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Comparison of different extraction techniques for the extraction of high valuable bioactive compounds from traditional medicinal plant

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Abstract

Introduction:

Perilla frutescens leaves is valuable as a medicinal plant as well as a natural medicine and functional food. *Perilla* leaves are an important source of essential oils and had antidepressive, anxiolytic, chemopreventive and strong antitumor-promoting activities.

Methods:

Four different isolation techniques, specifically ultrasound-assisted supercritical carbon dioxide (USC-CO_2) extraction, heat-reflux extraction (HRE), conventional supercritical carbon dioxide (SC-CO_2) extraction and hydrodistillation (HD) were employed to obtain essential oils from purple-leafed of *Perilla frutescens*. The essential oils were further analyzed using gas chromatography (GC) with flame ionization detection and gas chromatography with mass spectrometry (GC-MS). **Results**: The maximum yield of oil was extracted via USC-CO_2 extraction (1.62%, weight of the extracted oil/weight of feeding material) while utilizing less severe operating parameters, such as temperature (49 °C), pressure (28 MPa), organic solvent (0 mL) and the time consumed (105 min) by the process. The results revealed that the dominant component was perillaldehyde (> 40%) in total average content.

Discussion:

To evaluate the feasibility of supercritical separation processes and for establishing optimum operation conditions, a second-order kinetic model and a mass transfer model based on Fick's law were applied to appraise the kinetics and thermodynamics aspects of *perill*a oil extraction employing the USC-CO_2 systems. Additionally, the thermodynamic properties of essential oil in the supercritical fluid system were estimated following the theory developed by the second-order kinetic model and a mass transfer models.

Keywords: *Perilla frutescens*, essential oil, perillaldehyde, green extraction technique, ultrasound-assisted supercritical CO_2 extraction.

1. Introduction

Perilla frutescens (L.) Britton is an aromatic, annual herb of the Lamiaceae family that is widely used in food and traditional herbal medicine common in Asian countries, particularly China, Korea,and Japan (Jun et al., 2014;You et al., 2014; Zenget al., 2014; Tabanca et al., 2015; Hwang et al., 2017). Previous phytochemical studies have demonstrated that perilla leaves contain a variety of constituents classes, including phenolic acids, flavonoids, essential oil and vitamin (Tada et al., 1996; Ku et al., 2013; Liu et al., 2013;Verspohl et al., 2013; Zhou et al., 2014), which are known to have hepatoprotective, anti-inflammatory, antioxidant, antidepressive, anxiolytic, anti-aging, anti-hyperlipidemia, antitumor, antimicrobial, chemopreventive and neuroprotective activities (Huang et al., 2011; Kwon et al., 2014;Tang et al., 2014; Zhu et al., 2014; Chen et al., 2015; Park et al., 2015; Chen et al., 2016). Apart from medicine and food use, perilla leaves was also applied to produce perfume, soap, detergents and cosmetics (Vearasilp et al., 2015; Kawamura et al., 2016). Therefore, worldwide interest in the consumption and use of perilla leaves and products has dramatically and increased.

