JCHR (2021) 11(1), 17-22

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Using Response Surface Methodology for Assessment of Heating Effect on Reduction of Aflatoxin

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(Received: 27 January 2020 Accepted: 1 November 2020)

KEYWORDS

Aflatoxin;
Heating;
Response surface methodology (RSM)

ABSTRACT: Considerable attempts have been made for the complete elimination of aflatoxins (AFs), as potent health hazards to both humans and animals, or reduction of their content in foodstuffs with increasing the knowledge and awareness of these toxins. In spite of the fact that the most effective intervention is considered prevention, heating has been also applied for the inactivation of AFs in contaminated foodstuffs. In the present study, the adoption of response surface methodology was evaluated for the assessment of the effect of heating on the reduction of AFs. Despite various degrees of AF decrease in the samples by treatment, a significant reduction was observed in the heated samples at a temperature of 90°C for 240 min. According to the results of the current study, a 23.70 % reduction was reported in the amount of aflatoxin B_1 (AFB_1). Various treatment conditions demonstrated a significant difference in AFB_1 decomposition ($P<0.05$). In addition, AFB_1 degrading was reported to be depending on both time and temperature. After the statistical analysis of the obtained data, the third-order equation for the reduction of AFB_1 is presented as follows (A: Time; B: Heating): $AFB_1 = 12.28 + 6.24A + 4.95B + 1.12AB - 3.11A^2 + 1.55B^2$

INTRODUCTION

The prevention of mold contamination in some foodstuffs has become a public health problem over the previous decade. Carelessness in the observation of good agricultural practice rules (concerning cultivation or harvest) or desired states for the invasion of toxicogenic *Aspergillus flavus* strains may result in the production of extremely toxic aflatoxins (AFs) [1]. Mycotoxins as natural toxins are produced by some fungal species and related to morbidity or even mortality in animals, plants, and humans. Different chemical structures and low molecular weight are reported as the features of mycotoxins. These compounds are often observed in a great number of agricultural products and foodstuffs around the world. The contamination of human food or animal feed in various steps, such as production, harvest, transport, and storage of agricultural products, can be due to mycotoxins. However, particular geographical regions or climates have not been reported as endemic for these fungi. Indeed, fungal growth and toxin production occur only in case of the presence of appropriate environments and conditions [2].

Mycotoxins consist of AFs as the most important group, produced by different *Aspergillus* species, namely *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* [3]. The AF has been introduced among the cancer-

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DOI: 10.22034/jchr.2020.1891864.1095

causing agents by the International Agency for Research on Cancer [4]. Despite the fact that prevention is identified as the most effective intervention, the adoption of chemical, biological, and physical techniques has been investigated for the inactivation of AFs or reduction of their content in foodstuffs [5]. Several factors, primarily including process optimization enhancement, causing the reduction of the production cost are investigated by various laboratories [6] according to the classical technique of medium optimization through altering one independent variable while keeping all other variables at a fixed level. However, the aforementioned process is highly time-consuming and costly in case of a great number of variables.

Nevertheless, the restrictions of the single-factor optimization process can be removed using statistical experimental designs, such as the two-level factorial and response surface methodology (RSM) [7]. In this regard, one of the best approaches for the optimization experiment is RSM as an empirical technique adopted for multiple regression analysis using quantitative data obtained from properly designed experiments in order to simultaneously solve multivalent equations [8]. Therefore, the current study evaluated the effects of heating in a laboratory setting to propose an optimal heating condition and central composite design (CCD) for the significant reduction of cost and time.

MATERIALS AND METHODS

Preparation of samples

The samples of the present study were prepared by the use of a laboratory grinding mill and 40-mesh sieve. The samples were mixed using a mixer to obtain a uniform composition. Subsequently, the decreasing impact of heating on AF contamination in red pepper powder was evaluated in this study.

Extraction method

To this end, 10 g of the ground sample was mixed in 60 ml of 80% methanol for 30 min, using a shaker, and then filtered. Following that, the product was centrifuged for 30 min, and re-filtration was performed. Eventually, 0.25 ml of Tween was added to 5 ml of the filtered extract and stirred for 2 min.

Separation of aflatoxin using immunoaffinity column

To this end, 3.1 ml of the filtered extract was diluted in 9.9 ml of distilled water and filtered by means of a microfiber filter. Subsequently, 12.6 ml of the extract was utilized for the immunoaffinity column, which was preconditioned with 10 ml of phosphate-buffered saline (14 drops per min). Following passing the extract through the column, the column was rinsed twice with 15 ml of water and then dried. The AF was collected in the vial by 1.25 ml of methanol and diluted with 1.75 ml of deionized water. Eventually, 100 µl of the solution was injected into the high-performance liquid chromatography (HPLC) system.

