

Determination of total aflatoxin in rice consumption in Yasuj, Iran

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ABSTRACT

Aflatoxins are the secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* which are often produced by molds. The consumption of food contaminated with aflatoxin poison, which is associated with many humans and Aflatoxicosis, in some cases can lead to death. Nowadays, rice is the foodstuff for half of the world's population. Rice is exposed to fungal and aflatoxin contaminations like other cereal crops. This study aimed to investigate and compare the amount of aflatoxin and the level of toxin in rice samples in Yasuj. In this study, 45 rice samples collected from Yasuj supermarkets and the amount of aflatoxin contamination were tested by Elisa. The results showed that total aflatoxin contamination in all samples was lower than the national standard in Iran. The average amount of total aflatoxin in types such as Gerdeh, Champa, Shamim and Fajr, is 6.53- 6.14- 4.54 and 6.12 ng /g, respectively. Comparing the results of this study and other studies, it can be concluded that rice examined in this study is more desirable.

KEY WORDS: TOTAL AFLATOXIN, RICE, ELISA

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INTRODUCTION

Rice is one of the most important crops. Grain dark is the staple food for half of the world's population (Payan, 2011). Rice cultivation has existed in Iran since the Achaemenid (Khodabande, 2010). It is cultivated widely in certain areas of the world such as China, India, Japan, Uruguay and parts of South Africa (Payan, 2011). Many agricultural products from the cultivation to consumption are prone to contaminate with fungi. If the fungipollution-causing strains of toxin, it may produce mycotoxin in some stage of its growth as a secondary metabolite. (Reza, Tahereh, Alireza, & bahar, 2011) Liu *et al.*, 2015).

Despite numerous studies during the first half of the last century, only in the 1960s, it was found that metabolites of fungi are responsible for the illness and death of livestock. Currently, it is well established that toxic mold metabolites are responsible for many epidemics in human and animal population (Reza, Tahereh, Alireza, & bahar, 2011). Mycotoxins are a natural group of toxic compounds produced by several species of fungi. These compounds have different chemical structures and low molecular weight that are found in a large number of agricultural products and food. In general, many types of grains, oilseeds, nuts and dried fruit are prone to fungal pollution (Majid, *et al.*, 2008). The most important mycotoxins involve aflatoxins, patulin, ochratoxin A and zearalenone (Abdel-Wahhab & Kholif, 2010).

Aflatoxins are mycotoxins produced by the *Aspergillus* species and some species of *Penicillium* and *Aspergillus flavus* (Fink-Grenmels, 1999). Different types of aflatoxin are: AFP1, AFG2, AFG1, AFM2, AFM1, AFB2, AFB1 and AFQ1 that aflatoxin B1, biologically is the most active type between the known aflatoxins (Kazemi, Mohtadinia, Mahdavi, Akbari, & Salehpour, 2008). The most important factors in aflatoxin production include temperature, humidity, oxygen concentration, substrate, PH nutrients, microbial interactions, the presence or absence of inhibitors such as organic acids as well as mechanical damage (Amanlou, Rezaei Khkha, Ramazani, & Meyer, 2014, Liu *et al.*, 2015).

Kohgiluyeh and Boyer-Ahmad is one of the provinces, which has water resources and favorable climatic conditions for rice cultivation (Nader, Kobra, & Mohammad Javad, 2008). Six common varieties of rice in this province are Champa, Gerdeh, Tropical Champa, Shamim, Lenjan and Tarom (Cassel, Campbell, Draper, & Epperson, Aflatoxins: Hazards in grain/aflatoxicosis and livestock, 2001), (Arafa, Bloomer, Wilson, Simpson, & Harms, 1981). Since the review of total aflatoxin has not been conducted in this province and according to the importance of total aflatoxin monitoring in rice, this research evaluates the total aflatoxin in the rice produced in Kohgiluyeh and Boyer-Ahmad.

MATERIALS AND METHODS

In this study, the rice sample was randomly collected from Yasuj shops, according to the sampling method of crops.

