Middle-East Journal of Scientific Research 6 (3): 219-223, 2010
ISSN 1990-9233

Evaluation of Contamination Rate of Breakfast with Aflatoxin and Ochratoxin Using HPLC Method

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Abstract: Some factors may lead to contamination of breakfast grains with fungi including incorrect method of planting, harvesting, storing and transporting. The methods of prevention and control of highly Mycotoxin production depend on the kind of fungi and the products. In this research, the rate of contamination of breakfast grains with Aflatoxin and Ochratoxin was evaluated by the method of HPLC. 18 prepared samples from each group with different production date were selected; grinded and separately packaged, then they also were considered based on 3 stages of extraction, purification and determination. The diagnosis stage carried out with fluorescence detector: λme: 333, λxe: 460 and grain: 1000, Attn: 16 and determination of its amount was carried out with comparing the level under curve of each sample and the standards plus accounting the dilution factor. Findings: Results showed no Ochratoxin in examined samples. Aflatoxin B1, B2 were found in some samples, but their amount were under the Iran, Europe and USA's standards (5, 2-4 and 2, respectively) but the rate of Aflatoxin G1 and G2 were 0.00 in all samples. In conclusion, there is potential contamination of all kind of food with fungi and its poisons, global standards should be considered in food harvesting, transporting and storing until the consumption date.

Key words: Ochratoxin · Aflatoxin · HPLC · Grain chocolate breakfast

INTRODUCTION

Foods are supplied in different forms such as canned foods, diary, meat products, instant soups, breakfast grains, etc. . Many methods were used to reduce the rate of Mycotoxins with the aim of freeing consumptive foods to the lowest level of Mycotoxins [1]. In spite of developing countries, this matter is done by organizations with exposing laws that make productions with low Mycotoxins in developed countries.

Preventing and controlling methods of Mycotoxins mostly depend on the type of products, but some of general principles are executive. These methods include grain breeding before cultivation, caring while growing and appropriate drying after harvesting [2]. The contamination of foods and food resources by Mycotoxins , especially in tropical and semi tropical areas are unavoidable, whereas the heat and the humidity encourage the growth of Mycotoxins (*Aspergillus Penicillums*) and the production of poison is appropriate because of this and considering the harmful and inappropriate effects caused by poisoning with these Mycotoxins and for solving or getting these kind of effects to the least amount different strategies are used [3]. For removing the contamination of foods and contaminated food resources with Mycotoxins, different methods including chemical , biological and physical methods are used However, the ability of removing all of the poison must not damage the quality of food material [4]. In 1993, the World Health Organization has evaluated Aflatoxin carcinogenic potential, Ochratoxin, Fumonisin, Zearalenone and Trichothecen. In this evaluation

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Aflatoxins were counted as the first group of human carcinogenic, while Ochratoxin and Fumonisin were not known as human carcinogens . Metabolic activity in the liver shows that it is in a moderate rate from the point of sensitivity to poisoning and it is probable that to some extent it shows sensitivity to poisoning like carcinogenic [5]. In the case of excretion of M1 Aflatoxin from the pregnant females, it shows that the babies are also in danger. Therefore, it should be try to remove it from the food [6]. In 1967, 26 Taiwanese children involved with symptoms of food poisoning because of using mould rice and 3 of them died. The amount of B1 Aflatoxin in this contaminated rice was 200 microgram per kilogram. In 1974, 400 Indians were died because of hepatitis. The reason was the consumption of mould contaminated corn which contained 15 milligrams of Aflatoxin in each kilogram [7].

Research studies, proved that 25% of all of the annual foods are contaminated with fungus poisons that result to the economical compensation. Because of the importance of these two Mycotoxins in food products, different institutes showed some different standards for their existence in food product (0 to 50 nanograms in gram) the permitted contamination to B1 Aflatoxin in animals food product is 5 microgram per kilogram and for human food products is 2 microgram per kilogram according to Iranian standard. In the United State and Europe, the amount of Aflatoxin in food product, permitted is 20ppb and 2-4 ppb, respectively [8]. The standard amount of total Aflatoxin in Iran is 15ppb for B1 Aflatoxin in grains and 10ppb in raisins [9].

Mycotoxins have no protein structure, but have small molecules so acute Mycotoxicose is usually different from the poisoning caused by bacteria toxins. The symptoms of Mychotoxicose are very different because of their chemical structures and some effects like cancer or tumor will be seen.

In the present research , it was tried to examine the amount of the fungus poisons of Aflatoxin and Ochratoxin from breakfast grains.

MATERIALS AND METHODS

The Utilized Material and Instruments in this Plan Included: 1- pure Aflatoxin (B1, B2, G1 and G2) poisons. 2- Pure Ochratoxin A poison 3- methanol solvent MeOH 4- Acid acetic Solvent. 5- PBS solvent. 6- Tween 20 solvent. 7- Nacl solvent. 8- dynamic phase solver Acetic Acid (99:99:02) H2O: MECN.9-H2O-HPLC. 10-filter paper and GFF. 11- twice ionized water. 12- Fluorescence detector. 13- HPLC devise 14- Blender. 15- Immuno affinity column.

