Production of natural dyes from black rice bran extract on solid to solvent ratio and various of pH solvent

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Abstract. Black rice bran is a byproduct of black rice removal process. It has the potential to be a source of natural dyes. The objective of this study is to evaluate the effect of solid to solvent ratio (1:10, 1:15 and 1:20) and various pH solvent (3,4,5, and 6) on characteristics of natural dyes from black rice bran extract and determine the best treatment to produce natural dyes from black bran extract. Extraction process used maceration method at ambient temperature. The experiment was designed using factorial randomized block design, and if there was a treatment that had a significant effect, it was continued with a Duncan test. The result showed that the solid to solvent ratio, various pH solvent, and their interactions had a significant effect on yield, anthocyanin content, and colors (L*, a* and b*). The best treatment is solid to solvent 1:10 and the value of pH 3, with characteristic 9.45% yield, 596.94 mg/l anthocyanin, 810.56 mg GAE/100 g polyphenol, and color (L* value 51.53, a value 26.93, b value 42.13).

Keyword: natural dyes, black rice bran extract, anthocyanin, and color

1. Introduction
Dyes can be obtained from nature or made synthetically. Synthetic dyes are increasingly being applied to food and non-food products because they have a wide spectrum of colors, good stability, low price, are easy to obtain, and in small concentrations can produce the desired color. Excessive use of synthetic dyes can endanger the health of consumers [1]. Nowadays exploration of natural dyes (pigments) from various parts of plants is increasingly being done. The use of natural dyes is considered safer than the use of synthetic dyes. Some natural dyes that are widely used are green (chlorophyll), orange (carotenoids), yellow (xanthophyll), and red-purple (anthocyanin).

Anthocyanins are a group of phenolic compounds, that have a spectrum of red, purple, and blue colors and found in vacuoles in cells. Anthocyanins have glycoside bonds that are easily hydrolyzed, highly reactive, easily oxidized and reduced [2]. Anthocyanins are a group of naturally occurring phenolic compounds, which play an important role in the color quality of many flowers, fruits, vegetables and related products derived from them. Anthocyanin are well known as natural red dyes or natural red colorants [3], which make them important for the food industry [4]. Moreover, these natural
colorants are of interest due to their wide range of colors (i.e., deep red to deep blue), their high solubility in aqueous media, and their health benefits [5]. Research results from Mahmudatussa'adah et al. [6], anthocyanin extract in purple sweet potato varieties of Ayamurasaki at pH 1-14 had a spectrum of red, faded red, purple, blue, green, and yellow. Widarta and Arnata [7] showed that anthocyanin compounds in red rice bran could be extracted more with acidic conditions. Intensity and type of the color of anthocyanins are affected by the number of hydroxyl and methoxyl groups: if there are more hydroxyl groups, the color goes toward a more bluish shade; if there are more methoxyl groups, then redness is increased [8]. The fact that acidified extraction solvent has an important influence on anthocyanin extraction has also been observed in berries by Byamukama et al. [9]. Total levels of anthocyanin and antioxidant activity extracted with acidified solvents have a very good linear correlation. The higher the levels of anthocyanin, the higher the antioxidant activity will be [10]. Ethanol and organic acids are desirable because they are less toxic than methanol and hydrochloric acid [11].

One of the natural dyes from the byproducts of black rice process is black rice bran. This product has a red-purple color. Rice bran is the outermost layer of rice that is released during the process of polishing rice. Black rice bran has the greatest amount of anthocyanin when compared to white and red rice bran [12]. Phytochemical compounds contained in black rice bran are anthocyanins, antioxidants (-oryzanol), and phenolic compounds.[13]; [14]; [15]. The extraction process can be carried out to obtain anthocyanin contained in black rice bran. Anthocyanin is a polar pigment compound that will dissolve very well in polar solvents [16]. Widarta and Nocianitri [17], stated that the total levels of anthocyanin produced by black rice bran were 33.10 mg / 100 g of rice bran extracted with 96% ethanol solvent.

Tabanan Regency, is the largest producer of black rice in Bali. There are two kind of black rice in Bali, namely black rice and black glutinous rice. The research on the extraction of black rice bran as a source of natural dyes in Bali has never been done. Therefore, this study was conducted with the aim of determining the effect of solid-solvent ratio and various pH on the characteristics of black rice bran dye extracts and determining the best treatment (solid-solvent ratio and pH value) to produce black rice bran dye extracts.

