Reduction in Aflatoxin Contamination of Rice by Milling Procedures[1]

H. W. SCHROEDER, R. A. BOLLER, and **HUGO HEIN, Jr.,** USDA, Market Quality Research Division, College Station, Texas

ABSTRACT

Aflatoxins developed rapidly in rough rice inoculated with a toxin-producing strain of *Aspergillus parasiticus* and stored in a highly humid atmosphere (about 100% relative humidity) at 30°C. Maximum concentrations of the toxins were produced and accumulated after 15–33 days. More than 90% of the kernels were infected with *A. flavus* spp. within 3 to 6 days. After 21 days in storage, an average of about 60–80% of the toxins by weight were found in the combined bran and polish fraction after milling. The bran and polish fraction contained the toxins in a concentration more than 10 times that of the milled kernels.

In 1960, outbreaks of disease in fish and poultry in different parts of the world led to the discovery of the toxic nature of microcontaminants in some food and feed crops. These substances (aflatoxins) were subsequently found to be fungal metabolites produced principally by certain strains of species of the *Aspergillus flavus* group (1). Recently, a comprehensive review of the aflatoxins has been published (2).

In 1964, Schroeder (3) reported that some strains of *A. flavus* species produced a higher concentration of aflatoxins on rice than on peanuts. Unpublished data from this laboratory show that *A. parasiticus,* a species of the *A. flavus* group, is a consistent producer of large quantities of aflatoxins on a rice substrate. Subsequently, Shotwell *et al.* (4) in 1966 reported that milled rice was an effective substrate for the production of aflatoxin in the laboratory. Additional confirmation was published in 1966 and 1967 (5,6,7,8). Boller and Schroeder (6) surveyed the prevalence of toxin-producing strains of *A. flavus* species in rice grown in all of the major rice-producing areas of the United States. Their study indicated that about one-third of the isolates from rice were capable of producing significant amounts of the aflatoxins. Unpublished information from experiments in this laboratory suggested that much of the aflatoxin in a contaminated kernel of rough rice was removed by ordinary milling procedures. The studies cited above show that a potential hazard of aflatoxin contamination of rough rice does exist. However, if the concentration of the toxins is significantly reduced in milling, any possibility of danger to the consumer of milled rice would be eliminated or greatly reduced. The experiments reported in this paper were made to determine the probable effectiveness of commercial milling practices in removing aflatoxin contamination from rough rice.

MATERIALS AND METHODS

Aflatoxin contamination was induced in rough rice (Belle Patna variety) in the laboratory to provide rice containing the toxins over a range of

[1]Contribution of the Field Crops and Animal Products Branch, Market Quality Research Division, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box ED, College Station, Texas 77840, in co-operation with the Department of Plant Sciences, Texas Agricultural Experiment Station, Texas A&M University.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Fig. 1. Storage container for maintaining rice at a high relative humidity.

concentrations. In test 1, 56 samples of rough rice (150 g. each) were stored over water in screen baskets suspended in wide-mouthed 1-qt. Mason jars (Fig. 1). Forty-two samples were inoculated with dry spores of a strain of *A. parasiticus* isolated from rice and known to have the ability to produce aflatoxins (B_1, B_2, G_1, and G_2) on a rice substrate. Fourteen samples were treated similarly but without inoculation. All samples were incubated at 30°C. Four samples, three inoculated and one control, were removed from storage at 3-day intervals.

The moisture content of each sample was determined by the two-stage air-oven method (9). The prevalence of fungi infecting the seeds was determined by plating 200 seeds of each sample on malt-salt agar (7.5% NaCl) after surface-disinfection with a 1% solution of sodium hypochlorite. The remainder of each sample was air-dried at room temperatures to approximately 11 to 12% moisture content (wet basis). After each sample was shelled, 50 g. of brown rice from the noninoculated control was separated for aflatoxin analysis. The three inoculated samples were bulked and mixed thoroughly; a 50-g. aliquot was removed for aflatoxin analysis and a 100-g. aliquot was retained to be milled.

