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Cooked Rice Aroma and 2-Acetyl-1-pyrroline

Ron G. Buttery,* Louisa C. Ling, Bienvenido O. Juliano, and Jean G. Turnbaugh

The concentration of 2-acetyl-1-pyrroline has been determined in the steam volatile oils of 10 different varieties of rice. From these data the amount present in the cooked rice was calculated. This varied from less than 0.006 parts per million (ppm) for Calrose rice to 0.09 ppm for Malagkit Sungsong variety rice based on the dry weight of the rice. Odor panel evaluation described the odor of 2-acetyl-1-pyrroline as "popcorn"-like. Odor evaluation of the amount of popcorn-like odor in the different rice varieties ranked them in the general order of the concentration of this compound. Other odor quality evaluation tests confirmed the importance of this compound to the aroma of the more aromatic rice varieties.

Knowledge of the identities of the volatile aroma components of rice is important in the understanding of both human and insect perception of rice. Pest insects probably locate stored rice by keying in on the associated volatile (aroma) compounds. Volatile compounds found in cooked foods frequently occur in the "raw" foods also, although usually at a much lower concentration.

A considerable number of different varieties of rices are grown throughout the world. American consumers seem to prefer the more bland varieties such as the Texas Long Grain rice or Calrose rice. However, in Southeast Asia, India, and some Middle East countries, a number of more aromatic rices are highly favored and command much higher prices than the more bland varieties. A selection of these more aromatic rice varieties was studied by the authors and a potent aroma component, 2-acetyl-1-pyrroline, was identified for the first time (Buttery et al., 1982). The present work compares the concentration of this compound in the different rice varieties and reports some additional information on its chemical and odor properties.

The volatile components of rice have been studied previously by a number of researchers. Studies up until about 1978 were reviewed by Maga (1978). Some more recent studies include those of Yajima et al. (1978, 1979) and Tsugita et al. (1980).

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710 (R.G.B., L.C.L., and J.G.T.), and The International Rice Research Institute, Manila, Philippines (B.O.J.).

EXPERIMENTAL SECTION

Materials. Most rice varieties were obtained through the International Rice Research Institute (IRRI) in Manila, Philippines. These are 1981 crops of Azucena, IR841-76-1 (a line derived from Khao Dawk Mali 105), and Milagrosa from the Philippines, Basmati 370 from Pakistan, Hieri from Japan, Khao Dawk Mali 105 from Thailand, and Seratus Malam from Indonesia and the 1982 crop of Malagkit Sungsong from the Philippines. These were obtained as brown rices and milled in the laboratory removing ca. 10% of the outer layers. Calrose rice (milled) was obtained from local markets in Berkeley, CA, in 1982. Texas Long Grain rice (milled) obtained from the Comet Rice Mills in Houston, TX, and is probably Labelle variety.

Synthesis of 2-Acetyl-1-pyrroline. This followed very closely the method used by Büchi and Wüest (1971) for the synthesis of the related 2-acetyl-1,4,5,6-tetrahydropyridine. 2-Acetylpyrrole (1.7 g) in methanol solution (50 mL) was hydrogenated by using 5% rhodium on alumina catalyst (2.0 g) at room temperature under 10 psi of H_2 for 15 h with stirring. Filtration and removal of the solvent by distillation gave 1.8 g of the crude intermediate 2-(1-hydroxyethyl)pyrrolidine [cf. Hess (1915)]. This intermediate showed a mass spectrum with a molecular ion a 115 and other important ions at 70 ($M^+ - 45$), 68, 97 ($M^+ - 18$), and 82 ($M^+ - 33$) and infrared and ^1H NMR spectra consistent with the structure of this compound. The 2-(1-hydroxyethyl)pyrrolidine (1.8 g) was added to a stirred suspension of silver carbonate on Celite (16 g) in benzene (100 mL) under a nitrogen atmosphere. The mixture was refluxed under nitrogen for 15 h. Filtration removal of the silver carbonate and concentration by distillation to 5 mL gave a benzene solution of 2-acetyl-1-pyrroline which was

