Food Anal. Methods (2015) 8:569–577
DOI 10.1007/s12161-014-9934-3

Determination of Aflatoxins and Ochratoxins in Sicilian Sweet Wines by High-Performance Liquid Chromatography with Fluorometric Detection and Immunoaffinity Cleanup

Vita Di Stefano · Rosa Pitonzo · Giuseppe Avellone · Antonina Di Fiore · Lucio Monte · Agata Zofia Tabis Ogorka

Received: 29 May 2014 / Accepted: 1 July 2014 / Published online: 10 July 2014

Abstract Ochratoxin A is the only mycotoxins with legal limits in the European Union for wine, by contrast, there is no EU legislation imposed for Aflatoxins and Ochratoxin B. A reproducible and sensitive analytical method for six mycotoxins: Ochratoxin A, Ochratoxin B, Aflatoxins B_1, Aflatoxin B_2, Aflatoxin G_1 and Aflatoxin G_2 was developed by using immunoaffinity column cleanup and HPLC-FLD method in 30 sweet wines produced in Sicily. This typical type of wine is manufactured from grapes grown in warmer climates, which have a higher sugar content; also, the over-ripening and the drying process of the grapes before fermentation conditions are responsible for the particular susceptibility of these berries to contamination by mycotoxins producing fungi. Results revealed the presence of Ochratoxin A in 96.6 % and of Ochratoxin B in 83.3 % of the samples examined (mean 0.246 and 0.168 μg/l, respectively). None of the samples showed a contamination exceeding the EU limit (2 μg/kg). Nine of tested wines were found to contain Aflatoxins with concentrations ranging from below LOD values to 0.068 μg/l. The results showed a very low incidence of Ochratoxins and Aflatoxins in analyzed wines, confirming the high degree of quality and safety of Sicilian sweet wines.

Keywords Sweet dessert wine · Ochratoxin A · Ochratoxin B · Aflatoxins · IAC · HPLC-FLD analysis

V. Di Stefano · G. Avellone · A. Di Fiore
Department of Biological, Chemical and Pharmaceutical Science and Technology (STEBICEF), University of Palermo, Palermo, Italy

R. Pitonzo
Centro Grandi Apparecchiature (CGA) — UniNetLab, University of Palermo, Palermo, Italy

L. Monte
Regional Institute of Wine and Oil of the Sicilian Region (IRVOS), Palermo, Italy

A. Z. T. Ogorka
Faculty of Pharmacy–Toxicology, Jagiellonian University, Cracow, Poland

V. Di Stefano (✉)
Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), University of Palermo, Via Archirafi 32, 90123 Palermo, Italy
e-mail: vita.distefano@unipa.it

Introduction

Mycotoxin contamination of foodstuffs has become a top priority issue in human health. In terms of regulatory aspects, since the discovery of the Aflatoxins (AFs) in the 1960s, regulations have been established in many countries to protect consumers from intake of harmful mycotoxin-contaminated foodstuffs, as well as to ensure fair practices in the food trade (López-García 2010).

Mycotoxins, products of secondary metabolism of filamentary micromycetes, represent the most important contaminants of natural origin. Ochratoxins (OTs) are a family of toxic compounds produced mainly by *Aspergillus* and *Penicillium* fungi that occur as natural contaminants of different foods. Of these, the most important, due to its toxicity and occurrence, is Ochratoxin A (OTA). Different studies have demonstrated that it can cause nephrotoxic, hepatotoxic, carcinogenic, teratogenic and immunotoxic effects in animals (Pfohl-Leskowicz and Manderville 2007).

In humans, OTA has been associated with urinary tract tumors, and in 1993 the International Agency for Research on Cancer (IARC) evaluated it "as a possibly carcinogenic to humans" (Group 2B) (IARC 1993). It is widely spread in agricultural commodities such as grains, spices, coffee, grapes and fruits.

Wine, after cereals, represents the second largest source of OTA human intake (about 13 %), identified by SCOOP report (Miraglia and Brera 2002) and by Joint FAO/WHO Expert Committee on Food Additives (JECFA 2007). OTs contamination of grapes takes place in the field and it is caused mainly by *Aspergillus carbonarius*. OTA was first detected in wines by Zimmerli and Dick (1996). Since then, the presence of OTA in wines has been reported from a number of European and other countries wines, with an apparent increase in levels in wines originating from southern areas of Europe including Italy, Greece, Turkey, some parts of Spain and France (Otteneder and Majerus 2000; Varga and Kozakiewicz 2006; Remiro et al. 2013).

OTA is sometimes accompanied by the non-chlorinated analogue, Ochratoxin B (OTB), OTB methyl and ethyl esters (MeOTB and EtOTB, respectively), and OTA ethyl and methyl esters: Ochratoxin C (OTC) and methyl Ochratoxin A (MeOTA), respectively (Remiro et al. 2012). Their chemical structures are shown in Fig. 1.

The simultaneous presence of OTA and its analogues in wine samples was recently studied (Remiro et al. 2013; Valero et al. 2008). The co-occurrence of different mycotoxins in one same foodstuff could origin additive or synergic effects on human or animal health (Heussner et al. 2006).

Several toxicological studies are reported in literature about OTA, OTB, OTC and their analogues (O'Brien et al. 2001; Mally et al. 2005; Knasmüller et al. 2004; Dietrich et al. 2001; O'Brien et al. 2005; Xiao et al. 1996; Müller et al. 2003, 2004).

