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Analytical Methods

Sterigmatocystin presence in typical Latvian grains

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Abstract

Ninety five samples of different Latvian grains (wheat, buckwheat, barley, oats and rye) from the year 2006 and 120 samples from the year 2007 were analyzed for *Aspergillus* ssp. mycotoxin–sterigmatocystin (STC) content. 13.7% of the analyzed 2006 year samples were positive for STC with the concentration levels ranging from <0.7 to 83 μg/kg and 35% of the analyzed 2007 year samples were positive for STC with the concentration levels ranging from <1 to 47 μg/kg. A previously developed sensitive LC – Electrospray Positive Ionization – MS/MS method was applied for the analysis of STC in grains. Method includes sample extraction with acetonitrile/water solution, solid phase extraction (SPE) on Strata X SPE column, separation on reversed phase C_{18} column and STC detection by LC–MS/MS.

Keywords: Sterigmatocystin; Mycotoxin; Grains; LC–MS/MS; *Aspergillus* spp.; *Aspergillus versicolor*

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1. Introduction

STC is a mycotoxin produced by fungi of many *Aspergillus species* (Atalla, Hassanein, El-Beih, & Youssef, 2003; Mucke & Lemmen, 1998). Its molecular structure is similar to those of aflatoxin B_1 (Fig. 1). It is a precursor of aflatoxin B_1 in the biological transformation (Betina, 1989).

The steps involved in the biosynthesis of STC, as well as of aflatoxins, and the molecular characterisation of genes involved in the pathway are known and described in the literature (Sweeney & Dobson, 1999). Carcinogenic properties of sterigmatocystin were already studied and published (Purchase & van der Watt, 1970). The negative impact of STC on the DNA and on the tumor suppressor gene p53, in particular, was also studied (Tong-xi, Junichi, Kazuo, Wen-Yuan, & Shu-Ying, 2000). Relatively high levels (ppm range) of STC were detected in dwellings contaminated by *Aspergillus versicolor* caused by water flooding (Nielsen et al., 1999). Contamination of cereals with *Aspergillus* fungi is a serious health risk, due to the potential of STC production by these fungi. Very little data are available concerning monitoring of foodstuffs for STC. One of the few surveys in Europe was carried out in 1983 in the United Kingdom. Out of 523 analyzed samples (about 3%) 17 were found positive (Buckle, 1983). The drawback of this survey was that the method had a relatively high limit of detection (LOD) of 20 μg/kg. This fact did not allow any conclusion concerning lower contamination levels, which are also of interest. Another larger survey was done in Brazil (Valente Soarez & Rodriguez-Amaya, 1989), in the same decade, where sterigmatocystin was not detected at all. However, again the LOD of the method applied was high (35 μg/kg). These rather high LOD values that were obtained with conventional (non-mass spectrometric) methods are rather unsatisfactoral. In order to evaluate human exposure to this mycotoxin and, more importantly, to monitor food products for existing or future legal compliance, suitable and simple analytical procedures are required that allow the precise determination of STC. Methods have been described in the literature, which

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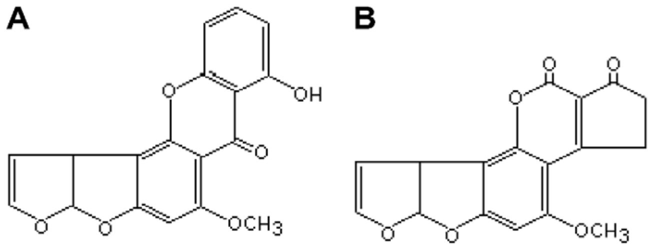

Fig. 1. Chemical structures of (A) sterigmatocystin and (B) aflatoxin B_1.

