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B I O D I V E R S I T A S Volume 21, Number 10, October 2020 Pages: 4591-4596 ISSN: 1412-033X E-ISSN: 2085-4722 DOI: 10.13057/biodiv /d211017 The quality of fermented goat milk produced by Pediococcus acidilactici BK01 on refrigerator temperature SRI MELIA1,?, INDRI JULIYARSI1, YULIANTI FITRI KURNIA1, YUDHA ENDRA PRATAMA2, DHIVA REZZY PRATAMA2 1Faculty of Animal Science, Universitas Andalas. Jl. Univ. Andalas, Limau Manis, Padang 25171, West Sumatra, Indonesia. Tel.: +62-751-71464, Fax.: +62-751-71464, ?email: srimelia75@ansci.unand.ac.id 2Animal Science Graduate Program, Universitas Andalas, Jl. Univ. Andalas, Limau Manis, Padang 25171, West Sumatra, Indonesia Manuscript received: 7 August 2020. Revision accepted: 11 September 2020. Abstract. Melia S, Juliyarsi I, Kurnia YF, Pratama YE, Pratama DR. 2020. The quality of fermented goat milk produced by Pediococcus acidilactici BK01 on refrigerator temperature. Biodiversitas 21: 4591 4596. This research aimed to determine the quality of chemical and microbiological milk of goats fermented with Pediococcus acidilactici BK01 with the storage time on refrigerator temperature. The method used is the experimental method of Completely Randomized

Design with five times the treatment of goat milk fermentation at the refrigerator temperature is over 0, 7, 14, 21, and 28 days with four repetitions. The results showed long-lasting storage of real effect ($P < 0.05$) on water content, pH, titratable acid, total lactic acid bacteria, and total plate count, but no noticeable effect ($P > 0.05$) on the protein levels and milk fat fermentation during the resulting storage. The value of moisture content during storage ranges between 85.88-84.92%, pH 4.48-4.28 , 3.69-3.49% fat content, 3.53-3.58% protein content, as well as the count of titratable acid ranging from 1.52-1.73%. The whole colonies of lactic acid bacteria reached between 9.106 log CFU/mL to 10.370 log CFU/mL, and the total plate count (TPC) value during storage experienced an increase from 2.653 log CFU/mL to 4.012 log CFU/mL. Based on the results of the study can be concluded that the milk of goat fermentation with P. acidilactici BK01 can maintain quality until the retention period of 28 days with the viability of lactic acid bacteria that meet the category as probiotics and still liked by the consumer. Keywords: Fermented milk, goat milk, lactic acid bacteria, Pediococcus acidilactici BK01 INTRODUCTION Fermented milk is one of the products processed by livestock through the fermentation stage involving lactic acid bacteria. Raw materials that are widely used to make fermented milk are cow's milk, buffalo milk, sheep's milk, and goat's milk. In developing countries, goat's milk has been widely consumed because it has therapeutic benefits, high nutritional value, and as a source of probiotic microorganisms (Selvajeyanthi et al. 2019). In the processing of milk fermentation, it utilizes goat milk because goat milk has many benefits for health including: can increase the absorption of Fe, efficient for the healing of asthma and tuberculosis, containing proteins, vitamin A, vitamin B (riboflavin), enzymes, and high minerals, has a great potential as a probiotic carrier (Cahyanti 2011), due to the presence of potentially lactic acid bacteria as probiotics (Melia et al. 2018) and does not cause diarrhea. With this specialty owned by goat Milk, it makes the milk a suitable medium for the growth of the decay and pathogenic microorganisms. Therefore, some ways to extend the shelf life of goat milk can be done by processing goat's milk into fermented milk (Kurnia et al. 2014). Fermentation of milk involves lactic acid bacteria, in which case it is used is Pediococcus acidilactici BK01. Excess strains of this P. acidilactici BK01 is a lactic acid bacteria. This is the result of isolation from Bekasam that has passed the selection as a probiotic bacteria (Melia et al. 2019). The selection of probiotic lactic acid bacteria among them can withstand the acidic condition of pH 2 and resistant to bile salts 0.3% and has antimicrobial activity against pathogenic bacteria, namely Escherichia coli and Staphylococcus aureus (Melia et al. 2018; Melia et al. 2019). Lactic acid bacteria can inhibit