THE INVESTIGATION OF OCHRATOXIN INCIDENCE IN PEPPER USING ELIZA METHOD

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Abstract: *Ochratoxian A (OTA) is a mycotoxian produced by several species of Aspergillus and penicillium. In this study , eighty six samples of different types of pepper powder purchased from Retail shops in Shahrekord-Iran were surveyed for the presence of ochratoxin A using ELISA method during Analytical results showed a Frequency of contamination of 52 % of total analyzed pepper samples. The percentage of contamination of samples was 19%, 33% and 0 % in red pepper, black pepper and white pepper Respectively. the highest frequency of positive samples (33%) and the most contamination sample (14.3 ng/g) was found in samples of black pepper.15 sample of 86 total samples(17.3%)exceed the maximum level of 5ng/g set by European regulations for OTA in Cereals and spices. evaluation of the data did not indicate a significant difference in contamination levels.*

Keywords: *black pepper, red pepper, white pepper, Ochratoxin A, ELISA*

Introduction

Mycotoxins are the second metabolites produced by specified fungus (Goryacheva et al, 2006). Ochratoxin A (OTA) is one of the important widespread mycotoxin in the world produced by A ochraceus , A carbonarius, A niger, P Verosocium (Pfohl-Leszkowicz et al,2008 and Zinedine et al,2006). International cancer research agency has been classified OTA in group B (IARC, 1993). Presence of OTA is proved through its determination in Blood, milk, cerebrospinal fluid and urine 3 4 6 Breitholtz-Emanuelson et al, 1993;

Breitholtz et al, 1991and Castegnaro et al, 2006).It is also toxic in some animals (Pfohl-Leszkowicz, 2009). The permission limit of it in cereal seeds, cereal products, seasons and dried fruits are 5,3 and 10 ng/kg respectively (EC, Commission Regulation (EC),2006).Peppers consider as OTA source to whom subject to raw peppers and harvesting process as well (Jalili et al,2010). Chromatographic methods are used to detect OTA, however owing to their being expensiveness replaced by ELISA method as a rapid detection method. This study aims at determination of OTA incidence in white, red, black peppers.

Material and methods

In this study 86 samples of red, white and black pepper powder purchased randomly from supermarkets in Shahrekord City. All purchased powder in 100g ethylene bags placed in appropriate temperature conditions in biotechnology Research Center of Shahkrekord, Islamic Azad University. 10ml of phosphoric acid added to 50 g of each sample mixed homogenously, 20 ml Dicholoromethane added, centrifuged 5 min and finally the supernatant(phosphoric acid) removed. Mixture filtered through a filter paper, then 12 ml of filtrate poured in a test tube, dried under the gentle flow of nitrogen at 50 ˚C, 2ml hexane added, centrifuged for 5min.50 µl of under layer diluted with 200µl buffer and finally 50 µl of diluted solution injected to kit. To trace OTA by ELISA, 100µl of buffer injected in A_1 and A_2 holes, then 50 µl diluted buffer injected in B_1 and B_2 holes by micro plate, 50 µl poured in $C_{1,2}$ to $H_{1,2}$ 50 µl of each sample solution added to holes of kit finally 25 µl Of conjugated solution (OchratxinA-HPR) to all holes except $A_{1,2}$ .Then 25 µl of antibody solution added to all holes except A_1. Micro plate placed in incubator at 37 ˚C for 1hr, then solutions removed from plate and holes washed 3 times using buffer, 100µl of substrate solution added and micro plate kept in ambient Temperature for 30 min. Finally 100µl of inhibiting solution added to each hole and their absorbance read by ELISA detector(STAT FAX 2000-UK) at 450nm.

Results

In this study 86 pepper samples investigated by ELISA aiming at OTA detecting. Results revealed that 45 samples of 86 samples contained OTA. Average, mean and standard deviation of OTA amount along with contamination range for both all samples and contaminated samples were 1.47, 0.31, 2.61, 0-10.4 and 2.81,1.08,3.04, 0.19-10.37 respectively.The amount of produced OTA depends on the kind of foodstuff, humidity and temperature, pH, the type of fungi. International Agency on Cancer Research has

classified OTA in Group B as cancer creating agent (IARC, 1993).OTA have genotoxicity, immunetoxicity, nephrotoxicity and teratogenic properties (Alvarez et al,2004 ; Juan et al, 2008; Matrella et al, 2006 and Zinedine et al, 2008). Besides some studies reported the effective role of OTA in creation of kidney fibrosis and urinary tract tumors (Pfohl-Leszkowicz and Manderville, 2007). Current study exhibited that 46 % of peppers in Shahrekord province of Iran are contaminated to OTA and contamination range was 0.19-10.37 ng/mg. Present study showed that 50 % of all types of pepper in Shahrekord city were contaminated to OTA and contamination level was 0.19-10.37. Jalli and Jinap(2010) reported that 47.5 % of 120 sample of Malaysian peppers Contaminated to OTA using HPLC method and contamination range was 0.15-13.58 ng/kg (Jalili et al,2010). OTA investigation using HPLC in pepper in Italy revealed a 3-10% contamination a 0.012-0.66 μg/kg as contamination range (Brera et al, 2010). Chung et al.(2009) reported a 32 % contamination to OTA in 300 samples in Honk Kong by HPLC method (Ghali et al, 2008). Overall results of current study and comparing it with similar studies revealed that food contamination condition differs due to geographical location, the sanitization level during cereal harvesting, storage and handling effect on fungi growth. Pepper grinding stage and accurate storage of them may consider as other affecting factors on OTA. Among 86 pepper samples contaminated to OTA, 15samples (17.4%) were more than permission limit (3ng). Regarding that amount is not too high; this contamination may have undesirable affections on consumer's body in long time as pepper is highly used season in Iranian diet. Hence observance of food safety principles during hygiene period of storage, handling of peppers in order to reduce contamination is recommended.

Table 1. OTA abundance , average and contamination level in peppers

Samples	Contaminated samples	Average	Standard deviation	Mean	Contamination range
86	45	1.47	2.61	0.31	0.0-10.37

Table 2.OTA abundance , average and contamination level in contaminated peppers

Samples	Average	Standard deviation	Mean	Contamination range
45	2.81	3.04	1.08	0.19-10.37

Table 3. OTA % of red , white and black pepper in Shahrekord city,Shahrekord Province of Iran

samples	N.O.	Positive OTA samples (%)	Negative OTA samples(%)
RED pepper	28	13	87
BLACK pepper	40	33	66
White Pepper	28	0	100
Total	86	46	53.9

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