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Original Article

SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF CITRONELLA OIL FROM *CYMBOPOGON WINTERIANUS* USING TAGUCHI ORTHOGONAL ARRAY DESIGN

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ABSTRACT

Objective: Optimum condition for the extraction of citronella oil from citronella (*Cymbopogon winterianus*) using supercritical carbon dioxide (SC-CO_2) was investigated.

Methods: In order to determine the optimum extraction condition, a Taguchi experiment with L_9 orthogonal array design was used. Effects of pressure, temperature and dynamic extraction time on citronella oil yield were investigated at levels ranging between 10-15 MPa, 35-45 °C and 60-180 min, respectively.

Results: The highest citronella oil yield (3.206%) was achieved at a factor combination of 15 MPa, 50 °C and 180 min. The obtained citronella oil yield from SC-CO_2 extraction was higher than that of percolation as the solvent extraction method using ethanol, which gave a citronella oil yield of 1.4%. The experimental oil yield at optimum condition was in accordance to the values predicted by a computational process using Taguchi method. Analysis of variance (ANOVA) with 95% confidence interval indicates that extraction temperature is the most significant factor in maximizing citronella oil yield, followed by dynamic extraction time and pressure.

Conclusion: Optimization process for oil yield from SC-CO_2 extraction of citronella (*Cymbopogon winterianus*) was successfully performed using Taguchi L_9 orthogonal array design. This study demonstrates that Taguchi method was able to simplify the experimental procedure of SC-CO_2 process.

Keywords: Supercritical carbon dioxide extraction, Citronella, *Cymbopogon winterianus,* Optimization, Taguchi

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INTRODUCTION

Citronella is a plant originated from India and Sri Lanka which belongs to the genus Cymbopogon. This plant is commonly found growing wild in most tropical Asian, American and African countries [1]. Oil of citronella is one of the most important essential oils obtained from different species of aromatic grasses. This oil is mainly used as a source of important perfumery chemicals due to its citronellal and geraniol contents, which are extensively used in soap, perfumery, cosmetic and flavouring industries throughout the world. Citronella oil can be used effectively as a plant-based insect repellent. The oil has also been known to possess antimicrobial, anthelmintic, antiparasitic, anti-inflammatory, anticonvulsant, antispasmodic and antioxidant properties [2].

Pharmacologically-active compounds in plants are usually present in low concentrations. Previously, various different extraction methods have been developed for the effective and selective extractions of these compounds from raw materials. Our laboratories have developed and investigated various extraction techniques and biological assays on natural products [3-7]. Current methods that are commonly used for the extraction of citronella are hydrodistillation, steam distillation and solvent extraction [8-9]. However, hydro- and steam distillation have several drawbacks, such as high-temperature operation which leads to the breakdown of thermolabile components and the hydration of chemical constituents, while the major disadvantage of solvent extraction is the presence of solvent residue in the product.

Extraction of essential oil-bearing plants with supercritical carbon dioxide (SC-CO_2) extraction is still considered as a new process on an industrial scale. Several studies have been done in recent years on the applications of SC-CO_2 on various essential oil-bearing plants such as black cumin, clove, cinnamon and ginger [10-13]. Carbon dioxide (CO_2) behaves as a lipophilic solvent. Thus, it is able to extract most non-polar solutes in plant and animal materials, including antioxidants, pigments, flavors, fragrances, fatty acids, and essential oils. Separation of CO_2 from the extract is simple and nearly instantaneous, leaving no solvent residue in the extract, as would be typical with organic solvent extraction. Unlike liquid solvents, the solving power of SC-CO_2 can be easily adjusted by slight changes in the temperature and pressure, making it possible to extract particular compounds of interest. In addition, CO_2 is inexpensive, available in high purity, FDA-approved and classified as a generally recognized as safe (GRAS) compound. CO_2 is also a good solvent for thermolabile compounds that are sensitive to high temperature because CO_2 has a relatively low critical point, with a critical temperature (Tc) and critical pressure (Pc) of 31 °C and 7.38 MPa, respectively [14].

Taguchi method has widely been used in many engineering applications since it is a powerful tool to design and investigate the parameters [15-16]. It is a technique among statistical experimental design methods that comprises a special design of an orthogonal array (OA) to study the entire parameter with a small number of experiments. Thus, by this method, it is possible to reduce both of the time and cost needed for experimental investigations and improve its performance characteristics [17]. The objective of this present study was to investigate the optimum condition for citronella oil extraction through SC-CO_2 extraction and to investigate the effect of extraction pressure and temperature and dynamic extraction time on citronella oil yield using Taguchi method. In addition, the yield and chemical profile of citronella oil extracts obtained by SC-CO_2 extraction were also evaluated and compared to those obtained by the solvent extraction method using ethanol.

