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Mycotoxin Detection in Food Products Based on Wheat Fractions

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Abstract

Study was undertaken to detect the presence of aflatoxin B_1(AFB_1) in commonly consumed wheat fractions i.e. semolina, porridge and refined wheat flour. Forty pooled samples of wheat fractions were collected from various localities of Ludhiana city. Raw samples of wheat fractions were qualitatively and quantitatively analyzed for AFB_1, contamination by PMC and TLC methods. Out of 40 samples, 28 (70%) were found to be contaminated with AFB_1. Positive screened wheat fractions were used for preparing *idli, upma, porridge, kulcha* and *bhatura* using fermentation-steaming, shallow frying, microwave, baking and deep frying cooking methods. Cooked products were again analyzed for AFB_1. Maximum reduction of AFB_1 was shown by microwave cooking of porridge at 150°C for 10 min., with mean destruction of 76.1% while deep frying (280°C for 30 sec) of *bhatura* and shallow frying (120°C for 3 min) of *upma* showed minimum destruction of 43.2 and 40.8 % respectively.

Keywords: Wheat products; Aflatoxin B_1; Detoxification; Baking; Microwave cooking.

Mycotoxins are compounds that are produced by fungi and cause illness or even death when food and feed containing them are consumed.[1] The mycotoxigenic fungi involved in the human food chain belong mainly to three genera: *Aspergillus, Fusarium* and *Penicillium*. While *Fusariuim* sp. are commonly destructive plant pathogens producing mycotoxins before or immediately after post- harvesting, *Penicillium* and *Aspergillus* sp. are more common contaminants of commodities and foods during drying and subsequent storage . [2]Of more than hundred toxic fungal metabolites isolated and identified so far, aflatoxins are unique in having high toxicity and carcinogenicity as well as being resistant to degradation under normal food processing conditions. Diet is the major route through which humans as well as animals are, exposed to aflatoxins. Exposure to aflatoxins in diet is considered an important risk factor for the development of primary hepatocellular carcinoma, particularly in individuals already exposed to hepatitis B.[3] Aflatoxins are also implicated with Indian childhood cirrhosis, Reye's Syndrome and Kwashiorkor .[4] The International Agency for Research on Cancer confirmed aflatoxins as a potential carcinogens .[5]

Cereals form the major bulk of all Indian diets. They are the major source of many nutrients .[6]Of all cereals, wheat occupies a unique position. Wheat (*Triticum aestivum*) has been shown to be naturally contaminated with different levels of aflatoxins during storage .[7] It was observed that level of aflatoxin contamination in grain sample was related with level of sanitation of mill storage places. A similar view had also been expressed by Kalyansundaram and Jayaraman 1996.[8] Wheat is milled to form semolina (suji/rava), porridge (dalia) and refined wheat flour (maida). Semolina is a coarsely ground endosperm and its chemical composition is similar to that of refined wheat flour. In India, it is also used in the preparation of large number of savoury and sweet preparations. Porridge is considered as breakfast cereal. It is

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made from wheat grain and requires cooking before serving. Scanty information is available regarding the presence of aflatoxins in commonly consumed food based on wheat fractions (i.e.semolina, porridge and refined wheat flour). Hence, the present study was undertaken to see the prevalence of aflatoxins in wheat fractions and further the effect of domestic processing on extent of detoxification of aflatoxins in cooked products.

Materials and Methods

A two stage sampling technique was used for selection of samples of wheat fractions i.e. semolina, porridge and refined wheat flour. First stage consisted of convenient sampling of 4 localities namely Ghumar Mandi, Chaura Bazaar, Khud Mahalla and Jawahar Camp. Second stage consisted of random selection of 40 samples (each weighing 1 kg/lot) of semolina, porridge and refined wheat flour. Samples were cleaned and stored in air tight containers at room temperature (37°C) and were further analyzed for aflatoxin B_1(AFB_1) using pressure mini column (PMC)[9] technique and thin layer chromatography (TLC) method. Positively screened wheat fraction samples were used for preparing *idli, upma, porridge, kulcha* and *bhatura* using various ingredients and processing methods given in Table 1.

