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Quantitation of Ochratoxin A in South African Wines

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The natural occurrence of the carcinogenic mycotoxin ochratoxin A (OTA) in wines sold in local retail outlets in South Africa and Italy was investigated by HPLC analysis with fluorescence detection following cleanup by immunoaffinity column. All 24 local South African wines tested (15 white and 9 red) were found to contain detectable levels (>0.01 μg/L) of OTA, with a mean of 0.16 μg/L in the white wines and a mean of 0.24 μg/L in the red wines. Results were subsequently confirmed by LC-MS analysis using positive ion electrospray ionization with collision-induced dissociation of the protonated molecular ion $[M + H]^+$ at *m*/*z* 404 and selected reaction monitoring of the resultant product ions $[M + H - H_2O - CO]^+$ at *m*/*z* 358 and $[M + H - H_2O]^+$ at *m*/*z* 386. Comparison with the fluorescence method gave a significant correlation ($r = 0.87$; $p < 0.01$). Although OTA contamination was present in all of the South African samples analyzed, levels were well below the suggested European Union limit of 0.5 μg/kg. The highest level found in a locally purchased wine was 0.39 μg/L in a blend of local and imported Spanish red wine. Of the eight Italian wines analyzed, only two red wines were contaminated above the suggested maximum level.

KEYWORDS: Ochratoxin A; wine; mycotoxin; food analysis; LC-MS

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced in temperate climates by a single *Penicillium* species, *P. verrucosum*, and in warmer and tropical climates by several *Aspergillus* species, of which *A. alutaceus* (formerly *A. ochraceus*, after which it is named) and *A. carbonarius* are the most important (*1*, *2*). It has been shown to be nephrotoxic, teratogenic, immunotoxic, and carcinogenic in several animal species, is the cause of Danish porcine nephropathy, and has been implicated in the etiology of Balkan endemic nephropathy (*2*−*5*). OTA produces kidney and liver cancer in rats and mice (*6*−*8*). The International Agency for Research on Cancer has classified OTA as possibly carcinogenic in humans (group 2B carcinogen) (*9*). OTA has been extensively found in human blood in Europe, Canada, north Africa, and Japan, raising concern over the implications for human health (*2*, *10*). OTA has been evaluated at the 37th, 44th, and 56th meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and a provisional tolerable weekly intake (PTWI) of 100 ng/kg of body weight has been established (*2*, *11*, *12*).

OTA occurs in cereals, coffee beans, nuts, cocoa, pulses, and dried vine fruit, as well as processed foods derived from these products (*4*, *13*). It has also been reported to occur in a number of associated beverages such as wine, beer, and grape juice. Wine is widely produced and consumed in both developed and developing countries. In Europe it has been estimated that wine is the second most important source of OTA in the diet after cereals and could account for as much as 15% of total OTA intake (*14*). The presence of OTA in imported and locally produced wines has been reported from a number of countries, mainly in western Europe and north Africa, including Algeria, Finland, France, Germany, Italy, Morocco, Portugal, Spain, Switzerland, Tunisia, and the United Kingdom (*15*−*28*). OTA is a frequent contaminant of wine, with an apparent increase in the levels in wines originating from the southern areas of Europe with their warmer climates (*15*, *22*). Given the analytical detection limits for OTA possible with modern methodology (∼0.01−0.001 μg/L), contamination rates of as high as 95−100% have been recorded in red wines originating in these southern areas (*15*, *18*, *20*). Contamination levels as high as 7.63 μg/L have been reported in southern Europe (*21*) with red wines frequently more contaminated than white wines from the same wine-growing region (*15*, *16*, *29*). Several countries have set regulatory levels for OTA in cereal-based commodities (*30*). However, no regulations for maximum levels in wine have been set, although Italian authorities have recently set OTA guidelines for beer at 0.2 μg/L (*31*). New regulatory levels have been discussed within the European Union for products such as wine for which a level of 0.5 μg/kg was originally proposed (*32*). Although recent Commission Regulation EC 472/2002 has set limits for OTA in cereals and dried vine fruit, limits for wine

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have not yet been agreed upon. No regulatory limits for OTA have been adopted in South Africa.

