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Response surface method applied to supercritical carbon dioxide extraction of *Vetiveria zizanioides* essential oil

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A B S T R A C T

Three operating parameters of supercritical carbon dioxide extraction (SCE) have been optimized by a response surface method using central composite design to obtain high yields of essential oil from roots of *Vetiveria zizanioides*. Analyses by multiple regression indicated that pressure has a major linear effect on oil yield, whilst temperature and time have a lesser impact. However, the effect of temperature in combination with pressure is significant. Overall, extraction yields increase with both pressure and temperature. The optimal SCE yield (1.38%) obtained at the operating conditions of 190 bar, 50 °C and 100 min was about four times higher than that achieved by hydro-distillation. In general, the quality of vetiver oil extracted by SCE is suitable for the food industry. In order for the SCE extracts to be acceptable in the perfumery industry, however, a high yield of vetiver oil would be required in association with high khusimol and low zizanoic acid contents.

1. Introduction

In recent years, there has been an increasing interest in essential oils extracted from various herbs and aromatic plants. This interest is due, in part, to the discovery of their multifunctional properties in addition to their classical roles as food additives and/or fragrances [1]. Newly discovered properties of essential oils include antibacterial, antifungal, antioxidant, and anti-inflammatory activities [2–4]. The pharmacological properties of essential oils extracted from plants have been the focus of interest from both academia and the pharmaceutical industry [5–8]. In addition, the insecticidal activities of essential oils are of interest to agricultural scientists and agri-businesses. Essential oils are now widely used as natural insecticides, cosmeceuticals, and aroma therapeutic agents.

A plant that is extensively used in the cosmetic, perfumery and food industries and has potential application in the pharmaceutical industry is vetiver (*Vetiveria zizanioides* L.) [9]. The plant, originating from India, is a tall, tufted, perennial, scented grass with a straight stem, long narrow leaves and a lacework root system that is abundant, complex, and extensive [10]. Since ancient times, vetiver grass has been used as a fragrant material and in traditional medicine because its roots contain essential oils with aromatic and biological properties [11]. The oil and its constituents are used extensively for blending in oriental types of perfumes as well as in other cosmetic and aromatherapy applications [12]. Recent research has demonstrated that extracts of vetiver roots possess several biological properties, such as antifungal [13], antioxidant [1], anticancer [14] and anti-inflammatory activities [15]. Vetiver oil can also be used for the treatment of patients with dementia-related behaviors to increase mental alertness and cognitive function [16]. These properties make vetiver extracts promising candidates for application in the pharmaceutical industry [9]. Additionally, vetiver can be used in food products as an aromatizing agent for canned asparagus and peas, and in some beverages [17–21].

Current methods for the extraction of vetiver oil are hydro-distillation, steam distillation and solvent extraction. However, hydro and steam distillation have several disadvantages, such as incomplete extraction of essential oils from plant materials, high operating temperatures with the consequent breakdown of thermally labile components, promotion of hydration reactions of chemical constituents, and require a post-extraction process to remove water. Solvent extraction overcomes the drawbacks of distillation, but has the major disadvantage of solvent residue in the extracts.

Supercritical fluid extraction has been used for the extraction of flavors and fragrances from natural materials [22]. Supercritical fluid extraction exploits the unique properties of materials, such as carbon dioxide, above their critical points to extract soluble components from matrices. Recently, there has been increased interest in supercritical and sub-critical extraction using carbon dioxide as a solvent. Carbon dioxide is an ideal solvent for the extraction of natural products because it is non-toxic, non-explosive, readily available

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and easy to remove from the extracted product. Supercritical CO_2 extraction (SCE) has several advantages over hydro-distillation, steam distillation and solvent extraction including operation at lower temperatures thus preservation of the thermally labile components in the extracts and elimination of the problem of toxic residual solvent in the products. Furthermore, the supercritical CO_2 extraction retains the organoleptic characteristics of the starting plant materials, which may not be preserved by the traditional extraction methods.

Several studies have been carried out on the SCE of vetiver essential oil [9,17,23]. However, only fixed operating conditions (200 bar and 40 °C) or variable pressure levels (100–300 bar) at fixed temperature (40 °C) have been employed in these studies. The effects of other operating conditions on SCE of vetiver essential oil were not fully investigated.

Several important factors determine the efficiency of SCE including the pressure, temperature and extraction time. To study the effect of multiple variables on output, the response surface method (RSM) is an effective technique [24]. The RSM is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [25]. The RSM has been successfully employed to model and optimize supercritical CO_2 extraction of oils from grape seed [26], cherry seed [27], walnut [28], hazelnut [29], apricot kernel [30], cottonseed [31], thyme [32] and Turkish lavender flowers [33].

The aim of this study was to optimize three operating conditions, namely temperature, pressure and time, to obtain oil extracts by SCE with high yield using the RSM. The effects of these conditions on the concentration of khusimol and zizanoic acid in vetiver SCE extracts were also investigated. In addition, the yield and chemical profile of the extracts obtained by SCE were compared with those achieved by hydro-distillation and hexane extraction.

2. Materials and methods

2.1. Plant material preparation

Fresh roots of *V. zizanioides* were supplied by the Vetigrass Company, Brisbane, Australia. Roots were washed to remove soils, and then they were air-dried at room temperature for 72 h. The dried materials were milled by a knife mill. The particles of milled roots were separated according to their particle sizes with a vibratory sieve system. Root particles in six size ranges were collected, aliquots of each size range were combined to obtain an estimated average particle size of 0.6 mm according to Table 1. The root particles were stored at −20 °C prior to extraction.

Table 1
Size ranges of roots particles.

Size range (μm)	Percentage
1000–2000	21
710–1000	25
500–710	25
355–500	19
250–355	8
<250	2

2.2. Soxhlet extraction

About 20 g of dried roots were loaded into a Soxhlet apparatus which was connected to a round flask containing 300 ml of hexane. The extraction was carried out at the boiling temperature of hexane for 5 h. After extraction, hexane was removed by evaporation at the boiling point. The evaporation was stopped once about 5 ml remained in the system this was then divided in halves: one was subjected to chemical analysis, whilst the other was evaporated to completely remove hexane for determination of extract weight. Experiments were conducted in triplicate.

