Food Chemistry 142 (2014) 299–305

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Optimization of ultrasonic-assisted extraction of bioactive alkaloid compounds from rhizoma coptidis (*Coptis chinensis* Franch.) using response surface methodology

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ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form 16 January 2013
Accepted 26 June 2013
Available online 17 July 2013

Keywords:
Alkaloids
Berberine
Ultrasonic-assisted extraction
Response surface methodology
Rhizoma coptidis

ABSTRACT

The optimum extraction conditions for the maximum recovery of total alkaloid content (TAC), berberine content (BC), palmatine content (PC), and the highest antioxidant capacity (AC) from rhizoma coptidis subjected to ultrasonic-assisted extraction (UAE) were determined using response surface methodology (RSM). Central composite design (CCD) with three variables and five levels was employed, and response surface plots were constructed in accordance with a second order polynomial model. Analysis of variance (ANOVA) showed that the quadratic model was well fitted and significant for responses of TAC, BC, PC, and AA. The optimum conditions obtained through the overlapped contour plot were as follows: ethanol concentration of 59%, extraction time of 46.57 min, and temperature of 66.22 °C. Verification experiment was carried out, and no significant difference was found between observed and estimated values for each response, suggesting that the estimated models were reliable and valid for UAE of alkaloids.

1. Introduction

Rhizoma coptidis (*Coptidis chinensis* Franch.), known as huanglian in China, is a traditional Chinese herb that has been widely used as a folk treatment for the removal of damp heat, fire, or toxicity for over 2000 years (Wagner, 2011). In recent years, rhizoma coptidis has been used as a food additive and supplemented into some products such as honey and other functional beverages. Seven kinds of alkaloids have been isolated and identified from rhizoma coptidis. Among them, berberine and palmatine, which are quaternary alkaloids with predominate bioactive effects (chemical structure shown in Fig. 1), have been confirmed as main constituents, comprising approximately 60% of the total alkaloid fraction (Xu, Gao, Zhang, Zhang, & Xu, 2007). According to recent studies (Kong et al., 2004; Kuo, Chi, & Liu, 2004; Kyoko, Kazuki, Yasuo, Toshimitsu, & Shinichi, 2007; Lee, Chen, Hsiang, Wu, & Ho, 2007), berberine and palmatine exhibit broad-spectrum antibacterial activities, anti-inflammatory effects, and good pharmacological properties such as inhibition of diabetes, cancer, and blood pressure.

A variety of extraction techniques have been utilised for the separation and analysis of alkaloids from rhizoma coptidis. Conventionally, alkaloids are extracted by heating reflux extraction, soxhlet extraction, or maceration at room temperature. Nowadays, advanced extraction methods have been developed and adopted for the extraction of bioactive compounds from natural plants. Ultrasonic-assisted extraction (UAE) is a rapid, effective extraction technique that uses ultrasonic waves to generate a cavitation in the solvent, which allows higher penetration of solvent into the raw plant materials (Romanik, Gilgenast, Przyjazny, & Kaminski, 2007). Compared with other modern extraction techniques such as microwave-assisted extraction, supercritical fluid extraction, and ion-pair extraction, UAE is more secure, economic, environmentally friendly, and easier to use. Despite modern extraction methods that greatly reduce extraction time and improve extraction efficiency, improper selection of process factors (extraction temperature, processing time, medium solvent, and solvent to sample ratio) or factor levels could result in low yield. At present, although various advanced techniques have been applied for alkaloid extraction from rhizoma coptidis, low yield remains an issue to be solved (Chen et al., 2008).

Thus, in the present study, response surface methodology was employed to investigate the effects of various process variables, including ethanol concentration, extraction time, temperature, and solvent to sample ratio, on the yields of total alkaloid content (TAC), berberine content (BC), palmatine content (PC), and antioxidant capacity (AC) from rhizoma coptidis subjected to UAE. The main objectives of this study were to determine the critical

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0308-8146/$ - see front matter
http://dx.doi.org/10.1016/j.foodchem.2013.06.136