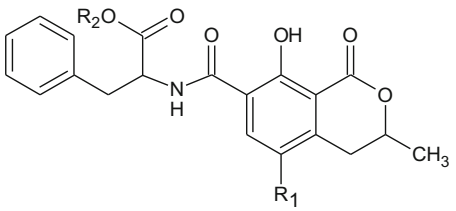
Currently, EU legislation permits a maximum of 2 μg/kg of OTA in wine and grape juice (Commission Regulation [EC] 1881 2006); this limit does not apply to liquor or dessert wines with more than 15 % alcohol content. On the contrary, there are no legal limits for OTB in food and wine.

The presence of OTA in wines has been extensively studied. Taking into account the levels encountered in different Mediterranean countries, the highest OTA concentration was found in samples from North Africa followed by Greece, Italy, Spain, France, Turkey and Croatia, although all below the maximum levels permitted (Remiro et al. 2013).

AFs are a group of an extremely toxic mycotoxins produced mainly by the filamentous fungi *Aspergillus flavus* and *A. parasiticus*. Major members are designated as AFB_1, AFB_2, AFG_1 and AFG_2. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic agents (Williams et al. 2004). AFB_1 is the most predominant and the most toxic of the group, classified as a Group I carcinogen (IARC 1993) and thus carcinogenic to human. It is one of the most potent genotoxic agents and strongest hepatocarcinogens identified which cause liver tumor (Hamid et al. 2013). AF's chemical structures are shown in Fig. 2.

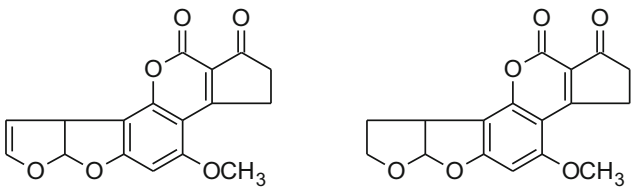
AFs and AF-producing strains (Fredj et al. 2007) have been detected in grape and musts occasionally (El Khoury et al. 2008; Aydogdu and Gucer 2009; Somma et al. 2012). Unfortunately, literature reports few studies regarding AF contamination in wines (Chunmei et al. 2013). The European Commission has established maximum permitted levels for AFs in cereals: 2 μg/kg for AFB_1 and 4 μg/kg for the sum of AFB_1, AFG_1, AFB_2 and AFG_2 (Commission Regulation (EC) 1881 2006). At present, the EU has not set a maximum allowable limit for AFs in wine, but this does not means that the problem can be ignored.

Italy is the world's second highest wine-producing country, behind France (OIV 2012), and Sicily is one of Europe's oldest viticultural regions. Today, Sicilian wines account for 17.5 % of the national total of Italian wine, and together with Puglia, Sicily is now the largest producer of Italian wine in

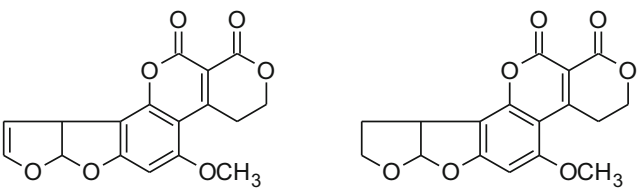


	R_1	R_2
Ochratoxin A	Cl	H
Ochratoxin B	H	H
Ochratoxin C	Cl	CH_3CH_2
Ochratoxin A methyl ester	Cl	CH_3
Ochratoxin B methyl ester	H	CH_3
Ochratoxin B ethyl ester	H	CH_3CH_2

Fig. 1 Chemical structures of Ochratoxin A and its analogues



Aflatoxin B_1 **Aflatoxin B_2**



Aflatoxin G_1 **Aflatoxin G_2**

Fig. 2 Chemical structures of the main Aflatoxins

Fig. 3 Location of sweet wines with designation of origin

Italy. Historically, Sicily has been world famous for the quality of its special sweet wines.

Sicilian sweet wines are made using the traditional method whereby grapes are grown on the vine as for a standard dry wine. Left to ripen for a little longer than is usual, providing weather allows, the grapes are harvested and laid out on mats or in racks and left for few weeks to dry. This process concentrates the sugars within the grapes to the point that the shrivelled, darkened grapes are almost raisin-sweet. It is at this point that fermentation and subsequent bottling takes place. This winemaking process leads to very fine wines, with higher alcohol content and extremely sweet. In Sicily, the geographical areas where sweet wines are traditionally produced are very limited. Noto, Siracusa and Pantelleria are famous for the production of Moscato and Passito, the Aeolian Islands for the production of Malvasia.

The choice of studying this type of wine comes because the traditional treatment of the grapes before fermentation (exposure of grapes to air, water reduction inside the berries and the simultaneous increase in the sugar concentration), makes the grapes more disposed to infection by bacteria and facilitates the development of molds which give rise to the formation of toxic metabolites.

The natural levels of OTA, OTB, AFB_1, AFG_1, AFB_2 and AFG_2 in 30 commercial sweet wines were investigated. Moreover, the simultaneous determination of the two classes of toxins is important because the presence of different mycotoxins in the same sample can produce synergistic effects on consumer health.

