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# Effects of wall materials on the physicochemical and antioxidant properties of microwave-assisted encapsulation of Basella rubra fruit extract

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## Abstract

The mature fruits of Basella rubra are a source of naturally occurring betalain pigments. However, the applications of using betalains in food industry are limited due to the stability. To overcome this problem, the use of encapsulation technique is employed. In this study, betalains were extracted from B. rubra fruits and encapsulated with different coating materials, maltodextrin (MD), gum arabic (GA) and a combination of maltodextrin and gum arabic (MD + GA), via microwave-assisted technique. MD encapsulation showed the highest of efficiency yield (60.31 ± 0.51 %). The encapsulation efficiency of all samples ranged from 82.13 ± 1.17 to 85.66 ± 0.20 %. The encapsulation of B. rubra fruit extract with GA showed the highest antioxidant activities, total betalains and total phenolic contents (TPC) ($P<0.05$). In addition, the lowest value of water activity was observed when using GA as wall material whereas the moisture content and % solubility of all encapsulated samples showed no statistically different ($P>0.05$). The different wall materials affected the L*, a* and b* values of encapsulated B. rubra fruit extract powder ($P<0.05$). Thermal study at 90°C demonstrated that using MD or GA as wall material could retain the highest content of betalains in B. rubra fruit extract after 30-min incubation whereas different wall materials had no effect on TPC and antioxidant activity. These findings suggest that GA encapsulation of B. rubra fruit extract through microwave drying showed the highest stability in terms of betalain contents, antioxidant activity and thermal stability. This process can be employed for the development of betalain food colorant from B. rubra fruit.

***Keywords***: Malabar spinach, maltodextrin, gum arabic, betalain, thermal stability

## 1. Introduction

Betalains are water-soluble nitrogen-containing plant pigments which composed of red-purple betacyanins and yellow betaxanthins (Gandia-Herrero *et al.*, 2016). A considerable interest of the feasibility of using betalain as natural food colorants as a replacement of synthetic dyes in food industry and their health benefits have been studied (Martins *et al.*, 2017). The most common edible source of betalains is red beetroot (*Beta vulgaris*) but it is restricted by its unfavorable flavor of earthy smell and high nitrate concentration (Herbach *et al.*, 2006). Therefore, the alternative sources of betalains other than the beetroot have been explored.

The ripened red-purple fruit of *Basella rubra* has increasingly gained the attention as the new source of betalains. It gives the high pigment yield with the trend of color similar to those found in beetroot (Pawar *et al.*, 2018). Even though the fruit of *B. rubra* is not usually consumed and considered as a waste, it has several health benefits including antioxidant and cytotoxic activities against human cervical carcinoma cells (Kumar *et al.*, 2015a). Thus, it has considerable potential to exploit as natural colorant and functional food ingredient. However, the current obstacle for the widespread use of betalains in food industry is their instability when exposed to various environmental factors including light, oxygen, moisture, pH, heat processing and storage conditions (Khan, 2016). In this regard, the stabilization of the betalains could be enhanced using encapsulation technology which is an efficient process to entrap active agents (like natural colorants) within other substances or wall materials (Desai and Park, 2005). This technology improved stabilization, bioavailability and shelf-life of natural compounds (Kumar and Giridhar, 2017). There are several methods that have been used to produce betalains-rich powder such as spray drying and freeze drying (Ravichandran *et al.*, 2014). Microwave drying is a new method which offers shorter processing time, high product quality, cost-saving and flexibility in producing a variety of dried products (Hangi and Amanifard, 2008). In this study, encapsulation process under microwave heating can be an alternative technique for producing encapsulated betalain powder from *B. rubra* fruit. To our knowledge, no published work about the application of microwave-assisted technique in the encapsulation process of betalains from *B. rubra* fruit.

