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Study of the chemical reduction of the fumonisins toxicity using allyl, benzyl and phenyl isothiocyanate in model solution and in food products

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ABSTRACT

Fumonisins (FBs) are bioactive compounds produced by several strains of *Fusarium* spp. which contain a polyketide structure similar to sphinganine. These mycotoxins contain a free amino group that could work as an electron donor and react with the electrophile carbon present within the isothiocyanate (ITC) group. The objective of this study was to determine the effect of ITCs (allyl, benzyl and phenyl) on the stability of FB_1, FB_2 and FB_3. Firstly, PBS solutions at three pH levels (4, 7 and 9) were prepared and added with pairs of one FB (1 mg/L) plus one ITC (1 mg/L). Then, gaseous ITC was used to fumigate corn kernels and corn flour contaminated with FBs produced by *Gibberella moniliformis* CECT 2987 *in situ*. Mycotoxin levels were evaluated using liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS), while products formed from the reaction of FBs and ITCs were examined by liquid chromatography coupled to mass spectrometry-linear ion trap (LC-MS-LIT). The reduction of FB_1 and FB_2 in solution ranged from 42 to 100% on a time-dependent manner. This variance was greatly influenced by pH. In general, lower pH levels eased the reaction between ITCs and FBs. ITC fumigation treatment (50, 100 and 500 μL/L) was able to reduce 53–96% of FB_1 levels, 29–91% of FB_2 and 29–96% of FB_3. Four reaction products between the bioactive compounds employed in this study were identified, corresponding to FB + ITC conjugates.

1. Introduction

Fumonisins (FBs) are mycotoxins produced by a variety of fungi from the *Fusarium* genus. These toxins are polyketides that are structurally similar to sphinganine. FBs were found to disrupt sphingolipid metabolism, which might lead to the fumonisin-induced mycotoxicoses (D'arco et al., 2009). Dietary exposure to these mycotoxins can cause various adverse health effects in animals and humans, including equine leukoencephalomalacia (ELEM) and porcine pulmonary oedema (Palacios et al., 2011).

Fumonisin B_1 (FB_1) is the most toxic and commonly occurring FB. This mycotoxin has been classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen to humans and its chronic consumption through contaminated corn has been related to esophageal cancer in humans (Wild and Gong, 2010). The maximum residue limit issued by the EFSA for total FBs in maize products for human consumption is 2 mg/kg (Meca et al., 2010). The cellular mechanisms behind FB_1-induced toxicity include the induction of oxidative stress, apoptosis and cytotoxicity. However, the role of production

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of ROS in FB_1-mediated toxicity is still obscure (Stockmann-Juvala and Savolainen, 2008).

The growth of *Fusarium* species in corn is a common occurrence and, therefore, the presence of FBs in corn-based products, such as corn grits, flakes, snacks and flour is a worldwide problem (D'Arco et al., 2009; Molinié et al., 2005). In addition, these toxins have also been found in wheat, barley, sorghum and rice (Mateo and Jiménez, 2000).

Some researchers have reported that FB_1 is degraded by heat treatment such as cooking, and the extent of mycotoxin degradation depends on temperature, exposure time, contamination level and the concentration of reducing sugars (Jackson et al., 1997). For instance, FB_1 reacts with glucose through its aliphatic primary amine (Maillard reaction) forming *N*-(carboxymethyl)fumonisin B_1 during heat treatment (Howard et al., 1998; Lu et al., 2002). The Schiff base initially produced by the reaction of FB_1 and D-glucose undergoes through the Amadori rearrangement forming a α-ketoamine, which is subsequently oxidized to *N*-(carboxymethyl) fumonisin B_1. This reaction was shown to produce FB_1 detoxification *in vitro* (Meca et al., 2010).

