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Small-Scale Induction of Postharvest Yellowing of Rice Endosperm

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ABSTRACT

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Rice endosperm often develop a yellow discoloration during commercial storage in conditions of high temperature and moisture, thereby reducing the value of the grain. This postharvest yellowing (PHY) appears to be coincidental with fungal presence. To study the yellowing process in a controlled manner, we developed a technique to induce PHY on a small, laboratory scale. Milled rice kernels were rinsed with water and incubated in clear test tubes or microfuge tubes at 65–80°C. This allowed direct observation of the color change and measurement using a colorimeter. Every rice cultivar tested (long and medium grain japonicas and indicas) showed some level of PHY, which increased with temperature yielding a maximum color change at 79°C. Most color change occurred within one day. The moisture parameters required for yellowing to occur were measured. Using sterilization and culture techniques, we found no indications of direct fungal involvement in the yellowing process.

Rice endosperm exposed to high temperatures and moisture during commercial storage often discolor, turning different shades of yellow, orange, or even a reddish color (Yap et al 1988; Dillahunty et al 2001). Rice containing discolored kernels has a lower commercial grade and value. The discolored rice is also known as or associated with damage, heat damage (Schroeder 1963), grain discoloration (Lee 1992), yellowing, stackburn, and postharvest yellowing (Dillahunty et al 2001). For clarity, we will refer to the process of rice endosperm becoming a uniform or nearly uniform yellow to reddish color in conditions of high temperature and moisture as postharvest yellowing (PHY).

Moisture level and temperature are the major factors involved in PHY, while oxygen and carbon dioxide concentrations have little or no effect (Bason et al 1990). PHY can occur whenever harvested rice is too moist or if the stored grain becomes wet, such as from rain while being transported in trucks or from leaks around storage bin door seals. The problem occurs in large storage bins and trucks, as well as in relatively small piles or containers. The portion of the rice that is wet becomes hot and develops a distinctly moldy smell. PHY has been correlated with fungus (Tisdale 1922; Singh et al 1982; Lee et al 1986), particularly *Alternaria* (or *Trichoconiella*) *padwickii* (Ou 1985), although other evidence points to an indirect role of fungi in PHY (Juliano 1985).

We developed a procedure to induce PHY on a laboratory scale to define the environmental effects on it. By conducting tests on a small-scale, more control of the environment was possible. One objective of this work was to determine whether fungi have a direct role in PHY. An overall understanding of the control and physiology of PHY would provide information on the means to reduce the incidence of PHY.

MATERIALS AND METHODS

General Postharvest Yellowing Procedure

A basic procedure was developed for the induction of PHY in test tubes. Milled rice was poured into a test tube (of any convenient size) to fill it to ≈1/5 full. The rice was then rinsed two times with deionized water. Then the test tubes were loosely capped and placed in an incubator (Sheldon 1525, Cornelius, OR) at 65 or 70°C (maximum temperature available for the incubator) for four to six days. After incubation, the tubes were cooled to and stored at room temperature on a bench top. Measurements could be made the next day or up to several weeks later. Details specific to each experiment are described.

Cultivar Test

Cultivars were selected to assess a diversity of rice. Bengal, Cypress, and Wells are tropical japonicas commonly grown in Arkansas (Mackill 1995), and Zhe 733 is an indica obtained from the Rice Research and Extension Center in Stuttgart, AR. L-205 and M-202 are japonicas commonly grown in California obtained from Kent McKenzie (California Cooperative Rice Research Foundation, Biggs, CA). Stg S is a local accession of weedy red rice (*Oryza sativa*) (Gealy et al 2002). Texmati (RiceTec, Alvin, TX) was obtained from a grocery store. Koshihikari is a japonica donated by Chris Isbell (Stuttgart, AR), and Indian basmati (Tilda, Rainham, UK) was purchased in an Oriental grocery. Kernels were incubated for six days at 65°C.

PHY of Rough, Brown, and Milled Rice

To determine the effect of three levels of processing of rice on PHY, three test tubes (13 × 150 mm) were each filled with 350 kernels, either of rough rice (with hulls), brown rice (hulls removed, unmilled), or white (milled) rice (cv. Wells), and then rinsed with water. Seeds were milled (Satake grain testing mill, Hiroshima, Japan) for 60 sec to completely remove any vestige of bran. The tubes were incubated at 70°C for six days. The rough rice was then hulled and milled and the brown rice was milled. After treatment, we used visual similarity to group the endosperm from each of the three levels of processing into three color categories: white and light yellow, medium yellow, or dark yellow.

