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Evaluation of Counter Current Horizontal Screw Extractor's Performance in Determining Leaching Equilibrium of *Clitoria ternatea* Anthocyanins

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Abstract. Anthocyanin compounds as natural coloring substances contained in *Clitoria ternatea* flowers have higher antioxidant activity than anthocyanins from other flower extracts. Obtaining anthocyanin extract from Clitoria ternatea flower can be done using the solid-liquid extraction method. The objectives of this study include making a leaching equilibrium diagram of *Clitoria ternatea* flowers and determining the optimum L/S ratio and the number of equilibrium stages in the counter current horizontal screw extractor. In the maceration process, a leaching equilibrium diagram is produced, which is then used to design and determine the number of equilibrium stages. The equilibrium diagram of anthocyanins from *Clitoria ternatea* flowers with a gradient slope to the right is in accordance with the literature so that the equilibrium diagram can be used in the design of the extraction unit and the calculation of the theoretical stage of the multi-stage extraction unit. Based on the equilibrium diagram, the Number of Transfer Units (NTU) of 4 stages and the Height of the Transfer Unit value of this process is 0.105 m.

Keywords: *Anthocyanin, Clitoria ternatea flower, counter current horizontal screw extractor, equilibrium curve, leaching*

Abstrak. Senyawa antosianin yang terkandung dalam bunga *Clitoria ternatea* memiliki aktivitas antioksidan yang lebih tinggi tinggi dibandingkan dengan antosianin dari ekstrak bunga lain. Untuk mendapatkan ekstrak_antosianin dari bunga *Clitoria ternatea*, dapat dilakukan menggunakan metode ekstraksi padat-cair. Tujuan dari penelitian ini diantaranya adalah membuat diagram kesetimbangan leaching bunga *Clitoria ternatea* dan menentukan ratio L/S optimum beserta jumlah tahap kesetimbangan pada *horizontal screw extractor*. Pada proses maserasi, dihasilkan diagram ketimbangan leaching yang kemudian digunakan untuk perancangan dan penentuan jumlah tahap kesetimbangan. Diagram kesetimbangan antosianin dari bunga *Clitoria ternatea* dengan kemiringan gradien ke arah kanan, hal ini sudah sesuai dengan literatur sehingga diagram kesetimbangan tersebut dapat digunakan dalam perancangan unit ekstraksi maupun perhitungan tahap teoritik unit ekstraksi multi-tahap. Berdasarkan diagram kesetimbangan yang dibuat, diperoleh *Number of Transfer Unit* (NTU) sebesar 4 tahap dan nilai *Height of Transfer Unit* (HTU) dari proses ini sebesar 0,105 m.

Kata kunci: *Antosianin, bunga Clitoria ternatea, counter current horizontal screw extractor, diagram kesetimbangan, ekstraksi padat-cair*

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INTRODUCTION

Color is the main attraction and an important assessment criteria for food, textile and cosmetic products. The food coloring industry market has grown rapidly in recent years and is expected to continue to grow by 10-15% annually (Cortez *et al*, 2016). Synthetic food colorants have several advantages, among others; diverse color types with a wide

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color range, guaranteed availability, stable, and reliable, strong coloring power, easy to obtain, cheap, and easy to use. However, the use of this kind of food colorant has a serious negative impact, especially to the human health because of its toxicity and carcinogenicity (Pujilestari, 2015).

Widespread access to information platforms has an impact on increasing public awareness of the environmental hazard and the side-effect of synthetic food colorants to the human health. This situation makes natural coloring agents much in demand by the food and baverage company and for household daily use. Natural coloring sources can come from nature such as plants and animals which have several advantages, including non-toxic compund, renewable, and biodegradable (Yernisa *et al*, 2013). The consumption of natural-colored pigments such as abthocyanins, carotenoids, betalains, and chlorophylls has been related to the reduction of diabetes, obesity and cancer (Li *et al*, 2016 ; Rodriguez *et al*, 2016; Cooperstone and Schwartz, 2016).

