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28831-1 , REV 28 AGT_6565-Article Text-27552-1-4-20200811	September 4, 2020

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The quality of fermented goat milk produced by *Pediococcus acidilactici BK01* on refrigerator temperature

Abstract. 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 5 times the treatment of goat milk fermentation at the refrigerator temperature is over 0, 7, 14, 21, and 28 days with 4 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%, 4.48-4.28 pH, 3.69-3.49% fat content, 3.53-3.58% protein levels, as well as the count of Total Titrated Acid ranging from 1.52-1.73%. The whole colonies of lactic acid bacteria reached between 237.8 x 10^8-12.75 x 108, and the total plate count (TPC) value during storage experienced an increase from 4.5 x 10^2 CFU/ml to 102.8 x 10^2 CFU/ML. Based on the results of the study can be concluded that the milk of goat fermentation with *Pediococcus 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 meet the standard of fermented milk Indonesian National Standard 2981:2009

Keywords: goat milk, *Pediococcus acidilactici BK01*, fermented milk, and lactic acid bacteria

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 *Pediococcus Acidilactici BK01* is a lactic acid bacteria. This is the result of isolation from Bekasam that has passed the selection as a probiotic bacteria. 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 *Escherechia coli* and *Staphylococcus aereus* (Melia, et al. 2018 and 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 inhibits *Listeria monocytogenes* (Pato et al., 2020.

Comment [L1]: Reference?

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 probotic (Holland et. al., 2011). Pediococcus acidilactici, produces a bacteriocin called Pediocin. Pediocin is able to inhibit the growth of positive gram bacteria and is also effective in inhibiting gram negative bacteria (Delves-Broughton, 2012) and Pediocin PA-1, is a peptide that is an antimicrobial and is used as a biopreservative 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.

MATERIAL AND METHOD

Fermented milk was made using the milk of the *Peranakan Etawa* and the starter of *Pediococcus 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.

Fermented Milk Production (modification of Melia et al., 2019)

Goat milk was pasteurized at a temperature of 65-67^{o}C for 30 minutes, then the milk temperature up to 37^{o}C (Donkor et al., 2006). Starter *Pediococcus Acidilactici BK01* added as much as 5%, next incubated for 12 hours at 37^{o}C temperature. Fermented milk of *Pediococcus acidilactici BK01* stored according to the treatment of 0, 7, 14, 21, and 28 days.

Proximate Analysis

Analysis of proximate from fermented goat milk was moisture content, proteins, fats, water content testing methods, proteins, fats, Titratable Acid, and pH were conducted following AOAC (2012).

Testing of pH and Titratable Acid

pH testing using HANNA Romania the calibrated pH meter with a buffer of pH4 and pH 7 (AOAC, 2012). The titratable acid was measured by mixing fermented milk with 10 mL of aquades, and it is calculated with 0.1 N NaOH using phenolphthalein indicator until it showed pink (Parmar, 2003)

Calculation of Total Plate Count and Total Lactic Acid Bacteria

Calculation of Total Plate Count (TPC) Paseephol and Sherkat (2009) and Total lactic acid bacteria and measured based on Harley and Presscot (2002).

Statistical Analysis

All the data obtained were analyzed statistically, which was done in as much as 4 repetitions. Data that has significant influence (P < 00.5) was continued with the Duncan's Multiple Range Test using SPSS software statistic 19.

RESULT AND DISCUSSION

Table 1. Proximate Analysis of Fermented Goat Milk of *Pediococcus acidilactici BK01*

Storage Time (days)	Water (%)	Protein (%)	Fat (%)
0	85,34^{ab}	3,53	3,69
7	84,92^{b}	3,50	3,66
14	85,88^{a}	3,55	3,53
21	85,57^{ab}	3,56	3,57
28	85,51^{ab}	3,58	3,49

Means within a column with different superscripts are signiûcantly different (P < 0.05)

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.

Water

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 increased on the storage on the 14th to 28th 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

Comment [U2]: How about proximate analysis????

Comment [U3]: This is a proximate analys

Comment [U4]: How much concentration starter (Pediococcus) which is inoculated in the milk

Comment [U5]: Why the other chemical components not analyzed (ash and carbohydrates)?

Comment [U6]: The significant result only on the day of 7 and 14.

Comment [U7]: Why would you use proximate analysis on protein during storag The Kjeldahl method calculates the total N s that any protein that may be degraded duri storage is also counted so that there is no changes the protein during storage.

Comment [U8]: Significant in water conte : between 7 and 14 d , why why do you say increased from day 14 to day 28???

This is supported by the statement of Herawati (2008), that changes in the water content of fermented milk can be influenced by the temperature and humidity of the room during storage and changed in water content in the product was a factor that is very influential to the decline in the quality of food products.

Comment [U9]: How about syneresis the fermented milk product that is important factor for decreasing the quality of fermented milk product during storage ??? Why don't you analyze syneresis?

Protein

Protein levels of fermented goat milk (table 1) during storage show no significant effect (P > 0.05) against the quality of the resulting protein. It was caused by the storage of fermented milk of *Pediococcus acidilactici BK01*, which was done at the refrigerator temperature until the storage of the 28th day has not influenced the resulting protein levels because it has not shown the sign will expire. From table 1 can be seen protein levels fermented milk until the storage of 28 days still meet the quality standard of Indonesian National Standard 2981:2009 fermented milk, ie, minimum 2.7%. These protein levels are lower than Melia et al. (2019), which is about 4%, this is likely due to the source of milk from different farms and the difference in the type of feed given, but the same as research with Güneş Bayır et al. (2019) which reported that the protein content of yogurt with the addition of cinnamon was 3.54%.

