Effects of Spray-drying Temperatures on Powder Properties and Antioxidant Activities of Encapsulated Anthocyanins from Black Glutinous Rice Bran

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ABSTRACT

The objective of this study was to determine the effects of spray drying temperature on the powder properties and antioxidant activities of encapsulated black glutinous rice (BGR) bran anthocyanins. The anthocyanins in BGR bran was extracted using acidified ethanol. The extract was encapsulated with maltodextrin (DE10) using spray drying at various inlet air temperatures (IAT; 140, 160 and 180°C). The results revealed that increasing IAT enhanced productivity with lower energy consumption, encapsulation efficiency, solubility, dispersibility, wettability, flowability and surface smoothness of the microcapsules. In contrast, total anthocyanin content (TAC), bulk density and color values (a*, C* and h°, respectively) of the microcapsules were decreased by increasing the IAT. Reducing power and DPPH radical scavenging activity of anthocyanin powders were not significantly different. In addition, anthocyanin powder produced using 180°C IAT showed the greatest encapsulation efficiency (96.72 ± 0.61%), solubility (87.42 ± 1.26%), dispersibility (86.45 ± 0.93%) and repose angle (23.50 ± 0.61 degree).

Keywords: Encapsulation, Anthocyanins, Black glutinous rice bran, Spray drying, Inlet air temperature

INTRODUCTION

Anthocyanins are the biggest group of water-soluble pigments that are widely distributed in fruits, vegetables and cereals (Mazza and Miniati, 1993). Their bright colors, which vary from orange-red to blue, are used as natural food colorants, replacing synthetic colorants due to their color varieties and safety (Mercadante and Bobbio, 2008). Moreover, properties of anthocyanins are related to human health, including antioxidant, anti-inflammatory and anti-cancer activity (Wang and Stoner, 2008). Specifically, cyanidin-3-glucoside has been reported to inhibit the growth of Lewis lung carcinoma cells in vivo (Chen et al., 2005).

Black glutinous rice (BGR), which is a traditional rice commonly cultivated in Thailand, is a rich source of anthocyanins pigments. The major portion of anthocyanins is found in the bran layer of the rice grain. The major anthocyanins in black rice varieties are cyanidin-3-glucoside and peonidin-3-glucoside (Ryu et al., 1998). Additionally, BGR has been reported to contain more anthocyanins than non-glutinous black rice and red rice (Sangkitikomol et al., 2008). Anthocyanins are unstable compounds that can be affected by many factors, such as pH, temperature, light, oxidation and solvents (Rein, 2005).

A method for improving the stability of anthocyanins is encapsulation by entrapping the pigment into the amorphous matrix of appropriate wall materials. Spray drying is an effective method to encapsulate natural food colorants (Cai and Corke, 2000). A spray-dried powder that has low moisture content is defined as being a good quality powder. The powder properties produced by spray drying depend on various processing conditions, such as feed viscosity, drying temperature, feed flow rate and the type of atomizer, as well as other variables. The process temperature is an important factor affecting the stability of anthocyanins (Idham et al., 2012). Cai and Corke (2000) found that higher inlet air temperatures (IAT) caused a greater loss of *Amaranthus* betacyanin and slightly affected the pigment stability during storage. Likewise, other powder properties, including moisture content, process yield, bulk density and wettability, are directly affected by IAT (Bhandari et al., 1993; Cai and Corke, 2000; Tonon et al., 2008).

Recently, many reports have described the use of spray drying to improve the stability of anthocyanins extracted from various sources. However, there are no reports on anthocyanins extracted from BGR bran. Therefore, the objective of this study was to determine the effects of the spray-drying temperature of the encapsulation process on the powder properties and antioxidant activities of anthocyanins microcapsules.

MATERIAL AND METHODS

Materials

BGR (*Oryza sativa* L. cv. Kum Doi Saket) was purchased from a local rice miller in Chiang Mai Province, Thailand. The rice was milled with a laboratory rice miller (McGrill type). The collected bran layer was packed in metalized bags and stored at -18ºC until use. Maltodextrin (DE10; CP Kelco, Lille Skensved, Denmark) was used as the wall material in this study.

Anthocyanins extraction

Briefly, 10 g of BGR bran was extracted with 350 ml of acidified ethanol (pH 1.5) with shaking for 9 hours. This was then filtered through Whatman filter paper no.1. The ethanol was removed from the extract using a rotary evaporator (Buchi, Labortechnik AG, Switzerland) under vacuum at 40ºC. Distilled water was added to the concentrated extract to adjust the total soluble solid to 5-degree brix, as measured using a hand refractometer (Master, Atago, Japan). The anthocyanin extract was kept at -18ºC until use.

