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A rapid method for the determination of the *Fusarium* mycotoxin beauvericin in maize

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Abstract The development of a rapid method for the determination of the *Fusarium* mycotoxin beauvericin (BEA) in corn and cereals is described. This method allows to prepare at least 5 times more samples simultaneously with similar analytical performance characteristics as previously published analytical methods.

Introduction

The *Fusarium* mycotoxin beauvericin (BEA) is a bioactive cyclodepsipeptide, which contains three D-α-hydroxy-isovaleryl and N-methyl-L-phenylalanyl residues in alternating sequence [1, 2]. Only a few publications are dealing with the toxicity of BEA to mammalian tissues and cells [3, 4]. Substantial toxicity of BEA to several mammalian cell lines is reported, which indicates that BEA may play a role in human and animal pathology through contamination of corn [4].

Well known producers of BEA are *Fusarium subglutinans*, *F. proliferatum* and *F. semitectum*, which are also present in naturally infected Austrian corn [3, 5–10].

Only a few analytical methodologies for BEA are published. All of them require laborious sample pretreatement steps [1, 2, 5–7, 11]. Thus, easy, rapid and still sensitive analytical methods for the determination of BEA in cereal grains are needed in order to assess toxicity and environmental occurrence.

Experimental

Sample preparation. For the spiking experiments different amounts of a 100 µg/mL standard solution of BEA were added to ground corn that contained no traces of BEA.

Instead of carrying out a defatting step, a 20 g amount of the ground corn sample was extracted with 80 mL of acetonitrile-water (84:16, v/v) for 3 min with a blender (T25, Ultra Turrax). After filtering the extract an aliquot of about 8 mL was transferred to a glass culture tube and was purified by pushing the flange end of the Mycosep #224 [12] into the culture tube until more than 4 mL had passed the column. Then 4 mL of this clear extract were transferred to vials and evaporated to dryness under a gentle stream of nitrogen at 50 °C. The residue was redissolved in 3 × 250 µL volumes of chloroform and applied to a silica solid-phase-extraction (SPE) column (Supel-clean LC-Si, 1 mL, Supelco). This column was preconditioned with 2 × 1 mL volumes of chloroform and was washed with 250 µL of chloroform after application of the "Mycosep-extract". The BEA was eluted with 600 µL of chloroform-methanol (97:3 v/v). The solution was evaporated under nitrogen at 50 °C and reconstituted to 250 µL with the HPLC solvent.

HPLC conditions. Analysis was carried out using a HPLC apparatus (HP 1090 Series II, Hewlett-Packard, Austria) equipped with an UV-DAD system. The column was a reversed-phase Vydac C18-column (250 × 4.6 mm i.d.). Acetonitrile-water (90:10 v/v) was used as the mobile phase with a flow rate of 1.2 mL/min under isocratic conditions. Both 192 nm and 209 nm were used as the detection wavelengths. Injections were made in duplicate and the injection volume was 20 µL. Quantification was performed by comparison of the peak area of BEA with a calibration curve of the peak area obtained with authentic standard.

Results and discussion

By means of this newly developed sample clean-up procedure, based on SPE steps, the sample throughput is enhanced by a factor of 5 (approx. 10 samples per day) compared with the usual laborious liquid-liquid-extraction procedures [1, 2, 5–7].

Furthermore, this clean-up enabled us to cut out the supplementary defatting step, which leads to severe losses of BEA as has been described by R. Krska et al. [1]. Therefore, a better mean recovery of 96.4% (n = 8) and precision (RSD = 5%) for BEA in the concentration range of 100–2000 µg/kg could be obtained from spiked samples [Fig 1]. A similar detection limit (LOD) of 46 µg of BEA per kg corn, compared with our recently described method [1], was reached using the wavelength of 209 nm for quantification. The LOD was calculated from the calibration curve obtained from spiking experiments [13].

