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Original Article

Optimization of a Starter Culture for the Production of Quality Yoghurt

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Specific microorganisms that determine the body, texture and flavor of final yoghurt is known as starter culture. The present study was carried out to optimize a starter culture for quality yoghurt production. Ten yoghurt samples were collected from different areas of Bangladesh and their organoleptic, biochemical and microbiological properties were assessed to find out the best possible 'starter'. For optimizing the starter, the effects of different parameters i.e. temperature, pH, incubation time and starter size on the production of quality yoghurt were investigated. After fermentation at different temperatures it was found that 45°C was the best for production of quality yoghurt in six hours, whereas other temperatures needed longer fermentation time. No yoghurt formation occurred within 24 hours at 50°C. This result indicated that at 45°C lactic acid bacteria show higher metabolic activity than the other temperatures. Quality yoghurt was found to be produced at pH ranging from 4.6 to 5 and beyond this pH range quality deteriorated. This study also revealed that both the quality of yoghurt and the fermentation time are dependent on the starter size. Smaller starter size lingers the fermentation period and also produces poor quality yoghurt. It was found that 5% starter was the most suitable one for the production of quality yoghurt. As most yoghurt producers in Bangladesh do not have any advance knowledge about the proper manufacturing conditions of yoghurt production, the output of this study will be helpful for them to produce quality yoghurt.

Key Words: Yoghurt, Fermentation, Starter culture, Optimization

Introduction

Yoghurt, a fermented milk product has become more popular worldwide for its important properties such as high soluble protein, rich nutrients, high calcium content, low-sugar and fat[1]. For its preparation, the milk is allowed to ferment with the microbial inoculums called lactic acid bacteria (LAB) which are used as a starter under controlled environmental conditions[2,3,4]. These bacteria ferment milk sugar lactose to lactic acid thus lowering the pH, and inhibiting the growth of pathogenic bacteria[5,6]. These bacteria also produce acetone which is the main flavoring agent of yoghurt[7]. Various types of factors influence yoghurt quality which may be nutritional (quality of raw milk), environmental (temperature, P^H, time of incubation) and size of starter[8]. Amount of active starter culture is an important factor which influences the quality of yoghurt. To activate the starter, suitable amount of inoculum, the time and the temperature of incubation are the important considerations. Aroma, flavor, stability, texture of final product and the rate of acidification of raw milk are the primary selection criteria of suitable starter culture[9]. In Bangladesh, the manufacturers usually follow the traditional method for making yoghurt without understanding the importance of above factors, and they have no knowledge about the microbiology of starter culture. Therefore, manufacturers cannot control the quality of yoghurt, and hence quality varies from one batch to another as well as from one region to another. The objective of the study is to determine an economic, efficient and scientific technique for manufacturing safe and quality yoghurt under the best controlled conditions.

Materials and Methods

Sample Collection

Ten samples of five well-known varieties of freshly prepared yoghurt were collected from six different manufacturers locating in different places in Bangladesh namely, Krishna Kabin of Mymenshing (KKM), Akboria Sweets of Bogura (ASB), Bonoful Sweet of Rajshahi (BSR), Matri Bhander of Comilla (MBC), and Tangail Sweets of Tangail (TST). The consistency, colour, taste, flavor and pH of the samples were recorded at the time of sampling. After the organoleptic evaluation the two best curd varieties and the worst one were selected for our study. Samples were collected aseptically according to the standard procedures and transported to the Microbiology laboratory in an insulated box with ice [10].

Organoleptic Evaluation

For organoleptic evaluation of yoghurt varieties, a 'taste panel' consisting of 15 panelists was formed that included undergraduate, graduate students, teaching and non-teaching staff members of University of Dhaka, Dhaka, Bangladesh. Hundred percentage scale was used ranging from excellent (score = 100) to very poor (score = 0) as extremes [11]. They were given a study questionnaire concerning the organoleptic features of

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yoghurt. Sensory properties of yoghurt samples such as taste, flavor, color, and texture were recorded.

