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The Development of Butterfly pea (*Clitoria ternatea*) Flower Powder Drink by Co-crystallization

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Abstract— **A method consist of co-crystallization, agglomeration, drying has been applied to develop a powder drink from butterfly pea flower (*Clitoria ternatea*) extract. The butterfly pea flower extract was concentrated by vacuum evaporation and incorporated with supersaturated sugar solution (more than 90 Brix), agglomerated and dried at 60^{o}C for 12 hours. The anthocyanin stability and antioxidant activity of the powder drink was evaluated for 28 days at three levels of temperature (27^{o}C, 40^{o}C, and 50^{o}C). The stability of anthocyanin decreased as the storage temperature increased. The half-life of anthocyanin in the powder drink at 27^{o}C, 40^{o}C, and 50^{o}C was 27.99, 16.53, and 9.81 days, respectively. Despite the anthocyanin significantly degraded, the decrease of antioxidant activity of the powder drink was not significant. Hence, the beneficial effect of the butterfly pea powder drink was retained.**

Keywords— **anthocyanin; butterfly pea; co-crystallization; stability; sugar**

I. INTRODUCTION

Butterfly pea (*Clitoria ternatea*), is a vine originating from South East Asia, which has spread throughout the tropics. The flower has been recognized to have health benefits such as antioxidant [1], antidiabetes [2, 3], antiinflammation [4], and anticancer [5]. The bioactive compounds of butterfly pea flower are nine types of polyacylated anthocyanin called ternatins and 15 types of flavonol glucosides [6].

One of the unique characteristics of butterfly pea flower is it bluish-purple at a low acidic condition, while other most anthocyanins are colorless. The combination of the exotic color and health benefits promoted butterfly pea flower as a functional drink. In Thailand, the drink – called *dok nam anchan* is very popular [7]. Recently, in Indonesia the butterfly flower drink is much easier to be found at many restaurants.

Prolonging the shelf-life and increasing the convenient of butterfly pea flower drink can be achieved by converting the extract to powder form. The use of several drying method including spray drying, vacuum drying and freeze drying to produce powdered butterfly pea flower extract has been reported [8, 9]. The highest remaining color of spray dried butterfly pea flower extract at pH 4 after 60 days is 48% [8]. The highest half-life of vacuum- and freeze-dried butterfly pea flower extract is 40.69 and 19.03 days, respectively [9].

The alternative method is co-crystallization using sucrose. The use of sugar was reported to improve the anthocyanin stability [10]. To the best of our knowledge, there is no work reported on the processing of powdered butterfly pea flower extract through co-crystallization. Hence, the objective of this research was to develop the butterfly pea flower powder drink and to determine its stability during storage.

II. MATERIAL AND METHODS

A. Material

The raw material used for this research was butterfly pea (*Clitoria Ternatea*) flowers. The flowers were obtained from a private plantation in Kembangan, West Jakarta, Indonesia, which were given the same treatment during plantation. The flower was harvested from the five months old plant in the morning of the day and directly proceed to the next step of the research. The material for formulation were citric acid (food grade, Brataco, Indonesia) and table sugar (Gulaku, local market). The chemical for analysis were hydrochloric acid 1 M and ethanol 96% (Merck, Germany), DPPH (2,2'-Diphenyl-1-Picrylhydrazyl) (Aktin Chemicals.Inc, China). The packaging materials/ metalized packaging used were the PET 12/VMPET12/VMPET12/ LLDPE40 (PT Bintang Toedjoe, Indonesia).

B. Extraction

Prior to the extraction, the effect of cell-wall destruction method (mortar and pestle, freezing and thawing, steam blanching for 6 minutes, and hot water blanching for 6 minutes) to the total anthocyanin of butterfly pea flower extract was studied. The statistical analysis (significant level or $\alpha = 0.05$) showed that there was no significant difference of the total anthocyanin affected by the method of destruction. By considering the practicality, the mechanical destruction using mortar and pestle was chosen.

The extraction of the mechanical destructed butterfly pea flower conducted by following the method of Marpaung et al. [11] with slight modification. The sample mixed with dilution of citric acid in distilled water and adjusted to pH 4.5. The ratio of solvent to sample was 4 ml/g. Afterwards, the mixture was then transferred into a water bath shaker that has been adjusted to 60 °C for 30 minutes. It was then filtered using a tea cloth and filter paper Whatman #41 to obtain the extract.

C. Formulation of the Butterfly Pea Flower Drink

The ingredients of the formulation used are butterfly pea extract, sugar, and citric acid. The trial and error conducted to obtain three best formula to proceed to the sensory evaluation (Table 1).

TABLE 1
THREE CHOSEN FORMULA OF BUTTERFLY PEA FLOWER DRINK FOR SENSORY EVALUATION

No	Sucrose (g)	Citric Acid (g)	Butterfly Pea Flower Extract (g)
1	70	0.46	80
2	58	0.46	80
3	58	0.7	80

Each formula added with distilled water to form 250 ml butterfly pea flower drink.