Analytical methodology for determining OTA in wine has generally involved sample cleanup with immunoaffinity columns and reversed-phase HPLC with fluorescence detection. Recent concerns in the European Union over OTA have led to the collaborative study of a method based on these principles that has been approved first action by AOAC International (*33*). Confirmation of the presence of OTA in various matrices has frequently been achieved by methyl esterification (*34*). The recent development of liquid chromatography−mass spectrometry (LC-MS) for detection of OTA now allows a more structurally specific confirmation to be achieved (*35*, *36*). The survey reported here was undertaken to investigate the possible presence of OTA in a variety of South African and Italian white and red wines. OTA was isolated on immunoaffinity columns and determined by HPLC with fluorescence detection. Results were confirmed both by methyl ester derivatization and by LC-MS analysis.

MATERIALS AND METHODS

Samples. Wine samples were purchased in local retail stores in Cape Town, South Africa (24 samples) and Cesena, Italy (8 samples) during 2000 and 2001 and stored at 4 °C prior to analysis. All information on the samples was taken from the bottle labels.

Reagents and Standard. OTA standard was purchased from Sigma (St. Louis, MO). Acetonitrile and methanol were of HPLC grade from Romil (Cambridge, U.K.). All other chemicals were of analytical grade from Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Millipore, Bedford, MA).

Determination of OTA. Wine samples were analyzed for the presence of OTA by immunoaffinity column cleanup and subsequent HPLC separation with fluorescence detection based on the method of Visconti et al. (*21*, *33*). Briefly, wine samples (10 mL) were diluted with a solution (10 mL) comprising sodium bicarbonate (5%) and polyethylene glycol (PEG 8000; 1%) and applied to an immunoaffinity column containing antibodies specific for OTA (Vicam OchraTest, Vicam, Watertown, MA). The column was washed with an aqueous solution (5 mL) containing 2.5% sodium chloride and 0.5% sodium bicarbonate, followed by water (5 mL). OTA was eluted from the column with methanol (2 mL). This eluate was evaporated to dryness under nitrogen at 50−60 °C and the residue reconstituted in the HPLC mobile phase (250 μL). The HPLC system consisted of a Waters 510 pump (Waters, Milford, MA), a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA) fitted with a 200 μL loop, and a Waters 470 scanning fluorescence detector set at excitation and emission wavelengths of 333 and 460 nm, respectively. An injection volume of 100 μL was used for all sample extracts. Separation was achieved on a 100 × 4.6 mm i.d. Luna reversed-phase column (Phenomenex, Torrance, CA) packed with 3 μm ODS-2, with a mobile phase of water/acetonitrile/acetic acid (99 + 99 + 2, v/v/v) pumped at a flow rate of 1 mL/min. Chromatographic data were captured and processed using Borwin chromatography software (JMBS Developpements, Le Fontanil, France). The repeatability of the method was verified with triplicate analyses of a red wine sample contaminated naturally at a level of 0.38 ± 0.01 μg/L (mean ± SD). The analytical recovery for OTA, determined on a red wine sample spiked at a level of 2 μg/L, was 97.3 ± 1.4% (mean ± SD; $n = 3$; 95% confidence level 93.8−100.8% recovery) with a limit of detection of 0.01 μg/L (signal/noise = 2:1).

Confirmation of OTA by Methyl Ester Formation. The presence of OTA was qualitatively confirmed in all South African wine samples by derivatization to its methyl ester (*34*). Briefly, the reconstituted sample extract (150 μL) or OTA standard was extracted with dichloromethane (3 mL). The lower organic layer was transferred to a small vial and evaporated to dryness, and the residue was redissolved in a 14% solution of boron trifluoride in methanol (0.5 mL). Esterification was performed by heating the capped vial for 15 min at 50−60 °C. The solvent was then evaporated, the vial cooled, and the final residue redissolved in HPLC mobile phase (150 μL). HPLC separation (100 μL injection) on the same analytical HPLC system qualitatively confirmed the presence of OTA in the wine samples by the loss of the peak corresponding to OTA (retention time of ~6.4 min) and the appearance of a peak at longer retention time (17 min) corresponding to the methyl ester of OTA.

