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Ochratoxin a Levels in Different Types of Bread and Flour

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Summary: Ochratoxin A (OTA) levels were determined in 132 different types of flour and bread (34 white flour, 14 wholemeal flour, 10 corn flour, 36 white bread, 28 wholemeal bread and 10 corn breads). The samples were collected from different bakers and markets in Bursa, Turkey and some unities of Turkish Army between February 2005 and May 2006 for analyses. OTA concentrations were determined by competitive enzyme-linked immunoabsorbent assay (ELISA) technique. Among the samples, highest mean concentration was detected in wholemeal flour samples as 9.30 µg/kg. OTA has been determined in 110 samples (83%). In 92 (70 %) of the samples the toxin levels were exceeded maximum tolerable limits (3 µg/kg). These findings indicate that flour and breads may have potential risk for the public health.

Key Words: Ochratoxin A; Flour; Bread.

Farklı Tip Ekmek ve Unlarda Okaratoksin A Düzeyleri

Özet: Bu çalışmada, 132 adet farklı tip un ve ekmek örneğindeki (34 beyaz un, 14 kepekli un, 10 mısır unu, 36 beyaz ekmek, 28 kepek ekmeği ve 10 mısır ekmeği) okratoksin A (OTA) düzeyleri belirlenmiştir. Örnekler, Bursa'daki farklı fırın ve marketler ile Türk Silahlı Kuvvetlerinin farklı birliklerinden, Şubat 2005-Mayıs 2006 tarihleri arasında toplanmıştır. Örneklerdeki OTA düzeyleri ELISA tekniği ile tespit edilmiştir. Örnekler arasında en yüksek değer 9.30 µg/kg olarak kepek ununda saptanmıştır. Örneklerin %83'nde (110 örnek) OTA tespit edilmiş ve %70'nde (92 örnek) toksin düzeylerinin tolerans limitinin (3 µg/kg) üzerinde olduğu belirlenmiştir. Un ve ekmek örneklerinde tespit edilen OTA miktarları, halk sağlı açısından risk oluşturabilecek düzeylerdedir.

Anahtar Kelimeler: Okratoksin A; Un; Ekmek.

Introduction

Ochratoxins are secondary metabolites of toxigenic species of *Aspergillus* and *Penicillum* fungi. They were first isolated in 1965 from *Aspergillus ochraceus*. Ochratoxins are composed of ochratoxin A (OTA), ochratoxin C (OTC), 4-hydroxyochratoxin A (4-OH OTA), ochratoxin B (OTB) and ochratoxin α (OTα). OTA is the most toxic member of the group[14]. *A. ochraceus, A. cabonarius* and *Penicillium verrucosum* are main producers of ochratoxins, and grows at moderate, high and cool temperature climates, respectively. In addition to the presence of OTA in different geographical regions, contamination by this mycotoxin occurs mostly under preharvest and postharvest conditions in cereals such as wheat, maize, rye, barley, and oats. It also occurs in peanuts, coffee beans, bread, rice, and dried fruits. Furthermore, the presence of OTA in food products such as coffee, wine, beer, grape juice, meat and meat products is due to its relative chemical stability during industrial processing[15,16].

OTA is genotoxic, carcinogenic, nephrotoxic, hepatotoxic, embryotoxic, teratogenic, and immunotoxic[2]. Therefore, the European Union Scientific Committee has recommended that

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OTA levels be reduced to below 5 ng/kg of body weight per day[5]. OTA has been discussed as a causative factor in human disease "Balkan endemic nephropaty" (BEN) and in the urinary tract tumors in humans. A similar nephropaty has been detected for some regions in France and Northern Africa[10]. High concentration of OTA have also been found to damage the intestinal mucosa in rats, dogs, swine, and chicken[8, 9]. It was reported that a single exposure to OTA resulted in a rapid inflammatory effect associated with desquamation, necrosis, and acute diarrhea[11].

The total production of field crops for human was approximately 22 million tons in Turkey in 2003 [17]. The consumption of bread and flour-based products are common in Turkey due to the feeding habits of the people. Generally, white bread is mainly preferred by Turkish people and most of people and bakers are not any information of OTA. Therefore, it is important to know the levels of OTA in flour and breads. There are limited data related to OTA levels in different types of flour and bread in Turkey. This study was carried out to determine the levels and incidence of the OTA in different types of flour and bread, and to assess the probable risks on human health.

Materials and Methods

The research materials consisted of 132 samples including white flour, wholemeal flour, corn flour, white bread, wholemeal bread and corn bread which were collected and analyzed between February 2005 and May 2006. The 85 samples were obtained from different bakers and markets in different districts of Bursa, and 47 samples including white flour and white bread samples were obtained from some unities of Turkish Army. The samples were stored in plastic bags at – 20 ºC until the analysis.