Water Requirements

Five milled kernels of Bengal or Cypress were placed in clear polymerase chain reaction (PCR) amplification tubes (200 µL) and predetermined amounts of water (0–50 µL) were added to each tube. The tubes were heated at 78°C for 114 hr in the thermocycler (PTC-200, MJ Research, Watertown, MA). Colorimeter (CR-10, Minolta, Osaka, Japan) measurements were made of the kernels through the tube.

Moisture Contents and Time Course

Milled rice of the cultivars Wells and L-205 (4 g each) were put in a preweighed glass test tube (16 × 100 mm), rinsed with water, lightly capped, and placed in the incubator at 70°C. The time course was arranged so that incubations were initiated on different days but all assays were completed on the same day.

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Each test was duplicated. At the end of the incubation period, the yellowed kernels were overlayed by incompletely yellowed kernels. The less yellowed kernels were easily removed as they remained separate and would pour out while the lower layer of yellowed kernels clumped together and tended to remain in the tube. The yellowed kernels were retained in the tube and dried overnight at 90°C in an oven. The final dry weights were obtained immediately upon removal from the oven on a Sartorius 2434

Fig. 1. Postharvest yellowing of milled rice of diverse cultivars. For comparison, kernels in the tube labeled white were not subjected to yellowing conditions.

balance (Goettingen, Germany) and the data were used to obtain the moisture content (MC). Colorimeter measurements were also obtained of the same dried, yellowed kernels to provide a time course of color changes.

Temperature Curve

Five milled kernels of Bengal or Cypress were placed in PCR amplification tubes. The kernels were rinsed by filling the tubes with water and then pipetting out all the free water. The tubes were capped and placed in a PCR thermocycler using a temperature gradient program from 61.5 to 81.6°C indefinitely, with a heated lid. After 88 hr, the tubes were removed. Colorimeter measurements were made of the kernels through the tube. Measurements of the two outermost columns of tubes in the thermocycler (lowest and highest temperature) were discarded. Four replicate tubes were prepared for each cultivar and temperature and three colorimeter readings were made of each tube.

Fungal Involvement

The ability of fungi to directly induce PHY was tested by inoculating each tube of milled kernels with three previously yellowed kernels. The previously yellowed kernels were marked with a red permanent ink pen for later identification, then added to the kernels of untreated rice to be incubated.

Yellowed rice endosperm were cultured on select media to determine whether any viable fungi could be detected. To provide surface sterilization but allow the survival of any internal fungus, a relatively benign surface sterilization technique was utilized. The kernels were first disinfected by dipping the kernels in 100% ethanol. These kernels were then placed onto sterilized potato dextrose agar (PDA) media, made by mixing 20 g of agar, the broth from boiling 300 g of diced potatoes in 1L of water for 1 hr, 10 g of dextrose, and water to make 1L of media. Rice dextrose agar (RDA) was made by substituting rice for the potatoes of PDA. A total of 40 kernels were cultured on PDA and RDA at 30°C.

Yellowed endosperm were examined for the presence of fungal hyphae by clearing and staining 50 g of yellowed kernels in 800 mL of water, 8 g of methylene blue (Harleco, Philadelphia, PA), and 40 g of KOH. The cleared endosperm were examined under a dissecting microscope.

To obtain fungus-free endosperm, Wells rice plants were grown from seed in a greenhouse and treated with a systemic fungicide at critical times during flowering. Panicle leaf sheaths were sprayed with 2.35 oz/gal of Quadris fungicide (Syngenta Crop Protection, Greensboro, NC) at the early boot stage, then when the first panicle exerted, and again two days later. Mature kernels were milled and then surface sterilized by soaking in undiluted commercial bleach for 2 min, then rinsing five times with sterile water. To induce yellowing, 4 g of rice was placed in a test tube, rinsed with sterile water, and incubated at 70°C for six days.

