

## Kinetic Study and Optimization of the Most Influential Factor on Batch-Extraction of Gingerol from Fresh Ginger (*Zingiber officinale*) Rhizomes by Using n-Hexane as a Solvent

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### Abstract

A solvent extraction of 6- gingerol from fresh ginger rhizome chips using n-hexane has been successfully carried out. This study aimed to investigate the effects of temperature, feed size, and feed mass on the yield of gingerol, to observe the kinetics of gingerol extraction process using n-hexane as a solvent, to find the most influential parameters in the gingerol extraction process and to determine the optimum conditions of the gingerol extraction process. The experiment was carried out for 60 minutes using two feed mass ( 50 and 75 g), two temperature (60 and 70°C) and two chips sizes (100 and 25 mm<sup>3</sup>) using 350 mL n-hexane. The second-order kinetics model was used to study the extraction kinetic parameters. The quick method was used to evaluate the most influential extraction parameters with respect to the yield of gingerol. Feed mass was found to be the most influential parameter in the gingerol extraction process. The optimum extraction conditions were found to be at 60°C, ginger rhizome chips with 25 mm size<sup>3</sup>, feed mass was 109.7g and 350 mL n-hexane, which resulted in crude extract with gingerol content of 517.11 ppm.

**Keywords:** extraction; ginger; gingerol; n-hexane; optimization

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### INTRODUCTION

Ginger (*Zingiber officinale*) is an Asia native plant which has been cultivated in numerous parts of the world including Jamaica, West Indies, and Africa (Alfaro *et al.*, 2013). Gingerol is one of the valuable

active chemicals in the ginger root in addition to zingiberene, shogaol,  $\beta$ -bisabolene,  $\alpha$ -farnesene,  $\beta$ -sesquiphellandrene,  $\alpha$ -curcumene, ginger oil, and resin. (Ravindran and Babu, 2005; Lu *et al.*, 2014; Gümüşay *et al.*, 2015; Stoilova *et al.*, 2007). Gingerol

is usually found in fresh ginger than in dried ginger (Ali *et al.*, 2008), due to its sensitivity to heat, both during storage and at processing (Chrubasik *et al.*, 2005). Gingerol is usually used as a cooking spice and also as a cross-linking agent for starch modification (Yavuz and Babaç, 2003). In addition, gingerol is also used as a mixture of medicines for cancer, migraine, nausea, and vitiligo (Shukla and Singh, 2007; Prasad and Tyagi, 2015; Liu *et al.*, 2013). Gingerol can be obtained through a liquid-solid extraction process by using a specific solvent and temperature.

Solid-liquid extraction or leaching is the process of removing specific components from a solid material through dissolution using a suitable solvent (Treybal, 1980). In this process, the targeted components move through the solid pores, diffuse out of the solid surface, pass the solid-liquid film and enter the bulk of solvent. Commonly, the conventional solvent extraction process requires several organic solvents, such as ethanol (Kaur and Kapoor, 2002) or methanol (Huda-Faujan *et al.*, 2009) to avoid oleoresin extraction. Unfortunately, conventional extraction faces some crucial limitations, such as lengthy extraction time, the requirement of costly and high purity solvents, evaporation of a large amount of solvent, poor extraction selectivity and thermal decomposition of thermos labile compounds (de Castro and Garcia-Ayuso, 1998). Some alternative extraction methods, such as supercritical extraction (Chen *et al.*, 1986), ultrasound (Balachandran *et al.*, 2006), enzyme (Chari *et al.*, 2013), or microwave (Liu *et al.*, 2014) have been successfully developed to enhance the gingerol extraction process. Although the yield of gingerol obtained from the latter extraction processes is higher than that of conventional extraction, the processes are not economical. To solve this problem, the optimization of operating conditions of conventional extraction is usually preferred.

