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Effect of Ethanol Addition as Extraction Solvent on The Content of Bioactive Materials in Dragon Fruit Skin Extract and Powder

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Abstract

This study aims to produce natural pigments for food prepared from dragon fruit skin by extraction and freeze-drying and to assess the effect of additional ethanol as extraction solvent on the process yield. During extraction stage, the effect of solvent (pure water and additional ethanol) on the yield of bioactive materials was assessed. Furthermore, during freeze-drying, the effect of maltodextrin addition as carrier agent on the quality of powder has also been evaluated. It has been found that the addition of ethanol as extraction co-solvent may give a positive effect on the yield of bioactive materials in the dragon fruit skin extracts, including the contents of anthocyanin, betacyanin, and total phenolic compounds. Regarding freeze drying, it was found that high recoveries of bioactive materials (84-92%) had been achieved, which indicates that freeze-drying may be suitable for drying such heat-sensitive materials. In addition, it was found that the addition of 10% maltodextrin as carrier agent may decrease the moisture content of the powder significantly, up to 8.16±2.12%, which is beneficial for its storage stability.

Keywords: *betacyanin; food additives; dragon fruit; extraction; anthocyanin.*

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INTRODUCTION

Dragon fruit plant is originated from Mexico, Central America, and North America. This plant was brought by the French in the 1870s to Vietnam and then spread to countries in Asia, including Indonesia. Red dragon fruit (*Hylocereus polyrhizus*) is the most commonly found dragon fruit in Indonesia. The red dragon fruit consists of fruit skin and flesh. Dragon fruit flesh has been used for consumption, like cut fruit, jam, juice, and chips. Dragon fruit skin, which is around 30-35% of the fruit weight, is rarely used and often becomes waste.

The dragon fruit skin has been reported to contain bioactive materials that can be utilized, such as antioxidants and pigments (Jamilah *et al.*, 2011). Pigments, such as betacyanin that is highly contained in dragon fruit skin, are used in the food industry to increase the product appearance. In addition, antioxidants serve as an antidote to free radicals in food, which is responsible to prolong the shelf life of food. Several antioxidant compounds that can be found in dragon fruit skin include vitamin C, flavonoids, tannins, alkaloids, steroids, and saponins (Noor *et al.*, 2006). The current consumer's concern about the health risks of synthetic pigments and antioxidants has increased the use of natural pigments and antioxidants in food, such as those produced from fruit and vegetable wastes like dragon fruit skin.

Extraction is an important process to produce antioxidant and natural pigment because it affects the amount of bioactive materials obtained from the skin. One of the things that can affect the extraction process is the type of the solvent used. Previous literature reported that bioactive materials have high solubility in water and ethanol (Hussain *et al.*, 2018; Chong *et al*., 2014). Water is preferable for extraction solvent of food additives due to its low cost, low health risk, suitability for food application, and compatibility with human bodies. However, previous literature also reported that the use of ethanol may result in an increase in the yield of bioactive materials. Ethanol may be capable to denature the enzymes contained in plant matrix, while water only dissolves components of the plant matrix (Hussain *et al.*, 2018). Thus, the effect of additional ethanol for extraction solvent should be investigated, in this case for extraction of pigment and antioxidant compounds from dragon fruit skin.

In addition, liquid extract from dragon fruit skin should be dried in order to prolong the shelf life and reduce the transportation cost. One of the drying methods that is suitable for heat-sensitive material like bioactive materials from dragon fruit skin is freeze-drying. Freeze drying used mild conditions, such as low temperature and low pressure, so the degradation rate is low. However, one of the challenges in drying of fruit waste extracts is the development of stickiness due to the high sugar and acid contents (Adhikari *et al.,* 2005), which occur due to the low glass transition temperature of the sugars and acids. Drying aids can be added to the drying feed in order to increase the glass transition temperature and to reduce the stickiness of the product (Suravanichnirachorn *et al*., 2018). One of the materials that can be used as drying aid is maltodextrin. The effect of maltodextrin addition during freeze-drying of the dragon fruit skin extracts has never been investigated before.

