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Superheated water extraction of glycyrrhizic acid from licorice root

Mohammad A. Shabkhiz [a], Mohammad H. Eikani [b,*], Zeinolabedin Bashiri Sadr [b], Fereshteh Golmohammad [b]

[a] Department of Agriculture, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran
[b] Department of Chemical Technologies, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

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ABSTRACT

Superheated water extraction (SWE) has become an interesting green extraction method for different classes of compounds. In this study, SWE was used to extract glycyrrhizic acid (GA) from licorice root. Response surface methodology (RSM) was applied to evaluate and optimize the extraction conditions. The influence of operating conditions such as water temperature (100, 120 and 140 °C) and solvent flow rates (1, 3 and 5 mL/min) were investigated at 0.5 mm mean particle size and 20 bar pressure. Separation and identification of the glycyrrhizic acid, as the main component, was carried out by the RP-HPLC method. The best operating conditions for the SWE of licorice were determined to be 100 °C temperature,15 mL/min flow rate and 120 min extraction time. The results showed that the amount of the obtained GA was relatively higher using SWE (54.760 mg/g) than the Soxhlet method (28.760 mg/g) and ultrasonic extraction (18.240 mg/g).

1. Introduction

Licorice (*Glycyrrhiza glabra*) belongs to the fabaceae family and grows mainly in southern Europe and Asia. Licorice root is extensively used in herbal medicine for its emollient, anti-inflammatory, anti-viral, anti-allergic, anti-oxidant, gastro-protective, and anti-cancerous properties. It is also widely used in food, confectionery, tobacco and pharmaceutical industry. Licorice extract is used in various products, such as cough syrups, baked goods, chewing gums, drinks, and tobacco to increase sweetness and/or foam (Hough & Hough, 1973; Kitagawa, 2002; Mukhopadhyay & Panja, 2008).

Licorice root contains numerous compounds, including sugars (up to 18%), flavonoids, sterols, amino acids, gums, starches, essential oils and saponosids (Karami et al., 2015; Kitagawa, 2002). The most important saponin in licorice root is glycyrrhizic acid (GA, $C_{42}H_{62}O_{16}$). It is formed by two parts: glucuronic acid (glycon) and glycyrrhetinic acid (aglycon). Its sweetness is about 50 times greater than sucrose. Also, reports show that its flavor remains in the mouth for a longer time (Kitagawa, 2002). These features have led to the creation of low-calorie sweeteners (Hough, 1973).

Several methods for separation of GA from licorice root have been investigated. The conventional methods for GA extraction from the licorice root use hot water extraction at ambient pressure and alkaline pH (ammonia solution) (Beasley, Howard, & Bell, 1979; Shen et al., 2007; Tian, Yan, & Row, 2008; Tianwei, Huo, & Ling, 2002), aqueous methanol (Jiang, Lua, & Chen, 2004; Sabbioni et al., 2005; Zeng, Zhang, Meng, & Zhi-Cen, 1990) and ethanol (Pan, Liu, Jia, & Shu, 2000). Other new methods like ultrasound and supercritical CO_2 extraction have also been studied (Charpe & Rathod, 2012; Hedayati & Ghoreishi, 2015).

Recently, advanced extraction technologies like subcritical or superheated water extraction (SWE) have been developed. SWE is a new technique based on the use of water at temperatures between 100 and 374 °C and pressure high enough to maintain the liquid state (Golmohammad, Eikani, & Maymandi, 2012; Mortazavi, Eikani, Mirzaei, Jafari, & Golmohammad, 2010; Soto Ayala & Luquede Castro, 2001). Under these conditions, water is less polar, and consequently the organic compounds would be more soluble than at room temperature. The most important advantages of SWE over other extraction methods are shorter extraction time, higher quality of the extract and lower costs. In addition, SWE is an environment friendly technique (Herrero, Cifuentes, & Ibanez, 2006). SWE is usually performed in two modes: static or dynamic. Extraction of active compounds from licorice root using superheated water has been previously studied in the static mode by Mukhopadhyay and Panja (2008) and Baek and Lee (2008).

The aim of this work is to investigate the SWE technique on licorice root in dynamic or flow mode. SWE of GA was carried out at different temperatures and flow rates. Then, the SWE results

* Corresponding author at: Department of Chemical Technologies, Iranian Research Organization for Science and Technology (IROST), P.O. Box: 33535111, Tehran, Iran.
E-mail address: eikani@irost.ir (M.H. Eikani).

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were compared to those obtained by ultrasonic assisted and Soxhlet extraction.

2. Materials and methods

2.1. Raw materials

Licorice root was supplied from the Medicinal Research Laboratory of Iranian Research Organization for Science and Technology (IROST, Tehran, Iran). The moisture content of the root was 5.70 wt%. The licorice root was cleaned by tap water, dried at 35 °C for one week and cut into small pieces (0.5–1 cm diameter and 0.5–1 cm length) (Wise Ven, Wisd Laboratory Instruments, Korea). The dried root was ground using a hammer mill (Tus, Khorasan Breaker Model T 8300. Iran) and passed through sieve No. 35 (0.50 mm). Before extraction, the samples were stored in polyethylene bags at −18 °C.

2.2. Chemicals and standards

Glycyrrhizic acid ammonium salt 95% pure was purchased from Sigma Aldrich (USA) and used as standard for the reversed phase (RP) HPLC analysis. Methanol, ammonia (25%), ethanol, and acetonitrile were supplied by Merck (Darmstadt, Germany). A doubly distilled water production unit (Fater Electric Co., Iran) was used to supply extraction water. Also, ethanol (96 wt%, Bidestan Co., Ghazvin, Iran) was used as solvent in the Soxhlet method.