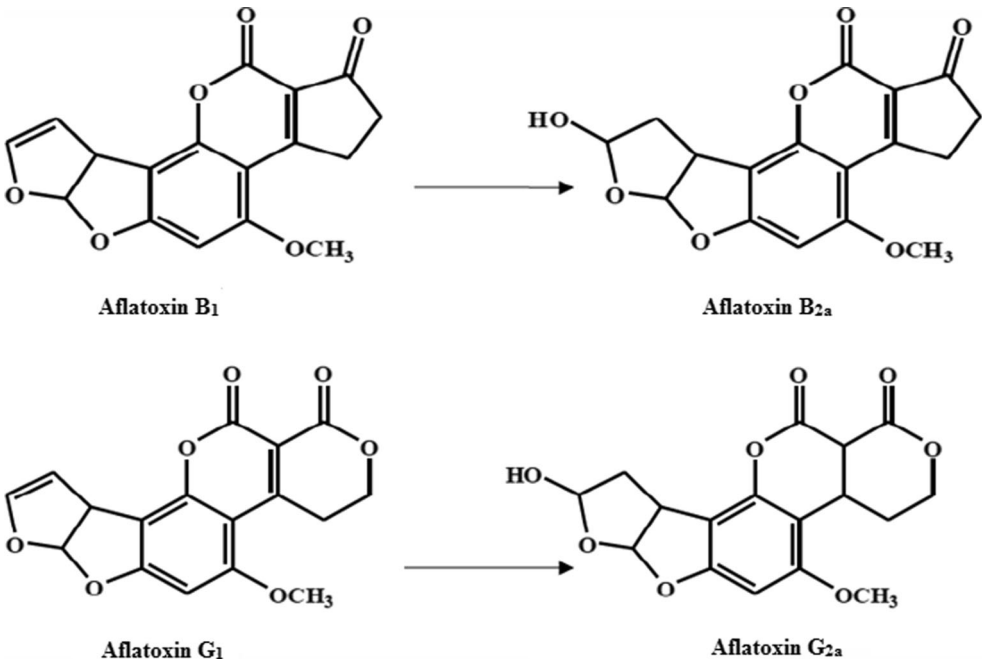


Fig. 4 Aflatoxins AFB_{2a} and AFG_{2a} by derivatization of AFB_1 and AFG_1

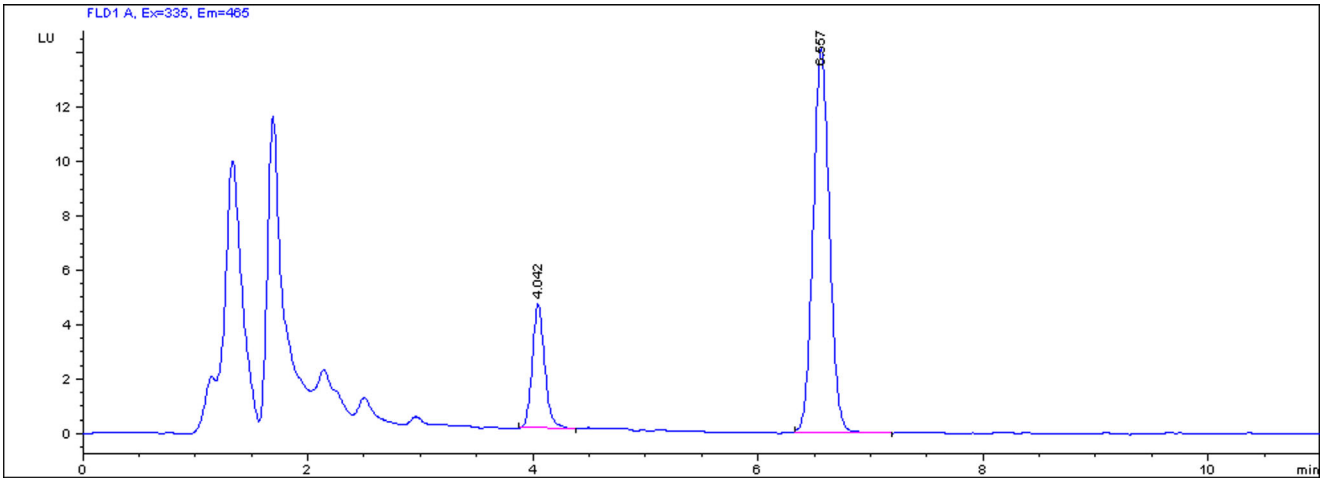


Fig. 5 Chromatogram of OTA (6.55 min.) and OTB (4.04 min.) standard solution 2 μg/l (injected volume 50 μl)

Materials and Methods

Samples

Sweet wines from five winemaking Sicilian regions were chosen for this study: Moscato of Siracusa, Moscato-Passito of Noto, Passito of Pantelleria, Malvasia of Lipari, Moscato of Partinico; their regional location is shown in Fig. 3.

Sweet dessert wines were purchased from local retails (bottled wine). A total of 30 samples belonging to the study areas were chosen. All wines were from 2007 to 2011 vintages. Their different alcoholic grade varied from 12.5 % to 14.5 % (v/v); their measured pH was in the range of 3.0–4.1.

All samples were stored at 4 °C until their analysis. All information on the samples was taken from the bottle labels.

Chemicals and Materials

Water, methanol and acetonitrile were HPLC grade. Glacial acetic acid, hexane, trifluoroacetic acid, sodium hydrogen carbonate and sodium chloride were all ACS grade. PEG 6000 and methanol were supplied by Merck (Darmstadt, Germany). Solvents and water were degassed for 20 min. using ultrasonic bath. The immunoaffinity columns used for OTs and AFs analysis were Ochraprep and Easy-extract, respectively, from r-Biopharm Rhone Ltd (Scotland, UK). OTA, OTB analytical standard solutions 10 μg/ml in acetonitrile, were purchased from Fluka and kept at −15 °C. Mix AFs standard solution at 1 μg/ml in acetonitrile (containing 250 ng of AFB_1, 250 ng of AFB_2, 250 ng of AFG_1 and 250 ng of AFG_2) were purchased from r-Biopharm Rhone Ltd and kept at −8 °C. The calibration standard solutions were made in mobile phase according to the concentration established, kept in security conditions, wrapped in aluminium foil, due to that mycotoxins gradually break down under UV light, held for less than 3 months and stored at −15 °C.