MATERIALS AND METHODS

Materials

The fresh stalks and leaves of citronella (*Cymbopogon winterianus*) were obtained from CV Pavettia Kurnia Atsiri (Subang, Indonesia).

Food-grade liquid CO_2 (purity of 99.99%) was supplied in cylinder tube by PT Inter Gas Mandiri (Cikarang, Indonesia). Ethanol 96% (technical grade) was purchased from PT Brataco Chemika (Cikarang, Indonesia). Analytical standard of citral and geraniol were purchased from Sigma-Aldrich,Co.(MO, USA).

Material preparation

Citronella was air-dried at room temperature for 1 w. Prior to the extraction process, dried citronella was manually chopped (±1 cm) and milled with a conical mill (Quadro Comil, Canada) with 040G screen and square impeller at 5000 rpm. These ground samples were packaged in a polyethylene bag and stored in a refrigerator. Moisture content of the sample before extraction was 14% wet basis (w. b).

Supercritical carbon dioxide (SC-CO_2) extraction

SC-CO_2 extraction was performed using a customized supercritical fluid apparatus described in the previous study [10]. SC-CO_2 extraction was carried out using a supercritical fluid extractor with CO_2 cycle system (KIST-Korea). A 1000 ml stainless steel extraction column loaded with approximately 50 g of dried and milled citronella was connected to the system. CO_2 pump (HKS-12000, South Korea) was cooled using circulating chiller (Lab. Companion, USA) and the pressurized CO_2 was delivered to the extraction vessel through a preheater. Temperatures of extraction column and preheater were controlled by a circulating heater (Lab. Companion, USA). The outlet of extraction column was connected to a back pressure regulator (Tescom, USA) to control the pressure in the extraction vessel. Extraction experiments were commenced when the system has reached the predetermined pressure and temperature. There were two stages of extraction which consisted of static and dynamic stages. The static stage was 60 min for all experiments, and the dynamic stage was varied. CO_2 flow rate was kept at 20 ml/min, measured at operating pressure and temperature for all experiments. SC-CO_2 was expanded across the separator vessel, and the extracts were collected in a glass vial after the experiment has been finished.

Solvent extraction (percolation)

Approximately 100 g of dried and grounded citronella were loaded into a 2500 ml custom-built percolator. Ethanol (96% v/v) with a total volume of 2600 ml was used as the solvent. A HKS-12000 Pump (Korea) was used to continuously deliver solvent through the system at a rate of 180 ml/min. After 6 h of extraction time, the solvent was then removed by evaporation process at a reduced pressure using Rotavapor R-215 (Buchi, Switzerland). The amount of remaining extract was determined as the yield of the experiment.

Gas chromatography (GC) analysis

Gas chromatography (GC) analysis was performed with a PerkinElmer GC Clarus 680 equipped with a flame ionization detector (FID) and an Elite-5 (5% phenyl, 95% dimethylsiloxane) capillary column (30 m×0.25 mm i.d×0.25 µm film thickness). Nitrogen was used as the carrier gas at a flow rate of 0.7 ml/min. 1 µl of sample was injected using the split mode (split ratio of 1:10). Injector and detector temperatures were 150°C and 320°C, respectively. Oven temperature was set at 80 °C, programmed to 120 °C at a rate of 3°C/min and further programmed at a rate of 20 °C/min to 310°C.

Taguchi orthogonal array

Taguchi method has widely been used in many engineering applications since it is a powerful tool to design and investigate the parameters. It is a technique among statistical experimental design methods that comprises a special design of an orthogonal array (OA) to study the entire parameters with a small number of experiments. Thus, by this method, it is possible to reduce both of the time and cost needed for experimental investigations and to improve the performance characteristics [18]. A Taguchi L_9 orthogonal array design was used to investigate the optimum condition in SC-CO_2 extraction process since it is the most suitable method for the investigated condition, i.e., three factors with three levels (values). The factors and levels are listed in table 1, whereas the structure of Taguchi L_9 orthogonal array design is shown in table 2.