One alternative source for natural food colorant is anthocyanins pigments from *Clitoria ternatea*Butterfly Pea flowers (*Clitoria ternatea*). Anthocyanins are water-soluble pigments which are part of the flavonoid family. Structurally, anthocyanins are found in the form of glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrrile salts and are composed of an aglycone also called anthocyanidin and a carbohydrate residue that may be glucose, xylose, galactose, arabinose, rhamnose or rutinose (Enaru *et al*, 2021). Due to their chemical structure, with a central core in the form of 2-phenylbenzopyrylium or flavylium cation (Figure 1), anthocyanins can be classified as polyphenols and secondary metabolites (Brooks and Celli, 2019).

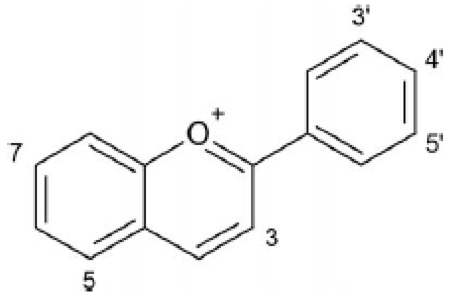

Figure 1. Core structure of anthocyanins in the form of 2-phenylbenzopyrylium or flavylium cation (Brooks and Celli, 2019).

Anthocyanins have special properties which have a wide range of pigmented colors, ranging from pink to red and from blue, purple to black. Anthocyanins consisted of over different 700 types of compound, but only 6 of them are found in fruits and vegetables. There are cyanidin which appears in the form of red-purple pigment, pelargonidin which appears in red and orange pigment, delphinidin which appears as a blue-reddish and purple color, pentanidin which appears in purple color and peonidin which appears in magenta and purple color. Delphinidin are the pigments who responsible for blue color extract from *Clitoria ternatea* flower, descrived as delphinidin-3-glucoside.

Counter current horizontal counter current screw extractor is one type of counter current leaching equipment that is widely used in the food industry. Horizontal screw on the extractor used, serves as a solids feed conveyor. The advantage of countercurrent counter current horizontal screw extractor is that, with the same liquid to solids ratio (L/S ratio), it can produce extracts with higher dissolved solids concentration than batch process. Counter current flow mode is widely applied in industry because it produces greater yields than other flow modes.

This research aimed to obtain an anthocyanin equilibrium diagram specifically from Clitoria ternatea. Equilibrium diagram consists of equilibrium lines between underflow (extract) and overflow (raffinate) concentrations for specific material. The purpose of making equilibrium diagram is to describe the relationship between the concentration of underflow and overflow as data needed in taking calculations in the design of the extraction unit. The method conducted inthis research was adapted to the previous study that was completed by Nurcahyo (2021) to determine equilibrium line of anthocyanin from Hibiscus sabdrariffah. This Clitoria ternatea anthocyanins equilibrium diagram is then used in determining the theoretical number of stages and stage efficiency in multi-stage leaching.

MATERIAL AND METHODS

Materials

The material used in this research was *Clitoria ternatea*butterfly pea flower from local farm in Blora Regency, Central Java with the moisture content of under 5% and water as the extraction solvent Hydrochloride Acid 37% (Merck, AR), Potassium Chloride (CP) and Sodium Acetate (CP) from local supplier were used for determination of anthocyanins concentration.

Instrumentation

Maseration leaching process was carried out in erlenmeyer glass by volume of 250 ml. Counter current leaching process is being conducted using horizontal screw extractor in the Food Technology laboratory of Politeknik Negeri Bandung. The scheme of the Screw Extractor is described in Figure 2 and the description is listed at Table 1.