2.3. Hydro-distillation

Dried vetiver roots (approximately 20 g) were hydro-distilled using a Clevenger-type extraction apparatus. The extraction was carried out over 12 h. After extraction, the extract was dehydrated by anhydrous sodium sulphate, then weighed. The extraction was repeated three times.

2.4. Supercritical CO_2 extraction

A 50 ml stainless steel extraction column loaded with approximately 10 g of milled roots was connected to the system shown schematically in Fig. 1. The CO_2 pump (ISCO Model 260D Syringe pump) was cooled to 4 °C and the pressurized CO_2 was delivered to the extraction vessel through a heating coil. The extraction column and heating coil were immersed in a water tank, the temperature of which was controlled by a circulating heater (Thermoline). The outlet of the extraction column was connected to an on–off valve that was placed upstream of a micro-metering valve. The extraction experiments were commenced when the system reached a pre-determined pressure and temperature. There were two stages of extraction: static and dynamic. The static stage was 30 min for all experiments, and the dynamic stage varied from 33 to 117 min. The flow rate of CO_2 was kept at 2 ml/min measured at operating pressure and 4 °C for all experiments. Supercritical CO_2 was expanded

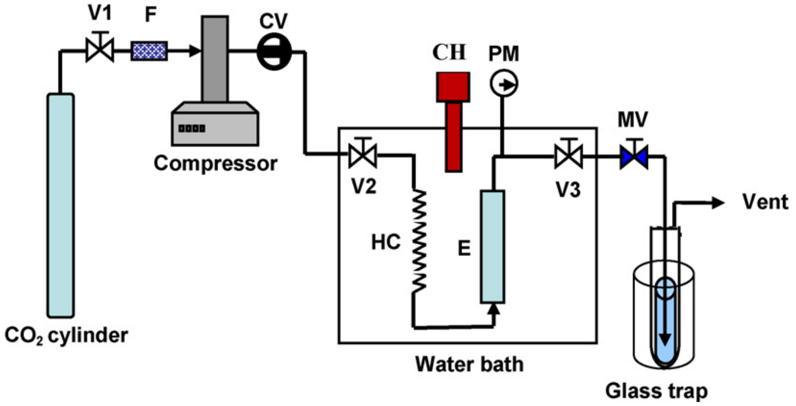

Fig. 1. Schematic diagram of SCF extraction. V1, V2, V3: stopping valve; F: filter; CV: check valve; HC: heating coil; E: extraction vessel; CH: circulating heater; PM: pressure meter; MV: micro-metering valve.

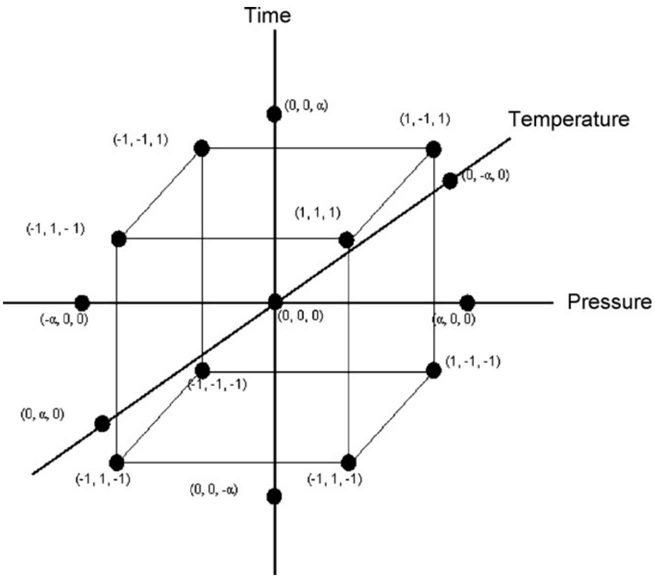

Fig. 2. Central composite design with three operating conditions of supercritical fluid extraction.

Table 2
Coded and uncoded levels of independent variables.

Independent variable	Coded levels				
	$-\alpha$	-1	0	1	α
X_1: pressure (bar)	69.3	100	145	190	220.7
X_2: temperature (°C)	36.6	40	45	50	53.4
X_3: time (min)	33	50	75	100	117

Note: α = [number of factorial runs]$^{1/4}$, in this study $\alpha = 1.6818$.

across the micro-metering valve, and the essential oil was collected in a glass tube. The glass tube contained 3 g of glass beads and was refrigerated between −20 and −5 °C in a cooling bath. The addition of glass beads aimed to increase the contact of CO_2 and essential oil with a cold surface in order to facilitate the separation of the oil and reduce the drag effect of CO_2. The extract was collected during the dynamic stage. After the experiment, the extract condensed along the tubing and valves was collected by washing with hexane. The hexane wash was then mixed with the extract collected in the glass tube. The mixture was made up to 5 ml with hexane, 1 ml was taken for GC and GC–MS analysis, the rest was placed in a fume cupboard to evaporate the solvent. The samples were then weighed.

2.5. Experimental design

A central composite design of the RSM is the most commonly used in optimization experiments. The method includes a full or fractional factorial design with center points that are augmented with a group of 'star points' (extreme values) to allow the estimation of the curvature [31]. As the distance from the center of the design space to a factorial point is defined as ±1 unit for each factor, the distance from the center of the design space to a star point is $\pm\alpha$ with $|\alpha| > 1$ (Fig. 2).

In this study, the central composite design was used to optimize three important operating variables of SCE (pressure, temperature and time) to achieve high yields of vetiver essential oil. Another variable of SCE, that is quantity of extracting medium (CO_2) used, was not considered directly in the experimental design. The justification for this is that mass of CO_2 used determines the process yield only in the cases where there is no mass transfer limitation and the exit CO_2 stream is always saturated with oil. These conditions do not necessarily occur in practice. The effect of the mass of CO_2 used was evaluated in separate experiments and the results are discussed in Section 3.2.

The operating conditions were varied at 5 levels (Table 2), and the design required 19 experiments with eight (2^3) factorial points, six extra points (star points) and five replications of the central point (Table 3).

The yield response (Y) was assumed to be affected by three independent variables (ξ_1: pressure, ξ_2: temperature, ξ_3: time) and is represented as follows:

$$Y = f(\xi_1, \xi_2, \xi_3)$$

Experimental yields were analyzed by a response surface method to fit a second-order polynomial equation:

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

where Y represents the response oil yield, β_0 is a constant, β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients, respec-

Table 3
Central composite design with coded and uncoded levels of independent variables, and experimental yield.

