

Impact of Storage of Freshly Harvested Paddy Rice on Milled White Rice Flavor

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ABSTRACT

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Between harvest and the start of drying, paddy rice may be held for more than 24 hr at moisture contents ranging from 16% to >26%. Microbes found on the freshly harvested rice grow under these conditions and produce a wide variety of volatile compounds that impact the flavor/aroma of the white rice obtained after drying and milling of the paddy rice. The contents of 10 volatile microbial metabolites were compared in white rice obtained from paddy rice harvested at differing moisture contents and immediately dried (0 hr) or held for 48 hr before drying. No increases in volatile microbial metabolite levels were observed in white rice obtained from paddy rice that was stored at 17–21% moisture contents for 48 hr. In white rice from paddy rice stored at $\geq 24\%$ moisture content, 3-methyl-butanol, 2-methyl-butanol, acetic acid, 2,3-

butandiol, and ethyl hexadecanoate increased markedly with time. Also in these samples, as determined by a descriptive panel, sour/silage and alfalfa/grassy/green bean flavors significantly increased ($P < 0.1$) in intensity. Sour/silage, the predominant off-flavor note in the stored samples, correlated highly ($r = 0.98$) with 2,3-butandiol. Ethanol concentration measurements on the paddy rice correlated highly with sour/silage ($r = 0.99$) and 2,3-butandiol ($r = 0.97$), and correlated well with several other volatile microbial metabolites. Carbon dioxide measurements taken on the paddy rice did not correlate as highly ($r = 0.78$) with sour/silage. Measurements of ethanol produced in paddy rice may serve as an indicator of off-flavor/aroma development in the resultant white rice.

Rice is typically harvested at moisture contents above those needed for safe long-term storage. For example, California medium-grain rice is typically harvested at $\approx 21\%$, but lots may range from 16% to >26% moisture content (wb). Delays in drying frequently occur, promoting growth of field microorganisms (bacteria, actinomycetes, and fungi) at these elevated moisture contents (Teunisson 1954a,b). Whereas some rice lots are delivered to the dryer within a few hours of harvest, others may be harvested in the late afternoon, held overnight at the field in a grain trailer, and delivered to the dryer the next morning. At the dryer, lots are accumulated in temporary storage before drying. It is possible, under normal handling, for rice to be held for >24 hr between harvest and the start of drying. Equipment breakdowns or other unexpected delays can cause even longer periods of high moisture storage.

A full range of microbes, including bacteria, fungi, and actinomycetes, are found on freshly harvested rice. DePadua and Hall (1966) evaluated the effects of up to 60 days of storage of rough rice at 18–26% moisture. Milled rice obtained from rough rice stored at 18% moisture in sealed containers for 60 days had no odor. The higher moisture samples had noticeable odor after drying and milling. Rice in unsealed containers was severely molded and damaged after 15 days.

Microorganisms produce a wide variety of volatile alcohols, ketones, alkanes, and terpenes during growth on cereal grains (Harris et al 1986). The most common volatile fungal metabolites are 3-octanone, 1-octen-3-ol, 1-octanol and 3-methyl-1-butanol, 2-methyl-1-propanol, 3-methylfuran, ethyl acetate, 2-methyl-isoborneol, and geosmin (Kaminski et al 1975; Abramson et al 1980; Tuma et al 1989; Schnurer et al 1999). These volatile compounds are responsible for many of the musty or earthy aromas associated with fungal spoilage (Harris et al 1986). Under anaerobic conditions, ethanol and carbon dioxide are produced by fermentation, in addition to these fungal metabolites. Monitoring ethanol and carbon dioxide may serve as an indicator of microbial activity.

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The objectives of this research were to 1) assess the impact of storing high moisture paddy rice on the flavor and aroma of the white rice obtained after drying and milling; 2) determine chemical indicators of off-flavor/aroma in the white rice resulting from microbial populations on the paddy rice; 3) determine whether measurements of ethanol and carbon dioxide produced in paddy rice could serve as indicators of off-flavor/aroma development in resultant white rice; and 4) find holding-time limits for freshly harvested paddy rice.

