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Optimization of Supercritical Carbon Dioxide Extraction of Essential Oils in Philippine *Cananga odorata* Hook Fil et. Thomson Flowers by Response Surface Method

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Essential oil was extracted from the flowers of Philippine *Cananga odorata var. genuina* grown in Pala-o, Iligan City by supercritical carbon dioxide (SC-CO_2). A statistical experimental design, first-order 2^3 factorial, was used to investigate the effects of three independent variables (pressure, temperature, and flow rate of CO_2) on % oil yield (w/w), % linalool (v/v) and % benzyl benzoate (v/v) on the extracted oil. An optimum oil yield (9.21 % w/w) was obtained under the following SC-CO_2 extraction operating conditions (98.61 bar, 39.58 °C, 2.99 mL/min). Gas chromatography was performed on the *ilang-ilang* oil extracted by SC-CO_2 extraction, laboratory, and commercial scale hydrosteam distillation. Degradation products were observed in the hydrosteam distillated sample.

Keywords: *benzyl benzoate;* ilang-ilang *oil; linalool; response surface methodology; supercritical fluid extraction*

INTRODUCTION

The Department of Science and Technology (DOST) has identified *Cananga odorata* Hook Fil et. Thomson (*ilang-ilang*) as one of the five most promising oil-bearing plants that is indigenous to the Philippines [1]. The *ilang-ilang* essential oil is highly in demand as it is an important ingredient in perfume and cosmetics industries. The wide applicability of *ilang-ilang* oil depends on the quality as well as the composition of the extracted essential oil, which is related to the extraction technique employed. The steam or hydrosteam distillation of essential

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oil is performed at an elevated temperature (~100 °C) that may cause chemical interchanges such as hydrolysis, thermal degradation, radical reactions, oxidation, esterification, or simply by the loss of the lightest boiling components due to direct evaporation [2]. As a result, the composition of the extracts differs from the original composition of essential oil in fresh plant material. Thus, the interest on Supercritical Fluid Extraction (SFE) has been receiving great attention in the past several years. The SFE offers the potential advantages of higher yields and better quality products due to low operating temperatures and leaves no toxic residues in the final product. Extraction with supercritical carbon dioxide ($SC-CO_2$) fulfills those advantages since $SC-CO_2$ is non-toxic, non-explosive, it can be removed from the extracted products without any residue and it is easy to reach its critical point (about 31.3 °C and 72.9 atm). In this case, the $SC-CO_2$ extracts closely resemble the aroma of the fresh plant material than that of hydrodistillated essential oils because of the mild operating conditions.

The SC-CO2 extraction has been used to obtain essential oils from different raw materials. The essential oils of aromatic plants such as savory, peppermint and dragonhead had been analyzed [3]. Reverchon [4] has successfully proposed the SFE plus fractionation separation of the essential oils from jasmine, rose and tuberose concretes. The extraction of essential oil from Turkish lavender flowers by SFE has also been studied [5].

This study reports SC-CO2 extraction of essential oil from freeze-dried ilang-ilang flowers. As an alternative process with respect to traditional extraction, there are many parameters that must be considered in the supercritical CO2 extraction of essential oil. These include the type of solvents, raw material-solvent ratio, the method of feeding the solvent, conditions of extraction (pressure, temperature and extraction time), effects of entrainer, conditions of separation, conditions of preparation of raw material such as particle size and moisture content [6]. In addition, the nature of the sample as regards both the matrix and the analyte also affects the SFE of essential oil. The ilang-ilang oil is composed of complex chemical composition such as esters, alcohols, sesquiterpenes, terpenes, phenol and phenol ethers, aldehydes, ketones and basic substances [7]. These components are affected by environmental and extraction conditions.

In this work, an experimental design based on surface response methodology was done to optimize the SC-CO2 extraction of essential oil from ilang-ilang flowers.