In encapsulation process, the very important step is to select a suitable wall material to retain the bioactive compounds, as well as encapsulation efficiency and their properties within the matrix (Bazaria and Kumar, 2017). The most commonly used wall materials in food industry are maltodextrin and gum arabic. Maltodextrin is an oligosaccharide produced by starch hydrolysis and has less than 20 dextrose equivalents (DE). Addition of maltodextrin reduces stickiness and agglomeration problems during storage (Gandia-Herrero *et al.*, 2013). It is safe, easily dissolved in water and inexpensive. Previous studies indicated that maltodextrin could improve the stability and retain antioxidant activity of spray-dried betalain powder (Gandia-Herrero *et al.*, 2013). Gum arabic, a processed product of *acacia* tree, is one of the most important coating materials due to good emulsifying and film-forming properties (Gharsallaoui *et al.*, 2007) as well as its low viscosity in aqueous solution (Gabas *et al.*, 2007). However, its application in the industry is very limited due to high cost, limited supply and quality variation (Madene *et al.*, 2006). The characteristics and properties of coating materials might affect the stability of bioactive compounds (Ravichandran *et al.*, 2014, Toledo-Madrid *et al.*, 2019). There is a lack of study to compare the performance of different coating materials on the retention of betalains and antioxidant activity of *B. rubra* fruit through microwave-assisted encapsulation.

Based on above reason, these studies aim to 1) investigate the feasibility of using microwave-assisted encapsulation technique 2) analyze physicochemical properties, antioxidant activity, thermal stability of encapsulated powder and 3) identify the optimal coating material that suitable for the encapsulation of *B. rubra* fruit extract using microwave-assisted technique.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

MD (DE 10-12) was purchased from Jining Xuli Chemical Co., Ltd. (China). GA was obtained from Jumbo trading Co., Ltd. (Zudan). Folin-Ciocalteu's phenol reagent was obtained from Lobachemie (Mumbai, India). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma-Aldrich (MO, USA). All other chemicals used were of analytical grade.

### 2.2 Preparation of *B. rubra* fruit extract

The red-violet ripened *B. rubra* fruits (8 weeks after flowering) were obtained from local supplier in Chonburi, Thailand. The fruits were washed with running tap water, air-dried at 30°C and stored in the freezer (-20°C) until use. Betalain pigments from *B. rubra* fruits were extracted with distilled water in a ratio of 1:10 (w/v) using electronic mixer (Panasonic MX-GM1011, Japan) with the maximum speed for 1 min. The mixture was filtered through cheesecloth to remove peel and seeds. Then the filtrate was centrifuged at 8,000 rpm for 20 min at 25°C (Sorvall® Biofuge primo R centrifuge, USA). The *B. rubra* fruit extract was stored at -20°C until use.

### 2.3 Quantification of betalain contents

The contents of betacyanin and betaxanthin in the extract was determined spectrophotometrically at 538 nm and 480 nm, respectively, using a UV-VIS spectrophotometer (Shimadzu, Japan) according to previous study (Stintzing *et al.*, 2005). The betacyanin and betaxanthin contents were calculated as below.

$$\text{betacyanins or betaxanthins (mg/100 g)} = \frac{A \times V \times DF \times MW \times 100}{\varepsilon LW}$$

where A = the absorbance at 538 nm for betacyanins and 480 nm for betaxanthins, V = extract volume (mL), DF = dilution factor, L (path length) = 1.0 cm, W = weight of powder (g), ε = Molar extinction coefficient of betacyanins and betaxanthins which equals to 60,000 and 48,000 L $mol^{-1}cm^{-1}$ and MW = 550 g/mol for betacyanins and 308 g/mol for betaxanthins.

### 2.4 Encapsulation of *B. rubra* fruit extracts using microwave-assisted technique

Maltodextrin (MD), gum arabic (GA) and maltodextrin:gum arabic (MD+GA) at the 1:1 ratio were tested. Encapsulation of betalain from *B. rubra* fruit was prepared by mixing 100 mL of extract with 20 g of coating materials. The mixtures were homogenized using a homogenizer (Ultra-Turrax T25, IKA Labortechnik, Germany) for 15 min at 13,500 rpm. Thirty mL of each mixture was poured into a 140 mm diameter x 20 mm height round glass plate (Anumbra, Czech Republic) and placed in a microwave oven for drying process. All samples were heated for 5 min with microwave power intensities at 800 watts (Phokphol, 2019). The dried material was scraped from the glass plate and ground in a mortar to yield a fine powder. The encapsulated samples were stored in high-density polyethylene bags at room temperature until use.

## 2.5 Determination of encapsulation efficiency (EE) and encapsulation yield (EY)

2.5.1 Determination of surface betalain content

Encapsulated powder 100 mg were dispersed in a 2 mL ethanol:methanol (1:1) solution, stirred on a vortex mixer for 1 min and centrifuged at 8,000 rpm for 15 min (Vergara *et al.*, 2014). The betalain content was spectrophotometrically quantified as described above.

