THE EFFECT OF DIFFERENT RATIO OF BACTERIA (LACTOBACILLUS BULGARICUS + STREPTOCOCCUS THERMOPHILUS AND BIFIDOBACTERUM LONGUM. ATCC15707) ON CHARACTERISTICS OF YOGURT AT DIFFERENT STORAGE PERIOD

M. Sabbah., A. M. Legowo, Y. B. Pramono

ABSTRACT: The main purpose of this research is to provide information about the effect of Bifidobacterium longum ATCC15707 on the characteristics of yogurt during different of storage period. The B.longum was obtained from Laboratory of Food and Nutrition Collection Culture Gadjah Mada University and a S.thermophillus + L.bulgaricus (1:1) was obtained from a commercial yogurt (Milkuat). This research was performed in Laboratory of Animal Science and Dairy Milk Processing Industry, Gadjah Mada University, used 8 L fresh milk cow, yogurt sample were stored at 4 ºC for 28 days. Research using Completely Randomized Design with Factorial pattern A x B (4 x 2). Factor A is the ration between yogurt starter ST+Lb and BL (T1 is control 4% from yogurt starter, T2 1:3 (v/v), T3 2:2 (v/v), T4 3:1(v/v)). Factor B is the storage period (1 and 21 days) for chemical, physical and organoleptic analyses and (1, 14, 21 and 28) for microbial. Parameters measured are microbiology (total lactic acid bacteria), chemistry (acidity, pH, total solids, fat, protein and lactose), physical (viscosity) and organoleptic quality (color, texture, taste, performance, sweetness, acidity, bitterness and rancidity). If there is a real effect of treatment was followed by Duncan Multiple Test and LSD test for organoleptic quality. Microbial counts were log transformed and statistically evaluated. The result indicated that the yogurt produce with 3% (S.thermophillus and L. delbrueckii subsp. bulgaricus) and 1% B.longum ATCC15707 during storage period (1 and 21 days) at 4 ºC effected significantly to increase acidity and total solid of yogurt more than other treatment, decrease pH value significantly different. Also this ratio between starter bacteria obtained the high score in the taste and texture from the panelists.

Keywords: yogurt, storage period, B.longum ATCC 15707

INTRODUCTION

The Bifidobacterium is presently the focus of renewed attention because of recent studies that underscore its importance for human health (Challa et al., 1997). Bifidobacterium provides countless essential nutrients, boosts immune function, protects against “unfriendly” bacteria, and supports a large network of cellular functions (Fuller and Gibson, 1997). Bifidobacteria use a unique pathway for carbohydrate metabolism the py-product consist of a mixture of acetic and lactic acids the pathway includes a unique enzyme (fructose-6-phosohate phosphoketolase), used and is a key diagnostic test to identify bifidobacteria (Chandan et al., 2006). As the bacteria grow, they use lactose as an energy source and produce lactic acid which lowers the pH and makes the yogurt’s taste as sour. Initially S. thermophilus ferments the lactose. L. bulgaricus, which is more acid tolerant, continues to ferment the remaining lactose. During this process the pH drops from 6.5 to around 4.5. This inhibits the growth of spoilage microbes. The presence of lactic acid causes the structure of the milk protein to change which gives yogurt its special thickened texture. The lactic acid also gives the yogurt its sharp taste. Other products of lactic acid fermentation such as acetaldehyde give the yogurt its characteristic aroma (Tamime and Robinson, 2003).

Lactic acid bacteria partially hydrolyze proteins and the amount of free amino acids in fermented dairy products increase. Moreover, pre-hydrolysis of these proteins may be useful for people who are lacking of digestion enzymes. The fat content of yogurt varies from 0.1% to 10% depending on the yogurt standards described by each country in the World (Tamime and Robinson , 2003). Generally, the mineral content of yogurt is similar to milk. Yogurt is an excellent calcium source for people suffering from lactose intolerance. Moreover, calcium supplied by yogurt may be better absorbed and utilized than calcium made available in other forms (Mckinley, 2005).

The research today focuses on methods of increasing viability of the live cultures, particularly the probiotics, as they are responsible for conferring the health benefits of yogurt. one of the way to improve yogurt both in terms of its potential as a healthy food and as an appetizing product by using Bifidobacterium longum ATCC 15707 this probiotics bacteria effected to reducing symptoms of lactose intolerance, improvement the taste of yogurt and effected on the physical of final product. in this study the measurement the influence of the different ratio between yogurt bacteria and B.longum on the microbial, chemical,

Submitted 9/12/2011, Accepted 20/2/2012. *M. Sabbah is with Department of Nutrition and Food Processing, Faculty of Agriculture, An-Najah National University, P.O. Box 7, Nablus, Palestine. A. M. Legowo and Y. B. Pramono are with Animal and Agricultural Sciences Faculty, Diponegoro University, Semarang, Indonesia. Direct inquiries to author A. M. Legowo (E-mail: anang_ml@yahoo.com).*

physical and sensory characteristics of yogurt product during different storage period.

