JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 8977−8982 **8977**

Occurrence of Ochratoxin A- and Aflatoxin B1-Producing Fungi in Lebanese Grapes and Ochratoxin A Content in Musts and Finished Wines during 2004

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This paper reports the results of an extensive survey on the occurrence of filamentous fungi isolated from wine-grapes in Lebanon and to test their ability to produce ochratoxin A (OTA) and aflatoxin B1 (AFB1) on CYA culture medium, in order to assess their potential for producing these mycotoxins on grapes. From the 470 grapes samples taken during season 2004, 550 fungi strains were isolated with 490 belonging to *Aspergillus* spp. and 60 belonging to *Penicillium* spp. All these isolated fungi starins were tested for their ability to produce OTA and AFB1. *Aspergillus carbonarius* shows that it is the only species able to produce OTA with a production percentage reaching 100% and a maximum concentration of 52.8 μg/g of Czapek yeast extract agar (CYA). In its turn, *Aspergillus flavus* was considered as the only AFB1-producing species with production percentage of 45.3% and a maximum concentration reaching 40 μg/g CYA. A total of 47 handmade musts produced from the collected grapes were also analyzed in order to correlate the presence of OTA in must and the occurrence of filamentous fungi on grapes; 57.4% were contaminated with OTA at low level with concentrations ranging between 0.011 and 0.221 μg OTA L^{-1}. The analysis of these must samples was not performed with regard to AFB1. Seventy samples of finish red wine were also assayed for OTA content. The results showed that 42 of the tested samples (60%) were found to be positive for OTA with low levels (0.012−0.126 μg OTA L^{-1}).

KEYWORDS: Ochratoxin A; aflatoxin B1; black aspergilli; *Aspergillus flavus*; grapes; wine

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin of considerable concern with regard to human and animal health. This mycotoxin possesses nephrotoxic, teratogenic, and immunosuppressive properties (*1*). In fact, in 1993, the International Agency for Research on Cancer (IARC) classified OTA as a possible human carcinogen (group 2 B) (*2*). Moreover, OTA is suspected to be involved in Balkan endemic nephropathy (BEN) (a fatal kidney disease occurring in some areas of south-eastern Europe) and in the high frequency of urinary tract tumors observed in some Balkan areas (*3*).

Aflatoxin B1 (AFB1) is a secondary metabolite of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (*4*). It is considered a potent carcinogen and teratogen, being highly toxic and mutagenic to both humans and livestock. Chronic exposure to low levels of AFB1 poses serious health and economic hazards (*5*, *6*).

The worldwide occurrence of OTA contamination of raw agricultural products has been well documented; such contamination occurs in a variety of food and feed, such as cereals, coffee beans, pulses (*7*−*10*), wheat, barley, maize, and oats (*11*, *12*), spices (*13*), and meat and cheese products (*14*). OTA has also been detected in beverages such as beer, and since 1996 it has been detected in wine (*15*) (second most important source of OTA intake after cereals, *16*) and grape juice (*15*). Several studies on wine and grape juice in France (*17*), Italy (*18*), Argentina, and Brazil (*19*) but also on dried vine fruit in Spain (*20*, *21*) showed that the occurrence of OTA was related to the grape contamination in the vineyard by several OTA-producing species of fungi, especially the black aspergilli, mainly *Aspergillus carbonarius* and the members of the *Aspergillus niger* aggregates. *A. carbonarius* is considered as the major OTA producer species in wine grapes (*16*). Numerous studies show

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10.1021/jf062085e CCC: $33.50
Published on Web 10/25/2006

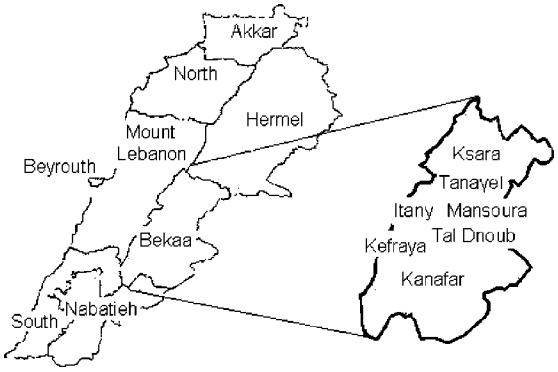

Figure 1. Origin of samples from Bekaa valley.

that molds can be found on grapes from veraison onward and sometimes even as soon as setting. Note that the mold development increases rapidly between veraison and maturation (*17*).

