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Quantification and Detoxification of Aflatoxin in Food Items

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Abstract. The present study was conducted to quantify and detoxify the aflatoxins in food items. For this purpose, total 30 samples of food were collected. The samples were quantified using thin layer chromatography (TLC) for the presence of aflatoxin level in food items. Out of them aflatoxins were not found in 10 samples. Remaining 20 aflatoxins +ve samples were treated with various chemical solutions i.e. 0.1% HCl, 0.3% HCl, 0.5% HCl, 10% citric acid, 30% citric acid, 50% calcium hydroxide, 0.2 and 0.3% NaOCl, 96% ethanol and 99% acetone for detoxification. The aflatoxins were reduced to 55.1%, 90.9%, 28.08% and 80.0% in Super Sella rice, Super Basmati rice, Brown rice and White rice, respectively. The aflatoxin level was reduced in maize grain, damaged wheat, peanut, figs and dates upto 31.3 %, 64.3 %, 63.6%, 42.7% and 19.8%, respectively. Aflatoxins were detoxified in cereals Dal Chana, Dal Mash, Dal Masoor), turmeric (Haldi) and *Nigela* seeds (Kalwangi) upto 70.5%, 83.0%, 46.2%, 82.09% and 36.9%, respectively. Reduction of aflatoxins was carried out 39.7 %,7.1 % 39.5% 82.0% and 62.0% in red chilli, makhana, corn flakes, desert (Kheer Mix) and pistachio. The significant results (p =0.042) of detoxification of aflatoxins in food items were obtained from present study.

Keywords: pepsin extraction, enzyme activity, stomach mucosa, buffalo

Introduction

Aflatoxins are toxic and carcinogenic metabolites produced by species of *Aspergillus,* especially *Aspergillus flavus* and *Aspergillus parasiticus.* The toxic effects include acute hepatitis, immune-suppression. In humans, the risks associated with aflatoxin consumption are well documented and the International Agency for Research on Cancer (IARC) has designated aflatoxin as a human liver carcinogen. Because of these toxic effects, the Food and Drug Administration regulates the aflatoxin concentration in food with aflatoxin. Commodities or food with aflatoxin exceeding 20 ppb (μg/kg) cannot move in trade (Wogan, 1999). Very little was known about mould metabolites prior to 1961. In that year some alarming reports of a mysterious disease of Turkey poults came from South East of England, tentatively named as Turkey X disease. In 500 such outbreaks about 1, 00,000 poults, mostly between three to six weeks of ages, died. Similarly 5000 partridges and pheasants from one farm and 14000 ducklings from another farm died (Asplin and Carnaghan, 1961). Reports from other places also accumulated on outbreak of acute poisoning of farm animals such as pigs (Loosmore and Harding, 1961) and calves (Loosmore and Markson, 1961). Bioassay test on duckling also helped in furthering and establishing the toxic factor (Sargeant *et al.,* 1961). Aflatoxins producing ability is confined to *A. flavus* and *A. parasiticus.* Strains of these two species are common and wide-spread and have been isolated from a number of different host materials. Colonies of *A. flavus* are green-yellow to yellow-green and that of *A. parasiticus* are dark green. The toxin is produced by mycelium and secreted into the medium or substrate, spores contain very little aflatoxin. Different strains of *A. flavus* produce varying amount of aflatoxin and same strains also produce varying amount of aflatoxins. Aflatoxin production is a genetical process depending on specific nutrient and environmental factors (Patterson, 1973). Investigation carried out by various researchers at Tropical Development and Research Institute, London indicated that *A. flavus* is found in the soil and air throughout the world. Both *A. flavus* and *A. parasiticus* are more prevalent in warmer climate and these moulds can be isolated from stores, dried stuff and tropical soil (Christensen, 1957). Aflatoxins naturally occur in rice (including brown, white, black, red and basmati) of

