Proximate Analysis and Antioxidant Activity of Red Rice (Oryza sativa L.) Milk

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Abstract. Plant-based milk has become a consumer interest in recent years due to the health-beneficial effect of the product. Red rice milk is one of the cereal-based milk that uses red rice (Oryza sativa L.) as the main ingredient that contains antioxidant activity. The utilization of red rice in Indonesia is very limited, only for replacing white rice. The potency of red rice to be developed as rice milk has been poorly studied about the antioxidant activity and proximate content in the red rice milk. In this research, red rice was used as the main ingredient. Proximate analysis and antioxidant analysis (DPPH and total phenolic content) were conducted on the red rice milk. This research aims to analyze the nutrients content in red rice milk and its antioxidant activity. Red rice milk contained 98.01% of water, 0.07% of ash, 0.13% of protein, 0.71% of fat, and 1.07% of carbohydrate. Red rice milk inhibited 53.37% of DPPH radical and contained total phenolic about 274.5 ppm. The result revealed the potential of red rice milk as a functional drink with antioxidants.

1. Introduction

In the past couple of years, people have notice and become more interested in products that contain a lot of beneficial health effects. Some people are searching for alternatives products that contain a similar appearance and consistency to cow’s milk. Therefore, plant-based milk products have become popular in recent years, such as soy milk and almond milk. Plant-based milk is the preferred choice among consumers who have a health reason and healthy food practice such as veganism [1]. The nutrient contents of cow’s milk, such as lactose and protein in milk, cannot be absorbed and digested by people who suffer lactose intolerance and allergy [2]. It has been proved that plant-based milk contains essential nutrients and many bioactive compounds proven to be advantageous for human health [3]. Soy milk and almond milk both are the most common commercially produced plant-based milk. The popularity of soy milk in Indonesia is due to the familiarity with soybean in Asian countries and the utilization of soybean in Indonesian cuisines such as tempeh and tofu [4]. However, in almond milk, the product is less prevalent in Indonesia because almond is expensive and imported from other countries. Another cereal-based milk that has the potential to be developed and commercialized is rice milk.

Rice (Oryza sativa L.) has become a staple food for Indonesian centuries ago as a source of carbohydrates. It was proved that rice poses pharmacological activities such as antioxidant and anti-inflammatory [5]. Several beneficial compounds which proven to contain biological activity are total...
phenolic compounds (TPC), flavonoids, γ-aminobutyric acid (GABA), α-tocopherol, and γ-tocopherol [6]. There are several rice varieties in Indonesia, including pigmented rice such as red rice. It has been reported that red rice contains higher anthocyanins and proanthocyanidins. Thus, they exhibit higher antioxidant activity compared to non-pigmented, such as white rice [7].

In this current point of view, red rice is only used as a replacement for white rice, and it is usually preferred for diabetic people. It poses a significant potential to be developed as a new food product. Therefore, research on red rice should be encouraged. To the best of our knowledge, so far in Indonesia, the development of rice milk is very limited. The only commercial product that uses rice as an ingredient for making the rice-based beverage, namely “beras kencur”, a traditional herbal drink made from a mixture of white rice, ginger (Zingiber officinale Roscoe), and aromatic ginger (Kaempferia galanga L.). There is no commercial rice-based milk product in Indonesia and no study for the nutritional content and antioxidant activity of red rice milk. Therefore, this research aimed to analyze the nutrients content in red rice milk and its antioxidant activity.

2. Materials and methods

2.1 Materials
Red rice was obtained from Bandar Organic Rice (Jakarta, Indonesia). The chemicals used in the analyses were purchase from Merck (Merck KGaA, Darmstadt, Germany). Folin-Ciocalteu reagent was purchased from Merck (Merck KGaA, Darmstadt, Germany), and DPPH (TCI Chemicals, Tokyo, Japan) was purchased from Indogen Indonesia.

2.2 Preparation of red rice milk
At first, about 100 grams of red rice was washed with clean tap water. Then, the rice was soaked into water for 3 hours. Afterward, the rice was put into a blender and mixed with hot water at 80 °C to the proportion of 3:10. Then, it was blended for around 2 minutes into rice slurry. The slurry was then filtered with the sterile filtered cloth to obtain the liquid. Then, the liquid was boiled at 100 °C for 10 minutes. Then, the boiled liquid was put into the sterile glass container, cooled at room temperature, and then stored at 4 °C, prior to use for analysis.

2.3 Water content analysis
The water content analysis was conducted according to AOAC method [8]. 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 to cool. Then water content was calculated following the equation:

\[
\text{Moisture content (\%)} = \frac{(W_1 - W_2) \times 100}{W_2},
\]

where: \(W_1 = \) sample before drying (g); \(W_2 = \) sample after drying (g)

2.4 Ash content analysis
The ash content of red rice milk was analyzed according to AOAC 923.03 [8]. 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 using burner. After dried out, the sample was put into the high temperature furnace with temperature between 500 °C to 550 °C for 5 hours. The ash content can be determined by this equation:

\[
\text{Ash content (\%)} = \frac{\text{Ash Weight/Sample Weight}}{\times 100}
\]
2.5 Protein content analysis
The protein content of red rice milk was analyzed using modified Kjeldahl method according to AOAC 981.10 [8]. Approximately 10 grams of the sample beverages was dried in oven at 105 °C overnight in Kjeldahl flask. Hydrolyzed with concentrated sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) with ratio of 1:10 to the total of the sample residue weight, with the addition of 1 gram of copper (II) sulphate anhydrate (CuSO\textsubscript{4}) and boiled at ±420 °C for 2 to 3 hours. After cooling, add distilled water to the samples before neutralization and titration procedure. Protein content can be determined following the equation:

\[
\text{Protein content (\%) = \left( \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times 14.007 \times 6.25 \times 100\%}{\text{Sample Weight} \times 1000} \right)}
\]

