Fatty acids and amino acids profile of organic black rice (*Oryza sativa* L.) milk

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**Abstract.** The popularity of plant-based milk has been increasing over the last few years to substitute animal milk. Cereal such as black rice (*Oryza sativa* L.) is a plant material that can be used to produce rice milk. Black rice has been reported to have high vitamin and mineral content and high fiber. Previous research also has shown the functionality of black rice, such as antioxidant, antihypertensive, and antihyperlipidemic. For this reason, black rice has the potency to be further processed into functional food such as rice milk. However, there is still a lack of basic information about the nutritional profile of black rice milk. Therefore, this research aimed to examine the fatty acids and amino acids profile of black rice milk. Fatty acid analysis was carried out using GC-FID. Amino acid content was analyzed using UPLC. The fatty acid profile analysis revealed that polyunsaturated fatty acid was the most abundant (0.1062%) in black rice milk, followed by saturated fatty acid (0.062%). The highest amino acid found in black rice milk was glutamic acid (0.0045 g/100 mL), aspartic acid (0.00269 g/100 mL), and arginine (0.0228 g/100 mL)

1. Introduction

The need to obtain nutritional benefits and, at the same time, combined with the sensorial acceptability are the main driving force of shifting in food choices [1]. These days, many people are more aware of their health and therefore influences their preferences to choose healthier food product. For example, an animal-based diet has been running for centuries and has negatively impacted health and the environment [2]. For a long time, milk has been described as a complete food with high nutritional contents and bioactive properties [3]. However, some people avoid consuming milk due to medical reasons such as lactose intolerance and allergy. Over the last few years, the emergence of new lifestyles, such as vegetarian and vegan, environmental issues, and antibiotic residues in milk have pushed researchers and food industries to develop alternative milk made from plant-based [4,5]. In several countries, the limited access to animal milk in some areas, high prices, and the contamination of pathogens are other reasons for realizing new milk analogs [6].

A liquid obtained from the extraction of a plant that resembles cow milk is called plant-based milk. It is produced by processing soluble material from grounded plant material with water to make a colloidal suspension or emulsion [5]. Although there are various preparation techniques to produce plant-based milk, the general processing method is the same such as soaking, grinding, filtration, mixing, homogenization, heat treatment, and aseptic packing [7]. The earliest use of plant-based milk substitute for cow milk was recorded in China through soybean milk [5]. Nowadays, several plant materials are used to make plant milk alternatives, such as legumes, cereals, seeds, nuts, and pseudocereals [8].
popularity of soybean is due to the nutrient content like protein that is comparable with cow milk and contains bioactive compounds such as isoflavones [8]. However, soybean also could cause allergy, and beany flavor limited the consumption of this product. Therefore, the use of cereal plants such as rice (Oryza sativa L.) is gradually increasing. Consumers perceive rice as a non-allergenic material and have the potency to be developed as rice milk [1].

Rice is a cereal grain widely cultivated in Indonesia and consumed by the majority of Indonesian as a staple food [9]. There are several types of rice in Indonesia, such as white rice, red rice, brown rice, and black rice. Black rice has been a significant interest in nutritional quality, bioactive compounds, and pigment [10]. It has been reported to contain higher macronutrients and micronutrients than white rice [11]. Previous studies also revealed that black rice exerted health benefits such as anti-inflammatory, antioxidant, and anti-cancer [12]. Based on that fact, there is an attempt to innovate a new black rice-based functional food. To successfully create the new food product, it is essential to know the behavior and characteristic of black rice milk. The data on the amino acids and fatty acids composition of black rice milk is lacking. Therefore, this study aimed to analyze the amino acids and fatty acids profile on black rice milk.

2. Materials and methods

2.1. Materials
Black rice var. Padi Hitam Mutiara (Mama Kamu, Karanganyar) was purchased from online marketplace. Chemicals used for fatty acid analysis are GC-MS grade, and for amino acid, analysis is HPLC grade.

2.2. Black rice milk preparation
The production of black rice milk was following the method mentioned in a previous study [16]. The first step of production was cleaning the black rice with water thoroughly and then soaking it for 180 minutes. After the soaking process, size reduction occurred by mixing the rice with water (ratio 3:10), then blending using a commercial blender for 120 seconds until the appearance turned into a slurry. Then, the slurry obtained was filtered and the liquid obtained was undergone heat treatment at 100 °C for 10 minutes. The cooked liquid rice slurry was then put aseptically into a packaging, cooled, and stored at 4 °C. The sample was then ready to use for analysis.

2.3. Determination of fatty acids profile of black rice milk
Analysis of fatty acid profile has followed the method in 18-6-1/MU/SMM-SIG. Fatty acids profile of black rice milk was analyzed using gas chromatography (GC) equipped with DB FastFAME column and detector used was flame ionized detector (FID). The carrier gas used in this analysis was helium. The injection of the sample occurred at 240 °C. The oven temperature was programmed from 50 and gradually increased up to 230 °C. The amount of sample injected was 1 µL in split mode. The interpretation of data was based on a comparison of retention time with fatty acid standards (C4–C24). The fatty acid content was calculated following the equation:

\[ \text{Fatty Acid Content (\%)} = \frac{\text{Area of Fatty Acid}}{\text{Total Area of Fatty Acids}} \times 100\% \]

2.4. Determination of amino acids profile of black rice milk
The determination of amino acids composition in black rice milk was conducted following the method in a previous study [12]. Ultra-performance liquid chromatography (UPLC) coupled with a C18 column was used to separate the amino acids. The results of separation were analyzed using a photodiode array detector (PDA). The sample was first hydrolyzed using 6 M HCl containing 0.5% (w/v) at 110 °C for 24 h. After hydrolyzed, the sample was dried at room temperature for 20 minutes using the mixture of triethylamine: water: ethanol (1:2:2, v/v). For the injection to UPLC, the sample was prepared by
dissolving the sample with buffer solution. The mobile phase was a mixture of AccQ. Tag UltraTM and aqua bikes. The column temperature was programmed at 49 °C, and a peak of the compounds was detected at 254 nm wavelength. The comparison of the peak with amino acid standards was conducted to quantify the amino acid.

