Application of anthocyanin natural colors from Butterfly Pea (Clitoria ternatea L.) extracts to cupcake

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Abstract: In this study, we compared the content of nutritional indicators including lipid, moisture, pH, protein, and ash between original and Butterfly Pea cupcake baked at 170°C for 20 minutes. Moreover, the sensory properties of Butterfly Pea cupcake favorite also are determined. There was an increase in the content of moisture, protein compared with those of the control cupcake, reaching 22.12 ± 1.87 and 3.69 ± 0.51, respectively. In contrast, the level of pH, ash, and lipid witnessed marginal decreases, reaching 7.08 ± 0.54, 1.01 ± 0.21, 12.10 ± 2.77 respectively. Notably, the Butterfly Pea cupcake had anthocyanin content of 2.58 ± 0.06 mg/L, which accounted for 41.82 ± 0.92% retention. The ability to decompose anthocyanin biological compounds is lower than 60%. The slight green color is determined to be influenced by the pH factor in the dough environment.

1. Introduction

Chemical composition from natural plants, like herbs, has been receiving significant public attention due to essential influences on human health [1–6]. Therefore, the extraction and application of natural color compound are increasingly considerate. Many studies have been conducted to evaluate the effect of the color compound in food processing like as Deepti Dabas and George Kean applied Betalains and Lycopene (natural red color) in hard candy. [7], Ricardo F. R. da Silva et al have extracted anthocyanin from Elderberry (Sambucus nigra L.) to apply in Croissant [8]. J. Fernández-López investigated the effect of natural extracts from rosemary, lemon and orange in beef meatball processing to evaluate their antioxidant and antibacterial [9].

Anthocyanin is one of the most natural color compounds which was widely used in food. Anthocyanin is the most significant water-soluble pigment in plants, including more than 540 types that differ in color such as orange, red, purple and blue. Anthocyanin occur in all tissues of plant such as leaves, stems, roots, flowers and fruits. Some fruits contain high level of anthocyanin such as eggplant, raspberry, cherry, blood orange. Therefore, the application of color compounds from anthocyanins is being studied in food and health purposes [10]. To replace artificial colorings, anthocyanins extracted from Butterfly Pea flower (BFP) was used widely because they also have pharmacological effects, anticancer, antioxidant ability. Butterfly pea flower (Clitoria ternatea) is a species of flowering plant in the legume family. The flower has a blue color, so their colors are applied
for various decorative items and natural coloring agents. Butterfly Pea flower’s extract is a blue color. Nevertheless, they can change color to light pink in weakly acidic and neutral aqueous. The previous study demonstrates that butterfly pea contains high levels of Anthocyanin and compounds with free radical scavenging activity such as phenolic groups, flavonoids. The main anthocyanin in BFP is delphinine glycoside which attributes to their blue color. The extract of BFF was used in cosmetics as hair dying. Moreover, it is also used as a confectionary coloring in the food industry. However, similar to other anthocyanin extracts from other plants, the blue color of BFF’s extract is susceptible to pH, light and temperature degradation so they are more difficult to handle and less stable which compare to synthetic colorants. Malaysia [4,11]. In recent years, many extraction methods have been used to increase anthocyanin yields so the investigation of the extraction of anthocyanin compounds had been conducted on different variety of plants in many different ways. A. Liazid et al. was investigated microwave-assisted extraction of anthocyanin from grape skin and found that anthocyanin could be extracted from grape in 5 mins, 100°C with 40% methanol in water [12]. Jeana K. Monrad used accelerated solvent extraction (ASE) methods to optimize and determine the effectiveness of the extraction process [13] or Tang-Bin Zou optimized ultrasound-assisted extraction of anthocyanin from Mulberry by using response surface methodology and found that 63.8% methanol contains 1 % trifluoroacetic acid (TFA), 43.2°C, 23.8 liquid to solid ratio and 40 min for the maximum yield [14].

Anthocyanin is an antioxidant compound which reduces the risk of colon cancer, prevents heart ischemia-reperfusion injury, anti-inflammatory effects, and to avoid diabetes and obesity. Therefore, adding anthocyanin to food increase nutritional value as well as the sensory factor in product. Moreover, the range of anthocyanins from BFP is wide (from red-orange to purple-blue) for easy application of product diversification. However, the past research revealed that anthocyanin content in butterfly extract is affected by pH, temperature, light, solvent extraction [10]. Thus, this study aimed to apply anthocyanins from BFP extract to cupcakes to investigate their degradation when heated above 170°C. Moreover, sensory appearance levels of Roselle cupcake favorite also are determined.

