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KINETICS OF ULTRASOUND-ASSISTED EXTRACTION OF ANTHOCYANIN FROM PURPLE ROSELLE CALYCES UNDER DIFFERENT PH CONDITIONS

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Abstract. This research presents that the higher temperature results on higher extracted anthocyanin. In addition, it was found that pH 2 was preferable for obtaining greater anthocyanin content. Employing the second order kinetics model in this research confirmed the good fitting of the model and experimental data.

Keywords: roselle, anthocyanin, extraction, ultrasound, kinetics.

1. Introduction

Bioactive compounds exhibit valuable properties for being an excellent natural source of bio-based product for additives in pharmaceutics (supplements and drugs), cosmetics and food. For this reason, the extraction of bioactive compounds from natural sources, such as a natural pigment, has recently increased. The previous studies have been conducted to extract various bioactive compounds from the natural source such as chlorophyll [1], betalains [2, 3], phenolic [4-6] and anthocyanin [7, 8]. Anthocyanin is a water-soluble natural pigment providing the color characteristic of red, purple and blue in most fruits and vegetables [6, 9]. The application of anthocyanin extracts from a natural source such as flowers, leaves, or fruits is of great interest for food and pharmaceutical applications. Anthocyanin has an attractive features acting as the natural source anti-inflammatories [10], colorants [11, 12], antioxidants and antimicrobials. In addition, the anthocyanin has the ability to stabilize food and potential to increase food shelf life [9].

Conventional extraction (CE) of anthocyanins, usually requires more extended time and has a low-efficiency characteristic [13, 14]. The CE is mostly based on the bath stirring extraction method or maceration extraction. Compared to the CE, ultrasound-assisted extraction (UAE) has been used to extract anthocyanin [7, 14, 15] and other phenolics [6]. This method has several advantages including reduced processing time and solvent volume. The UAE is preferable for its efficiency to extract bioactive compound with effective mass transfer and better solvent penetration through the plant cell walls *via* acoustical cavitation [16, 17]. In the UAE, ultrasonic waves develop cavity around the solute material wall, which releases the amount of energy at massive rate, assisting solvent in penetrating the cellular material [18].

The UAE process has been studied and widely performed to obtain extract from the natural source. However, optimization of anthocyanin extraction relies on adequate mathematical models to obtain better extraction results. Kinetics study is significant for implementation of anthocyanin UAE in industrial scale. Kinetics parameter obtained from the studies can minimize processing errors, improve the process efficiency and maintain the final product quality [19]. The kinetic studies of the natural source bioactive compound by UAE have been conducted such as anthocyanins extraction from *Aronia melanocarpa* [15], mix extraction of phenolic from berries [20], flavonoid from agro-food solid wastes [21], and phenolic from grape marc [22]. Kinetic studies by mathematical modelling are required to facilitate design and provide information on equipment scaling up for the industrial application [22].

Since anthocyanin is an easily degraded natural pigment, the extraction process should consider some factors promoted the anthocyanin degradation including light, oxygen, thermal, structure, anthocyanin concentration, and pH [23, 24]. This concern is principal to determine the actual condition of the extraction process. This work is focused on UAE of anthocyanin from roselle by using non-alcoholic solvent. Specifically, the examination of different pH and temperature on total anthocyanin extract and its effect on the kinetic parameter is studied. Total anthocyanin content in the extract for

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specific time can be predicted based on the kinetic model. Most of the studies on UAE kinetics showed that the second-order kinetic model gave suitable mechanism explanation [22, 25, 26].

2. Experimental

Kinetic models of anthocyanin extracted from purple roselle calyces were developed. Dry purple roselle calyces were obtained from Blitar, East Java, Indonesia. The study consisted of several steps including roselle calyces pretreatment, anthocyanin extraction, and kinetic models determination.

2.1. Extraction of Anthocyanin from Purple Roselle Calyces

Dry roselle calyces were ground using a blender and sieved to obtain roselle with 100 mesh in size. The ground roselle was stored at ambient temperature in a dark, dry container equipped by silica gel, to prevent roselle defection. Ratio of solvent (distilled water) and solute (roselle) was 1:5 w/w. UAE of the anthocyanins were carried out in an ultrasound cleaner (Krisbow, Indonesia) equipped with temperature regulator, temperature control and ultrasonic wave generator. The extraction condition was maintained constant at ambient pressure of 101 kPa. The temperatures of the extraction process were 313, 323 and 333 K. To study the effect of pH, a citric acid was added the solute-solvent solution to obtain pH of 1, 2, and 3. During the experiments, 1 ml of the sample was collected every 10 min (10, 20, 30, 40, 50, and 60 min) for further analysis.