2.5.2 Determination of total betalain content

Encapsulated powder (200 mg) was dispersed in 2 mL of water and mixed using a vortex mixer for 1 min and centrifuged at 8,000 rpm for 15 min (Vergara *et al.*, 2014). The betalain was spectrophotometrically quantified as described above.

2.5.3 Encapsulation efficiency (EE)

The encapsulation efficiency (EE) was calculated as below:

$$EE\ (\%) = \frac{\text{Total betalain} - \text{surface betalain}}{\text{Total betalain}} \times 100$$

2.5.4 Encapsulation yield (EY)

The yield of encapsulation during microwave-assisted drying was calculated as below:

$$EY\ (\%) = \frac{\text{Total mass of microcapsules after encapsulation}}{\text{Total mass of solids before encapsulation}} \times 100$$

## 2.6 Determination of total phenolic content (TPC)

TPC was measured using Folin-Ciocalteu colorimetric method as described (Singleton *et al.*, 1999). Briefly, 0.1 mL of sample was mixed with 2.9 mL of dd$H_2O$. Folin-Ciocalteu's phenol reagent (0.125 mL) was added. The mixture was incubated at room temperature for 5 min. Subsequently, 1.25 mL of saturated $Na_2CO_3$ (7% w/v) were added to the mixture and incubated at room temperature for 20 min. The absorbance of the mixture was measured at 765 nm. All data were determined in triplicate and expressed as mg gallic acid equivalent (GAE)/100 g of extract.

## 2.7 Determination of ferric reducing antioxidant power activity (FRAP assay)

FRAP assay was performed as previously described (Benzie and Strain, 1996). One-hundred µL of each sample was mixed with 3 mL of FRAP reagent (300 mM acetate buffer; pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$), and incubated for 15 min at room temperature. The absorbance was spectrophotometrically determined at 593 nm. The results were expressed as mg gallic acid equivalent (GAE)/100 g of extract.

## 2.8 Measurement of Instrumental color (L*, a*, b*)

The color of encapsulated powder was determined using colorimeter (Minolta Konica, Japan) with D65 illuminant and $10^0$ observer angle. Calibration was performed using the white tile. Results of six replicates were recorded according to CIELAB color parameters: L* (lightness), a* (redness-greenness) and b* (yellowness-blueness).

## 2.9 Determination of water activity, moisture content and solubility test

The water activity ($a_w$) was determined by using water activity analyzer (Aqualab, USA). The moisture content was analyzed using moisture analyzer (MB45 halogen, Ohaus, Switzerland). All samples were measured in triplicate.

The solubility of encapsulated powder was evaluated according to (Daza *et al.*, 2016) with some modification. Each sample (1.25 g) was mixed with 1.25 mL distilled water and incubated at 30°C for 30 min. Then the mixture was centrifuged at 3,000 rpm for 15 min. The supernatant was removed, transferred to porcelain dishes, and dried overnight in an oven at 105°C. All samples were measured in triplicate. Solubility was expressed as a percentage (%).

## 2.10 Thermal stability analysis

Non-encapsulated extract and encapsulated samples were placed in a water bath set at 90°C. The samples were collected at 0, 5, 10, 20, and 30 min to determine betacyanin, betaxanthin, TPC and antioxidant activity. All samples were measured in triplicate. The percentage of the remaining values was calculated from the following equation:

$$\text{Percentage of the remaining values} = \frac{A_t}{A_0} \times 100$$

where $A_t$ = concentration of betacyanin, betaxanthin, TPC, and antioxidant activity at a specified time, $A_0$ = concentration of betacyanin, betaxanthin, TPC, and antioxidant activity at time zero.

## 2.11 Statistical analysis

The results were expressed as the means ± standard deviation (SD). The one-way analysis of variance (ANOVA) was used to calculate significant differences, and multiple comparisons of means were assessed by Duncan's multiple range test (DMRT) using the statistic software SPSS version 17.0. $P<0.05$ was considered statistically significant.