MATERIALS AND METHODS

Samples

During the 2001 season, individual lots of paddy rice of cultivars M-202 and Akitakomachi were commercially harvested in California fields. Each lot was from a different field harvested on a different date. The rice was transferred from the harvester directly to plastic garbage cans that were transported for 1–2 hr to the laboratory. Three insulated metal boxes with gasketed lids each holding ≈ 227 kg (500 pounds) of rice were filled with replicates of each lot of rice (Fig. 1). A pretest comparison with a 10-ton (20,000 lb) grain trailer indicated that undried paddy rice had similar levels of gas concentration over time compared with the laboratory set-up. Rice was placed in the containers at arriving temperatures of 27 to 32°C (80 to 90°F). No attempt was made to

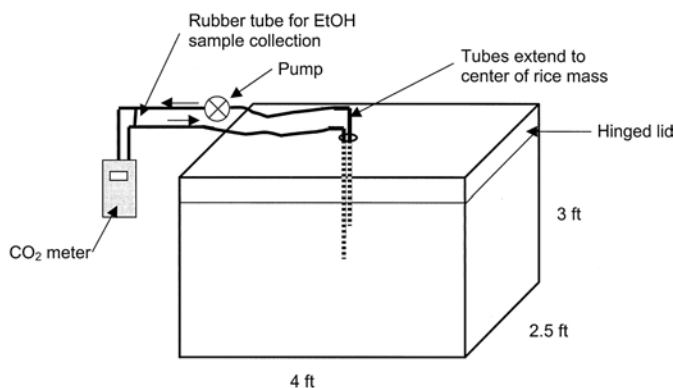


Fig. 1. Equipment for incubation tests.

¹ USDA-ARS Southern Regional Research Center, New Orleans, LA. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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control the initial rice temperature or its temperature during the tests because temperature is uncontrolled in commercial transportation and temporary holding. (Rate of rice temperature increase and maximum temperatures varied between replicates and there was no apparent correlation between any temperature index and initial rice moisture.)

Four lots of Akitakomachi and three lots of M-202 with moisture content ranges of 17–27% were tested. Moisture contents were determined by gravimetric analysis of samples dried for 72 hr at 103°C; water activity (a_w) was also determined (Aqualab, Decagon Devices, Pullman, WA). A recirculating gas sampling train was installed in the center of each batch. During the 48-hr test period, grain temperature and carbon dioxide (model 2820, Bacarach, Pittsburgh, PA) and ethanol (Alco Sensor IV, Intoximeters, St. Louis, MO) concentrations were measured every 8 hr. At each sampling period, ≈ 200 g of rice was collected from the center of each box using a commercial bullet-type grain probe. Samples were immediately placed in a screen-bottomed container and dried to $\approx 12\%$ moisture with air at 24 to 27°C (75 to 80°F). Larger (2,000 g) samples were collected at the beginning and end of the test. The large and small samples were used for identification of off-odor volatile compounds by gas chromatography/mass spectrometry (GC/MS). The large samples only were used for a sensory panel evaluation.

Samples were shelled with an impeller husker (Yamamoto model FC2K) and immediately milled using a laboratory two-pass mill (Yamamoto model VP31T). Milled samples were shipped overnight to the USDA-ARS, Southern Regional Research Center, New Orleans, LA. When received, samples were immediately preweighed into portions for identification of off-odor volatile compounds by GC/MS (Agilent 5973, Walnut Creek, CA) and sensory panel evaluation and then stored in glass jars at 4°C.

Analysis of Rice Volatile Compounds by Solid Phase Microextraction (SPME) and GC/MS

White rice samples (5 g) were placed in a 12-mL vial and sealed with a crimp-top fitted with a Teflon-lined septum and held at ambient temperature until analyzed in triplicate. For analysis, each sample was heated to 65°C for 15 min. Then volatile compounds were adsorbed onto a 1 cm carboxen/PDMS/DVB fiber for 15 min while the sample was maintained at 65°C. The SPME fiber was desorbed at 270°C into the GC injection port in splitless mode. The oven was held at 50°C for 1 min, then the temperature was increased at a rate of 5°C/min to 100°C, with a second ramp of 15°C/min to 300°C. Separation was achieved using a 30-m capillary column with a 1 μ m 5% diphenyl, 95% polydimethyl-siloxane film (DB-5, Supelco). The mass spectrometer was operated in scan mode at m/z 40–400 using electron ionization. Ten compounds were selected for quantization. Integration was performed on a target ion for each compound, and two qualifying ions were selected for each compound.

Sample Preparation for Sensory Analyses

Portions of white rice (600 g) were rinsed by covering the rice three times with cold water followed by straining to remove excess water. After rinsing, samples were transferred to preweighed rice cooker insert bowls and water was added to give a rice-to-water weight ratio of 1:1.4. The rice was soaked for 30 min and then cooked in a 5-cup rice cooker-steamer (Panasonic SR-W10G HP) to completion, followed by a 10-min holding period. Samples were taken from cookers as described by Champagne et al (1999). Cooking was staggered so that samples were analyzed at 20-min intervals.

