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The Effect of Incubation Time to The Physicochemical and Microbial Properties of Yoghurt with Local Taro (*Colocasia Esculenta*(L.) Schott) Starch as Stabilizer

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Abstract

The effect of incubation time by using three culture starters (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles*) and Taro (*Colocasia Esculenta*) starch as a stabilizer on the physicochemical and microbial characteristics of yoghurt were investigated. One of the problems in making yoghurt is the occurrence of syneresis caused by the unstable casein micelles. The addition of natural stabilizers is known to be able to solve the problem. In this research, local taro was added to the yoghurt as stabilizers followed by different incubation time (18-h, 24-h, 30-h, 36-h and 42-h). The results showed that incubation time had a highly significant effect ($p<0.01$) on viscosity, whey holding capacity (WHC), moisture content, carbohydrates, pH value, total acidity, and total lactic acid bacteria. During the fermentation process, the prolonged incubation time resulted in increased acidity, viscosity, WHC, and total lactic acid bacteria, while simultaneously decreased the pH value, moisture and carbohydrate content. The research concluded that 36 h incubation time produced the best yoghurt characteristics made with *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles* as culture starters and taro starch as a local Indonesia stabilizer.

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Introduction

Yoghurt is one of the fermented dairy products which contained beneficial bacteria for human health. Fermentation has become a popular food processing method as it yields more nutritional products and higher therapeutic value. Recently, the yoghurt market is aimed towards more sustainable product evaluation, while also oriented to the consumers'

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needs. Yoghurt quality is affected by several factors, which are incubation time and temperature,[1] starter cultures,[2,3,4,5] and stabilizer.[6] Each factor would then affect the yoghurt's pH, total acidity, viscosity, nutritional value, and microbial properties.[7,8]

The starter cultures will affect textural properties of dairy yoghurt during and after the fermentation process, thus each culture types will result in different yoghurt quality. The bacteria viability could also become unstable depending on several factors, including the types of used strains, the interaction between present bacterial species, fermentation time, and nutrients availability. The interaction between three lactic acid bacteria (LAB) culture starters (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles*) have shown a significant effect on the viscosity of homemade yoghurt.[9] The growth of starter culture is affected by several factors as well, such as chemical composition in milk, the total inoculum population, incubation time, the temperature during incubation and cooling time. The common yoghurt fermentation is done by incubating L. *delbrueckii subsp. bulgaricus* and S. *thermophilus* at 40-45°C for 2.5-3 h or at ambient temperature for 18 hours or more until it reached certain acidity level.[10] However, a longer fermentation time can be done up to 12 hours at a lower temperature (e.g. 30°C) to improve the yoghurt quality. However, at a lower temperature, the incubation would require a longer time.[11] Starter culture has interaction which is increasing the aroma compound. The lactic acid bacteria had the ability to produce exopolysaccharides and bioactive peptides. The produced exopolysaccharides could act as stabilizer, thickener, and emulsifier like other polysaccharides.

The addition of stabilizer on yoghurt production is aimed to prevent syneresis and producing favourable texture and stability during yoghurt processing and storage. Research done by Olorunnisomo[12] showed that natural stabilizers such as corn starch, milk powder, and baobab fruit pulp could give a significant effect to the chemical composition, sensory properties and total microbial of Zebu milk yoghurt. Furthermore, the addition of yoghurt stabilizers would reduce syneresis, increase the viscosity and WHC ($p \leq 0.05$), also enhanced the sensory acceptability of yoghurt made from camel milk. The total acidity and pH value of yoghurt made from camel milk were significantly affected by the stabilizer types. [13] The yoghurt characteristic can be improved by using a stabilizing agent. Taro starch is widely used in the food industry due to its ability to form gels on dairy product fermentations. Research on using local taro (*Colocasia esculenta)* starch as a stabilizer in yoghurt production is still limited, thus research to observe its potential should be done. The stabilizer was chosen based by its water solubility and stable viscosity properties, added with its availability and categorized as relatively easy to obtain food ingredient. This research aimed to determine the optimum incubation time on yoghurt fermentation which used 3 different culture starters and local taro starch as stabilizer based on the best characteristics of the produced yoghurt.

