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The Effect of Thermal Processing on the Degradation of Aflatoxin B_1 Within Matrix Iranian Traditional Pastries

Abstract

Food contamination with aflatoxins is a significant problem worldwide, and many countries have set limits for mycotoxin presence in feed and food commodities. In this study to evaluate the effect of temperature on the degradation of aflatoxin B1 in a food matrix, the concentration of aflatoxin B1 in 90 samples including pistachio nut, pistachio confection, and baklava bakemeat were evaluated by FL-HPLC after clean-up of extracts through Aflatest IACs. It is noteworthy that pistachio nuts containing aflatoxinB1 were used to produce pistachio confection and Baklava bakemeat. Also, the physical-chemical and microbial properties of samples and environmental factors affecting the concentration of aflatoxinB1 were surveyed. Results showed that there was no significant correlation between humidity and temperature of the production and packaging shop floors, moisture, and peroxide of samples with aflatoxin concentration. Only temperatures above 20 ˚C had a significant relationship with the probability of contamination to aflatoxinB1. But, with an increase in the rate of the humidity shop floor, peroxide, and moisture of product the probability of aflatoxinB1 contamination increased. Also, there was a significant relationship between mold counting and pH over 6.3 with the aflatoxin concentration of samples. The concentration of aflatoxin B1 in pistachio confection decreased by 19.81% and in baklava bakemeat by 36.93% compared to pistachio nuts. Regarding the different processes of baklava bakemeat and pistachio confection production, it seems that despite the effect of the food matrix, the temperature has an effective role in reducing the concentration of aflatoxin B1.

Keywords: *AflatoxinB1, Confection, Thermal processing, Pistachio, Baklava*

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1. Introduction

Several hundred different mycotoxins have been identified, but the most commonly observed mycotoxins that present a concern to human health and livestock include aflatoxinB1, fumonisins, patulin, etc. Aflatoxins are fungal microbial secondary metabolites known as the most severely toxicant and carcinogenic materials. Aflatoxins are mostly produced by *Aspergillus flavus* and Aflatoxin B1, B2, G1, and G2 are the main aflatoxins among the 26 types of toxins recognized by researchers. AFB1 due to its carcinogenicity most dangerous aflatoxin is known. AFB1 has been classified as Group 1 carcinogen by the International Agency for Research on Cance (IARC) (Li et al, 2015;; Restuccia et al, 2019; Rushing and Selim, 2019). Numerous studies have confirmed that children may be exposed to aflatoxinB1 through contaminated food and AFM1 through the milk of mammalian exposed to AFM1. There is no doubt about the potential risk of aflatoxin exposure in diets and thus extensive efforts are required to reduce it

Weather conditions such as humidity or temperature are some of the factors which affect the production of aflatoxinB1 in many kinds of food, particularly in pistachio nuts. Wu et al., (2016) report a very high concentration of aflatoxin B1 in Chinese peanuts that had been exposed in fields with an arid climate (Wu et al, 2016). Also, increasing fungal resistant and insect resistant hybrids/crops to action against pre-harvest infections and their outcome is a matter of worry (Kumar et al, 2017). Currently, different methods are available For reducing the foods' mycotoxins contents, including aflatoxinB1. Optical pulse, e.g., cause demolition and detoxification of aflatoxinB1. It has been reported that J/cm2 of pulse for 80 s reduces the AFB1 up to 75% and AFB2 up to 39.2%, while pulse for 15 s reduces AFB1 up to 90.3% and AFB2 up to 86.7% both in the chaff and the hard part of rice. (Wang et al, 2016). Ozone has an important effect on the degradation of aflatoxinB1. Of course, AFB1 and AFG1 are more sensitive to Ozone but AFB1 is sensitive to lower densities of Ozone, and AFG1 is sensitive to higher densities of Ozone (Agriopoulou et al, 2016; Babolhavaegi et al, 2018). Also, essential oils such as lavandins Grosso and Abrial, Origanum virens inhibited *Aspergillus parasiticus* growth and phenolic acids such as caffeic, chlorogenic, ferulic, and p-coumaric at a concentration of 20 mM are able to destroy aflatoxins (Loran et al, 2022). The CotA laccase from Bacillus subtilis, particularly with plant extracts as a mediator, can used to simultaneously degrade AFB1 and zearalenone in food and feed (Wang et al, 2019). It has been reported that the concentration of AFB1 in soya is reduced in 18 hours using an organic acid solution of citric acid, lactic acid, succinic acid, and tartaric acid normal up to 94.1%, 92.7%, 62%, and 95.1%. Moreover, the thermal processing at 100 °C and 150 °C for 90 minutes meaningfully reduces AFB1 up to 41% and 81.2%, respectively. The presence of moisture in foods causes an increase in the percentage of decomposition and demolition of aflatoxinB1 when exposed to heat; this occurs through lactone ring hydrolysis in effective densities of moisture and heat, or the presence of moisture in the environment stimulates the