2. Method

2.1. Sample preparation
Black rice bran from rice scrubbing in Tabanan was dried at 60°C ± 2°C until the water content reaches around 12%. Then crushed and sieved with 60 mesh sieve until it becomes powder bran. The bran that has passed 60 mesh sieve was then weighed as much as 20 grams for each treatment combination.

2.2. Research design
This study used a randomized block design (RCBD) of 2 factors. The first factor was the solid to solvent ratio consisting of 3 levels (A1 = 1:10, A2 = 1:15, and A3 = 1:20) and the second factor was the initial pH of the solvent consisting of 4 levels (B1 = 3, B2 = 4, B3 = 5, and B4 = 6) This experiment received 12 treatment combinations and grouped into 3 groups so that 36 unit experiments were obtained.

2.3. Sample extraction
The extraction process used maceration method with acidified ethanol solvent. Powder bran weighed at 20 grams each, and put in glass jars. Prepared 80% ethanol solvent with acidity according to treatment (pH 3, 4, 5, and 6). Acid control was done with citric acid. Powder bran plus acidified ethanol solvent according to the ratio of solid-solvents (1:10, 1:15, and 1:20). The glass jar was then tightly closed to avoid solvent evaporation.

Maceration process was carried out for 48 hours at room temperature (27-30°C). The mixture was then filtered and the filtrate obtained was evaporated until all the solvents have evaporated. The viscous extract that was attached was resuspended using ethanol pro analysis. The extract was transferred into a petri dish to air-dry so that the solvent completely evaporates.
2.4. Yield measurement

The yield is the quotient of the weight of the product produced divided by the weight of the powder of black rice bran multiplied by 100% [18]. The yield obtained is calculated by the formula:

\[
\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Powder weight (g)}} \times 100\%
\]  

(1)

2.5. Total anthocyanin content

Determination of total anthocyanin levels by using the pH differential method was conducted [19]. The principle of total anthocyanin analysis with a differential pH method was based on changes in the structure of anthocyanin (reversible) with changing pH. The crude extract sample was dissolved in two different buffer solutions. As much as 1 ml of extract was put into a 10 ml volumetric flask, then diluted using a solution of potassium chloride (KCL) pH 1 until the volume became 10 ml. A total of 1 ml of extract results were put into another measuring flask and sodium acetate (CH₃COO-Na) buffer solution pH 4.5 to the pitch mark, then left for 15 minutes then each was read at 510 nm and 700 nm in a UV-Visible spectrophotometer.

The wavelength of 510 nm is the maximum wavelength for cyanidin-3-glycosides, while the wavelength of 700 nm is to correct deposits that are still present in the sample. If the sample is really clear then the absorbance at a wavelength of 700 nm is 0. Samples that still contain sediment will cause light scattering so that the absorbance reading becomes biased. Absorbance is calculated using equation 2, to calculate anthocyanin levels calculated by equation 3. Anthocyanin structure in nature that is not entirely known and its complex structure causes the cyanidin-3-glycoside coefficient to be used as a standard coefficient in the anthocyanin total analysis equation due to the fact that this anthocyanin type is the most dominant in rice.

\[
A = (\lambda_{510} - \lambda_{700}) \text{pH 1} - (\lambda_{510} - \lambda_{700}) \text{pH 4,5}
\]  

(2)

Total Anthocyanin (mg/L) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times L}

(3)

Where:

- A : The absorbance value of the sample
- \(\varepsilon\) : Absorbance of molar Cyanidin-3 glycoside (26900 mol / cm)
- L : Wide cuvette (1cm)
- MW : Molecular weight of Cyanidin-3-glycosides (449.2 g / mol)
- DF : Diluent Factor

2.6. Phenolic content

Total phenolic content was analyzed using the Folin-Ciocalteau method [20] with a slight modification. 1 g of sample was dissolved into 10 ml of 95% ethanol, then filtered using filter paper and the filtrate was taken. 0.5 ml of extract was added with 0.5 ml of 95% ethanol and 2.5 ml of distilled water was homogeneous. Add 2.5 ml of Folin - Ciocalteau reagent, then homogenized. Stand the solution for 5 minutes, then add 0.5 ml of 5% Na2CO3 solution, then homogenized. Incubate the solution for 1 hour, measure the absorbance with a spectrophotometer at a wavelength of 517 nm. Sample data were compared with standard curves with gallic acid. Results are expressed in mg/g equivalent to gallic acid.

