

## Optimisation of Microwave-assisted Encapsulation of Black Mulberry (*Morus nigra*) Extract

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Black mulberry (*Morus nigra*) fruit extract contains high amounts of a bioactive compound known as anthocyanin. However, this compound is very sensitive to heat and light. Encapsulation is the most efficient method to protect the anthocyanin from harsh environments. In this study, a combination of gum Arabic and maltodextrin was used as wall materials to encapsulate black mulberry extract using a microwave-assisted technique. The main objective of this study was to optimise the microwave-assisted encapsulation of black mulberry to achieve an extract with the highest encapsulation efficiency (EE), total anthocyanin content (TAC), and antioxidant capacity (DPPH inhibition). The factors studied were material core-to-wall ratio (1:3–1:10), microwave power (450–630 W), and encapsulation time (2–4 min). The experiment followed a Face-Centred Central Composite Design. Based on the ANOVA results, only the core-to-wall ratio had a significant effect on EE, TAC, and DPPH while power only had a significant effect on DPPH. Based on the desirability function, the optimum encapsulation of black mulberry extract was 90.14 % EE, 23.03 mg/g dry basis TAC, and 85.37 % DPPH inhibition, obtained using the following microwave-assisted encapsulation parameters: core-to-wall ratio of 0.19 mL/mL, microwave power of 450 W, and time of 2.94 min.

### 1. Introduction

Observational and experimental trials have shown a significant correlation between fruit- and vegetable-rich diets and a reduced risk to certain chronic diseases. This result could be partly attributed to the existence of antioxidants in this type of diet, particularly phenolic compounds (Capanoglu et al., 2008). Black mulberry (*Morus nigra*) is one of the common mulberry species. Black mulberry fruits contain high concentrations of absolute phenolics, flavonoids, and ascorbic acid (Hojjatpanah et al., 2011). Anthocyanins, which give the dark purple pigment of the black mulberry fruit, are the main group of flavonoids in the fruit (Fazaeli et al., 2013). Earlier studies have shown a strong correlation between antioxidant activity and the content of anthocyanins in berries (Kalt et al., 1999). Future Market Insights reported that the mulberry market has been growing gradually from 2013 to 2017, and this market is expected to continue to grow until the year 2028. The global interest in the use of mulberry as a dietary supplement has increased every year. Black mulberry fruits are generally consumed fresh, but it can also be made into processed products e.g. juice, treacle, chutneys, and wines (Hojjatpanah et al., 2011). Processing stages can change and cause deterioration in the quality of natural antioxidants in fruits and vegetables. The heating process and multiple separation steps in processing can lead to oxidation, thermal degradation, and leaching; thus leading to lower antioxidant concentrations in processed products compared to fresh ones.

Since anthocyanin is a vulnerable compound, it is compulsory to encapsulate it because as a stable encapsulated product, anthocyanin can offer more therapeutic health benefits to consumers. Today, researchers and scientists have redirected their interests towards using the microwave technique for food encapsulation. In general, microwave-assisted encapsulation is preferred due to a shorter processing period, high product quality, low processing cost, and a broad capability to produce dried products (Haghi et al.,

2008). Microwave technology can reduce power consumption, curb waste products, minimise the impact on the environment, and ensure security (Zaidel et al., 2014).

Normally, encapsulation performance is better with a mixture of wall materials instead of a single material, so two or more wall materials are commonly used to achieve good encapsulation effectiveness (Nawi et al., 2015). There are a variety of organic and inorganic wall agent polymers that are available, including carbohydrates, gums, fatty acids, proteins, oils, and fats. Gum Arabic has multiple sugar heteropolymers that contain a small quantity of protein covalently connected to the carbohydrate chain, making it a useful film-forming agent and therefore efficient for encapsulating molecules. Encapsulation makes the cation of anthocyanins less vulnerable towards water molecule nucleophilic intrusion; thus enhancing the anthocyanin quality (Burin et al., 2011). Meanwhile, maltodextrin is normally used as another wall material because of its water-soluble features and its ability as an excellent textural modifier. Furthermore, it is less expensive than other edible hydrocolloids and it has a more delicate flavour and mouthfeel, so it does not affect the core material's original flavour (Takeiti et al., 2010). A mixture of carbohydrate and gum may provide maximum encapsulation effectiveness (Gharsallaoui et al., 2007). A study by Mahdavi et al. (2016) revealed that using wall materials made of a mixture of gum Arabic and maltodextrin resulted in the highest encapsulation effectiveness of the encapsulated product. Similar findings were found in the encapsulation of anthocyanins from roselle (Idham et al., 2012) and the encapsulation of anthocyanins from grape (Burin et al., 2011).