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different countries, including United State, Canada, Pakistan, India and Thailand (Bansal *et al.,* 2011). Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) also occur in freshly harvested corn grains in different regions of Brazil (Liliana *et al.,* 2009). The presence of aflatoxins in red chillis may be a great threat to the health of populations. Total 183 samples of red chilli were screened out for aflatoxin determination. 48 samples were positive for Aflatoxins B_1 with the range from 1.2 ppb to 968.3 ppb. Aflatoxin B_2 was detected only in 3 samples with the range of 0.3 ppb - 159.8 ppb. Aflatoxin G_1 and G_2 were absent in all chilli samples (Nisa *et al.,* 2012). Chronic poisoning of aflatoxin results in cancer (hepatocellular carcinoma) because liver is the target organ of aflatoxins. Acute intoxication of aflatoxins in human body is also lethal (Milita *et al.,* 2010). Many countries regulate aflatoxin levels in their foods. USA and EU (Europe Union) permit level lower than 20 ppb and Korea and Japan 10 ppb (Chiavaro *et al.,* 2001). Due to its importance different food items were selected for this study and different chemicals were used for detoxification of aflatoxins in these food samples.

Materials and Methods

This study was conducted in Food and Biotechnology Research Centre of PCSIR Laboratories Lahore. The food samples were prepared for aflatoxin analysis (Begum *et al.,* 1985). Aflatoxins were detected by Romers' method (Romer, 1975). Estimation of aflatoxins in toxic extracts was made by comparison with standard technique (AOAC, 2005). In this study, TLC technique was used for the determination of Aflatoxin in all samples.

Nature of samples. Samples of food such as Corn, Wheat, Wheat Flour, White Rice, Brown Rice, Super Basmati, Super Kernal Rice, Red Chilli, Pistachio, Cornflakes, Figs, Haldi, Garam Masala, Peanut, Kalwangi, Makhana and Dates were selected for the present study.

Sample collection. During research work food samples were collected from local market of the city and brought to the Laboratory for quantitative determination and detoxification.

Sampling. Since the aflatoxins are not uniformly distributed in commodities, grains were likely to have pockets of high aflatoxin concentration, firstly due to highly heterogeneous distribution of aflatoxins and secondly due to marketing in lumps of various sizes. To obtain most representative sample, a suitable sampling plan was adopted. These commodities were found stacked in jute bags and stored in house type godown. In order to obtain a more representative portion of these samples, 500 g were collected through a sample probe directly in plastic bags piercing jute bags diagonally from 2 to 3 places. They were passed through sample divider and reduced to approximately 200 g for the purpose of analysis and thus a greater homogeneity of contaminated portion was achieved. Each sample was then thoroughly mixed, ground and made into fine powder for experimental analysis.

Extraction. Extraction procedures and analytical methods vary from one commodity to another because of diverse chemical composition, preventing the development of any one method which could be applied uniformly to all products. However, extraction with chloroform is most suitable method for aflatoxins (B_1, B_2, G_1, G_2, M_1 and M_2).

Then test portion was taken from mixture. 50 g of ground sample was kept into 500 mL conical flask and 25 mL water and 150 mL chloroform was added into flask. Conical flask was shaken on wrist action shaker for 30 min and sample was filtered through filter paper. 50 mL chloroform was taken into beaker and put on steam bath for evaporation.

Chromatographic tank. The dilutions for spotting were got in micro liter. The 25 µL spot of test solution was applied on thin layer chromatography plate with micro syringe. Spot of 5 or 10 µL of aflatoxins (B_1, B_2, G_1, G_2, M_1 and M_2) standard was spotted on same plate as an internal standard. The plate was developed with anhydrous ether in thin layer chromatographic tank upto half then removed and dried. Then plate was redeveloped in the same direction in thin layer chromatographic tank with acetone-chloroform (1:9). Plate was removed and test solution spot was observed for presence or absence of aflatoxins under UV light. If preliminary plate would show that new concentration of test solution required then new concentration were prepared for spotting. Different 1 to 25 µL spots of test solution (3.5, 10.5, 24.9 µL) were spotted on new thin layer chromatographic plate and on the same plate 1 to 25 µL aflatoxins standard was spotted (Braicu *et al.,* 2008).

Detection and Estimation. Fluorescing intensities of sample spots were compared with those of standard aflatoxin spots. In case, fluorescing spot of sample lied between the standard spots, the average value of two standard spots was taken into consideration.