Ochratoxins and Aflatoxins Clean-up

We developed a method modified in comparison to that described by Visconti et al. (1999) because our

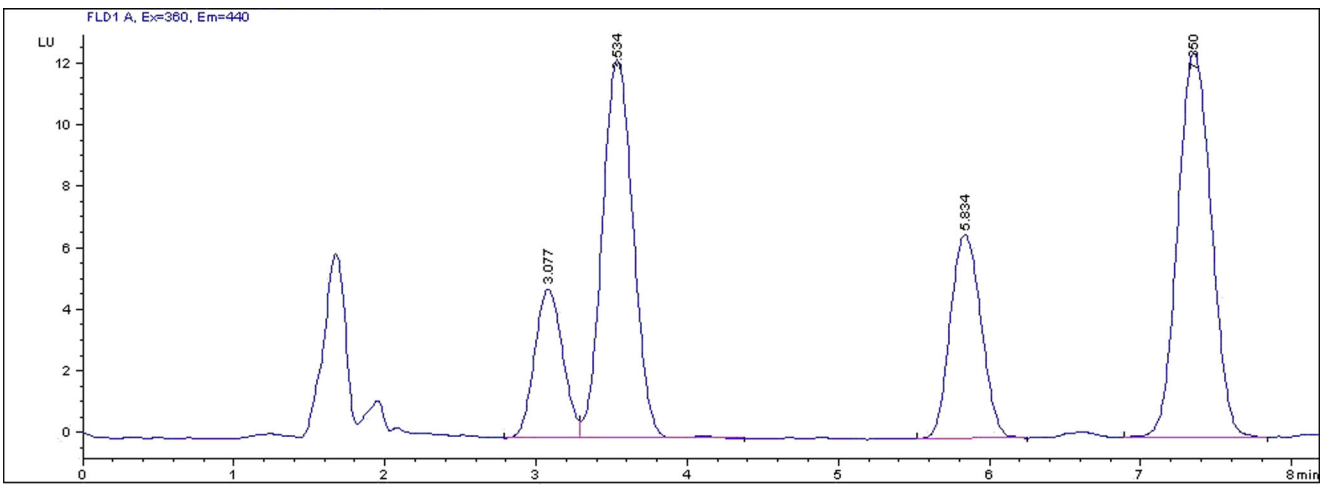


Fig. 6 Chromatogram of AFs (AFG_1, AFB_1, AFG_2 and AFB_2) standard solution 2 μg/l (injected volume 50 μl)

Table 1 Recovery results from the analysis of blank samples of Sicilian sweet wines spiked with OTA, OTB, AFB_1, AFB_2, AFG_1 and AFG_2 at two different levels

Spike level (μg/l)	OTA		OTB		AFB_1		AFB_2		AFG_1		AFG_2	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
0.5	95.1	3.1	92.4	5.4	87.4	7.6	79.1	6.3	83.4	4.9	75.2	4.3
2	93.4	2.7	89.2	7.9	79.3	5.9	81.5	8.0	78.7	6.1	77.4	5.8

purpose was to identify and quantify OTs and Afs simultaneously.

Sweet wine samples (10 ml) were diluted with 10 ml of water solution containing PEG 6000 (1 %) and $NaHCO_3$ (5 %), in this way the pH of the diluted wine was always within the optimal range (pH 7–8.5). The mixture was shaken vigorously and vortexed for 2 min. The IAC was placed on a SPE vacuum manifold (Visiprep, Supelco) and preconditioned with 3 ml PBS. Then, the mixture of the diluted sample (20 ml) was applied to the Ochraprep column (1–2 drops/s). The column was successively washed with 5 ml water solution containing NaCl (2.5 %) and $NaHCO_3$ (0.5 %) followed by 5 ml distilled water at a flow rate of 1–2 drops/s and dried with air. OTs were then slowly eluted from the IAC with 2 ml methanol/acetic acid 98:2 into a glass vial; the eluate was evaporated completely with nitrogen gentle stream and reconstituted with 500 μl of mobile phase before to HPLC analysis.

The extraction of the AFs was adapted from the OTs cleanup method with modifications. Ten milliliters of wine was taken on 10 ml of water solution containing PEG 6000 (1 %) and $NaHCO_3$ (5 %), shaken vigorously and vortexed for 2 min. The Easy-extract AFs column was preconditioned with 3 ml PBS, and the mixture (20 ml) was applied to the IAC (1–2 drops/s). The column was successively washed with 5 ml water solution containing NaCl (2.5 %) and $NaHCO_3$ (0.5 %) followed by 5 ml distilled water at a flow rate of 1–2 drops/s and dried with air. AFs were then slowly eluted from the IAC with 3 ml of acetonitrile into a glass vial. Also, in this case, the solutions obtained were dried under a gentle stream of nitrogen, but before HPLC/FLD analysis, the sample must be derivatized in order to increase the natural fluorescence of AFG_1 and AFB_1 (Fig. 4).

Precolumn Derivatization

The eluate containing AFs was evaporated to dryness with a gentle stream of N_2, redissolved with 400 μl of hexane and 100 μl of trifluoroacetic acid. The mixture was stirred for 1 min. and was made to stand for 5 min. Then the sample was added to 2 ml of a solution water/acetonitrile (9:1), stirred vigorously, then left until separation of the two phases and finally 1 ml of the lower phase containing AFs was drawn through the filters PTFE 0.45 μm and placed in a glass vial, ready for HPLC/FLD analysis. The solution containing derivatized AFs (AFB_{2a} and AFG_{2a}), must be readily analyzed in order to avoid deterioration due to their limited stability.

