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Effects of Microwave Power and Carrier Materials on Anthocyanins, Antioxidants, and Total Phenolic Content of Encapsulated *Clitoria ternatea* Flower Extract

Nurul Asyikin Md Zaki[1,2]*, Junaidah Jai[1], Mohd Hakim Syuwari Hasan[1], Nur Qistina Mohamad Kamarul Azman[1], Syafiza Abd Hashib[1], Nozieana Khairuddin[3], Norashikin Mat Zain[4] and Nurul Hidayah Samsulrizal[5]

[1]*School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, 40450 UiTM, Shah Alam, Selangor, Malaysia*

[2]*Food Process Engineering Research Group, Universiti Teknologi MARA, 40450 UiTM, Shah Alam, Selangor, Malaysia*

[3]*Department of Science and Technology, Faculty of Humanities, Management and Science, Universiti Putra Malaysia, Bintulu Sarawak Campus, 97008 UPMKB, Bintulu, Malaysia*

[4]*Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, 26300 UMP, Kuantan, Pahang, Malaysia*

[5]*Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200 IIUM, Kuantan, Pahang, Malaysia*

ABSTRACT

Clitoria ternatea, also famously known as the blue pea flower (local name: *bunga telang*), has attracted interest among researchers due to its plethora of biological and pharmacological properties. It is rich in anthocyanin and widely used as a natural food colourant. However, the poor stability of active compounds may affect the therapeutic benefits and limit their application in the pharmaceutical and food industries. Hence, this work aims to study the effects of microwave encapsulation on the anthocyanins, antioxidants, and total phenolic content of *Clitoria ternatea* flower extract (CTFE). Microwave-assisted encapsulation (MAEC) was carried out at three different powers (300, 450, and 600 W) with different formulations of Gum Arabic (GA) and Maltodextrin Dextrose (MD) as carrier materials from 40% to 70% w/v. The total phenolic content (TPC), antioxidant activity, and anthocyanins in encapsulates were analysed for the formulations. The

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E-mail addresses:
asyikin6760@uitm.edu.my (Nurul Asyikin Md Zaki)
junejai@uitm.edu.my (Junaidah Jai)
hakim25syuwari@gmail.com (Mohd Hakim Syuwari Hasan)
nqistinaa72@gmail.com (Nur Qistina Mohamad Kamarul Azman)
syafiza0358@uitm.edu.my (Syafiza Abd Hashib)
nozieana@upm.edu.my (Nozieana Khairuddin)
shikin@ump.edu.my (Norashikin Mat Zain)
hidayahsamsulrizal@iium.edu.my (Nurul Hidayah Samsulrizal)
* Corresponding author

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findings showed that increased microwave power increased TPC and antioxidant activity ($P<0.05$). However, adding carrier materials concentration above 60% reduced TPC and the antioxidant activity of microwave-encapsulated anthocyanin from CTFE. The best microwave-assisted encapsulation conditions of CTFE were found at 600 W microwave power with 50% w/v carrier materials GA/MD (ratio 1:1) concentration. The retention of anthocyanins, antioxidant activity, and TPC increased significantly ($P<0.05$) with increased microwave power and lower concentration of carrier materials. The MAEC approach to enhance the stability of anthocyanin in CTFE presents a high potential to expand its application as a high-value-added natural colourant.

Keywords: Active compounds, anthocyanins, blue pea flower, microwave encapsulation, total phenolic content

INTRODUCTION

Clitoria ternatea, or the blue pea flower, is popular as a colourant for food and delicacies. Additionally, *the Clitoria ternatea* flower has been widely used in traditional medicine, particularly as a supplement to enhance cognitive functions and alleviate symptoms of numerous ailments, including fever, inflammation, pain, and diabetes (Mukherjee et al., 2008). The blue pea flower is very popular among traditional Chinese and Ayurvedic medicine and has been consumed for centuries as a memory enhancer, brain booster, anti-stress and insomnia (Anthika et al., 2015; Salleh & Pa'ee, 2021; Mukherjee et al., 2008; Verma et al., 2013). The major phytoconstituents found in extracts from *Clitoria ternatea* flowers are pentacyclic triterpenoids such as taraxerol and taraxerone (Swathi et al., 2021). Furthermore, various phytochemicals such as kaempferol, quercetin, myricetin glycosides, and anthocyanins have been successfully isolated from *Clitoria ternatea* flowers (Jeyaraj et al., 2021). Thus, *Clitoria ternatea* flower extract (CTFE) has the potential to be used for functional food applications due to its plethora of biological and pharmacological properties.

