Separation and Purification Technology 165 (2016) 208–213

Contents lists available at ScienceDirect

Separation and Purification Technology

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

The mechanism for enhancing extraction of ferulic acid from Radix *Angelica sinensis* by high hydrostatic pressure

Jun Xi *, Shengwu Luo

College of Chemical Engineering, Sichuan University, Chengdu 610065, China

ARTICLE INFO

Article history:
Received 23 January 2016
Received in revised form 5 April 2016
Accepted 9 April 2016
Available online 11 April 2016

Keywords:
Solid-liquid extraction
High hydrostatic pressure
Mechanism
Radix *Angelica sinensis*
Ferulic acid

ABSTRACT

Radix *Angelica sinensis* contains a high amount of ferulic acid. However, the extraction of ferulic acid from Radix *A. sinensis* still encounters technical problems. Effect of high hydrostatic pressure on the yield of ferulic acid in Radix *A. sinensis* and its relevant mechanism were investigated in this paper. Compared with reflux extraction, high hydrostatic pressure significantly increased yield of ferulic acid, shortened extraction time, and reduced extraction temperature. The experiments results of swelling degree of matrix, solubility of ferulic acid, and microscopic examination of the treated materials indicated that an excellent matrix swelling effect, high solubility of ferulic acid and destruction of the residue tissue might be the key mechanisms for high extraction efficiency by high hydrostatic pressure. These results suggested that high hydrostatic pressure was an innovative technique of high efficiency and low operating temperature for ferulic acid extraction from plant materials.

1. Introduction

Radix *Angelica sinensis* (RA), commonly known as Danggui in China, derived from the dry root of *A. sinensis* (Oliv.) Diels, is a traditional medicinal and edible plant, which has long been used for treatment of menstrual disorders, dysmenorrhea, chronic constipation and other diseases [1]. In addition to its medicinal use, RA has also been widely used as a health food, a cosmetic and a dietary supplement in Asia, Europe and America [2]. The essential biologically active components of RA are alkylphthalides, coumarins, polysaccharides and ferulic acid [3]. Among these ingredients, the ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of the most important medical components possessing anti-oxidative properties by virtue of the phenolic hydroxyl group in its structure, which has been officially used as the marker component to characterize the quality of RA according to the current Chinese pharmacopoeia [4]. Previous investigations suggested that ferulic acid possesses many physiological functions, including antimicrobial and anticancer activities, and also protects against coronary disease, lowers cholesterol and increases sperm viability, ameliorates memory, enhances cholinergic activities and cerebral blood flow [5–8]. Thus, it is widely used in pharmaceutical, food and cosmetic industries.

The conventional technique for extracting the ferulic acid is to reflux RA for 4–5 h in 70% ethanol aqueous solution [9]. Unfortunately, the ferulic acid is heat-sensitive and its structure will be destroyed during long time heating in ethanol solution [10]. Moreover, the reflux method has a number of shortcomings, including long extraction time, great consumption of solvents, low extraction efficiency, and so on [11]. Therefore the reflux method is not good for extraction of ferulic acid. Developing a fast and efficient extraction method with low operating temperature has become a concern issue in the pharmaceutical and food industries.

High hydrostatic pressure (HHP) means cold isostatic superhigh hydraulic pressure that ranges from 100 to 800 MPa or even higher [12]. HHP is a food processing method which has shown great potentials in the food industry. Similar to heat treatment, HHP inactivates microorganisms, denatures proteins and extends the shelf life of food products [13,14]. The process of HHP is isostatic, i.e., the pressure is transmitted uniformly and instantly, and adiabatic, which means that no matter what the biomaterial shape or size is, there is little variation in temperature with increasing pressure (the temperature increases approximately 3 °C per 100 MPa, depending on the composition of the biomaterial) [15]. The mild operating temperature of HHP may lead to an enhanced extraction of active ingredient. This aspect of the process is of paramount importance with heat-sensitive components [16,17]. Thus, HHP appears to be a very interesting method to extract ferulic acid from plant tissue.

* Corresponding author.
E-mail address: xijun@scu.edu.cn (J. Xi).

http://dx.doi.org/10.1016/j.seppur.2016.04.011
1383-5866/

In recent years, it is reported that HHP has been successfully used in the extraction of anthocyanin, polyphenols, sulforaphane, fatty acids and antioxidant compounds from different plants [16–31]. These studies show that HHP has many advantages, such as shorter time (only about 5 min), higher extraction efficiency, lower power consumption. However, these studies have been only focused on the optimization of the extraction conditions or process factors, and few works have been done to comprehensively elucidate the effect mechanism of HHP for enhancing extraction performance until now. Therefore, a series of experiments are carried out to expound the mechanism in the present study.

