Journal of Food Safety and Food Quality 68, Heft 6 (2017), Seiten 125–146

Arch Lebensmittelhyg 68,
128–132 (2017)
DOI 10.2376/0003-925X-68-128

ISSN 0003-925X

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Evaluation of the effect of microwave on reduction of aflatoxin concentrations in contaminated red pepper powder

Bewertung der Wirkung von Mikrowellen auf die Verringerung von Aflatoxinkonzentrationen in kontaminiertem Paprikapulver

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Summary

Aflatoxins are the potent natural hepatotoxic, teratogenic, carcinogenic and immunosuppressive fungal agents with significant effects on humans and animals. The aim of the current study was to determine the efficacy of temperature in reducing the AF content in a laboratory setting to offer the optimal microwave conditions. In this experimental study, the effect of microwave on reduction of AF concentrations in contaminated pepper samples was evaluated in a laboratory setting. The treatment protocols showed some level of AF degradation, a significant reduction has been reported in the AF concentrations of the samples heated at 900 watts for 30–240 seconds. Moreover, a significant difference was observed between AF decompositions in different treatment conditions (P<0.001). Degradation of AFB1 was found to be time- and temperature-dependent. The findings of the present study indicated that microwave heating could cause a significant reduction in AF concentrations, compared to normal heating.

Keywords: Aflatoxin, Capsicum, Microwave

Zusammenfassung

Aflatoxine sind starke natürliche hepatotoxische, teratogene, karzinogene und immunsuppressive Pilzgifte mit bedeutsamen Wirkungen auf Mensch und Tier. Ziel dieser Studie war es, die Wirkung von Temperatur auf den Aflatoxingehalt unter Laborbedingungen zu bestimmen, um die optimalen Mikrowellenbedingungen aufzuzeigen. In dieser experimentellen Studie wurde die Wirkung der Mikrowellen auf die Reduktion von AF-Konzentrationen von kontaminierten Paprikapulver im Laborbereich untersucht. Die Behandlungsprotokolle zeigten einen gewissen Grad an AF-Abbau. Eine signifikante Reduktion der AF-Konzentrationen der Proben wurde bei 900 Watt für 30–240 Sekunden Erhitzung erzielt. Darüber hinaus wurde ein signifikanter Unterschied zwischen AF-Abbau bei verschiedenen Behandlungsbedingungen (P <0,001) beobachtet. Der Abbau von Aflatoxin B1 war zeit- und temperaturabhängig. Die Ergebnisse der vorliegenden Studie zeigten, dass die Mikrowellenerwärmung eine signifikante Reduktion der AF-Konzentrationen im Vergleich zur normalen Erwärmung bewirken können.

Schlüsselwörter: Aflatoxin, Capsicum, Mikrowellen

Introduction

Mycotoxins are secondary fungal metabolites, 400 different isolates of which have been discovered until now. However, only a few of these isolates possesses potential threat to human and animal health (Cancer, 1985). Aflatoxins (AFs) are the most important group of mycotoxins, produced by certain strains of fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B1 (AFB1) is one of the most potent natural hepatotoxic, teratogenic, carcinogenic and immunosuppressive agents with significant effects on humans and animals (Cancer, 1985). In 1987, the International Agency for Research on Cancer classified AF as a major cancer-causing agent (Cancer, 1987).

Some of the contributing factors for increased growth of Aspergillus species in herbs and spices are improper storage, drought and high humidity (Ozbey and Kabak, 2012). According to the statement of the European Union, maximum tolerable limits of AFs in spices are 10mg/kg for total AFs and 5mg/kg for AFB1 (EC, 2002). Human might be exposed to AFs directly (through the consumption of contaminated nuts, cereals, fruits and vegetables) or indirectly (by consuming the milk of animals fed with aflatoxin-contaminated feeds, where AFB1 or AFB2 are converted into AFM1 or AFM2 and secreted into the milk) (Stoloff et al., 1991).

Food processing, in particular cooking, affects the levels of natural AFs; however, the severity of exposure depends on the moisture content, type of matrix, natural composition and additives in foodstuffs (Scott, 1984). Several studies have denoted the destructive effects of various food processing methods on the stability of added or naturally contaminated AFs in food (Scott, 1984). The role playing-factors for the disintegration of aflatoxin are the matrix, physicochemical characteristics and additives of the food (Scott, 1984). Despite the destructive effects of food processing on AFs, a general resistance has been reported in these agents (Scott, 1984, Tabata et al., 1992).

