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Evaluation of Physicochemical Properties and Exopolysaccharides Production of Single Culture and Mixed Culture in Set Yoghurt

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Abstract

The Rheology of yogurt was influenced by the dry matter of milk and yogurt cultures. The purpose of this research was to determine the effect of using single culture (*L. bulgaricus, S. thermophillus)* and mixed culture (*L. bulgaricus* and *S. thermophillus)*, (*S. thermophillus, L. bulgaricus* and *Bifidobacterium)*as much as 3% for evaluation of physicochemical properties in set yoghurt such as pH value, acidity content, viscosity and syneresis, also to know total exopolysaccharides (EPS) production, total lactic acid bacteria (LAB) and microstructure of set yoghurt storage at refrigerator temperature (1 day, 2 and 3 days). The method of this research on first step was Randomized Completely Design, the variables measured on first step were pH content, acidity content, viscocity, and syneresis with 3 times replication. On second step used Randomized Completely Nested Design (3x4) with 3 times replication variables measured were pH stored, total lactic acid bacteria and total EPS production. The best treatment from second step continued testing of the microstucture with the aim of identifying structure components in set yoghurt. The conclusion on first step this research could decrease of pH value, increase the acidity, viscosity and inhibit syneresis set yogurt. On second step gave has decline in the average total LAB 1 log (CFU / ml) in set yogurt and has increasing of total exopolysaccharide production from each type of culture during.

Keywords *: Bifidobacterium*, fermented milk, *Lactabacillus, Streptococcus*

INTRODUCTION

Milk is a natural ingridients food that has a high nutritional value, The main constituent of milk are water (87.9%), protein (3.5%), fat (3.5 to 4.2%), vitamins and minerals (0.85%). Milk is highly perishable due to conditions of high nutritional value and high moisture content due to favorable conditions for microorganisms as a medium for microbial growth. In order to maintain required quality of milk and increasing the shelf life need one of effort to required there are pasteurized milk and fermentation technology.

Milk processing already much extending to improving shelf life and improve the nutritional value. Fermentation is one of a simple method from processing milk and has been widely recognized by the public. The processing yogurt in general involves two types of lactic acid bacteria as starter bacteria, *S. thermophilus* initiate the metabolic processes break down lactose into glucose and galactose (monosaccharides), whereas *L. bulgaricus* metabolizes most of the monosaccharides into lactic acid.

The research was produces of the set yoghurt. The Set yoghurt is one type of yogurt that has masses of midle-solid gel, made by inoculating a starter yogurt into the milk that has been pasteurized, curing and cooling which is done in the packaging [2]. Rheology of yogurt is highly influenced by the dry matter and yogurt cultures. Some cultures can improve the gel yogurt because can produces exopolysaccharide that can form a stable EPS (exopolysaccharides) matrix therefore useful as a stabilizer and natural thickener agent in yogurt products. EPS production, thickening properties, molecular mass and structural conformation are greatly affected by environmental factors. Depending on the strain and growth conditions, maximum yield of EPS may be achieved in the exponential or stationary growth phase. At the end of growth phase, there are some indications that EPS undergoes undesirable enzymatic degradation The types LAB culture can synthesize Extracellular polysaccharide or exopolysaccharide (EPS), which is a polysaccharide polymer that was secreted by the microbes out of the cell. Currently EPS-producing LAB (lactic acid bacteria) exploration is increasing due to the ability of LAB synthesize EPS is considered essential for health.

The study was performed using three different types of culture are two types of single culture and two kinds of mixed cultures. Thats all

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of LAB able to produce EPS, specifically *L. bulgaricus*, *S. thermophillus*, and *Bifidobacterium* individual treatment will be done incubation at 40 ° C for 24 hours and then evaluate the physical properties (pH, acidity, viscosity and syneresis) after all, the set yogurt back to treatment by saving them in the refrigerator at a temperature of 4-5 °C temperature According to [5] aims to inhibit or prevent damage, maintain quality and avoid toxicity due to excessive metabolic. Therefore, this study aimed to evaluation certain bacterial strains may even produce a single and mix types in various of these two types or three types. Furthermore, combinations of the single type and mixed types culture of EPS producing cultures improved not only the total EPS production but also physicochemical yoghurt texture, which will be measured from pH value and pH value after storage, total LAB and microstructure.