Experiment[a]	Coded variables			Uncoded variables			Mass of CO_2 used (g)	Yield (%)
	X_1	X_2	X_3	Pressure (bar)	Temperature (°C)	Time (min)		
1	−1	−1	−1	100	40	50	95	0.60
2	1	−1	−1	190	40	50	100	1.11
3	−1	1	−1	100	50	50	95	0.36
4	1	1	−1	190	50	50	100	1.19
5	−1	−1	1	100	40	100	190	0.72
6	1	−1	1	190	40	100	200	0.95
7	−1	1	1	100	50	100	190	0.46
8	1	1	1	190	50	100	200	1.38
9	−1.68	0	0	69.3	45	75	140	0.13
10	1.68	0	0	220.7	45	75	152	0.78
11	0	−1.68	0	145	36.6	75	147	0.84
12	0	1.68	0	145	53.4	75	147	1.03
13	0	0	−1.68	145	45	33	65	0.80
14	0	0	1.68	145	45	117	229	0.83
15	0	0	0	145	45	75	147	0.84
16	0	0	0	145	45	75	147	0.80
17	0	0	0	145	45	75	147	0.77
18	0	0	0	145	45	75	147	0.74
19	0	0	0	145	45	75	147	0.82

[a] Experiments were carried out randomly.

tively, x_i and x_j are the coded variables which are related to the original variable (ξ) as follows:

$$x = \frac{\text{original variable-midpoint of original interval}}{\text{interval of original range}}$$

The regression coefficients of the quadratic equation were determined using the Data Analysis Tool of Microsoft Excel 2003.

2.6. *Kinetic study*

The kinetics of SCE of vetiver oil were determined using a modified version of the apparatus depicted in Fig. 1 with a HPLC pump inserted between V3 and MV. The extraction procedure was similar to the one previously described. Extracts were collected at 10, 20, 40 and 60 min in order to determine the composition and yield of extracts obtained in the corresponding time interval. During the extraction, the HPLC pump was closed by an on–off valve. After a certain time, the extraction was stopped by closing V3, then the HPLC pump was opened and 3 ml of hexane were fed to remove accumulated extracts in MV and along the nozzle. The mixtures of extracts and hexane were collected in glass tubes which were removed from the glass trap. Half of the mixture was subjected to GC and GC–MS analysis, whilst hexane was evaporated from the second half and the mass of extract was recorded.

2.7. *Yield calculation*

After hydro-distillation, Soxhlet extraction and SCE, the extracted roots were dried in an oven at 50 °C for 96 h to determine the dry weight of the roots. The long drying time was to ensure the complete removal of moisture from the extracted roots. The completely dry roots were used to determine the yield of essential oil obtained from the different extraction processes as follows:

$$\text{yield} = \frac{\text{weight of collected oil}}{\text{dry weight of roots}} \times 100\%$$

2.8. *Gas chromatography and gas chromatography–mass spectrometry analysis*

The gas chromatographic (GC) analyses were performed with a Shimadzu GC 2010 gas chromatograph equipped with a flame ionization detector and an AT-5 (5% phenyl, 95% dimethylsiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium was the carrier gas used with flow rate of 1 ml/min. Samples of 1 μl were injected using the split mode (split ratio 1:10). Injector and detector temperatures were 220 and 250 °C, respectively. The oven temperature was set at 50 °C for 5 min, then programmed to 240 °C at a rate of 3 °C/min, and held at 240 °C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of n-alkanes (C_7–C_{24}) and the percentage compositions were obtained from electronic integration measurements based on area normalization.

The gas chromatography–mass spectrometry (GC–MS) analyses were performed on an Agilent Technologies 6890N Network GC system equipped with a HP-5MS (5% phenyl, 95% dimethylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and coupled with an Agilent Technologies 5975 mass selective detector. Helium was used as a carrier gas with a flux of 1.1 ml/min. The injector and detector temperature were set at 220 and 250 °C, respectively. The oven temperature was programmed at 50 °C for 5 min, and then increased to 240 °C (at the rate of 3 °C/min) which was held for a further 10 min. Sample volumes of 1 μl were injected using the split mode (split ratio 1:20). The retention indices were determined relative to a homologous series of n-alkanes (C_7–C_{24}) under the same operating conditions. The chemical components of vetiver extracts were determined by comparison of their GC retention indices and mass spectra with those reported in the literature [1,17,19,35–41], Adam library [42] and Wiley 5 library.

Table 4
Regression coefficients and corresponding *t* and *P*-values for vetiver oil yield.

	Coefficients	*t* stat.	*P*-value
Intercept	0.7897[a]	15.1184	0.0000
X_1	0.2618[a]	8.2685	0.0000
X_2	0.0253	0.7999	0.4443
X_3	0.0210	0.6644	0.5231
X_1X_1	−0.0963[a]	−3.0362	0.0141
X_2X_2	0.0749[a]	2.3616	0.0425
X_3X_3	0.0329	1.0373	0.3266
X_1X_2	0.1265[a]	3.0593	0.0136
X_1X_3	−0.0253	−0.6107	0.5565
X_2X_3	0.0418	1.0097	0.3390

[a] Means statistically significant at 5% level of significance.

3. Results and discussion

3.1. *Optimization of SCF extractions*

The effects of three operating conditions of SCE, namely pressure, temperature and time, on the extraction of vetiver essential oil were investigated using the RSM with central composite design. The experimental responses in term of essential oil yield are summarized in Table 3.