Table 1. OTA Levels in South African and Italian Wine Samples Determined by HPLC with Fluorescence Detection

wine sample	no. of samples	mean OTA (μg/L)	range of OTA (μg/L)
South African wines			
white wines, bottled	6	0.19	0.05–0.30
white wines, boxed	2	0.19	0.04–0.33
white wines, late harvest	7	0.12	0.06–0.18
red wines, bottled	4	0.29	0.18–0.38
red wines, boxed	5	0.21	0.07–0.39
Italian wines			
red wines	5	0.58	0.23–0.91
white wines	3	0.05	0.01–0.08

Confirmation of OTA by LC-MS. The presence of OTA in all of the Italian and the six South African wine samples purchased in 2001 was also confirmed by LC-MS analysis of the sample extracts. For this purpose, larger wine samples (50 mL) were separately purified on immunoaffinity columns by the above cleanup method. The final residue was redissolved in 300 μL of mobile phase comprising water/acetonitrile/formic acid (26 + 74 + 0.1, v/v/v). HPLC analysis was performed using a Spectra series P2000 binary pump and an AS 1000 autosampler equipped with a 20 μL injection loop (Thermo Separation Products Inc., Riviera Beach, FL). Isocratic reversed-phase HPLC was performed at a flow rate of 0.7 mL/min on a 150 × 4.6-mm i.d. Luna C_{18} column (Phenomenex, Torrance, CA) packed with 5 μm ODS-2. Positive ion electrospray ionization (ESI) mass spectrometry was performed using a Finnigan MAT LCQ ion-trap MS (San Jose, CA). The MS parameters were optimized by tuning the MS detector using ochratoxin A standard (1 μg/mL). During LC-MS analysis, the source and capillary voltages were 5 kV and 37 V, respectively, with the heated capillary at 220 °C. Nitrogen served as both sheath gas (80 arbitrary units) and auxiliary gas (20 arbitrary units). OTA was confirmed by monitoring the presence of its protonated molecular ion at m/z 404 and its subsequent collision-induced dissociation to product ions characteristic of OTA.

Statistics. Statistical analysis was performed according to standard analysis of variance (ANOVA) using the Systat 8 software package (SPSS Inc., Chicago, IL). OTA levels in 15 South African white wine samples were compared with levels in 9 red wine samples. Comparison of results achieved with the two analytical methods was performed on 14 pairs of results.

RESULTS AND DISCUSSION

Table 1 shows the levels of OTA in the South African and Italian wines sampled in this survey. All samples of South African wine showed contamination with levels ranging from 0.04 to 0.39 μg/L. The latter level was from a blend of local South African and imported Spanish red wine, raising the possibility that the high level was due to the imported component of the blend. A third of the South African samples ($n = 8$) had levels ≤0.1 μg/L, and all samples were below the suggested European Union regulatory limit (0.5 μg/kg). Although the red wines had mean levels of contamination above those of the white wines, a statistical comparison of the levels failed to find a significant difference ($p > 0.05$). Previous reports from other countries have indicated that red wine is more susceptible to OTA contamination (*15*, *16*, *29*). It has been postulated that this difference arises from the red wine processing conditions in which the elevated temperature and aerobic conditions associated with the dissolution of natural colorants produce