RESULTS AND DISCUSSION

Cultivar Test

Milled kernels from a diverse group of rice, including indicas, japonicas, and a weedy red rice accession, were subjected to PHY conditions. Every cultivar tested showed some degree of yellowing (Fig. 1) with variations in color, intensity, and proportion of kernels yellowing. Previous tests through four days also showed that different cultivars achieved different levels of color (Yap et al 1988).

Seed Condition

The advantages of using milled rice instead of rough or brown rice in tests to study PHY were the small volume of grain needed, the ability to visually track the color changes, and elimination of grain loss or sample mix-up during a final milling step. However, the percentage of kernels that became medium yellow and dark yellow was higher if the rice was incubated as brown or rough rice (≈75%) than if incubated as milled rice (30%) (Table I). The brown and rough rices also produced visibly darker yellow colors, results similar to those observed by Yap et al (1988). These results likely vary with cultivar and conditions as Yap et al (1988) showed that one line, IR 64, at high moisture, yellowed more when treated as milled than as brown rice.

Incubation of milled rice allowed the direct observation of discrete bands of PHY within the test tube (Fig. 1, see Texmati and Basmati). The band likely correlates with an ideal moisture level established along the height of the tube by evaporation and condensation. Although it is not known whether brown and rough rice discolor in a banding pattern similar to the milled rice because they must be milled to observe any color change, an equivalent banding probably does occur because white and light yellow kernels are found along with the darker kernels once the kernels have been hulled and milled (Table I). Although equal numbers of kernels were used in each test, the rough rice had a much lower volume after final milling because of the high number of grains breaking during milling after PHY, another disadvantage of using nonmilled rice for yellowing (Dillahunty et al 2001).

Role of Water

Preliminary observations indicated that the amount and distribution of water among the rice kernels in a tube was important. These observations included alterations in the yellowing pattern when kernels were soaked in water for 1 and 2 hr instead of just being rinsed, and when filter paper was inserted into the test tube along with the kernels to alter the distribution of water.

TABLE I
Postharvest Yellowing of White, Brown, and Rough Rice

	Percentage (w/w) of Kernels After Milling		
Kernel Form	**White/Light Yellow**	**Medium Yellow**	**Dark Yellow**
Milled	69.6	30.4	00.0
Brown	24.3	41.2	34.5
Rough	27.8	25.8	46.4

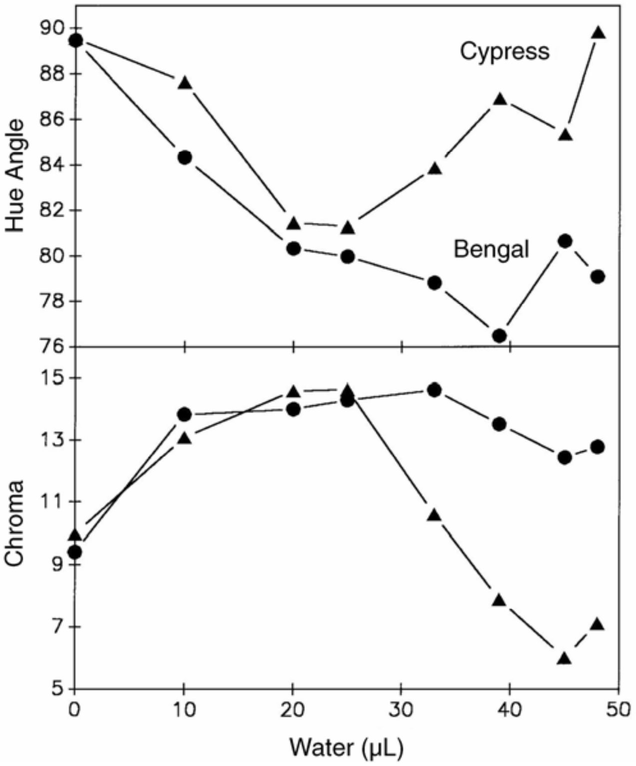

Fig. 2. Amount of water needed to induce postharvest yellowing of milled rice incubated in a thermocycler.

Two approaches were used to investigate the role of water in PHY. One approach was to add different amounts of water to a small number of kernels incubated in PCR tubes to determine the specificity for amount of water for yellowing to occur. Both Cypress and Bengal yellowed similarly at low water levels with a maximum level of chroma (color intensity) occurring with 10 µL of water but a minimum hue angle (closeness in color to yellow) with 20 µL (Fig. 2). When >25 µL water is added, Cypress and Bengal diverge in yellowing so that Cypress appears to have a narrower optimal range of water to achieve the greatest change in hue and chroma.