Table I. Concentration of 2-Acetyl-1-pyrroline Found in Cooked Rice Varieties in Terms of Dry Weight of Rice

variety	2-acetyl-1-pyrroline concn, ppm[a] milled rice	brown rice
Malagkit Sungsong	0.09	0.2
IR841-76-1	0.07	0.2
Khao Dawk Mali 105	0.07	0.2
Milagrosa	0.07	
Basmati 370	0.06	0.17
Seratus Malam	0.06	
Azucena	0.04	0.16
Hieri	0.04	0.1
Texas Long Grain	<0.008	
Calrose	<0.006	

[a] ppm = parts (weight) of compound per million (10^6) parts of rice (dry weight).

isolated by gas–liquid chromatography (GLC). This indicated an overall yield (from 2-acetylpyrrole) of 10%. The GLC column used was a 2 m long by 0.64 cm o.d. aluminum column packed with 15% Amine 220 on 60–80-mesh Chromosorb P.

The GLC-purified 2-acetyl-1-pyrroline was collected in 3 mm o.d. Pyrex tubes, sealed under vacuum, and stored at −20 °C.

Steam Distillation Continuous Extraction of Rice. The rice (500 g) was added to water (6 L) in a 12-L flask. A Likens-Nickerson steam distillation continuous extraction head was attached to the flask. Freshly distilled diethyl ether (125 mL) was used as the solvent in a 250-mL flask attached to the solvent arm of the head. The isolation was carried out at atmospheric pressure for 2 h. The ether extract was dried over sodium sulfate and concentrated on a warm water bath by using low hold up distillation columns to 0.15 mL.

Isolation of Basic Fraction. The ether concentrate from above was dissolved in 50 mL of hexane. This solution was then extracted with 3 N hydrochloric acid (3 × 25 mL). The combined acid extracts were washed with ether (1 × 50 mL). The washed acid extract was then neutralized with excess sodium bicarbonate under ether (100 mL) with ice bath cooling. The ether was separated and the aqueous layer extracted further (2 × 50 mL) with ether. The ether extracts were then combined, dried over sodium sulfate, and concentrated to 0.01 mL.

Capillary Gas Chromatography–Mass Spectrometry Analysis. This was carried out by using a 150 m long by 0.64 mm i.d. Pyrex glass capillary column coated with Carbowax 20M. The effluent from the capillary column was introduced into the mass spectrometer (a modified Consolidated 21-620 cycloidal type) by using a Llewellyn-Littlejohn type single-stage silicone rubber membrane molecular separator. The GLC column was temperature programmed by holding at 50 °C for 30 min after injection and then increasing from 50 to 170 °C at 1 °C/min and holding the temperature at the upper limit for 2 h longer.

Odor Evaluation. Odor threshold determinations were carried out as previously described for other compounds [cf. Buttery et al. (1981)] with Teflon bottles and tubing. Odor quality evaluations were carried out by using established panel procedures with 100-mL opaque Pyrex glass flasks.

RESULTS AND DISCUSSION

Earlier studies by the authors (Buttery et al., 1982) were carried out by vacuum steam distillation continuous extraction of the freshly cooked rice. However a more rapid and simpler method was desired for studying a large number of samples. In the present work a 2-h atmospheric steam distillation continuous extraction procedure was used on the uncooked rice. Cooking occurs during the isolation process. The amount of volatile oil obtained by this method was of the order of 5 parts per million (ppm). The volatile oils were analyzed by capillary GLC–MS, and the amount of 2-acetyl-1-pyrroline present was calculated from GLC peak areas. The concentrations found in the different rices is shown in Table I. It can be seen that the Malagkit Sungsong has the highest concentration with 0.09 ppm and the Calrose the least amount with 0.006 ppm. The mass spectrum and GLC retention data of the identified peak for each variety were consistant with that of a synthetic sample of 2-acetyl-1-pyrroline [i.e., molecular ion at m/e 111 (5), other major ions at 43 (100), 41 (50), 42 (24), 83 (11), 69 (11), 68 (8), 55 (2), 52 (0.9), 54 (0.2), 67 (0.2)].