TOTAL AFLATOXIN ANALYSIS BY ELISA

In recent years ELISA method is used for the determination of mycotoxins. The ELISA method as a general technique for the early diagnosis and detection of mycotoxins with advantages such as sensitivity, sample preparation and easy operation, safety, high accuracy and time saving can be noted. This chemical method used antigen-antibody technique. This method is sensitive, fast and relatively inexpensive (Institute of Standards and Industrial Research of Iran, 2001) (Khosravi, 1998). Total Aflatoxin competitive immunoassay kit EuroProxima B.V of the Netherlands was used to determine how much of the toxin existed in their food. Extraction and test methods for mycotoxins in the samples were performed according to the manufacturer's instructions.

SAMPLE PREPARATION

10 g of the rice powder sample was homogenized with 50 ml of acetonitril in room temperature for 30 minutes was completely shaken. The extract was filtered by No. 42 Whatman paper and then 50 ml of the filtrate was diluted with 150 ml of buffer solution. 100 ml of diluted extract was used per sink in the test. The rest was homogenized for 10 min at room temperature and then the resultant deposit was centrifuged. An aliquot (100 μ l) of the supernatant was diluted with 600 μ l of phosphate buffer at pH = 7.2. An aliquot of this solution (50 μ l) or standard solution (50 μ l), 50 μ l of the aflatoxin-peroxidase conjugate and 50 μ l of the mouse antibody solution against aflatoxin were added to each sink of the used plate. The determination was replicated three times. The samples were incubated for 30 min at room temperature in the darkness. The free and peroxidase-combined aflatoxins compete for the combining site with antibodies to mouse antibodies immobilized on the plate. Next, the plate was emptied and washed five times with phosphate buffer at pH = 7.2. Then, 50 μ l of tetramethyl benzidine and 50 μ l of urea peroxide were added and incubated again for 30 min in darkness. The reaction was terminated by adding 100 μ l of the stop reagent. The absorbance of the solution was measured at a wavelength of 450 nm, using an ELISA reading apparatus. The content of aflatoxins was calculated using the previously prepared standard curve.

Table 1. Analysis of total aflatoxin in the samples of rice in the city of Yasooj.					
Mim	Max	SD	Average	number of samples	Samples
6.42	6.63	0.09	6.53	7	Gerdeh
5.24	6.42	0.21	6.14	17	Champa
0.06	6.48	2.02	4.54	11	Shamim
5.09	6.9	0.58	6.12	9	Fajr

