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Optimisation of Anthocyanin Co-pigmentation from Butterfly Pea (*Clitoria ternatea*) Flower and its Application in Gummy

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ABSTRACT

In contrast to natural food colours, food manufacturers have increasingly used synthetic food colours to achieve attributes such as low cost, excellent appearance, high colour intensity, increased colour stability, and consistency. Furthermore, natural colourants such as anthocyanins have been linked to potential health advantages such as dietary antioxidants. Pea Flower (*Clitoria ternatea)* was utilized in this research because the high quantity of polyacylated anthocyanins known as ternatins in blue pea flowers which is a distinctive property of anthocyanins found in blue pea flowers. The purpose of this research is to improve anthocyanin thermal stability via co-pigmentation process from Butterfly Pea flower and to analyse the physicochemical features of gummy. The potential of Response Surface Methodology (RSM) for optimising anthocyanin co-pigmentation from Butterfly Pea (*Clitoria ternatea*) flower was investigated in this study. The effect of two test variables on the half-life of anthocyanin was studied in a specific range of pH 3-6 and anthocyanin to metal ratio (1:1 to 1:100). The data from the experiment were analysed using the RSM of MINITAB Software (Version 19), and the optimum half-life of anthocyanin of 191 minutes was established and verified. The optimal conditions were stated to be pH 3.75 and an anthocyanin:metal ratio of 1:75. A significant regression equation or model with a correlation value of 95.38% was also achieved at the 5% level. For the application of gummy, three types of gummies (synthetic blue incorporated gummy (F1), anthocyanin incorporated gummy (F2) and co-pigmented anthocyanin incorporated gummy (F3)) were produced to analyse its physicochemical qualities. The physicochemical qualities of F3 gummy were reported to retain the physicochemical since the pH values, water activity, moisture content, and textural properties were not significantly different ($p>0.05$). However, due to the % difference in polymeric colour present, the colour in terms of hue angle was noted to have a significant difference between F1, F2, and F3.

Keywords: Co-pigmentation, Natural colouring, Butterfly pea, Response surface methodology (RSM),Anthocyanin

INTRODUCTION

An important organoleptic feature that directly influences customer's approval and food choice is colour. In an attempt in maintaining or restoring the product colour consistency, food colouring pigments are commonly found in commercially available foods since they are unstable and can distort after processing (Renita et al.,

2023). However, synthetic food colourings, despite their widespread use, are increasingly being replaced by those derived from natural sources because of their possible health risks. This is because plant-based food colourings have greater organoleptic features and are healthier because of their higher quality and better organoleptic properties (Silva et al., 2022).

Consequently, numerous studies were done to generate safer and more effective food colourings as the need for naturally derived and plant-based food ingredients to replace synthetic additives increased over time. According to Md Zaki et al. (2020), anthocyanins, betacyanins, and carotenoids are some of the most researched plant-based pigments. This research solely focuses on the pigment of anthocyanins deriving from *Clitoria ternatea*, commonly known as Butterfly Pea which can be found in great profusion in most Asia tropical countries including Malaysia (Jamil et al., 2018). Previous studies have revealed that high antioxidant content of anthocyanins may act as defence mechanism against various degenerative diseases such as cancer and cardiovascular diseases as reported by Keppler and Humpf (2005). In addition, research suggests that anthocyanins act as an anti-aging agent, protects cells from harm, and promotes healthy eyes (Jamil et al., 2018).

In general, conventional extraction methods necessitate a longer extraction time and carry a high risk of heat destruction of specific bioactive chemicals (Zardo et al., 2019). Because of their highly reactive nature and low stability to food processing conditions, anthocyanins are readily destroyed (Li et al., 2021). However, a technique used in this research known as maceration is a low-cost and straightforward technique that does not introduce traces of organic solvent into the intended output. Additionally, the extraction of blue dye from *Clitoria ternatea* using the maceration process can be adjusted to provide optimal circumstances, such as a shorter extraction time and a lower extraction temperature with a higher extraction yield. Since thermal processing employed in the food industry tends to degrade anthocyanin, numerous studies have proposed various pre- treatments to boost the extraction yield and stability of such colour pigments. It has been claimed that chemical pre-treatments can increase the pigments' stability by associating them with co-pigments such as flavonoids, alkaloids, amino acids, organic acids, metals, phenolics, and anthocyanins increases their stability.

