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Analytical Methods

Supercritical carbon dioxide extraction of seed oil from *Opuntia dillenii* Haw. and its antioxidant activity

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ABSTRACT

Supercritical carbon dioxide extraction of seed oil from *Opuntia dillenii* Haw. and its antioxidant activity were investigated in this study. The effects of main operating parameters including extraction pressure, temperature, time and CO_2 flow rate on the extraction yield of seed oil were studied. The maximum extraction yield of 6.65% was achieved at a pressure of 46.96 MPa, a temperature of 46.51 °C, a time of 2.79 h and a CO_2 flow rate of 10 kg/h. The chemical composition of the seed oil was analysed by GC–MS. The main fatty acids were linolenic acid (66.56%), palmitic acid (19.78%), stearic acid (9.01%) and linoleic acid (2.65%). The antioxidant activity of seed oil was assessed by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test. Both methods demonstrated notable antioxidant activity of seed oil, which is nearly comparable to the references ascorbic acid and butylated hydroxytoluene (BHT). The antioxidant activity of the seed oil was also found to be concentration-dependent.

1. Introduction

Opuntia dillenii (Ker–Gawl) Haw. (Cactaceae), commonly known as pear bush, prickly pear, mal rachette or tuna, is a succulent shrub growing in semi-desert regions in the tropics and subtropics (Ahmed, EI Tanbouly, Islam, Sleem, & EI Senousy, 2005). It grows mainly in the south of China and is called Xian Ren Zhang in Chinese (Qiu, Chen, Pei, Matsuda, & Yoshikawa, 2002). The stem and fruit of *O. dillenii* are used in folk medicine for the treatment of diabetes (Perez de Paz & Medina Medina, 1988), gastric ulcers, inflammation (Park, Kahng, Lee, & Shin, 2001), etc. Analgesic (Loro, del Rio, & Pérez-Santana, 1999) and antihyperglycemic (Perfumi & Tacconi, 1996) effects have also been recorded for the fruit of this plant. Two parts of *O. dillenii* have been used as food, the fruit (prickly pears) and the "nopal". The prickly pears can be consumed freshly, after desiccation in sun, in marmalades or used as a colouring agent for foods, drinks and drugs. The nopal is consumed in Mexican regions as a constituent of salads (Chang, Hsieh, & Yen, 2008; Díaz Medina, Rodríguez Rodríguez, & Díaz Romero, 2007).

Chang et al. (2008) reported that methanolic extracts of *O. dillenii* fruit possessed notable antioxidant activity and inhibitory effect on low-density lipoprotein peroxidation, and the activities of seed extracts were stronger than those of peel and pulp extracts. Their results also demonstrated that the higher amounts of polyphenols and flavonoids in the seeds of *O. dillenii* may contribute to the stronger antioxidant activity of the seeds. However, the seeds of *O. dillenii* are rich in oil, and the high amount of unsaturated fatty acids may also possess potentially notable antioxidant activity. To the best of our knowledge, studies on the chemical composition of seed oil from *O. dillenii* and its antioxidant activity have not been reported yet. Moreover, the seeds of *O. dillenii* are the byproducts in the processing of its fruit for medicinal and food uses, and the total amount is huge. Hence, developing a high valued method for utilising the seeds of *O. dillenii* is of great importance. In fact, an interesting approach to enhance the value of seeds byproducts of some herbs is their use as potential sources of natural antioxidants (Chang et al., 2008; Simonetti, Ciappellano, Gardana, Bramati, & Pietta, 2002). The seed oil of *O. dillenii* possesses the potential as a high-quality edible oil of benefit to health, as well as providing valuable natural antioxidants for the pharmaceutical industry. Therefore, the objectives of the present study are to extract seed oil from *O. dillenii* and investigate its chemical composition and antioxidant activity.

Industrial seed oils are generally obtained by mechanical processes and organic solvent extraction (mainly hexane). The oil obtained by mechanical separation processes is of high-quality but, in most cases, the yield is lower. Hexane extraction achieves almost complete recovery of the oil, however, the solvent is dangerous to handle, and unacceptable as it is quite harmful to human health and the environment, which may restrict its use in food, cosmetic and pharmaceutical industries. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC—CO_2) is an alternative method for the extraction of oils from natural products and has received considerable attention (Gomes, Mata, & Rodrigues, 2007; Lu et al.,

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2007; Salgın, 2007). CO_2 is an inert, non-toxic, environmentally–friendly solvent and allows SFE at temperatures near room temperature and relatively low pressures. In combination with the fact that CO_2 immediately evaporates when brought to atmospheric conditions, the oil obtained is free from chemical and thermal degradation compounds and from solvent residue. The oils obtained by SC—CO_2 extraction are of outstanding quality and the yields are comparable with those by organic solvent extraction method (Friedrich & List, 1982; Gómez, López, & De la Ossa, 1996). In fact, CO_2 extracts are generally recognised as safe (GRAS) to be used in food products (Gerard & May, 2002). Therefore, SFE may serve as a very promising technology in food and pharmaceutical processing (King, 2000).