Physicochemical Analysis

The physicochemical properties such as surface tension, pH and lactose content of the selected yoghurt samples were analyzed according to the standard methods[12].

Quantitative Microbiological Assessment

The samples were processed immediately upon arrival using aseptic techniques. The total microbial load of the selected yoghurt samples were performed according to the standard conventional culture method[13]. Each sample was serially diluted in sterile distilled water. Aliquot of each dilution was cultured on Nutrient Agar (NA) for total bacteria, MacConkey Agar (MA) for coliforms, mFC agar for fecal coliforms, and Sabourad Dextrose Agar (SDA) for fungi, while deMan, Rogosa and Sharpe (MRS) agar was used for isolation of lactic acid bacteria (LAB) . The plates were kept in incubator at 37°C for 24 hours and then observed. Presumptive isolated lactic acid bacterial strains were obtained and sub cultured into the nutrient agar media followed by subsequent preservation in nutrient broth with 30% sterile glycerol at -20°C for further studies. Identification of presumptive lactic acid bacteria isolates was performed by using Gram staining, and different biochemical tests (i.e. Oxidase test, Catalase test, Indole test, Simmons's citrate test, Methyl Red (MR) test, Voges Proskauer (VP) test and various fermentation tests)[14].

Optimization of Temperature and starter culture concentration of the Selected Starter culture

The isolated lactic acid bacteria were inoculated into MRS broth and incubated at different temperatures (20, 25, 30, 37, 40, 45 and 50°C); and at different starter culture concentration (1, 2, 3, 4 and 5%).

Bench scale production of Quality Yoghurt to Identify Efficient Starter Culture

A bench scale trial for the production of yoghurt was performed [15]. For the preparation of starter culture, at first, 10 % skim milk was reconstituted in warm water and a homogeneous suspension was made. Then selected lactic acid bacterial isolates were combined and inoculated into that suspension. After incubating at 37°C for 18-24 hours, starter culture were examined for their ability of yoghurt production.

Result

Organoleptic Quality Assessment of Yoghurt Samples

For organoleptic evaluation of the yoghurt samples a 'test panel' consisted of 25 apparently healthy members included undergraduate, graduate students, teaching and non-teaching staff members of University of Dhaka, Dhaka, Bangladesh was made. Among the test varieties, organoleptically three most acceptable varieties (BSR01, ASB02, TST02) were found from Bogra, Rajshahi and Tangail while the worst one was from Comilla (Figure 1). The acceptable varieties were used for further study.

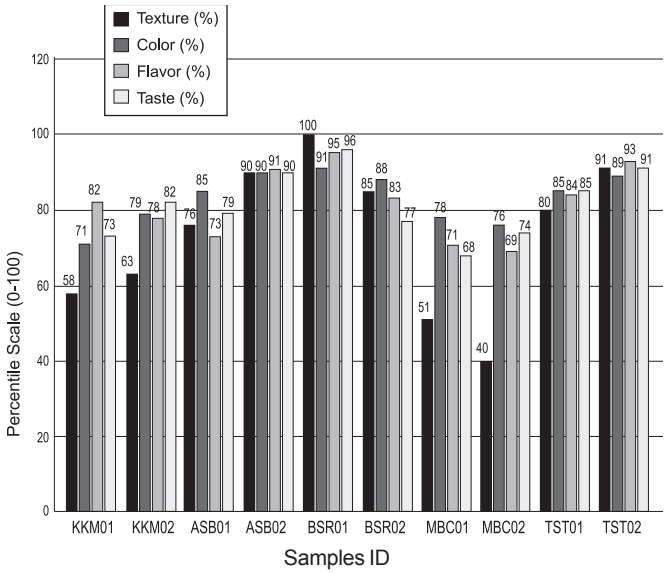

Figure 1. *Organoleptic Quality Assessment of the supplied Yoghurt Physicochemical Properties of Yoghurt Samples*

Yoghurt samples namely BSR01, ASB02, TST02 were found excellent in terms of physicochemical properties. All the recorded yoghurt samples were of high yoghurt tension except two samples from Rajshahi (Table 1). The pH range for traditional yoghurt was 3.8 to 5.3 with an average of 4.8. Lactose concentration varied between 2.0-3.5% (Table 1).

