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Analytical Methods

Simple and efficient methodology to determine mycotoxins in cereal syrups

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ABSTRACT

Consumption of cereal syrups is increasing nowadays. Mycotoxins may be found in syrups resulting from the use of contaminated raw material or invading microorganisms in the final manufactured product. However, these matrices have been scarcely explored regarding their mycotoxin content. A sensitive, simple and rapid method for the determination of ten mycotoxins (ochratoxin A, fumonisin B_1, fumonisin B_2, deoxynivalenol, fusarenon-X, T-2 and HT-2 toxin, citrinin, sterigmatocystin and zearalenone) in cereal syrups (rice, wheat and barley) has been developed and characterised using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) and a sample treatment based on QuEChERS procedure. Matrix-matched calibration curves were established and limits of quantification were below the limits usually established by current legislation in different foodstuff. The relative standard deviation of the whole analytical method was lower than 12% in all cases, while recoveries ranged from 70.2% to 100.6%, therefore fulfilling the current requirements for mycotoxins analysis.

1. Introduction

Cereal syrups are obtained by isolation of starch after wet milling of grains, hydrolysis and further purification. Different temperatures, pH, and kind of enzyme employed in the starch hydrolysis process produce syrups with different characteristics due to different carbohydrates profiles (maltose, glucose or fructose) (Serna-Saldivar, 2004). Cereal syrups are widely applied in food and pharmaceutical industry and their consumption is increasing nowadays. They are used as alternative to refined cane or beet sugar to soften texture, adding volume and increasing flavour of food. For instance, the starch sweeteners are replacing the traditional cane sugar in USA and the consumption of high fructose corn syrup, containing either 42% or 52% of fructose, is almost similar to that of sucrose (Gensberger, Mittelmaier, Glomb, & Pischetsrieder, 2012; Putnam, Allshouse, & Kantor, 2005). Reasons for this trend are a cheaper production and preferences in food and soft drink industries, since starch sweeteners are easier to handle than crystalline sucrose, readily dissolve in water and are easier to incorporate into soft drinks, imparting a fruit flavour to beverages and foods (Gensberger et al., 2012; Serna-Saldivar, 2004).

Mycotoxins are highly toxic natural secondary metabolites produced by filamentous fungi belonging mainly to the genera of *Aspergillus*, *Penicillium*, and *Fusarium* that grow in a wide range of agricultural goods before, during and after harvest (Krska & Molinelli, 2007). They have been considered as the most important chronic dietary risk factor, and many of them are classified as cytotoxic, carcinogenic, mutagenic, or immunosuppressive compounds (Cigić & Prosen, 2009). Cereals are among the mayor food commodities affected by mycotoxin contamination (Cigić & Prosen, 2009), involving a serious risk for public and livestock health. Mycotoxins may also be found in cereal syrups resulting from the use of contaminated raw material. Depending on the properties of different mycotoxins (mainly stability and water solubility), carry over from contaminated grains to different fractions during cereal processing may take place (Hazel & Patel, 2004), therefore increasing the chances of final food product contamination. Some studies carried out at laboratory scale to study the fate of mycotoxin contamination through the wet-milling of maize, concluded that very low levels of toxins were detected in the starch fractions (Lauren & Ringrose, 1997). However, a study carried out at plant scale demonstrated that about 30% of deoxynivalenol (DON) was transferred to the starch fraction in the commercial wet milling process of maize (Patey & Gilbert, 1989). In addition to the raw material (cereals) employed, another potential source of mycotoxin contamination of the final manufactured cereal syrups is the invasion by microorganisms. In the case of sugar cane molasses, a matrix with similar characteristics as cereal syrups, prolonged storage could cause mould growth (Scott, 1989). These moulds exhibit the potential to produce toxic metabolites (Northolt, Frisvad, &

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Samson, 1995). Moreover, mycotoxin contamination of molasses has already been described (Anfossi, Stracciari, & Biacchessi, 1990; El-Said, 2002).

From these studies it should be possible to suggest that accurate and reliable methods for the determination of mycotoxins in cereal syrups (a scarcely explored matrix) are required in order to ensure the quality of these products and preserve consumer health.

Different analytical techniques have been proposed to monitor the large range of mycotoxins with different chemical structures and in a diversity of matrices. Instrumental techniques include thin layer chromatography (TLC) (Heperkan, Güler, & Oktay, 2012), enzyme-linked immunosorbent assay (ELISA) (Dos Santos et al., 2011), capillary electrophoresis (CE) (Arroyo-Manzanares, Gámiz-Gracia, García-Campaña, Soto-Chinchilla, & García-Ayuso, 2010) or gas chromatography (GC) coupled with electron capture (ECD) (Cano-Sancho et al., 2011; Tanaka, Sago, Zheng, Nakagawa, & Kushiro, 2007; Valle-Algarra et al., 2005, 2009), flame ionisation (FID) (Wu & Smith, 2007) or mass spectrometry (MS) detections (Cunha & Fernandes, 2010). Nevertheless, the most popular technique for mycotoxins analysis by far is high performance liquid chromatography (HPLC) coupled with different detections such as UV/Vis (Cano-Sancho et al., 2011) and fluorescence (FL) (Arroyo-Manzanares, Gámiz-Gracia, & García-Campaña, 2012; Campone, Piccinelli, Celano, & Rastrelli, 2011) or MS (Campone, Piccinelli, & Rastrelli, 2011; Rubert, Soler, & Mañes, 2012). HPLC hyphenated to tandem MS (HPLC-MS/MS) or sequential MS (MS^n) detection has become the method of choice for mycotoxin determination in food analysis, due to the easy handling, high sensitivity and accuracy and the possibility to separate and detect a high number of analytes of interest in a single run without any derivatisation (Köppen et al., 2010). Ultra-high performance liquid chromatography (UHPLC), which uses <2 μm particle size of stationary phase, coupled to MS/MS is nowadays gaining attractiveness in contaminant analysis due to its inherent advantages, such as higher resolution and peak capacity, higher throughput and lower solvent consumption. Several methods have been reported for the multiclass analysis of mycotoxins by UHPLC–MS/MS (Beltrán, Ibáñez, Sancho, & Hernández 2009; Varga et al., 2013). However, so far there are few methods for mycotoxin determination in molasses, most of them based on TLC (El-Said, 2002; Trucksess, Flood, & Page, 1986).