Various investigations have further reported perilla leaves to be one of the rich sources of essential oils that exhibit significant pharmacological activities such as anti-inflammatory, antioxidative, neuroprotective, anticancer, anti-depression, anti-hyperlipidemia, and antimicrobial activities (Kang et al., 1992; Feng et al., 2011; Izumi et al., 2012; Buchwald-Werner et al., 2012; Tian et al., 2014) and are also used in perfumes, soaps, detergents, and cosmetics (Tian et al., 2014; Ha et al., 2015). In recent years, perilla essential oil is listed among generally regarded as safe (GRAS) food flavorings for use in baked goods, beverages, frozen dairy products, puddings, and processed vegetables and soups due to attractive flavors and nutritional values (Smith et al., 2001; Tabanca et al., 2015). Based on these reasons, it has focused on edible sources with high volatile material contents for manufacture of supplements with preventive and therapeutic effects. Therefore, the volatile constituents of perilla essential oil has been extensively investigated, which a wide variety of the composition of essential oils may be found perilla, with compounds varying among species, chemotypes, geographical location, growing environment, and collection zone (Huang et al., 2011; Liu et al., 2012; Zeng et al., 2014; Tabanca et al., 2015). However, many researches had been reported that the main volatile components of *Perilla frutescens* (red-leafed varieties of *Perilla frutescens*, Chinese name is Zisu) is perillaldehyde (Ye et al., 2009; Huang et al., 2011; Liu et al., 2012; Chang et al., 2014; Ji et al., 2014; Tian et al., 2014; Tabanca et al., 2015), which might be related to the antimicrobial, antioxidant, anticancer, vasodilative and anti-inflammatory properties (Huang et al., 2011; Igarashi et al., 2013; Ji et al., 2014; Tian et al., 2014; Xu et al., 2014; You et al., 2014; Fukushima et al., 2015). Besides, perillaldehyde is also used in food, flavor, perfumery, organic synthesis and pharmaceutical industry (Chang et al., 2014). In addition, Fukushima et al. reported that perillaldehyde is an index compound for quality control of *Perilla frutescens* in the Japanese Pharmacopoeia (Fukushima et al., 2015). For the extraction of the essential oil from perilla leaves, various extraction protocols, such as hydrodistillation or steam distillation (Seo et al., 2009; Huang et al., 2011; Liu et al., 2013; Chang et al., 2014; Tian et al., 2014; You et al., 2014; Zeng et al., 2014; Tabanca et al., 2015; Ha et al., 2015) organic solvent extraction (Seo et al., 2009; Liu et al., 2012), ultrasound-assisted extraction (Liu et al., 2012), microwave-assisted extraction (Liu et al., 2012), solid-phase microextraction (Ye et al., 2009; Huang et al., 2011; Tian et al., 2014), and supercritical carbon dioxide extraction (Huang et al., 2011; da Silva et al., 2015) have been reported. Among these techniques, ultrasound-assisted extraction (UAE) is a potential alternative technology to the conventional techniques due to its reduced cost and instrumental requirements (Wei et al., 2013; Yang et al., 2013). Some characteristics of UAE are high extraction efficiency, good reproducibility, simplified manipulation, low consumption of organic solvents and time and lower energy input (Yang et al., 2012; Yang et al., 2013). Ultrasonic enhancement during extraction is attributed to disrupt cell walls, reduce particle sizes, and enhance mass transfers of cell content via cavitation effects (Wei and Yang, 2014; Wei and Yang, 2015). In addition, SC-CO_2 has become of interest for its effective extraction of various compounds from natural materials to provide fairly clean and superior quality extracts in the food, pharmaceutical and biotechnology industries (Wei et al., 2015). The most important advantages of utilizing SC-CO_2 are its excellent mass transfer properties, facile separation of the solvent from the extracted material, use of less harsh operating conditions, reduced need for environmentally aggressive solvents (thus reducing their use and disposal) and ease of control by altering the temperature, pressure or use of a cosolvent. However, it has the limitation of needing high-pressure equipment, which is not only more expensive than that used for conventional extraction processes but also susceptible to failure of the mechanical stirring function, possibly resulting in a substantial decrease in the extraction efficiency due to the subsequent slow kinetics of the process (Wei et al., 2015).

In order to obtain the required extraction yields at shorter processing times or with higher economic efficiency, combinatory and hyphenated techniques are of growing importance because they often are the only way to achieve good extraction efficiency and required selectivity at the same or less severe operating conditions. Recently, there have been several attempts to apply a hyphenated process of UAE and SC-CO_2 extraction to extract compounds of interest from various raw materials (Yang and Wei, 2015; Yang and Wei, 2016). These novel procedures can further result in a substantial decrease in the overall cost of the operation. These enhancements might be due to the cavitational effects that intensify mass transfer to enhance the permeability of the solid matrix so that a greater amount of the solvents can penetrate into the inner areas (Yang et al., 2017). Furthermore, the turbulence and acoustic streaming induced by ultrasound can significantly increase the solid-liquid mass transfer coefficients to increase the yield (Wei et al., 2017).

To the best of our knowledge, there has been no report to date on the extraction of volatile oils from *Perilla frutescens* using a combined process (USC-CO_2) of UAE and SC-CO_2 extraction. Hence, the main purpose of this study was to develop effective extraction conditions for the extraction of volatile oils from *Perilla frutescens* using a combined procedure.. Furthermore, a second-order kinetic model and a mass transfer model based on Fick's law were applied to appraise the kinetics and thermodynamics aspects of perilla oil extraction employing the USC-CO_2 systems.