The second approach was to set up general PHY conditions in test tubes and measure the moisture content (MC) over the course of yellowing. The MC of both cultivars Wells and L-205 increased the first day and declined thereafter (Fig. 3).

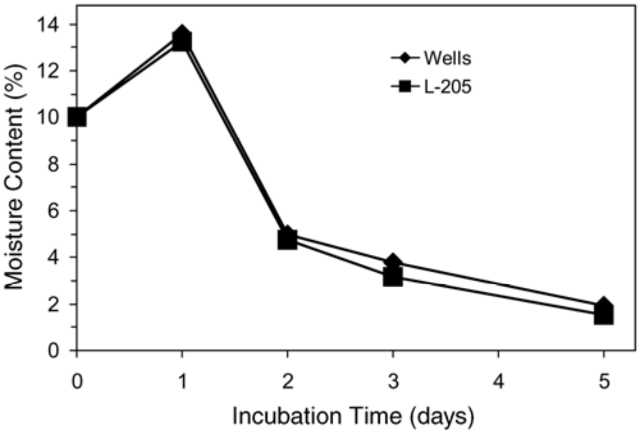

Fig. 3. Moisture content developed in milled rice during incubation for postharvest yellowing in test tubes.

From the two approaches, we conclude that yellowing in milled rice requires an optimal range of water addition (Fig. 2). Depending on the cultivar, yellowing may continue to occur at higher water levels. The greatest amount of PHY occurred in the first day of incubation (Fig. 2), the only time period that the kernels absorbed moisture (Fig. 3).

Time Course

PHY was most apparent within the first two days of incubation but continued to develop throughout the incubation period (Fig. 4). After the first day of incubation, the color continued to develop to the third day, primarily by an increase in chroma (color intensity). By the fifth day of incubation, the upper layers of Wells and L-205 were light yellow and yellow, respectively. The lower layers, from which measurements were made, were a dark, golden yellow for both cultivars. Yap et al (1988) showed that cultivars differed in the incubation time required for leveling off of the color change, as tested through four days.

Temperature Curve

A PCR thermocycler with a temperature gradient capability presented a convenient method to test the temperature range for inducing PHY by allowing a range of temperatures to be tested at one time in one device. But the small volume (200 µL) possible in a thermocycler demanded minimization of the number of kernels being incubated. Preliminary tests indicated that some cultivars would yellow with just one milled kernel at 65°C in a thermocycler. However, to obtain more consistent yellowing among cultivars, five kernels per tube were used. The chroma and hue angle changed in parallel for Bengal and Cypress (Fig. 5), indicating that these cultivars yellow similarly in regards to temperature. Similar patterns for yellowing were previously observed in these cultivars at temperatures up to 60°C (Dillahunty et al 2001). While the maximum reported temperatures for the induction of yellowing in previous studies were 60°C (Yap et al 1988) and

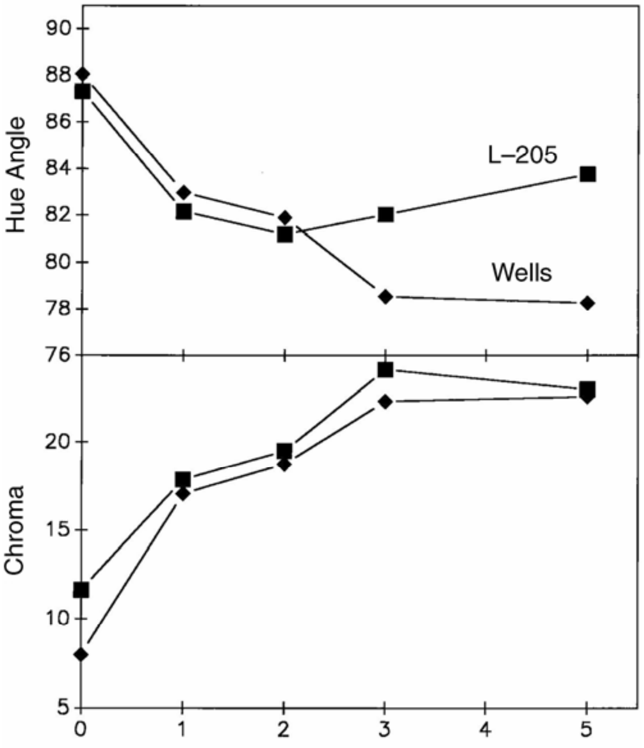

Fig. 4. Color changes during the time course of postharvest yellowing for milled rice during incubation in a test tube.

