Ergosterol Versus Dry Matter Loss as Quality Indicator for High-Moisture Rough Rice During Holding[1]

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ABSTRACT

Two varieties of long-grain and one variety of medium-grain rough rice were mechanically harvested, sun-dried, and rewetted for holding experiments. Small samples (~0.25 kg) of the three varieties were aerated slowly while held at four temperatures (18.3, 23.9, 29.4 and 35° C) and three moistures (16, 18, and 20%). Dry-matter loss (DML), ergosterol, and damaged kernels were determined with increased holding time. Ergosterol in the rough rice correlated well (r = 0.96) with damaged kernels in the milled rice. Rice grade dropped from U.S. No. 1 (damaged kernels ≤0.5%) to below U.S. No. 2 (damaged kernels ≥1.5%) as the ergosterol level increased more than 1 μg/g above its initial value. Rough rice DML correlated poorly (r = 0.62–0.63) with percent damaged kernels, and the resulting grade could not be predicted by DML. An empirical equation was developed to express ergosterol in high-moisture rough rice as a function of moisture content, temperature, and time. Ergosterol increase during the predrying holding period appeared to be a better indicator of quality loss in high moisture grain than DML.

Rice and maize often are harvested at 20–28% moisture to avoid breakage during the picker-sheller operation. However, fungal growth—which is affected by moisture content, temperature, and grain condition, including damaged kernels and foreign material—causes deterioration of high-moisture grain. The extent of fungal damage has been estimated by microbiological assay, germination of the grain, visual discoloration of kernels, chitin, ergosterol, and respired CO_2 or dry-matter loss (DML) (Bottomley et al 1950, Hummel et al 1954, Geddes 1958, Christensen and Kaufmann 1974, Seitz et al 1979, Pomeranz 1982).

Saul and Steele (1966) examined the relationship between DML and the United States standard grade of maize. To maintain maize above U.S. No. 2 grade, they recommended that no more than 0.5% DML occur in newly harvested grain (Steele et al 1969). In computer simulations, the limit of 0.5% DML has been used to predict the safe holding time or allowable drying time for high-moisture maize (USDA 1969, Thompson 1972, Brooker and Duggal 1982). In 1950, Bottomley et al reported little correlation between DML and the mold population in maize. More recently, Seitz et al (1982b) studied one lot of hand-harvested maize in which mold respiration contributed little to the first 0.5% DML. When the same lot of maize was inoculated with *Aspergillus flavus*, aflatoxin was produced before 0.5% DML occurred.

Ergosterol has been suggested as a convenient, sensitive, and reliable index for fungal invasion in maize, sorghum, and wheat (Seitz et al 1977, 1979, 1982a,b). In this study, we determined the DML and ergosterol in small samples (250–300 g) of high-moisture rough rice during slow aeration. The two indices of fungal invasion then were correlated with the U.S. grade of the milled rice.

MATERIALS AND METHODS

Rice

Long-grain (Newrex) and medium-grain (Nato) rough rice were mechanically threshed in 1980 at Beaumont, TX. The rice was cleaned in a small-grain cleaner (Cliper M-28, Burrows Equipment Co., Chicago, IL), sun-dried to ~18% moisture (wet basis), and kept frozen at −40°C for about one year before storage experiments. Another long-grain rough rice (Arkansas 007) was harvested at the Rice Experiment Station, University of Arkansas,

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Stuttgart, AR, in 1982. The Arkansas 007 rice was threshed by hand or by machine. Both lots were sun-dried to 14% moisture and cleaned in a Carter dockage tester. Prestorage moisture contents were determined with an electronic moisture meter (Motomco, Inc., Paterson, NJ).

