ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Growth and Antimicrobial Activity of *Lactobacillus acidophilus* in Probiotic Fermented Cheese Whey Beverages

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ABSTRACT

The research aims were to study the growth of *Lactobacillus acidophilus* and its antimicrobial activity in fermented cheese whey beverages added with combination of skim milk and sucrose at various concentrations incubated at various fermentation times. Cheese whey was added with skim milk and sucrose at a ratio of 3:9, 6:6, 9:3 (%) and fermented for 18, 24, 30, 36 hours on 37°C by *Lactobacillus acidophilus*. Antimicrobial activity was measured using turbidimetric assay method. Fermented beverage was added into culture media of *Staphylococcus aureus*. The growth of indicator culture was monitored by measuring absorbance every 3 hours at 600 nm. The result showed that the total viable *Lactobacillus acidophilus* in the fermented cheese whey beverages range between 9,8832 – 11,6959 log cycle (7,7x 10^9 - 4,9. x10^{11} cfu/mL) and there were inhibition against indicator bacteria (*Staphylococcus aureus*) indicated by the decrease of absorbance ratio of fermented beverages compared with the control media. Fermentation time of 24 hours and ratio of skim milk and sucrose 6:6 was the best treatment based on microbiological and physical-chemical parameter.

Keywords: Antimicrobial activity, fermented cheese whey, *Lactobacillus acidophilus*, probiotic

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ISSN: 0975-8585

INTRODUCTION

Whey is a by-product of cheese-making industry with the form of greenish yellow clear liquid obtained from the sieve screening and pressed curd during cheese making. Every one pounds of cheese from 10 litres of milk will produce 8-9 litres of whey [1].

In Indonesia, whey still has not been utilized even though cheese production in Indonesia is quite large. Data from Central Bureau of Statistics Indonesia in 1992 and 1994 respectively showed that the production of whey in Indonesia reached 21,708 litres and 270,414 litres per year [2]. However in laboratory scale, research on the utilization of whey for food production, has been carried out. Cheese whey has been used as functional fermented beverages [3]. This confirms that cheese whey can be used as an alternative for food products. Whey is also utilized to manufacture probiotic beverages using lactic acid bacteria (*Lactobacillus acidophilus*). Probiotic food is food products containing live bacteria (lactic acid bacteria) which have a positive effect to the "host" by improving the balance of microflora intestinal tract [4]. *Lactobacillus acidophilus* have already been tested as probiotic bacteria and naturally present in the digestive tract humans and animals and commonly used as starter cuture for food fermentation [5]. The research revealed that the probiotic bacteria remain alive in the digestive tract. The standard amount bacteria allowed to present in probiotics products is 10^7 CFU/ml live microorganisms in the final product [6].

Factors that determine the growth of bacteria is the availability of nutrition and an appropriate fermentation time. Whey cheese contains high enough protein (0.8 to 1.0%) and lactose (3.8 to 4.3%). The addition of skim milk containing protein and lactose are expected to affect the growth of lactic acid bacteria which can affect chemical composition of the product such as pH, total acid, total protein and total sugar. While the addition of sucrose as a cheap source of total solids can improve the texture, taste, and flavour [7]. Beside affects the texture of the product, sucrose also expected to improve the organoleptic quality of the product. During fermentation the activity of probiotic bacteria was indicated by the change of nutrients such as glucose and lactose in the medium into lactic acid which affect the aroma and taste of the product. However it is not yet known the effect of adding skim milk and sucrose during fermentation on the growth of probiotics (*Lactobacillus acidophilus*) and organoleptic characteristics of probiotic fermented cheese whey beverages.

The purpose of this study was to study the effect of addition of nutrients (skim milk and sucrose) to the growth of *Lactobacillus acidophilus* and antimicrobial activity during fermentation process of probiotic cheese whey beverages.

