Occurrence of ochratoxin A in wines in the Argentinian and Chilean markets

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This manuscript is dedicated to our valued colleague Prof. Rosa Lederkremer

Abstract
Wine contaminated with ochratoxin A (OTA) has been reported all over the world. Sixty-eight wine samples were analysed to assess OTA wine contamination in various regions of Argentina and Chile. In addition, some imported wines were analysed. Wine samples were collected at manufacturers' stock and retail markets in Argentina in 2003 and Chile in 2002. A high-performance liquid chromatographic method with fluorescence detection and two different immunoaffinity clean-up columns were employed, with recoveries higher than 90% (Argentina: LOD:0.008 µg/l, LOQ:0.015 µg/l; Chile: LOD:0.012 µg/l, LOQ: 0.04 µg/l). None of the analysed wines produced in Argentina or Chile were contaminated. The presence of OTA in wines would appear to be a lesser problem in Argentina and Chile than in other countries, but it still could contribute to OTA exposure of human populations and more studies of the occurrence of OTA in wine should be done.

Keywords: Mycotoxins, ochratoxin A, wines, immunoaffinity clean-up, high-performance liquid chromatographic method

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by some species of the genera *Aspergillu*s and *Penicillium*, and can contaminate a wide variety of foods.[1,2] According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA),[3] *OTA is produced by a single Penicillium species, P. verrucosum, by Aspergillus ochraceus and several related Aspergillus species, and by*

A. carbonarius, with small percentage of isolates of the closely related A. niger. OTA has been found in cereals and derived products (e.g. beer), legumes and pulses. It can also appear in other commodities such as coffee, cacao, nuts, spices, dried fruit, wine, etc., and in animal-derived products.[4]

Wine contamination with OTA has been reported all over the world.[3,5-15] However, no similar study has been performed on Argentinian and Chilean wines. The Codex Alimentarius Commission reported that wine is the second most important source of human exposure to OTA following cereals, giving a total dietary intake of 15%.[16]

Because OTA is known to have toxicological effects in humans and animals, such as nephrotoxic, immunotoxic, genotoxic and carcinogenic effects,[4,17,18] several countries have specific regulations for OTA content in a variety of commodities at levels ranging from 1 to 50 µg/kg for foods.

The JEFCA met in Geneva on February 6-15, 2001,[3] where it retained the previously established provisional tolerable weekly intake of 100 ng/kg body weight, pending the results of ongoing studies on nephrotoxicity and carcinogenicity mechanisms, and recommended a further review of OTA during 2004. The Commission of the European Community considered that *"it would be prudent to reduce exposure to ochratoxin A as much as possible, ensuring that exposures are towards the lower end of the range of tolerable daily intakes of 1.2–1.4 ng/kg b.w"*.[19] Recently, the European Commission proposed a maximal limit for OTA in wine of 2 µg/l.

The increased awareness of the potential risk for consumer health due to OTA exposure through wine consumption requires each country to carry out systematic measurements of OTA levels of the wines offered in the domestic market.

Because red wines tended to have higher OTA concentration than white wines,[15,16,20,21] the aim of this work was to obtain a preliminary overview of OTA contamination in red wines consumed in Chile and Argentina.

Results and Discussion

None of the red wines produced in Argentina or Chile analysed by us presented contamination. Other authors found two out of seven Argentinian red wines analysed with OTA at 0.028 and 0.042 µg/l; and two out of five Chileans wines contaminated with 0.028 and 0.07 µg/l.[15] Soleas et al.[11] reported that five out of 17 Argentinian and eight out of 42 Chilean red wines were contaminated at levels below 0.05 µg/litter.

On the other hand, Da Rocha et al.[22] showed that only 8 out of 48 isolates of *Aspergillus niger* produced OTA in the range of 32 to 77 µg/g, and none of the other *Aspergillus* species isolates from Argentinian grapes were OTA producers. Magnoli et al.[23] studied OTA production by 63 species of *Aspergillus* section *Nigri* isolated from wine grapes in Argentina. *A. niger* var. *niger* (19 strains out of 44), *A. niger* var. *awamori* (5 strains out of 15), and *A. faetideus* (1

strains out of 4), were OTA producers in the range of 0.002 µg/l to 0.045 µg/l. Both studies used YES medium at 30ºC during 10 days to test toxigenic capacity.[22,23]

With the goal of confirming the presence of OTA in wine, we also analysed imported wines from European regions in which contamination had been found.[6,8,13,14] Figure 1 shows the chromatograms obtained from an imported wine sample naturally contaminated with OTA (a) and the OTA methyl ester derivative (b).