Before the storage study initial levels of mechanically damaged kernels, impurities, germination, and ergosterol contents of the three rice varieties were assessed. The level of mechanically damaged kernels was determined visually on triplicate 50-g samples. Mechanically damaged kernels were defined as dehulled, hull-damaged, and broken kernels and were identified with the unaided eye. Impurities and immature kernels were also identified and separated from triplicate 50-g samples. Viability was measured from quadruplicate 25-kernel samples of surface-disinfected (1% sodium hypochlorite, 1 min) rice seed. The seed was enclosed in a wet paper towel, placed in a petri dish, and incubated at 24° C for seven days. Average germination of the rice seed at seven days was used as a measure of viability. The initial ergosterol in rice was determined on duplicate 50-g samples (Naewbanij et al 1984).

DML During Holding of High-Moisture Rough Rice

DML was measured by a slight modification of the method described by Kalbasi-Ashtari et al (1979). Rough rice was rewetted to ~16, 18, and 20% moisture (wet basis) and allowed to equilibrate for 48 hr at 23° C. Then grain (250–300 g, dry weight basis at a given moisture) was distributed into each of 16 jars fitted for aeration with inlet and outlet tubes. The sample jars were placed in constant-temperature chambers (18.3, 23.9, 29.4, and 35.0°C), and air (0.8–1.0 m^3/min · ton) was pumped through the rice. Before moving through the grain, atmospheric CO_2 was removed from the air by an absorption column filled with rings and saturated 36% aqueous potassium hydroxide. Using the modified Chung-Pfost equation (Pfost et al 1976) the a_w for rough rice at the experimental conditions was estimated. The CO_2-free air was saturated with water vapor as it passed through water held at the temperature (±0.5° C) needed to maintain the a_w of the rough rice being held at each moisture and temperature. On exiting a sample jar, air containing CO_2 evolved from that sample passed through a series of columns packed sequentially with silica gel, magnesium perchlorate, and granular asbestos particles coated with sodium hydroxide (Ascarite). The CO_2 absorbed on the Ascarite was determined gravimetrically at 24- and 48-hr intervals. The evolved CO_2 was converted to DML using the relationship that complete oxidation of 1 g of hexose (dry matter) produces 1.47 g of CO_2.

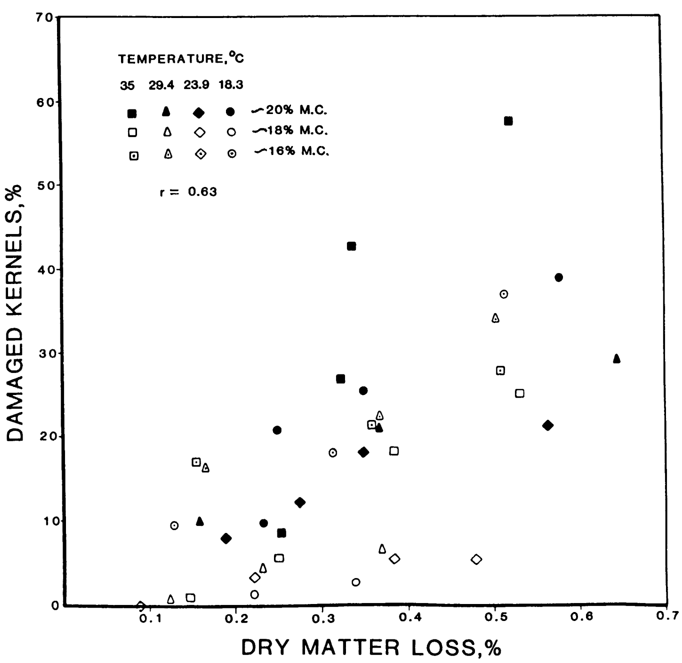

Fig. 1. Correlation between percent damaged (discolored) kernels and dry-matter loss in mechanically threshed Newrex (long-grain) rough rice.

During holding at a given moisture-temperature condition, quadruplicate sample jars from each rice lot tested were removed randomly from the storage chamber when the average accumulated DML reached ~ −0.18, 0.25, 0.38, and 0.50%. The final moisture in each sample jar was calculated from its dry-matter loss, initial moisture content, and initial and final mass. The moisture contents of all 16 samples removed from a storage cabinet were averaged, and the mean was designated as the storage moisture. The four samples removed at each DML level were air-dried to ~13% moisture, blended, and samples (2 × 50 g) were removed for ergosterol analysis. The remainder was milled for measurement of damaged kernels.

