OPTIMIZATION OF ESENTIAL OL EXTRACTION FROM VETIVERIA ZANIOIDES USING SUPERCRITICAL CARBON DIOXIDE

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The yield of supercritical fluid extraction of essential oil from the roots of *Vetiveria zizanioides* has been optimized with a response surface method with central composite design. Three operating parameters – pressure, temperature, time – were varied over 5 levels in a dynamic extraction process utilizing carbon dioxide as extracting medium. Supercritical fluid extraction produced yields up to five times higher than hydro-distillation and comparable to the yield of extraction with hexane.

Analyses of multiple regression indicated that the pressure the major linear effect on oil yield, whilst temperature and time had a lesser impact on the extraction yields. However, temperature had significant effect in quadratic form and interaction with pressure. At any extraction time, yields significantly increased with increasing pressure and temperature. The model predicts the theoretical yield when the extraction is operated at 220 bar and 54ºC for 40 minutes. The predicted yield is about seven times higher than the yield obtained by hydro-distillation and slightly higher than yield obtained by hexane extraction. Compared to hydro-distillation and extraction with hexane, supercritical fluid extraction has the distinctive advantages of being operated at low temperature and producing products free from residual solvent. The high yields and purity of the extracts obtainable through supercritical processing makes the technology attractive to the industry which is under pressure to produce "clean essential oil".

I. INTRODUCTION

In recent years, there is an increasing trend in research of essential oils extracted from various herbs and aromatic plants due to the continuous discoveries of their multifunctional properties other than their classical roles as food additives and/or fragrances. New properties of many essential oils, such as antibacterial, antifungal, antioxidant, and anti-inflammatory activities have been found and confirmed (Aruoma et al, 1996; Hammer et al, 1999; Güllüce et al, 2003). The pharmacological properties of essential oils extracted from plants have received the greatest interest of academic institutes and pharmaceutical companies (Loza-Tavera, 1999; Courreges et al, 2000; Carnesecchi et al, 2002 and Salim et al, 2003). On the other hand, the insecticidal activities of essential oils are more favored by agricultural scientists and agri-business. Consequently, many investigation and new findings have significantly prompted and expanded novel applications of essential oils which are now been widely used as natural insecticides, cosmeceuticals, and aroma therapeutic agents.

One such plant that is extensively used in perfumery industry is Vetiver (*Vetiveria zizanioides* L.) 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. Since the ancient time, Vetiver grass has been used as a fragrant material and in traditional medicine because its roots contain essential oils that have aromatic and biological properties. The oil and its constituents are used extensively for blending oriental types of perfumes and floral compounds, as well as in other cosmetic and aromatherapy applications. It is very persistent and one of the finest fixatives known. Vetiver oil is a main ingredient in 36% of all western quality perfumes and 20% of all men's fragrances (Lavania, 2003).

Currently, the methods used for extraction of Vetiver are mainly hydro-distillation, steam distillation and solvent extraction. However, the hydro and steam distillation have several drawbacks, such as operating at high temperatures leading to break-down of thermally-labile components, promoting hydration reaction of chemical constituents, requiring a post-extraction process to remove water and incomplete extraction of essential oils from plant materials. The solvent extraction does not have the same drawbacks as of distillation, but it has one major disadvantage that makes it less favourable for essential oil extraction: solvent residue in extracted essential oil.

Recently, an advanced method is used for extraction of flavours and fragrances from natural materials, namely supercritical fluid extraction (Caredda et al, 2002). Supercritical fluid extraction exploits the unique properties of gases above their critical points to extract soluble components from a raw material. Recently, there has been increased interest in supercritical and subcritical extraction which use 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 and easy to remove from extracted product. The supercritical CO_2 extraction (SCE) has several advantages over hydro-distillation, steam distillation and solvent extraction including: elimination of problem of toxic residual solvent in the products, operation at lower temperatures leading to less deterioration of the thermally-labile components in the extract. Furthermore, the supercritical CO_2 extraction retains organoleptic characteristics of the starting plant materials in extracts that do not occur in the traditional extraction methods.

