

Evaluation of the decrease in natural Aflatoxin concentration with temperature and time changes in contaminated red pepper powder

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Abstract

Background: With increasing knowledge and awareness of aflatoxins (AFs), as potent health hazards to both humans and animals, major efforts have been made to completely eliminate these toxins or reduce their content in foodstuffs. Although prevention is the most effective intervention, heating has been also used to inactivate AFs in contaminated foodstuffs.

Methods: The aflatoxin was extracted, using 80% methanol and then purified via immunoaffinity column. Measurements were performed, using high-performance liquid chromatography, equipped with a fluorescence detection system at excitation and emission wavelengths of 365 and 435 nm, respectively.

Results: The present findings indicated a 92.7% decline in the amount of AFB1 and a 94.8% reduction in AFB2. There was a significant difference in AF decomposition under different treatment conditions ($P < 0.05$).

Conclusion: Although treatment showed different degrees of AF reduction in the samples, a substantial decline was reported in samples heated at 150°C for 60 to 180 min. Also, degradation of AFB1 was found to be both time- and temperature-dependent.

Keywords: Mycotoxins, Aflatoxins, temperature, HPLC

Introduction

Mycotoxins are natural toxins which are produced by several fungal species and are associated with morbidity or even mortality in animals, plants, and humans. These compounds have diverse chemical structures and a low molecular weight. Mycotoxins are often found in a large number of agricultural and food products throughout the world. In different stages, such as production, harvest, transport, and storage of agricultural products, mycotoxins can result in the contamination of human food or animal feed. Nevertheless, these fungi are not endemic to specific geographical areas or climates. In fact, fungal growth and toxin production occur only if the environment and conditions are suitable (1).

AFs constitute the most important group of mycotoxins, produced by different *Aspergillus* species, i.e., *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (2). Among recognized AFs, AFB1 is considered as the most toxic of all (3). In fact, AF was included in the list of cancer-causing agents by the International Agency for Research on Cancer in 1987 (4).

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Improper storage, drought, or high humidity can lead to an increase in the growth of *Aspergillus* species in herbs and spices (5). The European Union has established maximum tolerable limits of 10 mg/kg and 5 mg/kg for total AFs and AFB1 in spices, respectively (6). Given the fact that AFs are a potent health hazard to both humans and animals, major efforts have been made to completely eliminate these toxins or reduce their content to significantly lower levels in foods and feedstuffs. Although prevention is recognized as the most effective intervention, use of chemical, biological, and physical methods has been investigated for inactivating AFs or reducing their content in foods. However, no scientifically designed experiments have documented the degrading effect of temperature on AF content in chili powder. Therefore, in this study, the effects of temperature were evaluated in a laboratory setting to suggest an optimal temperature condition.

Materials and Methods

Sample preparation

The samples were prepared, using a laboratory grinding mill and 40 mesh sieve passed. In order to achieve a uniform composition, the samples were mixed by a mixer. Then, the reducing effect of heating on AF contamination in chili powder was evaluated.

Temperature experiment

Based on the initial experiments, a temperature range of 60-150°C was applied in the samples. The samples were dried at 60°C to 150°C for 60 to 180 min in the oven. Afterwards, the samples were removed from the oven and cooled at room temperature; then, the amount of AF was evaluated.

Standards and reagents

All chemicals with the exception of methanol and acetonitrile were laboratory-grade compounds and purchased from Merck, Germany. Standards including AFG2, AFG1, AFB2, and AFB1 were purchased from Sigma (Germany).

Apparatus

Liquid chromatography (LC) was performed, using a reversed-phase high-performance LC (HPLC) system (Waters 2695, USA), equipped with a Gilson Workstation (Gilson GX-271 Aspec, USA) and a fluorescence detector (Waters 474, USA). The capital HPLC column was C18 (15 cm×4.6 mm, 5 µm). Moreover, aflatoxin immunoaffinity columns (IACs) were purchased from R-Biopharm (Darmstadt, Germany).

Extraction method

For this purpose, 10 g of the ground sample was mixed in 60 ml of methanol 80% for 30 min, using a shaker and then filtered; the product was then centrifuged for 30 min and re-filtered. Finally, 0.25 ml Tween was added to 5 ml of the filtered extract and stirred for 2 min.

