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Concentration of ochratoxin A in wines from supermarkets and stores of Valencian Community (Spain)

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Abstract

Ochratoxin A (OTA) is a mycotoxin produced by fungi species belonging to the genera *Aspergillus* and *Penicillium* being isolated in alcoholic beverages. The aim of this work is developed and applied a procedure for the analysis of OTA in wines. An analytical method based on immunoaffinity column (IAC) for clean-up, liquid chromatography with fluorescence detection (LC-FD), and LC-FD after of OTA methylation was used to determine the occurrence of OTA in wines. Recoveries of this mycotoxin spiked to red wines at 0.5 ng/ml level were >90% with an average of relative standards deviations of 4%. Furthermore, 116 wine samples from designation of origin (DO) and three samples from food stores of Valencian Community (Spain) were examined for the occurrence of OTA being the levels of this mycotoxin ranged from <0.01 to 0.76 ng/ml. Finally, the estimated daily intake of OTA in this study was 0.15 ng/kg bw per day.

Keywords: Wine; Ochratoxin A; Mycotoxins

1. Introduction

Ochratoxin A (OTA) is a mycotoxin produced by several fungi species belonging to the genera *Aspergillus* (e.g., *Aspergillus ochraceus* and *Aspergillus carbonarius*) and *Penicillium* (e.g., *Penicillium verrucosum*). This mycotoxin shows nephrotoxic, nephrocarcinogenic, teratogenic, and immunosuppressive properties [1]. Furthermore, it may be implicated in the human disease Balkan Endemic Nephropathy (BEN) and in the development of urinary tract tumors in humans [2]. In 1993, the International Agency for Research on Cancer (IARC) classified into group 2B as a possible human carcinogen [3].

In temperate areas such the Mediterranean region, the natural occurrence of OTA is detected in different kinds of foods and beverages including wines [1]. The use of immunoaffinity column (IAC) as clean-up methodology followed by liquid chromatography (LC) with fluorimetric detection has become the most popular procedures for OTA analysis in wines [4–8]. Several studies from African [9–11], American [12,13], and European [7,12,14–17] wines have demonstrated level of contamination of OTA. Valencian Community is a Mediterranean place which the production of wines with designation of origin (DO) is around 1 200 000 hl/year [18]. This designation of origin is designed by the Control Board, an independent agency of control and certification, according to the article 10 of the European Regulation 2081/92 [19], which will guarantee that the wine protected comply the requirements established of food safety and quality.

The objective of this work was to obtain data on the occurrence of OTA from 119 wines from Valencian Community (Spain) with designation of origin and other wines which is obtained from food stores in order to evaluate its potential contribution to the dietary OTA exposure of consumers of these wines. The relevance of this paper is based on the determination of OTA in wines together with the analysis of wines from Valencian Community. To date, the occurrence of OTA in wines from this place has not been carried out.

2. Experimental

2.1. Samples

Samples of dessert, red, rosé, and white wines from designation of origin from Valencian Community (Spain)

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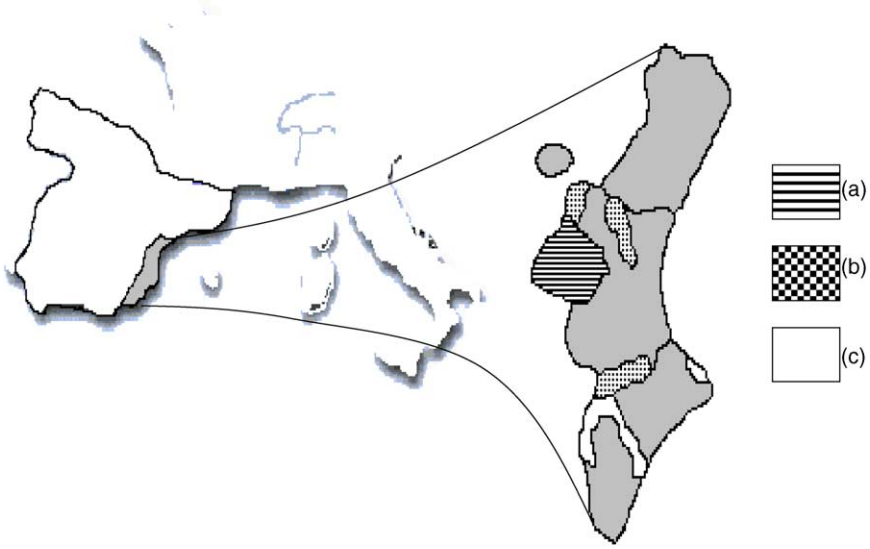

