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QUALITY, VIABILITY, AND ANTI-BACTERIAL PROPERTIES OF *LACTOBACILLUS FERMENTUM* NCC2970 IN PROBIOTIC FERMENTED GOAT MILK AT 4°C

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Abstract – This research studies the quality of goat's milk fermented with *Lactobacillus fermentum* strains NCC2970 to measure the viability of lactic acid bacteria (LAB) strain *Lactobacillus fermentum* and growth of the pathogen *Listeria monocytogenes* CFSAN004330 during 4 °C storage. Complete randomized design was used with 4 storage times; 1, 5, 10 and 15 days with 5 replicates. Storage time affected fermentation significantly ($P<0.05$), lowered water content and fat levels and increased the level of protein, total solids and viscosity. The water activity of the fermented goat milk was not affected significantly ($P>0.05$) by storage duration, but storage duration did significantly ($P<0.05$) lower the pH and increase titratable acid by 1.51% after 15 days. The viability of the LAB after 15 days of storage was 4.8×10^8 CFU/mL, which is still within the SNI and the Codex standard. Fermented goat milk inhibited the growth of *Listeria monocytogenes* strain CFSAN004330 for up to 9 days of storage from 1.1×10^6 CFU/mL to 4×10^3 CFU/mL. Based on the results of this research it can be concluded that goat milk fermented with *Lactobacillus fermentum* NCC2970 can maintain its quality up to 15 days if stored at 4 °C while maintaining the viability of probiotic LAB and is able to inhibit the growth of pathogenic bacteria.

INTRODUCTION

Goat milk consumption provides many benefits for human health and nutrition. Ohiokpehai, (2003) considers that goat milk has advantages over cow milk, especially as it has a smaller fat globule so it is easier to digest. Fast and clean handling is needed so that the milk does not quickly break down or become contaminated with pathogenic bacteria. However, this ability to sustain microorganisms also makes goat milk a candidate for fermented products.

Fermented drinks derived from raw milk using starter bacteria have a thicker texture and a slightly acidic flavor due to the actions of the bacteria. Fermented dairy products include yogurt, kefir and buttermilk. The fermentation process preserves the milk and increases the nutrition value.

Starters for fermented milk products contain LAB similar to those used to improve the quality of many foods (Nishino *et al*., 2018). LAB used to ferment milk also produces antimicrobial substances that act as natural antibiotics against pathogenic bacteria, help maintain digestive health and prevent various diseases, increase digestibility in lactose sensitive individuals and increase the safe storage time of the product.

Because of these benefits, much work to identify of LAB and probiotics that could potentially be developed to add value to food has been conducted (Smug *et al*., 2014). Jain *et al*., (2017) identified two strains of LAB, *Lactobacillus fermentum* isolated from buffalo milk and *Lactobacillus pentosus* isolated from goat milk as potentially useful probiotics and bio-therapeutic agents. Melia *et al*., (2018), also found LAB (*Lactobacillus fermentum L23*) from buffalo milk which potential as probiotic.

LAB are able to survive in the acidic conditions in the digestive tract, help the absorption of food by intestinal villi, boost immunity, kill pathogenic bacteria and improve nutritional value LAB used to fermented milk include *Lactobacillus bulgaricus*, *Streptococcus thermopillus*, *Lactobacillus fermentum*. Some research suggests LAB may be capable of

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reducing cholesterol The *Lactobacillus fermentum* NCC2970 isolated from goat's milk, is a non-pathogenic, gram positive, and does not form spores. It is facultative anaerobic, rod-shaped and extensively used in food products and food fermentation being highly beneficial as a probiotic for maintaining human health, protecting against a number of diseases. *Lactobacillus fermentum* is tolerant of acidity to pH 3 and can survive in up to 0.3 % of bile salts. The purpose of this research is to determine the quality of goat milk fermented with *Lactobacillus fermentum* NCC2970, the protective effect of this product against pathogenic bacteria and the viability of the LAB in the fermented milk during storage at 4°C.

MATERIALS AND METHODS

Milk from *Peranakan Etawa* goat milk (PE) from Padang, West Sumatra, Indonesia was fermented with 4% *Lactobacillus fermentum* strain NCC2970. With 5 replicate samples of the fermented milk were then stored at 4 °C for each of four storage durations; 0, 5, 10 and 15 days.