Because gingerol is a non-polar compound and is vulnerable to thermal decomposition, gingerol extraction should be carried out using non-polar solvents at low temperature. In this regard, hexane is one of the suitable solvents for gingerol extraction because it is non-polar and has a low boiling point (68°C). Wang *et al.*, (2009) used various solvent including hexane to extract gingerol in rat plasma. Ito *et al.* (2016) have extracted gingerol using hexane as a solvent for anti-osteoporosis remedy. Later, Kanadea and Bhatkhandeb (2016) also studied gingerol extraction using conventional soxhlet extraction at 80°C for 60 minutes.

This study aimed to investigate the effects of temperature, feed size, and feed mass on the yield of gingerol, to observe the kinetics of gingerol extraction process using n-hexane as a solvent, to find the most influential parameters in the gingerol extraction process and to determine the optimum conditions of the gingerol extraction process. Statistical approaches such as the Quick method (Sachs, 2012) and the

factorial experimental design were used to improve the yield of gingerol.

## MATERIALS AND METHOD

### Fresh Sample Preparation and Conventional Soxhlet Extraction Process

In this study, ginger rhizomes were purchased from a local market in Semarang - Indonesia. Then, the outer of ginger was peeled, the flesh of ginger was washed with de-ionized (DI) water and cut into thin slices (100 and 25 mm<sup>3</sup>). 6-Gingerol which the purity was 98%, was purchased from Chengdu Biopurify Phytochemicals Ltd. used as a standard compound. Technical grade n-hexane was purchased from the local chemical distributor in Semarang.

Two feed mass of ginger rhizome chips (50 g and 75 g) were extracted using 300 mL of technical grade n-hexane in an extraction flask equipped with mechanical stirrer, Liebig condenser, and thermometer. A waterbath heater with automatic temperature controller was used to maintain the extraction temperature. The experimental set-up can be shown in Figure 1. The extraction was conducted for 1 hour two different temperatures (50 and 60°C). Extract samples were withdrawn from the extraction flask every 10 minutes for gingerol content determination. The quick method was applied to determine the most influential parameters (temperature, feed mass, or feed size), which affects the extraction process. With the level was 2 and factor were 3, the total of the run was 8 (2<sup>3</sup>).

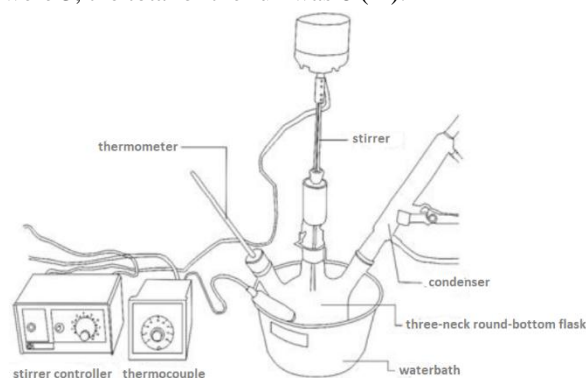


Figure 1. Experimental set-up of gingerol extraction process

### Gingerol Content Analysis

The crude extract sample was filtered, and the filtrate was collected for gingerol content determination using a UV-Vis Spectrophotometer (Optima SP-3000, Japan) at 200 nm (Huang *et al.*, 1997). The 6-gingerol standard solution of 5000, 1000, 200, 40, and 8 ppm concentrations was used to generate a standard calibration curve. Approximately 5 mL of filtrate sample was introduced into a cuvette and allowed to stand for 3 min at room temperature before reading its absorbance using UV-Vis Spectrophotometer.