the growth of pathogenic bacteria (Escherichia coli O157: H7 and Staphylococcus aureus ATCC25923) because of the presence of anti-bacterial compounds produced such as bacteriocin (Melia et al. 2017) and bacteriocin also inhibit Listeria monocytogenes (Pato et al. 2020). Pediococcus acidilactici is a strain of lactic acid bacteria that is often used in the processing of dairy products because of its ability to produce acids and is beneficial for health. These bacteria have antimicrobial activity against several other bacteria and have the potential as probiotic (Holland et al. 2011). P. acidilactici produces a bacteriocin called Pediocin. Pediocin can inhibit the growth of positive gram bacteria and is also useful in inhibiting gram negative bacteria (Delves-Broughton 2012) and Pediocin PA-1, is a peptide that is an antimicrobial and is used as a bio preservative alternative in the food industry (Yusuf 2018). Pediocin, including a type of

thermostabile protein, is active in preventing food spoilage and inhibiting pathogenic microorganisms such as L. monocytogenes, Enterococcus faecalis, S. aureus, and Clostridium perfringens (Gálvez et al. 2014; Juneja et al. 2012). The purpose of this research is to measure the chemical quality and microbiology of fermented milk made from the starter Pediococcus acidilactici BK01 during storage at refrigerator temperature. MATERIALS AND METHODS Fermented milk was made using the milk of the Peranakan Etawa and the starter of P. acidilactici BK01 as much as 5%. The study used a Completely Randomized Design of 5 treatment of storage fermented goat milk, i.e., 0, 7, 14, 21, and 28 days with four repetitions at refrigerator temperature (4oC). Fermented milk production Goat milk was pasteurized at a temperature of 65-67oC for 30 minutes, then the milk temperature up to 37oC (Donkor et al. 2006). Starter P. acidilactici BK01 added as much as 5%, next incubated for 12 hours at 37oC temperature. Fermented milk of P. acidilactici BK01 was stored according to the treatment of 0, 7, 14, 21, and 28 days (modification of Melia et al. 2019). Determination of chemical parameters The determination of chemical parameters from fermented goat milk was moisture content, proteins, fats, Titratable Acid, and pH. Moisture content Moisture content was measured by the drying oven method (AOAC 2005). As much as 2 grams of fermented goat milk powder samples were prepared and put into a porcelain cup, which had measured its empty weight with an analytical balance, then each sample and cup was put into the oven and dried at 105°C for 4 hours. After it reached 4 hours drying oven, the sample was removed from the oven. Next, it was put in a desiccator for 15 minutes and weighed with an analytical balance. The moisture content was determined by calculating the weight subtraction between the samples before and after drying, and then was divided by the sample's weight, and finally multiplying it with 100% (AOAC 2005). Protein content A sample of 0.5 gram was put into a Kjeldahl flask, then it was added with 10 ml of concentrated H2SO4 and 0.5-gram selenium, then it was destructed until the color turned into a clear green then followed by distillation. The distillation results were then titrated using 0.1 N HCl until the color changed into purple and determined the blank form. Protein levels were calculated using the formula as described in Chang and Zhang (2017). Fat content Samples were weighed 1.5 g in filter paper (as weight A) and wrapped. The wrapped sample was dried in an oven at 105 °C for 4 hours then weighed with analytical balance (as weight B). The sample was put into a fat flask and extracted by using ether solvent for 6 hours. After that, the sample was dried in an oven at 105 ° C for 1 hour, and it was weighed with analytical balance (as weight C). The percentage of total fat content was determined by calculating the difference weight between dried and extracted dried samples. Then it was divided by the sample weight (AOAC 2005). Testing of pH and titratable acid pH testing using HANNA Romania the calibrated pH meter with a buffer of pH 4 and pH 7 (AOAC 2012). The titratable acid was measured by mixing fermented milk with 10 mL of aquadest. and it is calculated Mangifera pajang Fibrous Polysaccharides", Journal of Food Science, 2012.">with 0.1 N NaOH using phenolphthalein indicator until it showed pink (Parmar 2003). Calculation of total plate count and total lactic acid bacteria The estimate of Total Plate Count (TPC) Mangifera pajang Fibrous Polysaccharides", Journal of Food Science, 2012.">Paseephol and Sherkat (2009) and Total Mangifera pajang Fibrous Polysaccharides", Journal of Food Science, 2012.">lactic acid bacteria and measured based on Harley and Presscot (2002). Sensory evaluation All fermented milk samples stored for 0, 7, 14, 21, and 28 days were