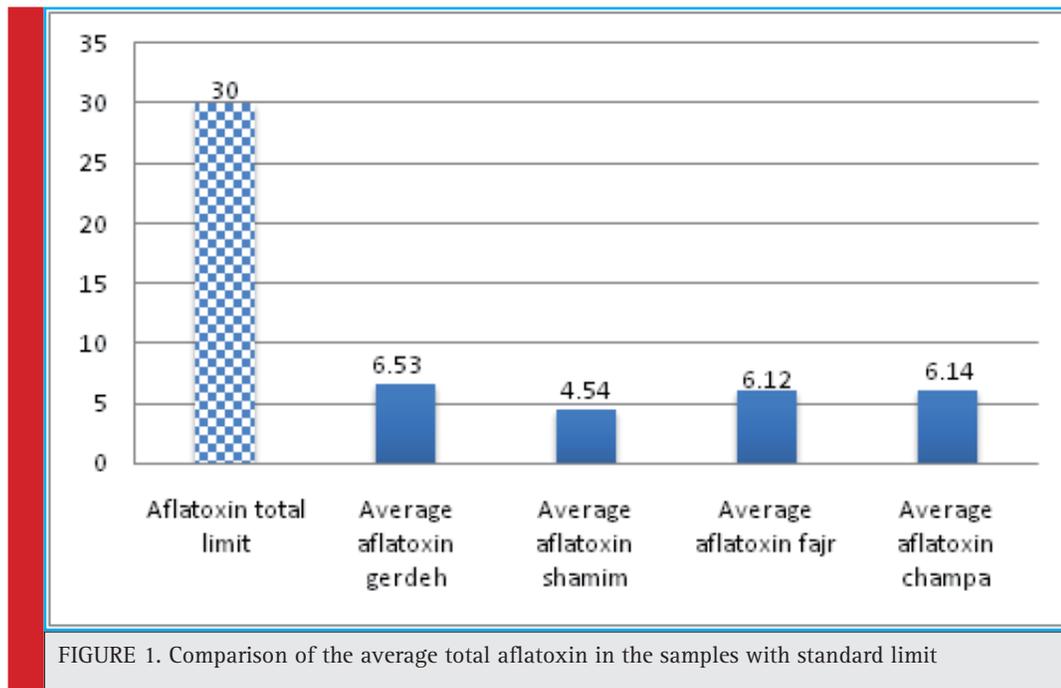
RESULTS AND DISCUSSION

The results are shown in Table 1. The findings of this study show that of the 45 samples all samples had total aflatoxin contamination, but it was lower than the standard limit. The limit of the toxin in different countries varies from 30 $\mu\text{g} / \text{kg}$ to 50 μg food. For example, in Iran it is 30 $\mu\text{g} / \text{kg}$ of food material (Food corporation of India, 2011)-(Jonathan, *et al.*, 2004).The maximum amount of aflatoxin 6.9 and the lowest amount of poison was 0.06 .The average amount of poison in the tested samples was measured 5.83 $\mu\text{g} / \text{kg}$. Average total aflatoxin concentration of toxin in the samples of rice Gerdeh, Champa, Shamim and Fajr, 6.53,6.14,4.54 and 6.12 $\mu\text{g} / \text{kg}$, respectively.The maximum data of The Gerdeh, Champa, Shamim and Fajr, sample, 6.62,6.42,6.68 and 6.9 $\mu\text{g} / \text{kg}$ respectivelyand minimum amount of them 6.42,5.24,0.06 and 5.09 respectively (Table 1). Figure 1 shows Average total aflatoxin in the rice samples.

Rice is among the food items in the basket of Iranians. Due to the high consumption of rice by the Iranians

considering quality and health, it can have an important role in maintaining and promoting overall health.However, due to the high volume of product, product diversity, different cultures, different weather conditions and other causes of contamination and harmful substances, a variety of factors exist. Among the most important and common infections, fungal infections and contaminated mold can be noted. Aflatoxins and Ochratoxin by a group of the secondary metabolites of fungi that are particularly *Aspergillus* arise Azqarch. Studies have shown that post-harvest processing as well as applying proper drying and storage to reduce pollution and prevent its spread areis is required (Rustom, 1997)-(Samarajeeva, Sen, & Cohen, 1990). In a study by Kazemi *et al* on rice East Azerbaijan, 23% of the samples were infected. (Department of Planning & Economic Information and Communication Technology Center, 2013-2014). In another study by Rezai on imported rice in Zabol, 6.27% of thesamples hadfungal infection (Center for Statistics and Information Technology Ministry of Agriculture , 2013-2014).

A recent study by (Liu *et al*, 2015) on the 370 rice schema from six provinces in China was conducted to determine aflatoxin and Ochratoxin A 5/63 percent and 9.4 percent, The results showed that the samples contained Ochratoxin a 4.1% of samples were aflatoxin and aflatoxin and 3.0% of samples above the limit Ochratoxin a Europe Union (Farsiani, Marziye, Mahsa, Mohamad reza, & Mahmoud, 2014).Figure 1 shows the present study.Most of that total aflatoxin investigate the prevalence of less than 30 ng per mg, based on Table 1,



the average total aflatoxin in samples examined 83/5 on the ng/mg. In a study by Elena Suárez-Bonnet *et al* (2013), based on the total aflatoxin contamination (B1, G1, B2, and G2) in rice from Mexico and Aspanya, the results showed that the average total aflatoxin of Spanish rice was 37 / 3 micrograms per kg, in the range of 6.1 to 1383 mg/ kg (Suárez-Bonnet, *et al.*, 2013).

CONCLUSION

Comparing the results of this study and studies in the country and other countries it can be concluded that the rice examined in this study is more desirable. However, the potential pollution. to the fungus and toxins resulting from it and universal standards in the harvest of all itemsshould beapplied to the provide perfect conditions for transportation and time- consuming maintenance. Another important point is that the cultivation and harvest time or until you quit at the end of bran or less is consumed, the possibility of less contamination is more. The role of temperature in mold growth and toxin production after harvesting isproven and it must be maintained at the right temperature as much as the mold growth is prevented.

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