Damaged Kernels in Milled Rice

Approximately 600 g of air-dried rough rice (~ 13% moisture) was hulled using a rice sheller (McGill Inc., Houston, TX). The brown rice was milled in a laboratory rice pearler (Satake Engineering Co., Inc., Tokyo, Japan) set to release 12–14% of bran from the brown rice. The high degree of milling ensured that all bran was removed from the kernel so the bran would not interfere with identification of discolored kernels.

Visual assessment of damaged kernels was made in duplicate 50-g samples of milled rice. Damaged kernels were off-color (usually yellow or slightly yellow kernels), and were classified as whole or broken as specified by the USDA (USDA 1983). Damaged kernel values were expressed as a percentage of sample weight.

Rice Grade

Rough rice grade was determined unofficially. The determination was based on the allowable fungus-damaged kernels in a milled sample as specified by the U.S. standards for rough rice (USDA 1983). The maximum levels of damaged kernels allowed for U.S. Nos. 1 and 2 rough rice are 0.5 and 1.5%, respectively.

Ergosterol in Rough Rice

Duplicate samples of rough rice (50 g) were ground for 1 min in a high-speed grinder (Mitey-Mill, model 228, Sturdee Health Products, Island Park, NY). The nonsaponifiable lipids were extracted as described by Seitz et al (1977), the extract was redissolved in 1.0 ml of benzene-acetonitrile (98:2, v/v), and an aliquot (250 μl) was subjected to preparative thin-layer chromatography (TLC, 20 × 20 cm, Brinkmann G-25 HR, Brinkmann Instruments, Westbury, NY). The plate was developed in benzene-acetonitrile (90:10, v/v), and the sterols were visualized using a light box. The opaque band (~ 2.5 cm wide) seen at R_f ~ 0.3 was scraped off, and ergosterol was assayed as described by Naewbanij et al (1984).

TABLE I
Properties of Rough Rice Before Holding

Rice Sample	Mechanically Damaged Kernels (%)			Immature Kernels (%)	Germination[a] (%)	Ergosterol[b] (μg/g)
	Dehulled	Split Hull	Total			
Newrex	2.3	11.3	13.6	1.8	40.0	2.45
Nato	0.7	6.4	7.1	1.7	80.0	2.82
Arkansas 007 (hand-threshed)	0.2	1.4	1.6	3.1	97.0	5.50
Arkansas 007 (mechanically threshed)	1.3	4.1	5.4	0.8	97.0	4.59

[a] Average of four determinations; each test was made on a 25-kernel lot.
[b] Average of two determinations using the thin-layer chromatography, ultraviolet method (Naewbanij et al 1984).

RESULTS AND DISCUSSION

Damaged Kernels Versus DML

During holding of three varieties of mechanically harvested rice (Table I), we found that DML of rough rice correlated poorly (r = 0.62–0.63) with damaged kernels in the milled rice. Figure 1 shows the poor correlation (r = 0.63) for one variety of long-grain rice. The level of damaged kernels was the limiting factor controlling rice grade, so DML correlated poorly with grade. Our results on rice agree with those of Bottomley et al (1950) on maize, if it is assumed damaged kernels arise from mold invasion. Those workers found no correlation between mold count and DML when maize was stored at 75–100% relative humidity and ~35° C.

In contrast to mechanically threshed rice, hand-threshed long-grain rice (Fig. 2) gave a high correlation (r = 0.97) between percent damaged kernels and DML. The kernels of hand-threshed rice are probably more uniform in structure than those of mechanically threshed samples.

Ergosterol Versus Damaged Kernels

Ergosterol measures the invasion of both field and storage fungi in grain, and an increase in ergosterol content above the initial concentration measures new fungal growth in grain during storage (Seitz et al 1977). We found a high correlation (r = 0.96) for the three varieties of rice studied between the increase in ergosterol content of stored rough rice and the percentage of damaged kernels in the milled rice (Fig. 3). The high correlation indicated that fungal growth was responsible for kernel discoloration in the stored rice. Milled rice of U.S. grade Nos. 1–5 may contain 0–6% damaged kernels (USDA 1983). Mold growth in rough rice equivalent to 0–6% damaged kernels in our work showed ergosterol increased above native levels (Fig. 3).

