Contribution of 2-Acetyl-1-pyrroline to Odors from Wetted Ground Pearl Millet†

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The odorous compound 2-acetyl-1-pyrroline (2AP) was the cause of an undesirable "mousy" odor that developed when raw pearl millet grits were wetted and slowly dried. The 2AP in millet was identified by comparing gas chromatography (GC) retention times, odor at the GC sniffing port, mass spectra, and vapor-phase infrared spectra with those of 2AP from Jasmine and Indian Basmati aromatic rices. Identification of 2AP in aromatic rices had been reported previously. The odor of 2AP at the sniffing port was similar to the undesirable odor in bulk samples of wetted millet grits and was the key factor in identifying 2AP as the odorous component. Both blue and yellow raw millet grits produced 2AP and the mousy odor.

INTRODUCTION

Pearl millet (*Pennisetum americanum*) is a staple food for millions of people in Asia and Africa. Pearl millet can be stored for long periods without deterioration in quality if the grains are intact and dry. When the grain is processed at elevated moisture levels (30% w/v), the quality of the resulting material deteriorates rapidly (Kaced et al., 1984; Reddy et al., 1986; Hanna et al., 1990). The off-odors that develop in ground pearl millet have been described as "mousy" and "acidic", resulting in an undesirable product (Reddy et al., 1986; Hanna et al., 1990). According to Kaced et al. (1984), lipid oxidation does not correlate with the onset of objectionable odors. Because pearl millet is an important food crop, there is a need to know the cause or origin of this undesirable aroma so procedures for preventing it can be developed.

The objective of this study was to identify the major cause of the undesirable mousy odor that develops during the wetting and drying of several types of pearl millet grits.

EXPERIMENTAL PROCEDURES

Blue-endosperm and yellow-endosperm millet samples were harvested in 1991 at the Kansas Agricultural Experiment Station at Hays, KS. Indian Basmati, Jasmine, and Texmati type aromatic rices were obtained by local purchase in Bryan, TX. Nonaromatic long-grain rice was obtained by local purchase in Manhattan, KS.

For optimized production of mousy aroma in raw millet grits, a procedure described by Reddy et al. (1986) was used. Each grit sample (40 g) was placed in a flat container, and then 30% (w/v) deionized water was added and thoroughly mixed into the sample. The mixture was air-dried at ambient temperature for 15 h with the aid of a laboratory hood or table fan. Dried samples were sealed in glass containers for at least 1 h prior to odor evaluation and chemical analyses.

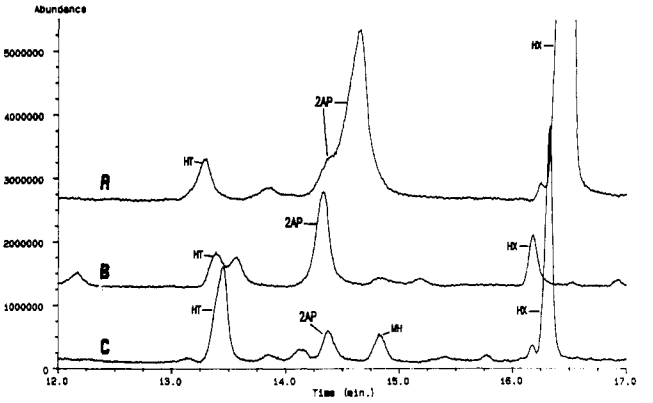

Figure 1. Total ion chromatograms obtained with the MSD in analyses of ground, wetted, yellow millet purged with helium (A); methylene chloride extract of Jasmine rice (B); and Jasmine rice purged with helium (C). Marked peaks are 2-acetyl-1-pyrroline (2AP), 2-(*E*)-heptenal (HT), 1-hexanol (HX), and 6-methyl-5-hepten-2-one (MH).