METHODS

Bacteria strain and cheese whey used

Lactobacillus acidophilus FNCC-0051 was obtained from PAU Food and Nutrition UGM, *Staphylococcus aureus* was obtained from the Laboratory of Biochemistry and Nutrition THP UB Malang. Acidic type cheese whey was obtained from Laboratory of

Product Technology Faculty of Animal Husbandry, University of Brawijaya. Total LAB (*Lactobacillus acidophilus*) was analyzed using the method of Lay [8].

Experimental Design

The research utilized Randomized Block Design (RBD), which arranged in factorial with two factors. The first factor is the length of fermentation in the level of 18, 24, 30, and 36 hours. The second factor is the concentration ratio of skim: sucrose with level of 3:9, 6:6, 9:3. Each treatment was repeated 3 times.

Liquid Starter Cultures Preparation

One streak of *Lactobacillus acidophilus* stock cultures was dipped in the 10 ml sterile MRS broth and incubated for 50 hours at the temperature of 37 ° C. Ten millilitres of MRS broth containing culture was transferred in 90 ml of pasteurized solution mixture of whey, 10% skim milk and 1% glucose and then incubated for 50 hours at 37 ° C to obtained ready-to-use starter culture liquid.

Probiotic Fermented Cheese Whey Beverages

Cheese whey was filtered then added with skim:sucrose of certain concentration ratio and 0.3 % NaCMC. The mixture then homogenized using a stirer for 15 minutes, pasteurized at 85^{o} C for 15 minutes. The mixture then allowed to cool down (40^{o}C) then added with the starter culture, and cultured at 37°C for a period as stated before.

Parameters Measured and Data Analysis

The total lactic acid bacterium was analyzed using the method described by Lay [8], pH was measured using the method of Apriyantono *et al*. [9], total acid was measured by Ranggana [10], total dissolved solids was measured by Apriantono *et al*. [9], the viscosity was analyzed using the method of Yuwono and Susanto [11], total sugar was measured using the method of Apriantono *et al*. [9], the levels of dissolved nitrogen was analyzed using the standard of AOAC [12], antimicrobial activity was analyzed using turbidimetry method. Organoleptic test including taste test, aroma, and appearance were also conducted.

RESULTS AND DISCUSSION

The Effect of Addition of Skim Milk and Sucrose into Cheese Whey before Fermentation

Chemical and physical composition of cheese whey after addition of skim milk and sucrose before fermentation was measured. The control used in this study is cheese whey solution without the addition of skim milk and sucrose.

It can be seen that the increase concentration of skim milk cause the increase in total nitrogen levels, dissolved nitrogen, pH, and viscosity. It is because most of the component of skim milk is protein with the total nitrogen of 5.435%. While the decrease in concentration

ISSN: 0975-8585

of sucrose led to a decrease in total sugar, total dissolved solids, and total acid. Total LAB should be relatively high in order to qualify as probiotic beverages which should have the number of active cells of at least 10^6 cells/mL products [13].

Microbiological Analysis of Probiotic Fermented Cheese Whey Beverages

The total number of *L. acidophilus* tends to increase at intervals 18-30 hours, and decreased at the fermentation time of 36 hours (Figure 1). The highest number of *Lactobacillus acidophilus* was showed by fermentation time of 24 hours and concentration ratio of skim:sucrose 6:6. It is due to the longer fermentation time the higher the number of lactic acid bacteria cells. At fermentation time of 24 hour *Lactobacillus acidophilus* achieve logarithmic phase of growth in which the bacteria cell divide rapidly, constant, and require more energy than the other phases. This energy is obtained from the addition of skim milk and sucrose concentration of 6:6 which seems to meet the needs of nutrition for *Lactobacillus acidophilus* to grow optimally. The logarithmic phase begins when growth of bacteria reach the maximum speed and during this phase the rate of growth always maximum [14].