Total anthocyanin content

The total anthocyanin content was determined by the pH differential method (Giusti and Wrolstad, 2001). The extract was diluted with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). All of the dilutions were measured at 510 nm and 700 nm using a UV-visible spectrophotometer (Thermo-Spectronic Genesys 10 UV scanning, Thermo Scientific, USA). The anthocyanin content was calculated by the followed equation and expressed as mg cyanidin-3-glucoside.

$$\text{Anthocyanin contents}\,(\text{mg / L}) = \frac{(A_{diff} \times MW \times DF \times 1000)}{\varepsilon \times \ell}$$

$$A_{diff} = (A_{510} - A_{700})_{pH\,1.0} - (A_{510} - A_{700})_{pH\,4.5}$$

where A_{diff} is difference of absorbance at various pH values, MW is the molecular weight (449.2 g/mol) of cyanidin-3-glucoside, DF is the dilution factor, molar absorptivity (ε) is 26,900 l/mol•cm and path length (ℓ) is 1.0 cm.

Encapsulation methods

Preparation of the feed mixture. The feed mixture was prepared by mixing maltodextrin (DE10) into 5-degree brix of the anthocyanin extract. This was then homogenized with a magnetic stirrer for 30 min at room temperature until the final solid content was determined to be 20-degree brix.

Spray drying conditions. A spray dryer (JCM Engineering Concept, Thailand) with IAT of 140, 160 and 180ºC was used in this study. The feed mixture was fed at a feed flow rate of 25 ml/min and spray dried through a nozzle atomizer. Then, the anthocyanin powder was collected and packed in a metallized bag. The obtained microcapsule was stored at -18ºC until analysis. In addition, thermal efficiency and productivity in percentage was calculated using the following equations (Hall and Hedrick, 1971):

$$\text{Thermal efficiency (\%)} = \left(\frac{T_{inlet} - T_{outlet}}{T_{inlet} - T_{room}} \right) \text{x } 100$$

$$\text{Productivity (\%)} = \left(\frac{\text{Powder Solid Weight}}{\text{Feed Solid Weight}} \right) \text{x } 100$$

Encapsulation efficiency

Encapsulation efficiency was determined using the method described by Idham et al. (2012) in order to evaluate the effectiveness of encapsulation. For surface anthocyanin contents (SAC), 100 mg of powder was dissolved quickly in 10 ml of 95% ethanol by vortex mixer (30 seconds), and then centrifuged at 6,000 rpm for 10 min. After that, the supernatants were collected and filtered before quantification. For total anthocyanin contents, 100 mg of powder was dissolved in 1.0 ml of distilled water and mixed by vortex mixer for 30 seconds, and then 9.0 ml of 95% ethanol was added and mixed by vortex mixer for 5 min. The suspensions were centrifuged at 6,000 rpm for 10 min, and the supernatants were collected and filtered before quantification. Encapsulation efficiency was calculated by the following equation:

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

where TAC is total anthocyanin content

Powder properties

Physical and chemical properties of the powder. The color of the powder was determined using a colorimeter (CR-410, Konica Minolta, Japan) in L^*, a^*, b^*, metric chroma (C^*) and hue angle (h^o). Moisture content was determined by following the method of AOAC (2000). Bulk density of the anthocyanin powders was determined by loading the powder into a 10 ml graduated cylinder to the 10 ml mark and then weighing it. The powder weight and volume were then used to calculate the bulk density (expressed as mass/volume). In addition, the microcapsule structure was also evaluated under scanning electron microscope (JSM-5200 model, JEOL, Japan).

Solubility. Solubility was determined by following the method described by Shittu and Lawal (2007) with some modification. One gram of anthocyanin powder was added to 10 ml distilled water in a 100 ml beaker and stirred continuously for 30 min. The suspension was transferred into a centrifuge tube and centrifuged at 6,000 rpm for 20 min. Supernatant was completely drained into an aluminum can and allowed to dry at 105°C for 24 hours. After drying, the weight of dried solid was used to calculate the solubility in percentage.