The rice was milled on a modified McGill Sample Mill No. 1 in two breaks: first break was 30 sec. with 15 lb. pressure and the second break was 30 sec. with 5 lb. of pressure, yielding the bran and polish fraction respectively. Because of the small amount of rice per sample in this test, the bran and polish were combined and the combined fraction is designated as the "bran fraction" in this paper. The bran fraction and 50 g. of the milled rice were assayed for aflatoxin content.

In the second test, 28 samples (240 g. each) of rough rice were stored under conditions similar to those described in test 1. Twenty-one samples were inoculated with *A. parasiticus* and seven were retained as noninoculated controls. The same procedure of handling, milling, and analysis of the samples was followed, except that each replicate sample was milled and the brown rice and milled rice were analyzed separately. However, the bran fractions of the three replicate samples were bulked and the bulked fractions were extracted and analyzed for aflatoxin content. This test was made to provide verification of the results of the first test and to provide an estimate of the variation among samples.

The extraction and thin-layer chromatography procedures described by Pons and Goldblatt (10) for detection and quantification of aflatoxins were used throughout these experiments.

RESULTS

Inoculation of rough rice with spores of *A. parasiticus* rapidly increased the prevalence of kernels infected by species of the *A. flavus* group. In both tests, isolations of *A. flavus* species increased from 11 or 15% to 98% or more within 3 to 6 days (Table I) and the incidence of isolations remained very high. The principal difference between the two tests in respect to the fungal flora was in the development of infections by species of the *A. glaucus* and *A. candidus* groups. In test 1, infection by *A. glaucus* spp. increased at a more rapid rate than in test 2. However, infection by *A. candidus* spp. increased more rapidly in test 2 (Table I).

TABLE I

PERCENTAGE OF RICE KERNELS INFECTED BY THE THREE MOST PREVALENT GROUPS OF *Aspergillus* SPP. AFTER INOCULATION WITH *Aspergillus parasiticus* AND STORAGE IN A RELATIVE HUMIDITY OF ABOUT 100% AT 30°C.

Test 1								Test 2			
Storage Time	*A. flavus*	*A. glaucus*	*A. candidus*	Storage Time	*A. flavus*	*A. glaucus*	*A. candidus*	Storage Time	*A. flavus*	*A. glaucus*	*A. candidus*
days	%	%	%	*days*	%	%	%	*days*	%	%	%
0	15	53	4	24	91	40	21	0	11	51	4
3[a]	....	....	....	27	95	57	22	3	99	6	1
6	98	33	1	30	99	34	41	6	98	19	2
9	97	77	1	33	88	38	39	9	97	49	9
12	94	80	2	36	94	36	49	12	96	59	17
15	100	52	3	39	65	34	54	15	97	60	15
18	99	52	9	42	81	35	67	18	97	45	30
21	92	69	9					21	98	61	42

[a] 3-Day sample lost in test 1.

The moisture content of the rice increased rapidly during the first few days in storage and differed significantly between the two tests. In test 1 (Fig. 2) the initial average moisture content was 12.8%; after 6 days it was 17.9%; and after 21 days 17.6%. Moisture contents in test 2 (not illustrated) were 12.6, 16.7, and 19.5% respectively.

