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RESEARCH ARTICLE OPEN ACCESS

Experimental Research of the Effects of Aflatoxins, Ochratoxin and Zearalenone Contamination of Imported Rice

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ABSTRACT
Aflatoxin, ochratoxin and zearalenone are major mycotoxins incereal grains like rice. Their existenceabove standard limits in food can result in serious and adverse effects like cancer. The aim of this study was to survey aflatoxins B1, B2 and G1 ochratoxin A, and zearalenone contamination of imported rice. In this study, 80 rice samples were randomly collected and their contamination with aflatoxin B1, B2 and G1, ochratoxin A, and zearalenone was determined using high performance liquid chromatography HPLC method. Ochratoxin A and zearalenone were found in none of the samples. However, 54.8% samples of imported rice and 22.2% samples of rice were contaminated with aflatoxin. The highest and the lowest amounts of aflatoxin were 2.46 and 0.34 ng/g in imported rice and 1.09 and 0.79 ng/g in the rice, respectively. The most abundant aflatoxin in imported and samples was aflatoxin B1. The findings of this study showed imported rice are contaminated with aflatoxin, especially aflatoxin B1, but contamination of the evaluated samples were lower than maximum tolerated levels established by National Standards Organization.

Key words: rice, aflatoxin, Ochratoxin, Zearalenone, HPLC

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I. INTRODUCTION

Rice is a semi aquatic, annual grass which can be grown under a broad range of climatic conditions. Rice and other agricultural commodities are susceptible to mould attack during the period of growth, storage and consumption. Buyukunal et al (2010). Therefore, the health of this product is of great importance (Aghili et al., 2012; Reddy et al., 2004). Aflatoxins, ochratoxins, and zearalenone are the most important mycotoxins thatare produced following the growth of different species of *aspergillus*, *penicillium*, and *fusarium* in different foods, (Unuson, 2006; Martins & Martins, 2004; Zinedin et al., 2007). Among aflatoxins, aflatoxin B_1 (AFB_1) is the most important of the aflatoxins, considered from the viewpoints of both toxicology and occurrence and has the highest potency as a toxin.(Mohammadi et al., 2012; Ito et al., 2001). These toxins are produced in foods in different stages of storage, production, or processing. Optimal conditions ofaflatoxin production are temperature more than 30°C and humidity more than 90% (Zinedine et al., 2007; Muscarella et al., 2007). Ochratoxinsare produced by *aspergillusspecies*, especially *aspergillusochraceus*.Type A isthe most toxic of ochratoxins found naturally in food. Zearalenone is also a toxin that is produced by *fusariumgraminearum* and *fusariumculmorum*.Cooking heat does not decrease the toxicity of these toxins. Mycotoxins are cytotoxic, genotoxic, mutagenic,teratogenic, and carcinogenic and cause acute renal and hepatic injury in high doses and hepatic cancer in low doses(Zinedine et al., 2007; Muscarella et al., 2007; Polychronaki et al., 2007). Many studies worldwide have shown the contamination of cereal grains and rice with these toxins. For example Reddy et al. assessedaflatoxins in rice samples of India in 2008 and confirmed their contamination withaflatoxin B1 (Reiter rt al., 2010; Behfar et al., 2008; Giray et al., 2007; Reddy et al., 2009; Tanaka et al., 2007; Nguyen et al., 2007). Most studies in Iran have focused on the contamination of the dairy products with aflatoxin.However, Mazaheri analyzed imported rice samples for aflatoxins (Mazaheri, 2009). In the military forces, only one study was conducted by Riazipour et al in on T2 toxin in rice using ELISA (Riazipour et al., 2009).

Our study was aimed to compare the, mycotoxins contamination in imported rice. Aflatoxins B1, B2 and G1, ochratoxin A, and zearalenonewere evaluated in the rice samples using High performance liquid chromatography (HLPC).

