

Microbiological Analysis of Mixed & Plain Ice Cream Samples Sold in Local Markets of Allahabad

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ABSTRACT

A total of thirty ice cream samples were collected from local market of Allahabad. The samples included two varieties, based on their flavours they were categorized in plain and mixed. Fifteen samples each from local manufacturers and brands were microbiologically examined. Dilutions of 10^{-3} and 10^{-5} for plain and mixed samples respectively were inoculated in Nutrient Agar plates for aerobic mesophilic count. The average aerobic mesophilic count was 2.0 cfu/g for the branded samples with a maximum bacterial growth of 10 cfu/g in the mixed ice cream samples. These results for locally manufactured ice cream samples were reported as, an average bacterial growth of 14.12 cfu/g with a maximum contamination of 33 cfu/g. Mac-Conkey's single and double strength broths were used for TCC and FCC. Average TCC for both flavours of industrially produced ice creams was 0.21 cfu/100ml, with average FCC of 0.09 cfu/100ml for mixed and 0.04 cfu/100ml for plain samples. Local samples gave a varied result with average TCC as 0.60 cfu/100ml for plain and 3.11/100ml for flavoured and average FCC was reported as 0.23 cfu/100ml for mixed samples and 2.50 cfu/100ml for plain samples. 20% out of total 15 plain samples of local ice cream were reported positive for the presence of *E. coli*. Thus good hygienic practice improves the hygienic quality of ice cream especially in all steps, post pasteurization and at the retail level.

Keywords: Ice cream, microbiological examination, MPN, TCC, FCC, *E. coli*.

INTRODUCTION

Ice cream, a milk-based product, is a good media for microbial growth due to high nutrient value, almost neutral pH value (~pH 6–7), and long storage period of ice cream. It is produced by freezing pasteurized mixture of milk, cream, and milk solids other than fat, sugars, emulsifiers, and stabilizers. Other ingredients include flavoring matters and water. Fruits, nuts, candies and syrups are optionally added into ice cream for flavor enrichment. It is sold in packages or in open containers at retail outlets/ice creams parlors, the open variety being distributed manually in scoops, cones, or sundaes across the counter^{6,8,12,18}. Quality of ice cream depends on extrinsic factors that include manufacture procedure, as well as intrinsic factors that include the proportion of ingredients used. Primary sources of microbial contamination of ice cream include water and raw milk, whereas secondary sources include flavoring agents, utensils and handling. Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream-mix, such as milk and milk powder, cream, flavouring and colouring substances and sanitizer^{16,2} and from contaminate air during processing⁹. Ice cream is one of the major products of the dairy industry and continues to dominate the interest of large segments of the population⁷. As most of the ice cream consumers are children of vulnerable age groups, it is required to be microbiologically safe¹⁸.

Therefore keeping in view the above facts, the present study entitled "Microbiological analysis of mixed and plain ice cream samples sold in local markets of Allahabad" was carried out with the following objectives.

1. To assess the quality of mixed and plain ice cream sample for total mesophilic bacterial count.
2. To detect the presence of total coliforms and fecal coliforms.
3. To identify the faecal coliforms present.

MATERIALS AND METHODS

Place of work

The present study entitled “Microbiological analysis of mixed and plain ice cream samples” was carried out in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-university), Allahabad.

Study sample

A total of 30 ice cream samples, 15 each for mixed and plain samples were collected from the local market of Allahabad.

Collection of samples

Packed ice cream samples purchased was aseptically stored in ice-bags and brought to the laboratory. The samples were kept at refrigeration temperature till further processing.

Bacteriological analysis

Total aerobic mesophilic bacterial count

10 gm of ice cream sample was taken aseptically in sterilized sample bottle and tempered in water bath at 40°C. 1 ml of ice cream sample was transferred to 9 ml of ringer’s solution and then subsequent serial dilutions upto 10^{-3} for plain and 10^{-5} for mixed sample were made. 1 ml from the dilution 10^{-3} for plain and 10^{-5} for mixed was poured in sterile petridish. To this cool and melted nutrient agar media was poured. The plates were further incubated at 37°C for 24-48 hours.