2.7. Color measurement

Color test using the ACCUPROBE Colormeter New York, USA. In the test using Colormeter, there are 3 values namely L, a, and b where L has an interval value between 0 - 100 for the color of brightness, ‘a’ has an interval value for green to red and b interval value for blue to yellow [21].
3. Results and discussion

3.1. Yield of extract
The results showed that solid to solvent ratio, variety of pH, and interaction between the two treatments had a very significant effect (p < 0.01) on the yield of black rice bran extract. The averages of yield of black rice bran extract is presented in Table 1. The highest yield was obtained from solid-solvent ratio 1:15 and pH 3 which was not different from the combination of treatments 1:10 and pH 3. The lowest yield was obtained from solid-solvent ratio 1:20 at the initial pH 6.

**Table 1.** Averages yield of black rice bran extract (%) on the variation of solid-solvent ratio and pH.

| Solid-solvent ratio | pH value | 3           | 4           | 5           | 6           |
|---------------------|----------|-------------|-------------|-------------|-------------|
| 1:10                |          | 9.45±0.29ab | 8.52±0.27cd | 8.34±0.36cd | 8.21±0.44cd |
| 1:15                |          | 9.71±0.21a  | 9.90±0.27abc| 8.63±0.21bc | 8.31±0.06cd |
| 1:20                |          | 8.53±0.31cd | 7.71±0.17d  | 6.71±0.23e  | 5.63±0.46f  |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results were ranked in ascending order a > b > c.

The results of this study were supported by DanGuo et al. (2012) which stated that the optimization of the extraction of anthocyanins from the fruit skin of Rhodomyrtus tomentosa (Ait.) Hassk var. Gangren, using 60% ethanol containing 0.1% (v/v) hydrochloric acid as solvent was solid-liquid ratio 1:15:7. This is in line with research conducted by Amalia et al. [22] that ethanol solvents acidified with 3% citric acid had the highest yield in buni fruit extracts.

3.2 Total anthocyanin
The results showed that solid-solvent ratio, pH, and the interaction of the two treatments had a very significant effect (p < 0.01) on the total anthocyanin of black rice bran extract. The averages of total anthocyanin values of black rice bran extract are presented in Table 2. The highest anthocyanin was obtained from a combination solid-solvent ratio 1:10 and pH 3 which was not different from the combination solid-solvent ratio 1:15 and pH 3. The lowest anthocyanin was obtained from the treatment combination the solid-solvent ratio of 1:20 at pH 6. Fatihinatullahibah et al. [23] stated that the treatment that best maintained the stability of anthocyanin pigment in teak leaf extract was the pH 3 treatment.

**Table 2.** The averages total anthocyanin (ppm) of black rice bran extract on the variation of solid-solvent ratio and pH.

| Solid-solvent ratio | pH value | 3           | 4           | 5           | 6           |
|---------------------|----------|-------------|-------------|-------------|-------------|
| 1:10                |          | 596.94±23.19a | 499.85±3.59b | 143.64±24.04e | 131.08±3.66ef |
| 1:15                |          | 578.23±3.25ab | 506.95±43.83b | 154.53±35.29e | 249.07±23.95d |
| 1:20                |          | 381.47±13.25c | 316.97±70.38cd | 259.76±27.08d | 52.79±3.33f  |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results were ranked in ascending order a > b > c.

The right amount of solvent and low pH conditions cause the extracted anthocyanin compound to be stable. According to Tananuwong and Tewaruth [24], the level of acidity of the solvent gave higher results to the total levels of anthocyanin monomers, because anthocyanins tend to be more stable under acidic conditions. According to the study of Moldovan et al. [25], the acidity of the extraction process greatly influences the stability of anthocyanin. This form of stability is related to the chemical structure of anthocyanin at pH 1-2 which is dominated by the form of the flavilium cation. Anthocyanins in the structure of flavilium kation at low pH is the most stable form of anthocyanin compounds. At pH 3 the structure of the flavilium cation will change to the structure of carbinol. In this phase the flavilium...
cation has been partially transformed into a colorless carbinol structure so that the red color fades. Research conducted by Joshi and Devi [26] stated that the extraction of Santa Rosa plums using 50% ethanol and 0.2% citric acid with a solid-solvent ratio of 1:10 produces the maximum total anthocyanin and the best sensory value. The use of large amounts of solvents will increase the evaporation time resulting in damage to anthocyanin compounds.

