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Evaluation of Ochratoxin A Contamination in Non Alcoholic Beers in Iran

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Abstract: Ochratoxin A is a mycotoxin produced by some species of fungi such as Aspergillus and Penicilium. It is found as a contaminant of a variety of animal and human foods. Ochratoxin A has teratogenic, carcinogenic, hepatotoxic and especially nephrotoxic effects on human and animals. According to the dietary recommendations in Iran, consumption of soft drinks is decreasing, while the consumption of nutritive beverages such as non alcoholic beer, is increasing. Therefore, this study was carried out to evaluate the content of Ochratoxin A in 70 malt beverage samples (35 local and 35 imported) which were collected randomly from supermarkets of Tabriz in Iran during July to September 2006. These samples were analyzed for levels of Ochratoxin A by ELISA technique. Results were compared with the European proposed maximum limit (2 ppb), for Ochratoxin A in beer. In general, all of the local and imported samples were contaminated with Ochratoxin A but the levels of contamination were under the maximum permitted levels. The mean Ochratoxin A concentrations in local and imported samples were 96.04±126.13 and 60.71±47.82 ng L^{-1}, respectively that the difference was not statistically significant ($p>0.05$). No significant differences were observed between different brands contamination. The detected range of Ochratoxin A in local samples was from 0.50-524.50 ng L^{-1}, while in imported samples was from 0.90-228.60 ng L^{-1}. The mean and range of Ochratoxin A contamination in local samples were higher than the imported samples. Although the Ochratoxin A concentrations of nonalcoholic beers in this study were under the maximum permitted levels, the long term continuous consumption may have considerable health problem despite the low levels of contamination.

Key words: Mycotoxins, Ochratoxin A, non alcoholic beer, ELISA

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by some species of fungi such as Aspergillus and Penicilium. Ochratoxin A is found as a contaminant of a variety of animal and human foods, including cereal and grain products, beans, spice, poultry, dried fruits, coffee, cocoa, wine, beer, fruit juices and milk (Obrien and Dietrich, 2005). Ochratoxin A has been identified in blood, bile and urine of humans and animals after consumption of contaminated food (Petkova *et al*., 2000) and has been implicated as one of the etiological agents in Balkan endemic nephropathy, a chronic renal disease involving progressive renal fibrosis and impaired renal function, where contamination with much Ochratoxin A is described (Vrabcheva, 2004). Ochratoxin A is also immunosuppressive, teratogenic, genotoxic, carcinogenic, hepatotoxic and mutagenic and affects blood coagulation, inhibit protein synthesis, promote membrane peroxidation, distrupt calcium homeostasis and inhibit mitochondrial respiration (Pfohl *et al*., 2002). These facts indicate that human exposure should be kept to minimum concentration. Ochratoxin A production is dependent on different factors such as temperature, water activity (a_w) and medium composition, which affect the physiology of fungal producers (Birzele *et al*., 2000) and can grow easily according to the unsuitable conditions of growth, harvest, transport and storage (Odhav and Naicker, 2002).

Barley is an important material for the production of malt for brewing purpose. Three major steps can be distinguished in the malting processes: Steeping, germination and kilning, The mycoflora and Ochratoxin A producers are determined at different stages of malting process (Gareis, 2001). Studies which carried out in Germany (Jiao *et al*., 2001; Thelman and Weber, 2002; Bresch *et al*., 2000; Wolf, 2000), Canada (Scott and Kanhere, 2003), Japan (Nakajima *et al*., 2001) and Southern Africa (Odhav and Naicker, 2002) revealed that the most samples were contaminated with Ochratoxin A.

If the above-mentioned mycotoxins producers were isolated in barley and malt, the malting process by-products such as barley rootlets would be contaminated by these same toxigenic species and their mycotoxin. In Islamic Republic of Iran, alcoholic beverages such as beer are not consumed as a religious beliefs, also nutritional recommendations suggest that soft drinks could be replaced with non alcoholic malt beverages, fruit juices and milk drinks, in addition, public believe that malt

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beverages consumption can prevent kidney stones formation due to diuretic effects. Therefore, the consumption rate is increased and there is no available information about the Ochratoxin A contamination in malt beverage samples (non alcoholic beer). So that this study carried out to evaluate the contamination of Ochratoxin A in this product.

