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Development of an immunochromatographic strip test for the rapid simultaneous detection of deoxynivalenol and zearalenone in wheat and maize

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ABSTRACT

A colloidal gold immunochromatographic strip (ICS) test based on competitive format was developed for the rapid simultaneous detection of deoxynivalenol (DON) and zearalenone (ZEN) in wheat and maize samples. For gold-based ICS test, antigen (DON-CBSA and ZEN-BSA) and goat anti-mouse IgG were respectively drawn on NC membrane as two test line (T_1 and T_2 line) and control line (C line), respectively. Monoclonal antibody (MAb)-gold conjugates (anti-DON MAb-gold and anti-ZEN MAb-gold) were sprayed onto the conjugate pad. To perform the test, 5 g of sample was extracted in a ratio of 1:5 with 50% methanol–water (*v/v*) by shaking for 3 min and the extract directly used without further cleanup steps. Matrix interference was eliminated by 2.5-fold dilutions of water extracts with buffer. The ICS test, which has cut-off levels of 100 ng/mL (1000 μg/kg) and 6 ng/mL (60 μg/kg) for DON and ZEN, respectively, and can be completed in 5 min. Analysis of DON and ZEN in wheat and maize samples revealed that data obtained from ICS test were in a good agreement with those obtained from ELISA and instrumental analysis (GC/MS for DON determination and HPLC for ZEN determination). The results demonstrate that the ICS test can be used as a reliable, rapid, cost-effective and convenient qualitative tool for on-site screening technique for the simultaneous determination of DON and ZEN in wheat and maize samples without special instrumentation.

1. Introduction

Mycotoxins are toxic metabolites produced under particular environmental conditions by fungi either in the field or storage of agricultural commodities (Shephard, 2008). Among the most prominent mycotoxins are Alternaria toxins, aflatoxins, ergot alkaloids, fumonisins, ochratoxins, resorcyclic acid lactones, and trichothecenes (Schenzel, Schwarzenbach, & Bucheli, 2010).

Deoxynivalenol (DON) and zearalenone (ZEN) are secondary metabolites produced largely by several species of *Fusarium* fungi, which are widespread in nature and commonly contaminate many cereal grains such as wheat, maize, corn, barley, and other cereal grains (Cetin & Bullerman, 2006). The simultaneous occurrence of DON with other *Fusarium* toxins, mainly type B trichothecenes and zearalenone, has been reported for various commodities (Kolosova, De Saeger, Sibanda, Verheijen, & Van Peteghem, 2007). DON, for instance, which is a potent protein synthesis inhibitor, is postulated to act as an inhibitor of plant defense response genes (Beyer, Verreet, & Ragab, 2005; Neuhof, Koch, Rasenko, & Nehls, 2008; Rotter, Prelusky, & Pestka, 1996). Several adverse effects of DON include dose-dependent induction of feed refusal, diarrhea and emesis in livestock, especially in swine (Kolosova et al., 2007).

ZEN is a nonsteroidal estrogenic mycotoxin and has been associated with early puberty, hyperplastic and neoplastic endometrium, and human cervical cancer (Shim, Dzantiev, Eremin, & Chung, 2009). In addition, several studies have been conducted and have shown that ZEN is cytotoxic and exhibits a genotoxic potential *in vitro* and *in vivo* through induction of micronuclei, chromosome aberrations, DNA fragmentation, cell cycle arrest, etc (Abbes et al., 2007; Abid-Essefi et al., 2003; Boussema-Ayed, Ouanes, Bacha, & Abid, 2007; Ouanes et al., 2003). As a result, most countries have set a legal limit for DON in cereals and many others for cereal products. For example, in the United States and China a maximum limit for the total amount of DON in cereal products for human consumption is set 1000 μg/kg, and the U.S. Food and Drug Administration has no legal regulations for zearalenone, but in China legal limits for zearalenone is set 60 μg/kg (Meneely et al., 2010; Shim, Kim, & Chung, 2009). To detection related mycotoxins, several analytical methods including HPLC, LC/MS, GC, GC/MS, and immunoassay have been developed over the

