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Determination of Aflatoxins in Dried Fruits of Afghanistan

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Abstract

Aflatoxins are carcinogenic and toxic to human beings. In this research work, the amount of aflatoxins contamination in seventy-two samples of dried fruits; Such as; Almonds and Raisins from processed raisins and almonds in Kabul city and Kabul customs, including twenty-one sample of almonds with shells, seven samples of pistachios with shells, six samples of almonds without shells, ten samples of pistachios without shells and twenty-eight samples of red raisins by using fluorometer is determined. Quantitative results show that 15 samples have 4 or less than 4 *ppb* aflatoxin, 21 samples have 5-10 *ppb* aflatoxin and the rest 35 samples have 10-25 *ppb* of aflatoxin. Similarly, in 27 samples of the almond amount of aflatoxins has been investigated, in all almond samples under investigation, none of the samples contained more than 22 *ppb* aflatoxin, which could be used as food. Ten samples are containing 4 *ppb* and less aflatoxin. Factors that play a role in the production of aflatoxins in almonds include Moisture, heat, microscopic effects of insects and organisms, storage time of almonds, infection shield and some other conditions. From the study, it was concluded that a continuous and strict national monitoring plan is needed to improve quality and safety of dried fruits supply in Afghanistan.

Keywords: Aflatoxins: Mycotoxins; Toxicity; Contamination; Fluorometry

Introduction

Dried fruits have become an essential part of food. Healthy life style needs provision of safe and good quality food. But unfortunately, food can be easily contaminated through the environment. Aflatoxins are toxic carcinogenic secondary metabolites produced by two type of fungi called *Aspergillus parasiticus* and *Aspergillus flavus* [1,2].

Mycotoxins are a type of chemical citrus that fungi (a type of fungus) produce it in the high growth stage on food; Such as grains of beans, peanuts, seeds of cotton, etc [3]. When foods contaminated with mycotoxins, including aflatoxins, are consumed by humans and animals they cause severe adverse health effects. Although a hundred years ago the health effects of spotted seeds caused by mycotoxins were known and the diseases caused by such seeds were partially exposed; in 1961, more information was provided about the adverse effects of aflatoxins, and the diseases caused by them were identified such as cancer of the liver and kidney etc. that are caused by aflatoxins (specifically aflatoxins mycotoxins B_1) has been diagnosed, therefore; for eradication of mycotoxins and specifically aflatoxins, the attention of the international community has been paid and the export and import restrictions of food have been created between the countries of the world [4,5].

Our country Afghanistan, which is also one of the countries that export fruits and food and imports some of them, needs to study and research food in terms of mycotoxins; Therefore, it is necessary to identify effective ways and methods of research for determining amount of

aflatoxins and methods to destroy the source of aflatoxins. Unfortunately, in Afghanistan not much work has been done so far [5,6].

Foods that are more contaminated by aflatoxins include various grains (wheat, corn, white rice, rice, mung bean, etc.), fruits (whole grains, seedless raisins, dates, etc.) Dairy products (yogurt, cheese, milk), baby food, medicines, etc [7,8].

Aflatoxins are potent toxins and are carcinogenic agents, mitochondria, amino suppressants, which are the second metabolic product of Sparagillus yellow and *Sparagillus parasiticus* in various food products. Approximately 18 different types of aflatoxins have been known, the most important of which are: G_1, G_2 B_1, B_2 M_1, and M_2. Aflatoxin B_1 is present in adequate amounts in food products and its pure amount has a solid crystalline yellowish-white color. Aflatoxins are well soluble in methanol, acetone, chloroform, and acetonitrile. Asparagus yellow products are specifically aflatoxins B_1 and B_2 [9,10]. *Asparagus parasiticus* aflatoxin, which is better than their B_1 and B_2 products. The four aflatoxins G_1, B_2, M_1, and M_2 may each be obtained separately from aspergillus yellow and *asparagus parasiticus* to a lesser extent than the other aflatoxins. As well as other aflatoxins; Such as M_1, G_1, parasitcol, and aflatoxicol are also among the products of asparagus yellow [11,12].

Aflatoxins M_1 and M_2 are more highly metabolic than aflatoxins B_1 and B_2. Animal milk, which is more commonly used as food, is contaminated by aflatoxins, physical properties of some aflatoxins are given in Table 1 [13].

