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The Effect of Adding Kimpul Starch (*Xanthosoma sagittifolium*) into Set Yoghurt with Different Incubation Time on the Chemical, Microbiological and Microstructural Quality

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***Abstract*—** *The purpose of this study was to know interaction between addition of kimpul starch (Xanthosoma sagittifolium) and different incubation time on pH value, protein content, total of Lactic Acid Bacteria (LAB), Total Plate Count (TPC), exopolysaccharide and microstructure of set yoghurt. This study used factorial experiment (4x4) and Completely Randomized Design (CRD) with 3 replications. First factor was addition of kimpul starch with various concentration (T0 =0%. T1= 1%, T2= 2% dan T3=3%). Second factor was different incubation time at room temperature (±23^{o}C) during (L1= 24 hour, L2= 32 hour, L3= 40 hour, L4= 48 hour), and continued with Duncan's New Multiple Range Test (DMRT). The observed variables were pH value, protein content, total of LAB, TPC, exopolysaccharide production and microstructure. The result of interaction between addition of kimpul starch and adjustment of incubation time was not significantly different (T>0.05) on pH, protein content, total of LAB, TPC and exopolysaccharide. The addition of kimpul starch was highly significant different (T<0.01) on protein content, total of LAB, TPC, and exopolysaccharide, but it was significant different (T<0.05) on pH. The adjustment of incubation time was highly significant different (T<0.01) on pH, protein content, total of LAB, TPC and exopolysaccharide. In conclusion, the best quality of set yoghurt combined from the addition of 3% kimpul starch and 24-hour incubation can improve the structure of set yoghurt in microstructure*.

***Keywords*—** *Chemical, Incubation, Kimpul starch, Microbiological, Set yoghurt*.

I. INTRODUCTION

Set yoghurt is a coagulation milk product with prebiotic property produced from lactic acid fermentation in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophiles* bacteria (Adolfsson *et al*., 2004). Hattingh and Viljoen (2001) explain that probiotic is a microflora lived well in singular or mixture form added into food in order to improve health. The addition of bacteria contributes to increase functional value of yoghurt (Borchers *et al*., 2002). *Lactobacillus acidophilus* is lactic acid bacteria had better resistance in digestive tract (Gomez & Malcata 1999) so that increase the quality of set yoghurt which is desired (Gardini *et al*., 1999).

Making process of set yoghurt frequently had a damage, it is caused by lowering of pH up to isoelectric point, it also can trigger the decreasing of water holding capacity. The decreasing of water holding capacity cause set yoghurt become resistant to have syneresis, it is physical damage such as separation of whey liquid from the gel that can reduce the quality of set yoghurt (Adams and Moss, 2008). One of evaluation to increase the quality of set yoghurt is by adding stabilizer to solve that problem (Agarwal and Prasad, 2013).

Kimpul tuber is a taro plant originated from America continent. Kimpul in scientific namely *Xanthosoma sagittifolium* (Rodriguez *et al*., 2009). Kimpul is an abundant source of carbohydrate, that can be utilized as starch source, and contained approximately 70-80% carbohydrate. One of advantages of kimpul is containing a bioactive compound--diosgenin. This compound is useful as anti-cancer, inhibitor of cell proliferation and has a hypoglycemic effect.

The duration or incubation time is one of parameter to determine the successful of lactic acid bacteria in producing primary metabolite and secondary metabolite during fermentation process (Athar, Shah and Khan, 2000). Lucey (2002) states an exorbitant temperature of fermentation can cause physical damage that is separation of whey or syneresis occurs in yoghurt. Temesgen (2015) reports that the addition of starch into yoghurt is beneficial to decrease physical damage. This is potentially to be developed considering that kimpul tuber is underutilized as starch source eventhough this plant contains an abundant of starch. Based on this explanation, it is encouraged to conduct a research in order to evaluate the chemical, microbiological, and microstructural quality of set yoghurt with addition of kimpul starch and adjustment of incubation time.