Measurement of aflatoxin by HPLC

By the use of HPLC equipped with a fluorescence detection system, the level of AF was determined using the C18 silica gel column and Kobra cell derivatization at excitation and emission wavelengths of 365 and 435 nm, respectively. The mobile phase included water-acetonitrile-methanol (30:20:60 v/v/v) containing 120 $Mg.L^{-1}$ of potassium bromide and 350 µl of 4M nitric acid, with a flow rate of 1 mL per min and an injection volume of 100 µL.

Optimization procedure

As a statistical method, RSM is adopted for the determination of the significance of parameters. The RSM is not only applied for the optimization of parameters in the process but also the investigation of the integrated effects of medium components. The CCD was utilized for the identification of important medium components. Two components were chosen as the variables of the present study, and each variable was shown at two levels of high (+) and low (−) in 12 trials, as presented in tables 1 and 2. Each row shows a trial; however, each column presents an independent (assigned) variable. The effect of each variable, sum of squares, mean square, F-value, P-value, and confidence level (%) were determined using Design-

Expert statistical software package (Version 7.0; State-Ease, USA), as shown in Tables 3.

Table 1. Applied variables in central composite design

Variable	Variable	+ values	− values
X1	Time (min)	10	240
X2	Heating (°C)	60	90

Table 2. Central composite design matrix of two variables of time (X1) and heating (X2) and noticed response

Run	Variable		Response	Predicted
	X1	X2	Experimental	
1	10	60	0.00	0.63
2	240	60	10.28	10.88
3	10	90	8.96	8.31
4	240	90	23.70	23.02
5	288	75	14.82	14.87
6	125	54	9.25	8.37
7	125	96	21.45	22.38
8	125	75	13.43	12.28
9	125	75	12.59	12.28
10	125	75	9.34	12.28
11	125	75	12.59	12.28
12	125	75	13.۴۳	12.28

RESULTS AND DISCUSSION

Heating is considered one of the physical techniques adopted for the reduction of AF contamination in edible foods. Therefore, this method can diminish the risks caused by the presence of AF in foodstuffs. As shown in tables 2 and 3 and Figure 1, heating reduces aflatoxin B_1 (AFB_1) content in red chili pepper powder. Various treatment conditions demonstrated a significant difference in AFB_1 decomposition ($P<0.05$). In addition, the process of degrading AFB_1 was dependent on time and temperature. After the statistical analysis of the obtained data, the third-order equation for the reduction of AFB_1 is presented as follows (A: Time; B: Heating):

$$AFB_1 = 12.28 + 6.24A + 4.95B + 1.12AB - 3.11A^2 + 1.55B^2$$

Table 3. Analysis of variance for response surface quadratic model

Source	Sum of squares	DF	Mean square	F-value	P-value
Model	392.15	5	78.43	31.85	0.0003
A-T	186.77	1	186.77	75.86	0.0001
B-H	196.35	1	196.35	79.75	0.0001
AB	4.97	1	4.97	2.02	0.2051
A^2	40.84	1	40.84	16.59	0.0066
B^2	15.57	1	15.57	6.32	0.0456
Residual	14.77	6	2.46		
Lack of fit	3.29	2	1.65	0.57	0.6039
Pure error	11.48	4	2.87		
Corrected total	406.93	11			

R-squared=0.9637; Adjusted R-squared=0.9334; Predicted R-squared=0.7889

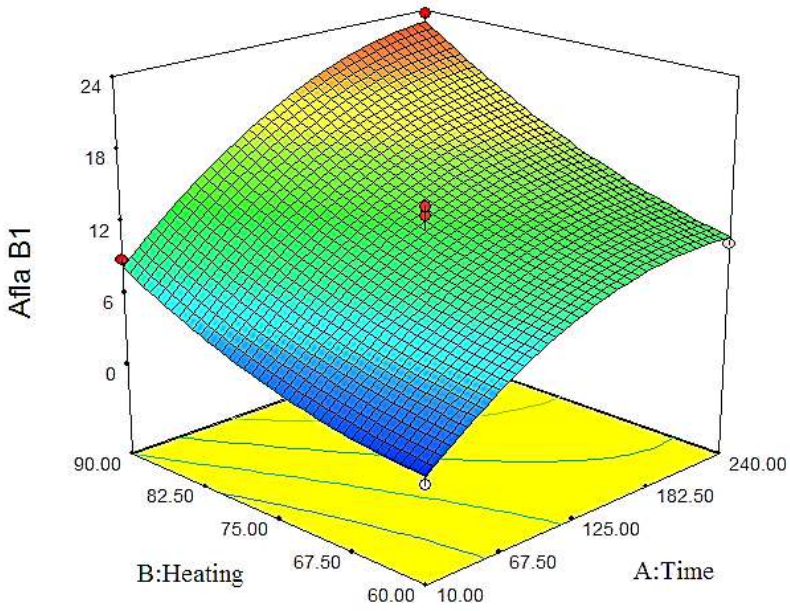

Figure 1. Three-dimensional response plot illustrating impact of heating on lowering aflatoxin B_1 contamination in red pepper powder

