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Study of ochratoxin A content in South Moravian and foreign wines by the UPLC method with fluorescence detection

Renata Mikulíková, Sylvie Běláková, Karolína Benešová *, Zdeněk Svoboda

Research Institute of Brewing and Malting, Malting Institute Brno, Mostecká 7, 614 00 Brno, Czech Republic

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ABSTRACT

In 2009–2010, 72 wine samples of Moravian and foreign origin were analysed for ochratoxin A contamination. A fast analytical method based on immunoaffinity column clean-up and followed by the ultra performance liquid chromatography coupled to fluorescence detection was used. LOD and LOQ values were 0.3 and 1.0 ng/L. Ochratoxin A was detected in 11% of Moravian wines and the detected OTA level ranged from 1.2 to 71.2 ng/L. In foreign wines, OTA level ranged from 1.6 to 227.0 ng/L. The values of OTA in all studied samples were significantly below the maximum allowable limit, 2.0 μg/kg, set by the European Union.

1. Introduction

Ochratoxins, secondary metabolites of toxigenic fungi (microscopic micromycetes), are dangerous contaminants of natural origin. They are produced by *Aspergillus* species (*A. ochraceus*, *A. sulphureus*, *A. sclerotinum*, *A. niger*, and *A. carbonarius*) in tropical and subtropical areas, by *Penicillium* species (*P. verrucosum*, *P. purpurascens*, and *P. commune*) in colder areas (Gupta, 2007). Ochratoxin A (OTA) is the most significant and widespread mycotoxin of the ochratoxin group. The main toxic effects of OTA are nephrotoxicity, hepatotoxicity, immunotoxicity, teratogenicity and neurotoxicity. OTA also has potential mutagenic and carcinogenic effects (Pfohl-Leszkowicz & Manderville, 2007; Ringot, Chango, Schneider, & Larondelle, 2006). It acts as a cumulative poison, with quick absorption and slow elimination. So far, only little information has been available on the combined effects of OTA and other mycotoxins (Ringot et al., 2006).

Ochratoxin A occurs in a number of food commodities both of plant and animal origin. The main OTA sources in food are cereals and cereal products (e.g. Duarte, Pena, & Lino, 2010; Kabak, 2009), followed by wine and wine grape products, including raisins (Aksoy, Eltem, Meyvaci, Altindisli, & Karabat, 2007; Ostrý, Ruprich, & Škarková, 2002). OTA also occurs in green and roasted coffee (e.g. Sugita-Konishi et al., 2006), legumes, spices and green tea. Contamination of brewing materials (Scott, 1996) by OTA is often associated with the presence of OTA in beer (e.g. Běláková, Benešová, Mikulíková, & Svoboda, 2011; Scott & Kanhere, 1995). The main animal sources are pork meat, blood and innards (Gareis & Scheuer, 2000).

OTA in wine and grape juice was first reported in Switzerland in 1996 (Zimmerli & Dick, 1996). Since then a number of studies focusing on OTA content in wine and wine grapes have been carried out. Summary review was given in studies from 2006 to 2007 (Mateo, Medina, Mateo, Mateo, & Jiménez, 2007; Varga & Kozakiewicz, 2006).

Higher OTA content was mainly found in red wines; rosé and white wines followed, OTA content depends on a winemaking process (Meca, Blaiotta, & Ritieni, 2010). The highest OTA content was detected in special and liquor wines (Valero, Marín, Ramos, & Sanchis, 2008). The European Commission set the maximum allowable limits (MALs) for wine to 2.0 μg/kg. (Off. Journal of the European Union, 2006). However, this limit does not apply to liquor or dessert wines with more than 15% alcohol content.

It is well known that the ochratoxin A occurrence and concentration in wines is highly affected by geographical and climatic conditions. Higher OTA content and higher number of contaminated samples was repeatedly detected in wines from the Mediterranean and other southern wine-producing areas. For example, OTA concentration in Italian wines varied from 0.01 to 4.0 μg/L (Brera, Soriano, Debegnach, & Miraglia, 2004), similarly, in Greek wines OTA level ranged from <LOD to 2.82 μg/L (Stefanaki, Foufa, Tsatsou-Dritsa, & Dais, 2003) and the maximum OTA value in Portugal wines was 2.4 μg/L (Pena, Cerejo, Silva, & Lino, 2010). Lower concentrations were measured in Spain, where OTA values

* Corresponding author. Tel.: +420 545 214 110/37; fax: +420 545 321 225.
E-mail address: benesova@beerresearch.cz (K. Benešová).

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varied from 0.06 to 0.316 μg/L (Lopez de Cerain, González-Penas, Jiménez, & Bello, 2002); similarly, OTA values in Turkish wines varied from 0.006 to 0.815 μg/L (Var & Kabak, 2007) and the maximum value in Croatian wines was 21.0 ng/L (Flajs, Domijan, Ivić, Cvjetković, & Peraica, 2009).

In central European wine regions, the OTA occurrence in grapes and wine was significantly lower. OTA was not detected in products from Hungary (Berente et al., 2005) and similarly, OTA was detected only in 1 of 116 wine samples from Austria (Eder, Paar, Edinger, & Lew, 2002). In Slovakia, content of OTA and other mycotoxins was analysed in wine grapes from South Slovakian, Nitrian and Small Carpathian regions with maximal content of 1.03 μg/kg (Mikušová, Ritieni, Santini, Juhasová, & Šrobárová, 2010). In 2005 a pilot study on the OTA occurrence in fresh grape juice, must and wine from South Moravia was performed. OTA content was under the limit of quantification (8 ng/L) in all samples (Ostrý et al., 2007).

