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Analytical Methods

Simultaneous determination of fumonisins B1 and B2 in different types of maize by matrix solid phase dispersion and HPLC-MS/MS

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ABSTRACT

This work involved the optimization and validation of a method, according to Directive 2002/657/EC and the Analytical Quality Assurance Manual of Ministério da Agricultura, Pecuária e Abastecimento, Brazil, for simultaneous extraction and determination of fumonisins B1 and B2 in maize. The extraction procedure was based on a matrix solid phase dispersion approach, the optimization of which employed a sequence of different factorial designs. A liquid chromatography-tandem mass spectrometry method was developed for determining these analytes using the selected reaction monitoring mode. The optimized method employed only 1 g of silica gel for dispersion and elution with 70% ammonium formate aqueous buffer (50 mmol L^{-1}, pH 9), representing a simple, cheap and chemically friendly sample preparation method. Trueness (recoveries: 86–106%), precision (RSD ≤19%), decision limits, detection capabilities and measurement uncertainties were calculated for the validated method. The method scope was expanded to popcorn kernels, white maize kernels and yellow maize grits.

1. Introduction

Maize (*Zea mays*) is a foodstuff that participates in a wide agro-industrial chain of production and processing of food and feed. One of the major problems of the maize chain is contamination by mycotoxin-producing fungi. This contamination can occur in the crop and worsen during harvesting, transportation, drying, processing and/ or develop during storage of maize and its derived products. (Galvão, Miranda, Trogello, & Fritsche-Neto, 2014).

Fumonisins are toxic mycotoxins produced by *Fusarium* fungi (Bezuidenhout et al., 1988). Among all identified fumonisins, B1 and B2 are the most toxic and could cause esophageal cancer in humans (Bordin, Rosim, Neeff, Rottinghaus, & Oliveira, 2014). The toxicity and occurrence of fumonisins in various foods has led the regulatory authorities, such as the Commission of the European Community, Food and Drug Administration of the United States and Agência Nacional de Vigilância Sanitária in Brazil, to establish maximum limits allowed in different foods. (Regulation EC/1881/2006; FDA Regulatory Guidance for Mycotoxins; RDC-ANVISA N° 7/2011).

In order to determine mycotoxin residues in various food matrices, several analytical methods have been proposed. The main sample preparation techniques that have been employed for analysis of mycotoxins, including fumonisins, are: (1) solid-liquid extraction (SLE) followed by clean-up with solid phase extraction (SPE) or by immunoaffinity column or by dispersive solid phase extraction (dSPE) (Beltrán et al., 2013; Abia et al., 2013; Wang et al., 2013; Szekeres et al., 2014; Liao et al., 2015; Petrarca, Rodrigues, Rossi, & De Sylos, 2014; García-Moraleja, Font, Manes, & Ferrer, 2015b; Jung et al., 2015; Ediage, Poucke, & De Saeger, 2015; Bryła, Szymczyk, Jedrzejczak, & Obiedzinski, 2015; Bryła, Roszko, Szymczyk, Jedrzejczak, & Obiedzinski, 2016; Petrarca, Rossi, & De Sylos, 2016), (2) liquid-liquid extraction (LLE) followed by clean-up with immunoaffinity column (Beltrán et al., 2013; Abia et al., 2013; García-Moraleja, Font, Mañes, & Ferrer, 2015a), (3) matrix solid phase dispersion (MSPD) (Rubert, Soler, & Manes, 2011; Rubert, Dzuman et al., 2012; Rubert, Soler, & Mañes, 2012; Serrano, Font, Ruiz, & Ferrer, 2012;Ye, Lai, & Liu, 2013; Blesa, Moltó, Akhdari, Mañes, & Zinedine, 2014), (4) dispersive liquid-liquid microextraction (DLLME) (Arroyo-Manzanares, Huertas-Pérez, Gámiz-Gracia, & García-Campaña, 2013) and (5) Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) (Arroyo-Manzanares et al., 2013; Azaiez, Giusti, Sagratini, Mañes, & Fernández-Franzón, 2014; Arroyo-Manzanares, Huertas-Pérez, García-Campaña, & Gámiz-Gracia, 2014; Pizzutti et al., 2014; Bolechová et al., 2015; Nielsen, Ngemela, Jensen, De Medeiros, & Rasmussen, 2015; Arroyo-Manzanares, Huertas-Pérez,