Fig. 1. Location of wines with designation of origin of (a) Utiel-Requena, (b) Valencia, and (c) Alicante from Valencian Community.

(Fig. 1) were purchased in supermarkets in Valencia and three white wine samples were obtained from food stores in a small village of Valencia. All samples were taken during July 2003 and stored at 4 °C until their analysis. All information on the samples was taken from the bottle labels.

2.2. Chemical and reagents

LC-grade acetonitrile, acetic acid, and methanol were supplied by Merck (Darmstadt, Germany). Clorhidric acid (HCl) was obtained by Scharlau (Barcelona, Spain) and phosphate-buffered saline (PBS) was bought from Sigma (St. Louis, MO, USA). Deionized water (<8 Ω cm resistivity) was obtained from a Milli-Q water purification system (Waters-Millipore, Milford, MA, USA). Solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath. The immunoaffinity column used for OTA analysis was Ochraprep from R-Biopharm Rhône Ltd (Scotland, UK). OTA standard was purchased from Sigma. OTA crystalline material was purchased from Sigma. The standard solutions were made in methanol according to the concentration established using a UV spectrophotometer ($\varepsilon = 55000/(\text{mol cm})$) kept in security conditions at −20 °C, wrapped in aluminium foil, due to that OTA gradually break down under UV light, and held for less than 3 months. Standard working solutions were prepared by appropriate dilution in the same solvent and stored in glass-stopped tubes at −20 °C.

2.3. Extraction procedure

Samples (10 ml) were diluted with 10 ml of PBS. The mixture was shaken for 5 min. The IAC was placed on a SPE vacuum manifold (Visiprep, Supelco) and preconditioned with 4 ml of PBS. Then, the mixture of the diluted sample (20 ml) was applied to the IAC column (1–2 drops per second), followed by a washing with 9 ml of PBS and 8 ml de-ionized water and then dried with air. The OTA was then slowly eluted from the IAC with 2 ml methanol into a glass vial; the eluate was evaporated to 1 ml with a gentle stream of N_2 and stored at 4 °C until LC analysis. Finally, 150 μl was injected onto the LC column.

2.4. LC analysis

A Shimadzu (Kyoto, Japan) SCL-GA system LC equipped with two LC-GA pumps, a Rheodyne Model 7125 injector (150 μl loop) and a SRF-535 fluorescence detector. A LC column Kromasil SC-18 (5 μm; 150 mm × 4.6 mm i.d.; Scharlau, Barcelona, Spain) was used with a mobile phase consisting of a mobile phase of acetonitrile/water/acetic acid (50:49:1, v/v/v) at a flow-rate of 1 ml/min. Detection of OTA was carried out using 333 and 470 nm as wavelengths for excitation and emission, respectively.

2.5. Confirmation

Confirmation of positive samples was performed according to the method of Zimmerli and Dick [6]. Briefly, 200 μl of the extract was diluted to 2.5 ml with methanol and then 0.1 ml concentrated HCl was added. The solution was left standing overnight at room temperature. Thereafter, the methanol was evaporated and the residue was taken up in 200 μl acetonitrile/water/acetic acid (50:49:1, v/v/v). OTA (90%) was methylated with method. The LC analysis was identical to that described above.