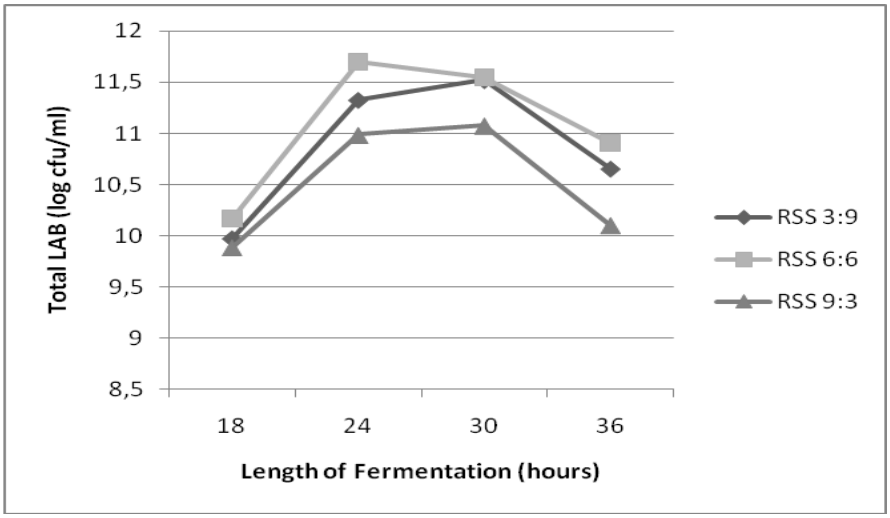

Figure 1: Effect of the Length of Fermentation Time and Concentration Ratio Skim: Sucrose on Growth of *Lactobacillus Acidophilus* in Probiotic Fermented Cheese Whey Beverages.

Antimicrobial activity expressed by the ratio of absorbance disparity at 0 time and 9th hours at TSB medium that has been added with sample compared to the medium without sample. When the ratio approaches or reach 1.000 means the sample does not able to inhibit the growth of *Staphylococcus aureus* in the medium, on the other hand the smaller the value the greater inhibition or the greater antimicrobial activity. In this research the highest inhibitory activity was showed by 24 hour fermentation and concentration ratio of skim: sucrose 6:6 (Table 2).

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Table 1: Chemical and physical Composition of Cheese Whey after Addition of Skim milk and Sucrose Before Fermentation

Treatment		Parameters							
Length of Fermentation time (hours)	Ratio Skim: Sucrose (%)	Total N (%)	Dissolved N (%)	Total Sugar (%)	TDS (°Brix)	pH	Tot. Acid (%)	Viscositiy (cp)	Tot. LAB (log cfu/mL)
18	3	0,3880	0,2334	11,6151	15,07	5,8	0,47	1,150	8,2673
	6	0,3904	0,2535	11,0946	13,93	6,2	0,45	1,270	8,1578
	9	0,3912	0,3043	10,6799	13,47	6,4	0,38	1,307	8,4702
24	3	0,3840	0,1832	11,4647	15,20	6,1	0,51	1,130	8,3423
	6	0,3880	0,2963	11,2550	14,00	6,3	0,45	1,234	8,3942
	9	0,3912	0,2962	10,9051	13,67	6,4	0,35	1,303	8,4586
30	3	0,3848	0,1882	11,5751	14,07	6,1	0,55	1,119	8,4610
	6	0,3872	0,2705	11,5447	13,80	6,3	0,47	1,239	8,3879
	9	0,3936	0,2825	10,4969	13,60	6,6	0,35	1,298	8,2628
36	3	0,3852	0,2015	11,5474	14,07	6,0	0,53	1,149	8,4481
	6	0,3884	0,2718	11,2978	14,00	6,2	0,46	1,236	8,4242
	9	0,3918	0,2922	10,9141	13,53	6,5	0,38	1,315	8,4615
Control		0,1694	0,1026	6,2150	5,67	5,5	0,57	0,8838	8,1987

Table 2: Average Antimicrobial Activity of Probiotic fermented Cheese Whey beverages in various length of fermentation time and concentration ratio of skim milk and sucrose

Treatment Combination		Average Antimicrobial Activity
Length of Fermentation time (hours)	Ratio Skim:Sucrose (%)	
18	3:9	0,683
	6:6	0,666
	9:3	0,630
24	3:9	0,726
	6:6	0,606
	9:3	0,618
30	3:9	0,892
	6:6	0,878
	9:3	0,721
36	3:9	0,982
	6:6	0,935
	9:3	0,863

