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Extraction of vetiver essential oil by ethanol-modified supercritical carbon dioxide

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ABSTRACT

In this study, the Response Surface Method with a Central Composite Design was applied to an investigation of the effects of ethanol concentration, pressure and temperature of ethanol-modified supercritical CO_2 extraction on vetiver oil yield and chemical components, and optimization of these conditions for the highest oil yield. An investigation of whether metals accumulated in vetiver roots would be co-extracted with essential oil by supercritical technology was also conducted. The results indicated that both pressure and concentration of added ethanol had significant linear effects on oil yield, while temperature and interactive effects were not significant. Oil yield increased with both pressure and amounts of added ethanol. The optimal conditions were determined to be 190 bar, 50 °C and 15% ethanol which produced 5.9% oil yield over three times the hydrodistilled yield, and nearly double that of pure SCE (without co-solvent). The operation of ethanol-modified SCE at lower temperature and pressure (100 bar, 40 °C and 15% ethanol) produced a similar yield (5.3%). This finding shows an alternative method for extracting high yields of vetiver essential oil without using very high pressure equipment. Chemical compositions of vetiver oil extracted within the experimental range of ethanol-modified SCE were not significantly different to those extracted by pure SCE. Another interesting result found in this study was that metals accumulated in vetiver roots were not co-extracted with essential oils by either ethanol-modified SCE or pure SCE. Therefore, SCE vetiver extracts are safe for a variety of applications in terms of heavy metal content in the products.

1. Introduction

Essential oil extracted from roots of vetiver grass (VG), *Chrysopogon zizanioides* (L.) Roberty, syn. *Vetiveria zizanioides* (L.) Nash, a perennial plant originating from India, has been used in perfumery and medicine for a long time owing to its aromatic and biological properties. Vetiver oil consists of a complex mixture of sesquiterpene alcohols and hydrocarbons with over 300 compounds. It is used for blending in oriental types of perfumes, cosmetics and aromatherapy. Recently, the discovery of new biological activities of vetiver oil and its components, such as antifungal, antibacterial, anticancer, anti-inflammatory and antioxidant activities, make vetiver extracts promising candidates for application in the pharmaceutical industry. VG extracts can also be used as aromatizing agents in the food industry [1]. Furthermore, VG extracts are considered as environmentally friendly insecticides due to their topical irritant activity on cockroaches and flies and powerful repellent and toxic activities against Formosan subterranean termites [2].

Vetiver grass has been successfully used for land protection, such as soil and water conservation, land rehabilitation and embankment stabilization, in the past 30 years. It is due to the fact that VG possesses outstanding morphological, agronomic and physiological characteristics [3]. It is a fast-growing plant with an abundant, complex, and extensive root system that tolerates extreme climatic and soil variations such as prolonged drought, flood, submergence, extreme temperature (−14 to 55 °C), soil pH (3.3–9.5), salinity, sodicity and high levels of Al, Mn and heavy metals. Recently, VG has been found to have great potential for phytostabilization of heavy metal contaminated soils as it can accumulate high concentrations of heavy metals in the roots, especially lead (up to 10,000 mg kg^{-1} of dry root), zinc (over 10,000 mg kg^{-1} of dry root) [4] and copper (1000 mg kg^{-1} of dry root) [5]. The high absorbability of VG raises the concern about the essential oil that is extracted from the roots being contaminated with heavy metals as VG may be grown on heavy metal contaminated soils.

Conventional extraction methods of flavor, fragrant and bioactive compounds from plant materials include hydrodistillation,

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steam distillation and solvent extraction. Supercritical fluid extraction (SFE), a novel and environmentally benign separation technology, represents a green alternative to the conventional extraction methods for the production of natural extracts [6]. SFE is operated at low or mild temperatures in the absence of air, hence avoiding thermal and oxidative degradation of natural compounds. This technology is capable of producing extracts with no solvent residues. Furthermore, SFE has been demonstrated to be a rapid extraction method compared to hydrodistillation and solvent extraction [1]. Carbon dioxide is the most commonly used supercritical fluid for extraction as it is an inexpensive, non-flammable and non-toxic solvent.

The extraction efficiency of natural compounds increases with their solubility in the supercritical fluid. However, since CO_2 is a non-polar solvent that cannot solubilise polar compounds, the extraction efficiency of natural polar compounds is low. The solubility of natural compounds in supercritical CO_2 could be improved by varying extraction temperature and pressure, and particularly by addition of polar co-solvents [7,8]. The manipulation of operating temperature and pressure regulates the CO_2 density which, in turn, determines the solvent power of CO_2 [9]. The addition of polar co-solvents to supercritical CO_2 forms a higher polarity supercritical mixed solvent leading to higher solubility of polar compounds in the mixed solvent than that in pure supercritical CO_2 [10,11]. Ethanol is often selected as a co-solvent in supercritical CO_2 extraction (SCE) of natural products as it is non-toxic and is miscibile with CO_2.

The Response Surface Method (RSM) with a Central Composite Design (CCD) has been demonstrated to be a powerful tool to investigate the individual and interactive effects of operating conditions of SCE, and to optimize these conditions for the highest oil yield from a wide range of natural sources, such as grape seed, cherry seed, walnut, hazelnut, apricot kernel, cottonseed, thyme, Turkish lavender flowers and sea buckthorn [1,12]. The extraction of vetiver essential oil by pure, non-polar supercritical CO_2 has been optimized by the RSM together with CCD in our previous study [1]. However, the application of RSM–CCD to optimize operational conditions of ethanol-modified SCE, and to evaluate the effects of operational parameters on vetiver oil yield and chemical composition, has not previously been reported in the literature.

The objectives of this study were to investigate the effect of three operating conditions of ethanol-modified CO_2 extraction, namely pressure, temperature and concentration of added ethanol, on yield and chemical composition of vetiver essential oil, and to optimize these conditions for the highest oil yield by using the RMS-CCD method. In addition, the capacity for SCE, either with or without modifier, to co-extract heavy metals with essential oil from vetiver roots was also investigated.

2. Materials and methods

2.1. Plant material preparation

Fresh roots of vetiver grass, over 4 years old, were collected at Gatton Research Station, Queensland, Australia. Root washing, drying and storage were conducted as previously described [2].

2.2. Extraction

2.2.1. Hydrodistillation

Hydrodistillation of vetiver roots (approximately 30 g) was carried out with three replications using a Clevenger-apparatus as described in Danh et al. [1]. The process was performed in 12 h.

2.2.2. Ethanol extraction

Ethanol extraction of vetiver roots (about 30 g) was carried out by using a Soxhlet apparatus which was connected to a round flask containing 500 ml of ethanol. The extraction was carried out at boiling temperature of ethanol for 5 h. After extraction, ethanol was removed by evaporation at the boiling point.

