

Study of Total Lactic Acid Bacteria (LAB) and Antioxidant Activity in Goat Milk Yoghurt Fortified by White Rice Bran Flour

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Abstract—Yogurt is one of functional fermented food products from milk created by the addition of Lactic Acid Bacteria (LAB) which has many health benefit. Goat milk yogurt has components of bioactive peptides with antioxidants potency. Antioxidant derivatives of bioactive peptides in goat milk yogurt include proline, histidine, tyrosine, and tryptophan. Rice bran is a by-product of rice mills which have good nutritional content and are rich in bioactive components, one of which has antioxidant activity. Antioxidant compounds found in white rice bran can be grouped into 8 groups: phenolic acids, flavonoids, anthocyanins, proanthocyanins, tocopherols, tocotrienols, γ -oryzanol, and phytic acid. The objective of this study was to observe total LAB of goat milk yoghurt fortified by white rice bran flour. This was a true experimental study with two factor treatments and five repetition. Yoghurt was made by adding starter which contained *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*. Total LAB was analyzed using Total Plate Count (TPC) and analysis of the antioxidant activity was done by 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Results showed that fortification by white rice bran flour in goat milk yoghurt could increase total LAB and antioxidant activity.

Keywords—antioxidant, goat milk, LAB, white rice bran, yoghurt

I. INTRODUCTION

Rice bran, a by-product, has garnered increasing attention as functional food in the last few years. This is related to rice bran functionality on body health (Tuarita *et. al.*, 2017). Rice bran is a by-product of rice milling, obtained from the outer layer of rice seed caryopsis (Henderson *et. al.*, 2012). Until now, rice bran development effort as functional food is still hindered by several problems, among them are the lack of awareness in the community on rice bran benefits, much of rice bran still used solely as livestock feed, the yet standardized rice bran quality and the lack of interest from downstream industry to develop rice bran. This becomes a particular challenge for rice bran development considering its promising potential for human health (Tuarita *et. al.*, 2017).

Rice bran role as functional food source with a potential to improve health can be seen from its bioactive components and fiber content. The type of food fiber

contained in rice bran are cellulose, hemicellulose, pectin, arabinoxylan, lignin, and β -glucan. Rice bran bioactive component consists of γ -oryzanol, ferulic acid, caffeic acid, tricene, kumaric acid, phytic acid, vitamin E isoforms (α -tocopherol, γ -tocopherol, tocotrienol), phytosterol (β -sitosterol, stigmasterol, campesterol), dan karotenoid (α -karoten, β -karoten, lutein, likopen) (Henderson *et. al.*, 2012).

Rice bran is rich with antioxidant and thus has the potency as a defense against free radical. Antioxidant compounds found in rice bran can be group into 8 groups: phenolic acid, flavonoid, anthocyanin, proanthocyanin, tocopherol, tocotrienol, γ -oryzanol, and phytic acid (Tuarita *et. al.*, 2017). The main antioxidant of rice bran is γ -oryzanol. Antioxidant γ -oryzanol is composed of the mix of esters ferulic acid and phytosterol. At least ten esters of ferulic acid has been identified in γ -oryzanol; cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, campestenyl ferulate, campesteryl ferulate, stigmastanyl ferulate, sitosteryl ferulate, 7-stigmatenyl ferulate, stigmateryl ferulate, campestanil ferulate, and sitostanyl ferulate. Among those compounds, cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, campestenyl ferulate, and sitosteryl ferulate are the most dominant compounds (Minatel *et. al.*, 2016).

The same findings were reported from goat milk studies. Goat milk has several advantages compared to cow milk, especially in health aspects. Goat milk is source of bioactive peptides, making it also has the potential to be developed as functional food with the right processing technology, such as fermentations. Lactic Acid Bacteria (LAB) possesses proteolytic enzyme that can hydrolyze bioactive peptide binding to precursor proteins to detach and thus produce processed milk product with relatively high bioactive peptide compounds. Among the biological functions of known bioactive peptides is antioxidant (Awemu *et. al.*, 2009).