The stability of polyphenols from CTFE has been reported to be very poor, especially in the human gastrointestinal tract. Therefore, it is important to develop a protective method to increase the stability of polyphenols and investigate their encapsulation under simulated gastrointestinal conditions. Microencapsulation of polyphenols from *Clitoria ternatea* flower petals using calcium alginate found that polyphenol degradation has been successfully reduced, while the biological activity increased after gastrointestinal digestion (Pasukamonset et al., 2016). According to Bringas-Lantigua (2011), encapsulation of CTFE may enhance the bioavailability of antioxidants and anthocyanins in the extracts. It is often used to protect the natural plant extract pigments from degradation and, thus, extend the shelf-life of these active compounds. Encapsulation is important and relevant in food and pharmaceutical industries as it protects food ingredients sensitive to degradation,

denaturation, and loss of volatile compounds. There are many encapsulation techniques, such as coacervation, spray drying, nanoencapsulation, microencapsulation, and microwave encapsulation.

Among the encapsulation techniques, microwave-assisted encapsulation is an alternative technology that uses microwave radiation to stimulate molecular motion and a constant dipole to rotate the molecules to generate volumetric heating. Time and energy consumption can be reduced significantly during microwave treatment due to its uniform heat distribution onto the material surfaces. Microwave technology is considered an economical method of preserving plant extract, as this method provides quality for the final product, is easy to operate, and could reduce water activity in the final products. Furthermore, the microwave-assisted technique has excelled in encapsulating natural colourants from dragon fruit (Zaidel et al., 2015), purple sweet potatoes (Nawi et al., 2015), as well as from hibiscus, lavender, and blackberry (Perez-Grijalva et al., 2018). The advantages of encapsulation via microwave technology are enhancement of antimicrobial activity, prolonged effects, and stability improvement towards the encapsulated plant extracts. However, the microwave technique is not widely used as there are still limited references on the microwave-assisted encapsulation (MAEC) process. Hence, this work aimed to evaluate the effects of microwave encapsulation on the anthocyanins, antioxidants, and total phenolic content of *Clitoria ternatea* flower extract.

METHODOLOGY

Materials

The dried blue pea flower was purchased from a local supplier around Klang Valley, Malaysia. Distilled water, maltodextrin, Gum Arabic, ethanol, hydrochloride acid, sodium hydroxide, potassium chloride, sodium acetate buffer, Folin-Ciocalteu phenol reagents, gallic acid, and sodium carbonate solution were purchased from Sigma Aldrich (USA).

Preparation of *Clitoria ternatea* Flower Extract

Clitoria ternatea flower was extracted by weighing 5 g each before being diluted in 100 ml of water (1:20) for 5 min and undergoing extraction. The extraction process was conducted using microwave-assisted extraction at 600 W power for 2 min. The *Clitoria ternatea* flower extract (CTFE) was then centrifuged at 7000 rpm for 15 min and ready to be further encapsulated using the microwave.

Microwave-Assisted Encapsulation of *Clitoria ternatea* Flower Extract

The carrier materials were mixed and diluted with water at various concentrations (40%, 50%, 60% and 70%) of Arabic Gum (GA) and Maltodextrin Dextrose (MD). The carrier

materials, GA and MD (1:1), were mixed with extracted sample (1:5) in the beaker and stirred for 20 min. The sample was then encapsulated in the microwave using the microwave-assisted encapsulation (MAEC) method using three power levels (300 W, 450 W, and 600 W) for 7 to 10 min until powder forms. The MAEC method was modified by Marsin et al. (2020).