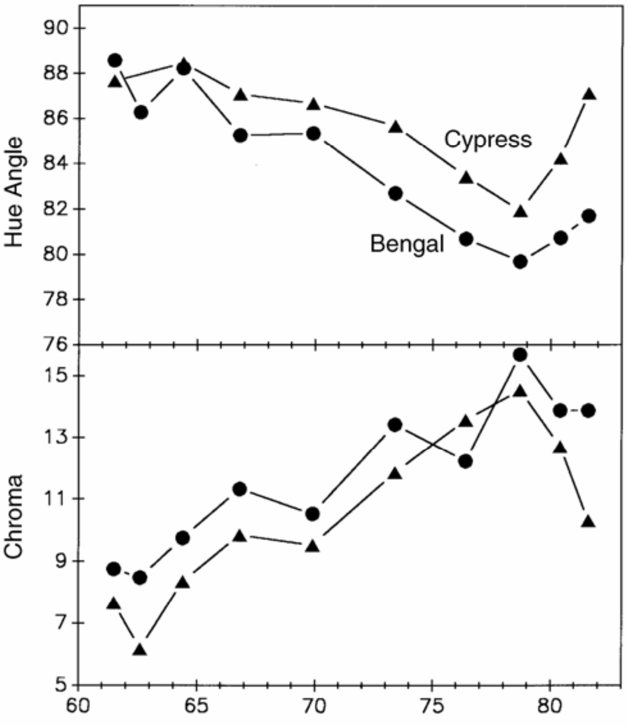

Fig. 5. Temperature gradient for postharvest yellowing of milled rice incubated in PCR tubes in a thermocycler.

66°C (Dillahunty et al 2001), we found that yellowing occurs through 81°C and that yellowing is optimized at ≈79°C (Fig. 5). Respiration rates of rough rice (at the moisture contents of the milled rice in this study), along with their associated microbes, increased with temperature to a maximum of 70–80°C (Dillahunty et al 2000).

Fungal Tests

Fungi have long been implicated in yellowing of rice (Schroeder 1963; Schroeder and Calderwood 1972; Ou 1985). We made a number of attempts to determine whether there is a direct fungal component to the yellowing process. If fungi are directly responsible for yellowing, they should be present on or in yellowed grains and thus be able to serve as an infection source to a fresh sample of rice being incubated so that greater yellowing would occur in the contiguous kernels. Seeding of a group of kernels to be incubated with already yellowed kernels did not result in yellowing of the contiguous untreated kernels (data not shown).

Fungi are numerous on and in rice hulls. But because we were yellowing milled rice, a direct fungal involvement would require the presence of living fungi internal to the endosperm. Mycelia were found on the underside of the pericarp of all rice seed examined (del Prado and Christensen 1952). However, the number and species of fungi found was greatly reduced by milling (Martínez-Bustos et al 1996) and only 0–18% (depending on moisture conditions) of milled rice yielded storage (internal) fungi (Fanse and Christensen 1966). Rice that had been yellowed as milled kernels were cultured on PDA and RDA general media. Only 15% of the kernels yielded fungal growth. We could find no fungal hyphae in cleared kernels of yellowed rice. If a fungal population within the endosperm resulted from external sources, its population would be greatest near the kernel surface. However, reducing any such fungal population by removing more tissue from the surface by increased milling did not affect yellowing (data not shown).

The life spans of fungi are not indefinite. Fungal mycelia were present in the pericarp of wheat that was over five years old but the mycelia were dead (Christensen 1951). After 12 months of storage, fungi in rice decline (Phillips et al 1988) or are virtually absent (Cogburn 1985). We have induced PHY in 5–10 year old rice kernels (data not shown) maintained in cold storage. If internal fungi in rice have the same mortality, fungi were not involved in that yellowing.

