



Development and validation of an ultrasound-assisted supercritical carbon-dioxide procedure for the production of essential oils from *Perilla frutescens*

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ABSTRACT

Four different isolation techniques, the ultrasound-assisted supercritical CO₂ (UscCO₂) extraction, traditional supercritical CO₂ (TscCO₂) extraction, steam distillation (STD) and heat-reflux extraction (HRE), were utilized to isolate volatile oils from the purple leaves of *P. frutescens*. The highest yield of volatile oils obtained by the UscCO₂ procedure was 1.62% (weight of extracted oil/dry weight of plant material), which was 13.7, 30.2 and 72.2% higher than the oil yields obtained by TscCO₂ extraction, STD and HRE, respectively. In addition, the content of perillaldehyde (49.76%, w/w) obtained by the UscCO₂ process was approximately 1.09, 1.43 and 1.23 times higher than the contents obtained by STD, HRE and TscCO₂ extraction, respectively. Moreover, the theoretical solubility of volatile oils in supercritical solvent was evaluated with the dynamic extraction method and then correlated with the Chrastil, Bartle and Kumar and Johnston models, with average absolute relative deviations (AARDs) of 0.71%, 0.74% and 3.56%, respectively.

1. Introduction

Perilla frutescens (L.) Britton is frequently used in foods and traditional herbal medicines (Tabanca et al., 2015; Torri et al., 2019). Recently, perilla essential oil was also categorized among the food flavorings that are generally regarded as safe (GRAS) for use as food additives owing to its attractive flavors and nutritional value (Tabanca et al., 2015). Therefore, the volatile constituents of perilla essential oil have been extensively investigated, and the composition of the essential oil varies substantially between species, chemotypes, geographical locations, growing environments, and collection zones. However, many studies have reported that the main volatile component of *P. frutescens* (red-leaved varieties of *P. frutescens*) is perillaldehyde (Chang et al., 2014), which might be related to the antimicrobial, antioxidant, anticancer, vasodilative and anti-inflammatory properties of the oil (Tian et al., 2014).

Steam distillation and hydrodistillation are widely utilized to obtain volatile oil from various raw matrixes and possess the advantage of convenience during operation (Gavahian, Lee, & Chu, 2018). However, the manipulation of these techniques for oil extraction is mainly limited due to the possible degradation of labile compounds, low extraction yield and long consumption time. Traditional supercritical carbon dioxide (TscCO₂) extraction has been considered an alternative to

traditional extraction procedures for the isolation of biological ingredients from various matrixes because it does not damage and lower the quality due to its low critical parameters (7.4 MPa, 31.1 °C). Furthermore, the excellent heat and mass-transfer characteristics, easy isolation of CO₂ from the extracts, employment of moderate extraction parameters, has abated the use of environmentally harmful solvents. However, this technique is limited by the need for a high-pressure apparatus that is responsive to the mechanical stirring failure, which may result in a substantial decrease in the procedure efficacy due to the subsequent slow kinetics of the process (Yang & Wei, 2018).

Recently, some researchers have made several attempts to apply a combinatorial procedure (UscCO₂) of ultrasound-assisted extraction (UAE) and TscCO₂ extraction to isolate interesting constituents from various plants (Yang & Wei, 2016). Owing to the effect of cavitation, the mass transfer of solvent and analytes is facilitated, and the extraction efficiency is enhanced. Hence, the primary objective of the current study was to determine the best parameters for the extraction of volatile oils from *P. frutescens* utilizing the aforementioned UscCO₂ procedure. The yield and main constituents of the volatile oil from *P. frutescens* were compared to those obtained with STD, HRE and TscCO₂ extraction. To determine the best operating conditions to estimate the feasibility of developing an UscCO₂ procedure, the theoretical solubility of volatile oils in the solvent using the UscCO₂ dynamic extraction method

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under different temperature and pressure conditions was further determined and correlated with density-based models.

2. Experimental

2.1. Raw material

Three different batches of dried *P. frutescens* (Pf1–Pf3) were procured from different local Chinese herbal shops (Taiwan). These plant materials were further triturated in a knife mill. The batches were then separated according to the mean particle sizes of 0.93, 0.73, 0.55, 0.36 and 0.11 mm. Subsequently, the moisture contents of Pf1–Pf3 analyzed by volumetric Karl Fischer titration were 10.27%, 9.58% and 8.71%, respectively. Helium (99.99%) and carbon dioxide (99.99%) were acquired from Yun-Shan Gas Co. Ltd. (Tainan, Taiwan). Limonene, perillaldehyde, (Z, E)- α -farnesene and caryophyllene were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and *n*-hexane was obtained from Merck Co. (Darmstadt, Germany).

2.2. Extraction of volatile oils by UscCO_2 and TscCO_2 procedures

UscCO_2 and TscCO_2 were performed with a semi-batch flow extraction procedure (Isco 260D, Lincoln, NE, USA). More details about the apparatus and its operating procedures have been provided elsewhere (Yang, Wang, & Wei, 2019a). Briefly, the dried and powdered leaves of *P. frutescens* (20 g), and 1-mm stainless steel balls were added into an extractor, which was later immersed in an ultrasonic bath (40 kHz, 185 W). After the desired pressure (10.5–33.0 MPa) and temperature (32–55 °C) were reached, a static stage was performed with ultrasound assistance for 15 min or without ultrasound assistance for 30 min, followed by a dynamic stage with/without ultrasound assistance (5–210 min) at a CO_2 flow rate of 0.13–0.76 g/min. The oils were collected in consecutive separators (4 °C), operating at 9 and 0.15 MPa in the first and second separator, respectively. Data are presented as the mean \pm standard deviation (SD). The SD was limited to \pm 6% to guarantee the accuracy of the data ($n = 4$).