Different methods of cooking (e.g., frying, roasting and baking in conventional microwave ovens) cause variable degrees of AF destruction (Scott, 1984, Tabata et al., 1992, Farag et al., 1996). Given the role of AF as a potent source of health hazards to humans and animals, the researchers have been concerned with the complete elimination of this toxin or significant reduction of its content in foodstuffs. Asprevention is the most effective strategy in this regard, the chemical, biological and physical methods have been assessed in terms of inactivating the AFs or content reductionin foodstuffs (Rustom, 1997).

To date, no studies have evaluated the effect of microwave on reduced concentrations of AFs in red pepper powder. So, this study aimed to determine the efficacy of temperature in reducing the AF content in a laboratory setting in order to suggest the optimal microwave conditions.

Materials and Methods

Study design

This is an experimental study conducted in the laboratory setting by testing the microwave effect of the AF content of the red pepper powder.

Sample preparation

The samples were prepared, using a laboratory grinding mill and 40-mesh sieve passed. In order to achievea uniform composition, a mixer was used. Afterwards, the microwave effect in reducing aflatoxin contamination in chili powder has been evaluated.

Microwave experiment

Based on initial experiments, 540–900 Watt microwave was radiatedon the samples. Power above 90 watt degrees showed negative impact on the taste and color of red pepper powder. The samples dried for a period of 30–240 sec for 540, 720 watt and 10–240 sec for 900 watt in the microwavebased on the given power. After the selected time, the samples were removed from the microwave and cooled at room temperature and the amount of aflatoxin was measured.

Standards and reagents

All chemicals with the exception of methanol and acetonitrile were laboratory-grade compounds and purchased from Merck, Germany. The standards including AFG2, AFG1, AFB2, and AFB1 were purchased from (Sigma, Hamburg,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 gof the ground sample was mixed in 60 ml of methanol 80 % for 30 min, using ashaker and was 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 andstirred 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 with10 ml of phosphate-buffered saline(rate of 14 drops per minute). After passing the extract through the column, the column was rinsed twice with 15 ml of water and then dried. The aflatoxinwas collected in avial, 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 derivatizationat excitation and emission wave lengths of 365 and 435 nm, respectively. Themobile-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 1mL per min and an injection volume of 100 µL.

Quality assurance

For evaluating the reliability of the results, in addition to using the validated methods, internal and external quality control experiments were performed. Regarding the inter-

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TABLE 1: *Type of samples.*

Sample	Type	AF contamination
Group 1	Artificially contaminated	B1 (24.933 ppb), B2 (1.215 ppb)
Group 2	Artificially contaminated	B1 (25.735 ppb), B2 (1.220 ppb)
Group 3	Artificially contaminated	B1 (25.733 ppb), B2 (1.157 ppb)

nal quality control, the accuracy and precision of the methods were verified. For this purpose, the 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. The recovery rate for AFB1 was 50–70 % and the average coeffcient of variation was 5.4 %. The aflatoxin level was corrected, according to the recovery value. LOD and LOQ for AFB1 were 0.033 ppb and 0.1 ppb, respectively.

Statistical analysis

In this study SPSS version 16 was applied for statistical analysis and in all measurements.

P-value less than 0.05 was considered statistically significant.

Results

The sample size of this study included 14 samples that we categorised in 3 groups. After data analysis, the contaminated samples with high levels of AFB1 were detected. The range of contamination in the three groups was between 24.933–25.735 ppb for B1 and 1.157–1.220 for B2. The detail of this data is presented in Table 1.

All treatment protocols showed some level of AF degradation. According to the results, AF contamination significantly reduced in the samples heated at 900 watts for 30–240 seconds ($P<0.05$). Heating of the samples at 720 watts for 240 seconds had the same effect on the reduction of AFs as 900 watts for 30 seconds, which slightly influenced the color and taste of red pepper powder.

Furthermore, microwave could decrease AF concentrations in red pepper samples. These results are provided in table 2 and figures 1 & 2.The results of this study were indicative of the reduction of AFB1 and AFB2 levels by 37.52 % and 33.86 %, respectively.