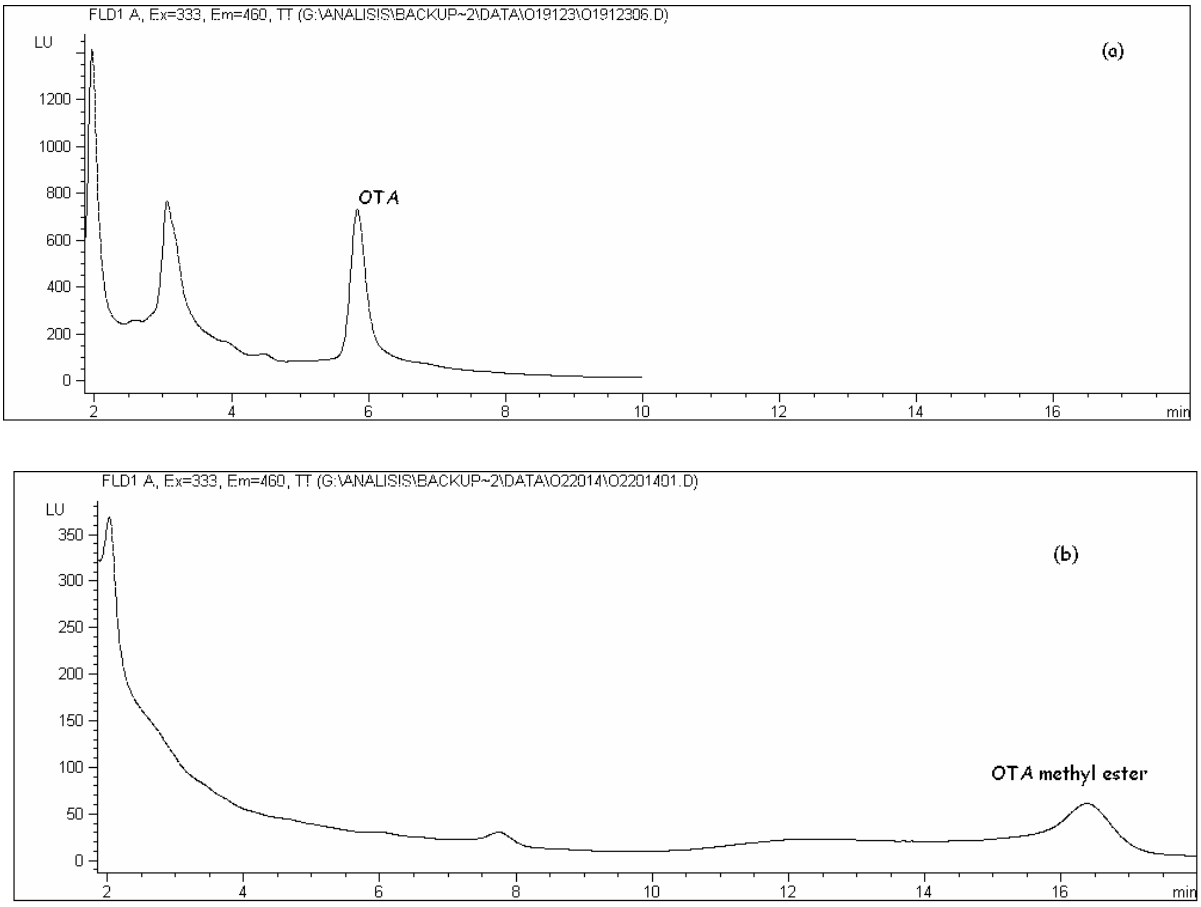

Figure 1. Chromatogram of: a) dessert wine sample with 1.32 µg/l OTA; b) OTA methyl ester derivative of the dessert wine.

Figure 2 shows the wine consumption trend from 1985 to 2001 for Argentina, Chile and the average of 15 European countries.

