

Supplementation of Indigenous Probiotic Bacteria into Yoghurt

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ABSTRACT

Traditional yoghurt bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, usually do not survive under acidic conditions and bile concentrations when they encountered in the gastrointestinal tract. Therefore, for yoghurt to be considered as a probiotic product, probiotic bacteria are incorporated as a dietary adjunct. The purpose of this research was to produce acceptable yoghurt supplemented with indigenous probiotic bacteria. This research was divided into three parts i.e. addition of indigenous probiotic (*Lactobacillus* sp. Dad13 and *Lactobacillus plantarum* Mut7) in standard formula, sensory evaluation of yoghurt on appearance, texture, flavor, and overall, and evaluation of selected probiotic culture for its viability and capability to grow with yoghurt starter. *Lactobacillus* sp Dad13 and *Lactobacillus plantarum* Mut7 were selected as indigenous probiotics cultures because they have the essential criteria for probiotic such as acid and bile tolerant, capable of suppressing enteric pathogenic bacteria, improving lactic acid bacteria population in colon and also have the ability to lower cholesterol levels. The results showed that *Lactobacillus* sp. Dad13 produced better flavor compared with yoghurt supplemented with *L. plantarum* Mut7 and traditional yoghurt without probiotic supplementation. However, supplementation of *Lactobacillus* sp Dad13 produced

lower sensory score on appearance, but similar in texture compared with traditional yoghurt. Supplementation of *Lactobacillus* sp Dad13 produced sensorically acceptable yoghurt and did not significantly different with traditional yoghurt. The number of total lactic acid bacteria (LAB) increased during fermentation from 2.45×10^7 CFU/ml to 2.31×10^8 CFU/ml. Combination of *L. bulgaricus* and *S. thermophilus* starter cultures produced highest lactic acid production. However, supplementation of *Lactobacillus* sp Dad13 did not statistically different on lactic acid production, pH, and LAB count. Supplementation of probiotic bacteria is important in order to make yoghurt have a better functional food properties.

Keywords : yoghurt, supplementation, indigenous probiotic, functional food

INTRODUCTION

Functional foods are foods with supposed to have specific health benefits. Probiotics are considered to be beneficial for health. Probiotics ("for life" in Greek) are live microorganism, present in foods, which have a beneficial effect on the intestinal microbiota (Quillien, 2001). According to Fuller (1989), probiotics are live microorganisms which actively enhance health of the host by improving the balance of microflora in the gut

when ingested live in sufficient numbers.

Since lactic acid bacteria used for the manufacture of various fermented dairy foods such as yoghurt do not survive in the gastrointestinal tract, a trend today is to incorporate probiotic bacteria such as *Lactobacillus acidophilus* and bifidobacteria into fermented foods.

Traditionally, yoghurt is manufactured using *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. These yoghurt bacteria are claimed to provide some health benefits, but they do not survive under acidic conditions and bile concentrations as usual encountered in the gastrointestinal tract (Shah, 2001). Therefore, for yoghurt to be considered as a probiotics product, probiotics are incorporated as a dietary adjunct.

Fermented milk could be made with probiotics bacteria, but the quality is not as good as yoghurt made with traditional yoghurt bacteria. Yoghurt made with probiotics bacteria also need a longer fermentation process. Thus the normal practice is to make yoghurt with both yoghurt and probiotic bacteria.

Ngatirah *et al.* (2000) have studied the ability several strains of lactic acid bacteria isolated from several local sources in assimilating cholesterol and deconjugating bile salts. They found variation among those strains in the ability to assimilate cholesterol and deconjugate bile salts. Isolates which have the highest ability to assimilate cholesterol and deconjugate bile salts were *Lactobacillus* sp Dad13 (isolated from traditional fermented buffalo milk) and *Lactobacillus plantarum* Mut7 (isolated from traditional fermented cassava). Therefore in this study, we used both isolates to be incorporated into yoghurt.

The purpose of this study was to produce an acceptable yoghurt supplemented with indigenous probiotic bacteria.