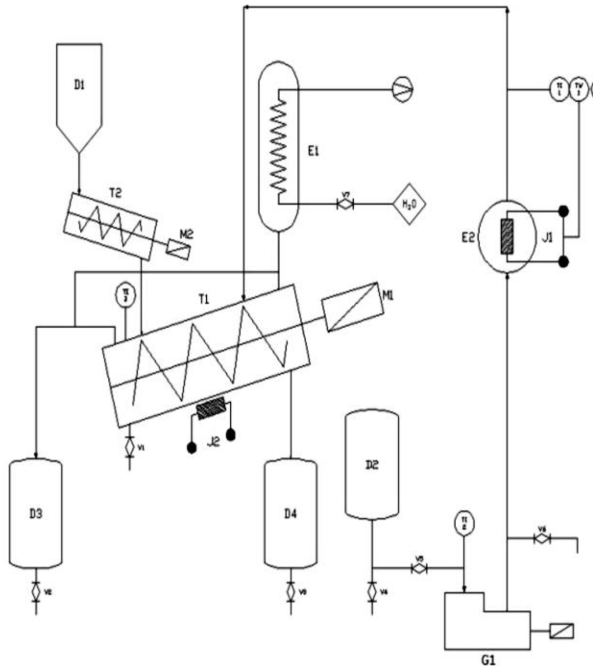

Figure 2. The scheme of counter current horizontal screw extractor

Table 1. Annotations

Code	Description
D1	Feed tank
D2	Solvent tank
D3	Extract tank
D4	Raffinate tank
E1	Condensor
E2	Pre-heater
J1	Hot stream for pre-heater
J2	Hot stream fo screw extractor
T2	Screw conveyor
T11	Temperature indicator of hot solvent
T12	Temperature indicator of solvent before entering the pump
G1	Solvent pump
M1	Motor for screw conveyor
M2	Motor for screw extractor

Procedure

Pretreatment

Fresh *Clitoria ternatea* flowers used for the preparation of equilibrium diagrams were first pre-treated which included the stages of cleaning impurities, size reduction and drying. Fresh *Clitoria ternatea* flowers are cleaned from leaves and stems that are carried away and then reduced in size. Next, the flowers were dried to reduce the water content. The drying process was carried out with a tray dryer in the Food Technology laboratory to observe the moisture.

Maseration

At this stage, first the maceration process is carried out where *Clitoria ternatea* flowers and water will be put into a 250 mL 98llustrate and then allowed to stand for 24 hours in a shaker. The L/S ratio (w/w) variations of water and *Clitoria ternatea* flower used are 15:1, 20:1, 25:1, 30:1, 35:1 and 40:1. The maceration process was carried out for 6 hours with a stirring speed of 150 rpm at room temperature. The product of this process was being separated using the 98 llust filter method to define the solution as the extract and the flower pulp as the raffinate. After the maceration process, spectrophotometric analysis was carried out so that the solution concentration was known.

Determination of anthocyanin concentration

The results of the *Clitoria ternatea* flower extraction were analyzed byg pH differential method using spectrophometer UV-Vis. The aim was to obtain the concentration of anthocyanins in the overflow solution and in the underflow. Two buffer solutions of pH 1 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) were prepared for monometric antocyanin concentration determination. Each of sample solution (1 mL) was diluted with each buffer solution and the absorban is measured at 520 nm for pH 1 and 700 nm wavelength for pH 4.5. The absorbance values of the underflow and overflow measurements of the leaching operation were interpreted as the concentration of anthocyanins in the *Clitoria ternatea* flower extract, expressed as the amount of delphinidin-3-glucoside, which is the most abundant anthocyanin contained in *Clitoria ternatea* flowers, using equation (1).

$$\text{Anthocyanin concentration} = \frac{A \times MW \times 1000 \times D}{\varepsilon \times l} \quad ....(1)$$

Annotation:

A	=	Absorbance
MW	=	Molecular Weight of delphinidin-3-glukosida (465,2g/mol)
D	=	Dilution factor
L	=	Cuvette lngths (cm)
ε	=	Molar absorptivity of delphinidin -3-glukosida, (29.000 L/mol.cm)

Determination of leaching equilibrium curve

In making the leaching equilibrium curve, the results of maceration of the above solution are separated between the extract (overflow) and the slurry (underflow). The results of maceration of the above solution are filtered, the filtrate is expressed as overflow and the juice is expressed as underflow. The overflow and underflow solution are then subjected to spectrophotometric analysis to determine their concentration. After the concentration is known, the overflow and underflow liquids are converted into mass fractions, then the N value is sought, then channeled into the leaching equilibrium diagram.

