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Survey and Determination of Aflatoxins in Iranian Wheat of Canvas Covered Store and Variations of them after Storage

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Abstract: The purpose of this experimental study was to determine Aflatoxins content of Iranian wheat and variations of them after storage. The store condition was canvas covered. In our study we selected Shahid Elmi Silo in Karaj, Iran. After evaluation silo, sampling plan was specified. Sampling was done in two steps from one of canvas covered stores with 25000 ton capacity, during one week on August and after 4 months storage, on December 2005. At the first, 50 samples were analyzed by HPLC to determine Aflatoxins content and then 50 samples were analyzed, by same method. The results showed that: at the first step of sampling, total Aflatoxin was detected just in three samples at amounts 0.23, 0.22, 0.53 ppb. At the second step total Aflatoxin was detected just in two samples at amounts 0.15 and 0.10 Ppb. According to the results of this study, contaminated wheat with aflatoxins was very low and the amount of contamination wasn't increased during storage.

Key words: Wheat, aflatoxin, storage, HPLC

INTRODUCTION

Wheat is one of the world's most important food crops. Foods made with wheat are a major part of the diet for over a third of the world's people. In fact, wheat can be found in some form at almost every meal such as: Bread, Cookie, Cake, Macaroni and Spaghetti.

In Iran bread is as a stable food and supplying people for energy, protein, minerals and vitamins. In 2007, production of wheat in Iran was almost 12 million tons and consumption of bread for every Iranian person was almost 157 kg in year. So wheat and bread have an important role in food safety in Iran.

Unfortunately, many agricultural commodities are vulnerable to attack by a group of fungi that are able to produce toxic metabolites called Mycotoxins. Among various Mycotoxins, Aflatoxins have assumed significance due to their deleterious effects on human beings, poultry and livestock (Reddy and Waliyar, 2005).

Aflatoxins are potent toxic, Carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus Aspergillus flavus and A. parasiticus on variety of food products.

Among 18 different types of Aflatoxins identified, major members are Aflatoxin B1, B2,G1 and G2.

Food products Contaminated with Aflatoxins include cereal, oilseeds, spices, tree nuts and milk (Shank, 1981).

Conditions that contribute to fungal growth and the production of Aflatoxins are: a hot and humid climate, kernel moisture of 13-20%, favorable substrate characteristics and factors that decrease the host plant's immunity (insect damage, poor fertilization and drought) (Charmley *et al*., 1995).

The problem of Aflatoxins contamination is most serious in tropical and sub-tropical countries such as Southeast Asian countries, where alarming levels of contamination in foods and feeds have been reported (Reddy and Waliyar, 2005).

A survey for various mycotoxins was carried out on samples of all wheat delivered to nine storage and marketing depots in south-eastern Queensland in 1983-1985. Aflatoxins B1, B2, G1 and G2 were detected in only one pooled sample of wheat, at a total Aflatoxins concentration of 0.003 mg kg^{-1} (Blaney *et al*., 2005).

Out of 223 samples collected from wheat growing belt of western U.P. (India), only 9 samples were found contaminated with Aflatoxin B, in the range of 8-40 ppb. In 1985, the entire 6 flood affected wheat samples collected from Punjab were found contaminated with Aflatoxin B1 in the range of 8-40 ppb (Goyal, 2000).

In other survey, 116 samples contain wheat, barley and sorghum collected from Swede, 20 samples were found contaminated with Aflatoxin B1, in the range of 50-400 ppb (Pittet, 1998).

Thus, in view of the above concerns and because of there isn't any research about determining Mycotoxins and especially Aflatoxins in Iranian wheat. So, this survey was aimed at determining Aflatoxins content of Iranian wheat and variations of them after storage in 2005.

MATERIALS AND METHODS

In our study we selected Shahid Elmi Silo in Karaj, Iran. This Silo was containing 6 stores (2 stores with 15000 ton and 4 stores with 25000 ton capacity).

Store was covered with canvas. After evaluating Silo, sampling plan was specified. Sampling was done in 2 steps from 1 of canvas covered stores with 25000 ton capacity, during one week on August and after 4 months storage, on December 2005 from the same point of sampling.

