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Encapsulation of Clitoria Ternatea/β Cyclodextrin

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Abstract: This research is to study the encapsulation of *Clitoria ternatea* with β-cyclodextrin. *Clitoria ternatea*, also known as butterfly pea, is an Asian plant known for its bright blue flowers. It has a high antioxidant content and is frequently used as a herbal tea and natural dye. Cyclic molecules called β-cyclodextrin have a hydrophilic exterior and a hydrophobic interior. By incorporating molecules into their cavities and forming inclusion complexes, cyclodextrin can change the chemical and physical characteristics of these molecules. The bioactive compounds in these *C. ternatea* flowers, on the other hand, are extremely sensitive to environmental changes. They do, however, require proper environmental protection to avoid damage to their bioactivity. Therefore, encapsulating *Clitoria ternatea* with β-cyclodextrin could help in creating barriers between the environment and sensitive bioactive components. The powder of *C. ternatea* was purchased online, mixed with β-CD with a ratio of 1:1 and was grounded using pestle and mortar for 30 minutes. The encapsulated *C. ternatea*/β-CD were then passed through different analyses like total flavonoid content, antioxidant activity and encapsulation efficiency only few to be named. Based on the findings done in this research, β-cyclodextrin increases the total flavonoid content in *Clitoria ternatea* and shows high antioxidant activity with encapsulation efficiency of 70.87 ± 13.67 %. It can be concluded that there were differences in physicochemical characteristics of encapsulated *C. ternatea*/β-CD when compared to *C. ternatea* as a control sample and the results show that β-CD has a good effect on *C. ternatea*.

Keywords: *Clitoria Ternatea*, B-Cyclodextrin, Encapsulation

1. Introduction

Clitoria ternatea, also known as butterfly pea, belongs to the Fabaceae family of plants. The hue of its flower, which is a vibrant deep blue with light golden patterns, is its most remarkable feature. *C. ternatea* powder is a natural purple and blue pigment that is used in the making of drinks and foods. The dark blue colour of *C. ternatea* powder derives from naturally occurring anthocyanins, which not only make a stunning colouring but also act as antioxidants [1]. Since *C. ternatea* contains bioactive

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compounds and they are sensitive to environmental changes, encapsulation technology can protect the bioactive compounds from the environmental stress. In the food industry, encapsulation helps in masking undesirable colour, flavour, or taste, preserving unstable constituents, incorporating additional nutritional and functional components, and releasing the ingredients at a controlled rate and time at specific locations. Encapsulation is the process of encapsulating food ingredients, enzymes, cells or other materials in a small capsule. This technique has been widely used in the food industry because the materials encapsulated in the capsule are protected from heat, moisture, and extreme conditions, enhancing their stability and viability [2].

Cyclodextrins, particularly beta ones, are employed in the pharmaceutical and food industry because of their potential to improve medication solubility and stability by forming complexes in the solid state [3]. β-CD is a cyclic oligosaccharide made up of seven glucose subunits linked by α-(-1,4) glycosidic linkages to produce a truncated conical structure; that form when starch is broken down and can be used as an encapsulating strategy [4]. Because of its polar cavity's ability to host molecules with molecular masses ranging from 100 to 400 g mol-1, which is most molecular mass range of most compounds of interest, the β-CD is the most widely employed of the CDs. However, the bioactive compounds in these *C. ternatea* flowers are extremely sensitive to environmental changes [5]. They do, however, require proper environmental protection to avoid their structural integrity and bioactivity from being damaged [6]. Encapsulation is a technique for encapsulating active compounds inside a carrier material, and it's a useful for improving bioactive chemical and living cell delivery into foods.

This method could create barriers between the environment and sensitive bioactive components, allowing for aroma and taste distinction masking undesirable odours, stabilising food ingredients and increasing bioavailability. Able to improve stability in finished products and during processing is one of the primary reasons for encapsulating active compounds [7]. The widespread *C. ternatea*'s use in conventional medicine has prompted scientists to investigate the pharmacological properties of extracts derived from *C. ternatea*. The extracts have been shown to have nootropic, diuretic, anti-inflammatory, antiasthmatic, antipyretic, analgesic, antilipidemic, antidiabetic, antioxidant, anti-arthritic and wound healing activities in several animal experiments [8]. As a result, the goal of this study is to learn the physicochemical characteristics of encapsulated *Clitoria ternatea*/β-cyclodextrin complex to provide high productivity and ensure that the finished products is of acceptable quality and able to give protection towards the active compounds.