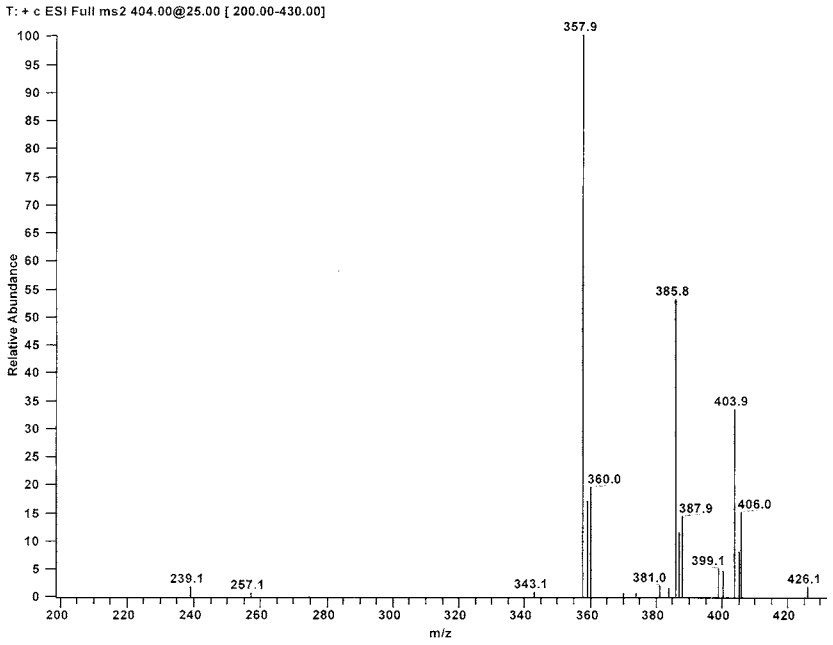

Figure 1. Positive ion electrospray mass spectrum of OTA standard showing the protonated molecular ion $[M + H]^+$ (*m/z* 404) of OTA and its product ions obtained after CID (collision energy of 25%) of the precursor ion *m/z* 404.

intensive contact with potential toxin-producing molds (*16*). OTA was also detected in all Italian wine samples (range = 0.01−0.91 μg/L), with two red wines having levels above the proposed European regulatory limit.

Little information is available in the literature on OTA occurrence in wines sold in South Africa. In their study on OTA in red table wines from various provenances, Zimmerli and Dick reported two South African wines to contain levels of 0.024 and 0.081 μg/L (*27*). A further two South African red wines analyzed in Germany showed one wine to be positive at a level of 0.05 μg/L (*29*). In another survey of wines from various wine-growing regions of the world, 18 South African white wines showed no detectable OTA (<0.05 μg/L), whereas only 2 of 13 red wines showed detectable levels (*37*). A recent survey of South African wines showed 10 of 16 red and 6 of 15 white wines to be contaminated with OTA (>0.01 μg/L) with median levels of 0.014 μg/L (maximum = 0.217 μg/L) and <0.01 μg/L (maximum = 0.195 μg/L), respectively, whereas 3 noble late-harvest wines were all contaminated with a median value of 1.732 μg/L (maximum = 2.672 μg/L) (*38*). The current study of a range of bottled and cheap box wines shows mean levels in white and red wines considerably above these previous results and confirms the need for monitoring possible contamination. In addition, the highest value reported here (0.39 μg/L) was from a box wine containing a blend of local and imported wine, which indicates that relatively cheap imports may be a source of concern, especially if regulations are imposed in the European Union. Although a number of late-harvest wines were also included among these samples tested, the mean level for these wines was lower than the other South African wines tested. Since the first reports of OTA occurrence in wine, a number of studies have reported OTA contamination in Italian wines (*21*−*23*, *26*, *27*, *29*). The values detected in the current study fall within the ranges previously reported in Italian wines. Previous studies have generally shown mean OTA levels in Italian white wines being <0.5 μg/L, although occasional samples can be above this level (*21*). In contrast to this, levels in red wines can be considerably higher, with some studies reporting mean levels >2 μg/L with a maximum reported level of 7.63 μg/L (*21*, *23*).

The specific cleanup achieved by the use of immunoaffinity columns resulted in well-defined chromatographic peaks at retention times identical to that of the authentic standard. As little information is available on the presence of OTA in South African wines, the OTA contamination detected in these wine samples was confirmed by two separate and independent means, namely, chemical derivatization to the corresponding methyl ester, which resulted in retention time shifts of the target analyte, and mass spectrometry. The mass spectrum of OTA revealed the most abundant protonated molecular ion of OTA $[M + H]^+$ at *m/z* 404, as well as a relatively small amount of its sodium adduct $[M + Na]^+$ at *m/z* 426. Collision-induced dissociation (CID) (collision energy of 25%) produced characteristic product ion spectra, with an abundant product ion $[M + H - H_2O - CO]^+$ at *m/z* 358 and lesser amounts of product ions $[M + H - H_2O]^+$ at *m/z* 386 and [M + H − phenylalanine]$^+$ at *m/z* 239 (**Figure 1**). The chemical structure of OTA contains a single chlorine atom, and hence the protonated molecular ion and its most abundant fragment ions each give rise to two ions in the mass spectrum differing in *m/z* value by 2 amu due to the natural abundance of the two chlorine isotopes, ^{35}Cl and ^{37}Cl. The observed CID fragmentation pattern of the protonated molecular ion of OTA corresponded to that previously noted for OTA in a triple-quadrupole instrument using argon as the gas in the collision cell (*36*). Selected reaction monitoring (SRM) in the positive mode was performed using the protonated molecular ion at *m/z* 404. A collision energy of 30% was used to fragment the parent molecule to the most abundant product ions at *m/z* 358 and 386. **Figure 2** shows the resulting chromatogram achieved under these conditions. Quantification was achieved by comparing the peak areas of OTA in the sample extracts with those of the standard under identical LC-MS conditions. All eight Italian wine samples and six South African wines purchased during 2001 were confirmed to be positive for OTA contamination by LC-MS analysis. Comparison of the quantitative analytical results obtained by fluorescence detection with