II. MATERIALS AND METHODS

Time and Location

This study had been conducted in December 2018 - February 2019. The research took place in Brawijaya Dairy Industry, Junrejo-Batu, Laboratory of Animal Product Technology of Animal Science Faculy, Laboratory of Quality and Food Safety Test of Agricultural Technology Faculty, Biochemical Laboratory of Mathematics and Natural Sciences Faculty in Brawijaya University Malang - Indonesia.

Dewiarum Sari, Imam Thohari, and Purwadi, "The Effect of Adding Kimpul Starch (*Xanthosoma sagittifolium*) into Set Yoghurt with Different Incubation Time on the Chemical, Microbiological and Microstructural Quality," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 225-230, 2019.

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Materials

The materials used in this research were set yoghurt which is made from cow milk with addition of kimpul starch. The substances used were fresh milk of cows obtained from Mitra Bhakti Makmur Cooperative in Junrejo-Batu, kimpul tuber obtained from traditional market in Tangerang, bacteria starter consist of *Lactobacillus bulgaricus*, *Streptococcus thermophillus* and *Lactobacillus acidophilus* obtained from Rumah Yoghurt in Junrejo-Batu.

Method

This study used factorial experiment method (4x4) with Completely Randomized Design (CRD). Two factors were used in this research. First factor was addition of kimpul starch with various concentration (T0 =0%. T1= 1%, T2= 2% and T3=3%). Second factor was adjustment of incubation time at room temperature (±23^{o}C) during (L1= 24 hour, L2= 32 hour, L3= 40 hour, L4= 48 hour) with 3 replications. The fresh milk and kimpul starch were pasteurized at 72^{0}C during 15 second (High Temperature Short Time method), then stir and measure using thermometer. Chill the milk until 43^{0}C, then add 2% bacteria starter of milk volume, and incubate based on different incubation time suit with the treatments at ±23^{0}C.

Variable of Study

The variable of this study were pH value, protein content, total of LAB, TPC, exopolysaccharide and *Scanning Electron Microscopy* (SEM). All variables were tested following procedure of AOAC (2005). The pH was tested by pH meter, protein content was tested by *kjeldahl* method, total of LAB and TPC were tested by pour plate method, and *Scanning Electron Microscopy* (SEM) was tested by following the procedure of AOAC (2005). While, exopolysaccharide was tested by centrifuge following procedure of Van Geel-Schutten *et al.* (1998).

Analysis of Data

The data obtained were analyzed by Analysis of Variance (ANOVA) based on factorial experiment (4x4) with Completely Randomized Design (CRD) and 3 replications. If it was significant different, it was continued by Duncan's New Multiple Range Test (DMRT). The purpose of this analysis was to know the effect of kimpul starch addition and adjustment of incubation time at room temperature to improve the quality of set yoghurt.

III. Results and Discussions

The Value of pH

The pH is an important parameter control of acidity flavor in yoghurt. The value of pH is important to reveal in order to maintain acidity condition that needed by lactic acid bacteria to grow. The result of pH test in set yoghurt can be seen in Table 1. The analysis of variance showed that addition of kimpul starch gave significant different effect (T<0.05) to the pH value, treatment of incubation time gave highly significant different effect (T<0.01) to the pH value. While, interaction between kimpul starch addition and adjustment of incubation time did not give significant different effect (T>0.05) to the pH value. The range value of pH in this study was around 3.77-4.54, it is still in the desired pH range of a yoghurt. The pH value which incubated in 24 hours and 32 hours was the appropriate pH value for yoghurt. This result is in accordance with Buckle et al. (1985), the study point out that the desired acidity of yoghurt is around 0.85%-0.90% and pH 4.0-4.5

The result showed that the average of pH value in set yoghurt with addition of kimpul starch was around 4.02-4.15. From Table 1 can be seen that the more addition of kimpul starch can decrease pH value, this is caused by activity of lactic acid bacteria which is increased as effect of substrate enhancement--glucose, so that increase production of lactic acid and make pH value down. This result conforms with Alakali *et al*. (2008), the study point out that addition of 0.5% corn starch as stabilizer resulting pH 4.42, while the control treatment had 4.50 as pH value. Adamberg *et al*. (2003) explain that pH had decline in that existence of lactic acid bacteria activity in breaking lactose of milk into glucose and galactose, then glucose changed into lactic acid.

