Chemical Characteristics And Microbiological Kefir Beverages From Bali Cattle During Storage

Ketut Suriasih, I Nyoman Sucipta,Wayan Citra Wulan Sucipta Putri, I Putu Surya Wirawan

Abstract: Bali cattle even without being fed booster are able to utilize low-quality forage, and do not experience growth disturbance. The potential of local bali cattle resources if maintained with good and sufficient feed will be a source of milk production in Indonesia that can still meet the needs of the community. Milk is a nutrient-rich food and is needed by all levels of society because it is healthy and smart. Meanwhile, Indonesian milk consumption is only 7.5 kg/capita/year, which is very far compared to other countries in the ASEAN region, because milk for most Indonesians is still a luxury / expensive item because 80% of national needs are still imported. Therefore the use of mammals other than dairy cows to produce milk needs to be considered.The microorganisms occurred in kefir grains, the chemical component of the milk used and the technology of manufacturing are factors that influence the microbiological and chemical characteristics of kefir during storage.The aims of the present study was to evaluate the microbiological and chemical attributes of refrigerated bali cattle milk kefir drink added different level of sucrose, during storage. Conclusions there was significant changes in microbial population of bali cattle milk kefir stored for 28 days. The number of lactic acid bacteria ranged from 109 cfu/ml at the beginning of the study and then decline to 108 cfu/ml on days 28. The pH of Bali cow's milk kefir also decreased from 4.3 at the start of the study and pH 3.7 on the 28th day.

Key words : Bali cattle, Chemical characteristics, Kefir, Microbiological

1. INTRODUCTION

Kefir is product of fermented milk originated from the Caucasian mountains, in Tibet or Monggolia. Originally kefir was made from sheep milk, while in Europe kefir was prepared from cows milk. There are many bali cattle find in Indonesia, especially in Bali, farmers used to raised Bali cows as well as male bali cattle for fattening. These Bali cows, if fed enough and good quality could produced milk for their calve and the farmers family. So that the milk can be processed into kefir. Kefir is a probiotic drink and functional food which shown some healthy effect such as: inhibit growth of pathogen[9], increase host immunity[10], retard growth of cancers cell. [2,8] and reduced blood cholesterol [3,10]. Kefir was produced through inoculation milk with kefir grains or mother cultures prepared from the grains. Kefir grains are gelatinous masses, consist of many lactic acid bacteria (lactobacilli, lactococci, leuconostoc), acetic acid bacteria and yeast, wrapped in casein and matrix of polysaccharide. The yeast will ferment milk sugar to ethanol and carbon dioxide which give a pricky sensation to kefir. The lactic acid bacteria occur in kefir such as Lactobacillus kefiranofaciens and L. kefir produced polysaccharide. The microorganisms occurred in kefir grains, the chemical component of the milk used and the technology of manufacturing are factors that influence the microbiological and chemical chaeacteristics of kefir during storage.The aims of the present study was to evaluate the microbiological and chemical attributes of refrigerated bali cattle milk kefir drink added different level of sucrose, during storage.

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2 MATERIAL AND METHODS

2.1. Kefir preparation

Kefir grains were bought from Dairy Laboratory of Faculty of Animal Husbandry, IPB university, Indonesia. The grains were refreshed by inoculating into full cream sterilised milk (3.5% fat content), and incubated at room temperature (27 to 28^{o}C) for 24 hour. The grains were separated by sieving on to plastic sieve, and used for kefir production. Bali cattle milk was obtained from Simantri farmers at Kelating Village, Tabanan Regency, Province of Bali, Indonesia. It pasteurized and used for kefir preparation. The pasteurized milk was inoculated with 5% (w/v) kefir grains obtained above, and incubated at 27^{o}C for 24 hour. After incubation, the fermented milk was sieved to separate the grains from the fermented milk (the kefir). The kefir was then divided into four batches and added 10% , 15%, 20% (w/v) and without (0%) sucrose to produced kefir drink. The kefir drink were then stored in refrigerator 5±1^{o}C for 7, 14, 21 and 28 days. Two replications were performed for all batches.