# 3. Results and Discussion

## 3.1 Physicochemical analysis of *B. rubra* fruit extract

The quantification of betalains from fresh *B. rubra* ripened fruit extracted with water resulted in total betalains of 156.37 ± 0.05 mg/100 g FW with 121.72 ± 0.36 mg/100 g FW of betacyanin and 34.65 ± 0.41 mg/100 g FW of betaxanthin. Based on the results, betacyanin was the dominant class of dyes in *B. rubra* fruit extract. The betacyanin value was consistent with previous study of *B. rubra* matured fruits (Kumar *et al.*, 2015b). The pH value was 6.01 ± 0.37, which was in agreement with previously reports of *B. rubra* fruits and *Opuncia indicus* fruits (Kumar *et al.*, 2015b, Saenz, 2000).

## 3.2 Encapsulation of *B. rubra* fruit extract with different wall materials

The encapsulation of *B. rubra* extract was performed using microwave-assisted technique with MD, GA and MD+GA. MD and GA are widely used as a coating material because of their ability to reduce the stickiness of the powder and nutrient binding properties (Mohd Nawi *et al.*, 2015). Colorant products of encapsulated *B. rubra* extracts are shown in Figure 1.

The encapsulation yield (EY) of MD, GA and MD+GA samples was 60.31 ± 0.51, 54.30 ± 0.53 and 45.70 ± 0.46 %, respectively (Table 1). The type of encapsulating agents showed a significant influence on EY of encapsulated *B. rubra* fruit extract (P<0.05). The powder encapsulated with MD exhibited the highest EY. Lower EY produced from GA and MD+GA might be caused by branched-chain structure of GA and its high hydrophilic nature which could possibly cause the particles to adhere to the glass plate during microwave drying process (Tonon *et al.*, 2009).

Encapsulation efficiency (EE) indicates the ability of coating materials to hold or enclose core materials inside the microcapsules (Desai and Park, 2005). EE also relates to the shelf-life of the pigment content in the powder (Idham *et al.*, 2012) and strongly depended on the type of coating materials and drying methods (Akhavan Mahdavi *et al.*, 2016). In this study, the results of the EE of MD, GA and MD+GA ranged from 82.13 ± 1.17 to 85.66 ± 0.20 % (Table 1). Using MD or GA alone provided the highest EE (P>0.05), whereas the EE value of MD + GA was the lowest (P<0.05). It might be because the time of microwave drying is not enough for the wall material (MD+GA) to entrap the betalain extract perfectly, resulted in the present of extract at the wall material surface (Abang Zaidel *et al.*, 2015). To our knowledge, there is no previous report on the betalain EE values of *B. rubra* fruit extract with microwave drying method. The EE of betalains found in the present work was comparable to or higher than other commonly used drying methods. Previous study indicated that the EE values of freeze-dried betalain from *Salicornia fruiticosa* using MD and GA as encapsulating agents varied from 86.50 to 92.30% (Mohamed *et al.*, 2018). Castro-Muñoz Saenz *et al.* (2009) reported EE values ranged from 18.07 to 57.30% of spray-dried betalain from purple cactus pear using a combination of MD and gelatin.

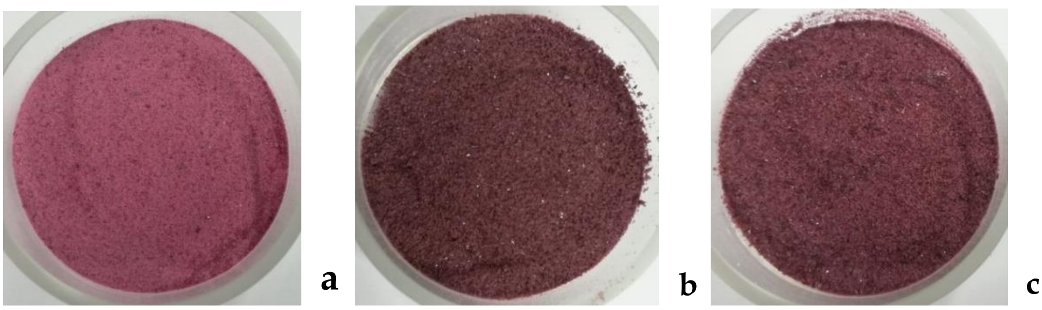



**Figure 1** Encapsulated *B. rubra* fruit extract using microwave-assisted technique using different wall materials (a) MD, (b) GA, and (c) MD+GA.