Fat

Fat is a component of milk that can provide higher energy than both protein and carbohydrates. Based on the results of the research known the highest fat content found in the 0-day storage was 3.69%, and the lowest on the 28th day of storage was 3.49%, but statistically showed a difference that was not significant (P > 0.05). This was in line with the protein results gained that up to 28 days of storage have not given a noticeable effect on the resulting fat levels. This fat content is almost the same as the fat content of yogurt with the addition of cinnamon, 3.2 - 3.3% (Güneş Bayır et al., 2019). If compared with the level of fat according to the quality standard fermented milk prescribed Indonesian National Standard 2981:2009, that was at least 3%, then the milk of fermented goat *Pediococcus acidilactici BK01* produced was worth consuming.

pH

The result of the analysis pH of fermented goat milk indicated a significant effect (P. < 0.01) between the duration of storage at the refrigerator temperature (Figure 1.). The longer the storage time was done, the pH value was decreasing. The decrease in the pH value was due to the activity of lactic acid bacteria derived from the starter *Pediococcus Acidilactici BK01* used in the manufacture of fermented goat milk. This bacteria will ferment lactose and will produce lactic acid, resulting in a decrease in pH. It is by the opinions of Costa et al. (2016) that lactic acid bacteria ferment lactose into glucose and galactose, then the glucose was converted into lactic acid.

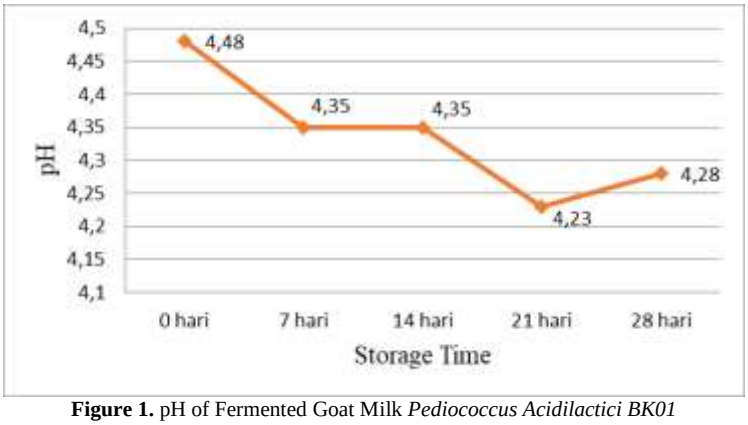

Figure 1. pH of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Also, a decrease in the pH of fermented goat milk during storage as the total acid increase of fermented milk was produced. The higher the whole level of fermented milk acid than the lower the pH, as seen in this research. According to Usmiati et al. (2011), the pH value of fermented milk will further decrease with the length of storage in cold temperatures. It was added by Melia et al. (2019) that the old storage of fermented milk using the starter Lactobacillus fermentum NCC2970 at a temperature of 4^{o}C able to lower the pH value.

The pH value obtained in this study ranged from 4.28 – 4.48. The results of this research were not much different from the Melia et al. (2019) research, where the pH of fermented milk is ranging from 4.0 − 4.9, so that it can be concluded that the pH value of milk fermented goat milk *Pediococcus acidilactici BK01* which was stored at the temperature of the refrigerator until the storage of the 28th day, still able to maintain its quality. Abdel-Hamid et al. (2018), the pH value of fermented milk made from Lb. casei ATCC 393 decreased during storage at cold temperatures of 4.69 to 4.04 for 28 days. Furthermore Bosnea, Kopsahelis, Kokkali, Terpou, and Kanellaki (2017) reported that Lb. probiotic yogurt casei ATCC 393 also decreased after 60 days of storage (pH 4.27 to 4.03). This is caused by the ability of Lb. casei ATCC 393 produces organic acids during storage (Terpou et al., 2017)

Total Titratable Acid (TTA)

The result analysis of total lactic acid (Figure 2.) showed the more extended the fermented goat milk product was stored, and then the total lactic acid was increasing as the pH decrease occurred. A low pH would be a suitable environment for *Pediococcus Acidilactici BK01* (which is a homofermentative type bacteria) to grow and produce metabolites of lactic acid. It was by the opinions of Mal (2013) and Magalhaes et al. (2011) stating that the length of storage will affect the total lactic acid and generally, lactic acid bacteria can be distinguished into two groups namely homofermentative and heterofermentative, where a homofermentative group of fermented glucose produces lactic acid as the only product, like *Pediococcus* and some *Lactobacillus.*