Leaching process using counter current horizontal screw extractor

The solvent flow rate used was taken from the results of the solvent flow rate calibration of 6.12 kg/h, while the solid flow rate used was taken from the results of the solid flow rate calibration of 3.92 kg/h.The overflow (extract) and underflow (juice) obtained from the leaching process were then subjected to spectrophotometric analysis to determine their concentration. The concentration of overflow and underflow is then channeled into the equilibrium diagram that has been made so that the number of leaching stages or NTU can be known.

RESULT AND DISCUSSION

Anthocyanin Concentration Measurement

The maceration process begins with the preparation of the solution with various concentration. This process was conducted under room temperature, with the stirring speed of 150 rpm for 6 hours. The stirring rotational speed and extraction time has a significant relationship to obtained extract concentration. The increasing rotational speed resulted the significant turbulency of the mixture and impacted to the higher mass transfer coeficient. Stirring exerted mechanical force on the mixture and maximized the contact of the solids and solvent, this condition resulted in the rise of the diffusion rate (Nurwahyuwono *et al*, 2021). With the higher diffusivity of solvent and the tenuous pores in solids, solvent easily penetrated the pores of solids so that solute contained in solids are easily extracted and equilibrium is reached faster (Foust, 1959). After the maceration process was complete, separation is carried out between the extract and the *Clitoria ternatea* flower pulp using vacuum filter methods. The extract was declared as the overflow stream and the *Clitoria ternatea* flower pulp juice was declared as the underflow stream for the anthocyanin concentration analysis test. While the *Clitoria ternatea* flower pulp is the remaining solid or insoluble solid.

Determination of anthocyanin concentration of underflow and overflow stream resulting from the maceration process of *Clitoria ternatea* flowers was carried out by spectrophotometric method. Anthocyanins contained in *Clitoria ternatea* flowers are expressed as delphinidin-3-glucoside which is measured at a maximum wavelength of 543 nm by spectrophotometric analysis (Wrolstad *et al*, 2005; Purwaniati, 2020). Then, the absorbance value was calculated using equation (2) to determine the concentration of anthocyanins for each L/S ratio variations in the overflow and underflow liquid as provided in Table 2.

Tabel 2. Anthocyanin concentration

No.	L/S Ratio	Overflow concentration (mg/l)	Underflow concentration (mg/l)
1	15/1	37.03	46.333
2	20/1	25.97	40.966
3	25/1	21.90	34.123
4	30/1	15.42	25.492
5	35/1	12.42	21.780
6	40/1	9.66	11.628

The given data explain that the highest anthocyanin content in *Clitoria ternatea* flower extract was obtained when an L/S (w/w) ratio of 15/1 was used with the concentration of 37.03 mg/l in overflow and 6.33 in underflow. This result is in accordance with research conducted by Pambudi *et al* (2019) regarding the solid-liquid extraction process of potassium carbonate (K_2CO_3) from palm oil mill boiler ash where the use of the smallest solvent and solid ratio produces the highest extract density. Density is inversely proportional to the volume of solvent, the smaller amount of the solvent used in the extraction process the higher dissolved mass that moves from solid to liquid. The high-density value decribes the high concentration of the extract.

Leaching Equilibrium Curve

The anthocyanin concentration data in Table 2 need to convert to weight fraction basis in oder to build the leaching equilibrium curve. The leaching equilibrium curve is used to describe the relationship of the N variable which means the ratio of solids and liquids to the mass fraction of anthocyanin as solute in the overflow liquid (xA) and anthocyanin as solute in underflow liquid (yA). The concentration of anthocyanins in the overflow and underflow stream at the equilibrium condition is the needed data in designing of the extraction appliance. The equilibrium condition also uses to determine the theoretical stage in multi-stage liquid solid extraction. To obtain the equilibrium curve, the anthocyanin concentrations obtained from all the L/S ratio were converted into mass fraction values and plotted on the curve as presented in Figure 3.

