RESEARCH ARTICLE | MAY 25 2021

The effect of starter concentration and incubation time on yogurt characteristics FREE

Aman Santoso ✉; Rini Retnosari; Vina Pramudita; Anugrah Ricky Wijaya; Tiara Nur Anisa; Eli Hendrik Sanjaya

AIP Conf. Proc. 2353, 030080 (2021)
https://doi.org/10.1063/5.0052694

The Effect of Starter Concentration and Incubation Time on Yogurt Characteristics

Aman Santoso[a], Rini Retnosari, Vina Pramudita, Anugrah Ricky Wijaya, Tiara Nur Anisa, and Eli Hendrik Sanjaya

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, Indonesia

[a]Corresponding author: aman.santoso.fmipa@um.ac.id

Abstract. The characteristics of yogurt are influenced by the concentration of the starter and incubation time. This study aims to determine the effect of variations in starter concentration and incubation time on the characteristics of yogurt. To achieve this objective, the study was carried out through several stages, namely sample preparation (pasteurization of cow's milk); incubation of cow's milk yogurt with variations in starter concentration and incubation time; characterization of the physical and chemical properties of yogurt. The physical properties were investigated through organoleptic tests of color, aroma, taste, and texture, along with pH and viscosity tests. In contrast, the chemical properties were analyzed from the total acid levels and protein levels. The results show that the variation in the treatment of yogurt production significantly affects the physical and chemical characteristics of the yogurt produced. The produced yogurt's pH is in the range of the standard value set by Food Standards Australia New Zealand (FSANZ). Besides, the amount of produced yogurt's total acid and protein concentration in this study are also met in the standard value set by the Indonesian national standard of yogurt. Moreover, the Mann-Whitney test results show excellent characteristics of yogurt produced. It has a soft texture, a distinctive aroma of yogurt, sour flavor, and white color.

INTRODUCTION

The consumption of milk and its processed products in Indonesia is still low, only around 11.8 L/capita/year. It is lower than in other countries such as Malaysia, which consumes 36.2 L/capita/year, and Myanmar that reaches 26.7 L/capita/year [1,2]. Low milk consumption in Indonesia is due to people with lactose intolerance who cannot consume fresh milk. Milk is a high nutritional value product that plays an essential role in improving community nutrition. However, milk utilization has a low economic value and is easily damaged due to the influence of pathogenic bacteria [3].

To increase the economic value and nutritional content, milk is processed into derivatives (dairy products) and combined with other food products. The use of technology in milk processing is very diverse, from conventional and simple to modern processing methods, especially for mass production. Along with science and technology development, many innovations have been created in milk processing with a fermentation process. Fermentation is one of the food processing to change food to be more durable, provide a delicious flavor, and increase the nutritional content of foods that are good for the health. Yogurt is a fermented milk product with a higher nutritional value than milk, and it can be used as a substitute for milk [4,5]. Besides having high nutritional value, the development of yogurt production in Indonesia, which increases year by year, can save Indonesia's cattle farmers.

Biologically, yogurt is a type of milk fermentation product assisted by Lactic Acid Bacteria (LAB). In general, yogurt production is facilitated by the mixed culture, which commonly is a mixture of *Lactobacilus bulgaricus* and *Streptococcus thermophilus*. Yogurt has a composition of 4-6% protein, 0.1-1% fat, 2-3% lactose, lactic acid 0.6-1.3% and pH 3.8 - 4.6. The quality of yogurt is influenced by several factors, namely the quality of milk, the homogenization process, the length of storage, the incubation temperature, the type of starter used, and the composition of the starter used [6].

International Conference on Life Sciences and Technology (ICoLiST 2020)
AIP Conf. Proc. 2353, 030080-1–030080-6; https://doi.org/10.1063/5.0052694
Published by AIP Publishing. 978-0-7354-4096-8/$30.00

Based on its production method, yogurt is classified into three types, namely yogurt set, Stirred yogurt, and Fluid Yogurt. Based on its flavor, yogurt is classified into three types, namely Plain Yogurt, Fruit Yogurt, and Flavored Yogurt [7]. Making yogurt involves several processes, namely pasteurization, inoculation of LAB, homogenization, and incubation. Inoculation of LAB and incubation is an essential process in making yogurt. Variations in the concentration of LAB starter affects the pH value, acidity, and viscosity of yogurt. Milk that has been inoculated with yogurt starter is then homogenized and incubated. The duration of incubation affects the acidity of yogurt. Increased incubation time is proportional to the total lactic acid produced [3,8].

