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Effect of microwave oven processing treatments on reduction of Aflatoxin B1 and Ochratoxin A in maize flour

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RESEARCH ARTICLE

ABSTRACT



The effect of microwave heat has been evaluated for reduction of Aflatoxin B1 and Ochratoxin A in artificially contaminated maize flour. Contaminated maize flour were heated in microwave for various times at two different power levels. The results suggest that Aflatoxin B1 and Ochratoxin A contamination can be reduced by heating samples using microwave oven. The exposure time to heat appears to have a great effect in reduction both of the toxins in maize flour samples. It is also necessary to pay attention on initial concentrations of Aflatoxin B1 or Ochratoxin A in studied maize flour samples.

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1. Introduction

Poor harvesting practices, improper drying, handling, packaging, storage and transport conditions contribute to fungal growth and increase the risk of mycotoxin production [1]. Moreover, mycotoxins are extremely stable and moderately heat-resistant compounds that remain almost intact after food processing [2]. Mycotoxins are fungal secondary metabolites found in many plant foodstuffs, particularly in cereals, fruits, nuts, kernels, spices, seeds, etc. animal fodder, and are toxic for humans and animals when ingested or inhaled with dust. The most relevant mycotoxins for food safety are Aflatoxins (AFs), Ochratoxin A (OTA), Patulin, Fumonisin, Zearalenone (ZEN), and Trichothecenes. These mycotoxins are produced by some species from the genera *Aspergillus*, *Penicillium*, and *Fusarium* and have multiple and combined toxic characteristics. These mycotoxins may be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, immunosuppressive, and/or estrogenic [3].

AFs and OTA are the most important naturally occurring mycotoxins in agricultural products [4]. AFs are difuranocoumarins composed from two furans and a coumarin ring [5]. AFs are highly carcinogenic and hepatotoxic and are primarily

found in peanuts, maize, nuts, spices, and in milk [6]. Among 18 different types of AFs identified, major members are Aflatoxins B1, B2, G1, G2, M1, and M2 (AFM1 and AFM2 are the hydroxylation products of AFB1 and AFB2, have been identified in milk and dairy products). Aflatoxin B1 (AFB1) is the most abundantly produced and the most toxic followed by G1, B2, and G2. AFB1 is classified by the International Agency of Research on Cancer as Group 1 carcinogen [7-9]. Aflatoxin s (AFs) are very stable and may resist quite severe processes like roasting, extrusion, baking, and cooking. For this reason, they can be a problem in processed foods, such as roasted nuts and bakery products [10].

OTA is a phenylalanine derivative of a substitute disocoumarin (R)-N-[5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl]-carbonyl-L-phenyl alanine. Its name is derived from *Aspergillus ochraceus*, the musty from which it was first isolated. OTA is the most toxic member of the Ochratoxins. This mycotoxin is structurally similar to the amino acid phenylalanine (Phe). Thus, it has an inhibitory effect on a number of enzymes that use phenylalanine as a substrate, in particular, Phe-tRNA synthetase, which can result in the inhibition of protein synthesis. It is a mitochondrial poison, causing mitochondrial damage, oxidative burst, lipid

Table 1. Effect of microwave heating on Aflatoxin B1 and Ochratoxin A concentration in contaminated maize flour at 50% power of high frequency output of 2450 MHz*.

Heating time (min)	Aflatoxin B1 ($\mu\text{g}/\text{kg}$)			Ochratoxin A ($\mu\text{g}/\text{kg}$)		
	T1 _{AFB1}	T2 _{AFB1}	T3 _{AFB1}	T1 _{OTA}	T2 _{OTA}	T3 _{OTA}
0.00	300.00 \pm 0.01	200.00 \pm 0.01	100.00 \pm 0.01	300.00 \pm 0.01	200.00 \pm 0.01	100.00 \pm 0.01
2.00	300.00 \pm 0.01	200.00 \pm 0.01	100.00 \pm 0.01	300.00 \pm 0.01	200.00 \pm 0.01	100.00 \pm 0.01
4.00	298.00 \pm 0.03	199.00 \pm 0.01	99.80 \pm 0.06	299.00 \pm 0.04	199.30 \pm 0.06	100.00 \pm 0.09
6.00	289.00 \pm 0.08	195.00 \pm 0.07	98.34 \pm 0.02	292.00 \pm 0.01	196.72 \pm 0.08	99.25 \pm 0.07
8.00	255.00 \pm 0.05	178.00 \pm 0.04	90.00 \pm 0.06	260.00 \pm 0.08	185.00 \pm 0.09	94.42 \pm 0.05
10.00	205.00 \pm 0.06	163.00 \pm 0.05	74.00 \pm 0.09	215.00 \pm 0.08	167.00 \pm 0.04	86.19 \pm 0.07