2. Materials and Methods

2.1 Sample Preparation

The powder of readily packed *C. ternatea* flower in an aluminium pouch with vacuum condition of 50 g was purchased online from E-commerce in Malaysia. The *C. ternatea* powder was mixed with β-CD powder with a ratio of 1:1 and was grounded using pestle and mortar for 30 minutes [9]. The encapsulated *C. ternatea*/β-CD were then stored in an amber bottle at room temperature until further use.

2.2 Functional Group Analysis

The functional group analysis was analysed using Fourier-Transform Infrared (FTIR) (IRAffinity-1S, Shimadzu, Japan). A small amount of *C. ternatea* powder, β-CD powder and encapsulated powder of *C. ternatea*/β-CD were taken to be placed in an IR chamber and analysed [10].

2.3 Colour Analysis

The calibrated spectrophotometer (MiniScan EZ 4500, HunterLab, USA) was used to determine the colour of encapsulated *C. ternatea*/β-CD flower powder and *C. ternatea* flower powder. The spectrophotometer was placed on a petri plate with 10 g of powdered sample [11]. The values of L*, a* and b* were measured. Equation (Eq. 1) were used to find colour difference of the samples.

$$\Delta E^* = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad \text{Eq. 1}$$

2.4 Moisture Content Analysis

C. ternatea powder and encapsulated *C. ternatea*/β-CD powder was placed and weighted for 2.5 g on an aluminium pan of rapid moisture analyzer (Bm-50 Series, Biobase, China). The moisture content of the samples was measured with 140° C with medium accuracy using a rapid moisture analyzer and expressed in percentage [12].

2.5 pH Level Analysis

The pH level was measured using a digital pH meter (MX-50, Japan). 4 g of the samples were weighed and diluted in 30 ml of distilled water. The pH meter was calibrated using buffer pH 3, 7 and 10 before measuring the samples pH [13].

2.6 Total Flavonoid Contents Analysis

The total flavonoid content of the encapsulated sample will be determined using an aluminium chloride assay. The samples' extract was prepared by weighted 5.3 mg of the samples and diluted it in 53 ml of distilled water. In a test tube, 1 ml of the samples was added followed by 3 ml of methanol. After that, 200 µl of aluminium chloride and potassium acetate were added to the solution. 5.6 ml distilled were added to solution and mixed together before being incubated at a rooom temperature in the dark for 30 minutes. After 30 minutes, the mixture was measured for 420 nm wavelength in UV-Vis spectrophotometer (Cary 60, Agilent, USA) [10].

2.7 Total Anthocyanin Contents Analysis

A pH-differential method was used for determining the total anthocyanin content (TAC). 1 ml of the extracted solution of the samples was added to a volumetric flask, and the remaining 10 ml were filled with pH 4.5 sodium acetate buffer and pH 1 potassium chloride buffer, respectively. For the powder analysis, 5 mL of each of the two different buffers were diluted with 0.5 g of the sample. The absorbance was determined at the wavelengths of 520 nm and 700 nm. Equation (Eq.2) was used to ascertain the pH 1 and pH 4.5 sample absorbance differences. And to determine the total anthocyanin content, equation (Eq. 3) was used [14].

$$A = (A520\,nm - A700\,nm)pH\ 1 - (A520\,nm - A700\,nm)pH\ 4.5 \quad \text{Eq. 2}$$

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad \text{Eq. 3}$$

2.8 Antioxidant Activity Analysis

The 2, 2-diphenyl-2-picrylhydrazyl (DPPH) technique was used to measure the sample's antioxidant activity. In 100 ml of methanol, 1.9 mg of DPPH was dissolved, and it was left to sit in the dark. to make a 0.1 M DPPH solution in methanol. 150 ml diluted extracts of 100 mg sample in 200 ml distilled water were added to a test tube with 3 ml of DPPH solution. After that, the mixture was left to incubate for 30 minutes at room temperature in the dark. Methanol was used as a standard for positive control. A UV-Vis spectrophotometer was used to measure the absorbance at 517 nm [10]. The percentage of antioxidant activity was calculated using the following equation (Eq. 4)

$$AA\% = \frac{abs\ control - abs\ sample}{abs\ control} \times 100 \quad \text{Eq. 4}$$

2.9 Encapsulation Efficiency

Using the equation (Eq. 5) formula suggested by [15], the effectiveness of encapsulating BPF colourant was determined. Encapsulation efficiency (EE) is the ratio of the total amount of anthocyanins in the powder to the amount of anthocyanins in the extract.

$$EE\% = \frac{total\ anthocyanin\ content\ in\ the\ powder}{total\ anthocyanin\ content\ in\ the\ extract} \times 100 \quad \text{Eq. 5}$$

2.10 Statistical Analysis

One-way analysis of variance (ANOVA) was used to collect and analyse the data, and Fisher's least squares test was used to detect significant differences with a p-value of 0.05. The data will be analysed, with the mean and standard deviation of the findings provided [10].

