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Research Article

Assessment of color stability and the functional properties of food colorant developed using *Clitoria ternatea* flowers

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Abstract

Color is one of the most important organoleptic properties of food which can influence on consumer preference. Due to health awareness, there is an increasing demand for natural food colorant over synthetic counterparts. Butterfly pea (Clitoria ternatea) flower is remarkable as one of the most vital anthocyanin sources that gives characteristic blue color. Present research study focused on the identification of a best method to extract anthocyanin from Clitoria ternatea flower without destroying its functional properties. Accordingly, 3 methods: direct extraction (T1), blanching extraction (T2) and blanching extraction with addition of natural preservative (T3) were selected to extract anthocyanin from C. ternatea flower. The functional properties were assessed using standard methods. The sensory evaluation was carried out to assess the color, taste, aroma and overall acceptability using a nine-point hedonic scale. Food colorant extracted from T2 had higher flavonoid content (173.97 ± 7.8 mg/L) and total anthocyanin content (67.6 ± 29 mg/L) compare to the other two treatments. However, sample from T1 contained strong antioxidant properties since it had the least IC50 (44.14 ± 2.9 %) while showing highest phenolic content (48.49 ± 4.0 mg/L). Out of the three treatments, food colorant from T3 had higher color stability, higher shelf life (28 ± 2 days) and was highly accepted in terms of overall acceptability and color by sensory panelists. Therefore, it could conclude that food colorant gel from T3 is the best alternative for artificial blue colorants which is a natural extract without any health concerns.

Keywords*: Anthocyanin, butterfly pea, food colorant gel, functional properties, natural food colors*

1. Introduction

Color is an important factor that enhances the appetizing value and consumer acceptance towards foods and beverages. In the food processing industry, development of food and beverages with an attractive visual appeal is an important factor to increase the consumers demand on quality (Rashid et al., 2021). Food

colorants are used in food processing to attract the consumer, to replace the color which is lost during food processing, regulates the color variation, decorative purposes and to increase the consumer acceptance of the food products.

Food colorants can be either natural or synthetic where synthetic colorants are chemical coal tar derivatives. Unfortunately, frequent usage of synthetic colorant leads to reduction of nutritional quality of foods. Therefore, many recent research findings proved that synthetic colorants were harmful or toxic substances to consume (Dilrukshi et al., 2019). Moreover, many of them have become toxic after prolonged use causing health problems such as indigestion, allergic reactions as asthma and urticaria, pathological lesions in the brain, tumors and cancer, paralysis, growth retardation and eye defects resulting blindness (Ashida et al., 2000).

Due to this limitation and worldwide tendency towards the consumption of natural products, the interest in natural colorants has increased considerably. Therefore, developing natural food colorants using locally available plants became a trend in present research studies. *Clitoria ternatea* is an underutilized, herbaceous flower in Sri Lanka and it has been known as a source of characteristic intense blue coloration due to presence of anthocyanins, which could be utilized as a natural coloring agent (Vankar and Srivastava, 2010). Butterfly pea has potential health benefits such as promoting cardio-vascular health and preventing hypertension, pyrexia and liver disorders, as well as a diuretic, digestive and sedative properties (Lakshan et al., 2019).

Recent research studies reported that butterfly pea is a good alternative source of natural antioxidants: delphinidin-3-5-glucoside, Phenolic compounds: anthocyanidins and Flavonoids: flavylium cation (AH+) (Rabeta and Nabil, 2013). Moreover, natural blue colorant from *C. ternatea* can be used to replace synthetic food colorant of brilliant blue (E 163). This study aimed towards the production of a natural blue colorant in both solid and liquid form from *C. ternatea* using an appropriate method to extract higher yield of anthocyanins while minimizing the degradation of phytochemical components of blue anthocyanin. Furthermore, through this study, expect to extend the shelf life of the natural food colorant using dehydration as a food preservation technique. In addition, this research was carried out to evaluate the functional properties of the natural blue colorant from butterfly pea flower and to incorporate an attractive, nutritional and healthy natural blue anthocyanin pigment to food products.

2. Material and Methods

2.1. Sample Collection and Sample preparation

Blue colored, fully bloomed, disease free, and undamaged healthy flowers of butterfly pea flower- double corolla type were collected from Badulla city, Sri Lanka

in January 2021. According to the herbarium chart, flower was authenticated as "*Clitoria ternatea"* from the reference book of "Flora of Ceylon" (Shaffer-Fehre, 2006). All flower samples were washed with distilled water to remove unwanted particles and to remove the dust. Then 30 g of fresh petals were weighed and stored at 4℃ in a refrigerator prior to further experiments, which were conducted not more than 3 days after harvest.

2.2. Extraction of water-soluble anthocyanin from *C. ternatea*

Three treatments were used in this experiment as direct method (T_1), blanching method (T_2) and blanching extraction with addition of preservatives (T_3). Three replicates were performed under each of the three treatments.