Preparation of the Samples: In this research, 18 different types of grains (wheat, corn, barley, rice) in 6 sub-group of 3 (A to C groups) from local manufacture companies with different expiry dates were choose and presented in the Table 1.

Experimental Methods: The experiment carried out in 3 stages of extraction, delivering and determination of the amount that described as following:

A) Extraction stage: pass through PBS solvent and mixed then 10 grams of each sample with ± 0.1 error weighted then added 50 micro liters of standard Ochratoxin A and G2, G1, B2, B1 Aflatoxins with 1000ppm viscosity to different samples.

Delivering 10+ 0.1 g sample, then adding 1 gram of NaCL and after that adding 100 ml extracting solvent (MeOH: H2O) and mixing them for 3 minutes with blender and filtering with normal filter paper and picking 5 ml of filtered solvent and adding 45 ml of PBS solvent and shaking it then put into GFF filter paper and picking the whole diluted concentrate along with making the temperature of the column to the temperature and the laboratory and passing of 20 ml of PBS solvent [8]. Again, added 36 ml of PBS solvent and dried the column with a gentle air through it for 10-15 second and adding 500 microliteres of MeOH: AOCH (98:02) to the columns and gathering it and then 1 minutes break and in this time adding 1000 micro liters of Me OH: AOCH (98:02) to the column and gathering it and then adding 1500 micro liters

Table 1: The identification of the samples with different ingredients

Producer Company	Product's name	Product's Ingredients
Dina Company	Chocolate Corn Chee-Puff (group A)	Mashed Corn- sugar- chocolate powder-
Panguan Company	Krasley Chocolate	salt- lecithin- dried milk- oil replaced by chocolate butter
(group B)	Wheat- barley- Rye- chocolate- flour-	Mashed rice, wheat and corn- sugar- cocoa
	cocoa- glucose- sugar- herbal oil	powder- malt concentrate- lecithin salt- vanilla-
Keivan Company (Kam)	Chocolate Bereshtook (group C)	monoastyarat glycerol

of H2O HPLC to vial and mixing it with vertex and washing the column with 20 ml of PBS solvent and injecting 100 micro liters to HPLC[5].

Amount determination stage: diagnosing with fluorescence detector λme = 333 and λxe = 460 and gain =1000 and attn=16 and the determination of the amount was done by comparing the under surface of the curve of all of the samples and the standards were done with counting of the coefficient.

RESULTS

Results showed that in any of the examined samples, Ochratoxin A was not found, but Aflatoxin B1 and B2 was found in some of the foods under the standard of Iran (ppb5) Europe (ppb2-4) and America (ppb20),respectively [8]. However, the amount of G1 and G2 Aflatoxins were 0.00 in all of the samples. The details of the results are presented in Table 2.

DISCUSSION

According to the FAO annual reports, more than 25 percent of all of the produced grains of the world are in danger of contamination with fungus [10]. Some kind of Aspergillus fungus are capable of producing Aflatoxins [10-12]. Among them fungi, *Aspergillus, flavones* and *A. parasiticus* are the most important producer of these poisons. Aflatoxins are special chemical combinations that during some frequent enzyme reactions by a few kinds of Aspergillus and Penicillium fungus at the time of growth in appropriate conditions are produced in most of the products [10-14]. Up to now 18 different types of Aflatoxins have been identified , but only B1, B2, G1, G2 Aflatoxins have been identified as food and food resources contaminators that among them B1 Aflatoxin has the most of toxicity rate [11,15,16]. These poisons result to the weakness of blood deficiency system and safety by the human cells and make them more sensitive to other infections [12,17]. Villa and Markaki [18] inspected B1 Aflotoxin and A Ochratoxin with the method of HPLC in break fast grains of the supermarkets of Athena, Greece and they found that in 56.3% of the sample there was AFTB1 that in 7 samples its rate was more than permitted rate of Europe. In 60% of the samples OTA was founded and 19 samples were contaminated to two Mycotoxins and also Molonie inspected some of breakfast grains in supermarket of France from the point of Ochratoxin A , citrinin and Fumonisin B1 and they found that in 60% of them its rate was more than