The regression line in Figure 3 indicates an increase of 0.41 μg/g of ergosterol for every 1% increase in damaged kernels attributable to mold growth. The intercept of the line gives ~ 0.25 μg/g of

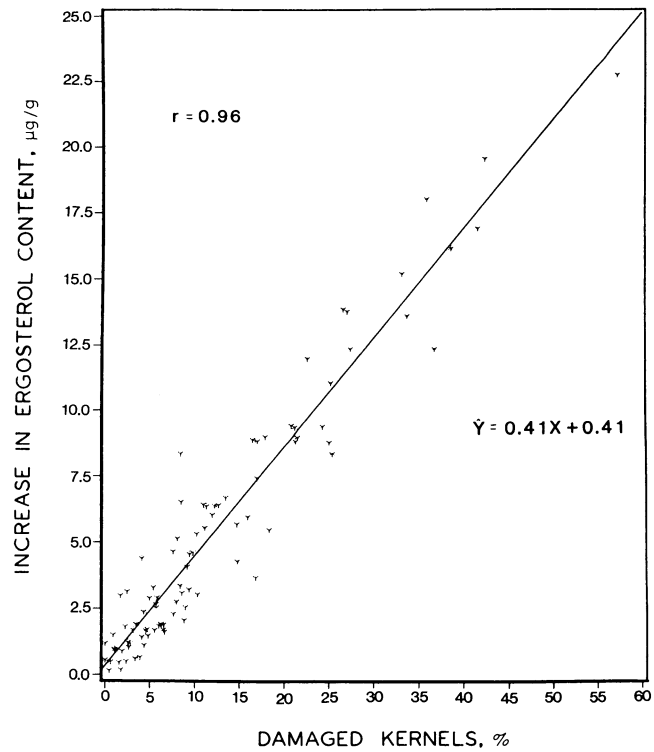

Fig. 3. Correlation between the increase in ergosterol content and the percentage damaged (discolored) kernels in stored samples of Newrex (long-grain), Nato (medium-grain), and Arkansas 007 (long-grain) rough rice. Initial ergosterol contents for Newrex, Nato, and hand- or mechanically-threshed Arkansas 007 were 2.5, 2.8, 5.5, and 4.6 μg/g, respectively.