Volatiles from millet and rice samples were collected and concentrated by using a Tekmar Co. (Cincinnati, OH; Model LSC 2000) purge and trap instrument equipped with a sample heater and a capillary interface module (Model 2530). The sample of millet or rice (about 30 g) was placed in a Tekmar glass sample tube, heated to 60 °C, and purged with helium for 10–20 min to transfer volatiles to a Tenax trap (3.18 × 305 mm). Excess water was then removed from the Tenax by purging the trap with helium for 8 min (increased up to 10 min if moisture content in the trap was high due to longer purge times). Collected volatiles were thermally desorbed at 180 °C and transferred via a heated fused silica tube to the capillary interface module where they were cyrofocused (−130 °C) at the top of the gas chromatography column. Rapid heating released the cyrofocused components in a narrow band for separation on a Supelcowax 10 column (30 m, 0.32 mm i.d.) in a Hewlett-Packard Model 5890 gas chromatograph (GC; Palo Alto, CA). Column head pressure was 86.1 kPa (12.5 psi) at 60 °C, which gave about 1.7 mL/min helium flow rate. Then the flow rate was held constant by automatically increasing pressure as the oven temperature increased. For best separation of 2AP and 6-methyl-5-hepten-2-one, the column temperature was held at 60 °C for 15 min and then increased at 20 °C/min to 225 °C for rapid removal of late eluting components (Figures 1 and 2).

Analyses were carried out with a Hewlett-Packard GC–IR–MSD system as follows. Components separated by the GC were detected and identified by first passing through a Fourier

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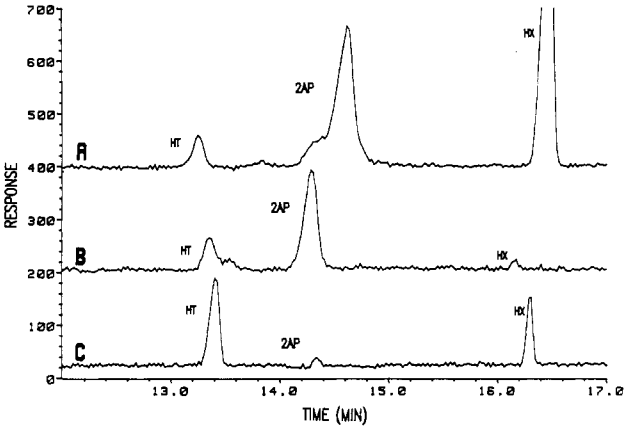

Figure 2. Total response chromatograms obtained with the IRD in analyses of ground, wetted, yellow millet purged with helium (A); methylene chloride extract of Jasmine rice (B); and Jasmine rice purged with helium (C). Marked peaks are 2-acetyl-1-pyrroline (2AP), 2-(*E*)-heptenal (HT), and 1-hexanol (HX). These chromatograms were obtained simultaneously with the total ion chromatograms shown in Figure 1.

transform infrared detector (IRD, Model 5965B) and then into a mass selective detector (MSD, Model 5970). The infrared (IR) spectra shown were recorded with resolution set at 4 cm^{-1}, whereas other spectra were usually recorded at 16 cm^{-1}. The IRD transfer line and flow cell temperatures were set at 130 °C for recording the spectra shown, but for many other analyses the temperatures were in the range 150–200 °C. The MSD ion source voltage was at 70 eV, and temperature was 250 °C. Identification of 2AP in millet was confirmed by matching retention time, mass spectrum (MS), and infrared spectrum with that of 2AP from rice. GC–MS data for 2AP from rice were previously reported by Buttery et al. (1983) and Lin et al. (1990). An authentic sample of 2AP was not available and is difficult to synthesize (Buttery et al., 1983).

To smell components eluting from the GC column, a simple sniffing port apparatus was used. A glass Y-splitter from Restek Corp. (Bellefonte, PA) was attached to the end of the 0.32 mm i.d. column. Attached to one arm of the splitter was a 0.25-mm fused silica transfer line leading to the inlet of the infrared detector. Attached to the other arm of the splitter was a 0.32-mm fused silica transfer line which carried the components out of the oven through the heated front inlet of the GC (the capillary interface module for the purge and trap instrument was placed over the rear inlet). An additional transfer line heater allowed extension of the line about 1 m away from the GC, where the person doing the sniffing could comfortably smell the end of the transfer line.