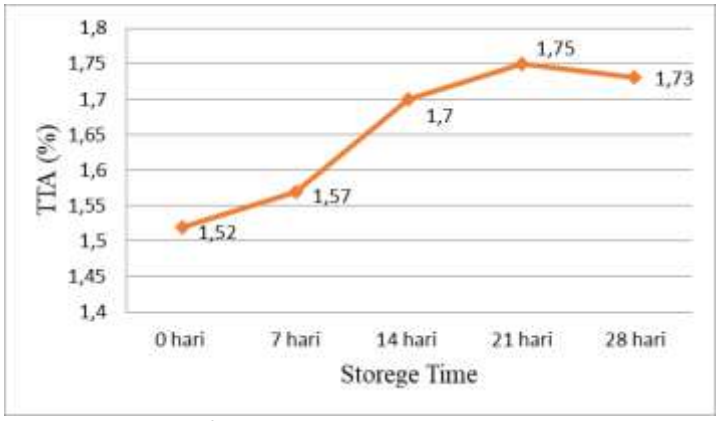

Figure 2. Titratable Acid of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Based on the results of the research of fermented goat milk *Paediococcus acidilactici BK01* stored at refrigerator temperature has qualified the quality of fermented milk Indonesian National Standard 2981:2009 IE 0.5-2. The value of T.T.A. fermented milk obtained in the research ranged from 1.52% − 1.73% for 28 days of the storage period. The results of the study were almost identical to the Melia et al. Research. (2019) on the quality, viability, and anti-bacterial properties of the *Lactobacillus fermentum* NCC2970 in goat milk fermentation at a temperature of 4^{o}C with a T..T. A rate of 0.80 – 1.52 during 15-day storage. Thus, the value of the results of this research has fulfilled the criteria as fermented milk. Abdel-Hamid et al. (2018) also reported that fermented milk made from Lb. casei 393, increased during storage for 21 days in cold temperatures. This is the same as the research of Sah, Vasiljevic, McKechnie), which uses Lb. casei 393 to produce probiotic yogurt. Dimitrellou et al. (2016), also explain the increase in titratable acid during storage for 28 days (0.7 to 0.9) along with a decrease in pH.

Table 2. Total Lactic Acid Bacteria and Total Plate Count of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Storage Time (days)	Total B.A.L. (Log CFU/ml)	TPC (Log CFU/ml)
0	10,376^{a}	2,653^{d}
7	9,798^{bc}	3,891^{b}
14	9,854^{b}	3,322^{d}
21	9,833^{b}	3,633^{c}
28	9,106^{c}	4,012^{a}

Means within a column with different superscripts are signiûcantly different (P < 0.05)

Comment [U10]: is it possible that the to
lactoic acid bacteria (LAB) is higher than the
TPC?
Whereas TPC is the total microorganism wh
LAB is part of the TPC

Based on the data in table 3, the duration of storage was significant ($P < 0.05$), decreasing the total lactic acid bacteria of fermented goat milk. The longer it was stored, the total lactic acid bacteria will decrease. Many dead lactic acid bacteria caused this, due to the more extended storage conditions (28 days), resulting in reduced nutrient availability for *Pediococcus acidilactici BK01* to produce lactic acid. Same with Abdel-Hamid et al. (2018), Lb. fermented milk casei ATCC 393, which is stored for up to 28 days in cold temperatures, has a number of lactic acid bacterial cells above 9 log cfu g^{-1}. Bosnea et al. (2017), Dimitrellou et al. (2016), and Sah et al. (2015) also reported a decrease in the number of bacterial cells after 60 days of storage at cold temperatures. Whereas Terpou et. (2017), stated the decline in bacterial cells occurred after 30 days of storage.

The total decrease in Lactic Acid Bacteria in this study was also closely related to the decline in pH occurring. This was by the opinion of (Prasanna et al., 2013), the decline in the number of lactic acid bacteria was closely associated with the reduction of pH products due to the accumulation of organic acids as a result of the metabolites of fermentation so that rapid pH decline will inhibit even stopping the growth of lactic acid bacteria itself.

The decrease in the amount of Lactic Acid Bacteria in this research was similar to study by Melia et al. (2019). Where fermented milk was kept, then lactic acid bacteria decreased to 4.8×10^8 CFU/ml. Further explained that it was caused by reduced lactose as a significant source of carbon by bacteria. Compared with Indonesian National Standard 2981:2009, the minimum amount of total Lactic Acid Bacteria in fermented milk is 10^7 CFU/ml.

Total Plate Count of Fermented Goat Milk

The number of aerobic bacterial colonies in fermented goat milk tended to increase with the more extended the storage period. The lowest amount of aerobic bacterial colonies in fermented goat milk was found on the 0-day storage day of 4.5×10^2 CFU/ml and the highest on 28-day storage of 102.8×10^2. This condition was in line with the total Lactic Acid Bacterial that the longer the storage was decreasing its number. It was closely related to the 4 phases experienced by lactic acid bacteria. The results of this research still meet the existing S.N.I. with the maximum limit of its bacterial contamination, according to S.N.I., is 1×10^6 CFU/ml.

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 12.75×10^8 CFU/ml with an acidity rate of 1.73 and pH of fermented milk goat reaches 4.28, with a value of TPC 102.8×10^2 CFU/ ml, protein content 3.57, fat content 3.49, and water content 85.51 that still fulfill Indonesian National Standard 2981: 2009 fermented milk

Comment [U11]: It is still acceptable for consumers, whereas you did not show the sensory analysis.

ACKNOWLEDGMENTS

This research was supported by research cluster publications to professors (Project No. T/8//UN.16.17/PPT.01.03/Pangan-PTU-KRP2GB/LPPM/2020) LPPM Andalas University.

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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 probotic (Holland et. al., 2011). Pediococcus acidilactici, produces a bacteriocin called Pediocin. Pediocin is able to inhibit the growth of positive gram bacteria and is also effective in inhibiting gram negative bacteria (Delves-Broughton, 2012) and Pediocin PA-1, is a peptide that is an antimicrobial and is used as a biopreservative alternative in the food industry (Yusuf, 2018). Pediocin, including a type of thermostable 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).