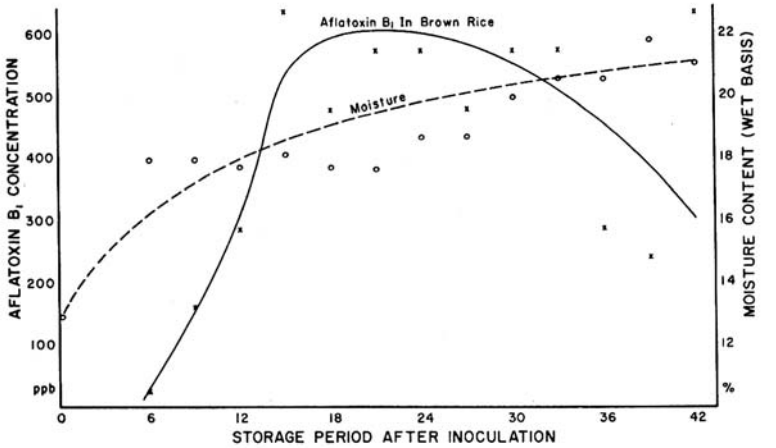

Fig. 2. Moisture content and accumulation of aflatoxin B_1 in rice inoculated with *Aspergillus parasiticus* as related to time in storage.

The general relation between moisture content and the production and accumulation of aflatoxin B_1 is illustrated by the results of test 1 (Fig. 2). The other principal aflatoxins (B_2, G_1, and G_2) followed the same trend of production and accumulation in general, although quantities differed greatly among the four toxins. Subsequently, only the data for aflatoxin B_1 will be reported in this paper, but the total quantities of aflatoxins found in these experiments were from about two to three times the quantities of B_1 reported.

Fungal deterioration of the rice in these experiments caused a significant decrease in the yield of milled rice (Fig. 3). Again, marked differ-

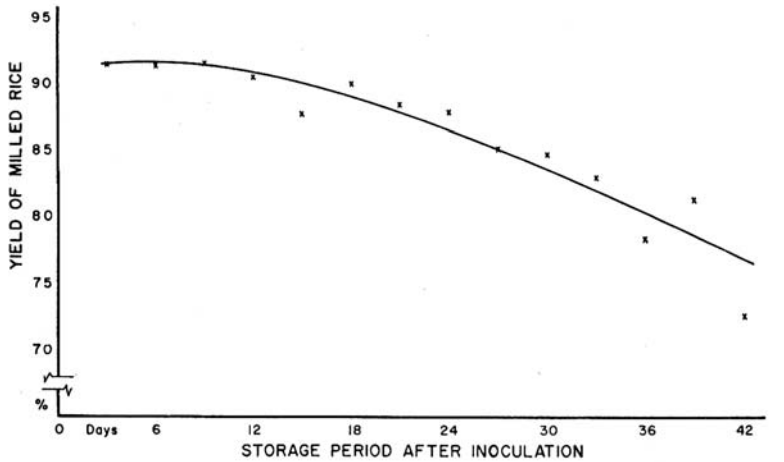

Fig. 3. Reduction in yield of milled rice in relation to time in storage at high humidity after inoculation with spores of *Aspergillus parasiticus*.

ences were observed between test 1 and test 2. Between 3 and 21 days in storage, the percentage of the kernel removed by milling increased from 11.1 to 13.5% in test 2 and from 8.0 to 11.3% in test 1.

The rate of aflatoxin B_1 production and accumulation in the rice and its pattern of distribution within the kernels varied in the two tests (Table II). The percentage of the toxin in the bran varied from 33 to >95%.

TABLE II

AFLATOXIN B_1 IN TWO FRACTIONS OF RICE AFTER MILLING IN RELATION TO TIME IN STORAGE AFTER INOCULATION WITH *Aspergillus parasiticus*

	AFLATOXIN B_1/100 g. BROWN RICE[a]					
	Test 1			Test 2		
TIME IN STORAGE	Milled Rice	Bran	Percent in Bran	Milled Rice	Bran	Percent in Bran
days	γ	γ	%	γ	γ	%
3	trace	trace	....	trace	0.19	>95
6	3.78	2.83	43	0.94	3.98	81
9	15.90	7.95	33	5.66	15.90	74
12	14.28	19.14	57	11.72	31.81	73
15	8.58	47.71	85	4.41	10.60	71
18	9.54	23.86	71	12.47	79.52	86
21	28.56	57.14	67	4.14	28.57	87
24	9.54	47.71	83			
27	8.58	47.71	85			
30	14.32	47.71	77			
33	9.55	47.71	83			
36	3.72	31.71	90			
39	7.66	47.71	86			
42	19.14	57.14	75			
Av. to 21 days	11.52	22.66	59	5.62	24.36	81
Av. 21–42 days	10.36	46.77	83			

[a] γ ÷ 100 = Concentration of aflatoxins in p.p.m.