Materials and Methods

Materials

The raw materials used in this research were fresh milk, 1.5% local taro starch, 4% skim milk, 3% LAB starters (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles*).

Experimental Design

This research was a laboratory experiment and using a completely randomized design. Four different treatments were used to determine the optimum incubation time to produce the best yoghurt characteristics. Each treatment was replicated 4 times, with first treatment (P1) was 18-h of incubation, second treatment (P2) was 24-h of incubation, third treatment (P3) was 30-h of incubation, fourth treatment (P4) was 36-h of incubation, and fifth treatment (P5) was 42-h of incubation. The measured and analyzed variables were yoghurt viscosity, WHC, moisture content, carbohydrate, pH value, total acidity, and total lactic acid bacteria (LAB).

Yoghurt Production

The fresh milk was pasteurized at 85°C for 30 min, followed by taro starch incorporation as much as 1.5%. After that, the temperature was cooled down to 43°C. The inoculation of a specific lactic strain of yoghurt was then done by adding LAB starter (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles*) as much as 3%. The incubation was done for 18, 24, 30, 36 and 42 hours at room temperature.

Yoghurt Quality Measurement

The physicochemical properties were analyzed according to[14] and microbiological properties were analyzed by describing the enumeration of lactic acid bacteria. Total microbial were expressed in Colony Forming Unit (CFU) per mL of yoghurt. A more detailed physicochemical properties measurement of the yoghurt is described as follows:

Viscosity Measurement

The yoghurt was homogenized before measured for the viscosity. The viscosity was measured by using Brookfield viscometer (Brookfield viscometer DVII, USA),[14] with a spindle no.6 at 2 rpm. The results are recorded after 50 s of shearing and presented in centipoises (cP) after.

Whey Holding Capacity (WHC)

The yoghurt WHC was measured as follows[15]: WHC (%). = (1 - W1/W2) × 100, where W1 is the weight of whey after centrifugation, and W2 is the yoghurt weight.

Moisture Content

The moisture content of yoghurt was determined according to AOAC[14] by drying the sample (10 g) at 105°C in the oven for 3h. The moisture content percentage was then obtained by dividing the weight loss after drying to the yoghurt fresh weight.

Carbohydrate Content

Carbohydrates were analyzed by using amylum test according to.[14]

pH value

The pH value was measured with a digital pH meter. The pH meter was firstly calibrated at pH 4 and 10 with buffer standards solution. Yoghurt as much as 50 ml was then placed in a beaker, the calibrated pH meter was then inserted and the obtained pH value was recorded.

Total Acidity

The total acidity was determined by 0.1% NaOH titration until the colour turned pink.

Total Lactic Acid Bacteria

Enumeration of total lactic acid bacteria was done by using the pour plate method. The MRS powder (62 g) was mixed with 1 L distilled water and autoclaved to make the MRS agar. The agar was then cooled until 45°C, and 15 mL of the MRS agar was then placed in a petri dish. The diluted yoghurt (1 mL) was then transferred in the MRS agar and mixed by gently tilting and swirling the dish. The plates were sealed with parafilm and left at room temperature to allow the agar to solidify. The plates were then inverted and placed in an incubator at 37°C for 48 hours. The lactic acid bacteria count was calculated as follows:

CFU/mL=(Total formed colonies x dilution factor)/(1 mL)

Statistical Analysis

The analysis of variance (ANOVA) was used to analyse the result. The Duncan's Multiple Ranged Test was then done to determine highly significant differences. The final results obtained were expressed as mean values ±standard deviation.