Optical reflection	Melting point	Molecular mass	Molecular Formula	Aflatoxins
B_1	$C_{17}H_{12}O_6$	312	$262-269^0C$	Blue
B_2	$C_{17}H_{14}O_6$	314	$286-289^oC$	Blue
G_1	$C_{17}H_{12}O_7$	328	$244-248^oC$	Green
G_2	$C_{17}H_{14}O_7$	330	$237-240^oC$	Green-Blue
M_1	$C_{17}H_{12}O_7$	328	299^oC	Blue-Purple
M_2	$C_{17}H_{12}O_7$	330	293^oC	Purple
B_2A	$C_{17}H_{114}O_7$	330	240^oC	-
G_2A	$C_{17}H_{14}O_8$		190^oC	-

Table 1: Some physical properties of aflatoxins.

Aflatoxins are present in different concentrations in food, which are received in different quantities in food of different countries of the world depending on the environmental conditions, and generally, in countries with temperate climates, their growth and development are high [4].

In the international trade of dried fruits, nuts, legumes, wheat, barley, corn, etc., foodstuffs are exported and imported if they contain a certain amount of aflatoxin not only food-exporting countries have problems with high-dose aflatoxins, but their importing countries also face similar problems [1].

The European Community has established the norm for the repatriation of fruits and foodstuffs that contains aflatoxins in large quantities which are not accepted by them commercially as they contain excess of aflatoxins Table 2 [14].

Number	countries	Group	Material	Amount of aflatoxin allowed	Considerations
1	Belgian	First	of all foods	4 *ppb*	European Commission Rules
2	Czechoslovakia	First	peanut	5 *ppb*	
3	Finland	First	Nuts and its products	4 *ppb*	
4	France	First	all food	4 *ppb*	
5	German	First	peanuts and all food	5 *ppb*	
6	Logranburg	First	all food	5 *ppb*	
7	Maldives	First	Peanuts	5 *ppb*	
8	Halind	First	Peanuts and all Food	5 *ppb*	
9	Norway	First	All food	5 *ppb*	
10	Polish	First	Top All Foods	5 *ppb*	
11	South Africa	First	all food	5 *ppb*	
12	Sorniam	First	Peanut and its products	5 *ppb*	
13	Sweden	First	all food	5 *ppb*	
14	Swisserland	First	Nuts and its products	5 *ppb*	
15	Russia	First	all food	5 *ppb*	
16	UK	First	Nuts and its products	5 *ppb*	Regulation of the European Committee
17	First	First	Peanuts	5 *ppb*	
18	Colombia	Second	Peanuts	0 *ppb*	
19	Denmark	Second	Peanuts and Bob food	0 *ppb*	Regulation of the European Committee
20	Japan	Second	Peanuts	0 *ppb*	
21	Singapore	Second	Peanut Vetil	0 *ppb*	
22	United States	Second	All food	0 *ppb*	
23	Australia	Third	Peanut and its crop	15 *ppb*	

24	Canada	Third	Peanut and its product	15 *ppb*	
25	Jordan	Third	all food	15 *ppb*	Export regulations
26	Hankang	Third	all food	15 *ppb*	Regulation of the European Committee
27	Greece	Fourth	All food	10 *ppb*	Regulation of the European Committee
28	Ireland	Fourth	All Foods	10 *ppb*	Export regulations
29	Israel	Fourth	All Foods	10 *ppb*	
30	Kenya	Fourth	Peanut	10 *ppb*	
31	Philippines	Fourth	Peanut	10 *ppb*	
32	Thailand	Fourth	edible oil	10 *ppb*	
33	Zombabi	Fifth	Peanut	23 *ppb*	
34	Styria	Fifth	All foods	08 *ppb*	
35	Brazil	Fifth	Flour and Peanuts	08 *ppb*	Export regulations
36	Italy	Fifth	Bob Food	08 *ppb*	Regulation of the World Health Organization
37	China	Fifth	Peanut	08 *ppb*	
38	India	Fifth	all food	06 *ppb*	

Table 2: The amount of aflatoxins in food products according to the norm of the European Union.

Material and Method

Collection of Samples

In this study, seventy-two samples of dried fruits; Such as: Pistachios, Almonds and Raisins from processed raisins and almonds in Kabul city and Kabul customs, including twenty-one samples of almonds with shells, seven samples of pistachios with shells, six samples of almonds without shells, Ten samples of pistachios without shells and twenty-eight samples of red raisins during the period from June 2020 to February 2021 were obtained and all 72 samples were subjected to determinations of aflatoxins.