The creation of fortified foods is the focus of current developments in the food industry. Natural pigments, which have certain functions and health benefits, are replacing artificial colourings in food preparation (Crupi et al., 2018). Gummy candies have become a suitable way to deliver functional pigments in food. Many consumers, from young children to the elderly, appreciate their aesthetic appeal. Furthermore, as opposed to the instability of pigments in an emulsified medium, gummy candies provide a system that is appropriate for the palatability and stability of pigment during storage (Otálora et al., 2019). The purpose of these co-pigments is to protect anthocyanins (flavylium ion) from the nucleophilic addition of water; otherwise, the flavalium ion would become pseudobase or colourless (Castañeda-Ovando et al., 2009). The aim of this study is to optimise co-pigmentation of anthocyanins from Butterfly Pea flower by using Response Surface Methodology (RSM) and physicochemical properties of gummy incorporated with Butterfly Pea flower colour.

MATERIALS AND METHODS

Materials

Fresh Butterfly Pea flowers were picked from a home-owned local garden in Dengkil, Malaysia. The Butterfly Pea flowers were thoroughly cleaned, and the green parts of sepal were cut and removed. Solvents and chemicals used in this experiment were of analytical grade and obtained from Evergreen Engineering and Resources and FC-Bios Sdn. Bhd., Malaysia.

Preparation of Butterfly Pea flower

The Butterfly Pea flowers were rinsed with distilled water to remove any debris and impurities. The flower samples were then kept in airtight glass containers and were frozen for 3 days prior to freeze drying process. They were then dried by freeze drying to remove water content for 5 days. The dried flowers were grinded by

using a mechanical blender to form powdered samples. The grinded samples were kept in airtight glass containers covered with aluminium foil to prevent exposure to the high humidity and light from the surroundings.

Extraction process

Maceration technique was used for the extraction of blue dye from the powdered samples. 2.5 g of Butterfly Pea Flower (BPF) powder was added into 50 mL dH_2O, and it was swirled by using an incubator shaker for 30 minutes at 25°C. The mixture was then centrifuged by a centrifuge machine for 30 minutes to separate the anthocyanin extract from the powder. The obtained strained and the press out liquid was further separated from unwanted impurities and residues by filtration. The liquid was then dried by freeze drying for 5 days to remove the water content. The solid yield of anthocyanin extract had been obtained.

Experimental design

A central composite design (CCD) consisting of 13 experimental runs with five replications at the central points were employed in this study using Response Surface Methodology (RSM). The experimental design was used to optimize co-pigmentation of anthocyanin independent variables namely pH (X1) and anthocyanin: metal ratio (X2). Half-life anthocyanin was measured as responses to the independent variables. The creation of design matrix, experimental data analysis and optimization were all commenced using Minitab statistical software version 19. All the design points were performed three times except the centre points. The experiments were run in randomized order to minimize the effect of unexplained variability induced by extraneous factors.

Table 1. The coded and uncoded values used in RSM for the optimization of anthocyanin co-pigmentation.

Factors	Codes	Levels				
		-α	-1	0	+1	+α
pH	X_1	3	3.75	4.5	5.25	6
Anthocyanin: Metal ratio	X_2	1:1	1:25	1:50	1:75	1:100

Calcium, the selected metal of choice as a co-pigment agent in this study was applied into response surface methodology to optimise the co-pigmentation condition on the stability of anthocyanin. The first factor which is the pH of Anthocyanin extract was adjusted between 3 - 6 by citric acid. With the co-pigment agent, second factor which is the ratio of anthocyanin-metal complex was added into the anthocyanin at the anthocyanin-metal ratio ranging from 1:1 to 1:100.

The samples were then mixed by vortex mixer and agitated in a water bath shaker at 25±1°C for 30 minutes. The thermal stability of co-pigment complexation was determined at 100°C. After heating for 0 (control), 5, 10, 20, 40 and 60 min, the samples were rapidly cooled in ice water for 5 minutes. Total anthocyanin content was then determined by pH-differential method. Stability of anthocyanins was reported in terms of degradation rate and half-life.