Aflatoxin B1 can occur in a wide range of plant products, including cereals such as maize, rice, and wheat but also in nuts, spices, figs, and dried fruits. This contamination occurs during growth, harvest, or storage of foods and feeds, especially when the conditions of temperature, relative humidity, and product moisture are favorable.

Several authors (*15*, *16*, *22*) reflected that wines from southern regions of Europe and the north of Africa contain high concentrations of OTA due to the climate being characterized by high humidity and high temperature. However, until this study, there was no available information about the contamination of grapes by the aflatoxin-producing fungi and/or aflatoxin.

In Lebanon, the situation was unknown due to the lack of data related to the mycoflora and the potential OTA- and AFB1-producing fungi on wine grapes. Our study aims therefore to screen for the presence and characterization of the filamentous OTA- and AFB1-producing fungi on wine grapes to evaluate the contamination rate of those fungi associated with grape during the growing season and to test in vitro their capacity to produce these mycotoxins.

MATERIALS AND METHODS

Study Area. Lebanon is located between 35° and 36°40′ longitude East and between 33° and 34°40′ latitude North on the eastern Mediterranean shores. It has a rectangular shape with 10.452 km^2 area. Seven winemaking regions were chosen for this study in the center and the south of the Bekaa plain, since it is a good representative sample (>75% of wine-grapes vineyards) for the whole situation in Lebanon according to the study (Ksara, Tanayel, Itany, Mansoura, Tal Dnoub, Kefraya, and Kanafar) (**Figure 1**).

Grape Samples. Grape bunches were collected from 27 Lebanese vineyards at veraison (mid-July, end of August) and from 20 of those vineyards at harvest (September, mid-October) located in the chosen winemaking regions during the year 2004. The sampling protocol consists of taking from each vineyard 10 bunches along two crossing diagonal transects. Bunches were kept in sterile bags and transported in cooled boxes (4 °C) to the laboratory (2−3 h) for analysis.

Culture Media. One of the culture media used for fungi isolation and identification was dichloran rose bengal hloramphenicol (DRBC) agar (Oxoid, Basingstoke, England), which contained (per liter of distilled water) 10 g of glucose (Fisher, Labosi Elancourt cedex, France), 5 g of meat peptone (Fisher), 1 g of KH_2PO_4 (Acros, Geel, Belgium), 0.5 g of $MgSO_4 \cdot 7H_2O$ (Acros), 25 mg of rose bengal; 2 mg of dichloran, 100 mg of chloramphenicol, and 15 g of agar (Difco, Fisher) (*23*). The other medium was Czapek yeast extract agar (CYA) (Oxoid), which contained (per liter of distilled water) 30 g of sucrose (Fisher), 1 mL of trace metal (Cu + Zn) solution (Fisher), 1 g of K_2HPO_4 (Acros), 10 mL of Czapek concentrate, 5 g of yeast extract (Difco, Fisher), and 15 g of agar (Difco, Fisher) (*23*).

Fungi Isolation. Five berries were randomly taken from each bunch and directly plated onto DRBC medium in Petri dishes. All plates were incubated for 7 days at 25 °C. After which all species belonging to the genera *Aspergillus* and *Penicillium* were isolated. For identification and morphological observations, isolated species were cultured on CYA medium and the identification was performed according to standard taxonomic systems based on the shape of conidiophores and conidia dimension observed with a binocular microscope with 100× magnification.

OTA- and AFB1-Producing Ability of Isolated Fungi. *Aspergillus* and *Penicillium* isolates were grown in Petri dishes containing CYA medium for 7 days at 25 °C. Three agar plugs were removed from different points of the colony for each culture, weighed, and collected into small tubes. A volume of 0.9 mL of methanol was added to each tube, and the tubes were left stationary for 60 min. Extraction was done after 20 min of centrifugation at 13 000 rpm for each tube, and the extracts obtained were filtered through a 0.45-μm Millipore filter into small vials and then analyzed and quantified by HPLC/FLD. The quantification of OTA and AFB1 was compared with that of standards of these two mycotoxins.