Obtained substance samples represent the set of materials under study for aflatoxin therefore, it is necessary to take samples from all parts of the material, for this purpose, the main lots or container should be emptied in a suitable place and its materials should be mixed. Warehouses should be divided into sub-warehouses (Table 3) the weight of each sub-warehouse should be 20% of the weight of the main warehouse. Obtained samples should be located in separate locations. Use plastic bags to transfer the amount of samples. Laboratory Standard Sample: A sub-sample weighing 10 kg shall be mixed and blended in accordance with the provisions of the 1998 (EC-53) Commission.

Warehouse weight per ton	Number of initial samples	Number of sub-samples
0.1 and more	10 samples	3kg
0.1-0.2	15 samples	4.5kg
0.2-0.5	20 samples	7kg
0.1-0.5	30 samples	8kg
1-2	40 samples	12kg
2-5	80 samples	18kg
5-10	80 samples	24kg
10-15	80 samples	30kg

Table 3: Number of initial samples taken from dried fruits.

Aflatoxins are light-sensitive; therefore, the exposure of the samples and the sample extracts to direct light was avoided before the preparation steps. First of all, the samples were divided to subsamples according to Table 3 and were ground as finely as possible to produce a homogeneous particle size by means of a high-speed blender [15].

Chemical and Apparatus

Dry fruits samples were obtained from the Kabul customs, Methanol and NaCl were purchased from Sigma Aldrich, distilled water obtained from a water purification system (Millipore Milli-Q Plus), methanol-water (60:40, v/v) solution was prepared, VICAM series-4 fluorometer was used for aflatoxins amount determination and Extracts were filtrated by a Whattman filter paper No. 4.

Extraction Procedure

Pour 25 g of the finely-ground test sample into an Erlenmeyer flask and add 5 g of NaCl. In Erlenmeyer flask, 125 mL methanol-water (60:40, v/v) solution is added. Close the lid of the Erlenmeyer flask and place it in a high place for one minute. The lid of the Erlenmeyer flask is then opened again and the solution is filtered through a prefolded filter paper. The resulting pure filtrate is collected, which is the extract. 20 ml of the pure extracted material is taken in a clean container, and was diluted with 20 ml of pure water, shaken vigorously to dissolve properly. 10 ml of dilute filtrate of the extracted material is poured into a Kuwait of fluorometer for aflatoxins detection [3,7,16].

Fluorometric Analysis

Then, a 10 mL of diluted filtrate was passed through an internal column of fluorometer at a flow rate of about 1-2 drops per sec. Then 10 mL of ultrapure water was passed through column at flow rate of 2 drops per sec. after that 1 mL methanol was passed through the column of fluorometer.

The 1 mL extracted and diluted sample is placed in fluorometer cuvette and fluorometer is turned on after 60 min. the concentration and amount of aflatoxin was recorded. In this research paper, the amount of aflatoxins in 72 sample of dried fruits of Afghanistan has been determined by a device called fluorometer [17,18].

Result and Discussion

Infection of fruits by aflatoxins (*sparagillus fungi*) and production of aflatoxins in fruits; for example, almonds, red raisins, etc. occur during the harvest and it comes after collecting and storing them. The degree of contamination of fruits by phoenixes is related to the condition of their storage such as temperature, humidity etc.

In this research work, seventy-two samples of dried fruits (raisins five samples, peeled almonds eight samples, without peel almonds two samples, peeled Pistachios one sample, a total of fifteen samples, contained 4*ppb* of aflatoxin and according to the norm set by the European Union Which are listed in Table (2), these materials can be exported to all countries of the world. The results are listed in Table 4.

Fifty-eight other samples by type of fruit (red raisins (16), peeled almonds (16), peeled almonds (2), without peel Pistachios (5) and peeled Pistachios (7) sample contained less than 15 *ppb* aflatoxins, which is according to the norm set by EU (2-8) and can be exported to the European Union to the countries of Hankang and New Zealand. From the total samples under study, three samples of fruits can be exported to the Gulf countries.

As can be seen, of the seventy-two samples of dried fruits, none contain more than 25 *ppb* of aflatoxin.

