MICROBIAL QUALITY ASSURANCE OF CURD DURING PRODUCTION AND STORAGE

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Thesis submitted in partial fulfilment of the
requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **"MICROBIAL QUALITY ASSURANCE OF CURD DURING PRODUCTION AND STORAGE"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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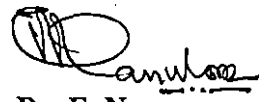
CERTIFICATE

Certified that the thesis entitled **"MICROBIAL QUALITY ASSURANCE OF CURD DURING PRODUCTION AND STORAGE"** is a record of research work done independently by Dr. Praseeda R., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Praseeda R.

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Introduction

1. INTRODUCTION

Milk is considered as a nutritious and balanced food for a mammal and has always been the first food of the newborn. At some stage in the course of human evolution, it was recognized that the milk of other animals was equally satisfying in meeting biological demands of man. Fresh milk turns sour on keeping and this property has been utilized by man for the preparation of countless palatable forms of fermented milk products, which constitute a vital component of the human diet in many regions of the world.

Curd is an Indian fermented milk product known for its refreshing taste, palatability and therapeutic values. From a biological stand point, fermented milk products are characterized by the accumulation of microbial metabolic products such as lactic acid, ethyl alcohol and a variety of other chemicals called flavour substances. Besides imparting nutrition, the fermented milk products help preserve the precious fluid milk which otherwise would deteriorate rapidly under high ambient temperatures of storage.

In the preparation of curd, milk is heated near to boiling, cooled to room temperature and inoculated with curd starter, usually the left over from the previous day's stock and incubated undisturbed at ambient temperature for four to six hours until it acquires a thick consistency. Curd is generally consumed in its original form as an accompaniment to the meal or it may be turned into a sweet or savoury lassi drink or as a dessert containing sugar and slices of fresh fruits.

In India, curd is largely made at home from mixed cultures set under varying time temperature combinations. Flavour and texture of curd may vary from house to house. However, lately, many dairy plants have standardised curd production using controlled culture composition under well-defined conditions of time and temperature of incubation.

India has emerged as the highest milk producing country in the world. Since independence, milk production had increased more than a five fold from a mere 17 in 1950-51 to 88.1 million tonnes in 2003-04. Out of 84.6 million tonnes of milk produced in India during the year 2001, 27.5 per cent was converted into ghee, 6.5 per cent to makhan, 6.9 per cent to dahi, 6.5 per cent to khoa and condensed milk, 37 per cent to milk powder, 1.9 per cent to paneer, channa and cheese, 0.6 per cent to ice cream and kulfi, 0.2 per cent to cream and 0.5 per cent to other products (Gupta, 2001).

Milk and milk products are highly perishable commodities and their shelf life is influenced by the initial microbial load. Curd is one of the most popular milk products and its quality is influenced by various factors such as type of milk, initial microbial quality of raw milk, proper maintenance of temperature during the heat treatment, proper adherence to hygienic practices during post heat treatment handling and maintenance of cold chain during its transportation and storage. During the manufacture of curd and its subsequent handling, various types of microbial contaminants gain entry from different sources such as milk, starter culture, water, air, utensils, personnel and others. Being a product which is consumed as such, the health hazards originating from consumption of contaminated curd are of immense public health significance.

A longitudinal study on the microbial quality of curd at various stages of production, processing, packaging and refrigerated storage provides an insight into the microbial contamination of the product during its production and also organoleptic changes during storage. The study also gives valuable information on the presence of certain bacterial pathogens. The compiled information will help the producers to get an idea about the major sources of contamination and to follow appropriate steps at various stages of production to reduce the microbial contamination and to prevent the changes in organoleptic quality during refrigerated storage of curd. This in turn will help the retailers in preventing economic loss due to product spoilage.

Considering the above facts, the present study was undertaken to assess the

I. Microbial quality of curd produced in the Dairy Plant and the effect of refrigeration on microbes during storage of curd by

1. Estimating the level of

a) Total viable count/g
b) Psychrotrophic count/g
c) Coliform count/g
d) Faecal streptococcal count/g
e) Yeast and mould count/g

and by detecting the presence of

a) *Escherichia coli*
b) *Staphylococcus aureus*
c) Salmonellae
d) *Pseudomonas aeruginosa*
e) *Bacillus* species

2) Evaluating the organoleptic qualities by detecting changes in

a) Flavour
b) Body and texture
c) Colour and appearance
d) Product acidity

3) Estimating the titratable acidity and pH

II. Microbial quality of four brands of curd retailed in Thrissur Corporation

III. Critical control points of microbial contamination of curd during its production at the Dairy Plant.

Review of Literature

2. REVIEW OF LITERATURE

2.1 MICROBIAL COUNTS

2.1.1 Total Viable Count

Sheikh *et al.* (1970) analysed 18 to 24 h old samples of dahi collected from six shops at Dacca. The total viable count of the samples varied from 22 x 10^6 to 365 x 10^6 organisms/ml.

Kahlon and Grover (1984) determined the bacteriological quality of 28 samples of dahi collected from halwais, vendors, dhabas, hotels, hostel messes and canteens of Punjab Agricultural University campus and of Ludhiana city. They reported that the samples had a mean total viable count of 9 x 10^5/g and the count ranged between 1 x 10^5 and 2.7 x 10^6/g.

Mohanan *et al.* (1984) analysed the microbiological quality of 60 samples of dahi prepared under household conditions at Bangalore. The study revealed that the samples had a mean total viable count of 246 x 10^6/ml and the count ranged between 5.6 x $10^7$and 5 x 10^8 cfu/ml.

Bacteriological quality of 100 samples of yoghurt made from ewe's milk obtained from local Mosul markets in Iraq was investigated by Khalaf and Shareef (1985) and reported that the samples had a mean total aerobic plate count of 9500/g.

Jayaram and Gandhi (1987) evaluated the microbiological quality of Dahi obtained from hotels, houses and an organized dairy. The standard plate count of the samples from the hotels ranged between 20 x 10^5 and 230 x 10^5/ml and the count of the samples from the houses ranged from 41 x 10^5 to 880 x 10^5/ml. The count of the samples obtained from the organised dairy was 6 x 10^5/ml.

Rajmany *et al.* (1989) evaluated the bacterial quality of 20 samples each of raw milk, khoa, curd, ice-cream, sweetened condensed milk, milk powder and processed cheese collected from local market of Udaipur city. They reported that the curd samples had a mean total viable count of 95.5 x 10^5 cfu/g and the count ranged between 25 x 10^5 and 196 x 10^5 cfu/g.

Bankole and Okagbue (1992) analysed the total aerobic bacterial count of 100 samples of Nono, a Nigerian cultured milk food traditionally produced by the Fulani tribe and reported that the samples had a mean count of 1.6 x 10^9 viable units/g.

Laye *et al.* (1993) evaluated the chemical and microbiological properties of plain nonfat yoghurt obtained from three sources, *viz.* A, B and C on day 2, 6 and 12 of refrigerated storage. The samples belonging to the sources A, B and C had a mean count of 7.9 x 10^9, 2.3 x 10^9 and 2.68 x 10^9 cfu/g respectively on day 2 of storage, whereas the count of the samples from the above sources on day 6 of storage were 7.4 x 10^9, 2.1 x 10^9 and 2.2 x 10^9 cfu/g, respectively. The corresponding count of the samples on day 12 of storage was 6.6 x 10^9, 2.02 x 10^9 and 1.9 x 10^9cfu/g.

Misra *et al.* (1993) evaluated the microbial quality of 50 curd samples collected from in and around Bhubaneswar. The study revealed that the samples had a mean total viable count of 12 x 10^6cfu/g and the count in the samples ranged between 6 x 10^6/g and 18 x 10^6cfu/g.

Mutukumira (1995) examined the chemical, microbiological and sensory properties of 10 batches of cultured milk, Amasi produced by small holder dairy farmers in Zimbabwe. Total viable counts of the samples ranged from 8.47 to 9.98 log cfu/g.

Samolada *et al.* (1998) studied the changes in the microbial flora of traditional fermented milk made from raw ewe's milk during the manufacture and storage at room temperature for five days. The mean total aerobic count of raw

milk was 5.7 log cfu/ml. The mean count of fermented milk on day one of storage was 7.19 log cfu/ml, which increased to 8.51 log cfu/ml on fifth day of storage.

Abdelgadir *et al.* (2001) determined the microbial quality of Sudanese fermented dairy product, Rob prepared from seven production sites in Khartoum. The mean aerobic mesophilic count of the milk samples collected in winter and summer was 6.65 $\log_{10}$ cfu/ml and 7.83 $\log_{10}$ cfu/ml. The count of the products after four hours in winter was 5.5 $\log_{10}$ cfu/ml whereas after 8 h fermentation, the count was 5.14 $\log_{10}$ cfu/ml. The corresponding counts of the samples collected during summer were 7.61 $\log_{10}$ cfu/ml and 6.91 $\log_{10}$ cfu/ml.

Adabesin *et al.* (2001) analysed the microbial quality, moisture content, pH and titratable acidity of fermented cereal mix, Fura da nono and Nono obtained from three sources, *viz.* A, B and C in Bauchi, a Nigerian city during 1998 and 1999. The study revealed that Fura da nono samples had a mean total viable count of 3.4×10^4, 3.8×10^4 and 3.2×10^4 cfu/ml, respectively from the three sources during 1998. The corresponding counts of the samples during 1999 was 3.5×10^4, 4.0×10^4 and 3×10^4 cfu/ml. Nono samples had a mean total viable count of 3.6×10^4, 3.6×10^4 and 3.4×10^4 cfu/ml, respectively from the three sources during 1998. During 1999, the count of the samples was 3.4×10^4, 3.5×10^4 and 3.2×10^4cfu/ml, respectively.

Fifteen samples of traditional fermented milks collected from individual households in South Africa and Namibia were tested and reported that the samples had a mean total plate count of 5.5×10^8cfu/g (Beukes *et al.*, 2001). The count of the samples ranged between 8.6×10^5 and 1.56×10^9cfu/g.

Birollo *et al.* (2001) evaluated the pH and cell counts of the whole set sweetened yoghurt obtained from an industrial manufacturer in Argentina. The samples were stored at 6°C and had a mean total cell count of 8.5 log cfu/ml on day one of storage and the count decreased to 8.3 log cfu/ml on day 10 of storage. The count of the samples on day 18 was 8.1 log cfu/ml.

Moreira *et al.* (2001) studied the microbial quality of 72 cartons of yoghurt consisting of 24 cartons of plain yoghurt and 48 cartons of fruit yoghurt, obtained from different supermarkets in Brazil during the months of April, May and June. Plain yoghurt from one of the sources had mean total count at the level of 3.7×10^7, 8.8×10^7 and 3.0×10^7 cfu/g, respectively. The corresponding counts from the other sources were 1.8×10^7, $1.2 \times 10^7$and 1.7×10^7 cfu/g.

Al-kadamany *et al.* (2002) studied the microbial counts of 80 packages of concentrated yoghurt, Labneh, each weighing 500 g. Labneh was produced by straining cow's milk set yoghurt in cloth bags. The samples of yoghurt were collected during storage at 5, 15 and 25°C. Initial count of the samples was 8 $\log_{10}$ cfu/g. Total plate counts increased by 2 log cycles on the third day of storage at 5 and 15°C and after four days, there was no increase in the count, till 11 days of storage at 5°C and 7 days at 15°C. Total plate counts of samples stored at 25°C increased by 0.5 log order for the first three days, and then remained stable.

Younus *et al.* (2002) compared the physico-chemical, microbiological and organoleptic quality of 25 samples of dahi and 10 samples each of various brands of yoghurt, viz. A, B and C collected randomly from the local markets of Rawalpindi and Islamabad during the month of October 2001 to March 2002. The study revealed that the dahi samples had a mean total viable count of 7.34×10^7 cfu/ml. The count in the yoghurt samples from the sources A, B and C were 5.61×10^7, 3.31×10^7 and 6.34×10^7 cfu/ml, respectively.

Molska *et al.* (2003) examined the microbiological quality of 61 samples of kefir and 92 samples of yoghurt purchased from retail market in Warsaw during the period between 1995 and 2001. The study revealed that the total number of bacteria in at least 90 per cent of yoghurt and 73 per cent of kefir were in the range of 10^7 to 10^9 cfu/g.

The microbial quality of 22 samples of Kule naoto, the traditional fermented milk product of Masai in Kenya were evaluated and found that the samples had a mean mesophilic bacterial count of 8.1 log cfu/ml (Mathara *et al.*, 2004).

Savadogo *et al.* (2004a) estimated the bacterial quality of 30 samples of traditional fermented milk collected from Northern Burkina, produced by Fulani individual households. The samples had a mean total viable count of 6.1×10^7 cfu/ml and the count ranged between 8.12×10^5 and 3.6×10^8 cfu/ml.

2.1.2 Coliform Count

Hudec (1968) examined the bacteriological quality of 427 samples of yoghurt in Czechoslovakia during the period from 1963 to 1967. The study revealed that 54.4 per cent of the samples had coliform counts of ≤10/g and 27 samples had coliform counts in the range of 20,000 to 30,000/g.

Keenan *et al.* (1968) analysed the bacteriological, physico-chemical and organoleptic quality of buttermilk obtained from 10 regional dairies and reported that none of the samples had coliforms.

Puhan *et al.* (1973) studied the microbiological status of 269 samples of commercial yoghurt purchased from small and large shops in Zurich, Switzerland during the period between December 1972 and March 1973. The study revealed that three per cent of the samples contained >10 coliform cells/ml.

Arnott *et al.* (1974) subjected 152 commercially produced yoghurts consisting of 15 plain and 137 fruit or flavoured yoghurts to microbiological examination and reported that the coliform count of the samples ranged from < 1 to 960/g. The organism was present in 13.8 per cent of the samples.

Mohanan *et al.* (1984) evaluated the microbiological quality of 60 samples of dahi prepared under household conditions at Bangalore and reported

that the samples had a mean coliform count of 51 viable units/ml and the count ranged between 0 and 100 viable units/ml.

Jayaram and Gandhi (1987) evaluated the microbiological quality of dahi obtained from hotels, houses and an organized dairy. None of the samples from houses and organized dairy had coliforms, whereas the samples from hotels had coliforms and the count ranged between 0 and 64/ml.

Abou-Dhonia *et al.* (1992) studied the chemical, microbiological and organoleptic properties of Labneh, made from homogenized reconstituted skim milk using nine combinations of starter cultures. The samples were examined on days 0, 10 and 20 of storage at 5°C and reported that none of the samples had coliforms.

One hundred and five samples of indigenously produced dairy products, *viz.* yoghurt, cream and soft cheese were randomly collected from various areas in Mosul City and analysed for the presence of coliforms (Al-Hadethi *et al.*, 1992). The average coliform count of yoghurt, cream and soft cheese were 5 x 10^4, 4 x 10^4 and 2 x 10^4 cfu/g, respectively.

Farid *et al.* (1992) determined the level of coliforms in 48 yoghurt samples obtained from shops and supermarkets in Minia, Central Egypt. Twenty three samples were collected on the day of manufacture and the remaining 25 samples were obtained two to three days after manufacture. Of the samples, 21.7 per cent of the former group and 40 per cent of the latter group had coliforms.

Medina *et al.* (1993) determined the microbiological quality of commercial cultured milk containing Bifidobacteria, manufactured in Spain and reported that none of the 20 samples examined had coliforms.

Mutukumira (1995) investigated the microbiological quality of 10 batches of cultured milk, Amasi, produced by small holder dairy farmers in the

Lancashire farming area of Zimbabwe. The study revealed that coliform count in the samples ranged from 3.41 to 6.90 cfu /g.

Samolada *et al.* (1998) studied the changes in the microbial flora of traditional fermented raw milk from ewe during the manufacture and storage at room temperature for five days and reported that the mean coliform count of raw milk samples was 3.96 log cfu/ml and the count increased to 6.42 log cfu/ml on day one of storage. On fifth day, the mean coliform count of the samples was 7.86 log cfu/ml.

Gupta *et al.* (2000) evaluated the microbiological quality of 29 samples of mishti doi sold in and around Calcutta and reported that the samples had a mean coliform count of 46.34 ± 15.35 cfu/g.

Birollo *et al.* (2001) studied the microbiological quality of whole set sweetened yoghurt stored at 6°C obtained from 10 industrial manufacturers in Argentina and reported that the product obtained from nine manufacturers were found free from coliforms on day one of storage, but the samples from one of the manufacturers had the organism at the level of 1.1 log cfu/ml. The counts on days 3, 5, 10 and 14 were 1.2, 1.0, 1.1 and 0.2 log cfu/ml, respectively. However the organism was not detected on 16^{th} and 18^{th} day of storage.

Younus *et al.* (2002) compared the physico-chemical, microbiological and organoleptic quality of 25 samples of dahi and 10 samples each of various brands of yoghurt *viz.* A, B and C collected randomly from the local markets of Rawalpindi and Islamabad during the month of October 2001 to March 2002.The study revealed that dahi samples had a mean coliform count of 4.39×10^3 cfu/ml. Yoghurt samples obtained from the source A was found free from the organisms. The samples belonging to the source B and C had a mean coliform count of 0.71×10^3 and 3.39×10^3 cfu/ml, respectively.

The microbiological quality of 61 samples of kefir and 92 samples of yoghurt purchased from retail network in Warsaw was evaluated during the period between 1995 and 2001 (Molska *et al.*, 2003). The study revealed that more than 86 per cent of kefir and 97 per cent of yoghurt analysed in the year 2001 were free from coliform bacteria.

Aly *et al.* (2004) investigated the sensory, chemical and microbiological quality of plain and carrot yoghurt made from cow's milk during refrigerated storage at 4 ± 2°C for three weeks. The mean coliform count of fresh plain yoghurt was 50 x 10^2 MPN/g. On 10^{th} day of storage, the count was 77 x 10^2 MPN/g which decreased to 11 x 10^1 on 21^{st} day of storage. Initial coliform count of 15 per cent carrot yoghurt was 30 x 10^2 MPN/g. The corresponding count on day one was 1.3 x 10^2 and coliforms were not detected on 3^{rd} day of storage. With 20 per cent carrot juice added to yoghurt, coliforms were not detected even on the first day of storage.

Savadogo *et al.* (2004a) estimated the microbiological quality of 30 samples of traditional fermented milk collected from Northern Burkina produced by Fulani individual households. The study revealed that the samples had a mean coliform count of 0.98 x 10^4 cfu /ml and the count ranged between 0.25 x 10^2 and 3.5 x 10^4 cfu/ ml.

2.1.3 Faecal Streptococcal Count

Aleksieva (1973) studied the bacteriological quality of 54 samples of yoghurt obtained 24 to 80 h after manufacture, during storage at 5 to 8°C and reported that enterococci titres ranged from 10 to 10000/g.

Arnott *et al.* (1974) analysed the microbiological quality of 152 commercially produced yoghurts in Ontario and reported that the enterococcal count ranged from <1 to 2200/g. Among the samples, 30.3 per cent had enterococcal count in the range of 1 to 100/g and 5.9 per cent of the samples had counts more than 100/g.

Tzanetakes *et al.* (1981) analysed 60 samples of traditional Greek yoghurt obtained from retail outlets of Thesaloniki, Greece and reported that enterococci were present in 30 per cent of the samples tested.

Bacteriological quality of 100 samples of yoghurt made from ewe's milk obtained from local Mosul markets in Iraq was investigated and reported that the samples had a mean faecal streptococcal count of 1000/g (Khalaf and Shareef, 1985).

Farid *et al.* (1992) determined the level of enterococci in 48 yoghurt samples obtained from shops and supermarkets in Minia, Central Egypt. On the day of manufacture, 23 samples were collected and the remaining 25 samples, two to three days after manufacture. Of the samples, 26 per cent of the former group and 56 per cent of the latter group had enterococci.

2.1.4 Psychrotrophic Count

Arnott *et al.* (1974) analysed the microbiological quality of 152 commercially produced yoghurts in Ontario and reported that psychrotrophic count in the samples ranged from <10 to 2.4 x 10^5/g.

Juffs and Babel (1975) investigated the effect of lactic cultures on the growth of psychrotrophic bacteria in milk stored at 3.5 and 7°C. Lactic acid producing Streptococci and *Leuconostoc cremoris* were the most effective in restricting psychrotrophic count. It was observed that the inhibitory effect of lactic culture decreased by the addition of catalase and thus suggesting that hydrogen peroxide was the inhibitor.

Gilliland and Martin (1980) investigated the growth of psychrotrophs in skim milk stored at 6°C, containing six strains of *Lactobacillus bulgaricus* at the level of 10^7/ml. During the study, it was observed that the growth of the psychrotrophs was not significantly affected by the count of lactobacilli at the level of 1 x 10^7/ml. However when the level of lactobacilli increased to 2.5 x

10^7/ml, there was significant inhibitory effect on the growth of psychrotrophs and the effect increased as the lactobacilli count increased.

Gilliland and Einell (1983) conducted an experiment in which cells of *Lactobacillus lactis* frozen cultures were added (1 x 10^8/ml) to refrigerated raw milk to determine the effect of growth of psychrotrophs during storage of milk at 5 or 7°C. The study revealed that some strains of *L. lactis* were significantly inhibitory towards psychrotrophs in refrigerated raw milk at both temperatures.

Varabioff (1983) analysed the bacteriological quality of 100 yoghurt samples from 13 manufacturers. The samples were analysed on receipt and during storage at 7°C, until expiry date from 14 to 29 days. The study revealed that psychrotrophic bacteria was present in six samples on arrival on zero day, but only in two samples at the end of the storage period.

Farid *et al.* (1992) determined the level of psychrotrophic bacteria in 48 yoghurt samples obtained from shops and supermarkets in Minia, Central Egypt. On the day of manufacture, 23 samples were collected and the remaining 25 samples, two to three days after manufacture. Of the samples, 21.7 per cent of the former group and 40 per cent of the latter group had psychrotrophs.

Microbial analysis of 20 commercial cultured milk manufactured in Spain containing Bifidobacteria was evaluated and reported that the samples had psychrotrophic count at the level of <10 cfu /g in all the samples (Medina *et al.*, 1993).

Samolada *et al.* (1998) studied the changes in the microbial flora of traditional fermented raw milk from ewe during the manufacture and storage at room temperature for five days and reported that the psychrotrophic counts of final product were higher by 1.7 log units than that in raw milk.

2.1.5 Yeast and Mould Count

Lembhe (1969) analysed samples of milk, cream, butter, dahi, khoa and condensed milk for total yeast count and lactose fermenting yeasts. The study revealed that in dahi samples, yeast counts were in the range of 0 to155, 000 /ml.

Puhan *et al.* (1973) assessed the microbiological status of 269 samples of commercial yoghurt (plain and flavoured) collected from shops in Zurich, Switzerland between December 1972 and March 1973. Of the yoghurt samples, 77 per cent were made from whole milk (≥3.5 % fat), 17.1 per cent from partially skimmed milk (≥ 2.0 % fat) and 5.9 per cent from skim milk (≤ 0.5 % fat). The maximum count of yeast and mould reported was 8500/ml, which was present in 10 per cent of the samples.

Arnott *et al.* (1974) analysed the microbiological quality of 152 commercially produced yoghurts in Ontario and reported yeast count in the range of < 2 to 3.2 x 10^5/g. The mould count was in the range of < 2 to 3 x 10^4/g.