Extraction, Detection, and OTA Quantification from Musts and Finished Wines. The 10 bunches collected from each vineyard were smashed and centrifuged two times at 4000 rpm for 10 min. A total of 47 must samples were obtained and frozen at −26 °C for analysis.

With the aim of estimating the OTA content in finished wines, 70 samples of Lebanese wines were purchased from the supermarket and stored in a refrigerator at 4 °C until analysis.

The extraction and detection procedure of OTA in must and wine was strictly derived from the European norm (EN 14133, V 03−128). A volume of 10 mL of must or wine was diluted in 10 mL of water solution containing PEG (1%) (Acros Organics) and $NaHCO_3$ (5%). The pH value of the result solution corresponds to the range 7.2−7.8. The solution was filtered through Whatman GF/A glass microfiber filter with porosity of 1.6 μm. The filtrate was then passed through an Ochrapep immuno affinity column (IAC) (r-Biopharm, St Didier Au Mont D'Or, France) at a flow rate of 1 drop/s. The column was successively washed with 5 mL of water solution containing NaCl (2.5%) and $NaHCO_3$ (0.5%) followed by 5 mL of HPLC-grade water at a flow rate of 1−2 drops/s and dried with air. OTA was eluted by 2 mL of HPLC-grade methanol through the IAC at a flow rate of 1 drop/s. The eluate, collected in an HPLC vial (Wheaton; 2 mL), was evaporated under nitrogen stream at 50 °C and reconstituted with 250 μL of mobile phase prior to HPLC analysis.

The Chromatographic System. *For OTA Detection.* OTA was detected and quantified by reversed-phase HPLC. The analysis was performed using a chromatographic system equipped with an autosampler (Agilent 1100, G1313A, ALS) and a fluorescence detector (Agilent 1100, G 1321A, FLD) set at 333 nm (excitation) and 460 nm (emission). The system was controlled by Chemstation software. A selected RP-18 column (5 μm particle size, 250 mm length × 4 mm internal diameter) (Lichrospher 100) was chosen at room temperature. The mobile phase (acetonitrile:water:acetic acid 99:99:2) (Scharlau) was pumped (Agilent 1100, Quat pump, G1311A) at a flow rate of 1.0 mL/min. The injection volume corresponds to 100 μL and the retention time was about 8 min. Under these conditions, the limit of detection of OTA was 0.01 ng mL^{-1}

For AFB1 Detection. The HPLC method for aflatoxin B1 analysis was adapted from Chan et al. (*24*). The apparatus consisted of a solvent delivery system with both fluorescence (λ_{ex} = 364 nm; λ_{em} = 440 nm) and UV detectors (λ = 225 and 362 nm). The spectra range is from 200 to 500 nm. The analytical column used was a 150 × 4.6 mm Uptisphere 5 μm C18 ODB fitted with a guard column of 10 × 4 mm. During analysis, the column temperature was maintained at 25 °C. An aliquot of sample (80 μL) was injected using an autoinjector (BIO-TEK, Milan, Italy). The mobile phase was 0.1% phosphoric acid (A) and methanol/acetonitrile (50:50) (B) delivered at flow rate of 1 mL/min. The retention time was about 6 min.

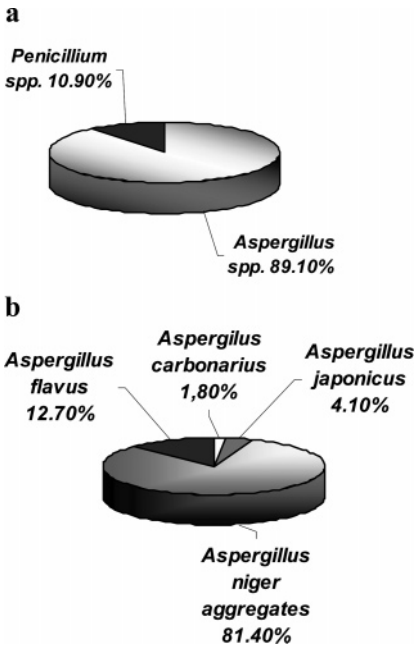

Figure 2. (**a**) Occurrence of *Aspergillus* spp. and *Penicillium* spp. from all maturation stages, (**b**) The isolation rates of species belonging to *Aspergillus* spp.