RSM verification process

The optimum condition predicted by the Minitab statistical software version 19 was used to repeat the co-pigmentation process. The verified value must be testified to be no significant difference at 5% level when compared to the predicted value of half-life anthocyanin by using independent t-Test SPSS Statistical Software version 20.

Preparation of gummy

First, gelatin powder was soaked in water for 30 min, after that samples with gelatin were melted by heating for 15 min at 60°C. Sugar following glucose syrup and blue colouring were added and dissolved in the mixture under heating. Obtained mixture with agar was further heated to 90°C under stirring. Citric acid was incorporated into gummy mass at the end of the process (boiling state). Obtained mass after mixing was poured into mould, and gummy was set at 22–24°C for 24 h to get a gel-hard form (Lele et al., 2018).

Three types of gummies were produced which are synthetic blue incorporated gummy (F1), anthocyanin incorporated gummy (F2) and co-pigmented anthocyanin incorporated gummy (sample). All three types of gummies were subjected to physicochemical analysis.

Physicochemical analysis on Gummy

The moisture content, water activity (aw), colour, pH, as well as texture profile analysis (TPA) of gummy incorporated with Butterfly Pea extract were determined for the physicochemical analysis.

Moisture Content

The moisture content of gummy candies was analysed by using forced draft oven method. The principle applied where sample was heated under specified conditions and the loss of weight was used to calculate the moisture content of the sample. First, aluminium cans with covers were dried in an oven at 105℃ for 3 hours. The empty weight of the oven-dried aluminium cans was measured. Then, 5 g of homogenised gummy samples were transferred into the aluminium cans and weighed. The samples were oven-dried at 105°C for 39 5 hours. The aluminium cans were then transferred into a desiccator and weighed quickly as soon as they cooled. The oven-drying process was repeated until the constant weight was observed (AOAC, 2020). The aluminium cans containing the dried samples were weighed after attaining room temperature. The percentage of moisture was calculated by using the formula:

$$Percentage\ of\ mositure\ (\%) = \frac{W1-W2}{W1} \times 100$$

where: W1 = Weight of sample before drying (g), W2 = Weight of sample after drying (g)

Water activity (aw)

Water activity analysis was conducted based on a method used by Suriya et al. (2017), in which the measurement was done by using AQUA LAB Series 4TE Water Activity Measuring Unit. The analysis was conducted by weighing about 2 g of the sample which then be placed into the sample container. The sample was spread evenly to cover the whole bottom part of the container. The container was placed in the machine to be read. The level of water activity (aw) was taken at 25℃.

Colour Analysis

The colour of gummy candies was measured by using Konica Minolta CR400 Chroma Meter based on the method adapted from Romo-Zamarrón et al. (2017). The colour analysis was conducted on the gummy 24 hours after production. The gummy candies were determined based on the CIE L*, a*, b* colour coordinates which L* is referring to lightness, a* is redness to greenness, b* is yellowness to blueness. The chromameter was set to be using D-65 lighting, a 2° standard observer angle and a light beam diameter of 8 mm. Before the analysis, the chromameter was calibrated by using a standard white plate first. Then, the colour of the gummy sample was being determined. Hue angle of the gummy candies was determined by incorporating the colour coordinates values according to formula as follows: h_{ab} = arctan (b*/a*)

pH Analysis

The pH analysis was used to determine how acidic or basic a compound was when dissolved in water (Vijayakumar & Adedeji, 2017). A pH meter was used to measure the pH of the gummy samples. The meter was calibrated initially with buffer solutions of pH = 7. The samples then were sliced into thin slices, combined with hot water (1:3, w:w), and repeatedly swirled until completely dissolved in water. About 5 g of samples were dissolved in 15 mL of hot water. Before measuring the pH, the hot solution was tempered at 25°C. All sample measurements were done in triplicate.

Texture Profile Analysis (TPA)

The textural analysis of the gummies was done by using STable Micro Systems TA.XTplus Texture Analyser together with the "Texture Exponent 32" software, this method was adapted based on a study by Kurt et al. (2022). The hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness of the gummy samples were measured at room temperature. The aluminium compression platen (P/75) which has a 75 mm diameter was used. Using a 50 kg load cell and a trigger force of 0.05 N, the test was carried out by measuring the force on compression. The pre-test, test, and post-test speeds were all equal to 0.5 mm/s, and the compression distance was 5 mm. There are two compression cycles carried out, separated by 5 s. For each sample, the measurements were triplicate.