2.2. Total Anthocyanin Analysis

Analysis of total anthocyanin was carried out according to the pH-differential method. This method was based on the color difference of anthocyanin pigments solution at pH 1 and pH 4.5. Absorbances of anthocyanin solutions were measured at specific pH using spectrophotometer UV Visible (Shimadzu UV Mini 1240) at 520 and 700 nm. To set the acidity, pH 1.0 buffer (potassium chloride, 0.025M), and pH 4.5 buffer (sodium acetate, 0.4M) were used. The absorbance of each sample was determined using Eq. (1) [27]:

$$A = (A_{515} - A_{700})\text{pH1.0} - (A_{515} - A_{700})\text{pH4.5} \quad (1)$$

The anthocyanin concentration was expressed through cyanidin-3-glucoside, and was calculated by Eq. (2) [27]:

$$Total\ anthocyanin = \frac{A \cdot M_w \cdot DF \cdot 1000}{e} \quad (2)$$

where A is the absorbance; M_w is a molecular weight of cyanidin-3-glucoside (M_w = 449.2 g/mol); DF is a dilution factor; ε is a molar extinction coefficient for cyaniding-3-glucoside (26900 l/mol·cm), and 1000 is a conversion factor from g to mg. Units of anthocyanin extracted were expressed as mg/l of cyd-3-glu equivalent [10].

2.3. Kinetic Studies of Anthocyanin Extraction

Kinetic model of anthocyanin extraction from purple rosella calyces was predicted according to a second-order reaction rate. This model has been used previously applied to investigate the kinetics model of oleanolic and ursolic acid [25], polyphenols [26] and phenolic [22]. The kinetics model was illustrated by Eq. (3):

$$\frac{dC_t}{dt} = k\left(C_s - C_t\right)^2 \quad (3)$$

where C_t is the concentration of extracted anthocyanin, mg/l, at a specific time t, min; C_s is the concentration of anthocyanin in the liquid extract at saturation, mg/l; k is the coefficient of second-order extraction rate, l/g·min.

The concentration at specific time extractions was determined by integrating Eq. (3) in the boundary conditions at C_t = 0 to C_t; t = 0 to t, and Eq. (4) was obtained.

$$C_t = \frac{C_s^2 \cdot k \cdot t}{1 + C_s \cdot k \cdot t} \quad (4)$$

Linearization of Eq. (4) was used to calculate kinetic parameters, as determined by Eq. (5):

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} = \frac{1}{h} + \frac{t}{C_s} \quad (5)$$

The h value calculated from this equation was determined by initial extraction rate (g/l·min) at time t (min), and C_t is 0. Other kinetic parameters were obtained from the intercept and slope value of the plotting of t/C_t *versus* time.

3. Results and Discussion

3.1. Effect of Temperature and pH on the Extraction Characteristics

Extraction by the UAE is widely used to extract various substances from natural sources. Some experiments confirmed that by using the ultrasonic wave for extracting a natural substance from plants provided more benefits than other methods, such as anthocyanin from blackberry and sweet cherry cultivar [14], extract of Boldo leaves [28],

antioxidant from *Jatropha integerrima* [29] and anthocyanin from purple sweet potatoes [16]. In addition, previous data comparison of ultrasonic-assisted extraction and conventional maceration extraction (ME) for the extraction of anthocyanin from red and purple roselle calyces confirmed that extractions by the UAE methods provided anthocyanin concentration about 16 times higher than the extraction using ME. The result of total anthocyanin extracted at various temperatures and pH is presented in Fig. 1.

