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Mycotoxin Contamination in Pseudo Cereals and their Detection Methods — A Review

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Abstract

Now a day the consumption of pseudo cereals is increasing day by day because of high protein content and good nutritional value. But these pseudo cereals, like cereals, nuts and oilseeds are having susceptibility to fungal growth and mycotoxins contamination. Various types of mycotoxins like aflatoxin B1, aflatoxin B2, aflatoxin G 1, aflatoxin G2, ochratoxin, are being determined in them and because of these substances it has become a great concern from the public health point of view. Various methods have been developed for their detection like thin layer chromatography (TLC), ELISA, gas chromatography (GC) or capillary electrophoresis (CE). However, the most popular technique is high performance liquid chromatography (HPLC) with UV/Vis, fluorescence (FL), or mass spectrometry (MS) detection. But recently ultrahigh performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (MS/MS) has become very popularQuEChERS-based methods have been recently used.

Keywords : mycotoxin, pseudo cereals, health effect, UHPLC, QuEChERC.

Introduction

Pseudocereals are plants that produce fruits or seeds, which are used and consumed as grains, though botanically neither they are considered to be grasses nor true cereal grains, but since they produce starch-rich seeds resembling to cereals because of which they are called pseudo cereals. Nowadays the interest on consumption of pseudo cereals is increasing due to their nutritional properties, such as protein content and quality as well as amino acids balance of amaranth and quinoa or phytochemicals concentration of buckwheat.[1] In addition, pseudo cereals are currently emerging as healthy alternatives to gluten-containing grains in the gluten-free diet necessary for celiac disease. Amaranth (*Amaranthus*), quinoa (*Chenopodium quinoa*) and buckwheat (*Fagopyrumesculentum*) are among the most consumed pseudocereals.
Mycotoxins are secondary metabolites producedmainly by fungi that are toxic to humans and animals. The most important toxigenic fungi belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium*.[2] The most important mycotoxins worldwide include aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxins.[3]

The presence of mycotoxins in food and feed may affect not only human health but also animal health, as they are responsible for causing different types of adverse effects such as estrogenic, gastrointestinal, and kidney disorders, induction of cancer, and mutagenicity. Furthermore, some mycotoxins are also immunosuppressive and reduce resistance to infectious diseases.[4]

The analytical methods that have been employed for the determination of mycotoxins contamination includes, liquid chromatography (LC) with fluorescence detection using a derivatization step,[5] LC with mass

spectrometry (MS)[6] thin layer chromatography (TLC)[7] or commonly used, gas chromatography mass spectrometry (GC–MS).[8] Although mycotoxin contamination in spelt (*Triticumspelta*) has not been extensively explored, spelt products have been included among others in several studies, as the determination of mycotoxins by enzyme-linked immunosorbent assay (ELISA).[9] Currently there is clearly an increased demand of the feeding industry toward the reduction of mycotoxin levels in cereals, nuts and also in pseudocereals as the demand of pseudocereals are increasing.

Brief Methodology

Taking into account the interest and the scarce data about the determination of mycotoxins in some of the pseudocereals, a developed and validated an analytical method for the simultaneous identification and quantification of 15 mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, FB1, FB2, T-2, HT-2, CIT, STE, F-X, NIV, DON, and ZEN) in pseudocereals.

Different analytical methods have been proposed for mycotoxin determination in food, such as thin layer chromatography (TLC)[11] ELISA[12], gas chromatography (GC)[13] or capillary electrophoresis (CE).[14] However, the most popular technique is high performance liquid chromatography (HPLC) with UV/Vis, fluorescence (FL),[15-17] or mass spectrometry (MS) detection.[18-20] Recently, ultrahigh performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (MS/MS) has become very popular, especially for multiclass determination of mycotoxins and for multiresidue determination with other contaminants.[21-24] Because of the complex structure of food particle matrices it is important to have an extraction and clean-up purification step which is required before analysis. Different methods for this clean up purification step have been proposed. The most common methodology implies solid-liquid extraction (SLE) followed by solid phase extraction (SPE) with immunoaffinity columns (IACs), which contain specific antibodies to the analyte of interest.[25] However, IACs are expensive and highly complex purification systems which has low recoveries for some mycotoxins and their use in multiclass analysis is limited because of their high selectivity.[26] As a consequence, simpler, more efficient, multiclass, and environmentally friendly extraction systems are demanded. Among the different proposals, the so-called QuEChERS (quick, easy, cheap, effective, rugged, and safe which is becoming increasingly popular treatment.

QuEChERS is a fast and inexpensive method widely used in the last years, mainly for the extraction of pesticides and presents some advantages such as its simplicity, minimum steps, and effectiveness for cleaning up complex samples.[27-28] It comprises two steps : (i) an extraction based on partitioning via salting-out, involving the equilibrium between an aqueous and an organic layer; (ii) a dispersive SPE (dSPE) for further clean-up using combinations of MgSO4 and different sorbents, such as C18 or primary and secondary amine (PSA). QuEChERS-based methods have been recently reported for the extraction of different mycotoxins in cereal and pseudo cereals and there products.[29-30] Taking advantage of UHPLC-MS/MS characteristics, we optimized a separation method that allows the determination of 15 mycotoxins in only four minutes. The studied mycotoxins are included in Regulation (EC) number 1881/2006 or considered as dangerous by the IARC.[31]

Analysis of Cereals and Pseudo Cereals

Cereals and pseudo cereals, now gaining popularity are a commodity of great interest, highly prone to microbial contamination because of their chemical composition. Matrices of concern are pseudocereals, such as amaranth, quinoa, and buckwheat. Though botanically they are not true cereal grains, they produce starch-

rich seeds consumed like cereals. Pseudocereals are also susceptible to fungal growth and therefore to mycotoxin contamination. Taking into account the interest and the scarce data about the determination of mycotoxins in some of the above mentioned matrices, a method was developed it was developed and validated for the simultaneous identification and quantification of 15 mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, FB1, FB2, T-2, HT-2, CIT, STE, F-X, NIV, DON, and ZEN) in pseudo-cereals, spelt, and white, red, and brown rice. As a sample treatment we proposed a simple salting out assisted solid liquid extraction (i.e. a QuEChERS-based extraction). No further clean-up was required, although matrix effect was higher than (20%) for some mycotoxins (aflatoxins, DON, and NIV). Thus matrix-matched calibration was applied. This methodology has proved to be a suitable and efficient choice for multiclass mycotoxin determination in these matrices, with LOQs below the contents currently regulated. It provides good recoveries (between 60.0% and 103.5%) and precision (RSD lower than 12% in all cases), allows extraction time reduction, and is environmentally friendly.[32]

Conclusion

As there is increased demand of the pseudo cereals world widely, the concern for the detection of mycotoxin is these have become an important issue. Various methods have been developed for detection of mycotoxin contaminants. UHPLC-MS/MS analytical methods for multiclass determination of mycotoxins based on QuEChERS and for sample treatment have been developed. These proposed methods are being used widely for the determination of mycotoxins not only in pseudo cereals but also in various other food commodities. They showed as general advantages in their efficacy, simplicity, versatility, and accuracy, as well as their low impact on the environment, shorter analysis time, and the relatively low-cost, compared with conventional IAC method. But as we know that though these methods have several advantages but sometimes the results may not always be correct.

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