Dispersibility. The dispersibility of the anthocyanin powder was determined by following the method described by Jinapong et al. (2008) with some modification. One gram of powder was added to 10 ml of distilled water in a 50 ml beaker, and stirred vigorously with a stirring rod until the powder was dissolved. The reconstituted powder was poured through a 150-µm sieve. After that, 1.0 ml of the sieved sample was transferred into a dried aluminum can and dried at 105°C for 4 hours. The dispersibility was calculated using the following equation:

$$\text{Dispersibility}(\%) = \frac{(10 + a) \times \text{TS}}{a \times \left(\frac{100 - b}{100}\right)}$$

where TS is the dried sample that passed through the sieve (%), is amount of powder (g), and b is moisture content of the powder (%).

Angle of repose. The repose angle of the anthocyanin powder was determined using the method described by Shittu and Lawal (2007) with slight modification. A glass funnel (250 ml) held on a ring stand was set over the white paper with height between the bottom of the funnel and the paper surface of 15 cm. The powder (5.0 g) was passed through a funnel to the paper below and piled as a triangle. The angle of the triangle base was measured from the cross section photograph. The obtained angle of repose was used to evaluate the flowability of the anthocyanin powder.

Antioxidant activity

Radical scavenging activity. The radical scavenging activity of anthocyanin powder was determined according to the method reported by Park et al. (2008) with some modification. Various concentrations of the samples were prepared using methanol. The final volume was adjusted to 3.0 ml, and then 0.1 mM DPPH radical solution (1.0 ml) was added and mixed by vortexing. The reaction was incubated in the dark for 30 min at room temperature. After that, the absorbance was measured at 517 nm against a methanol blank. Antioxidant activity was expressed as 50% inhibition concentration (IC_{50}). Butylated hydroxytoluene (BHT) was used for comparison. Radical scavenging activity was calculated using the followed equation:

$$\text{Radical scavenging activity}(\%) = \frac{\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}} \times 100$$

Reducing power. The reducing power was determined using the method reported by Oyaizu (1986). First, 2.0 ml (1 mg/ml) anthocyanin powder solution was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). Then, the mixture was incubated at 50ºC for 20 min. Next, 2.5 ml trichloroacetic acid (10% w/v) was added and centrifuged at 6000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. After a 30-min incubation, the absorbance was measured at 700 nm against a water blank. BHT was used for comparison.

Statistical analysis

All of the analysis was performed in triplicate. The data obtained was analyzed statistically using a statistical program (SPSS statistic version 20, IBM, USA). Differences were considered significant at $p \leq 0.05$ and the averages were determined by Duncan's new multiple range test.

RESULTS

Effect of IAT on thermal efficiency, productivity and moisture content of anthocyanin powders

IAT directly related to thermal efficiency and productivity, which increased when IAT increased, while the moisture content decreased (Table 1). The highest thermal efficiency (58.06%) and productivity (78.87%) were found in anthocyanin powder produced at 180°C IAT, while the highest moisture content (6.04%) was found in anthocyanin powder produced at 140°C IAT.

Table 1. Moisture content, productivity and thermal efficiency of spray drying.

Inlet air temperature (°C)	Thermal efficiency (%)	Productivity (%)	Moisture content (%)
140	55.79 ^{c} ± 0.39	72.95 ^{b} ± 1.88	6.04 ^{a} ± 0.05
160	57.40 ^{b} ± 0.16	75.42 ^{b} ± 1.16	4.41 ^{b} ± 0.16
180	58.06 ^{a} ± 0.26	78.87 ^{a} ± 1.12	2.88 ^{c} ± 0.37

Note: In each column, different superscripts represent significant differences (p ≤ 0.05).

Effects of spray drying temperature on encapsulation efficiency

Increasing the IAT caused the TAC to decrease (Figure 1A). The TAC of the anthocyanin powders decreased significantly by 8.96% when the IAT increased from 160 to 180°C. The lowest TAC (752.58 ± 2.13 mg/100 g DM) for the anthocyanin powder was observed at 180°C IAT.

The SAC decreased as IAT was increased. The lowest SAC (24.69 ± 4.66 mg/100 g DM) was observed at 180°C IAT (Figure 1B). The results showed that increasing IAT increased the encapsulation efficiency. The highest encapsulation efficiency (96.72 ± 0.61%) was observed at 180°C IAT, when most of the anthocyanin was entrapped in the structure of the maltodextrin matrix (Figure 1C).