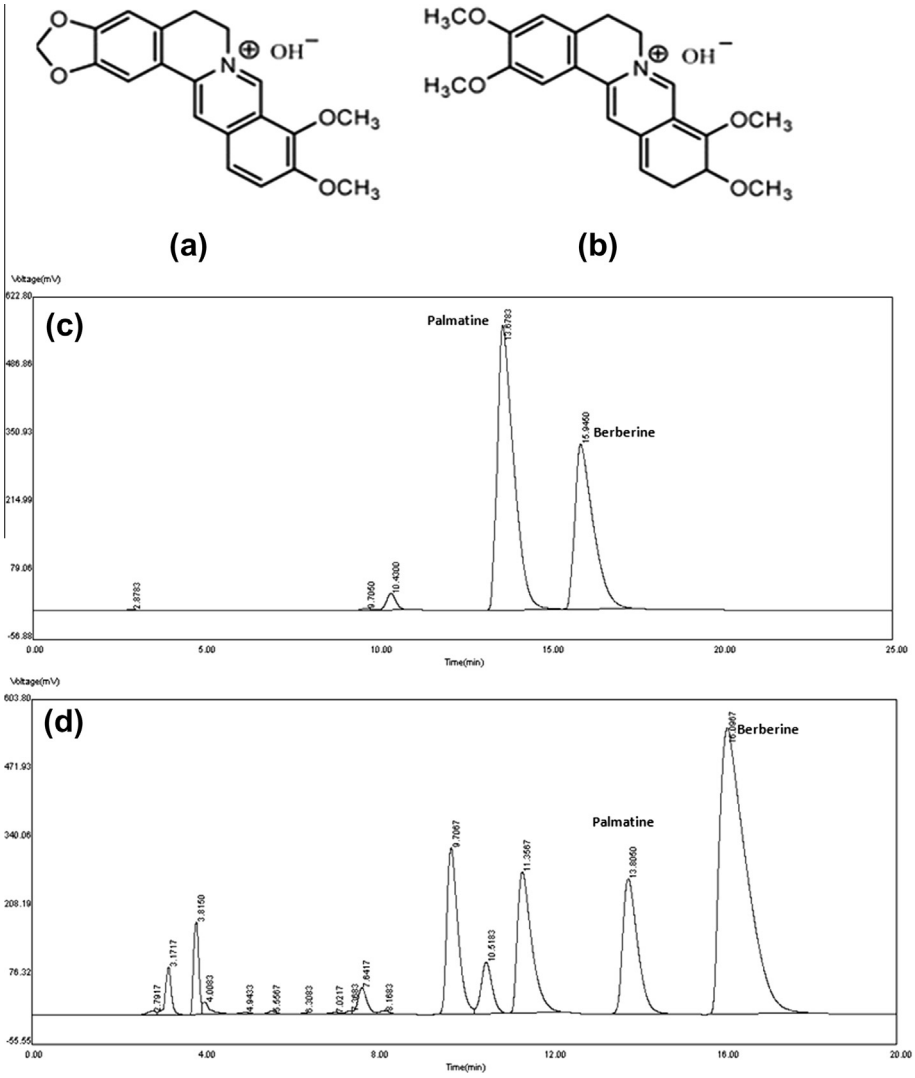

Fig. 1. Chemical structures for berberine (a) and palmatine (b), and HPLC profiles for standards (c) and sample (d). Mobile phase consisted of 25 mM KH_2PO_4 and acetonitrile at a mix ratio of 75:25, flow rate was 1.0 mL/min, and retention time was 30 min and absorption was set at 345 nm for 10 μL injection.

variables as well as the optimal extraction conditions that would allow maximum response yields in accordance with the response surface and contour plots. Further, different extraction methods were implemented in order to contrast extraction efficiency.

2. Materials and methods

2.1. Materials

Dried rhizoma coptidis, originally cultivated in Sichuang Province in China, was authenticated as *Coptis chinensis* Franch under the guidance of the Pharmacopeia of China (2005 version). Sodium acetate, potassium persulphate, ABTS, potassium dihydrogen phosphate, and standards of berberine chloride, palamtine chloride (purity >98%, w/w), and Trolox were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Ethanol (95%) and acetonitrile of HPLC grade were purchased from Duksan Pure Chemical Company (Ansan, Korea).

2.2. Preparation of rhizoma coptidis sample

Dried rhizoma coptidis slice was first screened in a 35 mesh sieve to remove sand, crushed to powder form in an electric grinder, and passed through a 40 mesh sieve with an aperture size of 250 μm. The powdered sample was packed in a polyethylene pail and stored in a freezer at −18 °C during the experiments.

2.3. Ultrasonic-assisted extraction process (UAE)

Ultrasonic-assisted extraction was performed in a sonication cleaning bath (model of JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd., Hwasung, Gyeonggi, Korea) operated at a frequency of 40 kHz and an ultrasonic input power of 250 W with a useable volume of 10 L (internal dimensions: 300 × 240 × 150 mm). The available range for extraction temperature was from 0 to 70 °C. Extraction process was carried out as follows: 1.0 g of rhizoma coptidis powder was placed in a 100 mL Erlenmeyer flask and mixed with 50 mL of aqueous ethanol solution, after which the flask was placed in the sonication bath and extraction carried out for the required time periods. Upon extraction, the mixture was filtered through a Buchner funnel under vacuum, after which the filtrates were collected in a volumetric flask. The final volume was diluted up to 100 mL with aqueous ethanol solution, and 2 mL extracts were injected through a 0.45 μm PTFE syringe filter for analysis.