This is presumably because the activity of the active cells of *Lactobacillus acidophilus* to produce antimicrobials compounds reached the highest after 24 hour fermentation time. During 24 hours of fermentation the bacteria reach logarithmic growth phase which is at this stage cells need more energy. This energy was obtained from the breakdown of the nutrients in the medium into organic acids such as acid lactate. Probiotic bacteria such as *Lactobacillus acidophilus* can produce organic acids such as lactic and acetic acid, hydrogen peroxide, and bacteriocins [15]. Decrease in pH in the medium due to the existence of organic acids produced by the bacteria which in turn cause the bactericidal and bacteriostatic effects.

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Chemical analysis

The longer the fermentation time along with the decrease of skim milk concentration and the increase of sucrose caused the total acid to increase. The highest total acid showed by the length of fermentation time of 36 hours and concentration ratio of skim milk:sucrose of 3:9 (Figure 2). *Lactobacillus acidophilus* breaks down lactose and protein in the medium to produce lactic acid. In the period of 36 hours, there is an accumulation of lactic acid in the product. Homofermentatif lactic acid grows in the media with the main source of carbon of glucose and lactose [16]. This will produce 90 % of lactic acid in the end.

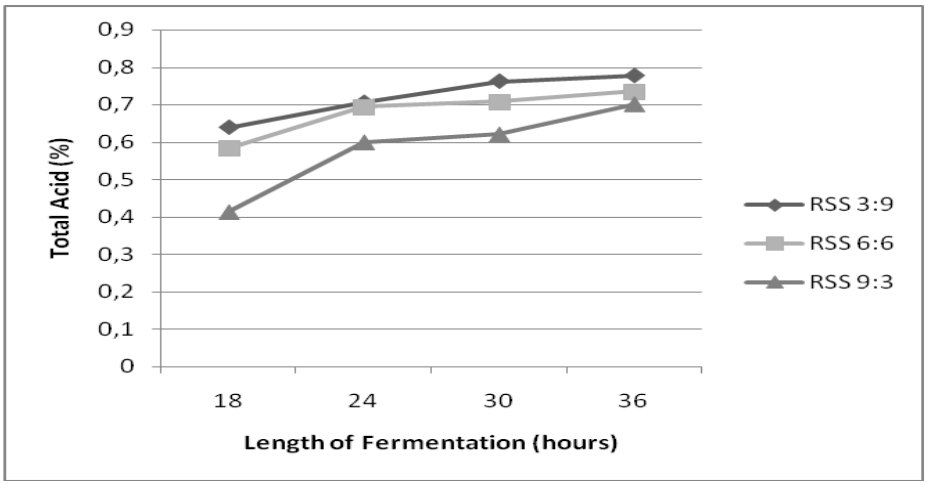

Figure 2: Effect of Length of Fermentation Time and Ratio Concentration of Skim: Sucrose on Total Acid Probiotic Fermented Cheese Whey Beverages.

The longer the fermentation time, the longer the time for the population of *Lactobacillus acidophilus* bacteria to grow and produce acid from lactose and sucrose, resulting in the accumulation of lactic acid and lowering the pH of fermented cheese whey beverage (Figure 3). The lowest pH obtained at fermentation time of 36 hours and the concentration of skim: sucrose 3:9. The optimum incubation temperature causes lactic acid ionization process is maximized resulting in increasing number of H ions are released [17]. The increase in free H ion is causing the pH to decrease.