2-Acetyl-1-pyrroline was extracted from rice by using a modification of a method suggested by Dr. K. R. Cadwallader (Louisiana State University, Baton Rouge, 1992, private communication). Five grams of rice and 8 mL of methylene chloride were placed in a culture tube (25 × 150 mm) with a Teflon-lined cap. The tube was heated in a water bath at 90 °C for about 1.25 h and then cooled in ice for 10 min. After the rice particles had settled, the methylene chloride extract was removed by using a Pasteur pipet, filtered through a drying tube (anhydrous sodium sulfate packed into a Pasteur pipet), and collected in an 8-dram vial. The extract was evaporated with aid of nitrogen flow to about 2 mL and then transferred to a tapered vial for evaporation to about 0.3 mL. The entire final extract was streaked onto a narrow piece (about 7 mm × 180 mm) of coarse filter paper and quickly placed in a Tekmar sample tube. The tube was immediately mounted on the purge and trap instrument and then purged with helium for 10 min (for the last 5 min with the sample tube heated to 60 °C), and finally the Tenax trap was purged with helium for 7 min to remove excess methylene chloride. The remainder of the purge and trap instrument settings were as given above.

RESULTS AND DISCUSSION

Chromatograms and spectra from the GC–IR–MS system, plus odors detected at the sniffing port, showed that an odorous component found in wetted, raw millet samples was identical to 2AP present in several types of aromatic rices. Significant portions of total ion chromatograms from the MSD and total response chromatograms from the IRD are shown in Figures 1 and 2 for (A) raw, wetted, yellow millet purged with helium; (B) methylene chloride extract of Jasmine rice; and (C) Jasmine rice purged with helium. The MS for the peaks labeled 2AP in Figure 1 were identical to each other (Figure 3) and were nearly identical to MS previously reported for 2AP from aromatic rice (Buttery et al., 1983; Lin et al., 1990). Similarly, the vapor-phase IR spectra for the peaks labeled 2AP in Figure 2 were identical to each other (Figures 4 and 5) and were consistent with condensed-phase spectra previously reported for 2AP (Buttery et al., 1983). In addition, the distinctive odor at the sniffing port when 2AP eluted was the same for each millet and rice sample and was similar to the odor of the bulk samples of the wetted raw millet grit samples. This observation, which was confirmed by four individuals familiar with the

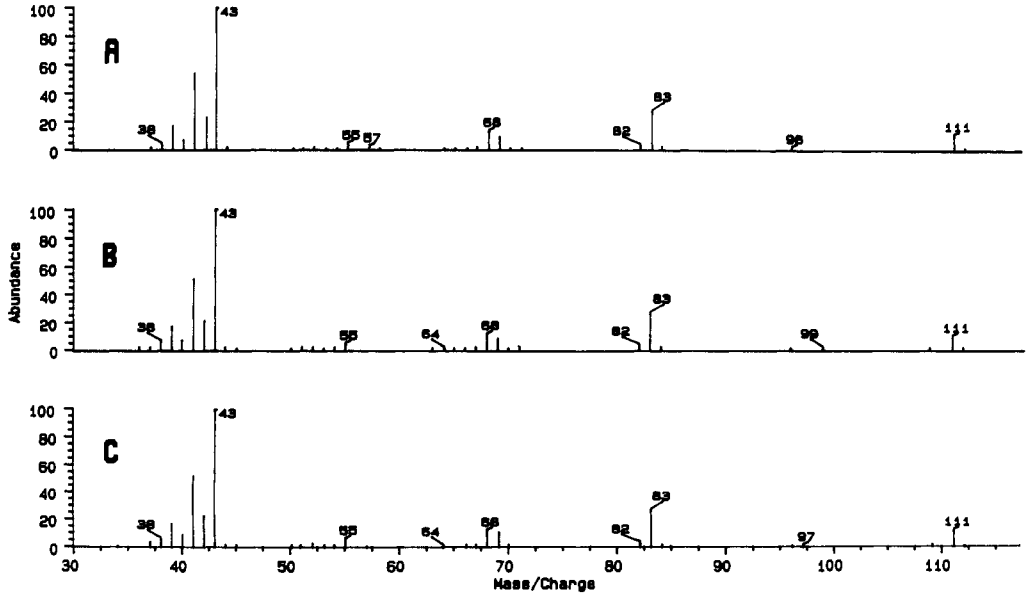

Figure 3. Each mass spectrum was from the 2AP peak in the corresponding A, B, or C total ion chromatogram shown in Figure 1.