2. Material and methods

2.1. Raw material

Three different batches of dried *Perilla frutescens* (Pf1- Pf3) were obtained from different local Chinese medicinal shops (Taiwan). and authenticated and deposited by Department and Graduate Institute of Pharmacology, Kaohsiung Medical University (Kaohsiung, Taiwan). All samples were triturated in a knife mill and were separated according to their sizes (standard testing sieve, series Tyler) with different mean particle sizes of 0.93, 0.73, 0.55, 0.36 and 0.11 mm. Subsequently, the moisture contents were 10.27%, 9.58% and 8.71% as determined by Karl Fischer volumetric titration. Carbon dioxide (99.99%) and helium (99.99%) were purchased from Yun-Shan Gas Co. Ltd. (Tainan, Taiwan), and *n*-hexane was bought from Merck Co. (Darmstadt, Germany). Limonene, Perillaldehyde, (Z, E)- α-farnesene and

Caryophyllene were procured as reference substances from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Volatile oils extraction by ultrasound-assisted SC–CO_2 (USC–CO_2) procedure

USC–CO_2 extraction experiments were performed with a semi-batch flow extraction apparatus. The solvent was delivered into a surge tank where the temperature was kept at 2.0 °C (±0.1 °C) by a thermostatic bath (Haake F3-K, Haake, Karlsruhe, Germany) in order to guarantee liquid CO_2 in the pump head section. The solvent was then pressurized by a high-pressure pump (ISCO 260D, Lincoln, NE, USA) and pumped to fill the extraction vessel until the working pressure was reached. The herbal sample (20.0 g, 0.36 mm) 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 the ultrasonic bath (fixed at the same position in the bath) to maintain a constant temperature throughout the extraction. More details about the SC–CO_2 extraction equipment and its operation have recently been described elsewhere (Yang and Wei, 2015b). The process of ultrasound-assisted extraction (static stage) utilized an ultrasonic bath with a working frequency of 40 kHz and 185 W of power (Branson B-33810E-DTH, USA).

After desired temperature (32–52°C) and pressure (10.0–33.0 MPa) were reached, a static extraction was performed for 15 min (with ultrasound assistance) and 30 min (without ultrasound assistance), followed by a dynamic extraction that varied from 5 to 210 min (with/without ultrasound-assisted) at the CO_2 flow rate of 0.13–0.76 g/min. The extracts were then collected in two cold separators placed in series. The optimum fractionation was achieved in both cases by operating at 9 MPa and – 4 °C in the first separator and at 0.15 MPa and 4 °C in the second. Finally, the expanded low-pressure CO_2 gas released from the extract passed through a wet-gas flow meter (type TG05, Ritter Co., Germany) into the atmosphere. The gas flow-rate was monitored with the rotameter (model p23/10082-03G, Aalaborg Co.), and the total volume of gas was measured with a wet test meter. The details were described in previous work (Wei et al., 2016).

To ensure the accuracy of the experimental data, this extraction process was repeated four times for the same sample. The standard error from replicate experiments was controlled to ± 6%. The data are presented in this study as means ±SD.

2.3. Essential oil extraction by hydrodistillation

The red leaves of P. frutescens were used to obtain the essential oils by hydrodistillation for 6 h in a Clevenger-type apparatus, until complete exhaustion. The yield was estimated on the basis of the dry plant weight from four replicates. Anhydrous sodium sulphate was used to dry the oils obtained.

2.4. Volatile oils extraction by Heat-reflux extraction (HRE)

The experimental apparatus and the extraction procedures have been described in detail in the literature (Wei et al., 2016a). Briefly, 20 g of dried powdered *P. frutescens* leaves was placed in a flask, and 400 mL of hexane was added. To prevent solvent loss during extraction, a condenser was connected to the flask. Then, 20 g of dried clove buds (0.36 mm) was extracted using HRE for240 min (four extraction cycles). After extraction, the solutions were carefully decanted, and the plant material was re-extracted using the same solvent. The yield (wt%) was defined as the oil mass versus the material mass.