2.2.1. Direct extraction method (T_1)

The collected petals were cut into small pieces and soaked in 100 ml of distilled water for 30 minutes. Then, mixture was blended for 30 seconds and filtered through 2 layers of muslin cloth to remove any coarse particles. After that, the pH of the Butterfly pea extract was measured using a benchtop pH meter (Model: 3001-TRANS INSTRUMENTS). The filtered *Clitoria ternatea* extract was centrifuged (Model: PLC-025- K- GEMMYCO) at 4000 rpm for 10 minutes to remove the fine suspended particles. Extract was again filtered with Whatman No: 01 filter paper and the filtrate were evaporated using a rotary vacuum evaporator (Model: RE 5-Pro LABFREEZ INSTRUMENTS,) at 45℃ temperature. The clear concentrated extract was lyophilized using a Benchtop Standard Freeze Dryer (Model: LBFD-A11, Labtron Equipment Ltd.UK), the final weight of the sample was measured and stored in a desiccator until use.

2.2.2. Blanching extraction method (T_2)

Petals of *C. ternatea* (30 g) were heated with 100 ml of distilled water at 40℃ for 6 minutes. Then, extract was filtered through 2 layers of muslin cloth and pH of the sample was measured. Filtered sample was centrifuged (4000 rpm, for 10 min) using a centrifugation machine (Model: PLC-025- K- GEMMYCO). After centrifugation, the supernatant was separated through Whatman No: 01 filter papers, and this was followed by a lyophilization.

2.2.3. Blanching extraction method with the addition of preservatives (T_3)

The extraction procedure was performed similar to blanching method with a slight modification. The food grade colorant was formulated as follows. Petals (30 g) were heated at 40℃ about 6 minutes. Briefly, the sediment of the water extraction was collected and filtered through the muslin cloth. The mixture was then centrifuged (4000 rpm, 10 minutes) using a centrifugation machine. After centrifugation, the

supernatant was separated and filtrate was evaporated (rotary vacuum evaporator, at 45℃) to get the concentrated sample. The mixture was then heated up to 50℃ (6 min) with 15 g of sugar to increase the viscosity in order to form a colored gel. Finally, 1 ml of lemon juice was added into the colorant gel to avoid the sugar crystallization and prepared colorant was stored in an airtight container at 4℃ in a refrigerator (Model: Sisil-ECO192WR) until use.

2.3. Quantification of the Total monomeric anthocyanin pigment content

Monomeric anthocyanin content was measured using pH differential method according to the AOAC official method (Lee et al., 2005).

Two different buffers were prepared; potassium chloride buffer with a pH value of 1.0 and sodium acetate with a pH value of 4.5. Each treatment (T_1, T_2 & T_3) was diluted (1:4) with each pH 1.0 and pH 4.5 buffers separately. After adding the test portion to the buffer, pH was measured using pH meter to assure the required buffering action in the prepared solution. Then absorbance of the test portion was determined at both 520 nm and 700 nm with UV- Visible Spectrophotometer (Model: EVOLUTION 201, THERMO SCIENTIFIC). Then total monomeric anthocyanin content was expressed using following equation (Lee et al., 2005).

$$\text{Anthocyanin pigment (mg/L)} = \frac{(A \times MW \times DF \times 1000)}{\Sigma \times 1}$$

Where,

A= (Abs 520 nm – Abs 700 nm) pH 1.0 – (Abs 520 nm – Abs 700 nm) pH 4.5
MW= Molecular Weight (449.2 g/mol for cyanidine-3-glucoside)
DF= Dilution Factor
Σ = 26900 L/mol.cm molar extinction coefficient
1= path length (cm)
10^3= factor for conversion from g to mg

2.4. Yield Analysis

The yield of natural food colorant was analyzed according to the following equation in order to analyze the best extraction method.

$$\text{Yield} = \frac{\text{Final weight of lyophilized freeze dried sample (g)}}{\text{Initial weight (L)}}$$

2.5. Evaluation of Antioxidant Activity

The DPPH scavenging activity of *C. ternatea* extract was evaluated according to the method described in (Kungsuwan et al., 2014) with some modifications. 1 ml of

sample (T_1, T_2 and T_3) was added to 3 ml of DPPH solution. Then mixture was shaken thoroughly and incubated at 30℃ for 20 minutes in dark environment. The absorbance values of samples were determined at 517 nm using UV-spectrophotometer. DPPH radical scavenging activity was expressed as IC_{50} / The half maximal Inhibitory Concentration (Kungsuwan et al., 2014).

$$\text{Radical scavenging Activity\%} = \{\frac{(\text{AB control} - \text{AB sample})}{\text{AB control}}\} \times 100$$

Where; AB = Absorbance

2.6. Determination of Total phenolic content

According to the (Cheok et al., 2013), the total phenolic content (TPC) of the anthocyanin extract of *Clitoria ternatea* was measured colorimetrically using the Folin-Ciocalteu (FC) method. Gallic acid standard curve was obtained according to the (Siddiqui et al., 2017). Each crude anthocyanin extract was dissolved in 70% ethanol to obtain 60 mg/L solution. Then, 1 ml of tenfold diluted FC reagent was added to 200 µl of crude anthocyanin extract and kept for 8 minutes. Then 800 µl of 7.5% (w/v) Na_2CO_3 (aq) was added and again kept for one hour. Absorbance values were measured at 765 nm with UV- spectrophotometer.

TPC of each crude anthocyanin extract was calculated using the calibration curve and expressed as mg of Gallic Acid Equivalents (GAE)/g.

