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Research Article

INCIDENCE OF AFLATOXIN CONTAMINATION AND ASSESSMENT OF PHYSICO-CHEMICAL PARAMETERS IN BREAKFAST CEREALS

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ABSTRACT
The best protection against Aflatoxins is monitoring their presence in feeds and foods. The aim of this work was to determine the level of Total Aflatoxins levels in samples of breakfast cereals. In breakfast cereal grains are Raw rice, Parboiled rice, Maize, Wheat, Ragi, Oats and cereal products are Atta, corn flakes, Maida, Macaroni, Noodles, Oat flakes, Rava, Riceflour, Vermicelli with regards to Maximum Permissible Limits in USFDA, European Union and Indian standards. Market samples (90 nos) of breakfast cereals were analyzed to determine the quantity of Aflatoxin contamination. The estimation of total Aflatoxin (B_1, B_2, G_1 and G_2) was done by adopting a analytical technique employing Thin Layer Chromatography (TLC) for screening and High Performance Thin Layer Chromatography (HPTLC) system for quantification. Among the total Aflatoxin, the Aflatoxin B_1 were dominant followed by Aflatoxin B_2, G_1, G_2. In breakfast cereals the tested samples showed 31.1% contamination with Aflatoxin above their respective Maximum Permissible Limit.

Key Words: *Aflatoxin, Breakfast Cereals, and Contamination Assessment*

INTRODUCTION
National and international institutions and organizations, such as the European Commission (EC), the US Food and Drug Administration (FDA), the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), have recognized the potential health risks to animals and humans posed by food- and feed-borne mycotoxin intoxication and the economic consequences of mycotoxin contamination were well demonstrated. Approximately 25-40% raw agricultural products worldwide are susceptible to invasion by the aflatoxigenic *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin has been found as contaminants in agricultural and food products especially in cereals and cereal products (Smela *et al.,* 2002), (Rawal *et al.,* 2010). They are hepatogenic, cause pulmonary interstitial fibrosis (Desai and Ghosh, 2003), liver cirrhosis, depressed immune response, tumour induction and teratogenesis (Thanaboripat *et al.,* 2004). Aflatoxin B1, the most potent one is metabolized into a variety of hydroxylated derivatives (Aflatoxin B_1, M_1, B_2) which are less toxic than the parent compound, although their presence in food is still a threat to human health. The aim of this investigation was to show frequency of appearance of Aflatoxins at cereals and to identify level of contamination of cereals and some of their products with these fungus metabolites- aflatoxins.

MATERIALS AND METHODS
36 samples of cereal grains and 54 samples of cereal products were randomly collected from the supermarkets and traditional bazaars of Chennai, Tamil Nadu. 6 samples are taken from each cereal grains and cereal products. As per AOAC Romer's all purpose method (1990) by HPTLC and were quantified with reference standards. The samples were first screened by TLC and then Quantified by HPTLC.

Aflatoxins are extracted from samples and the extract was dissolved in choloroform and used for TLC and HPTLC spotting. The dissolved residue was then spotted on to a silica gel plate of about 0.5mm thickness as 5 µl drops. The standard solution of Aflatoxin was also spotted on to the same plate as drops of 1, 3, 5 µl. The plate was developed in chloroform- acetone (1:9). After development, the plate was air-dried and observed under UV light. The fluorescence intensities of Aflatoxin spots of sample were compared with

those standard spots. The sample spot, which matches one of the standard spots, was selected. Standards were also used to compare the colour and Rf value of unknown sample streak on the plate. The amount of Aflatoxin was estimated.

Silica gel HPTLC plates in the format of 10×10 cm or 20×10 cm are used. The dried samples are applied as bands (spray-on technique) using Linomat-5 sample applicator. Prepared 9:1 ratio of Chloroform and Acetone and poured in twin-trough chamber (TTC) for development of plates. The spotted samples are developed in presaturated TTC up to 80mm from lower edge of plate. Transfer of reagent for derivatization of samples on a HPTLC plate may be accomplished by spraying.. The developed plates are dried by using dryer and sprayed with 20% H_2SO_4. After spraying the plates are dried. Finally the plates are scanned in CAMAG HPTLC Scanner-3 under 366nm wavelength to determine the levels of Aflatoxin contamination in the samples.

RESULTS AND DISCUSSION

Colour, odour, texture, insect infestation, presence of stone and fibre of cereal grains, cereal flours and cereal products are manually graded and presented in Table1a, 1b and 1c. Bulk Density for cereal grains are presented in Table.2. In cereal grains out of 24 samples 4 samples are found to be within the stipulated Standard. Moisture content of the cereal grains (24 Nos) are presented in Table. 3. Moisture content of all samples is found to be within the Standard level. Moisture content of the cereal products (54 Nos) are presented in Table.4. The moisture content of cereal products are found to be within the prescribed standard level.