Results and Discussion

Yoghurt Viscosity, WHC, Moisture Content, and Carbohydrates

The different incubation time showed a highly significant difference ($p<0.01$) to the yoghurt viscosity with local taro starch addition. Furthermore, yoghurt viscosity after incubated for 42 hours gave the highest effect, resulting in 2747.75±28.12^{d} viscosity, while the lowest viscosity was shown after 18 hours of incubation. The result also showed that yoghurt viscosity was increased along with incubation time up to 42 hours. This showed that incubation time affects the LAB starters' growth optimization and its yoghurt gel producing capability. The adding of Taro starch could effectively to reduce the amount of moisture content and to increase the viscosity.

The result showed that 1.5% taro starch addition as stabilizer increase the yoghurt viscosity. This caused by the availability of granule in *taro starch* which contained amylose and amylopectin which capable to bind water. The viscosity of yoghurt is affected by fermentation time. The longer fermentation time would produce thicker yoghurt viscosity. The addition of stabilizer would increase total solids content, water holding capacity, and thicker yoghurt. The result of this research is in accordance,[16] which reported that pectin addition as a stabilizer at 0.1%, 0.3%, and 0.6% gave significant ($p<0.01$) increase to the yoghurt viscosity. A similar result also showed on research which used different stabilizers in yoghurt made from camel milk. The starch used as stabilizer has increased the yoghurt viscosity, enhance its sensory properties, and inhibit syneresis. Furthermore, the materials were able to absorb water and swell many times its original size, which resulted in an increased viscosity.[13]

The different incubation time also gave a highly significant difference ($p<0.01$) on yoghurt WHC with local *taro starch* addition as a stabilizer. The highest WHC was shown after the 36 hours of incubation (91.45±0.07%), followed by 30 hours of incubation (89.28±0.21%), 42 hours of incubation (88.56±0.19%), 24 hours of incubation (87.13±0.06%), and lowest WHC was shown on the 18 hours of incubation (81.56±0.21%).

The factors affecting yoghurt WHC were total solids and stabilizer addition in the yoghurt. WHC correlates with the protein capability to hold water content in yoghurt structure,[17] while milk fat responsible to bind the water content.[13] The highest yoghurt WHC was shown after 36 hours of incubation, this could be caused by the *taro starch* functional properties which capable to bind water optimally. Amylose and amylopectin granules in taro starch were able to bind water, thus increase the yoghurt viscosity and improve the yoghurt texture.[18,19, 20]

Table 1: The Effect of Incubation Time on Viscosity, WHC, Moisture content, and Carbohydrates of Yoghurt

Incubation time	Viscosity (Cp)	WHC (%)	Moisture content (%)	Carbohydrates (%)
18-h	2510.25±39.84^{a}	81.56±0.21^{a}	87.3±0.46^{c}	5.01±0.24^{c}
24-h	2585.5±36.57^{b}	87.13±0.06^{b}	86.99±0.16^{c}	4.81±0.12^{bc}
30-h	2666.5±39.03^{c}	89.28±0.21^{d}	86.26±0.28^{b}	4.47±0.12^{b}
36-h	2717.25±23.39^{cd}	91.45±0.07^{e}	85.08±0.1^{a}	3.37±0.15^{a}
42-h	2747.75±28.12^{d}	88.56±0.19^{c}	84.93±0.15^{a}	3.35±0.15^{a}

Different superscripts within the same column indicate a highly significant difference ($p<0.01$). Value is expressed as means ± standard deviation.

The results also showed that incubation time decrease yoghurt moisture content from 87.3% after 18 hours of incubation, 86.99% after 24 hours of incubation, 86.26% after 30 hours of incubation, 85.08% after 36 hours of incubation to 84.93% after 42 hours of incubation. The declining moisture content was in line with the increase of total solid caused by LAB cell proliferation. The condition generally increases the nutritional content of the produced yoghurt.[8] The incubation time in soymilk gave a negative effect to the moisture content, from 93.45% at 0 hours of incubation then decreased to 92.70% after 72 hours of incubation, while the total solids, ash and protein content increased.