2.2.3. Supercritical fluid extraction

The ethanol-modified SCE of vetiver oil was carried out by using a modified version of the experimental apparatus reported in [1]. A micro-metering valve (MV1) and a static mixer (SM) were inserted in the previous system between valve V2 and heating coil HC (Fig. 1). A HPLC pump delivering ethanol was connected between MV1 and SM. CO_2 was pressurized in the compressor (ISCO Model 260D Syringe pump) at 4 °C to pre-determined pressures. MV1 was used to control the flow rate of the pressurized CO_2 from the compressor. The pressurized CO_2 and ethanol were pumped into an extractor via SM and HC at the different volume ratios of ethanol/CO_2 (vol%). The SM enhanced the mixing of ethanol and CO_2. This novel design ensured a constant volume ratio of ethanol/CO_2 was used in the whole extraction process. As the pressure in the extractor, pre-loaded with about 10 g of dry vetiver roots, was equal to the pressure in the CO_2 pump, the system was equilibrated for 15 min (static stage) at operating temperature and pressure. The system was then changed into dynamic operation mode during which the flow rate of CO_2 was kept at $2\,\mathrm{ml\,min^{-1}}$ measured at operating pressure and 4 °C for all experiments, and the flow rate of ethanol was adjusted corresponding to the specific volume ratio of ethanol/CO_2. The mixtures of vetiver extracts and

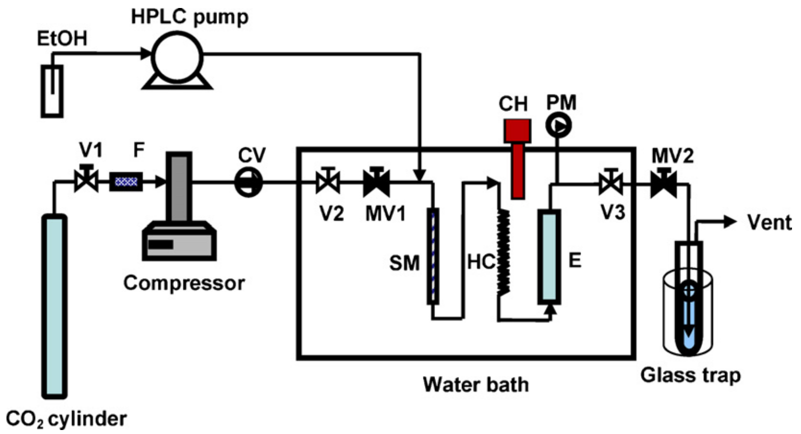

Fig. 1. Schematic diagram of ethanol-modified SCF extraction. V1–V3: stopping valve; MV1–MV2: micro-metering valve; HC: heating coil; E: extractor; SM: static mixer; CV: check valve; CH: circulating heater; PM: pressure meter.

Table 1
Coded and uncoded levels of independent variables.

Original variables	Coded levels				
	$-\alpha$	-1	0	1	α
X_1: pressure (bar)	NA (69.3)	100	145	190	NA (220.7)
X_2: temperature (°C)	36.6	40	45	50	53.4
X_3: volume ratio of ethanol/CO_2 (vol%)	1.6	5	10	15	18.4

Note. α = [number of factorial runs]$^{1/4}$, in this study α = 1.6818. NA: not applicable.

ethanol were collected in a glass tube during the dynamic stage. As the extraction stopped, the extract mixtures were made up to 10–15 ml with ethanol, and 3 ml of the mixture were used for GC and GC–MS analysis, while the rest was oven-dried at 50 °C for 4 h. The samples were then weighed.

The kinetics of ethanol-modified SCE of vetiver oil were performed at fixed operating conditions of 145 bar, 45 °C and different concentrations of added ethanol (0, 5, 10 and 15 vol%). The operating condition selected represented the central point of the optimization experiments. The extraction procedure was similar to the one previously described. Extracts were collected at 10, 20 and 30 min in order to determine yield of extracts obtained in the corresponding time interval.

The SCE of vetiver oil with pure CO_2 was performed at the optimal operating condition, 190 bar and 50 °C, determined in the study of Danh et al. [1], for comparison with the ethanol-modified SCE. The extraction involved the use of a modified version of the apparatus presented in Fig. 1 with the removal of the MV1 – HPLC pump – SM components. The procedure was similar to the one described above without addition of ethanol.

The yield of essential oil obtained from the different extraction processes was calculated by dividing the weight of collected extract for the dry weight of roots multiplied by 100%.

2.3. Experimental design

The RSM with a Central Composite Design was applied in this study to evaluate the effects of pressure, temperature and concentrations of added ethanol (vol%) on vetiver oil yield and chemical compositions. The design included 17 experiments with eight factorial points, four extra points (two for temperature, two for ethanol concentration) and five replications of central points (Table 1). The operating conditions were conducted at 5 levels, except pressure that was tested at three levels due to technical limitations. All experiments were carried out at conditions for which ethanol and CO_2 mixtures were completely miscible. The addition of co-solvent above 30 wt% is not recommended due to saturation of CO_2 with ethanol and formation of two phases [13]. The maximum addition of ethanol in this study was below the suggested level. However, the lower extra point of pressure operated at 69.3, 45 °C and 10 vol% ethanol did not form a homogeneous CO_2–ethanol system [14]. Furthermore, the upper extra point of pressure operated at 220.7 bar was over the pressure limitation of the HPLC pump.

The experimental yields were analyzed by the RSM–CCD to fit a second-order polynomial equation as described in the study of Danh et al. [1]:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i x_i + \sum_{i=1}^{3}\beta_{ii} x_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} x_i x_j \qquad (1)$$

2.4. Gas chromatography and gas chromatography–mass spectrometry analysis

The mixtures of vetiver extract and ethanol (300 μl) were added in 3 ml of hexane, and vigorously shaken for 3 min. The mixed solution was left to equilibrate for 3 min. A 1.5 ml aliquot of solution taken from the supernatant was used for chemical analysis. To confirm that the hexane had completely dissolved all of the vetiver oil components in the ethanol mixture, an additional experiment was set up. 200 μl of commercial vetiver oil was mixed with 2 ml of two different solvents, ethanol and hexane. 300 μl samples of oil mixture from each solvent were then added in 3 ml of hexane (3 replications). The following steps were carried out as mentioned above. The relative concentrations of all peaks identified by gas chromatography from two solutions were statistically compared. There were no significant differences in chemical compositions of vetiver oil mixed with hexane and ethanol (data not shown). These results indicated that all of the vetiver oil components in the ethanol mixture were dissolved in hexane.