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past decade (Kolosova et al., 2008). Chromatographic techniques are sensitive and are specific tools to simultaneously detect mycotoxins (Desmarchelier et al., 2010; Ediage, Di Mavungu, Monbaliu, Van Peteghem, & De Saeger, 2011; Monbaliu et al., 2010; Romero-Gonzalez, Martinez Vidal, Aguilera-Luiz, & Frenich, 2009; Schenzel et al., 2010), however, chromatographic methods are unsuitable for routine screening of large sample numbers, because they suffer from being time-consuming, laborious, and multicomplex. Most immunoassay has often been limited to laboratories equipped with tools and devices, and they are unsuitable for on-site detection.

Therefore, the development of a simple, rapid and low cost analytical methods for these mycotoxins was urgently in need. In the past few years, ICS test has increasingly gained interest in the field of food safety due to the potential for fast and simple on-site application. The ICS test offers a simple and rapid analysis and combines several benefits, such as a user-friendly format, long-term stability over a wide range of climates, and cost-effectiveness (Kolosova et al., 2007; Shim, Dzantiev, et al., 2009; Shim, Kim, et al., 2009). These characteristics make it ideally suited for on-site screening by untrained personnel. Immunochromatographic strips have been used for the detection of small molecular toxins such as ochratoxin A (Cho et al., 2005; Liu, Tsao, Wang, & Yu, 2008), fumonisin B_1 (Wang, Quan, Lee, & Kennedy, 2006), aflatoxin B_1 (Delmulle, De Saeger, Sibanda, Barna-Vetro, & Van Peteghem, 2005) and T-2 toxin (Molinelli et al., 2008). However, most of these studies are limited only for the detection of a single analyte in sample. One of the latest trends in analytical chemistry is the simultaneous detection of multiple analytes (Shim, Dzantiev, et al., 2009; Shim, Kim, et al., 2009). The merit of simultaneous detection methods is that they can reduce the time and cost per analysis, allow for simpler assay protocols, and decrease the sample volume required (Kolosova et al., 2007). Recently, a few immunochromatographic assay for the rapid simultaneous detection of mycotoxins has been reported (Kolosova et al., 2007; Saha, Acharya, Roy, Shrestha, & Dhar, 2007; Shim, Dzantiev, et al., 2009; Shim, Kim, et al., 2009). So far, there is only one literature about simultaneous detection of DON and ZEN. In this literature, the assay procedure could be accomplished within 10 min, and cut-off levels of 1500 μg/kg and 100 μg/kg for DON and ZEN, respectively, were observed (Kolosova et al., 2007). However, the cut-off levels of method are unsuitable for simultaneous detection of DON and ZEN in cereals for China legal limits for DON (1000 μg/kg) and ZEN (60 μg/kg).

In the current study, we improved the preparation of an ICS test for rapid simultaneous detection of DON and ZEN in wheat and maize samples. As confirmed by the results from ELISA and instrumental analysis, ICS test has been demonstrated to be simple and rapid simultaneous screening method for the detection of DON and ZEN on farm or at feed mills.

2. Materials and methods

2.1. Materials and chemicals

DON, ZEN, bovine serum albumin (BSA), Tween-20, sucrose, mycose, polyvinylpyrrolidone (PVP), sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS), polyethylene glycol (PEG MW = 1500 and 20,000) were purchased from Sigma Chemical Co. (St. Louis, MO). Goat anti-mouse IgG was obtained from Sino-American Biotechnology Co. (Luoyang, China). The anti-DON MAb and anti-ZEN MAb were prepared according to methods previously described (Liu et al., 2008). DON-CBSA and ZEN-BSA conjugate as well as the monoclonal antibodies against DON (anti-DON MAb) and ZEN (anti-ZEN MAb) were prepared in our laboratory (Xu et al., 2010). The anti-DON MAb and anti-ZEN MAb were purified using Protein-G Sepharose Fast Flow Columns (Amersham, NJ, USA). All other chemicals and solvents were of analytical grade or better and were obtained from Beijing Chemical Reagent Co. (Beijing, China). Deionized water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Stock solutions were prepared by dissolving 1.0 mg of DON in 1.0 mL of deionized water and 1.0 mg of ZEN in 1.0 mL of methanol, and then kept at −20 °C for further dilution.