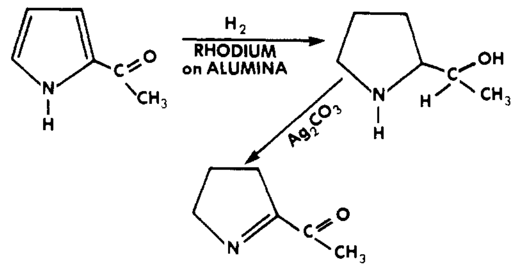

Figure 1. Synthesis of 2-acetyl-1-pyrroline.

Most other compounds previously identified in rice [e.g., Yajima et al. (1978)] were also found in the present work by using capillary GLC–MS. The 2-acetyl-1-pyrroline (Kovats' index 1320) was a clearly separate peak on the Pyrex Carbowax 20M GLC capillary at a position just before hexanol. A basic fraction of the steam volatile oil was also isolated in some cases by acid extraction and subsequent neutralization and reextraction. GLC analysis of this basic fraction gave an even better separation of the 2-acetyl-1-pyrroline, and this may be a more suitable method of analysis if less efficient GLC columns are used than that available for the present study.

The brown rice form of some of the rice varieties were also analyzed, and these data are also listed in Table I. It can be seen that 2-acetyl-1-pyrroline occurs at a higher concentration in the brown rice.

Quantitative Analysis. The figures in Table I are meant only to give a general idea of the variation of 2-acetyl-1-pyrroline with the different varieties. The GLC peak area method was used, making the usual assumption that all compounds, in the complex mixture, have the same response in the flame ionization detector. Only one sample from each variety was studied and no attempt was made to study the variation within any variety. The difference found between varieties such as Malagkit Sungsong (0.09 ppm) and Basmati 370 (0.06 ppm) cannot be considered meaningful for this type of study. The factor of about 10 times difference, between the more aromatic Asian rice varieties and the American Calrose and Texas Long Grain varieties, is certainly meaningful. The higher values found for brown rice indicates that there may be some variation depending on the degree of milling.

The method of isolation of the volatiles used in the present work gives a figure for the total 2-acetyl-1-pyrroline produced by the rice during the isolation period. In the normal cooking of a rice for food, much of this would be lost and the amount left in the rice would be considerably less. The method of isolation used in the present work was developed for its ease of operation. The authors' original method of isolation (Buttery et al., 1982) involved a vacuum steam distillation continuous extraction of the already cooked rice. This vacuum isolation method probably gives a more accurate figure for the amount actually present in the cooked rice. This figure was about $^1/_{10}$ that found by using the atmospheric isolation method.

2-Acetyl-1-pyrroline Synthesis. The successful synthetic method (Figure 1) described in detail under Experimental Section followed closely the procedure used by Büchi and Wüest (1971) for the synthesis of the related six-membered ring compound 2-acetyl-1,4,5,6-tetrahydropyridine. 2-Acetylpyrrole was used as the starting material. It was found necessary to use a large proportion of rhodium on alumina "catalyst" to complete the hydrogenation. Smaller ratios did not give complete hydrogenation. It is well-known that hydrogenation catalysts are deactivated to varying extents by nitrogen heterocyclic compounds. The Ag_2CO_3 oxidation was quite straightforward although a shorter reflux time might possibly give better yields. The 2-acetyl-1-pyrroline was a clear colorless liquid when first purified by GLC separation. It was immediately sealed under vacuum and stored at −20 °C but even under these conditions slowly turned to a red color which became darker the longer the storage. The authors speculate that a conjugated pyrroline polymer may be responsible, formed by condensation of the carbonyl groups with the 5-positions of other molecules, thus forming a long conjugated chain. For this reason 2-acetyl-1-pyrroline may be more stable in dilute solution. In the authors' experience this seemed to be true with dilute water solutions. The compound showed considerable instability to general gas chromatography conditions and could not be gas chromatographed by using the authors' silicone or Carbowax 20M packed columns. This may explain why it was not detected in the earlier studies of rice volatiles. It seemed reasonably stable when the Amine 220 packed column and the all-glass capillary GLC system were used.