Table 2: Mycotoxin final dense that examinated in spike type

		Final Density (ppb)				
Sample's Name	Sample's No.	OTA	AFTB1	AFTB2	AFTG1	AFTG2
Dina Chocolate Samples	D1	0.00	0.25	0.00	0.00	0.00
(Chee-Puff) D Group	D2	0.00	0.00	0.00	0.00	0.00
	D3	0.00	0.25	0.00	0.00	0.00
	D4	0.00	0.00	0.00	0.00	0.00
	D5	0.00	0.00	0.00	0.00	0.00
	D6	0.00	0.23	0.00	0.00	0.00
Total (percent)	-	0	73	0	0	0
Panguan Chocolate	E1	0.00	0.41	0.00	0.00	0.00
Samples (Krasley) E Group	E2	0.00	0.43	0.00	0.00	0.00
	E3	0.00	0.24	0.00	0.00	0.00
	E4	0.00	0.35	0.00	0.00	0.00
	E5	0.00	0.44	0.06	0.00	0.00
	E6	0.00	0.86	0.11	0.00	0.00
Total (percent)	-	0	94.13	5.87	0	0
Keivan Chocolate Samples (Kam)	F1	0.00	0.00	0.00	0.00	0.00
(Bereshtook) F Group	F2	0.00	0.49	0.00	0.00	0.00
	F3	0.00	0.00	0.00	0.00	0.00
	F4	0.00	0.00	0.00	0.00	0.00
	F5	0.00	0.00	0.00	0.00	0.00
	F6	0.00	0.00	0.00	0.00	0.00
Total (percent)	-	0	49	0	0	0
Spike Sample	-	5.00	5.44	1.06	5.00	1.00
Blank Sample	-	0.00	0.44	0.06	0.00	0.00
Total (percent)	-	0	95.87	4.13	0	0

the permitted amount in Europe 20%of the samples contained citirinin and Fumonisin B1 was found not only in corn flake, but also in the products that contained barley some of the samples contained all of three types of Mycotoxins [19]. Kabak [20] started to inspect the Ochratoxin HPLC in Turkey and found that in 38% of breakfast grains and in 17% of baby food based on grains OTA existed. But its density was lower than the permitted amount in Europe. Therefore it wasn't dangerous for human health. In the present research, generally AFB1=95.87% and OTA=0 % (in other word AFB1 was more than the others) it is worth to notify that in this research AFB2 was in the second rank with 4.13% which is a specification of this research. On the other hand, the variety of Aflatoxin in non-chocolate breakfast grains in Iran is more than Greece. In this research with inspection of the chocolate breakfast grains of three companies Keivan, Panguan and Shirin Gandomak we found that percentage of the existence of Aflatoxin B1 was 73, 94. 13 and, 49% and Aflatoxin B2 was 0.0, 5.87 and , 0.0% [20]. Flajs[21] started to inspect grape juice and red wines in Croatia with two methods of HPLC and ELISA and found that the amount of OTA in grape juice is more than the wine. Also with comparing HPLC and ELISA he couldn't clarity the low density of OTA[21]. Hashemi [22] started the evaluation of Aflatoxin M1 of consumptive pasteurized and sterilized milk of Babol city and found that the contamination of M1 in the milk of this district was more than the permitted amount . *Miahi started to segregate Aspergillus and Measuring the rate of the existing Aflatoxin in* fish powder, corn, residue soya and he found that the imported soybean, domestic produced corn and fish powder were more contaminated to Aspergillus fungus. The most contamination to Aflatoxin B1 was in domestic produced fish powder [9]. Alborzi [22] started to evaluate Aflatoxin M1 in pasteurized milk samples in Shiraz and found that 17.8% of all gathered samples contained Aflatoxin M1 more than the permitted amount [23]. Kamkar [25] started to inspect Aflatoxin M1 in the produced raw milk in Sarab and found that the average contamination rate to Aflatoxin M1 in fall and winter samples are considerably more than the summer and spring samples and the summer and spring samples were not different from each other [24]. Kamkar [25] evaluated the outbreak of Aflatoxin M1 in Feta cheese in Iran and found that the outbreak of Aflatoxin M1 in cheese samples may be dangerous for human health. The most of the food products which are used by human or animals is a good place for the growth of fungus and toxins [25]. These poisons make tissue changes that the most of it is done in liver and causes liver problems, cirrhosis and finally results to liver cancer [15]. Aflatoxicose decreases the growth and production and increases the time of blood coagulation and also has carcinogenic effects [12,15]. Aflatoxicose is shaped directly by eating contaminated foods to poison and indirectly by contaminated animal products such as milk, meat, eggs and prepared products from grains[26] . In 2004 severe Aflatoxicose caused to the death of 125 persons in Kenya by eating milk and contaminated foods to Aflatoxin [27,28]. Fischer and colleagues[29] segregated 20-75 micrograms in a kilogram of Aflatoxin from 51 percent of their studied corn samples [29]. We can say that in most types of food products there is an instinctive contamination to fungus and its poison. Some of the regions of our country like Khozestan province prepare good conditions for the growth of poison on food resources because of special climate conditions like heat degree and relative humidity rate and in this climate condition even the imported raw material become contaminated to these fungus and poisons. Because of the importance of Aflatoxin and its productive fungus, especially Aspergillus in human and animal health, this inspection was done to segregate and measurement of the rate of Aflatoxin and Ochratoxin existing in breakfast grain so that the relation among the contamination of the parts is inspected.

In conclusion, according to the Fungi producer Aflatoxin and controlling criteria of this fungi a positive harmony is seen among depopulation of Fungi producer Aflatoxin and controlling criteria with decreasing of Aflatoxin in productive place and we can use potential controlling criteria for the prevention of grain contamination to Aflatoxin.

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