Based on the previous gap in knowledge, this study aims to produce natural pigments by extraction and freeze-drying of dragon fruit skin. During extraction stage, the effect of solvent (pure water and additional ethanol) on the yield of bioactive materials was assessed. Furthermore, during freeze-drying, the effect of maltodextrin addition as carrier agent on the quality of powder has also been evaluated. The bioactive materials in the extracts and powder, including betacyanin, anthocyanin, and phenolic compounds have been quantified. In addition, the recovery of those bioactive materials and the moisture content of the freeze-dried powder have also been evaluated.

MATERIALS AND METHODS

Materials

Materials that were used in experiments are Folin-Ciocalteu's reagent (Merck), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich), and Na_2CO_3 (Bratachem, Indonesia). The dragon fruits that were used in experiments were freshly bought from a local supermarket (Superindo, Jatinangor, Sumedang, Indonesia).

Extraction

Dragon fruits were firstly washed, peeled, and crushed using a food processor (Kirin, Indonesia) until slurry was formed. The extraction was carried out by maceration at room temperature (25-30°C). The extraction solvent was varied: water and 10% ethanol, with a constant solvent to solid ratio of 3 mL/g. The extraction was carried out until 240 minutes. After each extraction experiment, the dragon fruit skin extracts were filtered using a filter paper (Whatman filter paper, Sigma Aldrich) and kept in the fridge for further freeze-drying process.

Freeze-drying

The freeze-drying experiment was carried out using an Alpha 1-2 LD Plus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH**,** Germany). The operation was done in vacuum (0.08-0.9 mbar) for 48 hours. The powder was then kept in fridge for further determination of the total phenolic contents, betacyanin, and anthocyanin contents.

Determination of betacyanin and anthocyanin contents

Diluted extracts were prepared and then the absorbance of the sample was measured by a spectrophotometer (Smart Spectrophotometer, Lamotte, USA) at wavelengths of 535 and 520 nm for betacyanin and anthocyanin, respectively. The amount of betacyanin and anthocyanin can be calculated by Equation (1).

$$Betacyanin\ or\ Anthocyanin\ \left({}^{mg}/_{g\ material}\right) = \frac{A \times DF \times Mr \times V_d}{\varepsilon \times L \times W_d} \quad (1)$$

where:

A = absorbance value

DF = dilution factor

Mr = molecular weight (550 g/mol for betacyanin and 449.2 g/mol for anthocyanin)
V_d = solution volume (mL)
ε = molar atenuation coefficient [60000 L/(mol .cm) for betacyanin and 26900 L/(mol.cm) for anthocyanin]
L = cuvette length (1 cm)
W_d = dragon fruit skin mass (g)

Equation (1) was used by assuming the anthocyanin as *cyanidin-3-glucoside* and the betacyanin as betanin (Giusti and Wrolstad, 2001; Kumar et al., 2015).

Determination of total phenolic compounds (TPC)

The TPCs of the extract and freeze-dried powder were determined with Folin-Ciocalteu's reagent (Shofinita and Langrish, 2016). Extract (0.1 mL) was mixed with demineralized water (0.2 mL), Folin Ciocalteu's reagent (1.5 mL), and 1.2 ml of 7.5% Na_2CO_3. The mixture was then kept for 30 minutes in dark. The absorbance of the sample was measured using spectrophotometry (Smart Spectrophotometer, Lamotte, USA) with a wavelength of 765 nm. Gallic acid solution was used as a standard and the TPC was stated as mg gallic acid equivalent (GAE)/g material.

Recovery of Bioactive Materials during Freeze-Drying

The recovery of the bioactive materials, including betacyanin, anthocyanin, and TPC was calculated by Equation (2).

$$Recovery\ of\ bioactive\ materials\ (\%) = \frac{Bioactive\ materials\ in\ freeze{-}dried\ powder\ \left(\frac{mg}{g\ DM}\right)}{Bioactive\ materials\ in\ extracts\ \left(\frac{mg}{g\ DM}\right)}\ x\ 100\% \quad (2)$$

Scanning Electron Microscopy (SEM)

The surface morphology of the powder was studied by a Hitachi SU3500 scanning electron microscope (Hitachi High Technologies America, Inc.). A small amount of the powder was placed on a carbon tape mounted on an aluminium stab and was coated with gold. The operating voltage of the instrument was set to be 10 kV for all the samples.