II. Methods

2.1. Plant material preparation

Fresh roots of *Chrysopogon zizanioides* were supplied by Vetigrass Company, Brisbane Queensland. Roots and leaves were washed to remove soils or sands, and then they were air-dried at room temperature for 72 hours. The dried materials were milled by a knife mill. The particles of milled roots were separated according to their particle sizes by using a vibratory sieve system. The average particle sizes of 0.6 mm were used for all experiments. The root particles and milled leaves were stored in -20^{0}C freezer before extraction.

2.2. Sohklet extraction

Twenty five grams of dried roots were loaded into Sohklet apparatus that was connected to round flasked containing 300 ml of hexane. The round flask was placed on thermal heater. The extraction were carried out at 80^{0}C (above boiling point of hexane) for 5 hours. After extraction, hexane was evaporated and extracted oil was weighted and analyzed. The experiment was repeated 3 times.

2.3. Hydro-distillation

Twenty five grams of dried roots were put into a 1 L flask (containing 0.5 L of distilled water) that was connected to Clevenger-type extraction apparatus, the mixture was heated to form the vapor that contained essential oils. The vapor condensed in the condenser and dropped into the collecting tube. The extraction was carried out in 12 hours. After extraction completes, water in collecting tube was removed first, then essential oil was collected. The collected essential oil was dehydrated by Sodium sulphate anhydrous, then weighted.

2.4. Supercritical fluid extraction

A 50 ml stainless steel extraction column loaded with 12 grams of dried root was connected to the system as presented in Figure 2.1. The CO_2 pump was cooled to 4^{0}C by ice circulating bath delivered the pressurized CO_2 to the extraction vessel through coiling tube. The extraction column and coiling tube were immersed into water tank where temperature was control by circulating heater (Thermoline). The outlet of extraction column was connected to on-off valve that was placed at upstream of micro-metering valve. Extraction process was optimized by using Central Composite Design of Response Surface Methodology with three independent variables: pressure, temperature and time of extraction (described later). Extraction started as the system reached a certain combination of pressure and temperature. There were two stages of extraction: static and dynamic. Static stage was 30 minutes for all experiments, and dynamic stage varied from 33 to 117 minutes. CO_2 flow rate was constantly kept at 2 ml/min for all experiments. Supercritical CO_2 was expanded across micro-metering valve, and then essential oil was collected in glass tube containing 3 g of glass beads that was placed in a cooling bath of salt solution and ice (−20 to −5 °C). The extract was collected during dynamic stage. After the experiment, the extract condensed along the tubing and valves was collected by washing with hexane that was then mixed with trapping solvent. The mixture was made up to 5 ml by hexane, 1 ml was taken for GC and GC-MS analysis, the rest was placed under fume cupboard to evaporate the solvent. Then extracted essential oil was weighted.

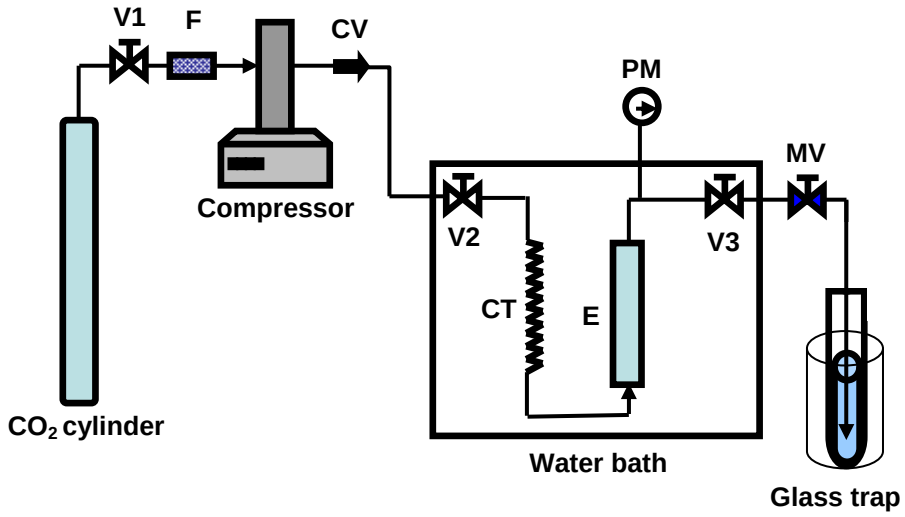

Figure 2.1: Schematic diagram of SCF extraction. V1, V2, V3: stopping valve; F: filter; CV: check valve, CT: coiling tube; E: extraction vessel; PM: pressure meter; MV: micro-metering valve.