The most suitable encapsulation method is determined from the physicochemical properties of the core material as well as the shell material (Ubbink and Kruger, 2006). Most importantly, the method chosen must be able to create uniformly distributed encapsulated spherical spheres with high encapsulation efficiency and high loading capacity. Besides, the method must also be able to function under simple conditions and is cost-saving. The common encapsulation techniques comprise spray-drying, freeze-drying, melt injection, melt infusion, emulsification, coacervation, crystallisation, fluid-bed coating, and microwave-assisted encapsulation (Vincekovic et al., 2017). Although in recent years some studies have investigated the encapsulation of anthocyanin extracted from diverse sources such as purple potatoes (Nawi et al., 2014) and red dragon fruits (Zaidel et al., 2015), none have investigated the encapsulation of black mulberry anthocyanin using microwave-assisted encapsulation. Therefore, the main aims of this study are to evaluate the potential of and the optimum extraction conditions for, the microwave-assisted encapsulation of black mulberry to achieve an anthocyanin-rich extract with high EE, TAC, and DPPH.

## 2. Materials and methods

### 2.1 Materials

Black mulberry fruit at the ripening stage (black colour) was purchased from Zenxin farm in Kluang, Johor. The chemicals used in this work were analytical grade (Sigma Aldrich, US).

### 2.2 Experimental design

Response Surface Methodology (RSM) was used to optimise the microwave-assisted encapsulation of black mulberry extract. Face-Centred Central Composite Design was applied in this study due to the limitation of the selected factors. The factors studied are listed in Table 1, together with their levels. This study selected the core-to-wall ratio, encapsulation time, and microwave power as the factors to be investigated based on the results of a preliminary study. The experimental design consists of 20 factorial experiments with 6 replicates at the centre point.

*Table 1: Factors studied in the encapsulation of black mulberry extract*

Factor name	Factor	Factor level		
		-1	0	1
Core-to-wall ratio (mL/mL)	A	0.1	0.2	0.3
Time (min)	B	2	3	4
Power (W)	C	450	540	630

### 2.3 Anthocyanin extraction

The fruits were dried at -40 °C for 48 h using a freeze-dryer (Alpha 1-2 LD plus, Belgium). The freeze-dried mulberry fruits were ground using a blender until the sample particle size reached 2 mm. The extraction was conducted using a solid-to-liquid ratio of (0.6 g/mL), 476 W microwave power, and a time of 11 min. All experiments were performed using water with 10 % ethanol as the extraction solvent. The temperature of extraction was fixed at 50 °C to prevent the degradation of anthocyanins. A MAS II microwave extractor (China) was used to assist the black mulberry extraction.

## 2.4 Microwave-assisted encapsulation

Gum Arabic and maltodextrin were used as the coating materials for encapsulating the black mulberry anthocyanin-rich extract. The mixture of gum Arabic, maltodextrin, and black mulberry extract was homogenised to achieve a uniform dispersion at 8,000 rpm using an IKA T-25D Ultra-Turrax homogeniser (Germany) for 5 min. Three mixtures with different core-to-wall ratios were prepared; 1:3 (v/v), 1:5 (v/v), and 1:7 (v/v). For encapsulation, the mixtures were placed in round glass plates and put inside a domestic microwave oven (Sharp, Japan). The mixtures were then dried and encapsulated at different combinations of power and time (Table 1). The process continued until the slurry changed into a powdery form (Abbasi and Rahimi, 2008).