2.3. Essential oil extraction by steam distillation (STD)

The essential oils of *P. frutescens* leaves were obtained by the STD process for 4 h in a Clevenger-type apparatus until complete exhaustion. The yield was estimated based on the dry plant weight from four replicates. Anhydrous sodium sulfate was used to dry the obtained oils.

2.4. Extraction of volatile oils by heat-reflux extraction (HRE)

Detailed operating procedures and the experimental apparatus were described in previous literature (Yang, Wei, Lian, & Huang, 2014). Briefly, the dried and powdered leaves of *P. frutescens* (20 g) were placed in a flask along with *n*-hexane (400 mL). A condenser was connected to the flask to avoid solvent loss during the extraction. Then, the powder was extracted using a 240-min procedure (four extraction cycles). After each extraction cycle, the solution was carefully decanted, and the sample was re-extracted using fresh portions of the same solvent. The yield (wt (%)) was represented as the oil mass versus the dry plant material mass.

2.5. Gas chromatographic and gas chromatography/mass spectrometry (GC/MS) analysis

Gas chromatographic analyses of the extracts were carried out on a gas chromatography with flame ionization detection (GC/FID) system (Shimadzu, model CG-14A, Kyoto, Japan) equipped with a DB-5 silica capillary column (30 m \times 0.25 mm \times 0.25 μm , J & W Scientific, Folsom, CA, USA). More details about the apparatus and its procedures have recently been described in detail in the literature (Wei, Xiao, &

Yang, 2016). Peak identification of the constituents in the oils was performed based on retention times and chromatographic behavior compared to those of the authentic standards. The concentrations of the constituents were estimated by comparison of their peak areas to those of the standards.

Qualitative analyses of the extracts were also performed by GC/MS. GC/MS chromatograms were obtained using a Thermo Finnigan PolarisQ Ion Trap with TRACE GC/MS system equipped with a fused-silica capillary column (30 m \times 0.25 mm, 0.25- μm film thickness, model HP-5 MS, Agilent Technologies Co., Ltd., Palo Alto, USA) and a mass spectrometer from the same company, which was operated in electron ionization (EI) mode (energy voltage, 70 eV). The column temperature was initially set at 60 °C for 2 min, then programmed to increase from 60 to 275 °C at 10 °C/min and held at 275 °C for 60 min. More details about the apparatus and its procedures have recently been described in detail in the literature (Wei, Xiao, et al., 2016).

The volatile constituents of perilla essential oil were identified by matching their mass spectra and retention times with those of the standards. Mass spectral correlations were made using the National Institute of Standards and Technology Mass Spectral Library and Wiley Mass Spectral Library.

2.6. Statistical analysis

All yield analyses were estimated assuming a dry-based plant weight. The average value and SD of the average value were evaluated by using the results of four experiments. More details about the analysis of variance (ANOVA) procedure were recently described in detail in the literature (Yang, Wang, & Wei, 2019b). To study the correlative results for perilla essential oil in the applied models, the objective function of the average absolute relative deviations (AARD(%)) between the calculated and experimental values and the coefficients of determination (R^2) were applied. AARD(%) and R^2 were evaluated by utilization the following equations, respectively:

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

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

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 average value of the yield, and n is the number of data. Lower AARD(%) values reflect stronger correlations.

3. Results and discussion

3.1. Influence of the CO_2 flow rate

To utilize the dynamic extraction process to estimate the theoretical solubility data, the CO_2 flow rate had to be adequately low to allow saturation/equilibrium to be reached. To estimate the influence of the flow rate on the yield of volatile oils, UscCO_2 was conducted over a static stage of 15 min followed by a dynamic stage of 5–210 min, at a given duty cycle of 75% (40 kHz working frequency and 185 W power), a mean particle size of 0.36 mm (Wei, Hong, & Yang, 2017), a pressure of 33 MPa and a temperature of 32 °C. The yields of volatile oils and the individual components (caryophyllene, limonene, perillaldehyde and (Z,E)- α -farnesene) are represented as the percent ratio (% w/w) of the mass of oil per mass of *P. frutescens* and the mass of compound per mass of extracted oil, respectively. In previous studies (Wei, Lin, Hong, Chen, & Yang, 2016), the overall extraction curves of volatile oils with scCO_2 were acquired by utilizing dynamic extraction and were represented as the volatile oils/biomass mass ratio (g of volatile oils/g of *P. frutescens*, %) as a function of the solvent/biomass mass ratio (g of CO_2 /g of *P.*

