STERIGMATOCYSTIN PRESENCE IN DIFFERENT LATVIAN BREAD SAMPLES

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Abstract

29 samples of different types of bread purchased from the local Latvian supermarkets were analysed for sterigmatocystin (STC) content. STC is carcinogenic and mutagenic mycotoxin produced by fungi of many *Aspergillus species* and it is a precursor of aflatoxin B_1 in the biological transformation.

Analysis of STC were done using previously developed method. Method includes sample extraction with acetonitrile: water solution (84:16, v/v), extract clean up on Strata X solid phase extraction (SPE) column, extract concentrating by evaporation under nitrogen and further redissolving in mobile phase, separation on reversed phase C_{18} high performance liquid chromatography (HPLC) column and STC detection by high performance liquid chromatography – tandem masspectrometry with electrospray positive ionization (LC – ESI^+ – MS/MS).

17.2 % of analyzed samples were positive for STC with the concentration levels ranging from 2.4 to 7.1 µg kg^{-1}.

From 6 rye bread samples only one (16.7%) was contaminated with STC, from 9 rye-wheat bread samples only one (11.1%) was contaminated with STC and 3 (21.4%) of 14 wheat bread samples were contaminated with STC. Totally 4 (80%) of 5 contaminated samples contained whole grains as the main ingredient.

So, whole grain bread can be a possible source of STC, however positive samples contain quite low levels of STC.

Key words: Sterigmatocystin, mycotoxin, bread, *Aspergillus spp.*, *Aspergillus versicolor*, LC-MS/MS

Introduction

STC is a mycotoxin produced by fungi of many *Aspergillus spp.* (Atalla *et al.*, 2003). Its molecular structure is similar to aflatoxin B_1 (AFB). It is a precursor of AFB in the biological transformation (Betina, 1989).

STC is a carcinogenic compound that has been shown to affect various species of experimental animals (Purchase *et al.*, 1970) and it is classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 1976). There are many reports about toxicity, carcinogenity, mutagenity, and teratogenity of STC (Sweeney *et al.*, 1999; Tong-xi *et al.*, 2000). STC is an important contaminant of building materials and dwellings (Engelhart *et al.*, 2002; Nielsen *et al.*, 1999). Natural occurrence in food and food products appears to be infrequent although only a limited number of surveys have been carried out. There are reports about the occurrence of STC in grains (Scott, 1972; Shannon *et al.*, 1976; Mills *et al.*, 1986; Rao *et al.*, 2000), in rapeseeds (Mills et al., 1986), in soybeans (Shannon *et al.*, 1976), in cheese (Francis *et al.*, 1987; Lund *et al.*, 1995; Abdalla *et al.*, 1996; Scudamore *et al.*, 1996), in spices (red pepper, caraway, cumin and marjoram) (ElKady *et al.*, 1995), in cocoa beans (Hurst *et al.*, 1987), in vegetables (Thurm *et al.*, 1976), in pistachio nuts (Sommer *et al.*, 1976), in coffee beans (Purchase *et al.*, 1973) and in feed (Scudamore *et al.*, 1997; Domagala *et al.*, 1997).

Neither country has legislation for STC, however some countries have set already relatively low maximum levels for STC (e.g., Czech Republic and Slovakia at the level 5 µg kg^{-1} for rice, vegetables, potatoes, flour, poultry, meat, milk, and 20 µg kg^{-1} for other foods) (Stroka, 2004) and soon after STC was recognized as a highly toxic compound, the California Department of Health Services used TD 50 values from the Cancer Potency Database to produce “no significant risk” intake levels for humans. The level resulting was 8 µg kg^{-1} body weight/day for a 70 kg adult (European Mycotoxin Awareness Network).

The aim of this study is to research samples of different type of bread, that are available in local supermarkets, for STC content using sensitive LC-MS/MS method (Versilovskis *et al.*, 2007).

Materials and Methods

Bread samples

Bread samples (6 rye bread, 9 rye-wheat bread and 14 wheat bread) were purchased from local supermarkets.

Before the analysis, the samples were crushed and than homogenized.

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 was purchased from Sigma (St Louis, MO, USA). Argon (AGA, Latvia) was used as a collision gas in the mass spectrometry.

