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IDENTIFICATION OF ANTHOCYANIDINS IN ANTHOCYANIN EXTRACT OF PURPLE SWEET POTATO (*IPOMOEA BATATAS* L), BLACK RICE (*ORYZA SATIVA* L), AND BLACK GLUTINOUS RICE (*ORYZA SATIVA* V. GLUTINOSA)

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ABSTRACT

Antosianin yang berasal dari berbagai komoditas mempunyai komponen penyusun dan kadar antosianin yang berbeda-beda. Komoditas lokal seperti: ubi jalar ungu, beras hitam, dan beras ketan hitam memiliki komponen struktur dan kadar antosianidin yang berbeda beda. Tujuan penelitian ini adalah untuk mengukur kandungan antosianin total dan mengidentifikasi antosianin tersebut secara kualitatif dan kuantitatif dari ekstrak ubi jalar ungu, beras hitam, dan beras ketan hitam. Metode penelitian yang digunakan adalah metode eksperimen dengan pendekatan kuantitatif berdasarkan rancangan acak lengkap (RAL) dengan tiga sampel berbeda yaitu beras ketan hitam, beras hitam, dan ubi jalar ungu. Penentuan kadar dan komponen penyusun antosianidin dari ekstrak ubi jalar ungu, beras hitam, dan ketan hitam, dilakukan dengan analisis identifikasi kualitatif dan kuantitatif dengan instrumen UHPLC-PDA (Ultra High Performance Liquid Chromatograph). Berdasarkan penelitian, total ekstrak antosianin pada ubi jalar ungu sebesar 0,6 mg/g bahan, jauh lebih rendah dibandingkan beras hitam (5,50 mg/g bahan) dan beras ketan hitam (6,00 mg/g bahan). Berdasarkan identifikasi ekstrak antosianin ubi jalar ungu, komponen utama antosianin adalah Delfinidin, Cyanidin, dan Malvidin dengan konsentrasi bahan masing-masing 0,12 mg, 0,12 mg, dan 1,20mg/100g. Sedangkan beras hitam dan ketan hitam teridentifikasi mengandung sianidin dan malvidin dengan konsentrasi bahan 0,73 dan 13,17 mg/100g serta 1,07 mg dan 17,6 mg/100g.

Keywords: anthocyanin; purple sweet potato; black rice; glutinous black rice; anthocyanins; anthocyanidins

ABSTRAK

Anthocyanins originating from different commodities have different constituent components and anthocyanidin levels. This will undoubtedly affect the anthocyanin content of total in foodstuffs. Local items such as; Purple sweet potato, black rice, and black glutinous rice have different structural components and levels of anthocyanidins. The purpose of this study was to measure the total anthocyanin content and assist anthocyanidins from the anthocyanin extracts of purple sweet potato, black rice, and black glutinous rice. The research method used was an experimental method with a quantitative approach based on a completely randomized design (CRD) with three different samples: black glutinous rice, black rice, and purple sweet potato. Determining levels and constituent components of anthocyanidins from purple sweet potato, black rice, and black glutinous rice extracts, qualitative and quantitative identification analyses were carried out using the UHPLC-PDA (Ultra high Performance Liquid Chromatographic) instrument system. Based on the study with pH-difference measurements, the total anthocyanin extract in purple sweet potato is 0.6 mg / g of material, much lower than black rice (5.50 mg / g of material) and black glutinous rice (6.00 mg / g of material). Based on the identification of the purple sweet potato anthocyanin extract, the main components of anthocyanidins were Delfinidin, Cyanidin, and Malvidin with concentrations of 0.12 mg, 0.12 mg, and 1.20mg / 100g of ingredients, respectively. Meanwhile, black rice and black glutinous rice were identified with cyanidine and malvidin with concentrations of 0.73 and 13.17 mg / 100g and 1.07 mg and 17.6 mg / 100g of ingredients.