In various regions of Iran, some generations and species of toxigenic fungi and AFs have been discovered in different kinds of food, air, and equipment. Based on the evidence, it was concluded that in order to reduce food-processing time, farm-management and food-storage functions are considered essential. Furthermore, these efforts can cause the prevention or minimization of toxins in agriculture, industry, and food production for the enhancement of human and animal health [9].

The findings of another study showed that contamination with AFs (range: 0.2-57.5 µg.kg^{-1}) was reported in 30.8% of the samples (n=37), out of 120 investigated samples. According to the results, all the aforementioned samples were contaminated with AFB_1 (range: 0.7-57.5 µg.kg^{-1}). A significant difference was demonstrated between the samples regarding moisture content through the investigation of the effective factors in the level of AF (P=0.046). Moreover, the packaging of the samples did not have a substantial effect on the level of AF (P=0.578) [10].

Another study was carried out on the effectiveness of different physical (i.e., ultraviolet irradiation, heating, and microwave), chemical (i.e., oxidation, bleaching, ammoniation, and sulphitation), and biological treatment techniques in the detoxification of AFB_1 in red chili powder. A maximum (83.32%) decrease in AFB_1 was produced using direct oven heating (at a temperature of 120°C) among the physical techniques. The inefficiency of other chosen chemical compounds in AFB_1 was shown except for oxidation with H_2O_2 causing degradation of 58.32%. The treatment of spiked chili powder with purified peroxidase resulted in biological detoxification of 66.2%. The aforementioned study demonstrated higher efficacy of the physical methods in comparison to other techniques in AFB_1 degradation [11].

Several factors, including the concentration of AFs, extent of binding between AFs and food constituents, heat penetration, moisture content, pH, ionic strength, and processing conditions, are effective in the efficacy and extent of a reducing technique [12]. The results of another study showed a significant reduction in the AFs content of nuts, corn, and oilseed meals resulting from roasting. Degrading AFs through roasting depended on both time and temperature. Higher than 95% of AFB_1 degradation in peanuts was reported by roasting at 150°C for 120 min. In the aforementioned study, naturally occurred AFs were more resistant to degradation by heating in comparison to artificially contaminated samples [13]. Heating at a temperature of 150°C for 120 min resulted in higher than a

95% reduction in the AF content of pistachio [14]. According to the literature, diverse treatment conditions caused a significant difference in the decomposition of AF ($P<0.001$). In addition, degrading AFB_1 was shown to be dependent on time and temperature [15].

Another study demonstrated that the mean AF contamination in the roasted samples (16.53 $\mu g.kg^{-1}$) was significantly higher in comparison to that reported for the raw nuts (7.25 $\mu g.kg^{-1}$) ($P<0.001$). Following the measurement of the moisture content of the samples and statistical analysis, a significant correlation was observed between the level of AF and amount of moisture in the samples ($P<0.001$) [16]. Based on the results of previous studies, AF degradation is under the influence of heating. On the other hand, heating at high temperatures (for a further reduction of AF in samples) causes the resulting product to be inedible. Therefore, the lowest possible temperature should be considered before performing toxicological studies and evaluating the effect of temperature on the samples for the prevention of damage to the final product.

CONCLUSIONS

The degradation of AFB_1 was shown to be dependent on both time and temperature. After the statistical analysis of the obtained data, the third-order equation for the reduction of AFB_1 is presented as follows (A: Time; B: Heating):

$$AFB_1 = 12.28 + 6.24A + 4.95B + 1.12AB - 3.11A^2 + 1.55B^2$$

According to the obtained results of the present study, time and heating are correlated with decreased concentrations of AFs. However, the application of high temperatures and long time for the reduction of higher concentrations of AFs may result in poor-quality products. Therefore, it is recommended to apply the lowest temperature and shortest time for the assessment of the effects of this method on contaminated samples in order to maintain the quality of the final product.

ACKNOWLEDGEMENTS

The author would like to express his gratitude to Pharmaceutical Sciences and Cosmetic Products Research Center, Kerman University of Medical Sciences, Kerman, Iran.

Conflicts of interest

The author declares that there is no conflict of interest.

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