ELSEVIER

Biochemical Engineering Journal 32 (2006) 79–83

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Dynamic microwave-assisted extraction of flavonoids from *Saussurea medusa* Maxim cultured cells

Min Gao [a,b], Bao-Zhen Song [a], Chun-Zhao Liu [a,b,*]

[a] *National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China*

[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China*

Received 27 June 2006; received in revised form 7 September 2006; accepted 8 September 2006

Abstract

An approach for automated, continuous and rapid extraction of flavonoids from *Saussurea medusa* Maxim dried cell cultures has been developed in a new-designed dynamic microwave-assisted extraction system. The main factors affecting the extraction process namely power of microwave irradiation, liquid/solid ratio, flow rate of solvent and irradiation time were optimized. The yield of flavonoids reached 4.97% in 60 min under the optimum microwave-assisted extraction conditions: 1200 W of radiation power, 50:1 (v/w) of the liquid/solid ratio, and 50 ml s^{-1} of solvent flow rate. The dynamic microwave-assisted extraction showed obvious advantages in short duration and high efficiency to extract flavonoids without causing degradation of target components from the *S. medusa* dried cell cultures in comparison with the dynamic solvent extraction without microwave assistance.

Keywords: Dynamic microwave-assisted extraction; Flavonoids; Dynamic solvent extraction; *Saussurea medusa* Maxim; Plant cell cultures

1. Introduction

Saussurea medusa Maxim is one of the most important traditional medicinal plants in China and officially listed in the Chinese pharmacopoeia. The most important bioactive compounds in this elite medicinal species are flavonoids including rutin, jaceosidin, hispdulin and so on. These flavonoids have shown significant scavenging of free oxygen radicals and antidecrepitude activity [1]. Owing to over-exploitation of the wild plants for commercial purpose and the difficulty of cultivation, *S. medusa* is now almost extinct and has been listed as the second grade national protected wild plant in China [2]. In view of these problems, production of bioactive flavonoids by *S. medusa* cell cultures has considerable importance not only in the protection of natural plant resources, but also in its potential commercial interest [3–5].

Extraction is the first step for the recovery and purification of bioactive phytochemicals from plant materials. A number of traditional extraction methods have been employed in the past few years, including solvent extraction, heat reflux extraction, Soxhlet extraction, and so on. These traditional extraction processes are time-consuming and laborious, and involve lengthy operation techniques, bulk amount of solvents and ultimately thermal decomposition of the target molecules at continuous high temperature. Microwave-assisted extraction of biologically active compounds has many advantages over these traditional extraction methods such as shortened extraction time and lower consumption of solvents. Numerous biologically active compounds have been extracted with application of microwave-assisted extraction, such as extraction of glycerrhizic acid from *Glycyrrhizia glaubra* root [6], extraction of notoginseng from cultured cells of *Panax notoginseng* [7], and extraction of camptothecin from *Nothapodytes foetida* [8].

Room temperature extraction and Soxhlet extraction have been employed to recover flavonoids from wild plant materials of *S. medusa*. However, microwave-assisted extraction of flavonoids from *S. medusa* cultured cells has not been reported. The objective of the current study was aim at checking the performance of a new constructed dynamic microwave-assisted device for the extraction of flavonoids from *S. medusa* cultured cells.

* Corresponding author at: National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China. Tel.: +86 10 82622280; fax: +86 10 82622280.

E-mail address: czliu@home.ipe.ac.cn (C.-Z. Liu).

1369-703X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2006.09.004

2. Materials and methods

2.1. Plant material

Calli of *S. medusa* were cultivated on Murashige and Skoog medium [9] supplemented with 0.5 mg l^{-1} 6-benzylaminopurine (6-BA), 2 mg l^{-1} naphthalene acetic acid (NAA), 30 g l^{-1} sucrose and 5 g l^{-1} agar for production of flavonoids. The medium pH was adjusted to 5.8 with 1 M NaOH before autoclaving. The cultivation was carried out in 250 ml flasks containing 50 ml of the above medium at 25 °C under 16 h light per day. Fresh calli were collected after 25 days, and then dried at 60 °C in an oven until a constant weight was obtained. The dried cells were grounded to 0.45 mm before extraction.

2.2. Apparatus and operation

The dynamic microwave-assisted extraction system was constructed of three stainless steel tanks (35 mm i.d. × 350 mm *H*) with 1.0 l working volume, and each tank was equipped with a microwave irradiation system (2450 MHz) with a maximum irradiation power of 2000 W (Fig. 1). The inner side of microwave cavity was constructed by quartz and microwave penetrated the quartz and then was absorbed by solvent and material. The stainless steel outside of the microwave cavity was used to let microwave reflect back in order to keep safety. A refrigerant system was put outside each microwave cavity to take away excess heat. The microwave-assisted extraction system was connected with 2.5 l container through a liquid pump ($H = 10$ m) with the addition of temperature measurement and time controlling.