Fruits under study	Number of sample	Aflatoxin in *ppb*	The number of groups in the country that accept this amount of aflatoxin in fruits				
			1	2	3	4	5
Red raisins	1	1 *ppb*	A	A	A	R	R
Red raisins	2	7 *ppb*	A	A	A	A	R
Red raisins	3	2 *ppb*		A	A	A	A
Red raisins	4	8 *ppb*	R	R	A	A	A
Red raisins	5	2 *ppb*	A	A	A	A	A
Red raisins	6	2 *ppb*	A	A	A	A	A
Red raisins	7	1 *ppb*	A	A	A	A	A
Red raisins	8	6 *ppb*	R	R	R	A	A
Red raisins	9	2 *ppb*	A	A	A	A	A
Red raisins	10	1 *ppb*	R	R	A	A	A
Red raisins	11	9 *ppb*	R	A	A	A	A
Red raisins	12	4.8 ppb	A	A	A	A	A
Red raisins	13	22 ppb	R	R	R	R	A
Red raisins	14	18 ppb	R	R	R	R	A
Red raisins	15	11 ppb	R	R	A	A	A
Red raisins	16	0.13 ppb	A	A	A	A	A
Red raisins	17	11 ppb	R	R	A	A	A
Red raisins	18	13.5 ppb	R	R	A	A	A
Red raisins	19	16 ppb	R	R	A	A	A
Red raisins	20	11 ppb	R	R	A	A	A
Red raisins	21	18 ppb	R	R	R	A	A
Red raisins	22	12.5 ppb	R	R	A	A	A
Red raisins	23	10 ppb	R	A	A	A	A
Red raisins	24	7.8 ppb	R	A	A	A	A
Red raisins	25	18 ppb	R	R	R	A	A
Red raisins	26	9.6 ppb	R	A	A	A	A
Red raisins	27	9 ppb	R	A	A	A	A
Red raisins	28	7.8 ppb	R	A	A	A	A
Almonds with shells	29	4 *ppb*	A	A	A	A	A
Almonds with shells	30	2 *ppb*	R	R	R	R	A

Almonds with shells	31	8 *ppb*	R	R	R	A	A
Peeled almonds	32	4 *ppb*	A	A	A	A	A
Peeled almonds	33	1 *ppb*	A	A	A	A	A
Almonds with shells	34	3 *ppb*	R	R	A	A	A
Almonds with shells	35	6 *ppb*	R	R	A	A	A
Peeled almonds	36	4 *ppb*	A	A	A	A	A
Peeled almonds	37	4 *ppb*	R	R	A	A	A
Almonds with shells	38	4 *ppb*	R	A	A	A	A
Almonds with shells	39	4 *ppb*	R	A	A	A	A
Almonds with shells	40	4.5 ppb	A	A	A	A	A
Almonds with shells	41	11 ppb	A	A	A	A	A
Almonds with shells	42	0.4 ppb	A	A	A	A	A
Almonds with shells	43	0.7 ppb	A	A	A	A	A
Almonds with shells	44	10 ppb	R	A	A	A	A
Almonds with shells	45	6 ppb	R	A	A	A	A
Almonds with shells	46	17.5 ppb	R	R	R	A	A
Almonds with shells	47	10 ppb	R	A	A	A	A
Almonds with shells	48	13 ppb	R	R	A	A	A
Almonds with shells	49	3.4 ppb	A	A	A	A	A
Almonds with shells	50	6.5 ppb	R	A	A	A	A
Almonds with shells	51	12 ppb	R	R	A	A	A
Almonds with shells	52	11 ppb	R	R	A	A	A
Almonds with shells	53	8 ppb	R	A	A	A	A
Almonds with shells	54	14 ppb	R	R	A	A	A
Almonds with shells	55	6.7 ppb	R	A	A	A	A
Skinless pistachios	56	2 *ppb*	R	R	A	A	A
Pistachio with skin	57	8 *ppb*	R	R	R	A	A
Pistachio with skin	58	2 *ppb*	A	A	A	A	A
Pistachio with skin	59	0 *ppb*	A	A	A	A	A
Pistachio with skin	60	8 *ppb*	A	A	A	A	A
Pistachio with skin	61	8 *ppb*	R	R	R	A	A
Pistachio with skin	62	3 *ppb*	A	A	A	A	A

Skinless pistachios	63	3 *ppb*	R	R	A	A	A
Skinless pistachios	64	6 *ppb*	R	A	A	A	A
Skinless pistachios	65	1 *ppb*	A	A	A	A	A
Skinless pistachios	66	1 *ppb*	R	R	R	R	A
Skinless pistachios	67	4 *ppb*	R	R	R	A	A
Skinless pistachios	68	7 *ppb*	A	A	A	A	A
Skinless pistachios	69	0 *ppb*	A	A	A	A	A
Skinless pistachios	70	0 *ppb*	R	R	A	A	A
Skinless pistachios	71	0 *ppb*	R	A	A	A	A
Skinless pistachios	72	9 *ppb*	A	A	A	A	A

Table 4: Shows the results of the determination of aflatoxins in researched seventy-two samples of fruits with the groups of countries to which these fruits can be exported.
Note: In above table, A is accepted and R is rejected based on the accepted norms of the European Community.

