Supercritical Carbon Dioxide Extraction of Glycyrrhizin from Licorice Root

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Abstract

As the development of the natural medicine, the glycyrrhizin extracted from the licorice root was found to have potential therapeutic value in the treatment of several diseases, especially available for anti-SARS virus [1-2]. Therefore, the purpose of this work was to find an optimal method to extract and refine the glycyrrhizin.

In this work, the extraction of glycyrrhizin by means of organic solvents and supercritical carbon dioxide (SC-CO_2) was investigated. The SC-CO_2 extraction apparatus was designed and set up by our own laboratory. The experimental conditions was as follows: pressure 10MPa-30MPa, temperature from 30？ to 60？, CO_2 flow rate about 10kg/h. The constitution of the solvent, and the particle size were all discussed in order to obtain the maximum extraction yield. The extraction of glycyrrhizin from licorice root with supercritical carbon dioxide was also compared with conventional extraction methods. Those methods include the extraction at room temperature (ERT), the traditional Soxhlet extraction, and the heat reflux extraction. The results show that the yield obtained by SC-CO_2 extraction was similar to that obtained by conventional solvent extraction. But the quality of the production extracted by SC-CO_2 was better than that of the production extracted by other methods. Subsequently, HPLC was used to analyze the chemical compositions of these productions.

Keywords: glycyrrhizin; licorice root; supercritical carbon dioxide; conventional extraction; HPLC

Introduction

Licorice is herbaceous triennial or perennial plant, with large roots. The roots and the stalks are used extensively fields, tobacco, food, flavoring, confectionery, perfumes, pharmaceutical industry [3], etc. Particularly in medicine licorice has a lot of virtue. Glycyrrhiza radix has been used as a Chinese traditional medicine for over 1000 years. It was used to relieve a cough, reduce phlegm, detoxifcation. At present, the medicine is frequently used to treat diseases [5,6,7] such as contagious hepatitis, bronchitic asthma, canker, ague, antivirus, and improve immunity. Especially, glycyrrhizic acid was found to have the ability to restain HIV reproduce in 1980s. Subsequently, the flavonoids were also found to have this function. Glycyrrhizin, which is also called glycyrrhizic acid, could be used treatment for sudden acute respiratory syndrome (SARS)[1,2]. Glycyrrhizin may protect the target cell from the SARS virus to attach to and invade. It also hinders virus reproductions and spread from one cell to another cell. As the development of the medicine, the demand of glycyrrhizin, flavonoids and other availability enlarge widely. The application of supercritical fluid extraction (SFE), particularly the use of liquid and supercritical carbon dioxide, has received much attention in Glycyrrhiza radix extraction in the last few years. This separation technique offers extraction yields comparable with those obtained by conventional extraction methods using organic solvent, followed by column separation and purification, to obtain pure compounds. The conventional extraction consumed large amounts of solvent, required lots of time, and polluted the environment. Since carbon dioxide has some advantages: chemically inert, nontoxicity, nonflammable, no pollution problem, and shorter concentration time. These advantages attract the researchers' interest in using supercritical carbon dioxide to extract Glycyrrhiza radix. For the extraction of polar or ionic compounds, organic solvents have been added as the cosolvent to increase the compounds' solubility in SC-CO_2. The extractions, which have been used extensively, have three components: glycyrrhizin, flavonoids, amylose.

There are many methods to extract glycyrrhiza radix, such as traditional extraction, heat reflux extraction, ultrasonic extraction, microwave-assisted extraction, SC-CO_2 extraction. These extraction methods have been compared in this paper.

2. Experiments

All reagents were of analytical grade. The roots of licorice were planted in Inner Mongolia province. Ethanol, acetone, and ammonia were purchased from chemical factory in ShenYang (LiaoNing province). Methanol and acetic acid (chromatogram grade) was purchased from chemical factory in TianJin. Carbon dioxide (SFE grade) was purchased from the gas station of Dalian University of Technology. The roots of licorice were smash by muller and separated the size into 20, 40, 60 and 80 mesh powder and dried to constant weight. Make different concentration of ethanol: 75%, 80%, 85%, 90%, and 95%. The traditional extraction methods were include decoction with water, heat reflux extraction, soak at room temperature, etc. Decoction with water method was adopted very early. The sample were extracted with 5?4?4 times water to heat reflux almost 2 hours respectively, combined three extractions until as 1/5 as the original extractions, and filtrated. The filtrate was concentrated. The process was the same as decoction method, except 12 times of the mixture, which was made up of 0.3% ammonia, 60% ethanol, and 39.7% water were instead of water. The time of reflux was 1.5 hours. The extraction was filtered and the excess solvent was evaporated with the rotary evaporator. The sample ware put into ammonia/water mixture (0.5:99.5 v/v), which were 12 times as the sample soaked at room temperature. After that, the solvent was concentrated and filtered. Added oil of vitriol into the solvent until there was some deposition separated out, when pH was 3. Washed the filtrate 3 times and dried at low temperature.