This study aims to examine the influence of Bifidobacterium longum ATCC 15707 with different ratio between (S.thermophilus + L. delbrueckii subsp bulgaricus) on the characteristics of yogurt was the product stored at 4ºC for 21 days. Benefits of this research is to provide information about the effect of Bifidobacterium longum on the characteristics of yogurt during different of storage period and. Bifidobacteria have a symbiotic relationship with its human host. Bifiobacteria are known to be probiotic, which means that it's a microbe that helps to protect its host and prevent disease.

MATERIALS AND METHODS

This research was held on April- May 2011 at the Laboratory of Animal Science and Dairy Milk Processing Industry, This laboratory holds a certificate of ISO 17025:2005, and Laboratory of Food Livestock Products (Milk and Eggs), Gadjah Mada University.

Material

The research material 8 liter fresh cow milk content 87.69% water, 12.31% total solid, 3% protein, 3.58% fat and 4.52% lactose. Traditional starter culture bacteria (Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus) obtained from the commercial yogurt (milkuat), and (Bifidobacterium longum ATCC 15707 are from American Type Culture Collection) obtained from Food and Nutrition Collection Culture (FNCC) Gadjah Mada University. Samples yogurt, aluminum foil, aquades, de-mann Rogosa Sharpe Broth (MRS-Broth), agar, penolptalin 1%, soduimhydroxide (NaOH) 0.1N, L-cystine, galactosamine, ammonia, ethanol 95%, ethyl ether, petroleum ether, hydrochleric acid (HCl),sulfuric acid (H2SO4), boric acid (H3BO3), potassium sulfate (K2SO4), Kjeldahl digestion tablets. The research equipment were autoclave, oven, incubator, refrigerator, thermometer, electric scales, Petri dish, glass beaker, erlenmeyer, pot, burette, test tube, micropipette, magnatic stir, pH meter model HANNA, colorimetric endpoint, mixer, desiccators, tongs, majonnier extraction flasks, mojonnier fat dishes, plastic pipette 1 and 0.5 ml, measuring flask.

Method

Preparation Bulk Starter Culture (ST+Lb) from Commercial Yogurt

Mother starter (ST+Lb) was prepared from commercial yogurt (milkuat) with grapes flavor content 1:1v/v two lactic acid bacteria (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus). The first 810 ml fresh cow milk obtained from the Gadjah Mada University was divided at the 2 Bottles 100 ml and 2 bottles 500 ml, after that the milk was sterilized in 110 ºC for 10 minutes, after that the milk cooled until 40 ºC. Secondly, first rejuvenation were taken 10 ml from commercial yogurt (milkuat) after shake it. Then inoculation in the 90 ml milk, after that put the first rejuvenation into the Incubator in 39 ºC, before the milk started conglomerate turn off, then took 10 ml to produced the second rejuvenation. The main goal of this rejuvenation was to remove flavor from commercial yogurt.

Step of processing mother starter and bulk starter (ST+Lb) from commercial yogurt (milkuat). Thirdly, the second rejuvenation were taken 10 ml from the first rejuvenation and inoculation in 90 ml milk sterile and made the same process like the past step. Fourth, inoculation 30 ml yogurt product from second rejuvenation to two bottles content 270 ml milk sterile for product bulk starter was used for inoculate milk , after that incubator in 39 ºC then one of bottle for acidity analysis each 15 minutes and the other bottle for the bulk starter storage in the refrigerator at 4 ºC. The fermentation was stopped when the acidity of yogurt mother starter in the range between 0.3-0.5 that meaning the total LAB more than 10-7 cfu/ml, bulk starter content (S.thermophilus and L. bulgaricuswas) with ration 1:1 ready to use for product yogurt.

Preparation Bulk Starter Bifidobacterium longum (BL)

Strain of Bifidobacterium longum ATCC 15707 American Type Culture Collection was obtained from the laboratory collection culture Gadjah Mada University. Firstly made pepton water, one gram pepton water for one liter distilled water after that sterilized it in 120 ºC for 20 minutes. The second made MRS broth, 1 g for 20 ml distilled water, and 0.1 g L-cystein to reduced O2, after that put it in the erlenmeyer flask and homogeneous all by magnetic stirring until all of the material dissolved in water. Thirdly sterilized of MRS broth in 121ºC for 15 minutes. Added growth factor galactosamine content of 2.5 g of galactosamine dissolved in 50 ml of distilled water and then sterilized the galactosemine by Millipore filter 0.2 µm.

