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Reduction of Aflatoxin B_1 Contamination in Pakistani Wheat Varieties by Physical Methods

Arshad Hussain*[a], Ghosia Lutfullah[b] and Shafqatullah[a]

[a]Food Technology Centre, PCSIR Laboratories Complex, Jamrud Road, Peshawar-25120, Pakistan
[b]Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

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Abstract. In the study of effect of physical treatments, such as washing and heating, on the AFB_1 contaminated wheat varieties, it was observed that the reduction of AFB_1 was directly proportional to washing time in all the varieties. The concentration of AFB_1 was reduced more by heating than washing. The level of AFB_1 in dried wheat decreased to more than 50% and 90% by heating in oven at 150 and 200 °C, respectively. However, the reduction of AFB_1 in wet wheat in which water (10%) was intentionally added was higher on heating at 100 °C for 30 min than that in the dried wheat.

Keywords: wheat, aflatoxin B_1, toxicity reduction, washing, heating

Introduction

Aflatoxins (AFs) are toxic secondary metabolites produced by species of *Aspergilli,* especially *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow on certain foods and feeds under favourable conditions of temperature and humidity and generate AFs before and/or during harvest, handling, shipment and storage (Peraica *et al.,* 1999; Bushby and Wogan, 1984). AFs are produced under optimum temperature and moisture conditions on *Aspergillus* contaminated carbohydrate-rich grains such as peanut, corn, cotton, and wheat (Jaimez *et al.,* 2000). AFs have the highest toxicity among mycotoxins and possess carcinogenic activity as well. They affect not only the health of humans and animals but also the economics of agriculture and food (Hwang *et al.,* 2004). Epidemiological studies have shown that AF exposure is associated with increased risk of hepatocellular carcinoma, particularly in combination with hepatitis B virus (IARC, 2002). The aflatoxin form has 17 members including B_1, B_2, G_1, G_2, M_1 and M_2, among whom aflatoxin B_1 is considered so far the strongest hepatocarcinogen agent. AFB_1, AFG_1, and AFM_1 have higher carcinogenic activity than AFB_2, AFG_2, and AFM_2, respectively.

Recently, contamination of mycotoxins in food including imported ones has received much attention because of the increase in international food trade under new trade treaties. Therefore, it is highly necessary to investigate means of toxicity reduction of mycotoxin contaminated raw material, as well as foods.

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). It is generally accepted that mycotoxin levels in food need to be reduced as low as technologically possible. Several physical, chemical and biological methods have been applied for the removal and inhibition of aflatoxigenic mould growth and subsequent aflatoxin biosynthesis. However, few of these have practical applications (Abrunhosa *et al.,* 2002).

Ammoniation, one of the chemical methods, was reported to detoxify AFs in various raw materials with high efficiency (Buser and Abbas, 2002). The level of AFs was reduced to over 40% by roasting and heating peanuts (Rustom, 1997). Buser and Abbas (2002) reported that an extrusion process is able to decrease the level of AFs to 33%. As a biological treatment, antagonistic microorganisms were used to reduce toxicity of AFB_1 (Cho and Kang, 2000). Studies on the reduction of aflatoxin by treatment with naturally occurring compounds such as, antioxidants have been investigated worldwide. These studies are usually carried out through the elucidation of metabolic pathways in aflatoxin forming microorganisms (Huwig *et al.,* 2001). A mushroom extract has been reported to inhibit AFB_1- 8, 9-epoxide formation (Lee *et al.,* 2003). The use of heat treatment has been investigated with the double aims of processing the food and partially or totally

*Author for correspondence; E-mail: arshadpcsir@yahoo.com

reducing mycotoxin contamination. Predominantly, in the case of peanuts and corn, the conventional practice of roasting has proved to reduce the contamination level by more than 40 % (Taha *et al.*, 2001).

The present study was undertaken for developing a process feasible for decontamination of AFB_1 by physical treatments with minimum possible detrimental effect on the nutritive value of wheat and thus convert the unusable contaminated commodity into usable form. In the present study, the reduction of aflatoxin B_1 contamination in wheat varieties was monitored employing physical treatment methods of washing and heating, which are generally undertaken in the home cooking, with a view to providing basic and valuable information about reduction of aflatoxin in wheat.

Materials and Methods

Collection of samples. Total 30 samples of different local wheat varieties such as Auqab, Ghaznavi-98, Saleem- 2000, Kyber-87, Pirsabak-2005 were acquired from NIFA Peshawar and Agricultural University Peshawar, Khyber Pukhtoonkhwa.