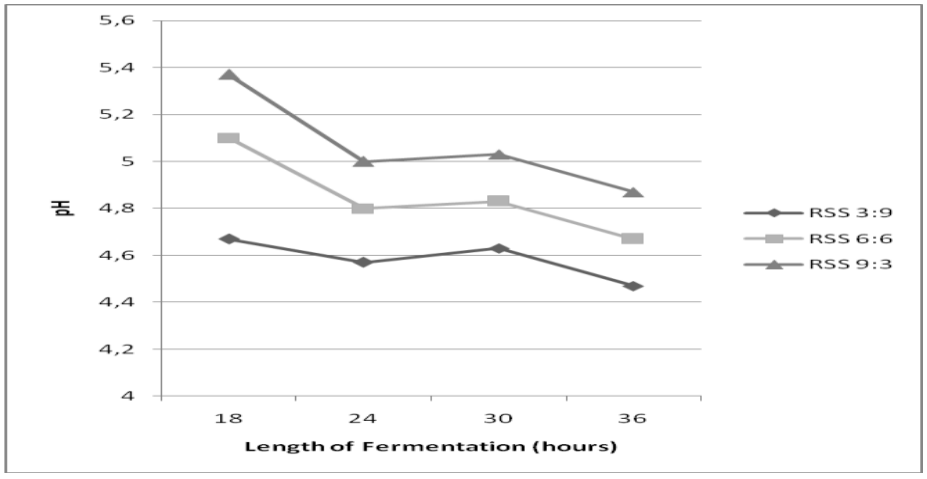

Figure 3. Effect of Length of Fermentation Time and Concentration Skim: Sucrose On The Ph Cheese Whey Fermented Probiotic Beverages.

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The highest total nitrogen obtained at 18 hours of fermentation time treatment with the concentration ratio between skim: sucrose 9:3, while the lowest value obtained in the treatment of fermentation time of 36 hours with a concentration of skim: sucrose 3:9. During fermentation there is a decrease in total nitrogen (Figure 4), it is thought because of the degradation of proteins into simpler compounds by microbes. The process of fermentation on protein degradation products will produce volatile compounds that allow a reduction in total nitrogen. Intermediate compounds and amino acid breakdown products vary widely [18]. There was also the release of alcohol and various gases such as carbon dioxide, methane, hydrogen, and ammonia. Ammonia is released in high amounts in further protein breakdown.

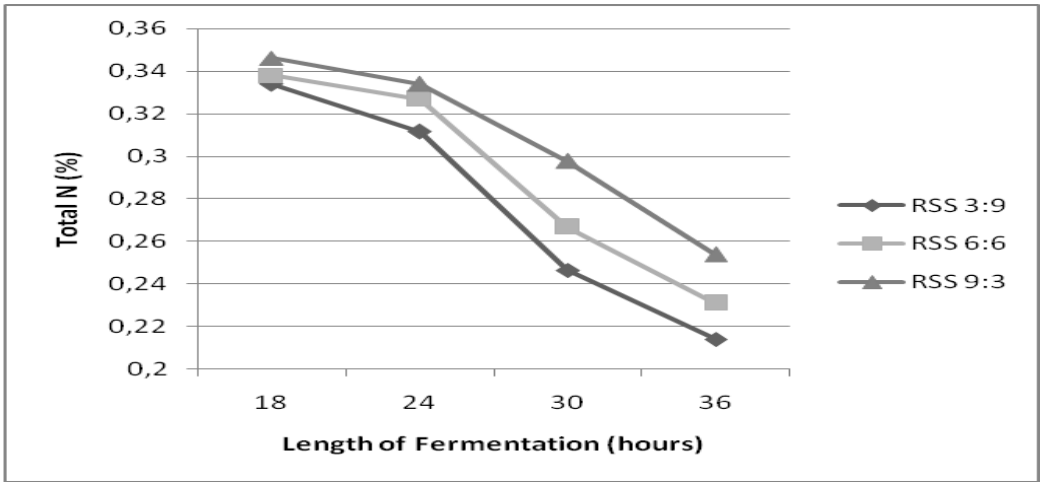

Figure 4. Effect of fermentation period and concentration Skim: Sucrose on Total Nitrogen of Cheese Whey fermented probiotic beverages

The longer the fermentation time with increasing concentrations of skim and decreased concentration of sucrose also cause the increase of dissolved nitrogen levels (Figure 5). During fermentation it is suspected that peptides and amino acids resulted from hydrolysis of proteins by protease enzyme activity increase. Moreover due to the increase of protein because of the addition of skim milk cause the reformation of nitrogen solute. A research found that the decomposition of protein is catalyzed by certain enzymes will eventually formed free amino acids [19].