3. Results and discussion

3.1. Method performance

The recovery for OTA on a red wine sample spiked at a level of a 0.5 ng/ml was 92 ± 4% (mean ± S.D., $n = 3$) with a limit of detection (*S*/*N*, 3:1) and quantification (*S*/*N*, 10:1) of 0.01 and 0.05 ng/ml, respectively. The Fig. 2c shows the LC-FD chromatogram obtained following the proposed method for a red wine fortified with 0.5 ng/ml. Precision was calculated in terms of intra-day repeatability ($n = 5$) and inter-day reproducibility (5 different days) on 0.25 ng/ml. The intra-day repeatability evaluated as R.S.D. ranged from 3 to 5%. The inter-day reproducibility was lower than 8% for all instances. Linearity was verified ($n = 5$) with six concentrations (0.05, 0.10, 0.25, 0.75, 1, and 5 ng/ml). The regression coefficients (r) were all >0.997. The results of the study reflected that the analysis gave cleanest chromatograms and recoveries were considered as valid for analyzing residues of OTA in wines according with European signification [20]. This method presents several advantages as is good repeatability, avoids the common emulsions formed when red wines are treated with liquid–liquid extraction and reduces drastically the time of analysis compared with other procedures [6]. In fact, the average time for wine sample preparation was about 10 min.

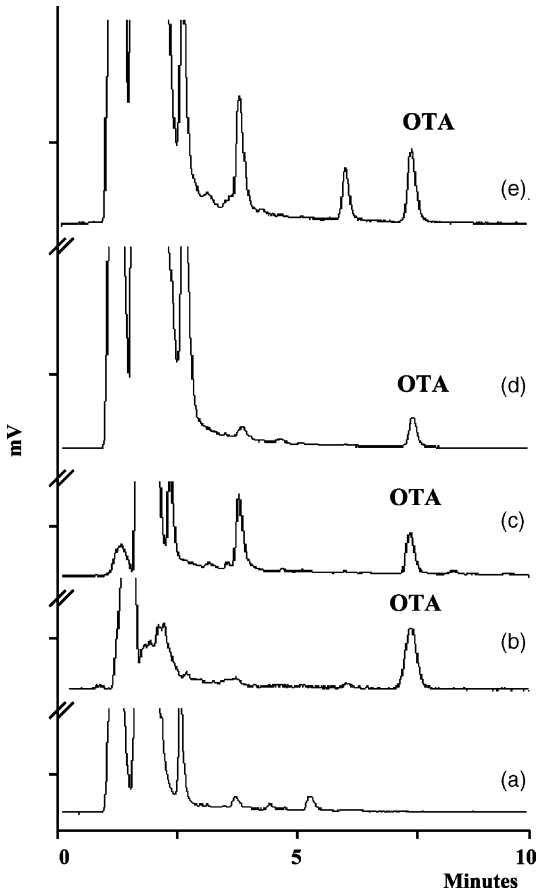

Fig. 2. LC-fluorescence chromatograms obtained of (a) non-fortified wine, (b) OTA standard solution (1 ng/ml), (c) red wine fortified with 0.5 ng/ml and two positive wine samples containing (d) 0.38 and (e) 0.76 ng/ml from dessert and white wines, respectively, from Valencian Community.

3.2. Occurrence of OTA

In the present study, a total 116 wine samples from designation of origin from Valencian Community and three samples from food stores were analyzed and their results are shown in Table 1. The levels of OTA ranged from <0.01 to 0.76 ng/ml. Fig. 2d and e show chromatograms obtained with positive samples found from dessert and white wines, respectively. The European Union have proposed 2 ng/ml of OTA in wine as maximum residue levels (MRLs) [21]. Neither of the studied samples shown levels above the European regulatory limit. On the other hand, level of OTA decreased in the order red > rosé > dessert > white for wines with designation of origin.