Processing of samples. Wheat varieties were artificially contaminated with aflatoxin B_1 (AFB_1). For washing, the contamination levels employed on wheat varieties [(Auqab, Ghaznavi-98) and (Saleem-2000, Kyber-87, Pirsabak-2005)] were 43 µg/kg and 46 µg/kg, respectively, for AFB_1. For heating treatment, the contamination levels employed on dried wheat samples [(Auqab, Saleem-2000) and (Ghaznavi-98, Kyber-87, Pirsabak-2005)] were 100 µg/kg and 55 µg/kg, respectively, for AFB_1, while on the wet wheat samples [(Auqab), (Saleem-2000) and (Pirsabak-2005)] were 28.6 µg/kg, 14.86 µg/kg and 18.56 µg/kg, respectively. Adequate amount of the standard dissolved in benzene were dripped on to 100 g portions of samples held in 600 mL beakers, taking precautions so as to avoid contaminating the wall. After 24 h the contaminated wheat was used for the aflatoxin reduction by physical methods of washing and heating.

Washing and heating. Wheat (20 g) contaminated with AFB_1 was put in 200 mL water and agitated to 155 rpm for 10, 20, 30 min using stirrer. Contaminated wheat was heated in an oven at various temperatures for 30, 60 and 90 min. The AFB_1 level of heated wheat was analyzed by TLC. Wet wheat samples were prepared by soaking wheat in water up to 10% moisture levels. Wet samples were heated at 100 °C for 30 min. Then, wheat was homogenized and analyzed for AFB_1 analysis according to standard method, by thin layer chromatography (AOAC, 2000). The analysis of AFB_1 in wet wheat samples was performed in the same way as for the dried ones.

Chemicals analyses. All the chemicals of analytical grade used in the present study were procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (St. Louis, USA).

Standard of aflatoxin B_1. Standard of aflatoxin B_1 (2.02 µg/mL) was purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solution of AFB_1 of concentration 1 µg/mL was prepared by diluting in benzene/ acetonitrile (98:2; v/v). This stock solution was then stored at 4 °C in refrigerator.

Determination of aflatoxin B_1. Aflatoxin B_1 was determined according to the standard method of AOAC (2000) by thin layer chromatography. Briefly, 50 g test sample was extracted with 250 mL acetone/ water (85:15 v/v) using blender for 3 min and filtered through Whatman filter paper No. 4 and 150 mL of filtrate was collected in 400 mL beaker. Then 170 mL of 0.02 N sodium hydroxide and 30 mL ferric chloride along with about 3 g basic copper carbonate were added to the filtrate in 400 mL beaker, mixed well and added to the mixture in 600 mL beaker. 150 mL of this solution mixture was filtered and transferred to a 500 mL separating funnel. To this 150 mL of 0.03% sulphuric acid was added and extracted twice with 10 mL of chloroform. Lower chloroform extract layer was transferred to another separating funnel and 100 mL of 0.02 M potassium hydroxide was added, swirled gently for 30 sec and left for layer separation. The chloroform extract layer was collected in a vial and 8 mL of it was evaporated to dryness at 45 °C under a gentle stream of nitrogen on a heating block. The residue was redissolved in 200 µL benzene/ acetonitrile (98:2 v/v) and subjected to thin-layer chromatography. Final identification and quantification of AFB_1 was performed by one-dimensional TLC on precoated silica gel plates (Merck, Germany). The plates were developed in a saturated chamber with chloroform/xylene/acetone (60: 30: 10; v/v/v). The samples spots were observed under long wave ultraviolet light (λ=365 nm) and determined by visual comparison with AFB_1 standard spots. Confirmation of the identity of AFB_1 was carried out with the spray of 50% sulphuric acid and using the trifluoroacetic acid

(TFA) reaction (Scott, 1984) and its amount was calculated as follows:

$$AFB_1\ (\mu g/kg) = S \times Y \times V / X \times W$$

Where:

S = Standard which matches the unknown (μL)
Y = Concentration of standard (μL/mL)
V = Final dilution of sample extract (μL)
X = Sample extract spotted giving florescent intensity equivalent to standard (μL)
W = Weight of the sample contained in final extract (g)

Statistical analysis. The statistical significance of the data was analyzed using Student's t-test. Each sample was analysed thrice and standard deviation was calculated (Steel *et al*., 1997). The data was expressed as average mean ± standard deviation (SD). Method of recovery varied from 94 to 104 % and the quantification limits was 1 μg/kg for AFB_1 (Simionato *et al*., 2003).

