

QUALITY OF GOAT MILK CHEESE WITH ADDITION OF RICE BRAN OIL RIPENED USING *Lactobacillus casei* AND *Streptococcus thermophilus*

Nurliyani*¹⁾, Indratningsih¹⁾, Widodo¹⁾, Endang Wahyuni¹⁾

¹⁾Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada
Jl. Fauna 3, Bulaksumur, Yogyakarta, Indonesia 55281
E-mail: nurliyani@ugm.ac.id

Submitted 13 December 2019; Accepted 29 February 2020

ABSTRACT

This study aimed to determine the effect of rice bran oil (RBO) addition on the microbiological, chemical, physical and sensory qualities of goat milk cheese ripened using *Lactobacillus casei* FNCC 0090 and *Streptococcus thermophilus* FNCC 0040. Cheese was prepared from goat's milk with a starter of *Lactobacillus casei* and *Streptococcus thermophilus* and rennet as coagulant. Cheese was divided into four groups: 1) ripened cheese without RBO stored for 0 d, 2) ripened cheese without RBO stored for 30 d, 3) ripened cheese + RBO stored for 0 d and 4) ripened cheese + RBO stored for 30 d. The results showed that the addition of RBO and ripening did not affect on the total number of lactic acid bacteria (LAB) of the cheese (their total number were in the range of $2.60-4.21 \times 10^7$ CFU/g). The acidity of cheese without RBO after ripening was higher ($p < 0.05$) than without ripening. Therefore, the pH value of cheese with the addition of RBO was higher than the cheese without RBO. The addition of RBO had no effect on moisture content of cheese, but the moisture content decreased ($p < 0.05$) after ripening. Meanwhile, the addition of RBO could reduce their soluble protein. Cheese added with RBO had a harder texture ($p < 0.05$) than cheese without RBO. Sensory quality of cheese was not influenced by the addition of RBO, but the saltiness of cheese with RBO was higher than without RBO and had no bitter in taste. In conclusion, RBO had no negative effect on the flavor and acceptability of the cheese and the growth of LAB, could increase cheese hardness, but could decrease the acidity and soluble protein in the cheese. However, the cheese with the addition of RBO ripened using starter consisting of *Lactobacillus casei* FNCC 0090 and *Streptococcus thermophilus* FNCC 0040 could be potentially classified as probiotic cheese which is beneficial for health.

Keywords: Cheese; microbiological quality; physicochemical quality; rice bran oil; sensory quality

INTRODUCTION

Dairy goat farms have started to develop, especially Ettawah crossbred goat in several regions in Indonesia. Generally goat milk is marketed in frozen form, while some are marketed in the form of fermented milk products such as yogurt, kefir and cheese on a small scale and a limited market. Recently, the development of functional food has also been growing, due to the increasing public awareness of the importance of maintaining the health. Sources of functional food can be originated from vegetable or animal sources. The use of vegetable oils that contain many essential fatty acids has been studied in various dairy products including cheese. Recently, to change the organoleptic properties, nutritional value and to decrease cholesterol and also to reduce the cost of cheese production can be use vegetable oil as a substitute for milk fat. It was reported that characteristic of physicochemical, textural and organoleptic properties in kashar cheese not influenced by palm oil (Kavak and Karabiyik, 2019).

Because of the characteristics of various cheese that has a higher pH and fat content, and a denser texture, the cheese can provide protection against the survival of probiotics in the digestive tract. Therefore, cheese can be used as a great vehicle for delivering probiotics compared to other products (Gheisari et al., 2014). Cheese is one of the dairy products that are rich in nutritional value, especially protein. In addition, there are various type of cheese in the world that consumed by many people, thus enabling the development of a market for probiotic cheese. The prerequisite for probiotic microorganisms is that they must

survive in sufficient quantities in food until they are ready for consumption and do not reduce their organoleptic properties. Organoleptic characteristics of dairy products such as aroma, taste, texture and appearance can be influenced by probiotic microorganisms, so that the organoleptic properties of dairy products is widely used as a subject for various study (Karimi et al., 2011). Consumption of cheese enriched with probiotics microorganism can improve the human health including enhancing the body's immune system, increasing oral health and digestive tract in the elderly and strengthen the intestinal immunity (Yerlikaya and Ozer, 2014).