Dubois *et al.* (1980) examined a sample of stirred yoghurt, in which there was gas formation and disagreeable smell and reported that the sample had high count of yeast at the level of 7 x 10^6/g. The contaminating yeast was identified as *Kluyveromyces bulgaricus.*

Suriyarachchi and Fleet (1981) purchased yoghurt samples from retail outlets in Sydney and examined to detect the presence of yeast in the samples. Out of the 128 samples studied, 45 percent exhibited yeast counts above 10^3 cells /g. A total of 73 strains were isolated. *Torulopsis candida* was the most frequently isolated species followed by *Kluyveromyces fragilis*, *Saccharomyces cervisiae*, *Rhodotorula rubra*, *Kluyveromyces lactis* and *Torulopsis versatilis.*

Mohanan *et al.* (1984) evaluated the microbiological quality of 60 samples of dahi prepared under household conditions at Bangalore. The yeast and

mould count of the samples ranged from 7 x 10^2 to 5 x 10^3 with an average of 2 x 10^3 cfu/ ml.

Hosono *et al.* (1989) examined 80 samples of fermented milk purchased from local markets in Indonesia and reported that the samples had mean yeast and mould count of 1.1 x 10^7 cfu/ml.

Bankole and Okagbue (1992) studied 100 samples of Nono, a Nigerian fermented product and reported that the samples had a mean yeast count of 1.5 x 10^7 cfu/g.

Microbial analysis of commercial cultured milk manufactured in Spain containing Bifidobacteria was evaluated by Medina *et al.* (1993). Yeast was detected in 65 per cent samples with counts ranging from 10 to 4100 cfu/g.

Sharma *et al.* (1993) analysed the yeast and mould count of 106 samples of dahi collected from local markets of Ludhiana city. The study revealed that log counts of yeasts were <1/g and >3/g in 40.6 and 17.9 per cent samples. Log counts of moulds were <1/g and >3/g in 51.8 and 11.3 per cent samples. Fusarium was the most frequent mould isolated and the most frequent yeast isolated was Torulopsis. Aspergillus was detected in eight samples.

Yamani and Abu-Jaber (1994) analysed the microbial quality of Labneh samples obtained from 18 producers in Amman, Jordan. The mean psychrotrophic and mesophilic yeast counts of Labneh samples after packing was 2.6 x 10^6 and 4.4 x 10^6/g, respectively. On 14th day of storage at 5°C, the psychrotrophic and mesophilic yeast counts increased to the level of 1.1 x 10^7/g and 1.4 x 10^7/g, respectively.

Samolada *et al.* (1998) studied the changes in microbial flora of traditional fermented raw milk from ewe kept at room temperature for five days. The mean yeast count of raw milk was 1.17 log cfu/ml. At room temperature, the

count was 0.59 log cfu/ml on day one of storage. On fifth day, the samples had a mean count of 7.86 log cfu/ml.

Gadaga *et al.* (2000) enumerated and identified yeasts in 30 samples of Zimbabwean fermented milk, Amasi, obtained from farms, households and collection centers. The study revealed that yeast counts of the samples ranged from <2 to 8.08 logcfu/g. The predominant isolates identified consisted of *Saccharomyces cervisiae* (22), *Candida lusitaniae* (11), *Candida colliculosa* (7) and *S. dairensis* (7).

Moreira *et al.* (2001) studied the microbial quality of 72 cartons of yoghurt, consisting of 24 cartons of plain yoghurt and 48 cartons of fruit yoghurt, obtained from different supermarkets in Brazil during the months of April, May and June. Plain yoghurt obtained from one of the sources during the period had mean yeast and mould count at the level of 790.25, 214 and 89.92 cfu/g, respectively. The corresponding counts of the samples obtained from the other sources were 43.95, 2.6 and 1.0 cfu/g.

Viajayalakshmi and Murugesan (2001) studied the organoleptic and microbiological quality of butter milk during storage at 6 to 8°C. The total fungal count on day one was 4×10^2 cfu/g, which increased to 1.4×10^3cfu/g on fifth day of storage.

Changes in the microbial counts of strained yoghurt, Labneh stored at 5, 15 and 25°C was studied by Al-kadamany *et al.* (2002). The mean initial count of yeast and mould was 4 $\log_{10}$ cfu/ml. The count increased during storage with counts ranging within 3 log cycles, irrespective of storage temperature up to 10 days.

Hattingh and Viljoen (2002) studied the survival of dairy associated yeasts in yoghurt and yoghurt related products stored at 5°C. Yeasts were inoculated at the level of 7 $\log_{10}$ cfu/ml in plain yoghurt and reported that the counts remained almost equal to the inoculum up to 32 days of storage.

Kavas *et al.* (2003) compared the microbiological and organoleptic characteristics of set type yoghurt produced by ultrafiltration using 100% goat milk (A), 70% goat-30% cow milk (B) and 50% goat-50% cow milk (C). The mean yeast and mould count of the products A, B and C were 6.76×10^2, 1.34×10^2 and 3.46×10^2 cfu/g and the count of these products gradually decreased to 1.2×10^2, 1.77×10^2 and 2.08×10^2cfu/g, respectively on seventh day of storage. On 14th day of storage, the counts of these products were 1.28×10^2, 1.25×10^2 and 1.65×10^2 cfu/g, respectively.

Viljoen *et al.* (2003) evaluated the microbial count of 32 samples of yoghurt, consisting of 16 plain yoghurts and 16 fruit yoghurts obtained from a local dairy plant in South Africa, during storage at 15 and 5°C. No yeast growth was observed in yoghurt samples incubated at 5°C during the first 10 day of storage. The yeast counts increased significantly after 15 days of storage and the mean yeast counts reached as high as 5 log cfu/ml. At 15°C of storage, yeast count was observed on the first day of storage and the mean count increased to the level of 6.78 log cfu/ml after 15 days of storage.

Aly *et al.* (2004) investigated the sensory, chemical and microbiological quality of plain and carrot yoghurt made from cow's milk during refrigerated storage at 4 ± 2°C for three weeks. The mean yeast and mould count of fresh plain yoghurt was 3.9×10^3cfu/g. On 10th day of storage, the mean count of the sample was 7.7×10^5cfu/g which increased to 2.8×10^8 cfu/g on 21st day of storage. Initial yeast and mould count of 15 per cent carrot yoghurt was 4.1×10^2cfu/g. The corresponding count on day five was 1×10^2 cfu/g and organisms were not detected on 10th day of storage. With 20 per cent carrot juice added to yoghurt, yeast and mould were not detected even on the first day of storage.

Mathara *et al.* (2004) assessed the microbial quality of 22 samples of Kule naoto, the traditional fermented milk product of Masai, Kenya and reported a mean yeast count of 5.9 $\log_{10}$ cfu/ml.

Savadogo *et al.* (2004a) examined the microbiological quality of 30 samples of traditional fermented milk collected from Northern Burkina produced by Fulani individual households. The study revealed that the yeast and mould count of the samples ranged from 1.83×10^3 to 3.7×10^6 cfu/ml with an average count of 2.6×10^4 cfu/ml.

2.2 ISOLATION AND IDENTIFICATION OF BACTERIA

2.2.1 *Escherichia coli*

Bhat and Reporter (1949) observed that *E. coli* behaved identically in both sweet and sour curd samples, indicating their adaptability; its lactose fermenting ability enabled it to multiply and remain active and motile for full three days, even in undiluted samples.

Tiwari and Singh (1964) studied the survival of *E. coli* in dahi contaminated before curdling and found that the organisms survived for 336 h at 22 to 25°C and for 576 h at 3 to 5°C.

Skountzos *et al.* (1973) examined a total of 455 yoghurt samples prepared from cow and ewe milk, obtained from Athens and Thessaloniki districts in 1971 and 1972. Of the samples, 11.1 percent of the industrially produced and 21.6 per cent produced by small enterprises had a high *Escherichia coli* count.

In a study, Prasad *et al.* (1980) could observe a shorter survival period of *E. coli* stored at room temperature than at refrigeration temperature, and he opined that it might be due to relatively faster acidity development in dahi at 25 to 26°C than at 4 to 5°C.

Khalaf and Shareef (1985) analysed the bacteriological quality of yoghurt in Mosul city, Iraq. *Escherichia coli* were detected in six percent of the samples tested.

Mohanan *et al.* (1985) investigated the growth and survival of *Escherichia coli* inoculated into milk at the level of 10^4/ml along with starter culture and incubated at 22 ± 5°C, 30°C and 37°C. It was observed that during the first 16 h of incubation, the viable cells of *E. coli* increased in samples incubated at 22 ± 5°C or 30°C, but the count decreased in samples incubated at 37°C. However after further incubation, the viable counts were drastically reduced particularly at 37°C and at the end of 48 h, the organism could not be detected in any of the samples.

Fate of two pathogenic and one non-pathogenic *E. coli* strains in two fermented milk products in Zimbabwe was determined by Feresu and Nyati (1990). The *E. coli* strains were inoculated at the level of 4 log cell number/ml. Out of the two products, the industrially fermented milk, Lacto, inhibited one of the pathogenic and the other non-pathogenic strains at 20°C for 24 h. The other pathogenic strain survived up to 120 h of storage at 20°C. In traditionally fermented milk, organisms multiplied to maximum 10^9 to 10^{10}/ml at 24 h of storage. The count decreased to the level of 10^5 to 10^8/ml and 10^4 to 10^7/ml at 120 h of storage of the fermented product at 20°C and 5°C, respectively.

Simango and Rukure (1992) investigated the survival of strains of bacterial enteric pathogens in two traditional fermented foods, *viz.*, Mahewa and sour porridge and in unfermented porridge. Enteropathogenic *E. coli* strains survived for 24 h after inoculation in Mahewa and sour porridge but the count decreased by 3 logs from an initial concentration of 10^6 to 10^7 cfu/ml. *Shigella* and enterotoxigenic *E. coli* strains were not detected after 24 h of storage.

Medina *et al.* (1993) examined the nature of microflora of 20 commercial cultured milk manufactured in Spain containing Bifidobacteria and reported that none of the samples had *E. coli.*

Microbiological quality of 50 curd samples was evaluated by Misra *et al.* (1993) and reported the presence of *E. coli* in 28 per cent of the samples.

Morgan *et al.* (1993) studied a case of verotoxin producing *Escherichia coli* 0157:H7 infection associated with the consumption of yoghurt. Out of 16 cases, 11 of them aged ≤10 years and five of the affected children developed hemolytic uraemic syndrome. The authors opined that this is the first reported case of developing hemolytic uraemic syndrome in children due to the consumption of yoghurt.

Dineen *et al.* (1998) examined the survival of *E. coli* 0157:H7 in the production process of yoghurt, sour cream and buttermilk. The commercial products were inoculated with *E. coli* 0157:H7 at the level of 10^3 cfu/ml. The organism was recovered for up to 6, 6 and 14 days in brand C, F and B yoghurt respectively, at levels of $<10^2$ cfu/ml. The pH of the yoghurt belonging to the brands C, F and B were 4.0, 4.2 and 4.0, respectively.

Chang *et al.* (2000) investigated the growth and survival of *Escherichia coli* 0157:H7 during the fermentation and storage of diluted cultured milk drink. *E. coli* 0157:H7 was inoculated into the cultured milk drink at the level of 5 logcfu/ml. However the level of the organism was reduced to non-detectable levels in the non-sugar added cultured milk drink (pH 3.5) prepared with *L. delbrueekii spp. bulgaricus* after one day storage at 7°C and for a period of <1 to 4 days with *L. casei,* respectively. Addition of sucrose in the fermented milk drink exerted a protective effect on the survival of *E. coli* 0157:H7 during the storage period.

Beukes *et al.* (2001) studied the microbiological profile of 15 samples of traditional fermented milk samples collected from individual households in South Africa and Namibia and reported that *Escherichia coli* was present in three of the samples. The *E.coli* counts of these samples were 4×10^3, 7×10^3 and 19×10^3/ml.

Soni *et al.* (2002) carried out serogrouping and pathogenicity study on *Escherichia coli* isolates by Congo red binding test. Out of the 17 serotypes

tested, 15 produced brick red colonies on the Congo red medium. A good correlation was observed between Congo red binding and pathogenic potential.

Soomro *et al.* (2002) examined 20 samples of dahi sold at Tandojam, Pakistan and reported the isolation of *E. coli* in 11 (55 per cent) of the samples.

2.2.2 *Staphylococcus aureus*

Saha and Ganguly (1957) reported an outbreak of Staphylococcal food poisoning which occurred in North Calcutta and affected 67 persons, due to the consumption of dahi. *Staphylococcus aureus* was isolated from the particular batch of dahi made from milk obtained from a suspected source.

Tiwari and Singh (1964) observed that there was no increase in the number of staphylococci during the first 18 h of incubation at 22 to 25°C in the preparation of dahi. They also reported that *S. aureus* was very resistant, surviving for 144 h at 22 to 25°C and for 912 h at 3 to 5°C.

Skountzos *et al.* (1973) examined a total of 455 yoghurt samples prepared from cow and ewe milk, obtained from Athens and Thessaloniki districts and reported that none of the samples had coagulase and DNAase positive Staphylococci.

Dincheva (1976) inoculated 24 h broth culture of enterotoxin producing strains of *Staphylococcus aureus* at the level of 4 to 6 x 10^6 cells /ml, along with one per cent 24 h culture of yoghurt starter. The samples were stored at 2 to 6°C and 18 to 22°C and examined at intervals. *Staphylococcus aureus* grew well at both temperatures and persisted for seven days at 2 to 6°C. At 18 to 22°C, *Staphylococcus aureus* strains died out when pH reached three.

Coagulase and TNase positive *Staphylococci* were detected in 3.5 per cent of samples collected from halwais, tendors, dhabas, hotels, hostel messes and canteens of Punjab Agricultural University and of Ludhiana City (Kahlon and Grover, 1984).

Khalaf and Shareef (1985) evaluated the bacteriological quality of 100 samples of yoghurt made from ewe's milk obtained from Mosul markets in Iraq and reported that 26 per cent of the samples were contaminated with *Staphylococcus aureus.*

Varadaraj and Ranganathan (1988) observed that *S. aureus* grew well in the presence of *Lactobacillus acidophilus* in milk and the count of the organism reached at the level of 10^8 cfu/ml from an initial level of 10^5 cfu/ml and produced TDNase to the extent of 18 to 20 mm diameter. However the same strains in the presence of *Lactobacillus bulgaricus* grew poorly and produced low level of the enzyme.

Rajmany *et al.* (1989) reported that coagulase positive S*taphylococci* was detected in 45 per cent of curd samples procured from local markets of Udaipur city.

Khedekar *et al.* (1990) compared the antagonicity of acidophilus cultures, viz. LB KV_3 and LB KI_4 with mixed strains of lactic cultures, LF-40 and yoghurt cultures (Lactobacillus – LBW and *Streptococcus thermophilus* – CH_1) to pathogenic staphylococci at 37°C and 15°C. Milk inoculated with LB KV_3, LB KI_4, yoghurt cultures and LF-40 showed reduction in *Staphylococcus aureus* after 8, 24, 8 and 36 h respectively on storage at 15°C after an initial incubation for 12 h at 37°C. At both the temperatures, viz. 37°C and 15°C, LB KV_3 had higher antibacterial activity against *Staphylococcus aureus* than LB KI_4. However yoghurt cultures were found superior to all other lactic cultures.

Abou-Dhonia *et al.* (1992) tested the chemical, microbiological and organoleptic properties of Labneh made from homogenised, reconstituted skim milk using nine combinations of starter cultures. The samples were stored at 5°C for 0, 10 and 20 days. They reported that Staphylococci were not detected in fresh or stored Labneh.

Ashenafi (1992) studied the growth potential of *Staphylococcus aureus* in ergo, a traditional Ethiopian fermented milk. Complete inhibition of test organism was observed within 24 h of the preparation of the product.

Misra *et al.* (1993) reported the isolation of 12 Staphylococcus from 50 curd samples collected in and around Bhubaneswar. Of the isolates, 60 per cent were coagulase positive.

Pazakova *et al.* (1997) studied the effect of yoghurt culture on the survival of *Staphylococcus aureus* added to milk in various concentrations during the fermentation and storage of yoghurt. When milk samples were inoculated with a concentration of 10^2 *S. aureus* cells, the organism was not recovered from yoghurt during fermentation and storage. The fermentation and storage of yoghurt was accompanied by increase in lactic acid and titrimetric acidity and decrease in pH value.

Estrada *et al.* (1999) conducted studies to assess the nature of growth of *Staphylococcus aureus* during fermentation of milk and subsequent storage at 4°C. Sterile skim milk was inoculated with four strains of *S. aureus*, each consisted of 10^6 cfu/ml and starter culture, at the level of 10^6 cfu/ml and incubated at 42°C for 8 h followed by refrigeration at 4°C. They observed that behaviour of four strains of *S. aureus* was similar. The organism survived the 8 h fermentation with lactic acid bacteria and the population began to decrease from the first day of storage and completely inhibited at 9 to 10 days.

Gran *et al.* (2003) determined the presence of selected pathogenic bacteria in 12 samples of raw milk, 27 samples of cultured pasteurized milk (CPM) and 21 samples of naturally soured raw milk (NSRM) sold at three small scale dairies in Zimbabwe. The mean count of *Staphylococcus aureus* in the samples from the sources were 5.2, 7.3 and 7.8 $\log_{10}$ cfu/ml, respectively. Typical *S. aureus* was present in 82 per cent of the samples.

2.2.3 *Salmonellae*

Tiwari and Singh (1964) studied the survival of *Salmonella paratyphi* in dahi during storage of the product at 22 to 25°C and 3 to 5°C. They observed that destruction of the organisms occurred in approximately 72 h of storage at the former temperature and 144 h of storage at the latter temperature.

Kulshrestha (1976) investigated the occurrence of Salmonella in 169 samples of milk products collected from various dealers at Bareilly city and reported the isolation of *Salmonella enteritidis* from 3 of the 22 dahi samples tested.

Rubin *et al.* (1982) determined the effect of lactic acid and pH on growth of *Salmonella typhimurium*, in yoghurt and reported that no viable counts were observed after 48 h when pH reached 4.5 and per cent of lactic acid was 1.5.

Kumari and Singh (1990) reported that *Salmonella* was not isolated from soy cheese slurries incubated at 30°C for 8 days, but when inoculated at the level of 20 x 10^6/g, the count increased sharply during the first 24 h followed by leveling off up to 5^{th} day of storage. After 5^{th} day, the count declined to 100 x 10^4/g.

Survival rate of *Salmonella typhimurium* in yoghurt and dahi stored at 5 to 7°C and 37°C was studied by Matta *et al.* (1991). The study revealed that the organism did not survive in dahi/yoghurt stored at 37°C for more than 25 h, but a few organisms survived even after 48 h storage at 5 to 7°C.

Medina *et al.* (1993) determined the microbial quality of 20 samples of commercial cultured milk containing Bifidobacteria in Spain and reported that none of the samples had Salmonella.

Beukes *et al.* (2001) assessed the bacteriological quality of South African traditional fermented milk and reported that none of the samples were contaminated with Salmonella.

Savadogo *et al.* (2004a) analysed the microbiological quality of 30 samples of traditional fermented milk collected from Fulani individual households in Northern Burkina. The study revealed that two of the samples were contaminated with Salmonella and the counts ranged between 5 and 20 cfu/ml.

2.2.4. *Pseudomonas aeruginosa*

Khalaf and Shareef (1985) investigated the bacteriological quality of 100 samples of yoghurt and reported the incidence of *Pseudomonas aeruginosa* in 12 per cent of samples.

Tayar and Sen (1993) studied the bacteriological quality of 78 yoghurt samples collected from markets in Bursa, Turkey and reported that 24 per cent of the samples revealed the presence of *Pseudomonas.*

Birollo *et al.* (2001) studied the viability of Pseudomonas species in the whole set sweetened yoghurt during refrigerated storage. Yoghurt samples were inoculated with the organism at the level of 10^5 to 10^6 cfu/ml and observed that the organism was not detected in the product after 2 to 5 days of storage.

2.2.5. *Bacillus Species*

Kroger (1975) opined that sporeformers such as *Bacillus subtilis*, if present in the milk, survive the heat treatment and germinate during incubation.

In a study conducted by Mukundan (1978) on the microflora of 48 samples of boiled milk, 162 isolates of *Bacillus* species were obtained. Among them, occurrence of *B. subtilis* was highest (52.47%) followed by *B. cereus* (14.2%), *B. pumilus* (12.35%), *B. licheniformis* (9.26%), *B. megaterium* (6.79%), *B. alvei* (3.7%) and *B. firmis* (1.23%).

Vitkov (1978) evaluated the *Bacillus cereus* count in dairy food products and reported that 64 batches of pasteurised milk samples had the count at the level of 10^1 to 10^3 /g.

Mohanan *et al.* (1984) analysed the microbiological quality of 60 samples of dahi prepared under household conditions at Bangalore and reported that the samples had aerobic spore count at the level of 0 to 30 per ml.

Mohanan *et al.* (1985) studied the growth and survival of *B. subtilis* and *B. cereus* inoculated into milk at the level of 10^4/ml along with starter cultures and held at storage temperatures of 22 ± 5°C, 30°C and 37°C. The study revealed that viable count of both the organisms reduced during the first 8 h at all the three temperatures of incubation and the organism could not be detected after 24h of incubation.

Sixty four cultures of aerobic spore forming bacteria were isolated from 180 raw and pasteurised milk samples and identified up to species level by Rajarathinam *et al.* (1985). *Bacillus subtilis* was the most predominant (14) isolates followed by *B. cereus* (12), *B. megaterium* (10) and *B. licheniformis* (9).

Jayaram and Gandhi (1987) studied the microbiological quality of dahi obtained from hotels, houses and an organized dairy. Spore formers were present at the level of 3 x 10^5 to 1.2 x 10^6/ml from hotels, 2.3 x 10^5/ to 1.28 x 10^6 ml from houses and 2 x 10^5 /ml from the organised dairy.

Misra *et al.* (1993) evaluated the microbial quality of 50 curd samples collected from in and around Bhubaneswar. During the study, bacilli were detected in four per cent of the samples.

Incidence of aerobic spore forming bacteria in lassi was determined by Pillai *et al.* (1993). Among the 52 isolates identified from 18 samples of lassi, occurrence of *B. subtilis* was highest (28.2%) followed by *B. megaterium*

(21.7%). *B. cereus* isolates were nonenterotoxigenic and incidence of isolation of the organism was 13.46 per cent.

Rangaswamy *et al.* (1993) reported the isolation of seven *Bacillus cereus* from 15 yoghurt samples collected from different sources in Victoria, Australia.

Balasubramanyam and Varadaraj (1994) studied the antibacterial activity of 10 market samples of dahi. During the study, 11 isolates were identified as *Lactobacillus*. The inhibitory activity of sterilised culture filtrate of each lactic acid bacteria against selected pathogenic and spoilage organism was determined by agar well diffusion assay and found that the isolates exhibited strong or moderate degree of inhibition of *Bacillus cereus* and *Bacillus licheniformis.*

Crielly *et al.* (1994) investigated the occurrence of *Bacillus* in milk and milk products and reported that *Bacillus licheniformis* was the most commonly isolated species from milk and its products whereas *Bacillus cereus* was found more often in raw milk. However, during the study, it was observed that in heat treated milk, the counts of *Bacillus cereus* was always very low.