RESULTS

Total Fungi Isolates from Grapes. During 2004 a total of 470 grape samples (270 from veraison and 200 from harvesting) were collected from seven wine-grape growing regions. Genera of *Aspergillus* and *Penicillium* were systematically isolated, counted, and identified. There was a total of 550 fungal isolates, with 490 (89.1%) belonging to *Aspergillus* spp. and 60 (10.9%) belonging to *Penicillium* spp. (**Figure 2a**).

On the basis of the morphology of the conidial head and the shape of the conidia, identification to species levels was carried out for all *Aspergillus* strains.

From the 490 *Aspergillus* strains identified, the most frequent were from section *Nigri* (87.3%), namely, the bisseriate species *A. niger aggregates* (81.4%) and *A. carbonarius* (1.8%) and the uniseriate species *Aspergillus japonicus* (4.1%). However, other isolates belonging to the *Aspergillus* genus other than the section *Nigri* are well-known as potential producers of mycotoxins, namely, *A. flavus*, the most known isolated species to be described as an aflatoxin producer (*25*), with an isolation rate of 12.7% (**Figure 2b**).

Black aspergilli were present in all vineyards during the two sampling periods. Their isolation rate found in the sampled grapes was 56.25%. Moreover, the percentage of the infected grapes−berries shows in general an increasing tendency during the development of the berry, achieving the highest levels at harvest time (**Figure 3**).

Mycotoxigenic Capacity of Fungal Isolates. From all fungal isolates, *A. carbonarius* was the single OTA producer with production percentage of 100% and maximum concentrations of 52.8 μg/g CYA. None of the others species of the *Nigri* section produced ochratoxin A at a detectable limit. Concerning *Penicillium* genus, none of the isolates was identified as an OTA producer. With regard to aflatoxin B1, *A. flavus* was considered as the single AFB1-producing species with production percentage of 45.3% and a maximum concentration reaching 40 μg/g CYA.

OTA Detection in Handmade Must. The results of the analysis made on 47 samples of handmade must are summarized in **Table 1**. Those results show that 27 samples (57.4%) were contaminated with OTA at a low level (<0.25 μg/L). The most contaminated ones were seven samples that reached a maximum concentration of 0.221 μg OTA L^{-1}. Twenty other samples showed traces of OTA with concentrations ranging from 0.011 to 0.075 μg OTA L^{-1}, whereas no OTA was detected in 20 of those handmade samples. None of the contaminated samples contains levels above the European MRLs fixed at 2 μg OTA L^{-1}.

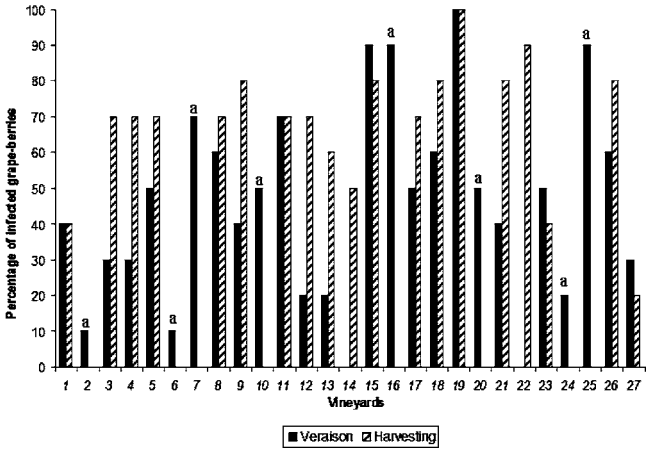

Figure 3. Percentage of infected grape-berries by black aspergilli in each vineyard at two stages of grapes maturation, veraison and harvesting. [a]Vineyards not sampled during harvesting.

Table 1. Contamination Rate of 47 Handmade Musts by OTA

concn range (μg OTA L^{-1})	max concn (μg OTA L^{-1})	no. of samples at maturation stage		total of samples
		veraison	harvesting	
0.1–0.5	0.221	3	4	7
<0.05–0.1	0.075	3	2	5
0.01–0.05	0.043	8	7	15
<DL[a]	–	13	7	20

[a] DL = detection limit.