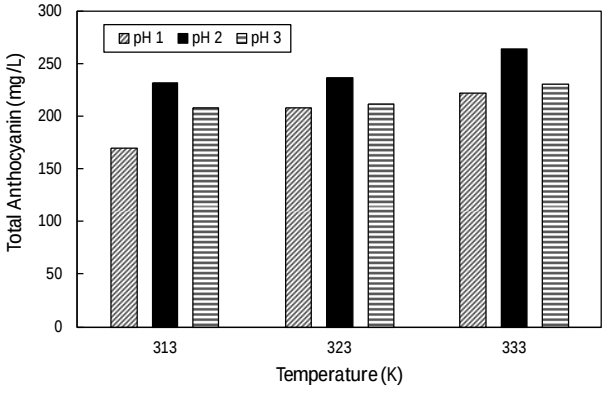


Fig. 1. Total anthocyanin extracted in various pH condition

Fig. 1 shows that the total anthocyanin at pH 3.0 is almost similar to those obtained at pH 1.0, indicating that anthocyanin from *H. sabdariffa* is preferably stable in an acidic pH range. The similar result reported that there was no significant difference of monomeric anthocyanin extracted at pH 2.0 and pH 4.5 [30]. Previous study showed that the extraction of anthocyanin from blackberry also gave the similar trend, where the addition of hydrochloric acid in ethanol showed a better result than 100% ethanol solvent [14]. In addition, another study presented that there was an apparent difference in the color of films containing anthocyanins and the film at pH 1–3 had a better reddish color [31]. Furthermore, acids were required in stabilizing anthocyanin in the form of flavylium cation. However, the acid level should not exceed the excess since the glycosidic bond hydrolysis or breaking linkages with metals or co-pigments is possible.

In addition, the figure shows a positive dependence of anthocyanin concentration extracted with the operation temperature. The anthocyanin concentrations increase as the temperature rises. The similar results are found for the extraction with different pH (1, 2, and 3). In general, there is a correlation between the temperature rise and the physical properties of the solute in the extraction process, such as acceleration of material softening and swelling, the decrease of the solvent viscosity, as well as the increase of the material solubility [22]. The viscosity of a substance tends to descend as the temperature rise, causing the increase in a diffusion coefficient [32]. With the increase of the diffusion coefficient, there will be a driving force of the solid mass to the solvent. As a result, the gradient coefficient rises and hence the concentration is more significant. The ultrasonic wave and heating introduce kinetic energy from outside into the liquid phase. This energy can modify the extraction process rate without affecting the thermodynamic equilibrium [33].

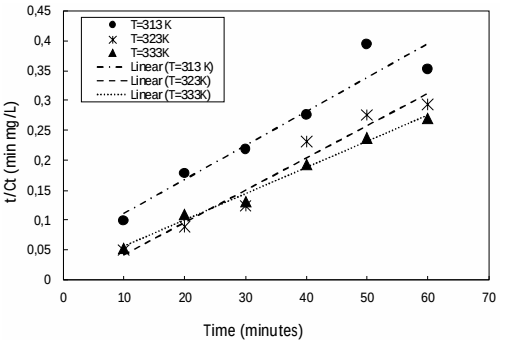
On the other hand, it is important to note that overheating can affect the anthocyanin molecules structures. The previous study indicated that the temperature above 333 K exhibited significant decline on the anthocyanin extracted. It was revealed that anthocyanin extracted from blueberry could be stable only at temperature below 333 K [34, 35].

3.2. Kinetic Study of Anthocyanin Extraction from Purple Roselle Calyces

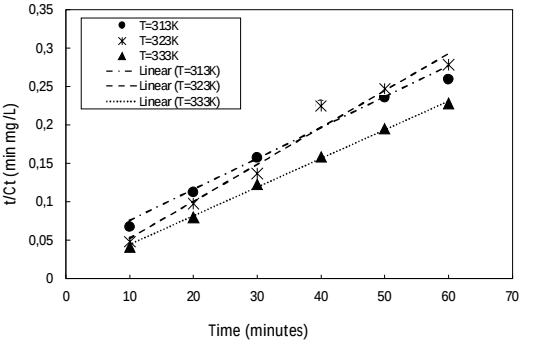
The study of anthocyanin extraction kinetic modelling was performed at various pH (1, 2, and 3) and various temperatures (303, 313 and 333 K). Kinetic model of anthocyanin from purple roselle extraction followed the second-order rate law. Fig. 2 illustrates the analysis of experimental data using the second-order model by plotting the value of t/C_t *versus* time.

As presented in the figure, linear regression fits the second-order kinetics of the experimental data, as it is indicated by the high correlation coefficients (R^2 = 0.91–0.99). The second-order kinetic model was also used to describe the UAE of polyphenol from *Picea abies* [26]. Qu *et al.* [36] also applied the second-order model to determine the solid-liquid extraction kinetics parameters of antioxidants from a pomegranate marc. Anna *et al.* [10] employed pseudo first-order model for describing the extraction of monomeric anthocyanin from the grape marc. The linearization of the second-order kinetic model fits all three variations of pH conditions (pH 1, 2 and 3). The kinetic parameters for each extraction condition are presented in Table 1.