When the media and pepton water had sterile, opened the ampoule content of B.longum, and then mixed it with adding 1ml pepton water , after that the Mixture was placed in 9 ml of MRS broth and mix together, then placed the test tube in the incubator in 39 ºC for 24 h, after 24 h took 10 ml MRS broth content of B. longum and injected it into the 90ml sterilized milk, then added 0,5 ml glycerol and 30 ml were taken from MRS broth content of B.longum and injected into the 300 ml sterilized milk then added 1,5 ml of glycerol, the glycerol added to increasing the shelf life of lactic acid bacteria under the cold conditions. After 2 h from incubation in 39 ºC analysis acidity by titration were taken 9 ml for yogurt then add 2 drop from p.p (Phenolptalein 1%) and titration by NaOH. The fermentation was stopped when the acidity of yogurt mother starter in the range between 0.3-0.5 that meaning the total LAB more than 10-7 cfu/ml. Finally, 300 ml bulk starter B.longum culture had prepare and ready to used to inoculate milk.

Yogurt Making

Eight litter fresh milk cow was pasteurized in 75 ºC for 5 second, after that cooled it until 45-44 ºC, then divided

it into 16 bottles size 500 ml each bottle was filled with 480 ml milk, then the milk was inoculated with 4% of starter culture. This experiment used two kind of starter culture, traditional starter culture (S.thermophilus + L.bulgaricus 1:1 v/v) and B.longum ATCC 15707. Each treatment was inoculated with different ratio between (S.thermophilus + L.bulgaricus) and B.longum ATCC 1570 (T1 4% ST+Lb), (T2 1% ST+Lb : 3% BL), (T3 2% ST+Lb : 2% BL) and (T4 3% ST+Lb : 1% BL) the inculcation of starter culture was obtained under the sterile condition, then the bottles was placed into the incubator in 38 ºC for 6 hour, then cooled the product in the room temperature for one hour, after that the yogurt product were stored in 4 ºC. Chemical, physical, microbial and Sensory characteristics of yogurt were analyzed in 1 and 21 days from the storage time, for one day of storage time used 8 bottles because each treatment content of 4 bottle 2 bottles for one day of storage time and 2 bottles for 21 days, but for the analysis of total lactic acid bacteria was held four time at 1, 14, 21 and 28 days of storage time because two times storage period not enough to know about the growth of lactic acid bacteria in yogurt product.

Experimental Design

The experimental design were used factorial design of A× B (4×2) factorial arrangement of treatments in a Completely Randomized Design (CRD) with repeated measures was utilized two replication. Differences among treatments were evaluated by analysis of variance. All analysis was performed using SAS. The Duncan test was used when analysis result significantly different between treatment. The treatment factor being gave in this research were (A) the use B. Longum and (Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus) with different ratio and (B) different time storage period (1, 21) days.

The variables observed in this study was total LAB, acidity and viscosity of yogurt product with different ratio of starter culture (S. thermophilus + L.delbrueckii subsp. bulgaricus) and B.longum ATCC 15707 (4:0, 1:3, 2:2, 3:1) and different time storage period 1 and 21 days for acidity and visvosity, but 1, 14, 21 and 28 days for total LAB. The procedures variables testing will be explained later.

Total Lactic Acid Bacteria (Total LAB)

Calculation of total lactic acid bacteria proceeded by dilution with 1:9 ratio ranging from - . The first dilution was done by using pipette 1 ml sample tube inserted into the first dilution of the second dilution was done by use pipette 1 ml sample was diluted in dilution first introduced into 9 ml sterile distilled water in a test tube, the third to the ninth dilution performed with In the same way as in the second dilution. Then performed the putting in cups using MRS media by way of 5,2 g of MRS broth and 2 g agar dissolved in 100 ml distilled water and then sterilized in an autoclave 121 for 15 minutes. Putting in cups made by way of 1 ml sample of the dilution - taken and entered into a petri dish. Then 10-15 ml of MRS medium for which has been cooled (temperature 47-50) was poured into the petri dish. Petri dish was moved further bleak .After solid plates were incubated upside down with a temperature of 37 for 14 hours.

Calculation of total lactic acid bacteria using standard plate count method is based on the assumption that every living bacterial cell will grow into a colony after incubated in culture medium and a suitable environment. According to (Fardiaz, 1993). Calculated total lactic acid bacteria were done by as follows: the selected and calculated cup containing the number of bacteria is between 30-300. Some colonies are incorporated into one is a collection of large colonies where the number of colonies in doubt, can be computed as a colony. A colony is visible as a thick line can be computed as a colony.