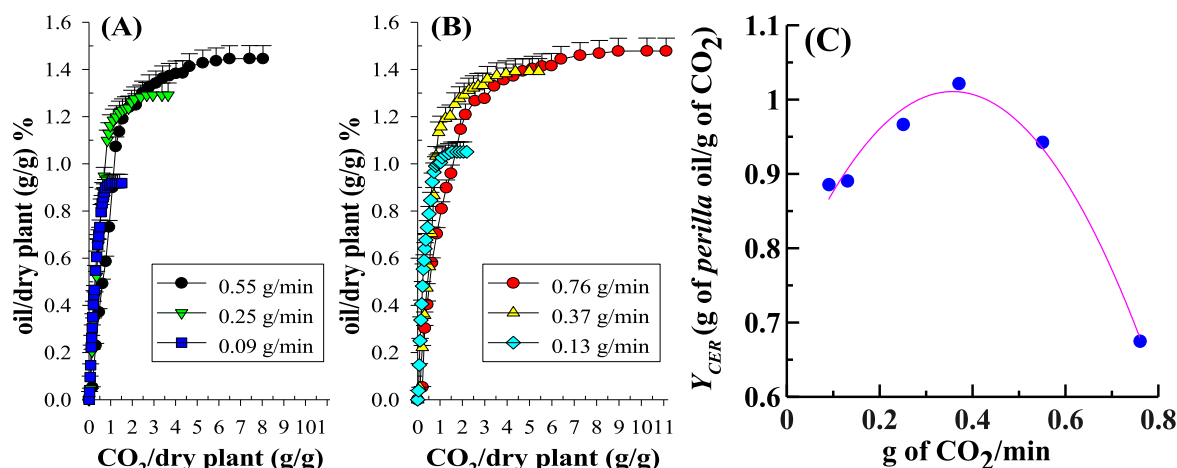


Fig. 1. (A and B) Overall extraction curves at various CO₂ flow rates and (C) the effect of CO₂ flow rate on the Y_{CER} (g of volatile oils/g of CO₂) value at 32 °C and 33.0 MPa for UscCO₂ extraction of volatile oils from *P. frutescens*.

frutescens) (Fig. 1A and B). Furthermore, the first linear segment of the extraction curves of the volatile oils was used to evaluate the solvent phase solute mass ratio at the bed outlet (Y_{CER}), which is illustrated in Fig. 1C. The results revealed the influence of flow rate on Y_{CER} at a temperature of 32 °C and pressure of 33 MPa. At a given pressure and temperature, the Y_{CER} increased with the increase in the flow rate from 0.09 to 0.37 g/min and approached a peak value at 0.37 g/min due to the increased extent of saturation and decreased mass-transfer limitations of the solvent at the vessel outlet. However, when the flow rate increased from 0.37 to 0.76 g/min, the value of Y_{CER} started to decrease due to the decreased extent of saturation of the solvent at the higher flow rate. Therefore, 0.37 g/min was determined to be an adequate CO₂ flow rate for estimating the solubility of volatile oils. The adequate CO₂ flow rate must low enough to ensure the supercritical fluid is saturated with solute at the extractor outlet, which the Y_{CER} should be at its maximum. However, the mass-transfer limitations of the supercritical system can be evaded at the adequate CO₂ flow rate. Consequently, the maximum value of Y_{CER} is validated as the theoretical solubility of the volatile oils, Y^* (g of volatile oils/g of CO₂).

3.2. Influence of dynamic stage

To evaluate the influence of dynamic stage on the yields of volatile oils, UscCO₂ extraction was performed with a static stage of 15 min, dynamic stage of 5–210 min, pressure of 28.5 MPa and temperature of 49 °C, and TscCO₂ extraction was performed with a static stage of 30 min, dynamic stage of 5–270 min, pressure of 33.0 MPa and temperature of 55 °C. Other experimental conditions, such as the mean particle size (0.36 mm) and flow rate (0.37 g/min), were set as previously mentioned.

The influence of dynamic stage on the yields of volatile oils is shown in Fig. 2. The same variation tendencies were found in both the TscCO₂ and UscCO₂ extractions, with the yields first increasing with increased extraction time and then reaching equilibrium. The yield of volatile oil increased effectively with the dynamic stage up to a plateau at 190 min for the TscCO₂ procedure and up to a plateau at 90 min for the UscCO₂ procedure, but an apparent variation with dynamic stage was not observed after that time point, i.e., there was no alteration in the yield of volatile oil when the dynamic stages for the UscCO₂ and TscCO₂ extractions increased from 90 to 210 min and from 190 to 210 min, respectively. Consequently, the proper dynamic extraction times for TscCO₂ and UscCO₂ extractions were determined to be 190 min and 90 min, respectively. Comparative analysis of oils extracted by using TscCO₂ extraction and UscCO₂ extraction (Fig. 2) revealed that the UscCO₂ procedure yielded 1.62% (w/w) of oil in a total extraction time