3. Results and Discussion

3.1 Functional Group Analysis

Most of the samples had the same functional groups (Table 1). In the *C. ternatea* sample, there are eight peaks detected with identified functional groups that are alkynes, phenols and alcohol, carboxylic acid, alkanes, alkenes, ketones, nitro groups, ethers and esters. As for β-CD, there are also 8 peaks detected with the identified functional groups of alkynes, phenols and alcohol, alkanes, alkenes, ketones, nitro groups, ethers and esters. The difference was found that β-CD is missing carboxylic acid as a functional group when compared to *C. ternatea*. In *C. ternatea*/β-CD, 7 peaks were detected with the same functional groups as *C. ternatea* but carboxylic acid and nitro groups are not present. Both *C. ternatea* and β-CD contain nitro groups but, in some way, when the two samples were inclusion together, the nitro groups are missing. Figure 1 shows the FTIR spectra of C. ternatea/ β-CD.

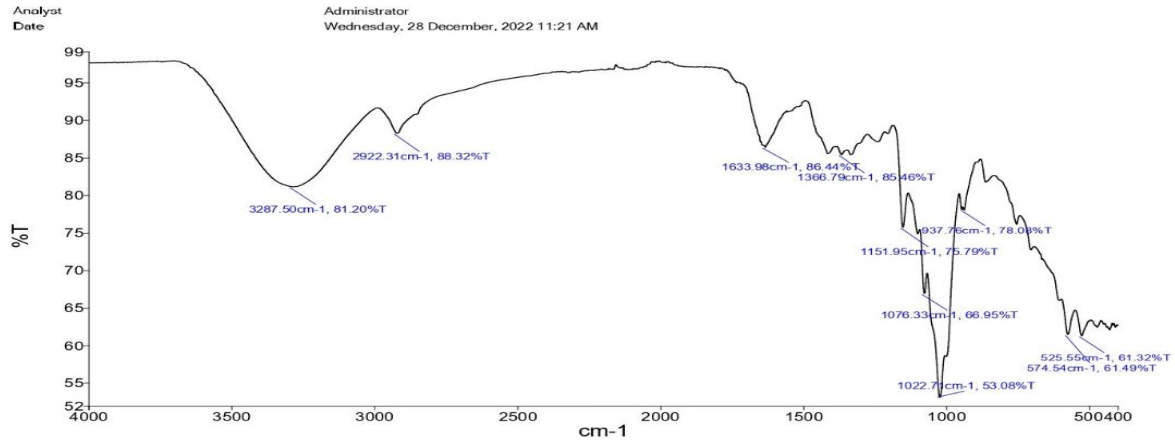

Figure 1: FTIR spectra of functional groups present in *Clitoria ternatea*/β-cyclodextrin

Table 1: The functional groups for *Clitoria ternatea*, β-cyclodextrin, *Clitoria ternatea*/β-cyclodextrin

Samples	No	Wavenumber (cm^{-1})	Frequency Ranges	Assignment	Functional Group
Clitoria ternatea	1	3271.195	3300 – 3200	≡C-H stretch	Alkynes
			3600 – 3100	Hydrogen-bonded O-H stretch	Phenols and alcohol
			3400 - 2400		Carboxylic acid
	2	2920.410	3000 - 2800	H-C-H asymmetric & symmetric Stretch	Alkanes
	3	2110.622	2200 – 2100	C≡C stretch	Alkynes
	4	1627.915	1675 – 1600	C=C-H symmetric stretch	Alkenes
			1750 – 1625	C=O stretch	Ketones
	5	1329.699	1400 – 1300	N=O bend	Nitro groups
	6	1234.991	1300 – 1000	C-O stretch	Ethers
	7	1147.589			Esters