Aflatoxin separation using IAC

For this purpose, 3.1 ml of the filtered extract was diluted in 9.9 ml of distilled water and filtered, using a microfiber filter. Then, 12.6 ml of the extract was used for IAC, which was preconditioned with 10 ml of phosphate-buffered saline (rate of 14 drops per min). After passing the extract through the column, the column was rinsed twice with 15 ml of water and then dried. Aflatoxin was collected in the vial, using 1.25 ml of methanol and diluted with 1.75 ml of deionized water. Finally, 100 µl of the solution was injected into the HPLC system.

Aflatoxin measurement by HPLC

Aflatoxin level was determined, using HPLC, equipped with a fluorescence detection system, using C18 silica gel column and Kobra cell derivatization at excitation and emission wavelengths of 365 and 435 nm, respectively. The mobile phase consisted of water-acetonitrile-methanol (30:20:60 v/v/v) containing 120 mg/L KBr and 350 µl HNO₃ 4M, with a flow rate of 1 mL per min and an injection volume of 100 µL.

Quality assurance

For evaluating the reliability of the results, in addition to using validated methods, internal and external quality control experiments were performed. Regarding internal quality control, the accuracy and precision of the methods were verified. For this purpose, AFB1, B2, G1, and G2 recoveries were recorded by analyzing a blank sample, spiked at 4 ng/g for AFB1 and AFG1 and 1 ng/g for AFB2 and AFG2, recovery rate for AFB1 was 50% - 70% and the average coefficient of variation was 5.4 %, The aflatoxin level was corrected, according to the recovery value. LOD and LOQ for AFB1 were 0.033ppb and 0.1ppb, respectively.

Results

After data analysis, the contaminated samples with high levels of AFB1 were detected. The range of contamination in the three groups was between 53.8 - 54.4 ppb for B1 and 6.1 - 6.2 for B2. The detail of this data is presented in Table 1.

Table 1. Type of samples used in the experiments

Samples	Type	AF contamination
Group 1	Artificially contaminated	B1 (53.8 ppb), B2 (6.1 ppb)
Group 2	Artificially contaminated	B1 (54.2 ppb), B2 (6.2 ppb)
Group 3	Artificially contaminated	B1 (54.4 ppb), B2 (6.2 ppb)

The treatment showed some degrees of reduction in AF contamination. The amount of AF contamination significantly reduced in samples which were heated at 150°C for 60-180 min. Although treatment showed different degrees of AF reduction in the samples, a substantial decline was reported in samples heated at 150°C for 60 to 180 min. The results related to the effects of heating on AF degradation in chili powder are presented in Table 2 and Figure 1, 2. The obtained results suggested a 92.7% decline in AFB1 contamination and a 94.8% reduction in AFB2.

Table 2. Effect of heating on degradation of AF contamination in chili powder

Time	60 min		120 min		180 min	
	B1	B2	B1	B2	B1	B2
60°C	4.5 ±3.6	10.6±5.7	6.1 ±2.9	11.6 ±4.3	7.1 ±3.8	13.9±5.1
90°C	18.7±2.4	24.1±3.7	20.8±1.6	26.7 ±3.9	25.1±1.5	28.8±4.1
120°C	39.7-6.2	43.9-2.6	51.9-2.4	59.4-1.9	62.3-4.8	68.6-3.2

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150°C	74.1-4.2	81.2-3.4	86.3-6.7	89.1-5.3	92.7-2.1	94.8-3.7
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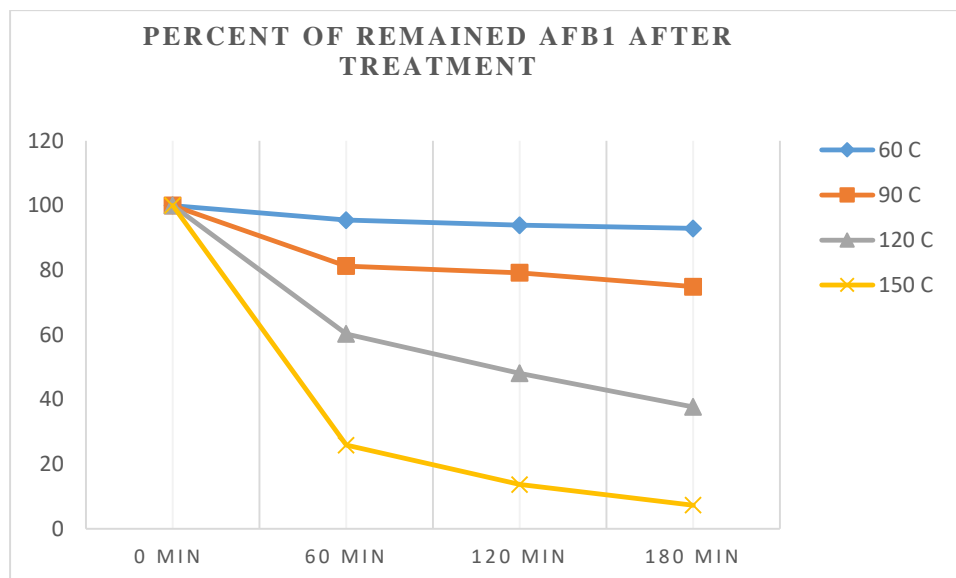