Kata kunci: antosianin; ubi ungu; beras hitam; beras ketan hitam; anthosianidin

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INTRODUCTION

Anthocyanins are included in the flavonoid group, a polyphenol group that plays a role in giving color to food. Anthocyanins are composed of an aglycone (*anthocyanidin*) which is esterified with one or more sugar groups (*glycone*). Most anthocyanins are found in the six anthocyanidin component structures: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Rinda, 2013). Anthocyanins, as exogenous antioxidants, are believed to have excellent antioxidant effects because they can inhibit various free radicals, especially in the physiological effects of the body (Prior, 2002). The total anthocyanin content determines the ability of anthocyanins as antioxidants and, most importantly, the active components contained in it, namely anthocyanidin (Khandelwal et al.,2014).

Previous research stated that black glutinous rice, purple sweet potato, and black rice are foodstuffs with a high source of anthocyanins compared to other vegetable sources such as corn, potatoes, and beans. Black glutinous rice has been known to contain anthocyanin group compounds that have several pharmacological activities, such as chemopreventive, antioxidant, anti-inflammatory, and antidiabetic effects (Ryu et al.,1998) (Zhang et al.,2005). The lack of glutinous rice has been known to contain anthocyanin compounds with several pharmacological activities, such as chemopreventive, antioxidant, anti-inflammatory, and antidiabetic effects (Ryu et al.,1998) (Zhang et al.,2005). Besides having a relatively low price, the potential for availability is significant and easy to find in Indonesia Yenrina et al., (2013); Husna et al., (2013). On the other hand, purple sweet potato, black glutinous rice, and black rice have been pr.

This study uses often found local food ingredients, such as purple sweet potato, black rice, and black glutinous rice. These three foodstuffs are often consumed because they are a source of carbohydrates. In addition, the three foodstuffs have a thick color, thus indicating the presence of anthocyanins. In previous research, black glutinous rice extract is known to have anthocyanidin content in the form of pelargonidin 3-glucoside using the thin layer chromatography method.

Based on the review of the background, the researchers wanted to know the levels and types of anthocyanins from the three foodstuffs because they have been widely used as food ingredients with health benefits related to their high anthocyanin levels. Based on the above background, this study aimed to determine the total anthocyanin content of the three foodstuffs, namely purple sweet potato, black rice, and black glutinous rice and identify the types of anthocyanin components and determine the Concentration of each kind of anthocyanidin component.

MATERIALS AND METHOD

The raw material used in this study was Purple Sweet Potato (*Ipomoea batatas* L.) Ayamurasaki variety obtained from organic farming in Sendangsari, Kulon Progo (D.I.Yogyakarta). Black Rice (*Oryza sativa*, L. indica) Toraja variety obtained from Organic Agriculture Malang (Surabaya). And Black Glutinous Rice (Oryza sativa Var. glutinosa) Setail variety obtained from the Department of Food Security (D.I.Yogyakarta).

Chemicals for sample preparation and analysis, such as ethanol 96%, citric acid (Technical), KCl buffer (pH 1.0), sodium acetate buffer (pH 4.5), ethyl acetate (Pro-Analysis), amyl alcohol (Pro-Analysis), HCl 2M, methanol-HCl 0.01%. (Pro-Analysis), methanol (MeOH, HPLC-quality) and 10% formic acid (HPLC gradient grade). Materials For Identification Standards, such as Pelargonidin-chloride, cyanidin-Florida, Delphinidin-chloride, and Malvidin- chloride (Sigma-Aldrich)Tool for Anthocyanidin identification Using Ultra High-Performance Liquid Chromatographic-Photodiode Array (UHPLC-PDA) system, Brand. PerkinElmer-UHPLC Flexar.

Anthocyanin Extraction

Before being extracted, the raw material is made into flour. Making purple sweet potato flour includes peeling, washing, slicing, and drying in a cabinet dryer at 50°C for 8 hours, then milling and sieving with a 60 mesh sieve. Meanwhile, the black rice and black glutinous rice are directly ground using a grinder and sieved through a 60 mesh sieve. Then extraction is carried out by maceration (once) to obtain anthocyanin extract, using 96% ethanol - 3% citrate as a solvent and ending with solvent evaporation (evaporation) with a rotary evaporator at a temperature of 40°C until all organic solvents evaporate.