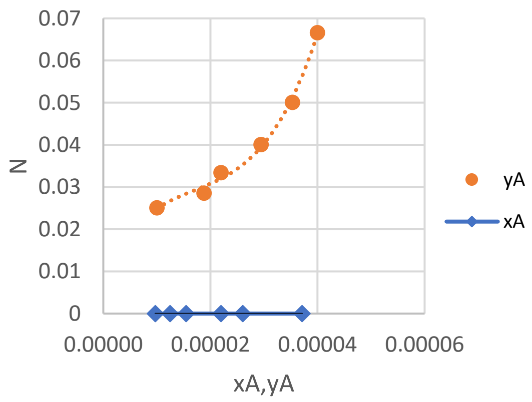

Figure 3. Leaching equilibrium curve of anthocyanin in *Clitoria ternatea* flowers

At the beginning of the maceration process, there were no anthocyanins contained in the solvent because the solvent used was pure water. The different anthocyanins concentration between water and *Clitoria ternatea* flower known as the driving force for mass transfer process. Equilibrium conditions are reached when the anthocyanin concentration in the overflow liquid is equal to the anthocyanin concentration in the underflow liquid. The anthocyanin concentration distributions in the overflow and underflow liquids will be connected by lines called tie-lines. At the ideal equilibrium condition, the tie-line should be a vertical line. In this study, the tie-line is obtained as presented in Figure 4.

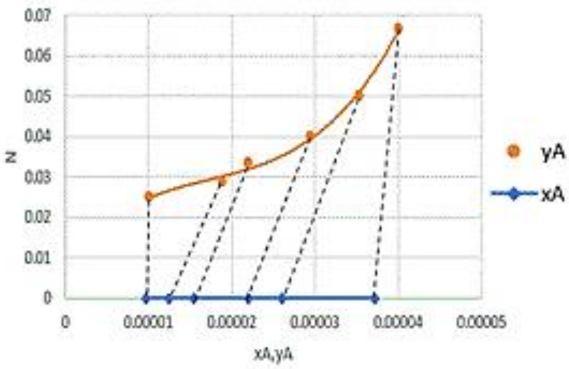

Figure 4. Tie-line in the anthocyanin leaching equilibrium curve

The given Figure 4 illustrate that the tie-line of the equilibrium curve is not vertical and represents the consistent slope to the right. This indicates that the anthocyanin concentration in the overflow liquid is not equal to the anthocyanin concentration in the underflow. In this study, the anthocyanin concentration in the overflow liquid is smaller than the underflow at all L/S ratios. This is very likely to happen considering that the vertical tie-line condition with zero gradient can only be achieved theoretically when the equilibrium time is infinite (Prastyo, 2010). Under actual conditions, the leaching equilibrium curve has a tendency to have a positive or negative gradient.

Solid-Liquid extraction of anthocyanin using counter current horizontal screw extractor

The leaching *Clitoria ternatea* flowers counter currently using a solid-liquid extractor is intended to find the number of equilibrium stages (Number of Transfer Units) and the equilibrium length (Height of Transfer Units) based on the previously obtained equilibrium diagram. At this stage, the flow rate of water as a solvent is 9.167 g/min with a flow rate of 0.1625 g/min and the process was carried out for 45 minutes.

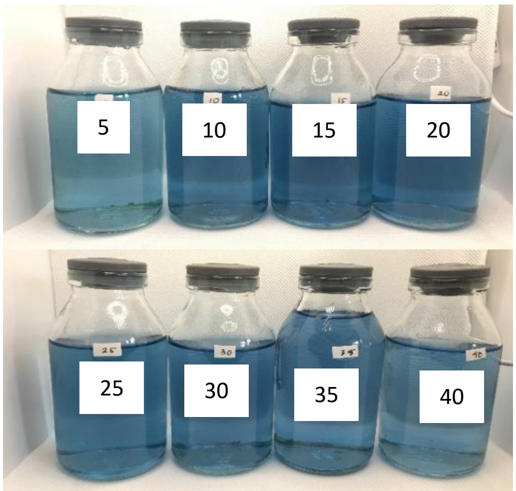

Figure 5. The color of extract samples vs operating time (minutes)

Figure 5 shows that the extract samples represented the blue pigment color. The representation of blue color happened because the leaching process used water as the solvent with neutral pH (7). *Clitoria ternatea* flower extract can represent different colors among the pH conditions as attached in Figure 5 below.