METHODOLOGY

Plant material. Fully matured and yellow flowers were handpicked from its tree, *Cananga odorata* Hook Fil et. Thomson, grown in Pala-o, Iligan City. The sample was prepared as follows: the petals of the flowers were separated from the cluster, washed and cleaned thoroughly with water to remove dirt particles and air-dried for five minutes to remove the water adhering in the sample. Then, the samples were packed thoroughly using clinging plastic wrapper, placed in the plastic container and finally frozen in the freezer. For subsequent freeze-drying, the frozen sample was placed in the glass container and frozen again in the freezer after which it was placed in the freeze dryer instrument for three to four days.

Chemicals

Absolute ethanol, linalool (99% purity), benzyl benzoate (99% purity), 1st , 2nd and 3rd grade of *ilang-ilang* oil from Anao, Tarlac, and commercial grade pure carbon dioxide.

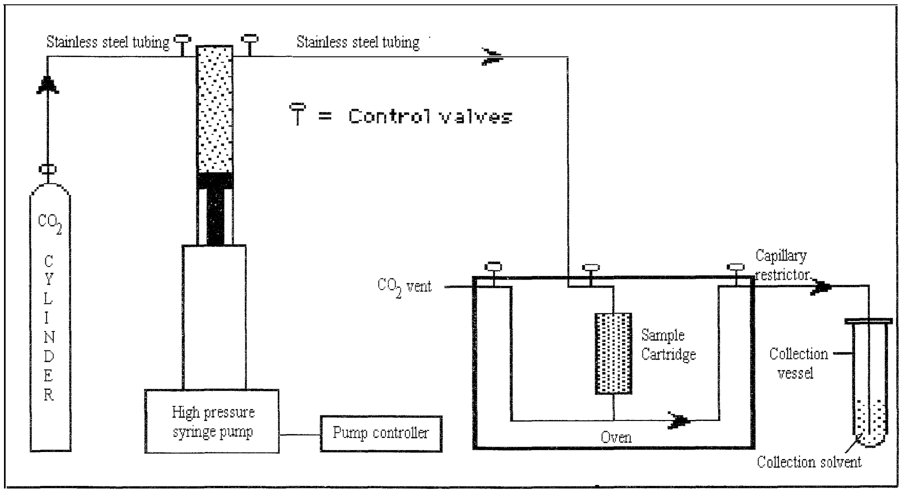

Fig. 1. *Schematic diagram of a supercritical fluid extraction system [8]*

Laboratory Scale Hydrosteam Distillation

Fifteen grams of freeze-dried sample were extracted by conventional hydrosteam distillation for three hours. This was done to evaluate the GC chromatogram of the hydrosteam distilled oil and compare with the SC-CO_2 extracted oil using the same nature of the starting material gathered in Pala-o, Iligan City.

Supercritical Fluid Extractor

All extraction was done using the supercritical fluid extractor model ISCO™ SFX 220. The schematic diagram of SFE system is shown in Figure 1 [8]. Gaseous commercial grade pure CO_2 was obtained from a steel cylinder equipped with dip tube via a control valve. The CO_2 was compressed by a high pressure syringe pump to a certain pressure (70-100 bar) which was regulated by the pump controller. The compressed gas passed through the stainless steel tubing and allowed to flow through the vertically mounted sample cartridge in the extractor body that was heated to a certain temperature (35-50 °C). The extract-laden gas from the sample cartridge passed through the control valve where the supercritical CO_2 is depressurized and circulated through the capillary restrictor where it was set to 60 °C to avoid plugging in the restrictor. The separated essential oil extract was collected in a collection vessel with 20 mL absolute ethanol as the collection solvent that was immersed in an ice-cold water bath. The CO_2 flow rate was regulated at 1 and 4 mL/min. The extraction was preceded with 30 minutes static before the dynamic extraction varying from 30-120 min. Another 30 min was allotted for flushing after every extraction. The extracted oil yield was calculated based on weight loss after extraction. The collected extracts trapped in 20 mL absolute ethanol were concentrated to 1 mL and were analyzed qualitatively by Shimadzu-14A gas chromatograph.