The value of extraction rate constant, k, is observed to be increased as the temperature rises. The change of k values and another kinetic parameter as the temperature change verifies that the temperature has a strong influence on the constants. By using this model, it is reported that the temperature has the most predominant effect on the C_s, h and k. In addition, this second-order kinetic model may be used to describe the extraction processes under different operating conditions and parameters of ultrasound-assisted extraction such as biomass particle size, extraction temperature, solvent/solid ratio, ultrasound amplitude level, and pulse duration/pulse interval ratio [36]. Further, plotting of experimental results against model prediction gives a good comparison with the average error in the range of 2–15 % as shown in Fig. 3.



a)



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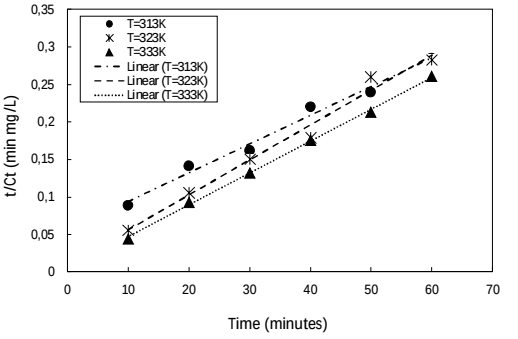
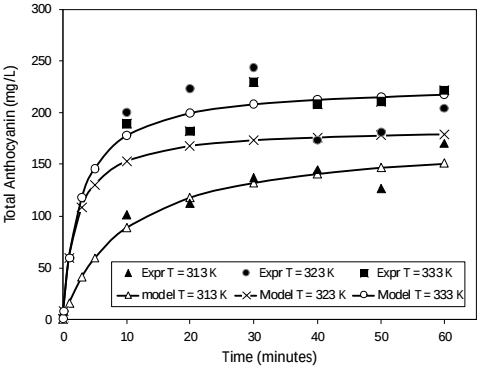
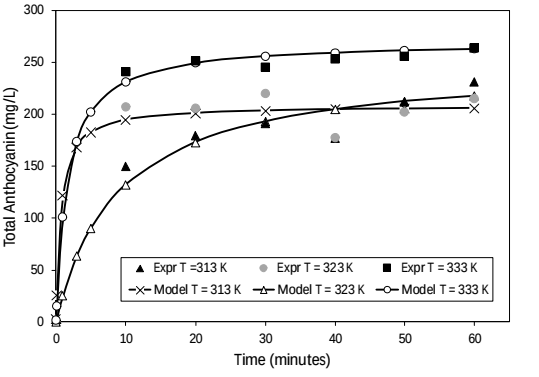


c)

Fig. 2. Validation of the second-order anthocyanin extraction kinetics of UAE from purple roselle calyces under various pH: 1 (a); 2 (b) and 3 (c)



a)



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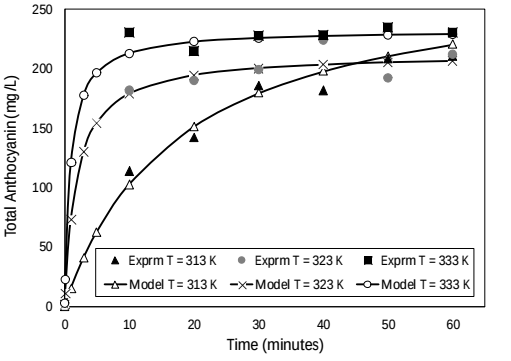

c)

Fig. 3. The comparison of experimental data with the practical calculation based on the second-order kinetic model at various pH: 1 (a); 2 (b) and 3 (c)

Table 1

Kinetic parameter of the second-order kinetic model of UAE of anthocyanin

pH values	T, K	$B = 1/C_s$	$A = 1/h$	$C_s = 1/b$, mg/l	$h = 1/A$	$k = h/C_s^2$
1	313	0.0057	0.0555	175.4386	18.018	$5.85 \cdot 10^{-4}$
	323	0.0054	0.0114	185.1852	87.719	$2.56 \cdot 10^{-3}$
	333	0.0044	0.0123	227.2727	81.301	$1.57 \cdot 10^{-3}$
2	313	0.0048	0.0034	208.3333	294.1176	$6.78 \cdot 10^{-3}$
	323	0.0040	0.0355	250.0000	28.1690	$4.51 \cdot 10^{-4}$
	333	0.0037	0.0062	270.2703	161.2903	$2.21 \cdot 10^{-3}$
3	313	0.0035	0.0626	285.7143	15.9744	$1.96. 10^{-4}$
	323	0.0047	0.0090	212.7660	111.1111	$2.45 \cdot 10^{-3}$
	333	0.0043	0.0040	232.5581	250.0000	$4.62 \cdot 10^{-3}$

