Comparison of Antioxidant Activity and Total Lactic Acid Bacteria (LAB) of Goat Milk Yoghurt, Goat Milk Yoghurt Fortified by Red and Black Rice Bran Flour

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Abstract. Yoghurt is a functional food which has various health benefit. Goat milk yoghurt contains bioactive peptides which have the potential as antioxidants. Rice bran, a by-product of rice milling process, is recently popular as animal feed. Rice bran provides good nutritional values and high bioactive compounds, such as antioxidant activity. The objective of this study was to determine the comparison of antioxidant activity and total Lactic Acid Bacteria (LAB) between goat milk yoghurt and goat milk yoghurt fortified by red and black rice bran flour. This was a true experimental study with randomization three factor treatments and five repetitions: (1) goat milk yoghurt without red or black rice bran flour fortification; (2) goat milk yoghurt with 4% red rice bran flour fortification; and (3) goat milk yoghurt with 4% black rice bran flour fortification. Yoghurt used in this experiment was a fermented milk product from Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus acidophilus. Analysis of the total LAB used Total Plate Count (TPC) test and analysis of the antioxidant activity used 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Data were analysed by Ms. Excell 2010. The addition of red and black rice bran flour to goat milk which were fermented together into yoghurt, has proven to increase antioxidant activity and total LAB, with the highest antioxidant activity and total LAB was the addition of black rice bran flour.

1. Introduction
Goat milk nutrient composition is high in lipid, protein, mineral, vitamin A, and vitamin B (riboflavin). Goat milk characteristic is more unique compared to goat milk for having smaller lipid globules and thus easier to be digested. Another unique characteristic is goat milk has “prengus” or goat aroma brought by its milk lipid containing capric acid, caprylate acid, and caproate acid [1]. An effort to lower goat smell in goat milk is required, which is hoped to increase consumer preference to consume goat milk. One of the efforts in lowering goat milk aroma is by fermentation [2].

Yoghurt is an example of milk product processed by fermentation with the help of LAB starter such as Streptococcus thermophilus and Lactobacillus bulgaricus [3]. Yoghurt as fermented milk product can be a nutrition source for human because fermentation can increase nutrition and palatability of goat milk [1]. Good quality yoghurt has sourness, aroma, and taste unique to yoghurt with the consistency of thick liquid to semi-solid [4]. Yoghurt quality improvement can be done by the addition of extract from fruit or vegetables, flour/cereals, and other materials. According to [5]
addition of other materials will complete the nutrition required by LAB during fermentation process and thus may increase its growth and development.

[6] conducted a study with the main purpose of increasing marketability and benefit of rice bran, where it was then processed into flour and further processed into yoghurt. In principle, rice bran is a by-product of rice milling which is rarely used in food products by the community. Several rice bran related researches today provide information of rice bran potency. Rice bran can potentially be used as functional food source for its bioactive compound and food fiber component [7]. Bioactive component contain in rice bran are: phenolic acid, flavonoid, anthocyanin, proanthocyanin, tocopherol, tocotrienol, γ-oryzanol, β-caroten, and phytate acid [8]. [7] reported that bioactive compounds contained in rice bran have the potency as antioxidant, with the highest among them being γ-oryzanol. Antioxidant activity in rice bran is highly influenced by rice variety and color pigment component in rice. The darker the pigment, the higher its antioxidant activity. [9] stated that black rice bran has the highest antioxidant activity, followed by red rice bran and brown rice bran.

This research used starter Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus acidophilus in yoghurt making. [4] provided information through their research that the three bacteria will stimulate one another and thus hastened their growth and development. These bacteria possessed lactase and protease enzyme. Lactase enzyme works by changing lactose into lactic acid. This turned yoghurt pH into acidic. In acidic pH condition, protein will coagulate, which turned the consistency thicker. Protease enzyme worked by hydrolyzing protein into simplest form of protein also called as bioactive peptides. Several bioactive peptides in yoghurt can potentially be antioxidant. Those known are: proline, histidine, tyrosine, and tryptophan. Antioxidant derivate of bioactive peptide can prevent cellular component damage by inhibiting the formation of radicals and showingscavenging radicaleffect[10]. The purpose of this research is to determine the comparison of antioxidant activity and total LAB between goat milk yoghurt and goat milk yoghurt fortified by red and black rice bean flour.

2. Research Method

2.1. Yoghurt production

Yoghurt production began by creation of working mother culture, which is the addition of starter powder (Yogourmet® contained Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus acidophilus) as much as 0.35 gram into 70 ml of pasteurized goat milk and incubated in 45°C temperature for 4 hours until it reached pH 4.4-4.5. The processed was followed by (1) yoghurt making by adding 3% working mother culture into 480 ml of pasteurized goat milk and (2) yoghurt making by adding 3% working mother culture and 4% red and black rice bran flour into 480 ml pasteurized goat milk and then incubated in 45°C temperature for 4 hours until they reached pH 4.5-5.

2.2. Lactic acid bacteria (LAB) total test

Goat milk yoghurt and goat milk yoghurt fortified by 4% red and black rice bran flour was tested for LAB by total plate count method. Total plate count method most often used to count the amount of microorganism inside food stuff. From desired yoghurt dilution, 1 ml yogurt was poured into petri dish and then 30 ml of liquid agar medium was poured after. After the agar medium solidified, the plates were incubated for 24 hours for bacterial colony growth, which then counted.

2.3. Antioxidant activity test

Goat milk yoghurt and goat milk yoghurt fortified by 4% red and black rice bran flour extracted by methanol were reacted with DPPH solution for 30 minutes. The antioxidant activity of goat milk yoghurt fortified by 4% red and black rice bran flour was measured as (%) discoloration.