These methods are generally extraction method and have been used in production. But that wasted too much water and organic solvent, which is do harm to circumstance and It takes a lot of time to the process.

Sonication

For glycyrrhizn, the procedures were adopted from an earlier report[7]. Briefly, 0.6g of sample were extracted with 20 ml of methanol/water mixture (70:30 v/v) at room temperature for 10 min and centrifuge at 2000 rpm for 10 min. The procedures were repeated three times. The extracts were combined; excess solvent was evaporated with the rotary evaporator and filtered.

Microwave-assisted extraction [8]

This method is very suitable for fast extraction. The extraction time only need 4-5min. Briefly, 10g sample, which was 50mesh were mixed with 100ml solvent which ethanol concentrations of 50-60%(v/v), ammonia concentration of 1-2% (v/v). The suspensions were irradiated under microwaves in pre-setting procedures (15s power on, 15s power off for three times to the desired temperature (about 85-90?) and then 3s power on for heating and 15s power off for cooling), but not allowed to super-boil. The extraction was filtered and excess solvent was evaporated with the rotary evaporator.

SC-CO_2 extraction

SC-CO_2 technology have been used to plant extract widely [11~18]. SC-CO_2 extraction was a very suitable method for licorice. Several reports have described [17]. Briefly, sample was accurately weighed, then extracted by the following methods.

The pulverized sample was packed into a sample cartridge. Different concentration of ethanol (75%, 80%, 85%, 90% and 95%) was as cosolvent. The extraction temperature was set at 40, 45, 50, 55? , respectively. Liquid carbon dioxide at high pressure (20, 25, 30,35MPa) was then allowed to flow into the sample cartridge. When the pressure reached the aim pressure opened the vent valve of the extractor and then the two separators. The crude extraction was obtained by reduced pressure evaporation.

HPLC conditions

For all experiments, the apparatus was equipped with a binary gradient pump, autosampler, column oven and diode array detector was used. The elution consists of mobile phase of (A) methanol, (B) water and (C) acetic acid and CH_3OH/3%HOAc was 33/67. Detection was at 254nm. Oven temperature was at room temperature and flow rate was set at 1.0ml/min. For all the experiments, 20μl of standards and sample extract were injected. The column used for separation was DOS (4.6mm i.d.*25cm).

3 Results and discussion

The main goal of this study was to obtain the best quality and the maximum yield of the glycyrrhizic acid and flavonoids by an optimal selection of SC-CO_2 parameters.

3.1 The effect of temperature and pressure on extraction yield

Extraction pressure is one of the most parameters. Different material has different condition, which is been determinate by the polar of the material. Nonpolar material is extracted at lower pressure, but polar is extracted at higher pressure. Since glycyrrhizic acid and flavonoids are all polar materials, the experiment's pressure is selected at higher pressure.

Temperature is another important parameters. The effect of temperature is very complex. At a certain pressure, if the temperature is been enhanced the molecular distance is increased and the affects among the molecules are decreased which cause the solubility reduced. Another aspect, enhanced the temperature can cause the vapor pressure of the extraction increase and increase the combine between molecules, so the solubility increased. However, high temperature can make the production quality decrease. So the whole experiment at lower temperature.

3.2 The effect of cosolvent on percentage extraction

The cosolvent is added in this experiment to increase the solubility of glycyrrhizin and flavonoids in SC-CO_2. Because SC-CO_2 is a nopolar solvent, so it makes glycyrrhizin and flavonoids hard to dissolve in it. The ethanol as cosolvent is added into SC-CO_2 in order to increase the solubility of glycyrrhizin and flavonoids. (see Fig2.)