Table 2 Repeatability (intra-day) and reproducibility (inter-day) results from analysis of blank samples with micotoxins at two different levels

		Intra-day repeatability (n=5)		Inter-day reproducibility (5 different days)	
	Theoretical concentration (μg/l)	Determined concentration (μg/l)	RSD (%)	Determined concentration (μg/l)	RSD (%)
OTA	2	1.97	0.04	1.89	0.16
	4	3.93	0.08	3.89	0.19
OTB	2	1.87	0.04	1.88	0.16
	4	4.04	0.04	3.96	0.14
AFG_1	2	1.97	0.04	1.89	0.16
	4	3.93	0.08	3.89	0.19
AFG_2	2	1.87	0.04	1.89	0.16
	4	4.04	0.01	3.96	0.14
AFB_1	2	1.97	0.04	1.89	0.16
	4	3.93	0.08	3.89	0.19
AFB_2	2	1.87	0.04	1.88	0.16
	4	4.04	0.04	3.96	0.14

RSD = relative standard deviation (n=3)

LC-FLD Analysis

OTs and AFs analysis was carried out in an Agilent Technologies 1100 liquid chromatographic system equipped with a Fluorescence detector (model G1321A), controlled by Chemstation software. A selected RP-18 analytical column (5 μm particle size, 150 mm×4.6 mm i.d.) (Phenomenex Luna C18 (2)100A) fitted with guard column was chosen. The mobile phase consisted of acetonitrile/water/acetic acid (99:99:2, v/v/v) for OTs analysis and acetonitrile/water (70:30 v/v) for AFs analysis. The injection volume was 50 μl, and the flow rate was 1.0 ml/min. Chromatography was performed at 40 °C, and the fluorescence conditions were as follows: excitation at 335 nm and emission at 465 nm for OTs analysis and excitation at 360 nm and emission at 440 nm for AFs ones for the entire analysis. In these chromatographic conditions, the retention times were 4.04 and 6.55 min. for OTB and OTA, respectively, as shown in Fig. 5, and 3.07, 3.53, 5.83 and 7.25 min. for AFG_1, AFB_1, AFG_2 and AFB_2, respectively, as shown in Fig. 6. The limit of detection (LOD) was determined based on the lowest quantity of analyte that can be clearly distinguished from background (S/N=3). LOD was about 0.01 μg/l for OTA, AFB_1 and AFB_2, 0.02 μg/l for OTB and AFG_2 and 0.03 μg/l for AFG_1. Quantification of mycotoxins analyzed was performed by measuring peaks areas of AFs and OTs at retention time and comparing them with the appropriate calibration curves.

Preparation of Standard Calibration Curve

From OTA and OTB standard solutions (1 μg/ml in acetonitrile), and from mix AFs standard solutions (250 μg/l each AFs in acetonitrile) were prepared six different calibration

Table 3 Ochratoxins and Aflatoxins analyzed occurrence in Sicilian dessert wines

Sample	Year of production	OTA (μg/l) ± SD	OTB (μg/l) ± SD	AFB_1 (μg/l) ± SD	AFG_1 (μg/l) ± SD	AFB_2 (μg/l) ± SD	AFG_2 (μg/l) ± SD
Moscato of Siracusa	2009	0.07±0.003	0.075±0.004	<LOD	n.d.	n.d.	n.d.
Moscato of Siracusa	2010	0.135±0.014	0.133±0.004	n.d.	n.d.	n.d.	n.d.
Moscato of Siracusa	2008	<LOD	<LOD	n.d.	n.d.	0.016±0.009	0.033±0.005
Moscato of Siracusa	2009	0.233±0.004	0.07±0.007	0.026±0.002	n.d.	0.013±0.012	n.d.
Moscato of Siracusa	2009	0.121±0.013	0.036±0.004	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2009	1.56±0.016	1.205±0.037	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2010	0.118±0.004	0.116±0.004	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2008	0.033±0.001	0.051±0.001	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2009	0.051±0.006	0.061±0.009	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2009	0.052±0.002	0.061±0.001	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2010	0.136±0.004	0.089±0.004	n.d.	n.d.	<LOD	<LOD
Moscato of Noto	2008	0.259±0.004	0.105±0.006	n.d.	n.d.	n.d.	n.d.
Moscato of Noto	2009	0.05±0.002	0.059±0.001	n.d.	n.d.	n.d.	n.d.
Moscato of Partinico	2007	0.088±0.004	0.078±002	<LOD	0.043±0.005	0.015±0.003	0.025±0.001
Moscato of Partinico	2008	0.074±0.003	0.065±0.02	0.035±0.003	0.041±0.024	<LOD	<LOD
Moscato of Noto	2009	0.825±0.007	0.423±0.208	n.d.	n.d.	<LOD	<LOD
Moscato Passito of Noto	2010	0.076±0.024	0.415±0.031	n.d.	n.d.	n.d.	n.d.
Moscato Passito of Noto	2010	0.141±0.003	0.081±0.002	n.d.	n.d.	n.d.	n.d.
Moscato Passito of Noto	2009	0.021±0.002	<LOD	<LOD	0.02±0.003	n.d.	n.d.
Passito of Noto	2010	0.322±0.015	0.226±0.013	n.d.	n.d.	<LOD	<LOD
Passito of Noto	2010	0.075±0.022	<LOD	0.021±0.031	<LOD	n.d.	n.d.
Passito of Pantelleria	2007	0.064±0.003	0.058±0.007	n.d.	n.d.	<LOD	0.027±0.006
Passito of Pantelleria	2008	0.22±0.013	0.051±0.006	n.d.	n.d.	<LOD	<LOD
Passito of Pantelleria	2009	0.857±0.011	0.217±0.014	0.017±0.004	<LOD	<LOD	0.021±0.012
Passito of Pantelleria	2010	0.754±0.027	0.214±0.035	n.d.	n.d.	n.d.	n.d.
Malvasia of Lipari	2007	0.08±0.003	<LOD	n.d.	n.d.	<LOD	<LOD
Malvasia of Lipari	2008	0.147±0.007	0.094±0.004	n.d.	0.068±0.015	n.d.	n.d.
Malvasia of Lipari	2011	0.07±0.001	0.063±0.015	n.d.	n.d.	n.d.	n.d.
Malvasia of Lipari	2011	0.049±0.015	<LOD	n.d.	n.d.	<LOD	<LOD
Malvasia of Lipari	2011	0.467±0.037	0.168±0.009	n.d.	n.d.	n.d.	n.d.