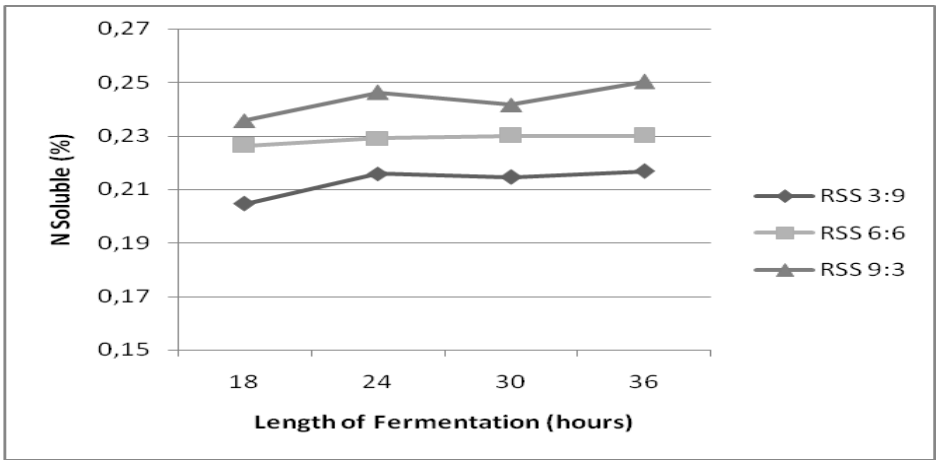

Figure 5. Effect of fermentation period and concentration Skim: Sucrose on Soluble Nitrogen of Cheese Whey fermented probiotic beverages

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Total sugar in the product decrease with longer fermentation time and increased concentrations of skim and reduced sucrose concentration (Figure 6). This is due to the activity of Lactobacillus acidophilus bacteria that converts sugars in a medium such as lactose from skim milk and sucrose into end products such as lactic acid. Total lowest sugar is shown by treatment of 36 hours of fermentation with skim and sucrose concentration ratio 9:3. In the bacterial cell lactose is hydrolyzed by the enzyme β-D-galactosidase into glucose and galactose [20]. Glucose is then converted to lactic acid through the EMP. Galactose can be reformed into glucose-6-phosphate via the Leloir pathway.

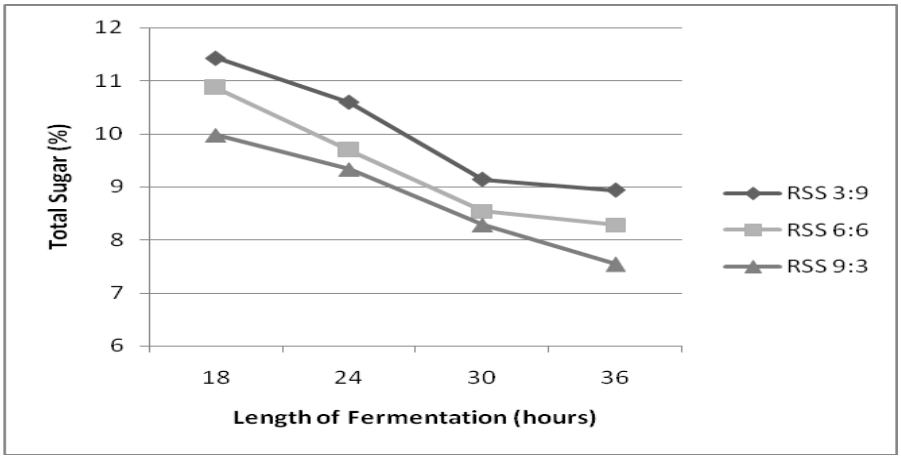

Figure 6. Effect of fermentation period and concentration Skim: Sucrose on Total Sugar of Cheese Whey fermented probiotic beverages