Noriega *et al.* (2003) compared the efficiency of carbonation of heat-treated skim milk as a method for inhibiting *B. cereus* growth during incubation at 37°C and cold storage at 4°C of fermented bifidus milk. Carbonated (pH 6.0) uncontaminated and contaminated (10 cfu/ml) milks were compared with respective non-carbonated controls. *B. cereus* multiplied actively during incubation at 37°C and growth was reduced by 2 log units at 12 h of incubation in carbonated samples (5.80 ± 0.57 log cfu/ml) as compared to the non-carbonated one (7.37 ± 0.01 log cfu ml^{-1}). However the counts of the organism decreased progressively in carbonated milk and were not detected beyond 14 days. The organism remained viable in non-carbonated milk until the end of storage (35 days).

Rossland *et al.* (2003) assessed the effect of *Lactobacillus* and *Lactococcus* cultures on the growth of *Bacillus cereus* NVH_{45} in reconstituted

skim milk. The study showed that the different *Lactobacillus* and *Lactococcus* cultures reduced the *B. cereus* population within 24 to 48 h. The difference in degree of inhibition was significant and the reduction in count varied from total inhibition to a decrease of 2 to 3 $\log_{10}$ cfu/ml.

Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk was studied by Savadogo *et al.* (2004b). Bacteriocin isolated from the sample exhibited antibacterial activity against *Bacillus cereus.*

2.3 PHYSICO-CHEMICAL CHARACTERISTICS

2.3.1 Titratable Acidity

Keenan *et al.* (1968) analysed the bacteriological, physico-chemical and organoleptic quality of buttermilk obtained from 10 regional dairies and reported that the titratable acidity of the samples ranged from 0.78 to 0.84 per cent lactic acid.

Sharma and Jain (1974) prepared dahi from cow milk and buffalo milk using pure cultures of *S. lactis*, *S. thermophilus* and *S. diacetylactis* and incubated at 30 or 37°C for 8 to 24 h. Titratable acidity was found to be higher in buffalo milk than cow milk. Acidity increased rapidly during the first 16 h of incubation. Highest acidity was observed in curd at 24 h. of production with 2.5 per cent *S.lactis* starter. The highest acidity of curd prepared from buffalo milk was 1.11 per cent lactic acid and for cow milk, it was 1.05 per cent.

Acidity of yoghurt starter in different types of milk was studied by Singh and Kaul (1982). They found that cultures grown in buffalo milk produced more acid and acetaldehyde and had higher proteolytic activity than cultures grown in cow or goat milk.

The relationship between initial acidity of plain yoghurt and the changes in acidity during refrigerated storage was investigated by Salji and Ismail (1983).

The results indicated that the samples with low initial acidity showed relatively the highest titratable acidity during one week of storage at 4°C. The initial acidity per cent of three samples were 0.79, 1.01 and 1.38 and the pH of these samples were 4.89, 4.18 and 3.82. After one week of storage at 4°C, the titratable acidity of the samples increased to 0.96, 1.10 and 1.48 per cent lactic acid and their pH values decreased to 4.27, 4.12 and 3.81.

Mohanan *et al.* (1984) analyzed the physico-chemical parameters of 60 samples of dahi obtained from private households and restaurants in Bangalore. The study revealed that the samples had a mean titratable acidity of 1.33 per cent lactic acid and the values ranged from 1.0 to 1.8 per cent.

Reddy *et al.* (1987) studied the titratable acidity of dahi and yoghurt during storage, at ambient and refrigeration temperatures and found that the titratable acidity values of the products increased during storage but the rate of increase was more in samples stored at ambient temperature.

The most important fermentative reaction used in dairy processing is the homofermentative conversion of lactose to lactic acid. For the preparation of high quality fermented products, rapid and consistent rate of lactic acid production is required (Frank and Marth, 1988). An optimum level of one per cent acidity is found to be important to prevent the growth of pathogens (Shaack and Marth, 1988). Yoghurt with an acidity of around 1g/100g lactic acid is a fairly inhospitable medium and really troublesome pathogens like Salmonella spp and *Listeria monocytogenes* will be incapable of growth (Tamime and Robinson, 2004).

Laye *et al.* (1993) estimated the titratable acidity of plain nonfat yoghurt made from three commercial sources *viz* A, B and C, during refrigerated storage. The acidity of the sample belonging to the source A remained the same as 1.09 per cent on day 2, 6 and 12. However the samples from source B had acidity at the level of 0.92, 0.94 and 1.1per cent on day 2, 6 and 12, respectively. The

samples from source C had an acidity of 1.30 per cent on day two and increased to the level of 1.32 and 1.37 per cent on day 6 and 12 of storage.

Sarkar *et al.* (1996) analysed 20 market samples of misti dahi obtained from different parts of West Bengal for their organoleptic, microbiological and chemical quality. The study revealed that the titratable acidity of the samples ranged between 0.36 and 1.17 per cent with an average of 0.92 per cent. The authors opined that by refrigerated storage or thermization, the acidity of misti dahi can be maintained at the desired level.

Mathur *et al.* (1999) reported that the curd produced from buffalo milk had an acidity of 1.71 per cent lactic acid on day one of storage and the value increased to 1.95 on day seven of storage.

Al-kadamany *et al.* (2002) determined the shelf life of 80 samples of concentrated yoghurt, 'Labneh'. The samples of yoghurt were collected during storage at 5, 15 and 25°C. The initial acidity of the samples was 1.9 per cent.

Kamruzzaman *et al.* (2002) determined the organoleptic and physico-chemical profile of dahi at room and refrigeration temperature. The acidity on zero day was 0.92 ± 0.01 per cent lactic acid. At room temperature, acidity increased to 1.42 ± 0.03. At refrigeration temperature, the acidity increased to 0.93 ± 0.03 on eighth day and on 12^{th} day, it was 0.94 ± 0.01 per cent.

Rao *et al.* (2002) analysed the chemical quality of 20 samples of dahi collected from the sweet meat shops and milk booths in Hyderabad city. All the samples of dahi had higher acidity than that prescribed by BIS. The mean titratable acidity of samples from sweet meat shops was 1.0420 per cent and that of the samples from milk booths was 1.0890 per cent. The authors opined that the higher acidity content in market dahi could be due to the use of different types of cultures, varied processing techniques and storage of dahi at high temperatures.

Younus *et al.* (2002) compared the physico-chemical quality of 25 samples of dahi and 10 samples each of various brands of yoghurt *viz.* A, B and C

collected randomly from the local markets of Rawalpindi and Islamabad during the month of October 2001 to March 2002. The study revealed that the dahi samples had a mean titratable acidity of 1.16 ± 0.32 per cent. The mean titratable acidity of the yoghurt samples from the sources A, B and C was 0.89 ± 0.02, 0.87 ± 0.04 and 1.13 ± 0.05 per cent, respectively.

The physico-chemical and organoleptic properties of conventionally made dahi from three sources *viz.* A, B and C and industrial yoghurt were compared by Soomro *et al.* (2003). The mean titratable acidity of industrial yoghurt was 0.88 ± 0.01 per cent whereas the dahi samples from the sources A, B and C had mean titratable acidity of 0.91 ± 0.02, 0.85 ± 0.01 and 0.90 ± 0.02 per cent, respectively.

2.3.2 pH

Keenan *et al.* (1968) analysed the bacteriological, physico-chemical and organoleptic quality of butter milk obtained from 10 regional dairies and reported that the pH values ranged from 4.29 to 4.56.

Kroger (1975) suggested that the final pH of good quality yoghurt vary from 4.1 to 4.2 after normal souring, which is optimal, but at a pH of above 4.5, the mix produced a weak coagulum.

The relationship between initial acidity of plain yoghurt and the changes in acidity during refrigerated storage of three samples was investigated by Salji and Ismail (1983). The initial pH of three samples was 4.89, 4.18 and 3.82. After one week of storage at 4°C, pH values of the samples decreased to 4.27, 4.12 and 3.81.

Durga *et al.* (1986) compared the changes in pH and acidity of yoghurt samples stored at room, refrigeration and freezing temperature. The initial pH of the samples was 4.9. The study revealed that pH values decreased to 3.9 and 4.6

on the ninth day of storage at room and refrigeration temperature, whereas the pH remained the same as initial value at freezing temperature.

Chemical and microbiological properties of plain non-fat yoghurt were determined on day 2, 6 and 12 of refrigerated storage by Laye et *al.* (1993). The samples were obtained from three commercial sources, viz. A, B and C, whereas control sample was prepared in the laboratory using yoghurt premix and standard culture. The pH of the control samples was 4.20 on second and sixth day of storage and reduced to 4.17 on day 12. The pH of samples belonging to the sources A and B were 4.30 and 4.50, respectively on the second day of storage, which increased to 4.37 and 4.58 on day 6 and 12, respectively. The pH of sample from source C remained the same as 4.35 throughout the storage period.

Changes in the microbial flora during the manufacture of traditional fermented raw milk from ewe, kept at room temperature for five days was studied by Samolada *et al.* (1998). The study revealed that the samples had a mean pH of 6.58 ± 0.20 on zero day and was reduced to 4.71 ± 0.40 on fifth day of storage.

Abdelgadir *et al.* (2001) analysed the physical and microbiological quality of Sudanese fermented milk, Rob from seven production sites in Khartoum. They reported the pH values of the samples from the seven sites of production as 3.87, 4.02, 3.55, 3.61, 3.37, 3.37 and 3.41, respectively.

According to Adabesin *et al.* (2001), the mean pH of "Fura da nono," a fermented cereal mixture in Bauchi, Nigeria obtained from three sources was 3.8, 3.5 and 3.6, respectively.

Al-kadamany *et al.* (2002) determined the shelf life of concentrated yoghurt, Labneh at storage temperatures of 5, 15 and 25°C. The mean pH of the fresh sample was four. On storage at 5°C, the pH decreased to 3.7 on 11^{th} day, whereas at 15°C, the pH was 3.65 on 7^{th} day of storage. At 25°C, the pH decreased rapidly to 3.6 on 3^{rd} day of storage.

Kamruzzaman *et al.* (2002) assessed the shelf life of dahi at room and refrigeration temperature. The pH value on day zero was 4.16 ± 0.07 at refrigerated storage. The pH value decreased to 4.10 ± 0.04 on sixth day of refrigeration. On 12th day, the value was 3.91 ± 0.01, after which the samples were found to be spoiled.

Younus *et al.* (2002) compared the physico-chemical, microbiological and organoleptic quality of 25 samples of dahi and 10 samples each of various brands of yoghurt, viz. A, B and C collected randomly from the local markets of Rawalpindi and Islamabad during the month of October 2001 to March 2002. The study revealed that the dahi samples had a mean pH of 4.54 ± 0.24. The mean pH of the yoghurt samples from the sources A, B and C was 4.55 ± 0.02, 4.57 ± 0.03 and 4.35 ± 0.03, respectively.

Soomro *et al.* (2003) compared the physico-chemical and organoleptic properties of industrial yoghurt and conventionally made dahi obtained from three sources, *viz.* A, B and C. The mean pH value of the yoghurt sample was 4.256 ± 0.0187 while mean pH values of dahi samples belonging to the source A, B and C was 4.31 ± 0.01, 4.31 ± 0.02 and 4.26 ± 0.02, respectively.

Aly *et al.* (2004) estimated the pH values of plain and carrot yoghurt prepared and stored at 4 ± 2°C for 21 days in the laboratory using cow's milk obtained from Fayoum district, Egypt. The pH value of fresh sample of plain yoghurt was 5.15. On the first day of storage, pH was 4.45 and later decreased to 4.15 on 21st day of storage.

Mathara *et al.* (2004) studied the physico-chemical and microbiological quality of 22 samples of Kule naoto, a traditional fermented milk obtained from different villages in Kenya and reported that the mean pH of the samples was 4.4 and the values ranged between 4.17 and 5.19.

Savadogo *et al.* (2004a) studied the physico-chemical quality of 30 samples of traditional fermented milk in Burkina Faso and reported that the pH of the samples ranged from 4.00 to 5.86 with an average of 4.70.

2.4 ORGANOLEPTIC EVALUATION

Iyengar *et al.* (1967) compared the quality of yoghurt produced from cow and buffalo milk and opined that consistency of the product made from buffalo milk was more acceptable than that produced from the cow milk, but the yoghurt produced from cow milk had higher flavour score. It was also observed that both the products could be stored at 5°C up to one week without any deterioration in quality.

Keenan *et al.* (1968) studied the organoleptic quality of buttermilk obtained from 10 regional dairies. Flavour score of 40 was considered as standard and the study revealed that flavour scores ranged from 34 to 39 and most of the samples lacked a well balanced culture flavour.

Organoleptic evaluation of dahi samples prepared under conditions simulating market environment was carried out in respect of both standard lactic cultures and the market sample of dahi (Sreenivasan and Ranganathan, 1972). During the study it was observed that in general, the samples examined after 24 h. of storage exhibited thick consistency and a mild pleasant flavour. Those samples examined after 72 h. of storage had an unpleasant smell accompanied by gassiness and slight bitterness.

Rangappa and Acharya (1973) reported that the initial acidity and physical properties of milk has a considerable effect on the texture and taste of dahi and yoghurt prepared from it. Milk stored for too long before seeding often gives rise to broken curd with poor taste.

Mohanan *et al.* (1984) found that the predominance of yeast, due to repeated transfers of starter cultures cause elimination of flavour producing organisms in dahi starter cultures.

Parnell-Clunies *et al.* (1986) compared the physical and sensory properties of yoghurt prepared by means of vat, HTST and UHT processing. The sensory scores of the product produced by all the three processing techniques were different. Fermentation was highest in yoghurt heated in vat, but its texture was grainy.

Sanyal *et al.* (1990) evaluated the shelf life of dahi stored at 30 ± 2°C and 4 ± 2°C using various additives. On sensory evaluation, experimental samples of dahi were acceptable up to 20 and 45 days while the control samples kept well only for two and seven days during storage at 30 ± 2°C and 4 ± 2°C, respectively. The scores decreased during storage and the decrease was more noticeable in the samples stored at former temperature than the latter. The scores for aroma and taste were almost similar at both the temperatures.

Natural yoghurt produced in three dairies was stored at 8°C and periodically monitored for the changes in chemical, physical and sensory properties and reported that deterioration in organoleptic quality was pronounced in all yoghurts after 25 days of storage (Eberhard *et al.*, 1993).

Gupta *et al.* (2000) studied the microbiological, chemical and ultrastructural characteristics of 20 market samples of mishti doi in and around Calcutta. Organoleptic examination of the samples revealed that except four, all had yeasty flavour. Only one sample had diacetyl flavour and three samples emanated foul smell.

The sensory properties of traditional acidic and mild, less acidic yoghurt were characterized by Ott *et al.* (2000). They opined that important flavour differences were found between the two classes of yoghurt which were due to acidity and not to different concentration of aroma compounds.

Viajayalakshmi and Murugesan (2001) studied the organoleptic changes in butter milk during storage at 6 to 8°C. The changes in organoleptic qualities of the butter milk were drastic, the product became sour and got separated into curd and whey and was acceptable till the third day of storage.

Bille and Keya (2002) compared yoghurt produced by heating milk to 90°C for 30 minutes in a vat and yoghurt fortified by the addition of skim milk powder. The study revealed that there was no significant difference between the two types with regard to viscosity and syneresis during the first four days after which the latter tended to whey off, became grainy in texture, and developed high acidity (2 to 2.5 per cent lactic acid). Sensory evaluation indicated that on an average, the unfortified sample of yoghurt made from milk preheated to 90°C for 30 min. was superior.

Al-kadamany *et al.* (2002) opined that flavour changes can be considered as a failure criterion to determine the shelf life of labneh compared to textural defect because textural defect was detected at a later stage than flavour change, during storage.

2.5 SHELF LIFE

Iyengar *et al.* (1967) conducted trials on the keeping quality of yoghurt made from cow and buffalo milk and the study did not reveal any appreciable difference in the keeping quality of the products made from them. They concluded that fermented milk products could be stored at 5°C up to a period of one week without any deterioration in quality.

Reddy *et al.* (1987) conducted an experiment to assess the shelf life of dahi and yoghurt from pre-concentrated milk and found that shelf life of dahi and yoghurt could be extended by four days of storage at ambient and refrigeration temperature than the products prepared with milk and stored at ambient temperatures.

Sanyal *et al.* (1990) studied the effect of preservatives for improving the shelf life of dahi and the results of sensory evaluation revealed that dahi stored at 30 ± 2°C and 4 ± 2°C were acceptable up to 20 and 45 days while control samples of dahi kept good only for two and seven days, respectively.

Al-kadamany *et al.* (2002) opined that the shelf life of concentrated yoghurt, Labneh ranged from 8.5 to 10.5 days at storage temperature of 5°C. The end of shelf life of Labneh was accompanied by an increase of 10 to 15 per cent of free whey, 0.5 to 0.6 per cent increase in lactic acid and a drop of 0.3 to 0.4 pH units.

Kamruzzaman *et al.* (2002) evaluated the shelf life of dahi during room and refrigeration temperature and reported that the product could be kept up to three days at the former storage temperature and 12 days at the latter

2.6 ANALYSIS OF CRITICAL CONTROL POINTS

Tzanetakis (1973) studied samples of starter culture used in the preparation of yoghurt and reported that the culture was infected with *Escherichia coli* and Enterobacter aerogenes. The study also revealed that the plastic cups used for coagulation of yoghurt were free of coliforms and surface of yoghurt samples was highly infected than that of the interior. During the study, it was concluded that environment constituted an important factor in the infection of the surface of yoghurt.

Microflora of 105 dahi containers collected from halwai shops in Kalyani, West Bengal was studied by Singh (1978). The total count of the rinse solution of the containers ranged between 0 and 500/ml, 500 and 1000/ml and 1000 and 3000/ml in 25, 57 and 23 containers, respectively. The coliform count in the rinse samples of 56, 30 and 19 mud pots were in the range of 0 and 10/ml, 11 and 50/ml and 51and 100/ml, respectively. Staphylococcal count in the rinse samples of 39 mud pots ranged between 0 and 10/ml. The count in the rinse samples of 40 mud pots ranged from 11 to 50/ml and the count in the rinse samples of 26 mud

pots varied between 101 and 500/ml. It was also observed that immersing the containers in boiling water for one minute reduced the total bacterial count by more than 90 per cent.

Birollo *et al.* (2001) evaluated the occurrence of bacterial contaminants in industrial processing lines of whole set sweetened yoghurt through a critical control plan. Coliforms, enterococci, yeasts and moulds were not detected in the milk after heat treatment, but a reduced contamination of 0.6 and 1.3 log cfu/ml (after starter inoculation and fermentation) of coliform bacteria was detected in the yoghurt. They opined that coliforms could be used as a measure of hygienic status in the processing and packaging of dairy products.

Goel *et al.* (2002) compared the effect of air washing and UV rays to minimize the microbial load in the air of work space of dairy plants. Air washing was given by a domestic cooler operated in the laboratory. Large numbers of trials were taken at different time of the day in the year 1998 and 1999. The study revealed that rate of reduction in the microbial load was highly correlated with the initial population of microorganisms of the work space. Higher rate of reduction was observed during UV sanitation treatment as compared to the air washing treatment.

Microbiological quality and hygienic practice during the production of naturally sour milk (NSM) made of unpasteurised milk and cultured milk (CM) made of pasteurized milk, were studied at three small holder dairies at Zimbabwe (Gran *et al.* 2002). Petrifilm™ was used for the determination of *Escherichia coli,* coliforms and aerobic mesophilic count (AMC). AMC Petrifilms used as contact plates showed that 52 per cent of the utensils at the dairies were not acceptably clean (>100 cfu/20cm^2). *E.coli* was found in 81 per cent of the samples of NSM and in all the samples of CM. Approximately 39 per cent of the NSM samples and 47 per cent of the CM samples contained more than 1000 cfu *E. coli*/ml.

Rajarajan and Nareshkumar (2003) studied the incidence of yeast and mould in dairy plant environment and reported that the count was higher in summer than in winter. The study also revealed that air samples obtained from the vat section had higher count while the storage section had the lower count.

Salustiano *et al.* (2003) evaluated the microbiological quality of air at the processing areas of a dairy plant by impaction technique and found that the yeast and mould count at the yoghurt processing area varied from 100 to 940 cfu/m^3 with an average of 294 ± 238.3 cfu/m^3. The mesophilic aerobic bacterial count ranged from 100 to 320 cfu/m^3 with an average of 212.2 ± 47.6 cfu/m^3. The authors opined that the counts were much higher than that of the standard prescribed by APHA (90cfu/m^3)

2.7 MICROBIAL STANDARDS

Netherlands Standard (1967) prescribed that the maximum product acidity of yoghurt should be 1.17g/100g lactic acid.

Davies *et al.* (1971) suggested that yoghurt at the point of sale should contain ≤ 100 viable yeast cells/ml.

Harrigan and Mc Cance (1976) suggested standards for plant in contact with products prior to pasteurization/heat treatment as 500cfu/$cm^2$for total count and <10 for coliforms as satisfactory, 500 to 2500 cfu/cm^2 as dubious and 2500 cfu/cm^2 total count and >100 coliforms as unsatisfactory.

The Indian Standards (1980a) prescribed that the maximum acidity for sweet dahi shall be not more than 0.7 per cent and for sour dahi, it shall be not more than one per cent. The maximum permissible coliform count of both sweet and sour dahi is 10/g and the yeast and mould count is 100/g.

The Turkish Standards Institute (TS 1330) prescribed that the coliform and yeast and mould count should not be more than 10 cfu/g and 100 cfu/g in yoghurt, respectively (Anon., 1989).

Indian Standards (1989) has prescribed specifications for yoghurt which state that specific lactic acid bacteria/g shall not be less than 10^6 and that *Escherichia coli, Staphylococcus aureus*, Salmonella and Shigella should be absent. The pH of yoghurt should be between 3.8 and 4.6.

International Dairy Federation, IDF (1992) suggested a minimum of 0.7 g lactic acid per 100g of yoghurt

The maximum standard plate count/L by rinse method for dairy equipments like milk can/pail, HTST pasteuriser and cream separator should not be more than 1000, 25000 and 25000, respectively (Yadav *et al.*, 1993).

The Government of British Columbia (2001) prescribed standards for industrial water used for dairy and food processing. *E.coli*, enterococci, *Pseudomonas aeruginosa* and faecal coliforms should not be present in 100 ml of raw drinking water without treatment.

Republic of Philippines Standards (2001) prescribed for yoghurt and other fermented milks state that *Salmonella* should be absent in five consecutive samples, coliforms should not be present in more than two out of five consecutive samples, the level of coliforms should not exceed 10 cfu/ml and coagulase positive *Staphylococci* should be less than 10 cfu/ml.

Microbiological specification of commercial cultures suggests that counts of mesophilic lactics, yeasts and moulds, coliforms, anaerobic spore formers and salt tolerant micrococci should not exceed 10 cfu/g. *E.coli, Streptococcus faecium* and coagulase positive *Staphylococci* should be <1 cfu/g. The culture must be free of *Salmonella*, Listeria and other pathogenic contaminants (Aneja *et al.*, 2002).

Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken to assess the microbial quality, the presence of bacterial pathogens and microbial contamination of curd during its production and the effect of refrigerated storage on microbial quality. Microbial quality of four different brands of curd *viz.* A, B, C and D retailed in Thrissur Corporation area was also assessed.

The microbial quality of fresh, retailed and refrigerated samples of curd was evaluated by estimating the total viable count (TVC), coliform count (CC), faecal streptococcal count (FSC), psychrotrophic count (PC) and yeast and mould count (YMC). All samples of curd were tested for the isolation of *Escherichia coli*, *Staphylococcus aureus*, Salmonellae, *Pseudomonas aeruginosa* and *Bacillus* species. The organoleptic qualities of curd such as colour and appearance, flavour, body and texture and product acidity and physico-chemical parameters such as titratable acidity and pH were also assessed.

In order to evaluate the critical control points of microbial contamination of curd during its production, samples such as milk, starter culture, air, water, hand washings of the workers, washings of equipments used in the preparation of curd were collected from various points of production and processing on the day of production of curd and were subjected to evaluation of the various microbial load and also the pH.

3.1 COLLECTION OF CURD SAMPLES

In the present investigation, a total of 180 freshly prepared curd samples belonging to 10 batches, each consisting of 250 g were collected from the Dairy Plant of the College of Veterinary and Animal Sciences. The samples were subjected to evaluation of the microbial quality, presence of *Escherichia coli*, *Staphylococcus aureus*, Salmonellae, *Pseudomonas aeruginosa* and *Bacillus* species and also critical control points of microbial contamination of the curd

during the various stages of production and the effect of refrigeration on the microbial quality of curd during 21 days of storage. The samples were also tested for the organoleptic and physico-chemical properties. The distribution of the samples, the day of examination and the number of samples examined on each day are shown in table 1.

Table 1. Distribution of the curd samples collected for analysis

Day of examination	No. of curd samples examined	
	Fresh	Refrigerated (4 ± 1°C)
0	20	-
3	-	20
5	-	20
7	-	20
9	-	20
12	-	20
15	-	20
18	-	20
21	-	20
Total	20	160

From each batch, 18 samples of curd were collected from the Dairy Plant immediately after packing. Each sample consisted of 250g and the samples were brought to the laboratory in insulated containers. Two samples were selected randomly and examined on zero day (day of collection). The remaining samples were stored under refrigeration (4 ± 1°C) and duplicate samples were taken randomly and examined on day 3, 5, 7, 9, 12, 15, 18 and 21 of storage.

During the investigation, a total of 80 curd samples each consisting of 500g belonging to four brands *viz.* A, B, C and D were collected from the retail outlets in the Thrissur Corporation area. From each brand, 20 samples were

collected. Only two samples belonging to a brand were collected on a day of collection and brought to the laboratory immediately for evaluation in an insulated container. From each brand the samples were collected 10 times and tested for microbial, organoleptic and physico-chemical qualities. Isolation and identification of bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, Salmonellae, *Pseudomonas aeruginosa* and *Bacillus* species were also carried out.

3.2 PROCESSING OF CURD SAMPLES FOR MICROBIAL EVALUATION

Each sample contained in a low density polyethylene sachet was opened aseptically before a Bunsen burner flame and 25 g was weighed and transferred to a sterile conical flask containing 225 ml of 0.1 per cent peptone water (diluent) and blended using a cyclomixer so as to mix the curd uniformly with the diluent to form 1 in 10 dilution. From this, 10ml of the inoculum was transferred into a conical flask containing 90ml sterile diluent and mixed thoroughly so as to form 1 in 100 dilution. Further 10 fold serial dilutions were prepared by transferring one milliliter of the inoculum into nine milliliters of the diluent. From each sample, dilutions were made up to 10^{-9} and selected dilutions were used for assessing various microbial counts. Refrigerated samples were thawed to room temperature and then processed as in the case of fresh samples.

3.3. MICROBIAL COUNTS

3.3.1 Total Viable Count

Total viable count (TVC) of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected dilution of each sample, one milliliter of the inoculum was transferred to Petri dishes of uniform size, in duplicate. To each of the inoculated plates, about 15-20 ml sterile molten standard plate count agar (Hi-media) maintained at 45°C was poured and mixed with the inoculum by gentle rotatory movement in clock-wise, anti-clock-wise, forward and backward directions. The inoculated plates were allowed to solidify

at room temperature and incubated at 37°C for 24 h. At the end of incubation period, Petri dishes with a bacterial count between 30 and 300 colonies were selected and the colony counts were taken with the help of a colony counter. The number of colony forming units (cfu) per gram of curd was calculated by multiplying the mean colony count of the duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/g.

3.3.2 Coliform Count

Coliform count (CC) per gram of sample was estimated as per the procedure described by Kornacki and Johnson (2001). Surface spread method was carried out to evaluate the bacterial load with violet red bile agar (VRBA) (Hi-media). From the selected dilutions of each sample, 0.1 ml of the inocula were transferred on to duplicate plates containing the medium and spread uniformly with a sterile L- shaped glass rod. The plates were incubated at 37°C for 24 h. At the end of incubation period, purplish red colonies with a diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. Number of organisms per gram of the sample was calculated by multiplying the mean count of the duplicate plates with the dilution factor and the count was expressed as $\log_{10}$ cfu/g.

3.3.3 Faecal Streptococcal Count

Faecal streptococcal count (FSC) was estimated as per the standard procedure prescribed by the Nordic Committee on Food Analysis (1968). Karl Friedrich (KF) streptococcal agar (Hi-media) was used for estimating the count. From the selected dilutions of each sample, 0.1ml of the inocula were transferred on to duplicate plates with the medium and was evenly spread with a sterile L-shaped glass rod. The plates were incubated at 37°C for 48 h. After the period of incubation, pink to dark red colonies with a diameter between 0.5 and 3 mm and surrounded by a narrow whitish zone were counted as faecal streptococci. The

number of organisms per gram of curd was calculated and expressed as described in coliform count.

3.3.4 Psychrotrophic Count

Psychrotrophic count (PC) of each sample was assessed by spread plate technique as suggested by Cousin *et al.* (2001). Surface spread method was carried out in duplicate plates containing plate count agar (Hi-media). Each plate was inoculated with 0.1 ml of the selected dilution of the sample as described in coliform count. The plates were incubated at 7 ± 1°C for10 days. At the end of incubation, the colonies were counted with the help of a colony counter. The number of colony forming units per gram of the sample was calculated as described in coliform count and the count was expressed as $\log_{10}$ cfu/g.

3.3.5 Yeast and Mould Count

Yeast and mould count (YMC) was estimated as per the method described by Beuchat and Consin (2001). Surface spread method was carried out in duplicate plates of potato dextrose agar (Hi-media) with 0.1 ml of selected dilution of the sample. The inoculum was evenly distributed on the plate as described in coliform count. Plates were incubated at 25°C for 2 to 5 days. At the end of incubation the colonies were counted with the help of a colony counter. The number of colony forming units per gram of the sample was calculated as described in coliform count and the count was expressed as $\log_{10}$ cfu/g.

3.4 ISOLATION AND IDENTIFICATION OF BACTERIA

Each sample was tested for the isolation and identification of *Escherichia coli, Staphylococcus aureus, Salmonella, Pseudomonas aeruginosa* and *Bacillus* species.

3.4.1 *Escherichia coli*

For the isolation of *Escherichia coli* from fresh samples of curd, a loopful of inoculum from each sample was inoculated onto duplicate plates of Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h (Indian Standards, 1980b). At the end of incubation period, three or four colonies with a dark center and a distinct indelible ink greenish black metallic sheen on deflected light were transferred on to nutrient agar slants and incubated at 37°C for overnight. The colonies were subjected to further characterisation and identification by cultural, morphological and biochemical reactions as described by Barrow and Feltham (1993) and are shown in flowchart 1. The isolates were subjected to Eijkman's test. Isolates were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

In order to isolate *Escherichia coli* from refrigerated samples of curd, 10 ml of the sample was added to 90 ml of trypticase broth and incubated at 37°C for 6 h for enrichment (Jay, 1996). From the enriched sample, a loopful of inoculum was streaked onto duplicate plates of EMB Agar. Further isolation and identification of the isolates were carried out as in the case of fresh curd samples.

3.4.2 *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, a loopful of the sample was inoculated on to Baird-Parker (BP) agar medium and was incubated at 37°C for 48 h (AOAC, 1990). At the end of incubation, colonies showing characteristic appearance (circular, smooth, convex, moist, 2 to 3 mm in diameter on uncrowded plates, grey-black to jet-back, frequently with outer clear zone and with buttery to gummy consistency on BP agar medium) were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature for further characterisation and

Flowchart 1. Isolation and identification of *Escherichia coli*

	Characteristics/Reactions
Sample	
↓	
Inoculated in EMB agar (37°C for 24 h)	
↓	
Nutrient agar	
↓	
Grams' staining reaction and cell morphology	Gram negative small rods
↓	
Mobility test	+
↓	
Growth aerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
Urease	-
↓	
ONPG	-
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate Utilization test	-
↓	
Carbohydrate utilization	
Lactose	+
Glucose	+
Mannitol	+
Inositol	-
Maltose	+

F-fermentation, + = positive, - =negative

identification following the procedure described by Barrow and Feltham (1993) and is shown in flowchart 2.

3.4.3 *Salmonella*

In order to isolate Salmonellae, 10 ml each of the sample was transferred into a sterile conical flask containing 90 ml of buffered peptone water and incubated at 37°C for 24h. At the end of the incubation period, 10ml each of the inoculum was inoculated in to 90ml tetrathionate broth (Hi-media) and to an equal quantity of selenite cystine broth (Hi-media). The contents of the flask were mixed thoroughly and the flask containing selenite cystine broth was incubated in a water bath at 43°C for 48 h and the flask containing tetrathionate broth was incubated at 37°C for 48 h. At the end of 24 and 48 h of incubation, a loopful of the culture from each of tetrathionate broth and selenite cystine broth was inoculated on to duplicate plates of Brilliant Green Agar (BGA) (Hi-media) and Mac Conkey Agar (MCA) (Hi-media) and incubated at 37°C for 24 h. At the end of incubation, colourless pink-white opaque to translucent colonies with a diameter of about 1-2 mm, surrounded by a pink or red hue on BGA and transparent colourless colonies with opaque center (Andrews *et al.*, 2001) were selected from Mac Conkey agar plates and were transferred to nutrient agar slants and incubated at 37°C for overnight and stored at refrigeration temperature for further characterisation of the isolates. The cultural, morphological and biochemical characteristics of the isolates were identified according to the procedure described by Edwards and Ewing (1972) and Barrow and Feltham (1993) and are shown in flowchart 3.

3.4.4 *Pseudomonas aeruginosa*

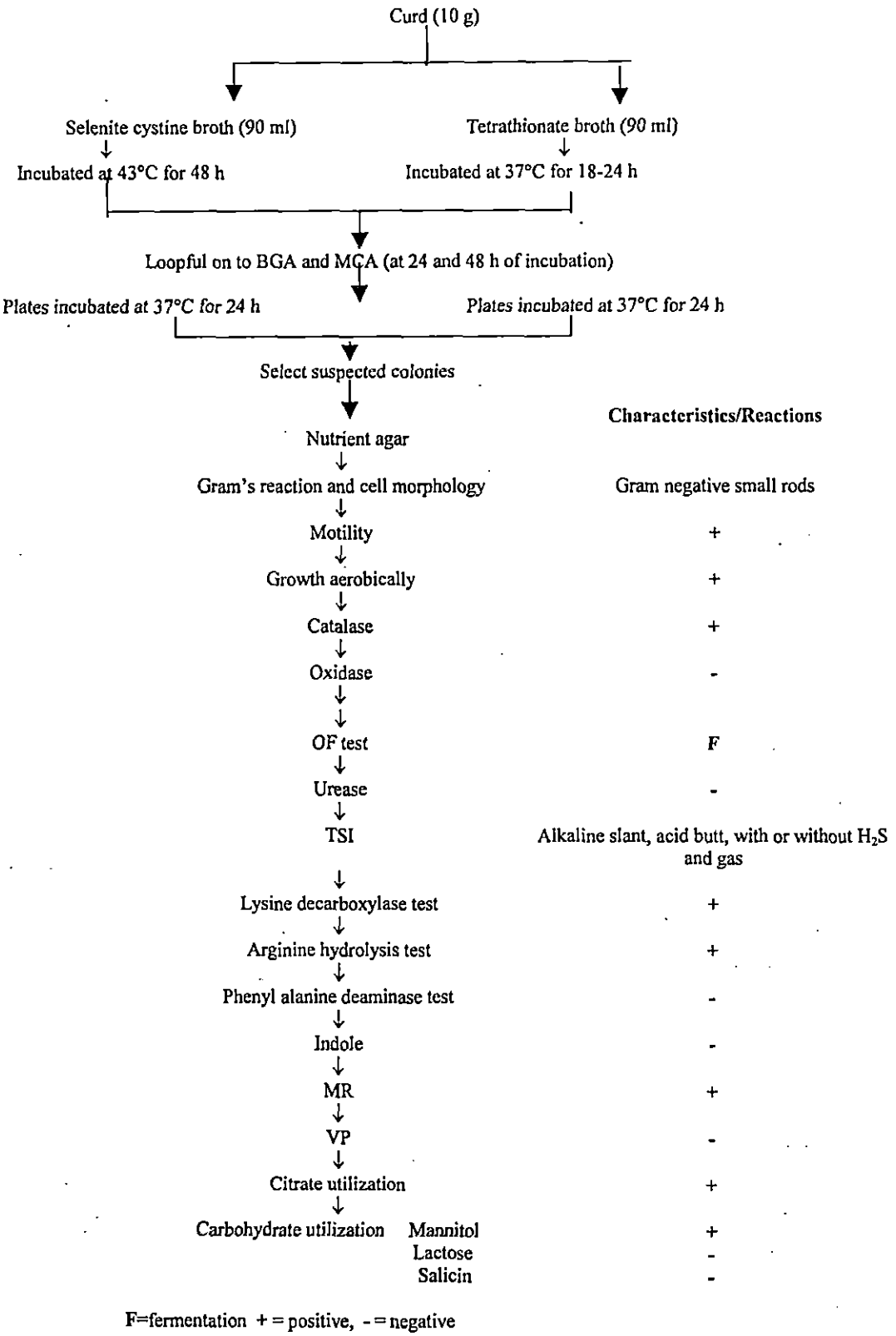
For the isolation of *Pseudomonas aeruginosa,* a loopful of the sample was streaked onto duplicate plates of Pseudomonas agar base (Hi-media) supplemented with Cetrinix (FD 029, Hi-media) and plates were incubated at 30°C for 24 h (Cousin *et al.*, 2001). At the end of incubation, pigmented/non-

Flowchart 2. Isolation and identification of *Staphylococcus aureus*

	Characteristics/Reactions
Sample	
↓	
Inoculated on to BP agar	
↓	
Nutrient agar	
↓	
Gram's staining reaction and cell morphology	Gram positive cocci in singles, pairs, cluster or bunch of grapes appearance
↓	
Motility test	-
↓	
Growth aerobically	+
↓	
Growth anaerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
VP	+
↓	
Arginine hydrolysis	+
↓	
Phosphatase	+
↓	
Gelatin liquefaction	+
↓	
Urease	+
↓	
Coagulase test	+
↓	
Thermonuclease test	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Mannitol (Aerobic)	+
(Anaerobic)	+

F = fermentation, + = positive, - = negative

Flowchart 3. Isolation and Identification of *Salmonella*



F=fermentation + = positive, - = negative

pigmented smooth circular colonies were transferred to nutrient agar slants and incubated at 30°C overnight. The isolates were stored at refrigeration temperature. The cultural, morphological and biochemical characteristics of the isolates were tested according to the procedure described by Barrow and Feltham (1993) and are shown in flow chart 4.

3.4.5 *Bacillus* species

For the isolation of *Bacillus cereus,* a loopful of the curd sample was inoculated onto duplicate plates of *Bacillus cereus* agar base (Hi-media) supplemented with Polymyxin B and incubated at 30 to 32°C for 24 h (Bennett and Belay, 2001). At the end of incubation period, peacock blue colonies surrounded by an egg yolk precipitate of similar colour were transferred to nutrient agar slants and incubated at 30°C overnight. The isolates were subjected to further characterisation and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flowchart 5.

For the isolation of aerobic spore formers, the samples were tested using standard plate count agar after heat treatment of suspension of samples at 80°C for 10 min followed by immediate cooling (Pillai *et al.*, 1993). The plates were incubated at 37°C for 24 h. Representative colonies that had developed on the plates after incubation were selected and inoculated onto nutrient agar. The isolates were identified by morphological, cultural and biochemical characteristics following the methods described by Barrow and Feltham (1993).

In vitro Pathogenicity Studies

1. ***Escherichia coli***

Congo Red Binding Assay

Congo red binding assay of the *Escherichia coli* isolates were carried out by the method given by Rajil *et al.* (2003). The medium used was Tryptone Soya Agar supplemented with 0.03 per cent Congo red dye (Nessler's) and

Flowchart 4. Isolation and identification of *Pseudomonas aeruginosa*

Curd sample		**Characteristics/Reactions**
↓		
Pseudomonas agar base (Cetrinix)*		
↓ at 30°C for 24 h		
Suspected colonies		
↓		
Nutrient agar slants		
↓ at 30°C for overnight		
Gram's reaction		Gram negative rods
↓		
Motility test		+
↓		
Oxidase		+
↓		
Catalase		-
↓		
OF – test		0
↓		
Indole		-
↓		
Arginine hydrolysis		+
↓		
Lysine		-
↓		
Ornithine		-
↓		
Citrate utilisation		+
↓		
Urease		+
↓		
Nitrate reduction		+
↓		
Carbohydrate utilization		
↓		
	Glucose	+
	Mannitol	+
	Maltose	-

O = Oxidative; + = Positive reaction; - = Negative reaction

* Supplement

Flowchart 5. Isolation and identification of *Bacillus cereus*

	Characteristics/Reactions
Curd sample	
↓	
Bacillus cereus agar	
↓	
Suspected colonies	
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram positive rods
↓	
Motility	+
↓	
Growth aerobic	+
↓	
Growth anaerobic	-
↓	
Catalase	+
↓	
OF	F
↓	
VP	+
↓	
Casein hydrolysis	+
↓	
Citrate utilisation	+
↓	
Starch hydrolysis	+
↓	
Urease	D
↓	
Nitrate	+
↓	
Carbohydrate utilization	
Glucose	-
Mannitol	-
Xylose	-
Mannitol	-

F=fermentation D = doubtful + = positive, - = negative

0.15 per cent bile salts (Loba Chemie). *E. coli* isolates were cultured on duplicate plates of the Congo red medium and incubated at 37°C for 24 h. After incubation, the cultures were left at room temperature for 48 h to facilitate annotation of results. Invasive *Escherichia coli* were identified by their ability to take up Congo red dye and production of characteristic brick red colonies.

2. ***Bacillus cereus***

Test for Hemolytic Activity

For testing the hemolytic activity, the bottom of a standard trypticase soy sheep blood agar plate was marked into 6 or 8 equal segments (Bennett and Belay, 2002). Each segment was labelled and inoculated about its center by gently touching the agar surface with a 2 mm loopful of culture. The plate was then incubated at 30 to 32°C for 24 h and checked for hemolytic activity as indicated by a zone of complete haemolysis surrounding the growth. *Bacillus cereus* was found to be strongly hemolytic.

3.5 CHARACTERISATION AND IDENTIFICATION OF ISOLATES

The isolates were identified by the following tests.

3.5.1. Primary Identification Tests

1. Catalase test

a) Slide test

A small quantity of colony was transferred onto a clear, grease free, glass slide and mixed well with a drop of three per cent hydrogen peroxide. Evaluation of effervescence with in a few seconds indicated a positive reaction.

b) Tube test

One ml of three per cent hydrogen peroxide solution was poured over the slope of a nutrient agar slant on which the isolate was grown. A positive reaction

was indicated by the development of effervescence immediately (Barrow and Feltham, 1993).

2. Gram's staining

The procedure for gram staining was as follows:

a. A thin smear of each isolate was made on a clean, grease free glass slide. Air-dried the smear and then heat fixed by passing over a flame.

b. The smear was then flooded with 0.5 per cent crystal violet in water and allowed to act for 30 sec.

c. Poured off the stain and washed with water.

d. Flooded the smear with Grams' iodine solution (one per cent iodine and two per cent potassium iodide in water) for 30 sec.

e. Poured off the solution and the smear was decolourised with a few drops of acetone and allowed to act for two to three sec.

f. Washed the smear and counter stained with dilute carbol fuchsin for 30 sec.

g. Poured off the stain from the slide, washed, dried and examined under oil immersion objective of the microscope (Barrow and Feltham, 1993).

3. Motility test

Motility of the organism was assessed by stabbing the isolate into the Hugh and Leifson's medium with a straight wire up to a depth of 5 mm. Motility was indicated by a spreading growth into the medium from the line of inoculations and growth of non-motile organisms was confirmed to the stab (Barrow and Feltham, 1993).

4. Oxidase test

A filter paper strip was moistened with a few drops of an aqueous solution of tetramethyl paraphenyline diamine dihydrochloride (1%). Each isolate was then smeared across the paper strip with a platinum loop. The appearance of a dark purple colour on the paper strip within 30 second indicated a positive reaction (Barrow and Feltham, 1993).

5. Oxidation – Fermentation test

Each isolate was inoculated into duplicate tubes of Hugh and Leifson's media by stabbing with a straight wire. One of the tubes was sealed with a layer of melted soft paraffin to a depth of about 3 cm above the medium. The tubes were incubated at 37°C for up to 14 days. A change in colour of the medium from green to yellow in the open tubes alone was taken as oxidation whereas a change in colour from green to yellow in both the tubes was regarded as fermentation. Absence of colour change in both tubes indicated no action on carbohydrates (Barrow and Feltham, 1993).

3.5.2 Secondary Tests

1. Aesculin hydrolysis

The organism was inoculated into aesculin broth and was incubated at 37°C and examined daily for five days. Blackening of the broth due to hydrolysis of aesculin indicated a positive reaction (Barrow and Feltham, 1993).

2. Arginine hydrolysis

The organism was inoculated into five ml of arginine broth and was incubated at 37°C for 24 h. At the end of incubation period, added 0.25 ml of Nessler's reagent. Arginine hydrolysis was indicated by the development of brown colour (Barrow and Feltham, 1993).

3. Casein hydrolysis

The casein agar plates were inoculated with the organism and incubated at 37°C for 48 h and examined. A clear zone around the colonies was taken as the positive reaction (Barrow and Feltham, 1993).

4. Carbohydrate utilisation test

Each isolate was inoculated into two test tubes containing peptone water with Andrade's indicator and one per cent of the appropriate sugar. One of the tubes contained an inverted Durham's tube. The inoculated tubes were incubated at 37°C and examined daily for seven days to detect the production of acid and/or gas. A change in colour of the medium to pink indicated acid production and the production of gas was indicated by the appearance of air bubbles in the inverted Durham's tube. Anaerobic condition of the medium was provided by adding a layer of sterile molten soft paraffin to a depth of about one centimeter above the medium (Barrow and Feltham, 1993).