TABLE 1. The pH of set yoghurt

Kimpul Starch (%)	Incubation Time				Average
	L1	L2	L3	L4	
T0	4.54±0.11	4.17±0.07	3.97±0.08	3.90±0.10	4.15±0.27^{c}
T1	4.52±0.05	4.14±0.12	3.93±0.05	3.84±0.04	4.11±0.28^{bc}
T2	4.52±0.07	4.05±0.04	3.89±0.11	3.82±0.06	4.07±0.29^{ab}
T3	4.41±0.14	4.04±0.07	3.87±0.09	3.77±0.16	4.02±0.28^{a}
Average	4.50±0.10^{c}	4.10±0.09^{b}	3.92±0.08^{a}	3.83±0.10^{a}	

* The different superscripts in the same row showed the highly significant different (T<0.05); The different superscripts in the same column showed the highly significant different (T<0.01)

The average pH value in set yoghurt with different incubation time had an average value around 3.83-4.50. The longer incubation time at room temperature can decrease pH value, this is caused by the number of microbe was higher during fermentation so that result in the lowering of pH because of the chemical change. The research of Arioui *et al*. (2017) state that addition of pectin in yoghurt with different concentration (0.1%, 0.3%, and 0.6%) fermented in 2 hours and 4 hours at temperature 45^{0}C had decreased pH value. The higher concentration of pectin that is 0.6% was resulted the lowest pH value, it was different with control treatment within 2 hours fermentation resulting pH 2.92, while 4 hour fermentation was resulted pH 4.49. Lactic acid bacteria (LAB) has β-galactosidase and lactate dehydrogenase (LDH) enzyme which can produce lactic acid from milk lactose (Innocente *et al*., 2016). Lan e*t al*. (2016) explain that lactose in milk will enter to LAB cell through permease, then β-galactosidase enzyme will cut off glycoside and bond into lactose in order to produce glucose and galactose so that the pH decreases automatically.

Protein Content

The analysis of variance showed that addition of kimpul starch and adjustment of incubation time gave highly significant different effect (T<0.01) to the protein content, while interaction between addition of kimpul starch and adjustment of incubation time did not give significant different

Dewiarum Sari, Imam Thohari, and Purwadi, "The Effect of Adding Kimpul Starch (*Xanthosoma sagittifolium*) into Set Yoghurt with Different Incubation Time on the Chemical, Microbiological and Microstructural Quality," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 225-230, 2019.

International Research Journal of Advanced Engineering and Science
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effect (T>0.05) to the protein content. The average value of protein content can be seen in Table 2.

The protein content resulted by this study was around 2.06%-2.22%. The addition of kimpul starch on making yoghurt can increase protein content, because kimpul starch contains protein about 0.77%, so that will increase protein content in set yoghurt compare to control treatment. The protein content in this study was lower than common yoghurt within approximately at 4.93% until 9.23% (Mahdian and Tehrani, 2007; Irvine and Hekmat, 2011). The protein content in set yoghurt is the total used of protein substance and protein inside of LAB. The higher total of microbe will produce more protein content, it is due to the several microbe component were protein. Irvine and Hekmat (2011) state that the higher viscosity of yoghurt was affected by coagulation process of protein, so the thicker the yoghurt, the higher the protein content.

TABLE 2. The protein content (%) of set yoghurt

Kimpul starch (%)	Incubation Time				Average
	L1	L2	L3	L4	
T0	2.03±0.13	2.09±0.04	2.14±0.06	1.97±0.12	2.06±0.10[a]
T1	2.17±0.08	2.05±0.04	2.15±0.08	2.10±0.10	2.12±0.08[ab]
T2	2.17±0.04	2.10±0.11	2.31±0.11	2.09±0.04	2.17±0.12[b]
T3	2.20±0.09	2.21±0.05	2.28±0.10	2.18±0.19	2.22±0.11[b]
Average	2.14±0.10[ab]	2.11±0.08[a]	2.22±0.11[b]	2.09±0,13[a]	

*The different superscripts in the same row and column showed the highly significant different effect (T<0.01)