RESULTS AND DISCUSSION

Optimisation of Anthocyanin Co-Pigmentation by Response Surface Methodology

In this research work, anthocyanin was extracted from Butterfly Pea flower. The impact of pH and anthocyanin: metal ratio on anthocyanin co-pigmentation condition were optimized by response surface methodology.

Optimisation of anthocyanin co-pigmentation condition

In this present study, response surface methodology was used to determine the optimum condition for the factors affecting anthocyanin co-pigmentation. The effect of the two (2) independent variables of X_1 (pH) and X_2 (anthocyanin: metal ratio) using five levels CCD on the half-life anthocyanin were determined using MINITAB software version 19.

The treatments with their respective actual variables level combinations and the obtained response were listed in **Table 2** for half-life anthocyanin. The **Table 2** showed the values of actual (experimental) and predicted response of each run. The highest actual and predicted half-life were 191 min and 185.20 min, respectively under specific condition of variable factors (pH of 3.75 and anthocyanin: metal ratio of 1:75), whereby the actual half-life anthocyanin showed a slight difference from predicted value. On the other hand, the lowest actual and predicted half-life also displayed slight differences, which were 79 min and 83.81 respectively at the predetermined variable factors condition (pH of 5.25 and anthocyanin: metal ratio of 1:25).

Table 2. Factors and comparison between actual (Y) and predicted (FITS) responses.

Run No	Factors		Responses	
			Half-life Anthocyanin (min)	
	X_1	X_2	Y_1	FITS 1
1	3.75	1:25	100	96.07
2	5.25	1:25	79	88.55
3	3.75	1:75	191	185.20
4	5.25	1:75	110	117.68
5	3.44	1:50	137	144.66
6	5.56	1:50	103	91.59
7	4.5	1:14.64	87	83.81
8	4.5	1:85.36	168	167.44
9	4.5	1:50	128	132
10	4.5	1:50	132	132
11	4.5	1:50	140	132
12	4.5	1:50	125	132
13	4.5	1:50	135	132

Note: X_1 = pH, X_2 = Anthocyanin:Metal ratio, Y_1 = Actual Half-Life Anthocyanin (min), FITS 1 = Predicted Half-Life Anthocyanin (min)

A regression analysis was carried out to fit mathematical models to the experimental data aiming at an optimal region for the response studied. By applying multiple regression analysis, the empirical relationship between the input variables and the response variable can be expressed in the following quadratic, second-order polynomial equation (Equation 1) in terms of uncoded values:

$$\text{Half-life anthocyanin} = -257 + 126.0X_1 + 5.29X_2 - 12.33X_1X_1 - 0.00510X_2X_2 - 0.800\,X_1X_2 \qquad \textbf{Eqn. 1}$$

From Equation 1, it was observed that the linear terms for pH and anthocyanin: metal ratio had positive effects on half-life anthocyanin (Y_1). In the quadratic terms X_1^2 and X_2^2 demonstrated negative effects on response Y_1. As for the interaction terms, X_1*X_2 had negative effects on the response Y_1.

Analysis of Variance (ANOVA)

The effect of experimental variables on the linear, quadratic and interaction terms were tested for adequacy and fitness by analysis of variance (ANOVA). The summary of the results obtained is shown in **Table 3**. By using lack-of-fit and coefficient determination (R^2), the adequacy of the model can be revealed. The significance of the equation parameter for test variables was assessed by an F ratio at a probability (p) of 0.05. The F value predicts the quality of the entire model while considering all design factors all at once.

Table 3. ANOVA of multiple regression models for the response variables.