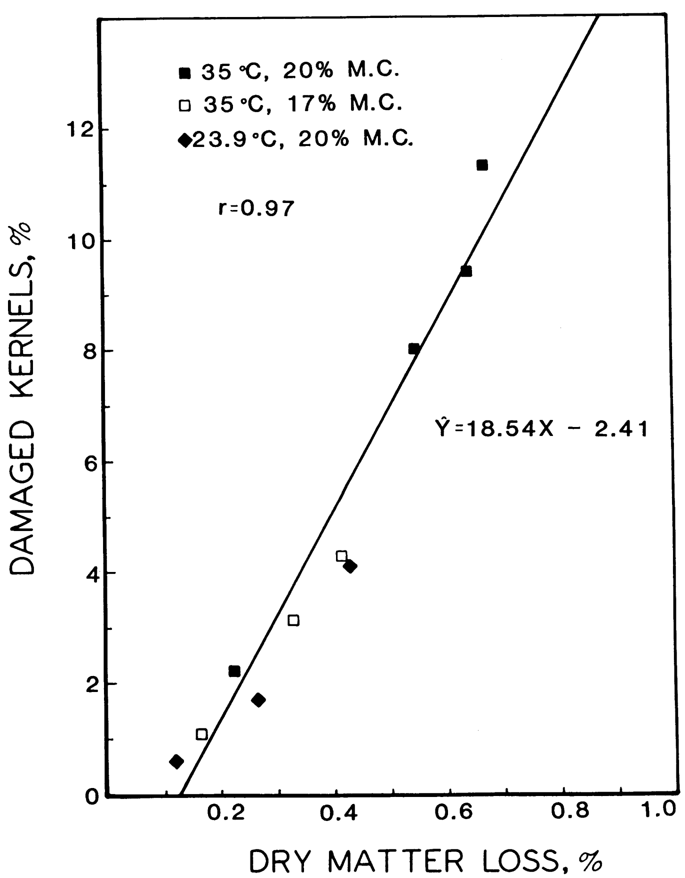

Fig. 2. Correlation between the percent damaged (discolored) kernels and dry-matter loss in hand threshed Arkansas 007 (long-grain) rough rice.

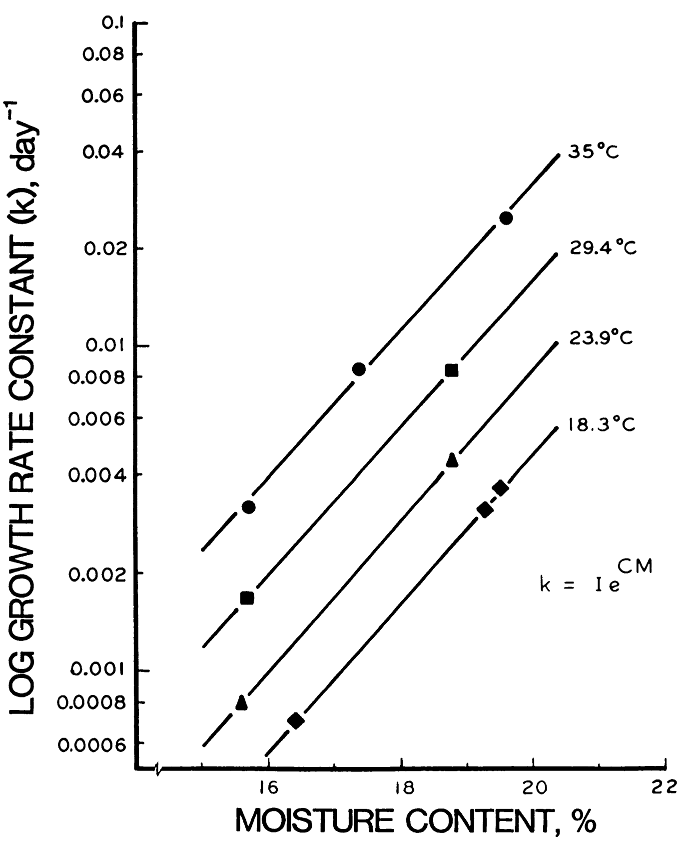

Fig. 4. Relationship between growth rate constant and moisture content (%, wet basis) of long-grain (Newrex) rough rice stored at 18.3, 23.9, 29.4, and 35° C.

ergosterol for rice with no damaged kernels. Because the damaged kernels in U.S. No. 2 rice must not exceed 1.5% (USDA 1983), an increase in ergosterol of $\geqslant 1\ \mu g/g$ above its initial concentration indicates a drop in rice quality below U.S. No. 2 grade. According to the U.S. grain standards, wheat and corn may contain 4 and 5%, respectively, of damaged kernels in U.S. grade No. 2. The increase in ergosterol accompanying 4–5% mold-damaged kernels in No. 2 wheat and corn might be determined more accurately than the increase in ergosterol accompanying 1.5% mold-damaged kernels in No. 2 rice.

Mathematical Model To Predict Increase in Ergosterol of Rough Rice

An empirical equation was derived to describe the increase in ergosterol concentration of rough rice in terms of its moisture, temperature, and storage time. The ergosterol content of rough rice was assumed to parallel mold growth, which is known to increase exponentially with time (Trinci 1974, Fiddy and Trinci 1976). Thus,

$$\ln \left| \frac{ERG}{ERG_o} \right| = kt \qquad (1)$$

where, [ERG] is the ergosterol content ($\mu g/g$) in grain at any storage time (t, days), [ERG_0] is the initial ergosterol concentration ($\mu g/g$), and k is the growth rate constant (day^{-1}).

