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Detection of Aflatoxin B1 in Some Canned Foods and Reduction of Toxin by Ultraviolet Radiation

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Abstract

This study was aimed to detect the presence of aflatoxin B1 in thirty nine samples of some canned foods (6 samples of beef meat, 4 samples of chicken meat, 6 samples of fish meat, 5 samples of mushroom and 18 samples of different types of legumes) which collected randomly from some Iraqi local markets using ELISA technique. Aflatoxin B1 was detected in thirty four samples and the concentration of toxin ranged from 2.5 ppb to 975 ppb.

UV radiation (365nm wave length) was used for detoxification of aflatoxin B1 from each type of tested samples with highest concentration (beef meat 975ppb, chicken meat 217 ppb, fish meat 75 ppb, mushroom 237.5 and legumes 207) at distance of 60 cm between UV source and tested sample for 30 minute exposure time. Results showed that UV radiation able to reduce aflatoxin B1 from 975, 217, 75, 237.5 ppb and 207 to 111, 30, 8, 44 and 23 ppb respectively and it is consider as an effective method which using for reduction of aflatoxin B1.

Keywords: Aflatoxin B1, canned foods, detection, detoxification, UV radiation.

الكشف عن سم الأفلا B1 في الأغذية المعلبة واختزال السم بأستخدام الأشعة فوق البنفسجية

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الخلاصة

هدفت هذه الدراسة للكشف عن وجود سم الأفلا B1 في تسعة وثلاثين عينة من من عينات الأغذية المعلبة (6 عينات من اللحم بقري، 4 عينات من لحم الدجاج، 6 عينات من لحم الأسماك، 5 عينات من الفطر و 18 عينة من البقوليات المختلفة) والتي تم جمعها بشكل عشوائي من بعض الأسواق المحلية العراقية بأستخدام تقنية ELISA. تم الكشف عن وجود السم في اربعة وثلاثين عينة وقد تراوح تركيز السم بين 2.5 جزء بالبليون الى 975 جزء بالبليون.

تم استخدام الأشعة فوق البنفسجية (بطول موجي 365 نانومتر) لغرض ازالة السمية لكل نوع من انواع العينات ذات التركيز الأعلى (لحم بقري 975 جزء بالبليون و لحم دجاج 217 جزء بالبليون و لحم السمك 75 جزء بالبليون والفطر 237.5 جزء بالبليون و البقوليات 207 جزء بالبليون) وكانت المسافة بين مصدر الأشعة والعينة هي 60 سم ومدة التعريض 30 دقيقة. وبينت النتائج ان الأشعة فوق البنفسجية قادرة على اختزال سم الأفلا B1 من 975 و 217 و 75 و 237.5 و 207 جزء بالبليون الى 111 و 30 و 8 و 44 و 23 جزء بالبليون على التوالي، وتعتبر الأشعة فوق البنفسجية وسيلة فعالة تستخدم لغرض اختزال سم الأفلا B1.

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Introduction

Mycotoxins are secondary metabolites which produced as a result of fungal attack [1], most of mycotoxins have a small molecular weights, and consider as a heat-stable compounds [2], more than 400 different types of mycotoxins are identified such as aflatoxins, ochratoxins, trichothecenes, patulin, fumonisins and zearalenone .

Aflatoxins are the most toxic class among mycotoxins, they consist of four major naturally-occurring compounds include aflatoxin B1, B2, G1 and G2. The B and G refer to the blue and green fluorescent colors produced by these compounds under UV light, while the subscripts 1 and 2 refer to major and minor components respectively, aflatoxin B1 is recognized the most acute and toxic class of aflatoxins for mammals and exhibits hepatotoxic, teratogenic and mutagenic properties, it is produced mainly by *Aspergillus flavus* and *A. parasiticus* [3, 4]. Aflatoxin B1 can be contaminate food and feed before and after harvest, during storage, transportation, and consumption [5]. Aflatoxin B1 contaminate a processed food and enter in general food supply and found in human and pet foods in addition to feed stocks for agricultural animals, the transformation of aflatoxin B1 can pass to the products into meat, egg and milk products by feeding animals with aflatoxin B1 contaminated food [6].

Studies have been looking for effective methods for detoxification, physical, chemical and biological methods have been used to destroying aflatoxins [7].

UV irradiation considered as a good physical method which used for destroying aflatoxins in food. Aflatoxin B1 has ability to absorb UV irradiation in different wave length and give a greatest absorption at 365 nm wave length, this wave length able to activate aflatoxin B1 and increase its susceptibility to degradation [8].

Our study aimed to investigate the presence of aflatoxin B1 in some canned foods and using of UV Irradiation for degradation

Materials and methods

Samples collection

Thirty nine samples of some canned foods (different types of canned meats, chickens, fishes, mushrooms and legumes) were collected randomly from some Iraqi local markets, Table-1, the collected samples were stored in the refrigerator until analyzed.

Table 1- Different canned foods samples with their origin.