chemical reactions in some mycotoxins and, consequently, changes their toxicity. Furthermore, the presence of proteins and other combinations in foods results in the maintenance and stability of aflatoxinB1 in heated food. This feature results from reduction of heat penetration and establishment of toxin through linking with proteins and other food ingredients (Lee et al, 2015). It has been also reported that a combination of non-aerobic fermentation and heat treatment can change the biological form of aflatoxinB1 in pea Pastries flour; of course, heat treatment and non-aerobic fermentation don't change the density of the amino acids in pea confection flour (Chen et al, 2015).

Flavanones existing in citrus fruits such as Naringin, Hesperidin, and NeoHesperidin, can prevent aflatoxin production by *A. flavus*. According to a report, 0.035-0.195 mmol of Naringin-NeoHesperidin mixture, 0.156-0.283 mmol of NeoHesperidin-Hesperidin mixture, and 0.206-0.037 mmol of Naringin-Hesperidin mixture can have a preventive effect on the production of aflatoxinB1 (Salas et al, 2016).

In addition to the above-mentioned methods, there is a new biological method that can prevent the growth of mold and the binding of aflatoxinB1 in different matrices.

The reduction in the growth of mold and production of aflatoxin may be caused by the competition of fungus and bacteria cells for survivorship in foods. Aflatoxins are most likely dependent on environmental conditions, and the dead cells have more capability to bind the AFB1 compared to the bacterial live cells. Lactobacilli have a special ability in binding to the B1 and M1 aflatoxins in foods. It seems that this is a reversible ability and the bounded aflatoxins will be freed again later (Zhao et al, 2018). Usually, microwave heating, cold plasma, electron beam, gamma irradiation, UV, pulsed light, and electrolyzed water are the newest methods in food and feed matrices for Aflatoxins reduction (Sadeghizadeh-Yazdi, 2019). Since confectionery products, particularly the pistachio confection and baklava bakemeat, are among the most commonly used food products in Iran, they can be an important source for transferring the aflatoxinB1 to the human body thus the present research is aimed to evaluate the concentration of aflatoxinB1 in pistachio confection and baklava bakemeat using HPLC-FL and to evaluate critical points of the process of pistachio Pastries production.

2. Materials and Methods

2.1. Sampling

Since Yazd province is the only producer of pistachio confection and baklava bakemeat in Iran, all the samples were gathered from this region. Regarding the Iranian sampling standard and considering the 95% confidence concentration and the primary study s=0.13, and also, concerning the 10% average estimation error (primary study) d=0.03, 90 samples of pistachio nut, pistachio confection, and Baklava bakemeat were collected.

Samples were randomly chosen from among the production factories in the city of Yazd. This research used low-quality pistachio nuts for the production of pistachio confection and Baklava bakemeat. Ranges of contamination in pistachio nuts with Aflatoxin B1 were equal to 15.37-437.21 (ng/g). To prepare the samples of pistachio confection and Baklava bakemeat, pistachio nuts were powdered and passed through a sieve with mesh-20, and then the samples were put in plastic bags and kept at -4 °C temperature until the time of test.

2.2. preparation of pistachio confection

To produce syrup, sugar was dissolved in water and the solution was stirred. The stirring motion also increases kinetic energy, which increases the temperature of the solution. The heat of the solution increases until boiling temperature and was stirred the solution well for 10 minutes at boiling temperature. Potassium tartrate was added to the boiling solution and heating continued until the Brix reaches 85%. Then natural flavors and pistachio powder were added to the solution and stirred until the temperature of the mixture reached 40°C and then was entered the stage of aging. Cutting was done at 15 ° C and finally labeled and packaged.

2.3. preparation of baklava bakemeat

The preparation step of the initial syrup was carried out according to the above method, and with this, the difference that the addition of natural flavoring and citric acid was done in 80% Brix.