2.4 Data analyses

After data were obtained, data processing was done by Microsoft Excel 2010 software. Data was presented as graphic which showed the average value of the 5 repetition of each treatment.
3. Results
Total LAB test was done by Total Plate Count method. The result of total LAB from goat milk yoghurt, goat milk yoghurt fortified by red rice bran flour, and goat milk yoghurt fortified by black rice bran flour was shown in Figure 1.

![Antioxidant Activity Graph](image_url)

**Figure 1.** Average Value of Antioxidant Activity

Lactic Acid Bacteria (LAB) is a type of bacteria important in processing probiotic beverages. The total number of Lactic Acid Bacteria (LAB) in a given probiotic beverage determine the quality of the said probiotic beverage [11]. Yoghurt is a probiotic beverage product obtained by fermenting milk with LAB. During its processing, a single culture or mixed culture of LAB can be used [12]. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are two type of bacteria most often used as yoghurt starter. If both bacteria used at the same time as yoghurt starter, they would form mutualism symbiosis. *Lactobacillus bulgaricus* produces amino acid and short peptides which induce the growth of *Streptococcus thermophilus*, which produces formic acid that support the growth of *Lactobacillus bulgaricus* [13]. [14] stated that according to SNI 2981:2009, the appropriate number of LAB in yoghurt is $10^7$ CFU/ml, while according to WHO/FAO, the minimum LAB requirement for probiotic is $10^6$-$10^8$ CFU/ml. The number of LAB in probiotic beverage consumed as health improvement drink is $10^5$-$10^9$ CFU/ml. From the result of this research it can be known that the average total of LAB from goat milk yoghurt made out of starter containing LAB *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* was $3.4 \times 10^6$ CFU/ml. The result showed that goat milk yoghurt is within good quality health beverage. The average total LAB of goat milk yoghurt fortified by red rice bran flour was $7.0 \times 10^5$ CFU/ml. The average total LAB of goat milk yoghurt fortified by black rice bran flour was $8.5 \times 10^5$ CFU/ml. The result showed an increase of LAB total count. [15] studied the comparison of LAB total in rice bran and skim milk fermented by probiotic LAB, which showed that rice bran fermentation media had higher number of LAB compared to skim milk fermentation media. This is assumed to be caused by more complete nutrition content in rice bran. In goat milk yoghurt fortified by red and black rice bran, nutrition source used by LAB during fermentation process was more complex, because of combined carbohydrate, protein, and fiber obtained from both goat milk and red or black rice bran flour. [5] stated that nutrition such as carbohydrate, protein, and fiber are used by LAB as energy source for growth, cell formation, and biosynthesis of metabolite products.

The result of antioxidant activity test from goat milk yoghurt, goat milk yoghurt fortified by red rice bran flour, and goat milk yoghurt fortified by black rice bran flour was shown in Figure 2.
Antioxidant is a chemical compound that can provide one or more electron to free radicals, which would inhibit the free radical[16]. Antioxidant compound in food stuff plays an important role as a defense factor of health[17]. From this research, it is known that the antioxidant activity of goat milk yoghurt fortified by red rice bran flour (45,20) and fortified by black rice bran flour (38,271) was higher compared to average antioxidant activity of goat milk yoghurt (77.103). A compound is considered to be very strong antioxidant if its IC₅₀ value < 50 µg/ml; strong if IC₅₀ value 50-100 µg/ml; moderate if IC₅₀ value 250-500 µg/ml; weak if IC₅₀ value > 500 µg/ml [18]. Thus, it can be concluded that the antioxidant activity contained in goat milk yoghurt fortified by red and black rice bran flour is classified as very strong antioxidant. This is in accordance with a research by [19], who studied the antioxidant activity of carrot extract is high, especially with 20% addition of carrot extract. This showed that the addition of natural compound such as fruit, vegetable, and grain, may increase antioxidant activity and thus can be utilized as antioxidant food source which can function as consumer’s defense against diseases caused by free radicals and oxidative stress[19] [15] [17]. Antioxidant activity of rice grain is strongly influenced by phenolic and anthocyanin compounds. The more phenolic and anthocyanin compounds, the higher its antioxidant activity. Compared to other rice grain, red and black rice grain has the highest content of phenolic and anthocyanin compound for it also has the highest level of pigmentation, especially in black rice grain. Phenolic compounds in rice bran are tocopherol, tocotrienol, γ-oryzanol, β-sitosteril ferulat, which bind with insoluble fiber [7]. Fiber degradation by LAB happened during fermentation process, forming simpler compound and causing covalent bond between phenolic compound and insoluble fat to break, and thus increase the antioxidant bioavailability of rice bran[15]. [17] stated that antioxidant from phenolic compound worked by lowering the concentration of radical oxygen and prevent initiation by capturing free radicals such as hydroxyl radicals. Goat milk itself contains various bioactive compounds, whose concentration can be increased by fermentation. Several bioactive compound in goat milk can lower DNA damage, especially those caused by free radicals. These bioactive compounds are lactopherin, like-laktoferin, and other peptides with antioxidant activity. These bioactive compounds are very heat sensitive, which may denaturize protein. However, fermentation process will produce novel peptides or bioactive peptides[20].

![Figure 2. Average Total Count of Lactic Acid Bacteria (LAB)](image-url)
4. Conclusion
Fortification by various different rice bran flour has increases antioxidant activity and total LAB of goat milk yoghurt, with the highest antioxidant activity and total LAB was fortified by 4% black rice bran flour.

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