Comment [p1]: Abbreviation needed

Comment [p2]: Italic

Comment [p3]: Italic, please check for all latin's name

Comment [p4]: Need to empahsized the importance of storage in fermented milk an also need some citation abot the storage of fermented milk

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.

MATERIAL AND METHOD

Fermented milk was made using the milk of the *Peranakan Etawa* and the starter of *Pediococcus 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.

Comment [p5]: Need explanation about t media that used for fermented milk and als the temerature of the refigerator due to the are many refrigerator with different type

Fermented Milk Production (modification of Melia et al., 2019)

Goat milk was pasteurized at a temperature of 65-67^{o}C for 30 minutes, then the milk temperature up to 37^{o}C (Donkor et al., 2006). Starter *Pediococcus Acidilactici BK01* added as much as 5%, next incubated for 12 hours at 37^{o}C temperature. Fermented milk of *Pediococcus acidilactici BK01* stored according to the treatment of 0, 7, 14, 21, and 28 days.

Comment [p6]: Reconsider the sentence

Comment [p7]: Which container that use for fermented milk, it will influnce the self li !

Proximate Analysis

Analysis of proximate from fermented goat milk was moisture content, proteins, fats, water content testing methods, proteins, fats, Titratable Acid, and pH were conducted following AOAC (2012).

Comment [p8]: Reconsider the sentences

Comment [p9]: All the detail prochedure needed and also the formulation

Testing of pH and Titratable Acid

pH testing using HANNA Romania the calibrated pH meter with a buffer of pH4 and pH 7 (AOAC, 2012). The titratable acid was measured by mixing fermented milk with 10 mL of aquades, and it is calculated with 0.1 N NaOH using phenolphthalein indicator until it showed pink (Parmar, 2003)

Calculation of Total Plate Count and Total Lactic Acid Bacteria

Calculation of Total Plate Count (TPC) Paseephol and Sherkat (2009) and Total lactic acid bacteria and measured based on Harley and Presscot (2002).

Statistical Analysis

All the data obtained were analyzed statistically, which was done in as much as 4 repetitions. Data that has significant influence (P < 00.5) was continued with the Duncan's Multiple Range Test using SPSS software statistic 19.

RESULT AND DISCUSSION

Table 1. Proximate Analysis of Fermented Goat Milk of *Pediococcus acidilactici BK01*

Storage Time (days)	Water (%)	Protein (%)	Fat (%)
0	85,34^{ab}	3,53	3,69
7	84,92^{b}	3,50	3,66
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Means within a column with different superscripts are signiûcantly different (P < 0.05)

Comment [p10]: significantly

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.

Water

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 increased on the storage on the 14th to 28th 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

This is supported by the statement of Herawati (2008), that changes in the water content of fermented milk can be influenced by the temperature and humidity of the room during storage and changed in water content in the product was a factor that is very influential to the decline in the quality of food products.

Protein

Protein levels of fermented goat milk (table 1) during storage show no significant effect (P > 0.05) against the quality of the resulting protein. It was caused by the storage of fermented milk of *Pediococcus acidilactici BK01*, which was done at the refrigerator temperature until the storage of the 28th day has not influenced the resulting protein levels because it has not shown the sign will expire. From table 1 can be seen protein levels fermented milk until the storage of 28 days still meet the quality standard of Indonesian National Standard 2981:2009 fermented milk, ie, minimum 2.7%. These protein levels are lower than Melia et al. (2019), which is about 4%, this is likely due to the source of milk from different farms and the difference in the type of feed given, but the same as research with Güneş Bayır et al. (2019) which reported that the protein content of yogurt with the addition of cinnamon was 3.54%.

Fat

Fat is a component of milk that can provide higher energy than both protein and carbohydrates. Based on the results of the research known the highest fat content found in the 0-day storage was 3.69%, and the lowest on the 28th day of storage was 3.49%, but statistically showed a difference that was not significant (P > 0.05). This was in line with the protein results gained that up to 28 days of storage have not given a noticeable effect on the resulting fat levels. This fat content is almost the same as the fat content of yogurt with the addition of cinnamon, 3.2 - 3.3% (Güneş Bayır et al., 2019). If compared with the level of fat according to the quality standard fermented milk prescribed Indonesian National Standard 2981:2009, that was at least 3%, then the milk of fermented goat *Pediococcus acidilactici BK01* produced was worth consuming.

pH

The result of the analysis pH of fermented goat milk indicated a significant effect (P. < 0.01) between the duration of storage at the refrigerator temperature (Figure 1.). The longer the storage time was done, the pH value was decreasing. The decrease in the pH value was due to the activity of lactic acid bacteria derived from the starter *Pediococcus Acidilactici BK01* used in the manufacture of fermented goat milk. This bacteria will ferment lactose and will produce lactic acid, resulting in a decrease in pH. It is by the opinions of Costa et al. (2016) that lactic acid bacteria ferment lactose into glucose and galactose, then the glucose was converted into lactic acid.