The EC adopted a directive prescribing sampling methods for Aflatoxins (Commission Directive, 1998). Bulk samples of wheat of 1500 tones or greater are required to be treated as sub lots of 500 tones. From each sub lot, 100 incremental samples of 300 g should be drawn to provide an aggregate sample weight of 30 kg. Samples were taken randomly throughout the consignments of wheat (Commission Directive, 1998; MAFF, 1997; Scudamore and Patel, 2000).

The samples were stored in plastic bags, adequately labeled and preserved in cool place, without direct sunlight until they were taken to the laboratory for Aflatoxins measurement.

Each aggregate sample thoroughly mixed and was ground. One kg as laboratory sample and 2 kg as Blank sample was taken and packed in plastic bags and kept in refrigerator with maximum 4°C. Blank samples were stored at -20°C.

Analytical methods: One hundred samples of wheat (in two steps) were received. At the first, 50 samples were analyzed by HPLC and Immunoaffinity column to determine Aflatoxins content, then 50 samples were analyzed, by same method. The method used for Aflatoxins was base on association of official analytical chemists (AOAC, 2000).

The principle of method: Test portion is extracted with CH_3OH-H_2O(7+3). Extract is filtered, diluted with water and applied to affinity column containing monoclonal antibody specific for aflatoxins B_1, B_2, G_1, G_2. Aflatoxins are isolated, purified and concentrated on column and removed from antibodies with CH_3OH. Total Aflatoxins are quantified by fluorescence measurement after reaction with bromine solution. Individual Aflatoxins are quantified by HPLC with fluorescence detection and post column iodine derivatization (AOAC, 2000).

RESULTS AND DISCUSSION

HPLC method validation and quality control: Recoveries obtained for analytical method fell within the acceptable range throughout the study and all results are corrected for recoveries that are given in Table 1 and 2. On-going control of the method was monitored using in-house naturally contaminated reference material.

The relative standard deviation for repeatability of the recoveries was estimated based on the following equations (ISIRI, 2003):

$$\bar{x} = \frac{\sum_{i=1}^{n}(x_{1+}x_{2+\ldots.}x_n)}{N}$$

$$S = \sqrt{\frac{\sum_{i=1}^{n}(x-\bar{x})^2}{N-1}}$$

$$RSD_r = \frac{S}{x} \times 100$$

$\bar{x}$	:	Average of test results.
N	:	Number of test.
$x_1, x_2, \ldots x_n$	:	Every test results.
S	:	Standard deviation.
RSD_r	:	Relative Standard Deviation for repeatability.

Determination of accuracy and precision of test method showed that recoveries for Aflatoxin B_1, G_1 was 70-110% and for Aflatoxin B_2, G_2 was 50-120%, so test method was accurated and because RSD_r of test method was below Horwitz equation RSD_r, precision of test method was acceptable.

The results of Aflatoxin concentration in wheat in 2 step of sampling are given in Table 3 and 4.

At the first step of sampling, total Aflatoxins was detected just in 3 samples at amounts 0.23, 0.22, 0.53 ppb. At the second step total Aflatoxins was detected just in two samples at amounts 0.15 and 0.10 Ppb. It was concluded that, contaminated wheat with Aflatoxins was very low and the amount of contamination, after 4 months, wasn't increased during storage.

Growth of the moulds and the production of mycotoxins are dependent upon a number of factors such as temperature and humidity during growth, harvesting, subsequent storage of crops (Whitaker *et al*., 1974).

Table 1: Percent of recoveries for Aflatoxin B_1, B_2, G_1, G_2 in spiked wheat

Sample code	Aflatoxin B_1 (%)	Aflatoxin B_1 (%)	Aflatoxin B_1 (%)	Aflatoxin B_1 (%)
1.1-1.7	99.69	119.07	166.68	49.60
1.8-1.13	106.82	100.44	110.47	41.81
1.14-1.20	120.12	121.22	132.38	52.71
1.21-1.28	101.98	103.04	117.21	83.03
1.29-1.36	105.26	113.14	118.94	92.40
1.37-1.39	118.06	117.37	109.40	97.38
1.40-1.41	99.23	91.68	102.04	81.15
1.42-1.44	116.87	107.93	119.21	99.29
2.1-2.7	77.17	81.68	95.19	61.41
2.8-2.15	65.21	74.77	73.08	93.14
2.16-2.24	69.76	86.75	80.14	96.77
2.25-2.33	76.15	82.06	94.20	93.10
2.34-2.42	78.14	80.26	90.40	91.25
2.43-2.50	85.65	81.66	92.63	94.10

