ACKNOWLEDGMENT

We thank R. G. Richards and R. W. Reed for their valuable technical assistance.

Registry No. STB, 41136-38-3; H_2O, 7732-18-5; benomyl, 17804-35-2; carbendazin, 10605-21-7.

LITERATURE CITED

Austin, D. J.; Briggs, G. G. *Pestic. Sci.* **1976**, *7*, 201.
Calmon, J. P.; Sayag, D. R. *J. Agric. Food Chem.* **1976**, *24*, 314.
Chiba, M. *J. Agric. Food Chem.* **1977**, *25*, 368.
Chiba, M. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 488.
Chiba, M.; Cherniak, E. A. *J. Agric. Food Chem.* **1978**, *26*, 573.
Chiba, M.; Veres, D. F. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1291.
Hall, D. J. *Proc. Fl. State Hortic. Soc.* **1980**, *93*, 341.
Kirkland, J. J. *J. Agric. Food Chem.* **1973**, *21*, 171.
Kirkland, J. J.; Holt, R. F.; Pease, H. L. *J. Agric. Food Chem.* **1973**, *21*, 368.
Koller, W.; Allan, C. R.; Kolattukudy, P. E. *Pestic. Biochem. Physiol.* **1982**, *18*, 15.
Singh, R. P.; Chiba, M. *J. Agric. Food Chem.* **1985**, *33*, 63.
White, E. R.; Bose, E. A.; Ogawa, J. M.; Manji, B. T.; Kilgore, W. W. *J. Agric. Food Chem.* **1973**, *21*, 616.
Zweig, G.; Gao, R. *Anal. Chem.* **1983**, *55*, 1448.

Received for review April 17, 1985. Revised manuscript received September 3, 1985. Accepted October 4, 1985.

Quantitative Analysis of 2-Acetyl-1-pyrroline in Rice

Ron G. Buttery,* Louisa C. Ling, and Thomas R. Mon

A relatively simple practical method has been developed for the quantitative analysis of 2-acetyl-1-pyrroline in rice samples. The rice analysis method uses a steam distillation continuous-extraction isolation procedure with an acid-phase solvent extraction. This is followed by regeneration of the basic volatiles and capillary or packed column gas chromatography analysis. Testing of the method, with a bland rice variety containing known added concentrations of 2-acetyl-1-pyrroline, showed that the method was sufficiently accurate for the purpose.

INTRODUCTION

With both bland and aromatic rice types, as with most food crops, it is necessary to continually breed new varieties for disease or insect resistance and other environmental factors. In breeding new varieties, however, there is the danger of changing other subtle desirable features such as the flavor characteristics. A simple chemical analytical method for rice samples, to evaluate the flavor of the new varieties, could be very useful. Some of us had previously shown that 2-acetyl-1-pyrroline was an important flavor component of cooked rice particularly the aromatic (basmati type) rice varieties (Buttery et al., 1983).

The chemical analysis of 2-acetyl-1-pyrroline in rice by conventional methods is, however, difficult because of the presence of other interfering compounds and the instability of this compound. The present study was carried out to develop a relatively simple, practical method for analysis of this compound in rice.

EXPERIMENTAL SECTION

Materials. Authentic 2-acetyl-1-pyrroline was synthesized as outlined by some of us previously (Buttery et al., 1983). A standard solution of this compound containing 290 parts per million (ppm) of 2-acetyl-1-pyrroline was made in benzene solution. This was stored at −20 °C and seemed quite stable over several months.

Collidine (2,4,6-trimethylpyridine), used as an internal standard, was Eastman Organic Chemicals No. 4815. It was used as a water solution containing 30.0 ppm collidine. This seemed quite stable at room temperature.

Other reagents were of good-quality analytical reagent grade. Diethyl ether was freshly distilled and was protected by the addition of ca. 0.001% Ethyl antioxidant 330.

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710.

Volatile-free water was obtained by boiling water in an open container removing about 10% of its volume. A volatile-free antifoam preparation was made by adding 20 mL of GE AF 60 Silicone antifoam emulsion to 600 mL of water in a wide-neck 1-L flask and concentrating the mixture to 200 mL by boiling.

All glassware was thoroughly cleaned and heated in an oven at 120 °C for several hours to remove all volatile contaminants. Aromatic rice variety samples were obtained from the International Rice Research Institute in the Philippines. Common rice samples were obtained from local markets.