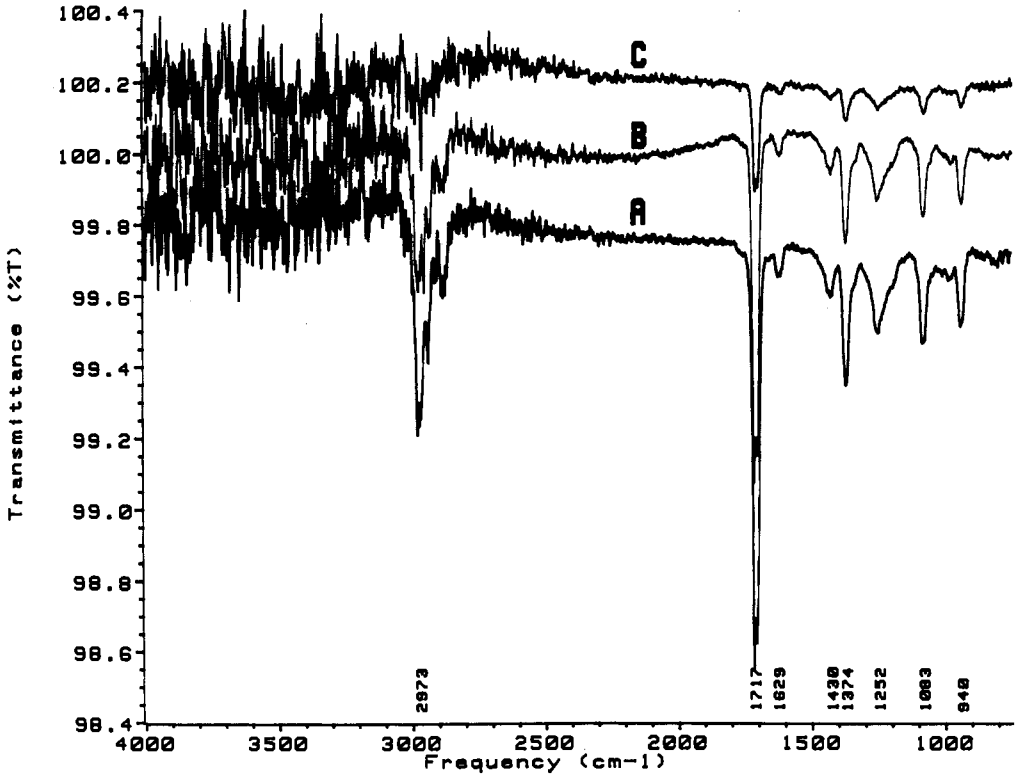

Figure 4. Each vapor-phase infrared spectrum was from the 2AP peak in the corresponding A, B, or C infrared response chromatogram shown in Figure 2.

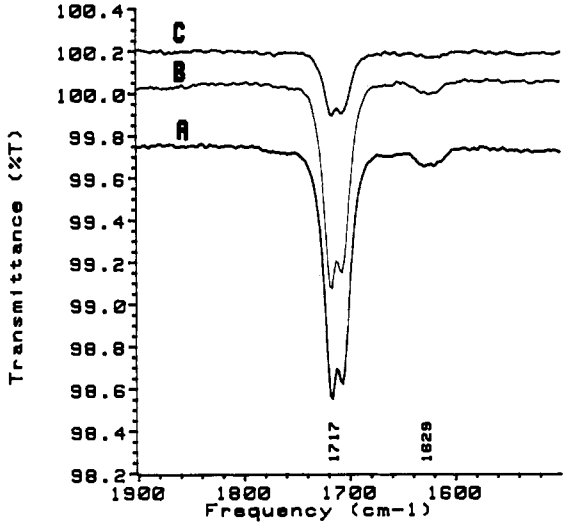

Figure 5. Portion of Figure 4 expanded to show that the splitting of the carbonyl absorbance was the same in all three IR spectra.

aroma, was the key factor that allowed us to determine when the odorous component eluted from the GC column.