Regardless the technique of choice, clean up step after extraction is usually required, although direct injection of the diluted extract in the UHPLC–MS/MS system, with no further clean-up, has also been reported for the determination of mycotoxins in different food commodities (Beltrán et al., 2009). Nowadays solid phase extraction (SPE) is by far the most popular technique used in routine analysis of mycotoxins. It can be performed either with non-specific sorbent (reverse-phase, normal-phase, ion exchange, etc.) or more widely reported, using very selective binding materials such as immunoaffinity columns (IAC) (Cigić & Prosen, 2009). The main drawback of SPE is its unsuitability for multiclass analysis, since each type of mycotoxin is extracted using certain conditions. In addition, IAC is an expensive and complex purification system which suffers from low recoveries for some mycotoxins, due to matrix complexity. QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe, is an effective simple method first introduced for cleaning up extracts from complex matrices in pesticide analysis (Lehotay, Anastassiades, & Majors, 2010). It involves a previous extraction based on partitioning via salting-out between aqueous and organic layer, and a subsequent clean up based on dispersive SPE (dSPE) by combining $MgSO_4$ and different sorbents (i.e. C18, primary secondary amine, etc.). This procedure has recently been applied for extracting mycotoxins from different cereals (Cunha & Fernandes, 2010) among other food commodities.

In this paper we present an analytical method for the simultaneous quantification of ten mycotoxins (ochratoxin A (OTA), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), T-2 toxin (T-2), HT-2 toxin (HT-2), citrinin (CIT), sterigmatocystin (STE), fusarenon-X (F-X), DON and zearalenone (ZEN)) in several cereal syrups with different carbohydrate content: wheat syrup (28.5% glucose, 10% fructose and 25.5% maltose in weight), barley syrup (8.5% glucose and 39% maltose in weight) and rice syrup (17% glucose and 39% maltose in weight). The study included some mycotoxins regulated under European legislation by Regulation (EC) No. 1881/2006 and subsequent amends (European Commission, 2006a, 2007, 2012) and others considered by the International Agency for Research on Cancer (IARC) as dangerous substances. The method is based on an extraction based on the first step of the QuEChERS methodology with no further clean up of extracts, followed by UHPLC–MS/MS determination. To the best of our knowledge, this is the first time that these matrices are analysed using these methodologies.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical reagent grade, solvents were of LC–MS grade and mycotoxins were of analytical standard grade. Formic acid used as mobile phase additive for LC–MS, methanol (MeOH), ammonium formate and individual standards of each mycotoxin were obtained from Sigma Aldrich (St Louis, MO, USA). Formic acid (analysis grade), supplied by Merck (Darmstadt, Germany), and acetonitrile HPLC grade (MeCN), supplied by Panreac (Madrid, Spain), were used for sample treatment.

Ultrapure water (18.2 MΩ cm^{-1}, Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout all the work.

Kits SampliQ QuEChERS consisting of either buffered QuEChERS extraction packed (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate and 0.5 g disodium hydrogen citrate sesquihydrate) or non-buffered QuEChERS extraction packed (4 g $MgSO_4$, 1 g NaCl) were supplied by Agilent Technologies Inc. (Wilmington, DE, USA).

Acrodisc 13 mm syringe filters with 0.2 μm nylon membrane (Pall Corp., MI, USA) were used for filtration of extracts prior to the injection into the chromatographic system.

2.2. Instruments and equipment

All experiments were carried out using an Agilent 1290 Infinity LC (Agilent Technologies, Waldbron, Germany) equipped with a binary pump, online degasser, autosampler (5 μL loop), and a column thermostat. The MS measurements were performed on a triple quadrupole MS API 3200 (AB SCIEX, Toronto, ON, Canada) with electrospray ionisation (ESI). A C18 Zorbax Eclipse Plus RRHD (50 × 2.1 mm, 1.8 μm) was used as chromatographic column.

A Universal 320R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany), a vortex-2 Genie (Scientific Industries, Bohemia, NY, USA) and an evaporator System (System EVA-EC, from VLM GmbH, Bielefeld, Germany) were also used for sample preparation.

The instrumental data were collected using the Analyst® Software version 1.5 with Schedule MRM TM Algorithm (AB Sciex).

2.3. Sample treatment

Cereal syrup samples (rice, wheat and barley) were purchased in local markets from Granada (Spain) and stored at room temperature.

A 2 g portion of cereal syrup and 8 mL of 30 mM NaH_2PO_4 pH 7.1 were placed into a 50 mL screw cap test tube with conical bottom, which was shaken by vortex for 10 s. Subsequently, 10 mL of 5% formic acid in MeCN was added to the tube, and it was shaken