evaluated for taste, aroma, and texture preferences by 25 staff members of the Animal Science Department. A five-point hedonic scale was utilized in this study (1 = dislike extremely; 2 = dislike slightly; 3 = neither like nor dislike; 4 = like slightly; 5 = like extremely). Statistical analysis All the data obtained were analyzed statistically using experimental design, five treatments, and four repetitions. Data that has significant influence ($P < 0.05$) was continued with the Duncan's Multiple Range Test using SPSS software statistic 19. RESULTS AND DISCUSSION The research results showed that the longer the storage at the refrigerator temperature showed a significant effect ($P < 0.05$) on water content, but showed no significant effect ($P > 0.05$) to the protein and fat value in the resulting fermented goat milk. Moisture content Based on Table 1, It was known that there was a difference in the milk content of fermented goat after stored up to 28 days ($P < 0.05$). The results of water content analysis during storage decreased to 84.92% up to 7 days of storage and not significant after 14 days. This was suspected because goat milk ferments during storage absorbed water from its environment. The longer the storage water content will continue to increase, even though at the start of water content, storage can be decreased. The results of this study are in line with Melia et al. (2019), which was the moisture content of fermented goat milk containing Lactobacillus fermentum strains NCC2970 which range from 85% after storage for 15 days. MELIA et al. - The quality of fermented goat milk 4593 This is supported by the statement of Herawati (2008), According to Usmiati et al. (2011), the pH value of that changes in the water content of fermented milk can be fermented milk will further decrease with the length of influenced by the temperature and humidity of the room storage in cold temperatures. It was added by Melia et al. during storage and changed in water content in the product (2019) that the old storage of fermented milk using the was a factor that is very influential to the decline in the starter Lactobacillus fermentum NCC2970 at a temperature quality of food products. of 4oC able to lower the pH value. The pH value obtained in this study ranged from 4.28 – Protein content 4.48. The results of this research were not much different Protein content of fermented goat milk (Table 1) during from the Melia et al. (2019) research, where the pH of storage shows no significant effect ($P > 0.05$) against the fermented milk is ranging from 4.0-4.9 so that it can be quality of the resulting protein. It was caused by the storage concluded that the pH value of milk fermented goat milk P. of fermented milk of P. acidilactici BK01, which was done acidilactici BK01 which was stored at the temperature of at the refrigerator temperature until the storage of the 28th the refrigerator until the storage of the 28th day, still able to day has not influenced the resulting protein levels because maintain its quality. Abdel-Hamid et al. (2018), the pH it has not shown the sign will expire. From Table 1, it can value of fermented milk made from Lb. casei ATCC 393 be seen as protein levels fermented milk until the storage of decreased during storage at cold temperatures of 4.69 to 28 days still meet the quality standard of Indonesian 4.04 for 28 days. Furthermore, Bosnea et al. (2017) National Standard 2981:2009 fermented milk, i.e., a reported that Lb. casei ATCC 393 also decreased after 60 minimum of 2.7%. These protein levels are lower than days of storage (pH 4.27 to 4.03). This is caused by the Melia et al. (2019), which is about 4%. This is likely due to ability of Lb. casei ATCC 393 produces organic acids the source of milk from different farms and the difference during storage (Terpou et al. 2017). in the type of feed given, but the same as research with Güneş Bayır et al. (2019), which reported that the protein Titratable acid (TTA)