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Gámiz-Gracia, & García-Campaña, 2015). High performance liquid chromatography (HPLC) or ultra performance liquid chromatography (UPLC) coupled to detectors such as UV–Vis spectrophotometric (Ye et al., 2013), fluorescence (Petrarca et al., 2014; Petrarca et al., 2016) and mass spectrometry (Rubert et al., 2011; Beltrán et al., 2013; Abia et al., 2013; Zhang et al., 2013; Arroyo-Manzanares et al., 2013; Wang et al., 2013; Azaiez et al., 2014; Arroyo-Manzanares et al., 2014; Pizzutti et al., 2014; Liao et al., 2015; Bolechová et al., 2015; Nielsen et al., 2015; García-Moraleja et al., 2015a; García-Moraleja et al., 2015b; Jung et al., 2015; Ediage et al.,2015; Bryła et al., 2015; Bryła et al., 2016), have been the most widely used techniques for fumonisin quantification.

Among the extraction and clean-up techniques abovementioned, MSPD has two important characteristics: extraction and clean-up in a single step and there is no need for solubilizing the solid and semisolid samples. These characteristics make MSPD advantageous for analyzing the mycotoxins in food samples, especially the solid ones (Barker, 2007). MSPD is especially advantageous for fumonisin determination in maize, once the distribution of these mycotoxins in the grain is not homogeneous and supramolecular structures with other maize components need to be broken to ensure efficient extraction (WHO/IARC, 2002). Thus, MSPD has been widely used for the extraction and clean-up of mycotoxins in food, employing chemically modified silica as dispersant and elution with either 100% or at high proportion organic solvents (Rubert et al., 2011; Rubert, Dzuman et al., 2012; Rubert, Soler et al., 2012; Serrano et al., 2012; Ye et al., 2013; Blesa et al., 2014).

In this work, an MSPD method was developed for the extraction and clean-up of fumonisins B1 and B2 in maize using silica gel as dispersant and elution with 70% ammonium formate aqueous buffer (50 mmol L^{-1}, pH 9) . The method was validated according to Directive 2002/657/EC and Manual of Analytical Quality Assurance of the Ministério da Agricultura, Pecuária e Abastecimento, Brazil (BRAZIL, Pecuária e Abastecimento, & Coordenação-Geral de Apoio Laboratorial, 2014). The following validation parameters: matrix effect, linearity, precision, trueness, decision limit, detection capability and measurement uncertainty, were evaluated. The scope of the validated method was expanded to popcorn kernels, white maize kernels and yellow maize grits.

2. Materials and methods

2.1. Chemicals

Standards of fumonisins B1 (FB1) and B2 (FB2) (minimum purity 98%, Sigma-Aldrich, St. Louis, USA and minimum purity 90%, Wako Pure Chemical Industries, Osaka, Japan, respectively) were used for stock solution preparations. Acetonitrile (ACN), methanol, acetic acid (99% w/w), formic acid (88% w/w), ammonium hydroxide (29% w/w) (HPLC grade, J. T. Baker, Mexico) and tetrahydrofuran (THF) (analytical grade, Dinâmica Química Contemporânea Ltda, Brazil) were used for mobile phase and/or extraction solution preparation. Silica gel (70–120 mesh, Fluka, USA) and silica chemically bound with octadecyl groups (C18 silica) (50 μm, 65A, Phenomenex, USA) were used for MSPD. Ultrapure water from Millipore Direct-Q3 UV purifier (Millipore, USA) was used for aqueous solution preparations.