The developed method is well suited for the analysis of BEA at µg/kg levels in corn and corn meal and is currently employed in the analysis of corn samples, which have been *Fusarium* inoculated or which are naturally contaminated with BEA. So far, we have analyzed 22 inoculated corn genotypes infected by *F. subglutinans* in duplicates and found BEA in concentrations between 5 to 75 mg/kg. The presented method proved to be rapid and well applicable to the analysis of contaminated corn samples.

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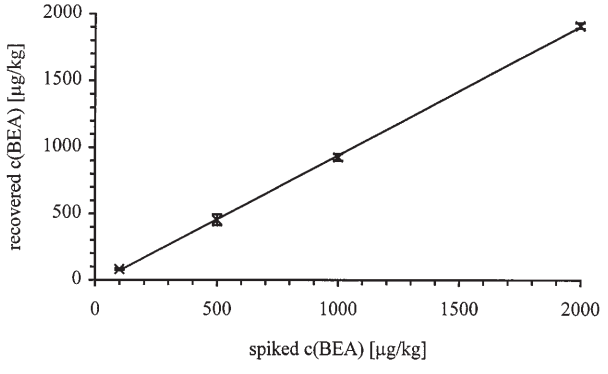

Fig. 1 Recovered BEA-concentration vs. added BEA-concentration in spiked corn

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Ultra-trace analysis of nitrite in food samples by flow injection with spectrophotometric detection

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Abstract A flow injection system with spectrophotometric detection is proposed for the determination of low levels of nitrite based on its catalytic effect on the oxidation of gallocyanine by bromate in acidic media. Various analytical parameters such as acidity, reagent concentration, flow rate, sample size, time, temperature, and interfering species were studied. The calibration graph was linear for 0.020–0.500 μg/mL of nitrite. The method is successfully applied to food samples. Up to 30 ± 5 samples can be analyzed per hour.

Introduction

The determination of nitrite is of great importance because of its harmful impact on human health [1–3]. A lot of methods have been proposed for the determination of nitrite in real samples, mainly based on the spectrophotometric detection of an azo dye formed by the reaction of nitrite with a Greece type reagent [4–7]. But these methods are time consuming and have low sensitivity with poor selectivity. Other methods are based on catalytic-kinetic reactions [8–11]. Using flow injection analysis (FIA) can eliminate disadvantages. Only few FIA methods have been used for the determination of nitrite by catalytic spectrophotometric principles [12–13], but their limits of detection are high (0.036 μg/mL [13] and 0.020 μg/mL [12]).

Here an FIA-method is proposed based on the catalytic effect of nitrite on the oxidation of gallocyanine (GC) by bromate in sulfuric acid by using the decrease in absorbance of gallocyanine at 530 nm.

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Experimental

Reagents

All chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled water was used throughout.

Standard nitrite solution (1000 μg/mL) was prepared by dissolving 492.8 mg dried (for 4 h at 105–110 °C) sodium nitrite (Merck) in doubly distilled water in a 100 mL volumetric flask. NaOH pellets were added to prevent liberation of nitrous acid and 1 mL of spectroscopic grade chloroform to inhibit bacterial growth. The stock solution was kept in a refrigerator for preservation. Working standard solutions were freshly prepared by diluting the stock solution with water.

Gallocyanine solution (5.0×10^{-4} mol/L) was prepared by dissolving 17.0 mg of gallocyanine (Serva) in 100 mL water.

Sodium bromate solution (0.20 mol/L) was prepared by dissolving 3.0794 g of $NaBrO_3$ (Merck) in 100 mL water.

Solution of sulfuric acid was prepared by diluting of appropriate amounts in water.

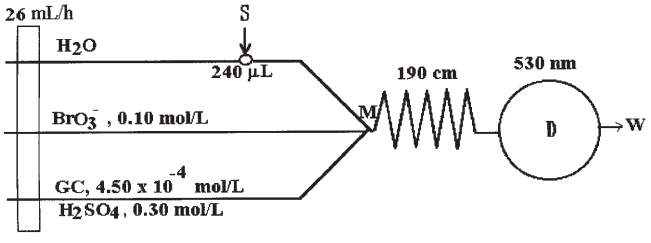

Fig. 1 FIA manifold used for the determination of nitrite