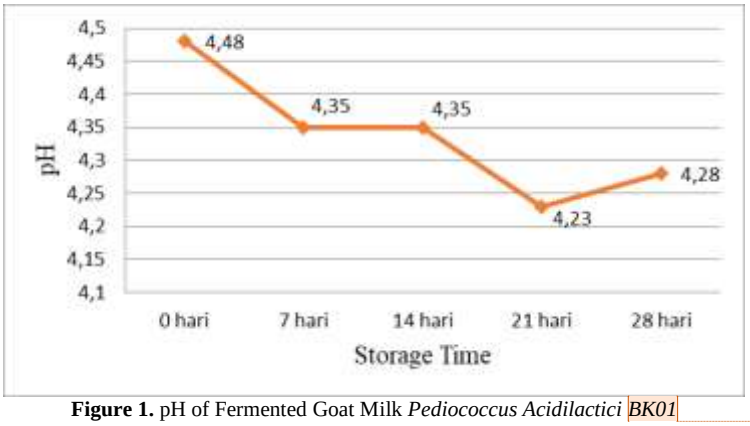

Figure 1. pH of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Also, a decrease in the pH of fermented goat milk during storage as the total acid increase of fermented milk was produced. The higher the whole level of fermented milk acid than the lower the pH, as seen in this research. According to Usmiati et al. (2011), the pH value of fermented milk will further decrease with the length of storage in cold temperatures. It was added by Melia et al. (2019) that the old storage of fermented milk using the starter Lactobacillus fermentum NCC2970 at a temperature of 4^{o}C able to lower the pH value.

Comment [p11]: please add more discussion from the result

Comment [p12]: Please change....hari. wi day in Figure 1

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The pH value obtained in this study ranged from 4.28 – 4.48. The results of this research were not much different from the Melia et al. (2019) research, where the pH of fermented milk is ranging from 4.0 − 4.9, so that it can be concluded that the pH value of milk fermented goat milk *Pediococcus acidilactici BK01* which was stored at the temperature of the refrigerator until the storage of the 28th day, still able to maintain its quality. Abdel-Hamid et al. (2018), the pH value of fermented milk made from Lb. casei ATCC 393 decreased during storage at cold temperatures of 4.69 to 4.04 for 28 days. Furthermore Bosnea, Kopsahelis, Kokkali, Terpou, and Kanellaki (2017) reported that Lb. probiotic yogurt casei ATCC 393 also decreased after 60 days of storage (pH 4.27 to 4.03). This is caused by the ability of Lb. casei ATCC 393 produces organic acids during storage (Terpou et al., 2017)

Total Titratable Acid (TTA)

The result analysis of total lactic acid (Figure 2.) showed the more extended the fermented goat milk product was stored, and then the total lactic acid was increasing as the pH decrease occurred. A low pH would be a suitable environment for *Pediococcus Acidilactici BK01* (which is a homofermentative type bacteria) to grow and produce metabolites of lactic acid. It was by the opinions of Mal (2013) and Magalhaes et al. (2011) stating that the length of storage will affect the total lactic acid and generally, lactic acid bacteria can be distinguished into two groups namely homofermentative and heterofermentative, where a homofermentative group of fermented glucose produces lactic acid as the only product, like *Pediococcus* and some *Lactobacillus.*

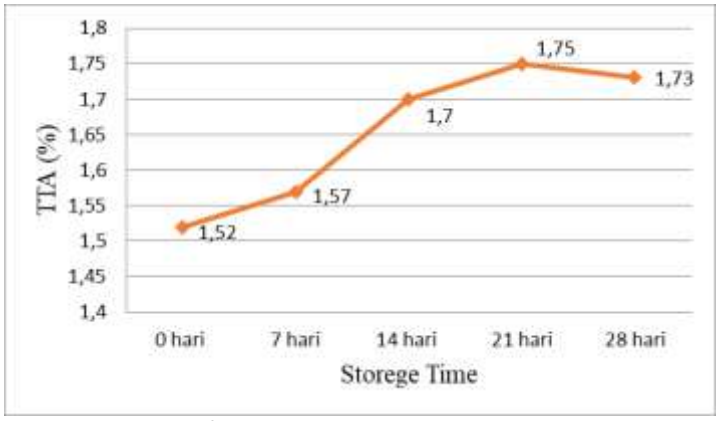

Figure 2. Titratable Acid of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Based on the results of the research of fermented goat milk *Paediococcus acidilactici BK01* stored at refrigerator temperature has qualified the quality of fermented milk Indonesian National Standard 2981:2009 IE 0.5-2. The value of T.T.A. fermented milk obtained in the research ranged from 1.52% − 1.73% for 28 days of the storage period. The results of the study were almost identical to the Melia et al. Research. (2019) on the quality, viability, and anti-bacterial properties of the *Lactobacillus fermentum* NCC2970 in goat milk fermentation at a temperature of 4^{o}C with a T..T. A rate of 0.80 – 1.52 during 15-day storage. Thus, the value of the results of this research has fulfilled the criteria as fermented milk. Abdel-Hamid et al. (2018) also reported that fermented milk made from Lb. casei 393, increased during storage for 21 days in cold temperatures. This is the same as the research of Sah, Vasiljevic, McKechnie), which uses Lb. casei 393 to produce probiotic yogurt. Dimitrellou et al. (2016), also explain the increase in titratable acid during storage for 28 days (0.7 to 0.9) along with a decrease in pH.