Table 1a: Physicochemical Parameters for Cereal grains

Parameters	Colour (%)		Odour (%)		Texture (%)		Insect (%)		Stone (%)		Fibre (%)	
	G	B	G	M	G	B	P	A	P	A	P	A
Raw Rice	83.3	16.6	66.6	33.3	100	0	0	100	50	50	50	50
Parboiled Rice	33.3	66.6	33.3	66.6	83.6	16.6	0	100	50	50	66.6	33.3
Wheat	100	0	83.3	16.6	83.3	16.6	16.6	83.3	33.3	66.6	66.6	33.3
Ragi	100	0	100	0	100	0	0	100	33.3	66.6	83.3	16.6

G–Good B–Bad M–Musty odour P–Present A-Absent

Table 1b: Physicochemical Parameters for Cereal flours

Parameters	Colour (%)		Odour (%)		Lumps (%)	
	G	B	G	M	P	A
Atta	100	0	33.3	66.6	33.3	66.6
Maida	100	0	100	0	16.6	83.3
Rice flour	50	50	100	0	33.3	66.6

G–Good B–Bad M–Musty odour P–Present A-Absent

Table 1c: Physicochemical Parameters for Cereal Products

Parameters	Colour (%)		Odour (%)		Texture (%)	
	G	B	G	B	G	B
Rava	100	0	100	0	100	0
Noodles	66.6	33.3	83.3	16.6	100	0
Macroni	100	0	100	0	100	0
Vermicelli	83.3	16.6	100	0	83.3	16.6
Corn Flakes	100	0	100	0	83.3	16.6
Oat Flakes	100	0	100	0	100	0

G–Good B–Bad

Table 2: Bulk Density for Cereal Grains

Cereal Grains	Bulk density (Mean±SE, n=6)	Range
Raw Rice	0.789±0.016	0.75-0.848
Parboiled rice	0.909±0.017	0.755-0.863
Wheat	0.742±0.022	0.651-0.807
Ragi	0.780±0.027	0.707-0.889

Table 3: Moisture Content for Cereal Grains

Sample	Moisture (Mean±SE, n=6)	Range
Raw rice	12.967 ±0.244	12.2-13.8
Parboiled Rice	13.133±0.184	12.4-13.6
Wheat	13.583±0.180	12.8-13.9
Ragi	10.200±0.213	9.8-11

Table 4: Moisture Content for Cereal Products

Sample	Moisture(Mean±SE, n=6)	Range
Atta	12.198±0.190	12.2-13
Maida	12.000±0.288	11.1-13
Noodles	11.142±0.238	10.25-11.75
Rava	12.017±0.370	10.5-13
Vermicelli	8.100±0.351	7.5-8.5
Rice Flour	11.350±0.232	10.6 - 12
Corn Flakes	4.067±0.102	3.8 – 4.5
Oat Flakes	11.050±0.333	10.1 - 12
Macaroni	11.900±0.309	10.6 -12.6

The recovery percentage for Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2 are 90%, 88%, 80% and 65% respectively. The levels of Aflatoxin contamination in breakfast cereals are showed in Figure 1 . 90 samples of breakfast cereals where collected and analyzed for Aflatoxin contamination. Out of 90 samples 28 samples (31.1%) were found to be Aflatoxin contaminated.

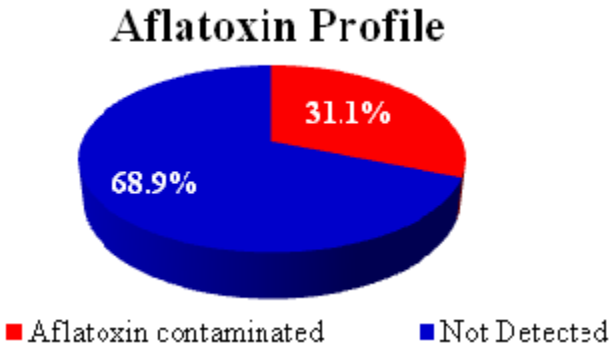
Out of 90 samples, 54 samples are cereal products and 36 samples are cereal grains. The results are presented in Table 5.

