

COMPARATIVE STUDY OF YOGHURT PREPARED BY USING LOCAL ISOLATED AND COMMERCIAL IMPORTED STARTER CULTURE

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Abstract

The present study was designed to evaluate the local isolated starter culture for the preparation of yoghurt. The yoghurt prepared from this starter culture was compared for various chemical and sensory characteristics with yoghurt prepared from commercial imported starter culture. Locally isolated starter culture and commercial imported culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in combination of 1:1 with dose levels of 1.50, 2.00, 2.50 and 3.00 %, were used for the preparation of yoghurt. These yoghurt samples were stored for 15 days in refrigeration and tested for chemical and sensory characteristics. Acidity of the both type of yoghurt samples was significantly affected by storage period and concentration of starter culture. Total solids decreased gradually during the storage in both types of sample. Lactose content of the samples manifested a decreasing trend but fat % did not change during storage. Yoghurt samples prepared from local isolated culture were found equally good as compared with yoghurts prepared from commercial imported starter cultures for their sensory attributes. Mix culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* may be prepared and used for yoghurt production in 1:1 ratio at the rate of 2.00-2.50 %.

Keywords: *L. bulgaricus* and *S. thermophilus*, starter culture, yoghurt

INTRODUCTION

The milk products that undergo extensive biochemical changes as a result of the action of microorganism are consumed in all parts of the world. Of these, yoghurt is so popular that it has assumed different forms e.g. stirred, set and frozen liquid yoghurt. The successful production of yoghurt depends upon the processing techniques i.e. correct selection of starter culture, heat treatment, inoculation and incubation temperature, preservation, handling and propagation of starter cultures that help to standardize and maintain uniformity in the quality of end product. The most common inoculating material used by the modern dairy plants is the culture comprising *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. These microorganisms grow together symbiotically and are

responsible for the production of good taste and aroma in yoghurt. The cultures are grown in milk in bulk starter vats and added to the basic mix to give a final concentration of 1-2% (v/v). An incubation temperature lies some where between 39 °C and 45 °C for the optimum acid production by the two species. In Pakistan, yoghurt is mainly prepared by traditional domestic and commercial methods. Yoghurt is prepared traditionally at home and by the shopkeepers. Under domestic and small-scale yoghurt production, traditional starter culture under uncontrolled conditions is used and this starter culture has low viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The time and temperature of incubation of inoculated milk vary from producer to producer. Yoghurt prepared by this procedure sometimes has high acidity which makes this product slightly sour and sometimes bitter. Though, it is cheaper but have short life with poor body characteristics and syneresis problem. Modern yoghurt manufacturing demands a high degree of aseptic mechanization. Commercial yoghurt is prepared by imported commercial starter culture/stock that is preserved in the form of liquid, as spray-dried, freeze-dried, and in frozen form. The decline in pH as a result of bacterial fermentation of lactose to lactic acid has a preservative effect on the product while at the same time the nutritional value and digestibility are improved [Bylund 1995]. Starter cultures are not developed on commercial basis in Pakistan and are imported from other countries. This situation has resulted into very high cost of the end product. In the last few years attempts have been made to improve the quality of yoghurt but this area needs to be further investigated. The present study was designed to optimize a combination of *L. bulgaricus* and *S. thermophilus* for the development of a starter culture to produce the yogurt of the same quality produced by using imported commercial starter culture.

MATERIALS AND METHODS

The present research was carried out at Institute of Food Science and Technology University of Agriculture, Faisalabad. Five different samples of local curd were collected from different areas of Faisalabad and brought to the Institute. Samples were prepared by serial dilution and were preserved at refrigeration temperature for isolation of bacteria. Special precautions were taken to sterilize all the glassware to be used in microbiological procedures. All media, Nutrient agar, Acetate agar, Rogosa agar, de Man, Rogosa and sharp agar (MRS) and broth, Neutral Red Chalk Lactose agar, β -disodium glycerophosphate agar, Yeast Lactose agar/broth used for microbial growth were prepared according to the methods of Cappuccino and Sherman [1996] and Harrigan and McCance [1976]. The pH of media was maintained by using N/10 NaOH and N/10 HCl.

Isolation of *Lactobacillus* and *Streptococcus* spp. from local sources involved the following steps:

- a) Preparation of normal saline
- b) Preparation of serial dilution
- c) Inoculation
- d) Incubation

The morphology of growth was observed according to the method as described by Malik [1995] and Awan and Rahman [2002].