Source	Degree of freedom	Adjusted sum of square	Adjusted mean square	F-Value	P-Value	Status
Regression	5	11081.9	2216.37	28.92	0.000	Significant
Linear	2	9810.2	4905.09	64.01	0.000	Significant
Square	2	371.7	185.84	2.43	0.158	Not Significant
2-Way Interaction	1	900.0	900.00	11.74	0.011	Significant
Error	7	536.4	76.63			
Lack-of-Fit	3	398.4	132.81	3.85	0.113	Not Significant
Pure Error	4	138.0	34.50			
Total	12	11618.3				
Half-life Anthocyanin (R^2 = 95.38%; Adjusted R^2= 92.08%)						

ANOVA is technique used to investigate the design parameters and to indicate which parameters are significantly affecting (contributing) the output parameters. In the analysis the sum of squares and variance are calculated. At a confidence level of 95%, the analysis of variance (ANOVA) indicates that the empirical model for the half-life of anthocyanin provides accurate predictions. The significance of each term at a confidence level of 95% was evaluated by the P-value. The P-value is the probability that the factors have no or an insignificant effect on the response. A greater F-value indicates that the RSM model fits the experimental data better. According to Baskaran et al. (2019), an insignificant lack of fit suggests a good model. The F-value for the lack of fit can be obtained by dividing the mean square of the lack of fit by the mean square of the pure error. The selected model in this study yielded a non-significant outcome ($p>0.05$) with a P-value of 0.113. Therefore, it suggests that the model is reliable and fits the experimental data well (Siti Roha et al., 2022). These F-values could be used to reject the null hypothesis. In addition, the two factors were highly significant to the regression model at 5 % level of significance. The adequacy of the models was justified through analysis of variance (ANOVA) and both independent variables (pH and anthocyanin: metal ratio) contribute significantly to the model. It was found 48 that model, linear, and 2-way interaction of test variables gave significant effect in the half-life of anthocyanins because $p < 0.05$ as shown in **Table 3.**

R^2 and R^2 (adjusted) are values derived statically that are used to assess the compatibility of the predicted model with the experimentally observed data. Consequently, the goodness of fit of the model is determined by the value of the determination coefficient, R^2, which illustrates the variability of observed values relative to their mean. R^2 is a criterion evaluation in which the correctness of the model in explaining the model is evaluated by its R^2 value. The closer the R^2 value is to 100% shows that the model will give better predicted values which are closer to the actual values for the response (Khor & Ramakrishnan, 2016). In addition, the empirical model must have an R^2 value of at least 75% to satisfactorily explain the majority of the variables. At the 5% significance level, a regression equation or model with a correlation value of 95.38 % was obtained. The highest predicted response was 191 minutes, as shown in **Table 3**. The correlation coefficient, R^2 = 95.38 %, which suggests that the experimental results might be accepted, indicating that just 4.62% of the total fluctuations are not explained by the model. This also indicates that the two independent variables account for 95.38% of the total variation.

A numerical response optimization technique was applied to determine the optimum condition of pH, and anthocyanin: metal ratio for the half-life of anthocyanin (**Table 4**). It was found that the optimum conditions for the target goal with a pH of 3.75 and anthocyanin: metal ratio of 1:75 were feasible to be carried out. Meanwhile, the optimum condition for the maximum and minimum goal was not feasible to be carried out.

Table 4. Comparison values of target and predicted responses for different optimum conditions and experiment feasibilities.

Goal		Lower	Target	Upper	Optimum condition		Predicted Responses (FITS 1)	F/NF
					X_1	X_2		
Target	**Half-life Anthocyanin (min)**	79	190	191	3.75	1:75	185.20	F
	FITS 1	83.81	185.19	185.20				
Maximum	**Half-life Anthocyanin (min)**	79	191	191	3.44	1:85	210.10	NF
	FITS 1	83.81	185.20	185.20				
Minimum	**Half-life Anthocyanin (min)**	79	79	14.10	3.44	1:15	66.46	NF
	FITS 1	83.81	83.81	14.12				

Where: X_1 = pH, X_2 = Anthocyanin:Metal ratio, FITS = predicted response (%), F = feasible, NF = not feasible

Surface plots

Surface plots for half-life anthocyanin at a feasible optimum condition are shown in Figures 1 and 2, respectively. The three-dimensional surface plot and two-dimensional contour plot show the effect of the feasible optimum on the pH and anthocyanin to metal ratio determined in the half-life of anthocyanin. Three dimensional graphs were generated for the pair-wise combination of the two test variables. Figures 1 and 2 highlight the roles played by the test variables and comparison between test variables. Figure 1 shows an elliptical contour plot which indicates there is a significant interaction effect between pH and ratio whereas Figure 2, the surface plot shows that the half-life of anthocyanins increased in the middle level of pH and ratio.