Table 2. Contamination Rate of 70 Samples of Lebanese Finished Wines by OTA

concn range (μg OTA L^{-1})	max concn (μg OTA L^{-1})	total of samples
0.1–0.5	0.126	2
<0.05–0.1	0.088	7
0.01–0.05	0.048	33
<DL[a]	–	28

[a] DL = detection limit.

Analysis of OTA Content in Wine. Of the 70 finished red wines samples originating from the Bekaa valley and purchased from the supermarket, 42 samples (60%) were found positive for OTA with low levels of contamination (range 0.012−0.126 μg OTA L^{-1}), while the mycotoxin was not detected in 28 of those samples (40%) (**Table 2**). None of the contaminated samples contains levels above the European MRLs fixed at 2 μg OTA L^{-1}.

DISCUSSION

Black aspergilli have been reported as the predominant mycobiota of grapes. In our study, results show that *Aspergillus* and *Penicillium* strains were present in all vineyards at all

maturation stages. Black aspergilli (namely *A. niger aggregates*, *A. carbonarius*, and *A. japonicus*) were the most common, and they constituted 87.3% of the total *Aspergillus* strains isolated from grapes at veraison and harvesting. These results are similar to those found in Mediterranean vineyards in France (*17*) Italy (*18*), and Spain (*30*).

The similar results obtained in general with regard to the *Aspergillus* spp. distribution can be explained by the fact that those vineyards belong to the same wine producer and thus they are subjected to the same cultural practices. Moreover, they are in the same geographic area with the same climatic conditions characterized by a very low humidity (30%) and a high temperature (34−38 °C) during the maturation period, even though they are distributed within seven grape-grown regions. The little difference in fungi occurrence stresses that even though they belong to the same climatic area, each vineyard possesses a microclimate that can affect the grape infection through the maturation stages, leading to a variation in the dynamics of the fungi occurrence.

As for the OTA production ability of fungi, our results obtained from Lebanese vineyards support highly that *A. carbonarius* is without a doubt the major producer of OTA within *Aspergillus* section *Nigri*. This fact is due to the extremely high percentage of its ochratoxigenic isolates, since for the 490 *Aspergillus* isolates, *A. carbonarius* showed that it is the only ochratoxigenic species with a production ability reaching 100% while none of the other species isolates shows an ability to produce this mycotoxin at a detectable limit. The results obtained from other surveys conducted in Spain (*31*, *32*), Portugal (*33*), Italy (*18*), and France (*17*) on OTA-producing fungi show also that *A. carbonarius* is considered as the main OTA producer and that its ochratoxigenic potential was not only occasioned by its intrinsic toxigenic character but also by its aggressiveness (*34*). In these countries, and to a lesser extent, *A. niger* was detected as a producer of OTA. However, its level of production remains very low compared to that of *A. carbonarius*.

However, the occurrence of an aflatoxin-producing fungus on grapes is of great importance, since *A. flavus* is considered as a nonhabitual member of the natural grape mycobiota given its low incidence on this fruit (*35*, *36*). The studies conducted in the other Mediterranean countries revealed a very low occurrence of this species in the vineyards (*35*, *36*). Our results showed that the isolation rate of the *A. flavus* was 12.7%, and 45.3% of its isolates was able to produce the AFB1 at a maximum concentration reaching 40 μg/g CYA. Magnoli et al. (*37*) have reported the occurrence of *A. flavus* in Argentinean grapes. In their study, the isolation rate of this species was about 17%, but no information has been given regarding the *A. flavus* production capacity of AFB1. Unfortunately, within the framework of our study, the occurrence of AFB1 in must and finished wines was not determined.

As for must, several authors reflected that the occurrence of OTA in grape juices and wines is linked to the presence of the ochratoxigenic fungi on grapes. Our must analysis shows that 27 samples (57.4%) were contaminated with low concentrations of OTA, reaching a maximum of 0.221 μg OTA L^{-1} (**Table 1**). So, independent of the growth stage considered, OTA was not always detected in grapes, even in those colonized by section *Nigri*. In other cases, some must samples were contaminated with OTA despite originating from vineyards that were not contaminated with *A. carbonarius* (**Table 3**). So the OTA production seems to be probably linked to other species even though those species did not produce the OTA at a detectable limit when isolated in the laboratory.