However, the bran contained more than 50% of the toxin in all but two of 21 determinations. The distribution of aflatoxin B_1 in brown rice, milled rice, and the bran followed about the same general pattern within each sample, regardless of the concentration of toxins or the length of time in which aflatoxins were produced and accumulated (Fig. 4).

DISCUSSION

Factors other than moisture and temperature influence the production and accumulation of aflatoxins and also affect the distribution of the toxins within the kernels (Table II). The make-up of the active mycoflora (Table I) is a significant factor. It is not clear at this time whether the activity of fungal species is a cause or an effect of some moisture and temperature variations. Certainly, there was no observable significant difference between the initial mycoflora of the rice utilized in test 1 and those in test 2. However, over comparable times under the same environmental conditions, the predominance of the principal species competing with *A. flavus* varied greatly between the two tests.

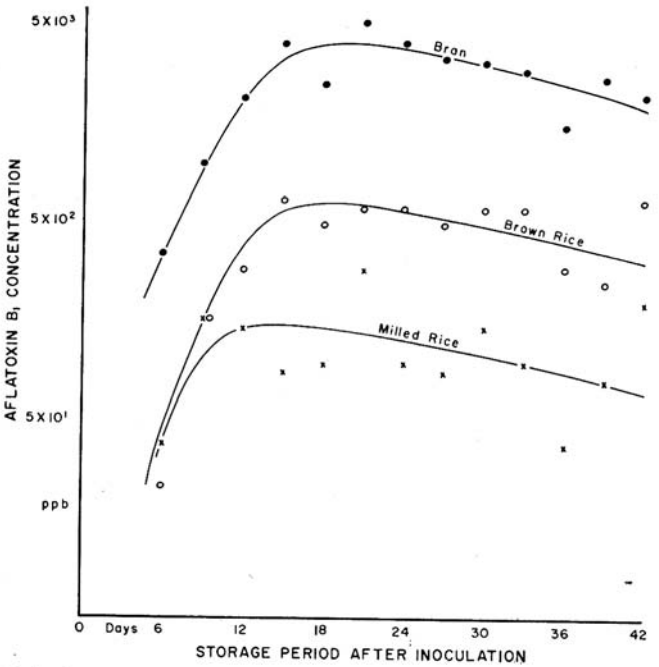

Fig. 4. Relative concentration of aflatoxin B_1 in brown rice and in the principal fractions after milling.

Increasing fungal activity was associated with an increase in the percentage of the kernel removed in milling (Fig. 3), and thus more toxins were also removed by milling. On the average, the concentration of aflatoxins in the bran fractions remained more than 10 times as high as in the milled rice fractions (Fig. 4).

Most of these data are concerned with rice containing unusually large amounts of aflatoxins. On the basis of the authors' experience, most naturally contaminated rice would contain aflatoxins at concentrations of <4 p.p.b. (trace) to 50 p.p.b. Furthermore, the toxins would probably be produced as a result of rapid growth of the fungus accompanied by limited penetration of the endosperm. In such circumstances, we would expect that most of the toxins would be in the fractions removed by milling. Thus, the analyses of the 3-day samples in test 2 (Table II) are probably most representative—that is, more than 95% of the toxins are in the bran layer.

Aflatoxins are slightly soluble in water. Possible diffusion from areas penetrated by fungi into undamaged portions of the endosperm may occur under some conditions, increasing the percentage of total toxins in the endosperm after milling. A more probable factor influencing this distribution pattern within the kernel is the growth of other microorganisms. In particular, other fungi can develop a competitive advantage, perhaps limited to the outer layers on the kernel, and metabolize a significant quantity of

aflatoxins. The ability of other fungi to metabolize aflatoxins has been demonstrated by Ashworth *et al.* (11).