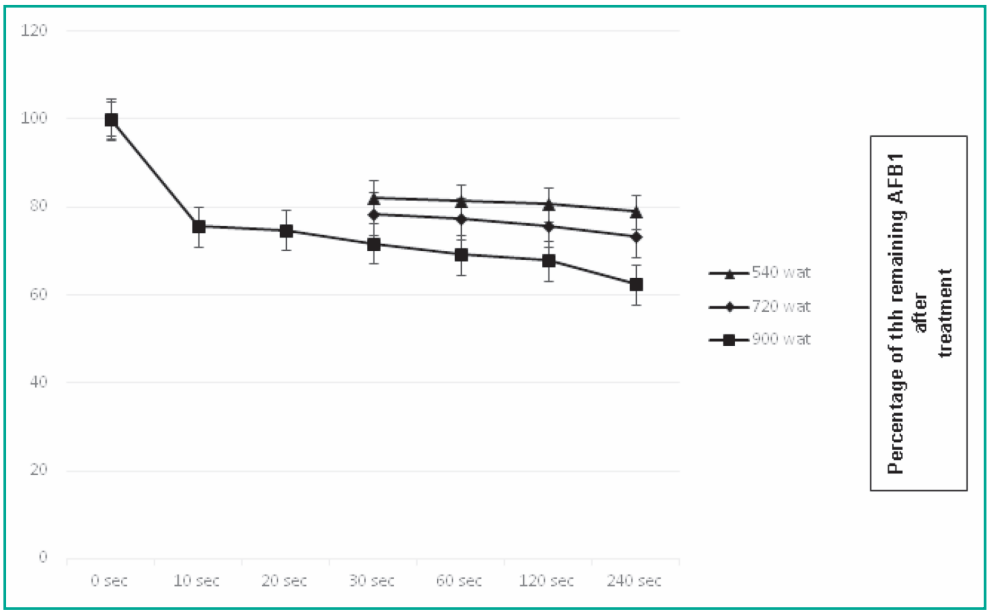

FIGURE 1: *Effect of microwave at 540, 720 and 900 watts on reduction of aflatoxin B1 in contaminated red pepper powder.*

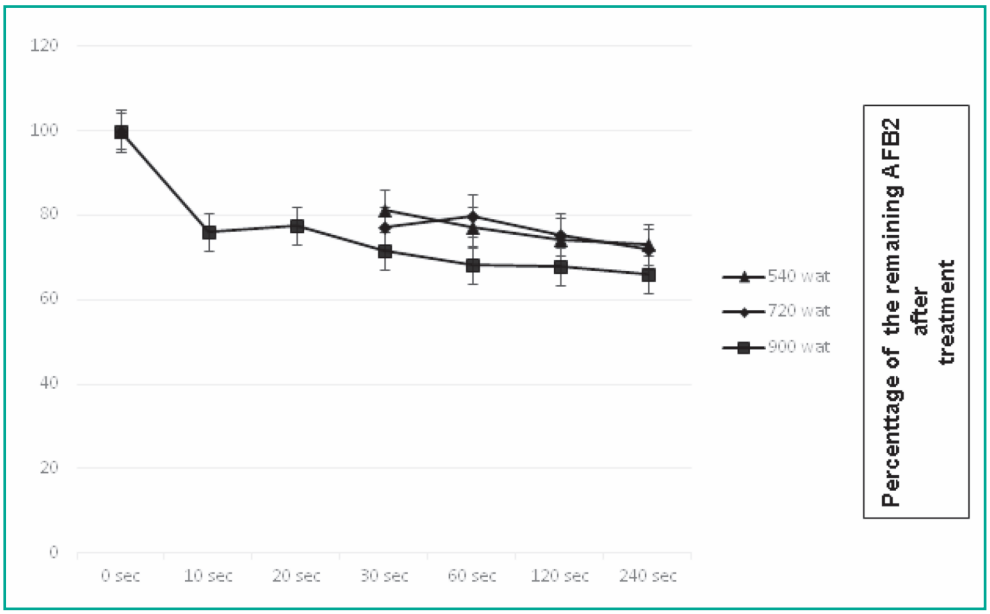

FIGURE 2: *Effect of microwave at 540, 720 and 900 watts on reduction of aflatoxin B2 in contaminated red pepper powder.*

TABLE 2: *Effect of microwave on reduction of aflatoxin concentrations in contaminated red pepper powder.*