The nutritional and antioxidant content in rice bran allows the use of bran in the food industry is quite promising, even though bran which is a byproduct of a rice milling, is generally only used for animal feed. Besides that it is important as a food ingredient (Ardali et al 2013., Abbas et al., 2016). Rice bran has been used in food as a full-fat, defatted bran, bran oil, and protein concentrate, and is used for baked goods, snacks, crackers, breads, cereals, pastries, pancakes, noodles, muffins, biscuits (Abbas et al., 2016). The percentage of rice bran ranges from 10% of the total grain with oil content of 18-22%. The oil is clear yellow at 20°C, odorless, acidic index <0.50 and density at 20°C in around of 0.920-0.930 (Chou et al., 2009).

Rice bran oil contains bioactive components that can reduce cholesterol, as antioxidants and antiinflammation such as oryzanol, phytosterols, tocopherol, tocotrienols, squalene, policosanol, and ferulic acid. Gamma oryzanol is an essential micronutrient in cooking oil obtained from RBO as a by-product of the rice milling

*Corresponding author:

Nurliyani

Email: nurliyani@ugm.ac.id

Department of Animal Product Technology, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna 3, Bulaksumur, Yogyakarta, Indonesia 55281

How to cite:

Nurliyani., Indratiningsih., Widodo., & Wahyuni, E. (2020). Quality of Goat Milk Cheese with Addition of Rice Bran oil Ripened Using *Lactobacillus casei* and *Streptococcus thermophilus*. Jurnal Ilmu dan Teknologi Hasil Ternak, 15 (1), 1-12

process. The presence of balanced fatty acids in RBO makes the oil popular (Al-Okbi *et al.*, 2014; Pal and Pratap, 2017). This study aimed to determine the effect of addition of RBO on the microbiological, chemical, physical and sensory qualities of goat milk cheese ripened with *Lactobacillus casei* and *Streptococcus thermophilus*. The cheese in this study is expected to provide information for the food industry to develop functional food that has health benefits.

MATERIALS AND METHODS

Cheese preparation

Goat milk cheese was prepared from goat milk obtained from Ettawah Crossbred goat at “Bumiku Hijau” Farm, Sleman, Jogjakarta. Rice bran oil was obtained from local market in Jogjakarta, Indonesia. Fluid microbial rennet was obtained from New England Cheesemaking Supply. *Lactobacillus casei* FNCC 0090 and *Streptococcus thermophilus* FNCC 0040 were obtained from Center for Food and Nutrition Studies, Universitas Gadjah Mada.

The steps in cheese making were: Goat milk as much as 4 L was divided into 4 groups. Group 1) ripened cheese without RBO stored for 0 d, 2) ripened cheese without RBO stored for 30 d, 3) ripened cheese + RBO stored for 0 d and 4) ripened cheese + RBO stored for 30 d. Milk was pasteurized by high temperature short time (HTST) method (72°C, 15 sd), then added with emulsifier (disodium hydrophosphate) as much as 0.3%. The temperature was lowered to 37°C, then a starter of 2.5% *Lactobacillus casei* FNCC 0090 (v/v) was inoculated 2 h earlier, then inoculated with starter culture of 2.5% *Streptococcus thermophilus* FNCC 0040 at 40°C for 1 h.

Pasteurized milk was added with rennet as much as 0.02% (v/v) of total volume, then incubated for 1 h until curd formed, and then cut the curd for draining whey. Separation of curd from whey was carried out by filtering it with cheese cloth, and then adding 2% NaCl, and left for 2 h in

refrigerator. Curd was pressed for 5 min with a weight of 5 kg, then wrapped in aluminum foil.

Cheese was stored in a refrigerator at 4°C for 0 and 30 d. After pressed, curd was weighed. The yield of curd in control cheese was 32.11%, and the yield of curd in the cheese with RBO was 25.53%. Cheese was analyzed for microbiological (total lactic acid bacteria), physical (texture), chemistry (pH, acidity, soluble protein, fat and moisture content), and sensory (texture, saltiness, acidity, bitterness, flavor, acceptance) quality.