5. Citrate utilisation test

A light suspension of the organism was made in normal saline and was inoculated with a straight wire onto the slope of Simmon's citrate agar. The inoculated medium was incubated at 37°C and examined daily up to seven days. The ability of the organism to utilise citrate as the sole source of carbon is indicated by a change in colour of the medium from green to blue and growth of the organism along the streak line (Barrow and Feltham, 1993).

6. Coagulase test

a) Slide test

A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. Macroscopic clumping with in few

seconds indicated a positive result and delayed clumping was considered as a negative reaction.

b) Tube test

Mixed 0.5 ml undiluted rabbit plasma with an equal volume of an 18 to 24 h broth culture of the test organism and incubated at 37°C and examined after one and four hours for coagulation. Negative tubes were left at room temperature overnight and re-examined (Barrow and Feltham, 1993).

7. Decarboxylase reaction

Each isolate was heavily inoculated with straight wire into three test tubes containing decarboxylase media. One of the tubes contained lysine and other contained ornithine. The third tube was taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a positive reaction, the medium first turned yellow and then became purple and the control tubes remained yellow (Barrow and Feltham, 1993).

8. Eijkman test

Each test organism was inoculated into tubes containing Mac Conkey broth with inverted Durham's tube, warmed to 37°C and incubated at 44 ± 0.1°C in a water bath for 48 h. Production of both acid and gas indicated a positive reaction (Barrow and Feltham, 1993).

9. Gelatin hydrolysis/liquefaction

Each isolate was inoculated into nutrient gelatin and incubated at 37°C up to 14 days. An uninoculated control tube was also set. The tubes were cooled every two to three days in a refrigerator for 2 h and then examined for liquefaction. A positive result was indicated by liquefaction of gelatin (Barrow and Feltham, 1993).

10. Hippurate hydrolysis

The slope of hippurate agar was lightly inoculated with the test organism and examined daily for seven days. Hydrolysis of hippurate was indicated by growth and the development of a pink colour due to alkali production (Barrow and Feltham, 1993).

11. Indole production

The isolate was inoculated into peptone water and incubated at 37°C for 48 h. At the end of incubation added 0.5 ml of Kovac's reagent mixed well and examined. A red colour in the reagent layer indicated a positive reaction (Barrow and Feltham, 1993).

12. Methyl red (MR) reaction

The MR-VP medium was inoculated with the isolate and incubated at 37°C for two days. Added two drops of methyl red solution at the end of incubation period and examined. Development of a red colour indicated positive reaction (Barrow and Feltham, 1993).

13. ONPG (O-nitrophenyl-P-D-galactopyranoside) test

Each isolate was inoculated into ONPG broth and incubated at 37°C for 48 h. The ß-galactosidase activity of the organism was indicated by the development of a yellow colour due to the production of O-nitrophenol (Barrow and Feltham, 1993).

14. Phenylalanine deamination

The phenylalanine agar slope was heavily inoculated with the test organism and incubated at 37°C for overnight. At the end of incubation, 0.2 ml of 10 per cent aqueous solution of ferric chloride was poured over the slope. A positive result was indicated by the development of a green colour on the slope and in the free liquid at the base (Barrow and Feltham, 1993).

15. Phosphatase test

The phenolphthalein phosphate agar was lightly inoculated with the test organism to obtain discrete colonies and incubated at 37°C for 18 h. At the end of incubation, 0.1 ml of ammonia solution (specific gravity –0.880) was placed in the lid of the Petri dish and the medium was inverted above it. Free phenolphthalein liberated by phosphatase reacted with the ammonia and phosphatase positive colonies became bright pink (Barrow and Feltham, 1993).

16. Starch hydrolysis

Starch agar plate was inoculated with the organism and incubated at 37°C for 5 days. The plate was flooded with Lugol's iodine solution. A clear zone around the culture indicated a positive reaction (Barrow and Feltham, 1993).

17. Thermonuclease test

The isolate was inoculated onto DNase agar plates and incubated at 37°C for 24 h. Flooded the surface of the plate with one per cent toluidine blue indicator solution. The presence of pink colour around the colonies indicated a positive reaction (Lancette and Bennett, 2001).

18. Triple sugar iron agar test

Each isolate was stab inoculated into the butt of triple sugar iron agar with straight wire and the slope of the agar was streaked with the wire. The inoculated tubes were incubated at 37°C for 24 h. The tubes were examined at the end of incubation for the development of an alkaline slant and an acid butt with or without the production of hydrogen sulphide (Edwards and Ewing, 1972).

19. Urease activity

Slopes of Christensens' urea agar was heavily inoculated with the test organism and incubated at 37°C. The tubes were examined after 4 h of incubation

and daily for 5 days. Development of a red colour in the medium indicated a positive reaction (Barrow and Feltham, 1993).

20. Voges-Proskauer reaction

The MR-VP medium inoculated with the isolate was subjected to methyl-red test. After completion of the test, added 0.6 ml of 5 per cent ά- naphthol solution and 0.2 ml of 40 per cent aqueous potassium hydroxide into the tube. After thorough mixing of the contents, the tube was kept in a slanting position and examined after 15 min and one hour. A positive reaction was indicated by the development of a strong red colour (Barrow and Feltham, 1993).

3.6 EVALUATION OF PHYSICO-CHEMICAL QUALITY

All samples of curd collected from the Dairy Plant and retail markets were subjected to evaluation of the physico-chemical characteristics such as titratable acidity and pH.

3.6.1 Titratable Acidity

From each sample of curd, 10 g was measured accurately into a clean, dry beaker and added an equal volume of freshly boiled and cooled water. Added 1 ml of phenolphthalein indicator solution to the mixed sample and titrated against 0.1 N NaOH solution drop by drop from the burette until a faint but definite and persistent pink colour was developed, which was the end point (Scott *et al.*, 2001).

3.6.2 Measurement of pH

From each sample of curd, 20g was transferred into a clean, dry 50ml beaker and the pH of the sample was measured using a digital pH meter (Elico LI 612 pH analyser).

3.7 EVALUATION OF ORGANOLEPTIC QUALITY

The samples were subjected to sensory evaluation by a four member panel using the score card prescribed by Ranganadham and Gupta (1987) with slight modifications in awarding marks to flavour, body and texture and product acidity. For every member of the panel, 15 to 20 g curd from each sample was served. Average score obtained from four members of the panel for each sample was used for statistical analysis. The score card used in the study is shown in appendix.

3.8 ASSESSMENT OF CRITICAL CONTROL POINTS DURING THE PREPARATION OF CURD

The samples of milk, starter culture, air, water, washings of personnel's hand, equipments and packaging materials were collected from different areas of the milk processing plant and evaluated the microbiological quality of these samples to determine the critical points of microbial contamination of curd during the various stages of production and are shown in table 2.

1. Raw milk

During the study, 10 samples of pooled raw buffalo milk were collected from the Dairy Plant on receipt of milk from the dairy farm. The milk in the can was thoroughly mixed with a plunger and transferred about 250 ml milk into a sterile conical flask. All samples were collected aseptically and brought to the laboratory in an insulated container and evaluated the microbial load of each sample according to the standard procedures. Only one sample was procured on the day of collection.

2. Pasteurised milk

In the present study, 10 pasteurised milk samples were collected immediately after pasteurisation of milk in a sterile conical flask. Each sample consisted of 250 ml. At a time, only one sample was collected.

Table 2. Details of sample collection for critical control point assessment

	Reception and storage of raw milk	
	↓	
Equipment wash	Pasteurisation	Before Air After
	↓	
Equipment wash	Cream separation	Before Air After
	↓	
Sample from vat	Thermal treatment (90°C,15-30 min)	Before Air After
	↓	
Storage can wash	Cooling to 40°C	
	↓	
	Starter inoculation	
	↓	
	Incubation at 28 – 32°C	
Inoculated sample after 1 h & 6 h of incubation	↓	
	Bulk storage at cold room	Air
	↓	
Package material wash	Packaging	
	↓	
	Cold storage	

3. *Skim milk*

A total of 10 skim milk samples, each consisting of about 250 ml were collected from the skim milk sprout of the cream separator. Before collecting the sample, skim milk was allowed to run for a few minutes and the sample was collected into a sterile conical flask and transferred to the laboratory for further processing. At a time, only one sample was collected.

4. *Heat treated skim milk*

During the study, 10 heat treated skim milk samples were collected in a sterile conical flask and brought to the laboratory for evaluating the microbial load. At a time, only one sample was collected.

5. *Starter culture*

In the present investigation, 10 starter culture samples, each consisting of 250 g were collected using a sterile spatula and transferred into a sterile conical flask. On the day of collection, only one sample was collected and brought to the laboratory for microbiological evaluation.

6. Milk after one hour of inoculation

A total of 10 samples of starter inoculated, cooled milk, after one hour of inoculation was collected into a sterile conical flask. Each sample consisted of about 250 ml and brought to the laboratory for microbial load evaluation. During each visit, only one sample was collected.

7. Milk after six hour of inoculation

In the present investigation, 10 starter inoculated milk samples, after six hours of incubation were collected into a sterile conical flask. Each sample consisted of 250 g and during each visit, only one sample was collected.

Processing of samples

In order to estimate the microbial load per milliliter or per g of the samples collected, after agitation, 25 ml or 25 g of each sample was transferred into 225 ml of 0.1 per cent peptone water (diluent). The sample was thoroughly mixed and further consecutive 10 fold serial dilutions were made by transferring one ml of inocula into nine ml of diluent. Dilutions were made up to 10^{-7}. The selected serial dilution of each sample of milk and starter culture was used to estimate the total viable count (TVC), Coliform count (CC), *Escherichia coli*

count (ECC) and faecal streptococcal count (FSC) as per the method described by Mortan (2001), Kornacki and Johnson (2001), Indian Standards (1980b) and Nordic Committee on Food Analysis (1968), respectively. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as $\log_{10}$cfu/ml or g.

8. Air

1. Total viable count

Direct exposure method prescribed by Evancho *et al.* (2001) was employed for the enumeration of air borne organisms before and after each process in different areas of curd production line. To estimate the bacterial counts in air, duplicate Petri dishes (90mm diameter) containing sterile nutrient agar medium was exposed for 15 min. The plates were brought to the laboratory in insulated container and incubated at 37°C for 48 h. The number of colonies developed in the duplicate plates was counted and the mean count of the plates was expressed as cfu/ft^2/min.

2. Yeast and mould count

In order to estimate the yeast and mould count, procedure described by Evancho *et al.* (2001) was followed. Duplicate plates of Potato Dextrose Agar (Hi-media) medium were exposed in the processing area before and after each process and the plates were brought to the laboratory in an insulated container and incubated at 25°C for 2 to 5 days and the mean count was expressed as cfu/ft^2/min.

9. Water

Collection of samples

a) Water in the storage tank

A storage tank within the premises of the dairy plant building was the source of water supply for the various operations in the plant. A clean sterile bottle of 250ml capacity was held by its bottom and plunged its neck downwards below the surface of the water. The bottle was then turned until the neck pointed slightly upwards. When the bottle was filled with water, it was raised above the surface of water and the stopper was replaced. The water samples were transported to the laboratory in an insulated container.

b) Tap water

Samples were collected from a tap in regular use at the dairy plant, in the pasteurisation room. Allowed the water from the tap to run to waste for about two minutes to flush the interior of the nozzle and discharge the stagnant water. In order to collect the sample, a sterile bottle of 250 ml capacity was held near the base of the tap and filled from a gentle stream of water from the tap, avoiding splashing and brought to the laboratory in an insulated container.

c) Hand washings

The samples for the estimation of bacterial load on the hands of personnel were collected from one randomly selected individual involved in processing operations. The plant was visited 10 times and on each visit the selected individual's hand was washed in 100 ml of 0.1 per cent sterile peptone water and the washing was collected into a sterile conical flask and brought to the laboratory in an insulated container.

Processing of water samples

Samples brought to the laboratory were agitated vigorously for about 25 times. In order to estimate the bacterial load per ml of water sample, 10 ml was transferred into 90 ml of sterile quarter strength Ringer's solution so as to form one in 10 dilution of the sample. Further 10 fold dilution of the sample was made by transferring one ml of inoculum to nine ml of the diluent. Dilutions were made up to 10^{-4}. The selected dilutions were used for the estimation of microbial counts as described by Indian Standards (1978).

10. Processing Equipments

Swab contact method and rinse method described by Evancho *et al.* (2001) was followed to collect samples from the double jacketed vat, cream separator, pasteurisation equipment and milk storage can for the estimation of bacterial count.

a. Double jacketed vat

To collect samples from the double jacketed vat, a sterile cotton swab was moistened with 0.1 per cent peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial with a rotating motion. The swab head was rubbed slowly and thoroughly over 100 cm^2 surfaces, which was marked with a sterile aluminium template (10 x 10 cm^2 interval areas). The swab was rubbed three times reversing the direction between strokes. After swabbing, the head of swab was cut with sterile scissors and transferred into sterile flask containing 100 ml of 0.1 per cent peptone water and brought to the laboratory in an insulated container. The flask was thoroughly agitated with the help of a cyclomixer at 8000 rpm for three minutes so as to extricate the bacteria attached to the cotton swab into the diluent.

b. Pasteurisation equipment / cream separator / milk storage can

Rinse method (Evancho *et al.*, 2001) was followed for the collection of samples from cream separator, pasteurisation equipment and milk storage can.

Pasteurisation equipment washing

Everyday, the HTST pasteuriser is thoroughly cleaned after pasteurization of milk by alkali treatment and thereafter with hot water circulation. Before pasteurisation the pasteuriser is again cleaned with hot water circulation followed by chilled water. A sample of one litre of chilled water washing was collected in a sterile conical flask and was brought to the laboratory in an insulated container.

Cream separator

One litre of sterile 0.1 per cent peptone water was poured into the processing equipment and was then collected from the skim milk sprout of the cream separator. Before collecting the sample, the sprout was opened and allowed to run for a few minutes and the samples were collected into a sterile conical flask and transferred to the laboratory in an insulated container.

Storage can

One litre of sterile 0.1 per cent peptone water was poured into the storage can and mixed thoroughly by agitation. The sample was transferred into a sterile conical flask and brought to the laboratory in an insulated container.

Serial 10 fold dilutions were prepared and estimation of bacterial counts was done as in the case of water samples. The bacterial count of the double jacketed vat was expressed as colony forming units (cfu) per cm^2 and that of cream separator; bulk milk storage can and pasteurisation equipment was expressed as cfu/ml.

11. Packaging materials

In order to assess the microbiological quality of low density polyethylene bags (250g capacity), 20 ml of 0.1 per cent sterile peptone water was poured into the bag and was agitated ten times by holding in both horizontal and vertical positions (Evancho *et al.*, 2001). The samples were then collected into a sterile conical flask by keeping the pouches upright.

The data obtained in the study were subjected to statistical analysis as per the procedure described by Rangaswamy (1995).

Results

4. RESULTS

In the present study, the effect of refrigeration with respect to microbial, physico-chemical and organoleptic qualities of curd samples were assessed and compared with that of the fresh sample. The samples were also tested for the isolation and identification of bacterial pathogens of public health significance. Critical control points of microbial contamination of curd in the production line were evaluated during the study. Microbial, physico-chemical and organoleptic qualities of four brands of curd samples retailed in and around Thrissur corporation were also assessed.

4.1 EFFECT OF REFRIGERATED STORAGE ON QUALITY OF CURD

4.1.1 Microbial Counts

4.1.1.1 Total Viable Count

The mean total viable count of fresh curd samples and the samples stored under refrigeration for 21 days is given in table 3 and Fig.1. The data obtained

Table 3. Mean total viable count of fresh and refrigerated curd samples

Days of storage	Mean ± SE (log_{10}cfu/g)
0	8.28 ± 0.16^{a}
3	8.87 ± 0.10^{b}
5	9.20 ± 0.19^{bc}
7	8.73 ± 0.28^{abcd}
9	8.32 ± 0.24^{abde}
12	8.12 ± 0.21^{ade}
15	7.22 ± 0.25^{f}
18	7.03 ± 0.38^{fg}
21	6.46 ± 0.28^{g}

Figures bearing the same superscript do not differ significantly
N=20 samples examined on each day

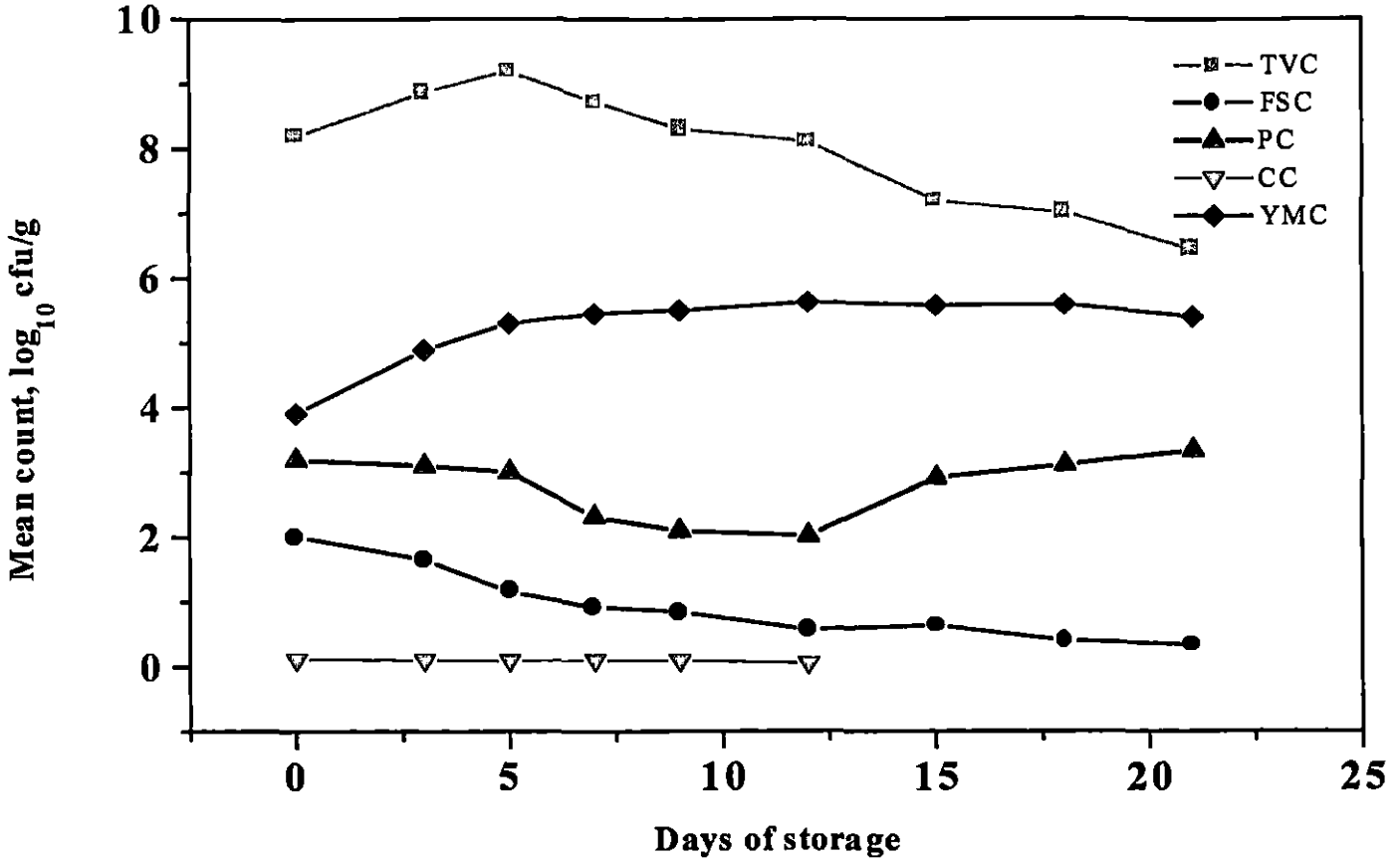


Fig 1. Variation in mean microbial count of fresh and refrigerated curd samples

were subjected to statistical analysis by paired 't' test. The mean total viable count of fresh curd samples increased significantly ($p<0.05$) from 8.28 ± 0.16 log_{10}cfu/g to 8.87 ± 0.10 log_{10}cfu/g on the third day of storage. But the increase was highly significant ($p<0.01$) on the fifth day of storage. Highly significant ($p<0.01$) difference was observed between the mean count of fresh samples and the count of the samples on day 15, 18 and 21 of storage. The mean count of the samples stored for 21 days was significantly different from the mean count of all samples, except the samples stored on 18th day.

Distribution of fresh and refrigerated samples of curd based on level of total viable count is shown in table 4. The count increased to the level of

Table 4. Distribution of fresh and refrigerated samples of curd based on level of total viable count

Days of storage	Total viable count (cfu/g)					
	10^5	10^6	10^7	10^8	10^9	10^{10}
0	-	-	2(10)	16 (80)	2 (10)	-
3	-	-	-	10 (50)	10 (50)	-
5	-	-	-	9(45)	10 (50)	1 (5)
7	-	-	3 (15)	11 (55)	5(25)	1(5)
9	-	-	-	14 (70)	6 (30)	-
12	-	-	8(40)	10 (50)	2 (10)	-
15	-	8 (40)	10 (50)	2 (10)		-
18	5 (25)	6 (30)	5 (25)	4 (20)	-	-
21	8 (40)	6 (30)	6 (30)	-	-	-

Figures in parenthesis indicate per cent

10^{10}cfu/gon the fifth and seventh day of storage and thereafter a gradual decrease in the count was observed as the period of storage increased. The count in 80 per cent of fresh samples was at the level of 10^8cfu/g. On the third day of storage, 50

per cent samples each had count at the level of 10^8 and 10^9cfu/g, respectively. One of the samples had count at the level of 10^{10}cfu/g on day 5 of storage and the level was maintained on day seven of storage. However, the count in 70 per cent of the samples was reduced to the level of 10^8cfu/g on day nine of storage. The count in 50 per cent samples each was at the level of 10^8 and 10^7cfu/g on days 12 and 15 of storage, respectively. On day 18 of storage, the count in 30 per cent samples was at the level of 10^6cfu/g and on 21st day of storage, 40 per cent samples had count at the level of 10^5cfu/g.

4.1.1.2 Coliform Count

Mean coliform count of fresh and refrigerated curd samples is given in table 5 and fig.1. The mean coliform count in freshly prepared curd was

Table 5. Mean coliform count of fresh and refrigerated curd samples

Days of storage	Mean ± SE (log_{10}cfu/g)
0	0.12 ± 0.12^{a}
3	0.11 ± 0.11^{ab}
5	0.10 ± 0.10^{abc}
7	0.11 ± 0.11^{abcd}
9	0.10 ± 0.10^{abcde}
12	0.06 ± 0.06^{abcdef}
15	ND
18	ND
21	ND

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

0.12 ± 0.12 log_{10}cfu/g. The counts showed a gradual decreasing trend till the 12th day of storage. The samples stored on days 15, 18 and 21 did not reveal the presence of organism.

