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Destruction of AFT by Ultrasound Treatment

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ABSTRACT

Aflatoxins are a group of mycotoxins with highly toxic، mutagenic, carcinogenic and immuno-suppressive properties. Aflatoxins are secondary metabolites produced by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*. The aim of this study was to evaluate effect of ultrasound on detoxification of aflatoxin total including aflatoxin B_1, B_2, G_1 and G_2. For this purpose standard vials of aflatoxin solutions with concentrations of about 17.7 ppb AFT were treated by Ultrasound irradiation at constant frequency of 20 KHz with intensities 20, 60 and 100% for 10, 20 and 30 minutes. Aflatoxin contents were analyzed by high pressure liquid chromatography (HPLC) method.
Results showed that the amount of AFT reduced about 41% at constant frequency of 20 KHz with intensities 60% for 10 minutes.

KEYWORDS: Aflatoxin, Ultrasound, Detoxification, HPLC

INTRODUCTION

Aflatoxins are secondary metabolites of certain strains of the fungi Aspergillus flavus and Aspergillus parasiticus [1]. They are a group of mycotoxins with carcinogenic, mutagenic, and immuno-suppressive properties [2]. Many chemical, physical, and biological methods have been proposed for the degradation of Aflatoxins. The most usual naturally occurring aflatoxins in foodstuff are aflatoxin B1, B2, G1 and G2 [3].

Mycotoxins have been more strictly monitored in the past decades due to their horrible effects discerned in humans and animals; strong toxic effects in humans and animals have been connected to these molecules, such as neurotoxicity, carcinogenicity, hepatotoxicity, cytotoxicity, mutagenicity, immunosuppressive, and effects of estrogenic [4, 5]. In peanuts *(Arachis hypogaea L.), Aspergillus sp.* correspond to the principal class of fungi that are collaborated to aflatoxin pollution, producing the types B1, B2, G1 and G2 [6]. Pistachio is the second non-oil export of Iran. For keeping the position of Iran in the global marketing and specification of pistachio, more efforts should be made. The main problem of pistachio exports is the Aflatoxin contamination [7].

Multiple strategies for the detoxification or inactivation of aflatoxins polluted feed-stuffs have been used such as physical separation, thermal inactivation, irradiation, microbial degradation and treatment with a diversity of chemicals [8]. There has been important debate over non-thermal effect of microwave (MW) radiation Non-thermal effect (MW specific athermal effect) was mentioned to have a considerable role in the inactivation of microorganisms in suspension [9].

For many years, the use of ultrasound inside the food industry has been a topic of research and progression. Power ultrasound has appeared as an alternative processing option to routine thermal approaches for sterilization and pasteurization of foodstuff products in last decade. Processing of ultrasound on its own or including heat and/or pressure is an efficient processing device for phytochemical retention and microbial inactivation. Benefits of ultrasound include higher yield, decreased processing time, and degrade energy consumption [10]. So the goal of this study was to examine the effect of ultrasound irradiation on amount of aflatoxin "in vitro" condition.

MATERIALS AND METHODS

All the chemicals were purchased from Merck chemical companies.

AFB_1, AFB_2, AFG_1 and AFG_2 with a purity higher than 98% were purchased from Sigma–Aldrich. Vials of aflatoxins samples including 1.5 ml standard solutions with constant concentrations of 8.1ng ml^{-1} AFB_1, 2.8ng ml^{-1} AFB_2, 5.1ng ml^{-1} AFG_1, 1.7 ng ml^{-1} AFG_2 (and 17.7ng ml^{-1} AFT).

Stock solutions of 1000 ng ml^{-1} for AFT were prepared in acetonitrile and left at 4 ˚C. The working solutions were prepared daily. HPLC-grade acetonitrile was obtained from Merk. High purity water used in research was obtained from a MilliQ purification system.

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Vials of aflatoxins samples were treated for ultrasound by Misonix Sonicator-XL2020 that has a fixed frequency at 20 KHz, and a variable power output with a maximum of 1000 W. To this end vials of aflatoxins were sonicated at various acoustic amplitudes 20, 60 and 100% for 10, 20 and 30 minutes and constant frequency of 20 kHz and then the residual AFT was measured by HPLC.

Aflatoxins were identified by confirming its retention time with standard aflatoxins by high-performance liquid chromatography (HPLC) technique. HPLC is the most regularly and greatly used method of mycotoxin analysis. HPLC reference methods that are completely sensitive and have rationally low levels of detection have been advanced for most of the major mycotoxins; thus, these are good methods for quantitative [11]. The HPLC equipment Waters - Alliance 2695 (United State) with a reversed-phase column (ODS-C18, 5µm, 4.6 mm×250 mm) with auto-sampler and fluorescence detector was used. All used chemicals were analytical or HPLC purity grade: sodium chloride, phosphate buffered saline (PBS), glacial acetic acid; acetonitrile andmethanol; poly ethylene glycol 6000 (Merck, Hohenbrunn, Germany); Aflatoxins (Sigma-Aldrich Chemie, Steinheim, Germany); immunoaffinity columns (R-Biopharm Rhone, Glasgow, Scotland). The analytical procedure was internally validated by means of calibration curve and recovery test. The calibration measurements were carried out with Aflatoxin standard solutions at concentrations 0.4, 1.2, 2.00, 2.80, 3.60, 5.60, 7.20 ng ml^{-1} for AFB_1 and AFG_1 and at concentrations 0.09, 0.24, 0.4, 0.56, 0.72, 1.12, 1.44 ng ml^{-1} for AFB_2 and AFG_2. The recoveries of aflatoxins using IAC columns for sample pretreatment were studied by spike standard solutions.