2. Methods and materials

2.1. Preparation of Butterfly Pea extract

In the extraction procedure, 10 g butterfly pea flower were mixed with various proportion of ethanol and distilled water based on the experimental design. Next, the mixture is placed in the microwave device, and irradiated under the different factors, including temperature, time and microwave power. Following that, Model LACE16 centrifuges the mixture at 6000 x g for 10 min. Before the estimate anthocyanin yield, the mixture was filtered with filter paper. The pH between 400 nm and 700 nm are applied to determine the anthocyanin yield.

2.2. Cupcake preparation

The mixtures are prepared including 20 grams of egg white, 21 grams of unsalted butter, baking powder, and sugar. 50-gram diluted BFP extracts (ratio 1:80 with concentrated BFP and pure water) replaced milk in Butterfly Pea cupcake formula. Then, it was sifted flour and a homogeneous blend. They are heated by the Electrolux 2-liter oven (EOT4805K model) with a maximum capacity of 1500W and dimensions of 299 x 472 x 350 mm to 170°C for 20 minutes.

2.3. Analytical method

2.3.1. Determination of Vitamin C

The ascorbic acid content in the sample determined according to Microfluorometric method based on the oxidation of ascorbic acid with 2,6-dichlorophenolindophenol (DCPIP) to dehydroascorbic acid and the colorless lenco derivatives [15]. The optimized reaction is at pH between 3 and 4 [11]. In this environment, a drop of excess blue DCPIP will make the solution turn pink.

A 5-gram of the sample was ground and extracted with metaphosphoric acid. A 5 ml of extract was
titrated with 2, 6-dichlorophenolindophenol (DCPIP). The titration stopped when a drop of excess blue DCPIP makes solution turn pink in acidic medium and last for 30 seconds. Indophenol solution was titrated with the standard ascorbic acid solution. The ascorbic acid content was expressed in mg per gram of dry matter (mg/g dry matter).

2.3.2. Determination of total Anthocyanins
The colorimetric methods measured the total anthocyanins content. Monomeric anthocyanin pigments reversibly change color in pH. Described by Giusti & Wrolstad. (2001) [16], after adjusted to pH 1.0 and 4.5 using 0.2 M KCl and 0.1 M acetate buffer, respectively, the absorbance was measured at 520 nm and 700 nm. The results were expressed as mg cyanidin-3-glucoside equivalent per volume of the sample (mg/L).

2.3.3. Determination of pH
According to the method of Von Elbe et al. (1974) [17], pH of control cupcake, Roselle cupcake and anthocyanins extract were determined by Consort multiparameter analyzer (model C3010T). The sample (0.5-gram) was ground. Then it filtered through filter paper and measured pH.

2.3.4. Determination of total Lipids
The mass of total lipid was measured by using the Soxhlet method [18] from the difference in the flask mass before and after extraction. 5 g of dried sample to a constant mass placed in the tube with diethyl ether solvent. The system is heated at 60°C within 48 hours. Lipid content was determined by mg per gram of dry matter (mg / g dry matter).

2.3.5. Determination of Protein
Total nitrogen was measured by Kjeldahl analysis [19]. 1 g of potassium sulfate is added to 1 g of solid sample to increase the boiling temperature of 3 ml of concentrated sulfuric acid. 0.1g copper sulfate and ferrous sulfate were added before the chemical samples sample. The catalytic effect of several drops of hydrogen peroxide accelerates acid oxidation. After 6 hours at 370°C, 10ml of fluid was calibrated with boric acid. Protein content was expressed in total percentage of nitrogen per gram of dry matter.

2.3.6. Determination of colour
Color measurements (CIE L*a*b* color space) were performed using 0.3NH Scanner Chroma colorimeter (NR60CP model). Lightness value L* have ranged from 0-100, with two components is a* (from green to red) and b* value (from blue to yellow).

2.3.7. Sensory evaluation
Sensory evaluation acceptance was performed by description methods of Amerin et al [20]. The results were statistical from 30 evaluation of consumers. The indicators of interest in the sample are color, texture, appearance, aroma, flavor and overall. Each of these indicators is evaluated at the 5-point scale. The chart and acceptance level description expressed the results.