Distribution of fresh and refrigerated samples of curd based on level of coliform count is shown in table 6. Ninety per cent of the samples examined

Table 6. Distribution of fresh and refrigerated samples of curd based on level of coliform count

Days of storage	Coliform count (cfu/g)	
	Nil	10^1
0	18(90)	2 (10)
3	18(90)	2(10)
5	18(90)	2(10)
7	18(90)	2(10)
9	19(95)	1(5)
12	19 (95)	1(5)
15	20(100)	-
18	20(100	-
21	20(100)	-

Figures in parenthesis indicate per cent

on zero day did not reveal the presence of coliforms. The same trend continued up to seven days of storage. However, 95 per cent of the samples stored on day 9 and 12 did not reveal the presence of the organism and none of the samples stored on days 15, 18 and 21 had the organism. The organism was present in 10 per cent of the samples examined on day 0, 3, 5 and 7 at the level of 10cfu/g whereas 5 per cent samples each had count at the above level on day 9 and 12 of storage.

4.1.1.3 Faecal Streptococcal Count

Mean faecal streptococcal count of fresh and refrigerated curd samples is given in table 7 and fig.1. A decreasing trend in faecal streptococcal count was observed during storage. The reduction in count on days five and seven was

Table 7. Mean faecal streptococcal count of fresh and refrigerated curd samples

Days of storage	Mean ± SE (log_{10}cfu/g)
0	2.01 ± 0.40^{a}
3	1.66 ± 0.34^{ab}
5	1.16 ± 0.24^{bc}
7	0.92 ± 0.22^{bcd}
9	0.85 ± 0.20^{cde}
12	0.57 ± 0.17^{def}
15	0.64 ± 0.17^{cdefg}
18	0.41 ± 0.21^{defgh}
21	0.34 ± 0.15^{defh}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

significantly ($p<0.05$) different from that of the count on the zero day. The count on 21st day reduced from that of zero day at a highly significant level ($p<0.01$). Counts on 18th and 21st days were lower than that of third day and the difference in count was highly significant ($p<0.01$). The counts on 21st day were significantly ($p<0.05$) lower than that of ninth day.

Distribution of fresh and refrigerated samples of curd based on level of faecal streptococcal count is shown in table 8. On the day of production, 30 per cent samples had count at the level of 10^3cfu/g, where as on the third day, only 10 per cent of the samples had the count at the above level. On fifth day of storage, 70 per cent of the samples had count at the level of 10cfu/g. The count in 80 per cent samples each, on seventh and ninth day of storage was at the level of 10cfu/g. As the storage days increased to18th and 21st day, 70 per cent each of the samples did not reveal the presence of faecal streptococci.

Table 8. Distribution of fresh and refrigerated samples of curd based on level of faecal streptococcal count

Days of storage	Faecal streptococcal count (cfu/g)			
	Nil	10^1	10^2	10^3
0	4(20)	4(20)	6 (30)	6(30)
3	4(20)	6 (30)	8 (40)	2(10)
5	4(20)	14(70)	2(10)	-
7	4(20)	16(80)	-	-
9	4(20)	16(80)	-	-
12	8(40)	12(60)	-	-
15	8(40)	12(60)	-	-
18	14 (70)	6 (30)	-	-
21	14 (70)	6 (30)	-	-

Figures in parenthesis indicate per cent

4.1.1.4 Psychrotrophic Count

Mean psychrotrophic count of fresh and refrigerated curd samples is given in the table 9 and fig.1. The mean psychrotrophic count of fresh curd samples was 3.20 ± 0.24 $\log_{10}$cfu/g. No significant difference was observed between the counts of fresh curd samples and those refrigerated up to seven days. Highly significant ($p<0.01$) difference was noticed between the counts of fresh curd and that stored for 9 and 12 days and between counts on 5th and 12th day stored samples. The difference between the mean count of the samples on the seventh day of storage and that of 15th and 18th day were significant ($p<0.05$), but the difference between the mean count of the samples stored on 7th and 21st day was at a highly significant ($p<0.01$) level. The mean counts of the samples on 18th day was increased at a highly significant ($p<0.01$) level as compared to that of the mean count of the samples on 12th day. The mean count of the samples on 18th and 21st day of storage were significantly ($p<0.05$) increased as compared to the

Table 9. Mean psychrotrophic count of fresh and refrigerated curd samples

Days of storage	Mean ± SE (log_{10}cfu/g)
0	3.20 ± 0.24^{a}
3	3.11 ± 0.24^{ab}
5	3.01 ± 0.09^{abc}
7	2.32 ± 0.32^{abcd}
9	2.10 ± 0.28^{de}
12	2.03 ± 0.29^{de}
15	2.92 ± 0.31^{abcef}
18	3.12 ± 0.17^{abcfg}
21	3.34 ± 0.23^{abcfgh}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

mean count of the samples on ninth day of storage. The reduction in the mean counts of the samples stored on 15th and 21st day was significantly ($p<0.05$) different from that of the mean count of the samples on day 12 of storage.

Distribution of fresh and refrigerated samples of curd based on level of psychrotrophic count is shown in table 10. The count in 15 per cent of the fresh curd samples was at the level of 10^4cfu/g, but the count in 55 per cent of the samples was at the level of 10^3cfu/g. The count at the above level was observed in 50 per cent of the samples on day 3, 15 and 21 of storage. Sixty per cent of the samples stored on fifth day had count at the level of 10^2cfu/g.The count at the above level was observed in 50 per cent of the samples stored on day 7, 12 and 18. An equal per cent of sample stored on day nine had count at the level of 10cfu/g.

Table 10. Distribution of fresh and refrigerated samples of curd based on level of psychrotrophic count

Days of storage	Psychrotrophic count (cfu/g)				
	10^1	10^2	10^3	10^4	10^5
0	-	6 (30)	11 (55)	3(15)	-
3	-	7 (35)	10 (50)	1(5)	-
5	-	12(60)	8(40)	-	-
7	5(25)	10(50)	5(25)	-	-
9	10 (50)	6(30)	4(20)	-	-
12	8(40)	10(50)	2(10)	-	-
15	-	8(40)	10(50)	2(10)	-
18	-	10 (50)	8(40)	2(10)	-
21	-	8 (40)	10(50)	-	2(10)

Figures in parenthesis indicate per cent

4.1.1.5 *Yeast and Mould Count*

Mean yeast and mould count of fresh and refrigerated curd samples is given in table 11 and Fig.1. Analysis of the data revealed a highly significant

Table 11. Mean yeast and mould count of fresh and refrigerated curd samples

Days of storage	Mean ± SE (log_{10}cfu/g)
0	3.91 ± 0.11^{a}
3	4.89 ± 0.12^{b}
5	5.30 ± 0.19^{bc}
7	5.45 ± 0.53^{cd}
9	5.50 ± 0.18^{cde}
12	5.63 ± 0.19^{cdef}
15	5.58 ± 0.19^{cdefg}
18	5.59 ± 0.19^{cdefgh}
21	5.40 ± 0.14^{cdefgh}

Figures bearing the same superscript do not differ significantly

($p<0.01$) increase in yeast and mould count on all samples subjected to refrigerated storage, compared to fresh curd samples. The difference between the mean counts of the samples on day 3 with that of days 7, 9, 15, 18 and 21 was significant ($p<0.05$). However, the mean count of the samples on day 12 showed a highly significant ($p<0.01$) increase as compared to that of the samples on the third day of storage

Distribution of fresh and refrigerated samples of curd based on level of yeast and mould count is given in table12. In fresh curd samples, 75 per cent had a yeast and mould count at the level of 10^3cfu/g and the maximum count was at

Table 12. Distribution of fresh and refrigerated samples of curd based on level of yeast and mould count

Days of storage	Yeast and mould count (cfu/g)			
	10^3	10^4	10^5	10^6
0	15(75)	5 (25)	-	-
3	-	15 (75)	5(25)	-
5	-	4 (20)	12(60)	4(20)
7	-	2(10)	14(70)	4(20)
9	-	4(20)	12(60)	4(20)
12	-	2(10)	13(65)	5 (25)
15	-	2(10)	14(70)	4(20)
18	-	4(20)	12(60)	4(20)
21	-	4(20)	16(80)	

Figures in parenthesis indicate per cent

the level of 10^4cfu/g. On the third day of storage, 75 per cent of the samples had count at the level of 10^4cfu/g. On day 5, 9 and 18 of storage, 60 per cent samples had count at the level of 10^5cfu/g. Seventy per cent of the samples on day 7 and 15 of storage had counts at the level of 10^5cfu/g. On 12^{th} day of storage, 25 per

cent samples had count at the level of 10^6cfu/g. However, the count in 80 per cent samples was at the level of 10^5cfu/g on day 21 of storage.

4.1.1.6 Relationship between the Microbial Counts

Total viable count

Correlation coefficient between mean total viable count and other microbial counts of fresh and refrigerated curd samples was calculated using Spearman's rank correlation test and are given in table13. Analysis of data revealed a positive and highly significant ($p<0.01$) association between the mean TVC and FSC on 21st day of storage. A significant ($p<0.05$) and negative association was observed between the mean TVC and PC on day nine of storage.

Table 13. Correlation between the mean total viable count and other microbial counts of fresh and refrigerated curd samples

Microbial counts	Mean total viable count (TVC)								
	Days of storage								
	0	3	5	7	9	12	15	18	21
CC	0.37	0.19	0.22	0.28	0.61	0.58	0.50	0.50	0.50
FSC	-0.35	-0.12	-0.08	-0.29	-0.08	0.08	0.04	0.27	0.74**
PC	-0.32	-0.08	0.15	-0.22	-0.63*	-0.02	0.15	0.18	-0.39
YMC	-0.50	-0.21	0.22	0.02	-0.07	0.05	0.08	-0.31	-0.50

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count,*=$p<0.05$, **$p<0.01$

Coliform count

Correlation between the mean coliform count and other microbial counts of fresh and refrigerated curd samples are given in table 14. Positive correlation was obtained between CC and all other counts. Between mean CC and FSC of samples on day 18 of storage, a positive and significant association ($p<0.05$) was observed. A similar association was observed between the mean CC and PC of samples on 12th day of storage.

Table14. Correlation coefficient between the mean coliform count and other microbial counts of fresh and refrigerated curd samples

Microbial counts	Mean coliform count (CC)								
	Days of storage								
	0	3	5	7	9	12	15	18	21
TVC	0.37	0.19	0.22	0.28	0.61	0.58	0.50	0.50	0.50
FSC	0.22	0.22	0.21	0.52	0.29	0.55	0.52	0.67*	0.56
PC	0.35	0.28	0.35	0.43	0.12	0.64*	0.50	0.50	0.50
YMC	0.52	0.33	0.09	0.09	0.09	0.25	0.50	0.51	0.50

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count, *=p<0.05

Faecal streptococcal count

Correlation between the mean faecal streptococcal count and other microbial counts of fresh and refrigerated curd samples are given in table 15.

Table 15. Correlation coefficient between the mean faecal streptococcal count and other microbial counts of fresh and refrigerated curd samples

Microbial counts	Mean faecal streptococcal count (FSC)								
	Days of storage								
	0	3	5	7	9	12	15	18	21
TVC	-0.35	-0.12	-0.08	-0.29	-0.08	0.08	0.04	0.27	0.74**
CC	0.22	0.22	0.21	0.52	0.29	0.55	0.52	0.67*	0.56
PC	0.07	0.11	0.18	0.29	0.38	0.33	-0.54	0.19	-0.06
YMC	0.16	-0.14	0.03	0.18	-0.01	-0.44	-0.10	-0.36	-0.06

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count,*=p<0.05, **p<0.01

Analysis of data revealed a positive and highly significant (p<0.01) correlation between the mean FSC and TVC of the samples on 21st day of

storage. However, a positive and significant ($p<0.05$) association was observed between the mean FSC and CC of the samples on day 18 of storage.

Psychrotrophic count

Correlation between the mean psychrotrophic count and other microbial counts of fresh and refrigerated curd samples are given in table 16. A negative

Table 16. Correlation coefficient between the mean psychrotrophic count and other microbial counts of fresh and refrigerated curd samples

Microbial counts	Mean psychrotrophic count (PC)								
	Days of storage								
	0	3	5	7	9	12	15	18	21
TVC	-0.32	-0.08	0.15	-0.22	-0.63*	-0.02	0.15	0.18	-0.39
CC	0.35	0.28	0.35	0.43	0.12	0.64	0.50	0.50	0.50
FSC	0.07	0.11	0.18	0.29	0.38	0.33	-0.54	0.19	-0.06
YMC	0.31	-0.37	0.34	0.08	0.28	0.15	0.63*	-0.40	-0.20

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count,*=$p<0.05$

but significant ($p<0.05$) relationship between the mean PC and TVC of the samples on ninth day of storage was noticed. The mean PC and YMC of the samples on 15th day of storage showed a positive and significant ($p<0.05$) association

Yeast and mould count

Correlation between the mean yeast and mould count and other microbial counts of fresh and refrigerated curd samples are given in table17. Analysis of data revealed a significant ($p<0.05$) and positive correlation between the mean YMC and PC of the samples on 15th day of storage.

Table 17. Correlation coefficient between the mean yeast and mould count and other microbial counts of fresh and refrigerated curd samples

Microbial counts	Mean yeast and mould count (YMC)								
	Days of storage								
	0	3	5	7	9	12	15	18	21
TVC	-0.5	-0.21	0.22	0.02	-0.07	0.05	0.08	-0.31	-0.50
CC	0.52	0.33	0.09	0.09	0.09	0.25	0.50	0.51	0.50
FSC	0.16	-0.14	0.03	0.18	-0.01	-0.44	-0.10	-0.36	-0.06
PC	0.31	-0.37	0.34	0.08	0.28	0.15	0.63*	-0.40	-0.20

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count,*=$p<0.05$

4.1.2 Isolation and Identification of Bacteria

Fresh and refrigerated samples of curd were subjected to isolation of pathogenic and spoilage bacteria. All isolates were identified by cultural, morphological and biochemical characteristics.

Escherichia coli

Escherichia coli were not detected from any of the fresh and refrigerated samples of curd.

Staphylococcus aureus

Staphylococcus aureus was isolated from 35 per cent of fresh curd samples. The organism was isolated from 25 per cent of the samples stored on day three and also from 20 per cent of the samples stored on day five. Of the samples stored for seven days, 2 (10%) revealed the presence of the organism. All isolates were coagulase and TNase positive.

Salmonella

Salmonellae could not be isolated from any one of the fresh and refrigerated curd samples.

Pseudomonas aeruginosa

None of the fresh and refrigerated curd samples revealed the presence of *Pseudomonas aeruginosa.*

Bacillus species

Bacillus species were isolated from fresh as well as refrigerated samples on all days of storage. Seventy per cent of the fresh samples and 25 per cent each of the samples stored for 15 and 18 days revealed the presence of the organism. However, none of the samples had *Bacillus cereus.* Different *Bacillus* spp. isolated from fresh and refrigerated curd samples are given in table18. A total of 74 *Bacillus* species were isolated from fresh and refrigerated curd samples. Of

Table 18. *Bacillus* species isolated from fresh and refrigerated curd samples

Species	Number of isolates	Number of positive samples								
		Days of storage								
		0	3	5	7	9	12	15	18	21
B.subtilis	41	6	6	6	6	5	5	4	2	1
B.coagulans	19	4	4	2	2	2	1	1	2	1
B.mycoides	2	ND	ND	1	ND	ND	ND	ND	1	ND
B.megaterium	5	ND	2	2	ND	1	ND	ND	ND	ND
B.pumilus	2	2	ND	ND	ND	ND	ND	ND	ND	ND
B.licheniformis	5	2	2	1	ND	ND	ND	ND	ND	ND
Total	74	14	14	12	8	8	6	5	5	2

ND: Not detected

the isolates, 55.40 per cent were *B. subtilis* and 25.67 per cent were *B. coagulans. B. megaterium* and *B. licheniformis* were present in 6.75 per cent each of the samples, and 2.70 per cent samples each were *B. mycoides* and *B. pumilus.*

4.1.3 Physico-chemical Quality

The physico-chemical quality of the samples such as titratable acidity and pH were estimated.

4.1.3.1 Titratable Acidity

Fresh and refrigerated curd samples were subjected to estimation of titratable acidity and the mean values are given in table19 and fig.2. Analysis of the data revealed that the reduction of the mean titratable acidity values of the samples stored on 21st day was highly significant ($p<0.01$) than that of the mean value of fresh curd samples. The mean value of samples stored on fifth day showed a highly significant ($p<0.01$) difference than that of the values of the samples stored on 18th and 21stday. The mean values of titratable acidity of the samples stored on day 5 and 15 were significantly ($p<0.05$) different. Highly

Table 19. Mean titratable acidity of fresh and refrigerated curd samples

Days of storage	Mean ± SE (% lactic acid)
0	1.85 ± 0.03^{a}
3	1.86 ± 0.05^{ab}
5	1.83 ± 0.04^{abc}
7	1.85 ± 0.06^{abcd}
9	1.88 ± 0.05^{abcde}
12	1.80 ± 0.03^{abcdef}
15	1.70 ± 0.04^{dfg}
18	1.70 ± 0.06^{aefg}
21	1.64 ± 0.03^{g}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

significant ($p<0.01$) difference was observed between the mean titratable acidity values of 21st day with that of days 9 and 12 of storage, respectively.

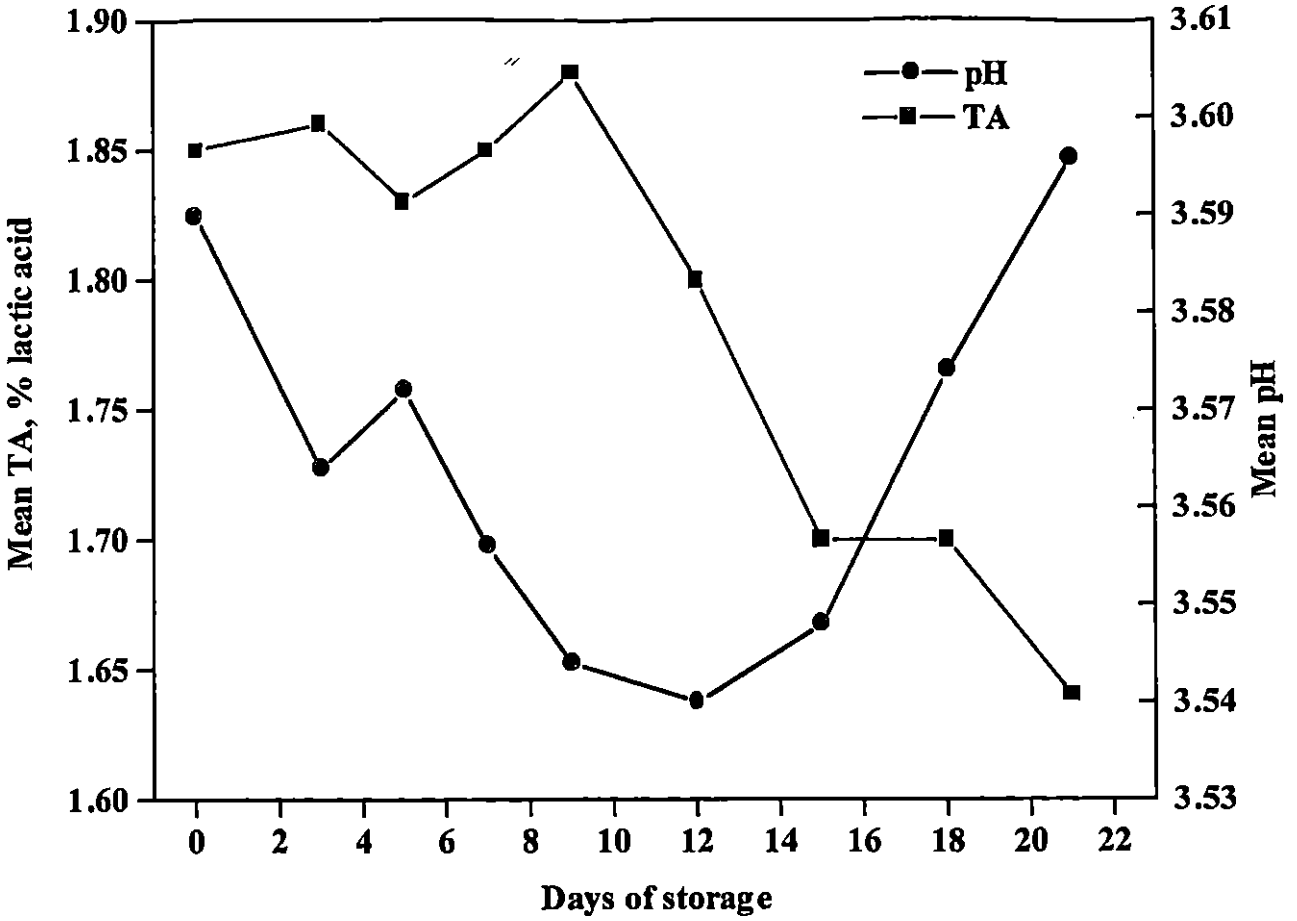


Fig 2. Variation in mean titratable acidity and pH of fresh and refrigerated curd samples during storage

4.1.3.2 pH

Fresh and refrigerated curd samples were subjected to the estimation of pH and the mean values are given in table 20 and fig.2. Analysis of the data by

Table 20. Mean pH of fresh and refrigerated curd samples

Days of storage	Mean ± SE
0	3.590 ± 0.010^{a}
3	3.564 ± 0.010^{b}
5	3.572 ± 0.006^{abc}
7	3.556 ± 0.008^{bd}
9	3.544 ± 0.010^{bde}
12	3.540 ± 0.010^{bde}
15	3.548 ± 0.010^{abde}
18	3.574 ± 0.007^{abcdf}
21	3.596 ± 0.009^{abcf}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

paired't' test revealed that compared to fresh samples, highly significant ($p<0.01$) decrease was observed in the pH of the samples stored for 15 days, except the samples stored for 5 days. The pH increased by 0.05 units on 21st day of storage when compared to 12th day. A highly significant ($p<0.01$) difference between the mean pH values of the samples stored on day zero and three were observed. The mean pH of the samples stored on day 12 showed a highly significant ($p<0.01$) reduction as compared to the mean pH of the samples stored on fifth day. The mean pH of the samples stored on day 21 had a highly significant ($p<0.01$) difference as compared with that of the mean pH of the samples stored on day 7, 9 and 12.

4.1.4 Organoleptic Qualities

Fresh and refrigerated samples of curd stored for 21 days were subjected to sensory evaluation. A semi-trained four member panel evaluated flavour, body and texture, colour and appearance and product acidity of the curd samples on 0, 3, 5, 7, 9, 12, 15, 18 and 21 days of storage.

4.1.4.1 Flavour Score

Mean flavour score of fresh and curd samples stored under refrigeration for 21 days were subjected to paired't' test and is given in table 21. Mean flavour

Table 21. Mean flavour score of fresh and refrigerated curd samples

Days of storage	Mean ± SE
0	34.35 ± 0.55^{a}
3	32.60 ± 0.45^{b}
5	31.80 ± 0.55^{c}
7	32.90 ± 0.77^{abcd}
9	32.15 ± 0.54^{bcde}
12	32.00 ± 0.49^{bcdef}
15	31.20 ± 0.75^{bcef}
18	30.20 ± 0.55^{g}
21	30.15 ± 0.61^{cg}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

scores between the fresh curd samples and the samples stored on third and fifth day revealed a highly significant ($p<0.01$) reduction in the scores. The mean score of the samples stored on day 18 and 21 also revealed a highly significant ($p<0.01$) reduction in the scores as compared to the score of the samples on zero day. A highly significant ($p<0.01$) difference was also observed between the

mean flavour score of samples on third and fifth day, from that of fresh curd samples. The mean flavour score of samples on 21st day of storage was significantly ($p<0.01$) different from the mean score of all samples stored on 0, 3, 7, 9, 12 and 15. The score of the samples on 18th day of storage was significantly ($p<0.05$) different from that of the scores of the samples stored on 9th and 15th day, but the reduction between the mean score of the samples stored on day 12 and 18 was highly significant ($p<0.01$).