Preparation of standards

A stock solution of a concentration of approximately 500 μg ml^{-1} 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 calibrated stock solution (50 μg·ml^{-1}) 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 spike samples for recovery experiments, and to prepare working standards 0.25 μg ml^{-1}, 0.1 μg ml^{-1}, 0.05 μg ml^{-1} and 0.005 μg ml^{-1} as equivalents to 25 μg kg^{-1}, 10 μg kg^{-1}, 5 μg $kg^{-1}$and 0.5 μg kg^{-1}.

Sample preparation

An amount of 25 g of homogenized sample was extracted with 16% of water in acetonitrile (100 ml) for 30 min using a horizontal shaker. After filtering through a filter paper, 10 ml of the raw extract was diluted with 20 ml water and purified using Strata X (500 mg) SPE column (Phenomenex, Torrance, CA, USA). Purifying procedure: column was conditioned with 6 ml methanol, followed by 6 ml of water prior to use, then 30 ml of diluted extract was loaded in the column, then column was washed with 35% acetonitrile in water, then with 35% methanol in water and STC was eluted with 4 ml of 100% acetonitrile. The resulting eluate was evaporated to dryness under nitrogen at 60 °C and re-dissolved in 250 μl 25% water in acetonitrile. The calibrants were prepared by spiking the blank matrix with the standard and prepared in the same way as the samples.

LC-MS/MS –analysis

A Waters Alliance 2695 liquid chromatograph (Waters) was connected to a MicroMass Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK). An electrospray ionization (ESI) probe in the positive mode was used in the analysis of STC. The mobile phase consisted of 0.01 % formic acid in acetonitrile and 0.01% formic acid in water (75:25, v/v) used in isocratic regime. The column used was a Phenomenex Luna C_{18}(2) (5 μm), 150x3.0 mm (Phenomenex, Torrance, CA, USA). The flow rate was 0.3 ml/min, column temperature was 30 °C and the injection volume was 50 μl. The parameters of the mass spectrometer were optimized using the STC standard. The best response was recorded with the following parameters: cone voltage 30 V, capillary voltage 3.5 kV, extractor 2 V, radio frequency (RF) lens 0.2 V, source temperature 120 °C and desolvation temperature 350 °C, cone gas flow 63 l h^{-1}, desolvation gas flow 553 l h^{-1}, collision energy 30 eV.

For the structural identification in multiple reaction monitoring (MRM) mode, the molecular ion [M+H] + (*m/z*=325) was fragmented within the MS to its daughter-ions (325>310 and 325>281) collision energy 30 eV, dwell 0.2 sec. Argon at pressure 3.5 bar was used as a collision gas. A calibration curve constructed using external standardization in matrix. The daughter-ion (*m/z*=281) was used for the quantification of STC. The ratio between peaks of STC obtained on two MRM channels (Peak area (325>310) / Peak area (325>281)) was used for confirmation of analyte. This ratio should be 0.69±0.14 for the compound to be confirmed.

Spiking for recovery studies

Spiked samples of different grains were prepared by adding 25 μl and 125 μl of the 5 μg ml^{1}

STC standard solution using a digital pipette to 25 g of sample in an 250 ml flask, whitch was left for 1 h at ambient temperature with occasional agitation to allow the acetonitrie to evaporate. These volumes of standard were equivalent to levels of 5 µg kg^{-1} and 25 µg kg^{-1} STC respectively. Six replicates at each concentration level were prepared from each commodity for recovery experiments. Recovery results for bread are shown in Table 1.

Table1

Recovery and precision results obtained from bread matrix

Spike level, µg kg^{-1}	Mean result (n=6), µg kg^{-1}	Standard deviation (SD), µg kg^{-1}	Relative Standard deviation (RSD), %	Mean recovery, %
5.0	4.8	0.2	4.6	96.0
25.0	25.8	1.6	6.2	103.1

Calibartion and Linearity

In LC-MS methods the matrix often causes the change of the response, because the matrix components disturb the ionization of the analytes (Tang *et al.*, 1993). Because of the matrix effect, the calibrants were always prepared in blank matrix.

