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Changes in moisture content, mycoflora and aflatoxin content of rice bran during storage

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Abstract. The changes in moisture content, storage mycoflora and aflatoxin B_1 (AFB_1) in bran from untreated or raw rice (Rr) and parboiled rice (Pbr) stored in small lots in polyethylene bags were studied at 15-day intervals up to 60 days, using five lots of each type of bran. Deterioration was more rapid with reference to all the three parameters, in Rr bran compared to Pbr bran, the former becoming completely overgrown and caked with fungi by the end of 60 days. *Aspergillus flavus* was the dominant fungus in Pbr bran, whereas *A. candidus* and *Trichoderma viride* were abundant in Rr bran. The frequency of incidence as well as concentration of AFB_1 increased with storage time in both types of bran, but the rate of increase as well as overall concentration were much higher in Rr bran. Thus raw rice bran is unsuitable for prolonged storage.

Key words: Aflatoxin, *Aspergillus*, Moisture, Parboiled rice, Rice bran, Storage fungi

Abbreviations: AFB_1 – aflatoxin B_1; MC – moisture content; Pbr – parboiled rice: Rr – raw rice

Introduction

Rice bran obtained from paddy (rough rice) after milling and polishing, being rich in nutrients like protein, lipids, vitamins and minerals, is used as an important food and feed source [1, 2]. Although rice bran is used as a main raw material for various commercial purposes, studies on the spoilage of rice bran by various factors are scant. India is the second largest rice producing country in the world with an annual production of over 50 million tons. Rice bran is a major by-product of the rice milling industry, and the annual production exceeds one million tons. Rice bran is commonly used in cattle feed. In the last decade or so, it is also being used as a source of edible oil. These usages necessitate the transportation and storage of bran for varying periods.

Our earlier studies showed that bran usually harbours abundant storage fungi, especially the potentially toxigenic *Aspergillus flavus* [3]. Further, storage of bran in the rice mill, even for a week, resulted in the formation of aflatoxin B_1 [4]. Therefore we determined the storability of bran from raw and parboiled rice with reference to moisture content, fungal numbers and aflatoxin production during storage.

Materials and methods

Storage and sampling of bran. Fresh samples of rice bran were collected during milling, from five commercial rice mills in and around Madras city. One sample each of parboiled (Pbr) and raw rice (Rr) bran were collected from each mill, in about 500 g lots, in clean polyethylene bags, tied tightly with rubber bands and stored at ambient temperature in the laboratory up to 60 days. The random

sampling method [5] was followed for collection as well as sample analysis, which was done at 0, 15, 30, 45 and 60 days of storage.

Determination of moisture content. The moisture content (MC) of the rice bran was determined by hot-air oven drying method. The percentage of MC was expressed on wet weight basis.

Analysis of storage fungi. The storage fungi of rice bran were analysed by the dilution plate technique [6], using a high-osmotic selective medium, Czapek-Dox agar containing 50% w/v sucrose. The plates were incubated at 30 °C up to one week. The individual species of storage fungi were counted separately, and their numbers were expressed as colony forming units per gram (cfu/g) of bran. The different species of *Aspergillus* and *Penicillium* were identified according to descriptions of Raper and Fennell [7] and Raper et al. [8] respectively.

Aflatoxin analysis. Extraction and assay of aflatoxin from rice bran were done by the method of Seitz & Mohr [9] using methanol as an extracting solvent. Because aflatoxin B_1 (AFB_1) was consistently present in most samples, we concentrated our studies on this toxin. Aflatoxin B_1 was assayed qualitatively by thin layer chromatography (TLC) along with a standard using chloroform: acetone (88:12) as a developing solvent, and quantified by a spectrophotometric method coupled with thin layer chromatography [10].

Statistical analysis. The statistical analysis (t test) was carried out to determine the significance of the experimental results [11].

