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RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Anthocyanin Microcapsule from *Clitoria ternatea*: Potential Bio-preservative and Blue Colorant for Baked Food Products

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Abstract
The stability of anthocyanins is greatly affected by pH and temperature, and thus their application in food products is limited. This study was aimed to develop an antibacterial food colorant using anthocyanin from *Clitoria ternatea* flowers by applying microencapsulation technology. Maltodextrin was used as a carrier agent in the spray drying process. The anthocyanin microcapsule was durable to the light exposure up to 21 days, and the best color stability was observed between the temperature range of − 20 °C to 4 °C. However, the color was sustained with approximately 50% of the stability index after 1 day exposure at 80 °C and 180 °C. On disk diffusion assay, the anthocyanin microcapsule manifested a broad-spectrum antibacterial activity against Gram-bacteria. According to minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assay, anthocyanin microcapsule exhibited bactericidal activity on all test bacteria, with the lowest MIC and MBC observed on *Escherichia coli*. The application of anthocyanin microcapsule on muffins has shown a significant inhibitory activity on foodborne bacteria. This study revealed the potential of *C. ternatea* anthocyanin microcapsule as a bio-preservative, which can prolong the shelf life of baked food products.

Keywords Anthocyanin · *Clitoria ternatea* · Antibacterial activity · Microencapsulation · Foodborne bacteria

1 Introduction

Color is the most imperative aspect of food production which significantly influences the consumers' preference. Today, natural colorants from plant origin, especially with the antibacterial activity, are in high demand due to the banning of several artificial food colorants such as Orange II, Fast Red, Amaranth, Auramine and Rhodamine [1]. Besides, the uses of synthetic colorants on food and beverages also cause the toxic and carcinogenic effects [2]. The safety of artificial blue colorant is highly controversial. The cytotoxic and genotoxic of Brilliant Blue FCF colorant on human blood lymphocytes cell cultures was reported [3]. Blue No 2 also causes hyperactivity and brain tumors on animal models. The adverse health effects of some artificial food colorants such as Patent Blue and Indigo Carmine were also recorded [4]. Therefore, it is necessary to look for a safer alternative for the food industry.

Food spoilage can be described as undesirable changes occurring on food due to microorganism development. Microbial attachment and growth are supported by air exposure, heat, light, and moisture [5]. Approximately, one-third of food which represented 1.3 billion tons per year, is not consumed and wasted worldwide. Therefore, food security can be assured by reducing the amount of food waste produced via food spoilage. Food colorants with antibacterial activity can be suitably added in food products to control the growth of food spoilage microorganisms and prolong the shelf life [6].

Clitoria ternatea is a perennial plant with elliptic leaves. The plant was traditionally used in the South East Asian community for multiple usages. The plant has vivid deep blue flowers, which is contributed by the presence of delphinidin anthocyanin [7]. In Thailand, the extracts of *C. ternatea* were added in cosmetic products due to their excellent

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antioxidant activity [8]. In India and Philippines, the young shoots, flower and fresh pod are consumed as vegetables or a side dish for the main course. As for Malaysia, the natural green and bright blue color from the leaves and flowers of *C. ternatea* are added in a variety of foods, especially in the making of the rice cakes [9]. In this study, *C. ternatea* flowers were selected because they are rich in blue anthocyanin. Besides, the flowers are reported as a therapeutic agent due to their excellent antidepressant, anticonvulsant, tranquillizing, memory enhancing, anticholesterol and antioxidant activities [9, 10].

Anthocyanins are water-soluble flavonoids with diphenyl propane skeleton ($C_6C_3C_6$) [11]. They are natural plant pigments imparting blue, red and purple colors to flowers, leaves and fruits. The most common types of anthocyanins are cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin. The improvement in encapsulation technology enhances the properties of anthocyanins. Thus, the role of anthocyanins as food colorant is becoming increasingly crucial. Besides contributing to the aesthetic and quality judgement of the food products, anthocyanins also showed potential biological activities [12]. However, anthocyanins are rarely added in food products prepared at a high temperature due to its poor heat stability. Moreover, their color and biological activities are greatly affected by environmental factors such as pH, temperature and light [7]. Free form anthocyanins (non-encapsulated) are also susceptible to the auto-oxidation, which limit their industrial applications and cause the products to be unstable upon storage [13]. Hence, in this present study, a development on antibacterial food colorant using anthocyanin of *C. ternatea* flowers was done by applying microencapsulation technology. The color of anthocyanin microcapsule was tested for its stability index and further compared with free form anthocyanin. Then, the antibacterial evaluation was performed according to disk diffusion assay, MICs and MBCs, prior to the application on muffins as a food model.