4.1.4.2 Body and Texture Score

Mean body and texture score of fresh and refrigerated curd samples are given in table 22. No significant decrease in mean body and texture score was

Table 22. Mean body and texture score of fresh and refrigerated curd samples

Days of storage	Mean ± SE
0	28.20 ± 0.53^{a}
3	27.75 ± 0.29^{ab}
5	27.90 ± 0.43^{abc}
7	29.60 ± 0.61^{acd}
9	29.35 ± 0.58^{ade}
12	29.20 ± 0.57^{abdef}
15	29.45 ± 0.67^{abdefg}
18	28.25 ± 0.50^{abch}
21	$28.75 \pm 0.80^{abcdefgh}$

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

observed between the fresh and curd samples stored at refrigeration for a period of 21 days. The mean score reduced to 27.75 ± 0.29 on the third day and then increased to 29.60 ± 0.61 on the seventh day. The mean body and texture score of curd samples stored between days 9 and 18, 12 and 18 and 15 and 18 differed at a

highly significant ($p<0.01$) level. Significant difference ($p<0.05$) was also observed in score of samples stored between days 5 and 9, 5 and 12 and 5 and 15. A significant ($p<0.05$) increase was observed between the score of the samples stored on seventh and ninth day as compared to that of samples on the third day of storage.

4.1.4.3 Colour and Appearance Score

Mean colour and appearance score of fresh and refrigerated curd samples are given in table 23. Mean colour and appearance score decreased significantly

Table 23. Mean colour and appearance score of fresh and refrigerated curd samples

Days of storage	Mean ± SE
0	8.05 ± 0.17^{a}
3	7.85 ± 0.17^{b}
5	7.70 ± 0.13^{abc}
7	7.95 ± 0.23^{abcd}
9	7.95 ± 0.12^{abcde}
12	7.80 ± 0.13^{abcdef}
15	7.75 ± 0.15^{abcdefg}
18	7.40 ± 0.12^{ch}
21	7.65 ± 0.26^{abcdefgh}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

($p<0.05$) in the samples stored on third day as compared to that of fresh curd samples. The mean colour and appearance score of the samples stored on 18th day differed significantly ($p<0.01$) at a higher level with that of the samples stored on day 0, 7, 9 and 15.

4.1.4.4 Product Acidity Score

Mean product acidity score of fresh and refrigerated curd samples are given in table 24. Mean product acidity score of curd stored on third and fifth

Table 24. Mean product acidity score of fresh and refrigerated curd samples

Days of storage	Mean ± SE
0	11.25 ± 0.20^{a}
3	10.70 ± 0.20^{b}
5	10.35 ± 0.22^{c}
7	9.75 ± 0.37^{bcd}
9	9.75 ± 0.37^{bcde}
12	9.55 ± 0.38^{bcdef}
15	10.25 ± 0.57^{abcdefg}
18	10.60 ± 0.39^{abcg}
21	11.50 ± 0.34^{ab}

Figures bearing the same superscript do not differ significantly

N=20 samples examined on each day

day was reduced at a highly significant ($p<0.01$) level than that of the mean score of fresh curd samples. The mean score of the samples stored on 21 days differed significantly ($p<0.01$) at a higher level from the mean score of samples stored on days 7, 9 and 12, respectively.

4.2 MICROBIAL, PHYSICO-CHEMICAL AND ORGANOLEPTIC QUALITIES OF MARKET CURD SAMPLES

4.2.1 Microbial Counts

A total of 80 curd samples belonging to four different brands were evaluated for their microbial, physico-chemical and organoleptic quality and also for the isolation and identification of certain bacteria of public health significance.

4.2.1.1 Total Viable Count

Total viable count (TVC) of curd samples belonging to four different brands collected from retail outlets in and around Thrissur Corporation was estimated. The mean counts of the samples are given in the table 25 and fig.3.

Table 25. Mean total viable count of market curd samples

Brands of curd	Mean ± SE ($\log_{10}$cfu/g)
A	8,40 ± 0.24
B	8.84 ± 0.33
C	8.93 ± 0.20
D	8.78 ± 0.23

N=20 from each brand

Analysis of variance test did not reveal significant difference between the mean total viable counts of samples from the four brands. However, the samples of brand C had the highest mean count, whereas the samples of brand A had the lowest mean count. Of the 80 samples tested, one of the samples from brand C had the highest count (9.97 $\log_{10}$cfu/g), while one sample each from brand A and B had the lowest count (7.30 $\log_{10}$cfu/g).

Distribution of market curd samples based on level of total viable count is shown in table 26. Of the 80 samples, 34 (42.50%) had a count at the level of 10^9cfu/g. The count in 16 (20%) and 30 (37.5%) samples was at the level of 10^7 and 10^8cfu/g, respectively. Sixty per cent of the samples of brand B had count at the level of 10^9cfu/g, but the count at the level of 10^8cfu/g was observed in 60 per cent of the samples of brand C. Only 20 per cent of the samples of the brand D had the count at the level of 10^7cfu/g.

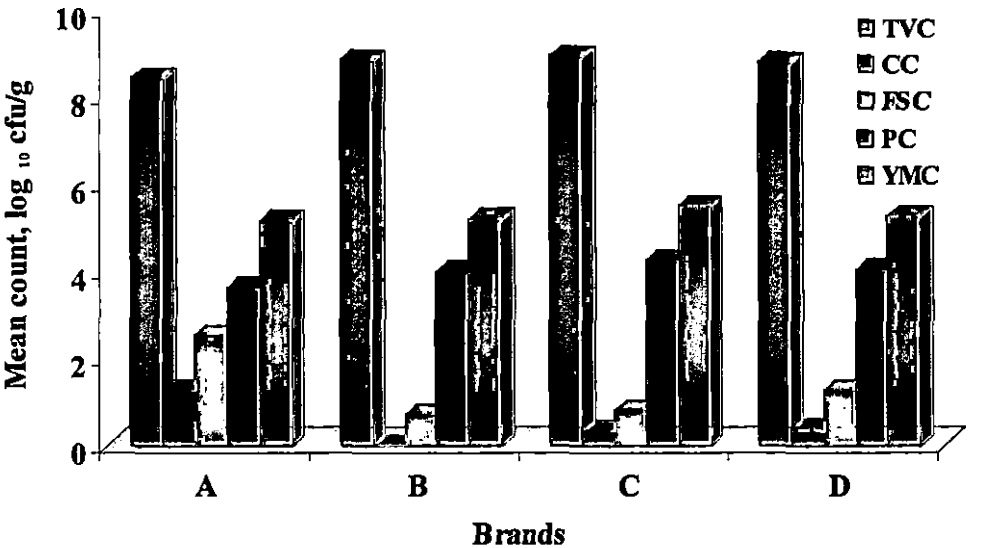


Fig 3. Comparison of mean microbial count of market curd samples

Table 26. Distribution of market curd samples based on the level of total viable count

Brands of curd	Total viable count (cfu/g)		
	10^7	10^8	10^9
A	6 (30)	8 (40)	6 (30)
B	6 (30)	2 (10)	12 (60)
C	-	12 (60)	8 (40)
D	4 (20)	8 (40)	8 (40)

Figures in parenthesis indicate per cent

4.2.1.2 Coliform Count

The mean coliform counts of the samples from the four brands are given in table 27 and fig. 3. The analysis of variance test revealed a highly significant ($p<0.01$) difference in the count of the samples from the of the samples from the

Table 27. Mean coliform count of market curd samples

Brands	Mean ± SE ($\log_{10}$ cfu/g)
A	1.258 ± 0.32^a
B	0.00 ± 0.00^b
C	0.335 ± 0.20^b
D	0.366 ± 0.21^b

N=20 from each brand $p<0.01^{**}$
Critical difference=0.6185

of the samples from the four brands. The samples of the brand A had the highest mean count, while none of the samples belonging to the brand B revealed the presence of organisms. Of the samples, one of the samples from brand A had the maximum count at the level of 2.8 $\log_{10}$cfu/g.

Critical difference test of the data revealed significant difference between the mean counts of samples of brand A and B, A and C and A and D.

Distribution of market curd samples based on the level of coliform count per gram is shown in table 28. All samples of brand B were found free of

Table 28. Distribution of market curd samples based on the level of coliform count

Brands	Coliform count (cfu/g)		
	Nil	10^1	10^2
A	2 (10)	12(60)	6 (30)
B	20 (100)	-	-
C	14 (70)	6(30)	-
D	14 (70)	-	6 (30)

Figures in parenthesis indicate per cent

coliforms. Out of 80 samples, 50 (62.5%) did not reveal the presence of the organism. However 12 (15 %) samples each from the brands A and D had the organism at the level of 10^2cfu/g.

4.2.1.3 Faecal Streptococcal Count

The mean faecal streptococcal count of samples from the four brands is given in table 29 and fig.3. Analysis of variance test revealed a highly significant ($p<0.01$) difference in the mean count of the samples from the four brands. The samples from the brand A had the highest mean count, while the samples from the brand B had the lowest. One of the samples from brand A had the count at the level of 3.67 $\log_{10}$cfu/g.

The data when subjected to critical difference test revealed a significant difference between the mean counts of samples of brands A and B, A and C and A and D.

Table 29. Mean faecal streptococcal count of market curd samples

Brands of curd	Mean ± SE ($\log_{10}$cfu/g)
A	2.518 ± 0.48^{a}
B	0.709 ± 0.33^{b}
C	0.806 ± 0.35^{b}
D	1.268 ± 0.42^{b}

N=20 from each brand $p<0.01^{**}$
Critical difference =1.082

Distribution of market curd samples based on level of faecal streptococcal count per gram is shown in table 30. Of the samples, 32 (40%) did not reveal the

Table 30. Distribution of market curd samples based on the level of faecal streptococcal count

Brands of curd	Faecal streptococcal count (cfu/g)			
	Nil	10^1	10^2	10^3
A	4 (20)	2 (10)	4 (20)	10 (50)
B	10 (50)	6(30)	4 (20)	-
C	12 (60)	4(20)	4 (20)	-
D	6 (30)	8(40)	2 (10)	4 (20)

Figures in parenthesis indicate per cent

presence of the organism. However 10 (50%) samples from brand A had the organism at the level of 10^3cfu/g. None of the samples of the brands B and C had the count greater than 10^2cfu/g whereas 60 per cent of the samples of the brand C did not yield the organism.

4.2.1.4 Psychrotrophic Count

The mean psychrotrophic count of samples from the four brands is given in table 31 and fig.3. Analysis of variance test did not reveal significant difference between the mean psychrotrophic counts of samples from

Table 31. Mean psychrotrophic count of market curd samples

Brands	Mean ± SE ($\log_{10}$cfu/g)
A	3.60 ± 0.28
B	3.97 ± 0.14
C	4.23 ± 0.26
D	4.03 ± 0.13

N=20 from each brand

the four brands. The samples of the brand C had the highest mean count, whereas the samples of brand A had the lowest mean count. Of the 80 samples tested, one of the samples from C brand had the highest count (4.90 $\log_{10}$cfu/g), while one sample from brand A had the lowest count (2.48 $\log_{10}$cfu/g).

Distribution of market curd samples based on level of psychrotrophic count per gram is shown in table 32. Of the 80 samples, 44 (55 %) samples had

Table 32. Distribution of market curd samples based on the level of psychrotrophic count

Brands of curd	Psychrotrophic count (cfu/g)		
	10^2	10^3	10^4
A	6 (30)	4 (20)	10 (50)
B	4 (20)	6 (30)	10 (50)
C	-	6 (30)	14 (70)
D	-	10 (50)	10 (50)

Figures in parenthesis indicate per cent

count at the level of 10^4cfu/g. The count in 14 (70%) samples of brand C was at the level of 10^4cfu/g. The counts in 8 (10%) and 28 (35%) samples was at the level of 10^2 and 10^3cfu/g, respectively.

4.2.1.5 Yeast and Mould Count

The mean yeast and mould count of the samples from the four brands are given in table 33 and fig.3. Analysis of variance test did not reveal significant

Table 33. Mean yeast and mould count of market curd samples

Brands of curd	Mean ± SE ($\log_{10}$cfu/g)
A	5.148 ± 0.24
B	5.186 ± 0.14
C	5.500 ± 0.14
D	5.259 ± 0.16

N=20 from each brand

difference between the mean counts of samples from the four brands. However, the samples of brand C had the highest mean count, whereas the samples of brand A had the lowest mean count. One of the samples from brand A had the highest count at the level of 6.70 $\log_{10}$cfu/g and another sample from the same brand had the lowest count 4.75 $\log_{10}$cfu/g.

Distribution of market curd samples based on level of yeast and mould count per gram is shown in table 34. The yeast and mould count of the samples

Table 34. Distribution of market curd samples based on the level of yeast and mould count

Brand	Yeast and mold count (cfu/g)			
	10^3	10^4	10^5	10^6
A	2 (10)	6 (30)	8 (40)	4 (20)
B	-	8 (40)	12 (60)	-
C	-	-	16 (80)	4 (20)
D	-	8 (40)	8 (40)	4 (20)

Figures in parenthesis indicate per cent

varied from 10^3 to 10^6cfu/g. The count in 44 (55%) samples was at the level of 10^5cfu/g. However, 12 (15%) samples had count at the level of 10^6cfu/g.The count of the samples of the brand C ranged between 10^5 and 10^6cfu/g and 80 per cent of the samples had count at the former level. None of the samples from brand B had counts at the level of 10^6cfu/g. Only 2 (2.5%) samples had count at the level of 10^3cfu/g.

4.2.1.6 Relationship between the Microbial Counts

The correlation coefficient between the microbial counts of market curd samples are shown in table 35. Analysis of the data revealed a positive and highly significant ($p<0.01$) association between TVC and PC. However, a non-significant but positive relationship was observed between mean CC and FSC and also between FSC and YMC of market curd samples. Negative and non-significant correlation was seen between TVC and CC, TVC and FSC, TVC and YMC, CC and PC, CC and YMC, FSC and PC and PC and YMC.

Table 35. Correlation coefficient of various microbial counts of market curd samples

Microbial counts	Microbial counts			
	CC	FSC	PC	YMC
TVC	-0.178	-0.192	0.439**	-0.105
CC		0.110	-0.176	-0.129
FSC			-0.072	0.007
PC				-0.216

TVC- total viable count, CC-coliform count, FSC-faecal streptococcal count ** $p<0.01$
PC-psychrotrophic count, YMC-yeast and mould count

4.2.2 Isolation and Identification of Bacteria

All curd samples collected from retail market were tested to isolate and identify *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa* and *Bacillus* species.

The pathogenic and spoilage bacteria isolated from market curd samples are given in table 36.

Table 36. Pathogenic and spoilage bacteria isolated from market curd samples

Bacteria	Number of positive samples			
	A	B	C	D
Escherichia coli	1	ND	3	ND
Staphylococcus aureus	1	ND	1	ND
Salmonellae	ND	ND	ND	ND
Pseudomonas aeruginosa	ND	ND	2	ND
Bacillus species	10	5	15	20

N=20 from each brand ND: not detected

Escherichia coli

Of the 80 curd samples tested, only four (5%) samples revealed the presence of *Escherichia coli*. Of the isolates, three were obtained from the samples of the brand C and one (5%) from the samples of brand A (Table 36). All isolates, one each from positive samples, were identified by cultural, morphological and biochemical tests. The isolates were positive for Eijkman's test.

The isolates were serotyped at National Escherichia and Salmonella centre, Kasauli. Of the four isolates, three fell into three serogroups and the remaining one was of rough type. One of the isolates was identified as O157 and

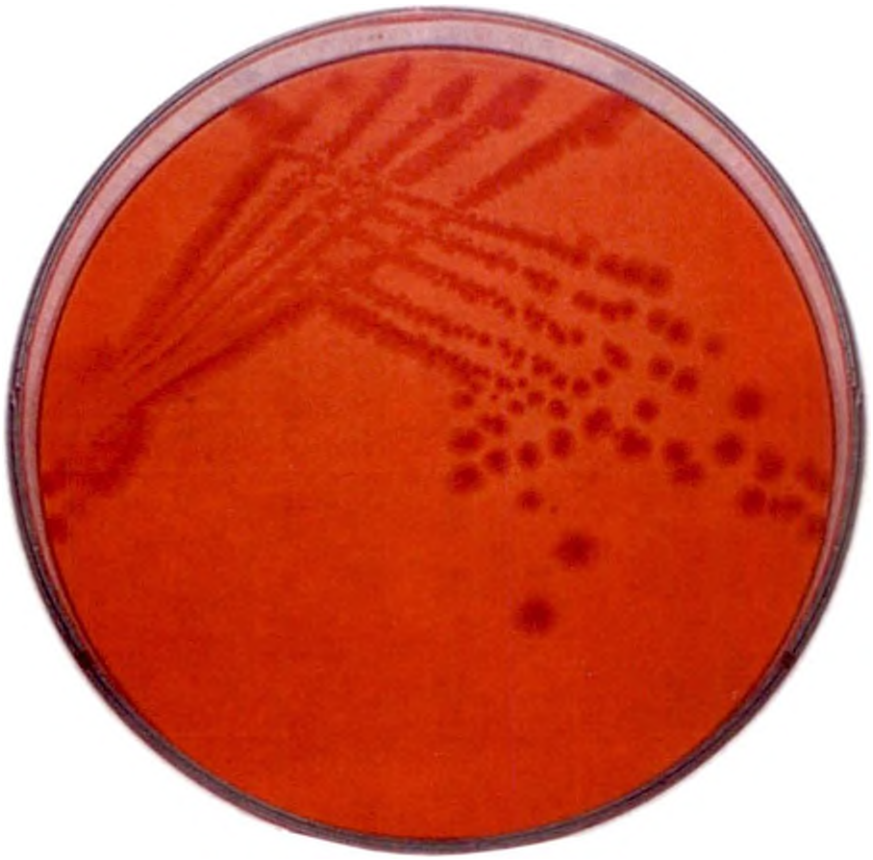


Plate 1. Congo red binding characteristic of *E.coli*

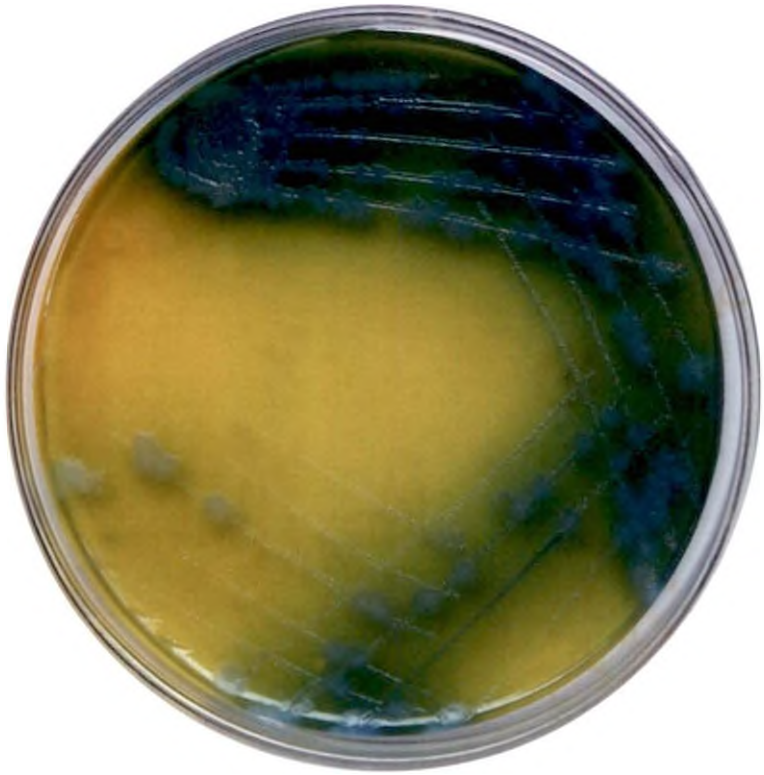


Plate 2. *Bacillus cereus* colonies on *B. cereus* agar

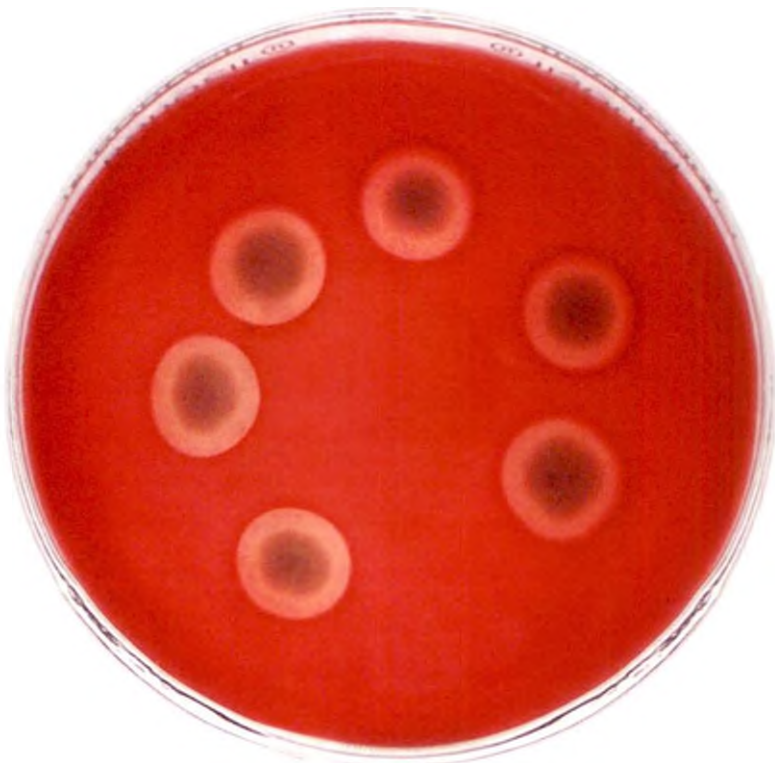


Plate 3. Hemolytic activity of *Bacillus cereus*

was isolated from the samples of Brand A while O5, O148 and the rough type were isolated from the samples of Brand C. All isolates were Congo red binding test positive, indicating their property of invasiveness and is illustrated in plate 1.

Staphylococcus aureus

All the samples were examined for the presence of *Staphylococcus aureus*, but the organism was detected in two of the curd samples, one (5%) each from brand A and C (Table 36). All the isolates were coagulase and TNase positive.

Salmonella

None of the curd samples obtained from retail market revealed the presence of the *Salmonella* (Table 36).

Pseudomonas aeruginosa

All samples were examined for the presence of *Pseudomonas aeruginosa*, but the organism was detected only in two (10%) samples of the brand C (Table 36). The samples from other brands did not reveal the presence of the organism.

