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Correlation of ochratoxin A level in wine with vine environment

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Abstract

The amount of Ochratoxin A in must and wine is strongly influenced by the pre- and post-harvesting conditions and the vinification techniques. The effect of pre- and post-harvesting conditions on Ochratoxin A biosynthesis depends mainly on the geo-climatic particularities of the grapes cultivation area. The pre-harvesting conditions that affect Ochratoxin A level in wine and related products are: climatic factors, vineyard location and grape variety. This paper refers to the correlation of these factors. The contamination with Ochratoxin A was analyzed in 99 wine samples (55 white wine and 44 red wine), 8 varieties produced in Vaslui, Vrancea, Prahova, Constanta, Arges, and Dolj vineyards, during 2007 – 2010. Ochratoxin A determination was performed by HPLC-FLD method with immunoaffinity clean-up. 32.7% of the white wine samples and 38.6 % of red wine samples were contaminated with levels ranging from 0.06 up to 0.99 ng ml^{-1} for white wine and from 0.19 up to 1.89 ng ml^{-1}. The mycotoxin level was correlated with the mean values of air temperature, air humidity, rainfall and shining during September of each production year.

Keywords: mycotoxins, HPLC determination, climatic factors

Introduction

In the recent years, world production of wine is facing severe effects induced by toxigenic fungi such as *Aspergillus carbonarius* and *Aspergillus niger*, responsible for Ochratoxin A (OTA) production. The presence of OTA in wine was reported for the first time in 1996 by B. ZIMMERLY & R. DICK [1]. In European population, wine is the second major source of human exposure to OTA, after cereals. OTA causes carcinogenic, immunotoxic, genotoxic, nephrotoxic effects on animals. It was classified, in 1993, by the International Agency for Research on Cancer as a group 2B agent (possible human carcinogen). The European Commission has fixed a maximum allowed limit of 2.0 ppb OTA in wine [2].

Ochratoxin A in wine is a problem starting from the vineyard (P. BATTILANI & al., [3]) and predominates in quantity and quality in red wines (54%) and rose (40%), rather than in white wines (25%) (H. OTTENEDER & P. MAJERUS, [4]). Fungal growth and OTA concentration depends on (1) up of the production region; the lower the latitude, the higher the frequency and the concentration (P. BATTILANI & al., [3], H. OTTENEDER & P. MAJERUS, [4]); (2) the contact between the berries and micotoxigen fungi is relevant; (3) injury or damage that allows penetration of the fungi in fruits and their susceptibility to infections that can occur during harvesting or as a result of extreme temperatures in the vineyards; (4) cultivation practices of the grapes including the use of fungicides and plant treatments and (5) winemaking techniques, including the type of maceration and fermentation conditions (A. LOPEZ DE CERAIN & al. [5]). Another important factor in the accumulation of OTA is the variety of the grapes (P. BATTILANI & al., [3]).

The importance of weather conditions on OTA biosynthesis was mentioned by A. PIETRI & al. [6] and A. LOPEZ DE CERAIN & al. [5] by the fact that fungi of the genus

Aspergillus, OTA producers, appreciate regions characterized by hot summers and dry, rather than temperate or cold climates.

The aim of this analytical study was to determine the level of OTA in Romanian wine and to explore any correlation with grapes cultivation region, grapes variety and climatic factors.

Materials and Methods

Reagents and Chemicals. All the chemicals used were analytical grade (sodium hydroxide, PBS) or HPLC grade (acetonitrile, methanol, acetic acid) and were purchased from Sigma-Aldrich (St. Louis, MO, USA0). A certified, precisely quantified, methanol standard stock solution of 1000 ng ml^{-1} OTA was purchased from R-Biopharm Rhone (Darmstadt, Germany). Highly purified water was generated by a Millipore Synergy 185 Ultra-Pure Water System from Millipore (Billerica, MA, USA).

Wine samples. Samples were collected from six geographical regions of Romania (Arges, Constanta, Dolj, Prahova, Vaslui and Vrancea). Four varieties of red bottled wine (Cabernet Sauvignon, Merlot, Pinot Noir and Feteasca Neagra) and four varieties of bottled white wine (Riesling, Sauvignon Blanc, Feteasca Alba and Feteasca Regala), produced between 2007 and 2010, were purchased from local stores.

Sample Preparation. For Ochratoxin A determination, 10 ml of wine sample were adjusted at pH 7.8 using 2M NaOH and then diluted with 10 ml PBS. After centrifugation at 1600g for 10 minutes, the samples were cleaned up using immunoaffinity columns OCHRAprep (R Biopharm Rhone). The elution was performed with 1.5 ml desorbtion solution (methanol and acetic acid 98:2 v/v) with a final dilution of 1.5 ml water. Prior to chromatograpfic analysis, the samples were filtered with 0.45 µm PTFE 12 mm syringe filter (Millipore, USA).

