

Determination of Zearalenone and Ochratoxin in Foodstuffs

¹Issa Gholampour Azizi and ²Masoumeh Azarmi

¹Department of Mycology, Faculty of Veterinary, Islamic Azad University - Babol Branch, Iran

²Department of Microbiology, Islamic Azad University - Babol Branch, Iran

Abstract: This study was conducted to determine Zearalenone and Ochratoxin in 60 samples of wheat flour from bakeries, 60 samples of rice from retailers and 54 spices (curry, pepper and cinnamon) samples. Contaminations were studied by competitive ELISA assay. The obtained results showed that among 60 wheat flour samples and 60 rice samples, 5 (8.3%) and 3 (5%) samples were contained Zearalenone above the limit of European community regulations (100 ppb), respectively. It was also observed that from 60 wheat flour samples, 45 (75%) samples and from 60 rice samples, 2 (5%) samples contained Ochratoxin above the limit of European community regulations (5 ppb). The achieved results revealed that from 54 spices samples, 25 (46.3%) and 24 (44.4%) samples contained Zearalenone and Ochratoxin, respectively.

Key words: Zearalenone • Ochratoxin • Rice • Spices • Wheat flour

INTRODUCTION

Mycotoxin contamination in agricultural products is seriously dangerous to humans and animals [1]. Fungi of *Fusarium* genus are the most prevalent toxin producing genus. The most important toxin of this genus is Zearalenone which is produced by *Fusarium graminearum* [2]. The toxin is a non-steroidal estrogenic compound which leads to no rut, pseudo-conception, infertility and a weak little fetus. The estrogenic and anabolic activities of Zearalenone in humans, animals and poultry have been proved. Feeding milk cow with Zearalenone-contaminated feedstuff helps this toxin to enter the milk which is dangerous to the public health. In human beings, the toxin has symptoms such as enlarging breasts in young girls, early maturity, hormones imbalance leading to the breast cancer and cervix cancer. Its acute and chronic toxicity, gene toxicity, immunological cytotoxicity, mutagenic effects and its carcinogenicity have also been reported [3-5]. The International Agency for Research on Cancer (IARC) has classified this toxin in the third sub-group of carcinogenic factors [6-8]. The European committee admitted the level of Zearalenone in corn up to 100 ppb [7].

Aspergillus ochraceus and *Penicillium viridicatum* in foodstuff produce Ochratoxin. These toxigenic fungi contaminate food products in different phases of

production and processing, especially in suitable heat and moist conditions. The toxin affects kidney and liver and by going through placenta causes teratogenic and immunosuppression. Its symptom includes low appetite, weight loss, faintness, depression, high thirst and increased urination. Ochratoxin is one of the major causes of death in human and animal in the contaminated regions [7]. Selouane *et al.* [9] detected the prevalence of Ochratoxin in grapes by 59% with 0.08-4 ppb concentration and reported the highest rate of Ochratoxin A production by *Aspergillus niger*. The permitted contamination limit of Ochratoxin in corn is 5 ppb [8]. Abdulkadar *et al.* [10] tested 106 samples of corn, nuts, spices, dried fruit and drinks for Ochratoxin and Zearalenone at Qatar supermarkets in 2004. They reported that 11 samples contained 0.20-4.91 ppb Ochratoxin and 13 samples contained Zearalenone with concentration of 0.18-6.81 ppb. Many countries have conducted inspection program and controlled mycotoxins for several years to promote public health. Schollenberger *et al.* [11] studied 214 plant origins on *Fusarium* toxin at German supermarkets which separated 38% Zearalenone.

Wheat and rice are the most important crops in Iran considering their culture, production and consumption. As these foodstuffs are essential food resources for human beings, their fungal contaminations play a crucial role in jeopardizing human health. But, still there is no

report available on this issue in Iran. Due to the effects of Zearalenone and Ochratoxin and their deteriorating effects on human health, conducting such a study to determine their level in wheat flour at bakeries, rice and spices in the region looks necessary.

MATERIALS AND METHODS

In this study, 60 wheat flour samples from bakeries, 60 rice samples from retailers and 54 spice samples (curry, pepper and cinnamon) were randomly collected in Mazandaran province, Iran in summer 2008. The contaminations of samples were tested for Zearalenone and Ochratoxin using ELISA assay by Agraquant kits supplied by Romer Company, Singapore. Data were analyzed by ANOVA utilizing the SPSS software package. The obtained results were compared with standard limits. There was an identical method to separate these two toxins. Zearalenone and Ochratoxin were extracted from samples by methanol 70%. Firstly, 200 µl enzyme conjugate was added to the uncoated wells and then 100 µl of the standard solutions and 100 µl of the samples were added to the wells. After that, 100 µl of the solutions were transferred to antibody-coated micro plate wells. They were incubated at the room temperature for 10 minutes. Zearalenone and Ochratoxin in the samples and standard solutions competed with enzyme conjugate in order to connect to the solid phase antibody. While using substrate in wells, a blue color was appeared, then using stop solution the blue color was changed to

yellow. Zearalenone and Ochratoxin concentration and absorbance were determined by ELISA Reader at 450 nm and 630 nm [12-15].