The chemical compositions of oil extracts were identified by gas chromatography coupled with mass spectrometry (GC–MS) and component concentrations were determined by gas chromatography (GC) [1].

2.5. Heavy metal analysis

To determine whether SCE co-extracted heavy metals together with vetiver oils, the roots of vetiver grown on soils contaminated with lead (Pb), zinc (Zn) and copper (Cu) from the study of Danh et al. [2] were used. Vetiver roots harvested from three different soils were blended together with ratio 1:1:1 by grinding. Vetiver roots were extracted by SCE with pure CO_2 and ethanol-modified SCE using the previously mentioned procedures. Concentrations of heavy metals in vetiver roots before and after extraction were measured by ICP–OES (PerkinElmer Optima 3000DV): 0.5 g of dried plant samples were ground to a fine powder, then digested with concentrated HNO_3 [2].

2.6. Statistical analysis

The regression coefficients of the quadratic equation were determined using the Data Analysis Tool of Microsoft Excel 2003. The data of heavy metal concentrations were analyzed by analysis of variance one factor. The means of different treatments were compared by Least Significant Difference (LSD) at the 5% significance level.

3. Results and discussion

3.1. Kinetic study

The cumulative yields of vetiver extracts over time are shown in Fig. 2. The total extraction time was 200 min. It can be seen that vetiver oil yields increased with the addition of ethanol. The extraction with addition of 15 vol% ethanol produced the highest yield that was nearly double the yield of SCE extraction with 0 vol% ethanol.

The extraction pattern of SCE of vetiver was independent of the ethanol content: the extraction rate was fast at the beginning and slower at the later stage. This trend was similar to the results obtained in our previous study on SCE of vetiver oil with pure CO_2

Table 2
Central Composite Design with coded and uncoded levels of independent variables, and experimental yield.

Experiment[a]	Coded variables			Uncoded variables			Yield (%)
	X_1	X_2	X_3	Pressure (bar)	Temperature (°C)	Ethanol (vol%)	
1	−1	−1	−1	100	40	5	3.58
2	1	−1	−1	190	40	5	4.78
3	−1	1	−1	100	50	5	2.47
4	1	1	−1	190	50	5	4.48
5	−1	−1	1	100	40	15	5.31
6	1	−1	1	190	40	15	5.77
7	−1	1	1	100	50	15	5.02
8	1	1	1	190	50	15	5.90
9	0	−1.68	0	145	36.6	10	4.29
10	0	1.68	0	145	53.4	10	4.73
11	0	0	−1.68	145	45	1.6	3.39
12	0	0	1.68	145	45	18.4	4.84
13	0	0	0	145	45	10	4.69
14	0	0	0	145	45	10	4.90
15	0	0	0	145	45	10	4.95
16	0	0	0	145	45	10	4.83
17	0	0	0	145	45	10	5.02

[a] Experiments were carried out randomly.

[1]. It can be explained by the distribution of oils within the root particles. The oil located on the surface of root particles is extracted in the early stage of the process, while oil located inside the intact cells is extracted in the later stage. The extraction of oil from intact plant cells occurs with a lower mass transfer rate than the extraction of oil located on the cells' surface.

In a previous study on SCE of vetiver with pure CO_2 [1], the extraction time did not have a significant effect on the oil yield within the experimental range of 37–117 min. Similarly, in the present study, the further extension of extraction time of the ethanol-modified SCE of vetiver oil past 90 min only produced very small increments of the yield. The results indicated that most of vetiver extracts were readily available for extraction by ethanol-modified SCE during the early stage.

3.2. Effect of operating parameters on oil yield and optimization of ethanol-modified SCE

The Central Composite Design combined with Response Surface Method were used to investigate the effects of pressure, temperature and concentration of added ethanol as well as their interactive effects on vetiver oil yields. The experimental yields of 17 runs and the analyses of their variances are summarized in Tables 2 and 3, respectively. By fitting the constant and coefficients into Eq. (1), the mathematical model was obtained:

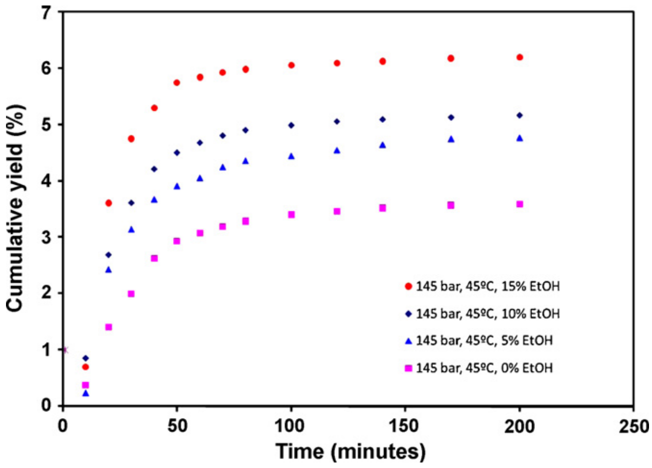

Fig. 2. The cumulative yield of vetiver extracts over time at the fixed operating conditions of 145 bar, 45 °C and different amounts of added ethanol (0, 5, 10 and 15%).

$$Y = 4.8771 + 0.5665X_1 - 0.0617X_2 + 0.6691X_3 + 0.1855X_1^2 - 0.1289X_2^2 - 0.2704X_3^2 + 0.1548X_1X_2 - 0.2341X_1X_3 + 0.1557X_2X_3 \qquad (2)$$

The results presented in Table 3 show that pressure ($P = 0.0037$) and the ethanol addition ($P = 0.0003$) had significant linear effect on the oil yield, while the temperature ($P = 0.5636$) had a negligible effect on the oil yield. The ethanol addition also presented a negative quadratic effect on yield ($P = 0.0457$). There were no significant interactive effects observed in this study. The mathematical model fitted the experimental data with $R^2 = 0.91$, indicating the calculated model to be able to explain 91% of the variability of experimental results. The greater similarity between the experimental and the predicted data shows the higher accuracy of the calculated model in the prediction of the extracted yields. Fig. 3 indicates a high correlation coefficient of the experimental and the predicted data up to 96%.

The empirical model demonstrated in Eq. (2) was used to generate the response surfaces of oil yields as function of pressure, temperature and amount of added ethanol within the experimental range. This model indicated that the oil yield increased with pressure and the level of added ethanol, while it showed a slight decrease with the increase of temperature.

Table 3
Regression coefficients and corresponding *t* and *P*-values for vetiver oil yield.