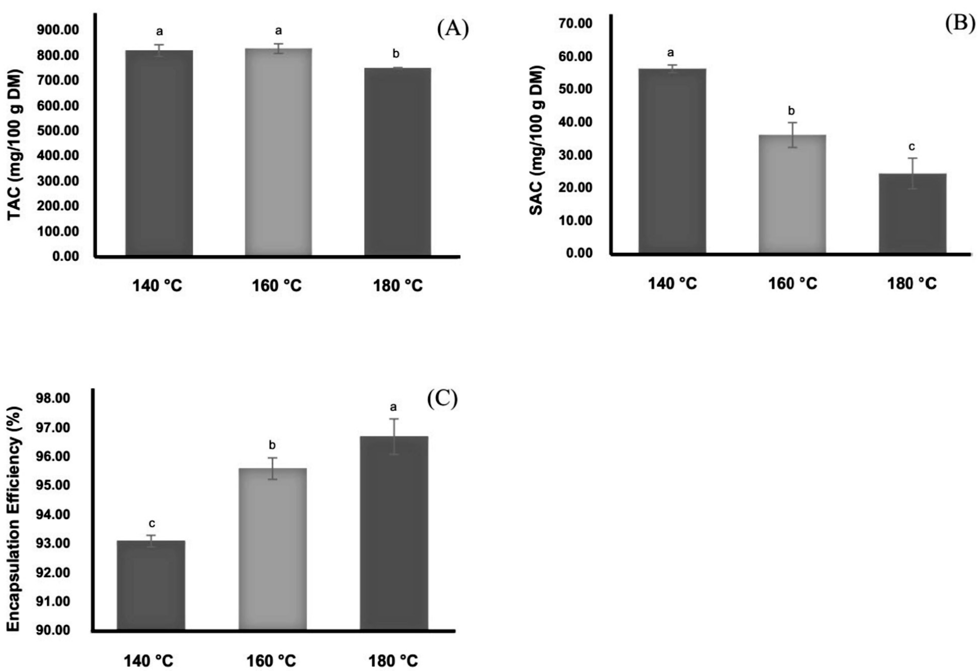

Figure 1. The efficiency of encapsulation process was performed at different IAT (140, 160 and 180°C). Different letters represent significant difference (p≤0.05).

Effects of spray drying temperatures on powder properties

Bulk density of the anthocyanin powder decreased significantly ($p \leq 0.05$) from 0.2795–0.2381 g/cm^3 as IAT increased (Table 2).

Table 2. Powder properties of anthocyanin powders produced with different IAT.

Inlet air temperature (°C)	Powder properties			
	Bulk density (g/cm^3)	Angle of repose (Degree)	Solubility (%)	Dispersibility (%)
140	0.2795 ^{a} ± 0.01	28.33 ^{a} ± 1.04	82.79 ^{b} ± 0.58	74.69 ^{b} ± 4.49
160	0.2552 ^{b} ± 0.01	26.00 ^{b} ± 0.50	84.60 ^{b} ± 0.98	77.26 ^{b} ± 2.09
180	0.2381 ^{c} ± 0.01	23.50 ^{c} ± 0.50	87.42 ^{a} ± 1.26	86.45 ^{a} ± 0.93

Note: In each column, different superscripts represent significant differences ($p > 0.05$).

Table 2 shows that the repose angle of the anthocyanin powders ranged from 23.50–28.33 degrees and decreased significantly ($p \leq 0.05$) as IAT increased. Anthocyanin powder produced at 180°C IAT showed the highest powder solubility (87.42 ± 1.26%) and dispersibility (86.45 ± 0.93%).

Table 3 shows the color parameters of the anthocyanin powders produced at different IAT. The hue angle of the powders ranged from 12.40–12.90 degrees, which corresponds to the magenta shade of red. The IAT had no effect on the L^* (lightness), while other color parameters (a^* = redness, C^* = chroma of the hue and $h°$ = hue angle) decreased significantly by increasing IAT ($p \leq 0.05$).

Table 3. Color parameters of anthocyanin powders.

Inlet air temperature	SAC (mg/100 g DM)	Color parameters of anthocyanin powders			
		L^{*ns}	a^*	C^*	$h°$
140	56.56 ^{a} ± 1.21	44.83 ± 0.44	34.02 ^{a} ± 0.18	34.90 ^{a} ± 0.18	12.90 ^{a} ± 0.07
160	36.40 ^{b} ± 3.79	44.88 ± 0.38	33.30 ^{b} ± 0.30	34.13 ^{b} ± 0.31	12.72 ^{b} ± 0.11
180	24.69 ^{c} ± 4.66	44.77 ± 0.39	30.81 ^{c} ± 0.06	31.55 ^{c} ± 0.06	12.40 ^{c} ± 0.06

Note: In each column, different superscripts represent significant differences ($p \leq 0.05$). ns represents non-significant difference ($p \leq 0.05$).

