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Occurrence and co-occurrence of mycotoxins in nuts and dried fruits from China

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ABSTRACT

In this study, 16 mycotoxins were analyzed in ten kinds of dried fruits and nuts sampled from four climate zones in China. The results showed that all 16 mycotoxins were detected at a contamination frequency of 124/253. The most frequent mycotoxin category detected was TCs with contamination levels ranging from <LOQ–473.16 μg/kg. Two samples contaminated with AFB_1 at levels that exceeded the maximum limit of China and EU. Significant differences of AFB_1, AFG_1, AOH, AME and ENNs contamination among the four climate zones were found. Specifically, the ENNs contamination level was significantly higher of samples from the semiarid to semi-humid zone than the arid zone, the semiarid zone and the humid zone. In the 124 positive samples, 66 samples were detected with two to eight mycotoxins. To the best of our knowledge, this is the first study of mycotoxin occurrence and co-occurrence in dried fruits and nuts from China that also included the emerging mycotoxins, ENNs and BEA.

1. Introduction

Nuts and dried fruits have become an increasingly attractive snack food because of containing essential amino acid, vitamin, mineral and rich dietary fiber which are beneficial for keeping health (Asghar et al., 2017). According to data from the European commission RASFF (Rapid Alert System for Food and Feed), nuts and their products have been one of the top safety-patrolled foods as a result of mycotoxin contamination (Miao & Zhou, 2014; Dai, Li, Liu, Liu, & Liu, 2015; RASFF, 2015). As for dried fruits, mycotoxin contamination has also been perpetually reported (Alghalibi & Shater, 2004; Juan, Zinedine, Molto, Idrissi, & Manees, 2008; Bircan, 2009; Azaiez, Font, Mañes, & Fernández-Franzón, 2015; Asghar, Ahmed, & Iqbal, 2016). Mycotoxins are fungal secondary metabolites mainly produced by *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Claviceps* spp., and *Alternaria* spp., and more than 400 species of mycotoxins have been described (Tolosa, Font, Mañes, & Ferrer, 2013). Due to the variable molecular structure of these mycotoxins, these metabolites exhibit a wide range of effects on human health, such as immunosuppressive disorders, hormonal teratogenic and mutagenic effects as well as carcinogenic effects on liver and kidney tissues (Pitt, 2013; Rychlik, 2017; Asam, Habler, & Rychlik, 2017). Sixteen mycotoxins are commonly found in dried fruits and nuts and they can be classified into four categories. The four categories consist of Aflatoxins (AFs: AFB_1, AFB_2, AFG_1 and AFG_2); Ochratoxins (OTs: OTA and OTB); *Alternaria* toxins (ATs) including tentoxin (TEN), alternariol (AOH), and alternariol monomethyl ether (AME); and Trichothecenes (TCs) including T-2 toxin (T-2), zearalenone (ZEA), emerging enniatins (ENNs: ENA, ENA_1, ENB, ENB_1) and emerging beauvericin (BEA) (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Andersen, Nielsen, Pinto, & Patriarca, 2015).

Several countries, including China, and international

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organizations have implemented mycotoxin limitation standards for dried fruit and nut production. According to Chinese National Food Safety standards (GB 2761, 2017), AFB_1 levels in cooked nuts and seeds cannot surpass 5 μg/kg while almonds and hazelnuts cannot contain AFB_1 more than 15 μg/kg. As for European Union (EU), both AFs and OTA are subjected to specific legal standards in dried fruits. The OTA levels cannot reach more than 10 μg/kg in dried vine fruit (raisins, etc.) and the AFB_1 levels cannot surpass 2 μg/kg in dried fruits while the total aflatoxin (AF_{tol}) levels cannot surpass 4 μg/kg ($AF_{tol} = AFB_1+AFB_2+AFG_1+AFG_2$) (EC, 2006). Newer regulations make an exception for dried figs whose AFB_1 levels can reach 6 μg/kg and AF_{tol} levels up to 10 μg/kg (EC, 2010). In the Ukraine, the maximum ZEA level for all nuts can be no more than 1000 μg/kg, while Armenia implemented a maximum T-2 level of 100 μg/kg for all fruits and their products (Li et al., 2017a). As for OTB, AOH, AME, TEN, ENNs and BEA mycotoxins, dried fruit and nut legal limitation standards have not been established.

There are numerous methods for sample preparation, multi-mycotoxin detection and analysis in mycotoxin detecting process (Turner et al., 2015; Li et al., 2009; Li et al., 2017a; Myresiotis, Testempasis, Vryzas, Karaoglanidis, & Papadopouloumourkidou, 2015). QuEChERS (quick, easy, cheap, effective, rugged, and safe) has been readily utilized for sample preparation of multi-mycotoxin detection in recent years because of its cost-effectiveness, ease of use and wide applicability (Myresiotis et al., 2015). Currently, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has quickly become the favoured detection technique due to its ability to measure various analytes quickly with high sensitivity and less sample preparation (Tan et al., 2016). Additionally, another option for multi-mycotoxin detection is ultra-high performance liquid chromatography (UHPLC) has been shown to separate analytes better by allowing for greater surface area and higher pressure to be used compared to HPLC (Turner et al., 2015).