Confirmation. Another very important step in the aflatoxins analysis was the fluorescing sample spots. This was carried out by spraying, evenly the thin layer chromatographic plate with aqueous sulphuric acid (50/50 v/v). After the spraying, thin layer chromatographic plate was allowed to dry and then viewed under UV light (365 nm).

Calculation. Concentration of aflatoxins (µg/kg) present in sample was calculated as follows.

$$\text{Aflatoxins (µg/kg)} = \frac{S \times Y \times V}{Z \times W}$$

Where:

S = volume in µL of aflatoxins standard of equivalent intensity to Z (µL of sample)
Y = concentration of aflatoxins standard in µg/ mL
V = volume in µL of solvents required to dilute final extract
Z = volume in µL of sample extract required to give fluorescence intensity comparable to that of S = µL of aflatoxins standard
W = weight in g of original sample contained in final extract

Treatment for detoxification. Fifty grams of grinded samples in which aflatoxins had been detected were kept in separate 500 mL conical flasks. Chemical solutions of 0.1% HCl, 0.3% HCl, 0.5% HCl, 10% citric acid, 30% citric acid, 05% calcium hydroxide, 0.2 and 0.3% NaOCl, 96% ethanol and 99% acetone were added into different flasks (Table 1). Conical flasks were shaken on wrist action shaker for 2 h and sample was filtered through filter paper and dried for two days.

Quantification after detoxification. Quantification of detoxified sample for aflatoxins was carried out by same method such as chloroform extraction, detection by thin layer chromatography, estimation through UV light and calculation by formula.

Statistical analysis. The statistical significance of the data was analyzed (p=0.042) using pair t-test (Steel *et al*., 1997).

Results and Discussion

Aflatoxins were detoxified by the treatment of different chemical solutions. For this purpose, total 30 samples were collected. These 30 samples were quantified using thin layer chromatography (TLC) for the presence of aflatoxins level in food items (Table 2). The aflatoxins were not found in 10 samples of food product, remaining 20 samples of food in which aflatoxins had been found were treated with chemical solutions.

Chemical solutions 0.1%, 0.3% and 0.5% of hydrochloric acid reduced aflatoxins to 39.7%, 55.1%, 90.9%, 39.5%, 62.0% and 82.0% in food items (Table 4) which are in line with work of Aly and Hathout (2011) who reduced aflatoxins 27.6%, 42.5% and 90% in food with concentrations of hydrochloric acid at different hours. Aflatoxins also reduced to 49.3%, 86.5% and 71.39% with concentration of 0.1%, 0.3% and 0.5% of hydrochloric acid which is same work as Aly and Hathout (2011) who did reduction of food items.

Aflatoxins reduced to 31.3%, 64.3%, 19.8%, 28.08%, 70.5% and 83.05% with treatment of 10% citric acid, 30% citric acid, 1% sodium bisulphate, 2% sodium bisulphate, 0.2% sodium hypochlorite and 0.3% sodium hypochlorite in food items and Aflatoxins reduced to 63.0%, 70.0%, 69.16%, 53.9%, 10.0% and 35.05% with treatment of 10% citric acid, 30 % citric acid, 1% sodium bisulphate, 2% sodium bisulphate 0.2% sodium hypochlorite and 0.3% sodium Hypochlorite in food items which are in line with work of Mukendi *et al.* (1991). They had detoxified

Table 1. Solutions for detoxification of Aflatoxins in food items

Food product	Chemical solution for detoxification
Red chilli	0.1 % HCl
	0.3% HCl
Red chilli	5 % NaOH
Super Sella rice	0.3 % HCl
Super Basmati rice	0.5 % HCl
White rice	5% $Ca(OH)_2$
Maize grain	10 % Citric acid
Wheat damage	30 % Citric acid
Peanut	99 % Acetone
Figs	96 % Ethanol
Dates	1 % Sodium bisulphate
Brown rice	2 % Sodium bisulphate
Makhana	5 % KOH
Dal chana	0.2 % NaOCl
Dal mash	0.3 % NaOCl
Corn flakes	0.1 % HCl
Kheer mix	0.3 % HCl
Pistachio	0.5 % HCl
Haldi	5 % $Ca(OH)_2$
Kalwangi	10 % Citric acid
Dal masoor	99 % Acetone