The NC membrane Prima 40 (P40) was obtained from Whatman (Middlesex, UK). The colloidal gold (40 nm in diameter), the sample pad, the conjugation pad and the absorbent pad were obtained from Jiening Bio. Co. (Shanghai, China). Immunoaffinity columns for ZEN (ZearalaTest) were supplied from Vicam (Watertown, MA, USA).

2.2. Apparatus

The equipment used for spraying and cutting strip tests was purchased from BioDot (Irvine, CA, USA). The BioDot system consisted of two BioJets Quanti 3000 and one Airjet Quanti 3000 attached on a BioDot XYZ-3000 (Irvine, CA, USA) dispensing platform. The guillotine cutter (model CM 4000) was supplied by BioDot (Irvine, CA, USA). Maxisorp polystyrene 96-well plates were purchased from Nunc (Roskilde, Denmark). Immunoassay absorbance was read with a Multiskan MK3 Spectrum purchased from Thermo. A Sigma 2K15 centrifuge from Sigma–Aldrich (St. Louis, MO, USA) was used for centrifugation of colloidal gold-MAb conjugates. UV–visible spectra were obtained by using an Ultrospec 4300 Pro UV/Visible Spectrophotometer (Amersham, NJ, USA).

For DON determination, the GC/MS method was carried out as previously described (Xu et al., 2010).

For ZEN determination, the HPLC system consisted of a Waters 510 solvent delivery pump (Waters, Milford, MA, USA), a 7725 manual injector system equipped with a 20 μL loop, a model 2475 multi-wavelength fluorescence detector (Waters, Milford, MA, USA). Fluorescence detection was performed with a 2475 multi-wavelength fluorescence detector with λex = 274 nm and λem = 440 nm. Chromatographic separation was achieved at room temperature using a 250 × 4.6 mm i.d., 5 μm Symmetry C_{18} column (Waters, Milford, MA, USA), with isocratic elution of acetonitrile–H_2O (70:30, *v*/*v*) at a flow rate of 0.80 mL/min. Aliquots of 20 μL standard or samples solutions were injected into the HPLC for the determination. All injections were repeated at least three times.

2.3. Preparation of colloidal gold-MAb conjugates

Anti-DON MAb and anti-ZEN MAb prepared in our laboratory were purified from mouse ascitic fluid using a caprylic acid and ammonium sulfate method (Guo et al., 2010). These MAbs were purified further by affinity chromatography using Protein-G Sepharose Fast Flow Columns (Amersham, NJ, USA). Each antibody was conjugated to colloidal gold (40 nm) as previously described (Xu et al., 2010). Briefly, the pH of the colloidal gold solution for anti-DON MAb or anti-ZEN MAb conjugation was adjusted to pH 7.0 with 0.1 M K_2CO_3. With gentle stirring, anti-DON MAb (100 μg/mL, 1.0 mL) or anti-ZEN MAb (100 μg/mL, 0.5 mL) was added dropwise to the colloidal gold solution (10 mL). After reacting for 30 min, 1 mL of 10% (*w*/*v*) BSA was added to block excess reactivity of the gold colloid, and further reacted for 15 min, and then the reaction mixture was centrifuged at 10,000 rpm (8497×*g*) at 4 °C for 25 min. The clear supernatant was carefully removed, and gold pellets were resuspended in 1 mL of conjugate storage buffer (5% sucrose, 0.2% BSA, 0.3% PVP, 1% mycose, and 0.05% sodium azide in 0.01 M PBS). From above the process, the anti-DON MAb-gold and anti-ZEN MAb-gold were obtained, and then stored at 4 °C until use.