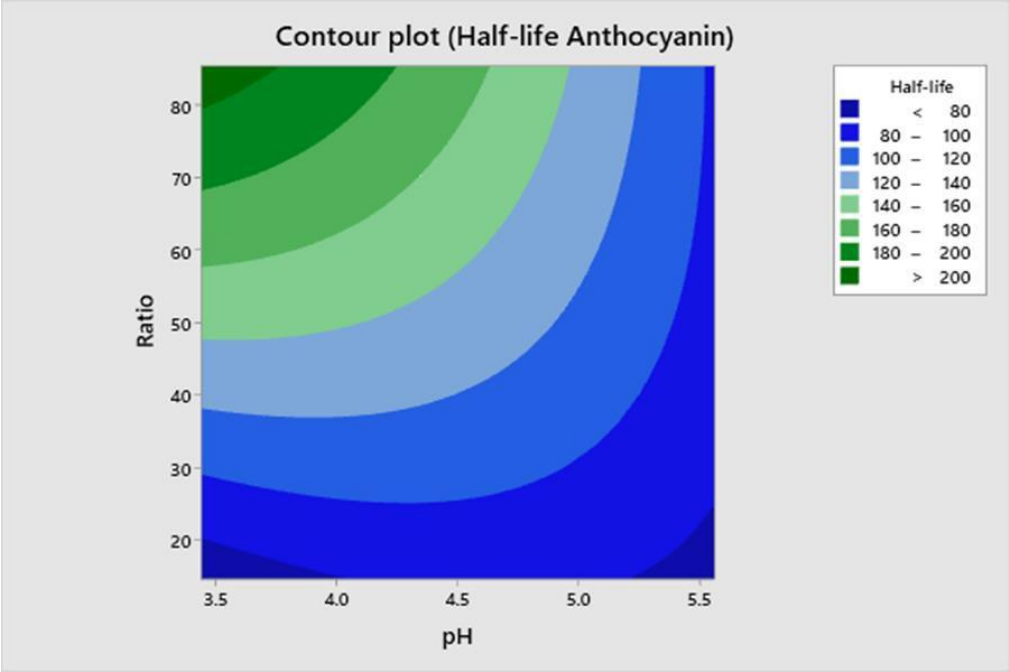

Fig. 1 Contour plot of half-life anthocyanin at optimum condition

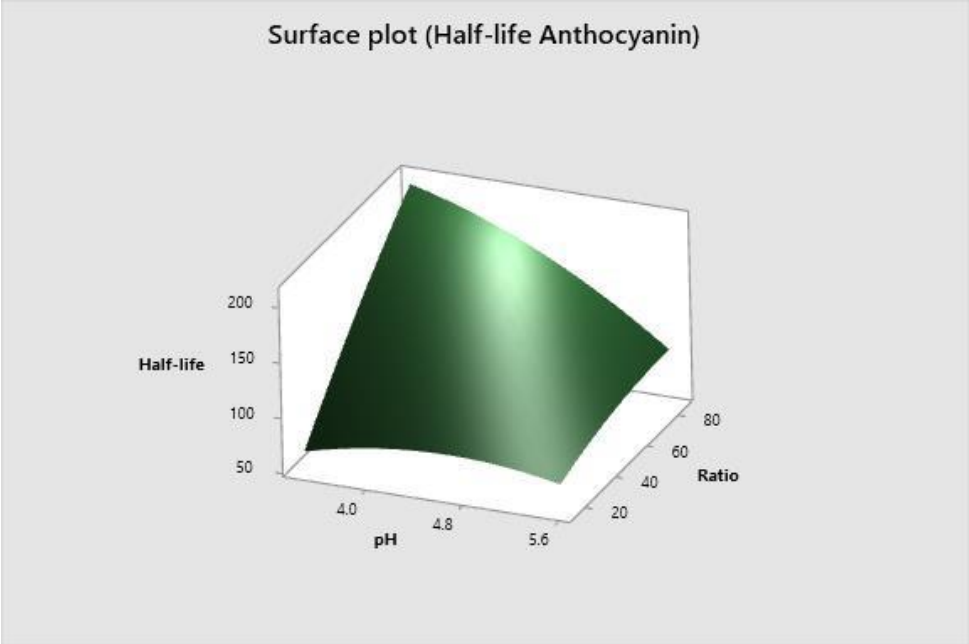

Fig. 2 Surface plot of half-life anthocyanin at optimum condition.

