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

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**AUTHOR CONTRIBUTIONS**

Nattapong Kanha assisted in conducting the experiments, performed the statistical analysis and data visualization and wrote the manuscript. Thunnop Laokuldilok designed and conducted all of the experiments and wrote the manuscript. All authors have read and approved of the final manuscript.

**CONFLICT OF INTEREST**

The authors declare that they hold no competing interests.

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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:** Anthocyanins, Encapsulation,Rice bran, Spray drying, 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).

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).

**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 -18 ºC 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 40 ºC. 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 -18 ºC 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 (ThermoSpectronic Genesys 10 UV scanning, Thermo Scientific, USA). The anthocyanin content was calculated by the followed equation and expressed as mg cyanidin-3-glucoside.

**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 180 ºC 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 -18 ºC until analysis. In addition, thermal efficiency and productivity in percentage was calculated using the following equations (Hall and Hedrick, 1971).

**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:



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 oC 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 oC for 4 hours. The dispersibility was calculated using the following equation.

**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 oC IAT, while the highest moisture content (6.04%) was found in anthocyanin powder produced at 140 oC IAT.

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

|  |  |  |  |
| --- | --- | --- | --- |
| Inlet air temperature (°C) | Thermal efficiency (%) | Productivity (%) | Moisture content (%) |
| 140160180 | 55.79 c ± 0.39 | 72.95 b ± 1.88  | 6.04 a ± 0.05 |
| 57.40 b ± 0.16 | 75.42 b ± 1.16 | 4.41 b ± 0.16  |
| 58.06 a ± 0.26 | 78.87 a ± 1.12  | 2.88 c ± 0.37  |

Note: In each column, difffknsdkfj kdjfksdjf ksdfkdjfkjsd ferent 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 oC 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).

**Effects of spray-drying temperatures on powder properties**

Bulk density of the anthocyanin powder decreased significantly (P 0.05) from 0.2795 – 0.2381 g/cm3 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/cm3)** | **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 fdsfjsd dfjsdfj sdfjdfjsd sdfjsdjf fsdf sdfjksdjf 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 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%).

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 Kldsjflakjslka akdjfald sjf asdjkfa lskdfja klsdjfkasdjf kasdjf akjsdf aksdfj asdkjf asdkjfTable 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 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 0.05). ns represents non-significant difference (P 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 1.** Microcapsule structure of anthocyanin powder that produced (A) 140 °C, (B) 160 °C and (C) 180 °C under scanning electron microscope.

**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% (IC50). A lower IC50 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).

**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 oC 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.

**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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**AUTHOR CONTRIBUTIONS**

Nattapong Kanha assisted in conducting the experiments, performed the statistical analysis and data visualization and wrote the manuscript. Thunnop Laokuldilok designed and conducted all of the experiments and wrote the manuscript. All authors have read and approved of the final manuscript.

**CONFLICT OF INTEREST**

The authors declare that they hold no competing interests.

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**REFERENCES**

AOAC. 2000. Official methods of the association of official analytical chemists. Association of Official Analytical Chemists, Washington D.C.

Bhandari, B.R., Senoussi, A., Dumoulin, E.D., and Lebert, A. 1993. Spray drying of concentrated fruit juices. Drying Technology. 11(5): 1081–1092.

Cai, Y.Z., and Corke, H. 2000. Production and properties of spray-dried *Amaranthus* betacyanin pigments. Journal of food science. 65(6): 1248–1252. https://doi.org/10.1111/j.1365-2621.2000.tb10273.x

Carr, R.L. 1976. Powder and granule properties and mechanics. In J. M. Marchello and A. Gomezplata (Eds.). Gas–solids handling in the processing industries. Marcel Dekker, New York.

Chen, P.N., Chu, S.C., Chiou, H.L., Chiang, C.L., Yang, S.F., and Hsieh, Y.-S. 2005. Cyanidin–3–glucoside and peonidin–3–glucoside inhibit tumor cell growth and induce apoptosis *in vitro* and suppress tumor growth *in vivo*. Nutrition and Cancer. 53(2): 232–243. https://doi.org/10.1207/s15327914nc5302\_12

Dorman, H.-J.-D., Peltoketo, A., Hiltunen, R., and Tikkanen, M.-J. 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chemistry. 83: 255–262. https://doi.org/10.1016/S0308-8146(03)00088-8

Ersus, S. and Yurdagel, U. 2007. Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray dryer. Journal of Food Engineering. 80: 805–812. https://doi.org/10.1016/j.jfoodeng.2006.07.009

Giusti, M.M., and Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV–visible spectroscopy. In R. E. Wrolstad (Eds.). Current protocols in food analytical chemistry (pp. F1.2.1–F1.2.13). John Wiley & Sons, New York. https://doi.org/10.1002/0471142913.faf0102s00

Hall, C.W., and Hedrick, T.I. 1971. Drying of milk and milk products. AVI Publishing, Connecticut.

