Anthocyanine pigment identification of north Sulawesi rice brand crude extracts, as potential natural antioxidant

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Anthocyanine pigment identification of north Sulawesi rice brand crude extracts, as potential natural antioxidant

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Abstract. Rice bran are by products of paddy to rice processing. Confirmed by many researches that rice bran contains various bio-active components, and specifically antioxidants where more than 100 variances of antioxidant. Aside of tocopherols and tocotrienols, oryzanols are active endemic compounds found in rice bran that functions as anti-inflammation even anti-cancer. Pigmented Paddy was reported to be a source of potential antioxidant because they contain high concentrations of polyphenols and anthocyanine that also has functions as anti-inflammation and also reduction of cholesterol in the blood. The purpose of this research was to identify active anthocyanine in crude extracts of Red Variety Rice Bran of North Sulawesi. Previous results have separated component fractions of the crude extracts of red variety rice bran of North Sulawesi based on their levels of polarity (buthanol, hexane and ethyl acetate). This separation process further continued by means of coloumn chromatography with a mobile phase of 1:1 hexane and acetone followed by a thin layer chromatography. Anthocyanine identification was determined by Liquid Chromatography / Mass Spectrometry - MS, where the ethyl acetate extract was confirmed as cyanidin-3-glucoside (C3G) with a molecular weight of 449,1367 kDa.

1. Introduction

Several areas in Indonesia, including North Sulawesi, are known to use rice bran only for livestock feed fillers, meanwhile observing the major components of rice bran it is apparent that rice bran has a strong potential to be utilized for other than just as filler ingredients in livestock feed, especially since it is well known that Indonesia has a large productivity concerning rice. A strong potential for the utilization of rice bran would be to develop edible oil as an alternative to unsaturated fatty acid containing edible oils (food oils). Oryzanol, an antioxidant unique to rice bran is known to a more powerful antioxidant than vitamin E in in-vitro oxidations of cholesterol [1].

The province of North Sulawesi has several sites of rice production center namely the Bolaang Mongondow Regency, Central Minahasa, South Minahasa, and Southeastern Minahasa that are actively producing several known varieties of rice (Superwin, Cigeulis, and the red rice). Rice production in North Sulawesi throughout 2015 reached 664.282 tons, while yielding about 10% rice bran, therefore the potential capacity of North Sulawesi's rice bran would range to 6.64 ton. It is most common that the use of rice bran to date has only been for animal feed (livestock feed). This research explores more on antioxidant potentials of rice bran from the varieties of rice cultivated in North

Sulawesi. The main expectation of this research is to place rice bran antioxidant as a natural alternative source which is applicable in food products as a source of unsaturated fatty acid containing edible oil.

2. Methods

The main object in this research is the red variety rice bran (Oryza glutinous) that is cultivated in Parepei village of Minahasa Regency - North Sulawesi.

2.1. Rice Bran Extraction

Rice bran extraction was carried out according to the method of [5] that was slightly modified. Red rice variety of rice bran flour samples at 5 kg each were macerated and extracted with 70% ethanol three times and kept overnight at room temperature. Maceration is carried out for 3 x 24 hours until all extracted ingredients are marked with changes in the color of the filtrate to be brighter. The filtrate obtained was then separated using a vacuum flask and filtered using Whatman No. filter paper. 40. The filtrate is then separated from the solvent by using a rotary vacuum evaporator at a temperature of 50^0C until all the solvents are evaporated and a crude extract of rice bran is obtained. The ethanol extracts were then fractionated with organic solvents (hexane, ethyl acetate and n-butanol) in accordance with their polarity level. Each extract was prefiltered with Whatman paper No. 42 and then evaporated by rotary evaporator (Buchi rotavapor) under vacuum to obtain the hexane, ethyl acetate and n-butanol crude extract of rice bran.

2.2. Rice Bran Anthocyanine Identification

Rice bran anthocyanine identification of the chosen variety utilizes Thermo *Liquid Chromatography/Mass Spectrometry-Mass Spectrometry* (LC/MS-MS) Orbitrap, using *cyanidin*-3-*glucoside (C3G)* as the standard.

3. Results and Discussion

The anthocyanine pigment identification that was carried out in The Laboratorium of Flavour Analysis, *Balai Besar Penelitian Padi*, Sukamandi, West Java. The sample utilized in this identification process were crude extracts of red variety rice bran which was separated to fractions of different polarity. The fraction used for anthocyanine identification was the fraction soluble in ethyl acetate, that was separated by chromatography with 60 F_{254} as the stationary phase and a mixture of hexane and acetone (1:1) was incorporated as the mobile phase. Pigment identification was then executed with *Liquid Chromatography / Mass Spectrometry* - MS.

