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Ultrasound-assisted extraction and quantitation of oils from *Syzygium aromaticum* flower bud (clove) with supercritical carbon dioxide

Yu-Chiao Yang[a], Ming-Chi Wei[b,*], Show-Jen Hong[a]

[a] Department and Graduate Institute of Pharmacology, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
[b] Department of Environmental Engineering & Science, Chia Nan University of Pharmacy and Science, Tainan 71710, Taiwan

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ABSTRACT

This study evaluated ultrasound-assisted supercritical carbon dioxide (USC–CO_2) extraction for determining the extraction yields of oils and the contents of eugenol, β-caryophyllene, eugenyl acetate and α-humulene from clove buds. Compared to traditional SC–CO_2 extraction, USC–CO_2 extraction might provide a 13.5% increase in the extraction yield for the oil while utilizing less severe operating parameters, such as temperature, pressure, CO_2 flow rate and the time consumed by the process. Our results were comparable to those obtained using the heat reflux extraction method, though the yield was improved by 20.8% using USC–CO_2. In kinetic studies, the USC–CO_2 extraction of clove oil followed second-order kinetics. The activation energy for the oil extraction was 76.56 kJ/mol. The USC–CO_2 procedure facilitated the use of mild extraction conditions, improved extraction efficiency and the quality of products and is a potential method for industry.

1. Introduction

Syzygium aromaticum (L.) Merr. & Perry belongs to the family Myrtaceae, which includes plants that grow prevalently in tropical regions. This plant has recently gained a great deal of interest by scientists and medical professionals because it exhibits many beneficial effects for human health. Its dried flower buds are commonly known as cloves and have been reported to be rich in volatile oil (approximately 15–20%) and to exhibit antiviral, antifungal, antioxidant, antitumor and insecticidal biological activities; in the food industry, clove is used as both a flavoring and a antimicrobial agent [1–3]. Clove oil is mostly composed of four compounds: eugenol, β-caryophyllene, α-humulene and eugenyl acetate [4,5]. Eugenol is the major compound in clove oil, representing over 50% of the total extracted composition [4,5], and has a strong biological and antimicrobial activity [6,7]. In addition, some studies have demonstrated that α-humulene has anti-inflammatory and antitumor activities [8,9]. Additionally, eugenyl acetate has exhibited various biological activities, such as hepatoprotective and antioxidant behaviors [7]. Furthermore, β-caryophyllene displays various biological properties, including antimicrobial, anticarcinogenic, anti-inflammatory, antioxidant, anxiolytic-like and local anesthetic effects [10]. Therefore, an effective method to prepare oils from clove buds would be useful for uses in foods or medicines for human consumption.

Conventional techniques, such as hydrodistillation, steam distillation or solvent extraction, may be used to extract the essential oil from cloves [4]; however, the disadvantages of these methods include the consumption of large volumes of organic solvent and energy, lengthy extraction procedures and the potential thermal degradation, hydrolysis and water solubilization of some of the fragrance constituents. Additionally, these conventional techniques have few adjustable parameters that control the selectivity of the extraction processes, resulting in less pure clove oil being extracted using these methods. Moreover, organic solvents may introduce contamination; solvent contamination is unacceptable because it is harmful to both human health and the environment, thus restricting solvent use in the food, cosmetic and pharmaceutical industries. Therefore, finding reliable alternative methods with better selectivity and efficiency to extract the essential oil of aromatic plants is critical for producing foods or medicines for human consumption. During natural product extraction, alternative leaching techniques, such as microextraction [11], ultrasonic-assisted extraction (UAE) [12–15] and supercritical carbon dioxide (SC–CO_2) extraction [16–19] have increased the extraction efficiency processes while reducing the leachant volumes and procedure times. Of these techniques, SC–CO_2 extraction is considered one of the best green technologies that can overcome the above-mentioned drawbacks of traditional extraction methods. Currently, SC–CO_2 extraction is a promising technology that is used during processing by the food, nutraceutical, pharmaceutical and cosmetic industries to extract oils or bioactive analytes from aromatic plants or various raw materials [18–20]. SC–CO_2 utilizes CO_2 pushed

* Corresponding author. Tel.: +886 6 2664911x6215; fax: +886 6 2667320.
E-mail addresses: s120702@mail.chna.edu.tw, ycyang@kmu.edu.tw (M.-C. Wei).

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beyond its critical point (7.38 MPa and 31.1 °C) and is as an ideal non-polar extraction solvent because it exhibits several positive features, including low surface tension, liquid-like density, and gas-like viscosity, as well as high dissolution power, compressibility and diffusivity. The low viscosities and high solute diffusibility characteristics of SC–CO_2 facilitate mass transfer during extraction, realizing a higher extraction rate than that obtained using conventional extraction methods. When using this emerging clean technology, it is further possible to avoid both using any toxic solvents and leaving residues in the product because CO_2 is a gas under ambient conditions, as well as an odorless, non-toxic, non-flammable, non-corrosive and inexpensive solvent that is easily separated from the extract and the solid matrix. Furthermore, because it has a relatively low critical temperature and pressure, it is attractive for extracting thermally labile compounds. In addition, the selectivity of SC–CO_2 extraction cans be adjusted by varying the temperature, pressure, static and dynamic extraction times, flow rate and additives to obtain fractions containing the desirable compounds; therefore, the extracts obtained using SC–CO_2 have superior quality relative to those obtained using conventional extraction methods. However, because their operating conditions utilize high pressures, SC–CO_2 extraction methods require high capital and operating costs. Additionally, because it involves high-pressure equipment, mechanical stirring is difficult to apply and may result in decreased extraction kinetics. Despite these small disadvantages, using SC–CO_2 as a solvent still has several unequaled advantages in food, cosmetic and pharmaceutical industries that may be enhanced further by properly pretreating the sample, such as sonication or elongated, passive entraining before the extraction [21]. Therefore, combinatory and hyphenated techniques have grown in importance because SC–CO_2 can achieve good extraction efficiency and selectivity under less severe operating conditions; there have already been several attempts to apply hyphenated extraction methods to obtain the desired compounds from various raw matrices [18,19,21,22]. Currently, applying ultrasound to assist a SC–CO_2 (USC–CO_2) extraction process has revealed important benefits due to the mechanical effects produced in the supercritical environment through the high amplitude vibrations, radiated pressure, streaming and agitation effects [22,23]. This pioneering method accelerated mass transfer to improve the extraction processes or accelerate the supercritical extraction rates. Previous studies have indicated that at less severe operating conditions (temperature, pressure, time and CO_2 flow rate), the yield of oils from various raw materials and their supercritical extraction times could significantly improve when ultrasound is coupled with SC–CO_2 extraction [22,24].