Total coliform count and fecal coliform count

For presumptive coliform test, single strength Mac-Conkey Broth in 10 and 5 ml volumes were used. To each of 5 tubes containing 10 ml broth with durhams tube, 10 ml of sample was inoculated. To each of 5 tubes containing 5 ml broth with durhams tubes, 1 ml sample was added and to the last 5 tubes containing 5 ml broth along with durhams tubes, 0.1 ml of sample was inoculated. The set of 15 inoculated test tubes were incubated at 37°C for 24-48 hours. The tubes were examined for acid and gas production. For confirmed coliform test the positive presumptive tubes were used. Other set of test tubes containing double strength Mac-Conkey Broth in 5 ml volumes were used. The double strength tubes were inoculated with 1 ml volumes from positive presumptive tubes. Again the test tubes were incubated for further 24-48 hours and examined for acid and gas production. For the completed test, the samples from positive double strength Mac-Conkey Broth were streaked onto Eosin-Methylene Blue agar media.

Identification of fecal coliforms

The following biochemical tests were done as prescribed in the Bergey’s Manual of Determinative Bacteriology.

Indole Test

This test was performed to find out which of the organism is able to oxidize trptophan into indole, pyruvic acid and ammonia. The isolated organism was inoculated into tryptone borth. The inoculated and control tubes were incubated at 37°C for 24-48 hours. After incubation, Kovac’s reagent was added to inoculate and control tubes. Development of cherry red colour at the top layer in the form of ring indicates positive test while absence of ring formation indicates negative test.

Methyl-Red Test

1 ml of inoculum was inoculated in test tubes containing MR-VP broth perform this test. The tube was incubated at 37°C for 24-48 hours. After incubation 5 drops of methyl red indicator was added to the tubes. Appearance of red colour was assigned as methyl red positive whereas colour change to yellow was assigned as negative test.

Voges-Proskauer Test

This test was performing to determine the capability of microorganisms to produce non acidic products such as ethanol and acetoin (acetyl methyl carbinol) from the organic acid.

The isolated microorganisms were inoculated in VP broth. All the inoculated control tubes were incubated at 37°C for 24-48 hours. After incubation, 12 drops of freshly prepared VP reagent I (naphthol solution), 2-3 drops of VP reagent II (40% KOH) was added to all the inoculated and control tubes. Development of crimson to pink (red) colour may be most intense at the surface, which indicates positive test while no change in colour indicates negative test.

Citrate Utilization Test

Citrate test was performed to determine the ability of microorganisms to utilize citrate as carbon source. The utilization of citrate depends on the presence of an enzyme citrase that break down citrate to oxalo acetic acid and acetic acid. The isolated microorganism was inoculated in Simmon's citrate agar slant and incubated at 37°C for 24-48 hours. After incubation, tubes was examined, change in colouration of slant from green to blue indicate positive test for citrate utilization.

Statistical Analysis

The data obtained were subjected to statistical analysis for which "t-value" of the investigated readings was calculated. This was done to determine whether the observations of the sampling results differ significantly from the expected result¹⁵.

RESULTS AND DISCUSSION

Total aerobic mesophilic bacterial count

Mixed samples

The total aerobic mesophilic bacterial count in branded and local sample investigated is listed in table 4.1. The branded samples (Fig: 4.1) were found to be within the limits of BIS standards³ (2.0×10^5 cfu/gm) indicating its superior quality. However the local samples of mixed ice cream showed the heavy contamination ranging more than 2.5×10^5 cfu/gm. The mean results indicate that the highest contamination was found in local sample 14.12×10^5 cfu/gm (exceeding the limit 2.5×10^5 cfu/gm)⁴.