In the next step, using confectionary flour, sugar, water, eggs, and oil, was prepared dough and after aging was spread on the bottom of the tray lubricated with oil. It is noteworthy that the flours used in the production of baklava bakemeat did not contain aflatoxin B1. Then the dough and syrup were mixed to form a three-layer cake. Then the three-layer cake was baked at 220 ° C for 20 minutes. At this stage, the syrup with 80% Brix and a temperature of 85 ° C was poured on the baked cake. At 180 ° C, pistachio powder was poured on the cake and was kept at this temperature for 10 minutes. Cutting was done at 15 ° C and finally labeled and packaged.

2.4. Chemical and reagents

Evaluation of the aflatoxinB1 concentration of the samples was done using materials, reagents, and detectors of MERCK (Germany), the HPLC machine was a WATERS (HPLC-FL 474, USA), the Immunoaffinity Aflatest Column (IAC) of VICAM Company (USA), and the aflatoxin standards were Sigma-Aldrich.

2.5. Determination of aflatoxinB1

AflatoxinB1 in samples was determined by high-performance liquid chromatography (HPLC) analysis according to the AOAC official method 999.07 with minor modifications (Cheraghali et al, 2007). First, 50 g of the sample was weighed by a scale with 0.1 mg sensitivity in a 500 ml-capped Erlenmeyer, and then 5 g of sodium chloride and 300 ml of the methanol-water mixture were added to it and stirred for 30 s. After that, 100 ml of hexane or cyclohexane (HPLC Grade) was added to it to remove fats. After preparing the standard aflatoxin solution, density was evaluated using a spectrophotometer. The working standard solution included 1000ng of B1 solved in Toluene-Acetonitrile (98 volumes of Toluene and 2 volumes of Acetonitrile). The content of the Erlenmeyer was transferred to a high-spin mixer and mixed for three minutes. The extract obtained from the sample was passed through a fluted filter paper and 10 ml of the extract was filtered through a glass microfiber filter.

2.5.1. Column preparation and purification

The reservoir was connected to the Aflatest IAC, and then we poured 10 ml of phosphate buffer saline (PBS) into the column and allowed it to pass the column at a 2 to 3 ml/min rate (velocity) without any external pressure. When 0.5 ml of PBS has remained in the upper part of the column antibody, we poured 60 ml of PBS into the reservoir and added 10 ml of the filtrated and transparent solution, which had been obtained in the previous phase, and mixed the contents with a spoon; then, we eluted the spoon with 1 to 2 ml of PBS and added the eluate to the reservoir. We allowed the extract to pass the column with a 2 to 3 ml/min flow rate without any external pressure. The column was eluted with 10ml water and was dried for 5 to 10s bypassing the air through the column with help of positive pressure. The aflatoxin was extracted from the column in two phases as follows: In the first phase, we poured 0.5 ml methanol into the column and allowed it to pass the column by the gravity force and without any external pressure. The extracted solution was gathered in a 3 ml volumetric flask. In the second phase, after one ml sample, 0.75 ml methanol was transferred to the column and the extracted solution was gathered in the same volumetric flask and the solution remained in the column was gathered in the volumetric flask under external pressure, then the volumetric flask was filled with water and the solution was stirred. It must be noted that whenever the obtained solution was not transparent, a filter (single-use filter made from cellulose or cellulose-nitrate with 0.45 µm hole-diameter) was used to make the solution transparent before injection to HPLC.

2.5.2. Determination of value using fluorescence detector and derivatization

Derivatization was done using Kobra Cell. In this method, in addition to regarding the recommendations of the manufacturing company, the flow velocity for the mobile phase was assumed 1 ml and the flow rate was assumed 100 µA, then 200 µl of the working standard solution was injected into the mixture. The limit of detection (LOD) was determined by spike samples. The spike concentration of AFB_1 pistachio nut was equal to 2 and for pistachio confection and baklava, bakemeat was 5 ng/g. The LOD for AFB_1 was 0.1 and 0.4 ng/g for samples, respectively.

2.5.3. Extraction and clean up

Samples were analyzed either using an HPLC method (the AOAC official method 999.07) with some minor modifications (Cheraghali et al, 2007). Regarding HPLC analysis, pistachio confection slurries were extracted with methanol/water/hexane (300 ml/75 ml/100 ml). After filtration, the extract was diluted with water and filtered through a glass microfiber filter. For clean-up of samples, Aflatest IACs were used. First, 10 ml PBS was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at a flow rate of ca. 1 drop/s. The column was washed with 15 ml water and dried by applying a little vacuum. Finally, AF was eluted with methanol by the following procedure. First, 0.5 ml methanol was applied to the column which was passed through by gravity. After 1 min, the second portion of 0.75 ml methanol was applied and collected. The eluate was diluted with water and analyzed by HPLC.