### Modeling of Gingerol Extraction Kinetics

The kinetics of 6-gingerol extraction was assumed to follow the second-order kinetic model, which usually used in solid-liquid extraction (Ho *et al.*, 2005; Rakotondramasy-Rabesiaka *et al.*, 2009). The mathematical expression of the model is shown in Eqs. (1):

$$\frac{dC_t}{dt} = k(C_s - C_t)^2 \quad (1)$$

where  $k$  is the second-order kinetic extraction rate constant,  $C_s$  is the extraction capacity or concentration of extract at saturation,  $C_t$  is a concentration of extract at any time. When  $t = 0$ , the value of  $C_t$  is 0, a new equation, Eqs. (2), can be obtained from the integration of Eqs. (1) following:

$$C_t = \frac{C_s^2 kt}{1 + C_s kt} \quad (2)$$

Linearization of Eqs. (2) can be shown in Eqs. (3) as the following:

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} \quad (3)$$

Since the initial extraction rate ( $h$ ) as  $C_t/t$  when  $t = 0$ , so the  $h$  can be determined by Eqs. (4) following:

$$h = kC_s^2 \quad (4)$$

All the experimental data were fitted to models, and the coefficient of determination ( $R^2$ ) was used as a criterion for adequacy of fit.

It is important to estimate the activation energy ( $E_a$ ) in the extraction process. The activation energy of gingerol extraction was calculated by relating the kinetic model shown in Eqs. (3) with the Arrhenius equation as shown in Eqs. (5) and (6)

$$k = C^{-E_a/(RT)} \quad (5)$$

$$\ln k = -\frac{E_a}{R} \left( \frac{1}{T} \right) - \ln C \quad (6)$$

where  $C$  is the gingerol concentration,  $k$  is the kinetic rate constant,  $E_a$  is the activation energy,  $R$  is the ideal gas constant, and  $T$  is the extraction temperature. The slope obtained from data fitting was  $E_a/R$ , and  $E_a$  was determined by multiplying the slope with  $R$  (8.314 J/mol.K).

## RESULTS AND DISCUSSION

### Gingerol Content in Fresh Peeled Ginger Calibration curve measurement

A calibration curve is essential for the determination of the gingerol concentration in the extract samples. The calibration curve was generated as a correlation between  $\ln$  concentration of gingerol vs absorbance as shown in Figure 2a. Figure 2 (a) reveals that the absorbance of the solution decreased linearly when  $\ln$  gingerol concentration increased. The correlation obtained was  $y = -0.0546x + 0.649$  and  $R^2$  was 0.956. The high value of  $R^2$  means that the standard solution data are robust enough to be used for determination of gingerol content. The gingerol

content from filtrate of extraction samples was read its absorbance in a UV-Vis Spectrophotometer. The true 6-gingerol content in the crude extract was calculated using the linear correlation obtained from the calibration curve.

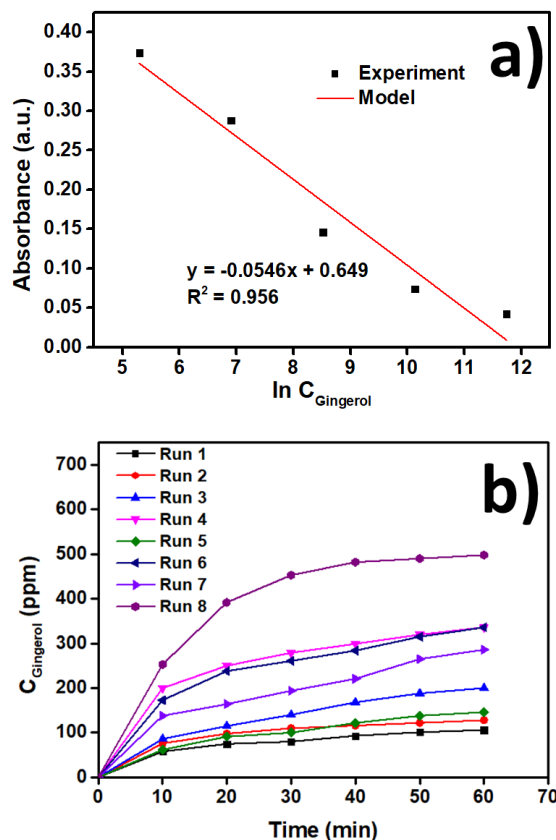


Figure 2. a) Calibration curve of gingerol concentration and b) concentration of extracted gingerol over the time