Table 3. Occurrence of *A. carbonarius* on Grapes and OTA Content in Grape-Derived Musts[a]

	presence of *A. carbonarius*			presence of OTA in must	
vineyard	veraison	harvesting	total of strains	veraison	harvesting
1	−	+	1	−	−
2	−	+	1	+	+
3	−	−		−	+
4	−	−		+	+
5	−	−		−	−
6	−	−		+	−
7	−	[b]		+	[b]
8	−	−		−	−
9	−	+	1	+	−
10	−	[b]		−	[b]
11	−	[b]		−	[b]
12	−	+	1	+	+
13	−	−		−	−
14	−	−		−	−
15	+	+	1	−	+
16	−	[b]		−	[b]
17	−	−		+	+
18	+	−		−	+
19	+	−		+	+
20	−	[b]		+	[b]
21	−	+	1	+	−
22	−	−		+	+
23	−	−		−	+
24	−	[b]		+	[b]
25	−	[b]		+	[b]
26	−	−		+	+
27	−	−		+	+

[a] +, presence; −, absence. [b] Sample was not taken.

Many authors mentioned that wines, and especially red wines, originating from regions with Mediterranean climates (southern Europe and north Africa) are more contaminated than those originating from temperate regions of central Europe (*15*, *26*), with maximum concentrations reaching 7.6 μg OTA L^{-1} (*27*), 4 μg OTA L^{-1} (*28*), 0.736 μg OTA L^{-1} (*29*), and 0.451 μg OTA L^{-1} (*15*). Our results (**Table 1**) showed that 60% of the tested samples were contaminated with low levels of OTA with a maximum concentration reaching 0.126 μg OTA L^{-1}. While Lebanon is considered a Mediterranean country, the low levels of OTA content in wine could be explained by the fact that all the tested samples originated from the Bekaa valley, which is separated from the Mediterranean sea by high mountainous chains culminating to 3000 m, where all the Lebanese wine-grape vineyards are located. This fact characterized the studied area as a semiarid climate with high temperature and a low humidity, which could not be favorable to the growth of OTA-producing fungi and/or OTA production.

For the present time, the low OTA levels detected can be probably explained by the fact that the low humidity (30%) is not favorable to OTA synthesis in the studied area or, to produce the mycotoxin, fungi require high humidity levels (above 70%). We can also consider that the majority of our *Nigri* section isolates belong to the *A. niger* aggregates (81.4%), which are considered as low producers/or nonproducers of OTA. So the occurrence of black aspergilli does not necessarily indicate OTA production. Thus, it appears that the nature of the fungi species coupled with extrinsic factors like the climatic conditions play a major role in mycotoxin production. Gaining an understanding of the influence of the physicochemical conditions on the growth and production of OTA by isolates originating from Lebanon requires further studies. This work and data collection will be pursued within our laboratory for the next years to come up with a conclusive explanation of this fact.

In conclusion, the results of this survey point out that black aspergilli are the predominant mycobiota of grapes, where *A. carbonarius* is considered as the main OTA producer that can play a major role in the OTA contamination of grapes and its derived. Analysis of musts and finished wines shows a low OTA contamination. However, none of the contaminated samples contains levels above the European MRLs fixed at 2 μg OTA L^{-1}.

More studies on fungi occurrence in grapes are needed in order to evaluate the different susceptibility to mycotoxin formation by different species of these fungi. We saw here a higher percentage of *A. flavus* than *A. carbonarius* in addition to the production of aflatoxin B1 by 45.3% of its isolates, which guided us to follow the existence of AFB1 in must and wine. So, field sampling will be continued, as well as the characterization of strains and data collection about the farming methods and the climate variation in order to determinate the interactions between mycotoxigenic fungal species present on grapes and to ensure mycotoxins-free grape products.

ABBREVIATIONS USED

OTA, ochratoxin A; AFB1, aflatoxin B 1; BEN, Balkan endemic nephropathy; HPLC-FLD, high-performance liquid chromatography with fluorescence detector; CYA, Czapek yeast extract agar; DRBC, dichloran rose bengal chloramphenicol agar; IARC, International Agency for Research on Cancer; ex, excitation; em, emission; MRLs, maximum residue limits.