SD standard deviation, *n.d.* not detected

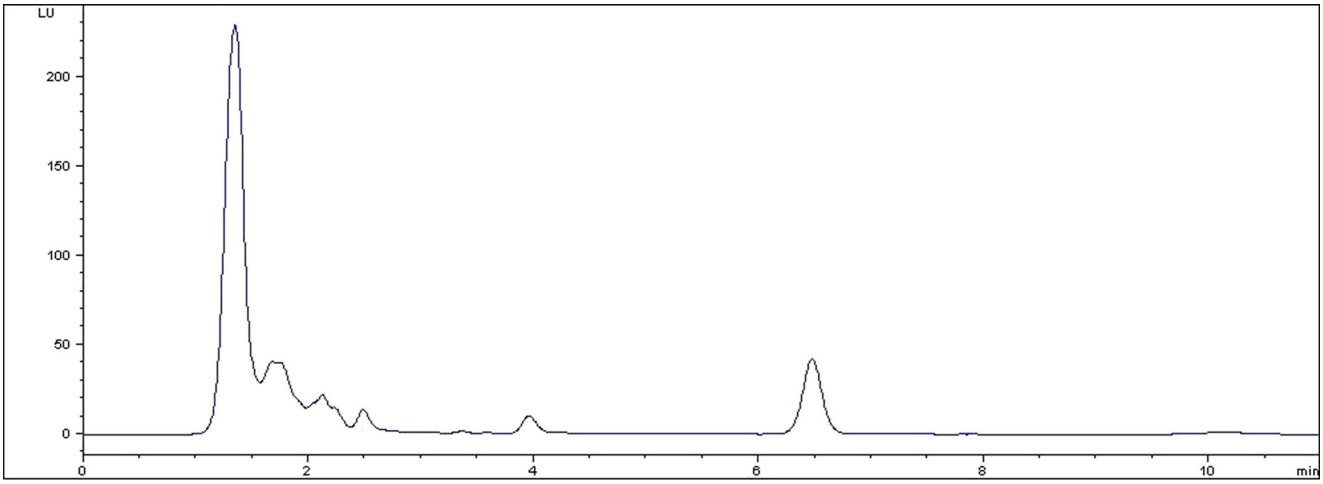


Fig. 7 Chromatogram of naturally OTs contaminated sweet wine sample

solution at concentrations of 0.5, 1, 1.5, 2, 4, and 8 μg/l using mobile phase for dilution.

Each solution at different concentration was prepared in duplicate and injected in triplicate, the mean was obtained from all measurements. Calibration curve was constructed as the dependence of the peak area on concentration of the standard. Calibration solutions were freshly prepared each day before the measurement. The regression coefficients (r^2) were 0.99 for all toxins analyzed.

Performance of the Analytical Method

Results of recovery experiments of the full analytical procedure (triplicate measurements) carried out on four mycotoxins-free sweet wines spiked with the six tested mycotoxins (OTA, OTB, AFB_1, AFG_1, AFB_2 and AFG_2) at two different levels (0.5 and 2 μg/l) are reported in Table 1.

Average recoveries of AFs and OTs ranged from 75 % to 87 % and from 89 % to 95 %, respectively. Values of relative standard deviation (RSD) ranged from 2.7 % to 8.0 %. Results of recovery experiments are in accordance with those reported in the literature (OIV-MA-AS315-10: R2011).

Precision was calculated in terms of intra-day repeatability (n=5) and inter-day reproducibility (5 different days) on two level of concentration (2 and 4 μg/l). The results of the tests are shown in Table 2.

Linearity was verified (n=5) with six concentrations, 0.5, 1, 2, 3, 4 and 8 μg/l. The regression coefficients (r^2) were 0.999 for OTA, 0.998 for AFG_1, 0.997 for OTB, AFB_1 and AFB_2 and 0.095 for AFG_2. The LOD of the method was about 0.012 μg/l for OTA, AFB_1 and AFB_2, 0.02 for OTB and AFG_2 and 0.04 for AFG_1.

The results of the study reflected that the analysis gave good repeatability, rapidity and precision. Recoveries were considered as valid for analyzing residues of OTA in wines according with European signification (Commission Regulation (EC) 401 2006).

The slope values of the regression curves obtained with standard solutions were then compared with the slope values obtained by the method of standard additions using the