Activation energy is determined based on Arrhenius equations. The parameter is obtained by linearizing Arrhenius equation according to Eq. (5). Based on this equation, ln$k$ is plotted as the y-axis, and $1/T$ is plotted as the x-axis. Then, the value of E_a/R is determined from the intercept of the linearization equation:

$$\ln k = \ln k_0 + \left(-\frac{E_a}{R}\right) \cdot \frac{1}{T} \tag{5}$$

At pH 3 Arrhenius plot of the extraction is presented in Fig. 4. Based on the figure, the relation of ln$k$, activation energy, and the temperature is written as Eq. (6) with $R^2 = 0.9038$.

$$\ln k = 28.145 - \frac{93.333}{831.4}\left(\frac{1}{T}\right) \tag{6}$$

According to the equation, the value of the activation energy is 93.333 kJ/mol. A positive value of activation energy indicates that the reaction is denoted as endothermic nature of the process. Since the activation energy is higher than 40 kJ/mol, the process is controlled by the solubilization reaction. It should be noted that the activation energy depends on several factors, including the process parameters of extraction, plant characteristics, bioactive compounds properties and structure, as well as selected model for modelling [37].

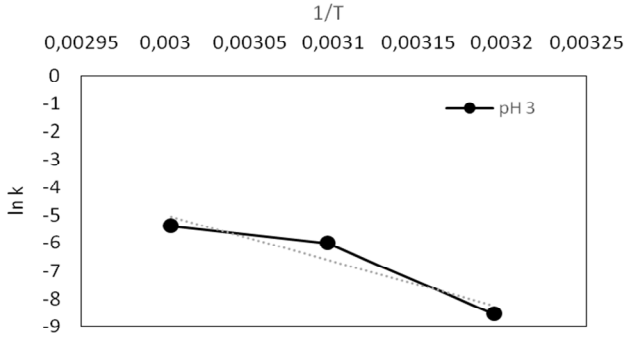

Fig. 4. Arrhenius plot
of anthocyanin extraction at pH 3

3.3. Comparison of Kinetic Parameters at Different pH

The activation energy calculated from the kinetic model at different pH is presented in Table 2.

Table 2

Activation energy of extraction on different pH condition

pH	$-E_a/R$	E_a, kJ/mol
1	-5151.9	42.833
2	-7184.1	59.728
3	-11226	93.333

Based on data presented in Table 2, it is obvious that the highest value of energy activation (93.333 kJ/mol) is achieved at pH 3. In contrast, the energy activation at pH 1 is the lowest (E_a = 42.833 kJ/mol). Activation energy is typically used to describe the energy required to reach the transition state of a reaction [13, 34]. As a consequence, the lower activation energy, the faster the reaction. In addition, the lower E_a in anthocyanin extraction indicates that extraction process is less susceptible to degradation.

4. Conclusions

Extraction of anthocyanin from purple roselle at various pH and temperatures was investigated. Extraction at pH 1–3 indicated that anthocyanin was stable in the acidic pH range. In addition, the extraction temperature in the range of 313–333 K provided higher total anthocyanin with the increase in temperature without indication of anthocyanin degradation at 333 K. The kinetics study confirmed that the second order kinetics model was suitable for the anthocyanin extraction from purple roselle.

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КІНЕТИКА УЛЬТРАЗВУКОВОЇ ЕКСТРАКЦІЇ АНТОЦІАНУ З ПУРПУРОВОЇ РОЗЕЛІЛИ ЗА РІЗНОГО pH СЕРЕДОВИЩА

Анотація. *Експериментально доведено, що антоціан краще екстрагується за вищих температур. Встановлено, що найбільший вміст антоціану досягається за рівня pH = 2. Застосовано модель кінетики другого порядку, яка добре узгоджується з експериментальними даними.*

Ключові слова: *розелла, антоціан, екстракція, ультразвук, кінетика.*