Titratable Acidity (TA)

Titratable acidity was obtained by titration method with 0.1 N NaOH, filled Erlenmeyer class with 9 ml of yogurt, then add 2-3 drop of indicator (pp) phenolphthalein. Yogurt was titrated with NaOH solution until the pink color the It is expressed as %lactic acid (Soukoulis et al., 2007).

Viscosity

Viscosity was measure by Brookfield viscometer model RVT. Temperature sample was 4ºC. Attach the spindle place the yogurt cup on the counter and lower the spindle through the surface of yogurt to level the notch on the spindle to the surface of yogurt, after that turn the viscometer on, then depress the lever after 25 seconds and then take the reading (Chandan et al., 2006).

RESULTS AND DISCUSSION

Total Lactic Acid Bacteria (LAB)

The results of total LAB see (Table 1) indicated the different ratio between (S. thermophilus + L. bulgaricus) and (B. Longum) at different of time storage period showed non significant different, between time of storage and the ration of LAB no interaction, ration between LAB were not give effect on the total LAB (cfu/ml). But the different of storage period 1, 14, 21 and 28 showed significant different ($p<0.05$), no different between the average of total LAB (cfu/ml) in 1, 14 and 21 days of time storage period (8.75, 8.50, and 9.15), respectively. In 28 days the total of LAB decreases that different with the other of storage time.

The result indicated the highest of average total LAB (cfu/ml) in 21 days at 4 ºC and the lowest in the 28 days of time storage at 4 ºC. That because the growth rate of bacteria depends on the amount of lactic acid produced, and thus on the pH. The bacteria grow faster at higher pH value. In the 28 days, the amount of lactic acid increased and pH decreased that affected to the decrease of total lactic acid bacteria in yogurt product.

The activity of lactic acid bacteria during the different storage time in this experiment is shown in (Figure 1). Found in the T4 with ratio 3:1% starter culture (ST+Lb) and (BL) respectively. The total of LAB in 14 days was higher than

Table 1. The Total LAB of Yogurt at the Different Ratio of Lactic Acid Bacteria during 1, 14, 21, and 28 Days of Storage period

Treatment (*ST+Lb*): (*BL*)	Total LAB log cfu/ml				
	Storage period (days)				
	1 days	14 days	21 days	28 days	Average
T1 4:0	8,32	7,92	9,48	5,89	7,90
T2 1:3	9,15	8,80	9,68	6,90	8,63
T3 2:2	9,07	8,27	9,38	5,83	8,13
T4 3:1	8,49	9,04	8,06	6,35	7,98
Average	8,75[a]	8,50[a]	9,15[a]	6,24[b]	

T1= Treatment 1 with 4% (*S.thermophilus+L.bulgaricus*), T2=Treatment 2 with 1% (*S.thermophilus+L.bulgaricus*) and 3% *B.longum*, T3= Treatemnt 3 with 2 (*S.thermophilus+L.bulgaricus)* and 2% *B.longum*, T4= Treatment 4 with 3% (*S.thermophilus+L.bulgaricus)* and 1% *B.longum*, [a,b]= Different letters within the same column differ significantly ($p<0.05$).

Table 2. The Titratable Aacidity of Yogurt at the Different Ratio of Lactic Acid Bacteria during 1 and 21 Days of Storage Period

	Treatment (*ST+Lb*): (*BL*)	Storage period (days)		Average
		1 days	21 days	
Titratable acidity (%)	T1 4:0	0,58[bc]	0,63[ab]	0,60
	T2 1:3	0,63[abc]	0,57[c]	0,60
	T3 2:2	0,59[bc]	0,61[abc]	0,60
	T4 3:1	0,60[abc]	0,64[a]	0,62
	Average	0,6	0,61	

[a,b,c,d] = different letters within the same column differ significantly ($p<0.05$). T1= Treatment 1 (control) with 4% (*S.thermophilus+L.bulgaricus*), T2=Treatment 2 with 1% (*S.thermophilus+L.bulgaricus*) and 3% *B.longum*, T3= Treatemnt 3 with 2 (*S.thermophilus+L.bulgaricus)* and 2% *B.longum*, T4= Treatment 4 with 3% (*S.thermophilus+L.bulgaricus)* and 1% *B.longum*.

Table 3. The Viscosity of Yogurt at the Different Ratio of Lactic Acid Bacteria during 1 and 21 Days of Storage Period.