Sample number	Brand	Origin
Meat samples		
1-	Beef hot dogs /Altaghziah	Beirut/ Lebanon
2-	Beef hot dogs /Maxima's	Brazil
3-	Beef luncheon meat /Altaghziah	Beirut/ Lebanon
4-	Beef luncheon meat /Maxima's	Brazil
5-	Beef luncheon /Hana	Amman / Jorden
6-	Corned beef/ Hana	Amman / Jorden
Chicken samples		
7-	Chicken hot dogs/ Altaghziah	Beirut/ Lebanon
8-	Chicken hot dogs diet/ Maxima's	Brazil
9-	Chicken luncheon meat /Altaghziah	Beirut/ Lebanon
10-	Chicken luncheon meat /Maxima's	Brazil
Fishes samples		
11-	Tuna /RIO mare	Thailand
12-	Tuna/ Sayad	Indonesia
13-	Tuna chunks /Durra	Thailand
14-	Tuna /Kasih	Jorden
15-	Tuna flakes/ Americana	Indonesia
16-	Tuna/ Heinz	Thailand
Mushroom samples		
17-	Mushroom /Kasih	Jorden

18-	Mushroom/ Teeba aljabal	Beirut/ Lebanon
19-	Mushroom slices /Maxima's	Brazil
20-	Mushroom pieces and stems /American garden	China
21-	Musroom sliced / Dilim mantar	Turkey
Legumes samples		
22-	Lima beans / Maxima's	Italy
23-	Lima beans with tomato sauce/ Chtoura	Lebanon
24-	Red beans/ Maxima's	Italy
25-	Lima beans with tomato sauce / Maxima's	Italy
26-	Chickpeas/ Kasih	Jorden
27-	Chickpeas / Durra	Jorden
28-	Chickpeas / American garden	UAE
29-	Chickpeas / Chtoura garden	Lebanon
30-	Fava beans / Durra	Jorden
31-	Fava beans / Chtoura	Lebanon
32-	Fava beans / Kasih	Jorden
33-	Fava beans / Americana	UAE
34-	Cooked peas / Heinz	UAE
35-	Peas and carrot / Kasih	Jorden
36-	Peas / American garden	UAE
37-	Peas and carrot/ Chtoura garden	Lebanon
38-	Black eye beans / Americana garden	UAE
39-	Fine green beans / Maxima's	Italy

Detection of aflatoxin B1 in collected samples using Enzyme Linked Immune Sorbent Assay (ELISA) technique

Detection of aflatoxin B1 using ELISA technique was performed using ELISA kit (W81110) which supplied by Shenzhen Lvshiyuan Biotechnology company, the extracted samples, aflatoxin B1 enzyme conjugate and aflatoxin B1 Antibody working solution were mixed and added to micro well. On removal of non-specific reactants, substrate (A and B) were added, then the micro wells measured optically using microplate reader at 450 nm for yellow color or at 650 nm for unstopped blue color to determine the OD value.

Determination of aflatoxin B1 concentration:

Percentage concentrations of aflatoxin B1 in test samples was calculated using aflatoxin B1 standard curve according to the following equation:

Percentage of absorbance value % = B / B0 × 100

B = the average OD value of sample or standard solution.

B0 = the average OD value of 0 ng/ml standard solution.

Detoxification of aflatoxin B1 using UV radiation

Samples Irradiation (Ultraviolet source)

The source of UV energy was UV lamp (VISION science Co., Ltd, Korea), the main wavelength of Lamp was 365 nm. Lamp was elevated to give a distance between lamp and sample (60cm) [9].

UV Treatment:

Two gram of contaminated samples (samples with highest concentration from each type of canned food) was exposed to UV radiation (365 nm) [9- 13] for (30 min) and the residual aflatoxin B1 content was measured at the end of exposure [9].

Statistical analysis:

All analytical determinations were performed at least in triplicate using SPSS program var. 11.5. Values of different parameters were expressed as the mean ± standard error using student T-test. A difference of $P \leq 0.05$ was considered statistically significant.

Results and discussion

Thirty nine samples of some canned foods were analyzed for presence of aflatoxin B1 (quantitatively) using ELISA kit. Results revealed that thirty four samples were contained aflatoxin B1, and the concentration of toxin ranged from 2.5 ppb to 975 ppb, Table-2.

Table 2- Detection of aflatoxin B1in some canned foods samples using ELISA technique

Sample number	Concentration of aflatoxin B1 (ppb)
1	225
2	215
3	150
4	50
5	975
6	22.5
7	150
8	150
9	212.5
10	217
11	37.5
12	22.5
13	25
14	24
15	75
16	50
17	25
18	175
19	237.5
20	62.5
21	72.5
22	2.5
23	12.5
24	75
25	200
26	20
27	25
28	70
29	207
30	25
31	0
32	50
33	62.5
34	0
35	25
36	0
37	0
38	0
39	33

Results observed that highest concentration in beef meat sample (beef luncheon/ Hana) was 975 ppb, [14] showed that concentration of aflatoxin B1 was 4 ppb in luncheon meat and 7 ppb in sausage. Results by [15] revealed that the average concentration of aflatoxin B1 (ppb) in sausage and luncheon were 9.03, 8.8 ppb respectively using HPLC technique, while [16] observed that highest mean values of aflatoxins residues (ppb) detected in luncheon samples were (3.71, 3.59, 5.24 and 6.77 ppb) using TLC technique.