In China, nuts and dried fruits may be a dietary source of mycotoxins that cannot be ignored, since they may be used as raw material for further applications in breakfast congee, baked goods, and tea (Wei et al., 2017). However, data are limited regarding mycotoxin occurrence in nuts and dried fruits of China. Current concerns mostly apply to AFs and OTA contamination in nuts, grapes and their products (Li et al., 2017a). Related contamination searches must be expanded to encompass all harmful mycotoxins that may reach to consumer tables. The aim of this study is to acquire the baseline knowledge about the contamination levels of the 16 mycotoxins in dried fruits (figs, longans, jujubes, raisins and persimmons) and nuts (walnuts, chestnuts, hazelnuts, pine nuts and almonds) produced in China. Considering the complex matrix of our study, QuEChERS is the most optimal choice for sample preparation and we employed UPLC-MS/MS analysis to quantify mycotoxin levels. To the best of our knowledge, this is the first report on multi-mycotoxin detection and contamination levels in dried fruits and nuts produced in China in which the emerging mycotoxins (ENNs and BEA) are included.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile, methanol and formic acid were obtained from Thermo Fisher Scientific (USA, purity ≥ 99.9%). Analytical-reagent citric acid was purchased from J.T. Baker (USA, purity ≥ 99.5%). Deionized water (<10 $M\Omega cm^{-1}$ resistivity) was obtained in the laboratory using a Milli-Q SP® Reagent Water System (Millipore, Beadford, MA, USA). Analytical-grade anhydrous $MgSO_4$ (purity ≥ 99.0%) and sodium chloride (NaCl, purity ≥ 99.5%) were obtained from Fengchuan Chemical Reagent Technology Co. Ltd (Tianjin, China). Syringe nylon filters were purchased from FINE Scientific Company (USA, 0.22 μm).

Standards of AFs, OTs, TCs and ATs were purchased from Pribolab (Singapore, purity ≥ 98.0%). Stock standard solutions of all mycotoxins were made in acetonitrile at the concentration of 200 μg/mL except ENNs and BEA which were made in methanol at the same concentration. All stock standard solutions were stored in the dark at −20 °C. Mixed standard stock solutions were prepared with the individual stock solutions in acetonitrile at a final concentration of 10 μg/mL of each mycotoxin. Mixed standard working solutions were prepared daily by diluting the mixed standard stock solution.

2.2. Instruments

The Xevo TQ UPLC-MS/MS (Waters, USA), CF16RXII high speed refrigerated centrifuge (Hitachi, Japan), Milli-Q Synthesis Ultrapure Water System (Millipore, USA), and mill and blender (JYL - C020, JOYOUNG COMPANY LIMITED, China) were used in this experiment. A reversed phase BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) was purchased from Waters Scientific (USA).

2.3. Sampling

A total of 253 samples were collected from local markets and supermarkets in 24 provinces, autonomous regions and municipalities in China. The 253 samples consist of 10 kinds of dried fruits and nuts: walnuts (n = 35), chestnuts (n = 33), hazelnuts (n = 20), pine nuts (n = 20), almonds (n = 25), dried figs (n = 20), dried longans (n = 15), dried jujubes (n = 35), raisins (n = 30) and dried persimmons (n = 20).

The edible parts of all samples were milled with blender by adding a certain amount of water in every 100 g edible section of samples. The specific volume of water added in each sample species is 50 mL for pine nut, 100 mL for dried longan, walnut, raisin, chestnut, 150 mL for dried fig, dried persimmon, almond, hazelnut and 200 mL for dried jujube, respectively. Subsequently, processed samples were preserved at −20 °C until analysis.

2.4. Analytical method

The analytical method was previously optimized in our laboratory (Wang et al., 2017) and validated according to the guidelines in the EU Commission Decision, 2002/657/EC (EC, 2002). The method validation and performance are shown as supplemented material.

2.4.1. Sample extraction

Specific weights of milled samples were placed into 50 mL PTFE centrifugal tubes. The specific weight of every sample species is following: 7.5 g for pine nut; 10.0 g for dried longan, walnut, raisin, chestnut; 12.5 g for dried fig, dried persimmon, almond, hazelnut and 15.0 g for dried jujube. 10 ml mixture of acetonitrile and citric acid (10 mMol citric acid in per liter of acetonitrile) was added to the 50 mL tubes and shaken for 3 min. Next 1 g of NaCl and 4 g of anhydrous $MgSO_4$ were added and the mixture was immediately shaken for 1 min. The tubes were centrifuged at 9000 rpm for 5 min. Subsequently, 5 mL of the acetonitrile layer was transferred to 15 mL centrifuge tubes containing 300 mg of C_{18} sorbent and the supernatant was filtered through a nylon membrane filter (pore size 0.22 μm) and transferred to sample vials for UPLC-MS/MS analysis.

2.4.2. Chromatographic analysis

The 16 mycotoxins were analyzed using the Waters Acquity