2.7. Determination of Total flavonoid content

Total flavonoid content was analyzed using the aluminum chloride colorimetric method (Cheok et al., 2013). Aliquot (1 ml) of anthocyanin extract was diluted with 3 ml of 70% ethanol and the mixture was allowed to react for 5 minutes. Then 200 µL of 10% $AlCl_3$, 200 µL of 1 M potassium acetate was added to the sample and the mixture was made to stand for 30 minutes further in the incubator. Finally, the reaction mixture was diluted with 5 ml of distilled water and the absorbance at 420 nm was recorded against the blank sample. TFC was calculated using the Catechin standard curve and expressed as mg catechin equivalents per gram of sample.

2.8. Evaluation of the color

Color of the natural food colorant sample was determined using a colorimeter (Model: PCE-RGB 2).

2.9. Evaluation of the color stability index

Color stability of the anthocyanin extracts obtained from T_1, T_2 and T_3 were analyzed for the natural food colorant incorporated king coconut beverage.

2.9.1. Effect of light on color stability

Effect of light on colorant stability was performed with samples (T_1, T_2 and T_3) inside the sealed glass bottles in day light condition. Samples were incubated at a temperature of 25℃ at predetermined time intervals (0/initial, after 7, after 14 days) in order to identify the color stability according to the light and dark condition. Then 5 ml of the samples were filtered and all the samples were assayed via UV-VIS Spectrophotometer (EVOLUTION 201, THERMO SCIENTIFIC) at 575 nm. Finally, the color stability index of the sample was calculated using the following formula (Roobha et al., 2011).

$$\text{Color stability index} = \frac{\text{Absorbance on sampling day}}{\text{Absorbance on Initial day}}$$

2.9.2. Effect of temperature on color stability

The color stability index of the anthocyanin extract obtained from T_1, T_2 and T_3 was evaluated after incorporating it to the king coconut beverage at different temperatures (4℃, 25℃) during predetermined time intervals (0/initial, after 7, after 14 days). The UV-VIS spectra were recorded for these three samples at 575 nm. The color stability index was calculated and expressed using the following formula (Roobha et al., 2011).

$$\text{Color stability index} = \frac{\text{Absorbance on sampling day}}{\text{Absorbance on Initial day}}$$

2.10. Shelf-Life Evaluation

According to (Yousuf et al., 2016) antibacterial and antifungal assay was performed for all the samples obtained from three extraction methods (T_1, T_2 and T_3).

$$\text{Colony Forming Units/ ml} = \frac{\text{Number of colonies} \times \text{total dilution factor}}{\text{volume of culture plated in ml}}$$

2.11. FTIR Analysis

The absorption spectra of extracted blue pigment samples (T_1 & T_2) were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with ATR. (ALPHA, PN:1005151/07, SN:201113) in the wavenumber range of 3996.73 cm^{-1} and 498.30

cm^{-1}. Then, background spectra were collected after each sample scanning and automatically deducted from sample spectra. An OPUS Spectroscopy software version 7.5 which is the leading spectroscopy software for state of the measurement, processing and evaluation of IR/FTIR, NIR and Raman Spectra was used for FTIR data analysis of the *Clitoria ternatea* natural food colorant sample (Johnson et al., 2022). The change in intensity and position in the characteristic peaks of different regions of the FTIR spectrum was used to determine the quality of the extracted food colorant powder in each two different extraction methods (T_1 & T_2).

2.12. Sensory Evaluation

Sensory evaluation was conducted for king coconut beverage samples, incorporated with colorant extracted from *C. ternatea*. The color, taste, aroma and overall acceptability were evaluated using a 9– point hedonic scale. Thirty untrained panelists were involved to evaluate the samples from different treatments which were arranged randomly to assess their organoleptic qualities.

2.13. Statistical analysis

All the statistical analyses were conducted using Minitab software 17 version. One-way ANOVA was employed to assess the statistical significance of the experimental data with Tukey's pairwise comparison to determine the differences among the treatments at the significance level of 0.05.

3. Results and Discussion

3.1. Extraction of anthocyanins from *Clitoria ternatea* petals

During the direct extraction method, anthocyanin was highly soluble in water and easily extracted from *C. ternatea* petals. According to (Rabeta and Nabil, 2013) the extractive value (w/w %) of *C. ternatea* is higher in water (18.21%) compared to its extractive value in alcohol (16.14%). Due to the high yield, direct extraction method was considered as the best convenient method. With that reasoning, aqueous extraction methods were utilized for the present research study.

Natural food colorant obtained by T_1 and T_2 was freeze dried as a food preservation method in order to produce powdered form to extend the shelf life of the product.
Colorant extracted from *C. ternatea* being a natural pigment which is in liquid form, it has low shelf life. Therefore, as another preservation method, colorant gel was produced using sugar and lime as natural food preservatives for T_3. Since, anthocyanin pigment is highly stable in acidic aqueous solutions, a low pH or acidic condition was maintained (Khoo et al., 2017).

3.2. Final crude yield comparison

Higher crude yield of 6.48 g/L ± 0.1 was reported in T_2 and lower crude yield of 3.84 g/L ± 0.1 was reported in the T_1. Accordingly, the blanching method seems to yield twice as much as the direct method as shown in Table 1.