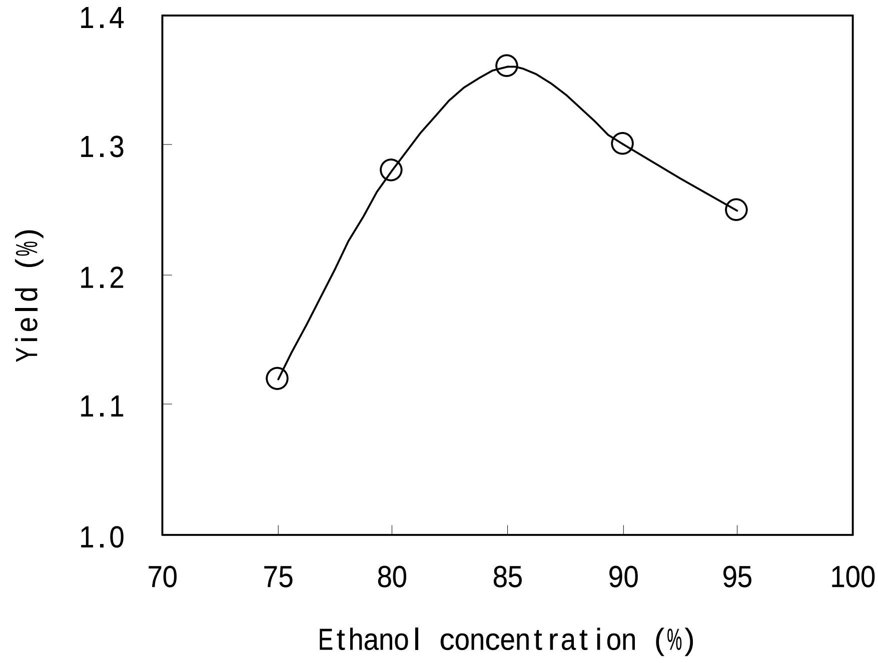

Fig.2 Effect of ethanol concentration on the yield

3.3 The effect of glycyrrhiza radix size on percentage extraction

Milling of the raw materials greatly improved the extraction efficiency. The smaller particle size should gain the greater yield. But too small particle size may be increase the flow resistance. Therefore the glycyrrhiza radix size should be 40-60 mesh. (see Fig.3.)

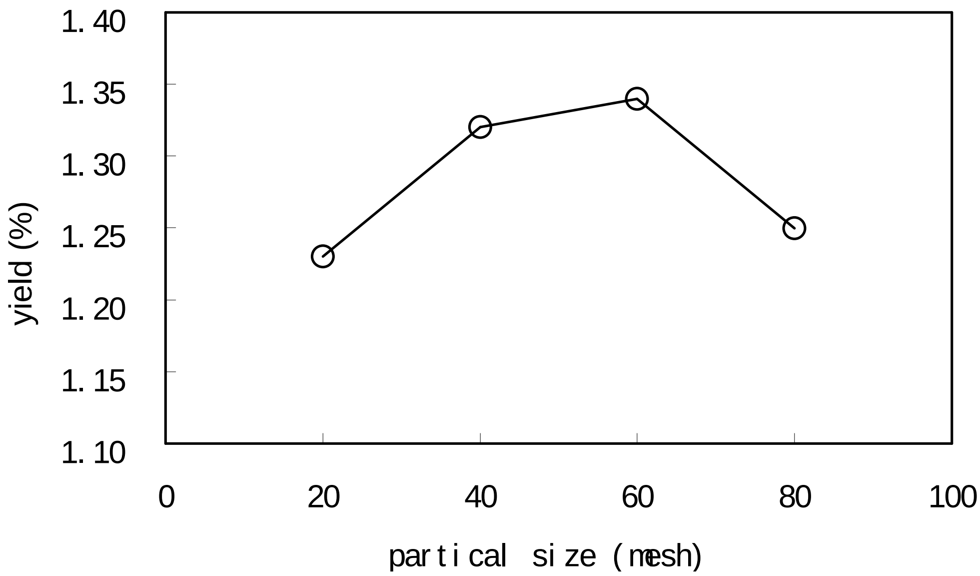

Fig.3 Effect of partical size on the yield

3.4 The effect of CO_2 flow rate on extraction yield

The amount of glycyrrhizic acid and flavonoids extracted was closely dependent on the flow at rates up to 10kg/h. The yields at flow rates of 10kg/h and 12kg/h appeared to be very similar. Lower solvent flow rates needed longer operating times to reach lower maximum extraction yields. (see Fig.4.)