Treatment (*ST+Lb*): (*BL*)	Viscosity (mps's)		
	Storage period (days)		
	1 day	21 days	Average
T1 4:0	2800	237.5	1518
T2 1:3	2000	306	1153
T3 2:2	2800	575	1687
T4 3:1	4150	600	2375
Average	2937[a]	429.62[b]	

[a,b] = Different letters within the same column differ significantly ($p<0.05$). T1= Treatmen t 1 with 4% (*S.thermophilus+L.bulgaricus*), T2=Treatment 2 with 1% (*S.thermophilus+L.bulgaricus*) and 3% *B.longum*, T3= Treatemnt 3 with 2 (*S.thermophilus+L.bulgaricus)* and 2% *B.longum*, T4= Treatment 4 with 3% (*S.thermophilus+L.bulgaricus)* and 1% *B.longum*

other treatment, after that the total L AB of T4 decrease directly until 28 days without increase in 21 days the same result with other treatment, though. In one days of T1 and T4 had same total LAB that because 1% of B.longum with 3% of (ST+Lb) gave effect to reduce the validity of the yogurt product. On the other hand T2 with ration 1:3% starter culture (ST+Lb) and (BL), showed high total LAB on the 1, 21 and 28 days of time storage respectively. Two and 3 % of starter culture from B.longum effected to increasing total LAB (cfu/ml) in yogurt product, that increase just slightly different with the control treatment content of 4% of (ST+Lb).

The results of the study of growth of lactic acid bacteria during refrigerated storage, presented in (Table 1). The different ration between (ST+Lb) and (BL), shown no significant differences $p<0.05$ on the total lactic acid bacteria in yogurt product. On the other hand, the increase of storage period affected to decrease total (LAB) significantly $p<0.05$. Ham et al. (2009) reported that after storage at 4 °C for 2 and 3 weeks, the number of lactic acid bacteria decreased slightly. The lowest total of LAB founded in days 28 was 6.24 log cfu/ml and the highest total LAB founded in days 21 was 9.15 log cfu/ml, for T1, T2 and T3 in the days 21 was high total LAB, but in the T4 the total LAB was to low too 8.06 log cfu/ml. The 2% of (S.thermophilus +

L.bulgaricus) and 2% of B.longume ATCC15707 from starter culture affected to increase the total LAB during storage period (1, 14, 21 and 28) days. The increase of acidity and decrease pH value affected to decrease the total LAB. Seeleel (2009) reported that the total LAB decreases because the pH decreases in the storage period.

Titratable Acidity (TA)

Acidity of yogurt at different ration of starter culture ST+Lb and BL during different time storage period are shown in (Table 2) and (Figure 2). Sample at days 1 and 21 were gave some variety of starter culture ST+Lb and BL between (0.57 and 0.64%). The highest acidity value obtained at days 1 was 0.60 treatments 4 and it being increased 0.64 at day 21. The highest acidity decrease was observed in treatment 2 (from 0.63 in 1 day to 0.57 in 21 days). Average of acidity in one day 0.60 and the acidity in 21 days of time storage 0.61. From this result, the acidity of yogurt at 1 and 21 days with different ration between ST+Lb and BL non significant different.

Average acidity of yogurt at different ratio of starter culture ST+Lb and BL (T1 4:0, T2 1:3, T3 2:2 and T4 3:1 v/v), respectively. This ratio showed non significantly difference between treatment (0.60, 0.60, 0.60 and 0.62), respectively. Highest acidity in T4= 0.62 with ratio 3% (ST+Lb): 1% (BL) v/v. The result showed that the acidity of yogurt affected significantly different ($p > 0.05$) with two factors, they were storage period 1 and 21 days and ratio of starter culture (ST+Lb) and (BL). There was some correlation between two factors that were the increase of time storage period of yogurt with 1% of bifidobacterum longum and 3% of (ST + Lb) affected the increase of yogurt acidity more than the control treatment.

Acidity of yogurt was produced from the metabolism of milk lactose by lactic acid bacteria. In one day of storage acidity of yogurt in treatment 3 and 4 had same result with the acidity of control treatment T1 that depend on the total lactic acid bacteria in the yogurt. Because of total lactic acid bacteria was increase, that being effected on the increased of yogurt acidity, that path was found in the T2 one days of storage with ratio 1:3 v/v (S. thermophilus + L. bulgaricus) and B.longum, respectively. The acidity increased in T2 because there were 3% of BL and lactic acids bacteria. In this part, BL effected on the metabolism of lactose because BL used an unique pathway for carbohydrate metabolism and its by-products consist of acetic mixture.