In the United States the bulk of the rice crop is harvested at moisture contents ranging from 18 to 24% (wet basis). The crop is dried low enough to prevent fungal growth in commercial dryers or on the farm with heated or unheated air passed through the grain, or with a combination of the methods. Calderwood and Schroeder (12) showed that aflatoxin, in significant concentrations, can accumulate in high-moisture undried rice in 2 to 3 days when the environment favors the growth of *A. flavus* spp. Boller and Schroeder (6) found that aflatoxin-producing strains of *A. flavus* spp. were associated with rice grown in all of the major rice-producing areas of the United States and that they make up a significant part of the normal mycoflora.

Fortunately, the conditioning of rice by artificial drying is an efficient and effective operation in the United States. There is no evidence that contamination of rice with aflatoxin is a serious problem at this time, and the data presented in this paper indicate that the consumer is further protected in that the concentration of the toxins in contaminated rice is greatly reduced by the milling process. It is essential, however, that the potential hazards of the problem be recognized and that the industry and responsible research agencies make every effort to maintain and improve the efficiency of conditioning, handling, and storage of rice.

Literature Cited

1. SARGEANT, K., SHERIDAN, A., O'KELLY, J., and CARNAGHAN, R. B. A. Toxicity associated with certain samples of groundnuts. Nature 192: 1096–1097 (1961).
2. WOGAN, G. N. Mycotoxins in foodstuffs. M.I.T. Press, Cambridge, Mass. (1965).
3. SCHROEDER, H. W. Factors affecting aflatoxin production *in vitro* by *Aspergillus flavus-oryzae* spp. Presented at meeting of Peanut Improvement Working Group, Washington, D.C., 1964.
4. SHOTWELL, O. L., HESSELTINE, G. W., STUBBLEFIELD, R. D., and SORENSON, W. G. Production of aflatoxin on rice. Appl. Microbiol. 14: 425–428 (1966).
5. SCHROEDER, H. W., and ASHWORTH, L. J., JR. Aflatoxins: Some factors affecting production and location of toxins in *Aspergillus flavus-oryzae*. J. Stored Prod. Res. 1: 267–271 (1966).
6. BOLLER, R. A., and SCHROEDER, H. W. Aflatoxin-producing potential of *Aspergillus flavus-oryzae* isolates from rice. Cereal Sci. Today 11: 342–344 (1966).
7. TABER, R. A., and SCHROEDER, H. W. Aflatoxin-producing potential of isolates of the *Aspergillus flavus-oryzae* group from peanuts (Arachis hypogaea). Appl. Microbiol. 15: 140–144 (1967).
8. SCHROEDER, H. W., and HEIN, H., JR. Aflatoxins: Production of the toxins *in vitro* in relation to temperature. Appl. Microbiol. 15: 441–445 (1967).
9. UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL MARKETING SERVICE. Methods for determining moisture content as specified in the Official Grain Standards for beans, peas, lentils, and rice (1959).
10. PONS, W. A., JR., and GOLDBLATT, L. A. The determination of aflatoxins in cottonseed products. J. Am. Oil Chemists' Soc. 42: 471–475 (1965).
11. ASHWORTH, L. J., JR., SCHROEDER, H. W., and LANGLEY, B. C. Aflatoxins: Environmental factors governing occurrence in Spanish peanuts. Science 148: 1228–1229 (1965).
12. CALDERWOOD, D. L., and SCHROEDER, H. W. Aflatoxin development and grade of undried rough rice following prolonged storage in aerated bins. U.S. Dept. Agr., ARS 52-26; 32 pp. (Jan. 1968).

[Received January 5, 1968. Accepted May 6, 1968]