Indian Journal of Science and Technology Vol.2 No.10 (Oct 2009) ISSN: 0974- 6846

Natural occurrence of aflatoxins and toxigenic fungi in rice bran oil and de-oiled bran

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Abstract: Samples of crude- and refined- rice bran oil and de-oiled bran collected from mills and local market places were analysed for the presence of aflatoxins B_1 and the causal fungi. Out of 20 crude rice bran oil and 20 refined rice bran oil samples analysed, 15 and 6 samples, respectively were positive for aflatoxins B1. The aflatxoin B_1 in the rice bran oil samples were in the range of trace to 956 ppb and averaged 618 ppb. Out of 30 samples analysed, 20 samples of de-oiled bran were positive and the toxin level ranged from 7 to 144 ppb with an average of 33 ppb. The storage fungi including toxigenic fungi present in the rice bran oil and de-oiled bran samples were *Aspergillus glaucus, A. flavus, A. niger, A . nidulans, A. candidus, A. fumigatus,* and *Penicillium* spp. and *Gliocladium viride.* In the rice bran oil samples, the fungi found were less in number (1 one to 14 cfu/g) with an average of 2 cfu/g. While, in the de-oiled bran, it was 1 to 130 X 10^3 cfu/g with an average of 33 X 10^3 cfu/g which include toxigenic *A. flavus*. About 60% of *A. flavus* from crude rice bran oil samples and 66.7% of *A. flavus* strains isolated from de-oiled bran samples were positive for aflatoxin production.

Keywords: Aflatoxins, mycotoxins, storage fungi, *Aspergillus flavus*, rice bran oil, de-oiled rice bran.

Introduction

The present investigation is based on our earlier study which indicated that the rice bran samples of raw rice and parboiled rice were contaminated with aflatoxins along with the elaborating fungi (Jayaraman & Kalyanasundaram, 1990; Jayaraman, 1991 & Vincent 1999). About 4 million tonnes of proteins, 5 million tonnes of edible oil and 75,000 billion calories, besides various quantities of vitamins and minerals have already been reported to produce from rice bran for consumption of human and domestic animals (Houston, 1972; Barber, 1978). The oil yielding potential of rice bran is exploited in India and used for cooking as well as for various industrial purposes. The lipid fraction of rice bran contains mainly oleic, linoleic and palmetic acids which are excellent nutrient source. Rice bran oil contains lowest hyphocholestrolaemic activity (Rukmini, 1985). De-oiled bran contains less fat and high protein that is used in the cattle- and poultry- feeds.

Therefore, the present study focused on the aflatoxin production in rice bran oil and de-oiled bran and also identified the causal fungi which is health hazard.

Materials and methods

Samples collection

Rice bran oil: The crude rice bran oil and the refined rice bran oil were collected from oil extraction mills and local market places. They were sampled in new PET bottles pre-cleaned with alcohol and brought to the laboratory for analysis of storage fungi and aflatoxins by standard procedures. The crude rice bran oil sampleas were collected from the rice bran oil extraction mills only (2 days old storage before refining). The refined rice bran oil (edible oil) samples were collected from mills as well as from local markets (various parts of TN, Karnataka & AP).

De-oiled rice bran or de-fatted rice bran: The unused polythene bags pre-cleaned with alcohol were used for the collection of de-oiled rice bran samples. After collection, the bags were tied with rubber bands tightly to avoid contamination and labeled. The de-oiled samples were collected from the rice bran oil extraction mills located in various districts of Tamil Nadu *viz*. Salem, South Arcot and Trichi. The samples were analysed in the laboratory within a week of collection and ascertain for the presence of aflatoxins and toxigenic fungi.

Enumeration of toxigenic fungi

The storage mycoflora of the rice bran oil samples were analysed on Czapek's Dox agar containing 50% sucrose by pour plate method. One ml aliquots of the oil samples were plated aseptically over the medium. The plates were swirled to distribute the inoculum uniformly entire surface area. To harbor the storage fungi which include toxigenic fungi, highly osmotic medium which contains 50% of sucrose in Czapek's Dox Media (Rao & Kalyanasundaram, 1983) was used. The media included antibiotic streptopenicillin in mentioned concentration to suppress any external contamination.