- i. Preparation of slide smear for staining
- ii. Heat fixation
- iii. Gram's staining

Gram's staining was performed according to the procedure described by Malik [1995]. All the colonies, which appeared after 48 hours incubation on the nutrient agar plates, were specifically examined for morphological characteristics. The required colonies were shifted onto the surface of rogosa agar and acetate agar plates (for *Lactobacillus*) and on the surface of neutral red chalk lactose agar and β -disodium glycerophosphate agar plates (for *Streptococcus*) for specific culture isolates. All the purified culture isolates were processed for the identification of organisms up to the species level. Sugar fermentation test and biochemical tests were performed for identification of selected pure culture isolates according to the method as described by Malik [1995].

SUGAR FERMENTATION TEST

All the selected isolates were examined for their ability to induce fermentation of different sugars including monosaccharides (glucose, fructose and galactose) disaccharides (maltose, lactose and sucrose) and manitol by the method of Harrigan and MacCance [1976] and Cappuccino and Sherman [1996].

BIOCHEMICAL TESTS

Biochemical tests were performed according to method described by Malik [1995] and Cappuccino and Sherman [1996].

Activated culture of 18-24 hours of the culture /isolate in MRS and yeast lactose broth (for *Lactobacillus* and *Streptococcus* spp. respectively) were separately subjected to motility examination through hanging drop technique as described by Harrigan and McCance [1976].

TOTAL BACTERIAL COUNT OF PURE CULTURE

The count of culture was done by two methods as described by Awan and Rahman [2002] and Cappuccino and Sherman [1996] i.e. (i) Direct microscopic count (Bread Smear Method) (ii) Serial dilution agar plate procedure for quantitative viable cells. Pure culture of *Lactobacillus* and *Streptococcus* was preserved in the form of liquid culture under refrigeration (4°C).

Yoghurt was prepared by using the following combination and dose levels (Table 1).

Table 1: Combination of *L. bulgaricus* and *S. thermophilus* as local starter culture for the preparation of yoghurt.

Treatments	Dose level of starter culture (%)	Ratio 1:1
T1	1.50	0.75, 0.75
T2	2.00	1.00, 1.00
T3	2.50	1.25, 1.25
T4	3.00	1.50, 1.50

Yoghurt was also prepared with commercial starter culture obtained from Local dairy by the same method as described above. The commercial starter culture consisted of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in 1:1 and dose level was kept exactly the same as in case of locally isolated culture.

Samples were analyzed for their chemical properties like pH, acidity, total solids, lactose and fat content by the method of AOAC (1990). Samples were subjected to sensory evaluation in order to check overall acceptability of yoghurt. Yoghurt samples were evaluated for texture, flavour, taste and overall acceptability by a panel of five judges using 9-point hedonic scale as described by Larmond [1977].

STATISTICAL ANALYSIS

Data thus obtained were subjected to statistical analysis by complete randomized design (2- Factor Factorial) and comparison of means was done by Duncan's Multiple Range Test [Steel *et al.*, 1996]

RESULTS AND DISCUSSION

The results pertaining to the effect of storage on pH under various starter culture treatments are shown in Table 2. A gradual and consistent decrease was noted in pH of yoghurt samples prepared by using locally isolated and commercial imported culture with respect to their concentration but culture type seemed not affecting pH of the samples significantly. Tamime and Robinson [1985] reported the reason for decrease in pH as a function of acidity which increased due to conversion of lactose into lactic acid during storage period. The results obtained are concordant with the findings of Wolfschoon [1983], and Masood [1997] who reported a decrease in pH values of yoghurt during storage. Acidity of the yoghurt samples from both types of cultures revealed that storage and concentration of the culture have highly significant effect on the acidity whereas their interactive effect was found to be non significant on acidity. The culture type did not show significant difference for acidity (Table 2). Increase in acidity was due to the formation of lactic acid produced by lactic acid bacteria present in yoghurt during storage period. O' Neil *et al.* [1979] and Shin *et al.* [1991] reported significant increase in acidity during storage of yoghurt. The effect of storage on total solids under various starter culture treatments is shown in Table 2. It is evident from the results that reduction in total solids throughout storage period might be due to change of lactose into lactic acid by lactose fermenting bacteria in yoghurt. O' Neil *et al.* [1979] also observed variation in total solids, acidity and fat % among different plain yoghurt samples. Treatments and storage period had significant effect on the total solids % of yoghurt samples prepared by locally isolated starter culture and commercially imported starter culture. However, interactive effect on total solid % remained non significant. These results are in line with the findings of Tamime and Robinson [1985] and Verman and Sutherland [1994]. Significant difference was observed for lactose content in all four treatments with different levels of starter culture (1.50, 2.00, 2.50 and 3.00 %), Lactose content decreased as dose of starter culture and storage period increased. The comparative study of four treatment groups showed that the final lactose content of yoghurt manufactured with 1.50% starter culture (T1) was higher followed by 2.00% starter culture (T2) and minimum lactose was found in treatment T4 (3.00% starter culture). The statistical results regarding the lactose content indicated that there was a significant variation among all the samples of yoghurt tested for lactose content. Goodenought and Kleyn [1976] reported that the decrease in lactose content during storage is due to production of lactic acid. The fat content of yoghurt however, displayed statistically non significant

