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Original Article

Mycotoxin monitoring for commercial foodstuffs in Taiwan

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ABSTRACT

Mycotoxins are toxic food contaminants that are naturally produced by certain fungi. They induce negative effects on human health by making food unsafe for consumption. In this study, analyses were performed to determine the levels and incidence of aflatoxins (AFs) in peanut products, tree nuts, spices, and *Coix* seeds; ochratoxin A (OTA) in wheat and roasted coffee, as well as OTA and AFs in rice; and citrinin (CIT) in red yeast rice (RYR) products. A total of 712 samples from nine different food categories were collected between 2012 and 2013. The samples were analyzed over 2 years for AFs, OTA, and CIT by methods recommended by the Ministry of Health and Welfare. These official analytical methods were extensively validated in-house and through interlaboratory trials. The analytical values of suspected contaminated specimens were confirmed by liquid chromatography – tandem mass spectrometry analysis to identify the specific mycotoxin present in the sample. We show that 689 samples (96.8%) complied with the regulations set by the Ministry of Health and Welfare. AFs were found in four peanut-candy products, one peanut-flour product, one pistachio product, one Sichuan-pepper product, and one *Coix* seed product. All had exceeded the maximum levels of 15 parts per billion for peanut and 10 parts per billion for other food products. Furthermore, 14 RYR samples contained CIT above 5 parts per million, and one RYR tablet exceeded the maximum amount allowed. Instances of AFs in substandard Sichuan pepper and *Coix* seeds were first detected in Taiwan. Measures were taken by the relevant authorities to remove substandard products from the market in order to decrease consumer exposure to mycotoxin. Border control measures were applied to importing food commodities with a higher risk of mycotoxin contamination, such as peanut, Sichuan pepper, and RYR products. Declining trends were observed in the noncompliance rate of AFs in peanut products, as well as that of CIT in RYR raw materials monitored from 2010 to 2013.

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1. Introduction

Mycotoxins are toxic metabolites produced by fungi. They are mostly found as natural contaminants of food products sold by supermarket chains and grocery markets, and are detrimental to human health [1,2]. Currently, more than 400 different types of mycotoxins have been identified. However, the most significant type presenting strong public-health concerns is aflatoxin (AF), followed by ochratoxin A (OTA), and other *Fusarium* toxins [3]. The occurrence of mycotoxins in food often corresponds to a geographical pattern. Worldwide food trading results in worldwide distribution of contaminated materials [4].

Aspergillus parasiticus and *Aspergillus flavus* represent the major proportion of AFs found in peanuts, nuts, seeds, spices, and various other crops and food products. Both of them contain AF-producing gene clusters. *A. parasiticus* produces AFB_1, AFB_2, AFG_1, and AFG_2. However, *A. flavus* only produces AFB_1 and AFB_2 [5,6]. AF production was observed in *A. parasiticus*, grown on media with glucose or lactose as the sole carbon source [7,8]. AF production was enhanced in the presence of sugar and unsaturated fatty acids in media [7,9]. AFB_1 is one of the most potent natural carcinogens known [10]. Regardless of the ingested dose level, its cumulative effect is to increase cancer risk and heighten the probability of liver cancer in patients suffering from hepatitis B or hepatitis C. Interestingly, children display the greatest susceptibility to AFs [11]. The International Agency for Research on Cancer (IARC) classified AFs as a group 1 human carcinogen [10]. The CODEX Alimentarius Commission (CAC) recommended that intake should be reduced to levels as low as reasonably possible for AF B, G, and M [12].

OTA is a common contaminant of grain storage in temperate regions. The gene clusters responsible for the biosynthesis of OTA have been identified in *Aspergillus* and *Penicillium* genera [13]. The presence of OTA has also been reported in food categories, such as rice, wheat, coffee, dried fruit, and spices. OTA exhibits nephrotoxic, carcinogenic, and immunotoxic properties. Its main target is the renal proximal tubule, where it exerts cytotoxic and carcinogenic effects [14]. The IARC classified OTA as class 2B, indicating that it is a possible human carcinogen [15]. OTA has a half-life of up to 840 hours in the human bloodstream with cumulative effect *in vivo* [11]. The CAC set a provisional tolerable weekly intake (PTWI) of 0.0001 mg/kg body weight (BW) [12].