Results

The average results of the 5 samples of Pbr bran and 5 of Rr bran are presented in Table 1 and Fig. 1. It may be seen from Table 1 that the fungal numbers in Rr bran, which were much higher than those of Pbr bran even at the time of collection, have registered a marked increase by 15 days of storage, the dominant species being *Aspergillus candidus*. Figure 1 shows a corresponding increase in MC as well as AFB_1 content of Rr bran, both being much higher than those of Pbr bran.

Changes in moisture content. The initial MC of the Pbr bran was 10.2%, and only slight fluctuations between 10 and 11% were seen during the 60 days of storage. But in Rr bran, the average MC increased from 11.7% to 13.6% after 30 days and to 13.9% after 45 days of storage. The increase of 1.9% in MC between 15 and 30 days of storage was found to be statistically significant ($p < 0.001$). By the end of 60 days, all the 5 samples of Rr bran were completely caked with fungi and hence considered unfit for handling. No determinations of MC were made in these samples (Fig. 1).

Changes in storage mycoflora. The total number of storage fungi even at the time of collection of samples were much lower in Pbr bran (average 0.4×10^3 cfu/g) than in Rr bran (average 22.9×10^3 cfu/g). A significant increase ($p < 0.05$ in Pbr and $p < 0.01$ in Rr) in numbers occurred in both types of bran after 15 to 30 days of storage. Subsequent fluctuations were not significant (Table 1). Among individual species, an increase in numbers was observed in *A. candidus*, *A. glaucus* and *A. terreus* in Pbr bran, but these were not significant individually. A significant increase ($p < 0.01$) was seen only in Rr bran with reference to *A. candidus* which was the dominant species in this bran, with a corresponding decrease in *A. fumigatus*. Besides the common storage fungi, *Trichoderma viride* occurred in considerable numbers in Rr bran, but as it is not generally regarded among storage fungi [12], it is not included in the data. The fluctuations in numbers of individual species are presented in Table 1 and their percentages in Figs. 2 and 3.

Natural occurrence of aflatoxin B_1. The frequency of occurrence of AFB_1 in rice bran increased with

Table 1. Changes in actual numbers of individual species of storage fungi in parboiled rice bran and raw rice bran at different intervals of the storage period

Nature of sample	Storage period (days)	Occurrence of individual species of fungi ($\times 10^3$ cfu/g) (average of 5 samples)								
		Total	*Ac*	*Af*	*Afu*	*Ag*	*Anid*	*Anig*	*At*	*P.*sp.
Parboiled rice bran	0	0.415	0.000	0.250	0.055	0.015	0.060	0.030	0.000	0.000
	15	1.235	0.250	0.135	0.185	0.300	0.075	0.045	0.180	0.060
	30	1.065*	0.170*	0.240	0.015	0.315	0.090	0.035	0.125	0.065
	45	0.810	0.140	0.235	0.065	0.200	0.020	0.012	0.075	0.045
	60	1.105	0.310	0.155	0.005	0.330	0.055	0.021	0.180	0.062
Raw rice bran	0	22.940	16.080	0.290	2.120	0.410	0.940	0.040	2.400	0.340
	15	49.520**	45.880**	0.240	0.440*	1.100	0.540	0.040	0.800	0.440
	30	49.300**	49.080**	0.720	0.540	1.920	0.380	0.120	1.380	0.280
	45	60.008	56.400*	0.220	0.540*	1.700	0.340	0.180	0.420	0.280
	60	Not done, because all the 5 samples completely overgrown and caked with fungi.								

*Less significant ($p < 0.05$); increase at 15 days in Pbr bran is not considered because of excessive variation between samples.
**Significant ($p < 0.01$).
Ac – Aspergillus candidus; *Af – A. flavus*; *Afu – A. fumigatus*; *Ag – A. glaucus*; *Anid – A. nidulans*; *Anig – A. niger*; *At – A. terreus*; *P.*sp. – *Penicillium* species.