The Moravian wine region includes around 97% of the total area under wine in the Czech Republic (Kraus, 2005). The Moravian wine region is divided into four sub-regions: Znojmo, Mikulov, Velké Pavlovice and Slovácko (Fig. 1). The total area of vineyards is 18,512 ha. Soil conditions are various with prevailing stony, skeletal, sandy and also clayey soils. The region has moderate inland climate with the average annual temperature is 9.42 °C, average annual rainfall 510 mm and average annual sunshine is 2244 h. Wet and fresh air blowing from the Atlantic Ocean slows down ripening of wine grapes contributing to the development of various aromatic and spicy substances. All these conditions imprint Moravian wines their unique character.

The aim of this study was to determine ochratoxin A content in wine samples from the Moravian wine production area using a new more sensitive UPLC method and compare the detected OTA levels with those found in foreign wines.

2. Experimental

2.1. Materials and reagents

OTA standard (solution, 10 μg/ml in acetonitrile), analytical and HPLC reagents were obtained from Sigma–Aldrich (Steinheim, Germany). The immunoaffinity columns Ochraprep were purchased from the company R-Biopharm (Germany).

Phosphate buffer (PBS) was prepared by mixing 800 ml of deionised water containing 19.1 ± 0.1 g of disodium hydrogen phosphate and 200 ml of deionised water containing 1.8 ± 0.1 g of potassium dihydrogen phosphate. The pH of the resulted phosphate buffer was adjusted to 7.4 with 2 M NaOH solution.

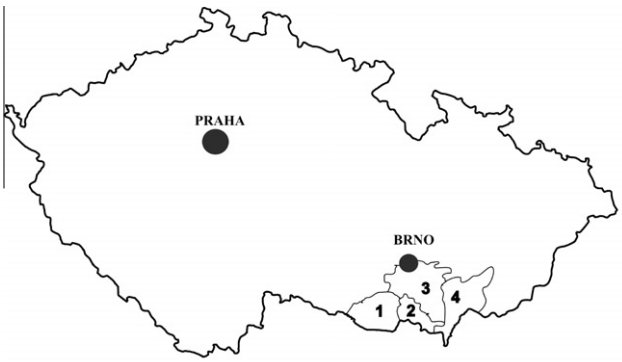

Fig. 1. The Moravian wine regions: 1, Znojmo sub-region; 2, Mikulov sub-region; 3, Velké Pavlovice sub-region; 4, Slovácko sub-region.

Mobile phase was prepared by adjusting 500 ml of deionised water to pH 2.0 with concentrated H_3PO_4. Before use it was filtered through the 0.22 μm nylon filter.

2.2. Samples

Two sets of samples were analysed: The first set included 46 wine samples from all four Moravian subregions. Wines were purchased in retail stores, specialised shops or delivered directly by a producer (wine grower). All of them were quality wines or special wines and archive wines. The effects of weather conditions in the harvest year, the fungicide treatments or production procedures were not determined. The other set contained 25 foreign wine samples (22 samples from Europe, two from Chile and one from South Australia). Wines were randomly purchased in the local stores during 2009.

2.3. Preparation of wine samples

NaOH (2 M) was added to 50 ml of the wine sample and pH was adjusted to 7.2. Then the sample was applied to the immunoaffinity column (OCHRAPREP) and washed with 20 ml of phosphate buffer. Ochratoxin A was eluted repeatedly (3×) with the mixture of 1.5 ml of methanol:acetic acid (98:2, v/v). The obtained eluate was concentrated on the rotary vacuum vaporiser, the residue obtained was transferred to 1 ml of methanol:water (50:50, v/v). The prepared sample was filtered prior to the analysis through a 0.22 μm nylon microfilter.

2.4. Preparation of standard calibration curve

OTA standard (Sigma–Aldrich) concentration was 10 μg/ml in acetonitrile (ACN). Concentration of stock solution prepared from this standard was 100 ng/ml in methanol:water 50:50, v/v. A nine-point calibration curve of methanol:water 50:50, v/v (at concentrations of 0.2, 0.4, 0.5, 1, 2, 4, 6, 8 and 10 ng/ml) was constructed. Fresh calibration solutions were prepared every day. Each solution was injected twice; mean was calculated from two measurements. Calibration curve was constructed as the dependence of the peak area on concentration of the standard. Ochratoxin A was identified by the comparison of retention time of a corresponding peak with the peak of the standard. The external standard method was used for the quantitative evaluation.

2.5. Instrumentation and OTA analyses

OTA standards and samples were analysed on the Acquity UPLC chromatographic system (Waters, USA). The system is equipped with a binary high-pressure gradient pump, vacuum degasser, autosampler with the Rheodyne injector, thermostat of columns and programmable PDA and a fluorescence detector. Data were collected and processed by the Empower software.

Analyses were performed on an Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 μm particle size) using binary gradient acetonitrile (ACN):water adjusted with H_3PO_4 to pH 2.0 (0 min 40% ACN, 2 min: 60% ACN, 2–2.2 min: 60% ACN, 2.5 min: 40% ACN). The flow rate of the mobile phase was 0.3 ml/min, the column temperature 40 °C, injection volume was 10 μl. The total analysis time was 5 min. The system was equipped with a fluorescence detector set to an excitation wavelength of 335 nm and emission wavelength of 440 nm.

2.6. OTA analysis by Mass Spectrometry

For the identification and confirmation of OTA in some selected samples, the High Performance Liquid Chromatography/Mass