	8	1020.440			
β-cyclodextrin	1	3257.576	3300 – 3200	≡C-H stretch	Alkynes
			3600 – 3100	Hydrogen-bonded O-H stretch	Phenols & alcohols
	2	2921.577	3000 – 2800	H-C-H asymmetric & symmetric stretch	Alkanes
	3	2114.935	2200 – 2100	C≡C stretch	Alkynes
	4	1638.276	1675 – 1600	C-C=C symmetric stretch	Alkenes
			1750 – 1625	C=O stretch	Ketones
	5	1332.336	1400 – 1300	N=O bend	Nitro groups
	6	1253.158	1300 – 1000	C-O stretch	Esters Ethers
	7	1151.605			
	8	1020.086			
Clitoria ternatea/β-cyclodextrin	1	3287.50	3300 – 3200	≡C-H stretch	Alkynes
	2		3600 – 3100	Hydrogen-bonded O-H stretch	Phenols & Alcohols
	3	2922.31	3000 – 2800	H-C-H asymmetric & symmetric stretch	Alkanes
	4	1633.98	1675 -1600	C-C=C symmetric stretch	Alkenes
			1750 – 1625	C=O stretch	Ketones
	5	1151.95	1300 -1000	C-O stretch	Esters Ethers
	6	1076.33			
	7	1022.71			

3.2 Colour Analysis

Looking at the L*a*b* values for each sample in Table 2, it can determine that the samples do not match in colour. These values indicate that *C. ternatea*/β-cyclodextrin is lighter, less yellow, more greener and bluer in colour than *C. ternatea*. To determine the differences between colours of the samples, calculations were made by subtract each of the standard value from sample value. From the calculation made, the values of $\Delta L^* = +20.45$, $\Delta a^* = -1.12$, and $\Delta b^* = -3.61$ were placed into the colour difference equation (Eq. 1), the total colour difference between the two samples is 20.80. The *p* value obtained from ANOVA for L*, a*, b* is 0.001, 0.000 and 0.000 respectively. It is less than significance level of 0.05 where there is significant difference of L*, a*, b* value of *Clitoria ternatea* and *Clitoria ternatea*/β-cyclodextrin. It appeared that the white colour of β-CD contributed to making the sample lighter and bluer in colour. *C. ternatea* powder appears to have bluish cyan hue with almost non intensity while *C. ternatea*/β-CD appears to have cyan-blue hue with pale intensity. Anthocyanins in *C. ternatea* is the phenolic pigments that cause the colour of *C. ternatea* to be deep lavender blue to violet. Five anthocyanins, three delphinidin derivatives and two cyanidin derivatives, were found to be the cause of the butterfly pea flowers' blue colour [16].

Table 2: The colour profile of the samples

Samples	L*	a*	b*
Clitoria ternatea	33.33 ± 3.55^b	-1.29 ± 0.11^a	-1.39 ± 0.21^a
Clitoria ternatea/β-cyclodextrin	53.78 ± 1.00^a	-2.41 ± 0.05^b	-5.00 ± 0.18^b

3.3 Moisture Content Analysis

From the Table 3, it is concluded that *C. ternatea* powder contains the moisture content of 9.94 ± 0.39 % while *C. ternatea*/β-CD contains the moisture content of 14.16 ± 0.28 %. The *p* value calculated from ANOVA is 0.000 which is less than 0.05. Therefore, an alternative hypothesis is accepted where there is a significant difference between the two samples. *C. ternatea* has lower moisture content while *C. ternatea*/β-CD has higher moisture content. This could be due to the moisture content of β-CD leading to the rise of the moisture content when encapsulated together with *C. ternatea*. Furthermore,

β-CD are also very accommodating to water molecules and the free CDs represent undefined hydrates at ambient temperatures, where the entrapped water has been proposed to stabilise the crystal lattice [17]. This might explain why the *C. ternatea*/β-CD moisture content is higher as the sample might have been exposed to the outside environment for quite some time before and after storage at ambient temperature. This also could lead to moisture migration between two components in the sample or between the sample and the outside environment and this can lead to the clumping of the sample where it actually happened towards both of the samples *C. ternatea* powder and *C. ternatea*/β-CD after storage. In a past study done by [10] the highest moisture content of the *C. ternatea* samples is the one that is encapsulated with gelatine with an oven dried encapsulation method at 14.33 ± 1.21 %. When comparing the result from the past study to this study, the moisture content of the sample is at the same range.