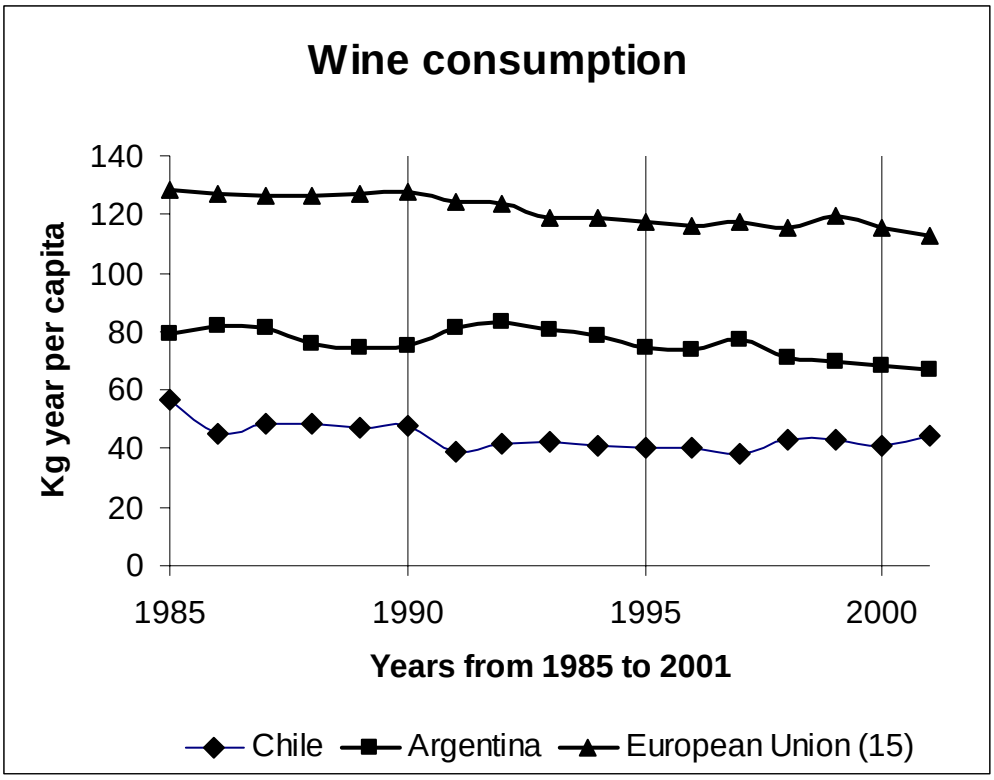

Figure 2. Wine consumption in Argentina, Chile and the European Union (15 countries).

The mean intake for those years was 121.34 kg year per capita for European countries, 76.06 kg year per capita for Argentina and 44.03 kg year per capita for Chile.[24] Taking into account the wine consumption in both South American countries, and based on the present results, it seems possible that wine intake is not an important OTA for the Argentinian and Chilean populations, in comparison with European people.

Although the number of imported wine samples analysed was limited, our results showed that OTA contaminations in European red wines was in the range of the SCOOP study.[20] From those countries which provided discriminated information to the SCOOP study, the mean concentration in red wine was 0.17 µg/l, and the mean level found in the sixteen European red wines analysed by us was 0.0315 µg/l. The Argentinian population may be more exposed than the Chilean population due to the consumption of imported wine, and because the average wine intake in Argentina is higher than in Chile (Figure 2).

Conclusions

This paper presents a preliminary report on OTA contamination of wines from Chile and Argentina. The presence of OTA in wines would appear to be a lesser problem than other countries, but it still contributes to OTA exposure. The results of this study confirm the

importance of continuing research in this direction, not only because of the serious human health concerns related to OTA exposure, but also due to the fact that both these countries export wines.

Experimental Section

General Procedures. Samples. Eighty-four samples of red wine~~s~~ were bought in manufacturers' stock and retail markets in the Argentinian cities of Luján and Buenos Aires (54 domestic and 16 imported wines) in 2003, and in the Chilean city of Concepción (14) in 2002. Table 1 shows details for all the South American wine samples. Information regarding the origin of the commercial samples was obtained from the bottle labels.

