Food Control 38 (2014) 178–183

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Incidence of citrinin in red yeast rice and various commercial *Monascus* products in Taiwan from 2009 to 2012

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A R T I C L E I N F O

Article history:
Received 30 May 2013
Received in revised form
7 October 2013
Accepted 13 October 2013

Keywords:
Mycotoxin
Citrinin
Red yeast rice
Monascus products
Taiwan

A B S T R A C T

Red yeast rice, produced by the fermentation of red yeast (*Monascus purpureus*) with white rice, has long been used in food colouring and meat preservation in Asia. Recently, powdered red yeast rice and its formulated products, used worldwide as dietary supplements, have the ability of reducing blood-lipid levels in humans. However, some *Monascus* strains could produce the mycotoxin citrinin as a secondary toxic metabolite so that commercial *Monascus* products are of a serious concern to the public now. The aim of this study was to obtain information about the occurrence of citrinin in red yeast rice and various commercial *Monascus* products in Taiwan from 2009 to 2012. A simple and rapid HPLC-fluorescence method was developed to detect citrinin in red yeast rice and *Monascus* products. The method's performance was acceptable in terms of recoveries, which ranged from 81.2% to 94.3% for citrinin-spiked samples at levels of 0.1, 1 and 10 mg/kg, and the relative standard deviation ranged from 2.5% to 5.7%. The limit of quantification of 0.05 mg/kg was achieved. The survey results showed that among total 302 samples, the incidences of citrinin contamination were 69.0%, 35.1% and 5.7% for red yeast rice (raw material), dietary supplements and red yeast rice processed products, respectively. The mean contamination levels were 13.3, 1.2 and 0.1 mg/kg for red yeast rice (raw material), dietary supplements and red yeast rice processed products, respectively. Such high citrinin contamination rate and level is worthy of note.

1. Introduction

Red yeast rice is the fermentation product of ordinary rice with *Monascus purpureus*. It is traditionally used for the colouring, flavouring and preservation of foods over one thousand year in East Asia and still commonly used in the Chinese cuisine. Red yeast rice has been used commercially to produce valuable secondary metabolites, such as monacolin K, γ-aminobutyric acid and 3-hydroxy-4-methoxy-benzoic acid (Su, Wang, Lin, & Pan, 2003). Among them, monacolin K is the same substance that is synthetically isolated from *Aspergillus terreus* and FDA approved as lovastatin, an inhibitor of hydroxylglutaryl-coenzyme A reductase and cholesterol synthesis (Bogsrud, Ose, & Langlet, 2010). Powdered red yeast rice and its formulated products, used worldwide as dietary supplements, have the ability of reducing blood-lipid levels in humans (Bliznakov, 2000). A number of studies have demonstrated that red yeast rice exerted biological and pharmacological effects including antioxidative effects, antihyperlipidemic effects, antidiabetic effects, anti-inflammatory effects, anti-Alzheimer's effects, antihypercholesterolemic effects, antihypertensive effects and anticancer effects (Hsieh & Tsai, 2003; Lee, Lee, Hwang, Lee, & Wang, 2013; Mohan-Kumari, Dhale, Akhilender-Naidu, & Vijayalakshmi, 2011; Shi & Pan, 2011).

However, some *Monascus* strains could produce the mycotoxin citrinin as a secondary toxic metabolite that was previously found mainly in *Aspergillus* and *Penicillium* genera (Blanc, Loret, & Goma, 1995). Citrinin is a known naturally occurring contaminant in stored food commodities such as corn, wheat, barley and rice. Citrinin-induced nephrotoxicity has been demonstrated in various cellular and animal models (Kogika, Hagiwara, & Mirandola, 1993; Lockard, Phillips, Hayes, Berndt, & O'Neal, 1980). Citrinin is nephrotoxic and a no-observed-adverse-effect level (NOAEL) of 20 μg/kg body weight per day was identified from a 90-day study in rats (EFSA, 2012). Possible toxicological mechanisms of citrinin in renal and liver mitochondria include interference with the electron transport system, alteration of Ca^{2+} homeostasis, and generation of oxidative stress (Aleo, Wyatt, & Schnellmann, 1991; Chagas, Oliveira, Campello, & Kluppel, 1995). Citrinin also induces chromosomal abnormalities in the bone marrow cells of mice (Jeswal, 1996) and shows aneuploidogenic potential in V79 cells (Pfeiffer, Grob, & Metzler, 1998). For these reasons, commercial *Monascus*

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http://dx.doi.org/10.1016/j.foodcont.2013.10.016

products are of a serious concern to the public. However, the specific legislation for citrinin is limited worldwide currently. In Japan, the maximum allowed level of citrinin in red fermented rice is 200 ppb. In Taiwan, the regulatory limits of citrinin in red yeast rice (raw material) and *Monascus* products are 5 ppm and 2 ppm, respectively (Taiwan FDA, 2009). The USFDA is not regulating manufacturers of red yeast rice products and as a result, many of these products may contain monacolin K and toxins such as citrinin (Childress, Gay, Zargar, & Ito, 2013).