Quantity Analysis of Aflatoxins in Raisins

The main factors of the presence of aflatoxins in raisins are related to carbohydrates, humidity, heat, air impact, and insect attack, etc., which are the fungi's that produce aflatoxins. If the humidity of the raisin storage environment reaches 20%, then the attack of the phoenixes will increase and the amount of aflatoxin will increase.

Table 5 shows the quantitative results of eleven samples of raisins. Five samples had less than 4 *ppb* of aflatoxin, seven samples 5-10 *ppb* of aflatoxin, and rest samples contain 10-22 *ppb* aflatoxin.

Fruits under study	Sample number	The amount of aflatoxin to *ppb*
Red raisins	1	11
Red raisins	2	7
Red raisins	3	12
Red raisins	4	18
Red raisins	5	2
Red raisins	6	2
Red raisins	7	1
Red raisins	8	16
Red raisins	9	2
Red raisins	10	11
Red raisins	11	9
Red raisins	12	4.8
Red raisins	13	22
Red raisins	14	18

Red raisins	15	11
Red raisins	16	0.13
Red raisins	17	11
Red raisins	18	13.5
Red raisins	19	16
Red raisins	20	11
Red raisins	21	18
Red raisins	22	12.5
Red raisins	23	10
Red raisins	24	7.8
Red raisins	25	18
Red raisins	26	9.6
Red raisins	27	9
Red raisins	28	7.8

Table 5: Amount of aflatoxins in raisin samples under investigation.

Considering Table 5 a graph of the suitability of raisin samples and the amount of aflatoxins in the raisin samples under study can be obtained Figure 1.

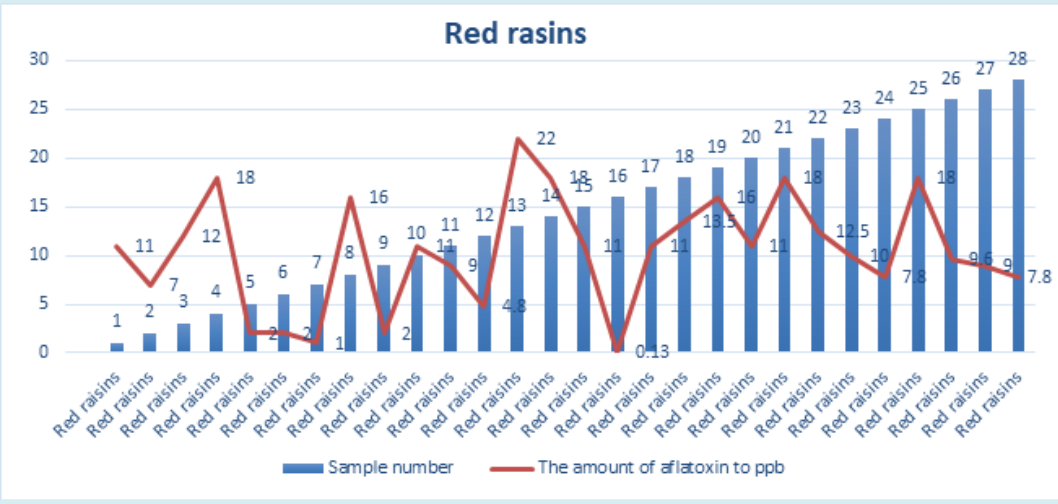

Figure 1: Graph of dependence between raisin samples and the amount of aflatoxins in them.

The graph of red raisin shows peaks with different ups and downs that reveal the amount of aflatoxin in red raisins is relatively high. Five samples have the lowest aflatoxin content and sample number 13 contains 22 *ppb* of aflatoxin. All of these samples are washed from raisins. The main reason for the high presence of aflatoxins in these samples is their high humidity and lack of attention to their storage after washing.