The different incubation time had average value around 2.09%-2.22%. The longer incubation time at room temperature can decrease protein content. The decreasing of protein content is due to the the higher LAB which is fermented lactose make the mobility of bacteria inhibited because the viscosity increased so that proteolytic bacteria are also inhibited. Hassan and Amjad (2010) explain that fermenting milk become yoghurt had trigger the occurrence of proteolysis activity--a protein changes into simpler amino caused by LAB. The soluble protein content was related to the proteolytic activity of bacteria which changes protein become amino acid and peptide, so that will increase protein content in yoghurt. However, the longer time of incubation will inhibit the mobility of bacteria and make viscosity higher. So if the activity of bacteria is inhibited then the activity of proteolytic will also be inhibited. One of the changes in the milk composition of yoghurt is the occurrence of protein denaturation due to the acid or low pH during fermentation process (Miller *et al*., 2000).

Total of Lactic Acid Bacteria (LAB)

The result of LAB total in set yoghurt can be seen in Table 3. The analysis of variance showed that addition of kimpul starch and adjustment of incubation time gave highly significant different effect (T<0.01) to the total value of LAB, while interaction between addition of kimpul starch and adjustment of incubation time did not give significant different effect (T>0.05) to the total value of LAB. The range value of LAB was around 7.05 – 7.42 log CFU/ml. This result is in accordance with Buckle et. al. (1985) that the final product of yoghurt usually contains 10^7 cell/ml for each type of bacteria.

TABLE 3. Total value of LAB (Log CFU/ml) in set yoghurt

Kimpul Starch (%)	Incubation Time				Average
	L1	L2	L3	L4	
T0	7.05±0.06	7.21±0.03	7.22±0.04	7.32±0.04	7.20±0.11[a]
T1	7.22±0.04	7.30±0.04	7.41±0.04	7.36±0.04	7.32±0.08[b]
T2	7.24±0.04	7.31±0.04	7.41±0.04	7.39±0.04	7.34±0.08[b]
T3	7.25±0.04	7.33±0.03	7.39±0.04	7.42±0.03	7.35±0.07[b]
Average	7.19±0.09[a]	7.29±0.06[b]	7.36±0.09[c]	7.37±0.05[c]	

*The different superscripts in the same row and column showed the highly significant different effect (T<0.01)

The average result of total value of LAB was around 7.20-7.35 log CFU/ml. The higher addition of kimpul starch will increase total value of LAB, this is due to the kimpul starch is beneficial as thickening in set yoghurt in that starch contains high carbohydrate. Vital *et al*. (2015) explain the strategy to increase the growth of proteolytic bacteria in making yoghurt is by adding nutrition as a prebiotic for LAB. Lan *et al*. (2016) explain that milk lactose in set yoghurt will also be fermented become galactose and glucose by lactase enzyme or phosphor galactosidase. Glucose from milk and starch will finally be overhauled by LAB become lactic acid so that the LAB total is higher.

The different incubation time had an average value of LAB around 7.19-7.37 log CFU/ml. The longer incubation time at room temperature can increase the total value of LAB. Because it also leads higher activity of LAB in breaking lactose from milk or starch so that will increase the total of LAB. Athar *et al*. (2000) state that the longer fermentation leads the total of LAB higher and decrease the lactose content because of the occurrence of primary metabolite formation. This is related to the aspects considered in the producing of fermentation product, that is to obtained the higher total of LAB in the end of fermentation process (Tamime, 2005).

Total Plate Count

The result of total plate count (TPC) in set yoghurt can be seen in Table 4. The analysis of variance result showed that addition of kimpul starch, different incubation time and both interaction did not give significant different effect (T>0.05) to the total value of TPC. The range value of TPC in this study was 7.25 – 7.48 log CFU/ml, it is in accordance with SNI (2009) that the minimum standard number of bacteria in yoghurt should contain 10^7 CFU/ml.