Comment [p14]: Please add more discussion

Table 2. Total Lactic Acid Bacteria and Total Plate Count of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Storage Time (days)	Total B.A.L. (Log CFU/ml)	TPC (Log CFU/ml)
0	10,376^{a}	2,653^{d}
7	9,798^{bc}	3,891^{b}
14	9,854^{b}	3,322^{d}
21	9,833^{b}	3,633^{c}
28	9,106^{c}	4,012^{a}

Means within a column with different superscripts are signiûcantly different (P < 0.05)

Based on the data in table 3, the duration of storage was significant ($P < 0.05$), decreasing the total lactic acid bacteria of fermented goat milk. The longer it was stored, the total lactic acid bacteria will decrease. Many dead lactic acid bacteria caused this, due to the more extended storage conditions (28 days), resulting in reduced nutrient availability for *Pediococcus acidilactici BK01* to produce lactic acid. Same with Abdel-Hamid et al. (2018), Lb. fermented milk casei ATCC 393, which is stored for up to 28 days in cold temperatures, has a number of lactic acid bacterial cells above 9 log cfu g^{-1}. Bosnea et al. (2017), Dimitrellou et al. (2016), and Sah et al. (2015) also reported a decrease in the number of bacterial cells after 60 days of storage at cold temperatures. Whereas Terpou et. (2017), stated the decline in bacterial cells occurred after 30 days of storage.

The total decrease in Lactic Acid Bacteria in this study was also closely related to the decline in pH occurring. This was by the opinion of (Prasanna et al., 2013), the decline in the number of lactic acid bacteria was closely associated with the reduction of pH products due to the accumulation of organic acids as a result of the metabolites of fermentation so that rapid pH decline will inhibit even stopping the growth of lactic acid bacteria itself.

The decrease in the amount of Lactic Acid Bacteria in this research was similar to study by Melia et al. (2019). Where fermented milk was kept, then lactic acid bacteria decreased to 4.8×10^8 CFU/ml. Further explained that it was caused by reduced lactose as a significant source of carbon by bacteria. Compared with Indonesian National Standard 2981:2009, the minimum amount of total Lactic Acid Bacteria in fermented milk is 10^7 CFU/ml.

Total Plate Count of Fermented Goat Milk

The number of aerobic bacterial colonies in fermented goat milk tended to increase with the more extended the storage period. The lowest amount of aerobic bacterial colonies in fermented goat milk was found on the 0-day storage day of 4.5×10^2 CFU/ml and the highest on 28-day storage of 102.8×10^2. This condition was in line with the total Lactic Acid Bacterial that the longer the storage was decreasing its number. It was closely related to the 4 phases experienced by lactic acid bacteria. The results of this research still meet the existing S.N.I. with the maximum limit of its bacterial contamination, according to S.N.I., is 1×10^6 CFU/ml.

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 12.75×10^8 CFU/ml with an acidity rate of 1.73 and pH of fermented milk goat reaches 4.28, with a value of TPC 102.8×10^2 CFU/ ml, protein content 3.57, fat content 3.49, and water content 85.51 that still fulfill Indonesian National Standard 2981: 2009 fermented milk

ACKNOWLEDGMENTS

This research was supported by research cluster publications to professors (Project No. T/8//UN.16.17/PPT.01.03/Pangan-PTU-KRP2GB/LPPM/2020) LPPM Andalas University.

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The quality of fermented goat milk produced by *Pediococcus acidilactici BK01* on refrigerator temperature

Abstract. 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 5 times the treatment of goat milk fermentation at the refrigerator temperature is over 0, 7, 14, 21, and 28 days with 4 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%, 4.48-4.28 pH, 3.69-3.49% fat content, 3.53-3.58% protein levels, as well as the count of Total Titrated Acid ranging from 1.52-1.73%. The whole colonies of lactic acid bacteria reached between 237.8 x 10^8-12.75 x 108, and the total plate count (TPC) value during storage experienced an increase from 4.5 x 10^2 CFU/ml to 102.8 x 10^2 CFU/ML. Based on the results of the study can be concluded that the milk of goat fermentation with *Pediococcus 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 meet the standard of fermented milk Indonesian National Standard 2981:2009

Keywords: goat milk, *Pediococcus acidilactici BK01*, fermented milk, and lactic acid bacteria

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).

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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.

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Analysis of proximate from fermented goat milk was moisture content, proteins, fats, water content testing methods, proteins, fats, Titratable Acid, and pH were conducted following AOAC (2012).

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Statistical Analysis

All the data obtained were analyzed statistically use, which was done in as much as 4 repetitions. Data that has significant influence (P < 00.5) was continued with the Duncan's Multiple Range Test using SPSS software statistic 19.

Comment [U1]: Experimental design ?

RESULT AND DISCUSSION

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Comment [U2]: Decimal : Coma or dot ?

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.

Water

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 increased on the storage on the 14th to 28th 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

Comment [U3]: this value is the same as t first day, there's really no increase

This is supported by the statement of Herawati (2008), that changes in the water content of fermented milk can be influenced by the temperature and humidity of the room during storage and changed in water content in the product was a factor that is very influential to the decline in the quality of food products.