GC analysis

GC analysis was carried out using a Shimadzu-14A equipped with a flame ionization detector (FID) and a CBP10 column (25 m and ID: 0.25 μm). The injector port temperature and detector temperature were set at 200 °C and the GC oven was programmed at 180 °C. The volume of sample injected was 0.4 μL.

Experimental Design

A statistical experimental design, first-order 2^3 factorial, was used to investigate the effects of the three independent variables (pressure, temperature and flow rate of CO_2) on percent oil yield (w/w), percent of two major components in essential oil, linalool (v/v) and benzyl benzoate (v/v), respectively. Extraction time was fixed at 90 min as a result of the preliminary experiment done on the effect of extraction time on the oil yield. At constant CO_2 flow rate of 4 mL/min, a plateau level was reached at extraction time of 90 and 120 min. These experimental results implied that at 90 min and 4 mL/min almost all of the essential oil in five grams freeze-dried *ilang-ilang* flowers was extracted. The values of pressure (80-100 bar), temperature (35-50 °C), and flow rate of CO_2 (1-4 mL/min) were selected and coded as x_1, x_2, and x_3, respectively. These experimental parameters were investigated at three levels (-1, 0, and 1). The relation between the coded and the original scales is given as follows [9]:

$$x_i = \frac{\text{orig. var. - ave of highest and lowest value}}{(1/2)\text{(highest value - lowest value)}} \quad (1)$$

Table 1 shows the parameters and the corresponding levels for the first-order 2^3 factorial design. The design matrix with corresponding responses on the % oil yield (y_1), % linalool (y_2) and % benzyl benzoate (y_3) is given in Table 2. Each run was randomized and done in triplicate trials.

Table 1. The coded and measured levels for the independent variables

SFE Parameters	*Level of x_i*		
	-1	0	1
x_1 (bar)	80	90	100
x_2 (^{0}C)	35	42	50
x_3 (mL/min)	1	2.5	4

Statistical analysis

The model proposed for each response was:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon \quad (2)$$

where y is the observable response variable; β_0, β_1,…… β_k are the unknown parameters and ε is the random error term. The analysis was done using the Statistical Analysis System (SAS). The graphical presentation of the first-order model for each response was made using Statistical Graphics System, Version 5.

RESULTS AND DISCUSSION

Experimental Design

The generated first-order model for % oil yield (y_1), % linalool (y_2) and % benzyl benzoate (y_3) responses using the freeze-dried *ilang-ilang* flowers are given in Equation 4, 5, and 6, respectively. The lack of fit is not significant at α = 0.05 level and this indicates that the first-order models adequately fit the experimental data.

$$y_1 = 4.297 + 2.79x_1 - 1.129x_2 + 1.406x_3 - 1.04x_1x_2 \quad (4)$$

$$y_2 = 0.137 + 0.053x_1x_2 \quad (5)$$

$$y_3 = 2.805 + 1.504x_1 + 0.721x_3 \quad (6)$$

The model given for y_1 indicates that pressure (x_1), temperature (x_2) and flow rate of CO_2 (x_3) were the major variables affecting the SC-CO_2 extraction of *Cananga odorata* Hook Fil et. Thomson flowers. The yield of *ilang-ilang* oil decreases with increasing temperature and increasing interaction between temperature and pressure. It increases with increasing pressure and CO_2 flow rate.

The model given for y_2 indicates that interaction between pressure and temperature is the major variable affecting the composition of linalool in the *ilang-ilang* oil. It increases with increasing interaction between pressure and temperature.

The model given for y_3 indicates that pressure and flow rate of CO_2 was the major variables affecting the composition of benzyl benzoate in the *ilang-ilang* oil. It increases with increasing pressure and flow rate of CO_2.