Carbohydrates

The incubation time also gave a negative effect on the carbohydrate content of yoghurt added with local *taro starch* as a stabilizer. At 18 hours of incubation, 5.01% of carbohydrate were produced, then continuously decreased to 4.81%, 4.47%, 3.37% and 3.35% after incubated at 24, 30, 36, and 42 hours respectively. The incubation time also significantly decrease ($p<0.01$) the produced yoghurt carbohydrate content. This can be caused by the carbohydrate utilization for bacteria metabolism, as carbohydrate provides energy for LAB growth and metabolism. A similar result also is shown on research done,[8] where the carbohydrate content was decreased from 1.52% at 0 hours of incubation to 0.60% after 72 hours of incubation.

Yoghurt pH and Total Acidity

The different incubation time and local taro starch addition as stabilizer gave a highly significant difference ($p<0.01$) to the yoghurt pH value. The highest yoghurt pH was shown after 18 hours of incubation (4.29±0.01), followed by 24 hours of incubation (4.17±0.02),[30] hours of incubation (4.10±0.01), which was not significantly different with the 36 hours of incubation (4.08±0.01), while the lowest yoghurt pH was achieved after 42 hours of incubation (4.03±0.02). The average pH value in this research has fulfilled the ideal yoghurt pH, which was around 4-4.5.

Table 2: Different Incubation Time on the Yoghurt pH Value and Total Acidity

Incubation time	Average pH	Average total acidity (%)
18-h	4.29 ± 0.01^{d}	0.81 ± 0.02^{a}
24-h	4.17 ± 0.02^{c}	0.87 ± 0.01^{b}
30-h	4.10 ± 0.01^{b}	0.97 ± 0.01^{c}
36-h	4.08 ± 0.01^{b}	1.01 ± 0.01^{d}
42-h	4.03 ± 0.02^{a}	1.04 ± 0.02^{e}

Different superscripts within the same column indicate a highly significant difference ($p<0.01$). Value are expressed as means±standard deviation

This research also showed that longer incubation time resulted in lower yoghurt pH. Incubation time correlates with the increase of total LAB in yoghurt, which resulted in higher lactic acid production. Lactic acid bacteria are capable to metabolize glucose into galactose and lactic acid in a series of the fermentation process. The condition thus resulted in higher lactic acid bacteria which lower the pH.[21]

Incubation during fermentation process is mainly divided into three phases, which are: 1) Lag phase where the bacteria are adapting to the medium, indicated by slow pH decline; 2) Log phase which indicated by faster pH decline; and 3) Phase where acidification rate decreased and become more stable with a little variation of increased pH[6].[23,24] The research done[8] showed that incubation time affects the Soymilk yoghurt pH, resulting in 4.09 pH after 72 hours of fermentation. Furthermore, factors which could affect pH value aside from incubation time were incubation temperature, starter culture and supplementation.[21,25,2, 26,27]

The different incubation time also gave significant effect ($p<0.01$) to the total acidity of yoghurt added with local taro starch as a stabilizer. The highest yoghurt total acidity was shown after 42 hours of incubation (1.04±0.02%), followed by 36 hours of incubation (1.01±0.01%), 30 hours of incubation (0.97±0.01%), 24 hours of incubation (0.87±0.01%), while the lowest yoghurt total acidity was shown after 18 hours of incubation (0.81±0.02%). The average yoghurt total acidity in this research has fulfilled the standard yoghurt quality, which is around 0.5-2.0%. Other research also showed that longer incubation time resulted in the higher total acidity.[2,23]