TABLE 4. Total Plate Count (TPC) (Log CFU/ml) of set yoghurt

Kimpul starch (%)	Incubation Time				Average
	L1	L2	L3	L4	
T0	7.25±0.04	7.44±0.05	7.41±0.10	7.44±0.03	7.39±0.10
T1	7.38±0.04	7.45±0.03	7.44±0.04	7.42±0.03	7.42±0.04
T2	7.40±0.05	7.41±0.11	7.43±0.06	7.45±0.12	7.43±0.08
T3	7.48±0.05	7.40±0.04	7.46±0.03	7.47±0.04	7.45±0.05
Average	7.38±0.09	7.43±0.06	7.44±0.06	7.44±0.06	

*The different superscripts in the same row and column showed the highly significant different effect (T<0.01)

This study showed that the total value of TPC was around 7.39-7.45 log CFU/ml. TPC in this study is affected by the existence of LAB in set yoghurt, so it is expected the bacteria

Dewiarum Sari, Imam Thohari, and Purwadi, "The Effect of Adding Kimpul Starch (*Xanthosoma sagittifolium*) into Set Yoghurt with Different Incubation Time on the Chemical, Microbiological and Microstructural Quality," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 225-230, 2019.

grew were dominated by LAB because the higher total of LAB can improve the quality of set yoghurt. Gustaw, Kordowska-Wiater and Kozioł (2011) explain that the content of starch was beneficial as a prebiotic during fermentation process and will be change become lactic acid by bacteria. Prebiotic is useful to support the growth and development of LAB.

The longer incubation time at room temperature leads higher activity of LAB to break lactose so that will increase the total of LAB and automatically will increase TPC. The average value of TPC with different incubation time was around 7.38-7.44 log CFU/ml. The ideal condition of growth media for LAB has homofermentative characteristic which is simultaneously breaking lactose become lactic acid and ethanol so that will increase total of acid and be produced rapidly (Bamforth, 2005). *Lactobacillus bulgaricus* will release amino acid such as valine, glycine and histidine which is needed for the growth of *Streptococcus thermophilus* (Helferich and Westhoff, 1980). *Lactobacillus acidophilus* will slowly grow during fermentation process in production of yoghurt (Shah, 2000).

Exopolysaccharide

Exopolysaccharide is polysaccharide in the outside of cell wall which is excreted by lactid acid bacteria. This substance plays an important role in set yoghurt in that function of improving physical properties, which useful as stabilizer. The result of exopolysaccharide test in set yoghurt can be seen in Table 5. The analysis of variance showed that addition of kimpul starch and adjustment of incubation time gave highly significant different effect (T<0.01) to the value of exopolysaccharide, while interaction between addition kimpul starch and adjustment of incubation time did not give significant different effect (T>0.05) to the value of exopolysaccharide.

TABLE 5. Total of exopolysaccharide (g/L) in set yoghurt

Kimpul starch (%)	Incubation Time				Average
	L1	L2	L3	L4	
T0	40,93±0,21	37,83±1,55	36,23±0,25	35,27±0,50	37,57±2,35^{a}
T1	40,67±0,12	38,30±0,10	37,47±0,64	35,70±0,70	38,03±1,91^{a}
T2	41,30±0,82	38,73±0,45	37,73±0,84	35,87±1,86	38,41±2,26^{ab}
T3	41,53±0,35	39,43±0,40	38,90±0,53	36,07±0,38	38,98±2,07^{b}
Average	41,11±0,52^{d}	38,58±0,94^{c}	37,58±1,12^{b}	35,73±0,94^{a}	

*The different superscripts in the same row and column showed the highly significant different effect (T<0.01)

The result showed that the total of exopolysaccharide was around 37.57-38.98 g/L. The more addition of kimpul starch in set yoghurt will cause LAB grow and develop rapidly so that trigger producing more exopolysaccharide. This result is emphasized by the study of Petry *et al*. (2000) that explain the synthesis of exopolysaccharide in nutrient media showed that exopolysaccharide was countinuously excreted several minutes after the growth and cell division stoped. The production of exopolysaccharide during logarithmic phase was very small, while in the optimal phase, approximately 0.75% carbohydrates will be converted to exopolysaccharide. Several bacteria strain will utilize carbohydrate as a growth media to produce exopolysaccharide. For example, such strain *Streptococcus mutans*, *Streptococcus bovin* and *Leuconostoc mesenteroides* have an ability to produce extracellular dextran (Tamime and Robinson, 2007).