Sensory Evaluation Protocol

Ten panelists trained in the principles and concepts of descriptive sensory analysis (Meilgaard et al 1991) participated in the study. The rice flavor lexicon included 12 unique flavor attributes that were determined by smelling and evaluation in the mouth

(Table I). The lexicon is based on the work of Goodwin et al (1996). The intensities were scored based on a universal scale for all foods (Meilgaard et al 1991); the maximum rating for rice flavor attributes is generally ≈ 5 . Each sample was presented to the panelists twice in separate sessions following a randomized design. The details of the procedure for presenting samples, standard (warm-up sample of commercial long-grain), and blind control (commercial long-grain) to panelists at each session are described by Champagne et al (1999).

Statistical Analyses

Mean panelist scores for each attribute in a session were determined and used in the statistical analyses. SAS Analyst, release 8.2 (SAS Institute, Cary, NC) was used to perform analysis of variance (ANOVA) and Tukey's HSD mean comparison tests.

RESULTS AND DISCUSSION

Ethanol and Carbon Dioxide Levels in Paddy Rice

Ethanol concentration showed a continuous, straight-line rise over the test period for paddy rice with moisture contents $> 18\%$. At moisture contents $< 18\%$, ethanol did not increase in the rice. At 21% moisture, ethanol rose at a rate of ≈ 0.001 mg/L/hr; but at 27% moisture, the rate of increase was 100 \times greater. Carbon dioxide levels tended to plateau after ≈ 24 hr for paddy rice at moisture contents in the 17–27% range. Typical patterns of ethanol and carbon dioxide concentrations over time for rice lots (Akitakomachi) at three moisture levels are shown in Fig. 2A and B, respectively.

The metabolic activity of microorganisms is affected by the a_w of its substrate. A linear function can be used to approximate the relationship between a_w and rice moisture for the range of moisture contents examined. Figure 3 depicts this relationship at 81°F (27°C) with $r^2 = 0.93$. Because rice is harvested based on moisture content and not a_w measurements, relationships between moisture content and carbon dioxide, ethanol, and volatile microbial metabolites are reported and related to a_w where appropriate.

TABLE I
Descriptive Sensory Analysis Attributes and Definitions
Used to Evaluate Cooked Rice Flavor

Sewer/Animal	An immediate and distinct pungent aromatic in the flavor characterized as sulfur-like and generic animal. The animal aromatic in the flavor can sometimes be identified as "piggy".
Floral	Aromatics associated with dried flowers such as lilac or lavender. This aromatic is characterized as spicy floral as in an "old fashioned sachet".
Grain/Starchy	A general term used to describe the aromatics in the flavor associated with grains such as corn, oats, and wheat. It is an overall grainy impression characterized as sweet, brown, sometimes dusty, and sometimes generic nutty or starchy.
Hay-like/Musty	A dry, dusty, slightly brown aroma/flavor with a possible trace of musty.
Popcorn	A dry, dusty, slightly toasted and slightly sweet aromatic in the flavor that can be specifically identified as popcorn.
Corn	The sweet aromatics of the combination of corn kernels, corn milk, and corn germ found in canned yellow creamed-style corn.
Alfalfa/Grassy/Green Beans	A dried, green, slightly earthy, slightly sweet aroma/flavor including grassy and fresh green bean aroma/flavor.
Dairy	A general term associated with the aromatics of pasteurized cow's milk. Most apparent just before swallowing.
Sweet Aromatic	A sweet impression such as cotton candy, caramel, or sweet fruity that may appear in the aroma and or aromatics.
Water-like/Metallic	Aromatics and mouthfeel of the minerals and metals commonly associated with tap water. This excludes any chlorine aromatics that may be perceived.
Sweet Taste	Basic sweet taste associated with sugar.
Sour/Silage	A sour fermented vegetation aroma/flavor, not decaying vegetation.
Astringent	The chemical feeling factor on the tongue described as puckering/dry and associated with tannins or alum.

Maximum carbon dioxide level at 24 hr and rate of ethanol rise were plotted against rice moisture (Figs. 4 and 5). Based on using ethanol as an indicator of microbial activity (Magan and Evans 2000), a threshold can be observed at $\approx 21\%$ moisture ($0.91 a_w$), below which no ethanol was detected, and microbial activity and the potential of off-odor development were very low.