Time/ Power	10 sec (Mean±SD)		20 sec (Mean±SD)		30 sec (Mean±SD)		60 sec (Mean±SD)		120 sec (Mean±SD)		240 sec (Mean±SD)	
	B1	B2	B1	B2	B1	B2	B1	B2	B1	B2	B1	B2
540 watts	–	–	–	–	17.74 ± 1.25	18.90 ± 1.80	18.69 ± 1.48	22.89 ± 3.03	19.35 ± 0.75	25.69 ± 7.08	21.00 ± 1.41	26.94 ± 6.02
720 watts	–	–	–	–	21.51 ± 1.30	22.93 ± 5.64	22.64 ± 1.54	20.12 ± 3.42	24.16 ± 0.68	24.59 ± 6.49	26.68 ± 1.17	28.10 ± 2.38
900 watts	24.42 ± 0.92	23.96 ± 1.18	25.29 ± 1.99	22.49 ± 2.57	28.28 ± 2.51	28.41 ± 2.30	30.80 ± 2.33	31.70 ± 5.17	32.23 ± 2.85	32.26 ± 2.55	37.52 ± 1.45	33.86 ± 1.26

Discussion

According to the results of the present study, microwave heating reduced AF concentrations in red pepper powder. Moreover, a significant difference was observed between AF decompositions in different treatment conditions ($P<0.001$). Microwave is one of the physical methods used to reduce AF concentrations in contaminated food.

In addition, it can lower the health risks associated with the presence of AF in the food products provided for consumers. Degradation of AFB1 was found to be time- and temperature-dependent.In line with the present study, in 1989. Betina et al. showed that AF was highly stable to dry heat, up to temperatures below the thermal decomposition temperature (Betina, 2010).

On the other hand, the results obtained by Rustamet al. (1997) revealed that the extent of AF destruction was related to the initial level of contamination, moisture content, and heating temperature and duration (Rustom, 1997). Correspondingly, prolonged heating may adversely affect the quality of the protein or availability of lysine (Scott, 1984).

In another study by Pluyer et al., it was reported that oven roasting at the temperature 150 °C for 30 minutes caused a 30–45 % reduction in the level of AFB1 in naturally contaminated peanuts. However, AF destruction was estimated at 48–61 % in artificially contaminated peanuts with similar treatment conditions (Pluyer et al., 1987). Moreover, Yazdanpanah et al. (2005) marked that heating at the temperature of 150 °C for 120 minutes led to AF degradation by more than 95 % in pistachios (Yazdanpanah et al., 2005).

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 (Vázquez-Durán et al., 2014). 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 (Hussain et al., 2011). In another study by Herzallah et al. (2008) showed that in feed samples subjected toMicrowave, aflatoxin B1 contents significantly ($p<0.05$) decreased by 32.3 % (Herzallah et al., 2008). In another study by Mobeen et al. (2011) showed that in Peanut feed samples subjected to Microwave, aflatoxin B1 decreased by 63.3 %(Zain, 2011).

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 red 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 H_2O_2 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 (Jard et al., 2011).

It reveals the importance of microwave testing for reduction the toxicity of the edible products. But as stated formerly few studies have considered this issue and it seems more controlled studies should be carried out to clarify the appropriateness of microwave heating and the scope of applications.

According to the results of the current research, time and power of microwave are correlated with decreased concentrations of AF. However, application of high microwave power and time to reduce higher concentrations of AFs may result in poor-quality products.

Study limitations

We carried out the study on the limited types of AFB and on the limited number of samples, that might be considered as the study limitation. Also we studies the issue on a single substance (red pepper powder) and study on more edible products might be needed to reveal the issue more appropriately.

Conclusion

Therefore, it is recommended that the lowest microwave power and time be applied to evaluate the effects of this method on contaminated samples in order to maintain the quality of final product. In conclusion, findings of the present study indicated that microwave heating could cause a more significant reduction in AF concentrations, compared to normal heating.

Acknowledgment

The authors are thankful from the chencellory of research of Kerman University of Medical Scieneses for funding and supporting the study. We also are grateful of the collaboration and commitment of our colleagues in the fungal laboratory of the university.

Author contribution

PKh and MM collaborated in the research protocol and study desing, MRH and GA helped us in the laboratory jobs doin the statistics and MLN prepared the manuscript first draft.

Conflict of interest

The authors declare no conflicts of interest.

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