Table 1. Red wine samples for Argentina and Chile

Winery N^o	Year Crop	Grape variety	Origin	
			Country	Region
1	1996	Merlot	Argentina	Mendoza
1	1999	Merlot	Argentina	Mendoza
1	2000	Cabernet Sauvignon, Merlot, Malbec	Argentina	Mendoza
1	2000	Malbec	Argentina	Mendoza
1	2000	Barbera	Argentina	Mendoza
1	2000	Cabernet Sauvignon	Argentina	Mendoza
1	2001	Unknown	Argentina	Mendoza
2	2000	Syrah	Argentina	Mendoza
2	2000	Cabernet Sauvignon	Argentina	Mendoza
2	2000	Merlot	Argentina	Mendoza
2	2000	Malbec	Argentina	Mendoza
2	2000	Cabernet Sauvignon, Merlot, Malbec	Argentina	Mendoza
2	2002	Unknown	Argentina	Mendoza
2	2002	Malbec	Argentina	Mendoza
2	2002	Burgundy	Argentina	Mendoza
3	2000	Syrah	Argentina	Mendoza
4	2000	Merlot	Argentina	Mendoza
4	2000	Syrah	Argentina	Mendoza
4	2001	Malbec	Argentina	Mendoza
4	2002	Pinot Noir	Argentina	Mendoza

Table 1. (Continued)

4	2002	Unknown	Argentina	Mendoza
4	2002	Unknown	Argentina	Mendoza
4	2002	Cabernet Sauvignon	Argentina	Mendoza
5	2000	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Malbec	Argentina	Mendoza
6	2000	Malbec	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
6	2002	Burgundy	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
7	2002	Cabernet Sauvignon	Argentina	San Juan
7	2002	Merlot	Argentina	San Juan
7	2002	Unknown	Argentina	San Juan
8	1998	Unknown	Argentina	Mendoza
8	1998	Sangiovese Merlot Malbec	Argentina	Mendoza
8	2001	Malbec	Argentina	Mendoza
9	2001	Burgundy Bonarda-Malbec	Argentina	Mendoza
9	2002	Unknown	Argentina	Mendoza
9	2002	Cabernet Sauvignon	Argentina	Mendoza
10	2000	Cabernet Sauvignon	Argentina	Mendoza
10	2001	Malbec	Argentina	Mendoza
10	2001	Malbec-Cabernet Sauvignon	Argentina	Mendoza
10	2001	Unknown	Argentina	Mendoza
11	2000	Merlot	Argentina	Patagonia
11	2000	Malbec	Argentina	Patagonia
11	2002	Unknown	Argentina	Patagonia
12	2002	Cabernet Sauvignon	Argentina	San Juan
12	2002	Unknown	Argentina	San Juan
13	2002	Merlot-Cabernet Sauvignon-Malbec	Argentina	Mendoza
13	2002	Unknown	Argentina	Mendoza
14	2002	Borgoña	Argentina	Mendoza

Table 1. (Continued)

14	2002	Cabernet Sauvignon-Malbec	Argentina	Mendoza
15	1999	Cabernet-Carmenère	Chile	Maipo Valley
16	1998	Cabernet Sauvignon	Chile	Itata Valley
17	1998	Cabernet Sauvignon	Chile	Rapel Valley
18	2000	Cabernet Sauvignon	Chile	Limarí Valley
19	2000	Merlot	Chile	Limarí Valley
20	2000	Cabernet Sauvignon	Chile	Maule Valley
21	2000	Merlot	Chile	Curicó Valley
21	2000	Cabernet Sauvignon	Chile	Curicó Valley
22	2000	Pinot	Chile	Pirque
23	1999	Cabernet Sauvignon	Chile	Lontué Valley
23	1999	Malbec	Chile	Lontué Valley
24	1999	Cabernet Sauvignon	Chile	Maipo Valley
25	1999	Merlot	Chile	Rapel Valley
26	2000	Cabernet Sauvignon	Chile	Colchagua Valley

Analysis for ochratoxin A. OTA was purchased from Sigma-Aldrich (USA). The standard solutions were made in benzene:acetic acid (99:1) according to the established concentration using a UV spectrophotometer at 333 nm (molar absorptivity: 5500). The required quantity was evaporated to dryness and dissolved in the mobile phase as indicated under chromatographic conditions.

Clean-up. Two different immunoaffinity clean-up columns were used in Argentina and Chile. Both procedures are briefly summarized.

Argentina. The extraction and quantification were based on Castellari et al.[25] with minor modifications. The column (Ochraprep, Rhône Diagnostics Technologies) was placed on an SPE vacuum manifold (Baker), and was first washed with 5 ml of PBS before use (PBS: dissolve 7.02 g NaCl, 0.201 g KCl, 1.14 g Na_2HPO_4, 0.26 g NaH_2PO_4, and 0.5 g NaN_3 in 1 l HPLC-grade water; adjust pH to 7.4).[26] Then 10 ml of wine, adjusted to pH 7.8 using 1 M NaOH, was diluted with 10 ml of PBS and introduced into the column at a flow-rate of about 1-2 drops per second. The eluted extract was introduced once more into the column. The column was successively washed with 10 ml of PBS and 10 ml HPLC-grade water at a flow-rate of about 3-4 drops per second and dried with air. OTA was then slowly eluted, using back-flushing three times in each fraction (4.5 ml and three 1.5 ml fractions), from the column with HPLC-grade MeOH at a flow-rate of about 1 drop per second. The eluted extract was evaporated into a silanised glass vial under vacuum at 40^{o}C and the residue was re-dissolved in 250 µl of mobile phase.