difference for treatment, storage and culture type as shown in Table 2. Non significant reduction in fat content at the end of storage period might be due to production of volatile fatty acids by yoghurt organisms. Tamime and Robinson [1985] reported that yoghurt organisms possess very weak lipolytic actions and the volatile acids in yoghurt are mostly derived from the hydrolysis of other components.

Table: 2 Chemical characteristics of yoghurt prepared by using different starter culture after storage of 15 days in refrigeration.

Chemical Characteristic	Culture Type	T1	T2	T3	T4
pH Value	LISC	04.44 ^a	04.39 ^b	04.34 ^c	04.32 ^d
	CISC	04.42 ^a	04.36 ^b	04.30 ^c	04.27 ^d
Acidity (%)	LISC	00.83 ^d	00.86 ^c	00.90 ^b	00.93 ^a
	CISC	00.85 ^d	00.90 ^c	00.94 ^b	00.96 ^a
Total Solid (%)	LISC	16.68 ^a	16.62 ^b	16.54 ^c	16.49 ^d
	CISC	16.64 ^a	16.59 ^b	16.53 ^c	16.49 ^d
Lactose (%)	LISC	02.95 ^a	02.88 ^b	02.81 ^c	02.74 ^d
	CISC	02.88 ^a	02.85 ^b	02.79 ^c	02.74 ^d
Fat (%)	LISC	03.48 ^a	03.48 ^a	03.46 ^a	03.46 ^a
	CISC	03.49 ^a	03.48 ^a	03.47 ^a	03.46 ^a

The mean values followed by the same letter in the column are non significantly different ($p < 0.05$)

LISC: Locally isolated starter culture

CISC: Commercially imported starter culture

T1= 1.50% starter culture

T2=2.00% starter culture

T3=2.50% starter culture

T4= 3.00% starter culture

SENSORY EVALUATION

The data relating to the effect of different treatments on the texture of yoghurt is shown in Table 3. Highest texture scores were recorded for yoghurt samples prepared with 2.00 and 2.50 % starter culture in both types of yoghurts and these scores were significantly different from yoghurt samples of 1.50 and 3.00 % starter culture preparations. The judges recorded similar level of scores for both type of yoghurts for flavour and taste but the concentration of 2.00 and 2.50 % showed higher acceptability for these sensory characteristics (Table 3). The results coincide with the findings of Shin *et al.* [1991]. Culture type however did not affect the sensory characteristics of the yoghurts. The results are in concordant with the findings of Chawala and Balachandra [1993]. Overall acceptability was found to non-significantly different for T1 & T4 and T2 & T3. The results are in concordant with that of Gupta and Prasad [1989] and Tamime and Robinson [1985] who used starter culture at the rate of 3.00 % and 2.50% respectively.

CONCLUSION

In the light of the findings of this research endeavor, it can be concluded that locally isolated bacterial culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for commercial production of yoghurt are equally effective as far as the chemical and sensory characteristics of the product are concerned.

Table 3: Sensory characteristics of yoghurt prepared by using different starter culture after storage of 15 days in refrigeration.

Sensory Characteristic	Culture Type	T1	T2	T3	T4
Texture	LISC	6.50 ^b	7.20 ^a	7.30 ^a	6.40 ^b
	CISC	6.80 ^{bc}	7.30 ^{ab}	7.50 ^a	6.50 ^c
Flavour	LISC	6.00 ^c	7.20 ^{ab}	7.40 ^a	6.50 ^b
	CISC	6.00 ^c	7.40 ^{ab}	7.70 ^a	6.60 ^b
Taste	LISC	6.70 ^b	7.20 ^{ab}	7.40 ^a	6.80 ^b
	CISC	6.90 ^b	7.40 ^{ab}	7.60 ^a	7.00 ^b
Acceptability	LISC	6.40 ^c	7.20 ^{ab}	7.40 ^a	6.60 ^{bc}
	CISC	6.60 ^c	7.40 ^{ab}	7.60 ^a	7.00 ^{bc}

The mean values followed by the same letter in the column are non significantly different ($p < 0.05$)

LISC: Locally isolated starter culture

CISC : Commercially imported starter culture

T1= 1.50% starter culture

T2=2.00% starter culture

T3=2.50% starter culture

T4= 3.00% starter culture

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