OTA concentrations were detected by competitive enzyme-linked immunoabsorbent assay (ELISA) technique (EL 312e Biotek, biokinetics reader, USA) according to the procedure described by manufacturer for creals (R-Biopharm) by using RIDASCREEN® Ochratoxin A-Test kits (Art. No.: R1301)[13]. There are not any recovery data of OTA in flour and breads while there are recovery rate in creals, feed, beer and pig serum. Therefore we determined recovery values for each sample material. The recoveries (when spiked with 10 ng/g and 20 ng/g OTA with *n*=3, R-Biopharm Ochratoxin A Standart) for white flour, wholemeal flour, corn flour, white bread, wholemeal bread and corn bread were ranged between 70% and 75%, between 100% and 120%, between 100% and120%, between 95% and 110%, between 80% and 90% and between 85% and 95%, respectively. All statistical analyses were performed using SPSS 10.0 Program. For statistical analysis of among the flours and breads multiple comparisons of variance (ANOVA) was used.

Results

The OTA levels in different types of flour and bread were variable. OTA not detected in the some of white flour and white bread samples were obtained from some unities of Turkish Army. Data regarding to OTA levels of the flour and bread samples were presented in Table I. OTA has been detected in 110 of 132 (83 %) samples.

Table I. OTA Levels in different types of bread and flour (µg/kg)

Tablo I. Farklı Tip Un ve Ekmeklerdeki OTA Düzeyleri (µg/kg)

Samples	*n*	Mean	S.E.M.	Minimum	Maximum
White flour	12	6.89	0.46	1.87	7.44
Wholemeal flour	14	9.30	1.33	1.07	16.70
Corn flour	10	6.39	1.10	3.86	15.67
White bread	11	6.38	0.48	4.36	9.75
Wholemeal bread	28	7.84	0.39	3.54	12.61
Corn bread	10	4.94	0.20	4.08	5.28
White flour *	22	1.39	0.55	nd**	8.60
White Bread	25	3.36	0.73	nd**	11.40

* the samples brought by Turkish army
** non detectable

Discussion

The fungus that produces ochratoxins can grow on grain stored at 15-19 % humidity and temperatures of ≥ 15 ºC[1]. The production of the toxin by the moulds is maximal at pH 5.5 in the presence of iron, copper and zinc[3]. Contamination of grain is possible during and especially after harvest[12].

The Codex Committee on Food Additives and Contaminants of the Codex Alimentarius Commission of the World Health Organization (CCFAC/WHO) recommendes a maximal residue level of 5 µg/kg OTA in grain and grain products that are used for human consumption[6].

In European Union and Turkey, the OTA level in cereals and cereal products are regulated with maximum residue levels as 5 μg/kg and 3 μg/kg, respectively[7,18].

In our study, the mean concentrations of white flour (6.89±0.46 μg/kg), wholemeal flour (9.30±1.33 μg/kg), corn flour (6.39±1.10 μg/kg), white breads (civilian 6.38±0.48 μg/kg and military 3.36±0.73 μg/kg), wholemeal bread (7.84±0.39 μg/kg) and corn bread (4.94±0.46 μg/kg) were higher than the maximum limits (3 μg/kg) set by CCFAC/WHO as well as EU and Turkey, except the mean value (1.39±0.55 μg/kg) of white flour samples obtained from Turkish Army. Wholemeal flour samples had the highest mean concentration of OTA as 9.30 μg/kg, although the mean concentration (7.84 μg/kg) of wholemeal bread samples was high. Both of the mean levels were higher than the limits (3 μg/kg) of CCFAC/WHO, EU and Turkey. All concentrations were also higher than the reported by Baydar et al.[4] (below 1 μg/kg in the 11 of 12 wheat-based flour samples, for two corn flour samples of 0.47 μg/kg and n.d.) in Turkey. The measured values in the 70 % of the total samples analysed in the study were higher than the maximum limit (3 μg/kg) set by EU and Turkey as well as CCFAC/WHO. Differences among the flour and breads were calculated. Statistically important difference ($p<0.05$) were observed between white flour samples and wholemeal flour samples, between wholemeal flour samples and corn flour samples. Similarly, important difference ($p<0.05$) were also observed between white bread samples and corn bread samples, between wholemeal bread samples and corn bread samples.

OTA levels can be reduced initially by cleaning the dust and broken grains from wheat. However, the reduction is small and probably depends on the condition of the grain when received. The bran and offal of wheat contain high levels of OTA. Therefore wholemeal flour contains much higher concentrations of OTA than white flour[15]. Similarly, OTA levels in the wholemeal flour and bread samples were much higher than the other types of flour and breads.

The findings indicate that the levels of OTA may affect the consumer and threaten the public health. The risk of OTA for consumer is high in Turkey because of the fact that the average daily consumption of wheat and wheat products by a Turkish adult is about twice as high as most Western countries[4].

OTA levels in flour and bread can be high due to the deficiency of the control on food processing in Turkey, although in Turkish Food Codex the maximum limits of OTA in grain and grain-based products has been established. In addition, there are growing concern to consume wholemeal-based products depending on dietary. The result of this study about the factual contamination of flour and breads with OTA implies that more emphasis should be given to the routine OTA inspection of grain, flour and breads in Turkey. In addition, govermental agencies need to inform and educate the farmers, bakers and consumers about the importance of OTA.

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