A given amount of *S. medusa* dried cell cultures and 2.0 l of 80% ethanol were put into the container, and then were mixed homogenously by a magnetic stirrer. The mixed extraction sample was recycled between the container and three extraction tanks by the liquid bump. The suspension was irradiated with 60 s of power ON to reach the desired temperature of about 80 °C, and then was irradiated periodically with microwaves in a pre-setting procedure (15 s of power ON for heating followed by 15 s of power OFF for cooling without allowing the suspension to super-boil).

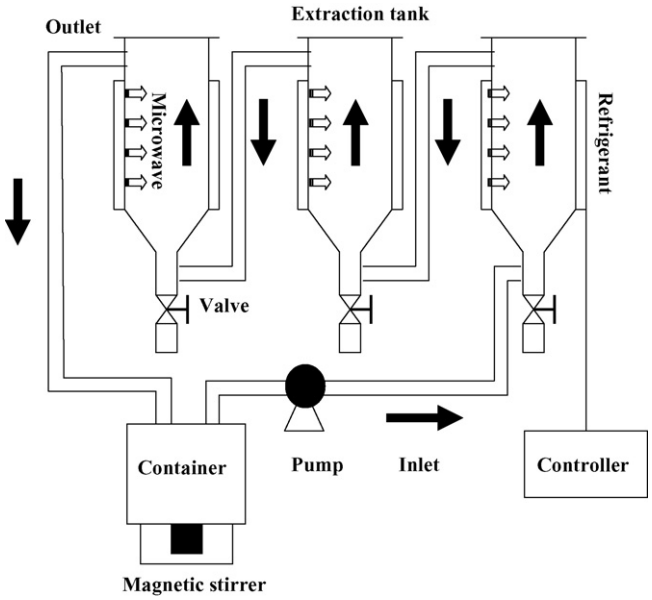

Fig. 1. Scheme of the dynamic microwave-assisted extraction system.

For dynamic solvent extraction, the same amount of *S. medusa* dried cell cultures and 2.0 l of 80% ethanol were put into the container, and then were mixed homogenously by a magnetic stirrer. The mixed extraction sample in container with a desired temperature at 80 °C heated by water bath was recycled between the container and three extraction tanks at flow rate of 50 ml s^{-1} without microwave assistance.

2.3. Analysis

The content of total flavonoids was determined by a spectrophotometric method [10,11]. Briefly, 1 ml extraction solution was pipetted into 5 ml test tube and diluted to 2 ml to which 0.15 ml 5% (w/w) $NaNO_2$, 0.15 ml 10% (w/w) $AlCl_3$ and 2 ml 4% (w/w) NaOH were added in the order stated. The absorbency value of the final solution was measured at 510 nm. Flavonoids content was determined using rutin as a standard. The yield of total flavonoids was defined as following: yield of flavonoids (w/w) = mass of total flavonoids extracted/mass of material (dried cell cultures of *S. medusa*) × 100%.

Qualitative and quantitative analysis of flavonoids in cell cultures of *S. medusa* was carried out by the HPLC method described by Liu et al. [12] with minor modification. Agilent 1100 HPLC system is composed of a quaternary pump with a degasser, a variable wavelength detector, an auto-sampler and 1100 ChemStation software. Sample analyses were performed on an Alltech C_{18} column (250 mm × 4.6 mm i.d., 5 μm) with a gradient elution of 0.1% phosphoric acid (A) and methanol (B) as follows: A–B (90:10) to A–B (10:90) in 50 min. The flow-rate was 0.8 ml min^{-1} and the effluent was monitored at 365 nm by UV detector. The reference standard of rutin was supplied by National Institute for the Control of Pharmaceutical and Biological Product with the purity no less than 98%.

All the experiments were repeated twice, and all values were the means of replicates ±S.D. One-way analysis of variance (ANOVA) was conducted to determine the statistically significant. Trends were considered significant when result of compared parameters differed at $P < 0.05$ significance level.

3. Results and discussion

The current research focused on the establishment of an efficient extraction process for flavonoids recovery from *S. medusa* dried cell cultures in a new constructed microwave-assisted extraction system, and the effects of microwave power, flow rate of extraction solvent and amount of plant material on yield of flavonoids were investigated as follows.