Figure 1. Effects of heating at 60, 90, 120 and 150°C on the reduction of AFB1 contamination in red pepper powder

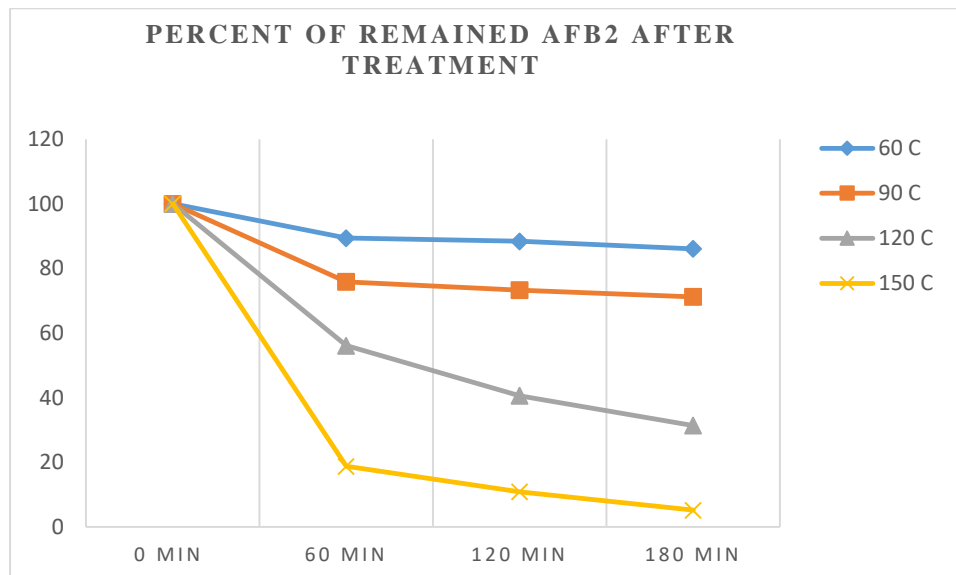


Figure 2. Effects of heating at 60, 90, 120 and 150°C on the reduction of AFB2 contamination in red pepper powder

Discussion

Heating is one of the physical methods used to reduce AF contamination in edible foods. Therefore, this method can diminish the risks caused by the presence of AF in foodstuffs. As shown in Table 2 and Figures 1 & 2, heating reduces AF content in chili powder. There was a significant

difference between AF decomposition under different treatment conditions ($P < 0.05$). Also, degradation of AFB1 was both time- and temperature-dependent.

Pluyer et al. also reported that oven roasting at 150°C for 30 min caused a 30-45% decline in AFB1 content in naturally contaminated peanuts. However, in artificially contaminated peanuts treated under similar conditions, destruction was reported to be 48-61% (7). In addition, Yazdanpanah et al. in 2005 showed that heating at 150°C for 120 min caused more than a 95% decline in AF content in pistachio (8). In an study by Duran and el al., In vitro cytotoxicity and genotoxicity induction by aflatoxin B1 (AFB1) from maize (ME) and tortillas (TE) produced by microwave nixtamalization were tested in monkey renal tissue. They concluded that the microwave nixtamalization procedure reduced aflatoxins and their in vitro toxicity and mutagenic activity (9).