The general dependence of k on moisture (M, % wet basis) and temperature of the rice was determined graphically. Figure 4 shows semilog plots of the least squares values of k plotted against moisture at each storage temperature. The family of lines in Figure 4 has identical slopes, showing that moisture had the same effect on the rate constant (k) at storage temperatures between 18.3 and 35.0° C. Each line in Figure 4 may be described by the equation $k = I \exp[C(M)]$, where, k day^{-1} is the rate constant, I is the intercept (day^{-1}), M is the moisture (%, wet basis), and C is the slope of the lines describing the effect of M on k.

When the logarithms of the line intercepts (I) in Figure 4 were plotted against temperature, the line in Figure 5 shows that the intercept can be described in terms of temperature (T, °C) and two new constants (A and B), that is $I = A \exp[B(T)]$. Substituting $A \exp[B(T)]$ for I in the equation $k = I \exp[C(M)]$ gave k as a function of temperature and moisture as follows:

$$k = A \exp[B(T) + C(M)] \qquad (2)$$

where, A, B, and C are constants. Finally, substitution of equation 2 for k into equation 1 gave equation 3,

$$\ln \left| \frac{ERG}{ERG_o} \right| = At \exp[B(T) + C(M)] \qquad (3)$$

After deriving the general form of equation (3), the values of the constants A, B, and C for long-grain (Newrex) and medium-grain (Nato) rice were determined using a nonlinear iteration program (Statistical Analysis System, SAS Institute Inc., Cary, NC). The values of A, B, and C given in Table II were significantly different at a 95% confidence level for the two rices. Those constants are different, perhaps, because of differences in grain structure and mechanical damage. Data obtained in this work were not sufficient to evaluate the effect of mechanical damage on ergosterol increase during storage.

TABLE II
Values[a] of Constants A, B, and C in Equation 3 for Ergosterol Content of Newrex (Long-Grain) and Nato (Medium-Grain) Rough Rice

	Constant		
Rice Variety	**A**	**B**	**C**
Newrex (long grain)	2.04×10^{-6}	0.11	0.44
Nato (medium grain)	6.50×10^{-7}	0.15	0.35
t value[b]	3.56	7.38	3.61

[a] Obtained using a nonlinear regression program (Statistical Analysis System, SAS Institute Inc., Cary, NC) from 35 mean observations on each rice variety.
[b] Constants for long- and medium-grain are significantly different ($P<0.05$).