Table 2: Accuracy and Precision of test method by HPLC for Aflatoxins in wheat

Aflatoxins	Amount of spike (nanogram/gram)	Recovery (%)	RSD_r
Aflatoxin B_1	2	98.19	7.54
Aflatoxin B_2	2	101.55	6.07
Aflatoxin G_1	2	111.34	8.81
Aflatoxin G_2	2	77.15	10.82

Table 3: Aflatoxins concentration of Iranian Wheat at the first step of sampling

Sample code	Aflatoxins concentration ($\mu g\ Kg^{-1}$)	Sample code	Aflatoxins concentration ($\mu g\ Kg^{-1}$)
1.1	N.d.	1.27	N.d.
1.2	N.d.	1.28	N.d.
1.3	N.d.	1.29	N.d.
1.4	N.d.	1.30	N.d.
1.5	N.d.	1.31	N.d.
1.6	N.d.	1.32	N.d.
1.7	N.d.	1.33	N.d.
1.8	N.d.	1.34	N.d.
1.9	0.23	1.35	N.d.
1.10	0.22	1.36	N.d.
1.11	N.d.	1.37	N.d.
1.12	N.d.	1.38	0.53
1.13	N.d.	1.39	N.d.
1.14	N.d.	1.40	N.d.
1.15	N.d.	1.41	N.d.
1.16	N.d.	1.42	N.d.
1.17	N.d.	1.43	N.d.
1.18	N.d.	1.44	N.d.
1.19	N.d.	1.45	N.d.
1.20	N.d.	1.46	N.d.
1.21	N.d.	1.47	N.d.
1.22	N.d.	1.48	N.d.
1.23	N.d.	1.49	N.d.
1.24	N.d.	1.50	N.d.
1.25	N.d.		
1.26	N.d.		

To protect consumer safety, International Organizations and every country based on local legislation, set limits for Aflatoxins.

In Iran, legislation indicates that wheat intended for human consumption must comply with limits of 5 ppb Aflatoxin B_1 and 15 ppb total Aflatoxins (ISIRI, 2002).

Any revision in this limit need to do research. Wheat is an strategic commodities in the world. In Iran, production of wheat was increased in recent years. So we can expert some of them.

Table 4: Aflatoxins concentration of Iranian wheat at the second step of sampling

Sample code	Aflatoxins concentration ($\mu g\ Kg^{-1}$)	Sample code	Aflatoxins concentration ($\mu g\ kg^{-1}$)
2.1	N.d.	2.27	N.d.
2.2	N.d.	2.28	N.d.
2.3	N.d.	2.29	N.d.
2.4	N.d.	2.30	N.d.
2.5	0.15	2.31	N.d.
2.6	N.d.	2.32	N.d.
2.7	N.d.	2.33	N.d.
2.8	N.d.	2.34	N.d.
2.9	N.d.	2.35	N.d.
2.10	N.d.	2.36	N.d.
2.11	N.d.	2.37	N.d.
2.12	N.d.	2.38	N.d.
2.13	N.d.	2.39	N.d.
2.14	N.d.	2.40	N.d.
2.15	N.d.	2.41	N.d.
2.16	0.10	2.42	N.d.
2.17	N.d.	2.43	N.d.
2.18	N.d.	2.44	N.d.
2.19	N.d.	2.45	N.d.
2.20	N.d.	2.46	N.d.
2.21	N.d.	2.47	N.d.
2.22	N.d.	2.48	N.d.
2.23	N.d.	2.49	N.d.
2.24	N.d.	2.5	N.d.
2.25	N.d.		
2.26	N.d.		

In world trade, this aim will be done with increasing quality of commodities, for example, decrease of mycotoxins limit based on Good Manufacturing Practice, Good Agriculture Practice and so on.

In view of the above concern, in our country there wasn't any research about mycotoxins in wheat. The present study is part of the continuous studies to clarify the level and incidence of natural occurrence of mycotoxins in Iranian wheat.

CONCLUSION

In summary, the contamination of cereals by mycotoxins is well documented in the scientific literature.

Most Iranian wheat of canvas covered store in karaj during the period of this survey (before and after storage) had no or low levels of Aflatoxins and they were below the tolerated standard level.

These data are required to support by same research in the other Silo in Iran.

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