Analyses by multiple regression were performed by using experimental oil yields to identify the constants and coefficients of variables, and to investigate the linear effect of variables as well as their quadratic and interaction effects by using the Student's '*t*' test and *P*-values. In general, the larger the magnitude of *t* and smaller the value of *P*, the more significant is the corresponding coefficient term [25]. Values of $P < 0.05$ indicate significant model terms. The estimated constants, coefficients of linear, quadratic and interaction effects are presented in Table 4. Fitting the constants and coefficients into Eq. (1), the following equation was obtained:

$$\begin{aligned} Y = {} & 0.7897 + 0.2618X_1 + 0.0253X_2 + 0.021X_3 - 0.0963X_{12} \\ & + 0.0749X_{22} + 0.0329X_{32} + 0.1265X_1X_2 - 0.0253X_1X_3 \\ & + 0.0418X_2X_3 \end{aligned} \qquad (2)$$

Among the three independent variables tested, only pressure ($P < 0.0010$) had a significant linear effect on the oil yield, whilst the effects of temperature ($P = 0.4443$) and time ($P = 0.5231$) on oil yield were very small. Pressure also showed a negative quadratic effect on yield ($P = 0.0141$). Although temperature did not have a significant linear effect, both its quadratic effect ($P = 0.0425$) and interaction effect with pressure ($P = 0.0136$) were important. The response equation fitted the experimental data with $R^2 = 0.92$, indicating that 92% of the variability in extraction yield of essential oil can be explained by the model presented in Eq. (2). The goodness-of-fit of the model to the experimental data shown in Fig. 3 has a correlation coefficient of 96%.

The response surfaces of oil yield as function of the independent variables within the experimental range were generated by using the empirical model presented in Eq. (2). The response surface equation indicates that oil yield increased with pressure, temperature and time.

The extraction yield is shown in Fig. 4 as a function of pressure and temperature at an extraction time of 50 min. The pressure had a significant positive effect on oil yield that can be explained by the increase in solvent power of supercritical CO_2 resulting from

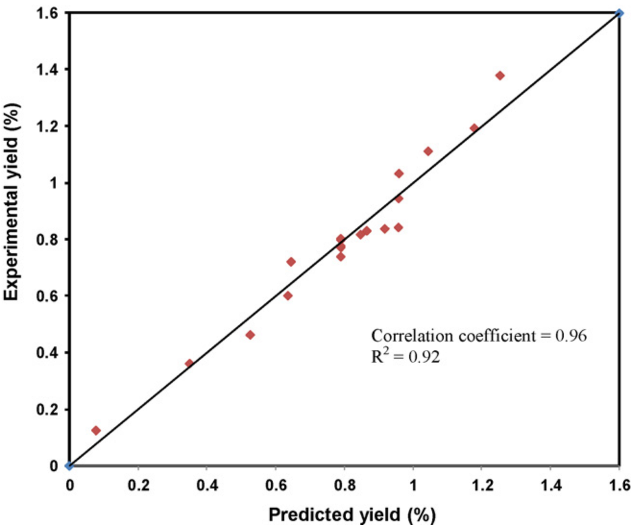

Fig. 3. Goodness-of-fit of empirical model.

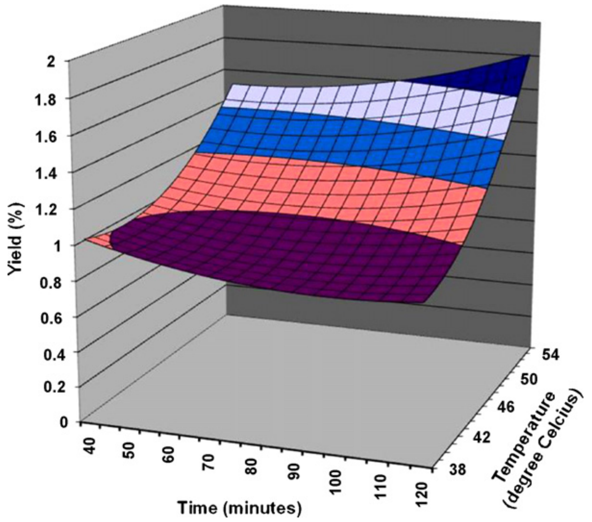

Fig. 5. Response surface plot showing the effect of temperature and time on oil yield at fixed pressure of 190 bar.

increments in density [43]. The increase in oil yield with pressure became larger as temperature increased. For example, as pressure increased from 100 to 200 bar, the oil yield increased from 0.64% to 0.89% at 40 °C and from 0.35% to 1.22% at 50 °C. Such increments in yields are due to the interaction between pressure and temperature.

At low pressures, increments in temperature resulted in the decrease of the oil yield, however at higher pressures the oil yield increased with temperature. Yield decreased from 0.64% to 0.35% when temperature increased from 40 to 50 °C at 100 bar, however at 220 bar the same temperature increment corresponded to a change from 0.89% to 1.28%. A similar trend was observed in a SCE study for the optimization of apricot kernel oil yield using the response surface method [29].

The effect of temperature on vetiver oil yield obtained from this study presents analogies to the influence of temperature on solubility in supercritical fluids. The solubility of solids in supercritical fluids is the combination of two competing effects: the increase of solid volatility and the decrease of the solvent density with temperature rise [44]. The effect of fluid density is dominant at pressures near the critical point: a moderate increase in temperature leads to a large decrease in fluid density and a consequent reduction in solute solubility. However, at higher pressure, the effect of increasing solid vapour pressure with temperature exceeds the effect of density reduction, hence the solid solubility increases with temperature [44]. The combined phenomena determine a region of retrograde behavior of the solubility of solids in supercritical fluids.

The effect of temperature and time on oil yield at 190 bar is shown in Fig. 5. Oil yield increased with temperature as increments in temperature enhanced the mass transfer rate [28,45,46].

The effect of pressure and time on oil yields at 50 °C is illustrated in Fig. 6. Again, pressure had the most significant effect on oil yield, whilst the extraction yield only slightly increased with time. The independence of extraction yields from time may be due to the extractible components being easily accessible to the solvent [47] and being available in limited amount.

According to the study of Lavania [48], oil cells of vetiver roots are found in the pith region that is located in the center of the roots (Fig. 7). Large sizes of root particles correspond to high mass transfer resistance as the CO_2 and the oil droplets must diffuse through thick layers of cells before freely mixing with the flowing solvent. The reduction of particle size increases the contact surface area between oil cells and solvent. In this study, dry roots were ground

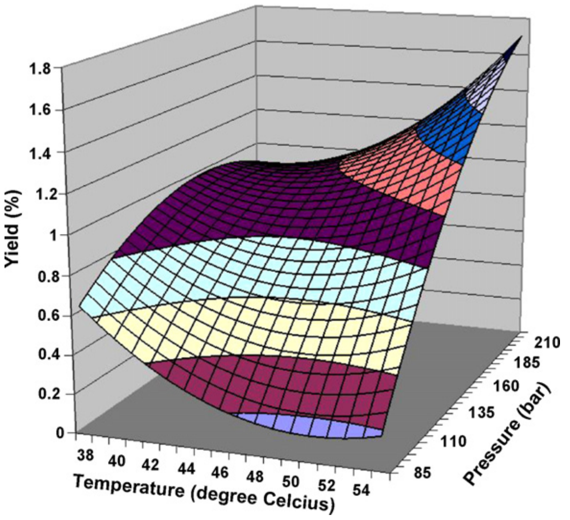

Fig. 4. Response surface plot showing the effect of pressure and temperature on oil yield at extraction time of 50 min.