Table 1: yield of the colorant extracted from *C. ternatea* flower

Method	Yield g/L
Direct extraction method	3.85 ± 0.1^{b}
Blanching extraction method	6.48 ± 0.1^{a}

Note: Means with different superscript letters differ significantly ($p < 0.05$)

3.3. Estimation of total monomeric anthocyanin content (TAC)

The monomeric anthocyanin content of butterfly pea extract is shown in the Table 2. The results of this study showed that, there is no any significance difference between anthocyanin content of different extracts from *C. ternatea* ($P > 0.05$).
In *C. ternatea*, highest anthocyanin content showed by the blanching extraction method (67.6 ± 29.0 mg/L) followed by the direct extraction method (59.95 ± 5.03 mg/L).

Increase of temperature may affect the polar solvents where total anthocyanin content of colorant gel (T_3) reported as 25.0 ± 5.03 mg/L might be due to the involvement of high temperatures of 50℃ – 70℃, that affects the color and anthocyanin content (Aprodu et al., 2020).

Table 2: Total anthocyanin content of colorant extracted from *C. ternatea* using three different extraction methods

Method	Total anthocyanin content (mg/L)
Direct extraction method	59.95 ± 5.03^{a}
Blanching extraction method	67.6 ± 29.0^{a}
Blanching extraction method with preservatives	25.0 ± 22.4^{a}

Note: Means with same superscript letters are not significantly different ($p < 0.05$)

3.4. Total phenolic content (TPC)

The total amount of TPC in each treatment were represented as mean mg GAE per gram of sample as shown in Table 3. Result was expressed as the regression equation of the gallic acid standard curve Y= 0.0693x + 0.1413 where, R^2 = 0.9938 (Lakshan et al., 2019). According to the above table, it shows significant difference of total phenolic content among three treatments ($P < 0.05$). Considering the three methods / treatments, TPC were as follows: T_1 (48.49 $\pm$ 4.00 mg/L) > T_2 (35.25 $\pm$ 16.05 mg/L) > T_3 (33.10 $\pm$ 11.74 mg/L).

Phenolics can be found in cell membranes, they are highly soluble in water and sensitive to thermal processing. Also, they may be lost by leaching (Rabeta and Nabil, 2013). Therefore, it can be assumed that the mechanism of the extraction method affects the extraction of phenolic compounds from the *C. ternatea*. During anthocyanin extraction, the use of freezing or high temperature and with the blending, cell rupturing of *C. ternatea* petals could release phenolic compounds (Rabeta and Nabil, 2013).

Folin-Ciocalteu assay only denotes crude estimate of the TPC present in an extract where it does not measure specific polyphenols, but many interfering compounds may react with the reagent, thus giving elevated apparent phenolics concentrations (Prior et al., 2005). Moreover, highly polar solvents with acid (citric acid) significantly reduces the amounts of TPC in other solvents. It might be the plausible reason for low TPC in *C. ternatea* food colorant gel obtained from the third treatment (T_3).

Table 3: Total phenolic content of the colorant extracted from *C. ternatea* using three different extraction methods

Method	Total phenolic content (mg/L)
Direct extraction method	48.49 $\pm$ 4.00 [a]
Blanching extraction method	35.25 $\pm$ 16.05 [b]
Blanching extraction method with preservatives	33.10 $\pm$ 11.74 [b]

Note: Means with different superscript letters differ significantly ($P < 0.05$)

3.5. Total flavonoid content (TFC)

Result was expressed as the regression equation of the calibration curve (Y = 0.0098x-0.0366, R^2=0.9723) and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg per g). According to the results, there was a significant difference in TFC ($P < 0.05$) among all treatments. As shown in Table 4, Blanching extraction method (173.97 $\pm$ 7.88 mg quercetin/g) showed higher flavonoid content than other extraction methods.

The concentration of flavonoids also depends on the polarity of the solvents used for extraction. Flavonoids are heat sensitive polyphenolic compounds. Therefore, the heat exposure greatly influences the flavonoid content in anthocyanin extracts (Kamtekar et al., 2014). Anthocyanin may contribute to the amount of TFC obtained from the plant samples as it is one of the important groups of water-soluble pigments in plants (Clifford et al., 2000).

Theoretically, the amount of TPC should be higher than that of total flavonoid content. In addition, the TPC measured by different methods might result in output difference (Katsube et al., 2004). Therefore, the sole Folin-Ciocalteu procedure cannot give the full phenolic constituent extracts. Hence, the reason behind the higher TFC over TPC could be that other phenolic contents cannot be quantified by this single Folin-Ciocalteu method. The overall phenolic contents that promote the quantification of flavonoid compound cannot be determined due to lower phenolic content than TFC. However, according to (Kaisoon et al., 2011), plant extracts with higher flavonoid do not necessarily contain high amount of TPC.