MATERIALS AND METHODS

In this study, 70 malt beverage samples (35 local and 35 imported) of different bottles and cans, which are corresponding to all of brands in supermarkets and traditional bazaars in Tabriz, Iran were collected randomly, during July to September. The samples were applied to the ELISA in order to get a quick idea of the Ochratoxin A levels-Monitoring of mycotoxins depend on precise and reliable analytical methods. However, there is no universal method for detection and quantification of mycotoxins in feeds or foods because the mycotoxins are chemically quite diverse, as are the matrices in which they occur. While analytical methods employ different separation/ detection techniques, such as ELISA, HPLC or TLC, all procedures require a suitable sample extraction step (Gilbert, 2000). The immunological based methods are preferable because they may reduce the time and expense required for analyses of these naturally occurring toxicant (Park *et al*., 2002). Additionally, the ELISA technique is highly sensitive, specific and can be automated to analyze numerous samples. So we used the ELISA technique to detect Ochratoxin A contamination.

Several countries have specific regulations for Ochratoxin A with maximum permitted levels ranging from 1-50 μg kg^{-1} for foods (Creppy, 2002). By European food regulations the maximum limit proposed for Ochratoxin A in beer is 2000 ng L^{-1} (2 ppb), (Baydar *et al*., 2005) that we compared our results with European limit.

Preparation of samples: The analytical grade chemicals were sodium Hydrogen Carbonate ($NaHCO_3$), Hydrochloric acid (HCL) and Dichloromethane (DCM). Ochratoxin A levels were determined by a commercially available Ochratoxin A ELISA kit (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany).

In order to determine Ochratoxin A by ELISA, 2.5 mL of 1 N HCL solution was added on 2 mL of each samples and shaken, then the sample mixture was extracted with DCM. Following the centrifugation (3500 g, 15 c, 15 min), DCM phase was collected and mixed with equal volume of 0.13 M NaHCO3 buffer (pH 8.1). The removed aqueous phase was diluted by the NaHCO3 solution (pH 8.1) and used for the Ochratoxin A ELISA kit.

The optical density was measured at 450 nm by using ELISA 96-well microplate reader (Sunrise, GmbH, Tecan, Austria).

Ochratoxin A contamination in each malt beverage sample was expressed as ppb. According to the manufacturer´s description, the detection limit for Ochratoxin A was 25 ng L^{-1} and recovery rate were more than 85%.

Statistical analysis: In the statistical analysis, initially, descriptive analyses were performed to describe the proportion of Ochratoxin A contamination in local and imported samples. Independent t-test were used for comparing the mean contamination of Ochratoxin A between the local and imported samples. One-sample t-test were used for comparing the mean contamination of Ochratoxin A by permitted values. And finally, Analysis of Variance (ANOVA), were performed for comparing the mean of Ochratoxin A contamination between different brands. Test results are statistically significant at $p<0.05$.

RESULTS

In this study 70 malt beverage samples were analyzed for levels of Ochratoxin A by ELISA technique. All of the local and imported samples showed detectable levels of Ochratoxin A as indicated in the Fig. 1. The mean Ochratoxin A concentration in local and imported samples were 96.04±126.13 and 60.71±47.82 ng L^{-1}, respectively. The difference between local and imported samples was not statistically significant ($p>0.05$). In according to ANOVA results, the mean contents of Ochratoxin A were not significantly different between several brands. The local samples contained Ochratoxin

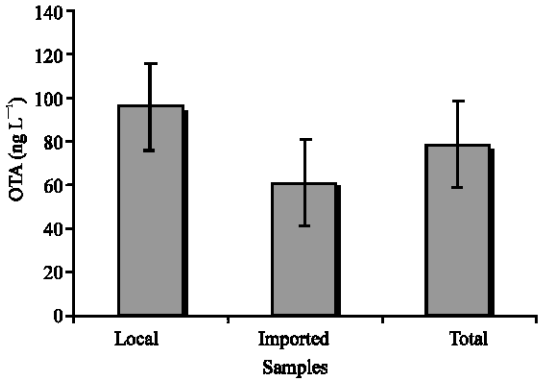

Fig. 1: The mean of Ochratoxin A concentration in total, local and imported samples. The difference between local and imported samples was not statistically significant

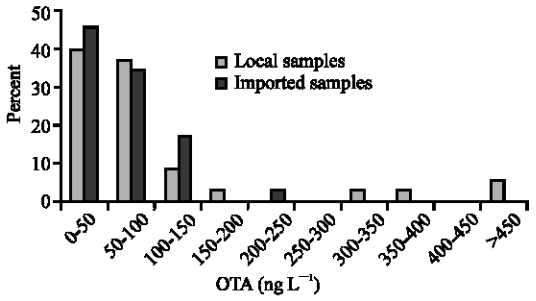

Fig. 2: The percentage of malt beverage samples in each concentration range

A ranged from 0.50-524.50 ng L^{-1} and the imported samples contained 0.90-228.60 ng L^{-1}, as shown in Fig. 2. Of local samples, 11.42% had Ochratoxin A concentration above the upper level of the imported samples. The mean and range of Ochratoxin A contamination in local samples were higher than the imported samples. From total local and imported products 25.7 and 40%, respectively had higher Ochratoxin A levels than the mean.