Total lactic acid bacteria analysis

Total lactic acid bacteria were determined on deMan, Rogosa and Sharpe (MRS) agar (Merck) media containing 100 ppm NaN₃ (Mundt *et al.*, 1967) and 100 ppm CaCO₃ (Hwanhlem *et al.*, 2011). To determine the number of bacteria, the cheese sample was weighed as much as 1.0 g, then dissolved into 9.0 mL of physiological NaCl solution. The dilution series were made to the final dilution 10⁻⁶. Dilution series 10⁻⁵ and 10⁻⁶ were pipetted as much as 0.1 mL, and then spread on the surface of each medium in Petri dish. Furthermore, the Petri dish was incubated for 48 h at 37°C. The total amount of lactic acid bacteria was calculated based on colonies growing on the surface of medium and expressed in log CFU/g sample (Roostita *et al.*, 2011).

pH and titratable acidity analysis

The pH value of cheese was measured using a pH-meter (HANNA-HI 98103), whereas the cheese acidity was determined by titratable acidity as lactic acid according to Hashim *et al.* (2009). The percentage of lactic acid was carried out by titration of 9 g of cheese using alkali (0.1 N NaOH) with phenolphthalein as an indicator to an end-point of faint pink color.

Moisture, soluble protein and fat analysis

The cheese samples were analyzed for moisture content in a drying oven at 105°C

and gravimetrically determined according to AOAC (1995). Soluble protein was determined by Lowry method (Plummer, 1987). Cheese samples of ± 0.5 g were diluted with distilled water to the range of protein content between 30-300 $\mu\text{g/mL}$, and added with 1.0 mL of Lowry B reagent (consisting of a mixture of 0.75 mL of 1% CuSO_4 solution; 75 mL of 2% K-Na-Tartrate; 15.0 mL of 2% Na_2CO_3 solution in 0.1 N (NaOH), then homogenized.

The mixture of solution was incubated at room temperature for 10 min, and added with 3.0 mL of Lowry A reagent (consisting of a mixture of Folin-ciocalteu reagent and distilled water in a ratio of 1:1), then homogenized. The mixture of solution was incubated at room temperature for 20 min. The absorbance was measured with a spectrophotometer (Spectronic 21D) at a λ of 750 nm. The levels of dissolved protein were obtained by entering the absorbance value of the sample .in the standard protein (bovine serum albumin) regression equation. Standard albumin solutions were prepared by dissolving 7.5 mg albumin with distilled water up to 25 mL, then obtained stock solution with a concentration of 0.3 mg/mL. Ten sets of albumin solution were prepared with a concentration range from 0.03-0.3 mg/mL or 30-300 $\mu\text{g/mL}$. Fat was analyzed by Babcock method (Lampert, 1975). A sample of 18 g was weighed in a

Babcock bottle and added 17.5 mL of concentrated H_2SO_4 (in an ice bath). Samples were centrifuged for 5 min. Warm water (60°C) was added to the sample to the mouth of the tube neck and centrifuged again for 2 min. Warm water (60°C) was added again until the fat was raised on the scale, then centrifuged again for 1 min. The scale on the tube was read (in water bath, temperature of $55\text{-}60^\circ\text{C}$).

Texture analysis

The measurement of cheese texture was done by cutting the cheese sample into a cube, then measuring it using Brookfield CT3 Texture Analyzer, No. M08-372-E0315 (Brookfield Engineering Laboratories, Inc., USA) at temperature of 27°C . Test standard using compression with probe type TA44 cylinder 4 mm D, pre-load 0.02 N, pre-load speed 50 mm/min and test speed 10 mm/min.

Sensory analysis

Sensory characteristics of cheese after 30 d ripening were carried out by 10 panelists of untrained students in Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada. The scale of evaluation for cheese according to (Jeon *et al.*, 2012) with modifications was listed in Table 1.

Table 1. Score of cheese evaluation

Score	Texture	Saltiness	Acidity	Bitterness	Flavor	Acceptance
1	Not very soft	Not very salty	Not very sour	Not very bitter	Not highly flavorful milk	Very dislike
2	Not soft	Not salty	Not sour	Not bitter	Not flavorful milk	Dislike
3	Rather soft	Rather salty	Rather sour	Rather bitter	Rather falvorful milk	Rather like
4	Soft	Salty	Sour	Bitter	Flavorful milk	Like
5	Very soft	Very salty	Very sour	Very bitter	Highly falvorful milk	Really like

Statistical analysis

The data of cheese quality, including microbiological, chemical, physical and sensory quality were presented as mean with standard deviations. Replication in each

treatment was 3 times. The mean between treatments were analyzed by two-way ANOVA (cheese treatment vs. ripening time treatment). If there were difference between treatments, it was continued with the

Duncan's Multiple Range Test (DMRT) and were considered to be significantly different if $p < 0.05$. Statistical analysis using the Statistical Package for Social Sciences (SPSS) program, version 17.