HPLC Analysis. HPLC separation was performed using Waters Alliance system with Inertsil ODS-3V, 5µm, 4mmx150mm analytical column and Inertsil ODS-3V, 5µm, 4mmx10mm guard cartridge. The mobile phase consisted in acetonitrile + acetic acid + water (51:2:47 v/v/v) and elution was realized at 1 ml min^{-1} flow rate. A fluorescence detector was used with excitation at 333 nm and emission at 443 nm. The injection volume was 100 µl, the run time 15 minutes and the retention time of OTA was about 8 minutes. Data were collected and analysed with Empower chromatography manager system.

Meteorological values. Temperature, total rainfall, relative humidity and shining period of each analyzed wine production year as mean of September were used as variance for statistical analysis. These data were obtained from National Institute of Meteorology and Hydrology.

Statistical analysis. The results were subjected to one-way analysis of variance (ANOVA) using OriginPro 8.5.1 statistical package program.

Results and discussions

Geographical location influence on OTA level in wine

Total level of Ochratoxin A was analyzed in 55 white wine samples produced during 2007 and 2010. Positive values were considered only those equal or higher than LOQ (limit of quantification) calculated on signal to noise base of 5. No detectable values (ND) were considered those lower than LOD (limit of detection) calculated on signal to noise base of 3., Only positive samples were used for mean and range of OTA values calculation. The results presented in table 1 show 32.7 % positive samples for white wine, with higher incidence in

Vrancea region (45 %). Looking at the range of OTA values it is obvious that the contamination level was low, with a maximum of 1 ng ml^{-1}.

Table 1. OTA level in white wine

Wine variety	Region of Origin	Number of Total Samples / Positive Samples	Mean of OTA values (ng ml^{-1})	Range of OTA values (ng ml^{-1})
Sauvignon Blanc	Arges	3/1	0.19	0.19
	Dolj	1/0	ND	ND
	Prahova	3/2	0.08	0.07-0.09
	Vaslui	1/1	1.0	1.0
	Vrancea	7/3	0.26	0.1-0.37
Riesling	Arges	2/0	<LOQ	<LOQ
	Constanta	1/1	0.14	0.14
	Vrancea	6/3	0.55	0.26-0.99
Feteasca Alba	Prahova	4/1	0.06	0.06
	Vaslui	1.1	0.1	0.1
	Vrancea	8/4	0.32	0.09-0.77
Feteasca Regala	Arges	5/1	0.07	0.07
	Prahova	4/1	0.36	0.36
	Vaslui	1/1	0.06	0.06
	Vrancea	8/3	0.08	0.07-0.10

Determination of OTA level in the 44 red wine samples resulted in 38.6 % positive samples, according to table 2. Looking at the range of OTA values it is obvious that the level of contamination was significantly higher, but below 2.0 ng ml^{-1}, the maximum admitted limit in wine. There were 5 samples with more than 1 ng ml^{-1}, all of them from Vrancea region (72% positive samples). All the samples coming from Dolj region had either not detectable contamination or below the Limit of Quantification (LOQ).

Table 2. OTA level in red wine

Wine variety	Region of Origin	Number of Total Samples / Positive Samples	Mean of OTA values (ng ml^{-1})	Range of OTA values (ng ml^{-1})
Cabernet Sauvignon	Arges	1/0	ND	ND
	Dolj	1/0	<LOQ	<LOQ
	Prahova	5/1	0.63	0.63
Pinot Noir	Dolj	1/0	<LOQ	<LOQ
	Prahova	1/0	ND	ND
	Vrancea	4/1	0.32	0.32
Merlot	Arges	2/0	<LOQ	<LOQ
	Prahova	5/0	<LOQ	<LOQ
	Vrancea	8/7	1.07	0.19-1.89
Feteasca Neagra	Arges	3/1	0.33	0.33
	Dolj	1/0	ND	ND
	Prahova	6/2	0.53	0.41-0.75
	Vrancea	6/5	1.26	0.24-1.71

The higher level of OTA in red wines than in white wines is correlated with the results reported by H. OTTENEDER & P. MAJERUS, [4]; A. FILALI & al. [7 P. MARKAKI et al,

[8]; C.A.R. ROSA & al. [9]; J. VARGA & al. [10]. According to H. OTTENEDER & P. MAJERUS, [4] and ROSA & al. [9], color gradient is connected to the time required for the wine to be extracted from grapes. Red wine needs a longer period to stay in contact with grapes skin, enabling mycotoxins extraction. Another hypothesis refers to the fact that white wines are clarified by the addition of clay, like bentonite or zeolite that could absorb OTA (A. HUWIG & al. [11]). The differences between the Ochratoxin A content in different wine types could also be related to the difference in vinification process (I. STEFANAKI & al. [12]).