* Values are mean of 3 replicates (n = 3).

peroxidation, and interferes with oxidative phosphorylation. In addition, OTA increases apoptosis in several cell types and OTA has been classified as a possible human carcinogenic (Group 2B) [10,11]. OTA is a stable compound that is not destroyed by common food preparation procedures. Temperatures above 250 °C for several minutes are necessary to reduce the concentration of this toxin [12].

There are many methods for reduction of AFs and OTA such as physical (Cleaning, heating, irradiation, adsorption), chemical (chemical compound, ozonization) and biological (applying bacteria, yeast and nontoxigenic *Aspergillus* strains) [13]. Many researches interested in reduction of AFs and OTA in food and feed. The present study was planned to assess the effect of microwave oven processing treatments on reduction of Aflatoxin B1 and Ochratoxin A in maize flour as long as maize flour has wide uses and maize is considered one of the most mycotoxins contaminated crops.

2. Experimental

2.1. Materials

Acetonitrile and methanol (HPLC-gradient grade) were obtained from Sigma-Aldrich (Germany). Standard AFB1 were obtained from Supelco (Spin). Standard OTA were obtained from Sigma-Aldrich (Germany). Immunoaffinity columns for AFs were broached from Alfa Test (Germany), immunoaffinity columns for OTA were broached from Germany, potassium dihydrogen phosphate from Merck (Germany), iodine, sodium chloride from Panreac (Spin). Maize flour samples were broached from local markets.

2.2. Apparatus

HPLC method was performed on a Shimadzu (Kyoto, Japan) liquid chromatography system, equipped with a model LC-20 AT pump and CTO-20A oven. The detector was a fluorescence detector (Shimadzu® RF-10 AXL, Kyoto, Japan) programmed to monitor at 365 nm for excitation and 435 nm for emission. AFB1 and OTA were completely separated using a stainless steel column of dimension (4.6 × 250 mm²) packed with symmetry C18 and 4 μm particle size (Merck, Germany). For the microwave application, a 2450 MHz microwave oven (Wattar, WST-61) was used.

2.3. Chromatographic conditions

The chromatographic experiments for determine OTA were carried out by using HPLC-FLD as follows; Mobile phase: Acetonitrile, water and acetic acid (60:39:1, v:v:v), Column oven temperature: 40 °C, Injection volume: 50 μL , Flow rate: 1 mL/min, Detection fluorescence: excitation at 333 nm, emission at 460 nm. The chromatographic experiments for determine AFB1 were carried out by using HPLC-FLD as follows; Mobile phase: Water, acetonitrile, and methanol (60:20:20, v:v:v), Column oven temperature: 40 °C, Injection volume: 50 μL , Flow rate: 1.3 mL/min, Detection fluorescence: excitation at 365 nm, emission at 435 nm.

2.4. Sample preparation and microwave heating

The study involved four maize flour samples, analyzed to be free of AFB1 by HPLC. One of the free samples of aflatoxin B1 (AFB1) was considered as control (C_{AFB1}). Three addition treatments, T1_{AFB1}, T2_{AFB1} and T3_{AFB1} were artificially contaminated with pure Aflatoxin of 300, 200 and 100 $\mu\text{g}/\text{kg}$ of AFB1. Aflatoxin s were dissolved in 5 mL of methanol of HPLC grade, and mixed with a mount of maize flour for 30 min. control and contaminated maize flour samples were then placed in plastic containers for 1 week. Maize flour samples were analyzed for Aflatoxin after storage. Two maize flour samples (about 100 g) in duplicate of each treatment (C_{AFB1}, T1_{AFB1}, T2_{AFB1} and T3_{AFB1}) were heated in microwave oven for 2, 4, 5, 6, 8, and 10 min at 50 and 100% power of high frequency output of 2450 MHz. For testing reduction of OTA in maize flour, it was done the same work mentioned above for AFB1.

