Note on Microbiological and Aflatoxin Analyses of Cereal Grains From the Tarai Plain of Southern Nepal

T. B. KARKI,[1] R. J. BOTHAST, and R. D. STUBBLEFIELD, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture,[2] Peoria, IL 61604

Over the past 20 years, a number of mycotoxins have been detected in cereal grains (Rodricks, 1976). Cereals consumed directly or used as ingredients must meet high standards regarding microbial quality and must be free of mycotoxins if a safe and wholesome food is to be ensured. Grain from Nepal is used primarily for food and, to a lesser extent, for beer and liquor fermentations.

Nepal has a heterogeneous topography, and most of the surplus cereal grains are produced in the plain of Tarai where temperature and humidity are relatively high. These grains are usually supplied to deficit hilly areas after six months' storage. Consequently, this preliminary study focused on the microbiologic and aflatoxin content of several grain samples collected from markets in Hitauda and its vicinity.

Samples (500 g, identified according to the kind of grain and geographic location of sampling) were shipped by air in plastic bags to the Northern Regional Research Center and stored in a freezer until examined. Counts of total aerobic bacteria, actinomycetes, and molds, as well as the percentage of kernels infected with mold, were determined according to procedures outlined by Bothast et al (1974). Fungi were isolated and maintained on malt extract agar slants. The *Aspergilli* and *Penicillia* isolates were then grown on Czapek's solution agar and malt extract agar plates. The dark molds (*Alternaria* and *Drechslera*) were grown on cornmeal agar. *Fusarium* isolates were grown on potato sucrose agar, and the mucoraceous isolates were grown on potato dextrose agar, Czapek's solution agar, and malt extract agar. All plates were inoculated at three points. Based on cultural and morphologic characteristics, the fungi were identified using appropriate monographs.

Aflatoxin assays were conducted according to the CB method approved by the Association of Official Analytical Chemists (1972). Aflatoxins B_1 and G_1 were chemically confirmed (Przybylski, 1975).

Bacterial counts tended to be higher than actinomycete or mold counts for all samples (Table I). Except for the single Ragi seed sample (*Eleusine coracana*), the count data are in close agreement with similar analyses obtained on U.S. grain samples (Hobbs and Greene 1976). The actinomycete count (10^5/g) on the Ragi sample is especially high.

Species of *Aspergilli* and *Penicillia* were the predominant surface molds isolated from Nepal corn, raw rice, and parboiled rice (Table I). Isolated *Aspergilli* were: *A. flavus, A. niger, A. versicolor, A. fumigatus, A. chevalieri, A. candidus, A. wentii,* and *A. sydowi.* Of

[1]Present address: Food Research Division, Department of Food and Agriculture Marketing Services, Bafarmahal, Kathmandu, Nepal. Direct all inquiries to the Northern Regional Research Center.

[2]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TABLE I
Microbiologic counts and Predominant Surface Molds on Grains

Samples	Counts per Gram: Bacteria	Actinomycetes	Molds	Predominant Surface Molds
Corn				
1.	4.0×10^3	1.4×10^2	8.3×10^3	*Penicillium islandicum,* Pink yeast, *Aspergillus flavus*
2.	1.2×10^4	1.0×10^1	8.0×10^1	*Aspergillus versicolor, Aspergillus niger, Rhizopus arrhizus, Penicillium* sp.
3.	9.4×10^3	1.5×10^1	2.2×10^3	*Aspergillus flavus, Aspergillus niger*
Raw Rice				
1.	2.1×10^4	1.3×10^2	8.5×10^1	*Absidia corymbifera; Rhizopus arrhizus*
2.	1.2×10^4	3.3×10^2	1.5×10^3	*Rhizopus arrhizus; Rhizopus* sp.
3.	4.8×10^4	4.1×10^2	1.9×10^3	*Rhizopus arrhizus; Rhizopus* sp.
4.	5.0×10^4	1.5×10^2	5.6×10^2	*Aspergillus candidus, Penicillium islandicum, Drechslera cyanodontis, Aspergillus fumigatus, Aspergillus flavus*
5.	2.2×10^4	6.0×10^1	4.5×10^1	*Penicillium islandicum, Rhizopus arrhizus, Absidia corymbifera*
6.	9.5×10^3	1.4×10^2	2.9×10^2	*Penicillium islandicum, Aspergillus versicolor; Aspergillus flavus*
7.	9.3×10^3	6.5×10^1	1.6×10^2	*Penicillium islandicum, Aspergillus sydowi*
8.	1.2×10^5	1.5×10^2	8.0×10^1	*Aspergillus candidus, Aspergillus sydowi; Mucorales*
Parboiled Rice				
1.	9.1×10^4	2.5×10^2	3.2×10^2	*Penicillium* sp., *Absidia corymbifera; Aspergillus flavus, Fusarium* sp., *Alternaria* sp.
2.	2.0×10^5	3.0×10^1	3.2×10^2	White yeast, *Penicillium chrysogenum* series close to *P. meleagrinum*
3.	1.9×10^3	5.0×10^1	3.5×10^1	*Penicillium islandicum, Aspergillus flavus*
4.	2.4×10^4	9.5×10^1	7.5×10^1	*Aspergillus flavus, Rhizopus* sp., *Aspergillus sydowi*
Wheat	5.6×10^4	2.9×10^2	3.5×10^3	*Penicillium chrysogenum* series close to *P. meleagrinum, Aspergillus candidus, Alternia* sp.
Ragi	3.8×10^6	5.5×10^5	3.0×10^4	*Phoma* sp., *Aspergillus sydowi, Drechslera cyanodontis, Penicillium* sp., *Fusarium* sp.