The influence of IAT on microcapsule structure is shown in Figure 2. Anthocyanin microcapsules produced at different IAT had different microcapsule surfaces, shapes and sizes. Higher IAT resulted in a smoother microcapsule surface. Cracks and pores were found in the anthocyanin microcapsule produced at 140°C IAT.

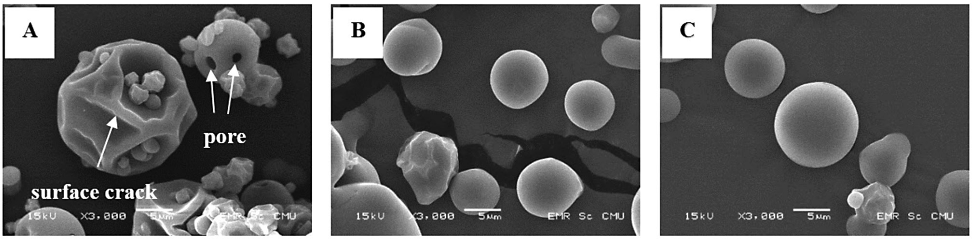

Figure 2. Microcapsule structure of anthocyanin powder that produced (A) 140°C, (B) 160°C and (C) 180°C under scanning electron microscope (3000).

Effects of spray-drying temperatures on antioxidant activities

The results revealed that the reducing powers of all of the anthocyanin powders ranged from 0.475–0.540 (Figure 2). IAT did not significantly influence the reducing power ($p > 0.05$) of anthocyanin powders. However, the reducing powers of all the anthocyanin powders were less than that of BHT (1.640 ± 0.05). The DPPH radical was used for determination of the radical scavenging activity, which was expressed as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50}). A lower IC_{50} value indicates increased scavenging activity. The DPPH radical scavenging activities of the anthocyanin powders were less than that of BHT (0.0257 ± 0.010 mg/ml).

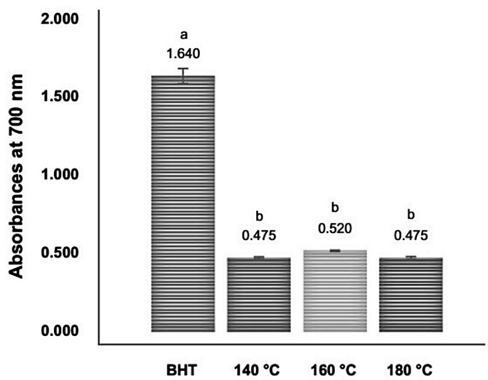

Figure 3. Effect of spray drying temperatutes on reducing power of the anthocyanin powders (1.0 mg/ml). Each column represents average ± standard deviation ($n = 3$). Different letters mean significant differences ($p \leq 0.05$).

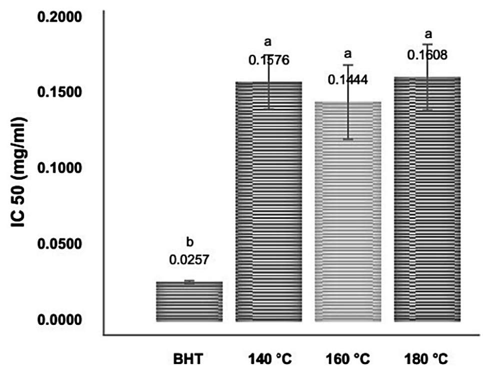

Figure 4. Effect of spray drying temperatutes on DPPH radical scavenging activity of the anthocyanin powders. Each column represents average ± standard deviation (n = 3). Different letters mean significant differences ($p \leq 0.05$).

DISCUSSION

Thermal efficiency is defined as the ratio between the energy required for moisture evaporation and the energy supplied to the dryer (Kaminiski et al., 1989). Thermal efficiency increased when IAT increased. Higher IAT increased the simultaneous mechanisms of heat and mass transfer more efficiently (Toneli et al., 2013), resulting in the higher drying rate and a lower moisture content of the obtained powder. At 180°C IAT, the obtained anthocyanin powder contained a lower moisture content, contributing to the smaller amount of sticky powder attached to the drying chamber, resulting in higher productivity (Kunapornsujarit and Intipunya, 2013). In addition, higher IAT reduced the energy cost, increased the productivity and resulted in a good quality powder with low moisture content.