### The 6- gingerol extraction profile

The gingerol concentrations from every experiment are shown in Figure 2b. It can be observed that the highest concentration of gingerol (498 ppm) was obtained when 6-gingerol was extracted at 60°C with a feed size of 25 mm<sup>3</sup>, and feed mass was 75 g. In contrast, the lowest concentration of gingerol was extracted from ginger rhizome chips at 50°C, with a particle size of 100 mm<sup>3</sup> and feed mass was 50 g. The particle size of ginger is a very important parameter in gingerol extraction. A larger particle size means the specific surface area of ginger rhizome chips is small. Under this condition, the physical contact between the solvent and 6-gingerol in the ginger rhizome chips was less intense, which led to lower the extraction yield and vice versa. Extraction temperature is one of important parameter in gingerol extraction. An increase in temperature means a higher supply of energy to extract the gingerol. In addition, a higher temperature also increases the solubility and diffusivity of gingerol in the solvent. As the physical and chemical bondings between the solvent molecules become weaker, the solvent viscosity decreases, and

make it is easy to diffuse into the ginger mass according to the Van't Hoff equation (Jiang *et al.*, 2015). Mass of feed in the extraction process is an important factor to be studied due to its availability with the solvent volume to effectively extract gingerol.

#### Determination of Gingerol Extraction Kinetics

In this study, the gingerol extraction kinetics was assumed to follow the second-order kinetic as shown in Eqs. (3), and the coefficient of determination ( $R^2$ ) of all sample was calculated. Figure 3a shows the gingerol extraction resulted in a linear line versus  $t/C_t$ , with the range of  $R^2$  for second-order kinetics was 0.902-0.984 as shown in Table 1. There are two stages during the extraction process of gingerol by using n-hexane: first, the driving force of n-hexane as solvent induces an important dissolution and scrubbing, and second, external diffusion of gingerol into the extract in a slower stage. The high value of determination coefficient confirms that the extraction process of gingerol by n-hexane works according to the model. Beside the value of  $R^2$ , the value of saturated extraction capacity ( $C_s$ ), the extraction rate constant ( $k$ ), and the initial extraction rate ( $h$ ) can be calculated by using equation of linear regression given in Figure 3a, where  $y = ax + b$ ,  $y = t/C_t$ ,  $x = t$ ,  $a = 1/C_s$ ,  $b = 1/kC_s^2$ , and complete results are given also in Table 1.

From Table 1, the value of  $k$  increased with increasing of temperature means temperature had the effect in extraction process as explained in the previous section. Surprisingly the  $k$  value of extraction process with ginger size was  $100 \text{ mm}^3$  higher than extraction process with ginger size was  $25 \text{ mm}^3$  (Run 1 and 2 compared to Run 5 and 6, Run 3 and 4 compared to Run 7 and 8). Generally, high  $k$ -value means the time required to reach solvent in a saturated condition is faster. But in this case, saturation may not mean n- contact surface area, so the concentration change over time is not significant. Therefore, the condition is considered to be saturated despite the capacity of n-hexane to extract gingerol is still available and there is a lot of gingerol in the deeper part of ginger. This condition also causes the high value of  $k$  in the extraction process on the low feed mass compared to the high feed mass (Run 1 and 2 compared to Run 3 and 4, Run 5 and 6 compared to Run 7 and 8).

In addition to the  $k$  value, the value of extraction capacity ( $C_s$ ) and the initial rate of extraction ( $h$ ) also increase with the increase in

temperature. The highest initial extraction rate is the extraction process of ginger with size  $25 \text{ mm}^3$ , feed mass 75 g, and temperature  $60^\circ\text{C}$ , while the lowest is the extraction process of ginger with  $100 \text{ mm}^3$ , feed mass 50 g, and temperature  $50^\circ\text{C}$ .