The anthocyanin extract was first stored in a freezer at -20ºC in a closed condition with aluminum foil before hydrolysis of anthocyanin acid.

Anthocyanin Acid Hydrolysis (Anthocyanin Extract Purification)

Preparations carried out before identification included hydrolysis of anthocyanins using 2M HCl with maximum heating of 80ºC in a water bath so that all anthocyanins were converted to the form of aglycones or anthocyanidins. The ethyl acetate fraction was removed, and the water fraction was extracted with amyl alcohol, then heated in a water bath at 80°C. After drying, the anthocyanidins were dissolved in methanol-0.01% HCl.(Harborne, 1989).

Anthocyanidin Identification

Identification of anthocyanidins using a Shimadzu UHPLC chromatography tool and referring to method 27 with qualifications such as the column used is C-18 with a PDA detector. The column oven temperature is set to 15°C. Gradient elution using 100% methanol (Solution A) and 10% formic acid (Solution B), and then the optimization method is set as follows:

- Linear gradient from 13.0% A and 87.0% B to 24% A and 76% B during the 0th minute to the 8th minute.

- Linear gradient from 24% A and 76% B to 100% A and 0% B during the 8th minute to the 14th minute.

The elution gradient was adjusted at a flow rate of 0.300 ml/min, followed by a pump pressure of 1870 to 1890 Psi. The injected volume is 20 L with a wavelength of 520 nm, which is then optimized for comparison solutions or anthocyanidin standards by making a standard curve using a concentration series of 10 to 60 ppm, while the criteria such as Pelargonidine chloride, Cyanidine chloride, Delfinidine chloride, and Malvidin chloride are dissolved in Methanol- 0.01%HCl.

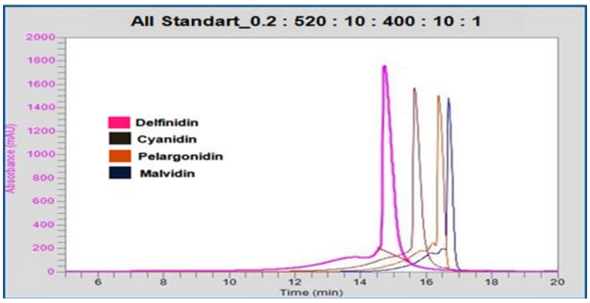

Figure 1. Chromatogram profile of anthocyanidins standard

Total Anthocyanin Analysis

Determination of the total anthocyanin content was carried out using the pH-difference method. The pH difference test was repeated three times. The anthocyanin extract was dissolved in KCl¬HCl buffer (0.0025 M, pH 1) and NaOAc buffer (0.4 M, pH 4.5) with a ratio of extract to buffer = 1: 5 (v/v). The absorbance of each solution was measured at a wavelength of 530 nm and 700 nm after being incubated for 15 minutes at room temperature. The results were entered into the formula:

Anthocyanin (mg/ml) = $\frac{A}{(\varepsilon \times 1)}$ x MW x DF

Note :

* A : Absorbance

A = [(A530-A700)pH1 - (A530-A700)pH4,5]

* MW : molecular weight (449.2)

* DF : Dilution factor

* ε : Koefisien ekstingsi molar sianidin 3-glikosida = 26900

Furthermore, the results of the above calculations are entered into the Lambert-Beer law: A = .L.C.ε with a molecular weight of cyanidin-3-rutinoside 26900 L/mol.cm.