Several milk bioactive peptides are known to play a role in body oxidative metabolism, which maintain the life of cells. Milk bioactive peptide antioxidant derivates consists of five to eleven hydrophobic amino acids, among them are proline, histidine, tyrosine, and tryptophan—

especially those that have been hydrolyzed my proteolytic enzyme activity. These bioactive peptide antioxidant derivates have the ability to prevent cellular component damage by inhibiting the formation of free radical and by exhibiting scavenging radical effect (Mohanty *et al.*, 2015).

Based on those facts, potency exploration in goat milk fermented into yoghurt product fortified or added by white rice bran is required to create it into natural functional food with LAB and antioxidant content. This experiment is hoped to strengthen national innovation system in developing natural functional processed based food product of goat milk fermentation fortified by white rice bran flour.

II. MATERIALS AND METHODS

A. 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% white 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.

B. LAB Total Test

Goat milk yoghurt and goat milk yoghurt fortified by 4% white 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.

C. Antioxidant activity test

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

III. RESULTS

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. Total LAB test was done by Total Plate Count method. The result of total LAB from goat milk yoghurt and goat milk yoghurt fortified by white rice bran flour was shown in Fig. 1.

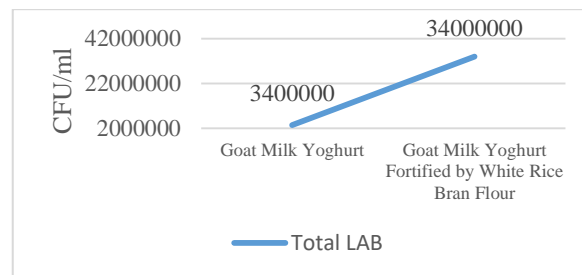


Fig. 1. Average Total Count of Lactic Acid Bacteria (Lab)

The result of antioxidant activity test from goat milk yoghurt and goat milk yoghurt fortified by white rice bran is shown on Fig. 2.

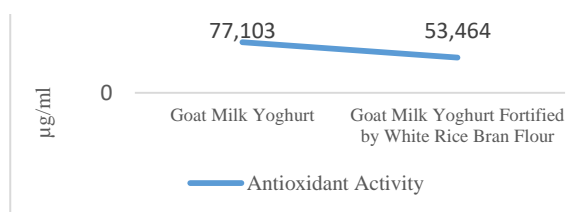


Fig. 2. Average Value of Antioxidant Activity

IV. DISCUSSION

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 (Agustine *et al.*, 2018). 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 (Rahman *et al.*, 2015). *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 *Lactobacillus bulgaricus* (Effendi *et al.*, 2009). Burton *et al.*, (2014) 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 10^6 - 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 3.4×10^6 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 white rice bran flour was 3.4×10^7 CFU/ml. The result showed an increase of LAB total count. Zubaidah *et al.*, (2010) 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 white 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 white rice bran flour. Zubaidah *et. al.*, (2014) 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.

Antioxidant is a chemical compound that can provide one or more electron to free radicals, which would inhibit the free radical (Husni *et. al.*, 2015). Antioxidant compound in food stuff plays an important role as a defense factor of health (Abbas *et. al.*, 2016). From this research, it is known that the antioxidant activity of goat milk yoghurt fortified by white rice bran (53,464) was higher compared to average antioxidant activity of goat milk yoghurt (77.103). A compound is considered to be very strong antioxidant if its IC_{50} value $< 50 \mu\text{g/ml}$; strong if IC_{50} value $50\text{-}100 \mu\text{g/ml}$; moderate if IC_{50} value $250\text{-}500 \mu\text{g/ml}$; weak if IC_{50} value $> 500 \mu\text{g/ml}$; (Werdhasari, 2014). Thus, it can be concluded that the antioxidant activity contained in goat milk yoghurt fortified by white rice bran flour is classified as very strong antioxidant. This is in accordance with a research by Samichah (2014), 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 (Samichah, 2004; Zubaidah *et. al.*, 2010; Abbas *et. al.*, 2016). 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, white rice grain has the lowest content of phenolic and anthocyanin compound for it also has the lowest level of pigmentation. Phenolic compounds in rice bran are tocopherol, tocotrienol, γ -oryzanol, β -sitosteril ferulat, which bind with insoluble fiber (Tuarita *et. al.*, 2017). 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 (Zubaidah *et. al.*, 2010). Abbas *et. al.*, (2016) 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 (Khan *et. al.*, 2019).

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