II. MATERIALS AND METHODS

The eighty rice samples(n=80) were randomly collected from the big storehouses and kitchens of Tehran according to the standard number 2581 of the National Standards. The samples were evaluated for mycotoxins (aflatoxins, ochratoxin A, and zearalenone) according to the standard number 6872 of the National Standards Organization using HPLC.

In this method, the toxin is first extracted using methanol/water (8:2) and the resulted extract is diluted with water to reach a specific dilution. The diluted extract is passed through immunoaffinity columns containing antibodies specific to each toxin; therefore, the toxin in the extract (antigen) binds to the antibodies in the columns. The toxin bound to the antibody in the column is washed by passing methanol through the columns, stored in a vial, and diluted with water. Reverse Phase HPLC (RP-HPLC) is used to determine the amount of the toxin, which is equipped with post-column derivatization. Post column derivatization brominates these toxins and converts them to compounds with more fluorescence than the primary toxins; therefore, the peaks become visualizable. The amount of the toxin is calculated through comparison of the area under standard curve or the height of the standard curve with that of the unknown sample, considering the dilution coefficient.

The labeled samples ground completely and 4 g transferred in the laboratory and ground them completely. In the next step, we weighed 4 (±0.1) g of the ground sample and transferred it to the Falcon tube and added 1g NaCl. After that, we added 4ml distilled water and 16ml pure methanol to the mixture. The Falcon tubes were shaken for 30 min and centrifuged for 10 min and then filtered using filter paper. We inserted 34ml PBS in the same number of Falcon tubes as the sample tubes, took 6ml from the sample Falcons and added to the Falcons containing 34ml PBS and shook the tubes firmly to mix. After preparing the toxin column, we took 10ml of the 40ml and added to the column. Then, we added 5ml PBS to wash the column and wind dried it for 10-15 seconds. In this stage, we placed the column on the 4ml vial and added 500 µL pure HPLC grade methanol (MeOH- HPLC) to the column on the vial. Then, we added 750µL HPLC grade methanol to the columns and wind dried the columns from above. In this stage, we added 1750µL acid 0.1% to the vial and placed the vials in the Vortex for some seconds. In the end, we used the 1ml vials for injection into the HPLC unit and recorded the results after a few minutes. The data were analyzedwith SPSS software using t-test.

III. RESULTS

The mean values of aflatoxins, ochratoxin A and zearalenone are shown in Table 1. Zearalenone and ochratoxin were found in none of the samples. Table 1shows that aflatoxin B1 is the most abundant mycotoxin in both imported and Iranian rice samples. Also, aflatoxins B1, B2 and G1 levels in imported rice were more than aflatoxin levels in other rice, however all values were lower than the maximum tolerated level (MTL) established by National Standards Organization (5 and 30 ng/gr for aflatoxin B1 and total, respectively). But no significant difference was observed between aflatoxins of imported and local rice samples (p>0.05).

Table 1- The mean values of AF (aflatoxin) B1, B2, and G1, OC (ochratoxin) A and ZE (zearalenone) in the imported and local rice samples (ng/g) (P>0.05)

Rice Type	Number of samples	AF B1	AF B2	AF G1	OC A	ZE
Imported rice	62	0.59±0.16	0.07±0.01	0.35±0.07	ND*	ND
Local rice	18	0.38±0.13	ND	ND	ND	ND
Total	80	0.41±0.16	0.07±0.01	0.35±0.07	ND	ND

ND: Not Detected

Table 2 reveals the percentage of contaminated samples of imported and local rice to aflatoxins B1, B2 and G1.

Table 2- The percentage of AF (aflatoxin) B1, B2, and G1 contamination (>LOD: Limit of Detection) in the imported and local rice types.