The highest concentrations of aflatoxin B1 in chickens meat samples (chicken luncheon meat/ Maxima's) and fishes meat samples (Tuna flakes /Americana) were 217 and 75 ppb respectively. Results by [17] conducted that the mean value of the total aflatoxins residues in the examined chicken luncheon was 0.87 ppb, while [18] found that the highest level of aflatoxin B1 in the examined smoked fishes samples was 4.66 ppb, also our results showed that the highest concentration of aflatoxin B1 among the collected mushroom samples (mushroom slice/ Maxima's) was 237.5ppb and 207 ppb among legumes samples (Chickpeas / Chtoura garden), [19] revealed that 5.53 ppb of aflatoxin B1 was found to be the highest concentration in canned Button mushrooms, while [20] showed that aflatoxin B1 presence in concentration 5 ppb in legumes.

Under specific environmental conditions toxigenic fungi produce aflatoxin B1, foods stored under high moisture/humidity (>14%) at warm temperatures (>20°C) and/or inadequately dried can potentially become contaminated. Warm (air temperature of 24C°–35 C°) and humid (moisture content of substrate between 25% and 35%) conditions lead to extensive mold growth and aflatoxinB1 production [21].

According to Food and Drug Administration (FDA), a maximum tolerable level of aflatoxin B1 is 20 ppb in foods, while the European Union (EU) permitted a maximum level of 4 ppb for total aflatoxins [22- 24], and when compare these levels with our results, we can clarify disqualification of most samples for human consumption.

Degradation of aflatoxin B1 using UV radiation

Degradation experiment was conducted by using the highest concentration of each type of collected samples, UV radiation was used for this target. Results showed that using of UV radiation (365 nm wave lengths) at a distance of 60 cm between UV source and sample for 30 minute can reduce the concentration of aflatoxin B1 significantly from 975, 217, 75, 237.5 and 207 to 111, 30, 8, 44, and 23 respectively, Table-3.

Table3- Degradation of aflatoxin B1 using UV radiation.

Sample number	Samples type	Concentration of aflatoxin B1 (ppb) before treatment with UV radiation	Concentration of aflatoxin B1 (ppb) after treatment with UV radiation(60 cm distance for 30 minute), (Each value expressed as Mean ± Standard Error (SE) of three replicates
5	Beef luncheon /Hana	975	111 *
10	Chicken luncheon meat /Maxima's	217	30 *
15	Tuna flakes/ Americana	75	8 *
19	Mushroom slices /Maxima's	237.5	44 *
29	Chickpeas / Chtoura garden	207	23 *

* = Significant ($P \leq 0.05$)

Results that mention in the table above showed the efficiency of UV radiation in decreasing of aflatoxin B1 from tested samples.

Study conducted by [25] showed that using of UV radiation at a distance of 15cm and 10 hr. exposure, aflatoxin concentration reduced to 99.1 % (350 ppb to 3 ppb), while [26] revealed that aflatoxin B in peanut oil was decreased from 51.96 to 7.23 ppb in 10 min using UV light, [9] observed that using of UV radiation at different distance and times can decreased the concentration of aflatoxin M1 in pasteurized canned milk samples.

UV radiation is one type of Ionizing radiation which may produce potential changes in molecules of the irradiated object [27].

For many years UV radiation has been discovered as an effective physical method to destroy aflatoxins for its photosensitive property [28], degradation rate was a function of the depth of penetration of the rays and thickness of film when operating conditions were held constant [29].

Photo degradation pathways of AFB1 might be complicated and accompanied by some complex chemical reactions, aflatoxin B1 might lose the C=O of the lactone ring and become to C16H14O4 firstly, because the lactone ring was the active site of aflatoxin B1, Then, the additional reaction and substitution reaction of small molecules such as R-NH2 and -NH2, which may due to the fact that the concomitant cracking reactions of the nitrogen-containing compound under UV irradiation occurred on the unsatisfied chemical double bond on the furan ring, and the right side five-membered ring of C16H14O4, while the OH groups had been replaced by NH2. Moreover, the H addition reaction definitely occurred on C=O e right side five-membered ring of C16H14O4, while the OH groups had been replaced by NH2.

After these complex reactions, the structure of C19H33N3O4 was formed. Thus, the P1 (C18H33N3O3) may be the metabolites of C19H33N3O4 after dropping the methoxy group (OCH3) under UV irradiation. Finally, as a result of the cracking of the five-membered ring in the middle of the compound of P1, the compound of P2 (C12H22N2O2, molecular weight: 226) formed [10].

Conclusion

Canned food can be contaminated by aflatoxin B1 if the storage conditions were unfavorable. UV radiation consider as a good method for destroying of aflatoxin B1 from contaminated samples.

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