Analysis of Total Phenolic Content

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method modified by Bei et al. (2018), in which 1 ml of extract was mixed with 0.5 ml of Folin-Ciocalteu reagent. After 5 min being kept in the dark at 26°C, 1 ml of sodium carbonate was added with 9 ml of distilled water. The absorbance was measured at 760 nm after 30 min incubation period in the dark. The results were expressed in mg of Gallic Acid Equivalent (GAE)/g of dry weight sample of BPF (mg GAE/g). All samples were analysed in triplicates. The formula of TPC is shown in Equation 1:

$$\mathrm{TPC} = \mathrm{C} \times \frac{\mathrm{v}}{\mathrm{m}} \quad (1)$$

where C is the rate constant, v is the pre-exponential factor, and m is the activation energy.

Analysis of Total Anthocyanin Content

The anthocyanin was measured using a pH differential method using a potassium chloride with pH 1.0 and a sodium acetate buffer with pH 4.5 (Nawi et al., 2015). An amount of 1 ml of extract was mixed with 9 ml of each buffer to produce 10 ml of solution, which was then incubated at 37°C for 15 to 60 min. All the experiments were carried out in triplicates. Both the solutions were measured at 520 nm and 700 nm, and the absorbance (A) and total anthocyanin were calculated using Equations 2 and 3:

$$\mathrm{A} = (\mathrm{A}_{520nm} - \mathrm{A}_{700nm})_{pH1} - (\mathrm{A}_{520nm} - \mathrm{A}_{700nm})_{pH4.5} \quad (2)$$

$$\mathrm{TAC} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF} \times 1000}{\varepsilon \times 1} \quad (3)$$

where: A is the absorbance at a specified wavelength, MW is the molecular weight for cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, and ε = 26,900 molar extinction coefficients in L/mol/cm.

Determination of Antioxidant Activity

The CTFE antioxidant scavenging activity was measured using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) following procedures of Alwi et al. (2017) with some

modifications. First, an amount of 1 ml extract was mixed with 1 ml of DPPH solution and 4 ml of 80% ethanol and incubated at 37°C for 30 min. All the experiments were carried out in triplicates. The absorbance was measured at 501 nm, and DPPH inhibition was determined using Equation 4:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (4)$$

where: A_o is the absorbance of the control solution (DPPH and ethanol solution without the CTFE sample), and A_1 is the absorbance of the sample.

Statistical Analysis

Statistical analysis was performed using Microsoft® Excel® for Microsoft 365 MSO (Version 2208 Build 16.0.15601.20072). A one-way analysis of variance (ANOVA) followed by a post hoc t-test was carried out to determine the statistical differences between encapsulated CTFE for each tested parameter. The significance level used was 0.05.

RESULTS AND DISCUSSION

Total Phenolic Content of Encapsulated CTFE

Figure 1 shows the total phenolic content (TPC) of encapsulated CTFE with different concentrations of carrier materials at various microwave powers. The results found a significant influence of GA/MD concentration on the total phenolic content ($P < 0.05$). The TPC increased gradually as the microwave power increased from 300 W to 600 W. However, at 50% GA/MD concentration, TPC showed no significant difference from 300 to 600 W. The highest TPC was obtained at 600 W and 40% concentration, which was 780 mg GAE/g. At high power, the microwave can speed up the encapsulation process.

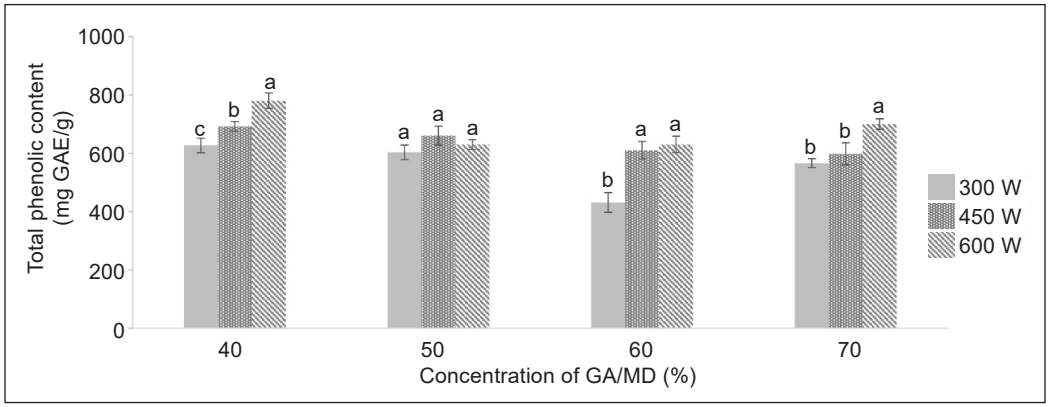

Figure 1. Total phenolic content of encapsulated CTFE at a microwave power of 300 W, 450 W, 600 W and GA/MD 40%, 50%, 60%, and 70% concentration. Bars with different letters indicate significant differences in microwave power within the same GA/MD concentration ($P < 0.05$).