3.3. Total polyphenols
The results showed that the treatment of the solid-solvent ratio, pH, and the interaction of the two treatments had a very significant effect (p < 0.01) on the total polyphenols of black rice bran extract. The averages of total phenolic values of black rice bran extract are presented in Table 3. The highest total polyphenols were obtained from solid-solvent ratio 1:15 and pH 3 which were not different from the solid-solvent ratio 1:10 and pH 3 and 1:10 with pH 4. The anthocyanin content is positively correlated with the total phenol content. The higher the anthocyanin content, the higher the total phenol content. In this research, the highest total anthocyanin and total phenol were found in the solid-solvent ratio 1:10 and pH 3.

Table 3. Averages total polyphenols (mg GAE / 100 g) of black rice bran extract on a variation of solid-solvent ratio and pH.

| Solid-solvent ratio | pH value | 3         | 4         | 5         | 6         |
|--------------------|----------|-----------|-----------|-----------|-----------|
| 1: 10              |          | 810.57 ± 9.10 a | 762.29 ± 35.49 ab | 739.87 ± 12.36 b | 508.64 ± 6.93 d |
| 1: 15              |          | 813.20 ± 4.12a | 602.29 ± 22.71 c | 603.36 ± 23.03 c | 569.52 ± 5.28 cd |
| 1: 20              |          | 713.99 ± 39.37 b | 615.65 ± 12.27 c | 556.24 ± 24.81 cd | 504.29 ± 38.51 d |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results are ranked in ascending order a > b > c

The results of this study were in accordance with research conducted by Guldiken et al. [27]. The extraction conditions that produce the optimum total phenol and anthocyanin in the black carrot are extraction temperatures of 50 °C, pH 3.5, solid-solvent ratio 1:10 (w / v), and ethanol / water ratio 75:25 (v / v). Predescu et al. (2016) also stated that maceration and ultrasound-assisted extraction methods led to the highest concentrations of phenolics and flavonoids, and the solid-to-solvent ratio of 1/10 (w: v) was the most effective.

3.4. Color measurement
There are 3 values used in the analysis, namely the values of L, a and b. The “L” value has an interval value of 0-100 which represents the brightness level. The more positive (+) “L” value, the brighter the color of the sample. The value of “a” is the value to indicate the interval of green to red. A positive (+) “a” value of the sample color will be redder, if the value of “a” is negative (-) then the color of the sample will be greener. Value “b” is the value that indicates the interval of blue to yellow. A positive “b” value (+) in the color of the sample will be more yellow and if the value of “b” is negative (-), the color of the sample will be more blue.

3.4.1. Brightness (L)
The results showed that the treatment of solid-solvent ratio, pH, and interaction between the two treatments had a very significant effect (p < 0.01) on the brightness of black rice bran extract. The averages value of the brightness of black rice bran extract is presented in Table 4. The highest brightness was obtained from a combination of the comparison of ingredients with solvent 1:20 and pH 6 and the lowest in the combination of 1:10 and pH 3.
Table 4. Averages L value of black rice bran extract on a variation of solid-solvent ratio and pH.

| Solid: solvent ratio | pH value | 3 | 4 | 5 | 6 |
|----------------------|----------|---|---|---|---|
| 1:10                 |          |   |   |   |   |
| 1:15                 |          |   |   |   |   |
| 1:20                 |          |   |   |   |   |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results are ranked in ascending order a > b > c

According to Jackman and Smith [28], an increase in the value of L * indicates the degradation of anthocyanin compounds due to a change from flavilium to chalcone form. Dark colors at the solid-solvent ratio 1:10 and pH 3 change to be brighter at a greater solid-solvent ratio and a higher pH. This degradation process was caused by several factors such as pH, temperature, sun exposure and oxygen. In the solid-solvents 1:20 and pH 6 was thought to be the equilibrium of the flavilium cation which turns into chalcone. The dark color became bright or less colourful [29]; [30]; and [31].

