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Brief communication

A chemical approach for the reduction of beauvericin in a solution model and in food systems

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ABSTRACT

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several *Fusarium* strains with a strong antibacterial, antifungal, and insecticidal activities.

This study evaluated the reduction of BEA added at 25 mg/kg in phosphate buffer saline (PBS) solutions at pH of 4, 7 and 10, or to different cereal products (kernels and flours) by the bioactive compounds phenyl isothiocyanate (PITC) and benzyl isothiocyanate (BITC). The concentration of the mycotoxin was evaluated using liquid chromatography coupled to the diode array detector (LC-DAD). In solution, BEA reduction ranged from 9% to 94% on a time-dependent fashion and lower pH levels resulted in higher BEA reduction. Cereal kernels and flours treated with gaseous PITC and BITC (50, 100 and 500 μL/L) presented BEA reduction from 9% to 97% and was dose-dependent. Among the crops, corn was the vehicle where BEA was mostly affected by the action of the ITCs, followed by wheat and rice, and lastly barley. Overall, PITC caused higher reduction of BEA and should be chosen over BITC as a fumigant to decrease the levels of this mycotoxin in grains and flours.

1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide mycotoxin which was originally isolated from *Beauveria bassiana* and later from *Paecilomyces fumosoroseus* and *Fusarium lateritium* (Logrieco et al., 2002). BEA has strong antibacterial, antifungal, and insecticidal activities (Jestoi, 2008), and has also shown significant cytotoxic activity toward various human cancer cell lines. The notable antimicrobial and cytotoxic activities of BEA have attracted research interest in its application as a potential antibiotic and anticancer agent for human health care (Jow et al., 2004).

BEA is considered a contaminant of cereals and derivate products (Zinedine et al., 2011). In particular, the presence of BEA in food and feed commodities has been reported in several countries of south and north Europe (Finland, Norway, Spain, Slovakia, Croatia, Switzerland and Italy), and also in USA, South Africa (Jestoi, 2008; Meca et al., 2010; Munkvold et al., 1998; Ritieni et al., 1997; Shephard et al., 1999; Zinedine et al., 2011) in very high concentrations (milligrams per kilograms) when compared with classical and legislated *Fusarium* mycotoxins such as fumonisins, trichothecenes or zearalenone.

Related to the methodologies employed for the reduction of this contaminant in food, there is only a US patent (Duvick and Rod, 1998) on the biological detoxification of the minor *Fusarium* mycotoxin BEA in the scientific literature. The inventors achieved 50% BEA reduction by employing a strain of *Norocardia glubera* as detoxification agent to wheat kernels with an initial contamination of the mycotoxin at 1000 mg/L. The microbial degradation using probiotic bacteria and bacterial and yeasts enzymes was also studied by Meca et al. (2013a,b).

Glucosinolates are metabolites found in plants belonging to the family *Brassicaceae* (Nielsen and Rios, 2000). These compounds are located within vacuoles and are released when the plant suffers mechanical damage (e.g. wounding, cutting). Once in the cytoplasm, they are hydrolyzed by the enzyme myrosinase (EC 3.2.1.147), resulting in the formation of three main groups of substances: nitriles, thiocyanates and isothiocyanates (Delaquis and Mazza, 1995; Luciano and Holley, 2009). The last group contains diverse compounds with strong antimicrobial activity and they have been used in food products (Lin et al., 2000; Nielsen and Rios, 2000; Obaidat and Frank, 2009). Benzyl and phenyl isothiocyanates have been researched for their antibacterial (Wilson et al., 2013) and antifungal properties (Smolinska et al., 2003). These compounds are strong electrophilic reagents and can react easily with nucleophiles such as amines, amino acids, alcohols, water, and sulfites during food treatment and under physiological conditions (Cejpek et al., 2000). Recently, it was found that allyl isothiocyanate (AITC) was able to react with the aminic groups of BEA both in aqueous solutions and in foods (Meca et al., 2012). This opened

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a new line of research, where isothiocyanates could be used to reduce the toxicity of some mycotoxins (Azaiez et al., 2013a,b). There are hundreds of isothiocyanates present in nature, and their reactivity is mainly determined by the chemical structure of their side chain (Verma, 2003). BITC and PITC are common isothiocyanates with strong reactive capacity. The aims of this study were: (a) to evaluate the possible reduction of BEA by BITC and PITC using a buffered aqueous solution at three different pH levels, and (b) to examine the reduction of BEA in cereals employing BITC and PITC fumigation.

2. Materials and methods

2.1. Materials

BEA (98% purity, MW = 783.95 g/mol), phosphate buffer saline (PBS) at pH 7, formic acid (HCOOH) PITC (94% purity, MW = 135.19 g/mol) and BITC (94% purity, MW = 149.21 g/mol) were obtained from Sigma–Aldrich (St. Louis, USA). Acetonitrile and methanol were purchased from Fisher Scientific (New Hempshire, USA). Deionized water (<8 MX 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.

2.2. Experimental solution preparation

Beauvericin (25 mg/L) was added to screw-capped tubes containing 10 mL of phosphate saline buffer at pH 4, 7 or 10. 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 injected into the LC system for BEA analysis.

