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QUALITY AND BACTERIA IDENTIFICATION OF YOGHURT INCUBATED UNDER ROOM TEMPERATURE USING MIXED STARTER

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Abstract

The two-stage study was conducted to establish the procedure of making yoghurt using liquid starter under room temperature, which is expected to have standard quality with the absence of non-lactate pathogen bacteria. On the first stage, ready-for-use pure starters of *Streptococus termophillus* (St) and *Lactobacillus delbrueckii subsp. bulgaricus* (Lb) were made and used for making the yoghurt to be further used as mixed starter at the rate of 5% with the St:Lb ratio of 1:1 (v/v). Incubation was done at 45°C for 3 hours (coagulation) and then kept refrigerated. Bacteria growth was observed every hour. Yoghurt production was done at the second stage using pasteurised milk which was divided into two parts, added with the mixed liquid starter and the pure starter (both from the first stage), respectively. Incubation was done either under room temperature for 20 hours or at 45°C for 3 hours. Observations were made on acidity (lactic acid equivalent), pH, yoghurt sugar, bacteria count and identification (St, Lb, *Pseudomonas sp, Salmonela* and *Stapylococcus*). Data were analysed using 2x2 analysis of variance (2 starters and 2 incubation), with 3 replications and further analysed using Duncan's new multiple range test. It was found that yoghurt could be made using either liquid mixed starter or pure starters. Incubation could be done under room temperature for 20 hours. The quality of yoghurt made using liquid mixed starter and pure starter were as follows: pH were 4.23 and 4.04, acidity were 1.205 and .965%, yoghurt sugar were 2.688 and 3.031%, St bacteria were $1.848x10^8$/ml and $1.432x10^8$/ml, Lb bacteria were $0.182x10^8$/ml and $0.168x10^8$/ml, respectively. No *Pseudomonas sp., Salmonela* and *Staphylococus* growths were observed during the room-temperature incubation. It is suggested to take into accounts the followings: step-by-step of the process of making the yoghurt, equipments and environment sanitations.

Key words: Yoghurt making, Room temperature incubation, Yoghurt bacteria

Introduction

Yoghurt is a fermented milk product made using bacteria activity of *Lactobacillus delbrueckii subsp. Bulgaricus* (Lb) and *Streptococcus termophillus* (St) under the incubation at 45°C for about six hours until the process of coagulation

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occurs. Most of households who would like to make yoghurt are facing the problems of not having any incubator, aside from the difficulty of getting starter. Making yoghurt under room temperature using liquid starter, therefore, becomes their choice. However, one of the problems is the possible presence and growth of pathogen bacteria in yoghurt making under room temperature. This study was intended to develop a procedure of making yoghurt with standard quality using liquid starter under room temperature, without the presence of any unwanted bacteria.

Materials and Methods

A two-stage experiment was conducted. In the first stage, pure starter and liquid mixed starter were prepared. The liquid starter was prepared using mixed sterilized milk added with 5% Lb and St, with the ratio of 1/1 (v/v). Incubation was done under 45°C until coagulation occurred, and then kept refrigerated. In this stage, observation was made every hour to see the bacteria growth.

In the second stage, two types of yoghurt were made using skimmed milk with those two types of starter (pure and mixed starters) produced in the first stage. Pasteurised skimmed liquid milk was prepared using 12% powdered milk in water at high temperature in a short time (83^0C, 15 seconds). Incubation was done at 45°C for three hours or at room temperature for 20 hours. Observations were made on the pH, acidity (lactic acid equivalent), yoghurt sugar, as well as the bacteria identification.

Data were analysed using 2x2 analysis of variance (two types of starter, two method of incubation), with three replications.

Results and Discussion

Bacteria Growth during Starter Preparation

Coagulation had occurred after three hours of incubation at 45°C, with the pH of 4.7, St and Lb bacteria counts of 42.5 and 4.75 million/ml, respectively. After four hours of incubation, the pH was decreasing down to 4.38, beyond the iso-electric point. Besides, Lb bacteria decreased down to 3.15 million/ml entering the death phase, while St bacteria was still at the growing phase (Figure 1). Therefore, the process was terminated after three hours of incubation.