	Coefficients	*t*-Stat.	*P*-Value
Intercept	4.8771*	29.0095	0.0000
X_1	0.5665*	4.2625	0.0037
X_2	−0.0617	−0.6061	0.5636
X_3	0.6691*	6.5748	0.0003
$X_1 X_1$	0.1855	0.9538	0.3719
$X_2 X_2$	−0.1289	−1.1566	0.2854
$X_3 X_3$	−0.2704*	−2.4264	0.0457
$X_1 X_2$	0.1548	1.1645	0.2824
$X_1 X_3$	−0.2341	−1.7615	0.1215
$X_2 X_3$	0.1557	1.1713	0.2798

* Means significant effect at $P < 0.05$.

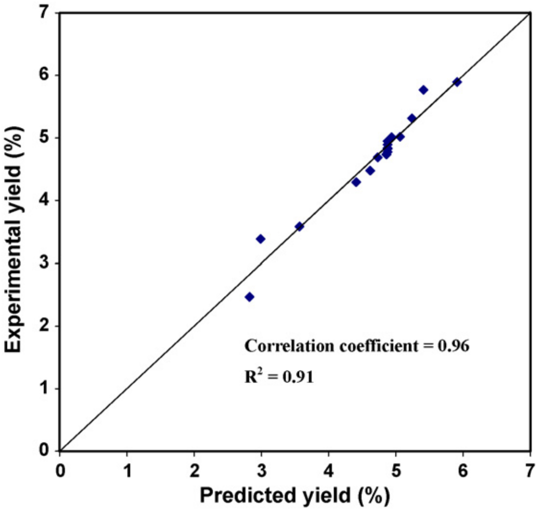

Fig. 3. Goodness of fit between the experimental and predicted yields.

The effect of pressure and ethanol on extraction yield at extraction temperature of 50 °C is illustrated in Fig. 4. It can be observed that the oil yield increased with pressure and with the addition of ethanol. Statistically, the pressure and ethanol concentration were shown to significantly affect oil yield. The oil yield depends on the solubility of vetiver oil components in the mixed solvent, whereby the higher solubility corresponds to a higher yield. The solubility of solutes in the co-solvent modified SCF depends on the mixed solvent density, the specific interactions and the difference between the local (around solute molecules) and bulk density [15].

The increase in concentration of ethanol and the raising of pressure resulted in an increase of the mixed solvent density [15]. The high density increases the magnitude of physical interactions, such as dipole–dipole, dipole–induced dipole and induced dipole–induced dipole interactions, between solute and solvent molecules, and consequently leads to an overall solubility enhancement [16,17].

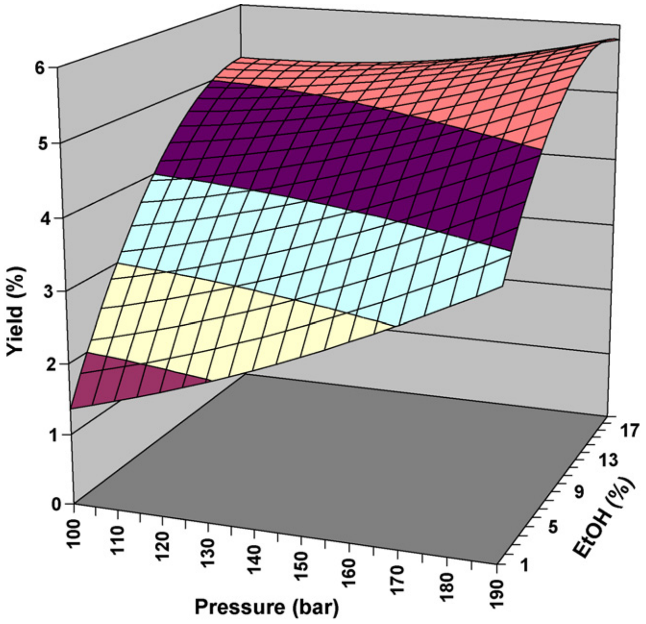

Fig. 4. The effect of pressure and the concentration of ethanol on oil yield at the extraction temperature of 50 °C.

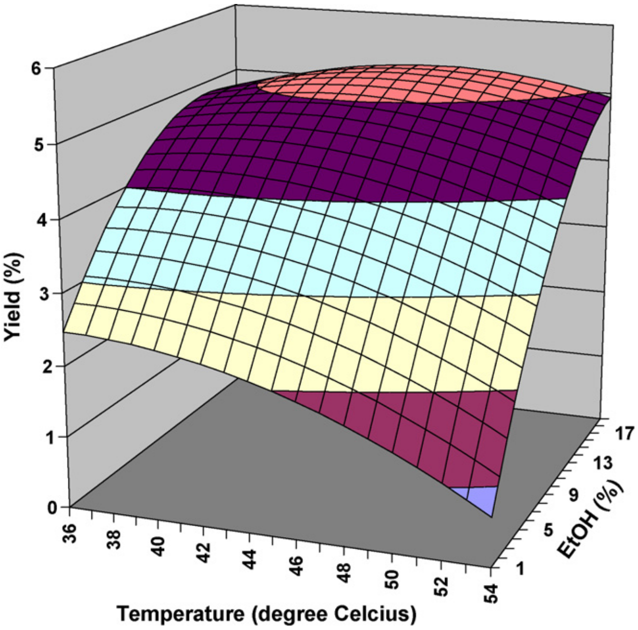

Fig. 5. The effect of temperature and the concentration of ethanol on oil yield at the extraction pressure of 100 bar.

The addition of polar co-solvent also promotes specific interactions, such as chemical association via hydrogen bonding, between the solute and co-solvent molecules resulting in further enhancement of solubility [16,17]. The increase in the amount of ethanol added may enhance the overall solubility of vetiver oil via the proportional increase in the number of hydroxyl groups available for hydrogen bonding between ethanol and the polar components of vetiver oil.

Furthermore, the increase in co-solvent concentration expands the difference between the local (around solute molecules) and bulk density. Near the critical point of a SCF solution, the solvent molecules cluster around solute molecules to form a local density that can be several times larger than the bulk density [18–22]. The greater the difference between the local (around solute molecules) and bulk density represents the higher solubility of solutes in the SCF solution [15]. The addition of co-solvent increases the density difference [15] that partly contributes to solubility enhancement.

The effect of ethanol addition on oil yield was more pronounced at lower pressure than higher pressure (Fig. 4). The increase in pressure results in a decrease in difference between the local and bulk density. As the pressure increases, the concentration of the co-solvent around solute will ultimately approach the bulk concentration [23]. The effect of pressure was significant at low concentrations of added ethanol, and became negligible at high ethanol concentration. This behaviour may be explained by the fact that the increase of pressure within the experimental range (100–190 bar) imposed a more significant effect on the local-bulk density gap at low modifier concentrations. The results have a great applicative potential for extraction of vetiver oil with high yields using ethanol-modified SCE at low pressure and high concentrations of added ethanol.