2.2. Microbiological analysis

Kefir samples (10 ml) were pippeted aseptically and diluted in 90 ml sterile saline solution (0.85% NaCl) and homogenized in a vortex mixer. Subsequent serial dilutions were prepared and viable numbers of the microorganisms enumerated using the foured-plate technique, plating in duplicate. A total of 1000 µl of each diluted sample were inoculated into De Man, Rogosa and Sharpe (MRS) (Oxoid CM361) and M17 (CM785) agar for enumeration of lactic acid bacteria; on oxytetracylin glucose yeast extract (OGYE) agar (Oxoid CM545) for yeast. Plates were incubated for 48 hours at 37^{o}C for lactic acid bacteria and 72 hours at 25^{o}C for yeast. Colony forming units were quantified from plates showed 30 – 300 colonies.

2.3. Chemical analysis

After storage at refrigerator (5 ± 1^{o}C) for 7, 14, 21 and 28 days, kefir samples were characterized in relation to their chemical composition. Protein percentage was analyzed according to Kjehldhal method (AOAC, 1999), lactose content was determined using methods No. 896.51 and 947.05 (AOAC, 1999), pH was measured using a digital pH-meter (ISTEK, Model 720P. Korea) calibrated with pH 4 and 7 buffers.

2.4. Statistical analysis

Analysis of variance (ANAVA) using 95% confidence intervals was run on each of the chemical and microbiological variables to determine possible differences among the samples for two factors "percentage of sucrose addition" and "storage time". Analysis were performed using the SPSS 21 virsion for windows.

3 RESULT AND DISCUSSIONS

3.1. Microbiological analysis

Fig.1. showed the changes in the microbial population of bali cows milk kefir during storage. Lactic acid bacteria were more frequently microorganisms group found in any kefir sample, ranging from 108 to 109 cfu/ml, followed by yeast, which ranging from 105 to 106 cfu/ml. The lactic acid bacterial counts in kefir stored for 28 days were higher than that reported by Farnworth et al. (2005) who claimed that, the population of lactic acid bacteria in kefir were 108–cfu/ml, and Farnworth and Mainville (2003) and Garcia-Fontan et al. (2006) of around 108 cfu/g.

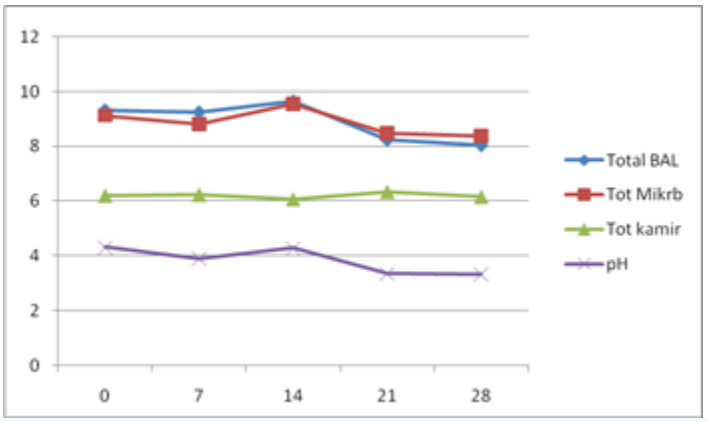

***Fig.1**. Average number of Lactic acid bacteria, total microbe and yeast and pH of bali cows milk kefir stored for 28 days at refrigerator*