DISCUSSION

In Islamic Republic of Iran, alcoholic beverages such as beer are not consumed as a religious belifes, so that we compared the content of Ochratoxin A in malt beverage samples (non alcoholic beer) with literature reports of other countries' beer samples.

In this study, all of local and imported samples were contaminated with Ochratoxin A while, Jiao *et al.* (2001) determined Ochratoxin A in 41% of 194 beer samples tested, Thelman and Weber (2002) reported Ochratoxin A in 27% of malt samples analyzed, Scott and Kanhere (2003) found Ochratoxin A in 26 of 41 beer samples tested (63%), Nakajima *et al.* (2001) determined the contamination of Ochratoxin A in 21 out of 22 japanese beers (96%) and 86 out of 94 imported beers (91%) and Odhav and Naicker (2002) found Ochratoxin A in 13 out of 29 traditional beer samples (45%).

One reason for differences between literature reports and our findings may be related to the methodology differences in Ochratoxin A analysis. The ELISA technique that we used to detect Ochratoxin A contamination, is more sensitive and specific than TLC which is a qualitative rather than quantitative technique has been used in previous studies.

The mean Ochratoxin a concentration in local and imported samples were 0.096±0.126 and 0.060±0.047 μg L^{-1}, respectively. Our results were similar to the findings of Bresch *et al.* (2000) who verified the presence of Ochratoxin A in Germany beer samples with mean value of 0.09 μg L^{-1} and Odhav and Naicker (2002) from Southern Africa, that they found Ochratoxin A in traditional beer samples with average value of 0.075 μg L^{-1}.

Jiao *et al.* (2001) reported the mean Ochratoxin A concentration in beer samples 0.10 μg L^{-1}. Thelman and Weber (2002) found Ochratoxin A in malt and beer samples tested with average concentrations of 0.92 μg kg^{-1} and 0.1 μg L^{-1}, respectively. Wolf (2000) verified the presence of Ochratoxin A in beer samples with mean value of 49.5 μg kg^{-1}. In this study, Ochratoxin A values were lower than of studies which is performed in Germany by Jiao, Thelman and Wolf.

In Canada, Scott and Kanhere (2003) found Ochratoxin A in beer samples tested with an average concentration of 0.061 μg L^{-1}. Nakajima *et al.* (2001) determined the mean value as 0.0125 μg L^{-1} in japanese beers and an average of 0.0101 μg L^{-1} in imported beers. The mean of Ochratoxin A contamination in our samples was higher than Scott and Nakajima.

Another reason for differences between literature reports and the results of this study may be related to the malt quality used for beer production.

Our local and imported samples contained Ochratoxin A ranged from 0.50-524.50 and 0.90-228.60 ng L^{-1}, respectively. The mean and range of Ochratoxin A contamination in local samples were higher than the imported samples.

However, the Ochratoxin A level in all local and imported samples, were lower than the proposed maximum limit of European food regulations for Ochratoxin A in beer which is 2000 ng L^{-1} (2 ppb), (Bennet and Klich, 2003). However, the continious daily intake of these products may have considerable health problem despite the low levels of Ochratoxin A.

Surveys carried out in several countries have demonstrated the human exposure to Ochratoxin A (Pitt, 2000; Richard *et al.*, 2001). These findings agree with those obtained from the surveillance of food commodities, which confirm that Ochratoxin A can occur in a wide range of food (Richard *et al.*, 2001; Van Egmond and Speijers, 2000). In a European assessment of the contribution of each food commodity to the mean total dietary intake of Ochratoxin A, cereals were the main source of Ochratoxin A intake (44%) and beer represented an estimated Ochratoxin A intake of 7% (32).

Mycotoxins have a significant economic and commercial impact, in addition both the productivity and nutritive value of the infected cereal and forage is affected, therefore, the industry should work further to reduce the growth of fungi and mycotoxin formation, aiming at diminishing Ochratoxin A content in their

products. During the production of beer, contamination of Ochratoxin A may transfer from barley to beer or overall process of beer production. Malt is a by-product of this process and contamination is occurred or increased in the three major steps of that (i.e., Steeping, germination and kilning), (Gareis, 2001; Gumus *et al*., 2004). The mycoflora and Ochratoxin A producers are determined at different environmental and cultivation variable, both pre-and post harvest, but the Ochratoxin A contamination mainly has been associated with post harvest conditions (Prado and Carvalho, 2003; Abramson *et al*., 2003). So for having a beer without mycotoxins, it is necessary to monitor fungal infection of barley (especially at the post harvest stages), which will used in malt beverage industries.