LITERATURE CITED

(1) Pitt, D. B.; Plestina, J. I.; Shepard, R.; Solfrizzo, G.; Verger, M.; Walker, P. J. P. Safety evaluation of certain mycotoxins in food. In *Joint FAO/WHO Expert Committee on Food Additives (JECFA)*; Food and Agriculture Organization: Rome, 2001; pp 281.

(2) IARC. Ochratoxin A. In *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans: Some Naturally Occurring Substances; Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*; International Agency for Research on Cancer: Geneva, Switzerland, 1993; Vol 56, pp 26−32.

(3) Pfohl-Leszkowicz, A.; Petkova-Bocharova, T.; Chernozemsky, I. N.; Castegnaro, M. *Food Addit. Contam.* **2000**, *19*, 282.

(4) Bennett, J. W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16.3*, 497−516.

(5) Karlowsky, P. Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. *Nat. Toxins* **1999**, *7*, 1−23.

(6) Mishra, H. N.; Das, C. A review on biological control and metabolism of aflatoxin. *Food Sci Nutr. Rev.* **2003**, *43* (3), 245−264.

(7) Jørgensen, K. Survey of pork, poultry, coffee, beer and pulses for ochratoxin A. *Food Addit. Contam.* **1998**, *15*, 550−554.

(8) Kuiper-Goodman, T.; Scott, P. M. Risk assessment of the mycotoxin ochratoxin A. *Biomed. Envir. Sci.* **1989**, *2*, 179−248. (ABSTRACT).

(9) Micco, C.; Grossi, M.; Miraglia, M.; Brera, C. A study of the contamination by ochratoxin A of green and roasted beans. *Food Addit. Contam.* **1989**, *6*, 333−339. (ABSTRACT).

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

(11) Speijers, G. J. A.; Van Egmond, H. P. World-wide ochratoxin A levels in food and feeds. In *Human Ochratoxicosis and its Pathologies*; Creppy, E., Castegnaro, M., Dirheimer, G., Eds.; John Liebbey Eurotext Ltd.: Paris, 1993; pp 85−100.

(12) Trucksess, M. W.; Giler, J.; Young, K.; White, K. D.; Page, S. W. Determination, and survey of ochratoxin A in wheat, barley and coffee−1997. *J. AOAC Int.* **1999**, *82*, 85−89.

(13) Hubner, M.; Vrabcheva, T.; Gareis, M. Simultaneous detection of ochratoxin A and B in spices and herbs by immunoaffinity column cleanup and HPLC. *Recl. Med. Veterinaire* **1998**, pp 507.

(14) Gareis, M.; Scheuer, R. (2000). Ochratoxin A in meat and meat products. *Arch. Lebensmittelhyg.* **2000**, *51*, 102−103.

(15) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. *Food Addit. Contam.* **1996**, *13*, 655−668.

(16) Cabañes, F. J.; Accensi, F.; Bragulat, M. R.; Abarca, M. L.; Castella, G.; Minguez, S.; Pons, A. What is the source of ochratoxin A in wine? *Int. J. Food Microbiol.* **2002**, *79*, 213−215.

(17) Bejaoui, H.; Mathieu, F.; Taillandier, P.; Lebrihi, A. Black aspergilli and ochratoxin A production in French vineyards. **2006**. In press.

(18) Battilani, P.; Pietri, A.; Bertuzzi, T.; Languasco, L.; Giorni, P.; Kozakiewicz, Z. Occurrence of ochratoxin A-producing fungi in grape grown in Italy. *J. Food Prot.* **2003**, *66*, 633−636.

(19) Da Rocha Rosa, C. A.; Palscios, V.; Combia, M.; Fraga, M. E.; De Oliveira Rekson, A.; Magnoli, C. E.; Dalcero, A. M. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. *Food Addit. Contam.* **2002**, *19*, 408−414.

(20) Abarca, M. L.; Accensi, F.; Bragulat, M. R.; Castella, G.; Cabañes, F. J. *A. carbonarius* as the main source of ochratoxin A contamination in dried vine fruits from the Spanish market. *J. Food Prot.* **2003**, *66*, 504−506.