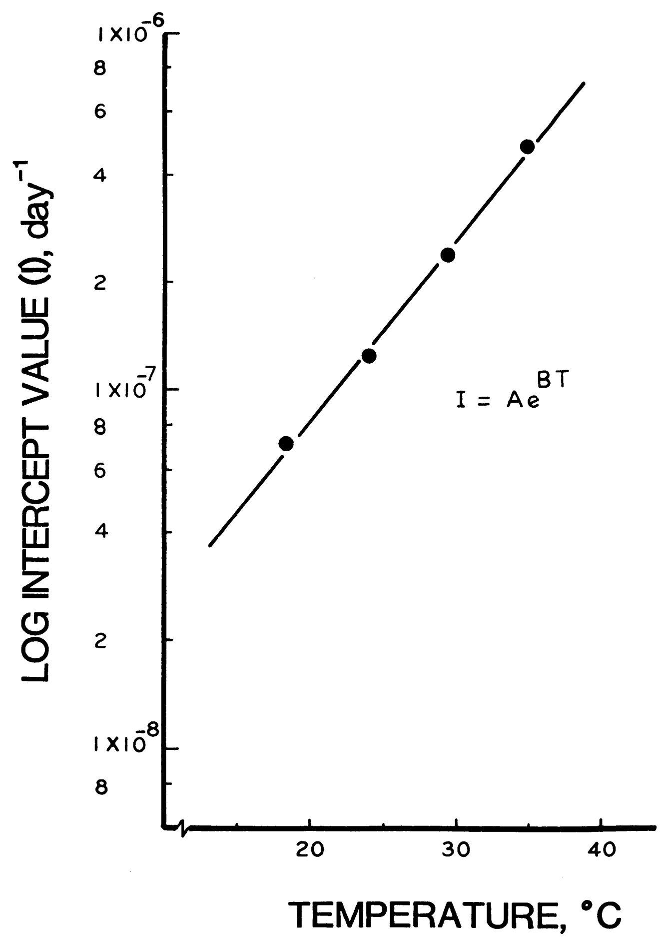

Fig. 5. Logrithmic relationship between the intercept values (I) from Fig. 4 and storage temperature (°C).

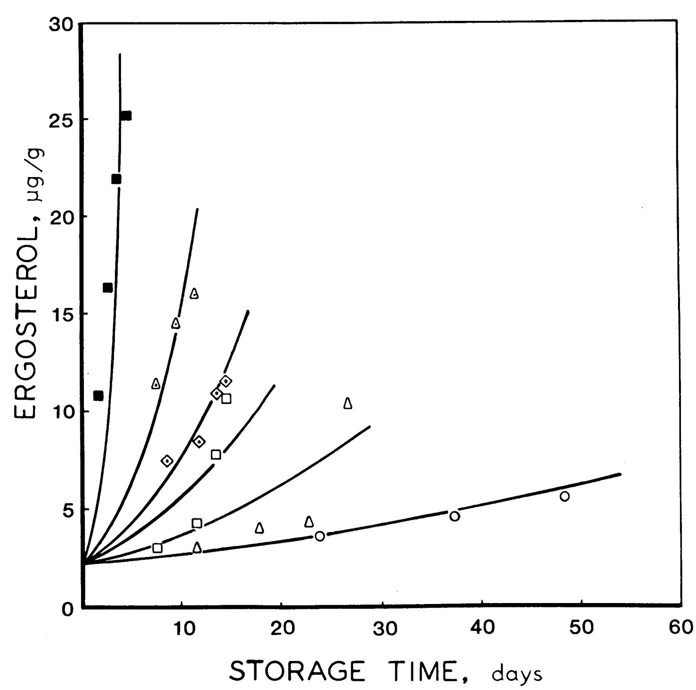

Fig. 6. Observed and predicted (——) ergosterol content using equation 3 and the constants in Table II for Newrex (long-grain) rough rice stored at 35°, 20.0% mc (■); 29.4° C, 18.9% mc (△); 23.9° C, 19.3% mc (◈); 35.0° C, 15.7% mc (□); 29.4° C, 15.7% mc (△) 18.3° C, 16.4% mc (o).

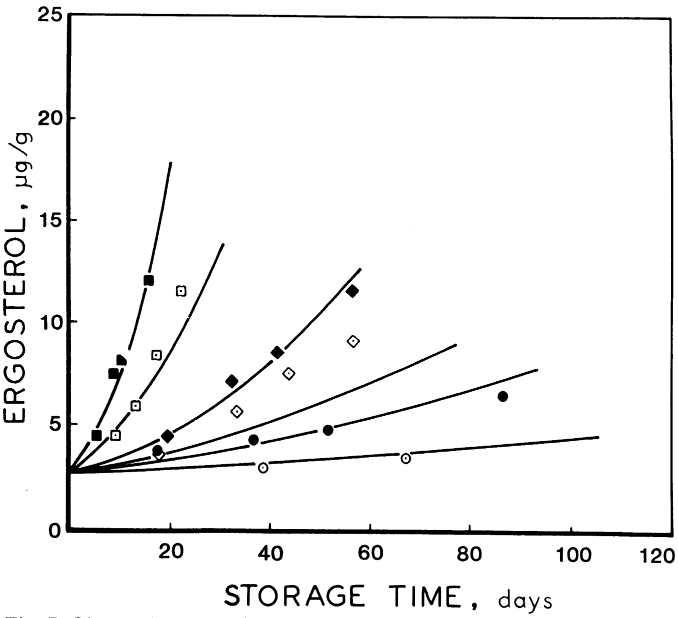

Fig. 7. Observed and predicted (——) ergosterol content using equation 3 and the constants in Table II for Nato (medium-grain) rough rice stored at 35°, 18.9% mc (■); 35°C, 17.3% mc (⊡); 23.9°C, 20.0% mc (◆); 23.9°C, 18.2% mc (◇); 18.3°C, 20.0% MC. (•); and 18.3°C, 17.5% mc (⊙).