2.5. *Analytical methods*

Gas chromatography analysis of the extracts was carried out a GC-FID system (Shimadzu, model CG-14A, Kyoto, Japan) equipped with a silica capillary column DB-5 (30m×0.25mm×0.25μm, J & W Scientific, Folsom, CA). The carrier gas was helium (1.7 mL/min, 99.9% purity, Yun-Shan Gas Co. Ltd., Tainan, Taiwan) 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.

Qualitative analysis of the extracts was performed by Gas chromatography/mass spectrometry (GC/MS). GC/MS was performed using a Thermo Finnigan PolarisQ Ion Trap with TRACE GC/MS 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 60 °C for 2 min, then programmed heating from 60 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 220 °C. The ionization energy was 70 eV with a scan time of 0.5 s and mass range of 40–500 AMU. Samples were run in ethyl acetate with a dilution of 5% (v/v).

The volatile constituents of perilla essential 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.

2.6. Statistical analysis

All yields and compositional analyses were calculated by assuming a moisture-free environment. The mean and standard deviation (SD) of the mean were calculated by using four experiments. An analysis of variance (ANOVA) was performed by using Tukey's method with a significance level of $P<0.05$ in Microsoft Office Excel 2010 (Microsoft CO., USA) and Origin software version 6.1 (Origin Lab CO., Northampton, MA, USA). To study the correlation results of perilla essential oil in the applied models, the objective function of the average absolute relative deviations ($AARD$%) between the calculated and experimental value and the coefficient of determination (R^2) were applied, which were computed by using the following equations, respectively.

$$AARD(\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{y_{p,i} - y_{e,i}}{y_{e,i}}\right| \tag{1}$$

$$R^2 = \frac{\sum_{i=1}^{n}(y_{p,i} - y_{e,i})^2}{(y_{p,i} - y_m)^2} \tag{2}$$

Where $Y_{p,i}$ is the calculated value corresponding to point i, $Y_{e,i}$ is the experimental value for experimental point i, Y_m is the mean value of the yield, and n is the number of experimental data. The lower $AARD$% values reflected better correlation results.

3. Theory

3.1 Kinetic behavior

Based on the second-order rate law, the analyte content (C) in raw materials for an extraction time (t) during the process may be represented as follows :

$$\frac{dC}{dt} = k(C_s - C)^2 \qquad (3)$$

In Eq. (3), the residual analyte for a unit of time (*dC/dt*) depends on the analyte concentrations in the liquid extract at a given extraction time *t* (*C*, mg/g), a second-order extraction rate constant (*k*, g/mg-min) and the reference concentration for the target compounds in the liquid extract (C_s, mg/g). The equation was outstandingly simple when correlating the experimental kinetic data for the solid solute into the UAE process, including only two parameters obtained using a simple error minimization method, such as the least squares method. The second-order rate law approximation with the initial and boundary conditions (*t*= 0 to *t* and *C*= 0 to *C*) yields the following theoretical equations:

$$\int_0^C \frac{dC}{(C_s - C)^2} = \int_0^t kdt \qquad (4)$$

$$C = \frac{C_s{}^2 kt}{1 + C_s kt} \qquad (5)$$

A linearized equation was obtained by rearranging Eq. (5):

$$\frac{t}{C} = \frac{1}{kC_s{}^2} + \frac{t}{C_s} = \frac{1}{h} + \frac{t}{C_s} \qquad (6)$$

where *h* (mg/g-min) is the initial extraction rate. When *t* approaches 0,

$$h = kC_s{}^2 \qquad (7)$$

The *t/C* values were plotted against time and fit to a straight line using a least-squares regression to estimate the *k*, *Cs* and *h* parameters.

As detailed above, the extraction temperature directly affects the second-order rate constants and may be described using the Arrhenius equation:

$$k = Ae^{-Ea/RT} \qquad (8)$$

where *A* is a temperature independent factor (g/mg-min), *Ea* is the activation energy (kJ/mol), *R* is the gas constant (kJ/mol-K), and T is the absolute temperature (K).