The resulting optimum operating conditions for all the responses (y_1, y_2, and y_3) are given in Table 3. Due to sample requirement for the physico-chemical analyses of *ilang-ilang* oil

Table 2. The 2^3 factorial design plus two center points with corresponding responses

Run	Random Order	Coded Variables			Responses		
					y_1	y_2	y_3
		x_1	x_2	x_3	Oil Yield (% w/w)	Linalool (% v/v)	Benzyl Benzoate (%v/v)
1	IV	-1	-1	-1	1.152 ± 0.389	0.1829 ± 0.018	0.4093 ± 0.091
2	V	1	-1	-1	8.439 ± 0.046	0.1106 ± 0.046	2.9587 ± 1.555
3	VI	-1	1	-1	1.001 ± 0.203	0.0895 ± 0.012	0.1054 ± 0.012
4	II	1	1	-1	3.386 ± 0.276	0.2621 ± 0.021	2.0121 ± 0.286
5	VII	-1	-1	1	3.245 ± 0.255	0.2213 ± 0.012	1.4476 ± 0.376
6	IX	1	-1	1	11.285 ± 0.352	0.1095 ± 0.011	4.5782 ± 2.936
7	III	-1	1	1	3.043 ± 0.384	0.0800 ± 0.008	0.3929 ± 0.067
8	I	1	1	1	7.656 ± 0.297	0.1440 ± 0.086	4.8344 ± 4.077
9	VIII	0	0	0	4.379 ± 0.420	0.1162 ± 0.018	3.1236 ± 0.779
10	X	0	0	0	4.214 ± 0.127	0.1587 ± 0.025	2.4854 ± 0.633

(~ 15 mL), it was decided to select a response with its optimum working conditions among the three responses. The pressures for the three responses do not vary much from each other (i.e. 98.61, 97.38 and 96.55 bar). The temperature for response y_1 has the lowest value at 39.58 °C. A lower temperature is preferred to avoid possible thermal degradation of labile compounds in *ilang-ilang* oil. In this case, the optimum operating conditions for response y_1 was employed in the succeeding SC-CO_2 of the essential oil of freeze-dried *ilang-ilang* flowers. The SFE technique gave the highest oil yield of 9.21 % (w/w) ± 0.27 compared with the oil yield of hydrosteam distillation (2-2.25% v/w) [7].

Physico-Chemical Analysis of the SC-CO_2 Extracted oil

On the basis of the standard specification for 2nd grade *ilang-ilang* oil given in Table 4, the optimized SC-CO_2 extracted oil passed the 2nd grade quality with an ester value of 84.56. The high acid value (26.7294) of the optimized SC-CO_2 extracted oil can be ascribed to the co-extraction of some fatty acids and fatty acids methyl esters as reported by Kerrola and Kallio

[10] during the supercritical and liquid extraction of coriander fruit at 90 bar and 40°C. The co-extraction of cuticular waxes also contributes to the high acid value as the waxes are located on the leaf's surface while the essential oil compounds are preferably located inside the cellular structure [11].

Table 3. Optimum operating conditions for % oil yield (y_1), % linalool (y_2) and % benzyl benzoate (y_3) responses

Optimum Operating Conditions	***Responses***		
	y_1	y_2	y_3
Pressure (bar), x_1	98.61	97.38	96.55
Temperature (^{0}C), x_2	39.58	46.36	41.59
Flow Rate of CO_2 (mL/min), x_3	2.99	1.84	3.62
% Yield (y_1 by weight loss difference; y_2 and y_3 by volume)	8.479	0.181	4.231

Gas Chromatography of Ilang-ilang *Oil*

The GC peaks in the chromatogram data of SC-CO_2 extraction (A), laboratory (B), and commercial (C) scale hydrosteam distillation are presented in Table 5. The constituents are classified into high, medium, and low volatile compounds according to their retention times (identified with relative peak position or number). The highly volatile compounds are detected earlier that have the lowest retention time (low peak number). The low volatile compounds have the higher retention times (high peak number).

SFE and Lab Scale Hydrosteam Distillation of Freeze-Dried Ilang-ilang Flowers

Chromatograms A and B indicate there is a difference in the constituents of high, medium and low volatile compounds but these components have not been identified in this study. This difference is attributed to the extraction techniques employed.