The method was linear for STC from 0.5 µg kg^{-1} to 25 µg kg^{-1}. A tolerance of ±10% accepted for the separate calibration points for good linearity. The regression line without matrix was y=13656x+212 (R^2>0.999) and regression line in matrix was y=4477x-176 (R^2>0.999). On this basis, the method considered linear for the analysis of STC.

Results and Discussion

From the analysed samples only one (16.7%) rye bread sample, one (11.1%) rye-wheat bread sample and three (21.4%) wheat bread samples were contaminated with STC. Four (80%) of five contaminated samples were prepared using whole grains.

Totally, 17.2% (5 samples) of the analyzed samples were contaminated with STC (Table 2).

Table 2

STC presence in different bread samples

Sample No.	Bread sample name	Sample type	Bakery	Result, µg kg^{-1}
1	"Hanzas" rye bread	R	Hanzas	n.d.
2	Real brown bread	R	Lāči	n.d.
3	"Saules" dark whole grain bread	R	Iļguciema	n.d.
4	"Rīgas" brown bread	R	Iļguciema	n.d.
5	"Fazer" whole grain rye bread	R	Druva	n.d.
6	Rye bread	R	Sono	**2.4**
7	"Hanzas" wheat-rye bread	RW	Hanzas	n.d.
8	Real fine rye-bread	RW	Lāči	n.d.
9	"Fit Life" Bread	RW	Lāči	n.d.
10	Fine rye-bread	RW	Iļguciema	n.d.
11	"Meistara" Rye bread	RW	Druva	n.d.
12	"Meistara" lime-pit fine rye-bread	RW	Druva	n.d.
13	Dinaburga	RW	Dinella	n.d.
14	"Saimnieks" whole grain bread	RW	Sono	**7.1**
15	Fine rye-bread	RW	Sono	n.d.
16	Fitness	W	Hanzas	n.d.

Sample No.	Bread sample name	Sample type	Bakery	Result, μg kg^{-1}
17	"Hanzas" wheat bread	W	Hanzas	n.d.
18	"Sendviču" rain bread with barley and rye flakes	W	Hanzas	n.d.
19	"Sendviču" grain bread with rye flakes	W	Hanzas	4.4
20	"Sendviču" grain bread with oat flakes	W	Hanzas	n.d.
21	Real wheat bread	W	Lāči	n.d.
22	"Saules" grain bread	W	Iļguciema	**3.2**
23	City's wheat bread	W	Iļguciema	n.d.
24	"Fazer" King's grain tosterbread	W	Druva	**5.6**
25	"Fazer" wheat tosterbread	W	Druva	n.d.
26	"Spēkotava" bran bread	W	Druva	n.d.
27	"Zeltene" Wheat bread	W	Dinella	n.d.
28	"Autumn" wheat bread	W	Dinella	n.d.
29	"Kurzemes" wheat bread	W	Sono	n.d.

R – rye bread; RW – rye-wheat bread; W – wheat bread; n.d. – not detected

However the concentration levels were quite low from 2.4 to 7.1 μg kg^{-1} and did not exceed maximum levels for this toxin set in Czeh Republic and Slovakia (20 μg kg^{-1}) but exceed level set in these republics for rice, vegetables, potatoes, flour, poultry and meat – 5 μg kg^{-1}. So, results of our research give possibility to suppose that levels of STC in grains from which these bread samples were prepared, were highly contaminated with STC. So, in case that STC is carcinogen it is not recommended to use contaminated products, because of possible chronic effects. Nevertheless, as mentioned above (in introduction section) "no significant risk" intake level for humans is about 8 μg kg^{-1} body weight/day for a 70 kg adult – it is approximately 480 μg per day! In this case, concentrations that were found in bread during this investigation cannot seriously affect consumer's health.
There are no available comparable results in literature for so wide range of bread samples.

Conclusions

This is the first research on STC content in different types of bread in Latvia. This study indicates about the occurrence of STC in bread, especially in whole grain bread. Although found contamination of bread is quite low, bread still can be a possible source of investigated toxin.
So, in the aspect of all mentioned above, monitoring of the presence of STC in bread and other food products in Latvia is clearly necessary.

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