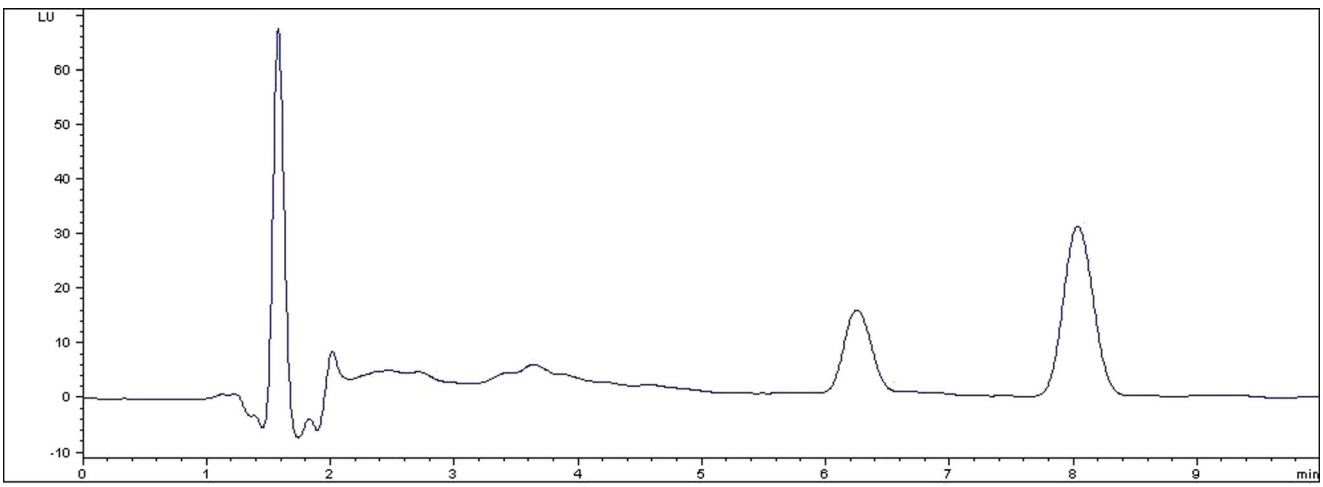


Fig. 8 Chromatogram of naturally AFB_2 and AFG_2 contaminated sweet wine sample

Student *t* test. In both studies, the slopes obtained were similar for a 95 % confidence limit, so it was concluded that the matrix effect was absent.

Results and Discussion

The analytical method was applied to 30 samples sweet wines originating from five sweet-wine-making Sicilian regions. The findings are reported in Table 3.

None of the analyzed wines contained OTA and OTB at a concentration in excess of the tolerated limit at 2 μg/kg.

The levels of AFs, if present, were very far from those currently enforced for other regulated foodstuffs, ranging from 0.10 to 8 μg/kg for AFB_1 and from 4 to 15 μg/kg for the sum of all four Aflatoxins (Commission Regulation (EC) 401 2006).

The six mycotoxins investigated did not appear simultaneously in studied samples.

Most of these samples were contaminated by OTs and the levels of OTA ranged from <LOD to 1.56 μg/l, while OTB ranged from <LOD to 1.205 μg/l. The highest OTs values were found in a sample of white Moscato of Noto. Only one sweet dessert wine contained OTA below the LOD while five samples contained OTB below the LOD value.

Results revealed the presence, to detectable values, of OTA in 96.6 % and of OTB in 83.3 % of the samples examined (mean 0.246 and 0.168 μg/l, respectively).

AFs showed a very rare presence. The concentration of at least one AFs has been clearly established in 30 % of tested samples, while about 23 % of the samples showed a contamination at values below LOD. AFB_1, AFG_1 and AFG_2 were in 13.3 % of the samples, while AFB_2 only in the 10 % of them. In all cases, levels of contamination were very low. Their mean values were 0.0247, 0.043, 0.0146 and 0.0265 μg/l for AFB_1, AFG_1, AFB_2 and AFG_2, respectively. The highest AFs value found was 0.068 μg/l for AFG_1.

AFs and OTs chromatograms of both natural contaminated are shown in Figs. 7 and 8.

Conclusion

This is the first report of OTA, OTB and the main AFs (AFB_1, AFB_2, AFG_1 and AFG_2) in dessert Sicilian wines.

OTs and AFs contents in Sicilian sweet wines tested in this survey suggested that a previously existing fungal contamination in the grape could occur, and this contamination, associated with the conditions of the drying process itself, led to the growth of the fungus and, consequently, of toxic metabolites.

This finding is related to the data provided by Valero et al. (2008) which demonstrated that it is after the harvesting, and particularly in the process of raising, when the *Aspergillus* species becomes predominant over other fungi present on the grape, due principally to greater tolerance of high temperatures and low activity of the water (a_w).

The use of IAC for clean-up followed by HPLC has shown to be a technique with good analytical performance for mycotoxins determination in wines. The choice of the method for the identification of tested mycotoxins which provides for their determination by HPLC/FLD is the most used method. The instrumentation is not very expensive and does not require the use of highly specialized staff. So this method can be developed in small laboratory inside wineries, for the control of wines prior to commercialization.

The application of this procedure to analyze 30 sweet wines from Sicily has demonstrated that none of them contained levels above the European MRLs, but the study suggests that there is a real risk of contamination by OTs and AFs in Sicilian sweet wines. In any case, due to their toxicity, a low but continued dietary exposure could contribute to a risk to humans, so the level of these mycotoxins in food should be as low as reasonably achievable.

However the incidence of OTA in wines produced in Sicily is definitively very low, compared with the findings in other wine producing countries. The four AFs were found separately in the different samples analyzed, with values still well below the lowest value reported in the EC Regulation no. 165 (2010) for foods.

The authors consider it necessary to continue the study in order to assess the mycotoxins contamination in the various years of production and to evaluate the effective influence of environmental parameters such as humidity, temperature, latitude and geographical location of the vineyard.

In conclusion, this study strongly confirms the excellent quality and safety of Sicilian sweet wines that are proven to be contaminated at levels far below the limits given in almost all samples analyzed.

Acknowledgments This work was financially supported by Ministero dell'Istruzione dell'Università e della Ricerca.