Quantity Analysis of Aflatoxins in Almonds

In the ten almond samples, Table 6. Three samples contain 10-20 *ppb* aflatoxin, and in all almond samples under investigation, none of the samples contain more than 22 *ppb* aflatoxin, which can be used as food. In six samples 4 *ppb* or less of aflatoxin was found.

Factors that play a role in the production of aflatoxins in almonds include humidity, heat, airborne effects of microscopic insects and organisms, storage time of almonds, infestation spores, and other conditions of the almond.

Fruits under study	Sample number	The amount of aflatoxin to *ppb*
Almonds with shells	29	4
Almonds with shells	30	22
Almonds with shells	31	18
Peeled almonds	32	4
Peeled almonds	33	1
Almonds with shells	34	13
Almonds with shells	35	16
Peeled almonds	36	4
Peeled almonds	37	4
Almonds with shells	38	4
Almonds with shells	39	4
Almonds with shells	40	4.5
Almonds with shells	41	11
Almonds with shells	42	0.4
Almonds with shells	43	0.7
Almonds with shells	44	10
Almonds with shells	45	6
Almonds with shells	46	17.5
Almonds with shells	47	10
Almonds with shells	48	13
Almonds with shells	49	3.4
Almonds with shells	50	6.5
Almonds with shells	51	12
Almonds with shells	52	11
Almonds with shells	53	8
Almonds with shells	54	14
Almonds with shells	55	6.7

Table 6: Amount of aflatoxins in almond samples under investigation.

The following graph shows the relationship between almond samples and the amount of aflatoxins in them Figure 2.

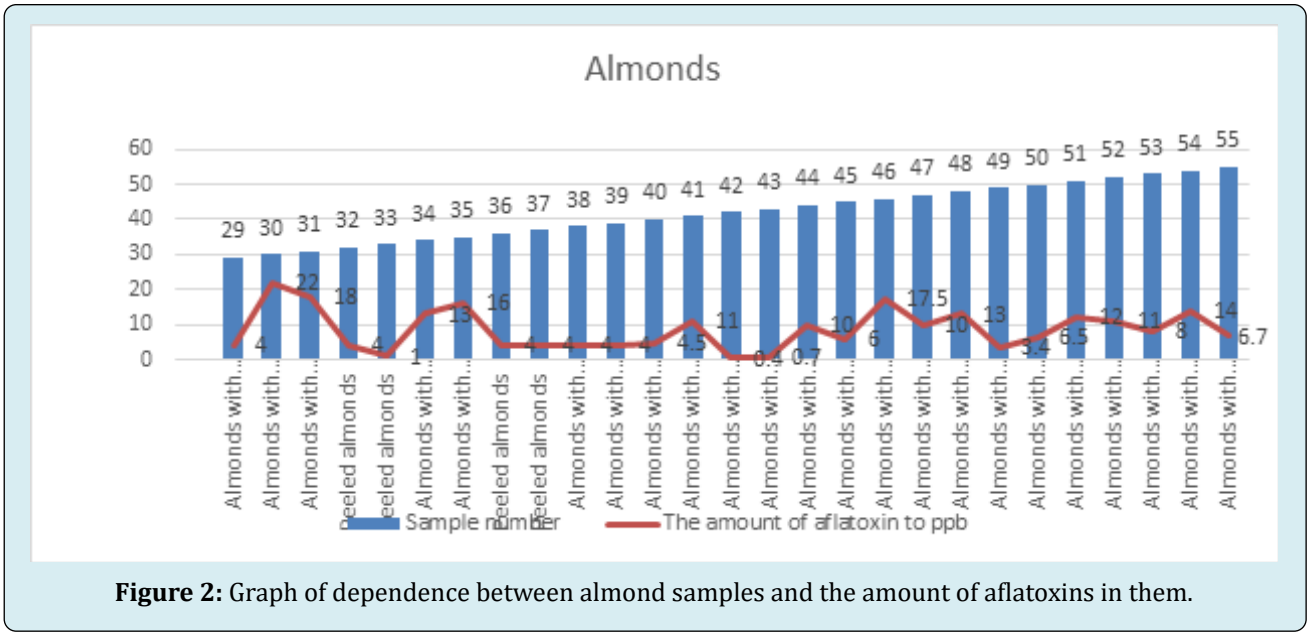

Figure 2: Graph of dependence between almond samples and the amount of aflatoxins in them.

Quantity Analysis of Aflatoxins in Pistachios

In seventeen samples of pistachios Table 7, one sample containing 4 *ppb*, three samples less than 10 *ppb* and twelve samples containing 10-20 *ppb* aflatoxins which can be used as food (Figure 3).