RESULTS AND DISCUSSION

The standard solutions of aflatoxins were used to find calibration/standard curve as delineated by the following regression equations for different aflatoxins, all data were showed in Table 1.

Table1. Equations and R- squared value for different aflatoxins standard curve

aflatoxins	Equation	R- squared
AFB_1	y = 2E+06x - 11361	0.9955
AFB_2	y = 3E+06x - 37051	0.9954
AFG_1	y = 88218x - 26181	0.9914
AFG_2	y = 1E+06x + 15177	0.9864

(Where y = area and x = amount of toxin.)

The results exhibited the linearity of the standard curve over the range studied. The coefficient of determination (R^2) was high for all the fractions. Figure 1 shows the calibration curve of standard solutions of AFB_1. The LOQ (limit of quantitation) amounts for AFB_1, AFB_2, AFG_1, AFG_2 and AFT were respectively 0.4, 0.08, 0.4, 0.08 and 0.96 µg/kg.

Concentration(ppb)	area
7.2	12682229
5.6	9305844
3.6	5923892
2.8	4904772
2	3002829
1.2	2231712
0.4	685731
Slope	1733874.775
Intercept	-113619.411

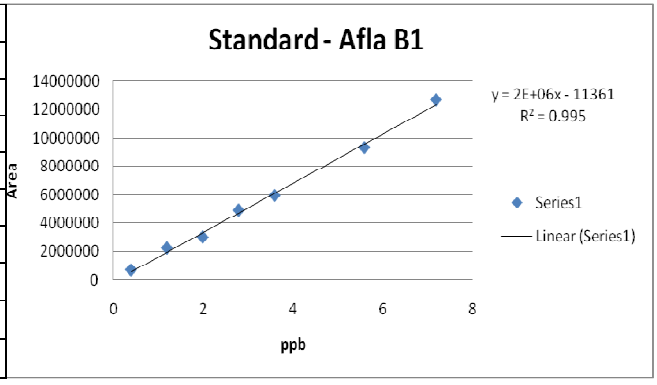

Fig.1. Calibration curve of standard solutions of aflatoxin B_1 by high-performance liquid chromatography analysis

Ultrasound irradiation was used in the present work in order to destroy aflatoxins were sonicated at various acoustic amplitudes 20, 60 and 100% for 10, 20 and 30 minutes and constant frequency of 20 KHz (Table 2 shows the number of treatments). Modern analysis of aflatoxins relies heavily on HPLC employing various adsorbents depending on the physical and chemical structure of the aflatoxins.

After treating the samples and analysis the aflatoxin contents by HPLC techniques, results which are given in Table 3 shows that the amount of AFB_1, AFB_2, AFG_1, AFG_2 and AFT maximum reduction is about 41% in treatment 4. Figure 2 shows the curves of the effect of treatments on aflatoxins.

Table 2.The number of treatments based on Ultrasound irradiation

amplitude	10 min	20 min	30 min
20%	T1	T2	T3
60%	T4	T5	T6
100%	T7	T8	T9

(T: Treatment)

The efficiency of a method depends to different factors, so the comparison will be more reliable when the impact of irradiation is investigated in a food system such as a feed stuff, but in such a system the intervening variables could not be controlled well. In a food system, we must use irradiation in a dose which does not affect on quality parameters of the system.

Nowadays power ultrasound emerges be as an optional processing to customary thermal approaches for sterilization and pasteurization of food products. Process of ultrasound on its own or in join with heat or pressure is an impressive processing tool for inactivation of microbial and phytochemical retention. The advantages of using ultrasound includes: lessen time of processing, increase throughput, and diminished energy consumption [10]. Rawson, Tiwari et al. (2010) examined the thermosonication's effect on the bioactive compounds of recently compressed watermelon juice [12]. Vilkhu et al (2008) reported that Ultrasound processing is also to increase distillation yield of bioactive substance by about 6 and 35% although depending on the processing situation [13]. Rawson, Tiwari et al. (2010) informed that sonication temperature played a important role in protection of bioactive mixture [12].