3.4.2. Reddish (a)
The results showed that solid-solvent ratio, pH, and the interaction between the two treatments had a very significant effect (p <0.01) on the reddish of black rice bran extract. The averages reddish of black rice bran extract is presented in Table 5. The highest reddishness was obtained from the solid-solvent ratio 1:10 and pH 6. The results showed that the highest reddishness was from the combination of treatments 1:20 and pH 6 (52787 ppm) and the lowest in the 1:20 solid-solvent ratio treatment and a pH 6 (52785 ppm).

Table 5. Averages a value of black rice bran extract on a variation of solid-solvent ratio and pH.

| Solid: solvent ratio | pH value | 3 | 4 | 5 | 6 |
|----------------------|----------|---|---|---|---|
| 1:10                 |          |   |   |   |   |
| 1:15                 |          |   |   |   |   |
| 1:20                 |          |   |   |   |   |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results are ranked in ascending order a > b > c

Color change in anthocyanin pigments due to changes in pH and the structure. Anthocyanin at pH 1-3 the dominant color was red due to the structure of anthocyanin-formed cation flavilium. At the higher pH value, flavilium cation changes to pseudobasa hemiketar carbinal, quinonoidal, and chalcon [29]; [32]; and [33]. The color anthocyanin becomes purple and blue at pH 4-7 because the structure of anthocyanin is in the form of carbinal, and some of it becomes quinoidal so it turns purple, and at pH >9 anthocyanin is in the chalcone structure which has a yellow spectrum [6] and [29]. The minus value on the redness measurement indicated that the extract color in the color spectrum shifts towards greenish color.

3.4.3. Yellowish (b)
The results showed that the solid-solvent ratio, pH, and the interaction between two treatments had a very significant effect (p <0.01) on the yellowish of black rice bran extract. The averages value of yellowish black rice bran extract is presented in Table 6. The highest yellowness is obtained from a combination of treatments ratio of materials with solvent 1:10 and pH 3 and the lowest is at a combination of treatments 1:15 and 1:20 at pH 6. Color degradation occurs from yellow to brownish yellow. The brownish yellow color may be caused by a heating process that is long enough to evaporate.
the extract at a large solid-solvent ratio.

**Table 6.** Averages b value of black rice bran extract on a variation of solid-solvent ratio and pH.

| Solid-solvent ratio | pH value | 3     | 4     | 5     | 6     |
|---------------------|----------|-------|-------|-------|-------|
| 1:10                |          | 42.63±0.09 a | 38.12±0.05 d | 36.50±0.03 g | 35.70±0.03 hi |
| 1:15                |          | 41.70±0.06 b | 37.60±0.03 e | 35.93±0.03 h | 32.13±0.05 j  |
| 1:20                |          | 38.79±0.01 c | 36.96±0.04 f | 33.31±0.04 i | 32.25 ± 0.01 j |

Note: Values are averages ± standard deviation of triplicate analysis; different letters in same column and row indicated significant difference (p < 0.05). Results are ranked in ascending order a > b > c.

In the research of Joshi and Devi [26] the highest value of b was 20 in plum extract using ethanol and citric acid 0.1% with a solid-solvent ratio of 1:8. This treatment contains a total of anthocyanin 180.00 mg/100mL. The highest value of b in this study was 42,633 having anthocyanin content of 596,944 ppm.

4. Conclusion
Solid-solvent ratio and pH of solvent greatly affect the yield, total anthocyanin, total polyphenol, and color of black rice bran extract. The higher the solid-solvent ratio and the higher the pH, the yield, total anthocyanin, total phenol. Colors a and b decrease, while the brightness increases. The highest yield, total anthocyanin, total polyphenol, reddish, yellowish and the lowest of brightness of black rice bran extract are on treatment solid:solvent ratio 1:10 and initial pH 3. Its characteristics are 9.45% of yield, 596.94 ppm of total anthocyanin, 810.57 mg GAE/100g of total polyphenol, and characteristic of color was 51.53 of brightness (L), 26.93 of reddish (a), and 42.63 of yellowish (b).

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