The increasing acidity is caused by activities of lactic acid bacteria (LAB) which break lactose and other sugars into lactic acid. Moreover, the decrease of lactic acid level correlates with the decreasing LAB activities in the yoghurt during storage. Lactic acid is one of the major lactose products in milk degradation due to bacterial fermentation. The production depends on the involved microorganisms, in which milk fermentation proceeds through the glycolysis pathway and yields lactic acid. Generally, the lactic acid content will be increased from 0 hours to 24 hours of incubation. The research done by22 showed that Homemade yoghurt with 12% (w/v) Sago Oligo Saccharides (SSO) showed the highest lactic acid content, followed by yoghurt with 20% and 8% (w/v) SSO respectively. All of the homemade yoghurt supplemented with SSO and inulin showed better acidity compared to the control group. The initial lactic acid concentration is ranged from 0.30 to 0.40%, while the final lactic acid content ranged from 0.66 to 1.10% for all samples. Furthermore, research[8] incubation time affect soymilk yoghurt total acidity. The highest total acidity was 1.82% after 72 hours of incubation. The increasing total acidity trend is caused by several organic and acetic acids accumulation yielded from LAB fermentation activity. The combination effect of n non-fat dry matter at 4, 8, or 12% in milk, different incubation temperatures (37°C, 40°C or 44°C) and different final pH (4.2 or 4.5) were investigated on the biochemical and microbial properties of the probiotic milk fermented with *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and yoghurt bacteria during and after fermentation. Samples with the higher dry matter have shown a slower declining rate of pH and redox potential, but an increased rate of acidity, longer incubation and higher final titrable acidity. Samples which were fermented at higher temperatures and lower final pH showed a longer fermentation and higher final titrable acidity. Moreover, samples with higher non-fat dry matter, lower incubation temperatures and higher final fermentation pH showed a greater probiotic bacteria viability.[2]

Total Lactic Acid Bacteria

The different incubation time highly affect ($p<0.01$) the total BAL in yoghurt added with local *taro starch* as stabilizer. The highest yoghurt total lactic acid bacteria was shown after 36 hours of incubation (9.55±0.21 CFU/mL), followed by 42 hours of incubation (8.95±0.21 CFU/mL), 30 hours of incubation (8.45±0.52 CFU/mL), 24 hours of incubation (5.83±0.40 CFU/mL), while the lowest was shown after 18 hours of incubation (3.43±0.46 CFU/mL).

Several factors which affect LAB viability include incubation time and temperature, pH, LAB strain, milk composition and supplementation.[2,3,23] The heterofermentative bacteria able to produce lactic acid, acetic acid and CO_2. Lactobacillus is homofermentative bacteria able producing lactate as metabolic product. The quality of yoghurt influenced by interaction between bacteria and may improve the sensory.[28]

The higher non-fat dry matter contents (12%), final pH at 4.5 with lower incubation temperatures (37^{0}C) showed the best probiotic bacteria viability. A significant relationship between the gradual increase acidity of yoghurt during the storage and the amount of lactic acid produced. The strategy used to improve the growth of probiotic bacteria in yoghurt was by adding local taro which would provide nutrients for the bacteria metabolism, thus resulted in higher lactic acid production as well. The survival of LAB is indicated by low pH condition in the media. Moreover, the increased prebiotics on the fermented milk had stimulated the metabolic activities of starter bacteria, thus resulted in an improved acidity development.[22]

Table 3: The Effect of Incubation Time on Total Lactic Acid Bacteria

Incubation time	Average LAB (10^9 CFU/mL)
18 h	3.43±0.46^a
24 h	5.83±0.40^b
30 h	8.45±0.52^c
36 h	9.55±0.21^d
42 h	8.95±0.21^{cd}

Different superscripts within the same column indicate a highly significant difference ($p<0.01$). Value are expressed as means±standard deviation

Conclusion

It could be concluded that 36 hours of incubation would produce the best yoghurt characteristics which were made by using three different lactic acid bacteria (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophiles*) and *taro starch* as a local stabilizer in Indonesia.

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Conflict of Interest

We declare no potential conflict of interest.

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