A response surface plot showing the effect of temperature and concentration of ethanol on oil yield is presented in Fig. 5. The concentration of ethanol had a significantly positive impact on oil yield over the experimental range of pressure. Temperature showed a small negative effect on the oil yield, and this impact became smaller as the concentration of ethanol increased. It is interesting to observe that at an ethanol concentration of 15 vol% the extraction temperature in the range of 40–50 °C produced the same oil yield.

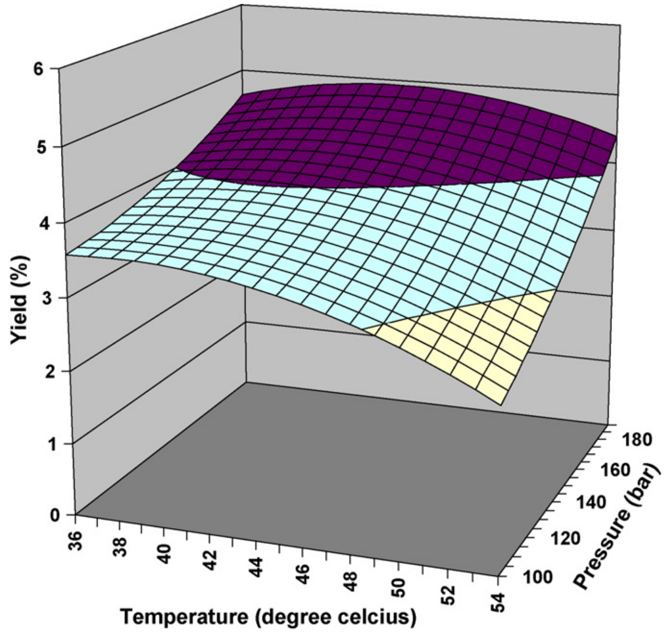

Fig. 6. The effect of pressure and temperature on oil yield at the ethanol concentration of 5%.

This finding can be applied to produce high yield of vetiver oil using ethanol-modifed-SCE at low temperature and high concentration of added ethanol.

A response surface plot showing the effect of pressure and temperature on oil yield at the fixed concentration of ethanol is shown in Fig. 6. The increase of pressure resulted in an increase of oil yield due to the increment in solvent density and power. The increase in oil yield with pressure was more significant at higher operating temperatures.

Increments in temperature resulted in a slight decrease of the oil yield over the tested range of pressure. The effect of temperature on oil yield observed in this work was different to one reported for SCE of vetiver oil by pure CO_2 [1] in which temperature effects depended on the pressure level. Specifically, increments of temperature resulted in the decrease and increase of oil yield at low and high pressures, respectively. The solubility of solutes in supercritical fluids increases with temperate owing to the increase of solid volatility. In the presence of co-solvent, the directly proportional relation between temperature and solid solubility may be interfered as temperature increases leading to the decrease of oil yield.

Pressure, temperature and concentration of added ethanol were optimized in order to obtain vetiver oil with high yields. The optimization of experimental conditions was carried out step-by-step [24]. Within the range of tested parameters, the optimal operating conditions were predicted at 190 bar, 50 °C and 15 vol% ethanol that produced the oil yield of 5.914%. The experimental yield at these conditions was 5.9%.

3.3. *Chemical components of ethanol-modified SCE extracts*

The chemical compositions of vetiver oil extracted by ethanol-modified SCE at different operating conditions are presented in Table 4. Zizanoic acid, khusimol, α and β-vetivone and isovalencenol were the main components of all ethanol-modified SCEs. The three operating parameters; temperature, pressure and ethanol concentration, had no significant impact on the identified chemical components (except for zizanoic acid) of vetiver extracts. Similar results were observed in the study of Danh et al. [1] that investigated the effect of pressure, temperature and time of pure SCE on vetiver oil composition. The addition of polar co-solvent to the non-polar CO_2 generates a solvent with higher polarity leading to the more efficient extraction of polar solutes [11,16,24,25]. However, ethanol-modified SCE of vetiver oil did not improve the selectivity toward any of the oil components. This behaviour indicated that the relative solubility of vetiver oil components are unchanged by the changes in solvent polarity resulting from the use of ethanol as a modifier. It may be explained by the fact that nearly all components of vetiver oil are readily soluble in non-polar supercritical CO_2 [1], hence the addition of ethanol (regardless of added amounts) could not make further differences. In addition, only a small percentage of vetiver oil components is represented by non-polar compounds, whilst the majority are polar compounds such as alcohols, carboxylic acids and carbonyl compounds, therefore the addition of ethanol has limited potential of selective extraction of oil components.

Zizanoic acid is the main compound in ethanol-modified SCE extracts. For perfumery application, it is an undesired component owing to its sensorial properties [26]. However, the quality of vetiver oil high in zizanoic acid can be improved by removal or separation of the acid followed by chemical transformation of the acid into khusimol [26].

3.4. *Comparison with hydrodistillation, SCE without modifier and ethanol extraction*

The chemical compositions of vetiver oil extracted by hydrodistillation, pure SCE and ethanol-modified SCE are presented in Table 4. There were no significant differences in chemical components of SCE (operated at 190 bar, 50 °C) and all ethanol-modified SCE experiments. It can be stated that SCE and SCE with ethanol as a modifier do not have specific selectivity on chemical components of extracted oils. However, chemical profiles of hydrodistilled oil extracts were different to those of both SCE processes. The content of alcohol, carbonyl compounds and hydrocarbon in hydrodistilled oil were higher than those of SCE and SCE with modifier. Carboxylic acids, undesired components for perfumery application, were present in higher fractions in SCE extracts than in hydrodistilled oil.

Yields of vetiver oils extracted by different processes are shown in Table 5. Hydrodistilled yield of vetiver oil in this study was over 5 times higher than that reported by Danh et al. [1], a difference that can be explained by the difference in the maturity of the vetiver roots used. The materials used by Danh et al. [1] were 7 months old, while the one used in this study was over 4 years old. The vetiver oil content in the roots was demonstrated to increase with time [27]. SCE yield in this study was over 2 times higher than that of hydrodistillation, but nearly four times higher in the study of Danh et al. [1]. The results imply that the efficiency of SCE compared to hydrodistillation is more pronounced with the vetiver roots having low oil contents.

Extraction with ethanol was highly unselective and produced yields as high as 15%. Extracts, however, contained high fractions of waxy compounds that are solid at room conditions. Overall, macroscopic and organoleptic evaluation of the ethanol extracts indicated that they were unsuitable for any valuable application without refining. As such, further testing of the ethanol extracts was not pursued.