2.5. Extraction of Aflatoxin B1 from samples

AFB1 was extracted from samples of maize flour treatment with microwave oven using 5 g of sodium chloride and 125 mL of methanol:water solution (30:70, v:v); the extract was filtered by filter 0.45 μm (PTFE), then 15 mL of filtrate was added into a conical flask with glass stopper, after that 30 mL of water was added and mixed and filtered the diluted extract through a glass paper [14]. The immunoaffinity column was prepared according to the supplier's instruction. Afterwards 1 mL of methanol was passed into the immunoaffinity column to elute the AFs, then 1 mL of water was added to the extracts.

2.6. Extraction of Ochratoxin A from samples

OTA was extracted from 10 g of sample by using 40 mL methanol:water (80:20, v:v). The extract was filtered, then 4 mL of filtered extract were diluted to 50 mL with phosphate-buffered saline (PBS), and applied to an OTA immunoaffinity column. The column was washed with water and the OTA was eluted from immunoaffinity column with methanol [15].

3. Results

In this research, the validated methods were used and the 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 and OTA recoveries were recorded by analyzing a blank sample, spiked at 4 ng/g for AFB1 and also 3 ng/g for OTA. Recovery rate for AFB1 was 75-80% and the average coefficient of variation was 4.9% and approximately the same for OTA. The AFB1 and OTA level was corrected, according to the recovery value. LOD and LOQ for AFB1 were 0.06 $\mu\text{g}/\text{kg}$ and 0.100 $\mu\text{g}/\text{kg}$, respectively. LOD and LOQ for OTA were 0.05 and 0.10 $\mu\text{g}/\text{kg}$, respectively. The linearity in the working standard solutions at three determinations of six concentration levels was reliable, 0.9996 for AFB1 and 0.9998 for OTA.

The effect of microwave heating on AFB1 and OTA concentration at 50 and 100% power of high frequency output of 2450 MHz is presented in Table 1 and 2.

Table 2. Effect of microwave heating on Aflatoxin B1 and Ochratoxin A concentration in contaminated maize flour at 100% power of high frequency output of 2450 MHz*.

Heating time (min)	Aflatoxin B1 (µg/kg)			Ochratoxin A (µg/kg)		
	T1 _{AFB1}	T2 _{AFB1}	T3 _{AFB1}	T1 _{OTA}	T2 _{OTA}	T3 _{OTA}
0.00	300.00±0.01	200.00±0.01	100.00±0.01	300.00±0.02	200.00±0.01	100.00±0.05
2.00	295.00±0.05	198.00±0.02	99.38±0.01	297.15±0.03	199.08±0.01	99.91±0.01
4.00	286.50±0.08	193.64±0.05	97.25±0.03	290.30±0.01	197.34±0.09	99.05±0.01
6.00	230.40±0.09	160.00±0.08	89.46±0.06	242.00±0.07	172.66±0.07	90.94±0.05
8.00	184.00±0.06	142.72±0.09	76.87±0.03	193.67±0.08	150.06±0.08	79.99±0.01
10.00	96.80±0.03	110.34±0.09	60.79±0.03	110.45±0.01	119.00±0.08	62.78±0.09

* Values are mean of 3 replicates (n = 3).

Table 3. Effect of microwave heating on aflatoxin B1(%) and ochratoxin A (%) in contaminated maize flour at 50% power of high frequency output of 2450 MHz*.

Heating time (min)	Aflatoxin B1 %			Ochratoxin A %		
	T1 _{AFB1}	T2 _{AFB1}	T3 _{AFB1}	T1 _{OTA}	T2 _{OTA}	T3 _{OTA}
0.00	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01
2.00	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.01
4.00	99.33±0.03	99.50±0.01	99.80±0.06	99.67±0.04	99.65±0.06	100.00±0.09
6.00	96.33±0.08	97.50±0.07	98.34±0.02	97.33±0.01	98.36±0.08	99.25±0.07
8.00	85.00±0.05	89.00±0.04	90.00±0.06	86.67±0.08	92.50±0.09	94.42±0.05
10.00	68.33±0.06	81.50±0.05	74.00±0.09	71.67±0.08	83.50±0.04	86.19±0.07

* Values are mean of 3 replicates (n = 3).