Table 2. Detection and estimation of Aflatoxins in food products

Sample No.	Food product	Aflatoxin	S	Y	V	Z	W	ppb
1.	Red chilli	B_1	0.5	2.02	0.5 (1000)	4.7	15.04	7.14
2.	Red chilli	B_1	2	2.02	0.51 (1000)	4.9	16.72	25.15
3.	Super Sella rice	B_1	2	2.02	0.51 (1000)	2.9	16.78	42.
4.	Super Basmati rice	B_1	5	2.02	0.99 (1000)	0.9	16.92	656.9
5.	Dal mash	Absent	_	_	_	_	_	_
6.	White rice	B_1	8.5	2.02	0.51 (1000)	24.9	16.70	125.53
7.	Pistachio	Absent	_	_	_	_	_	_
8.	Haldi	Absent	_	_	_	_	_	_
9.	Maize grain	G_1	0.5	2.03	0.51 (1000)	0.9	16.65	34.48
10.	Wheat damage	B_1	0.9	2.02	1470	0.9	16.67	174.32
11.	Peanut	B_1	8.5	2.02	0.51 (1000)	4.9	16.70	21.08
12.	Figs	B_1	1.0	2.02	0.50 (1000)	1.0	16.77	14.9
13.	Dates	B_1	0.5	2.02	1980	3.9	16.68	30.76
14.	Brown rice	B_1	2	2.02	0.51 (1000)	1.0	16.76	123.58
15.	Makhana	B_1	0.5	2.02	0.51 (1000)	14.9	16.73	2.09
16.	Dal chana	B_1	0.5	2.02	0.99 (1000)	14.9	16.72	8.02
17.	Figs	Absent	_	_	_	_	_	_
18.	Peanut	Absent	_	_	_	_	_	_
19.	Dal mash	B_1	0.5	2.02	0.5 (1000)	4.7	15.04	7.14
20.	Corn flakes	B_1	1.0	2.02	1 (1000)	9.5	16.27	13.0
21.	White rice	Absent	_	_	_	_	_	_
22.	Kheer mix	B_1	0.9	2.02	0.51 (1000)	4.9	16.70	11.32
23.	Pistachio	B_1	1.0	2.02	3.0 (1000)	2.9	16.51	167.17
24.	Haldi	B_1	1	2.02	1980	2.9	16.68	82.68
25.	Peanut	Absent	_	_	_	_	_	_
26.	Brown rice	Absent	_	_	_	_	_	_
27.	Kalwangi	B_1	4.0	2.02	0.51 (1000)	5.0	16.72	49.2
28.	Super Sella rice	Absent	_	_	_	_	_	_
29.	Super Basmati rice	Absent	_	_	_	_	_	_
30.	Dal masoor	B_1	2.0	2.02	0.91 (1000)	2.9	16.67	76.05

aflatoxins by comparing chemicals citric acid, sodium bisulphate, sodium hypochlorite (Table 3 and Table 4).

The present study showed significant detoxification in aflatoxins ($p = 0.042$) of food items when pair T-test was applied to quantified aflatoxins of food items before and after detoxification. It means that aflatoxins had been reduced statistically when aflatoxins were compared before and after detoxification.

Present study also showed that thin layer chromatography is a reliable method for detection and quantification of aflatoxins in food items before and after detoxification. (Okwu *et al.,* 2010, Olufunmilayo and Oyefolu, 2010).

Table 3. Detection and estimation in Aflatoxins in food products after detoxification