In the present study, SFE of seed oil from *O. dillenii* by SC—CO_2 was studied. The effect of the main operating parameters, namely extraction pressure, extraction temperature, extraction time and CO_2 flow rate, on the extraction yields of *O. dillenii* seed oil were investigated. The chemical composition of the seed oil was analysed by GC–MS. Furthermore, antioxidant activity of seed oil under optimised conditions was determined by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test.

2. Materials and methods

2.1. Plant material

Seeds of *O. dillenii* (Ker-Gawl) Haw. from Hainan province were supplied by Hainan Kangda Co., Ltd., Harbin, China and authenticated by Prof. Shao-Quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, PR China. The seeds were ground in a rotary mill and then sieved (20–30 mesh). The moisture content of the seeds was determined by drying 20 g of seeds at 70 °C to a constant weight in an oven for 72 h; moisture content 7.61% was obtained.

2.2. Supercritical fluid extraction

SFE of seed oil from *O. dillenii* was performed on an HA121-50-01 SFE device (Hua'an Supercritical Fluid Extraction Corp., Nantong, China), the schematic flow diagram was described in detail in a previous study (Wang, Pan, Sheng, Xu, & Hu, 2007). Carbon dioxide (purity 99.99%) was purchased from Liming Gas Corp. (Harbin, China). The operating methodology was as follows: liquid CO_2 supplied from a gas cylinder was cooled by ethanol to −5 °C before being pressurised to the desired pressure and passed into the device; the entire device was also pre-pressurised. For each experiment, approximately 300 g seeds of *O. dillenii* were loaded into a steel cylinder equipped with mesh filters (13 μm) on both ends to prevent the particles being flushed out. The loaded cylinder was then introduced into the extraction vessel, and CO_2 was let in. During the extraction process, the extraction pressure, extraction temperature and CO_2 flow rate were controlled by adjusting the valves on the front panel. When the scheduled time was achieved, the extraction vessel was depressurised and the oil was collected from the separation vessel. The oil obtained under the optimum condition was used for the following tests.

2.3. Experimental design

Orthogonal array design (OAD) was used to arrange the experiments and optimise the extraction process for seed oil from *O. dillenii*. OAD is a type of fractional factorial design in which orthogonal array is used to assign factors to a series of experimental combinations, the results can be analysed using a common mathematical procedure (Chen, Chen, Lee, Chan, & Hu, 2006; Evangelaras, Kolaiti, & Koukouvinos, 2006; Moore, McKay, & Campbell, 2006). The effects of extraction pressure, extraction temperature and time on the extraction yield were investigated. A L_{16} (4^5) orthogonal matrix with three factors, each factor containing four levels was selected to arrange the experiments. Temperatures (A) were 35, 40, 45 and 50 °C, times (B) were 1, 1.5, 2 and 2.5 h, and pressures (C) were 25, 30, 35 and 40 MPa. All the experiments were repeated in triplicate and the extraction yields were average values.

2.4. Preparation of fatty acid methyl esters (FAMEs)

Seed oil (10 g) was added into a 100 ml three-neck reaction flask with magnetic stirrer and mixed with methanol at a methanol/oil molar ratio of 6:1. Then the flask was placed in a water bath at 60 °C, and allowed to react for 40 min with KOH as catalyst at a concentration of 1% wt. of oil. After reaction, the mixture was brought to room temperature and treated with chloroform (2 ml) and 1 ml of water. The mixture was vigorously shaken; the organic phase was separated and 1.0 g Na_2SO_4 was added in; then analysed by GC–MS.

2.5. Gas chromatography–mass spectrometry analysis

GC–MS analysis was performed using an Agilent HP6890 N/5973 gas chromatography/mass spectrometer (Agilent, Santa Clara, CA, USA), equipped with an HP-5 silica capillary column (30 m × 0.32 mm i.d.; film thickness 0.2 μm). The column temperature was initially at 40 °C (held for 10 min) and then increased to 200 °C at 15 °C/min, held for 2 min, and finally increased to 230 °C at 10 °C/min. The mass spectrometer was operated in positive ion mode with ionisation energy of 70 eV. Injector and detector temperatures were 280 and 290 °C, respectively, ion source temperature was 230 °C. Helium was used as the carrier gas, the split ratio was 1:40. Mass units were monitored from m/z 35 to 425. The oil components were identified by comparison of their retention times and mass spectra with the NIST mass spectral library.

2.6. Determination of antioxidant activity

The seed oil obtained under the optimum conditions was subjected to screening for its possible antioxidant activity. The antioxidant activity was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test. All the data were the averages of triplicate determinations of three independent tests.

2.6.1. DPPH radical-scavenging assay

The scavenging activity of seed oil towards DPPH radical was determined by the method of Amarowicz, Pegg, Moghaddam, Barl, and Weil (2004). An aliquot of seed oil (100 μl) was mixed with 1.4 ml of ethanol and then added to 1 ml of 0.004% DPPH (Sigma–Aldrich) in ethanol. The mixture was shaken vigorously and then immediately placed in an UNICO UV-2100 spectrophotometer (UNICO, Shanghai, China) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ascorbic acid (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The radical–scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula:

$$\text{Inhibition percentage } (I_p) = 100(A_B - A_A)/A_B \text{ (Yen and Duh, 1994)},$$

where A_B and A_A are the absorbance values of the blank and of the tested samples, respectively, checked after 70 min.