## 2.5 Encapsulation efficiency

The Total Anthocyanin Content (TAC) and Surface Anthocyanin Content (SAC) were determined according to the method outlined in Mahdavi et al. (2016). To acquire the SAC, 100 mg of samples was weighed and added to 1 mL distilled water. The encapsulated black mulberry samples were ground using pestle and mortar. Then, 10 mL of ethanol was added and the samples were extracted for 5 min before being filtered. The surface anthocyanin extraction from the capsules was performed by rapidly washing the capsules with 10 mL ethanol in a vortex mixer for 10 s. Next, the mixture was centrifuged at 3,000 rpm for 3 min at 20 °C. After the phase separation, a clear supernatant was obtained, collected, and filtered over a 0.45 µm Millipore membrane size. The encapsulation efficiency (EE) was determined using Eq(1):

$$EE \% = \frac{(TAC - SAC)}{TAC} \times 100 \quad (1)$$

## 2.6 Total Anthocyanin Content

The Total Anthocyanin Content (TAC) of the encapsulated black mulberry extract was obtained following a pH-differential method using two different buffer systems as described in Huang et al. (2017). The first buffer was a potassium chloride buffer with pH 1.0 at 0.025 M, 125 mL of 0.2 M KCl, and 375 mL with 0.2 M HCl while the second was a sodium acetate buffer with pH 4.5, 0.4 M, 400 ml of 1 mL sodium acetate, 240 mL of 1 M HCl with 360 mL of water. To determine TAC, 0.1 mL sample solution was aliquoted into a 10 mL volumetric flask. Next, a buffer was added until it reached 10 mL of the whole solution. The absorbance of the solution was measured at 520 nm and 700 nm using a Jenway Genova UV-VIS spectrophotometer (United Kingdom). Distilled water was used as a blank in this measurement. The difference in absorbance between pH 1.0 and pH 4.5 samples was calculated using Eq(2):

$$Absorbance, A = (A_{520\text{ nm}} - A_{700\text{ nm}})_{pH1} - (A_{520\text{ nm}} - A_{700\text{ nm}})_{pH4.5} \quad (2)$$

The total anthocyanin content was calculated as cyanidin-3-glucoside (mg/g) according to Eq(3):

$$(mg.g^{-1}) = \frac{A}{\epsilon \cdot L} MW \times DF \times \frac{V}{W} \times 10^3 \quad (3)$$

where A is as described in Eq(2),  $\epsilon = 26,900$  molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside at 510 nm, L = path length = 1 cm, MW = the molecular weight of cyanidin-3-glucoside = 449.2 g/mol, DF = the dilution factor, V = the volume of extracting solution, and W = the weight of powder in g.

## 2.7 DPPH test

To evaluate the antioxidant activity of the encapsulated black mulberry extract, a DPPH assay (2,2-diphenyl-1-picrylhydrazyl) according to Sharma and Bhat (2009) was used. The extract, in the amount of 4.5 mL, was added into a 4.5 mL DPPH solution in a 15 mL tube. This tube was fully covered with aluminium foil to prevent exposure to light. The mixture was then shaken vigorously and kept aside for 30 min at room temperature. The absorbance of the mixture at 517 nm was measured using a spectrophotometer (Jenway Genova, United Kingdom). Eq(4) was used to calculate the DPPH percentage inhibited by the encapsulated black mulberry extract.

$$DPPH\ inhibition\ \% = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad (4)$$

## 2.8 Model verification

A verification test was performed to determine the reliability of the models. The models obtained for EE, TAC and DPPH were then validated using the methods mentioned in Section 2.3 to Section 2.5.

### 3. Results

#### 3.1 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) was conducted to evaluate the effects of the factors, the potential interactions between the factors, and the statistical significance of the EE, TAC, and DPPH models (Table 2). The adequacy of the models was evaluated by determining the coefficient of regression ( $R^2$ ), adjusted  $R^2$  value, and P-values. Based on a previous study, an  $R^2$  value greater than 0.75 indicates that the model has a good fit (Espada-belido et al., 2017). In this study, the values of  $R^2$  for EE, TAC, and DPPH were 0.9369, 0.8478, and 0.877. All values were within a satisfactory range, implying that the models were adequate. The P-value determines the significance of the model terms (Setyaningsih et al., 2012). P-values < 0.05 indicate the model is significant. The p-values obtained for EE, TAC and DPPH were 0.0015, 0.0015, and 0.0084. As shown in Table 2, the models (EE:0.817, TAC:0.2365, DPPH: 0.0783) and pure error values denoted good reproducibility of the experimental data. Table 3 shows the models for predicting EE, TAC and DPPH obtained using ANOVA.