content of yogurt with the addition of cinnamon was The result of titratable acid of fermented goat milk 3.54%. indicated a significant effect ($P < 0.05$) (Figure 2.) showed the more extended the fermented goat milk product was Fat content stored, and then the total lactic acid was increasing as the Fat is a component of milk that can provide higher pH decrease occurred. A low pH would be a suitable energy than both protein and carbohydrates. Based on the environment for P. acidilactici BK01 (which is a results of the research known the highest fat content found homofermentative type bacteria) to grow and produce in the 0-day storage was 3.69%, and the lowest on the 28th metabolites of lactic acid. It was by the opinions of Mal day of storage was 3.49%, but statistically showed a (2013) and Magalhaes et al. (2011) stating that the length difference that was not significant ($P > 0.05$). This was in of storage will affect the total lactic acid and generally, line with the protein results gained that up to 28 days of lactic acid bacteria can be distinguished into two groups, storage have not given a noticeable effect on the resulting namely homofermentative and heterofermentative, where a fat levels. This fat content is almost the same as the fat homofermentative group of fermented glucose produces content of yogurt with the addition of cinnamon, 3.2-3.3% lactic acid as the only product, like Pediococcus and some (Güneş Bayır et al. 2019). If compared with the level of fat Lactobacillus. according to the quality standard fermented milk prescribed Based on the results of the research of fermented goat Indonesian National Standard 2981:2009, that was at least milk Paediococcus acidilactici BK01 stored at refrigerator 3%, then the milk of fermented goat P. acidilactici BK01 temperature has qualified the quality of fermented milk produced was worth consuming. Indonesian National Standard 2981:2009. The value of TTA fermented milk obtained in the research ranged from pH 1.52%-1.73% for 28 days of the storage period. A decrease The result of the analysis pH of fermented goat milk in pH causes the increase in titratable acid during storage, indicated a significant effect ($P < 0.05$) between the which lactic acid bacteria ferment lactose to produce lactic duration of storage at the refrigerator temperature (Figure acid (Costa et al. 2016). The results of the study were 1.). The longer the storage time was done, the pH value almost identical to the Melia et al. Research. (2019) on the was decreasing. The decrease in the pH value was due to quality, viability, and anti-bacterial properties of the the activity of lactic acid bacteria derived from the starter Lactobacillus fermentum NCC2970 in goat milk P. acidilactici BK01 used in the manufacture of fermented fermentation at a temperature of 4oC with a TTA rate of goat milk. This bacteria will ferment lactose and will 0.80-1.52 during 15-day storage. Thus, the value of the produce lactic acid, resulting in a decrease in pH. It is by results of this research has fulfilled the criteria as the opinions of Costa et al. (2016) that lactic acid bacteria fermented milk. Abdel-Hamid et al. (2018) also reported ferment lactose into glucose and galactose, then the glucose that fermented milk is made from Lb. casei 393, increased was converted into lactic acid. during storage for 21 days in cold temperatures. This is the Also, a decrease in the pH of fermented goat milk same as the research of Sah, Vasiljevic, McKechnie), during storage as the total acid increase of fermented milk which uses Lb. casei 393 to produce probiotic yogurt. was produced. The higher the whole level of fermented Dimitrellou et al. (2016) also explain the increase in milk acid than the lower the pH, as seen in this research. titratable acid during storage for 28 days (0.7 to 0.9) along with a decrease in pH. 4,5 4,48 4,4 4,35 4,35 pH 4,3 4,28 4,2 4,23 4,1 0 7 14 21 28 Storage Time (days) Figure 1. pH of fermented goat milk Pediococcus acidilactici BK01 Table 1.