Table 3: Moisture contents, pH, total flavonoid contents, total anthocyanin contents, encapsulation efficiency, antioxidant activity of encapsulated *Clitoria ternatea*/β-cyclodextrin

Samples	Moisture Contents (%)	pH	Total Flavonoid Content (mg quercetin/ g)	Antioxidant Activity (%)	Total Anthocyanin Contents (mg cyanidin-3-glucoside/ L)	Encapsulation Efficiency (%)
Clitoria ternatea	9.94±0.39[b]	4.84±0.01[b]	97.27 ± 0.03[a]	87.45 ± 0.00[b]	24.096 ± 1.854[a]	-
Clitoria ternatea/β-cyclodextrin	14.16±0.28[a]	5.61±0.01[a]	97.29 ± 0.03[a]	91.56 ± 0.00[a]	9.268 ± 1.854[b]	70.87 ± 13.67[a]

3.4 pH Level Analysis

C. ternatea are known to serve as a cheap pH indicator. The flower extract has the ability to change colour. When it comes to the sample's extract colour, the flavylium and quinoidal forms of anthocyanin contribute nearly equally to the deep blue to purple colour of the sample at its normal pH range of 6.0 – 8.0. In a lower pH range, the flower extract will appear to be pink or light purple as more flavylium is formed. Whereas in higher pH range, the presence of both quinoidal and chalcone forms results in a green hue. According to Table 3 the pH level of *C. ternatea* is 4.84 ± 0.01 and is lower than *C. ternatea*/β-cyclodextrin with pH reading of 4.84 ± 0.01. The *p* value obtained from ANOVA for pH is 0.000 and it is less than the significance level of 0.05 which proved that there is significant difference between two samples in pH reading. This indicates that *C. ternatea* is more acidic than *C. ternatea*/β-CD but somehow both samples are in a lower range of acidic level. It appeared the β-CD helped increase the pH level of *C. ternatea* and makes it less acidic.

3.5 Total Flavonoid Contents Analysis

The total flavonoid content (TFC) in *C. ternatea* and *C. ternatea*/β-CD are almost the same and only have slight differences. The TFC values are 97.27 ± 0.03 and 97.29 ± 0.03 mg/g respectively. *C. ternatea*/β-CD seemed to be slightly higher in TFC than *C. ternatea*. Based on the ANOVA, the *p* value calculated is 0.519 which is more than the significance value of 0.05. Therefore, null hypothesis is accepted where there is no significant difference between the two samples. In a study in the past, the findings suggest that *C. ternatea* is a good source of flavonoids with strong antioxidant properties [18]. Based on the past study, the TFC determined of *C. ternatea* is 187.05 ± 3.18 mg/g at the optimum condition [19]. Compared to the past study, the TFC of *C. ternatea* in this study is lower. In another study, the TFC of *C. ternatea* encapsulated with gelatine by spray dried is 145.24 ± 2.44 and 45.24 ± 1.12 µg quercetin/100 mg by freeze dried [10]. These differences could be due to different species of *C. ternatea* being used as the flavonoids content may vary between species. Furthermore the encapsulation method in this study is different where in this study the encapsulation method used is the kneading method of purchased *C. ternatea* powder with unknown method of drying and β-CD. In a past study, it was shown that cyclodextrin (CD) inclusion complexation has a high potential for enhancing the flavonoids like natural products [20]. As for the kneading method, no heat was incorporated during

the *C. ternatea* inclusion with β-CD therefore the TFC are not affected as flavonoids are heat sensitive [21].

3.6 Antioxidant Activity Analysis

In Table 3, it can be seen that *C. ternatea* has high antioxidant activity which is 87.45 ± 0.00 % and encapsulation of *C. ternatea* and β-cyclodextrin has even higher antioxidant activity and it is 91.56 ± 0.00 %. The ANOVA results show that there is no *p* value available for antioxidant activity analysis as the standard deviation for both samples is zero which indicates that every data value is the same and is equal to the mean. The hypothesis of the samples cannot be determined as the p value cannot be obtained. *C. ternatea* itself is already known for being rich in several antioxidants. The high content of anthocyanin is credited to their antioxidant activity [22]. According to [23] because of their own structure, anthocyanins can directly combat free radicals through two different mechanisms, single electron factor and hydrogen atom transfer. Both mechanisms involve the anthocyanin producing its own free radical, but the latter is more stable and offers defence against oxidative damage brought on by the former. In a study done by [24], it was discovered that the anthocyanin extract from *C. ternatea* demonstrated potent anti-DPPH and anti-peroxyl radical antioxidant activity. [25] reported that the antioxidant activity was increased for 1:1 molar ratio of β-CD and bio active compounds particularly on DPPH assay. This could support why the antioxidant activity of *C. ternatea*/β-CD is higher as the ratio used in encapsulation for *C. ternatea*/β-CD is also 1:1.