Conflict of Interest Vita Di Stefano declares that he has no conflict of interest. Rosa Pitonzo declares that he has no conflict of interest. Giuseppe Avellone declares that he has no conflict of interest. Antonina Di Fiore declares that he has no conflict of interest. Lucio Monte declares that he has no conflict of interest. Agata Zofia Tabis Ogorka declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

References

Aydogdu H, Gucer Y (2009) Microfungi and mycotoxins of grapes and grape products. Trakia J Sci 7(Supplement 2):211–214

Chunmei J, Junling S, Qian H, Yanlin L (2013) Occurrence of toxin-producing fungi in intact and rotten table and wine grapes and related influencing factors. Food Control 31:5–13

Commission Regulation (EC) 188/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L364

Commission Regulation (EC) 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, L70/12

Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union, L 50/8

Dietrich DR, O'Brien E, Stack ME, Heussner AH (2001) Species- and sex-specific renal cytotoxicity of ochratoxin A and B in vitro. Exp Toxicol Pathol 53:215–225

El Khoury A, Rizk T, Lteif R, Azouri H, Delia ML, Lebrihi A (2008) Fungal contamination and B1 and Ochratoxin A in Lebanese wine-grapes and musts. Food Chem Toxicol 46(6):2244–2250

Fredj SMB, Chebil S, Lebrihi A, Lasram S, Ghorbel A, Mliki A (2007) Occurrence of pathogenic fungal species in Tunisian vineyards. Int J Food Microbiol 113:245–250

Hamid AS, Tesfamariam IG, Zhang Y, Zhang ZG (2013) B1-induced hepatocellular carcinoma in developing countries: geographical distribution, mechanism of action and prevention (Review). Oncol Lett 5:1087–1092

Heussner AH, Dietrich DR, O'Brien E (2006) In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxin on renal cells. Toxicol In Vitro 20: 332–341

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Int Agency Res Cancer, Lyon, France 56:489–521

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2007) Evaluation of certain food additives and contaminants: sixty-eighth report of the joint FAO/WHO expert committee on food additives. WHO Technical Report Series, 947

Knasmüller S, Cavin C, Chakraborty A, Darroudi F, Majer BJ, Huber WW, Ehrlich VA (2004) Structurally related mycotoxins, ochratoxin A, ochratoxin B, citrinin differ in their genotoxic activities, and in their mode of action in human derived liver (HepG2) cells; implication for risk assessment. Nutr Cancer 50:190–197

López-García R (2010) Mycotoxin management: an international challenge. In: Boisrobert C, Oh S, Stjepanovic A, Lelieveeld H (eds) Ensuring global food safety: exploring global harmonization. Academic Press, London, pp 209–216

Mally A, Keim-Heusler H, Amberg A, Kurz M, Zepnik H, Mantle P, Völkel W, Hard GC, Dekant W (2005) Biotransformation and nephrotoxicity of ochratoxin B in rats. Toxicol Appl Pharmacol 206:43–53

Miraglia M, Brera C (2002) Assessment of dietary intake of Ochratoxin A by the population of EU member states. In Reports on tasks for scientific cooperation. Report of experts participating in Task 3.2.7. Directorate-General Health and Consumer Protection, Rome, Italy

Müller G, Rosner H, Rohrmann B, Erler W, Geschwend G, Burkert B, Möller U, Diller R, Sachse K, Köhler H, Gräfe U (2003) Effects of the mycotoxin ochratoxin A and some of its metabolites on the human cell line THP-1. Toxicology 184:69–82

Müller G, Burkert B, Möller U, Diller R, Rohrmann B, Köhler H, Rosner H (2004) Ochratoxin A and some of its derivatives modulate radical formation of porcine blood monocytes and granulocytes. Toxicology 199:251–259

O'Brien E, Heussner H, Dietrich DR (2001) Species-, sex-, and cell type-specific effects of Ochratoxin A and B. Toxicol Sci 63:256–264

O'Brien E, Prietz A, Dietrich DR (2005) Investigation of the teratogenic potential of ochratoxin A and B using the FETAX system. Birth Defects Res B Dev Reprod Toxicol 74:417–423

OIV (2012) Statistical Report on World Vitiviniculture by International Organisation of Vine and Wine

OIV-MA-AS315-10: R2011. Compendium of International Methods of Analysis — OIV Ochratoxin A

Otteneder H, Majerus P (2000) Occurrence of ochratoxin A (OTA) in wines: influence of the type of wine and its geographical origin. Food Addit Contam 17(9):793–798

Pfohl-Leskowicz A, Manderville R (2007) Review on A: an overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res 51:61–69

Remiro R, Irigoyen A, González-Peñas E, Lizarraga E, López de Cerain A (2012) Quantification of ochratoxin A and five analogs in Navarra red wines. Food Control 27:139–145

Remiro R, Irigoyen A, González-Peñas E, Lizarraga E, López de Cerain A (2013) Levels of ochratoxins in Mediterranean red wines. Food Control 32:63–68

Somma S, Perrone G, Logrieco A (2012) Diversity of black Aspergilli and mycotoxin risks in grape, wine and dried vine fruit. Phytopathol Mediterr 51(1):131–147

Valero A, Marín S, Ramos AJ, Sanchis V (2008) Survey: ochratoxin A in European special wines. Food Chem 108:593–599

Varga J, Kozakiewicz Z (2006) Ochratoxin A in grapes and grape derived products. Trends Food Sci Technol 17:72–81

Visconti A, Pascale M, Centonze G (1999) Determination of ochratoxin A and domestic and imported beers in Italy by immunoaffinity column-linked HPLC. J Chromatogr A 888:321–326

Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr 80:1106–1122

Xiao H, Madhyastha S, Marquardt RR, Li S, Vodela JK, Frohlich AA, Kemppainen BK (1996) Toxicity of ochratoxin A, its opened lactone form and several of its analogs: structure–activity relationships. Toxicol Appl Pharmacol 137:182–192

Zimmerli B, Dick R (1996) Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. Food Addit Contam 13:655–668