Chemical analysis of fermented goat milk of Pediococcus acidilactici BK01 Storage time Water (%) Protein (%) Fat (%) (days) 0 85.34ab 3.53 3.69 7 84.92b 3.50 3.66 14 85.88a 3.55 3.53 21 85.57ab 3.56 3.57 28 85.51ab 3.58 3.49 Note: Means within a column with different superscripts are significantly ($P < 0.05$) Figure 2. Titratable acid of fermented goat milk Pediococcus acidilactici BK01 Table 2. Total lactic acid bacteria and total plate count of fermented goat milk Pediococcus acidilactici BK01 Storage time LAB. TPC (days) (Log CFU/mL) (Log CFU/mL) 0 10.376a 2.653d 7 9.798bc 3.891b 14 9.854b 3.322d 21 9.833b 3.633c 28 9.106c 4.012a Note: Means within a column with different superscripts are significantly ($P < 0.05$) Based on the data in Table 2, the duration of storage Total plate count of fermented goat milk was significant ($P < 0.05$), decreasing the total lactic acid The number of aerobic bacterial colonies in fermented bacteria of fermented goat milk. The longer it was stored, goat milk significantly ($P < 0.05$) increasing with the more the total lactic acid bacteria will decrease. Many dead lactic extended the storage period. The lowest amount of aerobic acid bacteria caused this, due to the more extended storage bacterial colonies in fermented goat milk was found on the conditions (28 days), resulting in reduced nutrient 0-day storage day of 2.653 log CFU/mL and the highest on availability for P. acidilactici BK01 to produce lactic acid. 28-day storage of 4.012 log CFU/mL. This condition was Same with Abdel-Hamid et al. (2018), fermented milk Lb. in line with the total lactic acid bacteria that the longer the casei ATCC 393, which is stored for up to 28 days in cold storage was decreasing its number. It was closely related to temperatures, has a number of lactic acid bacterial cells the 4 phases experienced by lactic acid bacteria. The results above 9 log CFU g-1. Bosnea et al. (2017), Dimitrellou et of this research still meet the existing SNI. The maximum al. (2016), and Sah et al. (2015) also reported a decrease in limit of its bacterial contamination, according to SNI, is 1 x the number of bacterial cells after 60 days of storage at 106 CFU/mL. cold temperatures. Whereas Terpou et al. (2017) stated the decline in bacterial cells occurred after 30 days of storage. Sensory evaluation The total decrease in Lactic Acid Bacteria in this study The sensory evaluation for P. acidilactici BK01 of was also closely related to the decline in pH occurring. fermented milk can be seen in Figure 3, which shows that This was by the opinion of (Prasanna et al. 2013), the the taste, aroma, and texture of fermented milk stored for decrease in the number of lactic acid bacteria was closely 28 days did not show a significant effect ($P > 0.05$). This associated with the reduction of pH products due to the means that storage time does not affect the taste, aroma, accumulation of organic acids as a result of the metabolites and texture of fermented milk. The level of consumer of fermentation. pH decline will inhibit even stopping the preference in terms of taste (3.59-3.70), flavor (3.70-3.80), growth of lactic acid bacteria itself. and texture (3. 70-3.84). The average result of the The decrease in the amount of Lactic Acid Bacteria in assessment by the panelists reached a score of 4. namely this research was similar to the study by Melia et al. like slightly. The same thing was shown by Ammar et al. (2019). Where fermented milk was kept, then lactic acid (2019), bio-yogurt with the addition of honey, is still bacteria decreased to 4.8 x 108CFU/mL. Further explained favored by consumers after being stored for 15 days. that it was caused by reduced lactose as a significant source Furthermore, Huang et al. (2020) reported the fat free of carbon by bacteria. Compared with Indonesian National buffalo set of yogurt with the addition of 3% polydextrose Standard 2981:2009, the minimum amount of total Lactic