Fig.4.1:- Aerobic mesophilic count in mixed Sample of branded ice cream

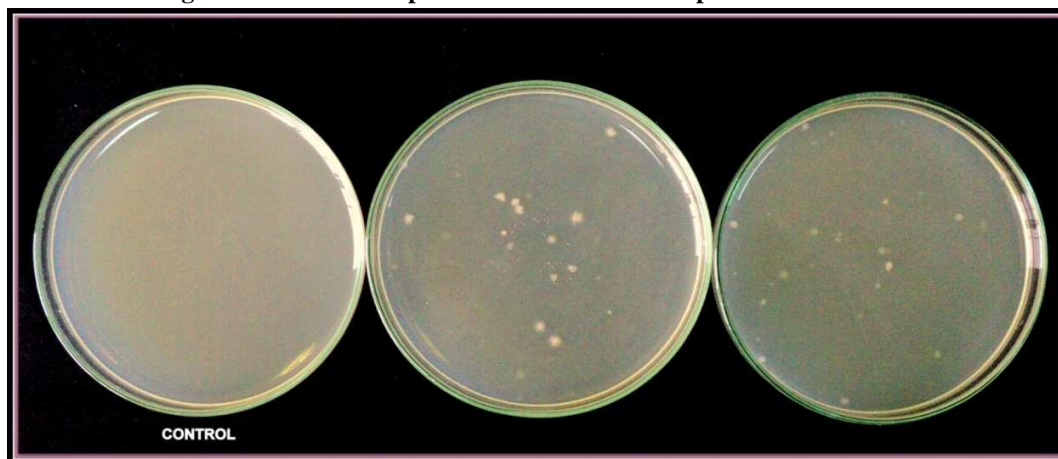


Table 4.1:- Aerobic mesophilic bacterial count of mixed samples.

Samples	Total no. of samples	Branded (10^5 cfu/g)		Local (10^5 cfu/g)	
		Range	Mean	Range	Mean
Mixed	15	(10 – 0)	2.0	(33 – 0)	14.12

t value = 1.38

Plain samples

Table 4.2 enlists the readings of total aerobic mesophilic bacterial count for plain ice cream samples. This was in accordance with the results for mixed samples. The branded samples showing superior quality

gave mean bacterial count of 35.12×10^3 cfu/g which was within the limits of standard, BIS³. The results for the local samples (Fig: 4.2) gave mean bacterial count of 273.45×10^3 which was higher than the readings of branded samples and much higher than specification, indicating inferior quality.

Fig.4.2:- Aerobic mesophilic count in plain sample of local ice cream

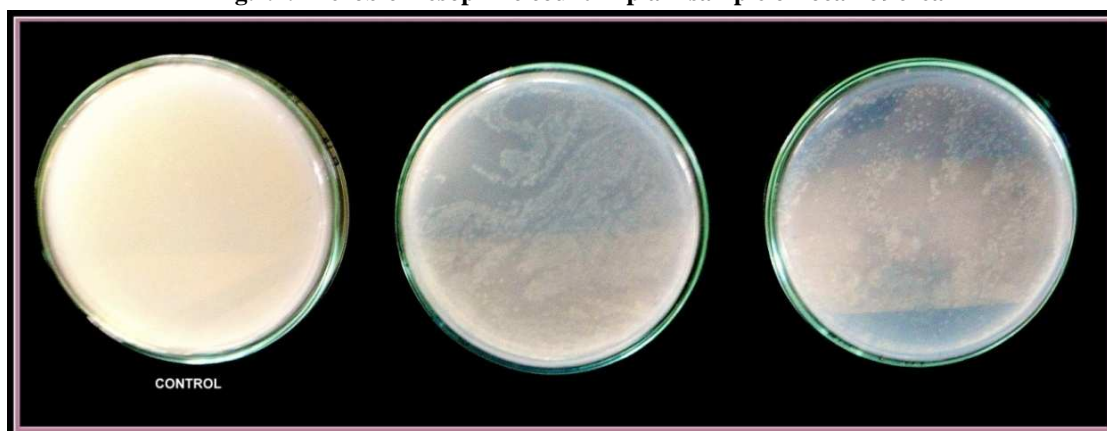


Table 4.2:- Aerobic mesophilic count of plain samples

Samples	Total no. of samples	Branded (10^3 cfu/g)		Local (10^3 cfu/g)	
		Range	Mean	Range	Mean
Plain	15	(105 – 9.5)	35.12	(600 – 2)	273.45