CONCLUSION

The mean Ochratoxin A concentration of non alcoholic malt beverages in this study was under the maximum permitted levels. Since, in our country it is highly recommended that consumption of nutritive beverages such as fruit juices, milk drinks and malt beverages (non alcoholic beer) must be increased for reduction of soft drinks intake and also public believe that malt beverages consumption can prevent kidney stones formation due to diuretic effects, therefore, it may be hazardous if the intake of contaminated malt beverages rises specially in patients with kidney diseases.

It is suggested that the contaminations should be monitored routinely for food safety during providing barley for industries and the overall process of malt beverage production, to reduce mycotoxins formation.

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REFERENCES

Abramson, D., K.K. Sinha and D. Bhatnagar, 2003. Mycotoxinformation and environmental factors. Food Addit. Contam., 20: 405-409.

Baydar, T., G. Girgin and A. Sevtap, 2005. Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in turkey. Ann. Agric. Environ. Med., 12: 193-197.

Bennet, J.W. and M. Klich, 2003. Mycotoxins. Clin. Microbiol. Rev., 16: 497-516.

Birzele, B., A. Prange and J. Kramer, 2000. Deoxynivalenol and Ochratoxin A in German wheat and changes of level in relation to storage parameters. Food Addit. Contam., 17: 1027-1035.

Bresch, H., M. Urbanek and K. Hell, 2000. Ochratoxin A in coffee tea and beer. Arch. Lebensmittelhyg, 51: 89-94.

Creppy, E.E., 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett., 127: 19-28.

Gareis, M., 2001. Contamination of German malting barley and of malt-produced from it with the mycotoxins Ochratoxin A and B.Arch. Lebensmittelhyg, 50: 83-87.

Gilbert, J., 2000. Overview of mycotoxins methods, present status and future needs. Nat. Toxin., 7: 347-352.

Gumus, T., K. Arici and M. Demirci, 2004. Asurvey of barley, malt and beer contamination with Ochratoxin A in Turkey. J. Inst. Brewing, 110: 146-149.

Jiao, Y., W. Blass, C. Ruhl and R. Weber, 2001. Ochratoxin A in foodstuffs (Vegetables, Cereals, Cereal products and Beer). Deut. Lebensm. Rundschau, 90: 318-321.

Nakajima, M., H. Tsubouchi and M. Miyabe, 2001. A survey of Ochratoxin A in domestic and imported beers in Japan by immunoaffinity and liquid chromatography, J.AOTAC. Int., 82: 897-902.

Obrien, E. and D.R. Dietrich, 2005. Ochratoxin A: The continuing enigma. Crit. Rev. Toxicol., 35: 33-60.

Odhav, B. and V. Naicker, 2002. Mycotoxins in South Africa traditional brewed beers. Food Addit. Contam., 19: 55-61.

Park, J.W., E.K. Kim, D.H. Shon and Y.B. Kim, 2002. Occurrence of Ochratoxin A in Korean barley and corn foods. Food Addit. Contam., 19: 158-162.

Petkova-Bocharova, T., I.N. Chernozemski and M. Castegnaro, 2000. Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria. Food Addit. Contam., 5: 299-301.

Pfohl-Leszkowicz, A., T. Petkova-Bocharova, I.N. Chernozemski and M. Castegnaro, 2002. Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causesand the potential role of mycotoxins. Food Addit. Contam., 19: 282-302.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. Br. Med. Bull., 56: 184-192.

Prado, G. and E. Carvalho, 2003. Ochratoxin A determination in beer by immunoafinity column clean-up and high-performance liquid chromatography. Cienc. Tech. Aliment., Campinas, 23: 58-61.

Richard, J.L., G.A. Bennett, P.F. Ross and P.E. Nelson, 2001. Analysis of naturally occurring mycotoxins in feedstuffs and food. J. Anim. Sci., 71: 2563-2574.

Scott, P.M. and S.R. Kanhere, 2003. Determination of ochratoxin A in beer. Food Addit. Contam., 12: 591-598.

Thelman, A. and W. Weber, 2002. Determination of Ochratoxin A in cereals, malt and beer after accumulation and separation on immunoaffinity columns and following high-pressure liquid chromatography with fluorescence detection. Deut. Lebensm. Rundschau, 93: 1-3.

Van Egmond, H.P. and G.I.A. Speijers, 2000. Survey of data on the incidence and levels of ochratoxin A in food and feed worldwide. Nat. Toxins, 3:125-144.

Vrabcheva, T., 2004. Analysis of ochratoxin a in food consumed by inhabitants from an area with Balkan endemic nephropathy: A 1 month follow-up study. J. Agric. Food. Chem., 52: 2404-2410.

Wolf, J., 2000. Ochratoxin in cereal and cereal products. Arch. Lebensmittelhyg, 51: 81-88.