MATERIALS AND METHODS

The material used in this study is set yogurt made from cow's milk that comes from Koperasi Mitra Makmur Bhakti "Junrejo" Malang. Pasteurized milk with a batch method with temperature 70-71 ° C for 15 minutes and inoculated process with the addition of culture containing *S. thermophilus, L. bulgaricus* and mixed (S. thermophilus and L. bulgaricus) and (*S. thermophilus, L. bulgaricus and Bifidobacterium*) as much as 3% of the total milk. Ripened milk or incubated at room temperature in the range 27-28 °C. Other materials used in this study is 70% alcohol, MRSA, pH buffer solution, TCA (*trichloroacid)* distilled water. The equipment used in this study include pH meter, analytical balance, viscometer, sentrifugator, scanning electron microscopy (SEM).

COLLECTED DATA METHODS

The research method used was laboratory experiments: on Phase 1: This study used a completely randomized design with 4 treatment are (P1) the addition of *L. bulgaricus culture* 3% after pasteurization, (P2) the addition of *S. thermophilus* culture 3% after pasteurization, (P3) the addition of mixed cultures (*S. thermophilus and L. bulgaricus*) 3%, (P4) the addition of mixed cultures (*S. thermophilus, L. bulgaricus and Bifidobacterium*) 3% after the pasteurization process is carried incubation at 40 °C during 24 hours, 3 replications. The variable measure of physicolchemical are pH value, acidity, viscosity and syneresis. On Phase 2: used with Completely Randomized Design nested with 3 x 4 treatments and 3 replications. The spesification namely: P (retention at refrigerator temperature), P1: 1 day P2: 2 days, P3: for 3 days. Q (3% addition of culture) Q1: *L. bulgaricus*: Q2: *S. thermophilus* Q3: *S. thermophilus and L. bulgaricus*, Q4: *S. thermophilus, L. bulgaricus and Bifidobacterium* as much as 3%*.* the parameter (physicochemical and exopolysaccharides methods) were used in this study and their citation.

DATA ANALYSIS

Data obtained processed with Microsoft Excel. Data were analyzed using ANOVA and Duncan's Multiple Range Test.

RESULT AND DISCUSSION

The pH value of Set Yogurt

The Results of ANOVA showed that the addition of single culture treatment (*L. bulgaricus, S. thermophillus*) with mixed cultures (*L. bulgaricus and S. thermophillus*) and (*S. thermophillus, L. bulgaricus* and *Bifidobacterium*) as much as 3% indicated provide highly was significant difference effect (P <0.01) on the pH set yogurt.

Table1. The Average of pH value

Treatment	U_1	U_2	U_3	Average
P1	3.70	3.69	3.82	3.73^b±0.071
P2	3.69	3.70	3.68	3.69^b±0.009
P3	3.66	3.68	3.67	3.67^a±0.011
P4	3.95	3.99	3.99	3.98^c±0.026

Description: different superscripts showed highly significant difference

Fermentation lactose to lactic acid that consists of more than one type is more quickly produce acid and lowering the pH. Occurs according to [3] LAB ferment the lactose into glucose and galactose, glucose subsequently converted into lactic acid. Based on the resulting product of the fermentation process, lactic acid bacteria can be divided into two, namely homofermentatif which produce lactic acid as the only fermentation product through the EMP (Embden Meyerhof Parnas) and heterofermentatif that in addition to producing lactic acid, also produces ethanol, acetic acid and CO_2 through fermentation pathways fosfoketolation. Increasing the pH value of yogurt due to a decline in the total amount of Hidrogen (H^+) ions that are triggered by a decrease in total acid [11].

