_2SO_4 plus rice. An explanation for this loss might be microbial and enzymatic activity in the aqueous extraction; for example, DMS can convert to dimethyl sulfoxide (DMSO) or dimethyl sulfone (DMSO2) when yeast or bacteria are present or at low pH (*1*, *2*,).

A 25% KBr solution was used to extract DMS from rough rice and its products, principally because the amount of DMS distributed into the headspace over KBr solution was much greater than over organic solvents, such as methanol, acetone, and toluene, but without the interference associated with organic solvents. In addition, gas volumes that can be injected into the GC are much larger than solvent volumes. This is especially important for the determination of natural levels of DMS. The time (~20−25 h) for completeness of extraction of DMS is similar to that found for fumigants (*15*, *16*). Determination of DMS was corrected for recoveries

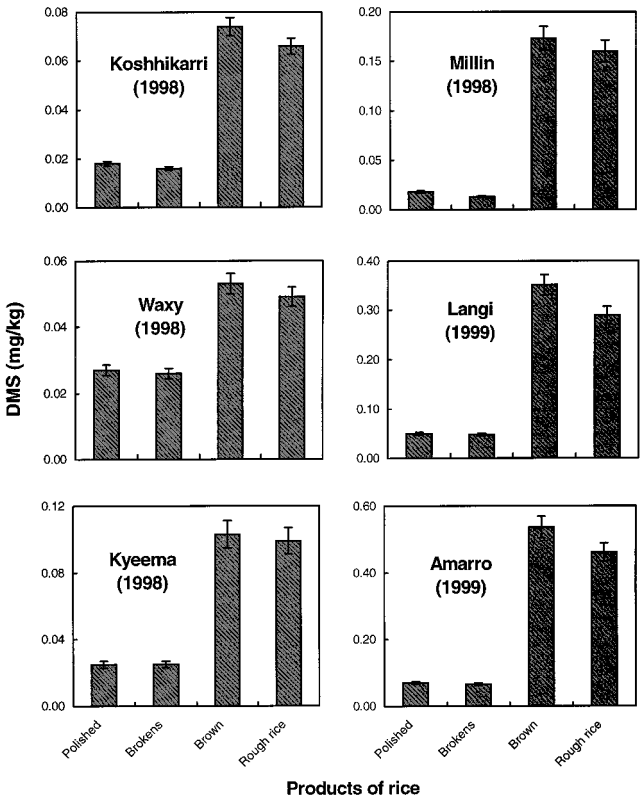

Figure 6. Natural occurrence of DMS in paddy, polished rice, brokens, and brown rice (error bars indicate the SD, $n = 2$).

of fortified sample using 25% KBr solution, and this fortified sample was used as a quantitative standard to calculate levels of DMS in rice samples. In this paper, only rough rice samples were used for fortification studies as the rough rice includes all products of the rice. Desmarchelier and Ren (*14*) discussed the use of fortified samples as standards. In fact, analysis of DMS is similar to analysis of grain fumigants, such as phosphine (PH_3), carbonyl sulfide (COS), methyl bromide (MeBr), and ethyl formate, which also degraded in the headspace over water and 20% H_2SO_4 plus rough rice (*15*, *16*).

Natural levels of DMS varied with rice products. This indicates that (1) the majority of DMS is not present in rice husks, because brown rice and rough rice contain the same level of DMS, and (2) the majority of DMS is present in rice germ or bran. It is possible that rice bran may play a role in preventing the oxidation of DMS in rice. The levels of DMS differed with varieties of rough rice and may correlate with differences in rice flavor; for example, two jasmine varieties of rice (Jasmine rice and SON 1022275) contained higher levels of DMS (3−30 mg/kg). Kokuzeicho-chokan (*17*) reported that old rice odors can be removed from sake by adding glucose oxidase to bring about decomposition of DMS. Results showing the loss of DMS with time during storage show that levels of DMS can indicate whether rough rice or its fraction (e.g., brown rice or polished rice) is fresh or aged and therefore may be an important indicator of quality in polished rice. However, it will be necessary to carry out further studies for the evaluation of each variety.

The presence of DMS in newly harvested rough rice and its products shows that DMS occurs naturally during growth in the rice field. It might be formed in two ways: (1) DMS may be absorbed during growth in the rough rice. It has been observed that there are large amounts of DMS (4.1−7.3 mg/m^2/year) in emissions from rice fields (*9*−*11*) and other types of wetland soils (*7*, *8*). Emission of DMS increased with application of organic manure and positively correlated with the total sulfur content in the soil (*12*). (2) DMS may be produced by enzymatic synthesis or biological hydrolysis (*2*, *5*, *18*, *19*). No rough rice or its products were found without DMS, but the levels were variable. This result indicates that levels of DMS should not be of concern with respect to the quality of rough rice and its products.