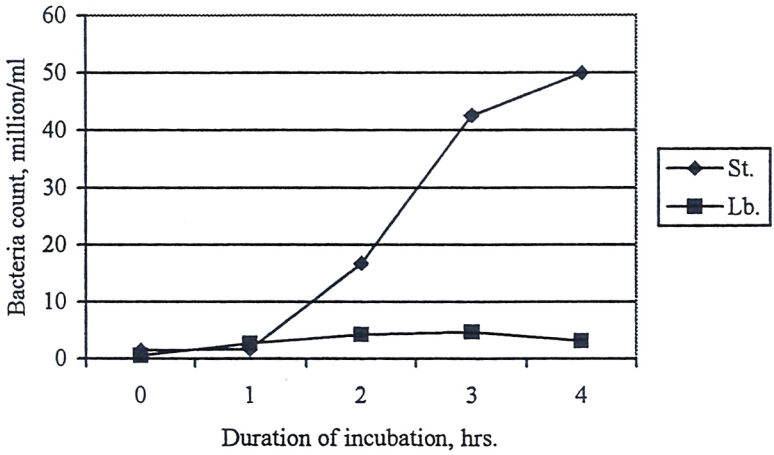

Figure 1. Bacteria growth in the yoghurt to be used as starter

This result was similar with that found by Purnami (1997) which stated that the incubation of yoghurt making for starter was terminated after three hours. At that time, the pH was 4.43, St bacteria count was 440.00 million/ml, in the growing phase, while Lb bacteria count was 10.02 million/ml, entering the death phase. Mahmudah (1997) found that Lb and St bacteria count were 81.00×10^3/ml and 55.20×10^8/ml.

Yoghurt Quality

PH and Acidity. Incubation temperature was found to affect ($P<0.01$) both pH and acidity. Types of starter were only affecting the acidity ($P<0.01$). There was an interaction effects ($P<0.01$) on both pH and acidity (Table 1 and 2).

Table 1. Average yoghurt pH at different incubation temperatures and starter types

Incubation temperatures	Starter		
	Mixed	Pure	Average
45°C	4.31	4.48	4.39[a]
Room	4.23	4.04	4.13[b]
Average	4.27	4.26	

[a, b] Averages with different superscripts differ ($P<0.01$)

Table 2. Average acidity (% yoghurt lactic acid equivalent) at different incubation temperatures and types of starter

Incubation temperatures	Starter		
	Mixed	Pure	Average
45°C	0.914	0.807	0.861[a]
Room	1.205	0.965	1.085[b]
Average	1.060[c]	0.886[d]	

[a, b] Averages with different superscripts at the same column differ (P<0.01)
[c, d] Averages with different superscripts at the same row differ (P<0.01)

The average pH of the yoghurt incubated under room temperature was lower compared with that incubated at 45°C (4.14 vs. 4.40). In another word, yoghurt incubated under room temperature was more acid than that incubated at 45°C (1.085 vs. 0.861%).

It is noted that the room temperature was ranging from 27 to 29°C, which is in the range of temperature appropriate for Lb and St bacteria growth (21 to 50°C and 20 to 53°C, respectively). The optimum temperature for both bacteria was in the range from 40 to 45°C (Buchanan and Gibbons, 1974). Since the room temperature was lower than the optimum temperature for bacteria growth, therefore the incubation under room temperature needed more time than that at 45°C (20 vs. 3 hours). According to Buckle *et al.* (1987), factors required for optimum bacteria growth were temperature, pH, nutrients supply, time, water activity and available oxygen.

Time of fermentation under room temperature was longer than that at 45°C, resulting in greater competition in longer time between both bacteria under room temperature. The more lactic acid was produced along with longer time, the more unwanted bacteria were killed and this can be observed through decreasing pH and increasing acidity (Frazier and Westhoff, 1978).

Types of starter did not affect the average pH. According to Salle (1982), acid lactic development could not easily decrease the pH, when there is a buffer in milk. All yoghurt, using either type of starter, was producing lactic acid and others such as citric acid that was functioning as buffer.

Types of starter affected acidity, where mixed starter resulted in higher acidity compared with pure starter (1.06 vs. 0.89%). This is due to faster bacteria growth in mixed starter, like what Larsen and Anon (1990) as well as Hui (1993) found. Bigiana and Hastowo (1992) found that there was bacterisin in yoghurt produced using mixed starter, which was a bacteristatic as well as bactericide.