Generally, yogurt production uses a mixture of 2 LAB starter cultures, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. However, based on several studies, these two bacteria do not last long in the digestive tract. This study used a commercial yogurt starter containing a mixture of 3 types of BAL, namely *Streptococcus themophillus*, *Lactobacillus bulgaricus,* and *Lactobacillus acidophilus*. The presence of *Lactobacillus acidophilus* is useful for improving the probiotic properties of yogurt products [1,9,10]. The utilization of commercial yogurt starter has several advantages, which are easy to obtain, good quality since they are always rejuvenated, and easier to apply directly to the community. If we use a pure bacterial starter, it is expensive, hard to get, needs special handling from experts, and can not be applied directly in the community. Based on these reviews, this research aims to investigate the influence of starter concentration variations and incubation time on the characteristics of yogurt from cow milk with commercial starters. To get optimum result, a mixture of 3 LAB, namely *Streptococcus themophillus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* so was used as a reference in making good quality yogurt to be applied in industry.

EXPERIMENT DETAILS

Materials

This study was carried out experimentally in a laboratory to determine the effect of variations in starter concentration and incubation time on yogurt characteristics from cow's milk with commercial starters containing a mixture of 3 LAB. The concentration variations were 2, 3, 4, and 5% of milk volume, while the incubation time variation was 6, 8, and 10 hours. The materials needed in this study were sodium hydroxide (p.a), sodium carbonate (p.a), folin-ciocalteu reagent, BSA (p.a), natrium tartrate (p.a), $CuSO_4.5H_2O$ (p.a), phenolphthalein (pp) indicator and distilled water.

Methods

This research consisted of 4 main stages. Stage 1 was sample preparation (pasteurization of cow's milk), while stage 2 was making cow milk yogurt with variations in starter concentration and incubation time. In stage 3, yogurt's physical characterization, including pH, viscosity, and organoleptic test for color, aroma, taste, and texture, were investigated. In stage 4, yogurt's chemical characterization, including analysis of total acid levels and protein levels, were characterized.

Cow Milk Sample Preparation

Fresh cow's milk was obtained from Mergosono, which was pasteurized by heating at a temperature of 72 °C for 15 seconds. 200 mL sterilized milk was inserted into 12 labeled Erlenmeyer (P21, P31, P41, P51, P22, P32, P42, P52, P23, P33, P43, P53), then cooled to 37 - 38 ºC.

Yogurt by Starters Concentration Variation and Incubation Time

Yogurt production was based on the procedures developed by Dibyanti [11]. Pasteurized milk was inoculated with commercial starter obtained from Home Industry in Batu City, Indonesia. It contained 3 LAB, namely *Lactobacillus bulgaricus*, *Lactobacillus acidophilus,* and *Streptococcus thermophilus*. The starter was varied in concentration with details as (1) P21, P22, and P23 were added 2% starter in milk (v/v); (2) P31, P32, and P33 were added 3% starter in milk (v/v); (3) P41, P42, and P43 were added 4% starter in milk (v/v); and (4) P51, P52 and P53 were added 5% starter in milk (v/v). The inoculated milk was homogenized by stirring for 5 minutes until homogeneous. The inoculated milk

was incubated in an incubator at 37 ºC with incubation time variations of (1) P21, P31, P41 and P51 incubated for 6 hours, (2) P22, P32, P42, and P52 incubated for 8 hours and (3) P23, P33, P43, and P53 incubated for 10 hours. The incubated milk was then transferred into sterile bottles and stored in a cooler at four ºC to stop the fermentation process.