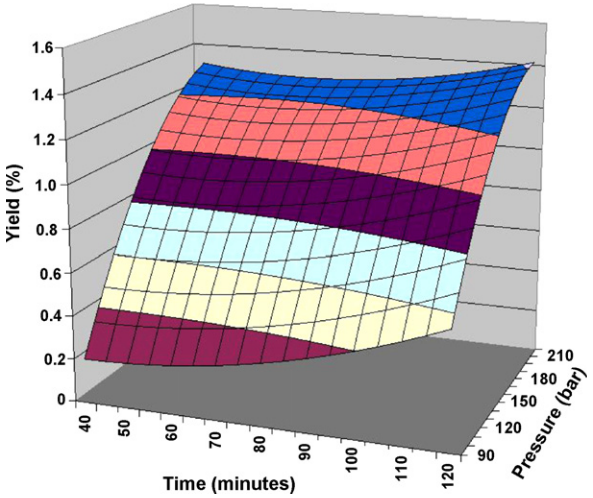

Fig. 6. Response surface plot showing the effect of pressure and time on oil yield at 50 °C.

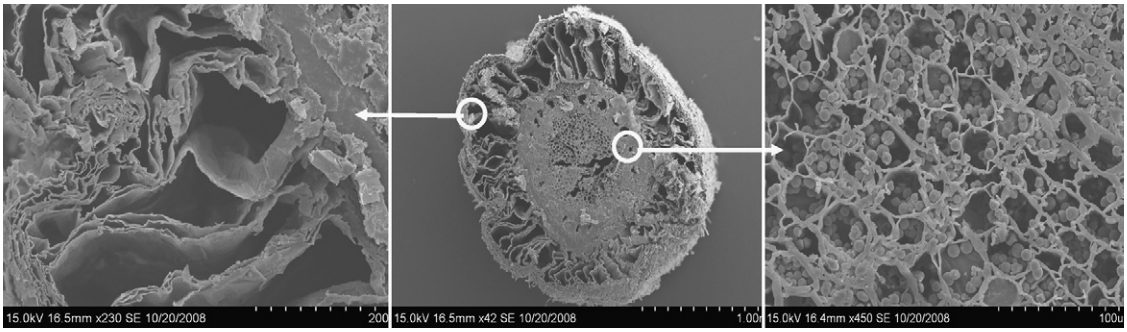

Fig. 7. Scanning electronic microscopy of dry vetiver root: localization of oil glands.

to an average particle size of 0.6 mm for all extractions and cell walls may have been disrupted. The combined effect of reduced mass transfer resistance and low oil content may have resulted in the extraction of most of the essential oil within a short time. The effect of time was further investigated with the kinetic study.

In the work of Talansier et al. [9], the maximum yield of vetiver oil obtained by SCE was produced at 40 °C and 200 bar. Consistently, the model developed in this study predicted the highest yields at 40 °C in the pressure range of 180–200 bar. Within the experimental conditions, the model predicted the highest yield (1.8%) at 220.7 bar, 54 °C and 117 min.

3.2. *Kinetic study*

The yields of vetiver oil as a function of the total amount of CO_2 used are shown in Fig. 8. It can be seen that the yield increased with increasing pressure and temperature. Yields close to the maximum were obtained at high pressure and temperature, whilst milder extraction conditions produced lower yields.

Extraction of vetiver oil can be divided into two phases. Initially, the increase in the total amount of CO_2 used resulted in a steep increase in the yield. However, when more than 120 g of CO_2 were used, the further passage of CO_2 led to lesser increments in oil yield. The results can be explained based on the distribution of oils within the root cells. At the early stage, the oil is extracted from the surface of the particles, and the solubility of the oil in supercritical CO_2 controls the mass transfer [28]. In the later stage, oil from the intact cells is extracted, and the mass transfer is controlled by the diffusion of oil within the particles [28]. The mass transfer rate is low and oil yield increments are insignificant compared to the fast extraction period [49,50].

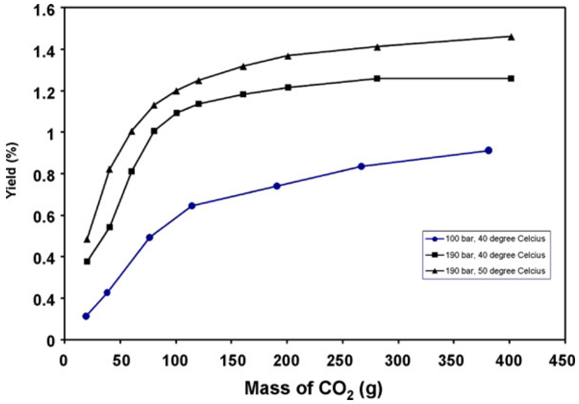

Fig. 8. Yield of vetiver oil extracted by SCE as a function of the total amount of CO_2 used at different operating conditions.

The reduced overall increment of the extraction yield in the second stage (less than 0.2%) may be explained by the limited content of oil in vetiver roots: almost all of the oil content was extracted by about 120 g of supercritical CO_2. At the experimental conditions, 120 g of CO_2 corresponded to extraction times of about 40 min. Consequently, the kinetic study explains why, within the experimental range of extraction time (37–117 min), time did not show significant effect on oil yield.

3.3. *Chemical components of SCE vetiver extract*

Chemical component profiles of vetiver extracts produced at different operating conditions are presented in Table 5. The identified components are grouped into four classes (hydrocarbons, alcohols, carbonyl compounds and carboxylic acids) according to their chemical functional groups. The composition of the extracts was almost unchanged (except for khusimol) over the experimental conditions indicating that the extraction process is not selective when a non-polar supercritical solvent is used [44]. The results are consistent with the study of Talansier et al. [9]. Zizanoic acid, khusimol, α- and β-vetivone and isovalencenol were the main components in all SCE extracts. The combined amount of these compounds accounted for about half of the extracts.