The increased of storage period at temperature 4 ºC the acidity yogurt in treatment 1, 3 and 4 were being increased just slightly different, because the activity of lactic acid bacteria inhibition at 4 ºC, and the increased of storage time period effected the decrease of total lactose of milk. But acidity at the different of storage time period in the treatment 2 with ratio 1:3 v/v (S. thermophilus + L. bulgaricus) and (B. longum) was decrease higher than other treatment because the ratio of (S. thermophilus + L. bulgaricus) more than B.longum. The increased of acidity affected the decreased the pH value. The result of titratable acidity yogurt at different ratio between (S.thermophilus + L.bulgaricus) and B.longume during storage at 4 ºC for 3 weeks presented in (Table 2), the titratable acidity is usually expressed as a percentage of lactic acid (Chandan at el., 2006). Titratable acidity of yogurt with different ratio between (S.thermophilus + L.bulgaricus) and B.longume during storage at 4 ºC for 3 weeks was significantly difference $p<0.05$ there are some correlation between ratio of starter couture and storage period of yogurt.

The 1%, 2% and 3% of B.longum ATCC15707 affected to increase acidity of yogurt. After 24 hour from storage the higher titratable acidity founded in T2= 0.63%, but the increase of storage period in the sample which content of 2% and 3% B.longum made the titratable acidity was decrease. After 21 days storage period the lowest titratable acidity found in T2= 0.57%, while the sample content of 1% B.longum the acidity was still increase in T4= 0.64% than the control treatment. Tamime and Robinson (2003) reported that the yogurt containing more D(-) lactic acid than L(+) lactic acid become highly acidic that can be occur on the increase storage period or increase the temperature 45 ºC or more, or by increase starter inoculation rate was more than 3%, or the starter contained more rods than cocci.

According to (Garvie, 1978; Hemme et al., 1981; Tamime and Robinson, 2003) reported In yogurt starter cultures, S. thermophilus and bifidobacterium produces mainly L(+) lactic acid and D(-) lactic acid is produced by L. delbrueckii subsp. bulgaricus. The T4 have the high acidity during storage period, that because amount of D (-) lactic acid it's higher than L(+) lactic acid with ration 3% of (ST+Lb) and 1% of B.longum ATCC15707 that because, the increase of ration B.longum ATCC15707 effect to increase the L(+) lactic acid.

The result of this research Similar with the Standar National Indonesia (SNI) (2009) reported the titratable acidity of yogurt must around range 0.5-2.0%. Titratable acidity is one of the most important parameters with respect to the shelf-life of fermented milk products and also a reasonable indication of the performance of the starter culture (Tamime and Robinson, 2003).

The catabolism of lactose by S. thermophilus, L. delbrueckii subsp. bulgaricus, and bifidobacterium results mainly in the production of lactic acid or lactic and acetic acids when bifidobacteria are used in the starter culture. During the manufacture of yoghurt, S. thermophilus grows faster than L. delbrueckii subsp. bulgaricus, and hence L(+) lactic acid is produced first followed by D(-) lactic acid. While the bifidobacteria produces L (+) acid as the result of lactose metabolism. Yogurt containing more D (-) lactic acid than L (+) lactic acid become highly acidic (Tamime and Robinson, 2003).

Viscosity

Result analysis of viscosity yogurt with different ratio of starter culture ST+Lb and BL (T1 4:0, T2 1:3, T3 2:2 and T4 3:1 v/v) at different of storage period shown in (Table 3) and (Figure 3). The result indicated that there was non significant

different between treatment. On the other hand, the different storage period gave significant different ($p<0.05$), but there were no correlation between ration of starter culture and storage period because non significant different ($p>0.05$). The apparent viscosity measurements of yogurt samples ranged between 237.5 and 4150 mpas's.

The highest viscosity was obtained for T4 was 4150 mpas's found in one day of storage yogurt, while the lowest viscosity was obtained for T1 237.5 mpas's. The result indicated that the highest average viscosity found in the 1 day of storage period 2937 mpas's, and the lowest viscosity found in 21 days of storage 429.62 mpas's. Reported that the increased of storage period affected to the decreased of yogurt viscosity. Robinson and Itsaranuwat (2006) goat's milk yogurt had a lower viscosity than yogurt made with cow's milk due to the low protein content of the goat's milk. This result similar with Tamime and Robinson (2003) founded that the cooling temperature will influence to reduce the final viscosity of the product. Cahdan et al. (2006) reported that the viscosity yogurt, around 15,000–25,000 cP

Influence of Total LAB, Acidity and Visicosity on the Characteristics of Final Yogurt Product

Total lactic acid bacteria, chemical and physical characteristic of yogurt product with different ration between yogurt starter and B.longum ATCC 15707 during different storage period 1 and 21 days show in (Figure 4) The result indicate that no correlation between ration of starter culture and storage period influence on the total lactic acid bacteria during 1 and 21 days at 4 ºC, the total lactic acid bacteria in 21 days was high than 1 days of storage period, but the total LAB influence significantly during the 1, 14, 21 and 28 days of storage period at 4 ºC.