In addition, according to the Eyring transition state theory (TST), the enthalpy (ΔH), entropies (ΔS), and Gibbs free energy (ΔG) of activation is giving as following:

$$\Delta Ha = E_a - RT \qquad (9)$$

$$Ln\frac{k}{T} = -\frac{\Delta Ha}{R} \times \frac{1}{T} + (Ln\frac{k_B}{h} + \frac{\Delta Sa}{R}) \qquad (10)$$

$$\Delta Ga = \Delta Ha - T\Delta Sa \qquad (11)$$

where, ΔHa is the enthalpy of activation (kJ /mol), E_a is the activation energy, ΔSa is the entropy of activation (kJ/ mol-K), ΔGa is the Gibbs free energy of activation (kJ/ mol), k_B is the Boltzman constant (1.38×10^{-23} J/ K), h is the Planck constant (6.6256×10^{-34} J /s).

3.2. Mass transfer kinetic model and thermodynamic assessment

Based on the basic assumptions, a one-dimensional non-steady state diffusion model derived from the Fick's second law can be approximately predicted the mass transfer of a solute from spherical particles and concentration differences will be only relevant in radial direction. The differential equation based on Fick's second law for the unidirectional diffusion of solute inside the porous particles of spherical geometry is shown below:

$$\frac{\partial M}{\partial t} = D_e(\frac{1}{r^2}\frac{\partial}{\partial r}(r^2\frac{\partial M}{\partial r}) \qquad (12)$$

where M is the analyte concentrations in the solid particle (mg/g), r is the distance to the centre of spherical particle (m), D_e is the analyte effective diffusion coefficient or diffusivity (m^2/s) and t is time (s).

The initial conditions (t=0, ∀r) for solving the Eq. (12) are:

For the solid phase at t = 0 : $M(0,r) = M_o; 0 \le \forall r \le R$ (13)

For the solvent phase at t = 0 : $C = 0; 0 \le \forall r \le R$ (14)

where M_o is the initial concentration of the analyte in the solid phase i.e. in the sample particles (mg/g), C

is the analyte concentrations in the liquid extract at a given extraction time t (mg/g) and R is the radius of sample particles (m).

The boundary condition at the central axis of sample particles (r = 0) is:

$$(r=0,\forall t),(\frac{\partial M(t,r)}{\partial r})_{r=0}=0 \qquad (15)$$

The boundary condition at the solid–liquid interface (r = R), the outcoming flux (J) of the analyte from the solid particle is equal to the incoming flux in the liquid phase as:

$$(r=R,t\rangle 0),(-D_e A\frac{\partial M(t,r)}{\partial r})_{r=R}=V_s(\frac{dC}{dt}) \qquad (16)$$

where A is particle area (m^2) and Vs is solvent volume (m^3 or L).

Using the initial and boundary conditions, the solution of Eq. (12) relate the dimensionless extent of extraction of solute with time in a series expression represented by following equation:

$$\frac{C}{C_\infty}=1-\frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n}\exp(-\frac{D_e n^2\pi^2 t}{R^2}) \qquad (17)$$

where C_∞ is the total amount of solute transferred after infinite time. After a certain time lapse or usually after the washing stage, only the first term of the series is important and Eq. (17) can be can written as:

$$1-\frac{C}{C_\infty}=\frac{6}{\pi^2}\exp(-\frac{D_e\pi^2 t}{R^2}) \qquad (18)$$

Using nature logarithm function to process both sides of Eq. (18), the following equation (Eq. (19)) is obtained:

$$Ln(\frac{C_\infty}{C_\infty-C})=Ln\frac{\pi^2}{6}+\frac{D_e\pi^2 t}{R^2} \qquad (19)$$

Since C_∞ is the equilibrium concentration for the target compounds in the liquid extract (C_s), Eq. (19) becomes:

$$Ln(\frac{C_s}{C_s-C})=Ln\frac{\pi^2}{6}+\frac{D_e\pi^2 t}{R^2} \qquad (20)$$

Consequently, the effective diffusion coefficient can be calculated from the slop the straight line when plotting the logarithm of ($C_s/(C_s-C)$) against time. To investigate the influence of temperature on the extraction process, the effective diffusivity values were fitted to the temperature dependence Arrhenius type equation:

$$D_e = D_0 \exp(-\frac{E_{AEd}}{R_g T}) \qquad (21)$$

where D_0 is the pre-exponential factor, E_{AEd} is the energy of activation for diffusion, R_g is the universal gas constant (8.314 J/mol- K) and T is the absolute temperature (K).