The mass and ^{1}H NMR spectra of 2-acetyl-1-pyrroline were described in the authors' previous publication (Buttery et al., 1982). The infrared spectrum (CCl_4 solution) showed major absorption maxima at 1695, 1620, 1435, 1370, 1340, 1250, 1080, 1000, 975, and 940 cm^{-1} in the 2000–600-cm^{-1} region. This together with the ^{1}H NMR (run in deuterated Me_2SO and separately in $CDCl_3$) had strongly supported the 1-pyrroline form rather than the 2-pyrroline form in comparison with the data obtained by Büchi and Wüest (1971) for the related 2-acetyl-1,4,5,6-tetrahydropyridine and 2-acetyl-3,4,5,6-tetrahydropyridine.

Possible Origin of 2-Acetyl-1-pyrroline. It seems reasonable that 2-acetyl-1-pyrroline has a similar origin to the bread (or cracker) aroma compound 2-acetyl-1,4,5,6-tetrahydropyridine found by Hunter et al. (1969). These authors produced the 2-acetyl-1,4,5,6-tetrahydropyridine in the laboratory by heating (75 °C) proline with glycerol and dihydroxyacetone. Proline or hydroxyproline might also be a precursor for 2-acetyl-1-pyrroline. Starting with hydroxyproline, conversion of the carboxy group to a methyl ketone group (common in food systems) followed by dehydration and migration of the double bond to the 1-position seems to provide a straightforward pathway. However, the authors were unable to carry this out in the laboratory by normal chemical means.

Formation of aroma compounds from proline and hydroxyproline have been extensively studied by Tressl et al. (1981). They did not, however, report finding 2-acetyl-1-pyrroline although they listed the related 2-acetyl-2-pyrroline as a possible product. Special conditions in rice may facilitate the formation of 2-acetyl-1-pyrroline.

Compounds formed in reasonable amounts by cooking, frequently can also be formed (in much lower concentrations) by long storage of the "raw" food at "room" temperatures (time–temperature effects). The typical aroma of the cooked aromatic rices has been described by rice technologists as being present in the "raw" rice. Preliminary analysis by the authors of raw rice volatiles could not confirm the presence of 2-acetyl-1-pyrroline, however.

Table II. Odor Threshold Determination of 2-Acetyl-1-pyrroline

concn, parts/10^9 parts of water	% correct judgments	total no. judgments
7	100	16
3.5	94	16
0.9	94	16
0.35	95	19
0.18	86	52
0.09	75	81
0.045	65	113
0.023	53	94

Table III. Most Often Used Panel Odor Descriptions of a 0.05-ppm Solution of 2-Acetyl-1-pyrroline and of Cooked Malagkit Sungsong Rice

0.05-ppm solution of 2-acetyl-1-pyrroline in water (22 judges)		cooked Malagkit Sungsong rice (23 judges)	
odor description	% of judges using description	odor description	% of judges using description
popcorn	82	popcorn	60
cooked oatmeal	45	cooked oatmeal	56
cooked rice	23	cooked rice	48
sweet	23	sweet	30
nutty	17	nutty	14

Aroma Evaluation. An odor threshold of 2-acetyl-1-pyrroline was determined in water solution by using established procedures [e.g., Guadagni and Buttery (1978)] with a trained panel consisting of 16 judges. As in previous threshold determinations the odor judges were presented with two Teflon squeeze bottles, one containing the solution and the other odor-free water. The task for each judge was to determine which of the coded bottles contained the odorant. Table II lists the results obtained. Plotting the data as outlined by Guadagni et al. (1973) gave a threshold of 0.1 part (mL) of compound per 10^9 parts (mL) of water. 2-Acetyl-1-pyrroline seems to be the most potent of the "cracker-like" group of odor compounds which includes 2-acetyl-1,4,5,6-tetrahydropyridine, 2-acetylpyrazine, 2-acetyl-2-thiazoline, and others [cf. Teranishi et al. (1975)].

The odor quality of the 2-acetyl-1-pyrroline was evaluated in a number of ways. In one study the judges were asked to describe the odor of a 0.05-ppm solution of this compound using as many descriptions as they could think of. An opaque bottle was used so that the judges could not see the contents. A similar test was carried out with Malagkit Sungsong variety of rice. The most often used descriptions are shown in Table III. It can be seen that "popcorn" was the term most used for both the 2-acetyl-1-pyrroline and the Malagkit Sungsong rice. The other most often used descriptions were also similar for both samples.