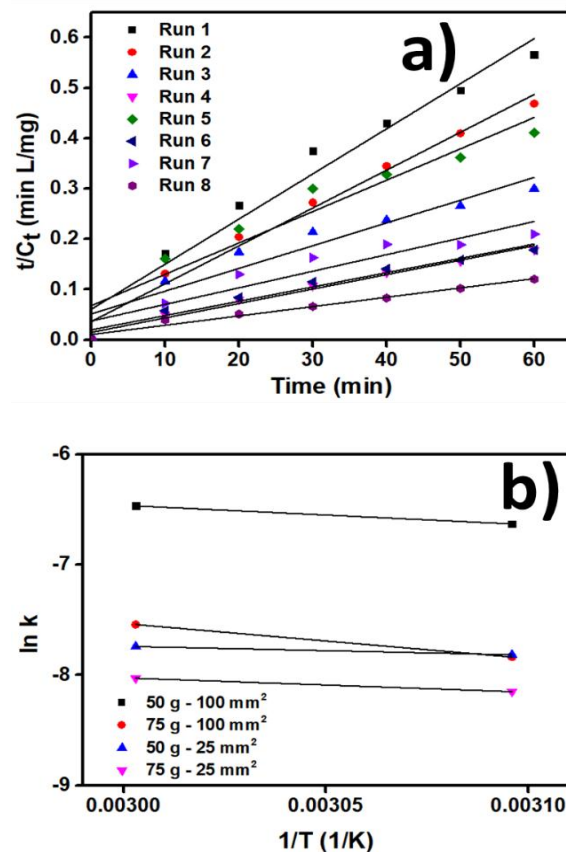


Figure 3. a) Kinetic model of the second-order extraction of gingerol from fresh peeled ginger in n-hexane and b) its Arrhenius plot for activation energy determination

#### Determination of Gingerol Extraction Energy Activation

Gingerol extraction activation energy should be investigated to determine the minimum energy required to do the extraction process. The values of  $\ln k$  were used to obtain the  $E_a$  and the value of  $\ln k$  vs time was plotted in a graph as expressed in Figure 3b. According to Eqs. (6), the slope of this linear regression is  $E_a/R$

Table 1. The kinetic reaction rate of gingerol extraction from fresh peeled ginger and its determination coefficient

Run	Feed Size (mm <sup>3</sup> )	Feed Mass (g)	Temperature (°C)	a (L.mg <sup>-1</sup> )	C <sub>s</sub> (mg.L <sup>-1</sup> )	b (min.L.mg <sup>-1</sup> )	k (L.mg <sup>-1</sup> .min <sup>-1</sup> )	h (mg.L <sup>-1</sup> .min <sup>-1</sup> )	R <sup>2</sup>
1	100	50	50	0.00895	111.73	0.061	0.00132	16.47	0.964
2	100	50	60	0.00751	133.16	0.036	0.001554	27.55	0.984
3	100	75	50	0.00451	221.73	0.052	0.000395	19.42	0.912
4	100	75	60	0.00282	354.61	0.015	0.00053	66.67	0.98
5	25	50	50	0.00523	191.20	0.068	0.000403	14.75	0.918
6	25	50	60	0.00294	340.14	0.020	0.000434	50.25	0.964
7	25	75	50	0.00329	303.95	0.038	0.000289	26.67	0.902
8	25	75	60	0.00185	540.54	0.011	0.000326	95.24	0.976

and the value of E<sub>a</sub> can be determined by multiplying slope with R (ideal gas constant). From calculations, the highest E<sub>a</sub> was extraction process with feed size of 100 mm<sup>3</sup> and feed mass 75 g, while the lowest was extraction process with feed size of 25 mm<sup>3</sup> and feed mass 50 g, with the values were 26327.53 and 6603.06 J/mol. Other configurations (feed size of 100 mm<sup>3</sup> – feed mass 50 g and feed size of 25 mm<sup>3</sup> – feed mass 75 g) have the E<sub>a</sub> value 14602.04 and 10870.56 J/mol, respectively. From that result, the increasing mass feed will also increase the activation energy. The larger size of feed needs higher energy activation compared to the smaller one because with a smaller surface area, n-hexane needs more energy to extract the 6-gingerol.