For perfumery applications, a high content of alcohols is desirable as this chemical characteristic is related to the quality of volatile oil components, whilst both hydrocarbons and acids are not desired because of their poor sensorial properties [17]. The content of hydrocarbons in all SCE extracts was very small being about 1%, because most of the hydrocarbons in vetiver oil are highly volatile and can escape together with CO_2 during the depressurization. It is interesting to observe that operation at low pressure and high temperature corresponded to high levels of alcohols in the extracts, whilst the content of acids was very low. Such extracts are ideal for use in perfumery. However, SCE yields at these conditions were low. Yields could be improved whilst preserving the selectivity of the process by using modifiers such as low molecular weight alcohols.

The supercritical CO_2 extracts can be used in the food industry as an aroma and antioxidant source. By sensory evaluation, SCE extracts have mild strength and no specific vetiver character, but have the smell of orris resinoid, and are deemed suitable as food aroma for potatoes or asparagus [17]. Moreover, two main components of vetiver oil, α- and β-vetivone, have strong antioxidant activities [1]. The concentrations of both α- and β-vetivone were nearly unchanged in all SCE experiments and accounted for around 10% of extracts, similar to hydro-distillation extracts. Consequently, the use of SCE extracts in food products offers further merit for consumers by providing a source of antioxidant that may help to maintain health and prevent diseases such as cancer and coronary heart disease.

Table 5
Chemical components of vetiver oils extracted by SCE at different operating conditions, hydro-distillation and hexane extraction.

KI	Compound	Aroma	Hy	Hex	1	2	3	4	5	6	7	8	Central point	9	10	11	12	13	14
1363	α-Ylangene [36]	0.12																	
1372	α-Duprezizanene [39]	1.38																	
1405	β-Funebrene [36]	0.38	0.12 ± 0.02																
1429	β-Copaene [36]	0.43	0.21 ± 0.01																
1441	Prezizaene [17,37,38]	3.54	0.14 ± 0.01																
1446	Khusimene [17,36–40]	1.65	0.17 ± 0.01																
1454	**α-Patchoulene**	1.12																	
1481	α-Amorphene [17,34,36,38–40]	2.92	0.31 ± 0.04																
1491	β-Vetispirene [17,36,39,40]	4.08																	
1493	δ-Selinene [36,40]	1.02																	
1497	γ-Amorphene [36]	0.38																	
1507	**Cuparene**	0.77																	
1511	δ-Amorphene [17,36,39,40]	2.78																	
1515	Nootkatene [36]	0.39					0.12				0.15								
1519	γ-Cadinene [17,36,37,40]					0.12	0.14	0.11		0.15	0.13								
1522	Valencene [17,34,36]	1.59																	
1529	δ-Cadinene [37,38,40]	0.2																	
1534	γ-Vetivenene [17,36,38–40]	3.62																	
1538	**10-epi-cis-Dracunculifoliol**					0.92	0.96	0.81		0.75	0.75	0.94	0.88 ± 0.10	1.22	1.08	0.7	0.92	0.87	0.95
1547	α-Calacorene [17,36]	0.42	0.11 ± 0.02																
1551	Elemol [34,36,37,39]						0.1				0.12								
1556	β-Vetivenene [34,36,38–40]	7.29	0.28 ± 0.02				0.2				0.16		0.12 ± 0.00	0.12	0.14	0.1	0.13	0.14	0.11
1589	Spathulenol [40]					0.21	0.21	0.21		0.14	0.14								
1596	Viridoflorol [40]					0.14	0.18	0.16		0.48	0.24	0.55	0.53 ± 0.04	1.8	0.65	0.56	0.53	0.59	0.59
1604	Khusimone [19,34,36–38,40]	0.68	1.34 ± 0.13	0.85 ± 0.04	0.69	0.97	1.09	0.86	0.6	0.83	0.89	0.98	0.91 ± 0.09	2.25	1.12	0.87	0.93	0.95	1.01
1646	**epi-α-Cadinol**		0.71 ± 0.02	0.34 ± 0.01		0.39		0.77		0.68		0.12							
1651	Pogostol [40]		0.48 ± 0.01	0.21 ± 0.01	0.33	0.23	0.2	0.23	0.33	0.11	0.32	0.15	0.39 ± 0.03	1.27	0.47	0.37	0.39	0.39	0.43
1661	Valerianol [40]		0.95 ± 0.02	1.87 ± 0.08	1.75				1.69										
1671	Epi-zizanone [34,39]	0.54	2.23 ± 0.04	0.97 ± 0.03	0.77	0.46	0.76	0.41	0.7	0.88	0.65	1.09	1.03 ± 0.05	3.74	1.25	1.05	1.06	1.1	1.16
1687	Khusinol [17,36]					1.12	0.15	1.02			0.28								
1700	**Eudesma-4(15),7-dien-1β-ol**			0.78 ± 0.02	2	0.82			1.85	2.31		2.16	2.40 ± 0.04	4.36	2.52	2.49	2.3	2.42	2.49
1701	Zizanal [34,38]	0.92								0.92		0.86	0.94 ± 0.03	1.76	0.99	0.96	0.88	1	0.97
1721	Juniper camphor [36,37,40]		1.35 ± 0.03			0.6		0.57											
1730	Vetiselinenol [34,36,39,40]		3.63 ± 0.50	1.27 ± 0.03	2.44	2.05	2.97	2.14	2.4	1.92	3.13	2.08	1.91 ± 0.06	3.8	1.88	1.8	1.75	1.68	1.89
1747	Khusimol [17,19,34,36–40]	7.8	25.8 ± 0.39	13.3 ± 0.69	14.57	15.7	23.31	14.81	13.32	16.94	20.53	15.45	16.9 ± 0.52	24.21	17.42	17.2	16.1	16.57	17.3
1759	14-Hydroxy-δ-cadinene [39]		1.03 ± 0.01	0.70 ± 0.02	0.68	0.77	1.12	0.75	0.65	0.86	1.02	0.78	0.87 ± 0.05	1.25	0.89	0.83	0.84	0.8	0.88
1795	Isovalencenol [17,34,36,37,39]		6.64 ± 0.13	3.43 ± 0.09	3.85	4.11	6.21	3.92	3.66	4.52	5.63	4.25	4.45 ± 0.18	6.59	4.61	3.95	4.19	3.95	4.59
1812	Nootkatone [17,34,36–40]		1.12 ± 0.04		1.62	1.38	1.28	1.36	1.54	1.49	1.64	1.39	1.41 ± 0.08	1.12	1.38	1.31	1.36	1.32	1.42
1818	Zizanoic acid [17,36,37]	2.44	9.60 ± 0.20	31.1 ± 0.06	34.84	25.88	14.71	26.58	34.37	26.52	21.4	25.88	23.8 ± 0.76	1.53	21.58	24.17	21.6	18.65	22
1824	β-Vetivone [17,19,34,36,37,39]	2.31	1.97 ± 0.05		1.88	2.41	2.29	2.27	1.9	2.29	2.18	2.48	2.48 ± 0.13	2.93	2.53	2.53	2.58	2.28	2.46
1833	Sesquiterne ketone [39]	0.61	2.31 ± 0.08	1.93 ± 0.06	3.09	3.35	2.5	3.54	3.44	2.85	2.67	3.54	3.17 ± 0.05	4.09	3.3	2.91	3.09	2.68	3.25
1845	α-Vetivone [17,19,34,36,37,39]	2.54	6.77 ± 0.11	5.42 ± 0.06	5.61	5.9	7.93	5.79	5.67	6.31	6.81	6.03	6.3 ± 0.20	11.93	6.5	6	6.21	5.8	6.46
1975	Hexadecanoic acid [34,37]		1.86 ± 0.2	1.92 ± 0.18	2.1	1.18	0.75	1.36	2.36	1.21	1.31	1.21	0.96 ± 0.12		0.83	0.83	0.84	1.14	0.86
Hydrocarbons		34.08	2.37	0.7	0.68	0.89	1.58	0.86	0.65	1.01	1.46	0.78	0.99	0.94	0.99	0.93	0.97	1.37	1.03
Alcohols		7.8	39.56	21.23	24.94	26.29	34.29	24.64	23.25	27.85	31.14	25.7	27.42	26.47	28.2	27.07	26.2	43.25	28.63
Carbonyl compounds		7.6	15.74	9.17	13.66	14.47	15.85	14.23	13.85	15.57	14.84	16.37	16.24	15.13	16.7	15.63	16.1	27.82	17.07
Carboxylic acids		2.44	11.46	32.98	36.94	27.06	15.46	27.94	36.73	27.73	22.71	27.09	24.71	19.79	22.9	25	22.5	1.53	22.41
Total identified		51.92	69.13	64.08	76.22	68.71	67.18	67.67	74.48	72.16	70.15	69.94	69.34	62.33	68.8	68.63	65.7	73.97	69.14