## 3.3 Total betalain contents, TPC and antioxidant activity of encapsulated *B. rubra* fruit extract

The effects of different encapsulating agents of *B. rubra* fruit extract using microwave-assisted technique on total betalain contents were evaluated. The results are shown in Figure 2. The encapsulated *B. rubra* fruit extract with GA retained the highest contents of betacyanin, betaxanthin and total betalains (P<0.05), compared to MD and MD+GA. The previous study showed that GA was a better wall material for encapsulation of betalain obtained from cactus pear peel extract, compared to MD (Toledo-Madrid *et al.*, 2019).

*B. rubra* fruits were reported to contain phenolic compounds and exhibit free radical scavenging activity (Kumar *et al.*, 2015a). In this study, TPC of sample encapsulated with MD, GA and MD + GA are presented in Table 1. GA retained the highest TPC (182.20 ± 1.75 mg GAE/100 g) of *B. rubra* fruit extract, followed by MD + GA (145.14 ± 5.15 mg GAE/100 g) and MD retained the lowest content of TPC (132.79 ± 2.23 mg GAE/100 g). In FRAP assay, GA had the strongest antioxidant activity, compared to MD and MD+GA (Table 1). This might be because of the good film-forming and stabilizing capacities of GA, which increased the stability of total betalain and phenolic contents in the encapsulated form (Gharsallaoui *et al.*, 2007). On the other hand, MD (DE10-12) contains long chain saccharides which is a poor barrier to oxygen during encapsulation (Bazaria and Kumar, 2017). In this study, GA offered good protection of betalains and phenolic compounds against oxidation during the drying process.

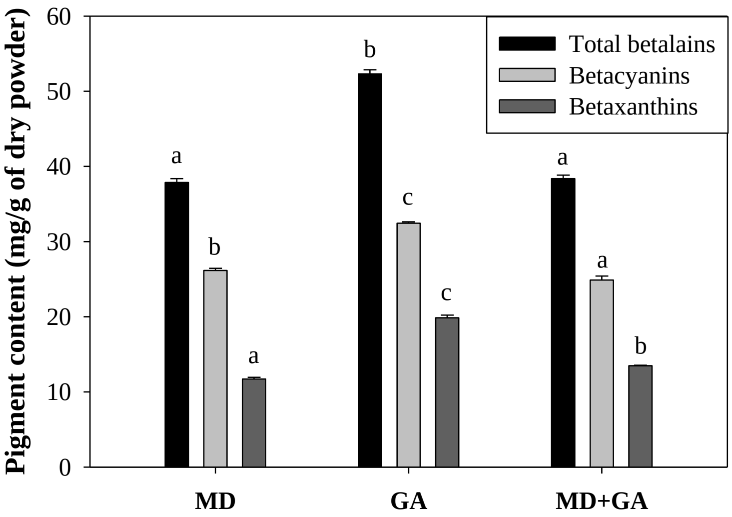



**Figure 2** Effects of different wall materials of *B. rubra* fruit extract encapsulation on total betalains, betacyanin and betaxanthin. Different letters indicate significant difference (P<0.05) between means of total betalains, betacyanin and betaxanthin of different wall materials.

**Table 1** Effects of different wall materials of *B. rubra* fruit extract encapsulation on % EY, % EE, TPC and antioxidant activity.

| Samples | % EY | % EE | TPC (mg GAE/100 g) | Antioxidant activity (mg GAE/100 g) |
|---|---|---|---|---|
| **MD** | 60.31 ± 0.51[c] | 85.66 ± 0.20[b] | 132.79 ± 2.23[a] | 41.28 ± 1.24[a] |
| **GA** | 54.30 ± 0.53[b] | 85.39 ± 0.15[b] | 182.20 ± 1.75[c] | 47.52 ± 1.26[b] |
| **MD+GA** | 45.70 ± 0.46[a] | 82.13 ± 1.17[a] | 145.14 ± 5.15[b] | 40.22 ± 3.11[a] |

**Note:** Different letters indicate significant difference (P<0.05) in the same column.

## 3.4 Color attributes of encapsulated *B. rubra* fruit extract

The values of L*, a* and b* of encapsulated *B. rubra* extract is presented in Table 2. All samples had positive a* values as red color which is attributed to their betacyanin content. The white color of MD contributed to the highest L* value of powder. On the other hand, using GA and MD+GA as encapsulating materials expressed lower values of lightness, it might due to the yellowish color of GA (Sonthong *et al.*, 2018). Therefore, the color of encapsulating agents influenced the final color of the colorant powder.