Table 2: ANOVA results of the response surface quadratic model EE, TAC, and DPPH

Source	EE			TAC			DPPH			
$R^2$ values	$R^2 = 0.9369$ ; Adj. $R^2 = 0.8801$			$R^2 = 0.8478$ ; Adj. $R^2 = 0.7108$			$R^2 = 0.8747$ ; Adj. $R^2 = 0.7619$			
Factor	DFSS	MS	P-value	SS	MS	P-value	SS	MS	P-value	
Model	9	8496.82	55.98	0.0015 <sup>a</sup>	503.85	55.98	0.0015 <sup>a</sup>	1241.45	137.94	0.0084 <sup>a</sup>
A	1	3.60	3.60	0.8070	1.24	1.24	0.6810	0.000	0.000	1.0000
B	1	136.90	136.90	0.1529	0.33	0.33	0.8302	19.04	19.04	0.4171
C	1	129.60	129.60	0.1632	0.25	0.25	0.8531	146.69	146.69	0.0407
$A^2$	1	2445.09	2445.09	0.0001	87.90	87.90	0.0051	215.61	215.61	0.0173
$B^2$	1	109.78	109.78	0.1961	10.55	10.55	0.2449	0.058	0.058	0.9636
$C^2$	1	21.84	21.84	0.38	8.55	8.55	0.2920	159.03	159.03	0.0345
AB	1	861.13	861.13	0.0031	160.38	160.38	0.0007	0.000	0.000	0.0818
AC	1	861.13	861.13	0.0031	0.080	0.080	0.9165	0.000	0.000	0.0818
BC	1	78.13	78.13	0.2697	0.016	0.016	0.9623	4.80	4.80	0.6797
Lack of fit	5	454.80	90.96	0.0817 <sup>b</sup>	24.54	4.91	0.2365	32.75	42.46	0.0783
Pure error	5	117.33	23.47	-	44.56	8.92	-	3.61	10.70	-

\*SS: sum of squares; DF: degree of freedom; MS: mean square

Table 3: The models of EE, TAC, and DPPH obtained in terms of coded values

Response	Regression equation
EE (%)	$88.53 - 0.60A + 3.70B - 3.60C + 10.38AB + 10.38AC + 3.13BC - 29.82A^2 - 6.32B^2 - 2.82C^2$
TAC (mg/g)	$22.24 - 0.032A - 1.40B - 0.67C + 0.080AB - 0.015AC - 0.46BC - 5.27A^2 - 2.29B^2 + 0.16C^2$
DPPH (%)	$84.91 - 0.40A + 1.80B - 2.10C + 5.38AB + 5.38AC - 0.62BC - 22.7A^2 - 7.27B^2 - 1.77C^2$

#### 3.2 Effect of core-to-wall ratio, time, and power on EE, TAC, and DPPH

This study showed that the effectiveness of microwave-assisted encapsulation depended mainly on the core-to-wall ratio (Table 2 and Figure 1).

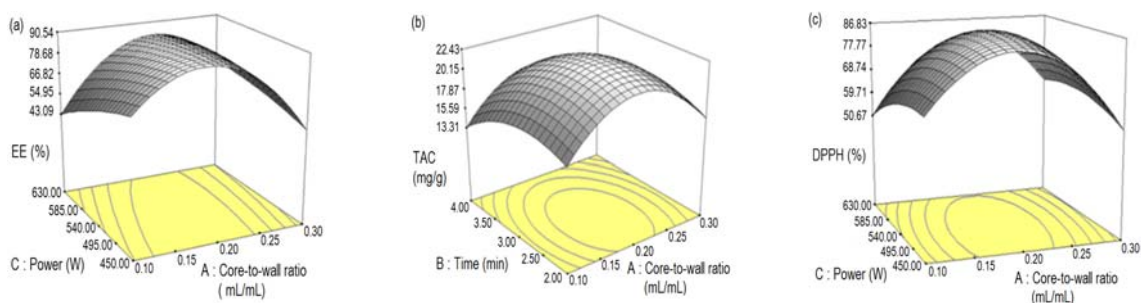


Figure 1: Response surface curves of the core-to-wall ratio, power, and time on (a) EE, (b) TAC, and (c) DPPH

