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# A Kinetic Study on Supercritical Carbon-dioxide Extraction of Indonesian *Trigona sp.* Propolis

# A Kinetic Study on Supercritical Carbon-dioxide Extraction of Indonesian *Trigona sp.* Propolis

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**Abstract**. This work is to investigate the supercritical $CO_2$ extraction of Indonesian *Trigona sp.* raw propolis. The kinetic study was conducted at $CO_2$ mass flowrate of 15 g/min, the temperature of 50 °C and pressure (250-350 bar). The process was run for 3 h and the extract was accumulated every 30 min. The proposed mathematical extraction models were Sovova, Logistic and Reverchon. The result shows that Sovova model gives a good fit between calculated and data value. The Sovova model also has lower SSE than Logistic and Reverchon model. The highest extract of 46 wt% was achieved at extraction time of 60 min, the temperature of 50 °C, the mass $CO_2$ flowrate 20 g/min and the pressure 300 bar. The extract constituent consisting of galangin, p-coumaric acid, ferulic acid, caffeic acid, cinnamic acid, kaempferol, and chrysin was detected by HPLC analysis. The antioxidant activity analyzed by DPPH method was expressed as $IC_{50}$ and the value is comparable to ascorbic acid.

## 1. Introduction

In recent decades, propolis has attracted increasing attention and use in foods and beverages, cosmetics, pesticides, and medicine (Oldoni, 2015). Propolis contains bioactive flavonoid and phenolic acid compounds providing antioxidants (Aga et al., 1994; Bankova et al., 2000; Sforcin and Bankova, 2011). Supercritical fluid extraction with $CO_2$ is well-known as an advanced alternative method in the extraction process (Biscaia and Ferreira, 2009; del Valle, 2015). A $CO_2$ supercritical fluid is mostly occupied as a supercritical solvent to extract natural bioactive compounds for bio-nutrition and pharmaceutical foods (de Melo et al., 2014; Garcia-Mendoza et al., 2015). In supercritical conditions, the fluid accommodates properties of gas and liquid. The supercritical fluid is dense like a liquid and viscous like a gas. Thus, supercritical fluid can diffuse rapidly into solid matrices and dissolve the bioactive compounds. These properties lead to the advantages of supercritical extraction such as regulating the solvent power, giving high selectivity and yielding high free-solvent extract (del Valle, 2015). Some studies on the extraction of propolis with supercritical $CO_2$ fluids have been published (De Zordi et al., 2014; Machado et al., 2016) and informs promising.

To perform a kinetic study on supercritical $CO_2$ extraction, some models are mathematically proposed. The model mostly was formulated based on the mass transfer model. In this work, the most common models proposed by Sovová (1994), Reverchon (1997), and Martinez et al. (2003) were applied. These models involve the mass balance partial differential equations. Further, this initial and boundary conditions were needed to solve the equation (Priyanka and Khanam, 2018).

Based on the literature review, the kinetic study on supercritical CO2 extraction of Indonesian Trigona Sp has never been performed. Thus, this work is to investigate the supercritical $CO_2$ extraction

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of Indonesian *Trigona sp.* raw propolis as well as to study the effect of process variables such as pressure, mass flow rate and temperature on extraction mass.

## 2. Methods

### *2.1. Chemicals*
The *Trigona sp.* raw propolis was obtained from the Institute for Non-Timber Forest Product Technology, Mataram, West Nusa Tenggara. It was grounded and sifted into 140 mesh. Ultra-high purity (UHP) Carbon dioxide ($CO_2$) was employed as an extraction solvent. Ethanol, gallic acid, ferulic acid, caffeic acid, cinnamic acid, CAPE, galangin, quercetin, and p-coumaric acid was also utilized for the HPLC standard (Sigma-Aldrich, Singapore).

### *2.2. Supercritical $CO_2$ extraction*
The supercritical extraction apparatus system (KIST-Korea) was prepared. Raw propolis of 50 g was milled and poured into the extraction chamber. Consecutively, the $CO_2$ fluid was fed into the extraction chamber with flowrate 10-20 g/min. The extractor was regulated until it reached a supercritical condition (P = 250 bar, T= 250 $^{o}$C and $CO_2$ flow rate = 20 g/min), then the process was run. The kinetic study was conducted for 3 h and the extract was accumulated every 30 min. The process variables were $CO_2$ pressure (250-350 bar).