Results and Discussion

Wheat samples (Auqab, Ghaznavi-98) and (Saleem-2000, Kyber-87, Pirsabak-2005) were artificially contaminated with AFB_1 43 μg/kg and 46 μg/kg, respectively. The effect of washing on the reduction of AFB_1 in wheat varieties is shown in Table 1. The reduction of AFB_1 in all the wheat samples was proportional to washing time.

The level of AFB_1 in wheat samples such as Saleem-2000, Kyber-87and Pirsabak-2005 decreased by 62%, 56%, and 58%, respectively, after 30 min washing. When wheat samples Auqab and Ghaznavi-98 were washed for 30 min, about 60% and 56% reduction in AFB_1 was obtained, respectively. There was no difference between wheat samples in terms of the reduction (%) of AFB_1 by washing. However, the value did vary according to the wheat variety. Due to low solubility of AFs in water, it is generally hard to remove AFs by washing. However, in this study about 40% of AFB_1, usually attached to the surface of wheat, could be removed by washing. The reduction degree of 10 and 30 min washing did not show a significant difference, which means it is very difficult to remove AFB_1 bonded or attached to wheat strongly. Washing of Saleem 2000 for only 20 min showed a significant difference ($P< 0.05$) compared to washing for 10 min. This result was in agreement with a previous report (Yeo and Kim, 2002). To investigate the effect of heating treatment on the destruction of AFB_1, all the contaminated wheat samples were heated in oven at various temperatures (50, 100, 150, 200 °C) for different periods (30, 60, 90 min). The effect of heating on the level of AFB_1 is shown in Table 2. Dried wheat samples were selected for heat treatment. Heating at 100 °C or lower did not lead to any marked reduction in AFB_1 levels, whereas at temperatures higher than 150 °C, the reduction of AFB_1 was much higher.

Heating the variety Auqab at 150 °C, caused AFB_1 destruction of about 60%, 75%, and 82% for 30 min, 60 min, and 90 min, respectively. In case of heating at 200 °C, over 90% of AFB_1 were destroyed. Compared to the heating at 50 °C, the heating at 100 °C for 60 min displayed a significant difference in AFB_1 reduction ($P<0.05$). However, heating of Auqab over 150 °C, showed a significant difference against heating at 50 °C, regardless of the heating time ($P < 0.01$ or $P < 0.05$). The effect of heating on Ghaznavi was almost similar to that on other wheat varieties. Greater decrease in AFB_1 was apparent in Saleem-2000 by heating at 150 °C. However, it is impossible to heat foods over 100 °C for reducing AFs level.

Table 1. Reduction of Aflatoxin B_1 in wheat varieties through washing for different time periods

Variety	Initial level of AFB_1 (μg/kg)	After 10 min		After 20 min		After 30 min	
		Final level (μg/kg)	Reduction (%)	Final level (μg/kg)	Reduction (%)	Final level (μg/kg)	Reduction (%)
Auqab	43	21.3 ± 1.7	50	21.2 ± 0.5	50	17.2 ± 2.6	60
Ghaznavi-98	43	25.2 ± 2.5	41	19.4 ± 0.4	55	18.9 ± 1.1	56
Saleem-2000	46	22.8 ± 1.2	50	18.2 ± 1.5*	60	17.1 ± 3.0	62
Kyber-87	46	25.0 ± 3.1	45	23.6 ± 2.4	48	20.0 ± 8.9	56
Pirsabak-2005	46	24.4 ± 3.0	46	21.9 ± 4.4	52	18.8 ± 3.0	58

The values are mean ± standard deviation (n = 3); * $P<0.05$; ** $P<0.01$.

Regarding the type of heating used to destroy AFs, it was reported that the use of oven for heating was more effective in reducing AFs than the use of other means of heating such as microwave (Soliman, 2002; Soliman *et al.*, 2001). For investigating the effect of moisture content on the reduction of AFB_1 level, the varieties, Auqab, Saleem-2000 and Pirsabak-2005 (10 % moisture content) were heated for 30 min at 100 °C (Table 3). The initial levels of AFB_1 in Auqab, Saleem-2000 and Pirsabak-2005 were 28.6 and 18.5 µg/L, respectively, and after heating the reduction of AFB_1 in these varieties was 40%, 47% and 43%, respectively. In comparison with the dried wheat samples, reduction of AFB_1 in wet samples of Auqab, Saleem-2000 and Pirsabak-2005 varieties was higher by 20%, 24% and 18%, respectively.