The data obtained from SC-CO_2 extraction process was subjected to signal-to-noise (S/N) ratio calculation. S/N ratio calculation is an evaluation of output performance stability which measures the level of performance and effect of noise parameters on performance. The experimental results should be transformed into S/N ratios, which mainly consist of three types, including smaller-the-better, nominal-the-best and larger-the-better. In this study, target values of 'larger is better' was used since the purpose of this study was to obtain the highest yield of citronella oil. The larger difference value (Δ) in the parameters indicates that the parameters will affect the process since any change in signal will cause a larger effect on the output factors being measured. S/N ratio is calculated using the following equation:

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad \text{……. (1)}$$

where y_i represents the experimentally observed value of the i^{th} experiment, and n is the number of the trial at the same level. Statistical analysis was performed using MINITAB v.15 (Minitab Inc., USA) statistical software package.

Table 1: Factors and levels used in experimental design

Level	Factors		
	Pressure (MPa)	Temperature (°C)	Dynamic time (min)
	A	B	C
1	10	40	60
2	12.5	45	120
3	15	50	180

Table 2: Standard for L_9 orthogonal arrays

Run	Independent variables		
	A	B	C
1	1	1	1
2	1	2	2
3	1	3	3
4	2	1	2
5	2	2	3
6	2	3	1
7	3	1	3
8	3	2	1
9	3	3	2

RESULTS AND DISCUSSION

Effect of SC-CO_2 extraction conditions on citronella oil yield

Based on experimental designs in the framework of Taguchi method and according to the array and orthogonal L_9, and considering the change of condition and different parameters which consisted as pressure (P), temperature (T) and dynamic extraction time (t), 9 experiments were performed in the present study. Summary of SC-CO_2 experiments using Taguchi method is shown in table 3. Experiment results showed that the obtained citronella oil yield was varied from 0.854-2.802%.

Table 3: Results of citronella oil using SC-CO_2 extraction

Run	Pressure (MPa)	Temperature (°C)	Dynamic time (min)	Yield (%)
1	10	40	60	0.854
2	10	45	120	1.972
3	10	50	180	2.132
4	12.5	40	120	1.324
5	12.5	45	180	2.174
6	12.5	50	60	2.802
7	15	40	180	2.454
8	15	45	60	1.528
9	15	50	120	2.448

Fig. 1 shows the main effect plot of citronella oil yield to different extraction factors and levels. Main effect plot was performed based on the average response for each combination of control factor levels. It can be seen in fig. 1 that an increased pressure from 10 to 12.5 MPa resulted in a significant increment of citronella oil yield, while further increasing pressure from 12.5 to 15 MPa resulted in a smaller citronella oil yield increment. Generally, using a higher pressure at isothermal conditions resulted in an increased solvent density and subsequently solvent power and solubility of the compounds. As the density increased, the distance between molecules was decreased, and interactions between compounds and CO_2 were increased, which led to greater solubility of compounds in CO_2 [19].

The effect of temperature on the yield of citronella oil obtained in this study presents analogies to the influence of temperature on the solubility of solid materials in supercritical fluids. Solubility of solids in supercritical fluids is the combination of two competing effects, which consists of the increased solid volatility and the decreased solvent density along with temperature rise [19-20].

Extraction time exhibited a positive impact on citronella oil yield as shown in fig. 1. The citronella oil yield was increased along with increased dynamic extraction time. The results showed that increased dynamic extraction time to 180 min was able to enhance the yield of citronella oil.