Table 5 Aflatoxin contamination in breakfast cereals

Type of Sample	Number of Samples	Contaminated sample	% of Contaminated Samples
Cereal Grains (Raw rice, Parboiled rice, Wheat & Ragi)	36	23	63.8
Cereal Products (Atta, Maida, Rice flour, Rava, Noodles, Macroni, Vermicelli, Cornflakes & Oatflakes)	54	5	9.26
Total	90	28	31.1

In cereal grains the percentage of total Aflatoxin contamination is higher than the cereal products. This could be due to improper post harvest technology and storage condition. Contamination can occur at any stage of food production from pre-harvest to storage (Wilson and Payne, 1994). Factors that affect aflatoxin contamination include the climate of the region, the genotype of the crop planted, soil type, minimum and maximum daily temperatures, and daily net evaporation (Wilson and Payne, 1994), (Ono and Sugiura, 1999) (Fandohan and Gnonlonfin, 2005). Aflatoxin contamination is also promoted by stress or damage to the crop due to drought prior to harvest, insect activity, poor timing of harvest, heavy rains

at harvest and post-harvest, and inadequate drying of the crop before storage (Hell and Cardwell, 2000), (Hawkins and Windham, 2005). Humidity, temperature, and aeration during drying and storage are also important factors.



The levels of Aflatoxin detected in cereal grains are shown in Table 6

Table 6: Total Aflatoxin in cereal grains (Mean±SE, n=6)

Sample type	Aflatoxin B_1 (µg/kg)	Aflatoxin B_2 (µg/kg)	Aflatoxin G_1 (µg/kg)	Aflatoxin G_2 (µg/kg)
Raw rice	4.02±1.74	-	-	-
Parboiled rice	6.81±1.45	-	-	--
Wheat	-	-	-	-
Ragi	-	-	-	-
Oats	10.00±1.29	5.16±0.16	-	-
Maize	32.5±4.78	6.5±0.84	5.00±0.25	5.00±0.00

- *Not Detected.*

Regulatory authorities in different countries have set the tolerance limits for aflatoxins that range from 0 to 50 µg/kg for controlling their levels in the food (FAO, 2004). In India, a tolerance limit of 30 µg/kg has been prescribed under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011, for all foods meant for human consumption (FSSA, 2011). The European Union (EU) has established with the Commission Regulation No. 1881/2006 severe limits for major mycotoxin classes in many products at high risk of contamination. As regards the Aflatoxins, the maximum levels (MLs) set by the EC in food for direct human consumption are 2 µg kg^{-1} for Aflatoxin B_1 and 4 µg kg^{-1} for the sum of Aflatoxins. The levels of Aflatoxin detected in cereal products are shown in Table 7.

Table 7: Total Aflatoxin in cereal products (Mean±SE, n=6)

Sample type	Aflatoxin B_1 (µg/kg)	Aflatoxin B_2 (µg/kg)	Aflatoxin G_1 (µg/kg)	Aflatoxin G_2 (µg/kg)
Atta	-	-	-	-
Maida	-	-	-	-
Noodles	-	-	-	-
Rava	-	-	-	-
Vermicelli	3.33±3.33	-	-	-
Rice Flour	-	-	-	-
Corn Flakes	1.66±1.054	1.66±1.054	-	-
Oat Flakes	6.66±4.944	4.166±1.054	1.66±1.054	1.66±1.054
Macaroni	-	-	-	-

- Not Detected.

In raw rice and parboiled rice the aflatoxins B_2, G_1 and G_2 are absent. The raw rice samples Aflatoxin B_1 was below the Indian & USFDA standards and above the EU standards. Ragi and wheat samples are free from total Aflatoxin. In oat samples the Aflatoxin G_1 & G_2 are absent but the presence of Aflatoxin B1 and total Aflatoxin were below the Indian standards and above the EU & USFDA standards. In maize the Aflatoxin B_1 & total Aflatoxin were above the Indian, EU & USFDA standards (Figure 2 & 3).