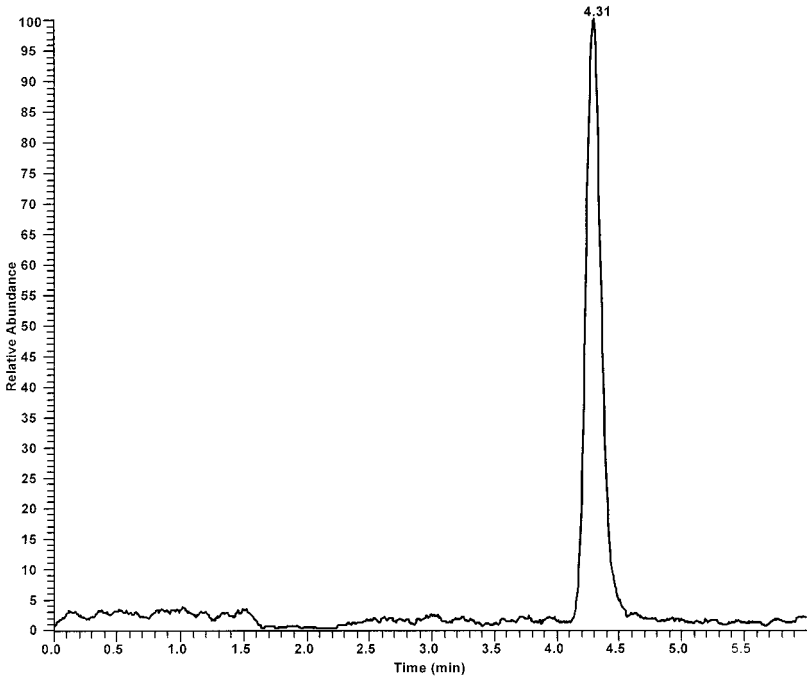

Figure 2. LC-MS chromatogram of a wine sample showing the peak for OTA obtained by selected reaction monitoring of the reaction products at *m/z* 358 and 386 produced by CID (collision energy of 30%) of the protonated molecular ion at *m/z* 404. The peak represents an on-column detection of 4.44 ng of OTA equivalent to a contamination level of 1.20 μg/L in wine.

those achieved using LC-MS showed a statistically significant correlation ($r = 0.87$; $p < 0.01$).

The recent 56th meeting of the JECFA confirmed a previous assessment of OTA, which set the PTWI for OTA at 100 ng/kg of body weight/week, which is equivalent to a tolerable daily intake (TDI) of 14.3 ng/kg of body weight/day (*2*). Assuming an adult body weight of 70 kg, this represents a daily intake of 1 μg of OTA. A Nordic expert group has suggested an even lower TDI value of 5 ng/kg of body weight/day (*14*). If it is further assumed that an individual's exposure to OTA is limited to wine consumption, then at a contamination level of 0.5 μg/L, a person would need to consume up to 2 L of wine per day to consume 1 μg of OTA. At this level of wine consumption, any increased risk from OTA is negligible in comparison to that from diseases associated with excessive alcohol consumption. However, OTA contamination levels of ≥ 5 μg/L have been occasionally reported in some red wine samples (*21*, *29*), and at this level, consumption of only a single wine glass of 200 mL per day would meet the JECFA PTWI level. Hence, concern over the potential OTA contamination of wine is justified, especially in traditional wine-consuming communities.

The application of recently developed analytical methods for OTA based on immunoaffinity column purification and HPLC separation facilitates routine monitoring for OTA contamination in wine. In addition, confirmation of contamination can be achieved either by economical chemical derivatization and use of the same chromatographic system or, where more sophisticated instrumentation is available, by application of LC-MS techniques involving recognition of characteristic ion fragmentation patterns. The presence of OTA in wines would appear to be a widespread problem in wine-producing regions of the world, and further investigations are needed into its natural occurrence in wine and the extent to which it contributes to the total OTA exposure in various populations, as well as the identification and occurrence of the producing organisms.