Effects of Moisture Content and Time of Storage on Volatile Microbial Metabolite Content

Moisture content. The contents of 10 volatile microbial metabolites were compared in white rice obtained from paddy rice (M-202 and Akitakomachi) harvested at different moisture contents and immediately dried (0 hr) or held for 48 hr before drying. The compounds selected included both common fungal metabolites found on stored grain and those found in fermented food products (e.g. wine). 2-Methyl-propanol, a fusel alcohol, is considered an important

flavor compound in yeast-derived foods and beverages (Schure et al 1998a) and has a solvent-like odor associated with it (Rychlik et al 1998). 3-Methyl-butanol and 2-methyl-butanol are breakdown products from Strecker degradation during fermentation and have malty and green almond-like odors, respectively (Rychlik et al 1998). 3-Methyl-butanol and 2-methyl butanol both have malty odors, whereas 1-octene-3-ol has mushroom-like odor (Rychlik et al 1998). 2,3-Butandiol and ethyl hexadecanoate are commonly produced by fermentation of carbohydrates during wine production and have sweet ethanol-like and waxy odors (Anonymous 2000), respectively. Acetic and iso-valeric acids have pungent, vinegar-like and rancid, cheese, animal, and sweaty odors (Rychlik et al 1998).

Cultivar type did not appear to influence the levels of volatile microbial metabolites. The levels of these volatile compounds in the white rice did not significantly increase ($P > 0.05$) with increase in harvest moisture content from 17 to 21% at storage times of 0 and 48 hr (data not shown). Comparing volatile microbial metabolites present in white rice obtained from paddy rice at 21 and 24% moisture at 0 hr, ethyl hexadecanoate was the only compound present at a higher level at the higher moisture content (Fig. 6A). Paddy rice at 24% moisture stored for 48 hr had significantly higher ($P < 0.05$) levels of 3-methyl-butanol, 2-methyl-butanol, 2,3-butandiol, and ethyl hexadecanoate in its white rice compared with those in white rice from paddy rice at 21% moisture held for the same time; the level of ethyl hexadecanoate was markedly higher (Fig. 6B). In white rice from 25% moisture paddy rice that was immediately dried, the levels of the volatile microbial metabolites were higher than in white rice from paddy rice at the lower moisture levels with acetic acid and iso-valeric acid being markedly higher (Fig. 6A). Paddy rice with 25% moisture content held for 48 hr had markedly higher levels of 2-methyl-propanol, 3-methyl butanol, 2-methyl-butanol, 2,3-butandiol, acetic acid, and iso-valeric acid in its white rice than in white rice from paddy rice at lower moisture contents held for the same time (Fig. 6B). In paddy rice immediately dried, increasing the moisture content to 27% resulted in large increases in the levels of 3-methyl-butanol,

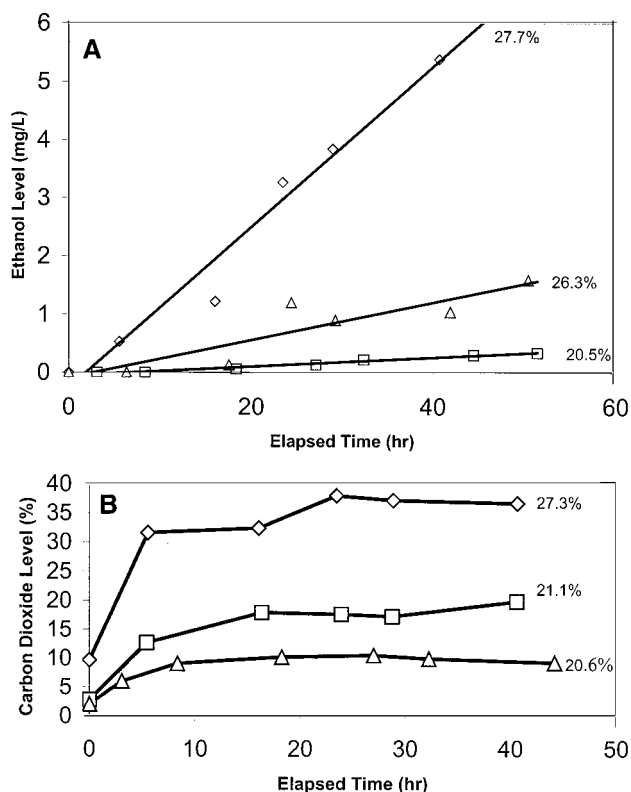


Fig. 2. A, Ethanol levels in incubation tests with three moisture levels of Akitakomachi rice. B, Carbon dioxide levels in incubation tests with three moisture levels of Akitakomachi rice.