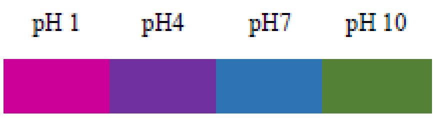

Figure 4. Anthocyanin color of *Clitoria ternatea* flowers at various pH (Angriani, 2019)

In addition to showing color, pH condition also affects the stability of anthocyanins in it. Based on research conducted by Angriani (2019) shows that *Clitoria ternatea* flower extract at pH 4-5 has a purple color with good stability and it can be stored for 2 months at room temperature. *Clitoria ternatea* flower extract at pH 6-7 has a faded color after a few days but lasts for up to 6 months at refrigerator temperature (Marpaung *et al.*, 2018 cited in Angriani, 2019). The extract samples were taken to be analyzed for anthocyanin concentration.

Calculation of the number and length of the equilibrium stage begins by determining the point L0 (mass flow rate of the solution in the slurry) and VN+1 (mass flow rate of the solution in the solvent) in the equilibrium curve so that point M (mixing) can be obtained. After that, continue by determining the LN point (mass flow rate of the solution in the raffinate) so that the V1 point (mass flow rate of the solution in the extract) can be obtained.

The next stage continues by determining the point Δ (equilibrium) by drawing a line from L0 to V1 then extended, and drawing a line from LN to VN+1, then extended, the intersection between the two lines is point Δ. To find the number of equilibrium stages, first draw a line from V1 to L1, then from L1 to point Δ. The intersection on the x-axis is then drawn to L2, then to point Δ and so on until it approaches point LN. For more details, it is presented in Figure 6.

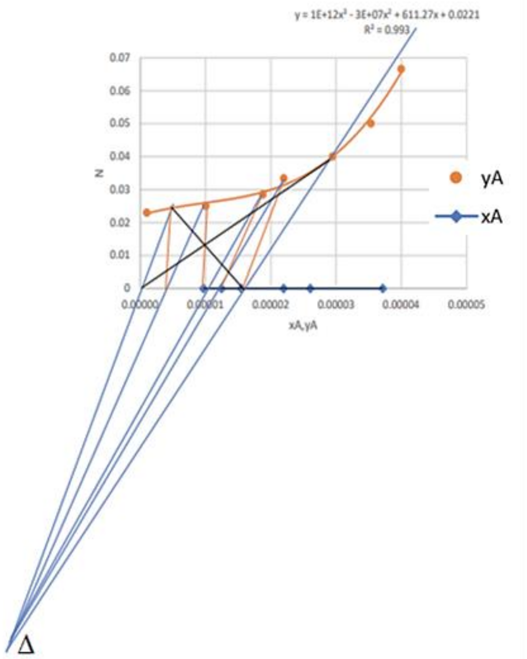

Figure 6. Determination of the number of equilibrium stages

After plotting on the equilibrium curve, 4 equilibrium stages connected by tie-line are obtained. The number of equilibrium stages is referred to as NTU (Number of Transfer Units). Then the calculation of the HTU (Height of Transfer Unit) value is carried out with the known results of the effective length measurement (Z) of 0.42 m. Based on the equation prepared in Perry's (1997), namely: Z = NTU. HTU, obtained HTU value of 0.105 m.

CONCLUSION

The leaching equilibrium diagram of for anthocyanin extraction from *Clitoria ternatea flowers* obtained in this study shows consistent tie-line slope to the right (positive gradient). This diagram can be used to describe the relationship between the concentration of underflow and overflow as data needed in taking calculations in the design of the extraction unit or the evaluation of the theoretical stage of the multi-stage extraction unit. The evaluation of a multi-stage counter current leaching process of anthocyanin extract using this diagram, resulted in the NTU of 4 stages and HTU of 0.105m.

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