To determine the effect of mass transfer within the sample on the extraction of the target compounds, the mass transfer Biot number (*Bi*) is usually employed to investigate the relative importance of internal and external resistances. The Biot number allow to predict whether the limiting factor is internal or external. Generally, the Biot number greater than 50 mean that internal transfer is limiting and thus the progress of extraction is mainly controlled by diffusion. Inversely, the system suffers from the external mass transfer limitation if it is less than 50. The mass transfer Biot number (*Bi*) can be determined according to the expression:

$$Bi = \frac{r_P K_T}{D_e} \qquad (22)$$

where K_T is the mass transfer coefficient (m/s) and r_p is the size of particle (m).

At the solid–liquid interface (r = R), the outcoming flux (*J*) of target compounds from the solid is equal to the incoming flux in the liquid and can be written in the following forms:

$$J = V_S(\frac{dC}{dt}) \qquad (23)$$

In the unidimensional system for a binary mixture, the incoming flux in the liquid at equilibrium can be simplified as:

$$J = AK_T \Delta C = AK_T (C_S - C) \qquad (24)$$

Combining Eqs. (23) and (24), with the simplified forms shown as follows:

$$V_s \frac{dC}{dt} = AK_T (C_S - C) \qquad (25)$$

Considering the initial and boundary conditions t = 0 to t and C = 0 to C, the integrated equation for Eq. (25) is written as:

$$Ln\frac{C_S}{C_S - C} = \frac{AK_T t}{V_s} \qquad (26)$$

The plot of ln $C_S/(C_S - C)$ against time was used for the calculation of the transfer coefficient (K_T). However, Eq. (26) can only be valid from t = 0 to t = t_R, where t_R is defined as:

$$t_R = \frac{1}{kC_s} \qquad (27)$$

4. Results and Discussion

To obtain the best efficiency of USC-CO_2 procedure, several extraction parameters need to be optimized, including the mean particle size, ultrasonic power, ultrasonic frequency, ultrasonic duty cycle, CO_2 flow rate, extraction temperature, extraction pressure and extraction time regarding the specific raw materials. Based on previous studies (Wei et al., 2017), a mean particle size of 0.36 mm and a static time of 15 min (with ultrasound assistance) were considered appropriate for the combined procedure and were held constant in the following experiments. In addition, the ultrasound-assisted extraction was also fixed at an ultrasonic frequency of 40 kHz, a power of 185 W and an ultrasound cycle of 75% (intermittent sonication).

To use the dynamic method to assess the experimental solubility data, the CO_2 flow rate was considered sufficiently low to enable saturation/equilibrium conditions to be obtained. To evaluate the influence of CO_2 flow rate on the yield of volatile oils, USC-CO_2 was conducted at a 15 min static stage followed by a 5–280 min dynamic stage with a mean particle size of 0.36 mm, a duty cycle of 75% (40 kHz working frequency and 185 W power), an extraction temperature of 32 °C and an extraction pressure of 33 MPa. The yield of volatile oils was expressed as the percent ratio (%, w/w) of the mass of oil per mass of *Perilla frutescens*. The extraction yields of the compounds (Caryophyllene, Limonene, Perillaldehyde and (Z, E)- α-farnesene) were expressed as the percent ratio (%, w/w) of the mass of compound per mass of extracted oil. According to the previous representative studies (Wei et al., 2016a, 2016b), the overall extraction curves (OECs) of volatile oils with SC–CO_2 were obtained using the dynamic method and were presented as the total mass of volatile oils (g volatile oils /g *Perilla frutescens*) as a function of extraction time (min) or as a function of the solvent/biomass mass ratio (g CO_2/g *Perilla frutescens*) (Fig. 1.). Furthermore, the solvent phase solute mass ratio at the bed outlet (Y_{CER}) can be determined from the slope of the initial linear portion of the overall extraction curves (OECs) of volatile oils (Fig 1), which are illustrated in Fig 2. The reported results are expressed in terms of the effect of CO_2 flow rate on Y_{CER} in the supercritical fluid system at a pressure of 33 MPa and a temperature of 32 °C. The value of Y_{CER} increased with increasing CO_2 flow rate from 0.09 to 0.37 g/min and approached a peak value at 0.37 g/min due to the increased saturated extent and dereased mass-transfer limitations of solvent at the extraction vessel outlet. However, when CO_2 flow rate increased from 0.37 to 0.76 g/min, the value of Y_{CER} started to decrease due to the decreased saturated extent of solvent at higher CO_2 flow rate. Therefore, 0.37 g/min was determined as an adequate CO_2 flow rate, which the CO_2 flow rate should be low enough such that the solvent is saturated at the extraction vessel outlet, but at the same time, mass-transfer limitations should be avoided. At saturation, the Y_{CER} should be maximum, and the corresponding CO_2 flow rate is suitable for measuring the solubility of volatile oils. Thus, the maximum value of the Y_{CER} is identified as the solubility of volatile oils, Y^* (g volatile oils/g CO_2).