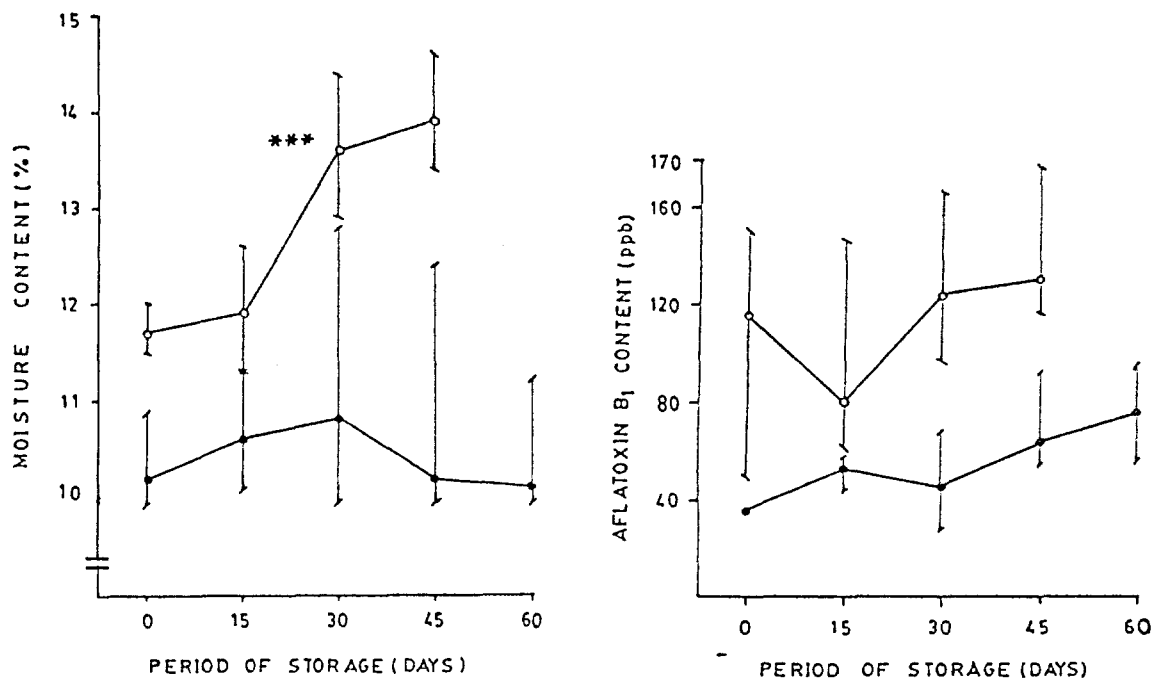

Fig. 1. Changes in moisture content and aflatoxin concentration in raw rice bran and parboiled rice bran during storage: range and average of 5 samples. ●——● Parboiled rice bran; ○——○ raw rice bran.
***Highly significant: $p < 0.001$