2.3. Experimental methods

2.3.1. Superheated water extraction system

The SWEs were carried out in a laboratory-built apparatus shown in Fig. 1. Detailed description of the apparatus is presented elsewhere (Eikani, Golmohammad, Salar Amoli, & Bashiri Sadr, 2013). However, the apparatus' high pressure pump was replaced by a more recent one (FWT Fluid and Water Technology, FX series, type: FXS C/A, Italy; Flow rate: max. 10 mL/min with 0.01 mL/min accuracy, max. pressure 20 bar). The suction port of the pump was changed accordingly, that is, it was placed and fitted in a (100 mL) balloon equipped with a SS316 HPLC microfilter (MF1). The other main parts of the apparatus were as follows: a fan-equipped temperature-controlled oven (Teb Azma Co., Tehran, Iran, up to 200 °C); a preheating coil (3 m length); a cylindrical extraction chamber (20 mL); and a heat exchanger. After the preheating coil, a three way line was made by using three high-pressure needle valves. Needle valves of NV1 and NV2 were placed in the inlet and outlet lines of the extractor, respectively. A needle valve on the by-pass line, NV3, performed three duties in three different modes of operation. It was opened at the preliminary pressurizing step, closed during the extraction runs, and closed again at the end of the experiments to wash and clean extract contaminated lines. The inlet/outlet pressures and inlet/outlet temperatures were measured using PI1/PI2 bourdon gauges and TI1/TI2 PT100 thermocouples, respectively. Pressure was maintained in the system using a back pressure regulator (Veriflo, Parker Instrumentation, Richmond, CA, max. pressure: 35 bar). All parts in contact with the solvent were made from stainless steel 316 L.

For all experiments, 1.0 g of ground licorice root (0.5 mm) was used and the extraction time was set to 120 min. The whole obtained extracts were dried at 50 °C, and then dissolved in 100 mL ammonia solution (8 g/L). 10 μL of that solution was used for RP-HPLC analysis.

2.3.2. Soxhlet extraction

5.0 g of licorice powder (0.50 mm) was packed in a filter paper and placed in the thimble holder of a Soxhlet apparatus and extraction was carried out for 4 h (Wang, Ma, Fu, Lee, & Wang, 2004). A mixture comprising 98 mL of ethanol 60% and 2 mL of ammonia solution was used as the solvent (Charpe & Rathod, 2012). For considering stoichiometry 1:5, 20 mL of the final extract was diluted to 100 mL using ammonia solution (8 g/L), and at the end 10 μL of that solution was analyzed by RP-HPLC.

2.3.3. Ultrasonic extraction

Ultrasonic extraction (USE) was carried out by an ALEX MACHINE 4 L Ultrasonic cleaning instrument (Alex Makina Co., Istanbul, Turkey). 1.0 g of powdered licorice root (0.50 mm) was mixed with 100 mL ammonia solution (8 g/L) and treated in an ultrasonic bath (40 kHz, 25 °C) for 30 min. The supernatant layer was centrifuged and then 1.0 mL of the layer was diluted to 6.0 mL using ammonia solution (8 g/L).The obtained solution was

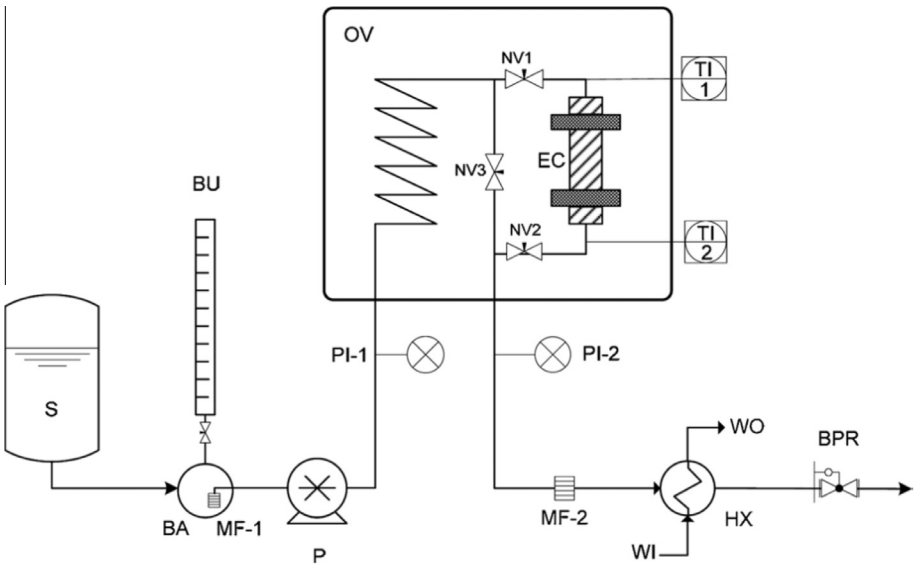

Fig. 1. The superheated water extraction system; BA: glass balloon; BPR: back pressure regulator; BU: burette; EC: extraction cell; HX: heat exchanger; MF: micro filter; NV: needle valve, OV: heating oven; P: pump; PI: pressure indicator; S: solvent vessel; TI: temperature indicator; WI: water inlet port; WO: water outlet port.