The optimum condition obtained from RSM was at pH 3.75 and anthocyanin:metal ratio of 1:75. The half-life of anthocyanins increased with the addition of co-pigment agent, calcium ion Ca2+ at a higher ratio in lower pH. Charurungsipong et al. (2020), reported that an increase in co-pigment ratio could slow down the rate of anthocyanin degradation. The low pH value of 3.75 and high ratio of 1:75 for the optimum condition may be supported by a study by Maylinda et al. (2019) where the authors mentioned that at low pH, the flavylium cationic anthocyanin was very dominant and the most stable. In acidic solution, the anthocyanins with metal co-pigment exist as four main structures, namely quinonoidal, the flavylium cation, the pseudobase or carbinol, and chalcone species (Houghton & Martin, 2021). In addition, a low pH increases the solubility of the metal, allowing it to bind more effectively with the hydroxyl group of anthocyanin. Nevertheless, according to Tang et al. (2019), increasing the pH can reduce the colour of 53 anthocyanins because, at high pH, the main structure of anthocyanin will be in the form of carbinol, which has lost its conjugated bond and thus does not absorb visible light. In accordance with Wahyuningsih et al. (2017), the metal ion chelation by anthocyanin was optimised at pH 3.75, resulting in the highest anthocyanin stability was proven.

Validation for the optimum condition of anthocyanin co-pigmentation of half-life anthocyanin was performed. The suitability of the model equation for predicting the optimum response value was evaluated for the optimum condition of anthocyanin co-pigmentation under conditions where the pH was 3.75 and anthocyanin: metal ratio was 1:75. The validation result obtained for half-life anthocyanin was 188 min. Optimization using actual experimental values was tested using the t-test (SPSS) (Siti Roha et al., 2022). There was no significant difference ($p > 0.05$) between predicted and verified values. Thus, it indicated that the model was significant and can be used to predict the optimization of anthocyanin co-pigmentation.

Physicochemical properties of gummy

The physiochemical characteristic of gummy was compared and evaluated based on its colour, content of moisture, water activity, pH and textural properties (**Table 5**). Both anthocyanin and co-pigmented anthocyanin incorporated gummy candies are acidic products, the values of colour obtained for F2 and F3 may be supported

by the fact that the colour of the anthocyanins in Butterfly Pea flower extract varies with pH as follows: red at pH less than 3.2, violet to blue at pH 3.2 to 5.2, light blue at pH 5.2 to 8.2, and dark green at pH 8.2 to 10.2. (Sutakwa et al., 2021). The reason for this colour change is due to structural changes in anthocyanin molecules caused by changes in hydrogen ions and the concentration of hydrogen ions in the medium. The presence of ion flavylium causes the red hue, ionic chalcone causes the green colour, and the neutral quinoidal base causes the blue colour (Salacheep et al., 2020).

However, it is worth noting that the values of hue angles for all samples are significantly different. F2 had a significantly higher hue angle value of 284±0.02 followed by F3 with a value of 273±0.01 and 220±0.04 reported on F1. As seen in Figure 4.6, F1 showed a bright colour of blue-green whereas F2 and F3 showed a shade closer to blue-purple. According to the hue values obtained, F1's value falls in the range of 198-234 (blue-green), F2 and F3 are within the range of 270-306 (blue-purple) as reported by Nikijuluw and Andarwulan (2013). Hue of Butterfly Pea anthocyanin extracts showed blue-purple color whereas synthetic blue showed blue-green colour, and this is because synthetic blue contains percentage of polymeric color higher than Butterfly Pea anthocyanin extracts (Nikijuluw & Andarwulan, 2013).

Table 5. Physicochemical properties of gummy incorporated with Butterfly Pea flower colour.