2.2. Stock and working solutions

Individual stock solutions were prepared at a concentration of 0.8 μg L^{-1} for FB1 and 0.6 μg L^{-1} for FB2 by dissolving the exact mass of each standard in ACN: ultrapure water (1:1 v/v) and were then stored at -10 °C. Working solutions were prepared by mixing the individual stock solutions and diluting them with ACN: ultrapure water (1:1 v/v) to a final concentration of 100 ng L^{-1} of FB1 and 50 ng L^{-1} of FB2. All solutions were stored at −10 °C.

2.3. Samples

Maize was used in the optimization and validation of the method. Popcorn kernels, white maize kernels and yellow maize grits were used to expand the scope of the method. All samples were ground to appropriate particle size and were provided by Laboratório de Controle de Qualidade e Segurança Alimentar of the Ministério da Agricultura, Pecuária e Abastecimento, Brazil. All samples were stored at −10 °C.

2.4. HPLC-MS/MS instrument

The HPLC-MS/MS analyses were performed in a triple quadrupole mass spectrometer with a turbo ion spray interface (API 5000, Applied Biosystems, USA) coupled to a HP Agilent Technologies 1200 series liquid chromatography system equipped with an autosampler and a quaternary pump (Agilent Technologies, USA). Both systems and data treatment were controlled by Analyst 1.5.1 software (Applied Biosystems, USA).

2.5. HPLC-MS/MS conditions

The optimum condition for the separation of FB1 and FB2 using a C18 column (100 × 3 mm, 2.7 μm, Poroshell, Agilent Technologies, USA) was obtained with the mobile phase: 0.1% v/v formic acid in ultrapure water (solvent A) and 0.1% v/v formic acid in ACN (solvent B). The chromatographic gradient was used as follows: from 0 to 3 min the percentage of solution B linearly increased from 20 to 90% and was maintained constant up to 3.4 min; from 3.4 to 3.5 min the percentage of solution B decreased to 20%, which was maintained up to 6 min. The mobile phase flow rate was 0.500 mL min^{-1}, the injection volume was 10 μL and the column temperature was maintained at 40 °C.

Electrospray ionization (ESI) conditions in positive mode were first optimized with direct infusion into the mass spectrometer to select the precursor and the product ions resulting from fragmentation, the declustering potential (DP) and the collision energy (EC) for each fumonisin (Table 1S). This optimization was conducted by direct infusion of standard solutions at 10 μg L^{-1} of FB1 and 5 μg L^{-1} of FB2 at a flow rate of 10 μL min^{-1}. Flow injection analysis (FIA) was used to optimize capillary voltage, curtain and nebulizer gas flow rates and source temperature. The experiments were conducted at a mobile phase flow rate (solvent A: solvent B, 1:1 v/v) of 0.5 mL min^{-1}. The following settings were also applied to the turbo ion spray source: capillary voltage, 4500 V; temperature, 650 °C; nebulizing gas (N_2), 40 (arbitrary units); curtain gas (N_2), 18 (arbitrary units); CAD gas (N_2), 4 (arbitrary units); entrance potential, 10 V. The fumonisins were evaluated employing the Selected Reaction Monitoring (SRM) mode. The most intense transition was used for quantification (352.4 *m*/*z* for FB1 and 336.4 *m*/*z* for FB2) and the other two transitions for confirmation (334.5 and 316.4 *m*/*z* for FB1; 354.3 and 318.3 *m*/*z* for FB2).

2.6. Preliminary studies for optimization of extraction of FB1 and FB2 by MSPD in maize

2.6.1. MSPD cartridge preparation

1 g of the maize sample and 1 g of the dispersant were weighed, transferred to a ceramic container and mixed. Polypropylene cartridges (15 mL) were mounted using a Teflon filter (20 μm) at the bottom, followed by glass wool and dispersed sample. The car-