Table 4: Total flavonoid content of the colorant extracted from *C. ternatea* using three different extraction methods

Method	Total flavonoid content (mg/L)
Direct extraction method	152.48 ± 9.18^{b}
Blanching extraction method	173.97 ± 7.88^{a}
Blanching extraction method with preservatives	131.46 ± 7.40^{c}

Note: Means with different superscript letters differ significantly ($P < 0.05$)

3.6. DPPH free radical scavenging activity

Clitoria ternatea flowers are rich in phenolic secondary metabolites such as anthocyanin and flavonoids. It may have higher antioxidant activity due to its redox properties and the chemical structure of anthocyanin. The antioxidant compounds present in the medium convert DPPH radical in to a more stable DPPH molecular product by donating an electron or a hydrogen atom. As a result, color changed from purple to pale yellow of reducing form of DPPH and this color change is measured by spectrophotometrically in order to quantify the antioxidant activity (Bueno et al., 2012). Here, DPPH radical scavenging was shown by the anthocyanin of *C. ternatea*, which were extracted by direct extraction method, blanching extraction method and blanching extraction with addition of natural preservatives method. Average DPPH radical scavenging activity of each three treatments were shown in Figure 1. In the present study, the antioxidant activity of the *C. ternatea* extract were as follows: direct extraction method (44.14 ± 3.29%), blanching extraction method (84.24 ± 30.5%) and blanching extraction with addition of natural preservatives method (90.78 ± 29.2%). According to the previous studies, the optimum antioxidant content was investigated using water extraction techniques which denoted IC_{50} values of 47% than the ethanol extraction technique (32%). Since, only water was used rather than any chemical for the extraction those aqueous anthocyanin extractions might have a greater percentage of inhibition of free radical formation (Chayaratanasin et al., 2015).

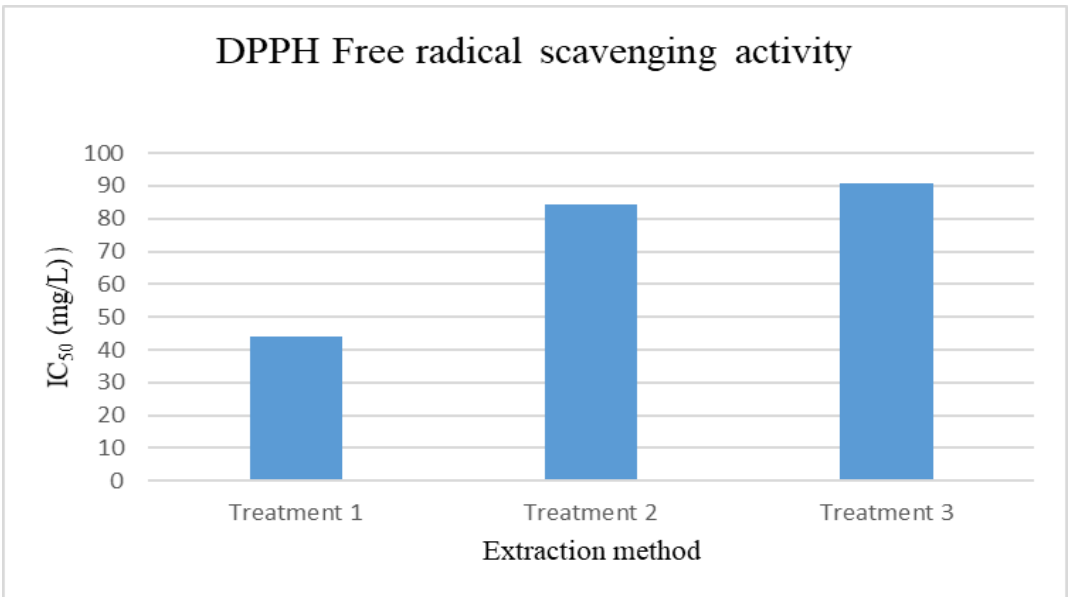

Figure 1: DPPH Free radical scavenging activity (IC_{50}) of extracts obtained from three different methods

Figure 1 revealed that the direct extraction method represents the lowest IC_{50} value (highest antioxidant activity) than other two methods (T_2 & T_3) (Rivero et al., 2020). Thus, it can be concluded that total anthocyanin content and total phenolic content are responsible for the antioxidant activity (Vankar and Srivastava, 2010).

3.7. Determination of the color stability

Color stability of the prepared natural food colorant was examined with the use of commercially available king-coconut beverage products.

3.7.1. Effect of light

Light condition has a role in destabilizing the anthocyanin molecular structure; with increase in temperature (Bakhshayeshi et al., 2006).
According to (Roobha et al., 2011), the highly reactive anthocyanin from *C. ternatea* can be easily degraded according to the different light conditions. The presence of light or the absence of light is an important parameter that influence the degradation of anthocyanin. According to the (Tantituvanont et al., 2008), light increases the flavylium cation construction, but in the absence of light the amount of chalcone in the extract containing anthocyanin was higher than its flavylium cation. Comparatively, from 1st day to 7th day, there was an increment of the color and after 7 days, color stability decreased gradually.
The results of this study, describes the light stress induced changes of the pigment against the prolonged storage period of anthocyanin of the natural food colorant from *C. ternatea*. Therefore, light is recognized to accelerate the degradation of anthocyanin and dark conditions are preferable for the color stability of anthocyanin as shown in Figure 2 & 3.