Isolation of the Volatile Basic Fraction from Rice. Water (6 L) was added to a 12-L round-bottom flask containing a large efficient magnetic stirrer. The flask was supported by a 1300-W (two-circuit) 115-V heating mantle. While stirring, 200 g of rice was added gradually (so as not to interfere with the stirring motion). Antifoam solution (50 mL) and the internal standard (5.00 mL of the standard 30 ppm collidine solution) were then added. A Likens–Nickerson type steam distillation continuous-extraction head (cf. Kontes No. K-523010-0000; Nickerson and Likens, 1966) was attached to the neck of the 12-L flask. A 250-mL round-bottom flask, containing 80 mL of dilute sulfuric acid (80 mL of water + 2.0 mL of concentrated sulfuric acid) and 120 mL of diethyl ether with a magnetic stirrer, was attached to the solvent arm of the head. To heat the water in the 12-L flask to boiling, 100 V was applied to the upper mantle circuit and 80 V to the lower circuit. On boiling, these were changed to 80 V on the upper circuit and 70 V on the lower circuit. Care should be taken to prevent any "burning" of the rice on the bottom of the 12-L flask as this produces interfering compounds (alkylpyrazines). The solvent was refluxed (and stirred) vigorously (80 V on the solvent mantle). After 2 h of the steam distillation continuous extraction, the solvent flask was removed and the aqueous layer separated,

using a 250 mL separatory funnel (Teflon stopcock), into a 500-mL wide-neck Erlenmeyer flask. This was then covered with diethyl ether (120 mL). Sodium bicarbonate (15 g) was added in portions over about 5 min. The neutral mixture was then poured into a clean 250-mL separatory funnel and the funnel stoppered and then shaken thoroughly. The upper ether layer was then separated and poured into a 250-mL wide-neck Erlenmeyer flask. Anhydrous sodium sulfate (40 g) was added to remove dissolved water. The dry ether solution was then filtered through a clean cotton plug (prewashed with ether) into a 200-mL pear-shaped flask. A short (17-cm-long) Vigreux distillation column was attached to the neck of the flask, a silicon carbide boiling chip added, and the ether concentrated (using a ca. 70 °C warm-water bath) to ca. 1 mL. This concentrate was then transferred to a graduated 2.5-mL micro test tube, a boiling chip added, and the ether concentrated to ca. 0.05 mL with a ca. 50 °C warm-water bath. This concentrate was used for the gas–liquid chromatography (GLC) analysis.

Gas–Liquid Chromatography Analysis. The GLC detector was of the flame ionization type. The main GLC column used for the analyses was a laboratory prepared, 150-m length 0.5-mm i.d. Pyrex glass capillary, wall coated with Carbowax 20-M. The inlet pressure was 15 psi of He. The column was held at 50 °C for the first 30 min after injection and then programmed at 1 °C/min from 50 to 170 °C. Sample size injected was 2 μL of the ether concentrate from above into a 170 °C injector connected directly to the column (no split). The gas chromatograph was a Hewlett-Packard series 5880A.

The analysis was also shown feasible with a commercially available capillary column. This was a J & W, 60 m by 0.326 mm i.d. (0.25-μm film) fused silica column wall coated with Durabond wax. This was programmed from 50 to 230 °C at 4 °C/min. Inlet pressure was 22 psi. Sample size was 0.2 μL, split $^1/_{50}$. Under these conditions, the retention time of 2-acetyl-1-pyrroline was 26 min and that of collidine was 29 min.

Use of a packed GLC column was also found possible. The packed column was a 1.2-m length by 3-mm o.d. Pyrex column packed with 80–100 mesh Chromasorb G coated 10% by weight with Carbowax 20-*m*. The column was kept constant at 90 °C with an inlet pressure of 6 psi of He. Sample size was 2 μL with a 170 °C injector. Under these conditions, the retention time of 2-acetyl-1-pyrroline was 22 min and that of collidine was 26 minutes.

RESULTS AND DISCUSSION

The procedure finally adopted for the quantitative analysis of 2-acetyl-1-pyrroline is described in detail in the Experimental Section.

General Approach. The initial step in the method was based on the method previously used by some of the authors (Buttery et al., 1983). This involved isolation of the volatiles using steam distillation continuous extraction with an apparatus of the Likens–Nickerson type (cf. Nickerson and Likens, 1966). Figure 1 shows a capillary GLC analysis of the steam volatile concentrate obtained by using this type of isolation with a Basmati rice sample. It can be seen that there are a very large number of volatile rice components. Because of its much higher odor potency, 2-acetyl-1-pyrroline (peak 62) is much more important to the total rice aroma than the other volatile components (cf. Buttery et al. (1983)). In measuring the area of the 2-acetyl-1-pyrroline peak it is difficult to avoid interference from other closely associated peaks, particularly with samples having a considerably lower concentrations of 2-acetyl-1-pyrroline than that shown in Figure 1. A step