Rice type	Number of samples	AF B1		AF B2		AF G1	
		N	%	N	%	N	%
Imported rice	62	34	54.8	2	3.22	2	3.22
Local Rice	18	4	22.2	0	0	0	0
Total	80	38	47.5	2	2.5	2	2.5

IV. DISCUSSION

Recently, occurrence of mycotoxins and in rice seems an issue ofgrowing concern. In the present study, among mycotoxins, Aflatoxin B1 and aflatoxinsB1, B2, G1 were detected in local and imported rice, respectively. Aflatoxin levels in imported rice were more than in local rice and all levels were lower than the maximum tolerated level (MTL), but the differences were not significant. It has been reported that 83% of the samples of the imported rice were contaminated with aflatoxin B1 (59 out of 71 samples) with a mean contamination of 1.89 ng/g. Moreover, regarding total aflatoxins, 83% of the samples were contaminated with a mean of 2.09 ng/g. Aflatoxin B1 contamination was above the local standard limits (5 ng/gr) in 2.8% (Mazaheri, 2009). In our study, the mean contamination was 0.412 ng/gr for aflatoxin B1 and 0.46 ng/gr for total aflatoxin and were not above the local standard limits in any of the samples. A study conducted by Hadian et al (2007) showed that 69% of the rice samples collected from Tehran chain stores were contaminated with ochratoxin A although the contamination was less than the standard limit while we detected ochratoxin A in none of our samples (Hadian et al., 2009). Riazipour et al (2009) measured T-2 toxin in local rice samples of ware houses of Tehran by ELISA and their results did not exceed the permissible limit and confirms results of our study (Riazipour et al, 2009). Mohammadi et al (2012) evaluated 152 samples of imported rice and reported that aflatoxin B1 contamination was not above the standard limits of Iran (5ng/gr) in any of the samples. They found that about 77% of the samples had contamination with total aflatoxin with a mean of 0.671 ng/gr, which was less than the maximum tolerated level (30 ng/gr)(Mohammadi et al., 2012). Among world studies, A study by Trung et al (2001) showed that 8% of the samples were contaminated with high levels of Ochratoxin A(Trung et al., 2001). Moreover, in a study conducted by Liu et al (2006), 92% of the rice samples were contaminated with aflatoxin B1(Liu et al., 2006). Toteja et al(2006) evaluated rice samples collected from 11 states from India and noticed that 17% of the samples were contaminated with Aflatoxin B1 above standard levels (30 µg/kg)(Toteja et al., 2006). Furthermore, a study which was conducted by Nguyen et al(2007) on rice in central Vietnam showed the high rate of aflatoxin B1 contamination (in more than 51% of the samples) followd by ochratoxin A (in 35% of the samples)(Nguyen et al., 2007). Comparison of our findings with those of Nguyen et al shows that both studies are similar in the order of the contaminating toxin (aflatoxin B1 followed by ochratoxin A) although the amount of contamination was much more thanresults of our study. Tanaka et al(2007) performed a study to evaluate the contamination of the Japanese rice with aflatoxin B1, B2, G1, and G2 using HPLC in 2007 and reported no contamination(Tanaka et al., 2007).According to a study by Siruguri et al (2012) on stored rice in India in 2012, contamination was below the standard limits of India (30 µg/kg) in all samples(Siruguri et al., 2012). Reddy et al (2008) also evaluated Indian rice in 2008 and reported that among 1200 evaluated samples, 67.8% were contaminated with aflatoxin B1 (from 0.1 to 308.0 µg/kg). Of all samples, only 2% had aflatoxin B1 contamination more than the standard level (30 µg/kg) (Reddy et al., 2009), while no sample had contamination above the standard levels in our study.

V. CONCLUSIONS

Mycotoxins have become a worldwide worry and they raise serious economic and sanitary problems. Mycotoxin exposure mainly occurs through the food chain. We found aflatoxin contamination in the imported and local rice samples, but contamination was within the standard levelsand the differences were not significant

(p>0.05). Appropriate transportation and storage conditions are very important factors for the low contamination of our samples.

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