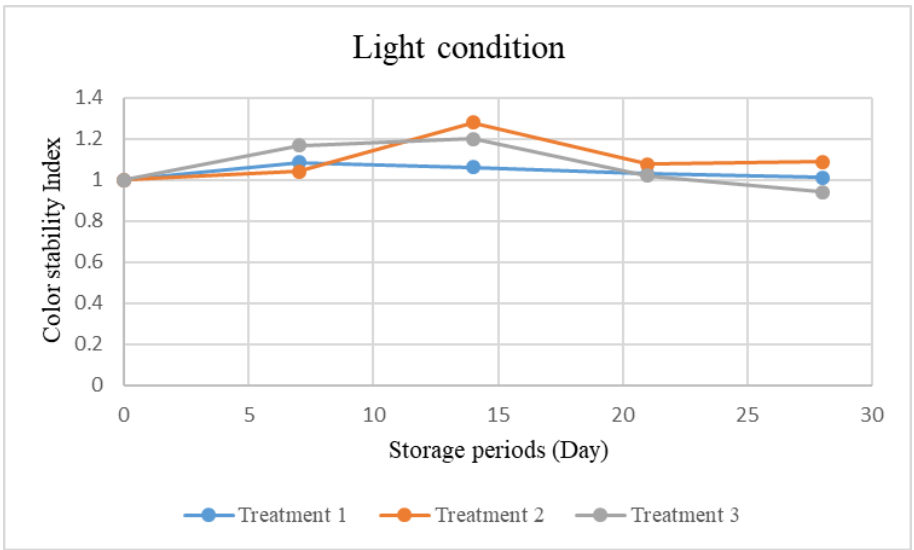

Figure 2: Color changes of the samples obtained from three methods at light condition

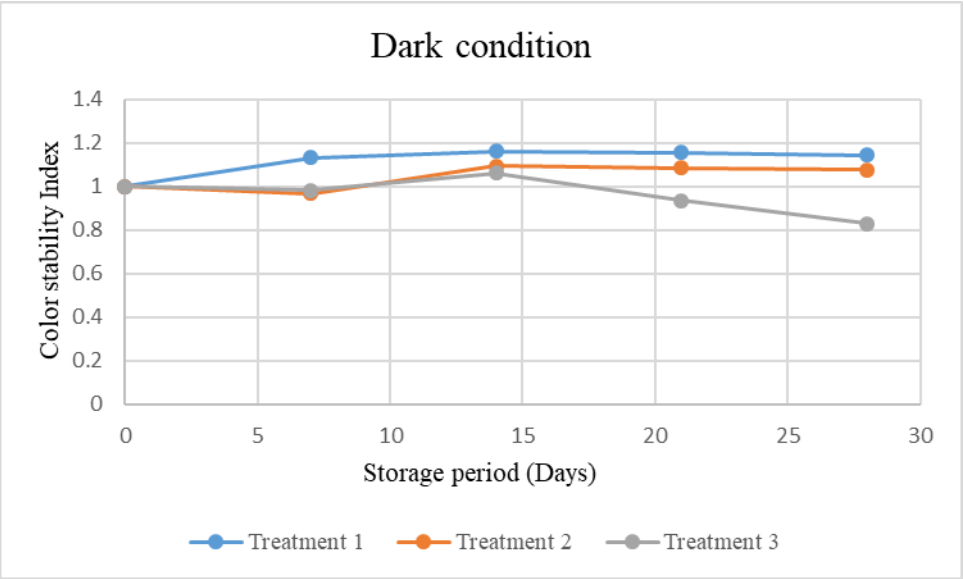

Figure 3: Color changes of the samples obtained from three methods at dark condition

3.7.2. Effect of temperature

Color stability index determined between the differences of temperature ranging 25℃ - 4℃ for certain time periods. As shown in Figure 4 & 5, the unstable anthocyanin pigment degraded at 25℃ and more stable at 4℃ resulting best stability index. Fast destruction of anthocyanin pigment at 25℃ could be due to the hydrolyzation of 3-Glycoside structure, which has a protective effect in unstable anthocyanin. The other suggestion is that the hydrolyzation of the pyrylium ring resulted in production of chalcone, which are responsible for brown color developed food containing anthocyanin (Lee et al., 2011). Therefore, an increase in temperature during storage will enhance the degradation rate of anthocyanin in natural food colorant obtained from *Clitoria ternatea* and as well as a brown color will be developed.

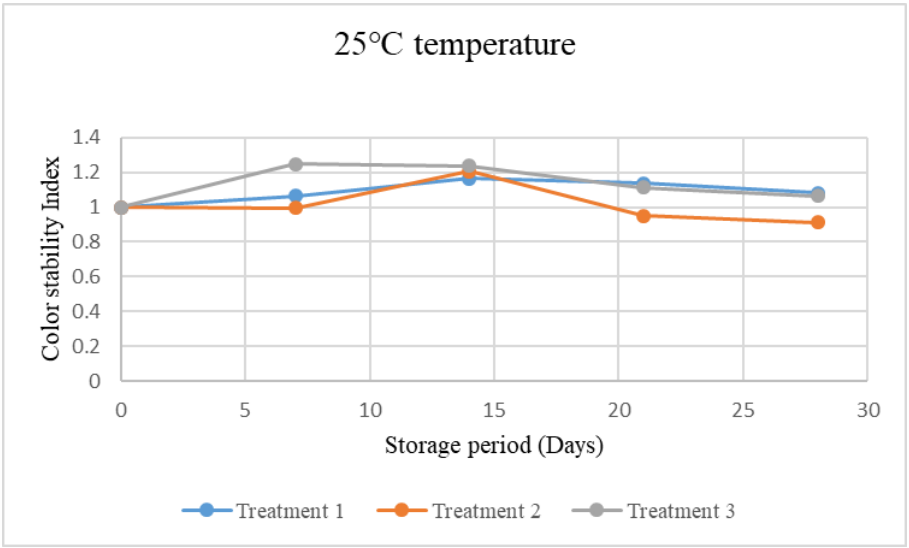

Figure 4: Color changes of the samples obtained from three methods at 25℃ temperature