SC–CO_2 has already been used to extract oils from clove buds [3–6], but this report details the first usage of a sequential ultrasound-assisted static extraction with an ultrasound-assisted SC–CO_2 dynamic extraction. Although in early studies, many empirical or theoretical models have been introduced to describe the kinetic processes used by SC–CO_2 while extracting oil from various raw materials [25,26]. A valid kinetic model would elucidate the extraction rate laws, thus facilitating the determination of the extraction mechanisms, enhancing the yield of the extraction process and reducing the operational costs and time. However, studying the clove oil yields should be accompanied by a thorough analysis of the kinetics that accounts for the effective power applied to the medium; this parameter was not included in any previous research and remains highly relevant for industrial applications. Therefore, the main objectives of this study are to apply a novel technique for extracting and determining the content of the oil from clove buds compared to the results obtained using conventional SC–CO_2 extraction and heat-reflux extraction techniques. The factors affecting USC–CO_2 extraction include the sample's particle size, the CO_2 flow rate, the extraction pressure, the extraction temperature, the dynamic extraction time and the static extraction time; these parameters were collectively studied to validate the optimal extraction conditions and study the kinetics of oil extraction from cloves.

2. Experimental

2.1. Materials

Dried clove buds was generously provided by Chuang Song Zong Pharmaceutical Co. Ltd. (Kaohsiung, Taiwan). The moisture content of the air-dried plant sample determined by Karl Fischer volumetric titration was 9.36%. Carbon dioxide with a purity of 99.8% was used as the solvent in this study and was purchased in the liquid form from Yun-Shan Gas Co., Ltd. (Tainan, Taiwan). Eugenol, β-caryophyllene, α-humulene and eugenyl acetate were also procured as reference substances from the Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification.

2.2. Analytical methods

2.2.1. Gas chromatography/mass spectrometry (GC/MS)

Qualitative analysis of oils was performed by Gas chromatography/mass spectrometry (GC/MS). GC/MS was performed using a Thermo Finnigan PolarisQ Ion Trap with TRACE GC/MS^n equipped with a fused-silica capillary column (30 m × 0.25 mm, 0.25-μm film thickness, model HP-5MS, Agilent Technologies Co., Ltd., Palo Alto, USA) and a mass spectrometer of the same company, which was operated in the EI mode (energy voltage, 70 eV). Column temperature set initially at 75 °C for 1 min, then programmed heating from 75 to 275 °C at 10 °C/min and subsequent holding at 275 °C for 60 min. The injector was maintained at 250 °C and helium was used as the carrier gas (1 mL/min; 1:10 split ratio). Ion source temperature was 200 °C. The ionization energy was 70 eV with a scan time of 0.5 s and mass range of 50–500 AMU. Samples were run in ethyl acetate with a dilution of 5% (v/v).

The main constituents of the oil were identified by matching their mass spectra and retention indices with those of pure compounds. Mass spectra correlations were done using NIST (National Institute of Standards and Technologies) Mass Spectra Library and Wiley Mass Spectra Library.

2.2.2. Gas chromatography

Quantitative analysis of the main constituents of the oil was performed by gas chromatography. Gas chromatography analysis of the extracts was carried out using a GC-FID system (Shimadzu, model CG-14A, Kyoto, Japan) equipped with a silica capillary column DB-5 (30 m × 0.25 mm × 0.25 μm, J & W Scientific, Folsom, CA, USA). The carrier gas was helium (1.7 mL/min) and the split ratio was 1:20. The injector and the detector temperatures were 220 and 240 °C, respectively, while column temperature was linearly programmed from 60 to 246 °C, at 3 °C/min, and from 246 to 280 °C at 5 °C/min. Solutions of samples in chloroform were injected in amount of 1 μL. The peak identification of target compounds within the extracts was performed on the basis of retention time and chromatographic behavior compared to those of the authentic standards. The quantity of the target compounds was calculated by comparing their peak area to that of the standards.

2.3. Ultrasound-assisted SC–CO_2 (USC–CO_2) extraction

The supercritical CO_2 extraction apparatus used was a semi-continuous flow, high-pressure system (Fig. 1). The herbal sample (5.0 g) was well mixed with 2-mm stainless steel balls and was then charged into the 43-mL extraction vessel (SS304, i.d. of 2.2 cm and length of 11.3 cm). The extraction vessel was later immersed into