Assay	Formulation		
	F 1	**F2**	**F 3**
Colour			
L	43.32 ± 0.04^{c}	46.68 ± 0.01^{b}	47.74 ± 0.01^{a}
a	7.63 ± 0.04^{a}	3.48 ± 0.02^{b}	3.28 ± 0.01^{c}
b	-9.17 ± 0.05^{c}	-5.77 ± 0.02^{b}	-4.95 ± 0.01^{a}
h	220 ± 0.04^{c}	284 ± 0.02^{a}	273 ± 0.01^{b}
pH	3.83 ± 0.01^{a}	3.81 ± 0.01^{a}	3.81 ± 0.01^{a}
Water activity (Aw)	0.71 ± 0.00^{a}	0.72 ± 0.00^{a}	0.73 ± 0.00^{a}
Moisture (%)	17.04 ± 0.11^{a}	16.99 ± 0.04^{a}	17.11 ± 0.04^{a}
Textural Properties			
Hardness	7653.75 ± 430.35^{a}	7220.73 ± 300.15^{a}	7507.48 ± 178.97^{a}
Adhesiveness	-18.86 ± 0.35^{a}	-18.48 ± 0.41^{a}	-18.78 ± 0.20^{a}
Springiness	0.96 ± 0.03^{a}	0.96 ± 0.03^{a}	0.99 ± 0.01^{a}
Cohesiveness	0.89 ± 0.01^{a}	0.89 ± 0.02^{a}	0.87 ± 0.01^{a}
Gumminess	5817.05 ± 156.33^{a}	5433.15 ± 386.29^{a}	5584.02 ± 253.20^{a}
Chewiness	4451.55 ± 375.46^{a}	4214.41 ± 319.46^{a}	4326.60 ± 132.14^{a}

Note: F1 = synthetic blue incorporated gummy, F2 =anthocyanin incorporated gummy, F3 = co-pigmented anthocyanin incorporated gummy. Data were written in mean±standard deviation (n=3).

Based on the results presented in **Table 5**, it can be identified that there was no significant difference in pH, water activity and moisture content between the gummy candies. The pH values for all samples were reported to be in the range of 2.0 to 3.5, which is the most favourable acidic condition in gummies as stated by Sumonsiri et al. (2021). Additionally, the low pH value was due to the presence of citric acid added for optimal gelation in the production of gummy (Williams et al., 2006). The reported ranges for water activity and moisture content for all samples were 0.50 to 0.75 and 8 to 22%, respectively (Ergun et al., 2010; McGill & Hartel, 2020). In general, the water content of sugar-based confections is determined by the boiling point relationship of the sugars contained in the formulation. The ultimate water content has a substantial effect on the texture of gummy candies, with more water activity and moisture content resulting in softer candies that are often preferred by consumers (Ergun et al., 2010; McGill & Hartel, 2020). Therefore, the addition of different colourings in this study retained the pH values, water activity and moisture content of gummy.

The mechanical properties of texture, specifically hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness, can be quantified. The texture of gummy candies is important since it determines their chewability. Furthermore, when it comes to the chewability of a gummy, each consumer has distinct expectations. The gummy samples showed small scale of hardness and high springiness (4.15±2.74) as shown by F1, F2 and F3, which are all preferred as they could give a good chewiness (4451.55 ± 375.46, 4214.41 ± 319.46 and 4326.60 ± 132.14 respectively) property (Ahmad et al., 2022). The preferable gummies, according to Mahat et al. (2020), should also exhibit good cohesiveness and springiness. These properties were noticed in all coloured formulated gummy candies. The springiness appeared to be 0.96 to 0.99, while the cohesiveness was between 0.87 and 0.89. In addition, the adhesiveness of the desired gummies should be as low as possible, since a high adhesiveness implies the possibility that they will adhere to the teeth, palate, and tongue. According to the provided data, all colourings have low adhesiveness and are therefore suitable to produce gummies (Sumonsiri et al., 2021).

CONCLUSION

Anthocyanin co-pigmentation of Butterfly Pea achieved higher half-life anthocyanin at an optimum condition by using pH of 3.75 and in an anthocyanin to metal ratio of 1:75. The incorporation of stabilised co- pigmented anthocyanin as a natural colouring will produce gummy with desirable physicochemical properties since there was no significant difference in terms of pH, water activity, moisture content and textural properties. This co-pigmented blue colour can be an alternative for synthetic blue colouring used in the food industry, and it may also be a safer option for consumers due to its minor difference in their physicochemical properties.

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