Chile. The extraction and quantification were based on Visconti et al.[7] with minor modifications.

A 10-ml wine sample was diluted with 10 ml of water containing PEG 6000 (1%) and $NaHCO_3$ (5%), mixed and filtered through a Whatman GF/A glass micro-fibre filter. A 5 ml aliquot of diluted extract was cleaned up through an Ochratest immunoaffinity column (Vicam) at a flow-rate of about 1 drop per second. The column was washed with 5 ml solution containing NaCl (2.5%) and $NaHCO_3$ (0.5%), followed by 5 ml distilled water at a flow-rate of 1-2 drops per second.

The OTA was eluted with MeOH (2 ml) into a glass vial. The eluted extract was evaporated under a nitrogen stream and re-dissolved in 250 µl HPLC mobile phase.

Apparatus and Chromatographic conditions. OTA (Figure 3) detection was achieved at 333 nm excitation, 460 nm emission wavelength for both countries. Injection volume of the samples was 100 µl. A calibration curve was established by injecting six standard solutions with OTA concentrations ranging from: 0.026 to 2.65 µg/l (R^2: 0.9997) and 0.1 to 10 µg/l (R^2: 0.9977) in Argentina and Chile[27], respectively. Recovery experiments were performed in both laboratories on OA-free wines samples spiked with different OA levels. A mean recovery of OA was greater than 90 %[27]. Results were not corrected for recovery.

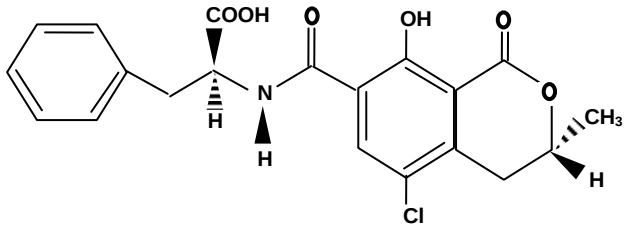

Figure 3. Chemical structure of Ochratoxin A.

Detection limits of the methods employed were 0.008 µg/l and 0.012 µg/l, and quantification limits were 0.015 µg/l in and 0.04 µg/l in Argentina and Chile[27], respectively.

Two different sets of equipment were used in both countries.

Argentina. Hewlett-Packard 1100 model equipped with fluorescence detector and Hypersil (125 x 4 mm) column packed with 5 µm BDS C-18 and Lichrocart guard column, packed with 5 µm RP-18. The computer program used for chromatographic analysis was Chemstation A.08.03.

The system was operated at 40^{o}C, with a mobile phase consisting of MeCN: water:AcOH (85:114:1 v/v/v) at a rate of 1 ml/min. The retention time of OTA was approximately 5.8 min.

Chile. Merck-Hitachi MODEL with fluorescence detector and Waters 746 Integrator and a Waters Symmetry RP-18 (150 x 3.9 mm, 5 µm) column and Symmetry guard column C-18 (3,9 x 20 mm, 0.5 µm).

The mobile phase was MeCN: water:AcOH (99:99:2 v/v/v) at a rate of 1 ml/min. The retention time of OTA was approximately 6.2 min.

Confirmation of OTA by derivatisation as the methyl ester. OTA standard solutions and samples were derivatised by forming the methyl ester of the mycotoxin. Slight modifications

were made to the Grosso et al. procedure.[28] Briefly, an aliquot of the MeOH elution phase from the immunoaffinity column was evaporated to dryness and re-suspended in 120 µl of a 12% MeOH solution of BF_3 (Baker C701-07). After heating for 15 min at 60 ºC, the derivative was analysed by HPLC using the same chromatographic conditions as for ochratoxin A. Retention time of the OTA methyl ester (Figure 4) was 16.3 min. Detection and quantification limits expressed as OTA were 0.017 µg/l and 0.028 µg/l, respectively.

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