Food Chemistry 170 (2015) 186–192

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Optimisation of ultrasound-assisted extraction conditions for maximal recovery of active monacolins and removal of toxic citrinin from red yeast rice by a full factorial design coupled with response surface methodology

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ARTICLE INFO

Article history:
Received 18 February 2014
Received in revised form 11 August 2014
Accepted 15 August 2014
Available online 24 August 2014

Keywords:
Ultrasonic-assisted extraction
Red yeast rice
Monacolins
Citrinin
Response surface methodology
Functional foods

ABSTRACT

This study optimised the ultrasound-assisted extraction (UAE) conditions to achieve maximal recovery of active monacolins with minimal contents of citrinin from red yeast rice (RYR). A central composite design after a full factorial design was utilised to examine the different UAE parameters. The studies revealed that HAc%, extraction time and EtOH% had significant influences on the recovery yield of monacolins, while HAc% and EtOH% were key factors for the elimination of citrinin. The resulting optimal conditions were as follows: ultrasound power of 250 W, HAc% of 7.7%, RYR amount of 0.2 g (solvent-to-solid ratio 40 mL/g), extraction time of 50.7 min, EtOH% of 57.2% and extraction temperature of 20 °C. Under these conditions, at least 94.7% of monacolins was recovered and 87.7% of citrinin was removed from RYR. This optimised UAE condition was further evaluated for potential industrial application in manufacturing of RYR as pharmaceuticals and nutraceuticals.

1. Introduction

Natural products as pharmaceuticals and nutraceuticals with positive effects on human health have been popular in our daily life. Red yeast rice (RYR), a well-known functional natural product from fermentation of steamed rice using fungus *Monascus purpureus*, has been widely used, especially in Eastern Asia, to improve digestion and blood circulation (Feng, Shao, & Chen, 2012; Zhu et al., 2012). RYR was found to contain a family of important secondary metabolites, such as monacolins, that are widely used in pharmaceutical and food industries (Hong, Seeram, Zhang, & Heber, 2008). Monacolins are known to be responsible for inhibiting hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, and thus regulate the cholesterol synthesis in liver. In clinic, monacolins have remarkable therapeutic effects on lipid profiles of hypercholesterolemic patients (Gordon, Cooperman, Obermeyer, & Becker, 2010). Monacolin K (a.k.a. lovastatin), one of the first commercially available HMG-CoA reductase inhibitors, was the major monacolin member in RYR. In addition, monacolins were also evaluated for their potential anticancer activities in colorectal cancer (Poynter et al., 2005) and breast cancer (Campbell et al., 2006) etc.

Monacolins have been of great interest to the food and pharmaceutical industries due to their inimitable benefits, an efficient process is therefore required to achieve maximal recovery of natural monacolins from RYR. However, due to the ubiquitous nature of several fungal species, citrinin was also formed during the fermentation process. Citrinin is a mycotoxin with hepatotoxicity and nephrotoxicity and is one of the most prevalent human contaminants in the food chain (Lee, Hung, et al., 2007), and must be limited to no-more-than 200 ppb in a daily administration (Nigovic, Sertic, & Mornar, 2013). Several clinical trials have found that 5 mg/day of monacolins in RYR has a comparable efficacy to 20 mg/day of pure monacolin K in lowering blood cholesterol (Chen, Yang, Uang, & Lin, 2013). Hence, the acceptable content ratio of monacolins and citrinin (M/C) in a final product must be great than 25 (5000/200), in order to be considered as effective and safe.

To achieve that ratio, some researches focused on general culture conditions and substrate evaluation for reducing citrinin while retaining monacolins formations in RYR (Lee, Chen, et al., 2007; Lee, Hung, et al., 2007). However, the selective culture conditions were not always suitable for every *Monascus* species, even

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http://dx.doi.org/10.1016/j.foodchem.2014.08.080
0308-8146/

though altering culture condition was able to reduce the citrinin formation. On the other hand, the use of the optimal culture condition on an industrial scale proves to be expensive and hard to manipulate for monacolins formation in RYR (Sun, Zou, Liu, & Xiao, 2011).

The development of a postprocess to selectively remove citrinin while retain monacolins from RYR product was proposed as an alternative solution to the above problem. Extraction is often utilised as an efficient and important technique in the recovery and purification of target substances from plant materials. Recently, various extraction techniques, such as heating, boiling, Soxhlet, accelerated solvent and ultrasound extraction, were developed for the recovery of monacolins from RYR (Liu, Guo, Duan, Wang, & Du, 2010; Nigovic et al., 2013) but the content of citrinin by-product was always neglected in these practices.

Ultrasound-assisted extraction (UAE) exhibited the best mass transfer, cell disruption, solvent penetration and capillary effect (Da Porto, Porretto, & Decorti, 2013) and it did not damage the temperature sensitive botanical materials (Peralta-Jiménez & Cañizares-Macías, 2013). Moreover, UAE was the simplest and most economical technique, and easy to scale up for industrial production (Delgado-Povedano & Luque de Castro, 2013; Wang et al., 2013).