RESULTS AND DISCUSSION

Total lactic acid bacteria

Table 2 shows that there was no significant differences between total lactic acid bacteria (LAB) in cheese with RBO and cheese without RBO after ripening for 30 d and also before ripening. Hence, RBO had no adverse effect on microbiological quality of cheese during ripening for 30 d. In the present study, rice bran oil that added as

much as 2.5% showed had no negative effect on the growth of lactic acid bacteria. In a previous study, the lactobacilli were the most abundant lactic acid bacteria, followed by enterococci and lactococci found in *Serrano* cheese (de Souza *et al.*, 2003). Lactobacilli microorganisms dominate during process of cheese production and ripening, mainly in the final stages of the process, which indicate that lactobacilli play a vital role in cheese manufacturing and are part of the lactic culture. However, the addition of salt in the process of cheese production can inhibit the growth of microorganism, thus reducing the ratio of lactococci and leuconostoc in the isolate of lactic bacteria (de Souza *et al.*, 2003).

Table 2. Average of total lactic acid bacteria in cheese with and without addition of rice bran oil (RBO) before and after ripening for 30 d

Cheese	Ripening (d)	
	Before ripening	After ripening
Without RBO ^{ns}	3.11 x 10 ⁷ CFU/mL or 7.41±0.31 Log CFU	2.97 x 10 ⁷ CFU/mL or 7.29±0.49 Log CFU
RBO ^{ns}	4.21 x 10 ⁷ CFU/mL or 7.25±0.85 Log CFU	2.60 x 10 ⁷ CFU/mL or 7.14±0.64 Log CFU

ns: not significant

Prerequisites as probiotic food according to the Japanese fermented milk and lactic acid bacteria beverages association are food ingredients must containing a minimum of 10⁶-10⁷ viable microorganisms per gram or milliliter (Ishibashi and Shimamura, 1993). In this study, total lactic acid bacteria in the cheese ripened with *Lactobacillus casei* FNCC 0090 and *Streptococcus thermophilus* FNCC 0040 and added with RBO showed in the range of 10⁷ CFU/g. Therefore, the cheese in this study could be potentially classified as probiotic food. According to Tarrah *et al.* (2018), two strains of *S. thermophilus* from dairy environment in Italy, namely M17PTZA496 and TH982 possess probiotic activity *in vitro*, although many *S. thermophilus* strain have very serious problem when passage through the human gastrointestinal tract. While, strain of *Lactobacillus casei* FNCC 0090 have been

known as probiotic (Suseno *et al.*, 2000; Aini and Hariani, 2019). Results are in accordance with a previous study that incorporated 1-3% RBO in yoghurt had a range bacteria in recommended level (10⁶-10⁷ CFU/g) (Abbas *et al.*, 2017).

Acidity and pH

Cheese added with 2.5% RBO after 30 d ripening had a lower pH (4.98) than unripened cheese. Cheese without RBO both before and after ripening, had no pH differences. Cheese without RBO after 30 d ripening had higher acidity compared to cheese without RBO before ripening and cheese with RBO both before ripening and after ripening. The pH and acidity of cheese were shown in Table 3.

There were no differences on the pH of the cheese before and after ripening. The increase in acidity of the cheese after ripening, due to the measure of acidity is

only expressed in lactic acid, whereas to determine of pH value is originated from several organic acids as metabolic products. The concentration of lactose, citrate and pH value of kefir will decrease and an increase in concentration of lactic acid, acetate, butyrate and propionate as well as glucose and galactose during fermentation process and storage of kefir at 4°C for 7 d (Leite *et al.*, 2013).

Moisture, soluble protein and fat

The average moisture and soluble protein content were not significantly different in cheese without RBO before and after ripening, whereas cheese with RBO after ripening had lower moisture content compared to cheese before ripening (Table 4). However, soluble protein content in cheese with RBO did not show any differences before and after ripening

(Table 4). Soluble protein of cheese without RBO both before and after ripening was higher than the cheese with RBO both before and after ripening. Therefore, RBO added into the cheese processing could decrease the soluble protein content, which might be caused by inhibition of the growth of proteolytic bacteria during cheese ripening. Table 2 shows a decrease in total number of LAB in the cheese with RBO more than without RBO after ripening, even though the effect was not significantly different. Rice bran is highly nutritious because it has various antioxidants that impart beneficial effects on human health such as γ -oryzanol, tocotrienols, ferulic acid, and phenolic compounds (Wanna *et al.*, 2016). However, the phenolic substances found to inhibit Gram positive and Gram negative pathogenic bacteria (Chen and Bergman, 2005).