stored for 21 days did not affect consumer acceptance. Acid Bacteria in fermented milk is 107CFU/mL. MELIA et al. – The quality of fermented goat milk 4595 Figure 3. Consumer acceptability scores of fermented milk In conclusion, extended storage in cold temperatures can affect the quality of milk of the fermented goat Pediococcus acidilactici BK01. The storage of goat milk fermentation for 28 days in cold temperatures can still maintain the number of lactic acid bacteria that meet the criteria of probiotics that are 9.106 log CFU/mL with a titratable acid of 1.73% and pH of fermented milk goat reaches 4.28, with a value of TPC 4.012 log CFU/ ml, protein content 3.57%, fat content 3.49%, and moisture content 85.51% that still liked by the consumer. ACKNOWLEDGEMENTS This research was supported by research cluster publications to professors (Project No. T/ 8 //UN.16.17/ PP1.01.03/Pangan-PTU-KRP2GB/LPPM/2020) LPPM Andalas University, Padang, Indonesia. REFERENCE Abdel-Hamid M, Romeih E, Gamba RR, Nagai E, Suzuki T, Koyanagi T, Enomoto T. 2018. The biological activity of fermented milk produced by Lactobacillus casei ATCC 393 during cold storage. Intl Dairy J 9: 1-8. Ammar ET, Ismail MM, Khalil AEWE, Eid MZ. 2019. Impact of fortification with honey on some properties of bio-yoghurt. J Microbiol Biotechnol Food Sci 2019: 503-508. AOAC. 2005. Official Methods of Analysis. Association of Official Analytical Chemists. Benjamin Franklin Station, Washington DC. AOAC. 2012. Official Method of Analysis: Association of Analytical Chemists. 19th Edition. AOAC, Washington DC. Bosnea LA, Kopsahelis N, Kokkali V, Terpou A, Kanellaki M. 2017. Production of a novel probiotic yogurt by incorporation of L. casei enriched fresh apple pieces, dried raisins, and wheat grains. Food Bioprod Process 102: 62-71 Cahyanti AN. 2011.Viabilitas probiotik Lactobacillus casei pada yoghurt susu kambing selama penyimpanan beku. J Teknologi Pertanian 12: 176-180. [Indonesian] Costa MP, da Silva Frasao B, da Costa Lima BRC, Rodrigues BL, Junior CAC. 2016. Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurt fermentation. Talanta 152: 162-170. Chang SKC, Zhang Y. 2017. Protein Analysis. In: Food Analysis. Springer, Cham. DOI: 10.1007/978-3-319-45776-536. Deives-Broughton J. 2012. Natural antimicrobials as additives and ingredients for the preservation of foods and beverages. In: Baines D, Seal R (eds.). Natural Food Additives, Ingredients, and Flavourings. Woodhead Publishing, UK. Dimitrellou D, Kandylis P, Petrović T, et al. 2016. Survival of spray-dried microencapsulated Lactobacillus casei ATCC 393 in simulated gastrointestinal conditions and fermented milk. LWT-Food Sci Technol 71: 169-174. Donkor ON, Henriksson A, Vasiljevic T, Shah NP. 2006. Effect of acidification on the activity of probiotics in yoghurt during cold storage. Intl Dairy J 16 (10): 1181-1189. Gálvez A, López RL, Pulido RP, Burgos MJG. 2014. Application of lactic acid bacteria and their bacteriocins for food biopreservation. Food Biopreservation, New York. GüneşBayır A, Bilgin MG. 2019. The effect of cinnamon on microbiological, chemical, and sensory analyses of probiotic yoghurt. Bezmialem Sci 7 (4): 311-316. Harley, Prescot. 2002. Laboratory Exercise in Microbiology. McGraw Hill Publisher, USA. Herawati H. 2008. Penentuan umur simpan pada produk pangan. Jurnal Litbang Pertanian 27 (4): 124-130. Holland R, Crow V, Curry B. 2011. Lactic acid bacteria Pediococcus spp. In: Fuquay JW (ed.). Encyclopedia of Dairy Sciences. Academic Press, USA. Huang L, Abdel-Hamid M, Romeih E, Zeng, Yang P, Walker G, Li L. 2020. Textural and organoleptic properties of fat-free buffalo yogurt as affected by polydextrose. Intl J Food Prop 23: 1-8. Juneja VK, Dwivedi HP, Yan X. 2012. Novel natural food antimicrobials. Annu Rev Food Sci Technol 3:

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