Table 2. Effect of heating for different time periods on reduction of AFB_1 in dried wheat

Temperature (°C)	Initial level of AFB_1 (µg/kg)	After 30 min		After 60 min		After 90 min	
		AFB_1 level (µg/kg)	Reduction (%)	AFB_1 level (µg/kg)	Reduction (%)	AFB_1 level (µg/kg)	Reduction (%)
Auqab	100						
50		98.0 ± 3.0	2	95.0 ± 3.3	5	82.0 ± 4.0	18
100		80.0 ± 1.9	20	69.2 ± 2.4*	31	57.3 ± 1.7*	42
150		40.0 ± 2.5**	60	25.0 ± 2.0**	75	17.6 ± 0.7*	82
200		4.0 ± 0.2**	96	11.0 ± 1.0**	90	7.0 ± 0.3**	93
Ghaznavi-98	55						
50		51.6 ± 3.6	6	52.7 ± 2.0	4	49.0 ± 0.4	11
100		45.6 ± 3.2	17	36.0 ± 1.6*	34	38.4 ± 2.8*	30
150		25.9 ± 2.3**	53	18.0 ± 1.8**	67	12.0 ± 2.9**	78
200		5.0 ± 0.7**	91	6.4 ± 0.3**	88	4.7 ± 0.8**	91
Saleem-2000	100						
50		95.0 ± 5.0	5	93.0 ± 3.3	7	82 ± 15.5	18
100		77.0 ± 1.9	23	70.0 ± 2.4*	30	62 ± 4.7*	38
150		38.6 ± 3.5**	61	24.0 ± 2.0**	76	12.0 ± 0.7*	88
200		5.0 ± 0.2**	95	9.0 ± 1.0**	91	6.0 ± 4.9**	94
Kyber-87	55						
50		52.0 ± 3.6	5	52.0 ± 2.0	5	47.2 ± 0.4	14
100		44.0 ± 3.2	20	45.0 ± 1.6*	18	35.0 ± 2.8*	36
150		24.2 ± 2.3**	56	18.9 ± 1.8**	66	12.2 ± 2.9**	77
200		6.4 ± 0.7**	88	4.2 ± 0.3**	92	3.7 ± 0.8**	93
Pirsabak-2005	55						
50		51.5 ± 3.6	6	50.0 ± 2.0	9	49.3 ± 0.4	10
100		46.5 ± 3.2	15	42.3 ± 1.6*	23	33.5 ± 2.8*	39
150		24.5 ± 2.3**	55	14.6 ± 1.8**	73	11.5 ± 2.9**	79
200		2.8 ± 0.7**	95	3.6 ± 0.3**	93	2.0 ± 0.8**	96

The values are mean ± standard deviation (n = 3); * = P<0.05; ** = P<0.01.

Table 3. Effect of heating at 100 °C for 30 min on reduction of AFB_1 in wet wheat

Moisture	Auqab			Saleem-200			Pirsabak-2005		
	Initial level of AFB_1 (µg/kg)	Final level (µg/kg)	Reduction (%)	Initial level of AFB_1 (µg/kg)	Final level (µg/kg)	Reduction (%)	Initial level of AFB_1 (µg/kg)	Final level (µg/kg)	Reduction (%)
10%	28.6	17.0 ± 3.0	40	14.8	7.8 ± 2.3	47**	18.5	10.5 ± 2.0	43*

The values are mean ± standard deviation (n=3); * = P<0.05; ** = P<0.01.

Heating of wet Saleem-2000 varieties showed a significant difference compared to the heating of the dried variety under the same conditions ($P < 0.05$). The relationship between moisture content in foods and destruction of AFs has been already reported by many researchers (Mendez-Albores *et al.*, 2004; Torres *et al.*, 2001). According to these reports, the increased moisture content enhances the destruction of AFs during cooking or baking. In addition, AFs are stable up to their melting points when heated without moisture (Mann *et al.,* 1967). Moisture is required to hydrolyze the lactone ring of the AFs when cooking at home at temperature of 85-95 °C (Samarajewa, *et al.,* 1990). For detoxifying of AFs, the most important step involves opening of the lactone ring of the AFs (Buser and Abbas, 2002).

Conclusion

In the present study of the effects of physical treatments such as washing and heating in oven on the reduction of aflatoxin B_1, reduction of AFB_1 toxicity was found to be directly proportional to washing time in all the studied wheat varieties. The level of AFB_1 decreased more by heating than washing. The reduction of aflatoxin by heating was more in case of wet wheat than in the dried wheat. The study provides a base for developing a method feasible for converting unusable contaminated wheat commodity in to usable form, through decomposition of aflatoxin B_1 by physical treatments and its reduction in wheat.

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