Protein

Protein levels of fermented goat milk (table 1) during storage show no significant effect (P > 0.05) against the quality of the resulting protein. It was caused by the storage of fermented milk of *Pediococcus acidilactici BK01*, which was done at the refrigerator temperature until the storage of the 28th day has not influenced the resulting protein levels because it has not shown the sign will expire. From table 1 can be seen protein levels fermented milk until the storage of 28 days still meet the quality standard of Indonesian National Standard 2981:2009 fermented milk, ie, minimum 2.7%. These protein levels are lower than Melia et al. (2019), which is about 4%, this is likely due to the source of milk from different farms and the difference in the type of feed given, but the same as research with Güneş Bayır et al. (2019) which reported that the protein content of yogurt with the addition of cinnamon was 3.54%.

Fat

Fat is a component of milk that can provide higher energy than both protein and carbohydrates. Based on the results of the research known the highest fat content found in the 0-day storage was 3.69%, and the lowest on the 28th day of storage was 3.49%, but statistically showed a difference that was not significant (P > 0.05). This was in line with the protein results gained that up to 28 days of storage have not given a noticeable effect on the resulting fat levels. This fat content is almost the same as the fat content of yogurt with the addition of cinnamon, 3.2 - 3.3% (Güneş Bayır et al., 2019). If compared with the level of fat according to the quality standard fermented milk prescribed Indonesian National Standard 2981:2009, that was at least 3%, then the milk of fermented goat *Pediococcus acidilactici BK01* produced was worth consuming.

Comment [U4]: if not significantly differe the values are the same

pH

The result of the analysis pH of fermented goat milk indicated a significant effect (P. < 0.01) between the duration of storage at the refrigerator temperature (Figure 1.). The longer the storage time was done, the pH value was decreasing. The decrease in the pH value was due to the activity of lactic acid bacteria derived from the starter *Pediococcus Acidilactici BK01* used in the manufacture of fermented goat milk. This bacteria will ferment lactose and will produce lactic acid, resulting in a decrease in pH. It is by the opinions of Costa et al. (2016) that lactic acid bacteria ferment lactose into glucose and galactose, then the glucose was converted into lactic acid.

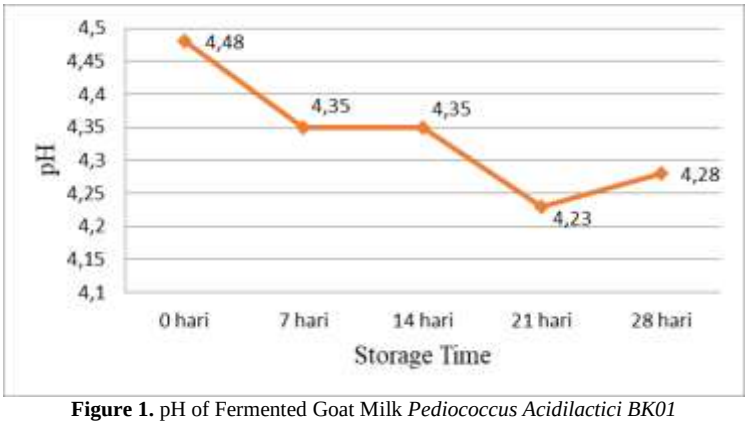

Figure 1. pH of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Also, a decrease in the pH of fermented goat milk during storage as the total acid increase of fermented milk was produced. The higher the whole level of fermented milk acid than the lower the pH, as seen in this research. According to Usmiati et al. (2011), the pH value of fermented milk will further decrease with the length of storage in cold temperatures. It was added by Melia et al. (2019) that the old storage of fermented milk using the starter Lactobacillus fermentum NCC2970 at a temperature of 4^{o}C able to lower the pH value.

The pH value obtained in this study ranged from 4.28 – 4.48. The results of this research were not much different from the Melia et al. (2019) research, where the pH of fermented milk is ranging from 4.0 − 4.9, so that it can be concluded that the pH value of milk fermented goat milk *Pediococcus acidilactici BK01* which was stored at the temperature of the refrigerator until the storage of the 28th day, still able to maintain its quality. Abdel-Hamid et al. (2018), the pH value of fermented milk made from Lb. casei ATCC 393 decreased during storage at cold temperatures of 4.69 to 4.04 for 28 days. Furthermore Bosnea, Kopsahelis, Kokkali, Terpou, and Kanellaki (2017) reported that Lb. probiotic yogurt casei ATCC 393 also decreased after 60 days of storage (pH 4.27 to 4.03). This is caused by the ability of Lb. casei ATCC 393 produces organic acids during storage (Terpou et al., 2017)

Total Titratable Acid (TTA)

The result analysis of total lactic acid (Figure 2.) showed the more extended the fermented goat milk product was stored, and then the total lactic acid was increasing as the pH decrease occurred. A low pH would be a suitable environment for *Pediococcus Acidilactici BK01* (which is a homofermentative type bacteria) to grow and produce metabolites of lactic acid. It was by the opinions of Mal (2013) and Magalhaes et al. (2011) stating that the length of storage will affect the total lactic acid and generally, lactic acid bacteria can be distinguished into two groups namely homofermentative and heterofermentative, where a homofermentative group of fermented glucose produces lactic acid as the only product, like *Pediococcus* and some *Lactobacillus*.

Comment [U5]: How are the results of th statistical analysis?
p>0.01 or p<0.01?