2 Materials and Methods

2.1 Collection of *C. ternatea* Flowers

The flowers of *C. ternatea* were collected from Jalan Tengkera, Melaka, Malaysia (GPS coordinates: N2 12.076 E102 144.326). The sampling was carried out manually by hand picking technique. The samples were placed in a plastic bag with zip-lock, prior to the cleaning and drying processes. All procedures were performed within 24 h of collection.

2.2 Extraction of Anthocyanin

The flowers were dried at 60 °C in an oven for 2 days until a constant weight was obtained. To extract the anthocyanin, *C. ternatea* flowers were soaked in distilled water containing 1 M acetic acid. The mixture was occasionally mixed, prior to the filtration process through a muslin cloth and Whatman No 1 filter paper [14]. The combination ratio of distilled water-acetic acid was set at 1:40 (w/v), and the pH was adjusted to pH 5.5.

2.3 Anthocyanin Test

To detect the anthocyanin, the extract was added with 2 N hydrochloric acid (Acros Organics, USA) and ammonia solution (Acros Organics, USA) at an equal volume (v/v). The presence of a blue violet solution was a positive indicator for anthocyanin [15].

2.4 Microencapsulation

Carrier agent (maltodextrin, dextrose equivalent, Sigma-Aldrich, USA) was combined with 6% (w/v) anthocyanin extract and homogenized by using a homogenizer (Heidolph, Germany) at 10,000 rpm for 5 min. Maltodextrin was gradually added until a concentration of 20% (w/v) was achieved. Spray drying was conducted using a Böchi Mini Spray Dryer (Switzerland) at 100% aspirator flow rate, an inlet temperature of 180 °C and feed rate of 6 mL/min. The spray-dried powder was kept in a desiccator until further use [14]. In this study, the encapsulated anthocyanin was enunciated as anthocyanin microcapsule; meanwhile, the crude extract was referred to free form anthocyanin.

2.5 Encapsulation Efficiency

The total anthocyanin content (TAC) and surface anthocyanin content (SAC) were determined [14]. For TAC analysis, 10 mg/mL of anthocyanin microcapsule was sonicated to break the microcapsule membrane. Then, 10 mL of ethanol was added to extract the anthocyanin content. For SAC analysis, the surface anthocyanin was extracted by quick washing with 10 mL of the ethanol in a vortex for 10 min, followed by centrifugation at 3100 g for 3 min. To quantify the amount of anthocyanin, the absorbance of the sample was measured at 575 nm with a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). The encapsulation efficiency was calculated using the formula below.

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{TAC} - \text{SAC}}{\text{TAC}} \times 100\%$$

2.6 Color Stability Index

2.6.1 Effect of Light

The experiment was done in clear vial glass (light condition). The samples were incubated at a temperature of 25 °C. At pre-determined time points (day 0, 1, 7, 14, 21, 28), 30 mL of each sample was withdrawn and filtered. Then, all samples were assayed for anthocyanin presence. Anthocyanin was detected via a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan) at 575 nm. The experiments were performed thrice on separate occasions. The color stability index of the sample was calculated using the formula below [16].

$$\text{Color stability index} = \frac{\text{Absorbance on sampling day}}{\text{Absorbance on Day 0}}$$

2.6.2 Effect of Temperature

The color stability index of the free form anthocyanin and anthocyanin microcapsule were conducted at different temperatures (− 20 °C, 4 °C, 25 °C, 50 °C, 80 °C and 180 °C). At pre-determined time points (day 0, 1, 7, 14, 21, 28), 30 mL of each sample was withdrawn and filtered. Then, all samples were determined for the anthocyanin presence according to the above-mentioned method and calculation.

2.7 Test Microorganisms

The foodborne test bacteria, including *Streptococcus* sp., *Staphylococcus aureus*, *Bacillus coagulans, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis,* and *Yersinia* sp., were used in this experiment. The cultures of the test bacteria were streaked on nutrient agar (Merck, USA) and incubated at 37 °C for 24 h. Then, to prepare the bacterial inoculum, the bacterial colonies were suspended in sterile distilled water. The turbidity of the bacterial inoculum was adjusted to match the turbidity of MacFarland 0.5 standard.