Table 3. Average acidity and pH of cheese with and without addition of rice bran oil (RBO) before and after ripening for 30 d

	Cheese without RBO		Cheese with RBO	
	Before ripening	After ripening	Before ripening	After ripening
Acidity	0.90 ^b ±0.07	1.13 ^c ±0.06	0.64 ^a ±0.11	0.88 ^b ±0.14
pH	4.88 ^a ±0.17	4.79 ^a ±0.22	5.08 ^b ±0.38	4.98 ^{ab} ±0.10

^{abc} Different letters in the same row indicate significantly different (p<0.05)

Table 4. Moisture, soluble protein and fat content of cheese with and without addition of rice bran oil (RBO) before and after ripening for 30 d

	Cheese without RBO		Cheese with RBO	
	Before ripening	After ripening	Before ripening	After ripening
Moisture (%)	52.60 ^b ±4.49	37.89 ^{ab} ±1.20	56.32 ^b ±2.08	26.23 ^a ±1.78
Soluble protein (%)	4.13 ^b ±0.07	4.15 ^b ±0.78	4.07 ^a ±0.78	4.03 ^a ±0.74
Fat (%)	16.50 ^a ±1.22	29.16 ^b ±4.15	20.33 ^{ab} ±2.21	36.23 ^c ±4.47

^{ab} Different letters in the same row indicate significantly different (p<0.05)

Lactic acid bacteria are a group of Gram-positive, non-spore forming, cocci or rods, catalase-negative, and fastidious organisms, with high tolerance for low pH (Mokoena, 2017). Intra and extracellular proteolytic activity including X-prolyl-

dipeptidyl aminopeptidase is classified as particular peptidase which split amino acid sequences containing proline, producing various peptides that generated in increased cell growth. Two strains of bacteria that have unique peptidase activity are

Lactobacillus acidophilus (L10 and La 4962), *Bifidobacterium* spp. (*B. lactis* B94 and *B. longum* Bl 536), and *Lactobacillus casei* (L26 and Lc 279), *Streptococcus thermophilus* (St 1342) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lb 1466) (Donkora *et al.*, 2007).

Other studies observed that the inhibitory effect of phenolic compounds on *Lactobacillus casei* BL23 has been determined, and phenolic compounds may disturb protein structure and also affect the properties of the cell membrane (Rivas-Sendra *et al.*, 2011). In the present study, there were no differences on the soluble protein before and after ripening, which might be caused by shorter ripening time and without additional proteases. Karaca and Güven (2018) reported that soluble nitrogen and 12% TCA soluble nitrogen contents of the cheese supplemented with protease were higher than the control cheese (without protease addition) after 90 d of ripening. Various factors that influence proteolytic activity are pH of curd, plasmin, chymosin, starter and non starter proteases, ratio of salt to water,

storage time, temperature and humidity (Park, 2001). The fat content increased when the moisture content decreased (Table 4).

This result in accordance with a previous study by Arslan *et al.* (2014), when the fat content clearly decreased, the moisture content increased, and also reported by Banks *et al.* (1994) in Cheddar cheese, Mozzarella cheese (Rudan *et al.*, 1999), and Indian cheese (paneer) (Kumar *et al.*, 2011) that as fat content decreased, moisture content increased. Fat content in the cheese with RBO was higher than without RBO (Table 4) as also reported in a previous study that corn oil substitution significantly affected the levels of fat in dry matter (Arslan *et al.*, 2014). Fat plays a major role in texture and flavor of the food products (Eswarapragada *et al.*, 2010).

Texture

The texture was not significantly different in cheese without RBO before and after ripening, whereas cheese with RBO after ripening had higher texture compared to cheese before ripening (Table 5).