The differences in microbial count were caused by different incubation temperature during kefir preparation. In this experiment, as well as at the study conducted by Suriasih et al. (2005), kefir preparation was hold at temperature of 28 ± 2°C, while kefir preparation reported by Farnworth and Mainville (2003) and Garcia-Fontan et al. (2006) were incubated at temperature of 22 – 25oC. Growth of microorganisms including bacteria were greatly affected by temperature of the environment. Lactic acid bacteria are mesophiles, grow at temperature range of 10° – 45°C, with optimum temperature of 30°–40°C. Within the optimum temperature range, growth of bacteria will increase as the environmental temperature raise, which result in fast growth rate and so that, greater microbial population. Beyond the optimal temperature, the microorganims will grow better if the environmental temperature was nearer to the optimum temperature. Incubation periods significantly affected the lactic acid bacteria counts of kefir samples. Increasing incubation periods from 24 to 48 and 72 hours, respectively reduced the number of lactic acid bacteria of 2.1% and 3.44 % (P<0.05). The reductions appeared due to the drop in pH of kefir samples. During kefir preparation, lactic acid bacteria will convert lactose of the milk to obtain energy for their maintenance and growth, and released metabolites, primarily lactic acid, result in a decrease in the pH of the environment (kefir). Longer incubation time, means more time available for the lactic acid bacteria to metabolized the lactose of the milk, and so that more lactic acid produced, which contributed to much lower pH surrounding the lactic acid bacteria. The dropped in pH of kefir samples below the optimum level, affect the intracellulair pH of the lactic acid bacteria, which inhibit the enzyme activity, ion transport and nutrient uptake, and so that retard the growth and then the counts of the lactic acid bacteria. The yeast counts (105–106 cfu/ml) of kefir samples were higher than that reported by Oner et al (2010). who reported that, the yeast counts of the kefir samples prepared using kefir grains were about 104 to 105 cfu/g. The higher counts of yeast in this study were caused by higher incubation temperature (28 ± 2°C), compared to 22°C in the study of Oner et al (2010).Higher temperature (within the optimum range) will increase metabolic activity of the yeast which result in faster growth rate and higher population of the yeast. This is in accordance with Todar (2009), who stated that yeast can grow at temperature range of psychrotroph and mesophile (< 7° – 45°C), with the optimum temperature range from 20°–30°C. Within the optimum temperature range, growth of yeast will increase, in parallel with the increase in environmental temperature due to the increase in metabolism and enzyme activity. Lengthening incubation periods from 24 to 48 and 72 hours significantly reduced the yeast counts of 2.9 and 5.1% (P<0.05) respectively. The reductions appeared due to the drop in the pH of the kefir samples. In general, yeast is an acidophilic organism and, so that, grow better under acidic condition. The optimal pH for growth of yeast can vary from pH 4 to 6. Deviation of the surrounding pH below optimal level, as such, in kefir samples incubated for 48 and 72 hours, in which the pH drop were 0.18 and 0.62 unit (or 0.83 and 0.44 unit from pH of kefir sample incubated for 24 hour), caused difficulties in enzymes activitiy, disturbed yeast metabolism, and so that, the yeast cell will not be able to grow normally, resulted in the drop of the yeast counts.

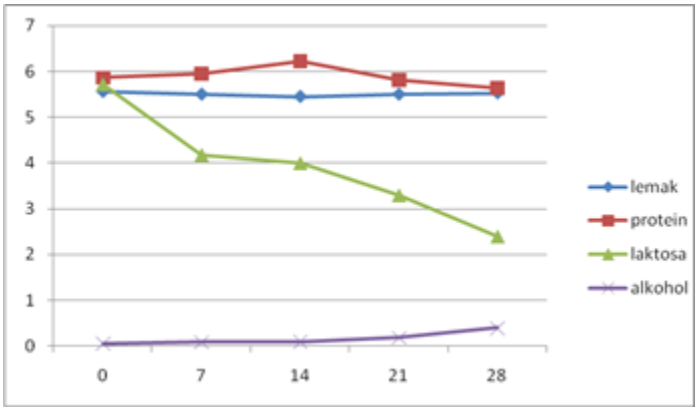

***Fig.2**. Average percentage of fat, protein, lactose and alcohol of bali cows milk kefir stored for 28 days*

3.2 Chemical Composition of kefir samples

Chemical composition of kefir depends on many factors such as kind of milk and technological conditions. Chemical composition of pasteurised Bali cattle milk used for kefir preparation composed of 5.50 ± 0.26% protein, 5.48 ± 0.16%

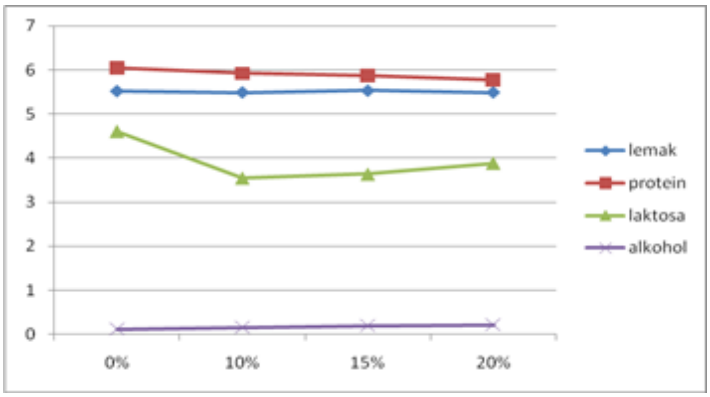

***Fig.3**. Average number of Lactic acid bacteria, total microbe and yeast and pH of bali cows milk kefir added sucrose*