2.8 Disk Diffusion Assay

The assay was conducted to screen the antimicrobial activity of the anthocyanin microcapsule [17]. The antibacterial activity of anthocyanin microcapsule was tested at 100 mg/mL by dissolving the substance in sterile distilled water. Then, 100 µL of the bacterial inoculum (10^6 CFU/mL) was spread on the Mueller–Hinton agar (Merck, USA) plate using a sterile cotton swab. A sterile paper disk containing 20 µL of the test substance was laid on the surface area of the inoculated medium. The disks containing chloramphenicol (20 µg/mL) and maltodextrin (20 µg/mL) were applied as a positive and negative control, respectively. The plates were incubated at 37 °C, 24 h. After the incubation period, the diameters of the clear zones were measured.

2.9 Broth Microdilution Assay

This assay was done to verify the MICs and MBCs of the anthocyanin microcapsule [18]. The anthocyanin microcapsule was set at a concentration range of 0.8–100 mg/mL. One hundred milliliter of bacterial inoculum and anthocyanin microcapsule was pipetted into a sterile 96-well microtiter plate. The bacterial inoculum was pre-prepared by diluting the inoculum in double strength Mueller–Hinton broth (Merck, USA). Then, the plate was incubated at 37 °C, 24 h. Next, to detect the bacterial growth, 40 µL of iodonitrotetrazolium violet (INT) (0.2 mg/mL, Sigma-Aldrich, USA) was added into each well. Subsequently, a loop full of the microbial culture from each well was streaked onto fresh nutrient agar (NA) (Merck, USA) plate to check the viability of the bacteria. The plates were incubated at similar conditions as the aforementioned procedure (37 °C, 24 h). MBC was recognized as the lowest concentration of the test substance which does not permit any visible growth on the plates, after the incubation period.

2.10 Preparation of Muffin

The ingredients used for the muffin's batter were 100 g white flour, 50 g butter, 50 g sucrose, 50 g eggs, 50 g milk, 3 g baking powder and 0.5 g salt. Sugar, eggs, milk, and butter were blended for 2 min at a speed of 4 (300 rpm) using a benchtop mixer (The Bakers ESM989, Taiwan). Then, baking powder, white flour and salt were then added and mixed for 20 s at 300 rpm. Two different formulations of batter were prepared; one was added with 5 g of spray-dried anthocyanin extract, while another one was added with 5 g of maltodextrin as control. The batter (10 g) was inserted into the muffin mold and baked at 180 °C for 25 min in a preheated oven (Berjaya Steel BJY-G30-1BD, Malaysia). The moisture content of the muffins was also determined with a moisture analyzer (A&D Model MS-70, USA).

2.11 Determination of Microbial Load on Food Model

The muffin samples were incubated in an incubator at 25 °C for 14 days. They were contaminated in an exposed condition. On a daily basis, the samples were harvested and mixed with 10 mL of 1% peptone water and crushed in a stomacher. The enumeration of total bacteria was approximated by viable cell count assay. Spread plates method was

employed by spreading 100 µL of diluents on plate count agar (PCA) (Merck, USA). The PCA plates were incubated for 2 days at 37 °C. The microbial load on the muffin sample was estimated per gram of sample [14].

2.12 Statistical Analysis

One-way ANOVA was employed to assess the statistical significance of the experimental data by using Tukey's HSD post hoc (SPSS, USA). Statistical significance was accepted at $p \leq 0.05$.

3 Results and Discussion

3.1 Encapsulation Efficiency of Anthocyanin from *C. ternatea* Flowers

Clitoria ternatea flowers contain natural color that can be easily extracted with water. Water was utilized as an extraction solvent in this study as previous study reported that aqueous extracts of *C. ternatea* showed better antibacterial activities, compared to ethanolic extract [19]. In this study, the blue extract was obtained through an extraction process using the acidic solvent at pH 5.5. This is a differential pH assay based on the structural transformations of the anthocyanin chromophore [15]. The extract changed to violet color, which indicates the presence of anthocyanin.

The encapsulation efficiency indicates the potential of maltodextrin to encapsulate or hold the anthocyanin inside the microcapsule. In the present study, the encapsulation efficiency reported for anthocyanin microcapsule was $87.34 \pm 5.9\%$. The observation was in agreement with the previous study [20]. This indicates that the maltodextrin and spray drying technique used in this study are suitable and efficient to encapsulate the anthocyanin from *C. ternatea*.

