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Comparison of extraction methods for analysis of citrinin in red fermented rice

Weiping Wang [a,*], Qi Chen [a], Xinfeng Zhang [a], Huashan Zhang [a], Qin Huang [a], Dongsheng Li [a,*], Jicheng Yao [b]

[a] Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Collaborative Innovation Center for Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China
[b] Wuhan Jiacheng Biotechnology Co., Ltd., Wuhan 430074, China

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ABSTRACT

There is no standard method for the extraction and analysis of citrinin in red fermented rice (RFR). In the study, five extraction methods were compared for their efficiency to analyse citrinin in RFR by HPLC–FLD (reversed phase high performance liquid chromatography with fluorescence detection), including, (1) ultrasonic extraction with EW solution (ethanol:water, 7:3, v/v); (2) ultrasonic extraction with TEF solvent mixtures (toluene:ethyl acetate:formic acid, 7:3:1, v/v); (3) shaking extraction with EW; (4) shaking extraction with EF solvent mixtures (ethyl acetate:formic acid, 1:1, v/v); (5) shaking combined with ultrasonic extraction in EW. Comparison of chromatograms of citrinin by HPLC–FLD with different extraction methods revealed that EW was the best extraction solvent. It was also found that shaking combined with ultrasonic extraction in EW was the most efficient extraction method to extract citrinin from RFR for qualitative and quantitative analysis.

1. Introduction

Red fermented rice (RFR) was obtained as cultures of fungal species of the genus *Monascus* on rice, which had long been used as a natural food colourant and medicine in East Asia for centuries (Baipong & Pinthong, 2003). Recently, it had also been used as a food additive and a dietary supplement in Europe and the U.S. However, another secondary metabolite, known as citrinin, a hepato-nephrotoxic mycotoxins for humans (Meister, 2003), was also found to be synthesized by *Monascus* strains (Blanc et al., 1995). It was reported that citrinin could cause 50% cell death to human embryonic kidney cell in a range of 1.8–4.7 mg/ml (Liu, Wu, Su, Chung, & Yu, 2005). According to animal tests, it was proposed that less than 2 ppm citrinin in *Monascus*-fermented products might be a safe concentration (Lee, Lee, & Pan, 2010). Natural occurrence of citrinin exists in widely-consumed-traditional RFR. Hence, to ensure the safety of RFR, it is important to accurately determine citrinin in RFR as well as their related products.

So far, there was no standard method for the analysis of citrinin in RFR. Xu, Jia, Gu, and Sung (2006) summarised the most important achievements on the analytical methods for citrinin published from 1980 to 2004. Commonly-used methods for analysing citrinin were thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) with UV or fluorescence detection (FLD), enzyme immunoassays (EIA) (Heber, Lembertas, Lu, Bowerman, & Go, 2001), as well as micro-fluidic electrochemical immunoassays (MFECIA) (Arévalo, Granero, Fernández, Raba, & Zón, 2011). Although the EIA method or MFECIA method was developed, it had not been fully-developed. Other methods used neutral column, aminopropyl column (Hartl & Stenzel, 2007) or polyamide column (Meister, 2004) to extract citrinin, but there were too many operating steps on the column chromatography, which might bring loss or bad chromatograms. Among these, the HPLC–FLD method had been successfully applied to the analysis of citrinin in grains and RFR (Huang et al., 2011; Meister, 2003, 2004; Schmidt, Brockmeyer, Knor, & Thielert, 2003). HPLC–FLD was considered as a quick method for qualitative and quantitative determination of citrinin.

The aforementioned methods for citrinin extraction were lack of consistency. Researchers had utilised different extraction solvents or techniques for extraction of citrinin from RFR. Although ultrasonic extraction technique and shaking extraction technique were mostly used to extract citrinin in reported articles (Carvalho et al., 2006; Jia, Xu, Zhou, & Sung, 2010; Li, Wu, Guo, Zheng, & Guo, 2012; Pattanagul, Pinthong, Phianmongkhol, & Tharatha, 2008; Zaied, Zouaoui, Bacha, & Abid, 2012), these methods differed

* Corresponding authors. Tel.: +86 27 59750481; fax: +86 27 59750482.
E-mail addresses: Wang1wei1ping1@163.com (W. Wang), Dongshengli86@163.com (D. Li).

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significantly in their extraction solvents and sample extraction procedures. Shaking extraction or ultrasonic extraction with EW solution (ethanol: water, 7:3, v/v) was a preferred method for determination of citirin contents in RFR (Carvalho et al., 2006; Li et al., 2012; Pattanagul et al., 2008). Pattanagul et al. (2008) and Carvalho et al. (2006) reported a shaking extraction technique with EW as extraction solvent. Li et al. (2012) treated RFR with ultrasonication using EW to extract citrinin. However, Zaied et al. (2012) extracted citrinin by shaking with acetonitrile-4% aqueous solution of potassium chloride (90/10: v/v). Jia et al. (2010) reported sonication extraction technique using acetonitrile/water (60:40, v:v) as extraction solvent. It was noteworthy that none of these methods compared their efficiency for the extraction of citrinin from RFR in their works.