RESULTS AND DISCUSSION

Extraction of dragon fruit skin

Figure 1 shows the anthocyanin contents of the dragon fruit skin extracts obtained with water and ethanol 10% as the extraction solvent. This result shows that a higher amount of anthocyanin was achieved for the variation with additional ethanol as co-solvent. This trend is similar to the previously reported studies regarding extraction of anthocyanin with similar solvents. It was reported that methanol and ethanol gave better anthocyanin yields from red currant, black currant, and grape, compared with water (Lapornik *et al.*, 2005). Anthocyanins are known to be soluble in both water and alcohol. Basically, anthocyanins are glycosides and acylglycosides of anthocyanidins. The acylglycosides of anthocyanidins have higher solubilities in alcohol, while the glycosides are very soluble in water (Khoo *et al*., 2017).

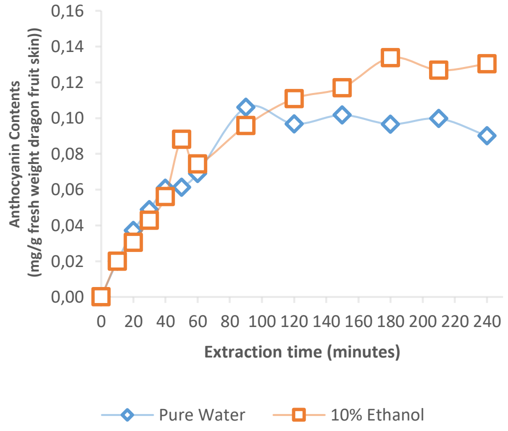

Figure 1. The effect of extraction solvent on the anthocyanin content of dragon fruit skin extracts.

The anthocyanin contents found in the dragon fruit skin extracts were between 0.02 and 0.14 mg/g fresh weight of dragon fruit skin. A previous study reported that dragon fruit skin had 0.045 mg anthocyanin/100 g DW (Lourdes *et al*., 2013), or if the dragon fruit skin contained 55% of moisture (from experiments), the anthocyanin content equals to 0.02 mg anthocyanin/g FW dragon fruit skin. The result in this study may be higher than the previous result, which may be attributed to the sources of the fruit, degree of ripening, and extraction technique (Shofinita *et al.*, 2015).

Figure 2 shows the betacyanin contents of the dragon fruit skin extracts obtained as affected by the use of different solvent: water and ethanol 10%. This result shows that additional ethanol as co-solvent may result in a higher amount of betacyanin in the dragon fruit skin extracts. Betalain pigments, which include betacyanin and betaxanthin, have high polarity and ionization in liquid solvent, which makes them soluble well in water and low molecular weight alcohol. A previous study found that betacyanin is soluble well in water and ethanol, and that the better extraction solvent is highly influenced by the varieties of the raw material. The author reported that betacyanin yield was higher by using water during extraction of betacyanin from *Amaranth gangeticus*, while a higher betacyanin yield was achieved by using ethanol for betacyanin from *Amaranth blitum* (Chong *et al.*, 2014). In addition, the betacyanin contents found in this study were between 0.01 and 0.07 mg/g fresh weight dragon fruit skin. A previous study reported that dragon fruit skin had 0.015 mg betacyanin/g FW dragon fruit skin (Harivaindaran *et al.*, 2008), which was in the range of betacyanin found in this study.

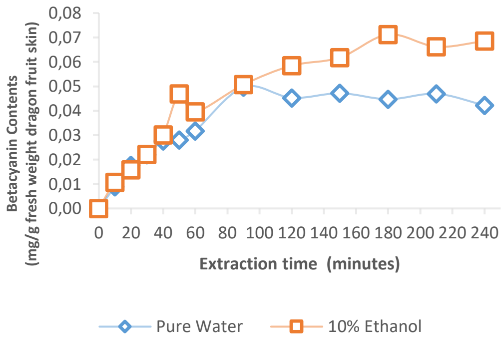

Figure 2. The effect of extraction solvent on the betacyanin content of dragon fruit skin extracts.