The significantly correlation between starter culture and storage period effected to increase acidity of yogurt when the ratio of B.longum ATCC 15707 was 2% and 1% but the 3% of B.longum effected to decrease acidity during storage period that because the catabolism of lactose by S. thermophilus, L. delbrueckii subsp bulgaricus, and bifidobacterium results mainly in the production of lactic acid, or lactic and acetic acids when bifidobacteria are used in the starter culture. During the manufacture of yoghurt, S. thermophilus grows faster than L. delbrueckii subsp. bulgaricus, and hence L(+) lactic acid is produced first followed by D(-) lactic acid. While the bifidobacteria produces L (+) acid as the result of lactose metabolism. Yogurt containing more D (-) lactic acid than L (+) lactic acid become highly acidic (Tamime and Robinson, 2003).

The result indicated that the ration between starter culture and different storage period influence significantly to increase total solid and decreased water content after one day of storage the total solid in the treatment content B.longum ATCC 15707 was less than the control treatment. On the other hand, increase storage period effected to increased total solid in the treatment content B.longum ATCC 15707 more than the control treatment. Hassan and Amjad, (2010) founded that with the passage of time, total solid mass could be increased. The increase in total solid contents could be due to loss of moisture. The increase of fat content and decreased water content affected to increased total solid, the increase storage period affected significantly to increase fat content these findings are in accordance with the result of Hassan and Amjad, (2010) who observed that the increase in fat content appeared to be due to acidic pH , and decreased protein content and lactose content.

The result found that increased ratio of B.longum ATCC 15707 influence significantly to decreased lactose content the T2 with ratio 3% B.longum ATCC 15707 and 1% yogurt starter influence to decreased lactose content more than the control treatment and also more than other treatment have less than 3% B.longum ATCC 15707, that because the B.longum have unique enzyme for metabolism lactose to lactic acid (Chandan, 2006).

After one day of storage yogurt the protein content was high than the fresh milk, that because increase protein content in yogurt depends on the proteoltic activity of lactic acid bacteria, which hydrolyses protein (caseins) into peptides and amino acid (Thomsa and Mills, 1981; Hassan and Amjad, 2010). That increase protein content influence to increase the viscosity of yogurt. Then the increased of storage period the protein content was decreased then the viscosity was decreased also, Chandan, (2006) indicated that the viscosity and texture characteristics of yogurt are primarily related to its moisture content and protein level. Apart from quantitative levels, protein fractions and their ratios play a significant role in gel formation and strength. Milk proteins, further, consist of caseins and whey proteins, which have distinct functional properties. Caseins, in turn consist of s1 -, -, and - caseins. The ratio of casein fractions and the ratio of caseins to whey proteins differ widely in the milks of various milch animals. Furthermore, pretreatment of milk of different species, prior to fermentation, produces varying magnitudes of protein denaturation. These factors have a profound effect on the rheological characteristics of fermented milks. Increased storage period at 4 ºC effected significantly to decreased the viscosity of yogurt, this result similar with Tamime and Robinson (2003).

Result of sensory analysis indicated that the texture and taste of yogurt gave significantly different shown in Figure. 3 result found that ration between yogurt starter and B.longum during different storage period 1 and 21 days given significantly influence on the texture and taste of yogurt 1 % of B.longum ATCC 15707 with 3% yogurt starter obtained high score for texture was 3.75 and taste was 3.34 this result high score than the control treatment with 4% yogurt starter. And also the taste of yogurt in T2 obtained the high score in taste but that non significant different with the T4, the texture of yogurt in the T2 with 3% B.longum significantly different with T4. That yogurt fermented with Bifidobacterium generally tends to taste milder in terms of acidity and flavor (Chandan et al., 2006).

The chemicals characteristic of yogurt affected on the taste and texture on yogurt Chandan, (2006) reported that

the fat content is increased; there is a significant improvement in flavor, viscosity, and taste. Generally the all of treatment content different ratio B.longum obtained high score than the control treatment content yogurt starter, that because the bifidobacteria produce more acetic acid than lactic acid. Therefore, if they are used in the culture makeup, the overall flavor profile will change as a result of higher acetic acid content (Chandan et al., 2006).