Developed products were dried at 60 ± 2°C, ground to powder and analyzed for AFB_1 to see the impact of cooking methods on AFB_1 destruction. Extraction for quantitative estimation of aflatoxins in raw and cooked samples was done by the method of Romer (1975).[10] Spotting of extracts was done on TLC plates along with standards of AFB_1

Table 1 Ingredients and methods used for preparation of germinated supplementary products

Product	Description of Recipe	Ingredients	Amount	Temperature and time	Cooking method
Idli	Mix semolina with curd and salt to taste. Mixture was made to pouring consistency by using water. Mixture was kept in a hot place for 2 hour S for fermentation. Then adding half teaspoon of eno salt, mixture was put in idli pan and steamed for 12-15 minutes.	Semolina Curd Water Eno salt	60g 25g 50ml ½ tsp	30° C, 2 h 120° C, 20 min	Fermentation Steaming without pressure
Upma	Semolina was shallow 'fried for 3 minutes with 1 tbsp. Of oil till it turned brown in colour. Then water was added and brought to boil. Seasoning was added and cooked till mass was dried.	Semolina Oil Water	60g 15g 180ml	120° C, 3 min	Shallow frying
Porridge	Broken wheat with water was microwave cooked for 10 minutes at 150°C	Broken wheat Water	60g 360ml	150° C, 10 min	Microwave cooking
Kulcha	Refined wheat flour was mixed with 3% yeast, 0.8% salt, 5% each sugar and butter and 56% water in the mixing bowl for 8 min. to prep. are dough. The dough was divided into three portions and kept for proofing for 20 min. at 30°C. After 20 min. pined out the proofed balls. Balls were kept in greased baking tray and proofed them for ± 20 min. Kulchas were baked at 240°C. for 10 min.	Refined wheat flour Compressed yeast Salt Sugar Butter Water	125g 3.75g 1g 6.25g 6.25g 70ml	30° C, 20 min 240° C, 10 min	Fermentation Baking
Bhatura	Refined wheat flour was mixed' with 3% yeast, 0.8% salt, 5% each sugar and butter and 56% water in the mixing bowl for 8 min. to prepare dough. The dough was divided into three portions and kept for proofing for 20 min. at 30°C. After 20 min. each portion was rolled into rounds of 8 inches diameter and deep fried in oil for 30 seconds at 280°C.	Refined wheat flour Compressed yeast Salt Sugar Butter Water	125g 3.75g 1g 6.25g 6.25g 70ml	30° C, 20 min 280° C, 10 min	Fermentation Deep frying

procured from Sigma Chemical Co. USA. Quantization of $AFB_1$was done by method of Coker *et al* (1984). [11]Aflatoxin levels in raw and cooked samples were detected using the following equation:

Where

S is the volume in µl of mycotoxin standard of equal intensity to Z µl of sample, Y is

$$\text{Aflatoxin (ug/kg)} = \frac{SxYxV}{W \times Z}$$

concentration of mycotoxin standard in µg/ml, Z is volume in µl of sample extract required to give fluorescence intensity comparable to that of 'S' µl of mycotoxin standards, V is volume (µl) of solvent required to dilute final extract and W is weight (g) of original sample contained in final extract.

Results and Discussion

Among the total 40 samples of wheat fractions, 28 samples (70%) were found to be contaminated with AFB_1 though there was no visual mould in any of the positive samples. AFB_1 contamination was observed at the rate of 39, 32 and 29%, respectively in semolina, porridge and refined wheat flour fractions. The results of Table 2 are in confirmation with findings of Breckenridge *et al* (1986)[12] who reported aflatoxin contamination in commonly consumed cereals like rice and wheat above the permissible limit of 20 ppb (FDA) and 30 ppb prescribed by PFA Act (1954).[13] Wheat has been shown to be naturally contaminated with different levels of aflatoxins during storage .[7]

Table 2: Screening of raw wheat fractions for aflatoxin B_1 (N=40)

Type of wheat fraction	n	No. of positive samples	Level of contamination
Semolina	13	11(39)	++++(4)
			++(3)
			+(4)
Porridge	14	9(32)	++++(3)
			+++(1)
			++(2)
			+(3)
Refined wheat	13	8(29)	++++(4)
flour			++(3)
			+(1)
		28(70)	

N : Total no. of samples, n : No. of samples of each fraction
Figure in parentheses [ ] represents % of positive samples
Figure in parentheses ( ) represents number of positive samples

\+ 26-115 µg/kg ++ 116-205 µg/kg
+++ 206-295 µg/kg ++++ 296-385 µg/kg

Semolina samples showed maximum incidence of contamination with moisture content ranging from 12.2-14.3 % with mean value of 13.1 %. The porridge samples had mean moisture content of 9.2% with range of 8.1-10.7% while the refined wheat flour samples had mean moisture content of 12.9% and ranged from 12.2-14.6%. The recommended safe moisture level for storage of wheat flour is 10% .[14] It was also reported that the most important factor in growth of AFB_1 production by *Aspergillus flavus* was the moisture and relative humidity surrounding the substrate. The safe moisture content of various food commodities at which fungus may not grow varies from one commodity to other therefore any increase in moisture content above the safe level for a particular commodity may support the growth of fungus. In the present investigation samples having moisture more than recommended safe level exhibited the presence of aflatoxin. Findings are also supported by Kumar *et al* 2002[15] who detected that 20% wheat flour samples above safe limit were positive to AFB_1