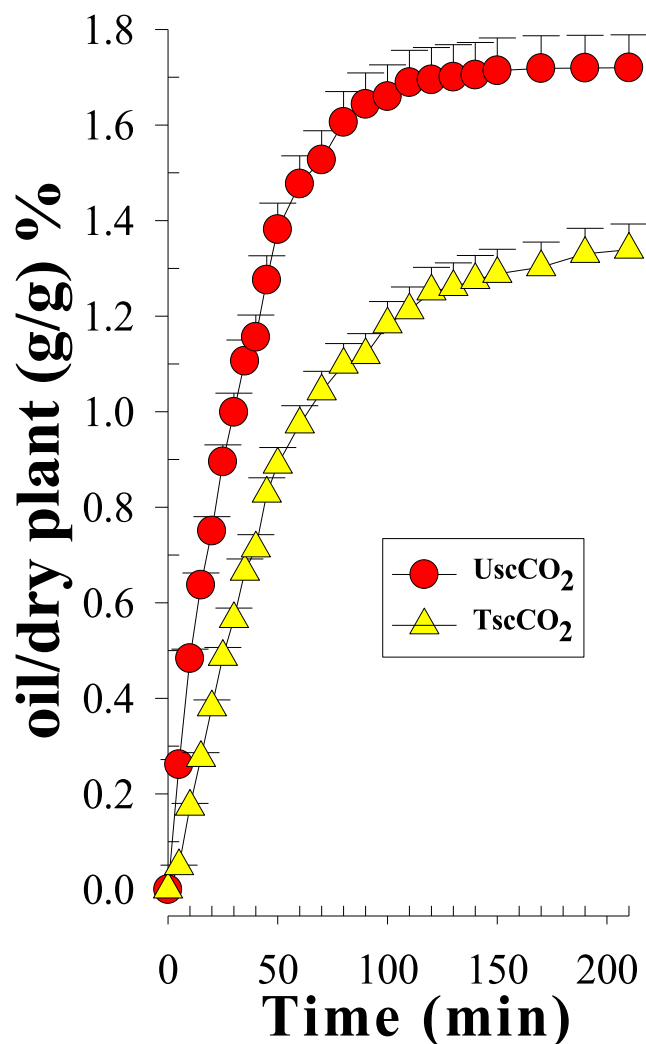


Fig. 2. Effects of ultrasonic energy and extraction time on the yield of volatile oils by using UscCO₂ (at 49 °C and 28.5 MPa) and TscCO₂ (at 55 °C and 33.0 MPa) extractions.

of 105 min, which is apparently higher than that of TscCO₂ extraction (1.34%). The results also demonstrated that the UscCO₂ procedure

afforded a higher yield of the desired volatile oil in less time and at a lower temperature and pressure. With ultrasound applied during the scCO₂ extraction, these intensifications might result from the effects of cavitation, thermal and mechanical, which could increase the swelling, hydration of raw matrixes, intermolecular movement and mass transfer, and induce magnification and rupture of the pores in the plant cell walls (Yang, Wang, & Wei, 2020). This application improves the energy effect and the mass transfer of active ingredient from the raw matrixes to the supercritical fluid system, which in turn results in the rapid release of active ingredient and allows the analytes to be extracted easily (Yang, Lin, & Wei, 2017). Similarly, some researchers highlighted the validated advantages of this novel procedure, including its high extraction efficiency, time saving nature and production of superior quality products for the biotechnology, food and pharmaceutical industries (Santos, Aguiar, Barbero, Rezende, & Martinez, 2015). A recent similar study also reported the extraction of clove oil using the UscCO₂ procedure, with the following variables greatly affecting the extraction efficiency of UscCO₂: pressure, temperature, static stage, dynamic stage, mean particle size of plant materials, flow rate of supercritical solvent, ultrasonic frequency, and duty cycle of ultrasound exposure (Wei, Lin et al., 2016).

3.3. Influences of temperature and pressure

To estimate the influences of temperature and pressure on the yields of volatile oils from *P. frutescens*, experiments were executed at pressures ranging from 10.5 to 33.0 MPa and temperatures ranging from 32 to 55 °C. The experimental data are illustrated in Fig. 3 and indicate the variations in the yields of volatile oils (g of volatile oils/g of dried *P. frutescens*, w/w%) with the CO₂ consumed (g of CO₂/g of dried *P. frutescens*). The effect of pressure on the yield, as depicted in Fig. 3A–E, showed that the extraction efficiency of the UscCO₂ procedure was critically related to the extraction pressure. As expected, the yield of volatile oils increased markedly when the pressure increased from 10.5 to 28.5 MPa (Fig. 3A–D) due to the increase in the density of carbon dioxide, which results in an increase in the number of CO₂ molecules and a reduction in the distance between the CO₂ and active ingredient molecules, thus increasing the interactions between the solute and supercritical solvent (Wei, Yang, & Hong, 2015). This increase leads to the more efficient release and extraction of volatile oils in the UscCO₂ technique. Similarly, most previous reports indicate that an increase in the operating pressure during scCO₂ extraction causes an increase in the yield of analytes acquired from various raw materials (Said, Pradhan, & Rai, 2014). Nevertheless, the effect of a pressure increase from 28.5 to 33.0 MPa on the yield of UscCO₂ extraction was relatively less significant (Fig. 3D and E). Such discrepancies may result from a reduction in the diffusivity of the solvent and an attrition in the free space in the matrix due to the higher condensation of the raw matrixes at a higher pressure of 33.0 MPa, which makes it difficult for molecules of the solvent to diffuse into the pores of the raw matrixes to dissolve the active ingredient. This result was probably because the decreases in carbon dioxide diffusivity and the solid void fraction should have a greater effect on the yields at 33.0 MPa than the CO₂ density increase. Similar variation trends were noted by Rudyk, Spirov, and Tyrovolas (2018), who extracted crude oil, and Kao, Chien, and Chen (2008), who extracted isoflavones from soybean cake. As a result, excess pressure (33.0 MPa) was not recommended in this study because of its relatively smaller impact on extraction yields. However, an increase in the yield of volatile oils was conspicuous with rising extraction pressure utilizing TscCO₂ (Fig. 3F–J), in which the yield of volatile oils peaked when the maximum operating pressure (33.0 MPa) was utilized. The results may be attributable to the predominance of the density effect in the TscCO₂ procedure, in which an increase in the density of the CO₂ at a higher pressure results in an increase in the solubility of the solutes.

