Separation and Purification Technology 110 (2013) 158–163

Contents lists available at SciVerse ScienceDirect

Separation and Purification Technology

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

Aqueous two-phase system for citrinin extraction from fermentation broth

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ARTICLE INFO

Article history:
Received 28 August 2012
Received in revised form 8 February 2013
Accepted 8 March 2013
Available online 21 March 2013

Keywords:
Citrinin
Penicillium citrinum
Aqueous two-phase system
Mycotoxin
Rotator disc contactor

ABSTRACT

Aqueous two-phase systems (ATPSs) were investigated for effective extraction of citrinin from *Penicillium citrinum* fermentation broth. The citrinin partitioning behaviour in each ATPS (polyethylene glycol–phosphate salts) was evaluated in terms of the following parameters: polyethylene glycol (PEG) molecular weight, pH and tie line length. The target molecule (citrinin) presented high affinity to the PEG rich phase in most of the experiments. The best recovery yield (98.4%) was obtained using PEG 4000 (20% w/w)/phosphate salt (16.7% w/w), pH 3.4 at room temperature (28–30 °C), but the best citrinin partitioning (36.41) was observed at pH 5.7 using the same ATPS. Continuous citrinin extraction studies were performed using the fermentation broth and this ATPS in a perforated rotating disc contactor (PRDC). Recovery up to 98.44% supports the possible use of this system on a laboratory scale.

1. Introduction

Citrinin [$C_{13}H_{14}O_5$, IUPAC: (3R,4S)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid], molecular weight of 250.25 g/mol, is an acidic lemon-yellow crystal with maximal UV absorption at 250 nm and 333 nm (in methanol), melting at 172 °C. It is sparingly soluble in water but soluble in dilute sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most of other polar organic solvents [1,2].

This mycotoxin is produced by several filamentous fungi of the genera *Penicillium*, *Aspergillus* and *Monascus*, which has been known as a natural contaminant in grains, foods, feeds, as well as biological fluids [3]. It is hepatonephrotoxic and implicated in disease outbreaks (nervous system, immunosuppression and carcinogenesis) in animals and humans [1].

The production of citrinin by Brazilian strain of *Penicillium citrinum* was studied by Pimentel et al. [4,5], using olive oil and yeast extract or ammonium sulphate as carbon and nitrogen sources, respectively. Their results showed that the *Penicillium citrinum* did not produce citrinin in olive oil and ammonium sulphate culture medium.

In order to be detected, citrinin must be extracted from the contaminated food or agricultural products and cleaned-up prior to Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), immunoassay, counter current chromatography (CCC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE). Immunoafinity clean-up techniques with high-resolution chromatography showed particular promise for mycotoxin [2]. Citrinin extraction using a method based on solvent partition was validated in breakfast cereals collected in French supermarkets. Only a few methods were studied based on the Association of Official Analytical Chemists International but none addressed complex mixtures as breakfast cereals. The techniques, in general, for mycotoxin extraction are sophisticated and expensive and there is no validated technique for analysis in the whole processed food [6].

An aqueous two-phase system (ATPS) has been studied as an alternative process for cost reduction and decreasing the number of purification steps [7]. ATPSs have been used for recovering biological products from different sources, because of several advantages: (a) providing environments gentle enough to preserve the functionality of biomolecules; (b) scaling up the operation is easy; (c) samples with solid particulates can be handled; (d) mass transfer is fast and (e) equilibrium can be reached in a matter of

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http://dx.doi.org/10.1016/j.seppur.2013.03.021

seconds. It is also a versatile and adaptable technique, because many parameters can be adjusted to obtain optimised conditions. Only certain salts and polyethylene glycol (PEG) are required in the system, which makes the technique also very economical [8–10]. This system also has great potential in downstream processing of large-scale continuous processes [11] because the ATPS are economically viable and can potentially reduce the environmental impact of wastewater [12].

Different types of columns have been described for use in ATPS, such as spray [13], packed bed [14], york-scheibel [15], rotating disc contactor [16], and perforated rotating disc contactor (PRDC) [17–19]. The perforated rotating disc contactor (PRDC) is well suited for systems with low interfacial tension between the phases such as ATPS formed by PEG–phosphate salts [19,20].

The purpose of this work is to study the parameters for citrinin extraction from *Penicillium citrinum* fermentation broth using aqueous two-phase systems (ATPSs) in discontinuous and continuous modes. However, due to the lack of references about the continuous extractions of small and non-proteic mycotoxin molecules using biphasic systems in PRDC systems, this work is original and pioneer at the moment.

2. Materials and methods

2.1. Materials

The Brazilian strain of *Penicillium citrinum* URM = 4216 (Universidade Federal de Pernambuco – UFPE, Brazil) was kindly supplied by Department of Mycology – UFPE. This strain was isolated from olive oil according to Pimentel et al. [4,5].

2.1.1. Chemicals and reagents

Polyethylene glycol (PEG) and citrinin were from Sigma Chemical Company (Missouri, USA). All other chemicals were of analytical grade from Merck (NJ, USA).