5. Conclusion

In this work, extraction of volatile oils from *P. frutescens* using a combined procedure of ultrasound-assisted and SC-CO_2 (USC-CO_2) extraction with various lengths of time (10-210 min), temperatures (32–55°C) and pressures (10.0–33.0 MPa) was investigated. Conventional extraction methods, such as traditional SC-CO_2 extraction, hydrodistillation and heat-reflux extraction, were conducted in parallel for comparison in this study. The results indicated that USC-CO_2 extraction was superior to the other extraction techniques, based on its higher extraction yield and extraction efficiency.

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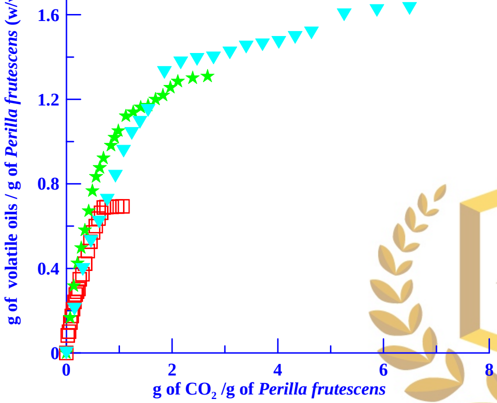
(a) 0.09 (□), 0.25 (★), and 0.55 (▼) g/min



(b) 0.13 (○), 0.37 (◆), and 0.76 (▲) g/min

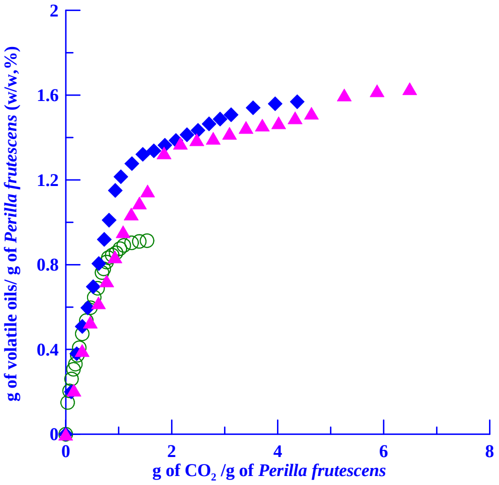


Fig.1. Overall extraction curves at various CO_2 flow rates for the USC−CO_2 extraction of volatile oils from *Perilla frutescens* at 32 °C and 28 MPa (0.09 (□), 0.13 (○), 0.25 (★), 0.37 (◆), 0.55 (▼), 0.76 (▲) g/min)

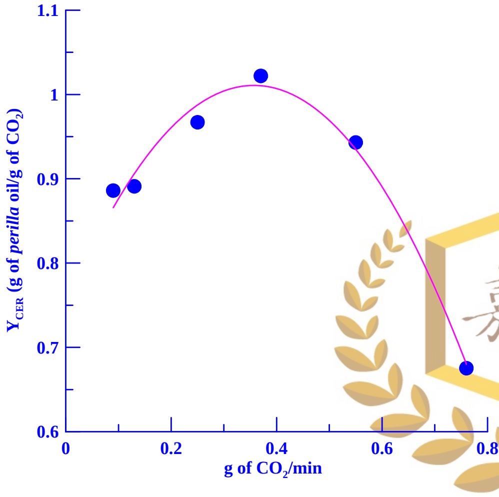


Fig.2. Effect of CO_2 flow rate on the Y_{CER} (g volatile oils /g CO_2) value at 32 °C and 28 MPa.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