lactose, 6.74 ± 0.43% fat. Technological process in kefir preparation will changes some of these chemical components. Chemical properties of kefir samples are shawn in Fig.3. and Fig.4. The data showed that, pH, protein and lactose content were significantly affected by incubation periods ($P<0.05$). Lengthening the incubation periods from 24 to 48 and 72 hours, significantly decreased the pH of kefir samples of 11.83% and 20.42%, respectively. pH of the kefir samples were a reflection of organic acids (primarily lactic acid) accumulation, yield from lactose metabolism by lactic acid bacteria grew in the kefir. The lower pH of the kefir samples incubated for 48 and 72 hours compared to that incubated for 24 hours appeared due to accumulation of lactic acid yield during the first and the second 24 hours fermentation (48 hours incubation period) and during the first, second and third 24 hours fermentation (72 hours incubation period). The result was in line with Motaghi et al. (1997) who reported that, kefir produced through 24 hours incubation period had the highest pH, followed by kefir produced through 48 and 72 hours incubation periods.

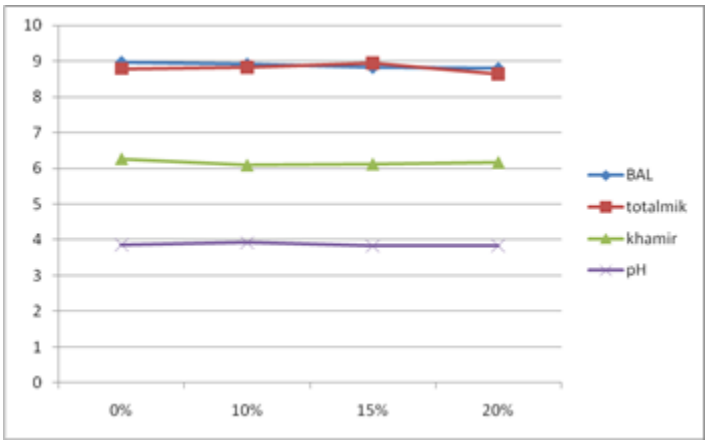

***Fig.4**. Average percentage of fat, protein, lactose and alcohol of bali cows milk kefir added various percent of sucrose*

Lactose content of kefir samples incubated for 48 and 72 hours were significantly lower than that of the kefir samples incubated for 24 hours. Increasing incubation period from 24 to 48 and 72 hours, significantly reduced the lactose content of the kefir samples of 10.92% and 4.32% ($P<0.05$), respectively. The decrease in lactose percentage due to longer incubation periods are supported by the finding of Purnomo and Muslimin (2012) who reported that, lactose content of kefir, decreased 0.70% and 0.07%, respectively during the first 24 hours fermentation. Lower lactose percentage in kefir prepared through longer incubation periods was in accordance with Motaghi et al. (1997) who found that, the reduction in sugar content of kefir was observed as a function of incubation time. This phenomena was caused by more lactose fermented by microorganisms occurred in kefir preparation, as more time available for fermentation activity, due to longer incubation period. In addition, lactose is a carbohydrate component of milk and, as such, the primary energy sources for growth of microorganisms occur in milk, especially for lactose fermenting microorganisms. With respect to protein content, it was found that lengthening the incubation period from 24 to 48 and 72 hours, significantly increased the protein content of the kefir samples. Significant increase was also occurred when the incubation period was lengthened from 48 to 72 hours. The increase in protein content due to longer incubation period was in accordance with Magalhaes et al. (2011), who reported that the protein content of kefir determined after 24 hours fermentation was higher than that before fermentation, due to the increase of microbial biomass, which also reveals that protein content of kefir samples consist of protein originated from the milk used for kefir preparation and biomass of the microbe grow in the kefir samples. It could be deducted that, protein content of 48 and 72 hours kefir samples come from protein of the milk and accumulation of biomass from microbe grow during 48 and 72 hours incubation periods. This accumulation caused the protein content of the kefir samples incubated for 48 and 72 hours were higher than that incubated for 24 hours.

4 CONCLUSION

There was significant changes in microbial population of bali cows milk kefir stored for 28 days. The Lactic acid bacteria numbers were ranging from 109 cfu/ml days 0 and then decline to 108 cfu/ml on days 28. The pH of bali cows milk kefir were also decline 4.3 on days 0 to 3.7 on days 28.

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