LITERATURE CITED

(1) Moss, M. O. Mode of formation of ochratoxin A. *Food Addit. Contam.* **1996**, *13* (Suppl.), 5−9.

(2) Benford, D.; Boyle, C.; Dekant, W.; Fuchs, R.; Gaylor, D. W.; Hard, G.; McGregor, D. B.; Pitt, J. I.; Plestina, R.; Shephard, G.; Solfrizzo, M.; Verger, P. J. P.; Walker, R. Ochratoxin A. In *Safety Evaluation of Certain Mycotoxins in Food*; WHO Food Additives Series 47; FAO Food and Nutrition Paper 74; WHO: Geneva, Switzerland, 2001; pp 281−415.

(3) Stoev, S. D. The role of ochratoxin A as a possible cause of Balkan Endemic Nephropathy and its risk evaluation. *Vet. Hum. Toxicol.* **1998**, *40*, 352−360.

(4) Pohland, A. E.; Nesheim, S.; Friedman, L. Ochratoxin A: a review. *Pure Appl. Chem.* **1992**, *64*, 1029−1046.

(5) Marquardt, R. R.; Frohlich, A. A. A review of recent advances in understanding ochratoxicosis. *J. Anim. Sci.* **1992**, *70*, 3968−3988.

(6) Schlatter, Ch.; Studer-Rohr, J.; Rasonyi, Th. Carcinogenicity and kinetic aspects of ochratoxin A. *Food Addit. Contam.* **1996**, *13* (Suppl.), 43−44.

(7) *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Ochratoxin A (CAS Registry No. 303-47-9) in F344/N Rats (Gavage Studies*); Technical Report Series 358, Boorman, G. A., Ed.; U.S. Department of Health and Human Services, National Institutes of Health: Research Triangle Park, NC, 1989; pp 1−141.

(8) *Ochratoxin A—Toxicological Evaluation of Certain Food Additives and Contaminants*; WHO Food Additives Series 35; WHO: Geneva, Switzerland, 1996; pp 363−376.

(9) *Ochratoxin A*; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, Vol. 56; International Agency for Research on Cancer: Lyon, France, 1993; pp 489−521.

(10) Hald, B. Ochratoxin A in human blood in European countries. In *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*; Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I. N., Bartsch, H., Eds.; IARC Scientific Publications 115; IARC Press: Lyon, France, 1991; pp 159−164.