Set yogurt made with additional cultures of *S. thermophillus* treatment had an average pH value of 3.73 ± 0.071 while the set yoghurt were made with additional *L. bulgaricus* cultures treatment had an average pH value of 3.69 ± 0.009, the addition of *L. bulgaricus* have a lower pH than yogurt made using with *S. thermophillus* culture because basically *L. bulgaricus* can grow optimally at a pH ranging from 5 for assistance activity of *S. thermophillus* whereas *L. bulgaricus* individually working would require a longer time to break up lactose into lactic acid therefore the pH value resulting from the addition of cultures of *S. thermophillus* treatment and *L. bulgaricus* culture has a pH value that was not different significantly because of the ability to break down lactose into lactic acid is almost same.

Set yogurt made with the treatment of mixed cultures (*L. bulgaricus and S. thermophillus*) has lower pH value 3.67 ± 0.011 due to the work of *L. bulgaricus* and *S. thermophillus* work in a symbiotic mutualism breaks down lactose into lactic acid so that the optimum pH of the yogurt into a range between 3.5-4 and were slightly higher treatment of mixed cultures (*S. thermophillus, L. bulgaricus* and *Bifidobacterium*) has a pH value that were slightly higher, 3.98 ± 0.026 this is due to the competition between the three types of culture in the use of lactose as the main carbon source, so that the lactic acid produced will be low.

Acidity of Set Yogurt

The Results of ANOVA that treatment of addition of a single culture (*L. bulgaricus, S. thermophillus*) with mixed cultures *(L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) as much as 3% showed was unsignificant effect (P> 0.05) on the acidity content of set yogurt, appeared on table 2.

Table 2. The average of acidity (%) set yogurt

Treatment	U1	U2	U3	Average
P1	1.10	0.95	1.34	1.13±0.196
P2	0.97	1.08	1.45	1.16±0.251
P3	1,.15	1.13	1.25	1.17±0.064
P4	1.06	1.24	1.20	1.16±0.094

The average acidity value of set yoghurt on additional culture *L. bulgaricus* with culture *S. thermophillus* range between 1.13 ± 0.196 and 1.16 ± 0.251 whereas L. bulgaricus and S. thermophillus slightly increased acidity values are 1.17 ± 0.064 and *S. thermophillus*, *L. bulgaricus* and *Bifidobacterium* decreased acidity values are 1.16 ± 0.094 and this is due to single culture and mixed culture there produced lactate was used by other cultures. The process of lactic acid produced, that is will be entering in the cells of lactic acid bacteria (LAB). on the bacterial cell, lactose will be hydrolysed by β-galactosidase enzyme into glucose and galactose. Glucose was produced by metabolized to pyruvate through Embden-Meyerhof cycle-Parnas (EMP) pathaway, and pyruvate will be metabolising by lactate dehydrogenased to lactic acid. Lactic acid and galactose will leave bacterial cells and accumulates in the medium (Milk). In set yogurt containing of Bifidobacterium culture has lower growth rate than other types of culture medium, especially on medium such as cow's milk than goat's milk, this showed the existence of metabolite utilization by the possible need to add Bifidobacterium [14].

Viscocity of Set Yogurt

The Results of ANOVA showed that treatment using single cultures (*L. bulgaricus*, *S. thermophillus*) with mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus, L. bulgaricus* and *Bifidobacterium*) was significant difference effect (P <0.01) in the viscosity of the set yoghurt on table 3.

