Optimization of Total Anthocyanin Extraction from Brown Rice (Oryza nivara)

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Abstract. Brown rice contains anthocyanin compounds. Anthocyanin is a natural pigment that gives red color to brown rice. Anthocyanin is an unstable compound, so it is necessary to optimize the extraction method to find out the proper extraction method in obtaining the largest anthocyanin level in brown rice. Optimization of extraction conducted in this study include solvent, addition of HCl and size of brown rice Measurement of levels in this study using the method of differential pH with visible spectrophotometer tool. The results showed that extraction using methanol-HCl solvent was the best extraction process. As for the size of rice particles does not affect the amount of extracted anthocyanins.

1. Introduction

Brown Rice (Oryza sativa L.) is the kind of food crop grain selected as a staple food or carbohydrate sources in developing countries. Broadly speaking, there are rice with various varieties like white rice, red rice and black rice. Brown rice has a low gluten so Brown rice can be used as a substitute for white rice which is being on a diet of sugar.

Brown rice has a lot of chemical components that have the potential for beneficial health like fiber, vitamins, gamma-aminobutyric acid (GABA), and gamma-oryzanol, which can reduce the risk of chronic diseases including hyperkolestrolemia, cardiovascular disease, obesity and diabetes type I. In addition, on brown rice anthocyanin compounds found that has the characteristic gives the red on red rice (Oryza nivara) is caused by a large amount of anthocyanin pigments found in the layer of rice [1].

It contains the anthocyanin phenolic compounds and provide natural colour present on fruit, flowers, leaves and vegetable. And is divided into three main parts, namely antosianidin, agloikon and glucosides. In addition, the relationships with compound is a compound that contains the flavonoids, which have the function as antioxidants that are believed to be able to cure degenerative diseases [2].

Stability of anthocyanin is influenced not only by the heating temperature on the process of processing, but also influenced by intrinsic and extrinsic factors in the product, such as pH, temperature, and concentration of the chemical structure of the relationships that exist, the presence of light, oxygen, enzymes, proteins, and the metal ion. Anthocyanin compounds are unstable against changes in temperature, therefore the need for optimization of extraction methods to obtain the highest levels of anthocyanin.

2. Materials and Methods

Sample used in this study is brown rice obtained from the market in Bandung. Sample used in the form of whole and fine brown rice, for fine brown rice begins with smoothing brown rice starting with
a blender. Characterization of brown rice is done by determining the total ash content and water content. Phytochemical screening is identification of flavonoids, identification of tannins and identification of quinones. Extraction was carried out by maceration method using ethanol, water, and methanol as solvent and smooth, and at 25°C. Then proceed with thickening extract using a rotary evaporator. Determination of the maximum λ extract and the determination of total anthocyanin by differential pH method that is pH 1.0 and 4.5.

2.1. Preparation of materials
Material preparation includes the collection of materials to be used, namely brown rice (Oryza nivara) obtained from one of the Market in Bandung.

2.2. Sample preparation
The sample used in the form of rice in whole form (grain) and in fine form by using a blander to obtain a fine red rice powder.

2.3. Phytochemical screening
Phytochemical screening consisted of flavonoid identification, quinone identification and tannin identification.

2.4. Identification of flavonoids
One gram of sample in 100 ml of hot water is boiled for 5 minutes and filtered. In 5 ml of filtrate magnesium powder was added and 2 ml of HCl-ethanol (1: 1), then shaken with 10 ml of amyl alcohol. Positive reactions are indicated by the formation of orange, yellow or red in the amyl alcohol layer [3].

2.5. Identification of quinons
One gram of sample in 100 ml of hot water is boiled for 5 minutes and filtered. 5 ml of filtrate were added a few drops of 1 N. NaOH solution. The formation of red showed a quinone. But there can be a false positive reaction with tannins. Then the examination continued with the addition of gelatin then the sediment was filtered and the diltrate was added with 1 N. NaOH

2.6. Identification of tannins
There are 2 methods used to test tannins. First, 1 ml of ethanol extract was added to 2 ml of water in a reask tube. 2 to 3 drops of dilute FeCl3 solution are added and observed for green to blue-green (fixed tannins) or black-colored colors (error tannins). Second, 2 ml of water extract are added to 2 ml of water, 1 to 2 drops of dilute FeCl3 solution are added. Dark green or blue green staining indicates tannins.

2.7. Optimization of the extraction method
Anthocyanin extraction from brown rice was carried out by maceration method at 25°C using 2 solvents including methanol and methanol HCl 1%. Maseration of samples by immersing 50 grams of brown rice powder and 50 grams of whole rice with 300 mL of 1% methanol and methanol HCl solvent at 25°C for 24 hours. Then filtered and taken the filtrate, do triplo. The extract obtained was concentrated using a rotary evaporator to obtain a thick extract.

2.8. Determination of λ maximum extract
Determination of the maximum λ of red rice extract was carried out by UV-Vis spectrophotometry method. As much as 1 mL of each extract of maceration 25 0°C, dissolved in 5 ml of methanol, then absorbance was measured at a wavelength of 400-800 nm.
2.9. Determination of Total Anthocyanin Levels with Differential pH Method
Determination of anthocyanin is carried out by differential pH method which is pH 1.0 and pH 4.5. At pH 1.0 anthocyanin is in the form of an oxonium compound and at pH 4.5 is a colorless carbinol. This can be done by making an aliquot of anthocyanin solution in water whose pH is 1.0 and 4.5 for absorbance then measured.