t value: - 1.99

In the present study the microbiological load of the industrially produced and packed ice cream samples was lower as compared to the local preparation (Fig: 4.3 & 4.4), which is in accordance with the findings of Boston and Akin^{5,11}.

Fig.4.3:- Aerobic mesophilic bacterial count of mixed samples

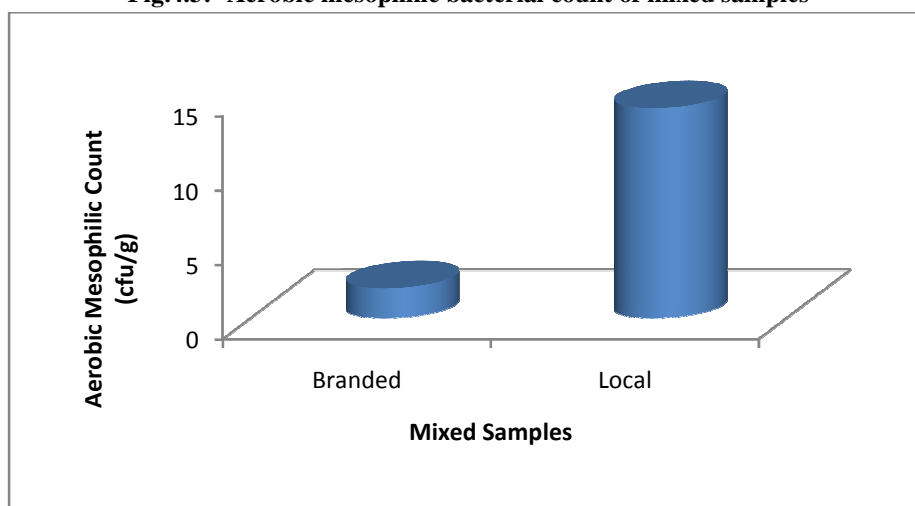
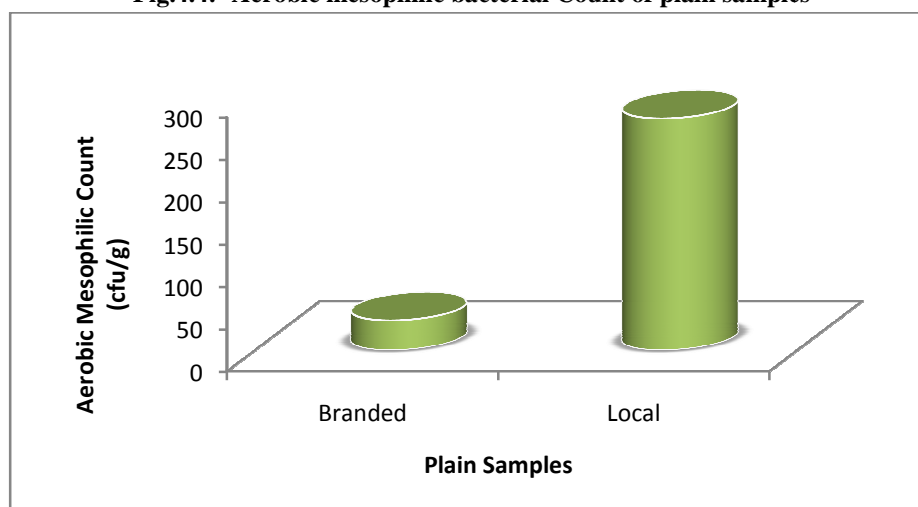


Fig.4.4:- Aerobic mesophilic bacterial Count of plain samples

Microbial quality examined in our investigation more or less comparable with the results of other co-works indicating high contamination in local samples, because of added ingredients increase the possibilities of microbial contamination and poor handling of retailers^{11,14,13}.