The higher values for red, rosé, and white wines were found of the most recently year of production and decreasing in accordance with the year. According to our results, Lopez de Cerain et al. [17] suggested that OTA is stable in wines for at least 1 year and that the concentration of OTA in raisins and consequently in wine varies from 1 year to the other depending on the meteorological conditions. In fact, several authors reflected that the wines from the Mediterranean region contain high concentrations of OTA due to the climate being characterized by high humidity and high temperature [10,14].

The higher value is obtained with white wines from food stores (0.76 ng/ml) versus wines with designation of origin. In some places, the vine grower sells his grapes to food stores which pay a part with money and the other with wine elaborated by themselves. This wine is called cold wine but is not wine with designation of origin. This food stores have not implemented food safety measures such as good agricultural practices (GAPs), good manufacturing practices (GMPs), and the hazard analysis and critical control point (HACCP) system [22]. It could explain the higher value for white wine in comparison with other wines. On other hand, wines of designation of origin are proposed by the Control Board which is an independent official authority in charge of prescribing and enforcing the regulations of a DO. These wines are given to a specific wine which meets certain requirements as its origin, the way it is processed, and its quality.

3.3. Daily intake

For Valencian Community wines, the average sample contamination of OTA was 0.25 ng/ml according to the data of our study. Assuming that wine consumption in this Community is about to 41.4 ml/day [23] and that an adult body weighs 70 kg, the estimated daily intake of OTA in this study was 0.15 ng/kg bw per day. This value represents 3 and 0.9%

Table 1
Occurrence of OTA in Valencian Community wine samples

Origin of samples	Year of production	No. of samples	No. of samples without residue[a]	No. of samples with residue	
				LOD–LOQ[b]	>LOQ (ng/ml)
Dessert wine					
Valencia (Moscatel)	2001	7	3	1	3 (0.28, 0.31, 0.40)
Alicante (Fondillón)	2001	6	2	1	3 (0.10, 0.19, 0.38)
Red wine					
Alicante	1999	8	8	–	–
	2000	9	6	1	2 (0.20, 0.29)
	2001	11	4	3	4 (0.10, 0.11, 0.20, 0.51)
Utiel Requena	1999	10	8	1	1 (0.06)
	2000	10	7	1	2 (0.20, 0.40)
	2001	8	4	2	2 (0.39, 0.53)
Valencia	2001	5	3	1	1 (0.38)
Rosé wine					
Utiel Requena	2000	11	6	2	3 (0.25, 0.32, 0.40)
	2001	10	3	2	5 (0.11, 0.24, 0.29, 0.33, 0.46)
White wine					
Valencia	2000	11	11	–	–
	2001	10	9	–	1 (0.09)
Food stores	2003	3	–	–	3 (0.32, 0.48, 0.76)

[a] <LOD, <limit of detection (<0.01 ng/ml).
[b] LOQ, limit of quantification (0.05 ng/ml).

of the tolerable daily intake (TDI) according to the Scientific Committee on Food of the European Commission (5 ng/kg bw per day) [24] and the WHO Committee of Experts on Food Additives (16 ng/kg bw per day) [25], respectively. In our study, the contribution to daily intake for these studied wines could be considered to be rather small in comparison with the daily intake values of OTA in France (2 ng/kg bw per day) [9], Greece (3.7 ng/kg bw per day) [15], Spain (0.3 ng/kg bw per day) [17], Sweden (0.2 ng/kg bw per day) [26], and Switzerland (0.7 ng/kg bw per day) [27].

4. Conclusions

The use of IAC for clean-up followed by LC-FD has shown to be a technique with good analytical performance for the OTA determination in wines. The application of this procedure to analyse 119 wine samples from Valencian Community has demonstrated that none of them contained levels above the European MRLs. Furthermore, the estimated daily intake of OTA has reflected to be rather small in comparison with other countries.

Acknowledgements

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