Table 1. *Physicochemical properties of the supplied Yoghurt samples*

Sample ID	Yoghurt Tension	pH	Lactose (%)
KKM01	Moderate	4.2	3.0
KKM 02	Moderate	4.3	2.9
ASB01	High	3.9	2.5
ASB02	High	4.5	1.85
BSR01	High	4.8	2.0
BSR02	High	3.9	2.1
MBC01	Low	3.6	3.5
MBC02	Low	3.9	3.4
TST01	High	4.0	2.5
TST02	High	4.6	1.9

Microbiological Quality Assessment

The total counts of viable heterotrophic bacteria were found in range between 2.9×10^4 and 4.0×10^5 cfu/g among the selected yoghurt samples. In addition, none of the samples were found positive for fecal coliform, and other food borne pathogens such as *E. coli*, *Salmonella* spp, *Staphylococcus* spp. and *Shigella*. However, two samples such as MBC01, MBC02 were positive in coliform test. In MRS agar medium, variable total counts of lactic acid bacteria among the various yoghurt samples were found (Figure 2).

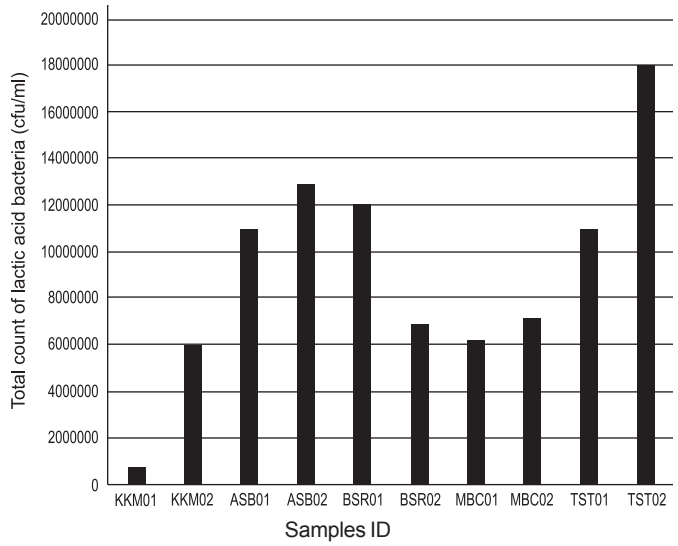

Figure 2. *The total counts of lactic acid bacteria from various yoghurt samples*

From Figure 2, it is clear that highest lactic acid bacterial count were found in BSR01, ASB02, TST02 samples, and assumptive lactic acid bacterial strains were isolated from these three samples. Based on the morphological and biochemical properties, assumptive four different strains of lactic acid bacteria were isolated namely, *L. bulgaricus, L. lactis, L. acidophilus* and *S. thermophilus* from ASB02, BSR0, TST02 samples. After organoleptic, physicochemical properties and microbiological quality assessment evaluation, three samples (ASB02, BSR0, TST02) were selected for further studies.

Screening of the Best Starter Culture

Four individual starter cultures namely St-1, St-2, St-3, St-4 were prepared using isolated lactic acid bacteria such as *L. bulgaricus, L. lactis, L. acidophilus* and *S. thermophilus,* respectively and six mixed starter cultures namely MC-1, MC-2, MC-3, MC-4, MC-5 and MC-6 were prepared by the mixture of St-1+ St-2, St-1+ St-3, St-1+ St-4, St-2+ St-3, St-1+ St-2+ St-3, St-1+ St-2+ St-3+ St-4, respectively.