Total coliform count and total fecal coliform count

The observations for total coliform count as well as for total fecal count are enlisted in table 4.3. The reading indicates difference in total coliform count and total fecal count between branded and local samples. Total coliform count for plain samples of local and industrially produced ice creams was found in the range of (0.01 to 24.00) cfu/100ml, and (0.02 to 0.63) cfu/100ml respectively. Similarly total fecal count range varies between (0.01 to 0.07) cfu/100ml and (0.01 to 24.00) cfu/100ml for branded and local samples (Fig: 4.5).

Mixed samples were also in accordance with the above results giving range of total coliform count between (0.02 to 0.79) cfu/100ml for branded samples and (0.05 to 1.4) cfu/100 ml for local samples. Total fecal count for mixed samples of branded and local are in the range of (0.01 to 0.49) cfu/100ml and (0.05 to 0.09) cfu/100ml respectively (Fig: 4.6)

Table 4.3:- Microbiological quality of ice cream sample with respect to TCC & FCC

Type of sample	Total coliform count (cfu/100ml)				Total fecal count (cfu/100ml)			
	Branded		Local		Branded		Local	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Plain	(0.63 – 0.02)	0.21	(24 – 0.01)	3.11	(0.07 – 0.01)	0.04	(24 – 0.01)	2.50
Mixed	(0.79 - 0.02)	0.21	(1.4 - 0.05)	0.60	(0.49 – 0.01)	0.09	(0.09 - 0.05)	0.23

Fig.4.5:- Mean coliform count of plain samples

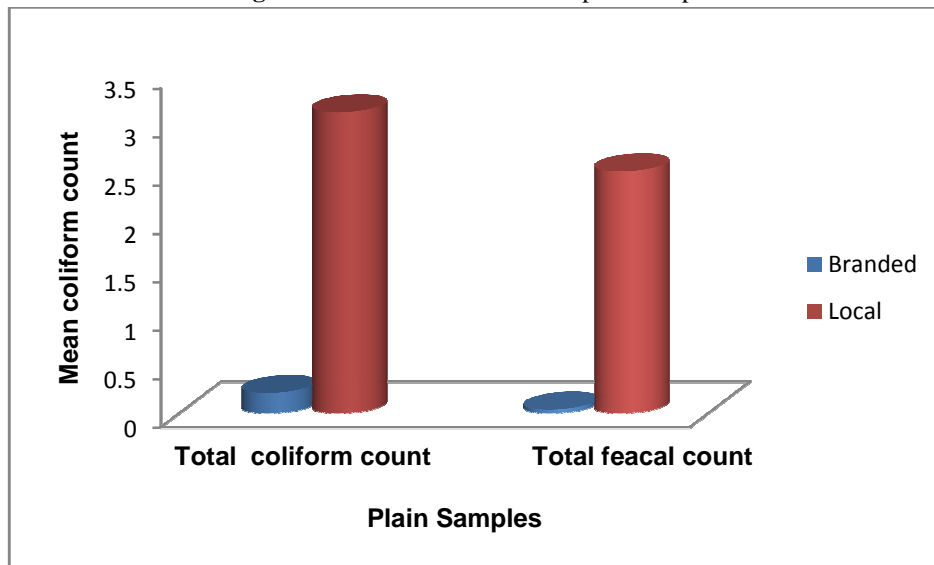
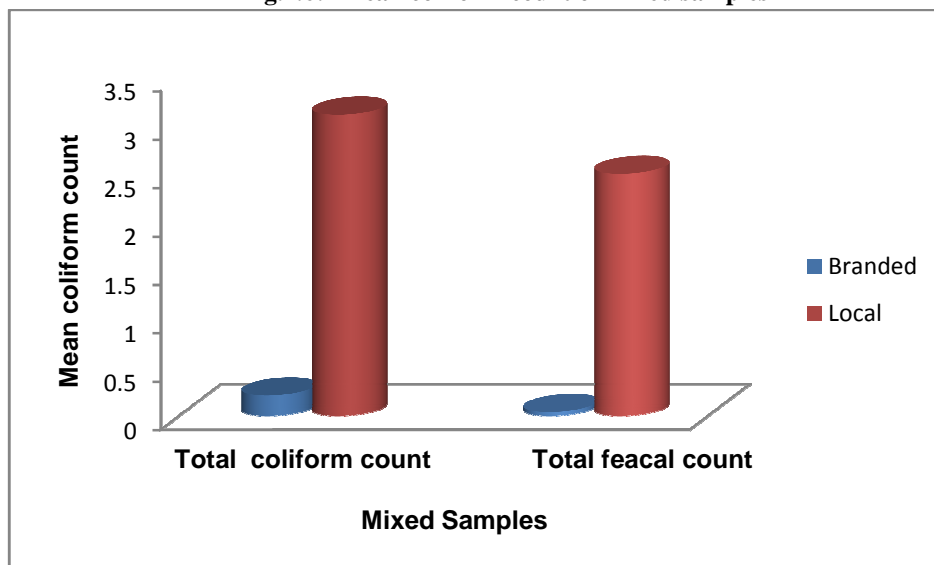