Sample No.	Food product	Aflatoxin	S	Y	V	Z	W	ppb
1.	Red chilli	B_1	0.9	2.02	0.99 (1000)	24.9	16.67	4.3
2.	Red chilli	B_1	0.9	2.02	0.51 (1000)	4.9	16.71	11.34
3.	Super Sella rice	B_1	0.5	2.02	0.91 (1000)	2.9	16.67	19.01
4.	Super Basmati rice	B_1	2.5	2.02	1975 (1000)	2.0	16.70	59.68
6.	White rice	B_1	2.0	2.02	0.51	4.9	16.72	25.51
9.	Maize grain	G_1	9.5	2.03	0.51 (1000)	24.9	16.71	23.67
10.	Wheat damage	B_1	1.0	2.02	0.515 (1000)	1.0	16.69	62.31
11.	Peanut	B_1	1.5	2.02	0.99 (1000)	24.9	16.77	7.2
12.	Figs	B_2	0.5	0.5	0.48 (1000)	0.9	16.56	8.54
13.	Dates	B_1	2	2.02	0.50 (1000)	4.9	16.72	24.66
14.	Brown rice	B_1	8.5	2.02	0.51 (1000)	5.9	16.71	88.87
15.	Makhana	B_1	0.5	2.02	0.51 (1000)	5.9	16.72	1.94
16.	Dal chana	B_1	1	2.02	487	24.9	16.68	2.36
19.	Dal mash	B_1	0.5	2.02	0.50 (1000)	24.9	16.69	1.21
20.	Corn flakes	B_1	0.5	2.02	0.51 (1000)	3.9	16.69	7.9
22.	Kheer mix	B_1	0.9	2.02	0.99 (1000)	24.9	16.77	4.3
23.	Pistachio	B_1	0.5	2.02	1980	3.9	16.68	30.7
24.	Haldi	B_1	6.0	2.02	0.51 (1000)	24.9	16.69	14.8
27.	Kalwangi	B_1	7.5	2.02	0.51 (1000)	14.9	16.72	31.0
30.	Dal masoor	B_1	3.0	2.02	0.50 (1000)	4.7	15.90	40.9

Table 4. Comparison of Aflatoxins estimation of food products before and after detoxification

Sample No.	Food product	Aflatoxin	Estimation before detoxification (ppb)	Chemical solution for detoxification	ppb after detoxification	Reduction in %age
1.	Red chilli	B_1	7.14	0.1 % HCl	4.3	39.7
2.	Red chilli	B_1	25.15	5% NaOH	11.34	54.9
3.	Super Sella rice	B_1	42.34	0.3% HCl	19.01	55.1
4.	Super Basmati rice	B_1	656.9	0.5% HCl	59.68	90.9
6.	White rice	B_1	125.53	5% Ca(OH)	25.51	80.0
9.	Maize grain	G_1	34.48	10% Citric acid	23.67	31.3
10.	Wheat damage	B_1	174.32	30% Citric acid	62.31	64.3
11.	Peanut	B_1	21.08	99% Acetone	7.2	63.67
12.	Figs	B_2	14.9	96% Ethanol	8.54	42.7
13.	Dates	B_1	30.76	1% Sodium bisulphate	24.66	19.8
14.	Brown rice	B_1	123.58	2 % Sodium bisulphate	88.87	28.08
15.	Makhana	B_1	2.09	5% KOH	1.94	7.1
16.	Dal chana	B_1	8.02	0.2% NaOCl	2.36	70.5
19.	Dal mash	B_1	7.14	0.3% NaOCl	1.21	83.05
20.	Corn flakes	B_1	13.0	0.1% HCl	7.9	39.5
22.	Kheer mix	B_1	11.32	0.3% HCl	4.3	62.0
23.	Pistachio	B_1	167.17	0.5% HCl	30.7	82.0
24.	Haldi	B_1	82.68	50% $Ca(OH)_2$	14.8	82.09
27.	Kalwangi	B_1	49.2	10% Citric acid	31.0	36.9
30.	Dal masoor	B_1	76.05	99% Acetone	40.9	46.2

Ultra-violet (UV) light, a standard procedure was used to differentiate the toxin from non-toxin forms of *A. spergillus* species.

Conclusion

It was concluded that significant detoxification i.e. 90.9% was observed when 0.5% HCl was used as detoxifying agent for aflatoxin B_1 in Super Basmati rice. Similarly, 83.05% detoxification of Aflatoxin B_1 was observed in Dal Mash, 82.09% in Haldi, 82% in Pistachio and 80% in White rice when 0.3% NaOCl, 50% $Ca(OH)_2$, 0.5% HCl and 5% $Ca(OH)_2$ were used, respectively.

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