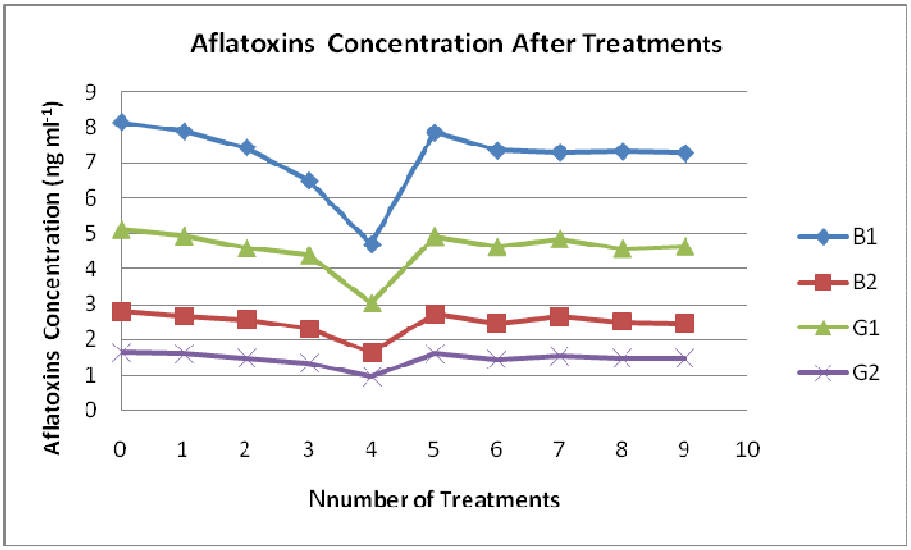

Fig.2. Aflatoxins concentration after treatments
(Treatment zero is primary concentration of Aflatoxins)

Table 3.Effect of Ultrasound irradiation on reduction rate of aflatoxins (ng ml^{-1})

Aflatoxin type	Aflatoxin conc. Before Treatment	LOQ* (ng ml^{-1})	Reduction rate (%)								
			T1	T2	T3	T4	T5	T6	T7	T8	T9
AFB_1	8.1	0.4	3.0	8.7	20.3	42.3	3.4	9.7	10.4	10.0	10.6
AFB_2	2.8	0.08	4.4	8.8	17.7	41.7	2.8	12.5	4.8	10.6	12.6
AFG_1	5.1	0.4	3.4	10.2	14.0	40.9	3.8	9.6	5.4	10.4	9.3
AFG_2	1.7	0.08	2.3	10.7	19	41.6	2.4	12.0	5.8	10.3	10.5
AFT	17.7	0.96	3.3	9.3	18	41.7	3.4	10.3	7.6	10.2	10.5

*LOQ: limit of quantitation T: Treatment

Electric power and mechanical power are the rate of doing work, deliberate in watts, and depict by the letter P. The expression wattage is used informally to mean "electric power in watts." The electric power in watts generated by an electric current I composed of a charge of Q coulombs every t seconds passing through a potential of electric (voltage) difference of V is

$$P = \text{work done per unit time} = \frac{VQ}{t} = VI$$

Where

Q shows electric charge in coulombs
t shows time in seconds
I shows electric current in amperes
V shows electric potential or voltage in volts

An alternate way to infer this formula is to note that voltage is delineated as $V = dW/dQ$, the amount of work that a unit charge (one coulomb) does when it moves between the two terminals, and the current is defined as $I = dQ/dt$, the number of coulombs flowing for each second, so

$$P = \frac{dW}{dt} \quad (\text{rate of work done per unit time})$$

$$= \left(\frac{dW}{dQ}\right)\left(\frac{dQ}{dt}\right) \quad (\text{work done per unit charge} \times \text{charge flowing per unit time})$$

$$= VI \quad (\text{voltage} \times \text{current})$$

Table 4 shows amounts of Energy and Power during ultrasound irradiation. Calculation of power and energy for each treatments indicated that increasing time of irradiation decreased power.

Table 4. Amounts of Energy and Power during ultrasound irradiation

amplitude		10 min		20 min		30 min	
20%	En	T1	55.4	T2	70.7	T3	87.3
	P		92.3		59		48.5
60%	En	T4	83	T5	145.8	T6	210.5
	P		138.3		121.5		117
100%	En	T7	110.5	T8	220.5	T9	320.2
	P		184.2		183.7		178

Conclusion

Ultrasound is well known to have a significant effect on the rate of various processes in the food industry. The advantages of using ultrasound si faster energy and mass transfer, reduced thermal and concentration gradients, reduced temperature and elimination of process steps.

Using ultrasound irradiation could remove significantly different aflatoxins in-vitro conditions (about 41%). Although this isn't a high rate of detoxification but the results of other research have indicated the use of ultrasound at a frequency of 20 KHz didn't have a great effect on food quality. But in model systems, there is some differences in the environmental conditions. The results of HPLC showed that ultrasound irradiation reduces aflatoxin in all treatments compared with the prototype and its toxicity is reduced. This seems to reduce the toxicity of aflatoxins is related to opening and hydrolysis of the lactone ring of aflatoxin, And produce nontoxic aflatoxin D1 or for the loss of one of the double bonds of the furan ring or loss furan ring as a result of cavitation effect on the structure of aflatoxins.

We suggest investigating the effect of different ultrasound irradiation in-vivo conditions to have possible and economical solutions to detoxify food and feed stuffs while the qualitative parameters of the systems preserved.

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