Table 3. The Average of viscosity (cP) set yogurt

Treatment	U_1	U_2	U_3	Average
P1	204	279	289	$257{,}3^a±46{,}45$
P2	215	279	287	$260{,}3^a±39{,}46$
P3	265	310	281	$285{,}3^a±22{,}81$
P4	1161	1240	1260	$1220{,}3^b±52{,}34$

Description: different superscripts showed highly significant difference

Increasing amount of lactic acid giving affect reduced value of pH yogurt. The pH reduced giving affect on milk proteins into the isoelectric point even viscosity varies depending on the acidity of each substrate. The potential to form a gel and decreasing in viscosity andp pH further, because the H^+ ions help glycosidic bond hydrolysis.

The rreatment of addition of different cultures showed difference viscosities. On Table 3 the average value of the viscosity on the set yoghurt with the addition mixed cultures (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) has the highest viscosity 1220,3±52,34 among treatment in this research due to the culture of *L. bulgaricus* and *S. thermophillus* doing symbiotic mutualism breaks down during metabolism in

Table 4. The Avarege Result of Second Step on pH Storage, Total LAB, and Total EPS production

Treatment	Culture3% (v/v)	pH Storage Value	Total LAB (Log CFU/ml)	Exopolysaccharides (mg/L)
P_1	Q_1	$4{,}60^{g}\pm0{,}210$	$5{,}40^{b}\pm0{,}692$	$24{,}63^{a}\pm0{,}971$
	Q_2	$4{,}42^{f}\pm0{,}040$	$6{,}17^{c}\pm0{,}011$	$47{,}10^{c}\pm6{,}194$
	Q_3	$3{,}88^{a}\pm0{,}020$	$6{,}18^{c}\pm0{,}005$	$34{,}56^{b}\pm3{,}899$
	Q_4	$4{,}05^{c}\pm0{,}015$	$6{,}69^{d}\pm0{,}025$	$34{,}33^{b}\pm0{,}550$
P_2	Q_1	$4{,}26^{e}\pm0{,}164$	$4{,}92^{a}\pm0{,}063$	$41{,}90^{c}\pm6{,}409$
	Q_2	$3{,}93^{b}\pm0{,}026$	$6{,}11^{c}\pm0{,}104$	$40{,}06^{bc}\pm4{,}650$
	Q_3	$3{,}82^{a}\pm0{,}030$	$6{,}17^{c}\pm0{,}017$	$36{,}10^{b}\pm0{,}173$
	Q_4	$4{,}21^{d}\pm0{,}005$	$6{,}69^{d}\pm0{,}026$	$30{,}93^{ab}\pm4{,}271$
P_3	Q_1	$4{,}24^{d}\pm0{,}096$	$4{,}90^{a}\pm0{,}095$	$78{,}03^{d}\pm24{,}28$
	Q_2	$3{,}89^{a}\pm0{,}104$	$6{,}12^{c}\pm0{,}103$	$48{,}66^{c}\pm9{,}699$
	Q_3	$3{,}89^{a}\pm0{,}045$	$6{,}08^{c}\pm0{,}090$	$57{,}16^{c}\pm8{,}658$
	Q_4	$4{,}46^{f}\pm0{,}100$	$6{,}58^{d}\pm0{,}015$	$40{,}36^{bc}\pm25{,}24$

Description: Different superscripts showed a highly significant difference (P<0.01)

lactose to lactic acid and needed presence of growth promoting factors from *Bifidobacterium* produced which also helps to protein coagulated. [7] reported that if the factors that cause changes in viscosity that is low pH, and denatured proteins. Increased viscosity values associated with the aggregation of casein micelles and gel formation as a result of biochemical and physico chemical changes during the fermentation of milk.

Syneresis of Set Yogurt

The Results of ANOVA showed that treatment using a single culture (*L. bulgaricus*, *S. thermophillus*) and mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) was significant difference effect (P<0.01) against of syneresis set yogurt on table 4.