Kernels considered free of internal fungi from fungicide-treated plants appeared to actually have the yellowing process enhanced. More of the kernels turned color (no light-colored band) and the colors were more yellow (Table II). Surface sterilization treatment reduced the appearance of discrete layers, perhaps because of the soaking involved in the sterilization process. In preliminary tests, soaking of the kernels in water for an hour before incubation resulted in a broader but lighter band of color.

Some of the research reported in the literature points to only an indirect role for fungi in PHY. Schroeder (1963) found no correlation between an increase in storage molds and the amount of heat damage. Phillips et al (1988) were also unable to find a correlation between fungal growth and yellowing. The proportion of fungi-infected rice could not be correlated with PHY (Martínez-Bustos et al 1996). Bason et al (1990) pointed out that yellowing occurs at temperatures where fungi are not active.

We propose that fungi are not a direct cause of the yellowing of rice endosperm and summarize the evidence in Table III. Fungi are likely to be an indirect cause of PHY in commercial rice storage by increasing the temperature of the grain in which fungi are growing (Juliano 1985). The respiration of oat, wheat, and barley seeds alone can only increase temperatures a few degrees, while the presence of fungi on or in the seeds can raise the temperature from ≈25°C to ≥50°C (Gilman and Barron 1930).

TABLE II
Postharvest Yellowing in Nonsterilized, Surface-Sterilized, or Fungicide-Treated Kernels Incubated in Test Tubes

Treatment	Color	Hue Angle	Chroma
Before treatment	White	85.6	9.0
Water rinse	White	89.1	15.6
	Yellow	83.6	20.8
Surface sterilization	Yellow Brown	75.1	17.4
Fungicide	Orange	76.4	20.5
	Light Brown	79.4	18.6
Fungicide & surface sterilization	Brown	69.0	18.1
	Light Brown	69.5	18.6

CONCLUSIONS

Although PHY is primarily a large-scale storage problem, the development of a small-scale system allows more control for testing the parameters involved in PHY. We have established the parameters that are important to replicate PHY on a small scale. The precise color and amount of time for the color change varies with cultivar, water, temperature, and probably numerous internal and environmental conditions.

Although rough and brown rice undergo PHY to a greater extent than milled rice, using milled rice allows for direct observation of the color changes. Most color change occurs within one day of incubation at 70°C with little change occurring after three days. Yellowing in cultivars varies and differences can be observed visually and quantified using a colorimeter. PHY also varied among cultivars according to the environment. For example, Wells yellowed in a test tube but did not visibly yellow under the minimal conditions of the PCR tube (data not shown). The genetic differences among the yellowing responses are another possible area of study.

Our induction of PHY in the absence of fungi strongly indicates that fungi do not directly cause PHY. Temperature and moisture are the environmental factors that most influence PHY. However, fungal (and other microorganismal) respiration in moist conditions can result in a temperature increase which, in this indirect fashion, results in PHY. A distinct optimal temperature for color change occurred at 79°C. The greatest change in the MC of the kernel

TABLE III
Evidence For and Against Fungal Involvement in Postharvest Yellowing (PHY)

For	Against
• Smell of yellowed rice in a storage bin is moldy • Apparent correlation between the level of fungal infection and PHY in rice storage	• PHY of milled rice in a test tube does **not** smell moldy • Milled rice (endosperm) can undergo PHY • Greater milling does not decrease PHY • Surface-sterilized milled rice undergoes PHY • Very few rice endosperm contain internal fungi • Fungi tend not to grow at 60–70°C • Fungi could be cultured from only a minority of milled, yellowed rice • Mycelia were not observed in milled, yellowed rice • Fungus-free seeds (from fungicide-treated plants) undergo PHY • Seeding rice with yellowed kernels does not induce PHY in surrounding grains • PHY occurs in a very tight ring in the test tube, more indicative of humidity specificity than seed/organism induction

occurred in the first day of incubation, the same time period for the bulk of the color change. The change in MC was only a few percentage points, all within the range of percent moisture for most harvested rice. And some PHY will even occur with a minimal amount of water (10 µL) in a relatively large (200 µL) space available in a tube for water vapor. Thus, temperature appears to be particularly critical in limiting discoloration. High temperatures, whether the temperatures are artificially produced or a result of fungal respiration, are likely to result in yellowing.

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Erratum

A correction was made to this article on December 6, 2005.
The correct version of Figure 3 is now inserted.