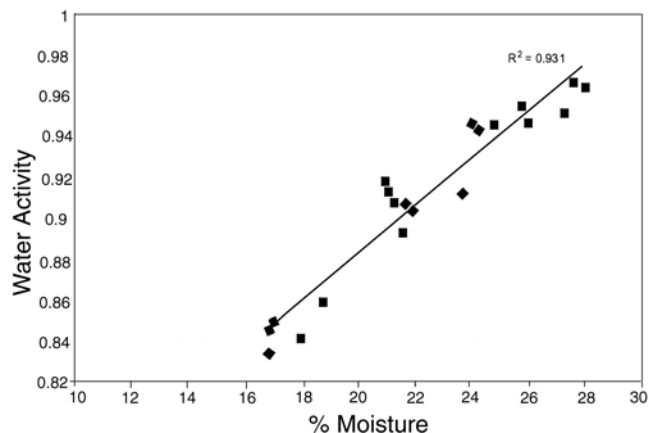


Fig. 3. Relationship between water activity and rice moisture. Akitakomachi rice (■) and M-202 rice (◆).

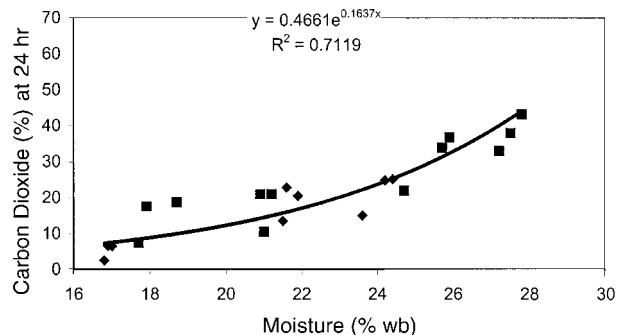


Fig. 4. Carbon dioxide concentration in a mass of rice after 24 hr in an insulated metal box. Data for Akitakomachi (■) and M-202 rice (◆).

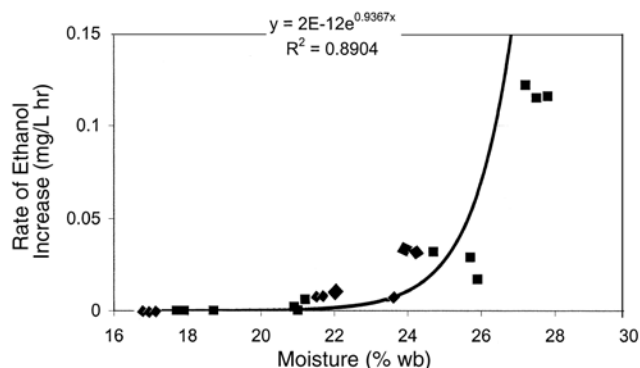


Fig. 5. Rate of increase in ethanol concentration in a mass of rice in an insulated metal box. Data for Akitakomachi (■) and M-202 rice (◆).

2-methyl-butanol, 2,3-butandiol, acetic acid, and iso-valeric acid in its white rice as compared with the levels of these compounds in white rice obtained from paddy rice at lower moisture contents (Fig. 6A). The level of 2,3-butandiol in white rice from paddy rice with 27% moisture content was 3× higher and the levels of acetic acid and iso-valeric acid were 2× higher than in those from paddy rice held for 48 hr at 25% moisture content (Fig. 6B).

Time. No increases in volatile microbial metabolite levels were observed in white rice obtained from paddy rice that was stored at 17–21% moisture contents for 48 hr (data not shown). When paddy rice was stored at 21% moisture, volatile microbial metabolite levels did not change or increased by small amounts in the white rice obtained at 0 and 48 hr, with the exception of the levels of 3-methyl-butanol and acetic acid, which largely increased with storage time (Fig. 6A, B). The data showed similar trends at 24, 25, and 27% moisture contents, except 2-methyl-butanol, 2,3-butandiol, and ethyl hexadecanoate also markedly increased with time (Fig. 6A, B).