3.2 Color Stability Index: Effect of Light Exposure

The highly reactive anthocyanin can be easily degraded, and thus the storage environment plays an essential role in attaining the color of anthocyanin. The presence of light is one of the de facto parameters that influenced the degradation of anthocyanins. Figure 1 shows the color stability index of free form anthocyanin and anthocyanin microcapsule for a storage period of 28 days, in the presence of light. In general, the *C. ternatea* anthocyanin was more durable to light after microencapsulation and its stability was up to 21 days. Both extracts exhibited indifferent color stability index from day 0 to 7 ($p \geq 0.05$). However, on day 14 onwards, there were notable changes in color stability indexes for free form anthocyanin ($p \leq 0.05$). As observed, the color stability index

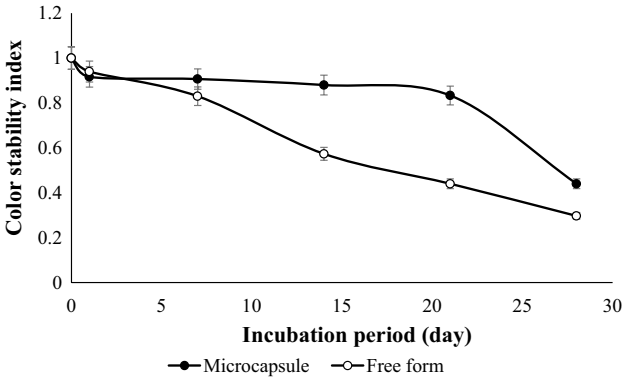

Fig. 1 The color stability index of free form anthocyanin and anthocyanin microcapsule for a storage period of 28 days, in the presence of light (condition: pH 5.5; temperature of 25 °C)

for free form anthocyanin was 0.57, which was lower than the anthocyanin microcapsule (color stability index at 0.83).

Our results were in consensus with previous studies [21–23]. Under light exposure, the changes in color stability indexes of anthocyanin were gradually parallel with the time increase. There was about 26.40–96.6% color destruction on 4 *Berberis* sp. once the extracts were exposed to light for 1 h [21]. Besides colorant, light can also influence the antioxidant activity. Based on 10 h observation, the antioxidant activity from mulberry fruit extracts was notably decreased with the presence of the light [22]. The microencapsulation of blueberry anthocyanin, which obtained via spray drying technique, has greater resistant to outdoor light than liquid blueberry extract alone. After being exposed to light for 10 days, the retention rate of blueberry anthocyanin microcapsule was approximately higher than 85%. Meanwhile, the free blueberry anthocyanin was lower than 65% [23].

In this study, *C. ternatea* anthocyanin microcapsule may get protection from wall material of maltodextrin. The component was able to prevent *C. ternatea* anthocyanin microcapsule against light-stress-induced changes of the pigments and prolonged the storage period of anthocyanin. Light is recognized to accelerate the degradation of anthocyanins. This event has been observed during the production of fruit juices and red wines. The anthocyanins sources with mono- or di-acylated derivatives are reported to be more stable than non-acylated. Thereby, a high amount of acylated form anthocyanins is preferable as a natural food colorant in industrial scale [24]. The isomerization reaction by cinnamoyl derivative can intensify the color produced by anthocyanins. This particular chemical exists in certain anthocyanins, and it can isomerize from *trans* to *cis* form. Other than color changes, the isomerization reaction also evokes resistance to hydration of the pyrilium ring in anthocyanin [25].

3.3 Color Stability Index: Effect of Various Temperatures

An increase in temperature during storage will increase the degradation rate of anthocyanin [26]. At − 20 and 4 °C, the free form anthocyanin and the microcapsule did not show any significant difference in the color stability index ($p \geq 0.05$) (Fig. 2). Both free form anthocyanin and anthocyanin microcapsule showed good stability at low temperature. The anthocyanin microcapsule was more stable than the free form anthocyanin. As observed the anthocyanin microcapsule exhibited no vast differences in color stability indexes between 25 and 50 °C. In general, after 1 day of the storage period, the free form anthocyanin recorded a more gradual decline at 25 °C onwards, compared to the anthocyanin microcapsule. The color of the anthocyanin microcapsule was able to stand at 80 °C and 180 °C for 1 day, with approximately 50% of stability index. This showed that the microencapsulation can protect the anthocyanin from degradation due to high baking temperature.

In this study, the difference in color stability index was more apparent with the increase of test temperatures. This incidence causes color loss and the appearance of brown-colored compounds [27] triggered by the formation of chalcone [21]. Based on our results, the maltodextrin components have overcome the color-changing issue and guarded the unstable anthocyanin against degradation at high temperature.