The de-oiled rice bran samples were analyzed for the presence of storage mycoflora and toxigenic fungi by serial dilution plating method (Jhonson & Curl, 1972). About 10 g was taken as a starter material and was diluted serially with sterile saline solution. The plates were incubated at 30 +/- 1°C for a week to develop culture mycelia followed by sporulation for identification. The *Aspergillus* species were identified after Raper and Fennel (1965) and the *Penicillium* species were after Raper *et al*. (1949). The quantitative pattern of storage mycoflora was expressed as number of colony forming units per gram (cfu/g) or per ml (cfu/ml). The fungal species were also maintained on agar slants of normal Czapek's Dox Agar with 3% sucrose for further work.

Analyses of aflatoxins

The reagents and solvents used for extraction and analysis in the present study were analytical grade of Emerck, Loba, BDH and Qualigens products. The standard aflatoxin B1 obtained as a free sample from National Institute of Public Health and Environmental Protection, Bilthovan, The Netherlands.

The standard AOAC method (Stoloff *et al*., 1971) was followed using Acetonitrile: Potassium chloride solution

Indian Journal of Science and Technology Vol.2 No.10 (Oct 2009) ISSN: 0974- 6846

(90:10) for extraction followed by identification and confirmation through thin layer chromatography (TLC).

The simple screening method for aflatoxin by Seitz and Mohr (1972) using methanol as a solvent and developing the TLC in chloroform : acetone (88:12) was also was used in addition to the above procedure.

The quantitative estimation of the aflatoxin from samples was made by the spectrophotometric method described by Nabney & Nesbitt (1965). Aflatoxin recovered in cold methanol from silica gel plate was read spectrophotometerically at 363 nm and 420 nm and the OD values were taken for calculation. The following formula was used for quantitative estimation.

$$\frac{D \times M \times 10^6}{E \times 200 \times t}$$ µg per 5 ml of solution

Where,

D- is the corrected OD at 363 nm (OD 363 nm- OD at 420 nm)

M- is the molecular weight of aflatoxins B_1 i.e 312

E- is the molecular extinction coefficient of aflatoxin B1 (22,000)

t- is the thickness of the cuvette in cm

Toxigenicity of fungal isolates

To screen the fungal isolates for production of aflatoxin *in vitro*, the fungi were cultured in slants of an agar medium containing 2% yeast extract and 15% sucrose (YES agar). Toxins were extracted from the molten agar with chloroform and assayed by TLC using toluene: ethyl acetate: 90% formic acid (6:3:1) solvent system (Bullerman, 1974). The fluorescent spots in blue and green TLC under short wavelength indicates the presence or absence of aflatoxin and the toxigenic property of the *Aspergillus flavus* strains.

Results

Aflatoxins

Out of 20 crude rice bran oil samples analysed, 15 were positive for aflatoxin B_1 in the concentration of 236 ppb to 956 ppb with an average of 618 ppb. Of the 20 refined rice bran oil samples analysed, 6 samples were found with aflatoxin B_1 contamination of trace to 28 ppb with an average of 20 ppb. In one sample of refined rice bran oil, aflatoxins B_2, G_1 & G_2 also were present in addition to aflatoxin B_1. Twenty of the 30 de-oiled bran rice bran samples collected were positive for aflatoxin B_1 in the range of 7 to 144 ppb with an average of 33 ppb. Table 1 indicates the details of samples analysed and the contamination levels with aflatoxins.

Toxigenic fungi

In the crude rice bran oil samples analysed , 15 out of 20 were contaminated with fungi including *Gliocladium viride, Mucor mucedo, A. flavus, A.glaucus, A.nidulans, A.niger and Penicillium sp*. in the order of dominance. Only 4 out of 10 refined rice bran oil samples were contaminated with fungi as *M. mucedo*. Out of 15 isolates of *A. flavus*, 9 were positive for aflatoxins. None of the refined rice bran oil samples were found to contaminate with *A.flavus*. Table 2 shows the presence of storage fungi and aflatoxigenic fungi in various samples studied in the present study.