(21) Heenan, C. N.; Shaw, K. J.; Pitt, J. I. Ochratoxin A production by *A. carbonarius* and *A. niger* isolates and detection using coconut cream agar. *J. Food Mycol.* **1998**, *1*, 67−72.

(22) Blesa, J.; Soriano, J.M.; Molto, J.C.; Manes, J. Concentration of ochratoxin A in wines from supermarkets and stores of valencian Community (Spain). *J. Chromatogr.* **2004**, *1* (2), 397−401.

(23) Pitt, J. I., Hocking, A. D., Eds. *Fungi and food spoilage*; Blackie: London, 1997.

(24) Chan, D.; MacDonald, S. J.; Boughtflower, V.; Brereton, P. Simultaneous determination of aflatoxins and ochratoxin A in food using a fully automated immunoaffinity column clean up liquid chromatography-fluorescence detection. *J. Chromatogr. A* **2004**, *1* (2), 13−16.

(25) Bau, M.; Bragulat, M. R.; Abarca, M. L.; Minguez, S.; Cabañes, F. J. Ochratoxigenic species from Spanish wine grapes. *Int. J. Food Microbiol.* **2005**, *98*, 125−130.

(26) Otteneder, H.; Majerus, P. Occurrence of ochratoxin A in wines: Influence of the type of wine and its geographical origin. *Food Addit. Contam.* **2000**, *17*, 793−798.

(27) Visconti, A.; Pascale, M.; Centonze, G. Determination of ochratoxin A in wine by means of immunoaffimty column clean-up and high-performance liquid chromatography. *J. Chromatogr.* **1999**, *864*, 89−101.

(28) MAFF. Survey of aflatoxin and ochratoxin in cereals in retail products. *Food Surveillance Sheet* **1997**, *130*, 1−8.

(29) Pietri, A.; Bertuzzi, T.; Pallaroni, L.; Piva, G. 2001. Occurrence of ochratoxin A in Italian wines. *Food Addit. Contam.* **2001**, *18* (7), 647−654.

(30) Belli, N.; Marin, S.; Duaigues, A.; Ramos, A. J.; Sanchis, V. Ochratoxin A in wines, musts and grape juices from Spain. *J. Sci. Food Agric.* **2004a**, *84*, 591−594.

(31) Abarca, M. L.; Accensi, F.; Bragulat, M. R.; Cabañes, F. J. Ochratoxin A-producing black aspergilli in dried vine fruits. In Book of abstracts "*Bioactive Fungal Metabolites*—Impact and Exploitation" Swansea: Wales, 2001; pp 38.

(32) Cabañes, F. J.; Accensi, F.; Bragulat, M. R.; Abarca, M. L.; Minguez, S.; Pons, A. *A. carbonarius* is a source of ochratoxin A contamination in wine. In *Book of abstracts "Bioactive Fungal Metabolites*—Impact and Exploitation; Swansea: Wales, 2001; pp 51.

(33) Serra, R.; Abrunhosa, L.; Kozakiewicz, Z.; Venancio, A. Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. *Int. J. Food Microbiol.* **2003**, *88*, 63−68.

(34) Battilani, P.; Pietri, A. Ochratoxin A in grapes and wine. *Eur. J. Plant Pathol.* **2002**, *108*, 639−643.

(35) Martinez-Culebras, P. V.; Ramon, D. An ITS-RFLP method to identify black *Aspergillus* isolates responsible for OTA contamination in grapes and wine. *Int. J. Food Microbiol.* **2006** (in press).

(36) Medina, A.; Mateo, R.; Lopez-Ocana, L.; Valle-Algarra, F.M.; Jimenez, M. Study of Spanish grape mycobiota and ochratoxin a production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Appl. Environ. Microbiol.* **2005**, *71*, 4696−4702.

(37) Magnoli, C.; Violante, M.; Combina, M.; Palacio, G.; Dalcero, A. Mycoflora and ochratoxin-producing strains of *Aspergillus* section *Nigri* in wine grapes in Argentina. *Lett Appl. Microbiol.* **2003**, *37*, 179−184.

Received for review July 24, 2006. Revised manuscript received September 20, 2006. Accepted September 21, 2006.

JF062085E