3.4 Antimicrobial Evaluation via Disk Diffusion Assay

The disk diffusion method was used to study the antibacterial activity of anthocyanin microcapsule. Table 1 summarizes the antibacterial activity of the anthocyanin microcapsule which was evaluated through disk diffusion assay. The anthocyanin microcapsule exhibited significant antibacterial

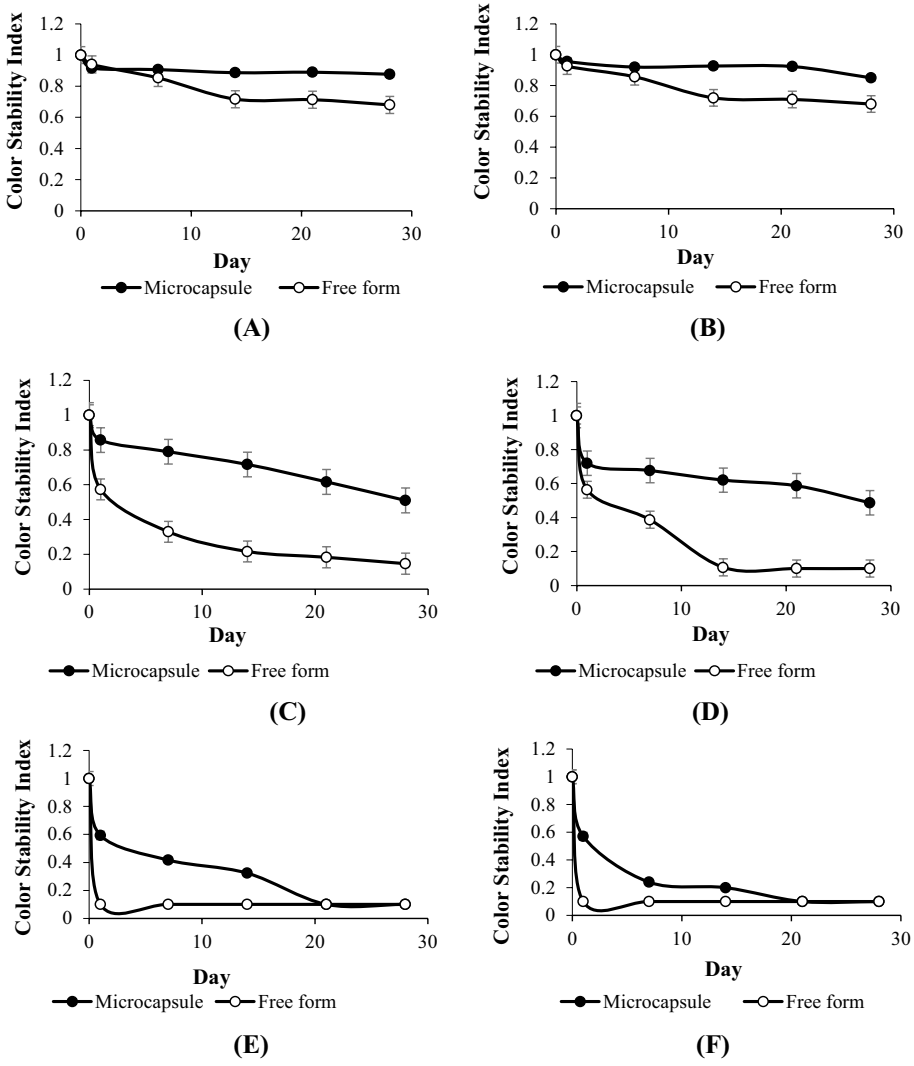

Fig. 2 The color stability index of anthocyanin microcapsule and free form anthocyanin at **a** − 20 °C, **b** 4 °C, **c** 25 °C, **d** 50 °C, **e** 80 °C, **f** 180 °C, in the presence of light (condition: pH 5.5)

Table 1 Antibacterial activity of anthocyanin microcapsule on disk diffusion assay. The microcapsule exhibited broad-spectrum activity on foodborne bacteria

Test microorganism	Diameter of inhibition zone (mm)		
	Anthocyanin microcapsule	Positive control	Negative control
Gram-positive bacteria			
B. cereus	39.0 ± 1.1	7.9 ± 0.6	–
S. aureus	35.0 ± 2.1	9.9 ± 0.6	–
Streptococcus sp.	35.3 ± 1.6	7.0 ± 1.0	–
B. coagulans	35.0 ± 1.2	18.0 ± 1.1	–
Gram-negative bacteria			
Yersinia sp.	37.7 ± 1.6	15.8 ± 1.2	–
P. mirabilis	37.3 ± 1.5	10.2 ± 1.5	–
P. aeruginosa	36.3 ± 1.2	11.4 ± 1.4	–
E. coli	39.7 ± 2.1	11.9 ± 1.6	–