(11) *Evaluation of Certain Food Additives and Contaminants*; 37th report of the Joint FAO/WHO Expert Committee on Food Additives; WHO Technical Report Series 806; WHO: Geneva, Switzerland, 1991.
(12) *Evaluation of Certain Food Additives and Contaminants*; 44th report of the Joint FAO/WHO Expert Committee on Food Additives; WHO Technical Report Series 859; WHO: Geneva, Switzerland, 1995.
(13) Kuiper-Goodman, T.; Scott, P. M. Risk assessment of the mycotoxin ochratoxin A. *Biomed. Environ. Sci.* **1989**, *2*, 179−248.
(14) Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants. *Position Paper on Ochratoxin A*, 31st session; The Hague, The Netherlands, March 22−26, 1999; CX/FAC 99/14.
(15) Otteneder, H.; Majerus, P. Occurrence of ochratoxin A (OTA) in wines: influence of the type of wine and its geographic origin. *Food Addit. Contam.* **2000**, *17*, 793−798.
(16) Majerus, P.; Bresch, H.; Otteneder, H. Ochratoxin A in wines, fruit juices and seasonings. *Arch. Lebensmittel.* **2000**, *51*, 95−97.
(17) National Food Agency, Finland, press release 22, Dec 4, 1998 (http://www.elintarvikevirasto.fi).
(18) Markaki, P.; Delpont-Binet, C.; Grosso, F.; Dragacci, S. Determination of ochratoxin A in red wine and vinegar by immunoaffinity high-pressure liquid chromatography. *J. Food Prot.* **2001**, *64*, 533−537.
(19) Ospital, M.; Cazabeil, J.-M.; Betbeder, A.-M.; Tricard, C.; Creppy, E.; Medina, B. L'Ochratoxine A dans les vins (Ochratoxin A in wines). *Rev. Fr. Oenol.* **1998**, *169*, 16−18.
(20) Woese, K. Ochratoxin A in grape juice and wine. *Mycotoxin Res.* **2000**, *16A*, 132−135.
(21) Visconti, A.; Pascale, M.; Centonze, G. Determination of ochratoxin A in wine by means of immunoaffinity column clean-up and high-performance liquid chromatography. *J. Chromatogr. A* **1999**, *864*, 89−101.
(22) Pietri, A.; Bertuzzi, L.; Pallaroni, L.; Piva, G. Occurrence of ochratoxin A in Italian wines. *Food Addit. Contam.* **2001**, *18*, 647−654.
(23) Castellari, M.; Fabbri, S.; Fabiani, A.; Amati, A.; Galassi, S. Comparison of different immunoaffinity clean-up procedures for high-performance liquid chromatographic analysis of ochratoxin A in wines. *J. Chromatogr. A* **2000**, *888*, 129−136.
(24) Filali, A.; Ouammi, L.; Betbeder, A. M.; Baudrimont, I.; Soulaymani, R.; Benayada, A.; Creppy, E. E. Ochratoxin A in beverages from Morocco: a preliminary survey. *Food Addit. Contam.* **2001**, *18*, 565−568.
(25) Festas, I.; Herbert, P.; Santos, L.; Cabral, M.; Barros, P.; Alves, A. Ochratoxin A in some Portuguese wines: method validation and screening in port wine and vinho verde. *Am. J. Enol. Vitic.* **2000**, *51*, 150−154.
(26) Burdaspal, P. A.; Legarda, T. M. Ochratoxina A en vinos, mostos y zumos de uva elaborados en espana y en otros paises europeos (Ochratoxin A in wines and grape products originated from Spain and other European countries). *Alimentaria* **1999**, *299*, 107−113.
(27) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Addit. Contam.* **1996**, *13*, 655−668.
(28) Ministry of Agriculture, Fisheries and Food, U.K. Food surveillance information sheet 130, Nov 1997.
(29) Majerus, P.; Otteneder, H. Nachweis und vorkommen von ochratoxin A in wein und traubensaft (Detection and occurrence of ochratoxin A in wine and grape juice). *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 388−390.
(30) *Worldwide Regulations for Mycotoxins 1995—A Compendium*; FAO Food and Nutrition Paper 64; FAO: Rome, Italy, 1997.
(31) Ministero della Sanita, Circolare 10; Rome, Italy, June 9, 1999.
(32) Scientific Committee on Food. *Opinion on Ochratoxin A*; CS/CNTM/MYC/14 Final, Annex II to Document XXIV/2210/98; EC: Brussels, Belgium, 1998.
(33) Visconti, A.; Pascale, M.; Centonze, G. Determination of ochratoxin A in wine and beer by immunoaffinity column cleanup and liquid chromatographic analysis with fluorometric detection: Collaborative study. *J. AOAC Int.* **2001**, *84*, 1818−1827.
(34) Nesheim, S.; Stack, M. E.; Trucksess, M. W.; Eppley, R. M.; Krogh, P. Rapid solvent-efficient method for liquid chromatographic determination of ochratoxin A in corn, barley, and kidney: Collaborative study. *J. AOAC Int.* **1992**, *75*, 481−487.
(35) Zöllner, P.; Leitner, A.; Lubda, D.; Cabrera, K.; Lindner, W. Application of a Chromolith SpeedROD RP-18e HPLC column: Determination of ochratoxin A in different wines by high-performance liquid chromatography−tandem mass spectrometry. *Chromatographia* **2000**, *52*, 818−820.
(36) Becker, M.; Degelmann, P.; Herderich, M.; Schreier, P.; Humpf, H.-U. Column liquid chromatography-electrospray ionisation-tandem mass spectrometry for the analysis of ochratoxin. *J. Chromatogr. A* **1998**, *818*, 260−264.
(37) Soleas, G. J.; Yan, J.; Goldberg, D. M. Assay of ochratoxin A in wine and beer by high-pressure liquid chromatography photodiode array and gas chromatography mass selective detection. *J. Agric. Food Chem.* **2001**, *49*, 2733−2740.
(38) Stander, M. A.; Steyn, P. S. Survey of ochratoxin A in South African wines. *S. Afr. J. Enol. Vitic.* **2002**, *23*, 9−13.

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