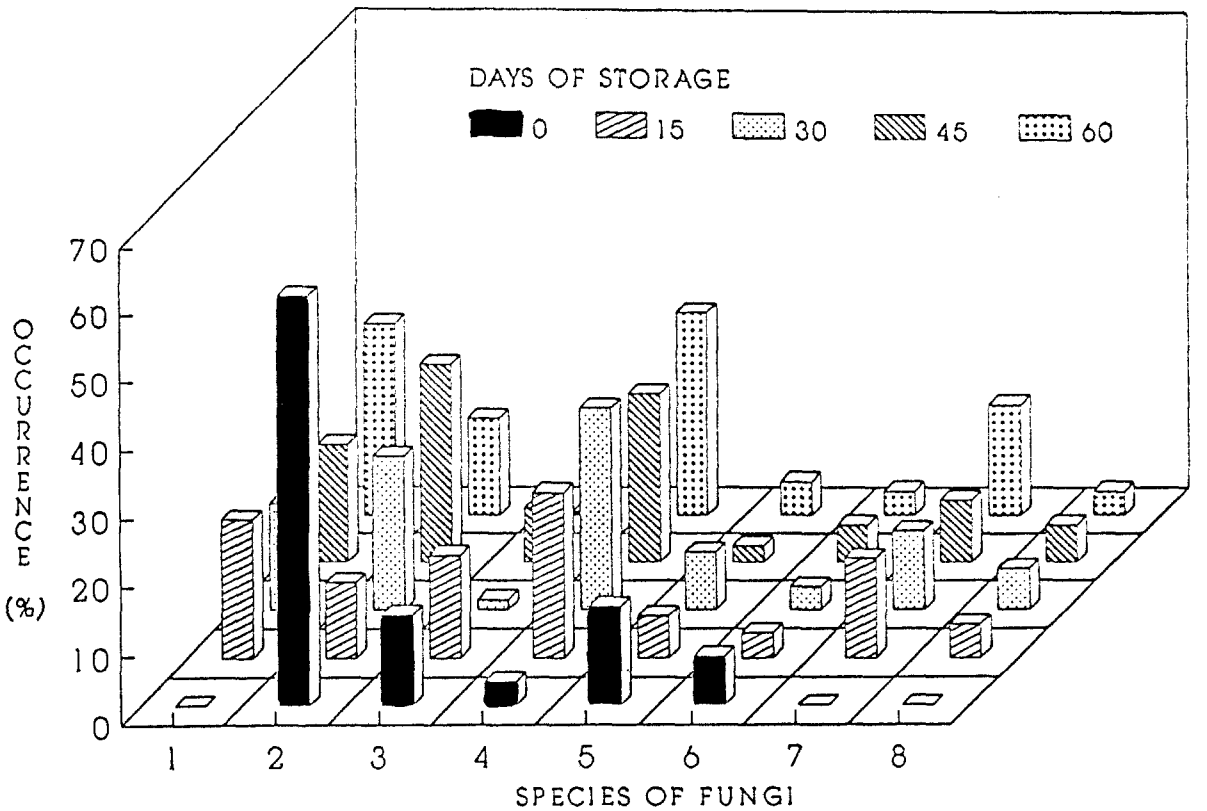

Fig. 2. Changes in comparative occurrence of individual species of storage fungi in parboiled rice bran at different stages of the storage period; occurrence represented as a percentage of the total. (1) *Aspergillus candidus*; (2) *A. flavus*; (3) *A. fumigatus*; (4) *A. glaucus*; (5) *A. nidulans*; (6) *A. niger*; (7) *A. terreus*; (8) *Penicillium* spp.

storage time. At the time of collection, AFB_1 was detected only in one sample of Pbr bran and 3 samples of Rr bran. All the 5 samples were positive for AFB_1 by the end of 30 days in Rr bran and 45 days in Pbr bran.

The aflatoxin content also differed in the two types of bran, being much lower in Pbr bran where it showed a gradual increase from an initial 35 ppb to 75 ppb at the end of 45 days. In raw rice bran it increased from about 115 ppb to a maximum of 140 ppb after 45 days (Fig. 1).

Discussion and conclusions

Several reports of storage changes in rice bran do occur in the literature, but these are mainly concerned with changes in free fatty acids, the increase of which is indicative of deterioration. Hence, measures are directed towards prevention of this type of deterioration [13]. In this respect, parboiled rice bran is generally reported to be more storable than raw rice bran, as the lipolytic enzymes are said to be inactivated during parboiling and there is no formation of free fatty acids during storage [14]. Apparently, little attention has been given to the growth of fungi, except for their possible role in lipolysis [15–17].

The present study shows parboiled rice bran to be more storable than raw rice bran even with respect to storage fungi and AFB_1. In fact, raw rice bran was totally unsuitable for prolonged storage in our ambient conditions. Whether the accumulation of free fatty acids be the cause or effect of fungal growth, the increase in moisture