The b* value represents yellowness-blueness. The results indicated that the b* values of MD, GA and MD+GA were close to zero, as yellow or blue pigments were not contribute significantly to the resulting color.

**Table 2** Effects of different wall materials of *B. rubra* fruit extract encapsulation on color attributes, water activity ($a_w$), moisture content and solubility.

| Sample | L* | a* | b* | $a_w$ | Moisture content (%) | Solubility (%) |
|---|---|---|---|---|---|---|
| **MD** | 57.02 ± 0.39[c] | 19.24 ± 0.26[c] | -1.54 ± 0.08[a] | 0.30 ± 0.03[b] | 2.40 ± 0.44[a] | 92.11 ± 0.58[a] |
| **GA** | 48.79 ± 0.45[a] | 11.02 ± 0.12[a] | 1.02 ± 0.17[c] | 0.20 ± 0.04[a] | 2.45 ± 0.07[a] | 92.82 ± 0.46[a] |
| **MD+GA** | 50.07 ± 0.25[b] | 13.01 ± 0.17[b] | 0.32 ± 0.06[b] | 0.29 ± 0.05[b] | 2.43 ± 0.49[a] | 92.42 ± 0.24[a] |

**Note:** Different letters indicate significant difference (P<0.05) in the same column.

## 3.5 Water activity ($a_w$), moisture content and solubility of encapsulated *B. rubra* fruit extract

Water activity ($a_w$) is an important indicator for storage and shelf life of dry products. As shown in Table 2, the $a_w$ of the samples ranged from 0.20 ± 0.04 to 0.30 ± 0.03. The powder of *B. rubra* extract encapsulated with GA showed the lowest of $a_w$ value ($P$< 0.05). The $a_w$ levels in all samples were lower than 0.6 which was previously determined to prevent microbial growth (Erkmen and Bozoglu, 2016).

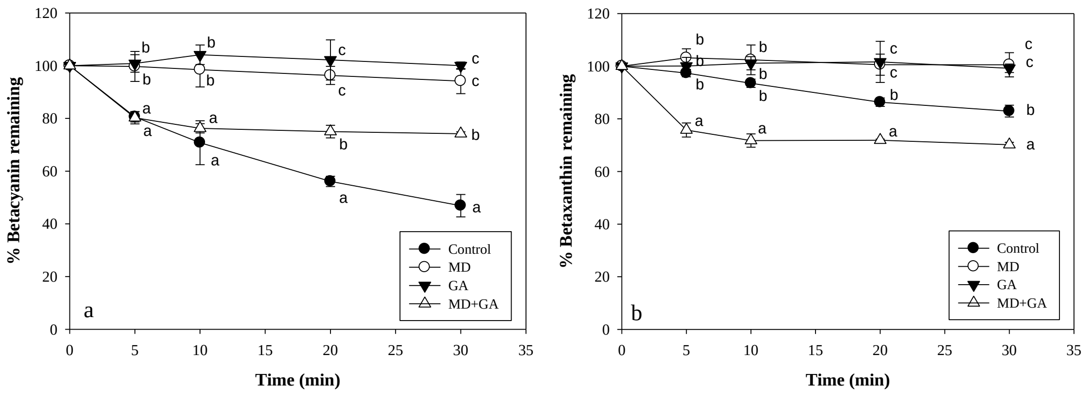
Moisture content indicates the water composition in a food system and relates to drying efficiency (Ferrari *et al.*, 2011). The values of moisture content of the colorant powder of *B. rubra* fruit extract were between 2.40 ± 0.44 and 2.45 ± 0.07% (P>0.05) (Table 2). These results were in agreement with previous study of spray-dried beetroot juice with MD and GA encapsulation (1.26 – 2.88%) (Bazaria and Kumar, 2017). Furthermore, the moisture content in this study was lower than those of spray-dried betalain from *B. rubra* fruit with MD encapsulation (3.2 ± 0.02%) (Kumar and Giridhar, 2017). Low moisture content prevents the caking and agglomeration of powder (Tonon *et al.*, 2009).

The % solubility was ranged from 92.11 ± 0.58 to 92.42 ± 0.24% which was not statistically different (P>0.05) because both MD and GA were highly soluble in water.