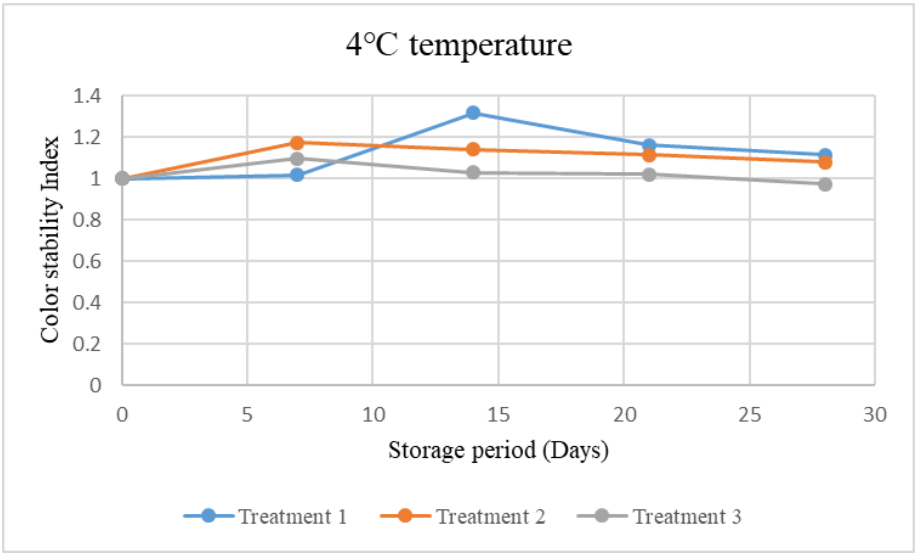

Figure 5: Color changes of the samples obtained from three methods at 4℃ temperature

3.8. Shelf-Life Evaluation

Determination of the Total Viable Plate Count (TVC) and yeast & mold counts in a food colorant are common, simplest and widely used microbiological techniques. According to the Table 05, results obtained in each treatment, assume that the

product is safe for human consumption prior to the sensory evaluation since the colony forming units are within the stipulated limits for 28 days of storage.

Table 5: Microbiological assays for total viable count of the treatments

Method	TVC for bacteria (CFU/mL)			TVC for fungi (CFU/mL)		
	initial day	after 14 days	after 28 days	initial day	after 14 days	after 28 days
Direct extraction method	0	2×10^1	1×10^2	0	2×10^1	1×10^1
Blanching extraction method	0	2×10^1	1×10^2	0	2×10^1	7×10^1
Blanching extraction method with natural preservatives	0	0	1×10^1	0	1×10^1	2×10^1

3.9. FTIR Analysis

The FTIR spectrum of *C. ternatea* extract from direct extraction / T_1 showed strong absorption bands at 3271, 1603, 1033 & 589 cm^{-1} (Figure 6). The absorption band in the 600-1000 cm^{-1} region indicates the presence of the aromatic rings.
The absorbance at 1043 cm^{-1} is due to the stretching vibration of the C-O-C esters whereas absorbance at 1178 cm^{-1} is described to the stretching of the pyran rings, which are typical of flavonoid compounds (Pereira et al., 2015).
Band at 1398 cm^{-1} corresponds to the C-O deformation of phenols whereas bands at 1603 and 1927 cm^{-1} are attributed to the C=C and C=O groups for aromatic rings, respectively.
The broad band at 3271 cm^{-1} was assigned to the sugar vibrations and phenol O-H groups (Vankar and Shukla, 2011). Therefore, the spectral analysis of the concentrated extracts showed the presence of O-H, C=O, C=C and C-O-C functional groups which are characteristics of the anthocyanins. Figure 6 and 7, both shows the same IR spectra. Hence, the food colorant from direct and blanching methods (T_1 & T_2) denoted the presence of anthocyanin.

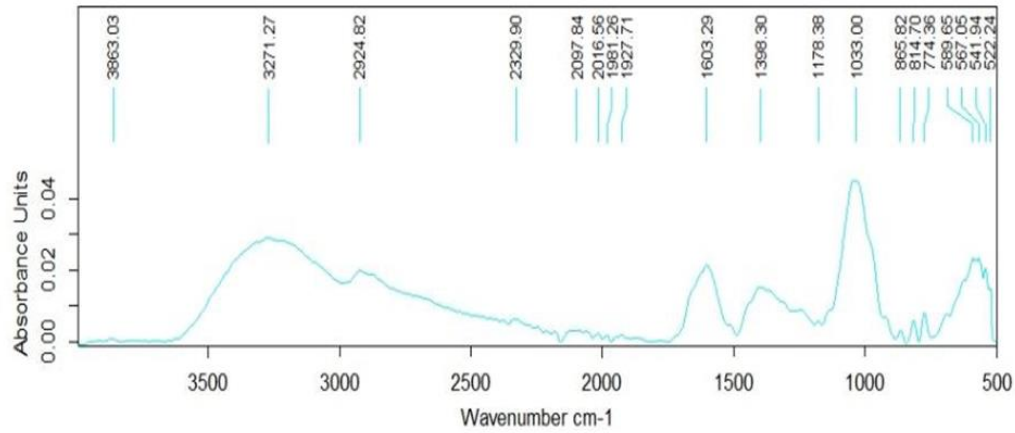

Figure 6: FTIR results for the sample obtained from direct extraction method (T_1)