RESULTS AND DISCUSSION

Total Anthocyanin Content

Based on the results in Table 1, which were analyzed by measuring pH difference (Adiari et al.,2017), the total anthocyanin content of black glutinous rice was 6.00 mg/g of ingredients, while black rice was 5.50 mg/g of elements. Previous research stated that black rice and black glutinous rice have a high total anthocyanin content, which ranges from 3.2 to 9.62 mg/g of material. Different varieties of rice will provide different levels of anthocyanins and yields. The types used in previous research were Thai rice Newdan53, Pamia97, Wongwan98, Newdan1-202, and Newand2-203(Ryu et al.,1998).

Table 1. Result analysis of anthocyanin total

No	Materials	Total Anthocyanins
1	Purple sweet potato	0,6 mg/g sample
2	Black glutinous rice	6,0 mg/g sample
3	Black rice	5,5 mg/g sample

The content of purple sweet potato anthocyanin extract is lower than black rice and black glutinous rice, which is 0.6 mg/g of ingredients. The same thing was also found in the total anthocyanin content of purple sweet potatoes in a previous study with the ayamurasaki variety with a content of 0.61 mg/g of ingredients (Zhang et al., 2005).

Anthocyanidins Identification

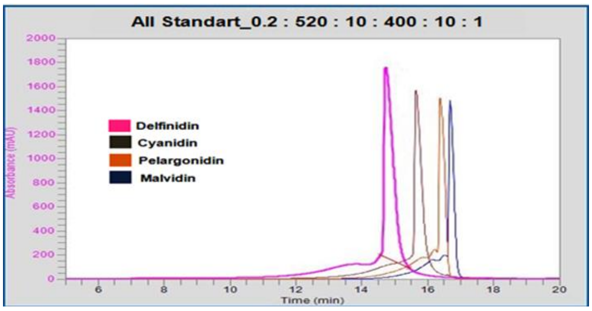

Figure 2. Chromatogram profile of anthocyanidins standard
Source : The compound of anthocyanin standard
Note: Peak 1 : Delphinidin (14.73 minutes), peak 2 : Cyanidin (15.65 minutes), peak 3 : Pelargonidin (16.38 minutes), and peak 4 : Malvidin (16.68 minutes).

Identification of Purple Sweet Potato Anthocyanidins

The results of the separation of purple sweet potato anthocyanidin extract with UHPLC-PDA detected at a wavelength of 520 nm can be seen in Figure 3.

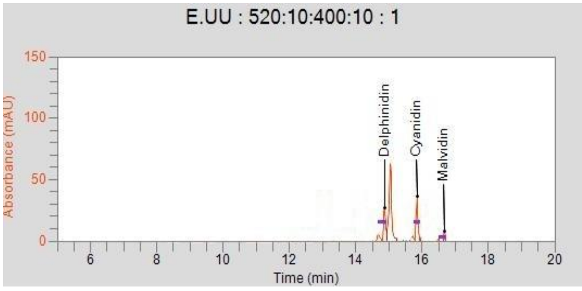

Figure 3. Qualitative identification of purple sweet potato anthocyanidins
Note delphinidin (14.863 minutes), cyanidin (15.842 minutes), and malvidin (16.670 minutes).

Based on the chromatogram separation results, the anthocyanidins identified from purple sweet potato were delphinidin, cyanidin, and malvidin. The three anthocyanidins that play an essential role in giving color to the chickenurasaki variety purple sweet potato in this study, where the difference between each type of anthocyanidin lies in the location and degree of methylation of the hydroxyl group. This result is also in line with previous research that the anthocyanidins found in purple sweet potato are delphinidin, peonidin, cyanidin, and malvidin, which play a role in the formation of purple sweet potato pigment. type of anthocyanidin Peonidin, because based on the identification of the main anthocyanin components in purple sweet potato with various cultivars such as: yamagawamurasaki, ayamurasaki, stokes purple, and purple okinawa, an anthocyanidin type peonidin was detected (Tsuda et al.,2002); Montilla et al.,(2011). It was suspected that peonidin appeared but was not detected due to the absence of a standard, thus referring to the statement in previous studies regarding the reference compounds that mainly were found were cyanidin and peonidin. According to supporting ideas from previous research, cyanidin and peonidin derivatives are one of the anthocyanins that contain the most and are used as a reference compound in general. In addition, based on previous research, it was stated that the type of anthocyanidin that was explicitly found in sweet potatoes was peonidin (Mahmudatussa'adah et al.,2014).