The operating parameters of 190 bar, 50 °C and 15% ethanol were determined to be the optimal conditions of ethanol-modified SCE in producing the highest oil yield. As discussed previously, the operation at low temperature, low pressure and high concentration of added ethanol produced high oil yield. Therefore, the yields produced at this condition (100 bar, 40 °C and 15 vol% ethanol) and the optimal conditions were used to compare with the yield obtained

Table 4
Chemical compositions of vetiver oil extracted by hydrodistillation, supercritical CO_2 and ethanol-modified supercritical CO.

KI	Compound	HYD	190 50 0	1	2	3	4	5	6	7	8	Central point	9	10	11	12
1403	Acoradiene	0.19 ± 0.04														
1426	Beta-copaene	0.31 ± 0.02														
1438	Prezizaene	0.79 ± 0.03	0.46 ± 0.02	0.38	0.48	0.46	0.51	0.45	0.55	0.44	0.57	0.45 ± 0.03	0.43	0.50	0.43	0.76
1443	Khusimene	1.05 ± 0.05	0.60 ± 0.03	0.50	0.58	0.62	0.66	0.54	0.67	0.53	0.70	0.57 ± 0.04	0.53	0.63	0.56	0.70
1451	Calarene	0.38 ± 0.01	0.17 ± 0.01		0.17	0.17	0.19		0.19	0.15	0.19	0.25 ± 0.05	0.15	0.18	0.16	
1459	Trans-isolimonene	0.32 ± 0.02			0.14		0.15		0.15					0.15	0.15	
1478	Alpha-Amorphene	0.89 ± 0.02		0.39		0.36	0.38	0.27		0.26	0.41	0.3 ± 0.06	0.26		0.27	
1488	Beta-vetispirene	0.56 ± 0.02				0.13	0.13			0.13		0.2 ± 0.00			0.16	
1490	Delta-selinene	0.80 ± 0.05		0.25		0.10				0.14						
1494	Gamma-Amorphene	0.26 ± 0.03								0.11						
1507	Cuparene (29.00)	0.28 ± 0.03														
1511	Delta-Amorphene	0.21 ± 0.00		0.17		0.12	0.12	0.19		0.18		0.25 ± 0.05			0.20	
1512	Nootkatene				0.35	0.39	0.41	0.36	0.42		0.42			0.39	0.37	0.44
1519	Alpha-cadinene	0.76 ± 0.02	0.40 ± 0.01	0.34						0.34		0.39 ± 0.04	0.35			
1544	Alpha-calacorene	0.26 ± 0.01				0.10										
1552	Beta-Vetivenene	1.49 ± 0.37		0.20	0.14	0.21		0.23		0.26		0.34 ± 0.08	0.18		0.29	
1595	Virodoflorol	0.57 ± 0.01	0.53 ± 0.04	0.42	0.54	0.55	0.55	0.53	0.59	0.45	0.61	0.53 ± 0.01	0.48	0.54	0.51	0.64
1601	Khusimone	1.38 ± 0.03	0.68 ± 0.01	0.98	0.68	0.67	0.70	0.69	0.72	0.67	0.78	0.71 ± 0.02	0.66	0.70	0.67	0.76
1648	Epi-alpha-Cadinol		0.51 ± 0.00	0.45		0.51	0.50	0.48	0.52	0.46	0.52		0.46	0.50	0.48	0.51
1651	Pogostol	0.85 ± 0.21	0.43 ± 0.01	0.53	0.42	0.45	0.42	0.44	0.44	0.48	0.47	0.41 ± 0.05	0.46	0.44		
1661	7-epi-alpha-Eudesmol	1.07 ± 0.01	0.77 ± 0.02	0.69	0.70	0.77	0.76	0.73	0.71	0.69	0.79	0.77 ± 0.03	0.70	0.77	0.73	0.78
1671	Epi-zizanone	2.50 ± 0.14	2.00 ± 0.05	1.81	1.92	2.01	2.06	1.92	2.04	1.84	2.11	1.95 ± 0.19	1.85	1.64	1.95	2.07
1683	Epi-nootkatol	1.90 ± 0.03	1.21 ± 0.04	1.13	1.17	1.24	1.23	1.19	1.24	1.13	1.29	1.23 ± 0.02	1.15	1.24	1.22	1.31
1687	Khusinol											1.33 ± 0.03				1.46
1700	Zizanal	1.72 ± 0.17	1.18 ± 0.13	1.18	1.15		1.20	1.18	1.22	1.11	1.23	1.11 ± 0.10		1.21	1.03	1.31
1722	Juniper camphor		0.98 ± 0.02	0.90	0.96	0.92	0.99	1.00	0.97	0.92	0.96	1.03 ± 0.03	0.95	0.95	0.95	1.08
1727	Vetiselinenol	3.65 ± 0.50	2.26 ± 0.09	2.10	2.63	2.21	2.36	2.72	2.47	2.63	2.45	2.29 ± 0.31	2.72	2.47	2.79	2.61
1745	Khusimol	14.3 ± 0.46	11.63 ± 1.53	11.74	11.80	12.13	12.18	11.95	12.41	11.32	12.63	11.31 ± 1.03	11.67	12.36	10.38	12.71
1769	14-Hydroxy-delta-Cadinene	0.42 ± 0.02		0.42	0.31	0.30		0.30	0.32	0.29	0.32	0.38 ± 0.15	0.29	0.32	0.57	
1792	Isovalencenol	7.26 ± 0.32	5.65 ± 0.23	5.35	5.33	5.54	5.66	5.53	5.78	5.09	5.67	5.33 ± 0.09	5.31	5.74	5.43	5.91
1809	Nootkatone	5.71 ± 0.86				4.62			1.54			4.78 ± 1.20				
1817	Zizanoic acid	0.68 ± 0.04	15.16 ± 1.11	7.04	12.65	13.78	13.50	15.11	13.61	15.76	13.33	11.18 ± 0.64	14.71	14.02	16.58	19.43
1820	Beta-Vetivone	2.62 ± 0.02	2.35 ± 0.14	8.86	2.25		2.46	2.41	2.31	2.12	2.32	2.24 ± 0.06	2.08	2.31	2.23	2.29
1830	Sesquiterne ketone	3.35 ± 0.14	3.00 ± 0.33	2.59	3.74	3.03	3.84	3.35	3.82	3.39	3.77	2.81 ± 0.08	3.79	3.94	3.68	4.10
1843	Alpha-vetivone	7.33 ± 0.07	6.40 ± 0.17	5.53	6.27	7.53	6.56	6.41	6.60	6.29	6.74	7.02 ± 0.13	6.17	6.57	6.59	7.92
1971	Hexadecanoic acid	0.25 ± 0.00	0.97 ± 0.06	0.91	1.16	0.61	1.04	1.06	1.00	1.20	0.97	0.86 ± 0.19		0.97	1.03	1.28
Hydrocarbons		8.97	1.63	2.65	2.18	2.95	2.56	2.34	2.30	2.82	2.62	3.12	2.20	2.16	3.16	1.91
Alcohols		29.60	23.97	23.30	23.55	24.32	24.65	24.56	25.13	23.17	25.39	24.22	23.90	25.00	22.50	27.02
Carbonyl compounds		24.60	15.61	20.95	16.01	17.86	16.84	15.95	18.24	15.42	16.96	20.61	14.55	16.37	16.14	18.46
Carboxylic acids		0.93	16.12	7.95	13.81	14.39	14.54	16.17	14.61	16.96	14.31	12.05	14.71	14.99	17.62	20.72
Total identified		64.11	57.33	54.85	55.55	59.53	58.59	59.02	60.28	58.38	59.28	59.99	55.36	58.53	59.42	68.11