Physical Characterization of Yogurt

The measurement of the pH of the sample was carried out according to the instructions for using the pH meter, with the buffer used of 4.0. The viscosity was measured using the Ostwald viscosimeter. 10 mL sample was inserted into the Ostwald viscosity and sucked it until the top mark. Count down the sample to the lower part of the sheet. The sample viscosity is calculated by Equation 1:

$$\eta : \frac{\eta \text{ distilled water}}{\eta \text{ sample}} = \frac{\rho \text{ distilled water} \times t \text{ distilled water}}{\rho \text{ sample} \times t \text{ sample}} \quad (1)$$

Note: η = viscosity (cP), ρ = density (g.mL^{-1}) and t = time (s)

Organoleptic tests, including color, aroma, taste, and texture, were tested by 30 panelists randomly. Panelists consist of undergraduate chemistry students, Universitas Negeri Malang. Each panelist gave a favorite level score of 1, 2, and 3. The data was collected for each parameter obtained from 30 panelists based on organoleptic yogurt criteria.

Chemical Characterization of Yogurt

The total acid analysis procedure was carried out by stages of 10 mL of the yogurt sample weighed and put it into Erlenmeyer 250 mL. Four drops of PP (Phenolphtalein) indicator were added and titrated with a 0.09 N NaOH solution until the solution's color became a constant pink color. The total acid in the sample was expressed in percent lactic acid, which could be calculated by Equation 2:

$$A = \frac{V_{NaOH} \times 0.009}{V_{sample}} \times 100\% \quad (2)$$

Note: A = Acid number
V_{NaOH} = volume of NaOH
V_{sample} = sample volume (mL)

Protein contents were analyzed using the Lowry-Folin method by spectrophotometry. The measurement began with making a standard solution BSA (Bovine Serum Albumin). The BSA solution with a concentration of 0.00; 0.04; 0.08; 0.12; 0.16; and 0.20 (mg/mL H_2O) was made by dilution from the standard solution, then 1 mL of each concentration was put in a vial bottle, and 1 mL of Lowry Reagent D was added and then homogenized with vortex for 5 minutes [12]. Next, 3 mL of Lowry E Reagent was added and stirred with the vortex. Then, homogeneous incubation at room temperature for 45 minutes was completed.

The measurement of density was carried out at 767 nm wavelength using a Vis spectrophotometer. 2 mL yogurt sample was dissolved into 100 mL distilled water, then shaken and filtered. About 1 mL of sample solution was taken and put into a vial bottle, then 1 mL of Lowry D Reagent was added and homogenized with vortex for 5 minutes. 3 mL of Lowry E Reagent was added and stirred with the vortex. After they were homogeneous, they were incubated at room temperature for 45 minutes.

RESULTS AND DISCUSSION

Fresh milk produced by farmers is shown in Figure 1. The pH value of cow's milk before the inoculated yogurt starter was 6.00. After the fermentation process, the pH decreases. The decrease in pH is due to the activities of *Lactobacillus bulgaricus, Streptococcus thermophilus,* and *Lactobacillus acidophilus* as LAB, which can convert lactose in milk to lactic acid [3,11]. In this research, the highest pH value of fermented milk is 4.00, which is observed in the yogurt sample with a starter concentration of 2% and an incubation time of 6 hours. It indicates that the yogurt produced met the standard set by FSANZ.

FIGURE 1. Farmer's Milk

FIGURE 2. Yogurt (Fermented milk)

This study shows that yogurt's pH value decreases by the increase of starter concentration used, as shown in Table 1. This is consistent with the other studies; the higher starter concentration increases acidity [3,13].

TABLE 1. Yogurt pH test results with starter concentration variation and incubation time

Concentration Starters	Incubation times		
	6 hours	8 hours	10 hours
2%	4.000	3.981	3.967
3%	3.967	3.942	3.922
4%	3.954	3.917	3.883
5%	3.937	3.903	3.841

The viscosity test was carried out using the Ostwald viscosimeter dan; the results are shown in Table 2. Based on the results of research conducted by [3], the viscosity of yogurt increases as the concentration of starter is added. A previous study states that at pH approaching 4.6, the solubility of casein is lost, so that hydrophobic interaction occurs between casein micelles from the main structure and consistency of yogurt [14]. This process affects yogurt's physical properties, including texture, viscosity, water binding capacity, and syneresis.