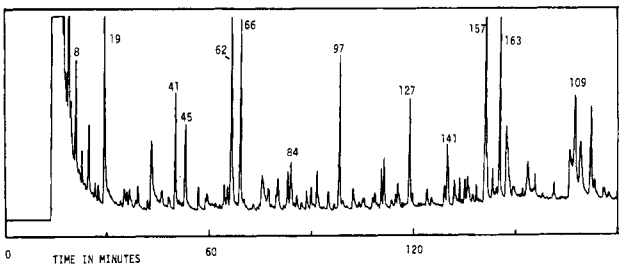

Figure 1. Capillary GLC analysis of the total steam volatile oil from basmati rice (using the 150 m × 0.5 mm i.d. Pyrex capillary wall coated with Carbowax 20-M. Peak 62 is 2-acetyl-1-pyrroline.

using dilute acid to extract only the basic components was therefore developed to lessen the effect of interfering components. A convenient and efficient way to extract the basic fraction is to use both dilute acid and diethyl ether in the solvent flask in the steam distillation continuous-extraction apparatus. In this way the basic components are extracted from the steam distillate and bound in the aqueous acid layer in the solvent flask.

Internal Standard. Use of internal standards is common in gas chromatography. With the present analysis it is convenient to add the standard to the rice and water in the 12-L distillation flask before beginning the steam distillation. The choice of the internal standard required that it (1) had related properties to the 2-acetyl-1-pyrroline (i.e. that it be basic, have a similar water solubility and similar volatility, etc.), (2) be a stable compound, (3) have a GLC retention time reasonably close to that of 2-acetyl-1-pyrroline, (4) be readily available from commercial sources. A number of compounds were tried. The most suitable compound that we could find was collidine (2,4,6-trimethylpyridine).

Recovery of Volatiles from Aqueous Acid. After the steam distillation continuous-extraction step the aqueous acid layer was separated from the ether layer in a separatory funnel. Most of the unwanted nonbasic volatile components remained in the ether layer (small percentages of the more water-soluble nonbasic components also occur in the aqueous acid layer but do not interfere to any appreciable extent). The basic components are regenerated from the aqueous acid by neutralization with excess sodium bicarbonate and reextracted with ether. To minimize manipulation time, only one extraction with ether was used. Extracting several consecutive times would undoubtably improve the transfer of material but would add considerably to the analysis manipulation time. A recovery factor relative to the internal standard seemed to be sufficient to compensate for this and other manipulation differences between the internal standard and 2-acetyl-1-pyrroline.

The relative recovery factor was determined by adding known quantities of both 2-acetyl-1-pyrroline and collidine to 6 L of water in the 12-L flask and carrying them through the whole process. This factor for the conditions described in the Experimental Section was determined to be 28.0% (with a standard deviation of 4.9% for nine GLC determinations and three steam distillation continuous-extraction procedures). It is necessary to multiply the results based on the internal standard then by the factor $^{100}/_{28}$ which equals 3.57 to get the amount of 2-acetyl-1-pyrroline actually present in the cooked rice. The equation used for calculating the concentration of 2-acetyl-1-pyrroline was

$$\text{concn (ppb)} = \frac{\text{area of AP peak}}{\text{area of TMP peak}} \times 150 \times 3.57 \times 5$$

where AP = 2-acetyl-1-pyrroline and TMP = collidine.

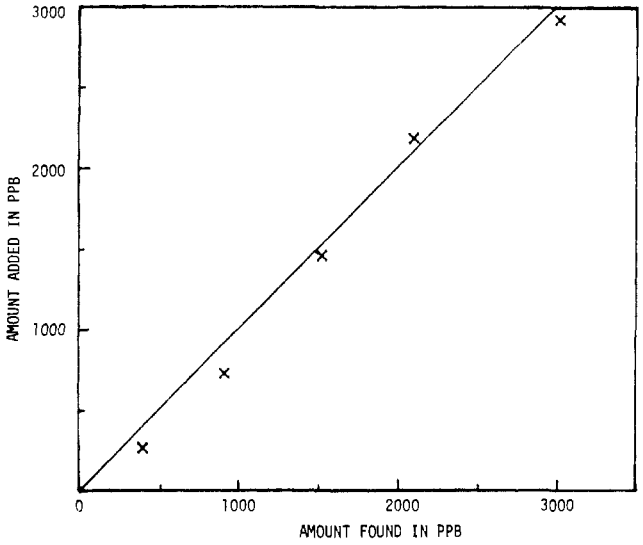

Figure 2. Relation between the amount of 2-acetyl-1-pyrroline found by the analytical method and that added to a bland rice variety. The solid line shows the relation if amount found = amount added.