Figures 1 and 2 show that, initially, the extraction yields of anthocyanin and betacyanin were similar for pure water and 10% ethanol. However, at extraction times above 80 minutes, the extraction yields were significantly different. During the initial stage of extraction, the solvent washes the solid particle surface, which facilitates the transfer of solute on the particle surface to the solvent. The later stage of extraction occurs by mass diffusion and is indicated by the reduction in the extraction rate.

Figure 3 shows the total phenolic contents (TPC) of the dragon fruit skin extracts obtained as affected by the use of different solvent: water and ethanol 10%. The TPCs found in this study were between 0.025 and 0.35 mg/g FW dragon fruit skin. These values are in the range of a previously reported study regarding the TPC value in dragon fruit skin extract, which was 28.16 mg GAE/ 100 g fresh skin (Nurliyana *et al.*, 2010). Figure 3 shows that additional ethanol as co-solvent may result in a higher amount of phenolic compounds in the dragon fruit skin extracts. This trend is similar to the previous study reported by Fathordoobady et al. (2016), who found TPCs of the 10% ethanolic extracts and pure water to be 123.75 and 118.76 mg GAE/100 g, respectively. As mentioned by the authors, the addition of small amount of ethanol (below 50%) may give effect of the dielectric constant of an ethanol-water mixture, which improves the solubility of phenolic compounds (Fathordoobady *et al.*, 2016).

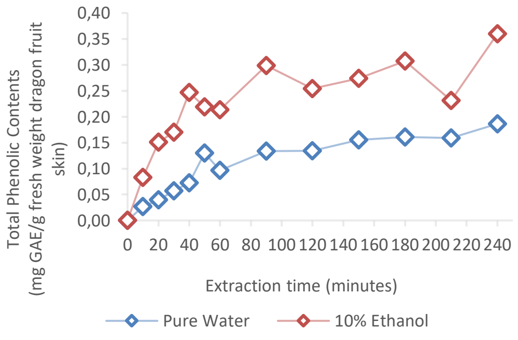

Figure 3. The effect of extraction solvent on the TPC of dragon fruit skin extracts

A previous study reported that gallic acid, as one of natural phenolic compounds, had a higher solubility in ethanol (23.73 g/100 g solvent) compared with in water (1.07 g/100 g solvent) at 298 K (Boas, 2017).

From extraction experiments, it was found that the addition of small amounts of ethanol may give a positive effect on the yield of bioactive materials in the dragon fruit skin extracts. Thus, the extracts with additional ethanol (10%) were prepared for further freeze-drying experiments.

Freeze drying of dragon fruit skin extracts

Effect of freeze drying on the recovery of bioactive materials

During freeze-drying, maltodextrin (10%-w) was added for some extracts. The addition of maltodextrin is expected to reduce the product stickiness due to the high sugar and acid contents associated with bioactive extracts (Adhikari *et al.* 2005). Dragon fruit skin was reported to contain some sugars, such as glucose, maltose, and fructose (0.86 - 4.15%) and also some organic acids, such as oxalic acid, malic acid, succinic acid, and citric acid (0.08 - 0.80%) (Jamilah *et al.*, 2011).

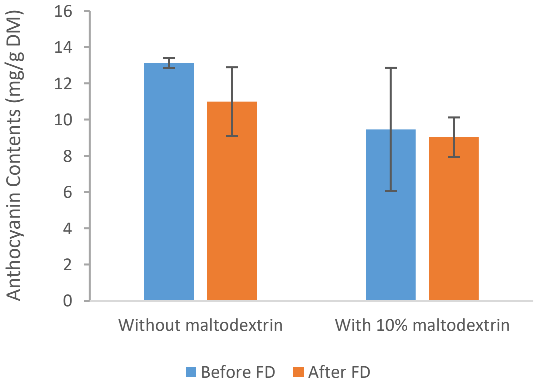

Figure 4. Anthocyanin contents in dragon fruit skin extracts before and after freeze drying of ethanolic dragon fruit skin extracts.