In another study by Hussain et al.(2011) showed that roasting resulted in a significant decrease in the AFs content of nuts, corn and oilseed meals. Degradation of aflatoxins by roasting was both time and temperature dependent. Roasting at 150 C for 120 min degraded more than 95% of AFB1 in peanuts. The author also reported that Aflatoxins in form of naturally occurrence were more resistant to degradation with heat compared to artificially contaminated samples (10). In another study by Herzallah et al.(2008) showed that in feed samples subjected to Microwave , aflatoxin B1 contents significantly ($p < 0.05$) decreased by 32.3% (11). In another study by Mobeen et al.(2011) showed that in Peanut feed samples subjected to Microwave , aflatoxin B1 decreased by 63.3% (12).

In another study by Jard et al.(2011) showed that efficacy of various physical (UV irradiation, heating, microwave); chemical (oxidation, bleaching, ammoniation, sulphitation) and biological treatments methods for detoxification AFB1 in chili powder. Amongst the physical methods, direct oven heating (at 120°C) produced maximum (83.32%) reduction of AFB1. With the exception of oxidation with H₂O₂ which produced 58.32% degradation, other selected chemical compounds were ineffective on AFB1. Biological detoxification of 66.2% was achieved by treating spiked chili powder with purified peroxidase. The author reported that the physical methods were more efficient over other methods in degrading AFB1 (13).

The effects of household processing on AFs content of maize products (boiled maize, porridge, roti, biscuits, muffins and idli) was studied. All processing methods (boiling, roasting, baking and steaming) destroyed AFs to a considerable extent. The percentage destruction ranged from 50-70% (14). The efficacy and extent of reduction method is depends on several factors, including AFs concentration, the extent of binding between AFs and food constituents, heat penetration, moisture content, pH, ionic strength, processing conditions and source of contamination (naturally or artificially) (10, 15).

The relationship between moisture content of foods and reduction of AFs has been demonstrated several times (16). According to these reports, by increased moisture content the destruction of AFs is increased during cooking or baking. Kabak and co-workers also reported that the moisture content is a critical factor in AFs reduction and in presence of water decontamination of food by heating is easier and more effective. They suggested that the presence of water helps in opening the lactone ring in AFB1 (by the addition of a water molecule to the ring) to form a terminal carboxylic acid. The terminal acid group thereafter undergoes heat-induced decarboxylation (17).

Hussain and coworkers reported that roasting resulted in a significant decrease in the AFs content of nuts, corn and oilseed meals. Degradation of aflatoxins by roasting was both time and temperature dependent. Roasting at 150 °C for 120 min degraded more than 95% of AFB1 in peanuts. The author also reported that Aflatoxins in form of naturally occurrence were more resistant to degradation with heat compared to artificially contaminated samples (10). In a related study a mean reduction of 66.5% was obtained by roasting, but the reduction seems to be heterogeneous (18).

In another study by Khazaeli et al. (2017) showed that among 120 tested samples, 37 (30.8%) were contaminated with aflatoxins (range: 0.2-57.5 µg/kg); based on the findings, all these samples were contaminated by AFB1 (0.7-57.5 µg/kg). Examination of factors affecting aflatoxin level showed a significant difference between the samples in terms of moisture ($P=0.046$). In addition, aflatoxin level was not significantly influenced by the packaging of the samples ($P=0.578$) (19).

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In another study by Khazaeli et al. (2017) showed that there was a significant difference in AF decomposition under different treatment conditions ($P < 0.001$). Also, degradation of AFB1 was found to be both time and temperature-dependent (20).

In another study by Khazaeli et al. (2014) showed that mean aflatoxin contamination in the roasted samples (16.53 $\mu\text{g}/\text{kg}$) was significantly higher than the raw nuts (7.25 $\mu\text{g}/\text{kg}$) (P -value < 0.001). After measuring the moisture content of the samples and Statistical analysis, there was a significant correlation between the aflatoxin and the amount of moisture in the samples (P -value < 0.001) (21).

Based on the results of previous studies, AF degradation is under the influence of heating. On the other hand, heating at high temperatures (for further AF decline in the samples) causes the resulting product to be unedible. Therefore, before performing toxicological studies and evaluating the effect of temperature on the samples, the lowest possible temperature should be considered to avoid damage to the final product.

Conclusion

According to the results of the current research, time and power of heating are correlated with decreased concentrations of AF. However, application of high heating and time to reduce higher concentrations of AFs may result in poor-quality products. Therefore, it is recommended that the lowest heating and time be applied to evaluate the effects of this method on contaminated samples in order to maintain the quality of final product.