The sensory evaluation was 9-scale hedonic test involving 30 selected untrained panelists. The significant different among formulas checked at significant level $\alpha = 0.05$ by non-parametric statistical analysis (Friedman's test and followed by Wilcoxon Sign-Rank test). The best formula was applied to the next research step.

D. Co-crystallization

Two stages were needed to convert the butterfly pea flower extract to powder. The first stage was to obtain the concentrated butterfly pea flower extract. 184 g of butterfly pea flower extract was concentrated to about 15 g by vacuum evaporation at 65°C.

The 100 g sucrose placed in a beaker glass and heated at 120°C and at the same time added with 50 g of water. The mixture continuously stirred until the sugar content greater than 90% Brix [12, 13]. The concentrated butterfly pea flower extract as the core material added and the mixture immediately placed in a water bath at a room temperature until the agglomerates are formed. This cooling done to prevent long-term exposure of the butterfly pea extract to high temperature, since this is known to provoke anthocyanin degradation. The second reason was to increase the supersaturation of the sugars, which facilitates faster crystallization.

The agglomerates of the product were spread on a tray and dried overnight in a hot air drier at 60°C until the moisture content of the product was about 3-5%. The dried product was pulverized to produce powder product. The product was then added with citric acid at amount equivalent to the percentage of amount of citric acid in the best formula to be dry mix powder, following the formula in Table 1.

E. Stability test

The butterfly pea powder drink was packed in a metallized packaging, stored at 27°C, 40°C and 50°C) without the presence of light for 28 days. The total anthocyanin and antioxidant activity of the powder was analyzed every 7 days. The degradation kinetics were evaluated by the first order reaction.

$$A = A_o.e\text{-}kt$$

$$t_{0.5} = \ln (2)/k$$

A was the final concentration, A_o was the initial concentration, k was constant of reaction rate (day^{-1}), t was the storage time (day), and $t_{0.5}$ was the half-life.

F. Total Anthocyanin

Total anthocyanin determines as delphinidin 3-glucoside by single pH Method [14]. The 1.5 mL extract adjusted with HCl 1 M to reach pH 1. The light absorbance scanned at visible region (400 – 700 nm) by a UV-VIS Spectrophotometer (Genesys 10uv Thermo Electron Corporation, U.S.A.) to find the λ_{max}.

$$A = (A_{\lambda max}) - (A_{700}) \times DF$$
$$TA\ (mg/L) = (A \times MW \times DF \times 1000)/(\varepsilon \times l)$$

MW was molecular weight of delphinidin-3-glucoside (465.2 g/mol), DF was a dilution factor, ε was molar absorptivity of delphinidin-3-glucoside (29000), and l was the cuvette width.

G. Antioxidant Activity

Antioxidant activity (AA) measured by DPPH (2,2'-Diphenyl-1-Picrylhydrazyl) radical scavenging activity [11]. The 1.27×10^{-3} DPPH solution prepared by diluting 10 mg of DPPH in 96% ethanol to final volume of 20 ml. This was the stock solution of DPPH. The absorbance of DPPH measured at a wavelength of 515 nm. For each analysis, 1 ml of the 1.27×10^{-3} DPPH solution diluted again with 10 ml ethanol 96%, resulting in 1.27×10^{-4} DPPH solution.

The sample was the butterfly pea powder drink diluted in water according to the formulation. Afterwards, 0.1 ml of the diluted sample was put into cuvette and mixed with 0.90 ml DPPH. The control solution made in the same way but by

exchanging the sample to 0.1 ml distilled water. The blank solution consists of 1 ml of ethanol. These solutions were each put into cuvettes and mixed thoroughly using a micropipette to make the DPPH reacting. The solutions were then kept in dark condition for 30 minutes before being measured to equilibrate, and then measure by a spectrophotometer at 515 nm. For each sample, duplicated reading of spectrophotometer was done. The antioxidant activity was calculated according to the following formula (with unit pro DPPH consumption):

$$\text{AA (\%)} = \frac{(absorbance\ of\ control - absorbance\ of\ sample)}{absorbance\ of\ control} * 100$$

H. Statistical analyses

The statistical analyses involved in this research were Friedman's test and Wilcoxon signed-rank test (OpenStat), One Factor ANOVA and trend analyses (Pearson's correlation and regression analysis) (Microsoft Excel® 2010 software, Microsoft Corporation). The significant level of all analyses was $\alpha = 0.05$.

III. Result and Discussion

A. Sensory Acceptance

Thirty panelists were involved in the hedonic scale (nine scale) sensory evaluation of three chosen butterfly pea drink formulas. Four attributes of the formula asked to the panelists were taste, aroma, color and overall. The non-parametric statistical analysis (Friedman's test and followed by the Wilcoxon signed-rank test) used to determine the difference among formulas ($\alpha = 0.05$). The best was formula 2 that consisted of 58 g sucrose, 0.46 g citric acid, and 80 ml butterfly pea flower extract per serving (Table 2).