3.7 Total Anthocyanin Content Analysis

Based on the Table 3, the total anthocyanin content (TAC) of *C. ternatea* and *C. ternatea*/β-CD have huge difference in which *C. ternatea* have higher TAC compared to *C. ternatea*/β-CD. To put together from the calculation of equation (Eq. 3), *C. ternatea* has a TAC of 24.096 ± 1.854 mg/L while *C. ternatea*/β-CD has a TAC of 9.268 ± 1.854. The *p* value obtained from ANOVA is 0.001 which is smaller than the significance value of 0.05. Where there is a significant difference between the two samples, the null hypothesis is thus rejected and an alternative hypothesis is accepted. The TAC value of *C. ternatea*/β-CD is lower compared to *C. ternatea* and this could be due to the anthocyanins were not fully extracted as the extractions of anthocyanins was influenced by the stability of the structures at various pH levels [26]. Further more the anthocyanin extracted from the samples is cyanidin-3-glucoside (c3g) and according to a study done by [27] it was found that c3g and β-CD complex was thermodynamically less stable.

3.8 Encapsulation Efficiency

Based on Table 3, the encapsulation of *C. ternatea* and β-cyclodextrin is 70.87 ± 13.67 %. The encapsualtion efficiency (EE) was calculated using the equation (Eq. 5). Based on the equation, the EE were obtained when the TAC in the powder is divided by the TAC in the extract. The TAC in the powder obtained during the experiment is 6.402 ± 0.096 and 9.268 ± 1.854 mg/L in the extract. It can be concluded tthat the encapsulation of *C. ternatea* and β-CD were as efficient as 70.87 ± 13.67 %. The ANOVA cannot be done due to there are only one factor for the dependent variable which is *C. ternatea*/β-CD is the only sample tested for encapsulation efficiency. For that reason, only means can be done on the samples to get the value of mean and standard deviation. In a study of encapsulation of *C. ternatea* powder with gelatine using freeze dried method, the EE obtained were 95.74 ± 0.05 and 63.38 ± 1.64 for the encapsulation done by convection oven dried [10]. In this study, the EE results are slightly different when compared to the past study [10], who uses a different encapsulation method on *C. ternatea*. However, based on another study [28], freeze-dried methods have higher encapsulation efficiency than the kneading method that was done for the encapsulation of *C. ternatea* and β-CD in this study. The efficiency of encapsulation is mostly determined by the biological material's ability to absorb the active substance.

4. Conclusion

In this study, it can be agreed that *C. ternatea* flower is rich bioactive compounds and rich in antioxidant as it has the antioxidant activity of 91.56 ± 0.00 % but is sensitive to environmental changes

and carrier like β-cyclodextrin managed to put the barrier between the *C. ternatea* bioactive compound and the surrounding environment. Although, not all of the analysis are succeed to prove it especially in TAC where the content is decreasing after the encapsulation between *C. ternatea* and β-CD happened. The content is 14.82 mg/L lower than *C. ternatea* who act as control sample. Not only that, this study also deduced that the result of the encapsulated *C. ternatea* were rise in moisture content as it probably seem not ideal as high moisture content can significantly affect the product's taste, texture, appearance, shape, and weight. But there also positive results shows from the encapsulation of *C. ternatea* with β-CD where it able to maintain the TFC in the *C. ternatea* flower. Last but not least, it can be concluded that the encapsulation of *C. ternatea*/β-CD was a success as it reached the encapsulation efficiency of 70.87 ± 13.67%. Because there are limitations to do this study, especially time and lack of suitable equipment and materials to evaluate the samples. A few further tests are required to understand more of the physicochemical properties of encapsulated *C. ternatea*/β-CD. As a result, the following proposal for further development have been made. For better encapsulation efficiency and results, spray dried method should be applied as it is one of the most popular microencapsulation techniques. A couple of more analyses can be conducted to understand more of the encapsulated *C. ternatea* like NMR analysis and morphological analysis using SEM spectroscopy. Total phenolic content also should have been conducted as the phenolic content exists in *C. ternatea* and it is closely related to flavonoid and anthocyanin content.

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