As depicted in Fig. 3A–J, these two different outcomes of temperature on the procedure were found in the TscCO₂ and UscCO₂

extractions of volatile oils from *P. frutescens*. At a lower pressure (10.5 MPa), the amount of volatile oils abated when the operating temperature increased from 32 to 55 °C, but at four higher pressures (18.5, 23.5, 28.5 and 33.0 MPa), the opposite trend was found. This trend should rather be explained by the crossover behavior of solubility isotherms, as mentioned by earlier studies (Tomita et al., 2014). At a lower operating pressure of 10.5 MPa, the density of supercritical solvent can be considerably deflated by raising the operating temperature, which causes a decrease in the efficiency of the procedures as the temperature rises. In contrast, at four higher operating pressures, the raising vapor pressure of the volatile oils, the diffusivity of the CO₂ and the molecular interactions of the CO₂ as a result of raising temperature may overcome the decreasing CO₂ density; consequently, there is a grand tendency toward higher procedure efficiency at higher temperatures. In addition, a high operating temperature also enhances the diffusion rate of CO₂, which is beneficial to the extraction procedure. However, the two apparently contradictory outcomes of operating temperature have a balanced relationship, which results in a crossover trend in the UscCO₂ dynamic extraction of perilla oil. Similar phenomena have been observed in the scCO₂ dynamic extraction of analytes (with or without cosolvent), such as rice bran oil (Tomita et al., 2014), crude oil (Rudyk et al., 2018) and Coenzyme Q10 (Couto, Seifried, Moquin, & Temellia, 2018). In addition, a temperature increase from 49 to 55 °C at the above-mentioned four higher operating pressures induced slightly reductions in the yield of the UscCO₂ procedure (Fig. 3B–E). The results are attributable to the decline in CO₂ density with rising temperature, which has a greater impact than the increased vapor pressure of volatile oils and the desorption of volatile oils from the active sites of matrix by supercritical solvent. In addition, cavitation intensity decreases with increasing temperature, which is also attributable to the decline in the yield of the UscCO₂ procedure. Therefore, 49 °C was chosen as the proper operating temperature for the UscCO₂ procedure. However, the TscCO₂ extraction method (Fig. 3G–J) was most favorable when using the highest tested temperature of 55 °C to obtain the highest extraction yield. These results indicate the merits of the UscCO₂ procedure, which can achieve a higher extraction yield and efficiently abate the operating time, temperature and pressure required relative to the TscCO₂ procedure.

3.4. Comparison of the different extraction methods

The data in Table 1 show that the highest yields of volatile oils and the main volatile components were achieved with UscCO₂ extraction at a pressure of 28.5 MPa and temperature of 49 °C for over 105 min. The highest yield of volatile oils obtained by the UscCO₂ procedure was 1.62% (weight of the extracted oil/dry weight of the plant material), which was 13.7, 30.2 and 72.2% higher than the yields of TscCO₂ extraction, STD and HRE, respectively. In addition, the content of perillaldehyde (49.76%, w/w) obtained by the current UscCO₂ process was approximately 1.09, 1.43 and 1.23 times higher than the content obtained by STD, HRE and TscCO₂ extraction, respectively. Furthermore, it can be seen from Table 1 that the UscCO₂ procedure not only produced higher yields of volatile oils and volatile components than HRE, TscCO₂ extraction and STD but also did not use an organic solvent and demanded less time, a lower pressure and a lower temperature. Therefore, the UscCO₂ procedure is beneficial for the extraction of volatile oils and volatile components from *P. frutescens* based on the yields and extraction efficiencies. Thus, this UscCO₂ procedure can operate on any medium, and it is superior to traditional extraction methods given its higher extraction yields, reduced time required and milder operating conditions. These characteristics of the UscCO₂ procedure allow it to meet the requirements for the development of the biotechnology, functional food and pharmaceutical industries.

