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Occurrence of patulin in various fruit products and dietary exposure assessment for consumers in China

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ABSTRACT

A quantitative method with broad applicability was developed and validated for patulin determination in fruit products by high-performance liquid chromatography (HPLC) with UV detection. Sample pretreatment was optimized for patulin extraction. 4.0 g of fruit products was extracted for patulin determination with acetonitrile and purified with a mixture of 1.0 g PSA and 3 g $MgSO_4$ plus a multifunctional cartridge to remove interferences. Under optimized conditions, the recoveries ranging from 75.6% to 118.5% were obtained at different spiking concentrations in various fruit products. The correlation coefficient of patulin from 5 to 1000 μg/L was approximately 1.00. The limits of detection (LODs) were from 2.6 to 7.5 μg/kg, and the limits of quantification (LOQs) were from 8.0 to 15.0 μg/kg for patulin in various fruit products. A total of 137 fruit products (97 dried fruits, 20 fruit juice and 20 jams) marketed and consumed in China were investigated. The incidence of patulin in fruit products was 30.7% (42/137) with a concentration ranging from 10.0 to 276.9 μg/kg. This study revealed that the dried figs, dried longans (seedless) and dried hawthorn products showed an average of patulin contamination of 87.6 μg/kg, 68.4 μg/kg and 5.1 μg/kg, respectively; the fruit juice of 5.4 μg/kg; the fruit jams of 5.0 μg/kg. The highest levels of patulin were found mostly in dried longans (seedless) and dried figs. Overall, 17.5% of total samples exceeded the maximum limit of 50 μg/kg set by the EU regulation. Chronic intake assessment indicated that only 0.29% (Hazard Quotient) acceptable daily intake (PMTDI, 0.4 μg/kg bw/day) was consumed through dried fruits, fruit juice and jams. Individual dietary risk assessment indicated patulin contamination in fruit products doesn't pose public health risks, but combined additive or synergistic toxic effects caused by multiple mycotoxin contaminations should not be ignored.

1. Introduction

Mycotoxins are secondary metabolites mainly produced by specific filamentous fungi under appropriate conditions of temperature and humidity (Nielsen, Mogensen, Johansen, Larsen, & Frisvad, 2009). As one of the most important mycotoxins, patulin is produced by a variety of molds (*Aspergillus*, *Penicillium* and *Byssochlamys*), and it has been found in rotting apples, grains, fruits, and vegetables (Pittet, 2001). As a neurotoxin, a number of studies have shown that patulin had chronically mutagenic, immunotoxic, genotoxic and gastrointestinal effects on rodents (Hopkins, 1993; Welke, Hoeltz, Dottori, & Noll, 2009). In addition, the amount of patulin is viewed as an effective index of unsound rotten apples in juice manufacture. Due to its toxicity and potential harm to human health, the Joint Expert Committee on Food Additives (JECFA) established a provisional maximum tolerable daily intake (PMTDI) for patulin of 0.4 μg/kg body weight/day (WHO, 1995). Therefore, investigation of patulin contamination in edible products and its dietary exposure assessment for consumers is increasingly needed.

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With the growing consumption of diverse fruit products for providing vitamins, minerals and other nutrients in human health, the safety of fruit products is becoming increasingly important. Currently, the occurrence of patulin in fruit products has been widely reported worldwide. Patulin was detected in 22.2% (10/45) of apple products with an average contamination 123 μg/kg, and 16.7% (1/6) of patulin contamination was found in pear products (Funes & Resnik, 2009). There was 50% (7/14) detectable rate for papaya from Portugal, with the highest patulin contamination of 118.3 μg/kg (Cunha, Faria, & Fernandes, 2009). The concentration of patulin detected in apple juice from Tunisia ranged from 0 to 167 μg/L (Zaied, Abid, Hlel, & Bacha, 2013), while much higher level of patulin contamination was found in apple juice from Tunisia with 889 μg/L in another report (Zouaoui, Sbaii, Bacha, & Abid-Essefi, 2015). Unfortunately, the presence of patulin contamination in fruit products is largely ignored in China, and published data related to patulin residues and risk assessment of fruit products for Chinese consumers are scattered.

In China, fruit products are sold in numerous forms, such as bottled or canned, shelled and unshelled, sundried or processed, loosely or tightly packaged, or so. Unfavorable processing and storage conditions could result in mold growth, particularly in small businesses and private factories. The contamination of patulin in various fruit products could be a potential hazard to consumers, especially for susceptible groups (e.g., children, oldie and gravida). Currently, both the Chinese government and the European Commission established the maximum limit (50 μg/kg) of patulin in fruit products and derived products (Commission Regulation, EC, 2006a; GB 2761–2011), while a stricter limit (10 μg/kg) of patulin was set in fruit products intended for infants and young children (Commission Regulation, EC, 2006a).