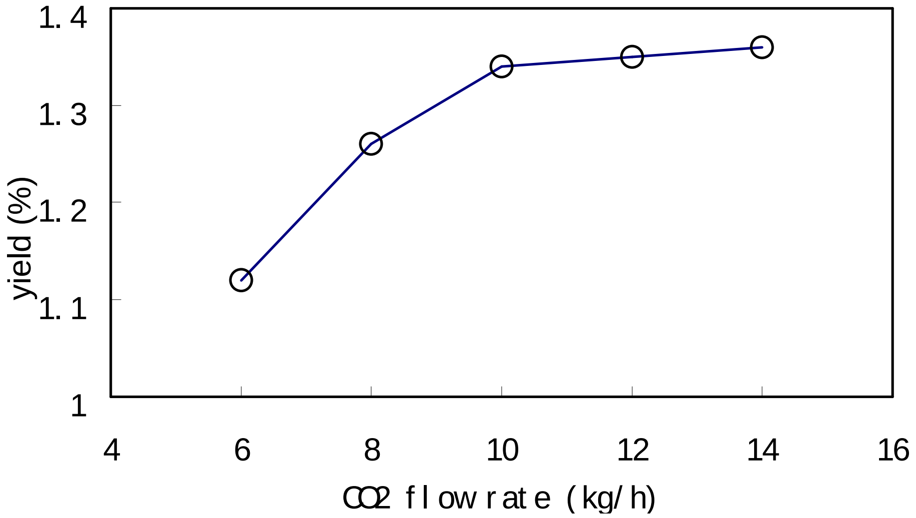

Fig.4 Effect of CO_2 flow rate on the yield

4. Conclude

The optimal extraction condition were as follows: pressure 30MPa, temperature 50? , CO_2 flow rate about 10kg/h, and the composition of the solvent was ethanol:water (17:3 wt%), the particle size was 40-60 mesh.

The SC-CO_2 extraction was better than traditional extraction, sonic extraction and microwave-assisted extraction and so on.

References

[1] Cinatl, J. et al., Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus, The Lancet, Vol.361**,** **2003**, p.2045.

[2] Helen R. Pilcher., Liquorice may tackle SARS, Nature, 13 June, **2003**

[3] R.L. Hall, Toxicants Occurring Naturally in Foods, National Academy of Sciences, Washington, DC, **1973**.

[4] Jung-Chung Lin., Mechanism of action of glycyrrhizic acid in inhibition of Epstein-Barr virus replication in vitro, Antiviral Research, Vol.59, **2003**, p.41

[5] Hoi-tak Chan, etc., Inhibition of glycyrrhizic acid on aflatoxin B1-induced cytotoxicity in hepatoma cells, Toxicology, Vol.188, **2003**, p.211

[6] Bart A. Poleger, etc., Physiologically Based Pharmacokinetic Modeling of Glycyrrhizic Acid, a Compound Subject to Presystemic Metabolism and Enterohepatic Cycling. Toxicology and Applied Pharmacology, Vol.162, **2000**, p.177

[7] E.S.Ong, Journal of Separation Science, Vol.25, **2002**, p.825

[8] Xuejun Pan, etc., Microwave-assisted extraction of glycyrrhizic acid from licorice root, Biochemical Engineering Journal, Vol.5, **2000**, p.173

[9] Sovova H,Kucera J,Jez J., Rate of the vegetable oil extraction with supercritical CO_2-II.Extraction of grape oil, Chemical Engineering Science, Vol.49, **1994**, p.415
[10] Fabio F,et al., Supercritical Carbon Dioxide Extraction of Evening Prim Rose Oil, JAOCS, Vol.68, **1991**, p.422
[11] Snyder J M, et al., Effect of Moisture and Size on the Extractability of Oils from Seeds with Supercritical CO_2, JAOCS, Vol.61, **1984**, p.1851
[12] Catalin Doneanu,Gheorghe Anitescu, Supercritical carbon dioxide extraction of Angelica archangelica L.root oil, Journal of Supercritical Fluids,Vol.12, **1998**, p.59
[13] Mei-Chih Lin,Ming-Jer Tsai,Kuo-Ching Wen, Supercritical fluid extraction of flavonoids from Scutellariae Radix, Journal of chromatography A, Vol.830, **1999**, p.387
[14] Jerry W.King, Ali Mohamed,Scott L.Taylor,T.Mebrahtu,Claudia Paul, Supercritical fluid extraction of Vernonia galamensis seeds. Industrial Crops and Products, Vol.14, **2001**, p.241
[15] Masaki Sato, Motonobu Goto, and Tsutomu Hirose, Fractional Extraction with Supercritical Carbon Dioxide for the Removal of Terpenes from Citrus Oil, Ind. Eng. Chem. Res., Vol.34, **1995**, p.3941
[16] A.Molero Gómez,C.Pereyra López,E. Martínez de la Ossa, Recovery of grape seed oil by liquid and supercritical carbon dioxide extraction: a comparison with conventional solvent extraction, The Chemical Engineering Journal, Vol.61,**1996**, p.227
[17] Fu Yujie, The study of extractions about glycyrrhizic acid, PhD thesis in Northeast University of Forest (China), **2002**.