Table 5. The Average of Syneresis Set Yoghurt

Treatment	U_1	U_2	U_3	Average
P1	0,52	0,49	0,49	$0{,}50^{b}\pm0{,}017$
P2	0,46	0,53	0,46	$0{,}48^{a}\pm0{,}040$
P3	0,46	0,54	0,43	$0{,}47^{a}\pm0{,}056$
P4	0,49	0,46	0,43	$0{,}46^{a}\pm0{,}030$

Description: different superscripts showed highly significant difference

Due to in each treatment of the set yogurt there is no addition of a stabilizer therefore the protein being hydrophilic and has a lower ability to binding water. The average of syneresis value in additional culture treatment and incubation time is fluctuating it is due to pH value and acidity content for each treatments was gave different affect for protein's ability to binding water, therefore gave affected the value syneresis of set yoghurt. Yogurt has a pH value range between pH isoelectric, casein has lower water holding capacity than normally pH yogurt already. According to [9] which states if the hydrogen bonds among water molecules, lower protein molecules and pores between casein molecules loosene therefore can be passed by free water molecules.

There has been 3 Differences kinds culture they are *S.thermophillus*, *L. bulgaricus* and *Bifidobacterium* will be giving different affect to the value of syneresis. For each kind of LAB having different ability to metabolize lactose into lactic acid therefore different affecting into protein coagulated ability due to might by changed of solubility casein and shrinkage of casein particles, on the acid milk gel particularly from lactic acid bacteria, there will be changing on the casein solubility and slow to change their proteolysis of casein particles become reactive to casein has properties like paracasein when the building blocks of protein networks shrink, all the networks will shrink proportionately so that the ability of the protein to bind to or concurrent water can be lost.

The pH Storage Value of Set Yogurt

The Results analysis of variance showed that storage treatment time in 1 day, 2 days, and 3 days at refrigerator temperature is nested on the use of single cultures (*L. bulgaricus*, *S. thermophillus*), Mixed cultures (L. *bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) was significant difference (P<0.01) on the set of storage pH of yogurt on table 4.

Yogurt if on leave or stored at room temperature therefor the microbes used condition arround to grow and make the process of fermentation continues and so, we need some effort for increasing shelf life that effort is storage with low temperatures for control microbial growth process further. At the low temperatures culture have been persists and slow metabolism. In these circumstances is used for maturation process yogurt that called with aging, and good conditions for producing secondary metabolites include exopolysaccharide that confers a benefit on product stability and giving positive effect for the human body.

Low temperature can lead to a reduction in the number of microbes that live in a food and cause denaturation of cell proteins. In all the treatments either single culture (*L. bulgaricus*, *S. thermophillus*), Mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (S. *thermophillus*, *L. bulgaricus* and *Bifidobacterium*) with storage time 24 hours, 48 hours, and 72 hours at refrigerator temperature has a pH value between 3.8 to 4.6. According to [13] reported that the decrease in temperature of about 43 °C to about 10 °C will be practically prevent further for growth of the starter and inhibits the acidity production. According to [13] reported that fermented milk products at a temperature 5 °C can be stored for 10 days but at a temperature 10 °C while the can last stored for 3 days. Cooling methode is the best effort for reached pH value 4.7 to 4.5. Cooling methode is also an effort to get a good temperature conditions for storage of yogurt. Yogurt can hold for 2 weeks in the cold conditions.

Total Lactic Acid Bacteria of Set Yoghurt

The Results analysis of variance showed that treatment storage time in 1 day, 2 days, and 3 days at refrigerator temperature is nested on the use of single cultures (*L. bulgaricus*, *S. thermophillus*), Mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) was significant difference (P<0.01) on the production of exopolysaccharide on table 4.

During at the storage period occurring decline in the average total LAB 1 log (CFU / ml) At 2 days storage in total BAL has decreased. [10] reported that the greater difference temperature storage giving effect of temperature optimal microba growth, the growth rate became slows and eventually stops. [10] stated if loss of viability cell and total LAB can also occurring of antimicrobial effects from diacetyl, acetic acid, and lactic acid, which appears on the product and sometimes due to bacteriocins. In the stationary phase to the death phase of bacteria storage process will perform secondary metabolites excreted metabolites out of the cell when environmental conditions have started unfavorable, while is one of the bioactive products.