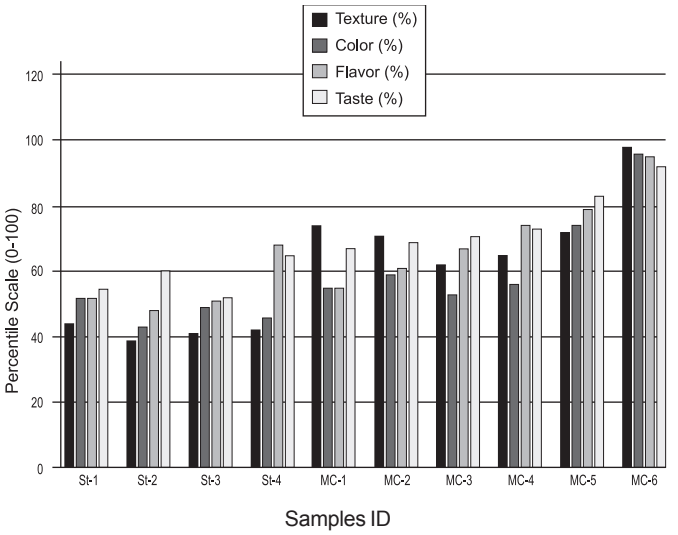

Figure 3. *Organoleptic properties of the newly produced yoghurts*

Fresh yoghurts were prepared by these different starter cultures, and the organoleptic, physicochemical properties of the newly produced yoghurts were evaluated by our selected expert test panelist (Figure 3 and Table 2).

Table 2. *Physicochemical properties of the newly prepared yoghurt*

Sample ID	Yoghurt Tension	pH	Lactose (%)
St-1	Low	3.2	3
St-2	Low	3.3	2.9
St-3	Low	3.9	2.5
St-4	Low	4.0	2.3
MC-1	Moderate	3.8	2.5
MC-2	Moderate	3.9	2.1
MC-3	Moderate	4.1	3.5
MC-4	Moderate	4.0	3.4
MC-5	High	4.0	2.5
MC-6	High	4.7	2.3

According to the test panel, it was found that yoghurt produced by MC-6 was excellent in terms of texture, color, flavor and taste. Based on the evaluation of organoleptic and physicochemical properties by the selected test panelists, it was clear that MC-6 was the best starter culture, and this culture was used in the subsequent yoghurt production in where different factors were optimized for the production of quality yoghurt.

Effect of Temperature in Yoghurt Production

Different quality yoghurts were produced at different incubation temperatures (Table 3). Best quality yoghurt was produced within a short period of time at 45°C. At low temperature, yoghurt formation period was usually lengthy, and quality of yoghurt was bad. For this experiment, fixed amounts of starter culture (5%) were mixed with pasteurized milk and then incubated at different temperature.

Effect of Starter Culture in Yoghurt Formation

Different concentrations of starter culture produce different quality yoghurt. Starter concentration also affect the incubation time and flavor of the final product.

In this experiment, about 1%, 2%, 3%, 4% , and 5% starter culture were used, and all samples were incubated at 45°C. It was found that best quality yoghurt was produced in 5% starter culture in a short time (Table 4).

Table 3. *Effect of temperature on the formation of yoghurt*

Temperature	Times after incubation						Quality of the final yoghurt
	2h	4h	6h	8h	12h	24h	
20°C	No change	No change	Thick layer produced	Thick layer produced	Layer become thicker	Yoghurt produced	Off flavor
25°C	No change	No change	Thick layer produced	Thick layer produced	Layer become thicker	Yoghurt produced	Moderate quality
30°C	No change	Thick layer produced	Thick layer produced	Layer become thicker	Yoghurt produced		Moderate quality
37°C	No change	Thick layer produced	Layer become thicker	Yoghurt produced			Good quality
40°C	No change	Thick layer produced	Layer become thicker	Yoghurt produced			Better quality
45°C	No change	Thick layer produced	Yoghurt produced				Best quality
50°C	No change	No change	No change	No change	No change	No change	