Fermentation of Goat Milk

The milk is heated at a temperature of 65-67 °C for 30 minutes. The temperature was lowered to 37 °C (Donkor *et al*., 2006) and 4% starter added. The mixture was then incubated at 37 °C for 8 hours in INFORS AG CH-4103 BOTTIMINGEN incubators.

Analysis of Fermented Milk

Total solids, protein, fat and water were measured according to AOAC, (2012) methods. Water activity was measured using 3 ml samples using an a_w Meter (Lab Master). Measurement of titratable acid was measured by titration (AOAC, 2012), pH was measured with a HANNA Romania pH meter calibrated with aqueous buffer at pH 4 and pH 7. The electrode was dipped in a 10 mL sample and the specific pH value recorded (AOAC, 2012). Viscosity measurement was made using Stormer Viscometer. 100 ml of sample was inserted into the test cell. The rotor is inserted into the sample and left spinning until the pointer of the scale needle stops, the final position of the needle showing the viscosity of the sample in dPa. s (AOAC, 2012).

Testing the viability of LAB

A LAB count was made on MRS agar (Merck) plates after 48 hours anaerobic incubation in an anaerobic jar (Merck) at 37 °C then after refrigerator storage of 1, 5, 10, and 15 days at 4 °C.

Testing the Growth of Pathogenic Bacteria

The CFSAN004330 strain of the bacterial pathogen *Listeria monocytogenes* (2.0 x 10^6 CFU/mL) was prepared in a Listeria broth (Merck) and 1 mL added to 9 mL samples of freshly fermented goat milk. Samples were stored at 4 °C and observed after 1, 3, 6, and 9 days. On the day of observation, the sample was diluted serially and cultured on Listeria agar (Merck). Then the amount of bacterial pathogen calculated. The measurement was carried out in triplicate.

Statistical Analysis

Statistical analysis is used to analyze water content, total protein, fat, solid, aw, pH, titratable acids, viscosity and LAB viability data. All analysis is done in triplicate. Where the analysis indicated a significant effect ($P<0.05$), this was further tested using Duncan's Multiple Range Test using the statistic 8 software for windows.

RESULTS AND DISCUSSION

The Composition of Fermented Goat Milk

Water Content Storage time at 4 °C has a significant effect on the moisture content of fermented goat's milk. In addition, the moisture content in the fermented milk is also influenced by the capacity of

Table 1. The composition of goat milk Fermented with *Lactobacillus fermentum NCC2970*

Storage (Days)	Moisture (%)	Protein (%)	Fat (%)	Total Solid (%)	Water Activity	Viscosity (dPa. s)
1	86.16^{a}	3.83^{c}	4.99^{a}	13.84^{c}	0.9498	1.2088^{d}
5	85.84^{ab}	4.10^{bc}	4.17^{b}	14.16^{bc}	0.9496	1.2850^{c}
10	85.52^{bc}	4.36^{ab}	3.60^{c}	14.48^{ab}	0.9498	1.3574^{b}
15	85.05^{c}	4.63^{a}	3.61^{c}	14.95^{a}	0.9478	1.4230^{a}

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

milk proteins to hold water, hence as protein content increases the amount of free water will decrease. According to Bezerra *et al*., (2012), this is related to the increase of syneresis as the pH is lowered to the isolelectric point of the proteins, resulting in a denaturisation of the proteins causing an instability in the casein micelle releasing liquid.

According to Wu *et al*., (2001), the water holding capacity is related to the ability of the protein to retain the structure of the fermented milk and other researchers consider that fat globules also play an important role. Additionally, Lee and Lucey, (2010), explains that the water holding capacity is influenced by an increase in protein levels as milk proteins are amphiphilic. Sodini *et al*., (2004), indicates that an increase in casein particles concentration leads to more interaction between casein micelles, which results in smaller pores, in a dense matrix, and and increased water holding capacity.

Protein: The protein content of the goat milk used in the manufacture of a fermented product affects the quality of the resulting product. The most important types of protein in the milk are casein in the form caseinate which plays a role in maintaining the stability of fermented milk. The fermentation of milk increases the digestibility of the protein because it breaks protein into smaller simpler units that are more easily broken down by digestive enzymes into the component amino acids (Dilna *et al*., 2015).