(–) No inhibitory activity

activity on all test foodborne bacteria ($p \leq 0.05$). A positive result was represented by the presence of the clear zone surrounding the paper disk. A diameter ranging from 35.0 to 39.7 mm was obtained from this experiment. The diameter averages displayed by both Gram-bacteria were insignificant, which signified a broad spectrum of the antibacterial efficiency. In addition, the negative control did not depict any inhibitory activity on all test bacteria. The result indicated that the antibacterial activity was contributed by the anthocyanin encapsulated in maltodextrin alone.

The results were in line with the previous study [14], where the anthocyanin extracts of *Clitoria ternatea* demonstrated an inhibitory effect on both Gram-positive and Gram-negative bacteria. However, the clear zone produced was narrower than our results, specifically 12.0–15.8 mm. The outcome has proven that the use of distilled water-acetic acid combination was better than ethanol-acetic acid combination, especially in extracting the anthocyanin from *C. ternatea*.

3.5 Bactericidal Effect of the Anthocyanin Microcapsule

In general, the MIC of anthocyanin microcapsule ranged from 1.56 to 25.00 mg/mL (Table 2). The broad range of MIC indicates the different susceptibility of test microorganisms to the anthocyanin microcapsule. A low MIC indicates that a little amount of substance is ample to inhibit the growth of the microorganisms. MBC is the most common assessment of bactericidal activity. Similar to MIC, the anthocyanin microcapsule showed an extensive range of MBC, specifically from 12.50 to 100.00 mg/mL. Based on this assay, Gram-positive bacteria were more susceptible

Table 2 The MIC and MBC of anthocyanin microcapsule recorded on broth microdilution assay. The microcapsule exhibited microbicidal effect on test microorganisms

Test microorganism	MIC (mg/mL)	MBC (mg/mL)
Gram-positive bacteria		
Streptococcus sp.	6.25	25.00
S. aureus	12.5	50.00
B. coagulans	6.25	12.50
B. cereus	6.25	12.50
Gram-negative bacteria		
Yersinia sp.	3.13	25.00
P. mirabilis	25.00	100.00
P. aeruginosa	6.25	25.00
E. coli	1.56	12.50

to anthocyanin microcapsule compared to Gram-negative bacteria. Besides, we also observed that the MBCs were notably higher than MICs for all test bacteria. The bactericidal activity of anthocyanin microcapsule was concentration-dependent, which means a higher concentration of test substance was needed to destroy the bacterial cells than to retard the bacterial growth. Mirroring the results on disk diffusion assay, the MICs and MBCs recorded by *E. coli* was the lowest among all test bacteria, with 1.56 mg/mL and 12.50 mg/mL, respectively. In general, the Gram-negative type of bacteria owns a double-layer membrane which is constructed from peptidoglycan and lipopolysaccharide [28]. The layer has made the bacteria resistant or less susceptible to any bioactive compounds [29].

3.6 Bacterial Load on Food Model

In this study, bacterial load was defined as a quantity of bacteria obtained from the muffins. Figure 3 shows the bacterial

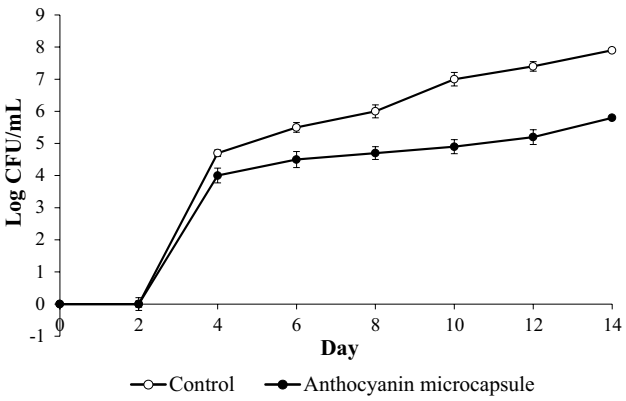

Fig. 3 A comparison of bacterial load on the muffin with and without anthocyanin microcapsule during 14 days of incubation period

load within 14 days of the incubation period. On day 0–4, there were no notable changes on bacterial load displayed by the muffins with anthocyanin microcapsule and without anthocyanin microcapsule (control) ($p \geq 0.05$). However, on day 6–14, a significant bacterial load obtained from the control ($p \leq 0.05$). The CFUs have been greatly decreased by the increase of anthocyanin microcapsule concentrations, which indicated a concentration-dependent growth-killing ability by the test compound.