Set yogurt with the treatment of mixed cultures (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) has a fairly high total LAB 6.6 compared with single cultures treatment *L. bulgaricus* had a total LAB as much as 5.08, *S. thermophillus* had a total BAL as much as 6.14 and Mixed culture (*L. bulgaricus* and *S. thermophillus*) had a total LAB as much as 6.15. According to [15] states that an aspect that should be considered in the fermentation products get a high total number of bacteria at the end of fermentation products. For example are some strains of *S. thermophilus* and *L. bulgaricus* can inhibit certain bacteria, especially probiotic bacteria during fermentation and storage of the product. Probiotic bacteria are affected by other bacteria during long time fermentation processed, but during the short fermentation of probiotic bacteria is hardly affected by other bacteria. Positive interaction occurs in some strains of probiotic bacteria such as *Lactobacillus acidophillus* and *Bifidobacterium longum* [15]. *S. thermophilus* and *L. bulgaricus* and *Bifidobacterium* are not mutually inhibit each other therefore set yogurt mixture is treated cultures (S. thermophillus, L. bulgaricus and Bifidobacterium) has the highest total LAB.

Total Production of Exopolysaccharide in Set Yogurt

The Results analysis of variance showed that treatment storage time as long as 1 day, 2 days, and 3 days at refrigerator temperature is nested on the use of single cultures (*L. bulgaricus*, *S. thermophillus*), Mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus, L. bulgaricus* and *Bifidobacterium*) was significant difference (P<0.01) on the production of exopolysaccharide set yogurt on table 4.

Due to the production of EPS was influenced by several conditions, such as growth medium, incubation time, incubation temperature and others condition. Besides that the amount of EPS produced by LAB species that is different is generally caused by heredity / genetic [11]. EPS production will have been occurred during the optimum maximum cell production [11]. In general, the EPS production started during the exponential phase and can reach a maximum cell production at the stationary phase [12]. In this research conducted in second step can produced the highest EPS production on the set yogurt treated 3 days storage at refrigerator temperature using a single culture of *S.* has an average of EPS production value is 48.8 mg/L more higher than set yoghurt produced by using additional a single *culture L. bulgaricus* and for treatment of additional mixed cultures (*L. bulgaricus* and *S. thermophillus*) the average value of EPS production as much as 127 mg/L lower than the set yogurt using (*S. thermophillus L. bulgaricus and Bifidobacterium*) has an average value of the highest EPS production as much as 105.6 mg/L, [16] reported that in yogurt, EPS production by the culture is very low, maximum produced as much as 500 mg/L but plays a major role in development of the final product texture. *S. thermophilus* is considered responsible for the production of EPS, although some strains of *L. delbrueckii subsp. bulgaricus* also EPS-producers.

A reciprocal interaction between the two species starter cultures can be seen in the production of EPS when both of them grow up together. Different than the set yogurt with treatment (*S. thermophillus, L. bulgaricus* and *Bifidobacterium*) *Bifidobacterium* strains in degrading polymers (oligo / polysaccharides). In EPS whose produced from *Bifidobacterium* strains generally have the ability saccharolitic better than *Lactobacillus* strain but in its ability to produce more EPS than the *Lactobacillus* group .

[1] reported that *Bifidobacterium longum* BB-79 is able to utilize lactose to produce EPS. [9]reported that the EPS produced by the microbes out of the cell as a product of secondary metabolites when the environmental conditions are not favorable. Synergistic interaction between the two cultures is also featured in the production of EPS produced and ultimately increase the viscosity of yogurt.

Microstructure of Set Yogurt

The results microstructure of set yogurt appears on Figure 1 that stored at refrigerator temperature treatment and the best 4 treatment exopolysaccharide production quantities in second step by SEM observation.