Many factors, including ultrasound power, extraction time and temperature, and solvent-to-material ratio etc., can influence the UAE process, individually and collectively (Xu et al., 2013), and it is difficult to single out main independent variables to optimise (Meziane, 2013). The conventional multivariable optimisation is usually based on the "one-factor-at-a-time" approach, which is unable to detect interactions among independent variables, and lack of complete information on effects of all determinants (Meziane, 2013; Xu et al., 2013). Response surface methodology (RSM) is a useful tool for evaluating multiple parameters and their interactions based on quantitative data (Li et al., 2011), and may effectively overcome the drawback of classic "one-factor-at-a-time" or "full-factors" approach.

The objectives of this study were (1) to establish an optimised simple, safe and low cost process of UAE for the food and pharmaceutical industries to extract maximum yield of monacolins with minimum citrinin content from RYR, and (2) to develop a rapid and accurate quality control method for the determination of monacolins contents using UHPLC-DAD–Q-TOF/MS. To the best of our knowledge, there has been no report on RSM application to the optimisation of UAE conditions for obtaining the high yield of monacolins with low content of citrinin in RYR.

2. Experimental

2.1. Materials and samples

The acetonitrile (ACN), formic acid (HCOOH) and trifluoroaceticacid (TFA) were of HPLC grade. All other reagents and chemicals were of analytical grade. Monacolin K was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Citrinin was supplied by Sigma–Aldrich (St. Louis, MO, USA). Monacolin K (2.2 mg/mL) was prepared using ACN/H_2O/HCOOH (53/47/0.1, v/v), while a stock solution of citrinin (0.8 mg/mL) was prepared by dissolving with ACN/H_2O/TFA (55/45/0.05, v/v). In order to prepare calibrators, a series of calibration curve solutions were prepared by appropriate dilution of the stock solution with corresponding solution. All of the standard solutions were stored at 4 °C until further use.

The RYR powder was kindly provided by Hangzhou Boda Biology Technology Co., Ltd., Zhejiang, China. A voucher specimen (SJTU 20131012) was deposited in the School of Pharmacy, Shanghai Jiao Tong University.

2.2. Analytical methods

2.2.1. UHPLC-DAD–Q/TOF-MS analysis of monacolins

In this study, ultra high performance liquid chromatography coupled with diode array detector–Q/TOF mass spectrometry (UHPLC-DAD–Q/TOF-MS) was used to identify and determinate monacolins. UHPLC was performed using Agilent 1290 series Rapid Resolution LC (Agilent Technologies, CA, USA) consisting of a vacuum degasser, auto sampler and DAD. An Agilent Zorbax SB C18 column (2.1 × 150 mm, 1.8 μm) maintained at 30 °C was used with an injection volume of 2 μL. Mobile phase A was a 0.1% formic acid/water solution (1/1000, v/v), mobile phase B was a formic acid/acetonitrile solution (1/1000, v/v), and the flow rate was 0.7 mL/min. The linear gradient conditions were: 0–3 min, 53% B; 3–5 min, 53%–70% B; and 5–6 min, 70% B. Monacolins were detected at 237 nm. The MS analysis was performed on a Micromass Q/TOF mass spectrometer (Micromass MS Technologies, Manchester, UK) via an electrospray ionisation interface and acquired in positive ion mode. The parameters in the source were set as follows: capillary voltage = 3 kV; source temperature = 150 °C; desolvation temperature = 550 °C; cone gas flow = 50 L/h and desolvation gas flow = 1000 L/h.

2.2.2. HPLC-FD analysis of citrinin

The content of citrinin was determined by the method of high-performance liquid chromatography with fluorescence detection (HPLC-FD) (Lee, Wang, & Pan, 2006). An Agilent C18 column (4.6 × 25 cm, 5 μm Agilent Technologies, USA) was utilised to separate citrinin from other peaks by isocratic elution with a mobile phase comprising 0.05% TFA in acetonitrile–water (55 + 45, v/v). Citrinin was detected by fluorescence detection with λex = 330 nm and λem = 500 nm.

2.3. Extraction procedures

The extraction process was performed with an ultrasonic device (SY-5200T, 55 kHz, 250 W, Shanghai Shenyuan Ultrasonic Instrument Co. Shanghai, China). The extraction of RYR was carried out using an ethanol (EtOH)/acetic acid (HAc)/water (H_2O) solution at different ratio (%, v/v) and a total volume of 8.0 mL. Different amount of the RYR powder and EtOH/HAc/H_2O solution were mixed in each extraction (the factor of solvent-to-solid ratio) and sonicated at various times and temperatures under several sets of designed UAE conditions. After the ultrasonic extraction, the sample was centrifuged at 13,000 rpm for 10 min to collect the supernatant for analysis.

2.4. Experimental design

Experimental design and study were performed using a full factorial design (FFD) followed with a rotatable central composite design (RCCD). Initially, the FFD was applied to identify the significant variables that influenced the yields of monacolins and citrinin from RYR. Immediately, the variables of significance resulted from FFD were investigated by RSM with RCCD. The software STATGRAPHICS plus 5.1 was employed to complete the full design and statistical analysis of data, and then RSM (CCD) was done by Design–Expert 8.5. The variables with confidence levels above 95% were considered as influencing the yield of target compounds from RYR significantly.

A two-level FFD was created to screen the main variables affecting the UAE procedures. This technique was a powerful tool for screening the key variables in a multivariable system. The number of experiments in FFD was given by $2^k + C$, where k was the number of variables, and C was the number of central points (Pinto, Melo, & Ferreira, 2014). In this study, the FFD screening experiments were