TABLE 2. Yogurt viscosity with starter concentration variation and incubation time

Concentration Starters	Incubation times		
	6 hours	8 hours	10 hours
2%	11.63	12.22	24.87
3%	12.55	14.84	28.69
4%	15.62	19.95	31.23
5%	15.92	24.18	34.67

Organoleptic tests covering texture, aroma, taste, and color were evaluated by 30 panelists, and the results are presented in Table 3. Organoleptic test data were analyzed by the Kruskal-Wallis test. The results show that the treatment of variations in concentration and incubation time has a significant effect ($p < 0.05$) on the texture, aroma, taste, and color of the yogurt synthesized.

The Mann-Whitney test results shown in Table 4 show that the yogurt produced has a soft texture, a distinctive aroma of yogurt, a very sour and white color. The sour taste is caused by the bacteria that ferment the lactose (milk sugar) into lactic acid, resulting in a distinctive yogurt flavor, sour taste, and thick texture due to the coagulation of milk protein by acid [15].

TABLE 3. Mode and hedonic scale of yogurt organoleptic test

Sample	Mean Rank			
	Texture	Flavor	Taste	Colour
P_{21}	162.85	194.05	167.60	162.05
P_{22}	157.20	145.03	133.98	139.80
P_{23}	134.60	166.82	113.98	158.35
P_{31}	157.20	139.60	180.37	144.90
P_{32}	202.40	204.27	198.50	233.95
P_{33}	202.40	179.77	227.63	211.70
P_{41}	211.93	218.57	201.25	208.45
P_{42}	213.42	219.93	224.92	206.60
P_{43}	203.60	193.38	161.25	195.00
P_{51}	192.30	149.82	193.98	161.60
P_{52}	172.38	174.32	166.72	182.00
P_{53}	15572	180.45	195.82	161.60

TABLE 4. Kruskal-Wallis test results on yogurt organoleptic test

	Parameter			
	Texture	Flavor	Taste	Color
Modus	2	2	3	3
Hedonic scale	Soft	Smells soft	Sharp acidity	White

Yogurt, which has the best texture and aroma, is P42, which is indicated by the mean rank of 213.42 and 219.93. In contrast, yogurt, which has the best taste, is P33 with the mean rank of 227.63, and yogurt, which has the best color, is P32 with the mean rank of 233.95. The results of the measurement of the total acid content in yogurt are shown in Table 5. According to the Indonesian national standard no. 2981: 2009, an excellent lactic acid level of yogurt (0.5-2.0%) has been achieved. The minimum total yogurt acid level is 1.22%, and the maximum was 1.40%. It indicates that the yogurt produced is within the standard range set by Indonesian national standard no. 2981: 2009.

TABLE 5. The lactic acid concentration in yogurt by variation of starter concentration and incubation time

Starter Concentrations	Incubation times		
	6 hours	8 hours	10 hours
2%	1.12	1.12	1.27
3%	1.18	1.24	1.31
4%	1.21	1.30	1.33
5%	1.23	1.38	1.41

The total acid level increased with the increase of starter concentration and incubation time. The increase in starter concentration is followed by the increase in acid levels since the rise in starter concentration indicated the increase in the number of microbes in the media. This escalation is also followed by an increase in microbial activity and microbial growth, which lead to the expansion in lactose, which changed to lactic acid as reflected by the acidic content of yogurt [1,11]. Moreover, the more bacteria produce lactic acid results in the increase in the acid formed. The protein content analysis in this study was carried out by the Lowry-Follin method. According to the Indonesian national standard no. 2981: 2009, the minimum protein content that must be present in yogurt is 2.7%, while according to the Food Standards Australia New Zealand (FSANZ, 2014), it should be 3%. In the results of this study, the minimum level of yogurt protein is 2.72%, while the maximum is 4.82%, as shown in Table 6.