### *2.3. Analysis*
The liquid product was separated from residue by centrifuging, filtered and prepared to analysis. The HPLC analysis was employed to identify the extract composition. Qualitative analysis of propolis extract was carried out using the HPLC Water Alliance, column C18, UV detector (280 nm), phase gradient of methanol and 1% acetic acid.
The antioxidant activity was measured using the DPPH method.

### *2.4. Kinetic model*
Three models were applied to investigate the extraction kinetic. These models are developed based on mass transfer phenomenon. In this section, the models are presented in the final equation terms, as seen in equation 1 for the Sovova model, equation 2 for the Logistic model, and equation 3 for the Reverchon model (Martínez et al., 2003).
Sovova Model:

$$m_{ext} = Y^*\left(1 - \exp(-Z)Q_{CO_2}t_{ext}\right) \quad (1)$$

Logistic Model:

$$m_{ext}(h = H, t) = \frac{m_t}{exp(bt_m)}\left\{\frac{1+exp(bt_m)}{1+exp[b(t_m-1)]} - 1\right\} \quad (2)$$

Reverchon Model:

$$m_{ext} = m_0\left[1 - \frac{6}{\pi^2}\sum_{n-1}^{\infty}\frac{1}{n^2}exp\left(\frac{-n^2\pi^2D_t}{r^2}\right)\right] \quad (3)$$

with $m_{ext}$ is the extracted mass (g), Y* is the solubility of the extract in the fluid phase (M/M), Z is CER time parameter, $t_{ext}$ is extraction time (min), h is adjustable parameter, $m_t$ is the initial solid mass (g), b is adjustable parameter (1/s), $t_m$ = adjustable parameter (s), $m_o$ is the initial component mass in solid (g), $D_t$ is the diffusion coefficient and r is the particle diameter.
The suitability of the model was determined based on the sum squared error (SSE). The model chosen was the model that had the smallest SSE value.

$$SSE = \sum\{[m_{ext}]_{calculated} - [m_{ext}]_{data}\}^2 \quad (4)$$

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## 3. Result and discussion

In the earlier study, the highest yield of propolis extract was achieved at a pressure of 250 bar and a temperature of 50 °C. Therefore, in this study, extraction was observed and studied at the optimum operating conditions. The profile of the supercritical $CO_2$ extraction of propolis was depicted in Figure 1. As shown in Figure 1, the extraction rate increases with the extraction time increasing during 60 min extraction time. Afterward, the extraction rate approaches constantly. It can be explained that at the beginning of extraction, the solubility of the extract in supercritical CO2 increases, but after 60 min, equilibrium begins to form and consequently the extraction rate decreases.

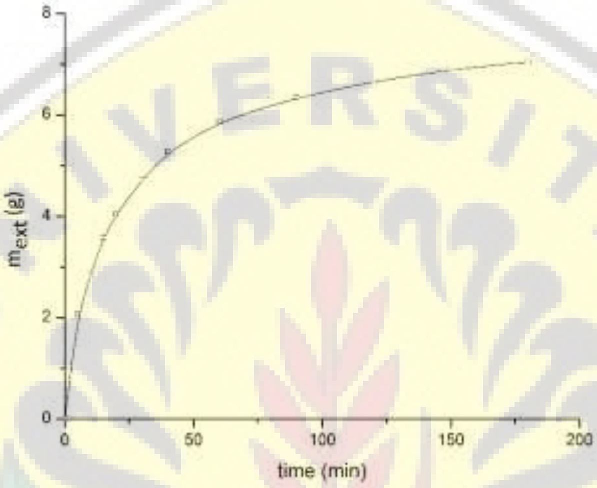


**Figure 1** Profile of propolis extraction by using SFE $CO_2$ method at 250 bar and 50ºC

The models proposed by Sovová (1994), Reverchon (1997) and Martinez et al. (2003) were fitted with the experimental data of *Trigona sp.* Propolis and shown in Figure 2. As shown in Figure 4, a model proposed by Sovová (1994) provided a better approximation with the experimental data than the Reverchon and the Logistic model. The SSE of Sovova model is 0.13 % which is less than Reverchon model of 0.21 % and Logistic model of 0,35 %.

