Antioxidant and Nutritional Analysis of Organic Black Rice (Oryza sativa L.) Milk

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Abstract. The consumption trend of the plant-based product has increased over the last few years, from health reasons to the unsustainability of consuming animal product. Plant-based product has been perceived by consumers as products to improve human health and well-being. Plant-based milk substitute such as rice milk is an example of the product and could be made from various rice varieties. Black rice (Oryza sativa L.) is one type of rice that is cultivated in Indonesia. Black rice has been reported to poses antioxidant activity, which is beneficial for health. For this reason, black rice has the potency to be further processed into a functional drink. However, the information regarding the antioxidant and nutritional content in the processed form is still lacking. This research aimed to analyze the antioxidant activity and nutritional content of organic black rice milk. Proximate analysis and antioxidant content (DPPH and total phenol) were conducted in this experiment. The results showed that black rice milk contained 92.36% water, 0.67% fat, 0.14% protein, 0.15% ash, and 6.69% carbohydrate. Black rice milk can inhibit 69.18% activity of 0.2 mmol DPPH radicals. It also contained total phenolic content of 32.75 mg GAE/100 mL.

1. Introduction
In the recent decade, there has been a renewed interest in an alternative new product. Consumers are shifting food choices from animal-based to plant-based, which is increasing, especially for healthier products [1]. Plant-based milk is an alternative product that gained renewed interest, and the market for this product is gradually expanding [2]. It has been led by consumers' awareness about health concerns related to cow milk consumption, such as lactose intolerance and allergy, negative perception of saturated fat content in cow milk, and sustainability issues [2,3]. For this reason, the demand for plant-based milk is projected to grow exponentially in the future, and the expected market value that reach more than 40 billion USD [4]. In the past, soybean-based milk is the most consumed of plant-based milk, as its nutritional content, such as protein, is the closest to cow milk. However, in recent years, the development of plant-based milk from other sources such as cereals, nuts, seeds, and pseudocereals has gained more attention [5].

Rice (Oryza sativa L.) is a strategic main food crop in Indonesia in the context of rice supply as a staple food. According to FAO, Indonesia is the third-largest rice producer globally, with 54 million tons annually [6]. Rice is considered the second most important crop for providing carbohydrates, proteins, vitamins, and minerals [7]. Rice is also preferred to be the main material for making rice milk due to its hypoallergenic form, compared to soybean milk. The development of rice milk has been
conducted by several researchers [7,8]. Different types of rice also can be used as material for making rice milk. Black rice (or purple rice), one of the rice varieties in Indonesia, is currently gaining popularity due to its health-beneficial effect. Anthocyanin is one of the bioactive compounds responsible for its color and other therapeutic effects such as antioxidant, anti-inflammatory and anticancer [9]. It is also reported to contain more nutrients content than white rice [10]. In terms of consumption, black rice is out of favor by people compared to other rice varieties such as red and white rice. Despite the strength and benefit of black rice, the development or utilization of black rice needs to be further expanded into a new product such as rice milk. To date, there is only a limited report of black rice development as a food product, especially about black rice milk development and its properties in Indonesia [9]. This research aimed to analyze the nutritional content and antioxidant activities of black rice milk.

2. Materials and Methods

2.1. Materials

Organic black rice var. Padi Hitam Mutiara (Mama Kamu, Karanganyar) was obtained from online marketplace. The chemicals used for analysis of nutrient content are technical grades (Merck KGaA, Darmstadt, Germany). For antioxidant determination, Folin-Ciocalteu reagent for total phenolic content analysis was purchased from Merck (Merck KGaA, Darmstadt, Germany), and DPPH (TCI Chemicals, Tokyo, Japan) was bought from Indogen Indonesia.

2.2. Black rice milk preparation

The preparation of black milk followed the method for rice milk production [11]. At first, about 100 grams of black rice were washed and soaked into water for 3 hours. Afterward, the rice was put into a blender and mixed with drink water at 80 °C to the proportion of 3:10. Then, it was blended for around 2 minutes to make a rice slurry. The slurry was then filtered with the sterile filtered cloth to separate the liquid. Then, the liquid was boiled at 100 °C for 10 minutes, put into the sterile glass container, cooled at room temperature, and then stored at 4 °C, prior to use for analysis.

2.3. Moisture content

The analysis of moisture content was done analysis was conducted according to AOAC method [12]. The empty container was put into oven at 105 °C for 3 hours and cooled into the desiccator. Then, approximately 10 grams of the sample beverages was put into container and then dried in oven at 105 °C. Then, the sample was transferred into desiccator until the weight is stable.

2.4. Ash content

The ash content of black rice milk was analyzed following the AOAC method [12]. For determination of ash content of high moisture product, the sample need to be dried out first. First, around 3 gram of sample was put inside porcelain cup and then dried out. After dried out, the sample was put into the high temperature furnace with temperature between 500–550 °C for 5 hours.

2.5. Protein content

The protein content of black rice milk was analyzed using modified Kjeldahl method [12]. Approximately 10 grams of sample beverages were put into a Kjeldahl flask. Hydrolyzed with concentrated sulfuric acid (H₂SO₄) with ratio of 1:10 to the total of the sample residue weight, with the addition of 1 gram of copper (II) sulphate anhydrate (CuSO₄) and boiled at ±420 °C for 2 to 3 hours. After cooling, add distilled water to the samples before neutralization and titration procedure.