Longer storage time at 4 ^{o}C significantly ($P<0.05$) increases fermented goat milk protein levels, which peaked at 4.63% after 15 days. All samples exceeded the SNI 01-2981-1992 standard that states that yogurt should contain a minimum of 3.5% protein. Increased levels of protein on storage are related to reduced levels of water due to evaporation.

Protein levels resulting from milk fermentation along with the proteins in the bacteria itself can contribute about 7% of the total milk protein in dairy product (Cruz *et al*., 2010). Fermented goat milk protein levels in this study were lower than that found by Ranadheera *et al*., (2012) of 5.39 ± 0.03% in goat milk yogurt made with Lactobacillus bacteria acidophilus LA-5, *Bifidobacterium animalis* subsp. lactis BB-12 and *Propionibacterium jensenii* 702 which may, in part, be related to the increased efficiency of a fermentation process utilizing more than one strain of bacteria.

Fat Longer storage time at 4 ^{o}C significantly ($P<0.05$) reduced the levels of fat. Fresh fermented goat milk contained 4.99% and this decreased to 3.61% by the 15th day of storage. This value is lower than that found by Ranadheera *et al*., (2012) who found 5.37 ± 0.17% fat content in their goat milk yogurt. Fermented goat milk fat content is also influenced by the fat content of milk prior to fermentation. In general yogurt contains no more than 1.7% fats (Surono and Hasono, 2002). Fat level decreases in storage is caused by the breakdown of fats by enzymes, the LAB break down loops to fatty acids during the process of fermentation. However, on longer storage, fat levels declined.

Total Solids Longer storage time at 4 ^{o}C significantly ($P<0.05$) increased the total solids from 13.84 for freshly fermented milk to 14.95% at 15 days. These figures are lower than the values found for stirred fruit yogurt (15.62%-16.12%) by Ranadheera *et al*., (2012). Total solid content is inversely proportional to the moisture content of goat's milk fermentation decreases to 15 days of storage. The percentage of total solids are influenced by the amount of water, protein and fat. An increase in total solids and protein positively influences the matrix density and decreases syneresis (Amatayakul *et al*., 2006).

Water Activity Water activity was not affected significantly by storage time at 4 ^{o}C ($P>0.05$) and ranged from 0.9478-0.9498. This figure is higher than Suharto *et al*., (2016), who measured 0.86. Water activity is influenced by complex chemical reactions and is related to the pH, viscosity and texture of the product.

Viscosity Longer storage time at 4 ^{o}C significantly ($P<0.05$) increased viscosity. The viscosity of the fermented goat milk ranged from 1.2088-1.4230 pa s. The best value for viscosity achieved in this study was 1.42 dPa.s. Which is relatively thin compared to the goat milk, yoghurt of Suharto *et al*., (2016) at 3.51 ± 1.60 pa s. This is because only pure goat's milk and one type of bacteria was used. Yogurt uses more than one type of bacteria that operate symbiotically resulting in a more completely fermented product with higher viscosity.

However, as the total solid content and the level of protein increase the matrix network will be stabilized (Nguyen *et al*., 2014). The adsorbed proteins are incorporated in the gel network during acid induced coagulation and can lead to improvements texture, decreased whey separation, increased gel firmness and better integration of fat globules (Tamime and Robinson, 1999). Increase in viscosity also occurs as there is an increase in interactions between the fat globule aggregations and the whey protein (Krzeminski *et al*., 2011).

Titratable Acid and pH Longer storage time at 4 °C significantly increased titratable acid. The pH of the fermented goat milk decreased significantly during storage, but did not fall below pH 4.0, which value is generally considered to be detrimental to the survival of probiotic organisms (Ranadheera *et al*., 2012). pH is influenced by the activity and growth of LAB during storage.

Preferred pH value in commercial yogurt is largely determined by the tastes of the consumer, but is generally around 4.5, as this degree of acidity maintains the quality of yogurt throughout its shelf life and provides the degree of sourness preferred by consumers (Hui *et al*., 2007). Acidity does tend to increase with storage time.