Note: aroma (reference oil); Hy (hydro-distillation); Hex (hexane extraction); bold words (putatively identified compounds).

Table 6
Regression coefficients and corresponding *t* and *P*-values for khusimol content.

	Coefficients	*t* stat	*P*-value
Intercept	16.911[a]	23.135	0.000
X_1	−1.483[a]	−3.348	0.009
X_2	0.857	1.933	0.085
X_3	−0.073	−0.164	0.873
X_1X_1	1.109[a]	2.499	0.034
X_2X_2	−0.371	−0.836	0.425
X_3X_3	−0.273	−0.616	0.553
X_1X_2	−2.291[a]	−3.960	0.003
X_1X_3	0.739	1.277	0.234
X_2X_3	−0.266	−0.460	0.656

[a] Means statistically significant at 5% level of significance.

3.3.1. Khusimol

Khusimol is the main and important component of vetiver essential oil, as it determines the oil quality through its desired woody odor [21]. From the multiple regression analysis of the khusimol level in SCE extracts (Table 6), pressure had significantly linear and quadratic effects, whilst temperature only had a significant interaction effect with pressure. The model presented in Eq. (3) had a correlation coefficient of 91% and $R^2 = 0.82$. The R^2 value was acceptable and indicated that the model accounts for 82% of the variability in the current experiment.

$$
\begin{aligned}
Y = {} & 16.911 - 1.483X_1 + 0.857X_2 - 0.073X_3 + 1.109X_{12} \\
& - 0.371X_{22} - 0.273X_{32} - 2.291X_1X_2 + 0.739X_1X_3 \\
& - 0.266X_2X_3 \qquad (3)
\end{aligned}
$$

The effect of pressure and temperature on khusimol concentration in SCE extracts is shown in Fig. 9. In general, high levels of khusimol in the extracts were obtained at high temperature and low pressure that corresponded to low CO_2 solvent strength, and thus, very low extraction yields (Fig. 4).

3.3.2. Zizanoic acid

Zizanoic acid is the main undesired component in SCE extracts. As it has poor sensorial properties [17], only extracts with low zizanoic acid content can be used in perfumery. Zizanoic acid levels in SCE extracts were quite high (from 15% to 35%) except in experiment 9 (Table 5). Although, the multiple regression analysis showed that pressure, temperature and extraction times as well as their interaction did not have significant effect on zizanoic acid content in SCE extracts, there was a general trend of achieving low zizanoic acid content at low pressure and high temperature (experiments 3, 9 and 13). Vetiver essential oil rich in zizanoic acid can be improved by removal or separation of the acid followed by chemical transformation of the acid into khusimol [17].

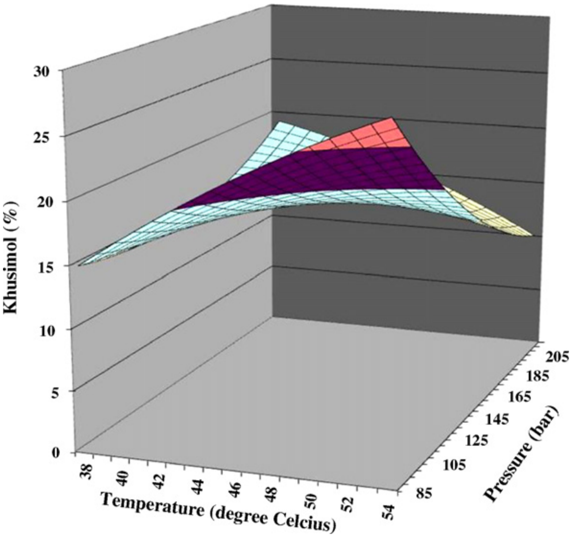

Fig. 9. Response surface plot showing the effect of pressure and temperature on khusimol content at the extraction time of 50 min.