2.5.4. AFB1 standard

After the preparation of a standard solution of individual AFB1, the concentration was determined using a UV spectrophotometer. These standards were used to prepare mixed working standards for HPLC analysis (Cheraghali et al, 2007).

2.5.5. Analysis of AFB1 by HPLC

AFB1 was quantities by reverse-phase HPLC and fluorescence detector with post-column derivatization (PCD) involving bromination (Cheraghali et al, 2007). PCD was achieved with a Kobra cell and the addition of bromide to the mobile phase. After dilution of AFB1 eluate with water, 100 µl was injected into HPLC. The analytical column used was C_{18}, 5 µm, 250 mm×4.6 mm i.d. Mobile phase was water: methanol: acetonitrile (54:29:17, v/v/v) with a flow rate of 1 ml/min. The fluorescence detector was operated at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. Each working day, a five points calibration curve was built for AFB_1 checked for the linearity, and used for quantification of AFB1 in samples.

2.5.6. Spiking process of samples

Internal quality control was performed in the evaluation of the reliability of the results of the AFB1 analysis. Regarding internal quality control, the accuracy and precision of the method were verified. In this regard, recovery of AFB_1 was recorded by analyzing blank samples spiked at 2 and 5 ng/g for AFB_1 (Table 1).

2.6. Physico-chemical and microbial properties

Physico-chemical and microbial properties (moisture, pH, peroxide, and mold counting) were evaluated according to the national standard of Iran to numbers 19696, 218, and 2395(ISISRI,2015; 2017; 2019). The peroxide measurement test in the pistachio nut was carried out in two steps. After extracting fatthe determination of peroxide content of extracting fat was carried out according to national Iranian standard no.4179, (animal and vegetable fats and oils - Determination of peroxide value-Iodometric (visual) endpoint determination)(ISIRI,2018). Method of the National Iranian Standard No. 672 (dry fruits –determination of the moisture content- test methods) was used to measure the moisture content of pistachio nuts (ISIRI,2015). Mold Counting is carried out according to national Iranian standard no.10899-2, (microbiology of food and animal feeding stuff - Horizontal method for the enumeration of yeasts and molds - part 2: colony count technique in products with water activity less than or equal to 0.95)(ISIRI, 2008). The Humidity/Dew point meter model HD-3008 was used to measure the temperature and humidity of the Production and packaging shop floor.

2.7 Method of Data analysis

The statistical data analysis was performed using the software SPSS v.24. The mean of the experiments done in triplicate and the significance of the difference between them were measured using the Tukey test at a confidence interval of 95%. Also, evaluation of the odds ratio (OR) was performed by Logistic Regression.

3. Results and Discussion

In this research were used Pistachio nuts contaminated with aflatoxin B1 for the production of samples.

After confirming the presence of aflatoxin in pistachio nuts, they were used in the preparation of baklava bakemeat and pistachio confection. Then, the effect of thermal processing on aflatoxin B1 amount and physicochemical properties and mold counting of baklava bakemeat and pistachio confection were evaluated. Temperatures 220°C for 20 minutes and above 100°C were used for the preparation of baklava bakemeat and pistachio confection. The concentration of aflatoxin B1 in pistachio confection decreased by 19.81% and in baklava bakemeat by 36.93% compared to pistachio nuts. The mean and standard deviation of AFB1 (ng/g) in baklava bakemeat and pistachio confection samples were 124.90±66.30 and 158.82±90.53.