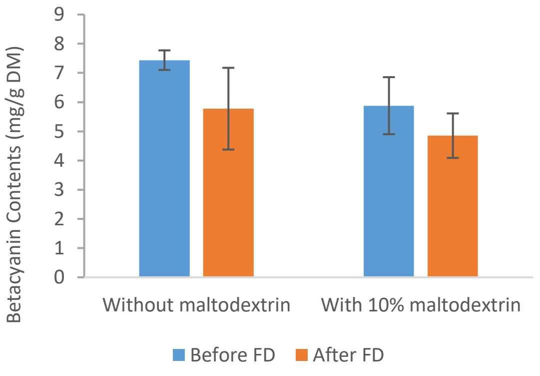

Figure 5. Betacyanin contents in dragon fruit skin extracts before and after freeze drying of ethanolic dragon fruit skin extracts.

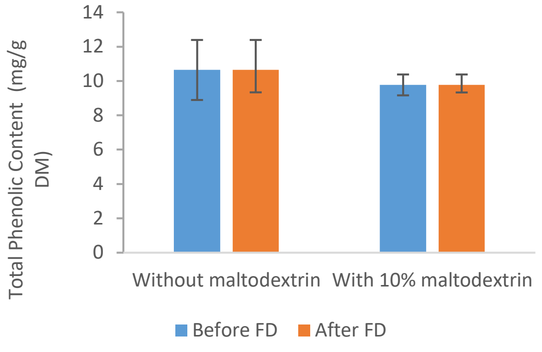

Figure 6. TPC in dragon fruit skin extracts before and after freeze drying of ethanolic dragon fruit skin extracts.

Figures 4, 5, and 6 show the effect of freeze-drying on the bioactive materials in dragon fruit extracts and powder, including anthocyanin, betacyanin, and TPC, respectively. This result shows that the addition of maltodextrin in the extracts may reduce the concentration of bioactive materials. This trend occurs because maltodextrin does not have significant amount of bioactive materials compared to the dragon fruit skin extracts, and thus its addition may lower the bioactive contents in the freeze dryer feed.

Figures 4, 5, and 6 also show that freeze-drying may affect the bioactive material contents in the materials, which may be occurred due to degradation. The bioactive material recoveries have been summarized in Figure 7. Figure 7 indicates that high recoveries of bioactive materials (more than 80%) can be achieved. Figure 7 also shows that there is no significant effect of maltodextrin addition on the recovery of bioactive materials during freeze-drying (P-value > 0.05).

Figure 7 shows that the anthocyanin recoveries found in this study were between 84 and 92%. High recoveries of anthocyanin can be obtained due to anthocyanin may be preserved inside the microcapsules during freeze-drying (Laokuldilok *et al.*, 2015). The anthocyanin recoveries found in this study is slightly higher than the previous studies. Franceschinis *et al.* (2014) previously reported 75% anthocyanin recovery during the freeze-drying of blackberry juice.

The betacyanin recoveries found in this study were between 83 and 88%, as shown in Figure 7. High recoveries of betacyanin during freeze-drying have also been reported by previous studies, such as Moßhammer *et al.* (2006) who reported 93% of betalain recovery during freeze-drying of cactus pear juice.

High TPC recoveries were also achieved in this study, which were between 85 and 88%. The high phenolic recoveries have also been reported before, such as during freeze-drying of tomato and ginger (Gümüşay *et al.* 2015). During freeze-drying, the loss of phenolic compounds may still occur even though mild condition is used, due to the changes in chemical structures and the binding of phenols to proteins during freeze-drying (Gümüşay *et al.* 2015).

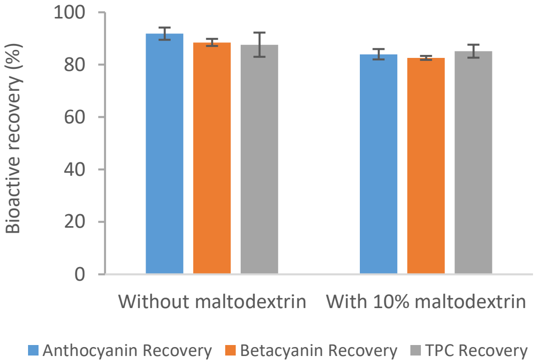

Figure 7. Bioactive materials recovery during freeze drying of ethanolic dragon fruit skin extracts.