2.5. Experimental design

Response surface methodology, or 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 (Montgomery, 2005). Central composite design is the most common design of RSM used in optimization experiments, it includes a full or fractional factorial design with center points that is augmented with a group of `star points' (extreme values) that allow estimation of curvature (NIST/SEMATECH e-Handbook of Statistical Methods, 2007). As the distance from the center of the design space to a factorial point is designed as ±1 unit for each factor, the distance from the center of the design space to a star point is ± α with $|\alpha| > 1$ (Figure 2.1).

In this study, central composite design was used to optimise three important operating conditions of supercritical fluid extraction (pressure, temperature and time) for high vetiver essential oil yield. The operating conditions were investigated at 5 levels (Table 2.1), and the design required 19 experiments with eight (2^3) factorial points, six extra points (star points) and five replication for central point (Table 3.1).

Table 2.1. Coded and uncoded levels of independent variables

Independent variable	Coded levels				
	-α	-1	0	1	α
X_1: Pressure (bar)	72.8	100	145	190	217.2
X_2: Temperature (ºC)	36.6	40	45	50	53.4

X_3: Time (minutes)	33	50	75	100	117

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

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

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

Experimental yields were analyzed by 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 β_0, β_i, β_{ii} *and* β_{ij} are regression coefficients of the equation and x_i and x_j are the coded variables linearly related to ξ_1. The coding of ξ_1 into x_i is expressed by the following equation:

$$x_i = 2(\xi_i - \xi_i^*)/d_i$$

where ξ_i = actual value in original units; ξ_i^* = mean of high and low levels of ni; and d_i = difference between the low and high levels of ξ_i.

The regression coefficients of quadratic equation were determined by by using Data Analysis Tool of Microsoft Excell 2000.

3.1.5. Yield calculation

Essential oil obtained from hydro-distillation, solvent and supercritical fluid extraction was deterimined as following

$$\text{Yield} = \frac{\text{Weight of collected oil}}{\text{Weight of dry materials}} \text{ x } 100\%$$

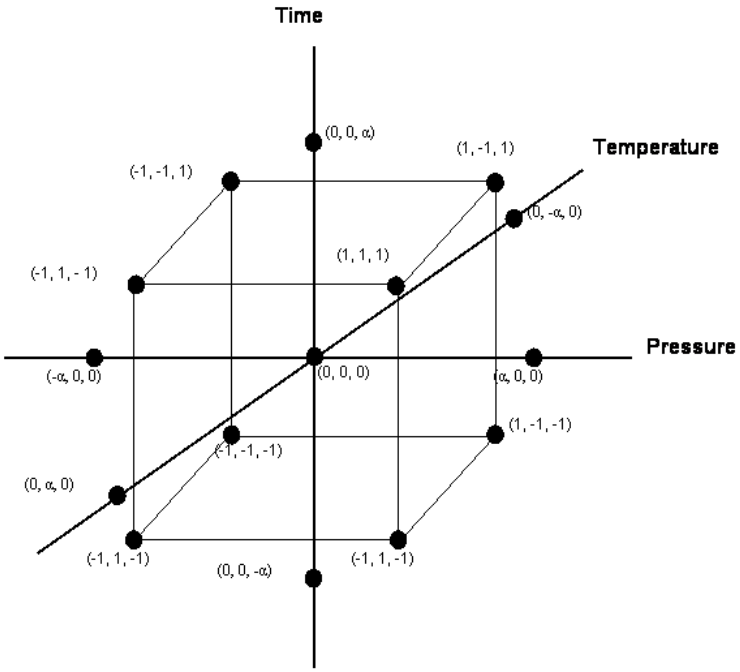

Figure 2.1. Central composite design with three operating conditions of supercritical fluid extraction: pressure, temperature and time.