Out of 30 de-oiled bran samples analysed for fungi, all the samples were found to be contaminated with different species of storage fungi with their population ranging from 1 to 130 X 10^3 cfu/g and with an average of 33 X 10^3 cfu/g. Among the species of fungi present, *A. flavus* and *Penicillium* sp. were dominant in occurrence. In general, *A. flavus* was recorded in almost in all the samples in low population. Of the 30 isolates of *A. flavus* from de-oiled bran 20 were positive for aflatoxin production.

Table 1. The natural occurrence of aflatoxin B1 in various samples collected

Description of sample	Natural occurrence of aflatoxin B1 in samples (ppb)					
	No. of samples collected	No. of samples positive	% of sample positive	Lowest level	Highest level	Average level
Crude rice bran oil	20	15	75	236	956	618
Refined rice bran oil	20	6	30	trace	28	20
De-oiled bran	30	20	66.7	7	144	33

Discussion and conclusion

Milling of paddy is the process where the husk and outer coating of rice is removed as bran and the polished rice is obtained for cooking as a food. In the subsequent milling process, the bran might become contaminated with stale bran present on the machinery as well as air of milling atmosphere with *A. flavus* and aflatoxin (Jayaraman, 1991). When bran is acquired for the purpose of extracting oil, there is generally a time lag between bran production, processing, marketing, transport and storage, which increases the chances of colonization of toxigenic fungi already present in bran as inoculum, a starter for toxin production. This might explain the high incidence of aflatoxins in crude rice bran oil samples found in this study. Although, most of the toxins were removed during refining process, this does not always happen, as 30% of refined oil samples showed up to 30 ppb of aflatoxin B1 (Table 1). Reduction of aflatoxin in oil refining process has been reported in Sesame, Corn germ and Olive oils and sometimes the high grade edible oils with up to 2,000 ppb of aflatoxins after refining (Isohata *et al*., 1996). This indicates that extreme care and proper precautions should be taken to eliminate the toxins during oil refining process.

Table 2. Natural occurrence of toxigenic fungi in various samples collected

Description of sample	Natural occurrence of aflatoxin B1 in samples (ppb)			
	No. of samples analysed	No. of species isolated	No. of species toxigenic	% of species toxigenic
Crude rice bran oil	20	15	9	60
Refined rice bran oil	20	0	0	0
De-oiled bran	30	30	20	66.7

Indian Journal of Science and Technology Vol.2 No.10 (Oct 2009) ISSN: 0974- 6846

Otherwise, these contaminated edible oils would pose a direct health hazard to man. Since the quality of rice bran oil is more suitable to maintain better body cholesterol level, which is recommended for keeping body in good health and reduce risks of coronary heart diseases.

Although most of the aflatoxins contained in rice bran are extracted along with the oil, some quantity of toxins still remained in the de-oiled bran, and further production might occur during storage because of the occurrence of toxigenic *A. flavus.* This could have deleterious effect on cattle and poultry, when the bran is fed either directly or as an ingredient of formulated feed. There are many reports of contamination of feeds with aflatxoin and other mycotoxins, from India (Shastry *et al*., 1965; Jayaraman & Kalyanasundaram, 1996).

The higher incidence of aflatoxigenic fungi and aflatoxin present in de-oiled bran (Table 2) would have contaminated the cattle-feed after formulation with various oil cakes and other agricultural commodities. The contaminated cattle-feeds may account for aflatoxin in the milk. Hence, precautionary measures are to be taken to control aflatoxin and decontamination of it in feed.

Acknowledgement

PJ is grateful to Dr. R. Rangarajan, the Chairman, K. V. D. Kishore Kumar, the Director and management team of Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College for their constant encouragement to do research and publications.

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