Idham, Z., Muhamad, I.I., and Sarmidi, M.R. 2012. Degradation kinetics and color stability of spray–dried encapsulated anthocyanins from *Hibiscus sabdariffa* L. Journal of Food Process Engineering. 35: 522–542. https://doi.org/10.1111/j.1745-4530.2010.00605.x

Jayanthi, P., and Lalitha, P. 2011. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. International Journal of Pharmacy and Pharmaceutical Sciences. 3(3): 126–128.

Jinapong, N., Suphantharika, M., and Jamnong, P. 2008. Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. Journal of Food Engineering. 84: 194–205. https://doi.org/10.1016/j.jfoodeng.2007.04.032

Kaminski, W., Zbicinski, I., Grabowski, S., and Strumillo, C. 1989. Multiobjective optimization of drying process. Drying Technology. 7: 1–16. https://doi.org/10.1080/07373938908916571

Kunapornsujarit, D., and Intipunya, P. 2013. Effect of spray drying temperature on quality of longan beverage powder. Food and Applied Bioscience Journal. 1(2): 81–89.

Mazza, G., and Miniati, E. 1993. Anthocyanins in fruits, vegetables and grains. CRC Press, London. https://doi.org/10.1201/9781351069700

Mercadante, A.Z., and Bobbio, F.O. 2008. Anthocyanins in foods: occurrence and physicochemical properties. In C. Socaciu (Eds.). Food colorants: chemical and functional properties (pp. 241–276). CRC Press, Boca Raton. https://doi.org/10.1201/9781420009286

Reiniccius, G.A. 2001. Multiple-core encapsulation – the spray drying of food ingredients. In P. Vilstrup (Eds.). Microencapsulation of Food Ingredients (pp. 151–185). Leatherhead Publishing, Surrey.

Nijdam, J.J., and Langrish, T.A.G. 2006. The effect of surface composition on the functional properties of milk powders. Journal of Food Engineering. 77(4): 919–925. https://doi.org/10.1016/j.jfoodeng.2005.08.020

Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 7: 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307

Park, Y.S., Kim, S.J., and Chang, H.I. 2008. Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. Korean Journal of Microbiology and Biotechnology. 36(1): 55–60.

Rein, M. 2005. Copigmentation reactions and color stability of berry anthocyanins. University of Helsinki, Helsinki.

Ryu, S.N., Park, S.Z., and Ho, C.T. 1998. High performance liquid chromatographic determination of anthocyanin pigments in some variety of black rice. Journal of Food and Drug Analysis. 6: 729–736. https://doi.org/10.38212/2224-6614.2893

Sangkitikomol, W., Tencomnao, T., and Rocejanasaroj, A. 2008. Comparison of total antioxidants of red rice, black rice and black sticky rice. Journal of the Nutrition Association of Thailand. 43: 13–21.

Selim, K.A., Khalil, K.E., Abdel-Bary, M.S., and Abdel-Azeim, N.M. 2008. Extraction, encapsulation and utilization of red pigments from Roselle (*Hibiscus sabdariffa* L.) as natural food colorants. Alexandria Journal of Food Science and Technology. Special volume conference: 7–20.

Shittu, T.A., and Lawal, M.O. 2007. Factors affecting instant properties of powdered cocoa beverages. Food Chemistry. 100: 91–98. https://doi.org/10.1016/j.foodchem.2005.09.013

Toneli, J.T.C.L., Monteiro, L.B., Briso, M.A.J., and Moraes Junior, D. 2013. Effect of the outlet air reuse on thermal efficiency of a pilot plant spray dryer with rotary atomizer. Chemical Engineering Transactions. 32: 241–246. https://doi.org/10.3303/CET1332041

Tonon, R.V., Brabet, C., and Hubinger, M.D. 2008. Influence of process conditions on the physicochemical properties of açai (*Euterpe oleraceae* Mart.) powder produced by spray drying. Journal of Food Engineering. 88: 411–418. https://doi.org/10.1016/j.jfoodeng.2008.02.029

Wang, L.S., and Stoner, G.D. 2008. Anthocyanins and their role in cancer prevention. Cancer Letters. 269: 281–290. https://doi.org/10.1016/j.canlet.2008.05.020