Isothiocyanates (ITCs) are products originated from the enzymatic hydrolysis of glucosinolates, which are sulphur-containing glucosides present in plants of the Brassicaceae family. These compounds contribute to the characteristic pungent taste of these vegetables (Engel et al., 2002), and have been reported as potent antimicrobials (Luciano and Holley, 2009). Allyl isothiocyanate (AITC), which is the most studied ITC, was found to inhibit the growth of yeast, mould and bacteria at very low levels (Isshiki et al., 1992). ITCs are characterized by the presence of a –N=C=S group, whose central carbon atom is strongly electrophilic (Zhang, 2004). This electrophilic nature enables ITC to readily bind to thiol and amino groups of amino acids, peptides and proteins, forming conjugates (Luciano and Holley, 2009), dithiocarbamate and thiourea structures (Cejpek et al., 2000). Fumonisins contain a free and readily available amino group. Therefore, ITCs could be good candidates to react these mycotoxins.

The objectives of this study were to a) evaluate the possible reduction of FBs by allyl (AITC), benzyl (BITC) and phenyl (PITC) isothiocyanates using a buffered aqueous solution at three different pH levels, b) examine the reduction of the FBs employing AITC, BITC and PITC in food systems (corn kernels and flour) contaminated with an FB-producer strain of *Gibberella moniliformis* (CECT 2987), and c) characterize the reaction products between ITCs and FBs.

2. Materials and methods

2.1. Materials and microbial strains

Fumonisin B_1, B_2 and B_3 (FB_1, FB_2, and FB_3) (98% purity), phosphate buffer saline (PBS) at pH 7, formic acid (HCOOH), AITC, BITC and PITC were obtained from Sigma–Aldrich (St. Louis, USA). Acetonitrile and methanol were purchased from Fisher Scientific (New Hempshire, USA). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath. The strain of *G. moniliformis* CECT 2987, was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain).

2.2. Solution model preparation

FB_1 and FB_2 (1 mg/L) were added to screw-capped tubes containing 1 mL of phosphate saline buffer at pH 4, 7 or 9. AITC, BITC and PITC at 1 mM were added to all tubes, which were subsequently tightly closed and shaken at 200 rpm and 23 °C. Aliquots were drawn at 0, 4, 8, 24 and 48 h and directly injected into the LC system for FB_1 and FB_2 analyses.

2.3. Strain and culture conditions

A solid corn medium was utilized in this study. The medium was prepared with 100 g of corn kernel and 2L of water. The mixture was boiled for 15 min and filtered through paper-filter Phenomenex 5 (Madrid, Spain) (De la Luz et al., 2007). Then, the solid fraction was autoclaved for 20 min at 121 °C, cooled and inoculated with a suspension of *G. moniliformis* CECT 2987 conidia (10^6 conidia/mL). Conidial concentration was measured by optical density at 600 nm and adjusted to 10^6 conidia/mL (Kelly et al., 2006). Fermentations were carried out at 25 °C for 30 days. At the end of the fermentation period, the kernels were dried in an oven (Memmert, Büchenbach, Germany) at 60 °C for 12 h. Half of the corn was ground into fine flour. Both kernels and flour were analyzed as described on Sections 2.5 and 2.6 to determine the FBs content before the treatment with ITCs.

2.4. Corn and corn flour

Two petri-dish bottoms (50 mm diameter) containing 2 g of corn kernels or corn flour contaminated with FBs produced by *G. moniliformis* were placed into 1 L Mason jars (Juvasa, Valencia, Spain). A 2.5 × 2.5 cm paper-filter soaked with ITC (allyl, benzyl or phenyl) was inserted into the jar at final concentrations of 50, 100 and 500 μl/L in the gaseous phase after volatilization. The control group did not receive any ITC treatment. Jars were hermetically closed and kept at room temperature (23 °C) for 48 h. Then, they were opened inside a fume hood and left for 30 min allowing the ITC gas to escape. Kernels and flour were used for further chemical analysis.

2.5. FB extraction procedure and clean-up

Fumonisin extraction was performed according to D'Arco et al. (2009). Briefly, 2 g of corn kernels or corn flour were extracted with 20 mL of methanol using an Ultra Ika T18 basic Ultraturrax (Staufen, Germany) for 3 min. The mixture was centrifuged at 4500*g* for 5 min and the supernatant was evaporated to dryness with a Büchi Rotavapor R-200 (Postfach, Switzerland). The residue was re-dissolved in 2 mL of extraction solvent. The extract was cleaned up using a Strata C18-E cartridge (6 mL, 1 g) (Phenomenex). The cartridge was firstly activated with