2.3. Food system model

Two Petri-dish bottoms (50 mm diameter) containing 2 g of samples (kernels and flours of barley, wheat, rice and corn) were placed into 1L Mason jars (Análisis Vínicos, Spain). Then, BEA from a standard solution (1000 mg/L dissolved in methanol) was added to the flour to achieve a final concentration of 25 mg/kg. A 2.5 × 2.5 cm paper-filter soaked with 50, 100 or 500 μl of a standard solution of BITC and PITC were inserted into the jars (1 L volume), delivering final concentrations of 50, 100 and 500 μl/L in the gaseous phase after total volatilization of the essential oils (both oils volatilized completely after a few hours at room temperature) (Luciano and Holley, 2009). The control group did not receive any 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 gases to escape. Flour and kernel samples were used for further analysis.

2.4. Mycotoxin extraction procedure

BEA was extracted according to the procedures described by Jestoi (2008). Briefly, 2 g of sample was extracted with 20 mL methanol using an Ultra Ika T18 basic Ultraturrax (Staufen, Germany) for 3 min. The mixture was centrifuged at 4500 g for 5 min and then 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, filtered through a 0.22 μM filter and injected into LC-DAD.

2.5. LC analysis

BEA was analyzed using a Shimadzu LC system equipped with LC-10AD pumps and a diode array detector (DAD) from Shimadzu (Japan). The DAD was set at 205 nm for BEA detection, and the separation occurred in a Gemini (150 × 4.6 mm, 5 μm) (Phenomenex, Torrance, CA, USA) column. The mobile phase was acetonitrile–water (70:30 v/v) at a flow rate of 1.0 mL/min isocratically. The injection volume was 20 μL. The identification of BEA was performed by comparing retention times and UV spectra of peaks of the extracted with those of the standard. Quantification of BEA was done using a calibration curve generated from serial dilutions of the standard (0.5–50 mg/L).

2.6. Statistical analyses

The data reported are the average values from a minimum of 3 experiments and represented by mean ± SEM. Differences among treatments were analyzed by Tukey's test. A P value of 0.05 was used as the cutoff for statistical significance.

3. Results and discussion

3.1. Method performance

Mean recovery of fortified cereal samples ($n = 3$) at levels of BEA (0.3–50 μg/g) was of 84.6%, with a relative standard deviations of 3.5%. The values obtained for recoveries and relative standard deviations of the method used are in agreement with the EU Commission Directive 2002/26/EC for methods of analysis of mycotoxins in foodstuffs (European Commission, 2002). Intra-day ($n = 5$) and inter-day (5 different days) variation values at were of 2.5–8.6% respectively. These values are below 15% which is the maximum variation for certification exercises for several mycotoxins. The detection limit (LOD) and the limit of quantification (LOQ) values were calculated according to $s/n = 3$ (signal/noise) and $s/n = 10$, and were of 0.17 and 0.50 μg/g respectively.

3.2. Influence of the pH on BEA reduction through reaction with the PITC and BITC

The reaction between BEA (25 mg/L) and the bioactive compounds, namely BITC and PITC (1 mM), was monitored by LC in PBS buffer solutions at three pH levels (4, 7 and 10). Samples were drawn after 0, 4, 8, 24 and 48 h. As shown in Table 1, the acid pH favored the reaction between the mycotoxin and the ITCs, where PITC was capable of reacting with 94.0 ± 3.8 of the mycotoxin after 48 h, whereas BITC caused an 81.2 ± 2.9% reduction of BEA at this same time-point. BEA reduction at neutral pH was of 71.9 ± 1.9 and 70.2 ± 2.5% for PITC and BITC respectively, while a 50% reduction was observed for both ITCs when the basic pH was employed. Allyl isothiocyanate (1 mM) presented a much faster reduction profile, where it was able to react with 80% of BEA (25 mg/L) after 4 h at pH 4 and 7, and the presence of the mycotoxin was undetectable after 48 h at both pH levels (Meca et al., 2012). Therefore, it seems that the short aliphatic chain of AITC facilitates the reaction of this compound with BEA in comparison with the more voluminous aromatic side chains of BITC and PITC.

3.3. BEA reduction in cereals

The same reaction observed *in vitro* between BEA and the natural compounds BITC and PITC also occurred when the essential oils were used to fumigate barley, rice, wheat and corn tainted with the mycotoxin. Kernels and flours were artificially contaminated with 25 mg/kg of BEA, and then exposed to gaseous BITC and PITC at 50, 100 and 500 μl/L during 48 h. Extracts of cereal flours and kernels were injected in the LC coupled to the diode array detection (DAD) and the BEA reduction rates were calculated. Fig. 1 shows the LC-DAD chromatogram of corn kernels extract before and after the treatment with 500 μl/L of PITC, where significant reduction of BEA is evident. Reduction levels for all three doses tested of BITC and PITC are presented on Fig. 1. Both compounds caused a pronounced reduction of BEA, and this activity was dose dependent. The highest reaction rate between BITC and BEA occurred when rice kernels were fumigated with the oil at 500 μl/L (92.5 ± 2.2% reduction), whereas the lowest percentage of BEA reduction (26.4 ± 1.1%) was detected when barley was treated with 50 μl/L of BITC. Considering all BITC doses, the average BEA reduction in cereal kernels caused by the presence of gaseous BITC was of 68.5 ± 1.2%. On the other hand, the mean BEA reduction in cereal flours when fumigated with BITC was 46.4 ± 2.0%, which is approximately 60% of the rate found for kernels. The highest and lowest reduction rates were found for rice treated with 500 μl/L BITC (65.5 ± 1.9) and barley with 50 μl/L BITC (9.2 ± 0.6%), respectively.