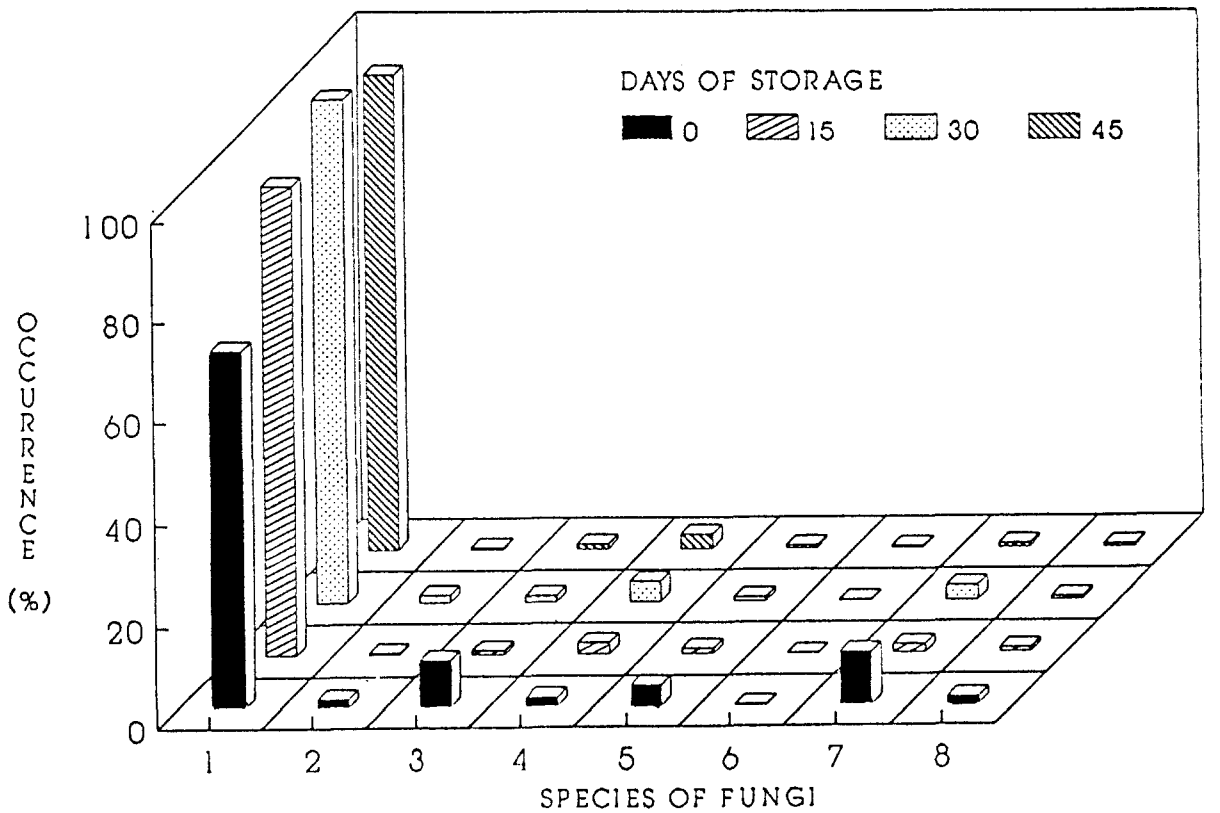

Fig. 3. Changes in comparative occurrence of individual species of storage fungi in raw rice bran at different stages of the storage period: occurrence represented as a percentage of the total. (1) *Aspergillus candidus*; (2) *A. flavus*; (3) *A. fumigatus*; (4) *A. glaucus*; (5) *A. nidulans*; (6) *A. niger*; (7) *A. terreus*; (8) *Penicillium* spp.

content would be definitely contributed to by the latter, eventually rendering the bran unfit for use because of excessive fungal growth.

Curiously, the higher incidence of AFB_1 does not correspond to a greater dominance of *A. flavus*, as all the five samples of raw rice bran showed a dominance of *A. candidus* whereas in parboiled rice bran *A. flavus* was dominant in all the five samples. Although the conditions of storage used in this study are not comparable with large scale storage, it should be noted by those who are involved in large-scale usage of bran for further processing, that suitable steps should be taken to prevent fungal growth and mycotoxin formation in raw rice bran. Also, the AFB_1 content of >100 ppb in this commodity is above the permissible level for animal feed.

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