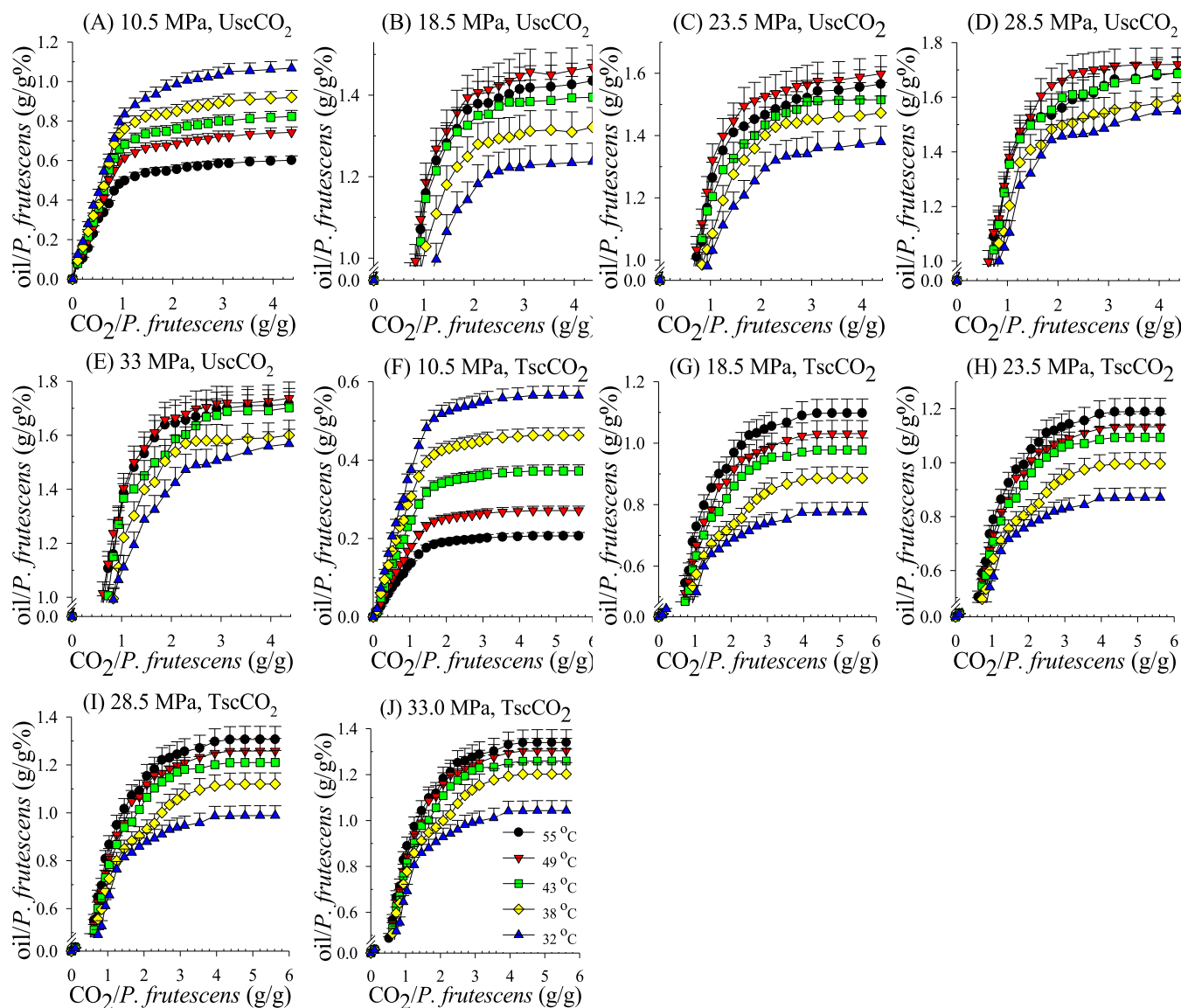


Fig. 3. The overall extraction curves for (A–E) UscCO₂ and (F–J) TscCO₂ dynamic extractions of volatile oils from *P. frutescens* at various temperatures and pressures and a CO₂ flow rate of 0.37 g/min.

3.5. Theoretical solubility of volatile oils

The theoretical solubility of volatile oils in supercritical solvent was derived from the UscCO₂ dynamic extraction curves of *P. frutescens* and is listed in Table 2. The solubility increased with pressure elevation (10.5–28.5 MPa) at all five operating temperatures; however, at the highest tested pressure of 33.0 MPa, the increase in solubility was minimal (Fig. 3A–E). Similar outcomes were found by Couto et al. (2018) and Yang and Wei (2015). This similarity is ascribed to the fact that the density of supercritical fluid rises with rising pressure, which increases the solubility of the solute in scCO₂ at higher pressures. While the pressure of scCO₂ increases (> 28.5 MPa), the solubility of volatile oils gradually begins to plateau, which allows the solubility to remain relatively constant.

When considering the effect of temperature on the solubility of volatile oils, a retrograde solubility phenomenon in the scCO₂ was observed at 10.5 MPa in which an increase in temperature reduces the solubility at pressures below the crossover region, while at pressures above the region, the theoretical solubility of the volatile oils increases with increasing temperature (32–49 °C). The existence of a retrograde

solubility behavior in scCO₂ has been discussed in many previous reports (Couto et al., 2018). The different effects of temperature on the solubility of solute in scCO₂ are due to the effects of temperature on both the vapor pressure of the solute and the density of CO₂. This reveals that at operating pressures lower than the crossover pressure, the density of solvent effect is dominant in the extraction, so the volatile oils are more soluble at low operating temperature. However, the effect of the vapor pressure, desorption, diffusion and mass transfer coefficients of volatile oils becomes dominant at pressures higher than the crossover pressure, which in turn allows the solubility to increase as temperature rises.

3.6. Correlations of volatile oil solubility data

The Bartle (Bartle, Clifford, Jafar, & Shilton, 1991), Chrastil (1982) and Kumar and Johnston (K-J) (1988) models are commonly used to correlate solubility data, specifically correlating the solubility of volatile oils in scCO₂ to the density of CO₂ and operating temperature. The correlations obtained by using the three semi-empirical density models for volatile oils are shown in Fig. 4. Furthermore, by fulfilling a multiple

Table 1

Comparison of the extraction conditions and yields obtained by various extraction methods.