Red yeast rice (RYR) made from *Monascus*-fermented cooked rice is a traditional cuisine in Taiwan, and is often used as food coloring and preservative. The lactone form of monacolin K produced by *Monascus purpureus*, also known as lovastatin, has an inhibitory effect on cholesterol synthesis [16]. Since lovastatin became a patented prescription drug, monacolin K-containing *Monascus* products can only be used as food or nonprescription dietary supplements [17]. However, some strains of *Monascus* contain gene clusters responsible for the biosynthesis of monacolin K and citrinin (CIT) [18,19]. The toxicological effects of such strains have been shown to arise through interference with mitochondrial electron transport and calcium homeostasis, leading to kidney swelling or necrosis in animal studies [20,21]. The IARC has designated CIT as group 3, indicating that it is not classifiable as a human carcinogen [22]. In a 90-day study, the nonobservable adverse effect level of CIT was determined to be 20 μg/kg of BW/day in rats [23]. The European Union (EU) recommended the level of no concern of nephrotoxicity as 0.2 μg/kg of BW/day [17].

Mycotoxin legal limits were established in several countries and international organizations worldwide, specifying the maximum limits for mycotoxins [12,24–27]. Taiwan set a total AF limit of 15 parts per billion (ppb) for peanut and maize, and 10 ppb for other foods. The EU set the lowest limits at 4 ppb for total AFs and 2 ppb for AFB_1. However, the United States regulates all foods at 20 ppb for AFs. CAC provisions stipulate 10 ppb for ready-to-eat nuts and 15 ppb for peanuts and nuts intended for further processing. For OTA, the limits were set at 5 ppb for coffee and wheat and rice categories, which is similar to the EU. As for CIT content requirements, RYR pigments, raw RYR, and RYR-based foods had limits set at 200 ppb, 5 parts per million (ppm), and 2 ppm, respectively, or below (Table 1).

It is difficult to remove mycotoxins from food products once contaminated. Previous studies have demonstrated higher incidences of AF contamination in peanuts, nuts, and spices, as well as CIT in RYR products [28,29]. In Taiwan, rice and wheat flour are staple foods, and the consumption of coffee has increased in the last decade. In order to protect the consumer's food safety, a market-monitoring plan was designed to investigate the levels of AFs in peanuts, nuts, and spices; OTA in coffee and wheat products; AFs and OTA in rice-based products; and CIT in RYR-based products. The results of this investigation will provide health authorities with an assessment of mycotoxin contamination in circulating foods in domestic markets, and serve as a reference for food management.

2. Methods

2.1. Samples

A total of 712 samples, including peanut products, nuts, dried fruit, wheat and rice products, *Coix* seeds, coffee, and RYR products, were collected from supermarkets, traditional markets, and grain dealers by 22 local health bureaus within their jurisdictions, between March 2012 and September 2013. The samples were sent to the laboratory, where they were grounded with a grinding mill (ZM 200; Retsch, Haan, Germany) and stored at 4°C prior to further analysis. For capsule-supplement samples, the capsule walls were removed before testing.

2.2. Chemicals

The AFB_1, AFB_2, AFG_1, AFG_2, and OTA standards were purchased from Supelco (St. Louis, MO, USA). The CIT standards were purchased from Fermentek (Jerusalem, Israel). The AflaTest-P and OchraTest columns were both purchased from Waters (Milford, MA, USA). Mass spectrometry (MS) grade methanol and acetonitrile were purchased from J.T. Baker (Center Valley, PA, USA). Sodium bicarbonate, sodium chloride,