The quadratic term of the core-to-wall ratio had a significant effect on EE, TAC, and DPPH (Table 2). The interaction term between core-to-wall ratio and power also significantly affected EE. In addition, the interaction term between core-to-wall ratio and time was significant for EE and TAC. As indicated by Hogan et al. (2001), the core-to-wall material ratio had a higher impact on powder characteristics and encapsulation effectiveness. Cakrawati et al. (2017) also highlighted the importance of core-to-wall ratio in encapsulation effectiveness. The interactions between the two factors can be observed in Figure 1. Referring to Table 2, it can be observed that there was a significant effect between the interaction of time and core-to-wall ratio on EE and TAC. This data is also justified by the results of Abbasi and Rahimi (2008). The type of core materials and microwave time influence the efficiency of the encapsulation procedure, as logically, a longer time will lead to the degradation of anthocyanin. Meanwhile, the power factor had a significant effect on DPPH, but had no significant effect on EE and TAC, which are the same results found by Padzil et al. (2018), who showed that 550 W and 330 W microwave power had no significant impact on the Total Monomeric Anthocyanin in a purple sweet potato that had been subjected to microwave-assisted encapsulation.

### 3.3 Microwave-assisted encapsulation optimisation via desirability function

Using the Desirability function in Design Expert (6.0.4, Stat-Ease, USA) software, the optimum conditions for the microwaved-assisted encapsulation of black mulberry extract was determined as a core-to-wall ratio of 0.19 mL/mL, 450 W microwave power, and an encapsulation time of 2.94 min (Table 4). These conditions were selected based on the highest desirability value of 84 %. The optimal value suggested for EE, TAC, and DPPH were 90.08 %, 23.03 mg/g, and 85.58 %.

*Table 4: Optimisation conditions for microwave-assisted encapsulation of black mulberry extract*

Core-to-Wall (mL/mL)	Time (min)	Power (W)	EE (%)	TAC (mg/g)	DPPH (%)	Desirability (%)
0.19	2.94	450.00	90.08	23.03	85.58	84.00

### 3.4 Verification of models

A model verification study was conducted to check the adequacy of the models obtained. Another set of experiments using the optimised conditions in Section 3.3 was conducted and the experimental values of EE, TAC and DPPH obtained were 92.18 %, 24.1 mg/g, and 88.5 % (Table 5). Although the experimental values of EE, TAC and DPPH were slightly higher than the predicted values, there was no significant difference between the predicted and experimental values (a percentage error below 5 %), indicating that the developed models were adequate in describing the relationship between factors and responses. Therefore, the result proves that the optimal condition recommended by the statistical software is reliable.

*Table 5: Verification Study of the EE, TAC, and DPPH models*

Test	Predicted value	Experimental value	Percentage error
EE (%)	90.08	92.18	2.27 %
TAC (mg/g)	23.03	24.1	4.48 %
DPPH (%)	85.58	88.5	3.29 %

## 4. Conclusion

In this study on the microwave-assisted encapsulation of black mulberry extract, core-to-wall ratio and power were found to significantly affect EE, TAC, and DPPH. On the contrary, the encapsulation time had no significant effect on EE, TAC, and DPPH for the tested range of 2–4 min. This microwave-assisted encapsulation method is environmentally friendly, low cost, and requires low energy and a short processing period. Moreover, it can also produce an encapsulated product with high encapsulation efficiency, high total anthocyanin content, and high DPPH inhibition. This work proves that the microwave is a competent instrument that can potentially be applied for industrial anthocyanin extraction-encapsulation. The underutilised black mulberry fruit, which is rich in anthocyanin, can be made into a natural colourant or functional ingredient in food and beverages.

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