The different incubation time had an average of exopolysaccharide around 35.73-41.11 g/L. The longer time of incubation at room temperature can decrease the value of exopolysaccharide. Factors that affect on decreasing of exopolysaccharide was medium growth, incubation time, type of strain, temperature and others. This result conforms with opinion of Pham *et al*. (2000) who stated that the longer incubation time can decrease production of EPS (exopolysaccharide), and total exopolysaccharide produced by LAB commonly caused by trait or genetic properties from bacteria strain. Production of exopolysaccharide was begun from exponential phase to maximum rate called stationary phase (Petry *et al*., 2000).

Microstructure

The microstructure in set yoghurt was observed using Scanning Electron Microscopy (SEM). Eisner (2006) explain that this tool is used to observe the structure morphology and characteristic of chemical composition. The following picture, can be seen in Figure 1, is the best observed microstructure in set yoghurt.

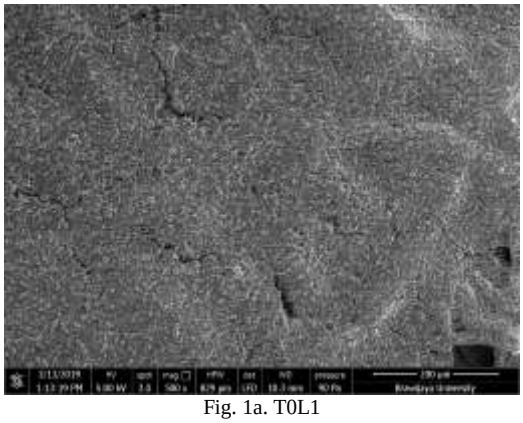

Fig. 1a. T0L1

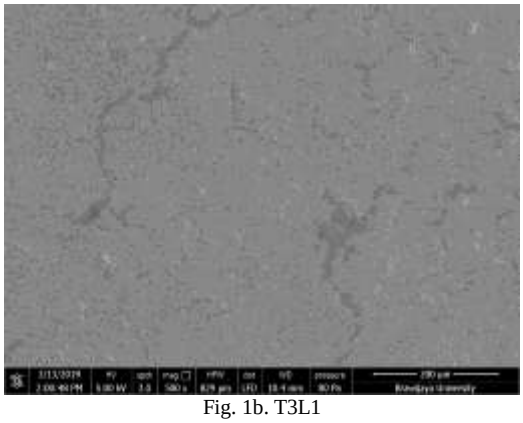

Fig. 1b. T3L1

From Figure 1 can be seen that T3L1 treatment added by 3% kimpul starch has tight structure compare to control treatment (T1L1 in Figure 1). This is due to the kimpul starch has a beneficial function as stabilizer so that will increase

Dewiarum Sari, Imam Thohari, and Purwadi, "The Effect of Adding Kimpul Starch (*Xanthosoma sagittifolium*) into Set Yoghurt with Different Incubation Time on the Chemical, Microbiological and Microstructural Quality," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 225-230, 2019.

viscosity and decrease syneresis. The more addition of stabilizer will increase the water holding capacity value, because starch is a complex carbohydrate that contained amylose and amylopectin which can bind water in yoghurt (Goncalvez *et al*., 2005). Phillips and Williams (2009) explain that granule starch has the ability to bind water. The granule starch will absorb water which lead swelling occur so that viscosity will increase and the texture of yoghurt becomes more compact. Malaka and Baco (2000) explain that LAB will form filaments that connect among molecules so that the texture of yoghurt becomes more compact by microstructure observation using *Scanning Electron Microscopy* (SEM), also can form the smooter gel structure because of having a little size of granule starch (Tattiyakul *et al*., 2006).

IV. Conclussion and Suggestion

In conclusion, the different concentration of kimpul starch addition can decrease pH value, but can increase protein content, total of LAB, TPC and exopolysaccharide. The different incubation time can decrease pH value, protein content, exopolysaccharide, but can increase total of LAB and TPC. The best treatment combined from the addition of 3% kimpul starch and 24-hour incubation has been able to improve structure of set yoghurt in microstructure.

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