Table 2. Titratable Acid and pH of *Lactobacillus fermentum* fermented goat milk

Storage (Days)	pH	Titratable Acid (%)
1	4.90^{a}	0.8012^{c}
5	4.38^{b}	1.1410^{b}
10	4.20^{c}	1.1600^{b}
15	4.04^{d}	1.5180^{a}

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

A lower pH is a result of the increase of titratable acid and is associated with the activities of LAB (Prasanna *et al*., 2013), which ferment lactose to produce lactic acid (Costa *et al*., 2016). *Lactobacillus fermentum* are homofermentative, producing lactic acid as the single fermentation product hence increasing acidity. Previous research by Ranadheera *et al*., (2012) found that an increase in acidity during storage has an influence on syneresis. This increase in pH could be due to better buffering from the increased protein (Li and Guo, 2006; Salaun *et al*., 2005).

Viability of Lactic Acid Bacteria in Fermented Goat Milk

The number of LAB significantly ($P < 0.05$) declines with storage at 4°C from 21.6 x10^{8} CFU/ml for freshly fermented milk to 4.8 x10^{8} CFU/mL after 15 days of storage. This indicates decreased viability (Ranadheera *et al*., 2012). According to Vinderola *et al*., (2000) the number of LAB colonies is influenced by changes in fat content in milk fermentation. The fat content is an integral part of the microstructures in fermented milk, and changes in fat content give rise to less favorable environmental conditions for LAB growth.

Table 3. The viability of lactic acid bacteria in fermented goat milk for storage at 4 °C

Storage (Days)	Lactic Acid Bacteria (x10^{8}) CFU/mL
1	21.6^{a}
5	18.6^{ab}
10	12.6^{bc}
15	4.8^{c}

Results are means of 3 replicates and those with different superscripts are significantly different ($P < 0.05$)

According to Fuller (1992) a probiotic should contain 10^{7}-10^{9} CFU/mL LAB to provide maximum health benefits. According to the CODEX (2003) a good yogurt should contain at least 10^{7} CFU/mL viable LAB. The International Dairy Federation stipulates that the minimum yogurt LAB count should be 10^{7}-10^{8} CFU/mL at the time of production, and these levels should be maintained for 30 days at a temperature of 4-7 °C to allow time for distribution and marketing (Surono and Hasono, 2002). The LAB count of the fermented goat milk in this study falls within this range after 15 days storage indicating favorable conditions for culture growth and potential as a probiotic.

The decrease in the number of colonies of LAB on storage could be associated with the reduction of lactose levels, the main source of nutrients for the LAB, as it is metabolized by the bacteria. Furthermore, as the fermentation proceeds the level of organic acid rises hence decreasing pH. Highly acidic conditions hamper the growth of the LAB (Shah, 2009). It is known that storage temperature also affects the viability of LAB. Storage at 4 °C maintains viability of LAB compared to higher storage temperature (Trisnawita *et al*., 2018).

The growth of pathogenic bacteria in goat milk Fermentation

The growth of bacterial pathogen *Listeria monocytoenes* was measured after 1, 3, 6, and 9 days of storage at 4°C. In table 4, it can be seen, after 9 days of storage, the number of *Listeria monocytogenes* strain CFSAN004330, decreased to 4 x 10^{3} CFU/mL. This proves that goat milk fermentation with L. *fermentum* strains NCC2970 successfully inhibited the growth of this bacterial pathogen. LAB produces antimicrobial compounds such as organic acids, hydrogen peroxide and b*acteriocins*. This property of LAB has been found by other researchers. Melia, *et al*., (2017), found the antimicrobial activity of LAB isolated from water buffalo milk inhibited the growth of *L. monocytogenes* and Fratini *et al*., (2016), discovered that the growth of *L. monocytogenes* 7644TM was inhibited in soya milk stored at a 4 °C.

Table 4. Growth of pathogenic bacteria (*Listeria monocytogenes* strain CFSAN004330) in Fermented Goat Milk

Storage (Days)	*Listeria monocytogenes strain CFSAN004330* (CFU/mL)
1	1.1×10^6
3	10×10^4
6	10×10^3
9	4×10^3

CONCLUSION

Goat's milk fermented using *Lactobacillus fermentum* strains NCC2970 meets the nutritional standards set by CODEX after 15 days storage at 4 °C. The viability of LAB remains high enough for the product to be regarded as a probiotic and the LAB effectively inhibit the growth of the pathogenic bacteria, *Listeria monocytogenes* strain CFSAN004330.

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