It is noteworthy that, factors such as the temperature of the process and the percentage of pistachio nuts used for sample production, were effective in reducing the aflatoxin levels of these products. However, due to the copyright law and secrecy of the formulation, the percentage of pistachio nuts consumption has not been mentioned. It should be noted that the concentration of pistachio nut used in pistachio confection was higher but the temperature used to produce it was lower than that of baklava bakemeat. In this study, mold total counts were performed according to Iranian National Standard No. 10899-2. The results showed that there was no significant relationship between the humidity of the production and packaging shop floors and the aflatoxin concentration of samples. But, increasingly humidity, the odds ratio of contamination to aflatoxin increases. Of course, only temperatures above 20°C showed a significant relationship with the probability of contamination with aflatoxin. There was no significant relationship between moisture, peroxide, and concentration of aflatoxinB1 in samples. But with increasing moisture and peroxide, the OR of contamination to aflatoxin increased, while there was a significant relationship between mold counting and concentration of aflatoxinB1 in the samples. Also, there was a significant relationship between pH over 6.3 and aflatoxinB1 concentration of samples (Table 2). In Table 2, Numerical values above and below the permissible limit (According to the national standards of Iran No. 218, 2395, and 3493) has reported as yes and no (ISIRI, 2017;, 2019;, 2017). Mean and standard deviation of moisture, mold counting, pH, and peroxide the samples were stored at ambient temperature in Table 3 it has been shown. The Interquartile range and Median of the mold counting were 1405 and 1150 for pistachio Nuts, 537.5 and 930 for pistachio confection, and 262.5 and 340 for baklava bakemeat. The concentration of aflatoxin B1 in the samples has been shown in Figure 1. The median and Quarterly range of aflatoxinB1 concentration in samples in Table 4 have been shown.

Table 1. Mean and standard deviation of recovery AFB_1 in samples

Sample	Spike concentration (ng/g)	Mean±SD	Recovery %
pistachio nut	2	1.9±0.09	92.55
pistachio confection	5	4.3±0.41	86.12
baklava bakemeat	5	4±0.37	78.12

Table 2. Odds ratio (OR), confidence interval, and p-value for independent variables

Variables	OR	CI (95%)	P-value
Temperature ˚C			
<15	1	-	0.100
15-20	2.200	0.363-13.338	0.391
>20	4.931	1.007-24.144	0.049*
Humidity (%)			
<50	1	-	0.190
50-60	0.968	0.264-3.546	0.961
>60	2.667	0.736-9.665	0.135
Moisture (%)			
<10	1	-	-
>10	2.358	0.806-6.899	0.117
Peroxide (meq/kg)			
NO	1	-	-
YES	2.170	0.705-6.675	0.177
Mold existence			
NO	1	-	-
YES	2.961	1.010-8.684	0.048*
pH			
<6.3	1	-	-
>6.3	3.138	1.069-9.211	0.037*

Table 3. The mean and standard deviation of moisture, pH, and peroxide of stored samples in ambient temperature during the twenty-eight days

Samples	Moisture	pH	peroxide
pistachio nut (n=30)	5.05±0.53	6.26±0.25	0.95±0.39
pistachio confection (n=30)	13.87±1.42	6.49±0.23	1.67±0.34
baklava bakemeat (n=30)	10.25±0.68	6.18±0.34	3.65±0.68

Table 4. Median and Interquartile range of aflatoxinB1 concentration in samples

Sample	Interquartile range	Median	Mean	SD
pistachio nut	163.12	211.93	198.06	112.21
pistachio confection	128.87	171.35	158.82	90.53
baklava bakemeat	103.46	120.77	124.9	66.30