Identification results indicated several spectrum peaks. Each spectrum read by the LC/MS-MS was compared to the standard compound. The spectrum peaks that was observed pictured molecular ions of [M+H]+ at M/Z 449,1367 at a retention time (RT) of 1,20, thus is expressed that the compound contained in the sample has a molecular weight of 449 kDa. Based on the C3G standard, the ethyl acetate extract of red variety rice bran also contains C3G, and along with C3G aglycons of anthocyanidine were also identified as cyanidines with [M+H]+ at M/Z 287,1220. Several other spectrums were present in the results (M/Z 305,0967, M/Z 511,1528, and M/Z 371,1794) but unfortunately due to the limitation of standards these peaks weren't identified.

Compounds corresponding to the spectrum peaks that were observed in the samples were the quantified by using C3G as standards in several levels of concentration. Standard solution exhibited a linier correlation between surface area (Y) with solution concentrations in different levels of concentrations (X) with a correlation coefficient of (R^2) = 0,99, and the curve was expressed in the model with the equation of $Y = 623{,}6X - 28740$ (Figure 1).

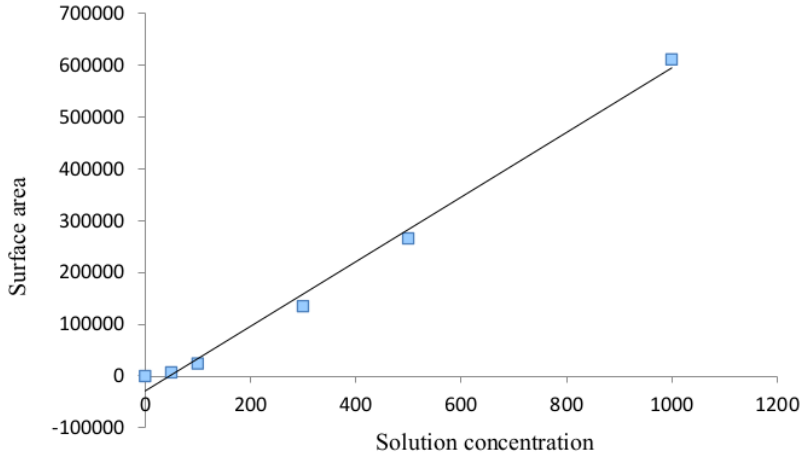

Figure 1. Correlation Coeficient of Surface Area and C3G concentrations

The following are the results of pigment quantification. Following are the quantification results of the colored pigments of anthocyanine from the ethyl acetate fraction of rice bran crude extract, with sample concentration of 48.01 ppb for sample 1 and 47.29 ppb for sample 2.

Table 1. Standard C3G Concentrations and Sample Concentrations

Sample	Concentrations (ppb)	Area
Standard C3G	0	0
Standard C3G	50	7245.11
Standard C3G	100	24293.70
Standard C3G	300	135134.88
Standard C3G	500	265807.74
Standard C3G	1000	611236.81
Sample 1	48.01	1203.36
Sample 2	47.29	754.62

Previous studies showed that C3G compounds plays the role as the major anthocyanine compound in rice that has black pigments in them, other anthocyanines have less concentrations, such as the malvidine-3-glucoside and peonidine-3-glucoside [9, 4], meanwhile anthocyanine compounds identified in a Korean variety of rice, another black pigmented variety and 3 other varieties of rice with red pigments are cyanidine-7-O-galactoside, cyanidine-3-O-glucoside, cyanidine-3'-O-glucoside, cyanidine-5-O-glucoside,cyanidine-3,7-O-diglucoside, cyanidine-3, 5-O-diglucoside and peonidine-4'-O-glucoside [2]. C3G has a strong positive effect on health that it plays the roles as antioxidants, anti-allergy, anti-inflammation and anti-cancer [6, 7, 8].

4. Conclusion

Results indicated 2 fractions of ethyl acetate of red variety rice bran using LC/MS-MS it was confirmed that pigments in the sample was cyanidin-3-glucoside with molecular weights of m/z 449,1367, and concentrations of 48.01 ppb for sample 1 and 47.29 ppb for sample 2.

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