Note: "±" indicated standard deviation of data.

Table 5
Yield of vetiver oil extracted by hydrodistillation, SCE with pure CO_2 and ethanol-modified SCE.

Methods	Operating conditions				Yields ± SD*
	Time (min)	Temperature (°C)	Pressure (bar)	Ethanol (vol%)	
Hydrodistillation	720	100	NA	NA	1.69 ± 0.07
SCE	105	50	190	0	3.74 ± 0.12
Ethanol-modified SCE					
Ex 5	105	40	100	15	5.31
Ex 8	105	50	190	15	5.90

Note: "*" indicated standard deviation of data.

by hydrodistillation and SCE (operated at 190 bar and 50 °C). The optimal condition (Ex 8) of modified SCE produced the highest oil yield as compared to other processes. This yield was over three times and nearly double that of hydrodistillation and SCE without modifiers, respectively. When SCE with ethanol as a modifier was operated at 100 bar, 40 °C and 15 vol% ethanol (Ex 5) it produced a yield that was 90% of the highest yield and 142% of SCE yield. In summary, operation at low temperature and pressure and at high concentration of added ethanol is recommended for the extraction of vetiver oil. Results indicate that high ethanol levels in the extracting medium determine favorable process yields, the combination of ethanol with CO_2 is essential to generate a product of commercial interest.

3.5. Heavy metals content in SCF extracts

In order to be accepted for application in the perfumery and food industries, vetiver extracts must not contain any toxic substances that may cause a health hazard to the consumers. However, vetiver plants have great potential for accumulating high concentrations of heavy metals, particularly Pb, Zn and Cu, in their roots. This characteristic may cause cross-contamination of vetiver oils extracted from the roots with heavy metals. Essential oils of vetiver grown on heavy metal contaminated soil extracted by hydrodistillation were shown to contain no heavy metals [2]. However, there are no studies reported in the literature about heavy metal contents in the essential oil of vetiver or other plant extracted by supercritical fluids.

A standard procedure of heavy metal analysis of plant materials requires 0.5 g of sample. However, the oil yields of pure SCE and ethanol-modified SCE were smaller than the suggested level (Table 6). Therefore, an indirect method was employed to determine the amount of metals in oil extracts by comparing metal contents in plant materials before and after extraction [28]. Metal contents in the roots of vetiver grown on Pb, Zn and Cu contaminated soils are shown in Table 6. All analyzed metals, particularly Pb, Zn and Cu, showed no significant differences in plant materials before and after extraction. The finding indicated that all metals were retained in plant materials during extraction. As accumulated in roots, metals tend to form metal-organic complexes that could not be dissolved by supercritical CO_2 or ethanol-modified supercritical CO_2. It can be concluded that vetiver oils extracted by SCE contained no or negligible amounts of metals. However, metal analysis on larger samples of vetiver oils is advisable to further confirm that oil extracted from VG grown on heavy metal contaminated soils using supercritical fluid technology can be acceptable in the market.

Table 6
Metal contents of vetiver roots before and after extraction by pure SCE and ethanol-modified SCE (data are presented as average ± standard deviation).

Metals ($mg\,kg^{-1}$ DW)	Before extraction	After extraction	
		Pure SCE[a]	Ethanol-modified SCE[b]
Ca	1890.0 ± 31.5 a	1896.7 ± 28.9 a	1904.5 ± 24.1 a
Cd	0.3 ± 0.0 a	0.1 ± 0.1 a	0.2 ± 0.2 a
Cu	243.0 ± 10.6 a	244.6 ± 5.4 a	245.4 ± 7.0 a
Fe	3218.5 ± 38.1 a	3264.4 ± 64.8 a	3277.5 ± 25.1 a
K	7205.6 ± 66.4 a	7311.8 ± 45.0 a	7309.7 ± 81.2 a
Mg	2861.2 ± 23.8 a	2873.5 ± 1.9 a	2858.6 ± 28.1 a
Na	3862.9 ± 69.0 a	3880.1 ± 28.1 a	3898.6 ± 31.5 a
Ni	18.7 ± 1.5 a	20.6 ± 0.9 a	19.7 ± 0.6 a
Pb	834.7 ± 12.6 a	853.8 ± 15.5 a	851.1 ± 8.5 a
Zn	868.2 ± 25.3 a	877.2 ± 19.5 a	880.1 ± 13.3 a
Oil yield (%)	NA	1.32 ± 0.32 a	2.1 ± 0.24 b

Note: the same letter in the same row indicated no significant difference at 5% level of significance. "±" indicated standard deviation of data.

[a] Pure SCE was performed at 190 bar and 50 °C.

[b] Ethanol-modified SCE was operated at 190 bar, 50 °C and 15% ethanol.

4. Conclusion

The application of the Response Surface Method coupled with the Central Composite Design allowed a full investigation of the effect of pressure, temperature and amounts of added ethanol on vetiver extracts by using SCE. Based on the statistical and graphic analysis, pressure and amounts of added ethanol were found to have the most significant influence on vetiver oil yield, while temperature and interactive effects of all tested parameters were not significant. The SCE with ethanol-modified CO_2 at 190 bar, 50 °C and 15 vol% ethanol produced the highest oil yield (5.9%), over three times and nearly double that of hydrodistillation and SCE with pure CO_2, respectively. Interestingly, the increment of pressure at high concentrations of ethanol resulted in a negligible increase in oil yield. In addition, the rise in temperature generally caused a slight reduction in oil yield. Therefore, the extraction at low temperature, low pressure and high concentration of ethanol (100 bar, 40 °C and 15 vol% ethanol) produced a similar yield (5.3%) compared to that of the optimal conditions. The chemical analysis of extracts showed no significant difference in chemical compositions (except for zizanioic acid content) of all SCE extracts. Heavy metal analysis of plant materials before and after extraction indicated that in all cases, SCE produced extracts free of metals. In short, ethanol-modified SCE performed at low temperature, low pressure and high concentration of ethanol has great application potential for producing high yields of vetiver oil with low pressure extraction apparatus. Vetiver extracts would be suitable for a wide range of applications, such as aromatherapy, food, and perfumery.