Out of 20 positively screened samples as semolina, porridge and refined wheat flour with 2+, 3+ and 4+ level of contamination, 12 samples were randomly selected for AFB_1 detection with both TLC and PMC methods (Table 3). Mean AFB_1 levels in semolina, porridge and refined wheat flour were 267.8+47.7 µg/kg, 272.6+30.9 µg/kg and 283.6+61.3 µg/kg, respectively, detected by TLC method whereas the corresponding mean value of AFB_1 in semolina, porridge and refined wheat flour were 212.5+37.5 kg/kg, 225+22.4 µg/kg and 225.0+35.4 µg/kg as detected by PMC method.

Results of effect of processing on AFB_1 destruction (Table 4) revealed that

Table 3: TLC and PMC method used for aflatoxin B_1 (AFB_1) detection in raw samples

Type of wheat fraction	Sample no.	TLC, µg/kg	PMC, µg/kg±25	Per cent variation
Semolina	1	340.4	275	19.2
	3	357.9	275	23.2
	10	204.7	175	14.5
	12	168.0	125	25.6
	Mean ±S.E.	267.8 ± 47.7	212.5 ± 37.5	20.6 ±2.43
	t value	0.91^{NS}		
Porridge	2	198.9	175	12.0
	4	321.7	275	14.5
	7	311.3	225	27.7
	8	196.0	175	10.7
	11	335.0	275	17.9
	Mean ±S.E.	272.6 ± 30.9	225 ± 22.4	16.6 ± 3.0
	t value	1.24^{NS}		
Refined wheat flour	5	343.6	275	20.0
	6	160.9	125	22.3
	9	346.3	275	20.6
	Mean ±S.E.	283.6 ± 61.3	225 ± 35.4	21.0 ± 0.7
	t value	0.83^{NS}		

NS: Non significant

Table 4: Effect of cooking methods on destruction (%) of AFB_1 in cooked .products

Name of product	Cooking method	Initial AFB_1 in raw sample, µg/kg	Residual AFB_1 in cooked sample, µg/kg	% destruction
Idli	Fermentation-Steaming	267.8	95.7	64.2
Upma	Shallow frying	267.8	171.1	40.8
Porridge	Microwave	272.6	61.8	76.1
Kulcha	Fermentation and Baking	283.6	84.4	65.3
Bhatura	Fermentation and Deep frying	283.6	159.4	43.2
t value 1-2	1.98^{NS}	2-4	0.23^{NS}	
1-3	3.12*	2-5	1.98^{NS}	
1-4	2.11**	3-4	4.21*	
1-5	0.64^{NS}	3-5	2.54*	
2-3	3.45*	4-5	2.22**	

*Significant at 5% level
** Significant at 10% level
NS Non-significant

Fermentation-steaming resulted in 64.2% destruction of AFB_1 in *idli* when fermented at 30°C for 2 h and then microwave cooking of porridge at 150°C for 10 min has significant impact on the AFB_1 destruction. The results indicated that baking of *kulchas* at 240°C for 10 min resulted in 65.3% destruction of AFB_1. The mean residual AFB_1 in cooked sample was 84.4±11.9 µg/kg. Saritha and Uma Reddy (1998)[16] showed 80% destruction of AFB_1 in bread made from refined wheat flour. The deep frying of *bhaturas* at 280°C for 30 sec resulted in 43.2% destruction of AFB_1. The mean residual AFB_1 in cooked sample was 159.4±31.7 µg/kg. Deep frying and shallow frying showed 43.2 and 40.8% destruction of

AFB_1 respectively, which was minimum as compared to other methods. Thus the residual level of AFB_1 in cooked products in *idli, upma, porridge, kulcha* and *bhatura* was found to be 5-8 times higher in comparison to permissible limit of 30 µg/kg (PFA 1954),[13] thus indicating that there is certain degree of exposure of population to the carcinogenic aflatoxin. Despite the fact that appreciable amounts of aflatoxins are lost during the process of heating, considerable quantities still remain to cause potential harmful effects on human health. So the present investigation warrants that cereal and cereal products should be tested for the presence of aflatoxins periodically.

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