allow the detection of STC at levels ranging from 2 to 100 μg/kg (Scudamore, Hetmanski, Clarke, Barnes, & Startin, 1996). However, it has been recognized that the surveillance of STC below ppb level was difficult in the past due to the weak fluorescence of this mycotoxin, and that a precise determination below this level requires mass spectrometric detection (Scudamore et al., 1996). The most frequently applied analytical methods so far, are based on thin-layer chromatography (TLC) with fluorescence detection (AOAC Official Method 973.38, 1995; Athnasios & Kuhn, 1977; Gimeno, 1979; Valente Soarez & Rodriguez-Amaya, 1989). Because of its weak native fluorescence, a derivatisation procedure is generally used to visualise the STC on the developed TLC plates (Athnasios & Kuhn, 1977). The most common derivatisation approach is spraying of the TLC plate with aluminium chloride ($AlCl_3$) solution, after development and heating of the plate. With such procedures, LODs were reported to be 50 μg/kg (Athnasios & Kuhn, 1977) and 140 μg/kg (Gimeno, 1979), in cereal grains. A validated official method is also based on TLC with $AlCl_3$ spraying, and is reported to have a limit of quantification (LOQ) of 100 μg/kg. Other methods are based on high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, and were applied for determination of STC in rice inoculated by *A. versicolor* at lower μg/kg levels (Neely & Emerson, 1990; Schmidt, Mondani, Ziegenhagen, & Dose, 1981) Application of HPLC with post column derivatisation with $AlCl_3$ has also been reported (Neely & Emerson, 1990), with results comparable to the official TLC method (AOAC Official Method 973.38, 1995). The lowest detection limit of 1.7 μg/kg for maize and 1.9 μg/kg for bread was reported when liquid chromatography with mass spectrometric detection (LC–MS) was applied (Scudamore et al., 1996). A method based on gas chromatography with mass spectrometric detection (GC–MS) reported an LOD of 5 μg/kg for STC in wheat (Salhab, Russel, Coughlin, & Hsieh, 1976).

In conclusion, currently the only methods with sufficient LOQs of sterigmatocystin at levels below 5 μg/kg are based on LC–MS and GC–MS.

For routine and monitoring purposes a reagent-free derivatisation procedure was also successfully applied for fluorescence induction of STC and method limit of detection was 2–11 μg/kg (Stroka, Dasko, Spangenberg, & Anklam, 2004).

Now are available LC–MS/MS based methods for routine and monitoring purposes with limit of detection 0.15 μg/kg in grains (Veršilovskis, Bartkevičs, & Miķelsone, 2007).

Neither country has legislation for STC, however some countries have set already relatively low maximum levels for sterigmatocystin (e.g., Czech Republic and Slovakia at the level 5 μg/kg for rice, vegetables, potatoes, flour, poultry, meat, milk, and 20 μg/kg for other foods) (Stroka et al., 2004) and soon after STC was recognized as a highly toxic compound, the California Department of Health Services used TD50 values from the Cancer Potency Database to produce ‘no significant risk’ intake levels for humans. The level resulting was 8 μg/kg body weight/day for a 70 kg adult (European Mycotoxin Awareness Network).

The aim of this study is to research typical Latvian grains (wheat, oat, rye, barley and buckwheat) from the years 2006 – 2007 for the presence and concentration of STC using sensitive LC–MS/MS method (Veršilovskis et al., 2007).

2. Materials and methods

2.1. Grain samples

Grain samples (50 wheat, 15 oats, 10 ryes, 10 barley and 10 buckwheat samples) were collected from different parts of Latvia in autumn 2006 and grain samples (20 wheat, 25 oats, 25 ryes, 25 barley and 25 buckwheat samples) were collected from different parts of Latvia in autumn 2007. After harvest, the grains were air-dried to a water content of less than 15% to avoid fungal growth during storage. Before the analysis, the samples were grounded with a laboratory-mill (Romer, Ras Mill, Union, MO, USA).

2.2. Chemicals and reagents

Methanol (HPLC-grade) and acetonitrile (HPLC-grade) was purchased from Merck (Darmstadt, Germany). Deionized water was purified with Millipore Milli-Q Plus system (Millipore, Molsheim, France). STC standard were purchased from Sigma (St. Louis, MO, USA). Argon (AGA, Latvia) was used as a collision gas in the mass spectrometry.

2.3. Preparation of standards

A stock solution of a concentration of approximately 500 μg/ml was prepared by dissolving 5 mg of STC in 10 ml of acetonitrile/methanol (50:50, v/v). An aliquot 500 μl of the stock solution was evaporated to dryness under oxygen-free nitrogen at ambient temperature and immediately redissolved in acetonitrile (5 ml). The STC concentration was established by UV spectrometry.

The calibrated stock solution (50 μg/ml) was used to prepare a standard stock solution of 5 μg/ml of STC, in acetonitrile/water (75:25, v/v). This solution was used to