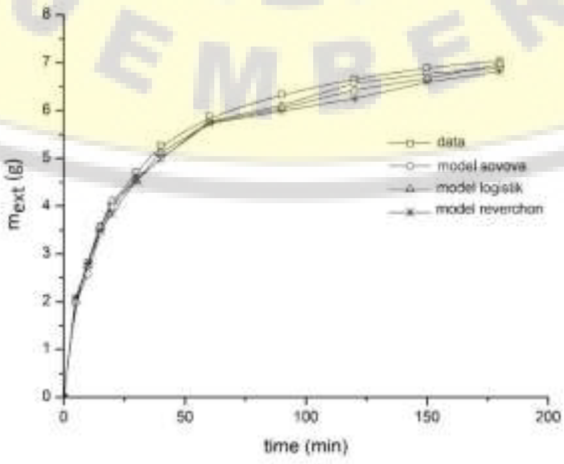


**Figure 2.** Relationship between extraction mass and extraction time on a different model (P=250 bar, T=250 °C)

Both Sovova and Reverchon models accommodated the mass transfer process in the model which considered the internal mass transfer. Consequently, the models fitted with the experimental data. Meanwhile, the Logistic model neglected the mass transfer resistance in supercritical extraction model. It caused the model slightly fitted with the experimental data.

It is well-known that in supercritical extraction, the pressure is mostly the significant variable that plays an important role. It is in line with our previous study. Thus, this work is also to check the effect of pressure on extraction mass. It was shown in Figure 3.

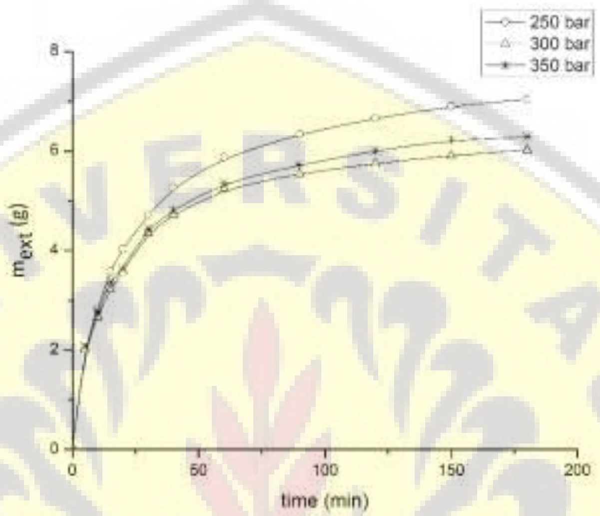


**Figure 3.** Relationship between extraction mass and extraction time on different pressure

In figure 3, it appears that the extraction carried out at a pressure of 250 bar gives a higher yield than at pressures of 300 and 350 bar. This can be caused because at higher pressures, the system is saturated with supercritical CO2 fluid, so it is unable to dissolve more extracts. Meanwhile, at the same pressure, increasing the extraction time will increase the extract yield.

The result of the qualitative analysis from HPLC analysis was presented in Table 1. Propolis produced by the supercritical method contains chemical compounds that function as antioxidants such as galangin, ferulic acid, caffeic acid phenyl ester (CAPE), p-coumaric acid, chrysin, caffeic acid, and cinnamic acid. Antioxidant test (DPPH test) also strongly indicates that the extract of propolis produced has very strong antioxidant properties, which is indicated by a very small $IC_{50}$ value (0.983 mg/g).

**Table 1**. Chemical composition of propolis extract

| Retention Time (min) | Compounds | Retention Time (min) | Compounds |
|---|---|---|---|
| 11.75 | Caffeic acid | 65.32 | Chrysin |
| 17.01 | p-coumaric acid | 66.46 | CAPE |
| 19.22 | Ferulic acid | 67.88 | Galangin |
| 31.80 | Cinnamic acid | | |
| 56.45 | Kaempferol | | |

## 4. Conclusion

This work summarizes that supercritical $CO_2$ extraction can be applied to extract the antioxidant compounds from Indonesian Trigona Sp raw propolis which provides the $IC_{50}$ value of 0.983 g/g. This value indicates that the extract shows promise as a super antioxidant.

This work also indicates that the Sovova model fits with the supercritical CO2 extraction of Indonesian Trigona Sp raw propolis. This model gives the SSE of 0.13 %.

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