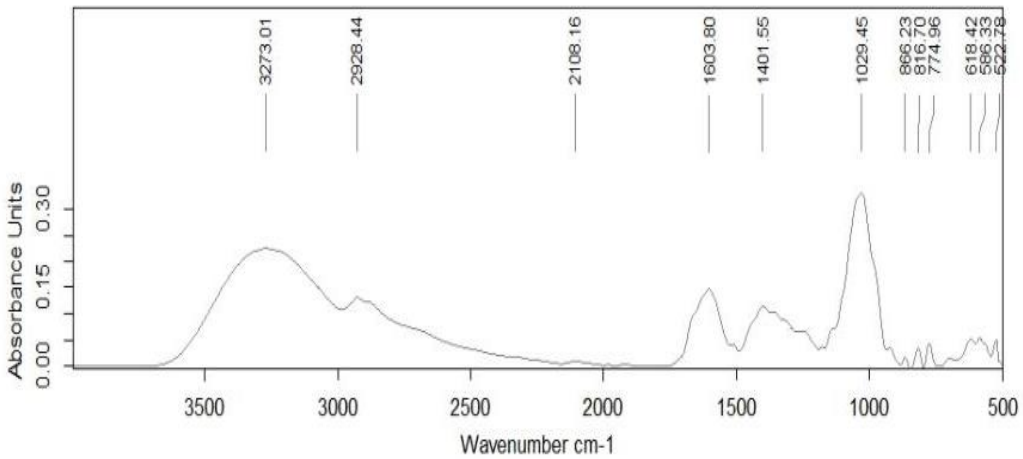

Figure 7: FTIR results for the sample obtained from blanching extraction method (T_2)

3.10. Color characteristics

The colorimetric parameters (L/ light transmission, C/ Chroma and H / Hue) allowed the characterization of the color of each phenolic extract.

According to Table 6, L, C and H obtained from direct extraction method (T_1) & blanching extraction method (T_2) resulted a hue range of cyan-blue color (219-240 nm) while blanching extraction with addition of preservative method (T_3) resulted a hue range of blue- magenta color (270-315 nm) (Pathare et al., 2013).

Table 6: Color characteristics of the food colorant: Lightness; L*, Chroma; C, Hue; H

Treatment	Method	L*	C	H
Treatment 1	Direct extraction method	5.75	40.77	225.75
Treatment 2	Blanching extraction method	18.94	23.148	219
Treatment 3	Blanching extraction method with preservatives	18.15	29.708	293.6

3.11. Sensory attributes of Anthocyanin incorporated king coconut beverage

The estimated median rank scores for sensory attributes such as aroma, taste, mouth feel, color, overall acceptability and the p values were summarized in table no. 7. According to the results of sensory evaluation, anthocyanin extract obtained from different extraction methods (T_1, T_2 and T_3) had a significant difference ($P < 0.05$) with respect to all the sensory attributes. Results of Friedman test denoted that food colorant from T_3 was most desirable and it might be due to the addition of sugar and lemon which gives a more acceptable flavor. Based on the sensory evaluation T_3 gained the highest mean rank for the assessment of overall acceptability, compared to the T_1 & T_2.

Table 7: Results of the sensory evaluation

Sensory attributes	Estimated median scores for T_1	Estimated median scores for T_2	Estimated median scores for T_3	Probability (P value)
Color	8	7	9	P = 0.000
Aroma	8	8	9	P = 0.000
Taste	7	7	8	P = 0.001
Mouthfeel	7	7	8	P = 0.000
Overall Acceptability	8	8	9	P = 0.000

Note: All data except for P values are expressed as median values.

4. Conclusion

The Present study revealed that, blue anthocyanins from *Clitoria ternatea* would be better to use as natural colorant materials for beverages. However, regards to the functional properties; anthocyanin content, phenolic content, flavonoid content, antioxidant activity which concerns the consumers with health benefits might pave the path to extract colorant using direct extraction (T_1) and blanching extraction (T_2) since their functional properties were conserved or preserved without any deterioration amidst the treatments. Sensory evaluation of colorant incorporated king coconut beverage formulations showed that T_3 was most preferred formulation at the day of preparation based on the organoleptic point of view compared to T_1 & T_2. Hence, artificial preservatives were not used for the development of the colorant to enhance shelf life, only lime juice was added since it contained natural citric acid as a natural food preservative. Therefore, the food colorant developed from T_3 (colorant jell) would be 100% natural and one of the best alternatives for synthetic food colorants regards to the sensory acceptance. But, in terms of conserving the functional properties, colorant developed from T_1 & T_2 would have the competitive advantage over the colorant developed from T_3.

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