In this study, the moisture content of the muffin was 22.42%, which is suitable to promote bacterial growth. The muffin samples with anthocyanin microcapsule were light blue. Physically, there was no visible microbial growth on the muffins with anthocyanin microcapsule. Plate count method was frequently conducted to estimate the number of viable microbial cells present in the sample, based on their ability to give rise to colonies under specific growth medium, temperature and time [30]. The microencapsulation by maltodextrin has prevented the anthocyanin from denaturation during the baking process. It has been proven since anthocyanin microcapsule successfully inhibited the microorganism on the muffins. The results revealed the potential of anthocyanin microcapsule as a bio-preservative for baked food products that rich in sugar and protein.

4 Conclusion

Any natural substances containing antibacterial activity have a tremendous value not only in pharmaceutical line but also in the food production. To replace synthetic dyes with natural colorants are not without challenges. It possesses an array of physicochemical stability tests, with respect to temperature, pH, light intensity, oxygen and several other factors. Thus, natural substances that able to withstand the physicochemical changes have a surplus-value in industrial application. In terms of color stability and antibacterial efficiency, *C. ternatea* anthocyanin-encapsulated maltodextrin was better than the free form anthocyanin. We successfully improved the color stability index of anthocyanins by microencapsulation once exposed to the light and heat. Besides, this microcapsule has a broad-spectrum antibacterial activity against foodborne Gram-bacteria. As for our future study, we plan to proceed with the in vivo investigation of *C. ternatea* anthocyanin microcapsule on the animal models.

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Compliance with Ethical Standards

Conflict of interest None.

References

1. Siti, A.A.M.; Noriham, A.; Manshoor, N.: Anthocyanin content in relation to the antioxidant activity and color properties of *Garcinia mangostana* peel, Syzigiumcumini and Clitoriaternatea extracts. Int. Food Res. J. **21**, 2369–2375 (2014)
2. Amelia, F.; Afnani, G.N.; Musfiroh, A.; Fikriyani, A.S.; Ucche, S.; Murrukmihadi, M.: Extraction and stability test of anthocyanin from buni fruits (*Antidesma Bunius* L) as an alternative natural and safe food colourant. J. Food Pharm. Sci. **1**, 49–53 (2013)
3. Amchova, P.; Kotolova, H.; Ruda-Kucerova, J.: Health safety issues of synthetic food colourants. Regul. Toxicol. Pharmacol. **73**, 914–922 (2015)
4. Kobylewski, S.; Jacobson, M.F.: Toxicology of food dyes. Int. J. Occup. Environ. Health. **18**(3), 220–246 (2012)
5. Rawat, S.: Food spoilage: microorganisms and their prevention. Asian J. Plant Sci. Res. **5**, 47–56 (2015)
6. Ferrari, C.C.; Germer, S.P.M.; Alvim, I.D.; Vissotto, F.Z.; de Aguirre, J.M.: Influence of carrier agents on the physicochemical properties of blackberry powder produced by spray drying. Int. J. Food Sci. Technol. **47**, 1237–1245 (2012)
7. Neda, G.D.; Rabeta, M.S.; Ong, M.T.: Chemical composition and anti-proliferative properties of flowers of *Clitoria ternatea*. Int. Food Res. J. **20**, 113–118 (2013)
8. Mukherjee, P.K.; Kumar, V.; Kumar, N.S.; Heinrich, M.: The Ayurvedic medicine *Clitoriaternatea*—from traditional use to scientific assessment. J. Ethnopharmacol. **120**, 291–301 (2008)
9. Yanti, Y.; Setiawan, T.; Lay, B.W.: Antibacterial, antibiofilm and quorum sensing inhibitory activities of *Clitoriaternatea* anthocyanin against *Streptococcus mutans*. Int. J. Infect. Dis. **73**, 143–144 (2018)
10. Rahman, A.S.; Saha, R.; Talukder, N.; Khaleque, S.M.A.; Ali, H.A.: Bioactivity guided cytotoxic activity of *Clitoriaternatea* utilizing brine shrimp lethality bioassay. Bangladesh J. Physiol. Pharmacol. **22**, 18–21 (2006)
11. Cisowska, A.; Wojnicz, D.; Hendrich, A.B.L.: Anthocyanins as antimicrobial agents of natural plant origin. Nat. Prod. Commun. **6**, 149–156 (2011)
12. Mozetic, B.; Trebse, P.; Simcic, M.; Hribar, J.: Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunusavium* L.). Lebenson Wiss Technol. **37**, 123–128 (2004)
13. Nielsen, I.L.F.; Haren, G.R.; Magnussen, E.L.; Dragsted, L.O.; Rasmussen, S.E.: Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography. Investigation of their pH stability and antioxidative potency. J. Agric. Food Chem. **51**, 5861–5866 (2003)
14. Leong, C.R.; Azizi, K.; Afif, M.; Taher, M.A.; Wahidin, S.; Lee, K.C.; Tong, W.Y.: Anthocyanins from *Clitoriaternatea* attenuate foodborne *Penicillium expansum* and its potential application as food biopreservative. Nat. Prod. Sci. **23**, 125–131 (2017)
15. Harsha, N.; Sridevi, V.; Lakshmi, M.V.V.C.; Rani, K.; Vani, K.N.D.S.: Phytochemical analysis of some selected spices. Int. J. Innov. Res. Sci. Eng. Technol. **2**(11), 6618–6621 (2013)
16. Malien-Aubert, C.; Dangles, O.; Amiot, M.J.: Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. J. Agric. Food Chem. **49**, 170–176 (2001)
17. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Disk Susceptibility Tests-Ninth Edition: Approved Standard M2-A9. CLSI, Pennsylvania (2006)
18. Tong, W.Y.; Ang, S.N.; Darah, I.; Latiffah, Z.: Antimicrobial activity of *Penicillium minioluteum* ED24, an endophytic fungus residing in *Orthosiphon stamineus* Benth. World J. Pharm. Pharm. Sci. **3**(3), 121–132 (2014)