TABLE 6. Protein levels with starter concentration variation and incubation time

Concentration Starters	Incubation times		
	6 hours	8 hours	10 hours
2%	2.87	3.73	3.26
3%	3.38	3.73	3.76
4%	3.83	4.61	3.92
5%	3.10	4.82	2.72

The protein content increased with the escalation in the concentration of the starter. However, at the incubation time of 10 hours, there is a decrease in protein content because it could be denatured in an acidic state. In the results of the study, the lowest protein content is found in the P53 yogurt sample. It is the yogurt sample with a treatment variation of the starter concentration of 5% and an incubation time of 10 hours. Yogurt P53 sample has the highest total acid content, which could cause protein denaturation so that the protein found in yogurt is low [16,17]. The level of yogurt protein is determined by the quality of fresh milk as the basic ingredient. The protein content in milk is related to the quality of yogurt produced; the higher protein content results in, the higher yogurt quality [18,19]. Moreover, yogurt production is affected by the type and concentration of the starter. The higher amount of microbes found in yogurt is proportional to the protein content since most of the components of microbial constituents were proteins [3,20].

SUMMARY

The pH of the produced yogurt is in the range of 3.84 to 4.00. Its total acid level and protein content range between 1.22%-1.40%, and 2.72%-4.82%, respectively. The Mann-Whitney test results show that the yogurt produced had a soft texture, a distinctive aroma, a very sour and white color. The addition of starter concentration and longer incubation time reduced pH value, increased viscosity, percentage of total acid level, and the protein content of the produced yogurt. Yogurt that has good quality has been generated by the treatment of adding a starter concentration of 4% and incubated for 8 hours.

REFERENCES

1. C. Chen, S. Zhao, G. Hao, H. Yu, H. Tian, and G. Zhao, Int. J. Food Prop. **20**, S316 (2017).
2. S. Buddies, Sci. Am. (2019).
3. S.H. Kim and S. Oh, Milk Dairy Prod. Hum. Nutr. Prod. Compos. Health 338 (2013).
4. S. Buddies, Sci. Am. (n.d.).
5. O. Adolfsson, S.N. Meydani, and R.M. Russell, Am. J. Clin. Nutr. **80**, 245 (2004).
6. Mercola.Com (n.d.).
7. R.C. Ray and D. Montet, *Fermented Foods. Part II, Part II,* (2017).
8. N. Wakhidah, G.J. M, and R. Utami, Proceeding Biol. Educ. Conf. Biol. Sci. Enviromental Learn. **14**, 278 (2017).
9. S. Manzi, J. Probiotics Health (n.d.).
10. A. Santoso, A.R. Wijaya, Y. Permatasari, T.N. Anisa, and Sumari, AIP Conf. Proc. **2231**, 040042 (2020).
11. P. Dibyanti, Pengaruh Penambahan Berbagai Konsentrasi Kultur Dan Waktu Inkubasi Terhadap Ph, Kadar Keasaman, Viskositas Dan Sineresis Set Yogurt, Sarjana, Universitas Brawijaya, 2014.
12. D. Serlahwaty and N. Sari, 8 (2015).
13. M.N.H. Siregar, L.E. Radiati, and D. Rosyidi, 8 (n.d.).
14. M. Djali, S. Huda, and L. Andriani, AgriTECH **38**, 178 (2018).
15. W.J. Lee and J.A. Lucey, Asian-Australas. J. Anim. Sci. **23**, 1127 (2010).
16. D.M. Beshkova, E.D. Simova, G.I. Frengova, Zh.I. Simov, and Z.N. Spasov, World J. Microbiol. Biotechnol. **18**, 365 (2002).
17. E. Obi T. and Obhioze Augustine Akpoka, (2019).
18. M. Djali, S. Huda, and L. Andriani, AgriTECH **38**, 178 (2018).
19. A.M. Jannah, A.M. Legowo, Y.B. Pramono, A.N. Al-Baarri, and B.M. Abduh, 5 (n.d.).
20. P. Morell, I. Hernando, E. Llorca, and S. Fiszman, Food Res. Int. **70**, 64 (2015).