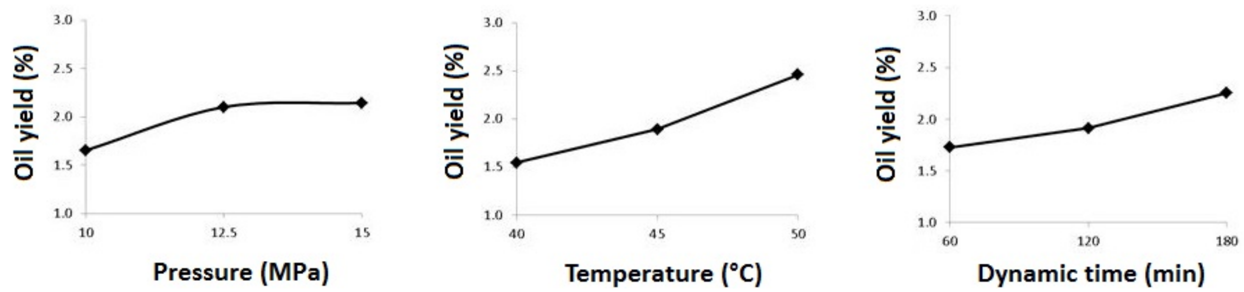

Fig. 1: Main effects plot of taguchi method for citronella oil yield

Optimum condition

The main effect plots of S/N ratios clearly depicted the optimum level of each significant factor on extraction yield as shown in fig. 1. In this study, since the target value was 'larger is better', the optimum condition for each factor was defined as the level that gives the highest point of means of each plot for citronella oil yield. Based on the results of Taguchi method, the highest citronella oil yield from SC-CO_2 extraction was obtained at a pressure of 15 MPa, the temperature of 50 °C and dynamic extraction time of 180 min. S/N ratio calculation results are shown in table 4. Greater difference value (Δ) of the average S/N ratio indicated that the control factors have a greater substantial effect on S/N ratio. Based on the S/N ratio calculation, the most influencing parameters in this process is extraction temperature, followed by dynamic extraction time and extraction pressure. Thereby, the influencing parameters in the extraction process follow the order: extraction temperature> dynamic extraction time>extraction pressure.

Analysis of variance (ANOVA) was carried out to evaluate the significance of extraction pressure, extraction temperature and dynamic extraction time on the yield of citronella oil using SC-CO_2 extraction, as shown in table 5. Results showed that different level of parameters exhibited no significant effect on citronella oil yield ($p>0.05$). However, it was found that temperature has a greater effect than dynamic extraction time and pressure on citronella oil yield. This result was in a good agreement to the S/N ratio calculation results.

Table 4: S/N value calculated for each parameter and level

Level	Pressure (MPa)	Temperature (°C)	Dynamic time (min)
1	2.103	1.533	1.923
2	5.184	5.243	4.795
3	5.956	7.660	7.007
Δ (max–min)	3.853	6.126	5.084
Rank	3	1	2

Table 5: Analysis of variance (ANOVA) of citronella oil yield

Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Pressure	2	13.02	13.02	6.508	0.62	0.618
Temperature	2	34.75	34.75	17.375	1.65	0.377
Dynamic time	2	16.20	16.20	8.098	0.77	0.565
Residual Error	2	21.04	21.04	10.519		
Total	8	85				

DF: Degree of freedom; Seq SS: Sequentials sums of squares; Adj SS: Adjusted sums of squares; Adj MS: Adjusted means of squares

Experimental validation

Experimental validation is the final step in the modeling process to investigate the accuracy and robustness of the established model. The final analysis involved a comparison between the predicted values of the established model and experimentally validated values. The predicted value at optimized condition is 2.93%, while the actual value at this condition is 3.206%. Percentage error found in this study was 9.1%. It was found that the average of percentage error was less than 15%, confirming and concluding that the methodology used in establishing the model in this scientific research was systematic. Citronella oil yield obtained in this study was higher than previous studies using SC-CO_2. In previous study, Silva *et al.* used SC-CO_2 to extract citronella essential oil. They varied the extraction temperature from 40 to 80 °C, as well as the pressure from 6.2 to 18 MPa. The results showed that the best citronella extraction yield was 2.18% at 60 °C and 15 MPa for 60 min [21].

Comparison with ethanol extraction (percolation)

In comparison to the solvent extraction method, the optimum yield from SC-CO_2 extraction was much higher than percolation (table 6). Moreover, in terms of dynamic extraction time, SC-CO_2 extraction was less time-consuming than conventional methods, and this extraction method does not need a further process to separate the remaining organic solvent in the obtained extract. Therefore, SC-CO_2 extraction exhibited a potential to be a better alternative for citronella oil extraction, since it offers the use of non-toxic, non-explosive, environmentally-friendly, cost-effective, time-saving and selectively-adjustable solvent in the extraction process.

Table 6: Citronella oil yields obtained by ethanol extraction and SC-CO_2 extraction at optimum condition

Methods	Yield (%)	Citral (%)	Geraniol (%)
SC-CO_2 extraction	3.206	0.508	3.620
Ethanol extraction	1.400	0.069	0.473

CONCLUSION

Process optimization of SC-CO_2 extraction on the yield of citronella (*Cymbopogon winterianus*) oil was successfully performed using Taguchi L_9 orthogonal array design. Optimum condition to obtain the highest oil yield (3.206%) was achieved at extraction pressure of 15 MPa, extraction temperature of 50 °C and dynamic extraction time of 180 min. The experimental oil yield at optimum condition was in a good agreement with the predicted computational results. S/N ratio calculation and ANOVA identified extraction temperature as the main factor that exhibited the most influential effect on SC-CO_2 extraction of citronella. Compared to the conventional method in this study (percolation using ethanol extraction), SC-CO_2 extraction process resulted in a higher yield of citronella oil with a shorter period of extraction time.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declared no conflicts of interest with respect to the authorship and/or publication.

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