Effects of Moisture Content and Time of Storage on Sensory Attributes

Cultivar type did not appear to influence the intensities of the sensory attributes. The intensities of the desirable flavor attributes floral, grain/starchy, popcorn, corn, dairy, sweet aromatic, and sweet taste in the white rice did not significantly change ($P > 0.1$) with increasing paddy rice moisture content in the 17–27% range for

storage times of 0 and 48 hr (data not shown). In paddy rice immediately dried, none of the undesirable flavors (sewer-animal, alfalfa/grassy/green bean, sour/silage, hay-like/musty, silver-like/metallic) in the white rice significantly ($P > 0.1$) changed with increase in moisture content of the paddy rice from which it was obtained. The undesirable flavor sour/silage significantly increased ($P < 0.001$) with increase in paddy rice moisture content at 48 hr of storage in white rice from the cultivars (Fig. 7). Alfalfa/grassy/green bean flavors (0.1 to 1.1) and astringent mouthfeel (0.2 to 0.7) in the white rice also significantly increased ($P < 0.0001$, 0.06) in intensity with increased paddy rice moisture content for rice held 48 hr and with 17–27% moisture content.

No significant changes ($P > 0.1$) in the intensities of the desirable flavor attributes floral, grain/starchy, popcorn, corn, sweet aromatic, and sweet taste were observed with time in white rice from paddy rice stored with 17–27% moisture contents for 48 hr (data not shown). Dairy flavor significantly decreased ($P < 0.05$) in intensity (from 0.8 to 0.4) in white rice from paddy rice stored with 27% moisture content. Undesirable sour/silage (Fig. 7) and alfalfa/grassy/green bean significantly increased ($P < 0.1$) in intensity in white rice from paddy rice stored with 24, 25, and 27% moisture contents for 48 hr. Astringent mouthfeel was significantly higher ($P < 0.05$) in white rice from paddy rice stored for 48 hr at 27% moisture content with intensity increasing from 0.4 to 0.7. Sewer-animal, hay-like/musty, and silver-like metallic flavors did not significantly change ($P > 0.1$) in white rice with time of storage of the paddy rice.

In addition to scoring the intensities of desirable and undesirable flavor attributes, panelists were asked to record comments concerning the samples. The panelists found the white rice samples from paddy rice stored for 48 hr with $\geq 24\%$ moisture contents to have noticeably high fermented corn/sour/silage aroma and taste. A fermented corn flavor note was associated with the sour/silage flavor. The mean intensity ratings of sour/silage in these samples were 1–4. Other aromas and tastes noted by the panelists in stored samples derived from paddy rice with higher moisture contents were nutty, peppery, and buttery.

Relationships Between Volatile Microbial Metabolites and Sensory Attributes

Correlations between sensory attributes, whose intensities were affected by paddy rice moisture content and time of storage, and volatile microbial metabolite levels in the corresponding white rice were examined to assess which compounds could serve as indicators for flavor/aroma. These correlations (r) with $P < 0.05$ were determined for the set containing samples in the 21–27% moisture