Normally, the yogurt culture, which is composed of Lb and ST, is responsible for the characteristic flavor and aroma of yogurt through the production of acetaldehyde, diacetyl, and acetic acid during the fermentation process. Lactic acid, being a nonvolatile substance, contributes to the acidic and refreshing taste of yogurt whereas the volatile by-products contribute to its pleasant and characteristic aroma. Of the volatile flavor components, acetaldehyde accounts for almost 90% (Tamime and Robinson, 2003)..

CONCLUSION

Result of this study found that the different ratio between yogurt starter and B.longum ATCC 15707, during storage period (1 and 21 days) had correlation in the acidity, of yogurt product, reject the null hypothesis, and found the storage period given significantly to decreased in the total LAB, and viscosity. According on this study the yogurt produce by 3% B.longum and 1% S.thermophilus+L.bulgaricus effected to increase the chemical characteristics of yogurt that's high acidity during storage period 1 and 21 days are 4 ºC.

Finally 1 % of B.longum and 3% of S.thermophilus + L.bulgaricus can be improvement the microbial, chemical and physical, characteristics of yogurt product more than other ratio and this is reflected positively on the improvement of the health benefits of milk such as lactose intolerance, diarrhea, cancers, and act.

Further research is recommended to evaluate the influence 5%, 6% of starter culture from yogurt starter and B.longum ATCC 15707 on characteristics of yogurt during 1, 2, 3 and 4 week of storage period.Further research is recommended to evaluate and compare characteristics yogurt produce from goat milk and sheep milk on the same condition in this research.

REFERENCES

Challa, A., D.R. Rao, C.B. Chawan and L. Shackelford. 1997. Bifidobacterium longum and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. J . Oxfod. 18 (3): 517-521.

Chandan, R.C. 2006. Enhancing Milk Composition, Physical and Processing Characteristics. In: Chandan, C.R..(Eds). Manufacturing Yogurt and Fermented Milks. 1nd Ed., Blackwell, Australia.

Chandan, C.R., H. Charles, K. Arun and Y.H. Hui. 2006. Manufacturing Yogurt and Fermented Milks. 1nd Ed., Blackwell, Australia.

Domagla, J. 2005. Texture of yoghurt and bio-yoghurts from goat's milk depending on starter culture type. Milchwissenschaft. 60 (3): 289-292.

Fardiaz, S. 1993. Analisis Mikrobiologi Pangan. Raja Grafindo Persada, Jakarta.

Fuller, R and G.R. Gibson. 1997. Modification of the intestinal microflora using probiotics and prebiotics Scandinavian. J. of Gastroenterology – Supplement. 22: 28-31.

Ham, J. S., J.H. Shin, Y. B. Noh, S. K. Jeong, G. S. Han, H. S. Chae, Y. M. Yoo, J.M. Ahn, W.K. Lee and C. Jo. 2009. Quality of Irradiated Plain Yogurt during Storage at Different Temperatures. Asian-Aust. J. Anim. Sci. 22 (2) : 289 – 295.

Hamann, W. T and E. H Marth. 1983. Survival of Streptococcus thermophilus and Lactobacillus bulgaricus in commercial and experimental yogurts. Journal of Food protection, 47 (10): 781 –786.

Hassan, A and I. Amjad. 2010. Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage African J. of Biotechnology Vol. 9 (20):2913-2917.

Mckinley, M.C. 2005. The nutrition and health benefits of yoghurt. Society of Dairy Technology. 58 (1):1-12.

Miller, G.D., D. Douglas, R.E. Molly, and M.A. David. 2000. Benefits of dairy product consumption on blood pressure in humans. J. Of the American College of Nutrition. 19 (2): 147–164.

Robinson, R.K. and P. Itsaranuwat. 2006. Properties of yoghurt and their appraisal. In Fermented Milks. A.Y. Tamime, Ed., Blackwell Publishing, Oxford,. pp. 76–94.

Seelee, W.,W. Tungjaroenchai and M. Natvaratat. 2009. Development of low fat set-type probiotic yoghurt from goat milk. As. J. Food Ag-Ind. 2 (4), 771-779.

Soukoulis, C., P. Panagiotidis, R. Koureli, and C. Tzia. 2007. Industrial Yogurt Manufacture: Monitoring of Fermentation Process and Improvement of Final Product Quality. J. Dairy Sci. 90: 2641–2654.

Tamime, A.Y. and R.K. Robinson. 2003. Yogurt: Science and technology, CRC Press, New York.

Thomas T. D and O. E. Mills. 1981. Proteolytic enzymes of dairy starter cultures. A review, J. Food Microbiol. 46: p. 245