Thus, the targeted bioactive compounds can be protected from heat exposure for a long time. However, it can also cause the decomposition of certain molecules and damage the bioactive compounds due to heat generated by the high power. For the comparison of the extract samples before and after encapsulation, it was observed that the TPC amount after encapsulation was higher compared to the number of fresh extracts. The encapsulation process exposed the extracts to microwave radiation, thus, increasing the temperature of the materials and the exposure to heat caused damage to the bioactive compounds. It was also reported that plant phenols could degrade at high temperatures during the encapsulation or extraction process (Sulaiman et al., 2017).

The amount of TPC decreased significantly ($P < 0.05$) as the carrier materials concentration increased. The decreasing pattern of TPC against the carrier material concentration was observed at a microwave power of 450 W. The coating agent protects the phenol compounds from degradation at constant microwave power. MD, as a polymeric coat, could assist in retaining bioactive compounds in the encapsulated extract (Sablania & Bosco, 2018). Besides, the encapsulation technique involves a defensive mechanism of the wall membrane that covers the particles of the encapsulated material to ensure that no active ingredients or phenolic compounds leak from the carrier materials (Mozafari, 2008). However, the TPC of CTFE with 40% GA/MD was the highest, although it contained the lowest carrier materials. It might be attributed to the accumulation and sedimentation of gum arabic in the suspension with a higher concentration of GA/MD prior to MAEC. The phenomenon could lead to reduced GA/MD in the sample and result in less encapsulation of TPC. This finding was similar to the encapsulation of isoflavone with milk, maltodextrin, and gum acacia (Mazumder & Ranganathan, 2020). Several factors could affect encapsulation, such as the chemical properties of carrier materials and extracts, emulsion characters, and encapsulation parameters.

Total Anthocyanin Content of Encapsulated CTFE

The total anthocyanin content (TAC) of encapsulated CTFE with a 40% concentration of GA/MD at various microwave powers is shown in Figure 2. This condition was chosen based on the highest TPC obtained during the MAEC. The highest TAC was obtained when the encapsulation was performed using 600 W at 7 min encapsulation time. It was observed that the amount of TAC increased as the microwave power increased

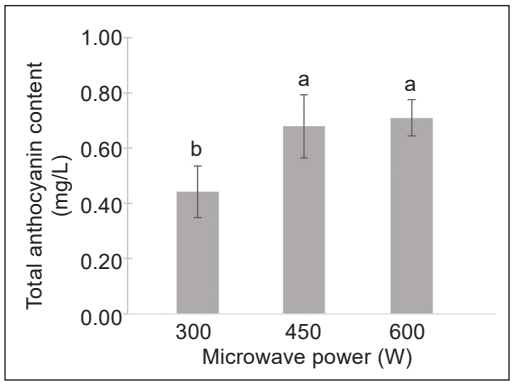

Figure 2. Total anthocyanin content of encapsulated CTFE with 40% concentration of GA/MD at microwave powers of 300 W, 450 W, and 600 W ($P < 0.05$)

for the encapsulation of CTFE with a 40% concentration of GA/MD. Low microwave power resulted in longer encapsulation time due to low heat generated throughout the process. Thus, anthocyanins monomeric may decompose during this long encapsulation time (Marsin et al., 2020).