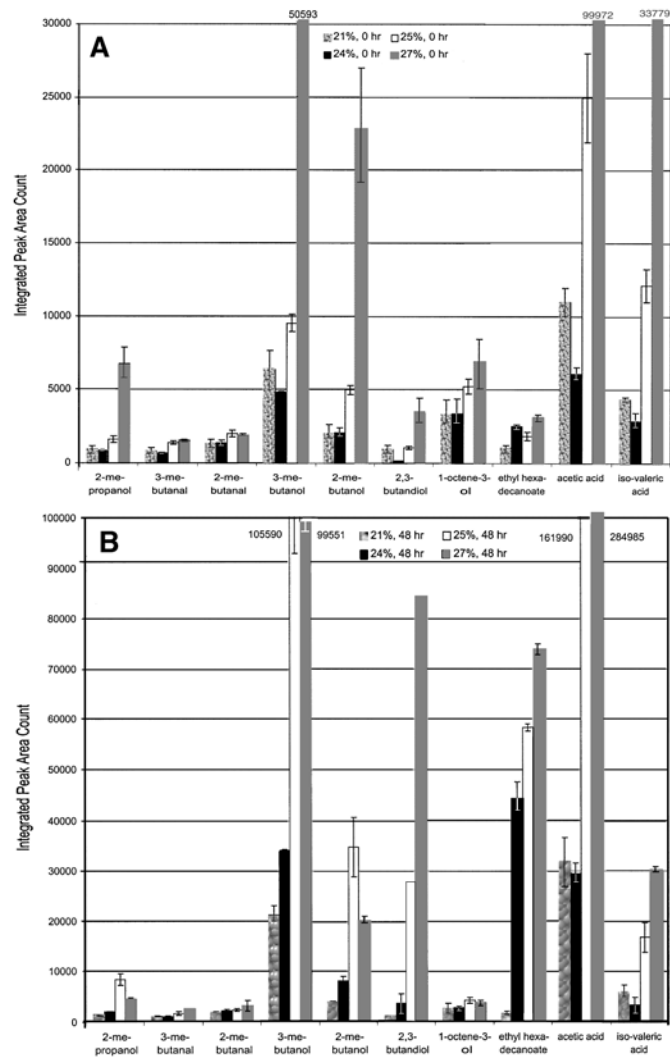


Fig. 6. Effects of moisture content of paddy rice that was immediately dried (A) or held for 48 hr (B) on levels of volatile microbial metabolites in corresponding white rice. Data for Akitakomachi (21, 25, 27%) and M-202 (24%).

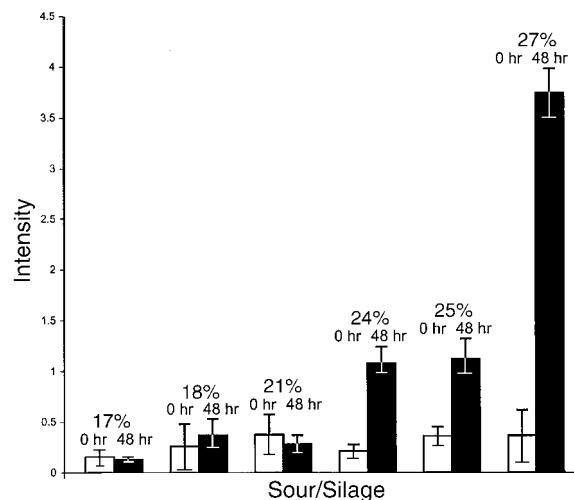


Fig. 7. Sour/silage flavor in white rice obtained from paddy rice at moisture contents of 17–27% that was immediately dried or held for 48 hr. Data for M-202 (17, 24%) and Akitakomachi (18, 21, 25, 27%).

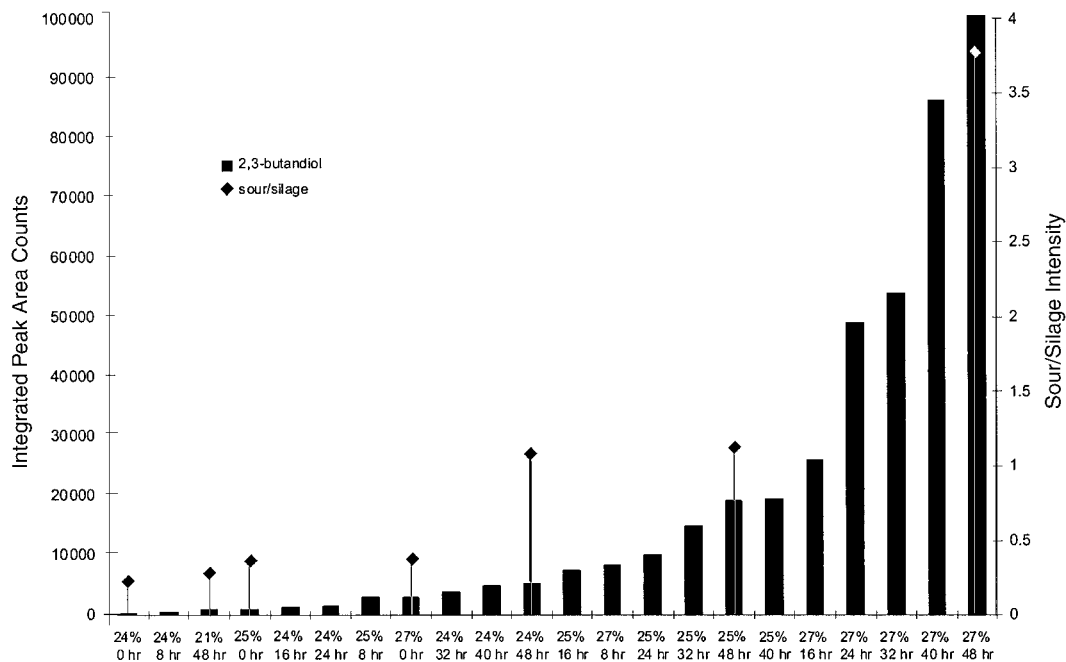


Fig. 8. Levels of 2,3-butandiol and sour/silage flavor in white rice samples obtained from paddy rice with moisture contents of 21–27% held 0–48 hr. Data for M-202 (24%) and Akitakomachi (21, 25, 27%).