Physical Analysis

Highest total dissolved solids (TDS) obtained at 18 hours of fermentation treatment and skim and sucrose concentration ratio 3:9, whereas the lowest value obtained in the treatment of fermentation time of 36 hours with a concentration of skim: sucrose 9:3 (Figure 7). The addition of sucrose led to an increase in total solids considering the addition of sucrose besides to improve texture, taste, flavour, and also a cheap source of total solids [7]. The decrease in total dissolved solids along with the duration of fermentation is suspected because of the amount of nutrients in the form of lactose and sucrose in the product utilized by lactic acid bacteria (*Lactobacillus acidophilus*) for metabolism. This is indicated by the decline in the total value of the sugar with the increasing fermentation time.

Highest relative viscosity values obtained at fermentation time of 36 hours with a concentration of skim: sucrose 9:3, while the lowest value obtained in a 24-hour fermentation treatment with skim and sucrose concentration ratio 3:9 (Figure 8). The longer fermentation increased the relative viscosity due to the high content of lactose and protein in the product. With the activity of Lactobacillus acidophilus that converts lactose into lactic acid causes the solution has a low pH value. This situation is thought due to lower pH values causes some whey protein fraction (the fraction of β-lactoglobulin especially) and skim milk protein (especially casein) becomes unstable and coagulate to form a gel. This is supported by prior finding that the milk protein will coagulate because of acid [21], and that the isoelectric pH of lactoglobulin is 5.1 to 5.6 [22].

ISSN: 0975-8585

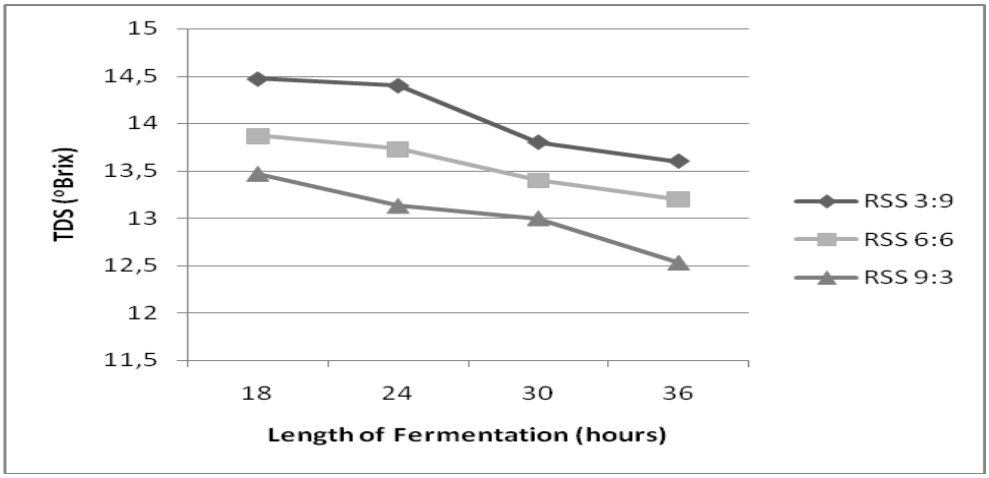

Figure 7. Effect of fermentation period and concentration Skim: Sucrose on Total Dissolved Solid (TDS) of probiotic fermented Cheese Whey beverages