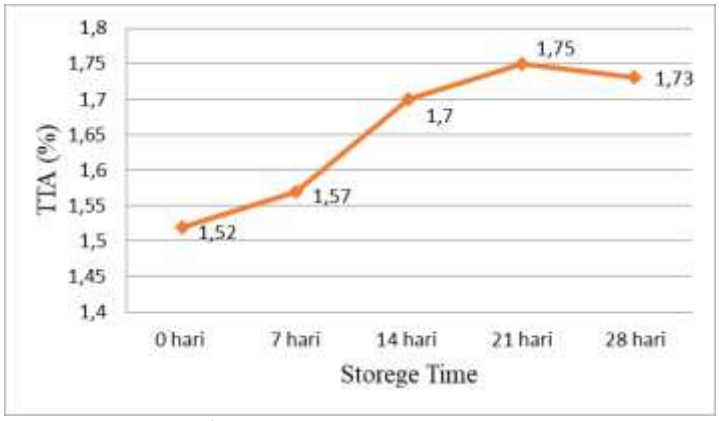

Figure 2. Titratable Acid of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Based on the results of the research of fermented goat milk *Paediococcus acidilactici BK01* stored at refrigerator temperature has qualified the quality of fermented milk Indonesian National Standard 2981:2009 IE 0.5-2. The value of T.T.A. fermented milk obtained in the research ranged from 1.52% − 1.73% for 28 days of the storage period. The results of the study were almost identical to the Melia et al. Research. (2019) on the quality, viability, and anti-bacterial properties of the *Lactobacillus fermentum* NCC2970 in goat milk fermentation at a temperature of 4^{o}C with a T..T. A rate of 0.80 – 1.52 during 15-day storage. Thus, the value of the results of this research has fulfilled the criteria as fermented milk. Abdel-Hamid et al. (2018) also reported that fermented milk made from Lb. casei 393, increased during storage for 21 days in cold temperatures. This is the same as the research of Sah, Vasiljevic, McKechnie), which uses Lb. casei 393 to produce probiotic yogurt. Dimitrellou et al. (2016), also explain the increase in titratable acid during storage for 28 days (0.7 to 0.9) along with a decrease in pH.

Table 2. Total Lactic Acid Bacteria and Total Plate Count of Fermented Goat Milk *Pediococcus Acidilactici BK01*

Storage Time (days)	Total B.A.L. (Log CFU/ml)	TPC (Log CFU/ml)
0	10,376^{a}	2,653^{d}
7	9,798^{bc}	3,891^{b}
14	9,854^{b}	3,322^{d}
21	9,833^{b}	3,633^{c}
28	9,106^{c}	4,012^{a}

Means within a column with different superscripts are signiûcantly different (P < 0.05)

Comment [U6]: usually a maximum of 2 decimal digits

Based on the data in table 3, the duration of storage was significant ($P < 0.05$), decreasing the total lactic acid bacteria of fermented goat milk. The longer it was stored, the total lactic acid bacteria will decrease. Many dead lactic acid bacteria caused this, due to the more extended storage conditions (28 days), resulting in reduced nutrient availability for *Pediococcus acidilactici BK01* to produce lactic acid. Same with Abdel-Hamid et al. (2018), Lb. fermented milk casei ATCC 393, which is stored for up to 28 days in cold temperatures, has a number of lactic acid bacterial cells above 9 log cfu g^{-1}. Bosnea et al. (2017), Dimitrellou et al. (2016), and Sah et al. (2015) also reported a decrease in the number of bacterial cells after 60 days of storage at cold temperatures. Whereas Terpou et. (2017), stated the decline in bacterial cells occurred after 30 days of storage.

The total decrease in Lactic Acid Bacteria in this study was also closely related to the decline in pH occurring. This was by the opinion of (Prasanna et al., 2013), the decline in the number of lactic acid bacteria was closely associated with the reduction of pH products due to the accumulation of organic acids as a result of the metabolites of fermentation so that rapid pH decline will inhibit even stopping the growth of lactic acid bacteria itself.

The decrease in the amount of Lactic Acid Bacteria in this research was similar to study by Melia et al. (2019). Where fermented milk was kept, then lactic acid bacteria decreased to 4.8 x 10^8CFU/ml. Further explained that it was caused by reduced lactose as a significant source of carbon by bacteria. Compared with Indonesian National Standard 2981:2009, the minimum amount of total Lactic Acid Bacteria in fermented milk is 10^7CFU/ml.

Total Plate Count of Fermented Goat Milk

The number of aerobic bacterial colonies in fermented goat milk tended to increase with the more extended the storage period. The lowest amount of aerobic bacterial colonies in fermented goat milk was found on the 0-day storage day of 4.5 x 10^2 CFU/ml and the highest on 28-day storage of 102.8 x 10^2. This condition was in line with the total Lactic Acid Bacterial that the longer the storage was decreasing its number. It was closely related to the 4 phases experienced by lactic acid bacteria. The results of this research still meet the existing S.N.I. with the maximum limit of its bacterial contamination, according to S.N.I., is 1 x 10^6 CFU/ml.

Comment [U7]: How are the results of th statistical analysis?
p>0.01 or p<0.01?

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 12.75 x 10^8 CFU/ml with an acidity rate of 1.73 and pH of fermented milk goat reaches 4.28, with a value of TPC 102.8 x 10^2 CFU/ ml, protein content 3.57, fat content 3.49, and water content 85.51 that still fulfill Indonesian National Standard 2981: 2009 fermented milk

ACKNOWLEDGMENTS

This research was supported by research cluster publications to professors (Project No. T/8//UN.16.17/PPT.01.03/Pangan-PTU-KRP2GB/LPPM/2020) LPPM Andalas University.

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