Fig.4.6:- Mean coliform count of mixed samples



So, on the basis of above observation that we got from the MPN method of examination (Fig: 4.7 & 4.8) for determining the total coliform count (TCC) and the faecal Coliform count (FCC) found more in local preparation as compared to industrially produced & packed ice cream samples.

Fig.4.7:- TCC (10⁵) in plain sample of local ice cream

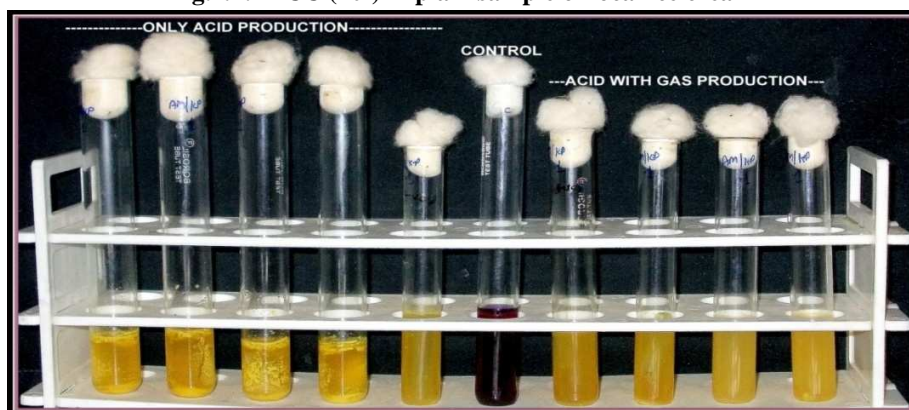
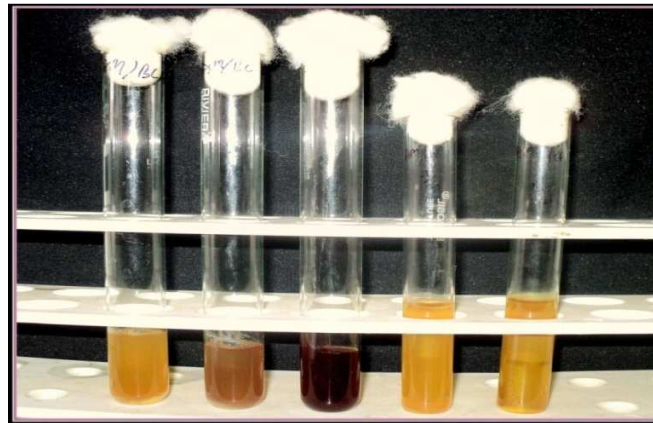