Effect of freeze-drying on the moisture content of the powder

Figure 8 shows the effect of freeze-drying on the moisture content of the freeze-dried powder. This result shows that the addition of maltodextrin may result in a decrease in the moisture content of the powder. This trend has also been reported by previous authors (Suravanichnirachorn *et al.*, 2018; Caliskan and Dirim, 2016). A higher concentration of maltodextrin in the freeze dryer feed may increase the solid contents in the extracts, and so decrease the amount of free water that is required to be dried (Caliskan and Dirim, 2016). Moreover, the addition of high molecular weight carrier agent such as maltodextrin may affect the microstructure of the product, such as a higher pore size (Harnkarnsujarit, Charoenrein, and Roos 2012). This may lead to a lower entrapment of moisture within the particle, and thus lower moisture content of the product. This decrease in the moisture content is beneficial for the storage stability of the powder because the degradation rate decreases in lower moisture content of material.

Surface Morphology of the Freeze-Dried Powder

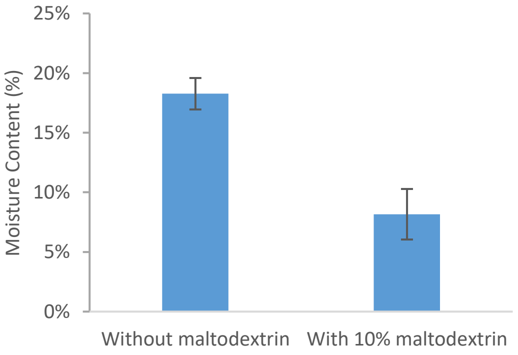

Figure 8. Moisture content of the freeze-dried powder as affected by maltodextrin addition.

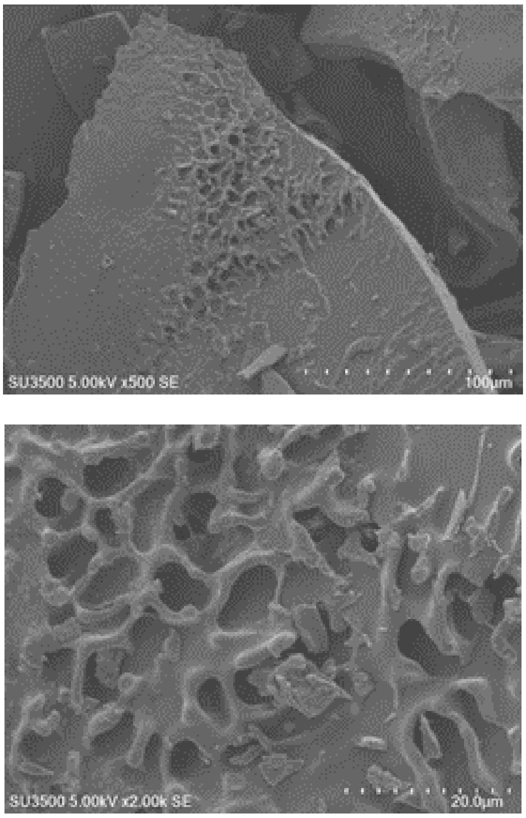

Figure 9. SEM result of the freeze-dried powder with 10% maltodextrin.

Figure 9 shows the SEM result of the freeze-dried powder, particularly for the powder with the addition of 10% maltodextrin. The image shows that the powder particles had irregular glanular powder shapes and had a rough surface. This trend have also been observed previously in the freeze-dried powders (Suravanichnirachorn et al. 2018). Pores could also be observed on the surface of the particle due to the formation of ice crystals during the freezing stage, which were then sublimated during the drying stage.

CONCLUSIONS

The addition of ethanol as co-solvent may give a positive effect on the yield of bioactive materials in the dragon fruit skin extracts, including the contents of anthocyanin, betacyanin, and total phenolic compounds. In addition, freeze-drying experiments have also been done for dragon fruit skin extracts. It was found that high recoveries of bioactive materials (more than 80%) had been achieved, which indicates that freeze-drying may be suitable for drying such heat-sensitive materials. In addition, the addition of 10% maltodextrin as carrier agent during freeze-drying may decrease the moisture content of the powder significantly, up to 8.16 ± 2.12%.

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