Table 4. *Effect of starter culture concentration on the formation of yoghurt*

Starter	Times after incubation						Quality of the final yoghurt
	2h	4h	6h	8h	12h	24h	
1%	No change	No change	No change	Thick layer produced	Layer become thicker	Yoghurt produced	Off flavor
2%	No change	No change	Thick layer produced	Thick layer produced	Layer become thicker	Yoghurt produced	Moderate quality
3%	No change	Thick layer produced	Thick layer produced	Layer become thicker	Yoghurt produced		Moderate quality
4%	No change	Thick layer produced	Layer become thicker	Yoghurt produced			Good quality
5%	No change	Thick layer produced	Layer become thicker	Yoghurt produced			Best quality

Discussion

As yoghurt is one of the most popular fermented milk products, usually served as dessert in Bangladesh, the focus of this study was to highlight the factors responsible for quality yoghurt production and to make the output of this study accessible to yoghurt producers to produce yoghurt scientifically and economically. For this purpose, ten traditional locally manufactured yoghurt samples were tested for organoleptic evaluation in terms of texture, color, flavor and taste. Three varieties namely, BSR01, ASB02, TST02 were found as highly acceptable by test panel. The physicochemical properties of these three yoghurt samples were also found excellent, and pH, lactose concentration were found in acceptable range. In addition, none of the samples were found to contain fecal coliform, and other food borne pathogens such as *E.coli*, *Salmonella* spp, *Staphylococcus* spp. *Shigella*. Conversely, Bhatt (1949) found that certain enteric pathogens were able to live for fairly long periods in yoghurt [16]. Among the tested yoghurt samples, highest lactic acid bacterial count was found in these three samples, and four different strains of lactic acid bacteria were isolated namely

L. bulgaricus, L. lactis, L. acidophilus and *S. thermophilus* from these samples. As these three samples were found excellent in various test by test panel, ten different starter cultures were made from isolated lactic bacterial strains of these samples. Consistently, it was published that all of these organisms are commonly found in the starter culture used for preparation of yoghurt [17].

Gupta *et al.,* 2000 described that the texture of the yogurt depends mainly upon the rate of development of the acidity, i.e. type of organisms present in the starter culture [18]. The wide variation in the quality parameter of yogurt can be attributed to the manufacturing conditions and type of organism used. Munzur *et al.,* 2004 described that the color of the yogurt depends on the color of milk or caramelized color obtained during heating of the milk or added coloring materials[19]. In this study, after evaluation of organoleptic and physicochemical properties by the selected test panelists, it was clear that MC-6 was the best starter culture, and this culture was used in the subsequent yoghurt production in where different factors were optimized for the production of quality yoghurt. It has been published in different studies that a mixed culture of *L. bulgaricus*, *L. lactis, S.lactis* and *S. thermophilus* was used for the production of good quality yoghurt [22,23]. In another study, it was reported that more acetaldehyde, the chief volatile flavor component of yogurt, is produced by *L. bulgaricus* when growing in association with *S. thermophilus* [24]. In a study, Rao and Dastur (1955) showed that good quality yoghurt should have a pH in the range of 4.6 to 5.0 [20]. Consistently, in our studies, pH was found 4.7 in newly prepared yoghurt produced by the starter culture MC-6. It was proved that lactose concentration varies between 2 and 3 in quality yoghurt[21]. Similarly, starter culture MC-6 produced yoghurt whose lactose concentration was 2.3. In addition, selected test panels scored the quality of the newly prepared yoghurt as excellent as the tension of this yoghurt was high. Best quality yoghurt was produced within a short period of time at 45°C. The similar results are reported by Anjum *et al*. (2007) and Wofschoon *et al*. (1983) [25,26]. At low temperature, yoghurt formation period was usually found lengthy, and quality was bad. For this experiment, fixed amounts of starter culture (5%) were mixed with pasteurized milk and then incubate at different temperature. 5% starter culture was found to produce best quality yoghurt.

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