Yoghurt Sugar. Incubation temperature affected (P<0.01) yoghurt sugar (Table 3). There was no effect of starter type on yoghurt sugar. There was an interaction effect of incubation temperature and type of starter on yoghurt sugar.

Table 3. Average yoghurt sugar at different incubation temperatures and types of starter

Incubation temperatures	Starter		
	Mixed	Pure	Average
45°C	3.244	2.827	3.036[a]
Room	2.688	3.031	2.860[b]
Average	2.966	2.929	

[a, b] Averages with different superscripts differ (P<0.01)

Average sugar in yoghurt incubated under room temperature was lower than that incubated at 45°C (2.86 vs. 3.04), which was in accordance with statistical result on average acidity. Higher acidity was possible due to greater amount of hydrolysed sugar.

Greater amount of average sugar in yoghurt produced using mixed starter was due to the presence of bacterisin that resulted in incomplete hydrolysis of yoghurt sugar. Any significant effects on yoghurt sugar shown in Table 3, could be explain from the hydrolysis process of sugar, which was related to different temperatures and types of starter.

***Streptococcus termophillus* Bacteria.** St bacteria count was not affected by incubation temperature, but affected (P<0.01) by type of starter (Table 4). This significant effect was in accordance with the finding of Larsen and Anon (1990), who stated that the rate of bacteria growth of mixed culture was higher than that of single bacteria culture. This was happened in both incubation under room temperature as well as at 45°C. St bacteria count produced with incubation under room temperature was greater (P<0.01) than that at 45°C, because the optimum temperature for St bacteria growth was in the range of 40 to 45°C (Buchanan and Gibson, 1974).

Table 4. Bacteria count of *Streptococcus termophillus* at different incubation temperatures and types of starter, 10^8/ml

Incubation temperatures	Starter		
	Mixed	Pure	Average
45°C	1,955	1.348	1,652
Room	1.848	1.437	1,643
Average	1,902[a]	1,393[b]	

[a, b] Averages with different superscripts differ (P<0.01)

Higher St bacteria was counted in the incubation at 45°C was due to shorter time (only three hours, compared to 20 hours).

***Lactobacillus delbrueckii subsp. bulgaricus* Bacteria.** What happened to the Lb bacteria in terms of different types of starter tended to be similar with what

happened to the St bacteria with similar explanation (Table 5).

Incubation temperature was found to affect the Lb bacteria count. Again, this had something to do with time duration of incubation, as well as the acidity.

Bacteria Identification of *Pseudomonas sp., Salmonella and Staphylococcus.*

There was neither *Pseudomonas sp., Salmonella* nor *Staphylococcus* bacteria found in the pasteurised skimmed milk. These bacteria species were not found in all yoghurt during incubation, either. However, for those who would like to produce home-made yoghurt, it is important to pay attention on the sanitation of the equipment and environment.

Table 5. Bacteria count of *Lactobacillus delbrueckii subsp. bulgaricus* at different incubation temperatures and types of starter, 10^8/ml

Incubation temperatures	Starter		
	Mixed	Pure	Average
45°C	0.161	0.153	0.157^a
Room	0.182	0.168	0.175^b
Average	0.172^c	0.161^d	

^{a,b} Averages with different superscripts at the same column differ (P<0.01)
^{c,d} Averages with different superscripts at the same row differ (P<0.01)

Conclusion

It was concluded that yoghurt could be made using either liquid mixed starter or pure starters. Incubation could be done under room temperature for 20 hours. The quality of yoghurt made using liquid mixed starter and pure starter were as follows: pH were 4.23 and 4.04, acidity were 1.205 and 0.965%, yoghurt sugar were 2.688 and 3.031%, St bacteria were $1.848x10^8$/ml and $1.432x10^8$/ml, Lb bacteria were $0.182x10^8$/ml and $0.168x10^8$/ml, respectively. No *Pseudomonas sp., Salmonela* and *Staphylococus* growths were observed during the room-temperture incubation. It is suggested to take the followings into accounts: step-by-step of the process of making the yoghurt, equipments and environment sanitations.

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