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Presence of Ochratoxin a in Red and White Grape Juice Commercialized in Iran

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Abstract: Ochratoxin A (OTA) is a mycotoxin that possess a risk to human health due to its nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic effects. Between September 2010 and March 2011, 85 samples of red and white grape juice were collected from supermarkets in the cities of Isfahan, Shahrekord, Tabriz and Uremia, Iran. All samples were analyzed for OTA contamination by ELISA. The recovery percentage of OTA in spiked grape juice samples at concentration of 5 ng/ml was found to be 87% as mean. Ochratoxin A was detected in only one sample (1.2%) of the red grape juice at levels concentration 1.6 ng/ml which was below the maximum tolerance accepted by the European Commission (5 ng/ml). This value reflects that the analysed samples have a minimal contribution to toxicological risk.

Key words: Ochratoxin A · Grape Juice · ELISA · Iran

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by certain toxigenic species of *Aspergillus* and *Penicillium*. Concerning grapes, black *Aspergilli* (i.e. *Aspergillus niger*, *Aspergillus carbonarius*) are known as potential producers of OTA. Specifically, *A. carbonarius* is the most dangerous species as most *A. carbonarius* strains (80-90%) display a high ochratoxigenic potential, which is mainly responsible for high toxin contamination [1-3].

Toxicological studies have demonstrated OTA's nephrotoxic, hepatotoxic, immunotoxic, teratogenic and carcinogenic activity towards several animal species, having been classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen to humans (Group 2B) [4]. OTA could be a risk factor for Balkan Endemic Nephropathy. The natural presence of OTA in food and foodstuffs is widespread, especially in temperate climates [5-7] and it is generally associated with a variety of products, such as cereals, coffee beans, cocoa beans, dried fruit and fruit juice especially grape juice. Many countries and international organizations have regulated the OTA content in several commodities. The European commission has enforced the limits of OTA in food and foodstuff with the following levels: 10.0 ng/g for the dried vine fruits, soluble coffee and some dried fruits, 5.0 ng/g for raw cereal grains, 3.0 ng/g for cereals and cereal products, 2 ng/g for grape products intended for human consumption, 0.5 ng/g for baby food and cereal-based food intended for young children [8].

Numerous methods for OTA determination in food have been described, including Enzyme-linked immunosorbent assay (ELISA) and thin layer chromatography (TLC) [9]. Liquid chromatography linked to fluorescence detection (HPLC/FD) was extensively used for OTA confirmatory Analysis [7, 10, 11]. However, immunological methods are preferred to chromatographic methods in routine and survey work. In addition, enzymatic immunoassay for the detection of OTA is fairly cheap, sensitive and quick.

There is no information about the natural occurrence of OTA in foodstuffs in Iran. The aim of this survey was therefore to determine concentrations of OTA in grape juice marketed in Iran.

MATERIALS AND METHODS

Collection of Samples: Between September 2010 and March 2011, 85 samples of red grape juice (n= 40) and white grape juice (n= 45) were collected from supermarkets in the cities of Isfahan, Shahrekord, Tabriz and Uremia, Iran.

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Method of Analysis: The quantitative analysis of OTA was performed using enzyme immunoassay: Ridascreen® ochratoxin A kit (R- Bipharm AG, Germany). The test is based on the antigen-antibody reaction.OTA extraction and tests were performed according to manufacturer's instructions. Each sample was extracted by dichloromethane with $NaHCO_3$ buffer (0.13 M, pH 8.1). The final extracts were diluted by distilled water and used for the specific ELISA kit. The optical density was measured at 450 nm using ELISA 96-well plate reader (Stat Fax 2000, England). All standard and sample solutions were analyzed in duplicate wells. The evaluation of ELISA data and the mycotoxin concentrations for samples were performed using software program (Ridasoftwin, Ridascreen®). Recoveries were determined by spiking negative samples of analyzed food at 5 ng/ml. According to the manufacturer's description, the detection limits for OTA by ELISA was 0.625 ng/ml.

RESULTS AND DISCUSSION

In present study, a total of 85 grape juice samples, including red and white grape juices, were obtained from markets in Shahrekord, Isfahan, Tabriz and Uremia, Iran. The samples were analyzed for OTA contamination by ELISA. The recovery percentage of OTA in spiked grape juice samples at concentration of 5 ng/ml was found to be 87% as mean. Ochratoxin A was detected in only one sample (1.2%) of the red grape juice at levels concentration 1.6 ng/ml which was below the maximum tolerance accepted by the European Commission (5 ng/ml) [8].

The presence of OTA in grape juices and wines was reported for the first time by Zimmerli and Dick [12]. Later surveys were carried out to evaluate the levels of OTA in grape juices and wines in Europe and South Africa. Data on OTA occurrence in wine showed levels of up to 7.0 ng/ml and higher levels in red wine produced in Europe [13-18] and from South Africa [19]. Zimmerli and Dick analyzed OTA in eight red and three white commercial grape juices; the white grape juices contained <5 ng/l, but the red ones ranged from <3 to 311 ng/l, with a mean of 188 ng/l [20]. Burdaspal and Legarda, analyzed 10 samples of grape juice and found OTA levels in the range of 15 to 102 ng/l [13]. Twenty samples of grape juice were analysed by Ministry of Agriculture, Fisheries and Food [21]; one sample contained no OTA and the others ranged from <20 to 2,050 ng/l, with a mean of 480 ng/l. Between 1995 and 98, many samples of grape juice were analysed in Germany [22]. Seven out of 38 white grape juices contained <10 ng/l of OTA, while in the rest of the samples the amount of OTA ranged from <10 to 1,300 ng/l. As regards red grape juices, 8 out of the 73 samples analysed were free of OTA, while the rest ranged from <10 to 5300 ng/l. In Rio de Janeiro, Brazil and from other parts of Brazil, Chile and Argentina, a total of 106 samples, including grape juices, pulp of frozen grapes, red, rose and white wines were obtained from markets and were analyzed for OTA contamination by HPLC using immunoaffinity columns. The limit of detection was 21 ng/l and the recovery of the method used was 80-90%. Ochratoxin A was detected in 29% and 12.5% of the grape juice and pulp of frozen grape samples respectively, at levels ranging from 21 to 100 ng/l [23].

Variation in the concentration of OTA, in grape juice samples reported in other studies may be a result of different sampling techniques employed, seasonal effects and/or laboratory methodologies employed in different studies (ElLISA, TLC and HPLC). Differences in OTA levels, probably due to different weather conditions, were also reported by Pietri *et al.* [15], Lopez de Cerain *et al.* [24] and Battilani *et al.* [25] between samples collected in the same regions but in different years.

Based on the data exposed in this paper, the analysed samples have a minimal contribution to toxicological risk. However, it is important to inspect and control grape productes for presence of OTA in a regular manner to evaluate the hygienic managements.

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