2. Materials and methods

2.1. Plant materials

The Radix *A. sinensis*, which came from Gansu province of China, were obtained from Tai-Ji Traditional Chinese Medicine Store (Chengdu, China) (Fig. 1). After being dried in a hot air oven at 40 °C for 24 h, the samples were ground into powder using a grinder and passed through 40 mesh sieve. The grinded samples were immediately vacuum-packed and stored at 4 °C for further use.

2.2. Chemicals and reagents

Ethanol used in the experimental work was analytical reagent grade chemicals (Beijing Chemical Reagents Company, Beijing, China). Water used for HPLC was purified with a Milli-Q Plus system (Millipore, USA). Acetonitrile and phosphoric acid of HPLC grade were obtained from J & K Scientific Ltd (Shanghai, China). Ferulic acid (purity ⩾ 99.6%), pharmaceutical grade standard, was purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). Other reagents were analytical grade and purchased from Chengdu Chemical Industry (Chengdu, China).

The HPLC system used was a LC-10AT vp HPLC system with a SPD-M10A vp diode-array detector (Shimadzu, Japan) and a Diamonsil™ C_{18} column (250 mm × 4.6 mm, 5 μm, Dikma, Beijing, China).

The pressure extractor was obtained from Shanghai Dalong Super-high Pressure Machine Co., Ltd. (Shanghai, China). Effective volume of vessel: 2 L; maximal working pressure: 600 MPa; inner diameter: 100 mm; pressure transmitting media: mixture of water and glycol (20:80, v/v). A laboratory-scale prototype of HHP system is composed of a pressure vessel, a pressure intensifier, a reversing valve, a piston pump and a thermocouple, as illustrated in Fig. 2 [16], which is a batch type for sample operation. The temperature inside the pressure vessel is regulated using a thermocouple with ±0.1 °C temperature control.

Fig. 1. The Radix *Angelica sinensis*.

2.3. Quantitative analysis of ferulic acid

The quantitative analysis of ferulic acid was determined using HPLC reported by Liu et al. [32] with minor modifications. The HPLC analysis was carried out on a Shimadzu LC-10AT vp HPLC system with a SPD-M10A vp diode-array detector (Shimadzu, Japan) and a Diamonsil™ C_{18} column (250 mm × 4.6 mm, 5 μm, Dikma, Beijing, China). All samples for HPLC analysis were filtered through a 0.45 μm membrane filter before injection. The flow rate was 1.0 ml/min and the column temperature was 35 °C. The mobile phase consisted of acetonitrile and 0.085% (v/v) phosphoric acid aqueous solution (19:81, v/v). The injection volume was 10 μL. The detector was set at 316 nm. All quantitative analyses were made by the external standard method, using ferulic acid as an analytical standard. The HPLC chromatograms of the ferulic acid standard and the extracts of RA were shown in Fig. 3. The HPLC method was validated and showed no statistical differences on a 95% confidence basis.

2.4. Extraction with different high hydrostatic pressure

Five-gram sample of RA powder was mixed with 100 ml of 70% aqueous ethanol, and placed in a sterile polyethylene bag. The bag was sealed after eliminating air from the inside and placed into a pressure vessel. After extraction in the pressure extractor at different pressure values (pressure gage: 100, 200, 300, 400, 500 MPa) and 25 °C for 10 min respectively, the mixtures were filtered through the quantitative filter paper (8–10 μm, Whatman, UK). The extraction solution was centrifuged at a speed of 4000 rpm for 10 min, and the supernatant was collected and stored at 4 °C in refrigerator for subsequent HPLC analysis. The extraction yields of ferulic acid in the samples were calculated by the formula:

$$\text{Yields (mg/g)} = \frac{m}{M} \quad (1)$$

where m was the extracted ferulic acid mass (mg), M was the mass of the dried sample (g).

2.5. Control extraction

Reflux extraction was carried out at normal atmospheric pressure by weighing 5 g of RA powder into a flask containing 70% aqueous ethanol using a solid/liquid ratio of 1:20 g/ml. Extractions were carried out in a water bath by agitation for 5 h. The temperature was kept at about 85 °C [9].

2.6. Swelling degree measurement of the samples

The swelling degree of samples was determined according to the method described by Dima et al. [33] with minor modifications. A quantity of 5 g of ground samples (40 mesh) was extracted with 100 ml of 70% v/v aqueous ethanol at different pressure values (pressure gage: 100, 200, 300, 400, 500 MPa) and 25 °C for 10 min, respectively. The swollen samples were removed from the solution using vacuum filtration, and then put back into 50 ml of the corresponding filtrate in a graduated test tube. The volume increment (v_1) was taken as the volume of the swollen samples. The initial volume of sample (v_0) was recorded as the increased volume after putting 5 g ground sample (40 mesh) into 50 ml of 70% v/v ethanol solution in a graduated test tube. For each sample, three measurements were performed. The swelling degree (SD) was calculated by the following equation: