Original Research

Occurrence of AF, AFB_1, OTA in Rice Commercialized in Eastern Turkey

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Abstract

A total of 100 rice samples were collected from five provinces (Kars, Agri, Erzurum, Igdir, and Ardahan) in eastern Turkey. Samples were investigated for total aflatoxin (AF), aflatoxin B_1 (AFB_1), and ochratoxin A (OTA) levels. The results show that AF levels in 65 (65.0%), AFB_1 levels in 35 (35.0%), and OTA levels in 38 (38.0%) of 100 samples were higher than the detection limits. AF and AFB_1 levels in all samples were at tolerable limits. OTA levels in 3 samples have been found higher than the legal limits. The highest OTA level in the samples was found in winter season due to climatic conditions, especially relative humidity. Additionally, rice is mainly contaminated by AF, AFB_1 and OTA. In Turkey, contamination in rice could be a relatively critical point for human health. Therefore, it is necessary to have an appropriate method for cereal preservation during distribution to consumers and markets.

Keywords: rice, total aflatoxin, aflatoxin B_1, ochratoxin A, climatic conditions

Introduction

Rice is a semi aquatic, annual grass that can be grown under a broad range of climatic conditions. The unique ability of the rice plant to grow on all kinds of land and water regimes, combined with its adaptation to a wide variety of climates and agricultural conditions, make rice the world's most important cereal crop [1]. Rice and other agricultural commodities are susceptible to mould attack during the periods of growth, storage, and consumption. Spoilage caused by mould growth can cause a decrease in quality and create a potential risk for public health by producing as secondary metabolites a diverse group of chemical substances known as mycotoxins [2].

Most common mycotoxins found in cereals are Aflatoxin (AF) and Ochratoxin A (OTA) that are produced by different species of *Aspergillus* and *Penicillium* [3]. AF are of great concern as they are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic carcinogenesis [4]. 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. Turkey has encountered the AF contamination problem in different foods exported and/or consumed in the country [5].

OTA is a ubiquitous mycotoxin that is suspected of being the main etiological agent responsible for human Balkan endemic nephropathy (BEN) and associated urinary tract tumors. It is frequently found in human blood due to its long elimination half-life (about 35 days in serum), as a consequence of its binding to plasma proteins, its enterohepatic circulation, and its reabsorption from urine. This makes OTA the most detected mycotoxin in human blood all over the world [6, 7].

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The main area of rice production is found on the Asian continent. Turkey is one of the major rice-producing countries, together with China, India, Indonesia, Brazil, Japan, and Italy [8]. Toxicological quality of rice could play an important role on consumer health. More precaution should be taken on quality controls in order to prevent toxicological hazards. The purpose of this study was to determine the occurrence of total AF, AFB_1, and OTA, together with total aerobic plate count and mould/yeast count in rice in a one-year study covering all seasons with different environmental conditions.

Experimental Procedures

Sample Collection

Rice samples were obtained from five provinces (Kars, Agri, Erzurum, Igdir, and Ardahan) of eastern Turkey at intervals between December 2005 and November 2006. They were collected from the same producers and retailers in each season. A total of 100 rice samples were investigated for toxicological (AF, AFB_1, and OTA) and microbiological quality. Samples were placed in sterile jars in 100 g portions and brought to the laboratory immediately. Samples used for microbiological determinations were stored in sealed plastic bags and kept at room temperature in a dark and dry place, and kept at 4ºC until analysis. Samples used for toxicological determinations were kept at -5ºC in bags while awaiting analysis.

Mycotoxin Analyses

AF, AFB_1, and OTA levels were determined in the rice samples by the enzyme linked immunoassay (ELISA) methods. Mycotoxin extraction and tests were performed according to manufacturer's instructions (R-Biopharm, Germany) using AF (Art. No. R4701) [9], AFB_1 (Art. No. R1201) [10], and OTA (Art. No. R1301) [11] RIDASCREEN test kits. Each sample was extracted using the confinable solvents system; methanol/water (70/30) for AF and AFB_1, and a dichloromethane with $NaHCO_3$ buffer (0.13 M, pH 8.1) for OTA. The optical density was measured at 450 nm using a Microtitre plate ELISA reader (ELX 800, Bio-tek Inst.). The mean values of the absorbance for the standards and the samples were evaluated according to the Rida®Soft Win program (RIDAVIN.EXE) distributed by Ridascreen (R-Biopharm). The mean recovery rate has been determined to be 85% for AF, OTA and 102% for AFB_1. According to the manufacturer's description, the detection limits for AF, AFB_1, and OTA by ELISA for cereals were 0.050, 1 and 0.025 μg/kg, respectively.

Microbiological Analyses

Total mesophilic aerobic (TMC) and mold/yeast (MY) counts were determined for each sample. In this plan, microbiological analyses were conducted according to the sampling method specified by Andrews et al. [12]. For this purpose, 10 g samples were aseptically taken and transferred into sterile plastic bags containing 90 ml maximum recovery diluents (Oxoid CM0317), homogenized for 1-2 min. Following homogenization, 10-fold serial dilutions were made in sterile maximum recovery diluents and inoculated onto specific culture media. TMC was enumerated in Plate Count Agar (PCA, Oxoid CM463) after incubation at 30°C for 48 hours [13]. MY were enumerated in Rose Bengal Chloramphenicol Agar (RB, Oxoid CM549) after incubation at 25°C for 5 days [14].