19. Tantituvanont, A.; Werawatganone, P.; Jiamchaisri, P.; Manopakdee, K.: Preparation and stability of butterfly pea colour extract loaded in microparticles prepared by spray drying. Thail. J. Pharm. Sci. **32**, 59–69 (2008)
20. Mahdavi, S.A.; Jafari, S.M.; Assadpoor, E.; Dehnad, D.: Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. Int. J. Biol. Macromol. **85**, 379–385 (2016)
21. Laleh, G.H.; Frydoonfar, H.; Jameei, R.; Zare, S.: The effect of light, temperature, pH and species on stability of anthocyanin pigments in four *Berberis* species. Pak. J. Nutr. **5**(1), 90–92 (2006)
22. Aramwit, P.; Bang, N.; Srichana, T.: The properties and stability of anthocyanins in mulberry fruits. Food Res. Int. **43**(4), 1093–1097 (2010)
23. Xu, Q.; Li, B.; Wang, D.; Luo, L.; Liu, G.; Zhou, Y.: Microencapsulation and stability analysis of blueberry anthocyanins. IOP Conf. Ser. Earth Environ. Sci. **252**, 052133 (2019)
24. Fan, G.; Han, Y.; Gu, Z.; Gu, F.: Composition and colour stability of anthocyanins extracted from fermented purple sweet potato culture. LWT **41**(8), 1412–1416 (2008)
25. George, F.; Figueiredo, P.; Toki, K.; Tatsuzawa, F.; Saito, N.; Brouillard, R.: Influence of trans-cis isomerisation of coumaric acid substituents on colour variance and stabilisation in anthocyanins. Phytochemistry **57**(5), 791–795 (2001)
26. Jiménez-Aguilar, D.M.; Ortega-Regules, A.E.; Lozada-Ramírez, J.D.; Pérez-Pérez, M.C.I.; Vernon-Carter, E.J.; Welti-Chanes, J.: Colour and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. J. Food Compos. Anal. **24**, 889–894 (2011)
27. Reyes, L.F.; Cisneros-Zevallos, L.: Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-and red-flesh potatoes (*Solanumtuberosu*m L). Food Chem. **100**, 885–894 (2007)
28. Silhavy, T.J.; Kahne, D.; Walker, S.: The bacterial cell envelope. Cold Spring Harb. Perspect. Biol. **2**(5), a000414 (2010)
29. Burt, S.: Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. **94**, 223–253 (2004)
30. Eyler, C.E.; Wu, Q.; Yan, K.; MacSwords, J.M.; Chandler-Militello, D.; Misuraca, K.L.; Goldman, S.A.: Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. Cell **146**, 53–66 (2011)