	STD	HRE	TscCO ₂ extraction	UscCO ₂ extraction
Herbal sample	Pf1	Pf1	Pf1	Pf1
Mean particle size (mm)	0.36	0.36	0.36	0.36
Plant weight (g)	100	20	20	20
Stirring rate (rpm)	–	300	–	–
Static extraction time (min)	–	–	30	15
Dynamic extraction time (min)	–	–	190	90
Total extraction time (min)	240	240	220	105
Extraction temperature (°C)	100	65	55	49
Extraction pressure (MPa)	–	–	33.0	28.5
Liquid/solid ratio (mL/g)	–	16	–	–
CO ₂ flow rate (g/min)	–	–	0.37	0.37
Extraction cycles	–	4	–	–
Duty cycle of ultrasound exposure (%)	–	–	–	75%
Yields% (RSD%) ^a				
oil ^b	1.13 ± 0.03 (2.65)	0.45 ± 0.02 (4.44)	1.34 ± 0.05 (3.73)	1.62 ± 0.06 (3.70)
limonene ^c	13.35 ± 0.52 (3.90)	10.14 ± 0.56 (5.52)	11.83 ± 0.46 (3.88)	15.17 ± 0.56 (3.69)
perillaldehyde ^c	45.62 ± 1.69 (3.71)	34.78 ± 1.32 (3.80)	40.54 ± 1.51 (3.72)	49.76 ± 1.95 (3.92)
caryophyllene ^c	9.43 ± 0.36 (3.82)	7.19 ± 0.28 (3.89)	9.59 ± 0.37 (3.86)	10.14 ± 0.39 (3.85)
(Z, E)-α-farnesene ^c	5.76 ± 0.23 (3.99)	2.53 ± 0.10 (3.95)	5.32 ± 0.21 (3.95)	5.98 ± 0.23 (3.84)
ACV% ^d	3.33	4.76	3.67	3.61

^a RSD: relative standard deviation or coefficient of variation (CV). $RSD(\%) = \frac{SD}{\bar{y}_m} \times 100\%$, $SD = \sqrt{\frac{\sum_{i=1}^n (y_{e,i} - \bar{y}_m)^2}{n-1}}$

^b Values of yields are written as the mean ± SD (g of oil/g of dry plant weight) of four replications.

^c Values of yields are written as the mean ± SD (g of constituent/g of oil) of four replications.

^d ACV: averaged coefficient of variation. $ACV(\%) = \sqrt{\frac{\sum_{i=1}^n (n-1) \times RSD_i^2}{m-1}} \times 100\%$,

where RSD_i is RSD for each component, and m is the number of components.

Table 2Solubility data for *P. frutescens* oils obtained by UscCO₂ dynamic extraction.

T (°C)	P (MPa)	ρ ^a (kg/m ³)	Y* _{oil} ^b × 10 ³ (g oil/g CO ₂)
32	10.5	760.051	8.055 ± 0.331
	18.5	867.666	8.990 ± 0.347
	23.5	904.672	9.546 ± 0.388
	28.5	933.114	10.221 ± 0.441
	33.0	953.445	10.234 ± 0.426
38	10.5	667.094	7.722 ± 0.317
	18.5	834.302	9.497 ± 0.370
	23.5	877.501	9.923 ± 0.457
	28.5	909.376	10.763 ± 0.442
	33.0	931.632	10.783 ± 0.445
43	10.5	578.482	6.954 ± 0.278
	18.5	804.654	10.075 ± 0.403
	23.5	854.039	11.212 ± 0.449
	28.5	889.141	11.917 ± 0.477
	33.0	913.159	12.058 ± 0.461
49	10.5	441.899	6.217 ± 0.248
	18.5	766.348	10.604 ± 0.424
	23.5	824.725	11.863 ± 0.474
	28.5	864.279	12.189 ± 0.459
	33.0	890.625	12.194 ± 0.468
55	10.5	361.884	4.846 ± 0.194
	18.5	725.117	10.355 ± 0.415
	23.5	794.638	11.295 ± 0.473
	28.5	838.944	12.016 ± 0.469
	33.0	867.733	12.149 ± 0.471

^a ρ: density of the scCO₂.

^b Y*_{oil}: the theoretical solubility of volatile oil in scCO₂.

linear regression, the correlated results of the solubilities of volatile oils in scCO₂ with the three density models are displayed in Table 3. Average absolute relative deviations (AARD(%)) smaller than 3.6% (0.71–3.56%) indicate that these three density-based models reliably describe the solubility of volatile oils in scCO₂.

Furthermore, from the regression parameters, the heat of the

solution (ΔH_T), vaporization (ΔH_{vap}) and solvation (ΔH_{solv}) for binary (volatile oils and supercritical solvent) systems can be evaluated. The data in Table 3 reveal that the ΔH_T values acquired from the Chrastil and K-J models are 14.509 and 13.968 kJ/mol, respectively, and ΔH_{vap} acquired from the Bartle model is 34.328 kJ/mol. This result reveals that vaporization is an endothermic process. Furthermore, the solute–solvent heat of solvation acquired from the difference between ΔH_{vap} and ΔH_T is –20.090 kJ/mol, which indicates that solvation is an exothermic process. This phenomenon might result from the fact that ΔH_{vap} is higher than ΔH_T for the volatile oils.