The analytical methods of citrinin reported in current studies include enzyme-linked immunosorbent assay (ELISA), thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) and capillary chromatography (Abramson, 1999; Lee, Wang, & Pan, 2006; Mornar, Sertic, & Nigovic, 2013; Nigovic, Sertic, & Mornar, 2013; Yu, Lee, & Pan, 2006; Zheng, Xin, & Guo, 2009). Citrinin has a conjugated, planar structure that gives it natural fluorescence ability (Fig. 1), which makes it feasible for qualitative and quantitative determination of citrinin by using a fluorometer. Thus, HPLC with fluorescence detection is the main analytical method now. In the last few years, liquid chromatography coupled with mass spectrometry (LC–MS) has also been an effective analytical tool for the analysis of citrinin, especially for confirmation.

Although citrinin is a possible contaminant of *Monascus* fermentation products such as red yeast rice dietary supplements, little information is available on the incidence of citrinin. The surveillance data of citrinin in red yeast rice and *Monascus* products is limited in the literature. The aim of this survey is to investigate the incidence of citrinin contamination in commercial red yeast rice and various kinds of *Monascus* products in Taiwan.

2. Materials and methods

2.1. Chemicals and reagent

Citrinin standard was purchased from Sigma–Aldrich (St. Louis, MO, USA). LC grade acetonitrile and methanol were purchased from Merck Co. (Darmstadt, Germany). The acidifier, formic acid, was purchased from J.T. Baker (USA).

2.2. Preparation of standard solution

The stock solutions of citrinin were prepared by dissolving 5 mg of citrinin in 10 mL of methanol and stored at −20 °C. The standard solutions were freshly prepared from appropriate dilutions of the stock solution with methanol (2.5, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/mL).

2.3. Sampling

A total of 302 samples of several kinds, including 84 red yeast rice (raw material), 77 dietary supplements (tablets or capsules

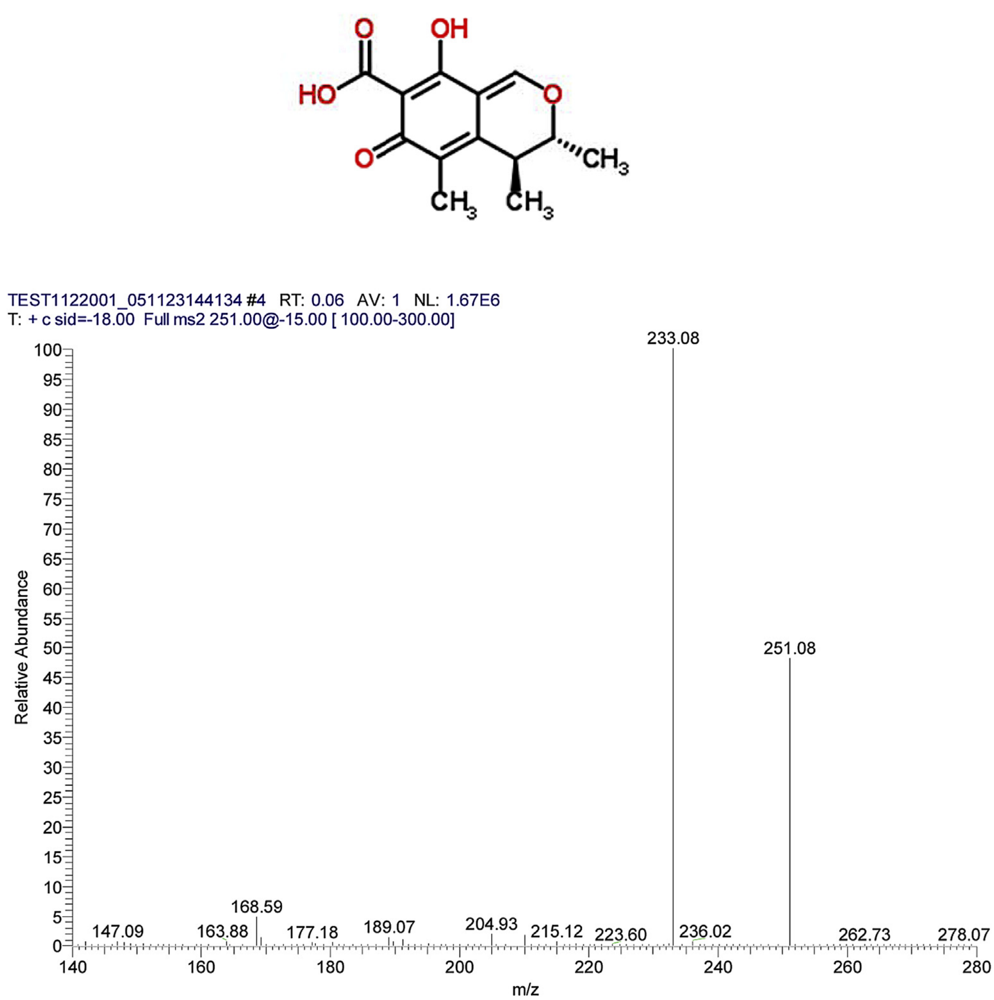

Fig. 1. Chemical structure and MS spectra of citrinin.