In this study, 40 of the 99 wine samples were positive: 6 in 2007, 13 in 2008, 7 in 2009 and 14 in 2010. Their distribution according to wine variety and production year is presented in figure 1. Although the number of analyzed samples was rather the same for each year, the number of positive samples was almost twice for 2008 and 2010.

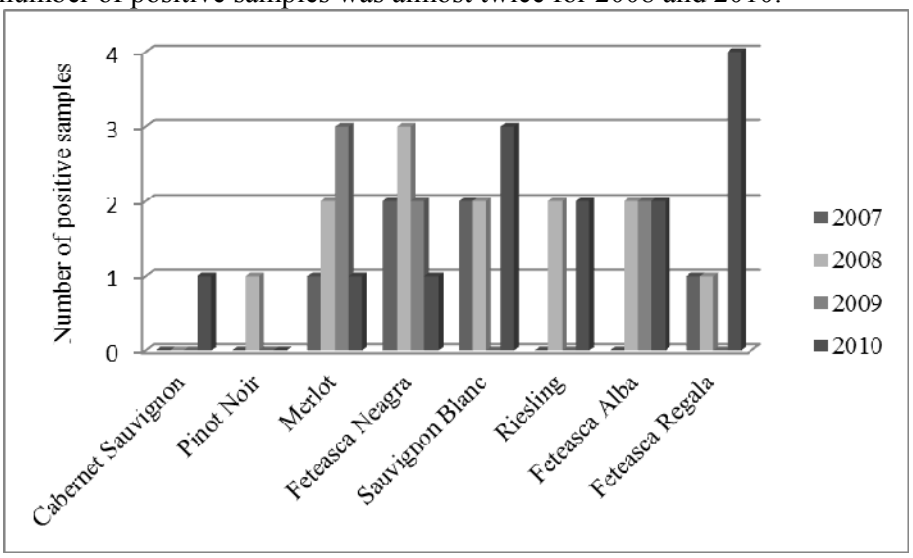

Fig 1. Distribution of positive samples according to the production year and wine variety

Weather conditions influence on OTA level in wine

It is well-known that the quality of wine depends a lot on the quality of grapes, which, in turn, depends on weather conditions. Variations in the biochemical composition of grapes are essential, but it is also true that the same wine can be produced year after year with the corrections of the industrial winemaking methods. Unlike this, mycotoxin contamination is rather difficult to solve.

Recent studies have identified the weather conditions, especially temperature and humidity, that are important for toxigenic fungi growth and OTA production by these species during pre- and post-harvesting and during transport. The ecological conditions for fungi optimum growth have been shown to be 30-35ºC and for OTA production 15-25º C (N. MAGAN & D. ALDRED, [13]). So, higher temperature and humidity during pre- and post-harvesting seems to increase the risk of OTA contamination. But lower temperatures extend the ripening period in autumn when the weather is easily changing and the possibility of rainfall increases.

This study aimed to make a correlation between OTA level in vine and the values of air temperature, air humidity, rainfall and shining during September of each production year (2007-2010). Table 3 presents the values of Pearsons'correlation coefficient (r) and the coefficient of determination (r^2) calculated by the linear regression for each wine variety. Cabernet Sauvignon and Pinot Noir are missing from this table because there was only one positive sample for each of them.

Table 3. Correlation of OTA level with weather conditions

Wine variety	Temperature		Rainfall		Relative humidity		Shining period	
	r	r^2	r	r^2	r	r^2	r	r^2
White wine								
Sauvignon Blanc	0.3634	0.0237	0.4880	0.1293	0.1679	0.1146	0.1521	0.1372
Riesling	0.1723	0.1644	0.0905	0.1907	0.3217	0.2154	0.0908	0.1017
Feteasca Alba	0.2895	0.0994	0.1204	0.2319	0.2617	0.2295	0.1079	0.0886
Feteasca Regala	0.1219	0.1493	0.2122	0.1460	0.4563	0.1847	0.2242	0.1294
Red wine								
Merlot	0.3532	0.0503	0.4051	0.0306	0.1164	0.0818	0.0716	0.0539
Feteasca Neagra	0.1729	0.1087	0.0965	0.1742	0.4127	0.1163	0.2475	0.1028

The obtained data revealed a weak correlation of weather conditions with OTA level in analyzed wine samples since the all Pearsons' correlation coefficient are below 0.5. Regarding shining period influence on OTA level, there is almost no linear relationship between the two variables. No real linear correlation between OTA level and weather conditions may be explained by the possible prevention and/or correction actions during harvesting and wine processing.

Conclusions

The presence of OTA in wine depends on many factors. Geographical origin of wine and grape variety are important, with higher values for red wine than for white wine.

Although it is known that weather conditions (especially temperature and humidity) affect toxigenic fungi development, in this study the obtained values do not confirm the correlation with mycotoxin contamination. That means OTA level in wine is determined also by other factors, such as agro-technical practices and vinification technology.

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