MATERIALS AND METHODS

Strains and culture conditions

Lactobacillus sp. Dad13, *Lactobacillus plantarum* Mut7, *L. bulgaricus* FNCC 0041, and *Streptococcus*

thermophilus FNCC 0040 were obtained from Food and Nutrition Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University. Culture stocks were kept in 10% glycerol and 10% skim milk with the ratio 1 : 1, kept in sterile 1,5 ml polyethylene screw cap tubes, at -40 °C. These strains were rejuvenated in MRS broth (Merck, Oxoid) at 37 °C for 24 h.

Preparation of yoghurt

Commercial pasteurized homogenized low fat milk and skim milk powder were used to make yoghurt. Commercial pasteurized homogenized low fat milk was added with skim milk powder, sucrose, and flavoring agent. The mixture was then pasteurized at 85 °C for 30 min, cooled to 40 °C prior to inoculation. The starter used was 10% of the total yoghurt mixture. Standard yoghurt was treated with different indigenous probiotics i.e. *Lactobacillus* sp. Dad13 and *L. plantarum* Mut7. The ratio of *S. thermophilus* and *L. bulgaricus* was 1:1, while *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus* sp Dad13 or *L. plantarum* Mut7 was 2:1:1. Enumeration of the Lactic Acid Bacteria were conducted before and after incubation at 43 °C for 6-7 hours. Sensory evaluation was conducted on appearance, texture, and flavors by ten panelists to evaluate products acceptability.

Co-culture of lactic acid bacteria

To study whether *Lactobacillus* sp Dad13 can survive and compete with *S. thermophilus* and *L. bulgaricus*, single culture (*S. thermophilus* or *L. bulgaricus* or *Lactobacillus* sp Dad13) and mixed cultures (*S. thermophilus* + *L. bulgaricus*; *S. thermophilus* + *Lactobacillus* sp Dad13; *L. bulgaricus* + *Lactobacillus* sp Dad13; and *S. thermophilus* + *L. bulgaricus* + *Lactobacillus* sp Dad13) were grown in 10% skim milk. Enumeration of the viable cells were conducted before and after incubation at 37 °C for 18 h. Approximately 10 colonies of each plates were taken and observed under microscope for the morphological tests.

Enumeration procedures

Serial dilutions were prepared with dilution blanks composed of 0,1% peptone (Difco) in distilled water. The viable cells were counted using pour plates technique with MRS agar + CaCO₃. The MRS agar was prepared by dissolving 1,5% agar and 1% CaCO₃ in MRS broth prior to sterilizing (15 min at 121 °C). The plates were incubated at 37 °C for 48 h.

RESULTS

The yoghurt formulation was shown in Table 1. Addition of flavoring agent was addressed to obtain the more acceptable yoghurt formula by the unaccustomed consumers. From the three variations of yoghurt tested overall the best formula was obtained in yoghurt made with *S. thermophilus* and *L. bulgaricus* (control) with the overall score of 16, followed by yoghurt supplemented with *Lactobacillus* sp Dad13 and the third was obtained in yoghurt supplemented with *L. plantarum* Mut7. *Lactobacillus* sp Dad13 was isolated from fermented buffalo milk ("dadih") so it was more compatible to incorporated into yoghurt because it comes from the same inhabitant, while *L. plantarum* Mut7 was isolated from fermented cassava.

Sensory evaluations were conducted to know the differences in appearance, texture, and also flavors from 3 types yoghurt compared to commercial yoghurt. From Figure 1 it was shown that highest mean appearance score was in yoghurt made with *S. thermophilus* and *L.*