2.3.8. Data Analysis
All experiments were conducted in duplicate. The mean and standard deviation of the results were calculated using Microsoft Excel program. Experiment data was analyzed using one-way analysis of variance (ANOVA) test in SPSS program (Statistical Package for the Social Sciences) with the level of significance at 5%.
3. Result and discussion

3.1 Color measurements for control and BFP cupcake

Figure 1 showed BFP cupcake with a 80 time dilution. As a sensory perception, the cake has slight green. To explain that, pH value is 7.08 ± 0.54, the color system of anthocyanin is changed in the direction of vivid blue to slight blue, combination original color of the cake will create the color system as shown in figure 1. The previous study shows that the anthocyanin pigment produced intense red, violet, blue, blue-green, green, and yellow at pH 0.05 - 12.0 [21]. At the same time, the brightness L*, a*, b* in Table 1, the control value of a* b* achieved -2.01 ± 0.02 and 22.79 ± 0.01, respectively which shown the yellow color. In contrast, the BFP cupcake is -8.24 ± 0.01 and 15.43 ± 0.01 which represents the green color.

![Figure 1. Sample control (A) and Butterfly Pea cupcake with ratio 1:80 dilution (B)](image)

**Table 1. Lightness of cupcake**

| Sample        | L*        | a*        | b*        |
|---------------|-----------|-----------|-----------|
| Control       | 51.28 ± 0.16b | -2.01 ± 0.02b | 22.79 ± 0.01b |
| BFP cupcake   | 42.83 ± 0.07a | -8.24 ± 0.01a | 15.43 ± 0.01a |

Means ± standard deviation are not significantly different (p>0.05) in each row. DM = dry matter.

3.2 The nutritional characteristics

The results of nutritional characteristics of control and BFP cupcake are showed in table 2 describes the nutritional values in cupcakes, indicators of pH and anthocyanins content in the BFP extract. Table 2 show the pH value of control and BFP cupcake achieved at 7.45 ± 0.27b and 7.08 ± 0.54b, respectively. The BFP cupcake changes in color due to the process of mixing the extract into the cake. Most valuable nutritional ingredients in BFP cupcake including ash (1.01 ± 0.21a), moisture (22.12 ± 1.87a) and lipid (12.10 ± 2.77a) were not significantly different. The results showed that the percentage of remaining anthocyanin in BFP cupcake achieved 41.82 ± 0.92. It can be explained that the starch can bind and retain the anthocyanins during high-speed mechanical mixing.

**Table 2. Nutrition contain in control and BFP cupcake**

| Measurement              | Diluted BFP extract | Control | BFP cupcake |
|--------------------------|---------------------|---------|-------------|
| **pH**                   | 5.71 ± 0.25a        | 7.45 ± 0.27b | 7.08 ± 0.54b |
| **Ash %**                | None                | 1.21 ± 0.15a | 1.01 ± 0.21a |
| **Moisture %**           | None                | 15.84 ± 4.43a | 22.12 ± 1.87a |
| **Lipid**                | None                | 14.92 ± 1.99a | 12.10 ± 2.77a |
| **Protein**              | None                | 2.82 ± 0.61a | 3.69 ± 0.51a |
| **Anthocyanin mg/L**     | 6.17 ± 0.02a        | None    | 2.58 ± 0.06b |
| **Retention %**          | None                | None    | 41.82 ± 0.92 |
3.3 Sensory appearance levels
Figure 2 shows the level of evaluation of BFP cupcake and control based on 5 grade. Evaluate the color, texture, shape, aroma, flavor and overall criteria in the 30 untrained panelists. The parameter of Roselle cupcakes such as color, appearance, aroma and flavor had higher acceptance scores than control cupcakes. However, the acceptance scores about texture of untrained panelists in BFP sample is quite lower than the control sample. When adding BFP extracts into the cupcake, the color of the cake is changed, which increases the excitement, novelty, and level acceptance of the consumers comparing with a traditional cupcake. Moreover, the BFP extract improves the aroma and the flavor of the cupcakes. Nevertheless, the porosity of the BFP cupcake was lower than the control cupcake. This result was quite similar with the result of A.R. Abdel-Moemin when investigating Roselle calyxes extract in cupcake processing [23]. The level of acceptance in BFP cupcake achieved at 3.81 ± 0.6, which was higher than control cupcake (3.18 ± 0.5). Therefore, the BFP cupcake favorite level is better than the traditional cupcakes.