Table 4. Effect of microwave heating on aflatoxin B1(%) and ochratoxin A (%) in contaminated maize flour at 100% power of high frequency output of 2450 MHz*.

Heating time (min)	Aflatoxin B1 %			Ochratoxin A %		
	T1 _{AFB1}	T2 _{AFB1}	T3 _{AFB1}	T1 _{OTA}	T2 _{OTA}	T3 _{OTA}
0.00	100.00±0.01	100.00±0.01	100.00±0.01	100.00±0.02	100.00±0.01	100.00±0.05
2.00	98.33±0.05	99.00±0.02	99.38±0.01	99.05±0.03	99.54±0.01	99.91±0.01
4.00	95.50±0.08	96.82±0.05	97.25±0.03	96.77±0.01	98.67±0.09	99.05±0.01
6.00	76.80±0.09	80.00±0.08	89.46±0.06	80.67±0.07	86.33±0.07	90.94±0.05
8.00	61.33±0.06	71.36±0.09	76.87±0.03	64.56±0.08	75.03±0.08	79.99±0.01
10.00	32.28±0.03	55.17±0.09	60.79±0.03	36.82±0.01	59.50±0.08	62.78±0.09

* Values are mean of 3 replicates (n = 3).

Also, the percentage of reduction of AFB1 and OTA contamination in artificially contaminated maize flour by microwave heating at medium and high power level for 2, 4, 6, 8 and 10 min is shown in Table 3 and 4. Although, almost all treatment protocols showed some degree of AFB1 and OTA degradation, heating samples at high power level of microwave oven for 8 min and at medium level of microwave oven for 10 min caused the best reduction on level of AFB1 and OTA in tested samples. Effect of microwave heating at 100% power for 10 min on reduction of AFB1 and OTA in artificially contaminated maize flour can be seen in Table 2 and 4, it is noticed to be the higher reduction of AFB1 and OTA as compared to other treatments, but there was some changes in appearance of maize flour. The results in Table 1 and 2 appear that degradation of AFB1 and OTA is also dependent on their initial concentrations in samples, this might be due to availability of more active toxins for destruction during microwave heating.

4. Discussion

The results presented in this study have been shown a relatively degradation of AFB1 and OTA. Moreover, the degree of AFB1 and OTA reduction was found to be dependent on the duration of exposure to microwave heating and the initial concentrations of AFB1 or OTA in samples, and the destruction of Aflatoxins AFB1 and Ochratoxin A OTA were found to increase by increasing the power level of microwave oven too.

The effect of microwave heating on degradation of AFB1 and OTA was found to be relatively efficient just at longer time exposure and high level power of microwave oven; this could be due to the resistance of most mycotoxins to heat [16]. Also, the results were in agreement with those of Samarajeewa *et al.* [17] who found that 200 °C was required for degradation of 60% of AFs in olive oils. Also, the results in the present study were relatively in agreement with those of Ployer *et al.* [18], they found that microwave heating was equally effective in destroying 48 to 61% of AFB1 in peanuts. There were some

agreements with the study which was done by Hussain and Lutfullah, too [7]. Herzallah *et al.* had studied about of artificially contaminated feeds, and they found that microwave heating was not high efficient to decontaminate of AFs [19]. They explained that the lower efficacy of microwave heating in decontamination of AFs which could be due to the result of shorter heating time, which was 10 min, as well as the feed particle geometrical dimension, which influences decreasing the penetration depth of the microwaves through the feed, this could be due to the AFs of naturally contaminated feeds being embedded within the feed commodities, on the contrary to the feed artificially contaminated with AFs that are not fully embedded within the commodities. Therefore, the natural AFs might be less likely to be degraded with radiation treatments, because they are within the commodity protected from radiation versus being on the surface of feeds when artificially applied [19]. Depending on that, results can be explained the results in the present work.

5. Conclusion

It was sum up from the present study that AFB1 and OTA level reduction rate increase with increase in exposure time, microwave oven power level, and the initial concentrations of AFB1 or OTA in studied maize flour samples.

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Disclosure statement

Conflict of interests: The authors declare that we have no conflict of interest.

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Sample availability: Samples of the compounds are available.

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