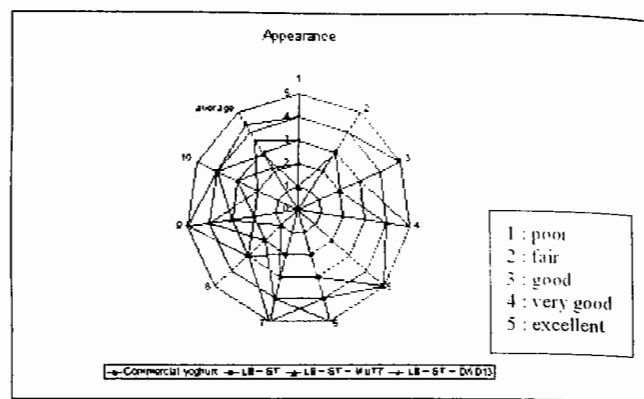


Figure 1. Sensory evaluation on appearance

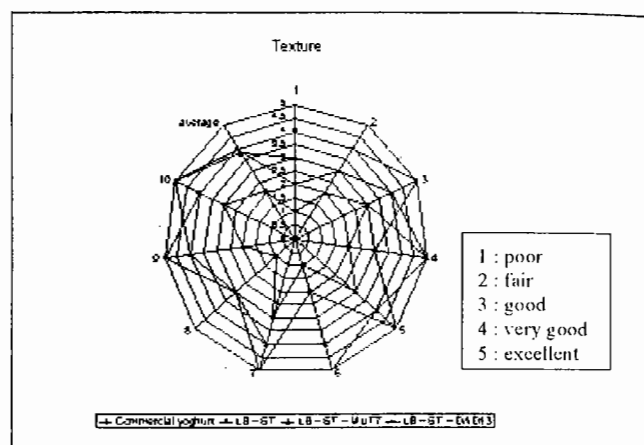


Figure 2. Sensory evaluation on texture

bulgaricus, followed by yoghurt supplemented with *Lactobacillus* sp. Dad13. Yoghurt supplemented with *Lactobacillus* sp. Dad13 did not significantly different compared to traditional yoghurt.

Table 1. Yoghurt formulation

Composition	Percentage	Formula
Commercial pasteurized homogenized low fat milk	90% (v/v)	90 ml
Starter	10% (v/v)	10 ml
Additive		
Skim milk powder	2% (w/v)	2 g
Sucrose	10% (w/v)	10 g
Flavoring agent	0,3% (w/v)	0,3 g

Figure 2 indicated that highest average of texture score was obtained in yoghurt made with *S. thermophilus* and *L. bulgaricus*, and also yoghurt supplemented with *Lactobacillus* sp Dad13. There was no significant difference between the 3 types of yoghurt. Among the panelists, there was a big variation in score assessment of texture. This variation maybe due to low frequency of the panelists in consuming the product so that the assessment was less precise.

From Figure 3 it was shown that the highest average of flavors score was obtained in yoghurt supple-

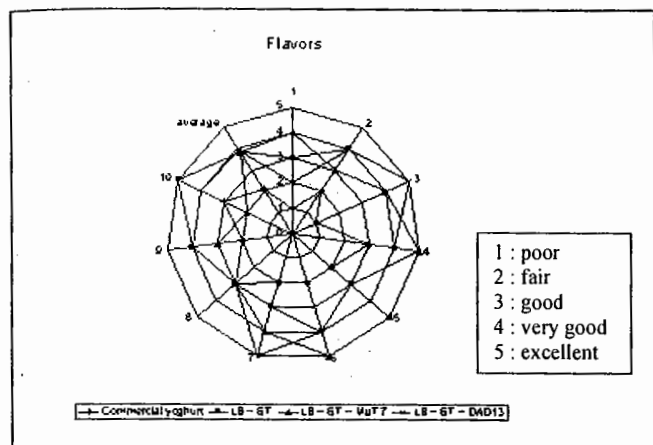


Figure 3. Sensory evaluation on flavors

mented with *Lactobacillus* sp Dad13, followed by yoghurt made with *S. thermophilus* and *L. bulgaricus*, and also yoghurt supplemented with *L. plantarum* Mut7.

Yoghurt have to be made by a mixed starter cultures *S. thermophilus* and *L. bulgaricus* because both culture were giving a good appearance, texture, and flavors properties (Tamime And Robinson, 1985). Fermented milk with only *Lactobacillus* sp Dad13 could be made, but the incubation period was long and the product quality was less satisfactory.

Since some probiotic strains grow very slowly in milk, traditional yoghurt cultures (*L. bulgaricus* or *S. thermophilus*) or mesophilic mixed strain culture are often used together with probiotic strains to speed up the acidification process (Dave and Shah, 1997).