2.6. Fat content

The fat content of black rice milk was analyzed following AOAC method mention [12] with slight modification. Approximately 10 grams of sample beverages were put into a petri-dish with filter paper on it. The samples were dried in 105 °C oven for 2 hours. Afterwards, the filter paper was wrapped with
another new filter paper. The wrapped sample then placed into Soxhlet apparatus, then undergo extraction procedure for 4 hours.

![Diagram of black rice milk production](image)

**Figure 1.** Flowchart of black rice milk production

2.7. **Carbohydrate content**
The carbohydrate content of black rice milk was analyzed by using the carbohydrate by difference method [9].

2.8. **2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay**
The DPPH scavenging activity of black rice milk was analyzed according to [13]. Around 1 mL of sample is added to the mixture of 7 mL of methanol pro analysis and 2 mL of DPPH 0.1 M solution. The mixture is kept at room temperature in dark condition for 30 min. Then, aliquot mixture was put into cuvette, the absorbance was measured using UV-VIS spectrophotometer at 520 nm.

2.9. **Total phenol assay**
Total phenol analysis was performed according to AOAC SMPR 2015.009 for estimation of total phenolic content using the Folin-Ciocalteu Assay [14]. Gallic acid was used as the standard. The sample was measured using UV-VIS spectrophotometer at the absorbance of 765 nm.
3. Results and Discussion

3.1. Nutrient composition of black rice milk

Table 1 presents the result of nutrient components analysis in black rice milk. Black rice milk contained 92.36% of moisture, 0.15% of ash, 0.67% of fat, 0.13% of protein, and 6.69% of carbohydrate. The nutrient content of black rice milk is low compared to the cow milk in the past research [21], especially in protein (4.09%), fat (3.50%), and ash (1.05%) content [15]. Exception to carbohydrate content, where the carbohydrate value in black rice milk (6.69%) is higher than cow milk (4.78%) [16]. The findings in this research are in accordance with the previous findings which said that plant-based milk generally has a lower protein, and fat content [16].

To achieve similar nutritional composition and sensory profile like cow milk, fortification with added ingredients usually added such as addition of sugars and starch [17], protein-containing plants [18], plant-based oils [19], vitamins, and minerals [20]. The black rice milk contained low number of calorie due to low number in fat, protein, and carbohydrate. Generally, high number of calorie content of plant-based milk is usually attributed to the addition of sugar and other carbohydrates ingredients [17]. In this research, black rice milk was produced without the addition of sugar and other carbohydrates-based, therefore, can be considered as a reduced calorie beverage. According to Food and Drugs Administration [26], a food that considered as a reduce or less calorie if provide 25% fewer calories than the reference product (in this case, cow milk).

| Composition | Black Rice Milk | Cow Milk [21] |
|-------------|-----------------|---------------|
|             | Wet Bases (%)   | Dry Bases (%) | Wet Bases (%) | Dry Bases (%) |
| Moisture    | 92.36           | -             | 87.35         | -             |
| Ash         | 0.15            | 1.96          | 1.05          | 8.30          |
| Fat         | 0.67            | 8.77          | 3.50          | 27.67         |
| Protein     | 0.13            | 1.70          | 4.09          | 32.33         |
| Carbohydrate| 6.69            | 87.57         | 4.01          | 31.70         |
| Energy (kcal)| 33.31           | -             | 63.9          | -             |

3.2. Antioxidant activity of black rice milk

Table 2 presents the antioxidant activity and total phenol content of black rice milk. The antioxidant activity of black rice milk was evaluated using DPPH assay. The result showed that black rice milk contained inhibitory activity of DPPH for 69.18%. The inhibitory activity of black rice milk is mainly attributed to the bioactive compound such as anthocyanins. Anthocyanin is a water-soluble pigment that contributed to the color and antioxidant activity of black rice [22]. The solubility in water also makes the black rice milk contained antioxidant activity. The total phenol content of black rice milk was 32.75 mg GAE/100 mL. Besides anthocyanins, another phenolic compound such as ferulic acid has also been reported to exert antioxidant activity in black rice. Probably, this compound also contributed to the antioxidant activity in black rice milk. Compared to another plant-based milk, the total phenolic content of black rice milk is lower than soymilk (61.4 mg/100 mL) [23], but higher than hazelnut milk (5.15 mg/100 mL) [24], and coconut milk (8.21 mg/100 mL) [25].
Table 2. Antioxidants of Black Rice Milk & Other Plant-Based Milk

| Analyses                        | Black Rice Milk | Soy milk [23] | Hazelnut milk [24] | Coconut milk [25] |
|---------------------------------|-----------------|---------------|--------------------|-------------------|
| DPPH (% inhibition)             | 69.18 ± 3.62    | 17.00 ± 0.00  | -                  | -                 |
| Total phenolic content (mg GAE/100 mL) | 32.75 ± 6.80  | 61.40 ± 1.20  | 5.15 ± 5.46        | 8.21 ± 0.13       |

4. Conclusion
Black rice milk is cereal-based milk that has the potency to substitute cow milk. Black rice milk has a low-calorie content due to a low amount of carbohydrate, fat, and protein. Black rice milk showed some inhibitory activity to DPPH and contained phenolic compounds. The results showed that the nutritional value is quite low compared to cow milk, in terms of protein content. Therefore, further research should focus on the formulation of black rice milk to improve its nutritional content.

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