3.4. Comparison with conventional extraction methods

Chemical profiles of vetiver oil extracted by hydro-distillation, hexane extraction and SCE are presented in Table 5. No significant differences were found in the oils extracted by n-hexane and SCE by GC and GC–MS analysis. Similar findings were reported for the extraction of walnut oil [27]. Hexane and CO_2 are non-polar solvents so they exhibit similar behavior for extracting chemical compounds from plant materials. However, the chemical profiles of hydro-distillation extracts were significantly different from those of hexane and SCF extracts. Water is a polar solvent with different extracting properties compared to non-polar solvents. Hydro-distillation extracts were richer in hydrocarbons and alcohols than hexane and SCE extracts whilst having lower levels of acid compounds. The carbonyl compounds in hydro-distillation and SCE extracts were similar and higher than in hexane extracts. Extracts obtained by hydro-distillation are suitable for application in the perfumery industry due to their low acid and high alcohol content, whilst SCE extracts could be suitable for the food industry.

The extraction yields of vetiver oil by the distillation method vary depending on the area of origin of the material, growing conditions and maturation of plants. Extraction yields of vetiver oil by hydro-distillation are between 0.5% and 2% on a dry weight basis [51]. Yields obtained by hydro-distillation in this study were relatively low (Table 7), as vetiver was grown hydroponically and roots were collected from young plants (7 months old).

Soxhlet extraction using hexane as a solvent produced the highest yield (1.91%) whilst hydro-distillation the lowest (0.31%). Solvent extraction of plant materials produces oleoresin, which contains not only the volatile compounds but also waxes, colour pigments and albuminious materials with semi-solid consistency [52]. Hexane is a very powerful solvent, and was used to determine the content of oil extracted from cotton kernel [31] and artemisinin in *Artemisia annua* L. [53]. Vetiver hexane extract can be defined as 100% recovery of essential oil from the roots. In this study, hexane extracts contained small solid particles that may be chemical compounds with high molecular weights, hence were not dissolved in hexane at room temperature. The presence of these particles explained the highest yield obtained by hexane extraction as compared to SCE extraction and hydro-distillation. Therefore, the maximum content of vetiver essential oil in this study must be lower than 1.91%.

Table 7
Yields obtained by hydro-distillation, solvent and supercritical fluid extraction of vetiver essential oil.

Methods	Operating conditions				Yield (%)
	Temperature (°C)	Time (min)		Pressure (bar)	
		Static	Dynamic		
Hydro-distillation	100	N/A	720	N/A	0.31 ± 0.01
Soxhlet extraction	70	N/A	300	N/A	1.91 ± 0.19
SCF extraction					
Experimental	50	30	100	190	1.38
Predicted	54	30	117	220.7	1.80
Confirmed	54	30	117	220.7	1.43 ± 0.08

Note: N/A means not applicable.

In the range of tested pressure, temperature and time, the yield obtained at the operating conditions of 190 bar, 50 °C and 117 min was the highest (1.38%). The maximum yield predicted by using the model presented in Eq. (2) was 1.8% at 220.7 bar, 54 °C and 117 min. The predicted yield together with the highest experimental yield were used to compare yields obtained by Soxhlet extraction and hydro-distillation (Table 7). The experimental and predicted yield from SCE extraction were much higher than that of hydro-distillation, nearly four and six times, respectively. The predicted yield was 94% of the hexane extract. However, three extractions operated at this condition produced yields of $1.43 \pm 0.075\%$ which is about 80% of the predicted yield. The result can be explained by considering that the model did not take into account the limited content of essential oil in vetiver roots. As mentioned previously, the maximum content of vetiver essential oil must be lower than 1.91%. Furthermore, kinetic experiments at 190 bar and 50 °C produced yields of 1.37% and 1.46% in 100 and 200 min, respectively. Prolonging extraction to 300 min resulted in negligible yield increments (data not shown). In summary, the maximum yield of vetiver essential oil obtained by SCE in this study was around 1.4% and corresponded to an almost complete extraction of the oil components from the plant material. The yield obtained at 190 bar, 50 °C and 100 min (1.38%) was similar to the yield ($1.43 \pm 0.08\%$) achieved at 220.7 bar, 54 °C and 117 min (Table 7) which corresponded to the maximum produced yield. Therefore, in the range of tested pressure, temperature and extraction time, the extraction conditions of 190 bar, 50 °C and 100 min can be considered as the optimal condition for economic reasons.

In terms of extraction time, SCE was the quickest process being about 80% and 50% less time consuming than hydro-distillation and Soxhlet extraction, respectively. Moreover, SCE was operated at lower temperature than the other two methods. Both conditions contribute to the feasibility of operating SCE on large scale.

A direct comparison between the results obtained from SCE processing in this study and the earlier studies [9,14] is not possible since vetiver oil yields and components are significantly affected by growing locations, environmental factors (temperatures, nutrients, etc.), age of plants and genetic variation [11,37,54]. This study used the root materials collected from the Monto variety grown in Australia for 7 months, whilst the earlier studies used roots collected from unknown varieties grown in Brazil with unknown age.

4. Conclusion

The analysis of a response surface method of experimental design showed that pressure has the most significant effect on vetiver oil yields produced by SCE, whilst temperature and time have minor impact. However, temperature has moderate quadratic effect and interaction effect with pressure. Oil yield increases with pressure and temperature, with the optimal operating conditions for maximum yield (1.38%) being 190 bar, 50 °C and 100 min. Pressure has a significantly linear effect on the content of khusimol. In addition, the interaction effect of two operating conditions was significant on this compound. Extracts with high content of khusimol and low content of zizanoic acid, ideal for use in perfumery, corresponded to low yields. To be applied in the perfumery industry, the yield should be improved whilst preserving the process selectivity. The addition of modifiers to the process may address this point. SCE extracts obtained with high yield are suitable for application in the food industry.

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