Patulin can be detected by multiple analysis methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC-MS) according to specific detection requirements, and each method has its merits and faults (Barreira, Alvito, & Almeida, 2010; Beltran, Ibanez, Sancho, & Hernandez, 2014; Vaclavikova et al., 2015). Because of low ionization properties of patulin, high investment and operating costs, LC –MS and GC-MS haven't been widely used for patulin determination. Currently, HPLC coupled to UV detection method has still been the most widely used technology for patulin analysis, especially for apple-based products. However, considering different physicochemical properties of fruit products, an optimal pre-treatment method with broad applicability and accurate quantitation characteristics is needed to develop for patulin determination.

Although some surveys were conducted to determine the occurrence of patulin in different food products, especially in apple related products, only a few attempted to assess its potential risks to human health (Guo, Zhou, Yuan, & Yue, 2013; Piqué, Vargas-Murga, Gómez-Catalán, Lapuente, & Llobet, 2013). Dietary risk assessment associated with patulin contamination in dried fruit products except for apple related products has never been reported previously in China, to our best knowledge. So determination of patulin in a variety of fruit products marketed in China is urgently needed for assessing its food safety risks. The present study aims to develop a suitable analytical method to monitor patulin contamination by optimizing extraction solvents and purification processes. The occurrence of patulin in various fruit products in China was investigated, and dietary risk assessment was also estimated for Chinese consumers.

2. Materials and methods

2.1. Chemicals and reagents

Patulin standard (purity > 99.0%) was purchased from Romer labs (Union City, Missouri, U.S.A.). 5-Hydroxymethyl-2-furfural (5-HMF) was obtained from Dr. Ehrenstorfer GmbH (Augsburg. Germany). HPLC grade of acetonitrile and ethyl acetate (purity > 99%) were the products of Tedia (Fairfield, Ohio, U.S.A.). HPLC grade of formic acid (purity > 99%) and acetic acid were obtained from Anaqua Chemicals Supply Inc. Ltd. (Houston,Tex.,U.S.A). Pectinase (≥3.800 units/ml) was obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA). Propylethylenediamine (PSA) and Diatomite Earth (DE) were purchased from Bonna-Agela Technologies Inc. (Tianjin, China). Analytical grade $MgSO_4$ (purity 99.0%) was purchased from Guangdong Guanghua Sci-Tech co., Ltd. (Shantou, China). Ultrapure water was produced from a Milli-Q system (Bedford, MA, USA).

2.2. Preparation of standard solutions

Standard stock solutions (100 mg/L) were prepared by diluting commercial products of patulin in ethyl acetate. The interval standard solutions were accurately prepared by pipetting 1.0 mL of patulin (100 mg/L) into a glass tube and dried by nitrogen gas at 45 °C. Then the residue was re-dissolved in water (pH 4.0 adjusted by acetic acid), and the final volume was made to 100 mL (1.0 mg/L). A serial of standard working solution was prepared in concentrations of 5, 10, 50, 100, 200, 400, 800, and 1000 μg/L with the mixture of acetonitrile and water (10:90, v/v) with pH 4.0 in brown bottles before analysis. The standard stock solution was storied at −20 °C and protected from light, and the interval standard solutions were stored in a refrigerator at 4 °C.

2.3. Sample pre-treatment optimization

Acetonitrile and acetonitrile containing (0.1% or 0.5%) formic acid or acetic acid (0.1% or 0.5%) were selected for the screening optimum extraction solvent. Two absorbents (PSA and DE) and a PriboFast® 228 multifunctional cartridge (Pribolab Pte. Ltd., Singapore) were applied for the optimization of the purification process. In this study, different amount of absorbents (PSA: 0.5 g, 1.0 g and 2.0 g; DE: 1.0 g) mixed with 3 g $MgSO_4$ plus the multifunctional cartridge were investigated for the purification process. Blank dried hawthorn spiked with patulin at concentration of 120 μg/kg was used for the optimization of the extraction solvent and purification process. Blank apple juice spiked with patulin at concentration of 120 μg/kg was used for the optimization of the extraction solvent.

Patulin was extracted and purified previously with some modifications (Boonzaaijer, Bobeldijk, & van Osenbruggen, 2005). The detailed procedure of the sample pre-treatment optimization process was as the following. As for dried hawthorn, 4 g of homogenized samples was transferred to a 50 mL Teflon centrifuge tube with 4 mL water, along with 75 μL pectinase. The tube was put in a water bath for 2 h at 40 °C for enzymatic reaction; as for apple juice, 4 g of sample was transferred to a 50 mL Teflon centrifuge tube without pectinase treatment. The remaining procedures for the two types of fruit samples were the same as the following: 20 mL extraction solvent was added into the test tube and vortexed for 2 min. The tube was centrifuged for 3 min at 6793 g under room temperature, and the supernatant was transferred to a 50 mL Teflon centrifuge tube containing a mixture of PSA or DE and $MgSO_4$. The above tube was quickly shaken by hand for 1 min for the first time clean-up. After centrifugation of the above extract for 3 min at