References

[1] L.T. Danh, R. Mammucari, P. Truong, N. Foster, Response surface method applied to supercritical carbon dioxide extraction of *Vetiveria zizanioides* essential oil, Chem. Eng. J. 155 (2009) 617–626.

[2] L.T. Danh, P. Truong, R. Mammucari, N. Foster, Economic incentive for applying vetiver grass to remediate lead, copper and zinc contaminated soils, Int. J. Phytorem. 1, First published on: 23 August 2010 (iFirst) (2010).

[3] L.T. Danh, P. Truong, R. Mammucari, N. Foster, Vetiver grass, *Vetiveria zizanioides:* a choice plant for phytoremediation of heavy metals and organic wastes, Int. J. Phytorem. 11 (2009) 664–691.

[4] R. Antiochia, L. Campanella, P. Ghezzi, K. Movassaghi, The use of vetiver for remediation of heavy metal soil contamination, Anal. Biochem. 388 (2007) 947–956.

[5] M. Castillo, R. Fonseca, J.R. Candia, Report on the pilot study on the use of vetiver grass for Cu mine tailings phytostabilisation at Anglo American El Solado mine, Chile. Fundacion Chile (report in Spanish), 2007.

[6] A. Caredda, B. Maringiu, S. Porcedda, C. Soro, Supercritical carbon dioxide extraction and characterization of Laurus nobilis essential oil, J. Agric. Food Chem. 50 (2002) 1492–1496.

[7] E. Ruckenstein, I. Shulgin, The solubility of solids in mixtures composed of a supercritical fluid and an entrainer, Fluid Phase Equilib. 200 (2002) 53–67.

[8] T.Q. Armando, K. Oro, K. Shunsaku, M. Takashi, Recovery of oil components of okara by ethanol-modified supercritical carbon dioxide extraction, Bioresour. Technol. 97 (2006) 1509–1514.

[9] N.L. Rozzi, R.K. Singh, Supercritical fluids and the food industry, Comp. Rev. Food Sci. Food Saf. 1 (2002) 33–44.

[10] J.M. Dobbs, J.M. Wong, K.P. Johnston, Non-polar co-solvents for solubility enhancement in supercritical fluids, J. Chem. Eng. Data 31 (1986) 303.

[11] J. Ke, C. Mao, M. Zhong, B. Han, H. Yan, Solubilities of salicylic acids in supercritical carbon dioxide with ethanol cosolvent, J. Supercrit. Fluids 9 (1996) 82.

[12] X. Xiang, G. Yanxiang, L. Guangmin, W. Qing, Z. Jian, Optimization of supercritical carbon dioxide extraction of sea buckthorn (*Hippopha thamnoides* L.) oil using response surface methodology, LWT, Food Sci. Technol. 41 (2008) 1223–1231.

[13] I. Hasbay-Adil, H.I. Cetin, M.E. Yener, A. Bayındirli, Subcritical (carbon dioxide + ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts, J. Supercrit. Fluids 3 (2007) 55–63.

[14] J.S. Lim, Y.Y. Lee, S.C. Hai, Phase equilibria for carbon dioxide–ethanol–water system at elevated pressures, J. Supercrit. Fluids 7 (1994) 219–230.

[15] U. Kopcak, R.S. Mohamed, Caffeine solubility in supercritical carbon dioxide/co-solvent mixtures, J. Supercrit. Fluids 34 (2005) 209–214.

[16] S.S.T. Ting, S.J. Macnaughton, D.L. Tomasko, N.R. Foster, Solubility of naproxen in supercritical carbon dioxide with and without cosolvents, Ind. Eng. Chem. Res. 32 (1993) 1471.

[17] S.S.T. Ting, D.L. Tomasko, S.J. Macnaughton, N.R. Foster, Chemical–physical interpretation of cosolvents effects in supercritical fluids, Ind. Eng. Chem. Res. 32 (1993) 1482.

[18] C.A. Eckert, D.H. Ziger, K.P. Johnston, S. Kim, Solute partial molar volumes in supercritical fluids, J. Phys. Chem. 90 (1986) 27–38.

[19] O. Kajimoto, M. Futakami, T. Kobayashi, K. Yamasaki, Charge-transfer-state formation in supercritical fluids: (N,N-dimethy1amino) benzonitrile in CF_3H, J. Phys. Chem. 92 (1988) 1347.

[20] H.D. Cochran, L.L. Lee, Solvation structure in supercritical fluid mixtures based on molecular distribution functions, ACS Symp. Ser. 406 (1989) 27.

[21] P.G. Debenedetti, Clustering in dilute, binary supercritical mixtures: a fluctuation analysis, Chem. Eng. Sci. 42 (1987) 2203.

[22] I.B. Petsche, P.G. Debenedetti, Solute–solvent interactions in infinitely dilute supercritical mixtures: a molecular dynamics investigation, J. Phys. Chem. 93 (1989) 7075.

[23] C.R. Yonker, R.D. Smith, Solvatochromic behaviour of binary supercritical fluids: the carbon dioxide/2-propanol system, J. Phys. Chem. 92 (1988) 1261.

[24] R.H. Myers, D.C. Montogomery, Response of Surface Methodology, Process and Product Optimization using Designed Experiments, 2nd ed., Wiley, New York, 2002.

[25] J.M. Dobbs, J.M. Wong, K.P. Johnston, Nonpolar cosolvents for solubility enhancement in supercritical fluids, J. Chem. Eng. Data 31 (1986) 303.

[26] J. Martinez, P.T.V. Rosa, C. Menut, A. Leydet, P. Brat, D. Pallet, M.A.A. Meireles, Valorization of Brazilian vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) oil, J. Agric. Food Chem. 52 (2004) 6578–6584.

[27] D.R. Massardo, F. Senatore, P. Alifano, L.D. Giudice, P. Pontieri, Vetiver oil production correlates with early root growth, Biochem. Syst. Ecol. 34 (2006) 376–382.

[28] V.D. Zheljazkov, L.E. Craker, B. Xing, Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil, Environ. Exp. Bot. 58 (2006) 9–16.