### The Influential Parameter in Gingerol Extraction Process

#### Determination of the most influential parameter in gingerol extraction process

To find the most influential parameters (either feed size, feed mass, or temperature) in the 6-gingerol extraction process, the quick method was employed. Based on the experimental data, the highest effect value was feed mass effect with the value of 75.5, higher than the effect value of temperature and feed size with the values were 70 and 62, respectively. The value of interaction between temperature and feed mass was 17, while the value of interaction between temperature and feed size was 30.5. Accordingly, the value of interaction between feed size, feed mass, and the temperature was -11.5. Surprisingly, the effect value of interaction between feed mass and feed size was 0, means there is no interaction between the feed size and feed mass in the extraction process. From the abovement data, feed mass becomes the most influential parameter in gingerol extraction process.

#### Optimization of the most influential parameter in gingerol extraction process

Since the most influential factor on gingerol extraction had been found, the optimization of the most influential was conducted to obtain the highest gingerol content during the experiment. To test this hypothesis, fresh peeled ginger with size 100 mm<sup>3</sup> with various mass (25, 50, 75, 100, 125 g), was extracted with 300 mL of n-hexane with a temperature of 60°C for 1 hour. From the extraction using 25, 50, 75, 100, and 125 g ginger rhizome chips, gingerol

content of 208, 336, 498, 502, 506 ppm, respectively, were obtained. A simple model was used to find the highest gingerol content, and the results are shown in Figure 4. From that figure, a highly fitted quadratic equation can be obtained, which is  $y = -0.433x^2 + 9.499x - 3.857$  with the value of R<sup>2</sup> was 0.991. The highest gingerol can be achieved from this study is 517.11 ppm using the feed mass is 109.7 g. A higher ginger rhizome chips mass as a feed for gingerol extraction process may lead to the insufficient amount of solvent for extraction and reduce the gingerol concentration. A true extraction of gingerol has been carried out under the optimum condition of feed mass (more less 110 g) and the resulted gingerol concentration was 523 ppm, only higher 1.14% than the proposed model.

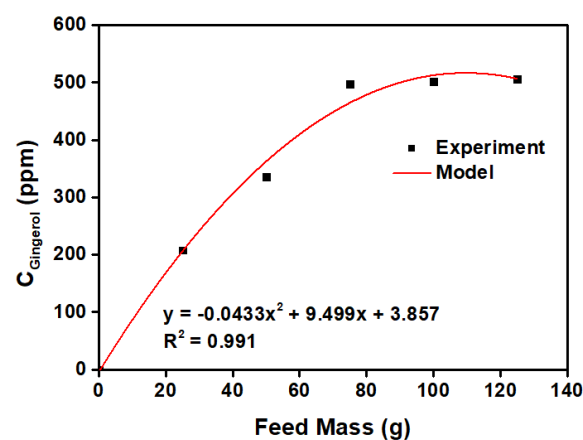


Figure 4. Actual and model of resulted gingerol from various feed mass of ginger

### CONCLUSION

The batch conventional extraction of 6-gingerol from ginger rhizome chips using n-hexane has been conducted. The highest gingerol concentration in the crude extract was 498 ppm when extraction was carried out at 60°C, feed mass was 75 g, and feed size was 25 mm<sup>3</sup>. Feed mass was found to be the most influential parameter in the gingerol extraction process. The optimum extraction conditions were found to be at 60°C, ginger rhizome chips with 25 mm size<sup>3</sup>, feed mass was 109.7 g and 350 mL n-hexane, which resulted in crude extract with gingerol content of 517.11 ppm. In addition, the second-order

kinetic model describes the kinetics gingerol extraction very well.

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