RESULTS

The obtained results revealed that among 60 wheat flour samples, 5 (8.3%) samples were contaminated with Zearalenone which was above the limit of European community regulations (100 ppb). Similar result was observed in 3 (5%) samples of 60 rice samples. The concentration of Zearalenone in wheat flour samples and rice samples were 1-107 and 0-106 ppb and the average concentration were 33.44 and 21.78 ppb, respectively. It was observed that from 60 wheat flour samples, 45 (75%) samples and from 60 rice samples, 2 (5%) samples contained Ochratoxin to above the limit of European community regulations (5 ppb). The concentration of Ochratoxin in wheat flour samples and rice samples were 0.5-9.3 and 0.1-5.3 ppb and the average concentration were 5.97 and 1.94 ppb, respectively (Table 1).

The analysis of the spices samples for contamination showed that from 54 spices samples, 25 (46.3%) and 24 (44.4%) samples contained Zearalenone and Ochratoxin, respectively. The concentration of Zearalenone and Ochratoxin were 35-261 and 1-18 ppb and the average concentration were 119.02 and 5.68 ppb, respectively (Table 2, 3). There was not a significant relationship between these mycotoxins contamination levels and types of the analyzed foodstuffs.

Table 1: Distribution of Zearalenone and Ochratoxin contamination in wheat flour and rice samples

Toxins	Samples	No	No. p.	%	Mean ± SE	SD	Max	Min
Zearalenone	wheat flour	60	5	8.3	33.23±4.32	33.44	107	1
	Rice	60	3	5	11.17±2.81	21.78	106	0
Ochratoxin	wheat flour	60	45	75	5.97±0.30	2.34	9.3	0.5
	Rice	60	2	5	1.94±0.18	1.40	5.3	0.1

SE: Standard Error of Mean, SD: Standard Deviation, No: Number, ppb: µg/kg

No. p: Positive Number, European community regulations for Zearalenone is = 100 ppb and for Ochratoxin = 5ppb

Table 2: Distribution of Zearalenone contamination in spices samples

Samples	No	No.p.	%	Mean±SE	SD	Max	Min
curry	18	9	50	119.06±15.57	66.05	254	35
pepper	18	5	27.8	97.68±14.52	61.60	250	35
cinnamon	18	11	61.1	140.22±17.46	76.06	261	35
Sum	54	25	46.3	119.02±9.31	68.42	261	35

Table 3: Distribution of Ochratoxin contamination in spice samples

Samples	No	No.p.	%	Mean±SE	SD	Max	Min
curry	18	9	50	6.94±1.31	5.58	18	1
pepper	18	7	38.9	3.94±0.50	2.14	9	1
cinnamon	18	8	44.4	6.14±1.25	5.29	16	1
Sum	54	24	44.4	5.68±0.64	4.70	18	1

DISCUSSION

Zearalenone are estrogenic mycotoxin which have cellular, sexual and carcinogenic effects [3, 4]. Ochratoxin affects kidney and liver and also has teratogenic and immunosuppressive effects on human [7]. *Fusarium* and *Aspergillus* are found in food storage places which produce mycotoxin at suitable moisture and temperature. The amount of mycotoxin in rice is usually lower than wheat [16]. Although it is difficult to prevent mycotoxin formation in wheat prior to harvesting due to the high temperature and moisture, it is possible to attain favorable result by suitable storage [7]. Decreasing fungal growth and mycotoxin formation in wheat and rice is essential since it is consumed by both humans and animals. Food contaminations with Zearalenone and Ochratoxin have been considered by numerous researchers: In Bulgaria, Zearalenone was measured in 91 crop samples (19 barley, 54 maize and 18 wheat) that were 11.1, 21.1 and 1.9, their mean concentration were 29, 80.6 and 10 ppb and their maximum contamination were 36.6, 148 and 10 ppb, respectively [17]. In 2004-2005, Zearalenone and Ochratoxin were tested in 209 samples (spices, dried fruit, corn, wheat and barely) at Tunisian supermarket. Ochratoxin contamination was 59.8% with the mean concentration of 3.5-5.3 ng/g, but Zearalenone was detected in 15% of the samples with the mean concentration of 10.4-11.8 ng/g [14]. Whereas, in current study positive percentage in wheat, rice and spices was 8.3, 5 and 46.3% for Zearalenone and 75, 55 and 44.4% for Ochratoxin, respectively.

The results of the study showed that Zearalenone and Ochratoxin contamination level in food was high. It is a serious public health problem, because all age groups including babies and children extensively consume the products. Therefore, keeping toxins in low levels in foodstuff is of great importance. To reach this goal, foodstuff should be kept away from probable contamination. There are various methods to control mycotoxins. One of the methods is to prevent mycotoxin formation at stores by reducing moisture and preventing them from damage [18]. Considering acute and chronic effects of Zearalenone on human and also Ochratoxin neuropathy and liver cyto-toxicity, it is necessary to constantly control them in food [1]. As there is no reduction in the level of mycotoxin after cooking [16], it would be feasible to pass some regulations to decrease mycotoxigenic moulds in food. There should be some standards for suitable storage of rice and wheat; because,

these products may get contaminated which endanger human health; therefore, it is crucial to pass some regulations to reduce mould contamination.

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