Appearance Figure microstructural set yogurt with 2000 x magnification above showed the presence of EPS had been seen on the set yogurt cultures treated of single single culture (*L. bulgaricus*, *S. thermophillus*), and Mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) against storage time 1 day, 2 days, and 3 days at refrigerator temperature.

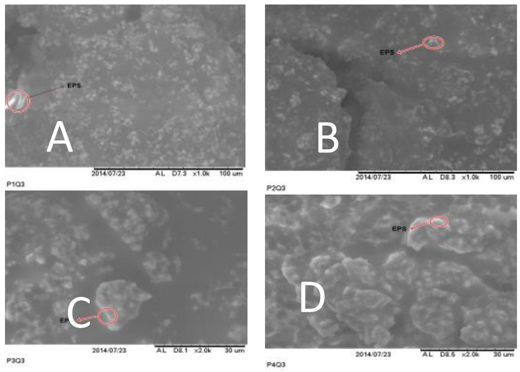

Figure 1. Strructure of exopolysaccharide in yogurt at stored refrigerator (A: 0, B: 1 day, C: 2 days, D: 3 days).

On the microstructure test under SEM observation showed that the protein network in yogurt with coarse structure of relatively large spherical aggregates in the network forming large pores in Figure A, B and D are clearly visible exopolysaccharide production than that seen in the red marks on the image A, B, C, D.

EPS produced by a variety of bacteria during the fermentation process, made into solid particles and produce a larger protein aggregates. [4] reported that the influence of stirring yogurt on process made with exopolysaccharide different from yogurt without exopolysaccharide, because it does not show in syneresis directly, although structural damage increases. Shear-induced microstructure in yogurt made with exopolysaccharide producing cultures demonstrated consists of compartmentalized protein aggregates between channels containing exopolysaccharide. In figure A highly visible of exopolysaccharide due to the best treatment for 3 days storage at refrigerator temperature by using a culture of *Lactobacillus bulgaricus* 3% of 78.03 mg/L is the highest number of other treatments, therefore from the appearance on the figure D highly visible exopolysaccharide few therefore pores and protein aggregates seems so obvious it is due to by the use of mixed cultures of *S. thermophillus*, *L. bulgaricus* and *Bifidobacterium* are in the process of forming lactic acid fermentation is working optimally and produce enough protein coagulant so that the viscosity of set yoghurt made with mixed cultures of *S. thermophillus, L. bulgaricus* and *Bifidobacterium* are very high. High lactic acid content due to more proteins coagulated and more consistent set ogurt increased so that the test microstructure is clearly visible pores and protein aggregates.

Microstructure test through by SEM observation showed that the culture will be a connecting filaments between each molecule of casein, so that texture of yogurt to be more compact with better rheological properties [8].

CONCLUSION

The production of set yogurt with additional of single cultures (*L. bulgaricus, S. thermophillus*), mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) as much as 3% was influenced of physicochemical properties value, appeared by decrease of pH value, increase the acidity, viscosity and inhibit syneresis set yogurt whereas the additional of using single culture (*L. bulgaricus, S. thermophillus*), mixed cultures (*L. bulgaricus* and *S. thermophillus*) and (*S. thermophillus*, *L. bulgaricus* and *Bifidobacterium*) as much as 3% on storage at refrigerator temperature for 1 day, 2 days and 3 days could be able increase in pH storage value, during at the storage period has decline in the average total LAB 1 log (CFU / ml) in total LAB set yogurt and has increasing of total exopolysaccharide production between set yogurt made from single cuture and mixed culture, were appeared on microstructure testing, EPS produced by a variety of bacteria during the fermentation process, made into solid particles and produce a larger protein aggregates. Although it was apparent from our work that thevarious used single type and mixed types culture made EPS exhibited different physicochemicals characteristics, their interactions with milk components during fermentation may be more dominant in governing the final texture of yoghurt. The various used single type and mixed types culture can be able was influenced physicochemicals and EPS play an important role in the development of yoghurt texture.

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