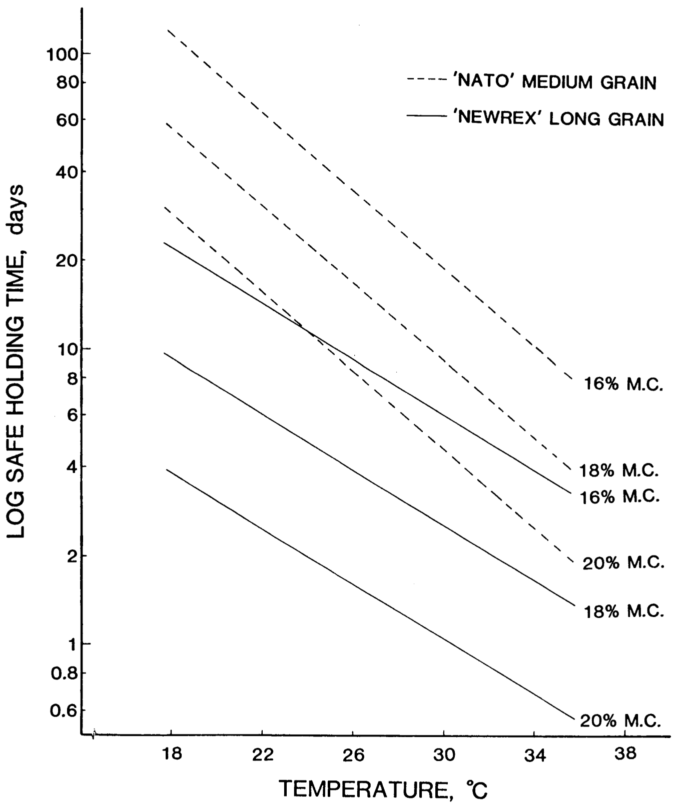

Fig. 8. Predicted maximum permissible holding time for a sample of Newrex (long-grain) and Nato (medium-grain) rough rice at various moistures and temperatures.

Figures 6 and 7 show the observed and predicted ergosterol using the sets of constants in Table II for long-grain (Newrex) and medium-grain (Nato) rice stored at temperatures of 18.3–35.0°C and moistures of 15.6 to 20%. At all 12 combinations of temperature and moisture used on Newrex and Nato rices, the theoretical and experimental values of ergosterol with storage time were the same at a 95% confidence level.

Maximum Holding Time Predicted for Rough Rice

The empirical equation 3 could be used to predict the safe holding time for long- and medium-grain rough rice at a given moisture and temperature if a 1 $\mu g/g$ increase in ergosterol content was used as the criterion for maximum permissible holding of freshly harvested rough rice. An increase of $\geqslant 1$ $\mu g/g$ would lower the grain grade below U.S. No. 2. Figure 8 shows the predicted maximum times for holding long- and medium-grain rice based on the materials used in this study. Before equation 3 can be used generally to predict the holding time for any sample of rough rice, more information is needed on how the constants A, B, and C vary between samples. Furthermore, more ergosterol data must be correlated with the quality of rice.

CONCLUSIONS

Ergosterol, but not DML, is a useful index of rice quality change during storage of high-moisture rough rice. An increase in ergosterol of $\geqslant 1$ $\mu g/g$ from its initial concentration indicates a drop of rice quality below U.S. No. 2 grade.

The increase in ergosterol level in stored rice may be expressed in an equation as a function of moisture, temperature, and storage time. In computer simulations of drying and holding of grain, ergosterol appears to be better than DML as a predictor of quality during holding of high-moisture rough rice.

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