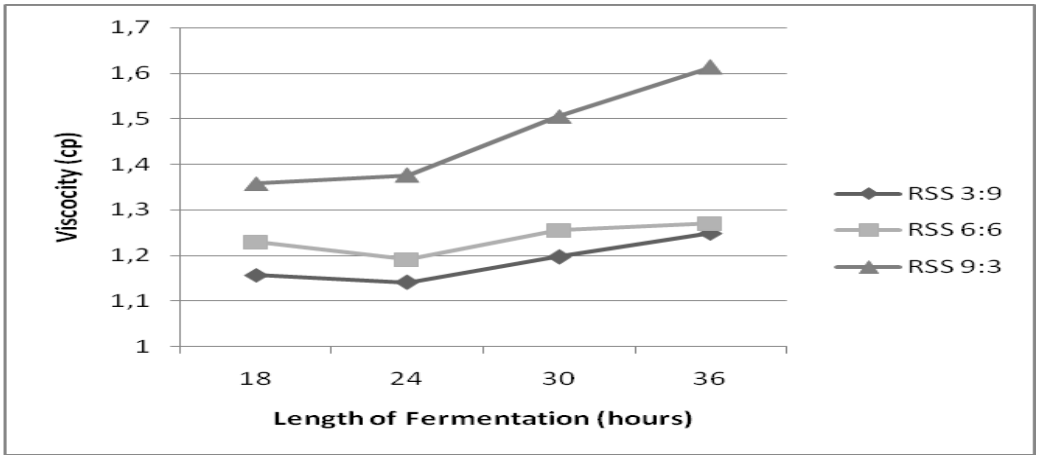

Figure 8. Effect of Fermentation Period and Concentration Skim: Sucrose on Viscocity of Probiotic Fermented Cheese Whey Beverages

Organoleptic Analysis

The mean value of the panellists on the taste preferences of probiotic beverages fermented whey cheeses ranged from 2.10 (do not like) - 5.30 (kind of like). Highest level of preference for flavours found on the product with fermentation time of 30 hours with skim and of sucrose concentration ratio 3:9 and the lowest value found in products of fermentation treatment for 36 hours and the concentration of skim: of sucrose 9:3. This is because the increasing of sucrose added cause an increase in sweetness which is preferred by panellists. Value preferences of panellists on the product with 36-hour fermentation time decreases, this is because with the longer of fermentation time the more optimal the metabolism of nutrients fermented into lactic acid. Lactic acid formed will give a distinctive flavour to the fermented cheese whey beverage, but the excess acid in the product fermented for 36 hour was likely not preferred by the panellists.

Skim milk added in the product has a relatively high content of lactose ie 52.3% and lactose content of 3.8 to 4.3% was also found in fresh cheese whey. Lactose is the main carbohydrate that can be used by the starter culture (*Lactobacillus acidophilus*) as a carbon and energy source for growth. Lactose is converted to glucose and then hydrolyzed by the

enzyme dehydrogenase produced by starter and produce lactic acid. Lactic acid produced causes a decrease in milk pH or increasing the acidity and cause the characteristic flavour of yogurt.

The mean value of the panelists's favourite flavour of cheese whey fermented probiotic beverages ranged from 3.45 (somewhat like) - 4.45 (neutral).The mean value of the preference panellists to the appearance probiotic fermented cheese whey beverages ranged from 3.50 (somewhat like) - 4.35 (neutral).

CONCLUSIONS

The growth of lactic acid bacteria (*Lactobacillus acidophilus*) in probiotic fermented cheese whey beverages reaches the range 9.8832 to 11.6959 log cfu / mL. The highest mean total *Lactobacillus acidophilus* demonstrated by product fermented for 24 hours and the concentration skim: sucrose 6:6, and decreased at 36 hours of fermentation. Testing of antimicrobial activity of probiotic fermented cheese whey beverages showed the inhibition against indicator bacteria *Staphylococcus aureus*. The highest inhibitory activity demonstrated by products with a 24-hour fermentation time and concentration of skim: sucrose 6:6.

The best combination of treatment of concentration ratio of skim milk and sucrose and variation in the fermentation time of probiotic fermented cheese whey beverages based on parameters physical, chemical, and microbiological shown by treatment fermentation for 24 hours and the concentration skim: sucrose 6:6. Whereas for organoleptic parameters demonstrated by treatment with fermentation time of 30 hours and skim the concentration: sucrose 9:3.

ACKNOWLEDGEMENTS

We are grateful to the PAU Food and Nutrition Gadjah Mada University for providing the bacteria strain *Lactobacillus acidophilus* FNCC-0051 and to the Laboratory of Product Technology Faculty of Animal Husbandry, University of Brawijaya for providing cheese whey. We also would like to thanks to Dr. Ir. Estri Laras Arumingtyas, MSc.St. for reviewing this paper.

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