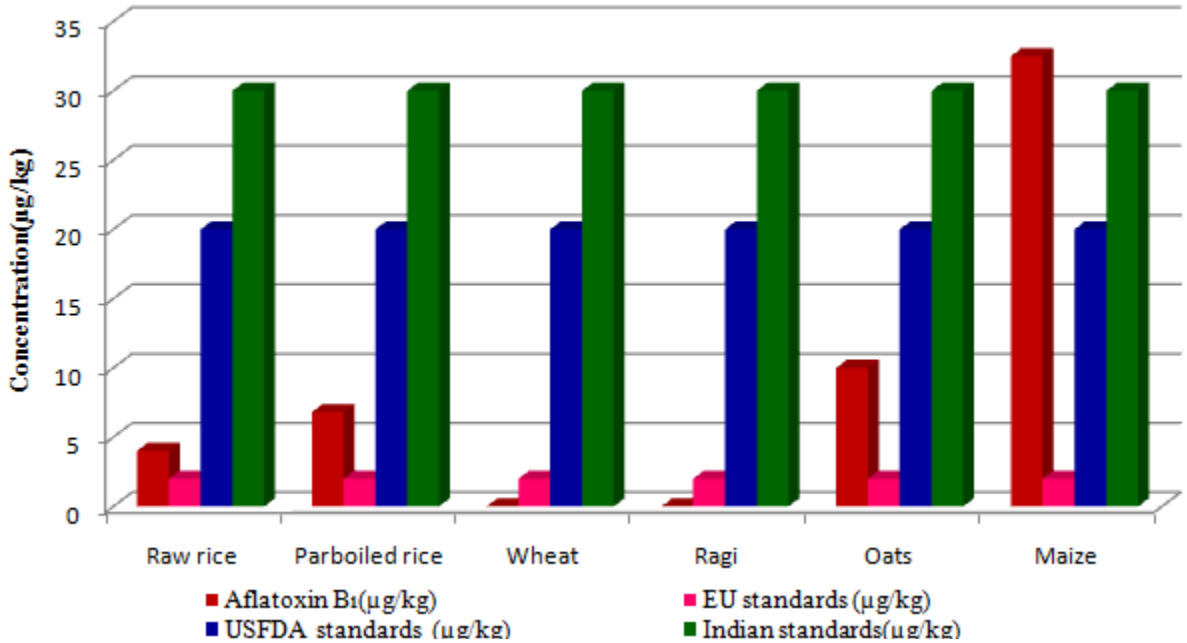


Figure2: Detection of Aflatoxin B1 in Cereal grains Vs Regulatory standards

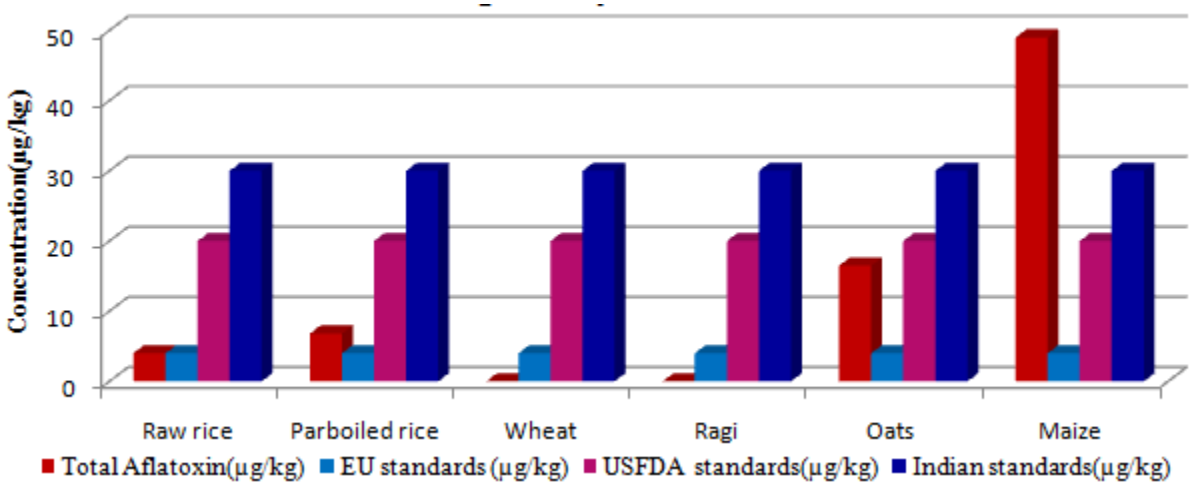


Figure 3: Assessment of Total Aflatoxin in Cereal grains compared with Regulatary Standards

In cereal products Atta, Maida, Macaroni, Noodles, Rava, Rice flour are free from total Aflatoxin contamination. In oat flakes out of 6 samples 2 samples are contaminated with total Aflatoxin and above the EU, Indian and USFDA Standards. Out of 6 samples in 1 sample, Aflatoxin B_1 was below the Indian and USFDA Standards. In corn flakes out of 6 samples 2 samples are contaminated with Aflatoxin B_1 & B_2 and below the Indian & USFDA Standards and above the EU Standards. In vermicelli out of 6 samples

1 sample is contaminated with Aflatoxin B_1 and above the Indian Standards, below the EU and USFDA Standards (Figure 4 & 5).