4. Conclusions

In this work, extraction of volatile oils from *Perilla frutescens* using a combined process of ultrasound-assisted and scCO₂ extractions with various extraction durations (10–210 min), temperatures (32–55 °C) and pressures (10.5–33.0 MPa) was investigated. Conventional extraction methods, such as TscCO₂ extraction, STD and HRE, were carried out in parallel for comparison in this study. The results revealed that the current UscCO₂ technique is superior to the other procedures due to its higher extraction efficiency. With ultrasound applied during the scCO₂ extraction, these intensifications might result from the effects of cavitation, thermal and mechanical, which could increase the swelling, hydration of raw matrixes, intermolecular movement and mass transfer, and induce magnification and rupture of the pores in the plant cell walls. Furthermore, the theoretical solubilities of the volatile oils in the binary system were determined at temperatures ranging of 32–55 °C and pressures ranging from 10.5 to 33.0 MPa. Theoretical solubility was further correlated with three semi-empirical density models. The results show that the experimental solubility data of volatile oils were satisfactorily correlated with the three density models with an overall AARD% of 0.71–3.56%.

CRedit authorship contribution statement

Yu-Chiao Yang: Conceptualization, Data curation, Writing - original draft, Methodology, Software, Validation. **Chia-Sui Wang:**

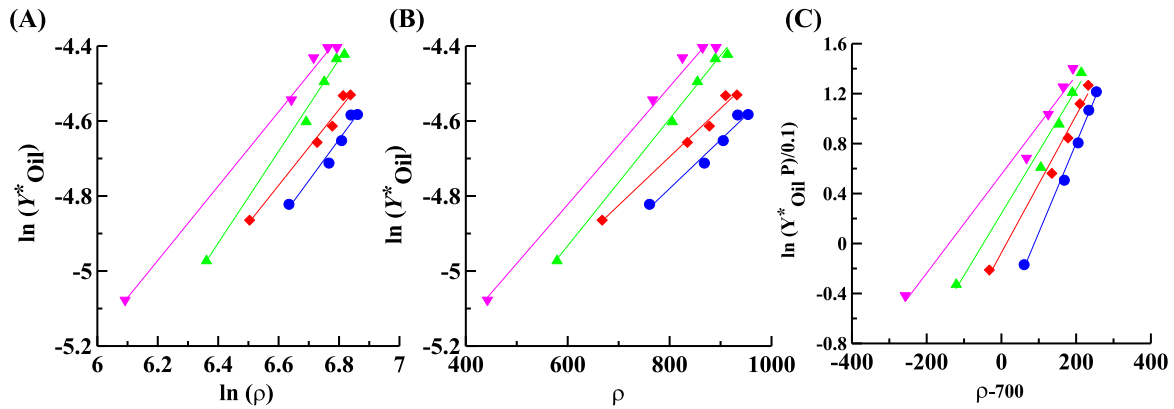


Fig. 4. Plots of (A) $\ln(Y^*_{Oil})$ versus $\ln(\rho)$ using the Chrastil model, (B) $\ln(Y^*_{Oil})$ versus ρ using the K-J model and (C) $\ln(Y^*_{Oil} P/0.1)$ versus $(\rho-700)$ using the Bartle model for oil extraction from *P. frutescens* at various temperatures and pressures of 10.5–33.0 MPa (●: 32 °C, ◆: 38 °C, ▲: 43 °C and ▼: 49 °C).

Table 3

The fitted solubility parameters of three density-based models, and the approximate heat of vaporization, total heat of solution and heat of solvation for *P. frutescens* oils obtained by UscCO₂ dynamic extraction.

Models	n^d	Parameters	AARD ^e (%)	ΔH (kJ/mol)
Chrastil ^a	20	a_0 (−6.31)	0.71	$\Delta H_{T,C}^f$ (14.51)
K-J ^b	20	b_0 (−0.44)	0.74	$\Delta H_{T,K-J}^g$ (13.97)
Bartle ^c	20	c_0 (13.12)	3.56	ΔH_{vap}^h (34.33)
				ΔH_{solv}^i (−20.09)

^a Chrastil model: $\ln(y^*) = a_0 + a_1 \ln(\rho) + \frac{a_2}{T}$

^b K-J model: $\ln(y^*) = b_0 + b_1 \rho + \frac{b_2}{T}$

^c Bartle model: $\ln\left(\frac{y^* P}{P_{ref}}\right) = c_0 + c_1(\rho - \rho_{ref}) + \frac{c_2}{T}$

where y^* is the theoretical solubility, P is the pressure (MPa); P_{ref} is 0.1 MPa, ρ is the density of the scCO₂ (kg/m³), ρ_{ref} is a reference density (700 kg/m³), T is the temperature (K), and a_0 , a_1 , a_2 , b_0 , b_1 , b_2 , c_0 , c_1 and c_2 are the fitted parameters.

^d Number of data points used in the correlation.

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

^f Total heat of solution obtained from the Chrastil model, $\Delta H_{T,C} = -a_2 \times 8.314$ J/mol-K.

^g Total heat of solution obtained from the K-J model, $\Delta H_{T,K-J} = -b_2 \times 8.314$ J/mol-K.

^h Heat of vaporization obtained from the Bartle model, $\Delta H_{vap} = -c_2 \times 8.314$ J/mol-K.

ⁱ Heat of solvation, $\Delta H_{solv} = \frac{1}{2}(\Delta H_{T,C} + \Delta H_{T,K-J}) - \Delta H_{vap}$

Writing - review & editing, Visualization, Validation, Supervision.
Ming-Chi Wei: Conceptualization, Methodology, Visualization,
Investigation, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare no competing financial interest.

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