TABLE 2
THE SENSORY ACCEPTANCE OF THREE FORMULAS OF BUTTERFLY PEA FLOWER DRINK

Formula	Taste	Aroma	Color	Overall
1	5.23± 1.28^{c}	6.07± 1.41^{a}	4.80± 1.24^{b}	5.50± 1.17^{c}
2	7.33± 1.18^{a}	6.33± 1.40^{a}	5.70± 1.49^{a}	7.30± 0.88^{a}
3	6.77± 1.22^{b}	6.13± 1.04^{a}	5.63± 1.16^{a}	6.57± 1.04^{b}

The overall acceptance was mostly affected by the taste. The relatively mild sweet and sour taste was most preferred.

B. Powder Drink

The purpose of this process is to produce a butterfly pea powder drink. Production of the butterfly pea powder drink was made possible through vacuum evaporation and co-crystallization. First, the extract that has the highest acceptance level was being evaporated to increase the soluble solid of the extract. The initial weight of the extract was 184.5 ± 2.12 g and the final weight after evaporation was 16.9 ± 1.22 g.

The concentrated extract entered the co-crystallization. Through co-crystallization, the sample formed agglomerates. The agglomerate was dried at 60^{o}C for 12 hours to reach of 3.5-5% moisture content [13]. The dried material was pulverized and mixed with citric acid following the best formula based on the previous sensory evaluation. The total anthocyanin and antioxidant activity of the powder drink after diluted in 250 ml water was 16.98 ± 1.22 (mg/L) and 34.2 ± 2.34%, respectively.

C. Stability Testing

The anthocyanin stability and antioxidant activity of butterfly pea powder drink during four weeks of storage were observed at three different temperatures. Anthocyanin is one of the most heat-sensitive flavonoid [15]. As predicted, the anthocyanin stability of the powder drink decreased as temperature increased (Fig. 1).

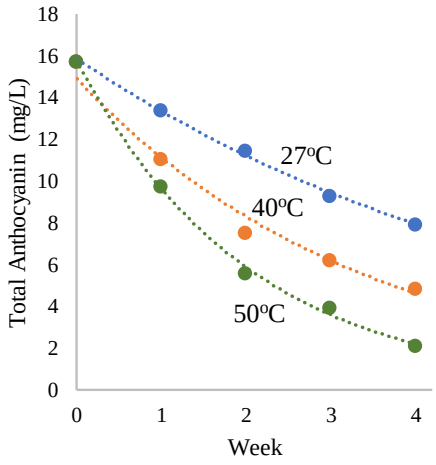

Fig. 1. Total anthocyanin of butterfly pea flower powder drink during storage at three different temperatures.

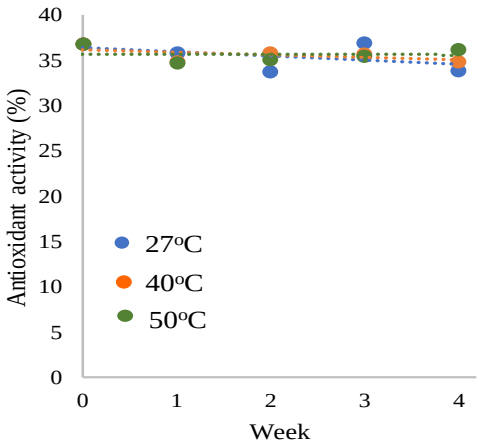

Fig. 2. Antioxidant activity of butterfly pea flower powder drink during storage at three different temperatures.

The anthocyanin degradation can be modelled by first order degradation kinetics (coefficient of determination, r > 0.95). The degradation rate of the powder drink at 27^{o}C, 40^{o}C, and 50^{o}C was 0.0248, 0.0419, 0.0707 per day, respectively. Meanwhile, the respective half-life was 27.99, 16.53, and 9.81 days. This anthocyanin stability was comparable to the spray dried, vacuum dried, and freeze dried butterfly pea flower extract powder [8, 9] and much more higher than butterfly pea flower extract at pH 7 [16].

In contrast to the total anthocyanin, the antioxidant activity of the butterfly pea flower drink remained stable during storage as seen in Fig. 2 which also supported by the regression analysis (r < 0.2, p-value > 0.05). The possible explanation of the high stable antioxidant activity is as follow. Thermal degradation of an anthocyanin initiated by the ring opening of the C-ring of anthocyanin, followed by the deglycosilation, and end up by the cleavage of C-ring to form a benzaldehyde and benzoic acid derivative [17]. Both degradation product reported to have antioxidant activity [18]. Hence, despite the anthocyanin partly decreased, the functional effect of the butterfly pea flower drink was maintained.

IV. CONCLUSION

The butterfly pea flower powder drink has been successfully developed through the co-crystallization of concentrated butterfly pea flower extract with supersaturated sugar solution (> 90 Brix), followed by agglomeration and drying at 60^{o}C for 12 hours. The anthocyanin content of the drink was 16.98 ± 1.22 mg per liter.

The half-life of the powder drink was 27.99 days at 27^{o}C temperature and decrease as the increase of storage temperature to 40^{o}C and 50^{o}C. However, the antioxidant activity remained stable. This result supported the potentiality of butterfly pea as a source of functional drink.

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