The factor 150 is the number of micrograms of internal standard added, and the factor 5 is needed to convert to parts per billion (ppb).

The major difference in the recovery of 2-acetyl-1-pyrroline and the internal standard seems to occur during the steam distillation step. The reasons for this are not known although they may be partly due to the instability of 2-acetyl-1-pyrroline. Measurement of the relative ether/water partition coefficients for 2-acetyl-1-pyrroline and collidine showed that they were in the ratio of 0.74/1.0, the collidine being slightly less soluble in water.

Testing of the Method. The method was tested by taking samples of a common American rice containing less than 10 ppb 2-acetyl-1-pyrroline and adding known amounts of 2-acetyl-1-pyrroline. This was then taken through the analytical procedure as described in the Experimental Section. The results found are plotted in Figure 2 against the added amounts. The solid line shows where the values should occur if found = added concentrations. It can be seen that there is some scatter around the line, but the method seems accurate enough for the purpose, considering the small concentrations involved and the unstable nature of 2-acetyl-1-pyrroline.

Studies with Rice Samples. The 2-acetyl-1-pyrroline is formed from the rice during the cooking process, and what the method actually measures is not the concentration in the rice at any particular time but the total amount of 2-acetyl-1-pyrroline produced from the given weight of rice during the heating (2-h) period. For this reason it is important to keep the degree of heating and the heating period the same for the different samples. Figure 3 shows GLC analysis using the laboratory-constructed 0.66-mm i.d. Pyrex capillary column. The peak corresponding to 2-acetyl-1-pyrroline is denoted by AP and that of the internal standard collidine by TMP. Sample A is for basmati rice and samples B for a very bland U.S. rice.

Table I lists the results found for several varieties of rice. It should be pointed out that we have not demonstrated

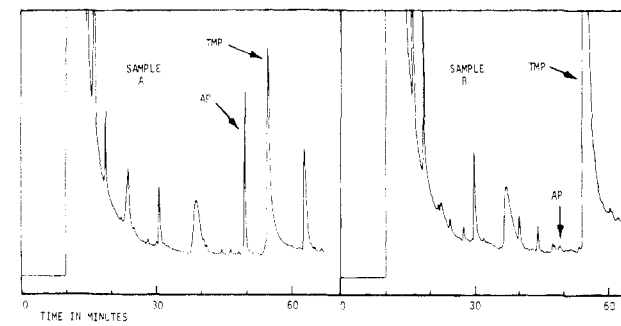

Figure 3. GLC analysis of the basic fraction from basmati rice (sample A) compared to that of the basic fraction of Texas long-grain rice (sample B) using a similar GLC column as for Figure 1. AP = 2-acetyl-1-pyrroline. TMP = collidine (2,4,6-trimethylpyridine) internal standard.

Table I. Concentration of 2-Acetyl-1-pyrroline Found in Some Samples of Cooked Rice

rice sample	concn,[a] ppb
Malagkit Sungsong (brown)	760
basmati 370 (brown)	610
IR 841-76-1 (brown)	560
Texas long grain (Bluebell var.; polished)	6

[a] ppb = parts per billion (10^9).

that the method is accurate for the low value found for Texas long-grain rice. However, it would be expected to be of the right order. In previous analyses of these rice varieties by the authors (Buttery et al., 1983), the low efficiency of recovery of 2-acetyl-1-pyrroline in the steam distillation continuous-extraction step had not been taken into account. When the recovery factor is applied to the old figures, however, they do agree fairly well with the present analyses.

Other Types of GLC Columns. The GLC columns used by the authors for the main part of this work were laboratory constructured and are thus not generally available. Preliminary studies showed that analysis using a commercially available capillary column (60 m × 0.326 mm Durabond wax, fused silica) was also quite feasible.

A short packed Pyrex column (1.2-mm length × 3-mm o.d. Pyrex packed with 10% Carbowax 20-M on 80–100 Chromasorb G) also gave satisfactory separation. A longer (e.g., 7-m low-loaded, 1% Carbowax 20-M) packed column would undoubtably give better separation.

Because of the instability of 2-acetyl-1-pyrroline it is advisable to use injector temperatures of 150–170 °C. Higher injector temperatures seemed to cause some decomposition.

Registry No. 2-Acetyl-1-pyrroline, 99583-29-6.

LITERATURE CITED

Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. *J. Agric. Food Chem.* **1983**, *31*, 823.
Nickerson, G. B.; Likens, S. T. *J. Chromatogr.* **1966**, *21*, 1.

Received for review June 10, 1985. Accepted for review October 30, 1985. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.