Statistical Analysis

The data were subjected to analysis of variance, whereas differences between means were tested for significance by Duncan's multiple range tests in the general linear model of statistical software SPSS [15].

Results and Discussion

The count ranges, averages, and dispersions of AF, AFB_1, and OTA levels are shown in Table 1. AF levels in 65 (65.0%), AFB_1 levels in 35 (35.0%), and OTA levels in 38 (38.0%) of 100 samples were higher than the detection limits. AF and AFB_1 levels in all samples were within the legal limits of the Turkish Food Codex (TFC) [16] and European Commission Regulations [17]. Only OTA levels in 3 samples were found higher than the tolerable limits.

Rice is an important food crop worldwide, along with wheat and corn and has been a major food in several countries. Rice provides 27.0% of dietary energy supply and 20.0% of dietary protein intake all over the world [18]. A number of studies have been examined on the presence of mycotoxins throughout the world (Table 2).

Detection of AF in rice is a public health concern where the commodity is consumed as a staple and also is used as an ingredient in animal feed, but where post-harvest and quality control systems are inefficient [29]. In this study, highest AF and AFB_1 levels were 3.26 and 1.86 μg/kg, respectively. In a previous study, Ghali et al. [24] tested 16 samples and 12.5% of the samples were contaminated with AF with an average level of 4.7 μg/kg. Reddy et al. [32] observed that AF presence in 3 out of 48 rice sample were higher than the acceptable limits. In the respect of maximum AF results in this study, they were lower than Bandara et al. [33] and Ghali et al. [24] but higher than Liu et al. [34].

In another study, Toteja et al. [35] analyzed 1,511 parboiled rice samples in India and 38.5% of samples were contaminated with AFB_1. Cespedez and Diaz [28] reported that AFB_1 was found in 36.3% of samples. AFB_1 percentages in this study were similar to these results. However, Osman et al. [27] found that 48.2% of rice samples were contaminated with AFB_1. Sales and Yoshizawa [29] demonstrated that AFB_1 was detected in 74 of 78 (94.8%) rice samples. In Vietnam [4], 51.0% of 100 rice samples

Table 1. Results of mycotoxin analysis of rice samples.

Seasons	Mycotoxins	Range (µg/kg)	Average* (µg/kg)	SL[a] (µg/kg)	Dispersion (µg/kg)		
					ND[b]	ND-SL	>SL
Winter (-7ºC, %70.3 R)[b] (n=25)	AF	1.12-3.26	1.93	4	-	25	-
	AFB_1	ND-1.85	1.32	2	13	12	-
	OTA	ND-3.02	1.11	3	12	12	1
Spring (7.1ºC, %66.7 R) (n=25)	AF	ND-3.00	1.73	4	17	8	-
	AFB_1	ND-1.86	1.02	2	17	7	-
	OTA	ND-1.56	0.85	3	17	8	-
Summer (21.9ºC, %47.4 R) (n=25)	AF	ND-3.01	1.47	4	18	7	-
	AFB_1	ND-1.70	1.00	2	21	5	-
	OTA	ND-3.02	0.58	3	17	7	1
Autumn (9.8ºC, %57.8 R) (n=25)	AF	0.06-3.20	1.83	4	-	25	-
	AFB_1	ND-1.76	1.14	2	14	11	-
	OTA	ND-3.02	0.73	3	16	8	1
Year (n=100)	AF	ND-3.26	1.74	4	35	65	-
	AFB_1	ND-1.86	1.12	2	65	35	-
	OTA	ND-3.02	0.81	3	62	35	3

AF – Total Aflatoxin; AFB_1: Aflatoxin B_1; OTA: Ochratoxin A.
ND – Levels were below minimum detection limits.
* Average takes into account only the samples above ND.
[a] SL: Satisfactory limits by TFC and EC.
[b] Climatic parameters have been obtained from the Turkish State Meteorological Service.
R – Relative humidity.

Table 2. Occurrence of AF, AFB_1 and OTA in rice reported in previous studies.