Table 4. Physico-chemical analysis of optimized SC-CO_2 extracted oil

Test	*International Standard Specification (2nd Grade)**	*SC-CO_2 Extracted Oil*
Color	pale to dark yellow	brownish yellow
Specific Gravity @ 20°C	0.923-0.929	0.9318
Refractive Index @ 20°C	1.5060-1.5100	1.49834
Acid Value	under 3	26.7294
Ester Value	75-100	84.56

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Table 5. Classification of *ilang-ilang* oil constituents according to volatility

Chromatogram	*Retention Time (mins)/Peak Number*		
	High	Medium	Low
A	(2.1-3.1) / 2-11	(3.2-4.3) /12-19	(4.4-5.3)/20-24
B	(2.1-3.1) / 2-6	(3.2-4.3) / 7-15	(4.4-5.3)/16-20
C	(2.1-3.1) / 1-10	(3.2-4.3) /11-17	(4.4-5.3)/18-22

Evidence of possible degradation of thermosensitive compounds was depicted in chromatogram B. These peaks were not seen in the remaining chromatogram for *ilang-ilang* oil extracted by SC-CO_2. The occurrence of these peaks for the hydrosteam distilled oil in the laboratory scale is due to the distillation process which is operated at high temperature at approximately 100 °C compared with the optimum SC-CO_2 extraction operating temperature of 40 °C. The experimental evidence confirms that SC-CO_2 extraction avoids degradation of thermosensitive compounds as proven by other researchers [12,13] who studied the SC-CO_2 extraction of the fragrances compounds in other vegetable matter.

Laboratory and Commercial Scale Hydrosteam Distillation of Ilang-ilang Flowers

Chromatograms B and C showed the constituents of *ilang-ilang* oil using varying nature of starting material from different plant source. In chromatogram B, freeze-dried flowers obtained in Pala-o, Iligan City was extracted in a lab scale hydrosteam distillation. A commercial scale hydrosteam distillation (chromatogram C) was used in extracting the *ilang-ilang* oil using fresh flowers from the plantation in the municipality of Anao, Tarlac.

On the basis of the number of peaks and peak height intensity, more were observed in chromatogram C compared with that of chromatogram B. This difference is due to the nature of the starting material given the same extraction technique used.

SFE of Freeze-Dried and Fresh Ilang-ilang Flowers

The nature of starting material affects the GC chromatograms of the high, medium, and low volatile constituents of *ilang-ilang* oils extracted in lab (freeze-dried flowers) and commercial scale hydrosteam distillation (fresh flowers). SC-CO_2 extraction of fresh *ilang-ilang* flowers was done in order to compare it with the SC-CO_2 extraction of freeze-dried *ilang-ilang* flowers.

More peaks with high peak heights were observed in chromatogram A versus chromatogram B, which can be ascribed to the nature of the starting material of *ilang-ilang* flowers. The moisture contents of fresh and freeze-dried *ilang-ilang* flowers were 81.08% (± 0.331) and 8.48% (±0.030), respectively. Dunford and Temelli [14] stated that at high moisture content, the moisture is considered to act as barrier for permeation of CO_2 into a sample matrix, thus reducing the contact of SC-CO_2 phase with the sample solution. On the other hand, the drying technique can significantly affect the SC-CO_2 extracted oil because it can induce selective elimination and/or decomposition of some compounds [15].

CONCLUSION

Essential oil was extracted from the flowers of Philippine *Cananga odorata var. genuina* grown in Pala-o, Iligan City by supercritical carbon dioxide (SC-CO_2) using optimum parameters derived from surface response method. An optimum oil yield of 9.21 % w/w was obtained under the following SC-CO_2 extraction operating conditions: 98.61 bar, 39.58 °C, 2.99 mL/min. GC analysis of the extracts indicates degradation products were minimized in SC-CO_2 extraction compared with hydrosteam distillation.

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