ACKNOWLEDGMENT

We thank Drs. E. J. Wright, C. P. Whittle, and M. J. Lacey for helpful advice. We give special thanks to David Rees for information on DMS in brewing procedures.

LITERATURE CITED

(1) Wilson, R. J. H.; Booer, C. D. Control of the dimethyl sulphide content of beer by regulation of the copper boil. *J. Inst. Brew.* **1979**, *85*, 144−148.
(2) Anness, B. J.; Bamforth, C. W. Dimethyl sulphide—A review. *J. Inst. Brew.* **1982**, *88*, 244−252.
(3) Christensen, K. R.; Reineccius, G. A. Gas chromatographic analysis of volatile sulfur compounds from heated milk using static headspace sampling. *J. Dairy Sci.* **1992**, *75*, 2098−2104.
(4) Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramamlt) using GC/MS techniques and odour dilution analyses. *Nahrung—Food* **1998**, *42* (6), 371−375.
(5) Hansen, J. Inactivation of MXR1 abolishes formation of dimethyl sulfide from dimethyl sulfoxide in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **1999**, *65* (9), 3915−3919.
(6) Mazza, G.; Cottrell, T. Volatile components of roots, stems, leaves, and flowers of *Echinacea* species. *J. Agric. Food Chem.* **1999**, *47*, 3081−3085.
(7) Chapman, S. J.; Kanda, K. I.; Tsuruta, H.; Minami, K. Influence of temperature and oxygen availability on the flux of volatile sulphur compounds from wetlands: a comparison of peat and paddy soils. *Soil Sci. Plant Nutr.* **1996**, *42* (2), 279−288.
(8) Cooper, W. J.; Cooper, D. J.; Saltzman, E. S.; Mello, W. Z., de; Savoie, D. L.; Zika, R. G.; Prospero, J. M. Emission of biogenic sulphur compounds from several wetland soil in Florida. *Atmos. Environ.* **1987**, *21* (7), 1491−1495.
(9) Minami, K.; Kanda, K. I.; Tsuruta, H.; Oremland, R. S. Emission of biogenic sulfur gases from rice paddies in Japan. *Biogeochemistry of Global Change*: *Radiatively Active Trace Gases*; 1993; pp 405−418.
(10) Kanda, K. I.; Tsuruta, H.; Minami, K. Emission of dimethyl sulfide, carbonyl sulfide, and carbon disulfide from paddy fields. *Soil Sci. Plant Nutr.* **1992**, *38* (4), 709−716.
(11) Kanda, K. I.; Minami, K. Measurements of dimethyl sulfide (DMS) emission from lysimeter paddy field. *Jpn. J. Soil Sci. Plant Nutr.* **1991**, *62* (2), 35−40.
(12) Yang, Z.; Kong, L.; Zhang, J.; Wang, L.; Xi, S. Emission of biogenic sulfur gases from Chinese rice paddies. *Sci. Total Environ.* **1998**, *224* (1−3), 1−8.
(13) Breeden, D. C.; Juvik, J. A. An extraction method for the determination of dimethyl sulfide in cooked corn. *J. Food Compos. Anal.* **1992**, *5* (2), 134−138.
(14) Desmarchelier, J. M.; Ren, Y. L. Analysis of fumigant residues? A critical review. *J. AOAC Int.* **1999**, *82* (6), 1261−1280.
(15) Desmarchelier, J. M.; Allen, S. E.; Ren, Y. L. Modifications of a method for determining multifumigant residues. *J. AOAC Int.* **1998**, *81* (3), 638−644.

(16) Le Trang Vu; Ren, Y. L. An improved method of analysis for, and natural levels of ethyl formate in stored grains. *J. Stored Prod. Res.* **2000**, submitted for publication.
(17) Kokuzeicho-chokan. Sake odour improvement. Jpn. Unexamined Patent JP 57 170 180A, 1982.
(18) White, F. H.; Wainwright, T. The measurement of dimethyl sulphide precursor in malts, adjuncts, worts and beers. *J. Inst. Brew.* **1976**, *82*, 46−48.
(19) Kavanagh, T. E.; Derbyshire, R. C.; Hildebrand, R. P.; Clarke, B. J.; Meeker, F. J. Dimethyl sulphide formation in malt-effect of malting conditions. *J. Inst. Brew.* **1976**, *82*, 270−272.

Received for review May 16, 2000. Revised manuscript received October 20, 2000. Accepted October 27, 2000. We thank the Grain Research Development Corp. (GRDC), Co-operative Research Center for Sustainable Rice Production (Rice CRC), and the Stored Grain Research Laboratory (SGRL) agreement for financial assistance.

JF000607Z