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

3. Results and discussions

The fatty acid contents of black rice milk are expressed as a percentage of g total fatty acids per 100 mL of sample (Table 1). The result showed that polyunsaturated fatty acids (PUFAs) were the most abundant fatty acids in black rice milk with a total of 0.1062%, followed by monounsaturated fatty acids (0.00951%), and saturated fatty acids (0.062%). Linoleic acid (0.0982%) and docosadienoic acid
(0.008%) were the compounds of PUFAs observed. In a previous study, saturated fatty acids such as stearic acid, palmitic acid, and myristic acid, were the dominant fatty acid constituents and responsible for 50% of cow milk's total fatty acid content [13]. Compared to cow milk, the fatty acid content in black rice milk is higher in terms of unsaturated fatty acid. It is recommended to reduce the consumption of saturated fatty acid-containing products like milk, as excessive intake of saturated fatty acid has been proven to increase the risk of heart diseases [14].

Table 1. Fatty acid profile of black rice milk

| Saturated Fatty Acid | % (w/v) | Monounsaturated Fatty Acid | % (w/v) | Polyunsaturated Fatty Acid | % (w/v) |
|----------------------|---------|----------------------------|---------|---------------------------|---------|
| Butyric acid         | - *     | Myristoleic acid           | -       | Linoleic acid             | 0.0982  |
| Caproic acid         |         | Pentadecenoic acid         | -       | Linolenic acid            | -       |
| Capric acid          |         | Palmitoleic acid           | -       | Eicosadienoic acid        | -       |
| Undecylic acid       |         | Heptadecenoic acid         | -       | Eicosatrienoic acid       | -       |
| Pelargonic acid      |         | Oleic acid                 | 0.00951 | Arachidonic acid          | -       |
| Enanthic acid        |         | Eicocyanic acid            | -       | Eicosapentaenoic acid     | -       |
| Caprylic acid        |         | Erucic acid                | -       | Docosadienoic acid        | 0.008   |
| Lauric acid          |         | Capric acid                |         | Docosahexaenoic acid      | -       |
| Tridecyllic acid     |         | Undecylic acid             |         |                           |         |
| Myristic acid        |         |                            |         |                           |         |
| Pentadecylic acid    |         |                            |         |                           |         |
| Palmitic acid        | 0.0538  |                            |         |                           |         |
| Margaric acid        |         |                            |         |                           |         |
| Stearic acid         | 0.0062  |                            |         |                           |         |
| Nonadecylic acid     |         |                            |         |                           |         |
| Arachidic acid       | 0.0022  |                            |         |                           |         |
| Heneicosylic acid    | n.d.    |                            |         |                           |         |

Note: - * = not detected

The amino acid profiles of black rice milk are reported in Table 2. Amino acid content is expressed as g of amino acid content per 100 mL of black rice milk. The most abundant amino acids in black rice milk are glutamic acid (0.00455 g/100 mL), aspartic acid (0.0269 g/100 mL), serine (0.0202 g/100 mL), and leucine (0.0201 g/100 mL). The number of amino acids in black rice was lower compared to animal milk [15]. It is expected that the low protein content in black rice milk contributed to the low number of amino acids. A previous study reported the low protein content of rice milk [16], complementing the findings in this study. Generally, plant protein is low compared to cow milk and affects the low nutritional quality such as amino acid [17].
Table 2. Amino acid profile of black rice milk (g/100 mL)

| Amino acid    | Black Rice Milk | Cow Milk [15] |
|---------------|-----------------|---------------|
| Lysine        | 0.0142          | 0.261         |
| Threonine     | 0.0122          | 0.149         |
| Valine        | 0.0138          | 0.220         |
| Isoleusine    | 0.0090          | 0.199         |
| Leusine       | 0.0201          | 0.322         |
| Tyrosine      | 0.0119          | 0.159         |
| Phenylalanine | 0.0144          | 0.159         |
| Histidine     | 0.0085          | 0.089         |
| Arginine      | 0.0228          | 0.119         |
| Aspartic acid | 0.0269          | 0.250         |
| Serine        | 0.0202          | 0.179         |
| Glutamic acid | 0.0455          | 0.689         |
| Proline       | 0.0120          | 0.319         |
| Glycine       | 0.0171          | 0.070         |
| Alanine       | 0.0187          | 0.113         |

n.d. = not determined

4. Conclusion
Analysis of the fatty acid profile of black rice milk showed that the major fatty acid was polyunsaturated fatty acids (linoleic acid and docosadienoic acid). Analysis of the amino acid profile of black rice milk showed that glutamic acid was the highest, and tyrosine was the lowest amino acid content.

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