content range at 0 and 48 hr storage times. Sour/silage was the predominant off-flavor note in the stored samples, correlated highly (0.98) with 2,3-butandiol. Correlations were also good between sour/silage and 3-methyl-butanal (0.87), 2-methyl-butanal (0.87), and acetic acid (0.89). Alfalfa/grassy/green bean (0.94) correlated well with 3-methyl-butanol. Astringent mouthfeel correlated well with 2-methyl-butanol (0.70).

Carbon dioxide measurements taken in the bulk samples with 21–27% moisture content at 0 and 48 hr did not correlate as highly (0.78) with sour/silage, the predominant off-flavor. Using the Alco Sensor IV, Intoximeter, ethanol was either not detected in the samples at 0 hr or at very low levels. Ethanol concentration measurements taken on the 21–27% moisture content samples at 48 hr and ethanol slopes at these moisture contents correlated highly or well with sour/silage (0.99), astringent (0.96), 2-methyl-butanol (0.99), 3-methyl-butanol (0.95), 2,3-butandiol (0.97), acetic acid (0.90), and iso-valeric acid (0.90).

Chemical Indicators and Holding Time Limits

As discussed above, panelists found the fermented corn/sour/silage flavor/aroma to be noticeably high in white rice samples obtained from paddy rice with 24% and higher moisture that was held for 48 hr. The high correlation of 2,3-butandiol to sour/silage suggests that it may be a good indicator for this off-flavor. However, this high correlation does not imply that 2,3-butandiol was responsible for the sour/silage flavor/aroma. At best, based on its aroma (sweet ethanol-like), 2,3-butandiol probably played a small role in the sour/silage flavor/aroma.

Levels of 2,3-butandiol were measured in samples taken at 8-hr intervals to determine whether the compound increased linearly over the 48-hr period or leveled off during that time frame. 2,3-Butandiol displayed a linear increase as a function of time at each moisture level in the 24–27% range with coefficient of determination (r^2) of 0.93–0.98.

Figure 8 shows the levels of 2,3-butandiol in white rice samples obtained from paddy rice with moisture $\geq 24\%$ that was held for 0–48 hr. Also included is the level in white rice from paddy rice with 21% moisture that was held for 48 hr. Samples with 2,3-butandiol intensities equal or larger than that associated with noticeably high fermented corn/sour/silage aroma and taste (mean

sour/silage flavor intensity scores ≥ 1) can be concluded to likely be off-flavor. Thus, the holding time limits at 24, 25, and 27% moisture contents (corresponding to a_w 0.94, 0.95, and 0.96) would be <32–40, 16, and 8 hr, respectively. This generally agrees with the recommendation that rice at $>23\%$ moisture can be safely stored for <1 day (Matsuo et al 1995). Data indicate that white rice from paddy rice held up to 48 hr with moisture contents $<24\%$ would have levels of 2,3-butandiol less than those associated with noticeably high fermented corn/sour/silage aroma and taste. Whereas 65% of panelists detected, identified, or measured sour/silage flavor in white rice obtained from paddy rice with 24% moisture that was held for 48 hr, only 20–30% of the panelists detected, identified, or measured sour/silage flavor in the white rice obtained from paddy rice with 21% moisture (a_w 0.91) held for 48 hr and from that with 24, 25, and 27% moisture that was immediately dried.

Carbon dioxide measurements on the wet, paddy rice did not correlate highly enough with sour/silage flavor/aroma to serve as an indicator of off-flavor development. However, as discussed above, ethanol measurements on the paddy rice correlated highly ($r = 0.99$) with sour/silage flavor/aroma intensities determined for the 0 and 48 hr samples. Likewise, ethanol measurements taken every 8 hr correlated fairly well with 2,3-butandiol measurements (0.90). Therefore, it appears that ethanol measurements taken on paddy rice could indicate off-flavor/aroma in the corresponding white rice. Paddy rice with ethanol concentrations >0.4 mg/L that has been handled following the protocol described in this study would likely yield white rice that has undesirably high sour/silage flavor/aroma. Further studies need to be conducted to determine how well ethanol measurements taken on paddy rice in commercial storage bins correlate with off-flavor in white rice and concentration limits.

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