Fig.4.8:- FCC (10^5) in plain sample of local ice cream

Further the confirmatory test proved the presence of *E. coli* in 3 plain samples of local ice cream by the existence of metallic sheen on the EMB plates (Fig: 4.9). Coliforms being non spore formers should be susceptible to pasteurization. Their post-pasteurization presence in ice cream may be due to faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The level of presence of these organisms in food has been described as index of food hygiene^{10,17}.

Fig.4.9:- Confirmed test on EMB-plate with metallic sheen

The presence of high level faecal coliform contamination represents a public health risk due to the possible presence and transmission of pathogens via faecal route and a common houseflies¹.

Identification of coliforms

On the basis of IMViC test (Fig: 4.10), isolates of 3 local samples confirmed positive results, which revealed that the isolates were of pure culture of *E. coli*. (Table 4.4).

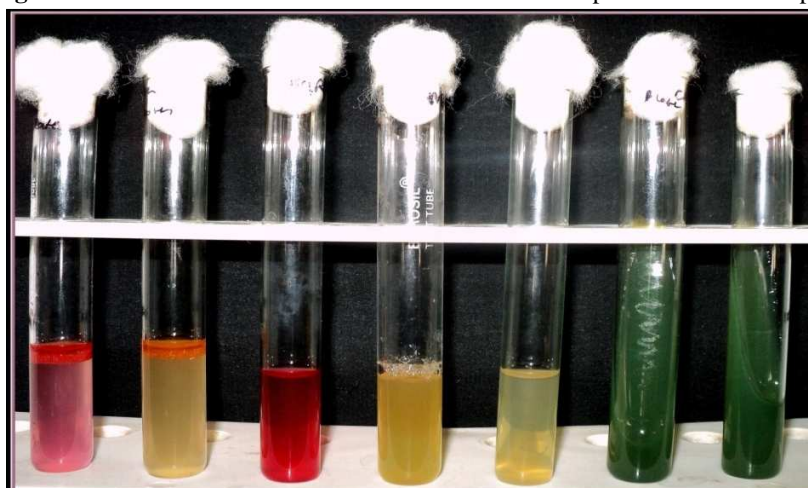
Fig.4.10:- IMViC Test for isolated *Escherichia coli* from plain ice cream sample

Table 4.4:- Biochemical identification of *Escherichia coli* isolated from plain ice cream sample

Type of sample	No. of samples	Biochemical test			
		Indole production	MR reaction	VP reaction	Citrate use
Plain	3				
(n = 15)	(20%)	+	+	-	-

CONCLUSION

The present study revealed that in comparison to overall microbial quality of local (mixed) ice cream samples that of with branded (mixed) sample was poorer. The counts of microorganisms above the recommended criteria and the presence of some groups of pathogenic bacteria may pose a risk for public health particularly for children and vulnerable elderly people. It is clear from the previous and present study that there is a necessity for developing the hygienic status of locally produced ice cream in domestic or catering premises. Thus, good hygiene practices should improve the hygienic quality of ice cream especially in all steps, post pasteurization and at retail level. An increased level of staff training and transfer of knowledge, especially in relation to food safety, handling maintaining and cleaning of machine & tools may improve the situation. The mandatory adoption of a food safety management system based on HACCP should improve the quality of ice cream. The quality of the raw material prior to process, manufacturing and storage of the products under appropriate conditions should be given a priority and also in order to prevent the infections & toxification resulting from pathogenic microorganisms. Workers and sales people in both ice cream producing, catering premises should be regularly checked for sustaining the favourable hygienic status.

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