2.6 Fat content analysis
The fat content of red rice milk was analyzed using modified crude fat analysis AOAC 945.16 [8]. 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. Wrap the filter paper with another new filter paper and tied. The sample then placed into Soxhlet apparatus, then undergo extraction procedure for 4 hours. Fat content was determined following the equation:

\[
\text{Fat (\%) = \left( \frac{\text{Fat content}}{\text{Sample Weight}} \right) \times 100}
\]

2.7 Carbohydrate content analysis
The carbohydrate content of red rice milk was analyzed by using the carbohydrate by difference method [9]. The carbohydrate content was determined following the equation:

\[
\text{Carbohydrate (\%) = \left( 100 - (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash}) \% \right)}
\]

2.8 DPPH scavenging activity
The DPPH scavenging activity of red rice milk was analyzed according to [10]. Around 1 mL of sample is added to 7 mL of methanol p.a. 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. DPPH scavenging activity was calculated using the following equation:

\[
\text{DPPH-scavenging effect (\%) = \left( \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \right) \times 100\%}
\]

where: Acont is the absorbance of control reaction and Atest is the absorbance of the samples

2.9 Total phenolic content analysis
Total phenol analysis was performed according to AOAC SMPR 2015.009 for estimation of Total Phenolic Content Using the Folin-Ciocalteu Assay [11]. Gallic acid was used as the standard. The sample was measured using UV-VIS spectrophotometer at the absorbance of 765 nm.

\[
\text{Total Phenols = \left( \frac{(A - b)/m}{Df} \right)}
\]

where: A the absorbance of the sample test solution at 765 nm; b = the y-intercept of the calibration curve; Df = the dilution factor (10); m = the slope of the calibration curve.
3. Results and discussion

3.1. Proximate composition of red rice milk

The analysis of main food components to obtain considerable data such as moisture, ash, protein, fat, and carbohydrate is conducted in red rice milk. The determination of the percentages of these components is termed a proximate analysis. The results of the proximate analysis and energy content of red rice milk are shown in Table 1.

| Composition      | Wet Bases (%) | Dry Bases (%) |
|------------------|---------------|---------------|
| Moisture         | 98.01 ± 0.08  | 4943.51 ± 209.05 |
| Ash              | 0.07 ± 0.01   | 3.74 ± 0.59   |
| Protein          | 0.13 ± 0.01   | 6.89 ± 0.25   |
| Fat              | 0.71 ± 0.02   | 35.91 ± 1.08  |
| Carbohydrate     | 1.07          | 53.77         |
| Energy (kcal)    | 11.19         | -             |

The results showed that red rice milk has high moisture, low protein, fat, and carbohydrate content. The high moisture content (98.01%) is understandable as the product is a beverage. The low protein content (0.13%) and fat content (0.71%) of red rice milk are in accordance with previous findings that cereal-based milk substitutes are low in protein and fat content [12]. The fat content in red rice milk is in accordance with the red rice samples, as it poses low-fat content.

Although it could be considered a cow’s milk substitute, red rice milk contains an insignificant protein (0.13%). Another finding supports this result, which determines the low protein content in rice extract (0.3%) [13]. The number could be considered low compared to commercial plant-based milk such as almond and cashew milk. However, it should be noted that commercial plant-based milk has been fortified with additives and compounds to improve the protein value of the milk [14].

Contrary to the carbohydrate content obtained in the previous result [15], the carbohydrate content in red rice milk is shallow (1.07 %) due to the filtration process during rice milk production. The removal of solid particles in rice milk is probably responsible for the low carbohydrate content. Low carbohydrate, protein, and fat content impacted the low energy level in red rice milk (11.19 kcal). Usually, rice milk is high in carbohydrate content due to adding sugars to improve the consumer acceptance of the product [16]. Small quantities of oil or fat-based ingredients may be added to the rice milk to increase the lipid content and improve product mouthfeel [17].

3.2. Antioxidant activity of red rice milk

The antioxidant activity of red rice milk was determined using DPPH and total phenol methods. The result of antioxidant activity of red rice milk was shown in Table 2. It can be seen from Table 2 that red rice milk had the ability to inhibit 53.37% of DPPH radicals and total phenolic content of 274.5 ppm. It is shown that red rice milk exerts antioxidant activities. Red rice has been proven to contain more antioxidant activity compared to white rice. It could be attributed to the malvidin and cyanidin-3-glucoside, the anthocyanin pigments contained in red rice [18,19]. These compounds could act as hydrogen donors and reducing the free radical activity [20]. As anthocyanin is a water-soluble pigment, it contributes to the antioxidant activity of rice milk.

| Analyses                  | Results             |
|---------------------------|---------------------|
| DPPH (% inhibition)       | 53.37 ± 0.83        |
| Total phenolic content (ppm) | 274.5 ± 22.17      |
4. Conclusion
Red rice milk is a cereal-based milk that become alternative beverage to animal milk. Red rice milk has a low-calorie content due to low amount of carbohydrate, fat, and protein. It is shown that red rice milk has an antioxidant activity. It possesses a DPPH radicals scavenging activity and total phenolic content. This result could give a new insightful information that red rice milk has a potential as functional drink with antioxidant activities. Further research should focus on the improvement of the product formulation, especially to improve the nutritional property such as protein content of red rice milk and organoleptic properties of the product.

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