## 3.6 Thermal stability of betacyanin, betaxanthin, TPC and antioxidant activity of encapsulated *B. rubra* fruit extract

Temperature is one of the most important factors that influences betalain stability and antioxidant activity during food processing and storage (Herbach *et al.*, 2006). The increasing of temperature caused the degradation of betacyanin (red color) to betalamic acid (bright yellow color) and unstable form of cyclodopa-5-O-glycoside (colorless) (Herbach *et al.*, 2004). The degradation of betalains that are used as food additives is the major concern for food industry and consumers. Thermal degradation of encapsulated betalain-rich extract was studied at 90°C for 5, 10, 20, and 30 min. The percentages of betacyanin and betaxanthin after the treatment are shown in Fig. 3(a) and (b), respectively. In this present study, betacyanin and betaxanthin contents of non-encapsulated *B. rubra* fruit extract (control) were degraded faster (P<0.05) than other encapsulated colorant powders. However, no difference was observed between MD and GA (P>0.05). Betalains are heat sensitive pigments. Therefore, encapsulation with MD or GA improved betalains stability against high temperature. The combination of MD and GA showed the lowest level of remaining pigments which might be related to the decreasing of %EE value.

Heating temperature of 90°C for 30 min did not affect TPC of non-encapsulated extract and all encapsulated powders (P>0.05) (Figure 3(c)). In contrast, the levels of the antioxidant activity in all encapsulated samples were significantly higher than the non-encapsulated extract (Fig 3 (d)). It could be due to the rapid degradation of unprotected betalain pigment during heating. The remaining antioxidant activities of encapsulated *B. rubra* fruit extract by different types of encapsulating agent showed no significant difference (P>0.05). These results are similar to previous report (Toledo-Madrid *et al.*, 2019). In this study, the encapsulation process could protect antioxidant properties of *B. rubra* fruit extract against thermal treatment

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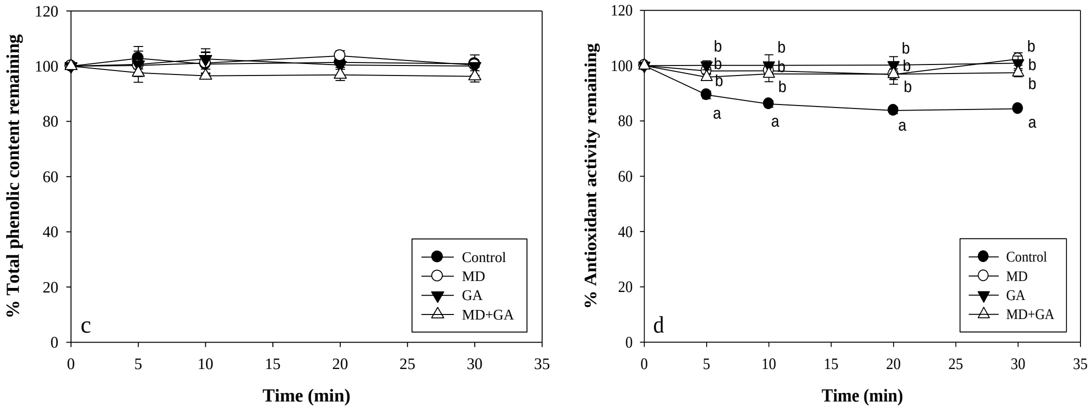


**Figure 3** Effects of different wall materials of *B. rubra* fruit extract encapsulation on thermal stability at 90°C of (a) betacyanin, (b) betaxanthin, (c) TPC, and (d) antioxidant activity. Different letters indicate significant difference (P<0.05) at the same time of heating.

## 4. Conclusion

The findings of this study showed the feasibility of using microwave-assisted technique to produce encapsulated betalain-rich extract from *B. rubra* fruit. Encapsulation with MD or GA alone exhibited the highest level of EE. The encapsulation of *B. rubra* fruit extract with GA exhibited the better quality due to the highest retention of betalains, total phenolic content and antioxidant activity, as well as the lowest value of water activity, which influences the stability of powder. The color measurement showed that different wall materials had the impact to the encapsulated powder. Encapsulation of the *B. rubra* fruit extract with MD or GA improved the thermal stability of betalain and antioxidant activity over non-encapsulated extract. This might be a good reason to further use encapsulated colorant powder from *B. rubra* fruit in food processing. In this study, GA is the suitable encapsulating agent for *B. rubra* fruit powder produced by microwave drying. Future studies need to be investigated the effects of storage time on the stability of encapsulated colorant and the application in food system.

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