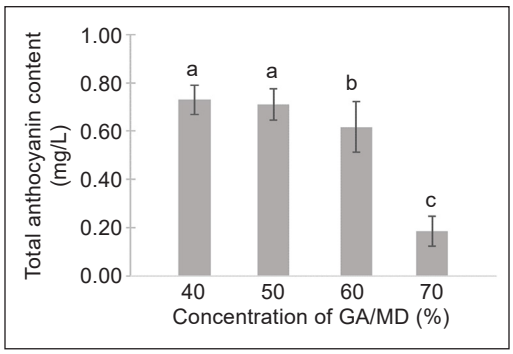

Figure 3. Total anthocyanin content of encapsulated CTFE with different concentrations of GA/MD at 600 W ($P < 0.05$)

Figure 3 shows the TAC of encapsulated CTFE with different concentrations of carrier materials at 600 W microwave power. The results found a significant effect of GA/MD concentration on TAC values on the encapsulated CTFE ($P < 0.05$). In this study, based on the overall TAC obtained at various concentrations and microwave power, the highest TAC was obtained when the CTFE was encapsulated with 40% GA/MD (0.73 ± 0.06). This finding could be attributed to using GA/MD as the carrier material that covers and protects the extracted compounds from the environment. Encapsulation reduces the core reactivity of the environmental factors and the transmission rate of the core material to the outside environment by forming a physical barrier between the core compound and the other external compound of the product (Jyothi et al., 2010). Nonetheless, TAC was reduced when the GA/MD was increased. An increase in GA/MD concentration decreased the total anthocyanin content in blue pea powder due to adding dried solids to the mixture (Hariadi, 2018). The ratio of carrier materials to the CTFE increased, thus, possibly causing the accumulation and sedimentation of the carrier materials in the suspension with a higher concentration of GA/MD. It affected the protective characteristic of anthocyanin and necessarily decreased its content.

The TAC of the initial extract was 0.9184 mg/L. The encapsulation resulted in a huge loss of anthocyanins from the initial extract due to the heat exposure to CTFE during MAEC. However, the encapsulation of CTFE is important to protect the anthocyanins from degradation during storage. Factors such as storage with access to light and air did not cause significant degradation of anthocyanins in encapsulated samples due to efficient coating by carrier materials (Pieczycolan & Kurek, 2019). Furthermore, the degradation of anthocyanins occurs on the surface, and the encapsulated extract is adequately protected against the transfer of oxygen through the density of the matrix and the distance from the degradation factor (Tonon et al., 2010).

Antioxidant Activity of Encapsulated CTFE

Figure 4 shows the percentage of inhibition demonstrated by encapsulated CTFE at various microwave power for encapsulation with a 40% concentration of GA/MD. The highest

inhibition was exhibited by encapsulated CTFE at 600 W, which accounted for 78%. The lowest inhibition was observed as 70% at 300 W. These findings demonstrated that using the microwave to encapsulate CTFE can retain most of the antioxidant activity. Therefore, the antioxidant activity of CTFE peaks at a microwave power of 600 W. However, when it reaches the optimum condition, the value decreases or becomes negligible up to a certain point due to exposure to a higher temperature. A study on the antioxidant activity of dried ginger reported an increase in microwave power of 0.6 W/g–0.9 W/g. However, it decreased when the microwave power was further increased to 1.2 W/g, which may be related to the degradation of active substances under high microwave power (Zeng et al., 2023).

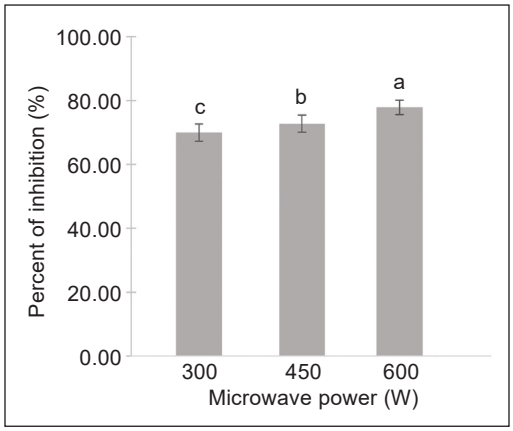

Figure 4. Antioxidant activity of CTFE encapsulated with 40% concentration of GA/MD at microwave powers of 300 W, 450 W, and 600 W ($P < 0.05$)

High microwave power results in a faster encapsulation and may retain higher antioxidants than low microwave power (Marsin et al., 2020). The encapsulation rate may be lower due to low microwave power. It will affect the encapsulation efficiency of the end product. The heat penetration will also be low, thus causing longer encapsulation time; prolonged exposure to heat during the encapsulation process results in the degradation of antioxidants. However, a single microwave process may also cause non-uniform heating and a low penetration rate of microwave energy (Ng et al., 2020).