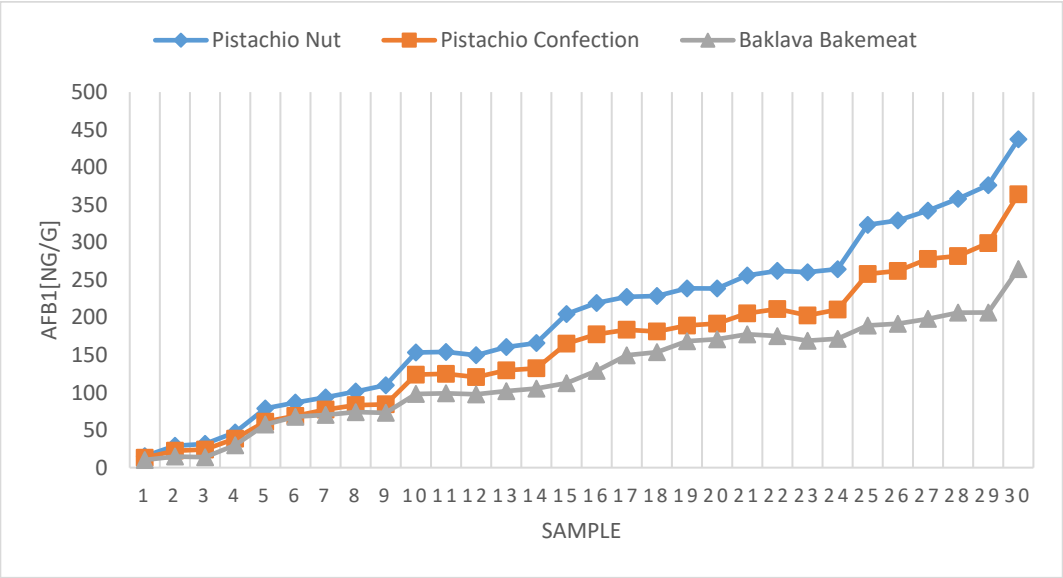

Figure 1. The concentration of Aflatoxin B1 (ng/g) in samples

Based on Food and Agriculture Organization (FAO) reports, annually, about 20% of the foods produced in the world are contaminated by mycotoxins; of which aflatoxinB1 has a greater share than the others (Wu et al, 2016). Although aflatoxinB1 is known as one of the most carcinogenic natural combinations for some animals, it is not yet clear whether it has the same effect on humans or not. It has been reported that the estimated absorption concentration for aflatoxin in cereal-based foods is equal to 0.003-0.852 $\mu gkg^{-1}bwd^{-1}$, and the AFB_1 has got the highest absorption concentration (Blankson and Mill-Robertson, 2016). Since the pistachio confection and baklava bakemeat are the traditional confectionery products of Iran and are not produced in other countries, it is not possible to compare the research findings with other similar cases; therefore, the comparison was performed between the research data and the results obtained from measurement of aflatoxin in pistachio and other products. Cheraghali et al. (2007) evaluated the concentrations AFB1, B2, G1, and G2 in different pistachios of Iran using HPLC method and found out that 11.8% of the samples had AFB1 above the maximum tolerated concentration (5 ppb), and the average concentration of AFT was 7.3 ppb, which was less than the maximum tolerated concentration (MTL) and the maximum permitted concentration according to the Foods Codex Commission (15 ppb) and only 7.5% of the samples had contamination above the MTL. The present research's findings are consistent with the findings of Cheraghali et al in terms of the presence of contamination with aflatoxinB1 in pistachios, but it seems that the lower concentration of contamination in their samples was because they were evaluated samples for exporting to other countries. As mentioned, pistachios nuts used for the production of samples, were contaminated with aflatoxinB1 and were of poor quality and thus were more likely to have aflatoxin contamination compared to the samples studied by Cheraghali et al

Set et al. (2010) evaluated the contamination concentration of bulk-sold pistachio and red pepper powder in Turkey using the HPLC method. They found out that in 17.1% of the bulk red pepper powders and 23.1% of the packaged red pepper powders the AFT was above the permitted concentration and the AFB1 concentration was equal to the permitted concentration and only one packaged sample had 89.99 ppb of AFB1. The concentration of contamination with AFT and AFB_1 in bulk pistachios was about 0.007-7.72 ppb. The reason for the difference between the research results and the contamination concentration of pistachios can be due to the effective factors of aflatoxin production (moisture, pH, peroxide, and mold counting) which were previously mentioned (Set & Erkmen, 2010;Priesterjahn, 2020). Also, the level of aflatoxin reduction in foods depends on several factors such as food ingredients, pH, and matrix (Pankaj et al, 2018).

Var et al. (2007) evaluated some samples of halva in terms of contamination with AFB1 using the TLC method. They discovered that the AFB_1 concentration in simple halva samples and the cocoa halva samples was less than LOQ and the average concentration of AFB_1 in samples containing pistachio was 7.5 ppb (Var et al, 2007). The results of War et al were almost consistent with the present research findings. Chun et al. (2006) evaluated the aflatoxin concentration in fruit Pastries and their products. They reported that 10.6% of the samples have different values of aflatoxin and maximally up to 28.2 ppb, which is consistent with the research findings (Chun et al, 2006). Ghali et al. (2009) evaluated the concentrations

B1, B2, G1, and G2 in sorghum and pistachio samples and reported that 52.5% of the pistachio samples were contaminated and their average contamination was 21.8 ± 38 ppb. The difference between the research findings with findings of the above researchers might be related to the sample preparation method, because Ghali et al. have used the reverse phase real-time sensitive method for evaluation of aflatoxin concentration, furthermore they have evaluated the pistachio samples while in the present research the samples are pistachio confection and baklava bakemeat which include other ingredients such as sugar, flour, etc, and it seems that the other constituents of the formulation intervented in the accurate diagnosis of aflatoxin (Ghali et al, 2009).