Table 2. The Concentration of purple sweet potato (sp.Ayamurasaki) anthocyanidins

Materials	Time retention (minute)	Anthocyanidin	Concentration (mg/100 g)
Purple sweet potato	14.863	Delphinidin	0.12
	15.842	Cyanidin	0.12
	16.670	Malvidin	1.20

Based on the quantitative identification of the concentration levels of the three types of purple sweet potato anthocyanidins above, it can be seen that delphinidin and cyanidin are lower than the concentrations of malvidin, this can be seen from the retention time and color pigment. Pelargonidin, peonidin, and cyanidin cause the red color of the anthocyanin pigment (Laksmiani et al.,(2019). The results in Table 2 are slightly different from the study, which stated that the percentage concentration of cyanidin anthocyanidins was higher than that of delphinidin, petunidin, pelargonidin, peonidin, and malvidin in the purple sweet potato cultivar Violette, amounting to 74 %(Lachman et al.,2020).

The high and low monomeric content of anthocyanidins can be influenced by many factors such as location, soil conditions, nutrition, sweet potato varieties, temperature, light, etc. High malvidin shows more red–bluish color spectrum than other color spectrums but tends to be in a broader range. little or the same (Mahmudatussa'adah et al.,2014). The number of monomeric anthocyanins was different from sweet potatoes from different locations, but the color pattern, spectra, and antioxidant activity were relatively the same (Mahmudatussa'adah et al.,2014).

Identification of Black Rice Anthocyanidins

The results of the black rice anthocyanidin extract chromatogram separation using the UHPLC-PDA system detected at 520nm can be seen in Figure 3.

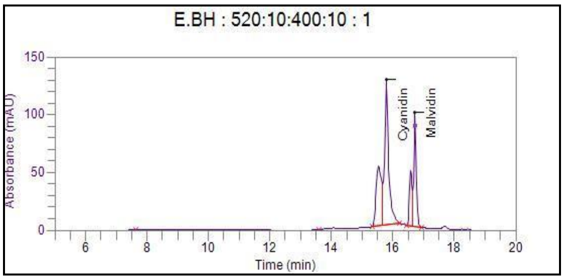

Figure 4. Chromatogram profile of black rice anthocyanidins
Note: Cyanidin (15.781 minutes) and Malvidin (16.724 minutes).

Purple sweet potato (Table 1). Identification of component types and levels of anthocyanidins was made by comparing the retention time and peak area of black rice anthocyanidins with standard anthocyanidins. Based on the chromatogram separation results, the anthocyanidins identified from black rice were cyanidin and malvidin with concentrations of 0.73 mg/100g and 13.17 mg/100g of material (3 replications), which were higher than the cyanidin and malvidin content in sweet potatoes.

The presence of two different peaks indicates the potential for identifying other types of anthocyanidins, but they cannot be placed and read due to the absence of standards

Table 3. Concentration of black Rice anthocyanidins

Materials	Time Retention (Minutes)	Anthocyanidin	Concentration (mg/100 g)
Black rice	15.781	Cyanidin	0.73
	16.724	Malvidin	13.17

The results of the identification in this study are from previous studies that total malvidin in pigmented rice, such as black rice, has a range of malvidin amounts from 2.10 to 1045.12 mg/100g ingredient22. In addition, the discovery of several malvidin contents was also stated by other researchers22, who also identified malvidin in black rice, which was in the range of 3.2-3.3 mg/100g. Meanwhile, the Concentration of malvidin in the previous findings was 17.6 mg/100 g, which was higher than the Concentration of malvidin in this study.23 In addition to malvidin, cyanidin was also detected in this study. This is supported by other findings that malvidin, pelargonidin, and cyanidin are black rice's significant substances, and each of these anthocyanidins has natural abilities for health (Zhang et al.,2005).