![Sensory evaluation of the cupcake](image)

**Figure 2.** Survey chart of sensory evaluation of BFP cupcake favorite (level 1: dislike extremely; level 2: dislike moderately; level 3: Neither like nor dislike; level 4: like moderately; level 5: Like extremely)

4. Conclusions
The coloring agents are applied in food to create diversity as well as improve the quality of product. However, the use of artificial coloring agents will adversely affect consumers’ health, so the natural color compounds are widely used to replace synthetic colour. Meanwhile, Butterfly Pea is a blue flower which contains a lot of anthocyanin content. Anthocyanins act as potential sources of eco-friendly natural food dyes and their significant effects on human health. The addition of BFP extract can improve the quality of cupcake products as well as support health benefit. In this study, the content of lipid, moisture, pH, protein, and ash in the Roselle cupcake are examined, achieved 12.10 ± 2.77, 22.12 ± 1.87, 7.08 ± 0.54, 22.12 ± 1.87, 1.01 ± 0.21 respectively. The survey values are not different, separate the color space L* a* b* BFP cupcake -8.24 ± 0.01 and 15.43 ± 0.01 with slight green color. The anthocyanin content retained about 40% increases the nutritional value in the cake, achieved 2.58 ± 0.06 mg/L. The non-training evaluation committee limits this research and it is difficult to control the desired color system of the original BFP.

References
[1] Mai H C, Nguyen T S V, Nhan T H, Le D C N and Bach L G 2019 Processes 7 90
[2] Tran Q T, Le T T T, M O, Do T L, Vu M H, Nguyen D C and Bach L G 2019 Molecules 24 895
[3] Pham P T T, Nguyen T H, Thi T V, Nguyen T T, Le T D, Vo D M H, Nguyen D H, Nguyen C K, Nguyen D C and Bach L G 2019 Polymers (Basel). 11 177
[4] Tran T H, Ha L K, Nguyen D C, Dao T P, Nhan L T H, Nguyen D H, Nguyen T D, Vo D V N, and Bach L G 2019 Processes 7 56
[5] Minh N P, Thu T M, Tham N H, Bach L G 2018 J. Glob. Pharma Technol. 10 186–92
[6] Le V T, Bach L G, Pham T T, Le N T T, Ngoc U T P, Tran D H N 2019 J. Macromol. Sci. Part A Pure Appl. Chem. 6 1–7
[7] Journal O, Dabas D and Kean G 2014 s Adv Food Technol Nutr Sci Open J 10–6
[8] Colouring A N B, Silva R F R, Barreira C M, Heleno S A, Barros L, Calhelha R C and Ferreira I C F R 2019 Molecules 1–13
[9] Ferna J, Kuri V, Zhi N and Pe J A 2005 Meat Sci. 69 371–80
[10] Lee P M and Abdullah R 2011 Int. Conf. Biotechnol. Food Sci. 7 49–53
[11] Morton J F 1966 Florida State Hortic. Soc. Univ. Miami.
[12] Liazid A, Guerrero R F, Cantos E, Palma M and Barroso C G 2011 Food Chem. 124 1238–43
[13] Auromoustakos A N D Y M 2010 J. Agric. Food Chem. 2862–8
[14] Zou T, Wang M, Gan R and Ling W 2011 Int. J. Mol. Sci. 3006–17
[15] Puwastien P, Siong T E, Kantasubrata J, Craven G, Feliciano R. R and Judprasong K 2011 Asean 188
[16] Giusti M M and Wrolstad R E 2001 Curr. Protoc. food Anal. Chem. F1.2.1-F1.2.13
[17] Von Elbe J H, Maing I and Amundson C H 1974 J. Food Sci. 39 334–7
[18] Manirakiza P, Covaci A and Schepens P 2001 J. food Compos. Anal. 14 93–100
[19] Bradstreet R B 1954 Anal. Chem. 26 185–7
[20] Amerine M A, Pangborn R M and Roessler E B 2013 Principles of sensory evaluation of food (Elsevier)
[21] Abdullah R, Lee P M and Hung L K 2010 Int. Conf. Sci. Soc. Res. 254–8
[22] Wang W and Xu S 2007 J. Food Eng. 82 271–5
[23] Abdel-Moemin A R 2016 Food Sci. Hum. Wel. 8 01-08