The encapsulation process might affect the polyphenol activity of the extract, which in turn affects the antioxidant activity. It was found that MAEC provided high retention of antioxidant activity, which might be due to the shorter time at a lower temperature process, which reduced the degradation of phenolic compounds (Parthasarathi et al., 2013). Furthermore, polyphenols extracted from apple pomace and fermented apples using microwave-assisted extraction at 60°C obtained higher antioxidant activity than samples extracted at different temperatures (30, 40, 50, 70, and 80°C) (Ajila et al., 2011).

Encapsulation Efficiency of Encapsulated CTFE

Figure 5 shows the encapsulation efficiency of encapsulated CTFE with various concentrations of GA/MD at 600 W. The best encapsulation efficiency was 40% and 50% of GA/MD concentrations at 600 W microwave power, with 76% and 78% values, respectively. This percentage is high enough to confirm that GA/MD is an appropriate combination for carrier material encapsulating CTFE. The encapsulation efficiency of

Clitoria ternatea flowers is 60.22%, 63.38% and 95.74% for ultrasonic spray drying (outlet temperature 100°C), convection oven drying (80°C), and freeze-drying (-80°C), respectively (Liew et al., 2020). The encapsulation was unstable above 50% concentration of carrier materials. Therefore, the encapsulation efficiency decreased when encapsulation was done with carrier materials above 50% concentration (Marsin et al., 2020) which might be due to the accumulation and sedimentation of excess carrier materials in the suspension because the solubility of arabic gum is 43–48% in water. It shows that the encapsulation has reached saturation at 50% concentration of GA/MD and limits its capacity.

The higher encapsulation efficiency can be attributed to the electrostatic ionic interaction between magnesium and potassium cations in gum arabic polysaccharides. A similar study on the encapsulation of anthocyanin from *Syzygium cumini* found that arabic gum provided higher encapsulation efficiency than chitosan, maltodextrin, and sodium alginate (Abdin et al., 2021). Arabic gum forms a dry coating around the core material, preventing contact between the core material and air, whereas maltodextrin develops an amorphous glass structure during encapsulation (Yadav et al., 2020).

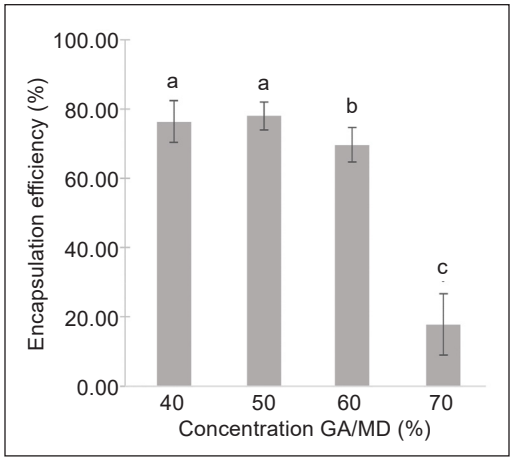

Figure 5. Encapsulation efficiency of CTFE encapsulated with 40%, 50%, 60%, and 70% concentrations of GA/MD at 600 W ($P < 0.05$)

CONCLUSION

In conclusion, the best concentration of carrier materials for encapsulating CTFE with arabic gum and maltodextrin dextrose is 50%, which provides higher encapsulation efficiency, shorter time, and stable preservation of bioactive compounds. Based on the findings, the optimum condition for encapsulation efficiency was achieved at 600 W of microwave power and 50% of GA/MD concentration. Microwave power, exposure time, and concentration of carrier materials significantly affect the retaining of anthocyanins, antioxidants and total phenolic content of *Clitoria ternatea* flower extract. Anthocyanin retention increases with microwave power and lower concentration of carrier materials. Therefore, the microwave-assisted encapsulation approach to enhance the stability of anthocyanin in CTFE presents a high potential to expand its application as a high-value-added natural colourant.

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