Chromatograms shown in Figures 1 and 2 were obtained by using the same column and oven temperature program, but component elution times varied due to differences in column loadings. To obtain acceptable IR spectra of 2AP, it was necessary to load the column with as much volatile sample as possible. Thus, millet and rice samples had to be purged for extended periods of time, and even with extended dry-purge times, there were relatively high amounts of water and other early eluting components on the column. The degree of overloading varied among samples and thus caused the retention time shifts shown in Figures 1 and 2. In particular, as indicated by the infrared detector, excess water was the apparent cause of the retention time shift and abnormal shape of the 2AP peak obtained from the analysis of the yellow millet sample. Mass and IR spectra obtained through the shoulder part of the 2AP peak were identical to those found through the main part of the peak.

By purging rice with helium it was difficult to collect enough 2AP for recording a strong IR spectrum, although an acceptable spectrum was obtained (spectrum C in Figure 4). The methylene chloride extraction procedure provided a means of obtaining a better IR spectrum of 2AP from rice. It was clear that the 2AP component observed in the methylene chloride extract of Jasmine rice had the same GC retention time, odor at the sniffing port, and IR and MS spectra as those observed for 2AP obtained with direct helium purge of Jasmine rice samples. This supported the contention that the 2AP components obtained by the two different analysis procedures were the same. Helium purge and methylene chloride extraction of Indian Basmati rice gave the same results as those observed with Jasmine rice. Helium purge of Texmati rice also indicated the presence of 2AP. As expected, however, helium purge of bland (nonaromatic) rice showed no evidence of 2AP.

Analyses of blue millet vs Indian Basmati and Jasmine rice produced results similar to those of the yellow millet described above. When these analyses were conducted, the IRD resolution was set at low resolution (16 cm^{-1}) rather than at the high resolution (4 cm^{-1}) used for the spectra shown in Figures 4 and 5. With the low-resolution setting, the splitting of the carbonyl absorbance illustrated in Figure 5 was not resolved, but the compound could still be identified as 2AP by comparison to the spectrum from Indian Basmati rice.

A potential interfering compound, 6-methyl-5-hepten-2-one, was present in millet and rice samples. The mass spectra of these two compounds contain many common ions. 2AP does not have ions that are distinctly different from ions in the MS of 6-methyl-5-hepten-2-one, so even with the use of selected ion monitoring it is important to

have the compounds separated. A distinguishing feature of the MS of 6-methyl-5-hepten-2-one is the presence of mass (m/z) 108 which is not present in the MS of 2AP. With the Supelcowax 10 column, 2AP eluted slightly ahead of 6-methyl-5-hepten-2-one. Holding the column temperature at 60 °C for the first 15 min of the run allowed for good separation of these compounds; similar findings were reported by Tanchotikul and Hsieh (1991). The concentration ratio of 2AP to 6-methyl-5-hepten-2-one was much higher in the methylene chloride extract than when rice was purged with helium.

From the evidence cited above there was little doubt that 2AP caused the mousy odor that developed in wetted, ground, raw millet. It is interesting that 2AP in millet caused an undesirable mousy odor, whereas its presence in aromatic rices has been described as "popcorn"-like and considered a desirable trait by at least some people. Also, 2AP was deemed to be a positive character impact compound in fresh bread flavor and aroma (Grosch and Schieberle, 1991). Differences in odor desirability and character are probably influenced by relative concentrations of 2AP, as well as other volatile components, in the food products. It appeared that the concentrations of 2AP in the wetted millet grit samples were higher than those in the aromatic rices.

The origin of 2AP during the wetting and drying of ground raw millet is not yet known. Proline apparently plays an important role in formation of 2AP in bread (Grosch and Schieberle, 1991) and should be considered as a possible precursor in the wetted millet. Investigations concerning various factors that affect formation of 2AP in millet are in progress.

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