The present study aimed to find a more efficient extraction method for qualitative and quantitative determination of citrinin using HPLC–FLD. Optimum conditions for chromatographic separation and extraction efficiency were investigated. The development of the method was based on the comparison of ultrasonic or shaking extraction with EW and other solvent mixtures.

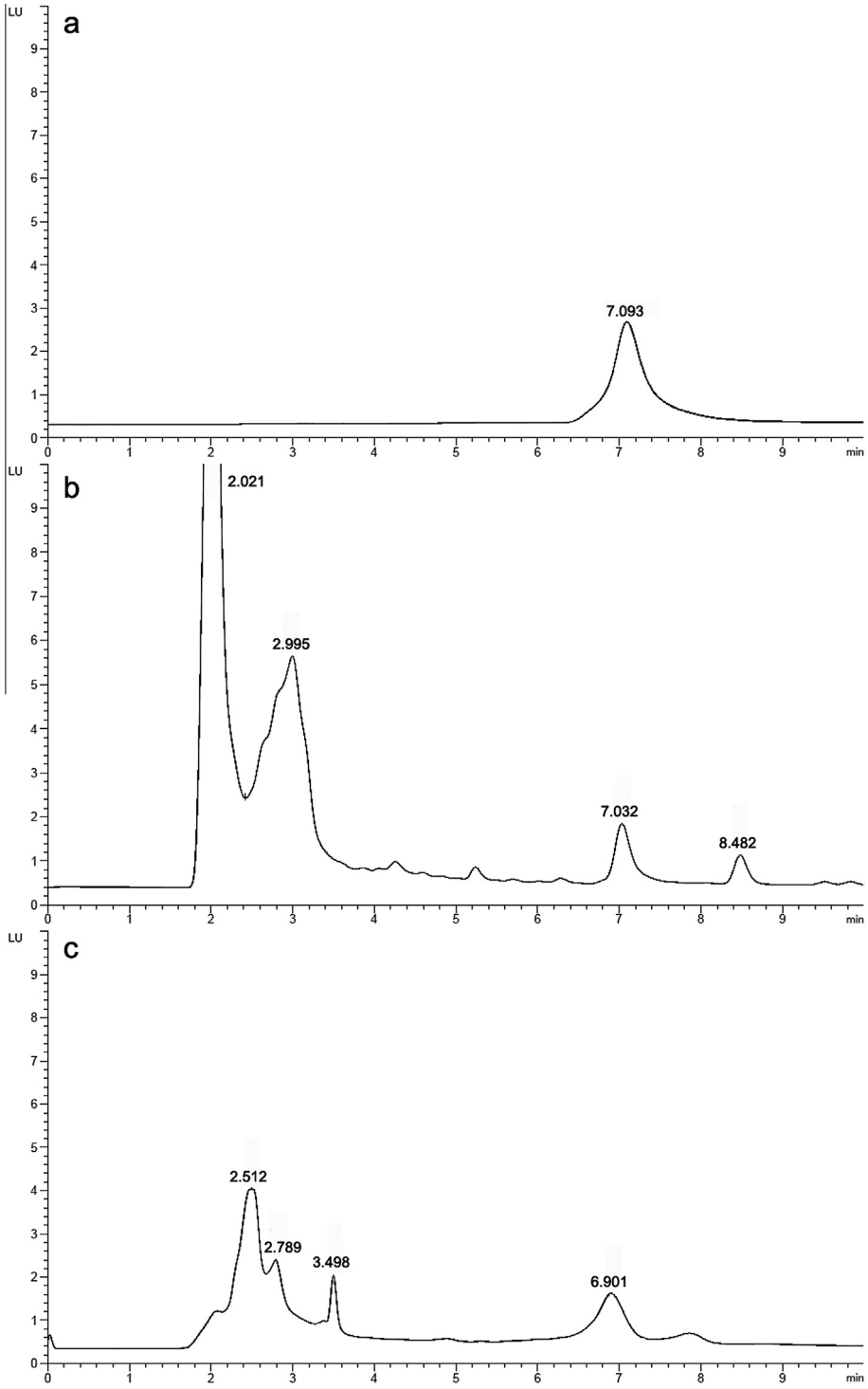

Fig. 1. Chromatograms of citrinin standard (a), and citrinin extracted by ultrasonication with EW (b) or TEF (c) from red fermented rice by HPLC.