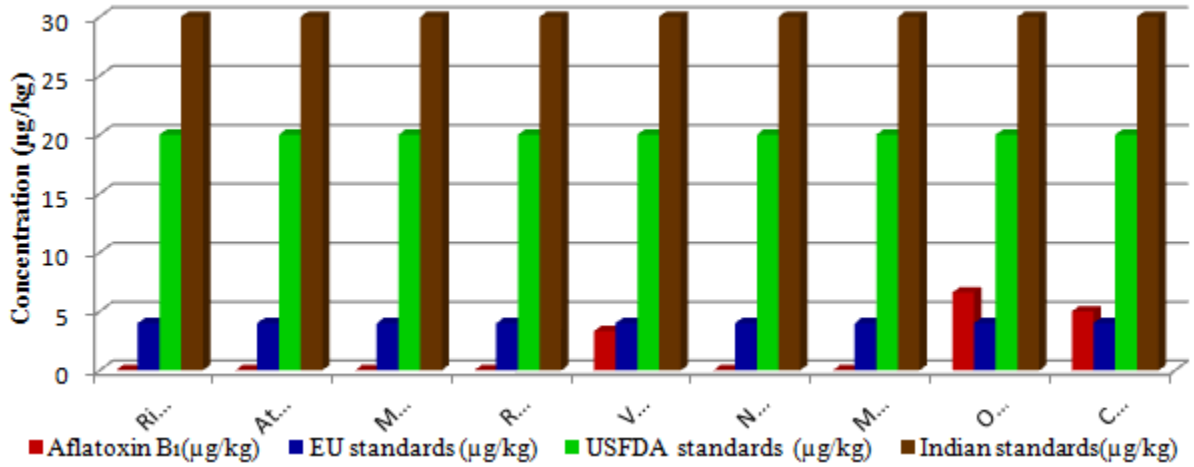


Figure 4: Level of Aflatoxin B1 in Cereal products Compared with Regulatory Standards

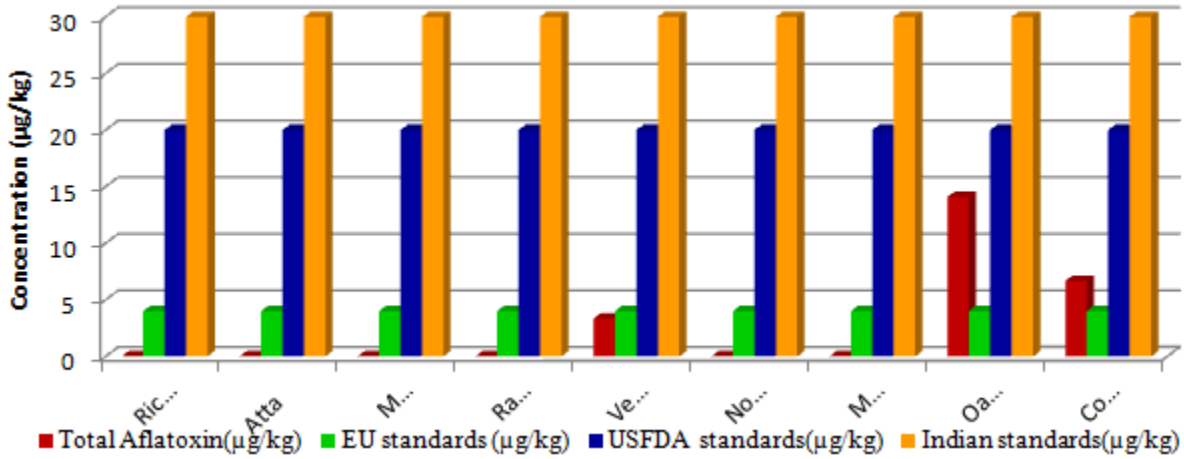


Figure 5: Assessement of Total Aflatoxin in Cereal products Vs Regulatory Standards

In the present study total Aflatoxin in breakfast cereal and its products were analyzed. Among the total Aflatoxins, the Aflatoxin B_1 were dominant followed by Aflatoxin B_2, G_1 and G_2. In breakfast cereals the tested samples showed 31.1% contamination with Aflatoxin above their respective MPL values. In cereal grains the 63.8% of total Aflatoxin contamination is higher than the cereal products (9.26%) which might be due to improper post harvest technology and storage condition. The study warrants for the further improvement of Good Agricultural Practice (GAP) and the need for accredited laboratory as per regulatory norms to assess the Aflatoxin contamination. The present study necessitates the periodical monitoring of post harvest surveillance of mycotoxin in processed foods, studies in compliance with regulatory norms.

CONCLUSION

The results of the present study demands strengthening and further enhancement of post harvest technology in crops by implementing Hazard Analysis Critical Control Point (HACCP) 'from form to fork' to provide quality and safe food for enhancing food safety and global security. To attain this, quality control and food safety in food production areas are necessary.

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