Iqbal et al. (2011) measured the concentration of aflatoxin in Pastries and cereals, which had been collected from the local supermarkets, after being imbued with A. flavus through the TLC method (thin layer chromatography). The aflatoxin concentration in cereals and Pastries was 14-45 ppb and 5-17 ppb, respectively. Based on the limit of aflatoxin by the FAO (50 ppb) all of the samples were recognized as consumable. Besides, long-term storage (18 months) than short-term storage (2-3 months) causes an increase in aflatoxin concentration. So, order to minimize the aflatoxin concentration requires avoiding storing the Pastries and cereals in warm and moist conditions. Studies have shown that the concentration of aflatoxin in hot peppers is directly related to the storage temperature so aflatoxin concentrations are 61% higher in hot peppers stored at 25 and 30 °C than in those stored at 20 °C. A gradual increase in temperature during prolonged storage with aeration may be the main reason for increases in aflatoxin concentrations (Iqbal et al, 2011). Moisture in foods increases the percentage of decomposition and elimination of aflatoxinB1 against heat and this is accomplished by hydrolysis of the lactone ring at the effective concentrations of humidity and temperature. Also, the presence of proteins and other compounds in the food induce preserves and stabilize aflatoxinB1 against thermal treatment, that this property is due to reduced heat penetration and toxin fixation by binding to proteins and other components of the food sample.

In this research, there was no significant relationship between the humidity of the production and packaging shop floor and the aflatoxin content of samples, and only temperatures above 20 °C had a significant relationship with the probability of contamination with aflatoxin. Research findings did not match Iqbal et al.'s results because we used low-quality nuts to evaluate the effect of the production process on the concentration of aflatoxinB1 in Pastries.

Diella et al (2018), 124 samples (almonds, apricot kernels, chestnuts, hazelnuts, peanuts, pistachios, walnuts and Brazil nut) evaluated in terms of aflatoxin concentration by using HPLC method. They found out that twenty samples (16.1%) were contaminated with Aflatoins of which 55% were non-adapted, according to Reg. 165/2010. The mean aflatoxin concentration (µg/kg) of total Aflatoins and AFB1 were 16.6 and 15.1, respectively. Pistachios appeared more exposed to AF pollution than the other nuts, with levels of total Aflatoins ranging from 8.8 to 387.3 µg/kg and of AFB1 from 8.2 to 354.5 µg/kg. The most of polluted samples was from Asia and AF pollution was different in the various Asiatic sub-regions aside of the type of nuts, and samples from Western Asia were the least contaminated (Diella et al, 2018).

In terms of contamination to aflatoxin, the research findings were consistent with Diella et al results, but their results indicate that the type of Pastries, process conditions, and formulation are important factors that affect the aflatoxin concentration. Zhao et al. (2018) have reported that there has a linear relationship between pH value and AFB_1 concentration in the range of 0.2–20 µM. The research findings were adapted to Zhao et al results (Zhao et al, 2018). In this research, there was a significant relationship between pH value and AFB1 concentration of samples. In fermented products such as cookies, besides temperature, the concentration of yeast and the fermentation time and PH plays a basic role in reducing aflatoxinB1 (Noroozi et al, 2020).

One of the limitations of the study was that the probability concentration of aflatoxin B1 transferred from other components of the formulation was not evaluated. Of course due to the reduced concentration of aflatoxin B1 in samples, if aflatoxin B1 has been added to the samples by other ingredients, it has been degraded by thermal treatment and this can indicate the destructive effect of heat on aflatoxin B1.

4. Conclusion

Regarding the different thermal conditions of baklava bakemeat and pistachio confection production, it seems that despite the protective effect of formulation components on aflatoxin B1, the temperature can have an effective role in reducing the concentration of aflatoxin B1. So that the results of this study showed, that the temperature can degrade by 19.81% and 36.93% of aflatoxin B1 in pistachio confection and baklava bakemeat.

According to the risks of long-term use of this toxin and its carcinogenic potential, it is necessary to have perfect and accurate control on factors such as temperature and moisture either during the plantation and harvest phase or during the storage of raw materials. Moreover, using processed pistachio nuts (roasted or radiated) is useful in avoiding the probable risks of this toxin. Also, the results of this research showed that thermal processing has a significant effect on reducing aflatoxin B1 in the baklava bakemeat and pistachio confection.

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Compliance with ethical standards

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