The encapsulation process occurred during the drying step. When the water was evaporated, wall material was formed as a matrix structure to enwrap the anthocyanin core. The TAC of anthocyanin powders was decreased by the higher IAT. Tonon et al. (2008) reported that IAT was a factor affecting the açai TAC in microcapsules, due to the high sensitivity of this pigment to degradation at high temperature. In addition, Cai and Corke (2000) also reported that higher IAT (> 180°C) was not suitable for spray drying of betacyanin pigments. The SAC is the content of anthocyanin located at the surface of the microcapsule and not entrapped by wall material. Thus, the SAC directly exposed to the high temperatures showed greater loss when the higher IAT was used.

Encapsulation efficiency is a parameter indicating the efficiency of the encapsulation process. Increasing IAT increased the encapsulation efficiency, thus indicating that a higher amount of the anthocyanin core was protected from the thermal processing and storage conditions (Selim et al., 2008; Idham et al., 2012). Therefore, 180°C IAT encapsulated and protected more anthocyanin content inside the wall.

Anthocyanin powder produced using 180°C IAT showed the lowest bulk density, which indicated that this powder had the largest particle size (Reiniccius, 2001; Nijdam and Langrish, 2006; Tonon et al., 2008). This powder also contained more occluded air within the particles and a larger number of spaces between the particles, which resulted in a greater possibility for oxidative degradation and reduced the storage stability (Cai and Corke, 2000). The angle of repose is a measure of the powder flowability of particulate solids (Shittu and Lawal, 2007). The results indicate that all anthocyanin powders were great flowing particulate solids, because the repose angles were lower than 35 degrees (Carr, 1976).

The powder produced at 180°C IAT showed higher solubility and dispersibility than the powders produced at lower IAT (Table 2). The powders produced at lower IAT formed lumps more often than those produced at higher IAT. The spherical shape with higher surface moisture content resulted in lumps, making it more difficult to dissolve in water (Kunapornsujarit and Intipunya, 2013). Moreover, a higher IAT also produced powders with low sedimentation volumes and that were less likely to form sediments at the bottom of the containers (Shittu and Lawal, 2007).

The highest *a** and *C** values were observed at 140°C IAT. This was related to the SAC. Anthocyanin located at the surface of the wall contributed to the color of the powders. The results indicate that the color of the anthocyanin power was not related to the anthocyanin content trapped inside the wall.

The microcapsule structure of anthocyanin powders was influenced by the IAT. Increased IAT resulted in a smoother microcapsule surface because the faster |moisture evaporation led to the formation of a smooth and hard crust (Tonon et al., 2008). The cracks and pores were found in the anthocyanin microcapsule formed at 140°C IAT. The cracks and pores can induce the core oxidation during the storage (Cai and Croke, 2000). Therefore, higher IAT improved the properties of the microcapsule structure.

Anthocyanin is a powerful natural antioxidant. To evaluate the antioxidant properties of anthocyanin powders, the reducing power and DPPH radical scavenging activity was measured. Reducing power has been used to evaluate the ability of natural antioxidants to donate electrons (Dorman et al., 2003). The antioxidants react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), and then react with ferric chloride to form a ferric ferrous complex that has a maximum absorption at 700 nm (Jayanthi and Lalitha, 2011). Therefore, a higher absorbance at 700 nm indicates a higher reducing power. IAT did not significantly influence the reducing power ($p > 0.05$) of anthocyanin powders, and all powders showed a lower reducing power than the synthetic antioxidant BHT. However, the results demonstrated that all of the anthocyanin powders were electron donors to free radicals, which stabilized the radical chain reaction. IAT did not affect DPPH radical scavenging activities of anthocyanin powders, which were also less than that of BHT. According to a report by Ersus and Yurdagel (2007), the DPPH radical scavenging activity of anthocyanin powder from black carrots had a high correlation with their anthocyanin content. However, the IC_{50} of all anthocyanin powders was not significantly different ($p > 0.05$) due to the slight difference in the TAC between these powders.

CONCLUSION

The IAT is a factor that influences anthocyanin microcapsule properties. Increasing IAT resulted in increases in thermal efficiency, productivity, encapsulation efficiency, solubility and dispersibility, while powder moisture content and color values (a^*, C^*, and $h°$) decreased. The anthocyanin powder produced at 180°C IAT showed the best microcapsule properties, having the highest productivity, the lowest energy consumption, the best powder properties and the highest number of smooth surface microcapsules. However, both antioxidant activities (reducing powder and DPPH radical scavenging activity) of all of the anthocyanin powders were not affected by IAT.

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