In another quality test, groups of two or three samples of the cooked rice varieties listed in Table I were presented to the (21–23) judges. The judges were asked to rank the samples in terms of the greatest popcorn-like aroma. Common samples were used so that the comparison could be overlapped and thus the whole group could be ranked in terms of the sample with the most popcorn-like aroma

Table IV. Ranking of Cooked Rice Samples in Terms of Those Having the Greatest Popcorn-like Aroma (at the Top of the Table) to Those Having the Least (at the Bottom of the Table)

Malagkit Sungsong	
Milagrosa	
Khao Dawk Mali 105	greatest popcorn aroma
IR841-76-1	
Basmati 370	
Seratus Malam	
Azucena	
Hieri	least popcorn aroma
Calrose	
Texas Long Grain	

to that with the least. The order found is that shown in Table IV. It can be seen that this order is in general agreement with the concentrations of 2-acetyl-1-pyrroline found in these varieties shown in Table I. Malagkit Sungsong was ranked as having the most popcorn-like aroma and Texas Long Grain the least.

In a third type of quality evaluation the two varieties of rice, Calrose and Malagkit Sungsong, were compared in a difference test. One variety of cooked rice (2 × 50 mL) was placed in two opaque identical flasks (100-mL stoppered Erlenmeyer). One of these flasks was labeled "control". The other variety of cooked rice (50 mL) was placed in a third identical opaque flask. The three coded flasks were placed in the panel booth side by side. The judges' tasks were to smell each flask and match one of the two coded unknown samples with the sample marked control. With 41 total judgements, the correct sample was matched 83% of the time. This is highly significant data that the panel can tell the difference between the odor of the two cooked rice varieties. The two rice varieties were next compared by using the same test except that 25 mL of a 0.05-ppm solution of 2-acetyl-1-pyrroline was added to each Calrose sample (25 mL of odor-free water was added to each Malagkit Sungsong sample). In this case, in 40 judgements, the correct sample was matched only 62% of the time. This is only slightly better than pure chance where the correct sample would be matched 50% of the time. These results support the fact that the main difference between the odors of the two varieties is the much greater concentration of 2-acetyl-1-pyrroline in the Malagkit Sungsong variety.

It might be noted that Malagkit Sungsong (a waxy rice variety) is used in the Philippines for the preparation of flattened parboiled brown rice wherein this popcorn-like odor has been synonymous to good quality.

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Registry No. 2-Acetyl-1-pyrroline, 85213-22-5; 2-acetylpyrrole, 1072-83-9; 2-(1-hydroxyethyl)pyrrolidine, 63848-93-1.

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A Direct Titrimetric Method for the Rapid Estimation of Water-Extractable Sulfur Dioxide in Corn Grain

Steven R. Eckhoff and Martin R. Okos*

A method is presented for the rapid measurement of water-extractable sulfur dioxide in corn. The method differs from standard alkali or distillation procedures in that bound SO_2 is not measured. Sulfur dioxide in ground corn samples is extracted in a buffer solution and titrated with iodine directly, without filtration. Average standard deviation of the method was 21.3 ppm, as determined from samples containing SO_2 levels ranging from 162.3 to 1197.0 ppm (weight basis). The method requires no specialized equipment and is suitable for control or research purposes. Direct comparison was made with the distillation procedure of the Manufacturing Confectioners' Alliance and the FMF (Pearson, 1977).

Treatment of high-moisture shelled corn with small amounts of sulfur dioxide inhibits microbial growth during low-temperature grain drying (Eckhoff et al., 1980; Van Cawenberge et al., 1982). The procedure, called the trickle-SO_2 procedure, involves the intermittent injection of SO_2 into the drying air which is carried into the bin where it acts upon the indigenous microflora. In order to develop appropriate SO_2 application procedures and to

Department of Agricultural Engineering, Purdue University, West Lafayette, Indiana 47907.

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