2.2. Methods

2.2.1. Citrinin production

The Brazilian strain of *Penicillium citrinum* was maintained at 28 °C on slants of potato-dextrose-agar (PDA) made up of potato (14 g/L), dextrose (20 g/L) and agar (20 g/L). The fermentation broth (500 mL), containing citrinin, was prepared in Erlenmeyer flask (2 L) containing PD medium and using pre-inoculum (10^7 - cells/mL for 50 mL of PD medium), both incubated at the same conditions, 72 h at 28 °C and orbital shaker (100 rev/min). The citrinin was produced in the stationary phase of fermentation and its concentration was determined according to Pimentel et al. [5]. The fermentation broth was filtered and centrifuged for 15 min at 12500*g* to remove the biomass completely and the supernatant was used for ATPS extraction processes as described below.

2.2.2. Preparation of aqueous two-phase systems

In order to obtain different tie-lines (Table 1), the ATPS was prepared with appropriate quantities of 50% (w/w) PEG solution, 40% (w/w) potassium phosphates (K_2HPO_4 and KH_2PO_4) solutions, which pH was adjusted to (3.4; 5.7; 7.0 and 9.8) using, when necessary, hydrochloride acid or potassium hydroxide solution at room temperature (28 ± 2 °C). Distilled water was added to final system amount of 6 g. After vortex shaking for 3 min, the fermentation broth (0.5 mL, 8.3%) was added and the mixture was centrifuged (1500*g* for 10 min) to separate the phases [21]. Samples from the phases were removed for measuring citrinin concentration. The extraction experiments were carried out at room temperature (28–30 °C) and in duplicate.

Table 1
Global composition of the aqueous two-phase system (ATPS) used for citrinin extraction from *Penicillium citrinum* fermentation broth, considering the final concentrations of PEG and potassium phosphate in each tie-line.

Tie-lines	PEG (%w/w)	Potassium phosphate (%w/w)
1	10	10
2	15	15
3	20	16.7

2.2.3. Perforated rotating disc contactor (PRDC)

Perforated rotating disc contactor was made using a Perspex column (32 mm internal diameter and 170 mm height). Three perforated discs (30 mm diameter) and drilled with 20 holes with 2 mm diameter (disc free area of about 9%) were mounted on a central shaft and equally separated. The column was maintained at 25 ± 1 °C throughout all the experiments, and the disc rotational speed was 220 rpm [11,17,19].

2.2.4. Continuous citrinin extraction using PRDC

The PRDC column was initially filled with 90 mL of the phosphate phase containing fermentation broth, with final citrinin concentration of 4–5 μg/mL. The dispersed phase (inlet PEG solution) and the continuous phase (inlet phosphate salt solution mixed with fermentation broth citrinin) were fed upward and downward. The extracted phase (outlet PEG solution enriched with citrinin) and the raffinate phase (outlet exhaust salt solution) were withdrawn at the top and the bottom of the column, respectively (Fig. 1). The column was operated counter-currently in a continuous mode for no less than 5–10 h to reach steady-state conditions. These conditions were assumed to take place in the system when no significant variations in the mass transfer coefficient, purification factor and separation efficiency were detected for at least 1 h. For this purpose, these parameters were measured every 10 min using samples from the raffinate and extracted phases. The four flow rates were kept constant by peristaltic pumps, and no liquid phase was recycled [17,19].

The system was tested at flow rates of 1 mL/min and 2 mL/min for continue and disperse phases, respectively, using a rotating speed of 140 rev/min. Samples for further citrinin concentration measurement from continuous and dispersed phases were withdrawn at intervals from 10 up to 70 min.

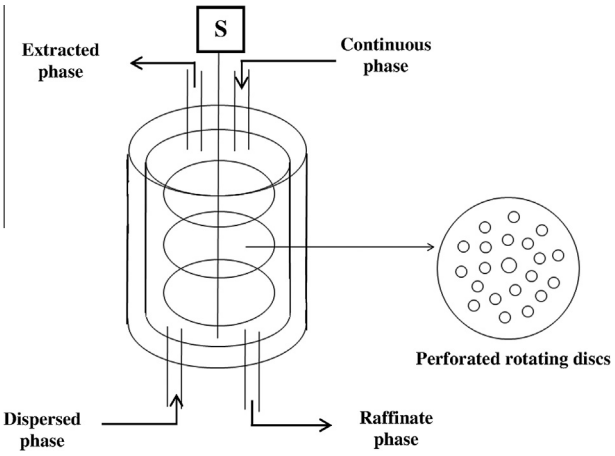

Fig. 1. Schematic diagram of the perforated rotating disc contactor (PRDC) used for the continuous citrinin extraction by two-phase PEG–phosphate system. Continuous phase, inlet phosphate salts solution containing crude-toxin (fermentation broth); dispersed phase, inlet PEG solution; extracted phase, outlet PEG solution enriched with crude-toxin; Raffinate phase, outlet exhaust salt solution. Shaft stirrer (S).