Table 5. Texture (N) of cheese with and without addition of rice bran oil (RBO) before and after ripening for 30 d

Cheese	Ripening	
	Before	After (30 d)
Without RBO	8.52 ^a ±0.88	8.49 ^a ±0.89
With RBO	9.11 ^a ±1.02	14.80 ^b ±2.16

^{ab} Different letters in the same row and column indicate significantly different ($p < 0.05$)

The texture of the cheese is influenced by various factors including: the manufacturing process, variations in the method, changes in composition and biochemical during ripening.

Texture can be detected by the sense of sight, hearing, touch, and kinesthetic, because texture is a sensory and functional manifestation of the structural, mechanical and surface characteristics of food (Enab *et al.*, 2012). The increase in texture of the cheese after ripening in the present study, may be caused by low proteolytic activity. In addition, the salt that added in cheese

processing also inhibits the proteolytic activity during ripening. According to Fredrick *et al.* (1986), the hardness of cheese was influenced by proteolysis, where proteolytic activity was inhibited by salt (Guinee and Fox, 1983).

In the present study, the harder cheese texture was found in the cheese supplemented with RBO at higher fat content after ripening due to a decrease in moisture content. Characteristics of cheese texture are influenced by the interaction and cross-linking of the casein protein matrix and fat phase due to the effect of fat

plasticization (Madadlou *et al.*, 2007). Texture which is one of the physical characteristics of cheese in addition to the melt properties and color can be influenced by the initial milk composition as raw material of cheese, processing procedures, and ripening conditions (Lucey *et al.*, 2003).

Sensory quality

Sensory evaluation showed that there were no significant differences of cheese before ripening on texture, acidity, flavor, and acceptability. The salty level of cheese with RBO was higher than without RBO,

while cheese with and without RBO had no bitter taste (Table 6). Because the texture of cheese with RBO after ripening has greater hardness (Table 5) and tends to have a lower moisture content (Table 4) than cheese without RBO, the cheese feels more salty (Table 6), although the sensory analysis of the texture showed no significantly different with score of 3.00-3.07 (rather soft). The cheese saltiness without RBO had a score of 2.73 (rather salty), while cheese with RBO had a score of 3.80 (salty). However, rice bran oil did not cause bitter taste and could not affect on cheese flavor (Table 6).

Table 6. Descriptive score and acceptance of cheese with and without addition of rice bran oil (RBO) before and after ripening for 30 d

Sensory	Cheese without RBO	Cheese with RBO
Texture	3.00±0.85	3.07±1.03
Saltiness	2.73 ^a ±0.46	3.80 ^b ±0.56
Acidity	2.93±0.80	2.47±0.74
Bitterness	1.80 ^b ±0.56	1.67 ^a ±0.62
Flavor	2.67±0.92	2.67±0.98
Acceptance	3.00±0.85	3.07±0.88

^{ab} Different letters in the same row indicate significantly different ($p < 0.05$)

In a previous study, there were no significance difference in flavor (odor and taste) of the kashar cheese supplemented with palm oil and the control cheese, due to palmitic and oleic acids in the palm oil can not contribute to cheese odor and taste as much as short- and medium-chain fatty acids (Kavak and Karabiyik, 2019). Therefore, oleic, linoleic and palmitic acids - rich RBO (Oluremi *et al.*, 2013), also had no effect on cheese flavor. Food quality parameters and representation of consumer preferences for food that are very important are sensory properties. Some previous studies, supplementation of probiotics in cheese does not seem to affect its sensory characteristics (Albenzio *et al.*, 2013). In addition, Escobar *et al.* (2013) reported that probiotic supplementation of panela cheese had no effect on perceived taste or appearance. However, cheese produced with *Streptococcus thermophilus* plus

Lactobacillus casei showed highest sensory quality compared to combination of other probiotic bacteria, mainly in taste or appearance (Yerlikaya and Ozer, 2014).

CONCLUSION

Rice bran oil (RBO) had no negative effect on the flavor and acceptability of cheese and the growth of lactic acid bacteria, could increase cheese hardness, but could reduce the acidity and soluble protein in the cheese. However, the cheese with the addition of RBO ripened by using starter of *Lactobacillus casei* FNCC 0090 and *Streptococcus thermophilus* FNCC 0040 could be potentially classified as probiotic cheese which is beneficial for health.

Conflict of Interest

There are no conflict of interest including personal researchers or other

relationships with other personal or organizations that can influence their work.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Faculty of Animal Science, Universitas Gadjah Mada through “Hibah Penelitian Pascasarjana”.

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