We conducted co-culture experiment to study whether *Lactobacillus* sp Dad13 could compete and survive if it was grown with *S. thermophilus* and *L. bulgaricus*. From Table 3 it was seen the increasing lactic acid production, pH, and increasing bacteria population after 18 h incubation. Mixed cultures of *S. thermophilus* and *L. bulgaricus* capable of yielding higher acid production compared to others. Sensory evaluation was correlated with the result in which yoghurt made with mixed cultures of *S. thermophilus* and *L. bulgaricus* yield the highest flavors score. Total lac-

Table 3. The percentage of lactic acid production, pH, and LAB count before and after incubation at 37 °C for 18 h

No.	Treatment	Percentage of lactic acid production (%)	pH	Total LAB (CFU/ml)	
				before fermentation	after fermentation
1	Control (no culture added)	0.180	6.34 ± 0,01	0	0
2	St	0.648	4.68 ± 0,07	2.02 X 10 ⁷	3.11 X 10 ⁸
3	Lb	0.612	4.76 ± 0,17	1.74 X 10 ⁷	2.61 X 10 ⁸
4	Dad13	0.612	4.79 ± 0,08	1.61 X 10 ⁷	2.14 X 10 ⁸
5	St + Lb	0.720	4.55 ± 0,07	1.58 X 10 ⁷	3.11 X 10 ⁸
6	St + Dad13	0.648	4.61 ± 0,01	1.73 X 10 ⁷	3.10 X 10 ⁸
7	Lb + Dad13	0.612	4.81 ± 0,13	1.90 X 10 ⁷	2.59 X 10 ⁸
8	St + Lb + Dad13	0.648	4.65 ± 0,04	1.59 X 10 ⁷	2.88 X 10 ⁸

St = *Streptococcus thermophilus*; Lb = *Lactobacillus bulgaricus*; Dad13 = *Lactobacillus* sp. Dad13

tic acid bacteria population after incubation at 43 °C for 6-7 hours in yoghurt made with *S. thermophilus* and *L. bulgaricus* was higher than in yoghurt supplemented with *Lactobacillus* sp. Dad13.. This indicated that combination of both cultures produced best symbiosis. According to morphological tests, it was shown that *Lactobacillus* sp Dad13 could survive and compete with *S. thermophilus* and *L. bulgaricus* since we observed rod-shaped bacteria either in combination of *S. thermophilus* and *Lactobacillus* sp Dad13 or combination of *S. thermophilus*, *L. bulgaricus* and *Lactobacillus* sp Dad13.

Growth and survival properties vary between different bacterial strains (Dave And Shah, 1997; Hull *et al.*, 19A84), therefore the combination of probiotic and traditional starter cultures is important to study. The strains used in the preparation of yoghurt must be checked carefully in order to know their survival properties. In general, lactobacilli survive better than bifidobacteria due to their greater tolerance of oxygen and to low pH. *L. bulgaricus* may affect the survival of probiotics due to the amount of acid and hydrogen peroxide produced (Dave and Shah, 1997; Rybka and Fleet, 1997). Stimulatory effect on the growth of bifidobacteria in the presence of *L. bulgaricus* have been observed and were suggested to be due to proteolytic activities of the lactobacilli which increased the availability of valine, glycine, and histidine (Dave And Shah, 1997). *S. thermophilus* has not been reported to inhibit probiotic microbes to the extent observed with *L. bulgaricus*, and may sometimes stimulate their growth or survival due to consumption of oxygen (Ishibashi And Shimamura, 1993). Enhance growth of *S. thermophilus* in the presence of probiotic strains has also been observed (Dave And Shah, 1997). The study of the survival of commercial strains of *L. acidophilus* and *L. rhamnosus* GG together with a mesophilic lactococcal culture showed that strain GG was stable in this environment, whereas the number of viable cells decreased in the case of some of the *L. acidophilus* strains (Nighswonger *et al.*, 1996).

CONCLUSION

Supplementation of traditional yoghurt with indigenous probiotic *Lactobacillus* sp Dad13 produced sensorically acceptable yoghurt and did not significantly different with traditional yoghurt on appearance, texture, flavors, and overall properties. It also did not statistically different on lactic acid production, pH, and LAB count. Supplementation of probiotic bacteria is important to make yoghurt as better functional food.

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