TABLE II
Incidence of Mold Growing From Surface-Disinfected Grains From Nepal

Sample	Media[a]	Mold Infection (%)
Corn		
1.	A	68
	B	54
2.	A	38
	B	46
Raw Rice		
1.	A	0
	B	0
2.	A	0
	B	0
3.	A	0
	B	0
4.	A	0
	B	0
5.	A	2
	B	0
6.	A	2
	B	0
7.	A	0
	B	0
8.	A	0
	B	0
Parboiled Rice		
1.	A	8
	B	2
2.	A	0
	B	4
3.	A	0
	B	0
Wheat	A	98
	B	100
Ragi	A	46
	B	42

[a]A = Czapek's agar containing 20% sucrose; B = malt extract agar.

TABLE III
Aflatoxin Content in Cereal Grains From Nepal (ppb)

Sample	B_1	B_2	G_1	G_2
Corn				
1.	8.8	5.0	...	...
2.	37.5	5.0	...	...
3.	19.3	2.3	57.6	9.7
Raw Rice				
1.	10.0	...	...	...
2.	5.0	...	...	...
3.	Trace[a]	...	...	...
4.		Not detected		
5.	Trace	...	...	...
6.	15.0	...	...	...
7.	Trace	...	...	...
8.		Not detected		
Parboiled Rice				
1.	3.8	1.8	...	...
2.	2.5	...	...	...
3.	Trace	...	...	...
4.	12.5	...	...	...
Wheat			Not detected	
Ragi		Not detected		

[a]Trace = less than 2.5 ppb.

the *Penicillium* spp. isolated, only *P. islandicum* was identified. The *Mucorales* identified were *Rhizopus arrhizus* and *Absidia corymbifera*. *Mucorales* and *Drechslera* spp. also were identified from raw rice, and *Alternia* spp. and *Rhizopus* spp. from parboiled rice. *Alternaria* spp. were present along with *Penicillium* spp. and *A. candidus* on the single wheat sample (Table I). Species of *Phoma, Aspergillus, Drechslera, Penicillium,* and *Fusarium* were isolated from Ragi.

Fungi grew from 68 and 54% of the surface-disinfected kernels of corn on Czapek's agar containing 20% sucrose and malt extract agar, respectively (Table II). The percentages dropped, however, on corn sample 2 to 38 and 46%. Species of *Fusarium, Penicillium,* and *Aspergillus* were frequently observed. *Aspergillus flavus* infection was 4 and 21% for lthe respective corn samples. Essentially no mold grew from surface-disinfected grains of raw and parboiled rice (Table II). Mold infection was higher, however, in the wheat and Ragi samples (Table II), averaging 99 and 44%, respectively. Species of *Alternaria* and *Drechslera* were predominant.

In Nepal, corn is harvested at high moisture levels (18–21%) and stored on the cob with husk for about six months before being shelled and consumed. Consequently, it is not surprising that all three samples of corn were aflatoxin positive, ranging from 8 to 37.5 ppb of B_1 (Table III). One sample contained all four aflatoxins: B_1, B_2, G_1, and G_2. Within the purview of 12 rice samples, low levels of aflatoxin (ranging from trace to 15 ppb of B_1) were detected. A trace to 12.5 ppb was detected in the four parboiled rice samples. No aflatoxin was found in the samples of wheat and Ragi.

The results of this preliminary study imply that the microbiologic counts on cereals from Nepal are satisfactory and that aflatoxin may be a hazard, especially in corn. Only 17 cereal samples were evaluated, however, and selected from food markets in one area of Nepal. Certainly a more extensive survey is warranted to evaluate truly the quality of Nepalese grain.

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