Fruits under study	Sample number	The amount of aflatoxin to *ppb*
Skinless pistachios	23	12
Pistachio with skin	24	8
Pistachio with skin	25	12
Pistachio with skin	26	10
Pistachio with skin	27	8
Pistachio with skin	28	8
Pistachio with skin	29	3
Skinless pistachios	30	25
Skinless pistachios	31	16
Skinless pistachios	32	11
Skinless pistachios	33	11
Skinless pistachios	34	14
Skinless pistachios	35	17
Skinless pistachios	36	10
Skinless pistachios	37	10
Skinless pistachios	38	10
Skinless pistachios	39	19

Table 7: Amount of aflatoxins in pistachios samples under investigation.

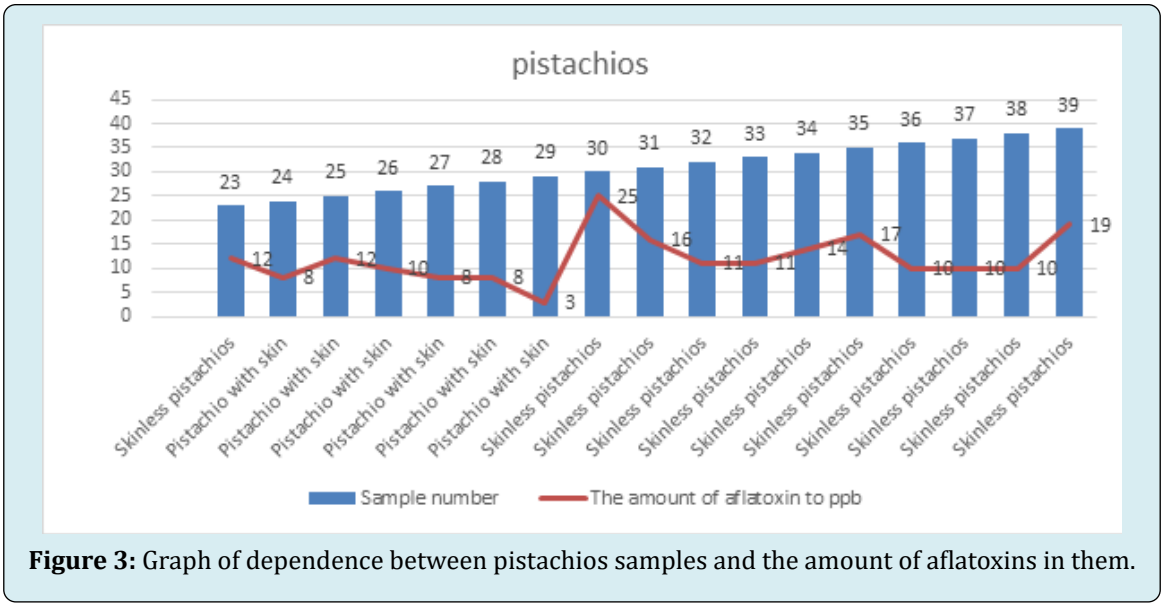

Figure 3: Graph of dependence between pistachios samples and the amount of aflatoxins in them.

Comparative Analysis of Aflatoxins in Raisins, Almonds and Pistachios

Contamination of raisins, almonds and pistachios by aflatoxins- toxic secondary metabolites in Afghanistan has been a dangerous phenomenon; food can be easily contaminated through the environment their consumption can lead to serious health damage. Graph Figure 4 below shows the Comparative contamination of raisins, almonds and pistachios by aflatoxins.