2.10. Measurement and calculation of total anthocyanin concentration
The extract was weighed as much as 25 mg, dissolved in 5.0 ml of methanol which was added with 1% HCl. A total of 1.0 ml of extract solution was added with 5.0 ml in vial 1, added a buffer solution of KCl pH 1.0 and 2 vials added with sodium acetate pH 4.5 solution stir until dissolved. The solution is allowed to stand for 30 minutes - 1 hour (operating time). Each absorbance was measured at a maximum wavelength of 700 nm with a methanol solvent containing KCl and sodium acetate.

3. Results and discussion
Phytochemical screening is useful to determine the content of compounds contained in the ingredients, the results are influenced by the selection of solvents and the extraction methods used. The content of bioactive compounds in a plant experiences differences due to several factors, including environmental factors such as height of place, type of soil, climate and formation of secondary metabolites in plants which are influenced by temperature, pH, water activity and light intensity. Phytochemical screening was carried out namely identification of flavonoids, quinones and tannins.

Red rice extract is positive for flavonoids, where the results of this screening are in accordance with the research of [4], that rice has phytochemical components of flavonoids and phenolic acids. Flavonoid compounds in the sample showed that the sample contained anthocyanins. Anthocyanin levels in rice determine the intensity of dark colors and correlate with antioxidant activity. Flavonoids are one of the active compounds as tyrosinase inhibitors [5].

The extract was added with 1 mL of 10% Fe (III) chloride solution. If it forms dark blue, blackish blue or greenish black indicates the presence of polyphenol and tannin compounds. Testing of polyphenols / tannins carried out by adding 10% FeCl3 is expected to cause dark blue, black blue or greenish black. Discoloration does not occur with the addition of FeCl3 due to the absence of hydroxyl groups present in tannin compounds.

In phytochemical screening it is known that all positive samples containing flavonoids are characterized by the formation of orange, yellow or red in the amyl alcohol layer. In the quinone analysis also showed positive results where the change in the color of the sample which was initially faded red to solid red, and in the tannin analysis also showed a positive result where the resulting color was blackish blue.

The extraction method used in this study is maceration. The maceration method was chosen because the compounds contained in rice are anthocyanins that are not heat-resistant or thermostable, and the maceration method can extract large amounts of samples. In addition, the maceration method is an extraction method which is the easiest way to do it, but requires a large amount of solvent. In this study water solvents, 1% HCl water, ethanol, methanol and methanol HCl 1% were used due to the physical and chemical properties of anthocyanins seen from the solubility of anthocyanins in polar solvents such as methanol, acetone, or chloroform more often with water and acidified with hydrochloric acid or formic acid. Anthocyanin is stable at pH 3-5 and temperature of 50 °C, has a molecular weight of 207.08 gr / mol and the molecular formula C15H11O3. This maceration process is carried out at temperatures below 50oC to avoid anthocyanin damage in the sample. Water is a polar descent that is often used daily in life. However, when used to extract extracted brown rice does not extract the desired compound. this is because water is not a solvent commonly used to extract, and it is easy to decompose because water is a medium that is easy to grow by bacteria. seen from the results of the maximum lambda scanning, no anthocyanin wavelength was obtained so it was not followed by the determination of anthocyanin levels. Ethanol is a polar solvent that is often used to extract a compound or can be called a universal solvent. When used to extract anthocyanins in brown rice does
not get a wavelength of 515-545 nm, this can be caused by cyanidin-3-glucoside compounds can not be extracted in large quantities by using ethanol and the samples cannot be determined anthocyanin levels. Using different solvents also aims to determine which solvents the highest amount of anthocyanin can be withdrawn. In addition, it is known that anthocyanin is stable at acidic pH, therefore the addition of 1% HCl is used as a solvent. Figure 1 is color of solution from maceration extract.

**Figure 1**: Macerated brown rice extract

Determination of the maximum λ extract aims to determine the maximum absorbance of anthocyanins contained in the extract. Determination of the maximum λ extract was carried out by Uv-Vis spectrophotometry method with a wavelength of 400-800 nm. Figure 2 is the spectrum of maximum λ extract was carried out by Uv-Vis spectrophotometry method with a wavelength of 400-800 nm.

**Figure 2**: Sample Spectrum

Extraction using methanol solvent produces a wavelength in the range 515-545 nm, where the wavelength is the length of the cyanidin-3-glucoside. Anthocyanin seen from the appearance of red, red, purple and blue has a maximum wavelength of 515-545 nm. One of the factors that influence the color of anthocyanins is the change in pH. Acidic properties will cause anthocyanin color to become red, while alkaline properties cause anthocyanin to turn blue. In addition to changes in pH, pigment concentration, the presence of mixtures with other compounds, the number of hydroxy and methoxy groups also affect anthocyanin color [6].
From the data obtained it can be seen that samples added with 1% HCl at maceration have higher levels than those not added 1% HCl. This can happen because anthocyanins dissolve more easily in acidic conditions. At lower pH than 2, anthocyanins are cations (flavilium ions); but at a slightly acidic pH of vacuol cells, other quinoid forms are also present. Quinoid forms are rapidly oxidized by air and damaged. Therefore, anthocyanins are safest when done in a slightly acidic solution. Conditions that are getting more acidic near pH 1 will cause more anthocyanin pigments to be colored in the form of colored flavilium or oxonium cations and absorbance measurements will show greater numbers of anthocyanins [7].

The process of refining the sample also helps release anthocyanins that are bound to brown rice. Factors that influence the solubility of a solid in a liquid are particle size, where the smaller the particle size the greater the solubility of a material.

4. Conclusions
Based on the results of the research, it can be concluded that the sample of red rice in fine form with maceration extraction using 1% methanol-HCl as solvent has the highest anthocyanin content.

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