References

1. Murphy PA, Hendrich S, Landgren C, Bryant CM. Food mycotoxins: an update. *Journal of food science*. 2006;71(5):R51-R65.
2. Tosun H, Arslan R. Determination of aflatoxin B1 levels in organic spices and herbs. *The Scientific World Journal*. 2013;2013(10):1-4.
3. Oancea S, Stoia M. Mycotoxins: a review of toxicology, analytical methods and health risks. *Universitatis Cibiensis Series E*. 2008;7(1):19-36.
4. Cancer IAFRo, Cancer IAFRo. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. France: International Agency for Research on Cancer Lyon; 1987.
5. Ozbey F, Kabak B. Natural co-occurrence of aflatoxins and ochratoxin A in spices. *Food Control*. 2012;28(2):354-61.
6. Commission EC. Commission Regulation (EC) No. 472/2002 of 12 March 2002. Brussels,, Belgium: L. 2002;75:18-20.
7. Pluyer H, Ahmed E, Wei C. Destruction of aflatoxins on peanuts by oven-and microwave-roasting. *Journal of Food Protection*. 1987;50(6):504-8.
8. Yazdanpanah H, Mohammadi T, Abouhossain G, Cheraghali AM. Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts. *Food and Chemical Toxicology*. 2005;43(7):1135-9.
9. Vázquez-Durán A, Díaz-Torres R, Ramírez-Noguera P, Moreno-Martínez E, Méndez-Albores A. Cytotoxic and Genotoxic Evaluation of Tortillas Produced by Microwave Heating during Alkaline-Cooking of Aflatoxin-Contaminated Maize. *Journal of food science*. 2014;79(5):T1024-T9.

10. Hussain A, Ali J, Akhter S. Degradation of aflatoxins by roasting in contaminated peanuts. *Pakistan Journal of Biochemistry and Molecular Biology (Pakistan)*. 2011.
11. Herzallah S, Alshawabkeh K, Fataftah AA. Aflatoxin decontamination of artificially contaminated feeds by sunlight, γ -radiation, and microwave heating. *Journal of Applied Poultry Research*. 2008;17(4):515-21.
12. Mobeen A, Aftab A, Asif A, Zuzzer A. Aflatoxins B1 and B2 contamination of peanut and peanut products and subsequent microwave detoxification. *Journal of Pharmacy and Nutrition Sciences*. 2011;1(1):1-3.
13. Jard G, Liboz T, Mathieu F, Guyonvarc'h A, Lebrihi A. Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. *Food Additives & Contaminants: Part A*. 2011;28(11):1590-609.
14. Reddy U, Rani P. Effect of processing on detoxification of aflatoxins in maize. *Indian Journal of Nutrition and Dietetics*. 2000;37(2):59-63.
15. Hwang J-H, Lee K-G. Reduction of aflatoxin B1 contamination in wheat by various cooking treatments. *Food Chemistry*. 2006;98(1):71-5.
16. Mendez-Albores A, De Jesús-Flores F, Castaneda-Roldan E, Arambula-Villa G, Moreno-Martínez E. The effect of toasting and boiling on the fate of B-aflatoxins during pinole preparation. *Journal of food engineering*. 2004;65(4):585-9.
17. Kabak B, Dobson AD, Var II. Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Critical reviews in food science and nutrition*. 2006;46(8):593-619.
18. Perez de Obanos A, Gonzalez-Penas E, Lopez de Cerain A. Influence of roasting and brew preparation on the ochratoxin A content in coffee infusion. *Food additives and contaminants*. 2005;22(5):463-71.
19. Khazaeli P, Mehrabani M, Heidari MR, Asadikaram G, NAJAFI ML. Prevalence of aflatoxin contamination in herbs and spices in different regions of Iran. *Iranian journal of public health*. 2017;46(11):1540.
20. Khazaeli P, Mehrabani M, Heidari MR, Asadikaram G, Najafi ML. Evaluation of the effect of microwave on reduction of aflatoxin concentrations in contaminated red pepper powder. *JOURNAL OF FOOD SAFETY AND FOOD QUALITY-ARCHIV FUR LEBENSMITTELHYGIENE*. 2017;68(6):128-32.
21. Khazaeli P, Najafi M, Bahaabadi G, Shakeri F, Naghibzadeh tahami, A.(2014). Evaluation of aflatoxin contamination in raw and roasted nuts in consumed Kerman and effect of roasting, packaging and storage conditions. *Life Sci J*.10:578-83.