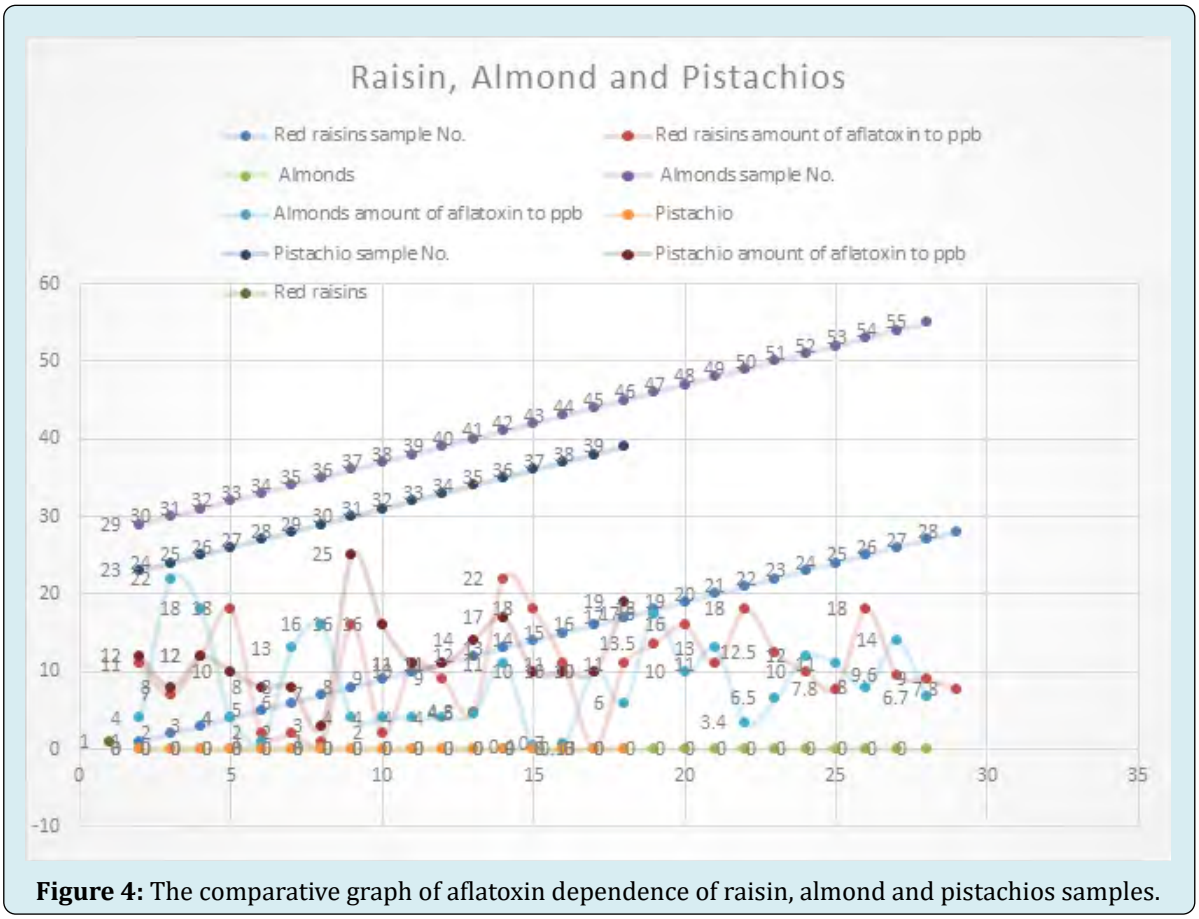

Figure 4: The comparative graph of aflatoxin dependence of raisin, almond and pistachios samples.

The Comparative graph of aflatoxins Contamination in fruits; Such as: Almonds, pistachios and raisins reveals the amount of aflatoxin that in pistachios is more than almonds and raisins and in raisins the amount of aflatoxin is less than almonds.

Conclusion

72 samples of dried fruits; Such as Almonds and red raisins that were investigated for amount of aflatoxine; shows that contamination of aflatoxins in raisins, Almonds and pistachios is in different quantities, aflatoxin contamination is high in pistachios than almonds and raisins, in raisins it is less than almonds. The amount of aflatoxin in almonds with shell is less than peeled almonds. The amount of aflatoxin in skinned pistachios is less than skinless pistachios; because pistachio cover prevents pistachio kernel contamination.

The factors that cause increase of aflatoxin in fruits are humidity and heat; therefore, dried fruits should be protected from moisture, if dried fruits are washed, they should be dried completely. In seventy-two researched samples (raisins, almonds and pistachio) that were processed for export, which were taken from Kabul customs, the amount of aflatoxins in them was determined; It was found that the amount of aflatoxin in none of samples was more than 25 *ppb*; Contamination of dried fruits, nuts, and legumes occurs in farms, during harvests and their untimely collection in these cases the amount of aflatoxin in them is observed in large quantities. The increase amount of aflatoxins from the norm set by international organizations and the European Union for fruits causes liver and kidney cancer.

The amount of aflatoxins in different quantities in food products is related to physical and chemical treatment and is also related to how they are kept from moisture, heat during collection and storage in warehouses.20 of barley, and 20 of wheat

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