Identification of Black Glutinous Rice Anthocyanidins

The results of the chromatogram of the anthocyanidin extract of black glutinous rice using the UHPLC-PDA system detected at a wavelength of 520 nm can be seen in Figure 5. The type and level of anthocyanidins were identified by comparing the retention time and peak area of black glutinous rice anthocyanidins with standard anthocyanidins.

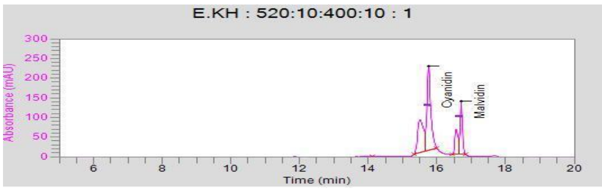

Figure 5. Chromatogram profile of black glutinous rice anthocyanidins
Note: Chyanidin (15.756 minutes) and Malvidin (16.709 minutes).
Note: Cyanidin (15.756 minutes) and Malvidin (16.709 minutes).

Based on the chromatogram separation results, the anthocyanidins identified from black glutinous rice were cyanidin and malvidin with levels of 1.07 mg/100g and 17.6 mg/100g of material, respectively, with a total anthocyanin of 600 mg/100g of material.

Table 4. The concentration of Black Glutinous Rice anthocyanidins

Materials	Time retention (minutes)	Anthocyanidins	Concentration (mg/100 g)
Black Glutinous Rice	15.756	Sianidin	1.07
	16.709	Malvidin	17.6

The three main ingredients in this study, namely purple sweet potato, black rice, and black glutinous rice, all identified as cyanidin and malvidin. However, the anthocyanidin delphinidin was identified only in purple sweet potato, while based on the Concentration of cyanidin and malvidin levels in black glutinous rice, cyanidin and malvidin content were higher than those two ingredients which can be seen in table 4. The most common anthocyanin compound found was pelargonidin (orange). , cyanidin (orange-red), peonidin (orange-red), delphinidin (blue-red), petunidin (blue-red), and malvidin (blue-red) (Priska et al.,2018). The color caused by anthocyanins depends on the environment's acidity (pH). Anthocyanins will react easily if mixed with strong acid. The resulting color will be more concentrated when bound to acid (Park et al.,2008). Differences may also influence the emergence of Delphinidin in pH and different environmental conditions.

Table 5. Anthocyanidins content from purple sweet potato, black rice, and black glutinous rice

Materials	Delphinidin (mg/100g)	Cyanidin (mg/100g)	Malvidin (mg/100g)
Purple sweet potato	0.12	0.12	1.20
Black rice	-	0.73	13.17
black glutinous rice	-	1.07	17.60

Table 5 shows that the anthocyanin components, namely cyanidin and malvidin, in black glutinous rice are higher than in black rice. The results obtained are thought to be due to the anthocyanin pigment in black glutinous rice not only found in the bran, but also in the endosperm of rice. However, the anthocyanin pigment is concentrated in the bran (Kano et al.,2005). The identification of anthocyanidins in black glutinous rice showed that the Concentration of malvidin was higher than cyanidin. This result is reinforced by previous research that the anthocyanidin components of black glutinous rice Heugjijubyeo variety are cyanidin, peonidin, pelargonidin, and malvidin (Park et al.,2008).

CONCLUSION

Based on the results of this study, it can be concluded that the total anthocyanin content of the three ingredients, namely purple sweet potato, is 0.6 mg/g ingredients, black rice is 5.50 mg/g ingredients, and black glutinous rice is 6.00 mg/g ingredients. The anthocyanin content (*Malvidin* and *Cyanidin*) of black glutinous rice is higher than that of black rice. The anthocyanin component Delphinidin was only found in sweet potatoes but not in black rice and glutinous rice. The stability of anthocyanins influences the different types of anthocyanins and their content to pH (acid and base), the environment, and the species of raw materials used.

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