Country	Number of Samples	Mycotoxin	Incidence (Detectable Samples)	Range*	References
South Korea	88	AFB_1 OTA	5 (5.6%) 8 (9.0%)	1.8-7.3 2.1-6.0	Park et al. [19]
Brazil	68	OTA	2 (2.9%)	6.0-9.0	Simionato et al. [20]
Portugal	42	OTA	6 (14.2%)	0.09-3.52	Pena et al. [21]
Morocco	20	OTA	18 (90.0%)	0.02-32.4	Zinedine et al. [22]
Vietnam	100	OTA	31 (31.0%)	0.08-2.78	Nguyen et al. [4]
Côte D' Ivore	10	OTA	10 (100.0%)	9.0-92.0	Sangore-Tigori et al. [23]
Tunisia	16	AF AFB_1 OTA	2 (12.5%) 0 4 (25.0%)	2.0-7.5 - 0.8-2.3	Ghali et al. [24]
Spain	64	OTA	5 (7.8%)	4.3-27.3	Gonzalez et al. [25]
United Kingdom	40	OTA	3 (7.5%)	1.0-19.0	Scudamore et al. [26]
United Arab Emirates	500	AFB_1	241 (48.2%)	1.2-16.5	Osman et al. [27]
Columbia	22	AFB_1	8 (36.3%)	1.0-52.8	Cespedez and Diaz [28]
Philippines	78	AFB_1	74 (94.8%)	0.025-11.0	Sales and Yoshizawa [29]
India	170	AFB_1	0	-	Sundaram et al. [30]
Japan	74	AFB_1	2 (3.0%)	0.3-3.6	Tabata et al. [31]

* Range takes in to account only the samples above detection limits.

and in India [36] 67.8% of 1,200 samples were detected positive. These results showed that AFB_1 occurrence in rice in Turkey could be a relatively critical point for human health. The result of this study also indicated that there was no statistical effect of temperature and relative humidity obtained from the Turkish State Meteorological Service [37] (TSMS) on aflatoxin (AF, AFB_1) occurrence in rice samples. These findings are in agreement with the findings of Simas et al. [38], who investigated the presence of mycotoxins in brewer's grain and found no correlation with climatic factors. However, Nyguen et al. [4] reported that toxins in rice were greatly affected by the season of the year, particularly the rainy season that proved to be a major risk factor for the presence of AFB_1.

Rice is usually harvested at very high moisture levels (35-50%). Therefore, mycotoxin-producing moulds could contaminate the grain and produce important quantities of OTA during storage [19]. In this study, the OTA contamination in rice ranged between 0.042 and 3.02 µg/kg with an average of 0.82 µg/kg. Pena et al. [21] found OTA concentrations ranging from 0.09 to 3.52 µg/kg in Portugal. In a previous study, high levels of OTA were reported by Trung et al. [39], Gonzalez et al. [25], and Scudamore et al. [26], which showed the levels of OTA ranging between 21.3-26.2 µg/kg, 4.3-27.3 µg/kg and 1.0-19.0 µg/kg, respectively. These differences can be due to the analysis method and extraction procedures. Castegnaro et al. [40] observed that the OTA amount detected decreases with increasing pH of extraction and in alkaline medium OTA is converted to an open-ring OTA that could no longer be recognized by the antibodies.

However, detected OTA percentages were higher than the results by Park et al. [19] and Simionata et al. [20], and lower than the results by Zinedine et al. [22] and Sangare-Tigori et al. [23]. These could be due to regions, geographical conditions, storage conditions, and climatic effects. Zinedine et al. [22] stated that cereals, spices, and derived products could be affected by mycotoxin contamination due to the climatic conditions, especially humidity and temperature of the region. In this study, the highest OTA level in the samples was found in winter, with an average of 1.11 µg/kg and significantly higher than in summer ($p<0.05$). These may be related to the highest relative humidity values. Cousin et al. [41] reported that OTA is the most important mycotoxin in cooler climates.

Penicillium verrucosum and *Aspergillus ochraceus* is the main component responsible for producing OTA. These species differ in their ecology, in the commodities affected, and in the frequency of occurrence in different geographical regions. *P. verrucosum* grows only at temperatures below 30°C and down to 0.80 water activity [42]. The obtained data from TSMS confirmed that it can be the source of OTA in rice in this study.

The results of microbiological analysis showed that rice samples were lower than the legal limits of Food Safety Authority of Ireland (FSAI) [43] (5 and 6 log cfu/g for TMC) and TFC [44] (5 log cfu/g for TMC and 4 log cfu/g for MY). According to the results from this study, the highest rates of TMC (3.60 log cfu/g) and MY (2.78 log cfu/g) counts have been detected in the summer, which has the highest temperature values in all seasons among the provinces. The lowest rates of TMC (3.27 log cfu/g) and MY (1.58 log cfu/g) counts were in the winter, which has the lowest temperature values in all seasons. Temperature is considered most critical factor for the microbial quality of food at the stage of storage and consumption. Microbial growth is seen mainly parallel with temperature increase. Higher results were reported by Chang et al. [45], and Piernas and Guiraud [46]. These variations may be related to processing and storage conditions.

Conclusions

Mycotoxins have become a worldwide preoccupation and they raise serious economic and sanitary problems. Mycotoxin exposure mainly occurs via the food chain. One of the main contributors to mycotoxin intake is cereals and cereal products. In our previous survey on Turkey, the results indicate that rice is mainly contaminated by AF, AFB_1 and OTA. Relative humidity was found as an affective factor on the occurrence of OTA. The Turkish population consumes great amounts of cereals and cereal-based products. Therefore, it is necessary to have an appropriate method for cereal preservation during distribution to consumers and markets. In conclusion, a survey of a large number of rice samples will be needed to fully assess and evaluate exposure of the population to mycotoxins by determining the presence of the toxins.

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