Bacillus species

All the samples of brand D revealed the presence of *Bacillus* species. The organism was isolated from 15 (75%), 10 (50%) and 5 (25%) samples belonging to brands C, A and B, respectively (Table 36).

Different species of *Bacillus* isolated from market curd samples are given in table 37. Out of the 99 isolates obtained from 50 samples of four brands, *B. subtlis* was the highest (52.52%) followed by *B. cereus* (14.14%) (plate 2), *B. licheniformis* (12.12%), *B. megaterium* (10.11%), *B. pumilus* (8.08%) and *B. coagulans* (3.03%). Hemolytic activity of *Bacillus cereus* isolates was carried out and is illustrated in plate 3.

Table 37. *Bacillus* species isolated from market curd samples

Species	Total number of isolates	Number of isolates			
		A	B	C	D
B. subtilis	52	4	2	18	28
B. cereus	14	3	2	6	3
B. licheniformis	12	5	1	2	4
B. megaterium	10	3	3	1	3
B. pumilus	8	4	0	2	2
B. coagulans	3	0	0	1	2
Total	99	19	8	30	42

4.2.3 Physico-chemical Quality

The physico-chemical quality of the samples such as titratable acidity and pH were estimated.

4.2.3.1 Titratable Acidity

The mean titratable acidity of the curd samples from the four brands are given in table 38. The analysis of variance test revealed a highly significant difference ($p<0.01$) in the titratable acidity of curd samples from the four brands.

Table 38. Mean titratable acidity of market curd samples

Brands of curd	Mean ± SE (% lactic acid)
A	1.643 ± 0.10^{a}
B	1.632 ± 0.04^{a}
C	1.460 ± 0.09^{a}
D	1.123 ± 1.10^{b}

N=20 from each brand $p<0.01^{**}$
Critical difference=0.2581

The samples of brand A had the highest mean titratable acidity, while the samples belonging to brand D had the lowest. Of the samples, one of the samples from brand A had the highest acidity (2.16 % lactic acid), while the lowest (0.76 % lactic acid) was observed in a sample from brand D.

The data when subjected to critical difference test revealed significant difference ($p<0.05$) between the mean titratable acidity of the samples of A and B, B and D and C and D.

4.2.3.2 pH

The mean pH of curd samples from the four brands are given in table 39. The analysis of variance test revealed a highly significant difference ($p<0.01$) in the pH of the samples from all the four brands. The samples of brand D had the highest mean pH, while the samples belonging to brand B had the lowest mean pH.

Table 39. Mean pH of market curd samples

Brands of curd	Mean ± SE
A	3.679 ± 0.02^{b}
B	3.621 ± 0.01^{b}
C	3.702 ± 0.06^{b}
D	3.840 ± 0.06^{a}

N=20 from each brand $p<0.01^{**}$

Critical difference=0.125

Critical difference test revealed a significant difference between the mean pH of samples from brands A and D, B and D and C and D. On the other hand, no significant difference was observed between the mean pH of samples from brands A, B and C.

4.2.4 Organoleptic Evaluation of Market Curd

The four different brands of curd from retail market were also subjected to sensory evaluation. The analysis of sensory scores of the market curd was carried out by Kruskal – Wallis test and no significant variation was found among the brands for flavour, body and texture and colour and appearance.

4.2.4.1 Flavour Score

The mean flavour score of curd samples from the four brands are given in table 40. The samples of the brand C had the highest mean flavour score

Table 40. Mean flavour score of market curd samples

Brands of curd	Mean ± SE
A	28.30 ± 0.65
B	27.15 ± 1.20
C	28.65 ± 0.65
D	28.50 ± 1.18

N=20 from each brand

(28.65 ± 0.65) and the lowest score was observed in the samples of brand B (27.15 ± 1.20). But there was no significant difference between the flavour score of the samples of different brands. High acid taste was reported for two samples of brand A and four samples of brand B. One of the samples from brand C had yeasty and another had abnormal flavour. Bitter flavour was noticed in two samples of brand B.

4.2.4.2 Body and Texture Score

The mean body and texture score of curd samples from the four brands are given in table 41. The mean body and texture score was highest in the samples of the brand A (27.30 ± 0.96) and the mean lowest score was observed

in the samples of the brand C (24.25 ± 0.70). Kruskal-Wallis test did not reveal significant difference between the mean score of the samples of various brands. Wheying off was noticed in four samples of brand A and two samples of

Table 41. Mean body and texture score of market curd samples

Brands of curd	Mean ± SE
A	27.30 ± 0.96
B	25.20 ± 1.40
C	24.25 ± 0.70
D	26.20 ± 0.49

N=20 from each brand

brand D Thin body was mainly noticed in samples of brand C and one of the samples produced gassiness. Grainy texture was noticed in two of the samples of brand A.

4.2.4.3 Colour and Appearance Score

The mean colour and appearance score of curd samples from the four brands are given in table 42. The mean colour and appearance score of the samples of various brands did not differ significantly. The mean score was highest for samples belonging to brands B and D and the samples of the brand A had the lowest score.

Table 42. Mean colour and appearance score of market curd samples

Brands of curd	Mean ± SE
A	7.10 ± 0.18
B	7.55 ± 0.17
C	7.40 ± 0.48
D	7.55 ± 0.24

N=20 from each brand

4.2.4.4 Product Acidity Score

The mean product acidity score of curd samples from the four brands are given in table 43. Kruskal-Wallis test revealed significant difference ($p<0.01$)

Table 43. Mean product acidity of market curd samples

Brands of curd	Mean ± SE
A	9.50 ± 0.27^{b}
B	9.65 ± 0.50^{b}
C	11.25 ± 0.42^{a}
D	10.90 ± 0.18^{a}

N=20 from each brand ** ($p<0.01$)

between the mean score for product acidity in the samples of various brands. Least score for product acidity was observed in the samples of brand A (9.50 ± 0.27) and the maximum score for samples of brand C (11.25 ± 0.42). The data when subjected to critical difference test revealed a highly significant ($p<0.01$) difference between the mean score of samples of brands A and C and between A and D. Also highly significant ($p<0.01$) difference was observed between the mean score of the samples of the brands B and C and B and D. No significant difference was noted between the mean scores of samples of brands A and B and C and D.

4.3 ASSESSMENT OF CRITICAL CONTROL POINTS IN THE PRODUCTION LINE OF CURD

4.3.1 Changes in the Mean Microbial Count during Various Stages of Production of Curd

The changes in the mean microbial count during various stages of production of curd are given in table 44. The TVC of raw milk was 5.96 ± 0.22 $\log_{10}$cfu/ml. The heat treated skim milk had a count of 2.55 ± 0.34 $\log_{10}$cfu/ml

and the count increased to 5.97 ± 0.27 log_{10}cfu/ml in inoculated milk after one h. Highly significant ($p<0.01$) difference was observed between the mean TVC of heat treated milk and that of curd (zero day). TVC of inoculated milk after one and six h were also found to be significantly different ($p<0.01$) from that of fresh curd at a higher level.

Table 44. Mean microbial count during various stages of production of curd

Characteristics	Microbial counts(log_{10}cfu/ml or/g)			
	TVC	CC	FSC	YMC
Raw milk	5.96 ± 0.22^{a}	1.50 ± 0.27^{a}	2.13 ± 0.69^{a}	1.75 ± 0.15^{a}
Pasteurised milk	5.19 ± 0.14^{b}	0.91 ± 0.41^{ab}	0.34 ± 0.21^{b}	1.39 ± 0.16^{ab}
Skim milk	4.71 ± 0.29^{bc}	1.13 ± 0.36^{bc}	1.59 ± 0.42^{ac}	1.32 ± 0.11^{abc}
Heat treated skim milk	2.55 ± 0.34^{d}	ND	0.59 ± 0.38^{abcd}	ND
Starter inoculated skim milk after one h	5.97 ± 0.27^{abc}	0.98 ± 0.23^{abce}	1.39 ± 0.63^{abcde}	3.58 ± 0.27^{d}
Starter inoculated skim milk after six h	7.19 ± 0.05^{e}	0.75 ± 0.31^{bef}	1.58 ± 0.11^{acef}	3.70 ± 0.22^{de}
Curd (zero day)	8.28 ± 0.16^{f}	0.12 ± 0.12^{f}	2.01 ± 0.40^{acef}	3.91 ± 0.11^{de}

Figures bearing the same superscript in the same column do not differ significantly
TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, ECC: *Escherichia coli* count YMC: yeast and mould count

The mean coliform count of raw milk was 1.50 ± 0.27 log_{10}cfu/ml. The mean coliform count of pasteurized milk was 0.91 ± 0.41 log_{10}cfu/ml. Coliforms were not detected in heat treated skim milk. But starter culture inoculated milk after one h had coliforms at the level of 0.98 ± 0.23 log_{10}cfu/ml. However, fresh curd samples had coliform count at the level of 0.12 ± 0.12 log_{10}cfu/g. Significant ($p<0.05$) difference was observed in the mean count of coliforms in the milk after one h of inoculation and fresh curd samples. Highly significant ($p<0.01$)

difference was noted between the mean count in raw milk and skim milk with that of fresh curd.

The mean FSC of heat treated skim milk was 0.59 ± 0.38log_{10}cfu/ml and the count increased to 1.39 ± 0.63 log_{10}cfu/ml and 1.58 ± 0.11 log_{10}cfu/g at one and six h of starter inoculation. The count of fresh curd was 2.01 ± 0.40 log_{10}cfu/g. A highly significant ($p<0.01$) difference was observed between the mean FSC of pasteurized milk with that of six h starter inoculated milk and fresh curd samples. Significant ($p<0.05$) difference was observed between the mean count of heat treated skim milk with that of six h starter inoculated milk and fresh curd samples.

Heat treated skim milk was free of yeast and mould. But after one h of starter inoculation, the count was at the level of 3.58 ± 0.27 log_{10}cfu/ml and the count of fresh curd samples was 3.91 ± 0.11 log_{10}cfu/g. Highly significant ($p<0.01$) increase in the count was observed between fresh curd, starter inoculated milk after one and six h with that of raw milk, pasteurized milk and skim milk samples.

4.3.2 Changes in Mean pH during Production of Curd

The changes in the mean pH during various stages of production of curd are given in table 45. The mean pH of raw and pasteurized milk were significantly ($p<0.05$) different. Highly significant ($p<0.01$) difference in mean pH was observed between raw milk, heat treated skim milk, inoculated skim milk after one and six hour and fresh curd samples.

Table 45. Mean pH during various stages of production of curd

Characteristics	pH (Mean ± SE)
Raw milk	6.55 ± 0.01^{a}
Pasteurised milk	6.68 ± 0.03^{b}
Skim milk	6.55 ± 0.03^{a}
Heat treated skim milk	6.83 ± 0.001^{c}
Inoculated skim milk after 1 h	6.07 ± 0.01^{d}
Inoculated skim milk after 6 h	3.93 ± 0.01^{e}
Curd (0day)	3.59 ± 0.01^{f}

Figures bearing the same superscript do not differ significantly

4.3.3 Starter culture

Samples of starter culture were subjected to the estimation of pH and various microbial counts and are given in table 46. The starter culture had a mean pH of 3.75 ± 0.01and the mean TVC was 6.43 ± 0.21 $\log_{10}$cfu/g. The mean YMC of the starter culture was 3.70 ± 0.21 $\log_{10}$cfu/g.

Table 46. Mean pH and microbial count of starter culture

Raw material (starter culture)	Mean ± SE
pH	3.75 ± 0.01
TVC($\log_{10}$cfu/g)	6.43 ± 0.21
CC	ND
FSC	ND
YMC ($\log_{10}$cfu/g)	3.70 ± 0.21

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count, YMC: Yeast and mould count, ND: Not detected

4.3.4 Air

The mean total viable count and yeast and mould count of air samples obtained from the various areas of pasteurisation, cream separation and thermal

treatment before and after the production of curd and the cold storage room of the dairy plant are given in table 47. The mean TVC and YMC of air samples from

Table 47. Mean microbial count of air samples in dairy plant

Sampling area		Mean ± SE (cfu/ft^2/min)	
		TVC	YMC
Pasteurisation room	Before	27.6 ± 1.52	12.7 ± 1.2
	After	69 ± 1.75	13.5 ± 0.93
Cream separation room	Before	20.4 ± 2.84	4.8 ± 1.2
	After	45.2 ± 4.5	7.83 ± 1.5
Thermal treatment room	Before	70.5 ± 2.3	4 ± 0.35
	After	96.3 ± 1.6	12.3 ± 0.92
Cold storage room		10 ± 1.3	19 ± 1.64

TVC: total viable count, YMC: yeast and mould count

the areas of pasteurisation, cream separation and thermal treatment were higher after processing of the samples. In the cold storage room, the mean TVC and YMC of air samples was 10 ± 1.3 and 19 ± 1.64 cfu/ft^2/min.

4.3.2 Water

The mean total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) of water samples collected from the storage tank, tap and hand washing samples of personnel engaged in processing line are given in table 48. Among the samples, hand washing had the highest mean total viable count whereas storage tank water samples had the lowest count. Tap water samples and hand wash of personnel had coliform count. *E. coli* and faecal streptococci were detected in hand wash samples.

Table 48. Mean bacterial count of water used in dairy plant

Source	Bacterial count (log_{10}cfu/ml)			
	TVC	CC	ECC	FSC
Storage tank	3.50 ± 0.10	-	-	-
Tap water	4.01 ± 0.25	1.03 ± 0.06	-	1.02 ± 0.19
Hand wash of personnel	4.53 ± 0.24	1.19 ± 0.38	0.05 ± 0.05	0.49 ± 0.23

TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, ECC: *Escherichia coli* count

4.3.3 Equipments

The hygienic status of the equipments used in the processing plant was also evaluated. Bacterial counts of pasteurizer, cream separator, double jacketed vat, storage can and packaging material were estimated and the mean count is given in table 49. Among the equipments, cream separator had the highest total

Table 49. Mean bacterial count of processing equipments used in the dairy plant

Equipments	Bacterial count (log_{10}cfu/cm^2 or per ml)			
	TVC	CC	ECC	FSC
Pasteuriser	4.49 ± 0.22	0.31 ± 0.26	-	1.01 ± 0.42
Cream separator	4.95 ± 0.07	0.36 ± 0.24	-	1.03 ± 0.32
Double jacketed vat (log_{10}cfu/$cm^{2)}$	4.53 ± 0.15	1.16 ± 0.16	-	0.46 ± 0.21
Storage can	3.92 ± 0.18	1.02 ± 0.09	-	1.21 ± 0.05
Packaging material	3.20 ± 0.18	-	-	0.10 ± 0.10

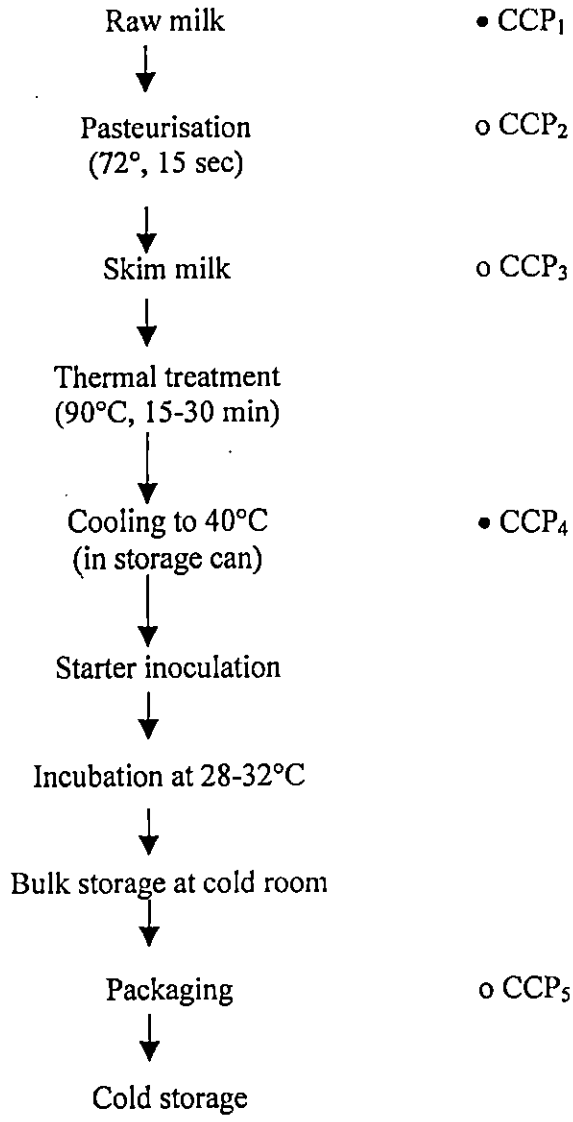
TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, ECC: *Escherichia coli* count

viable count and the lowest count was observed in the packaging material. Double jacketed vat had the highest coliform count followed by storage can.

E. coli was absent in all the samples tested. Coliforms and *E. coli* were not detected in any of the packaging material samples.

The critical control points of bacterial contamination during preparation are given in flowchart 6. Assessment of critical control points in the production line of curd identified the lack of good quality raw milk, potable water, unsatisfactory personnel hygiene of the workers, improper sanitary conditions of the processing equipments and high bacterial and fungal count of air samples at various sites in the plant. The quality of the product can be improved by use of raw milk with good microbial quality, strict adherence to personnel hygiene, use of disinfectant solutions before and after processing operations, cleaning and sanitizing of equipments and regular maintenance of the processing environment of the plant.

Flowchart 6. Critical control points in the preparation of curd



• Potential site for major contamination
o Potential site for minor contamination

CCP_1	-	Use raw milk with good microbial quality
CCP_2	-	Ensure proper pasteurization by adhering on to the recommended time-temperature combination
CCP_3	-	Periodic cleaning and sanitization of equipments to minimize the bacterial load and strict adherence to personnel hygiene
CCP_4	-	Use of properly cleaned and dried cans for storage
CCP_5	-	Use of good quality, sterile packaging material

Discussion

5. DISCUSSION

In the present study, the effect of refrigeration on microbial, physico-chemical and organoleptic qualities of curd samples were assessed and compared with that of the fresh samples. All samples were also tested for the isolation and identification of bacterial pathogens of public health significance. Critical control points of microbial contamination of curd in the production line were evaluated during the study. A total of 80 curd samples belonging to four different brands were obtained from the retail outlets and evaluated for their microbial, physico-chemical and organoleptic quality and also for the isolation and identification of certain bacteria of public health significance.

5.1 EFFECT OF REFRIGERATED STORAGE ON QUALITY OF CURD

5.1.1 Microbial Counts

5.1.1.1 Total Viable Count

Fresh curd had a mean total viable count of 8.28 ± 0.16 log_{10}cfu/g (Table3). A significant ($p<0.05$) increase of total viable count was noticed up to fifth day of storage. Thereafter, the count slightly decreased and reached 6.46 ± 0.28 log_{10}cfu/g on 21st day of storage. The high count of the samples may reduce its keeping quality and the chance for the presence of bacterial pathogens is also high. The decrease in count later might be attributed to the reduction of lactic acid bacteria. The findings of the present study could not be compared with similar research findings due to lack of data. However, in similar fermented milk product like yoghurt, Laye *et al.* (1993) observed a reduction in total viable count during the storage period of 12 days. Al-kadamany *et al.* (2002) also reported that total plate counts increased by 2 log cycles on the third day of storage at 5°C and after four days, there was decrease in the count, till 11 days, in case of concentrated yoghurt samples. The sanitary quality of foods such as sauer kraut,

fermented milks and related products can not be ascertained by total plate count, since these products are produced by activities of microorganisms (Jay, 1996).

5.1.1.2 Coliform Count

The mean coliform count of fresh curd samples was 0.12 ± 0.12 $\log_{10}$ cfu/g (Table 5). The organism could not be recovered from 90 per cent of the samples up to seventh day of storage and in 95 per cent of the samples on ninth and 12th day. After 12th day of storage, coliforms were not detected in the samples. The fresh curd samples of the present study revealed that 90 per cent met the coliforms limit prescribed by Indian Standards (1980a) and Republic of Philippines Standards (2001) which state that the count should not be more than 10/g. The finding is in agreement with that of Mutukumira (1995) who had recorded coliform count in the range of 3.41 to 6.90cfu/g in cultured milk.

The presence of coliforms in fermented milks is considered as an indicator of poor hygienic status followed during their production (Kornacki and Johnson, 2001). Coliform count is used to assess the overall quality and hygienic condition prevailing during processing of food. However, coliforms are highly sensitive to acidic environment in yoghurt and other fermented milks (Birollo *et al.*, 2001).

5.1.1.3 Faecal Streptococcal Count

The mean faecal streptococcal count of fresh curd samples was 2.01 ± 0.40 $\log_{10}$cfu/g and 20 per cent of the samples were free of the organism on the zero day and thereafter a gradual decrease in the count was observed during storage (Table 7). The count in 60 per cent of the samples was at the level of 10cfu/g on 15th day of storage. The present study showed cell viability for enterococci at low pH. The finding of Birollo *et al.* (2001) in case of yoghurt was similar to the present study, who reported that enterococci can remain viable up to 42 days of cold storage. Classical enterococci are considered better indicators of food sanitary quality than coliforms, especially for frozen foods (Jay, 1996).

The organism can grow between 0 and 50°C. The presence of the organism throughout the storage period indicate that enterococci are good indicators of post-processing contamination of curd and poor hygienic practices followed during its production.

5.1.1.4 Psychrotrophic Count

The mean psychrotrophic count of fresh curd samples was 3.20 ± 0.24 $\log_{10}$ cfu/g (Table 9). The count decreased during the first 12 days of storage, thereafter a little increase in the count was observed. On zero day, 55 per cent samples had count at level of 10^3 cfu/g and the count in 15 per cent samples was at the level of 10^4 cfu/g.

Decrease in psychrotrophic count was reported by Juffs and Babel (1975). The reduction in count may be due to the inhibitory effect of the lactic cultures present in the curd samples. Gilliland and Martin (1980) also observed that lactobacillus strains at the level of $>10^7$/ml had significant inhibitory effect on the growth of psychrotrophs and the effect increased as the lactobacilli count increased. The increase in psychrotrophic count after 12^{th} day of storage may be due to the reduction in the total bacterial count including starter bacteria.

5.1.1.5 Yeast and Mould Count

The mean yeast and mould count of fresh curd was 3.91± 0.11 $\log_{10}$cfu/g (Table 11). The count gradually increased from third day of storage and reached 0.5 log higher than the initial count on seventh day of storage. The finding is in agreement with the count in dahi recorded by Sreenivasan and Ranganathan (1972). Yeasts and moulds are active at low pH and are the important spoilage microorganisms, because they can develop in the acidic conditions of the product. The organisms are the most acid-tolerant in foods and may cause organoleptic changes and packaging deformation due to gas production (Birollo *et al.*, 2001). The high count could be attributed to contamination from the air and starter culture itself, since the latter showed yeasts and moulds at higher level. The

product studied did not meet the standards prescribed by Indian Standards (1980a). A serious risk of gas production and off flavour development may not be manifested until the yeast population reaches $1x10^5$cfu/ml. Such counts can be achieved only within a 2 to 3 week shelf life (Tamime and Robinson, 2004).

The use of yeast free starter cultures, application of strict hygienic measures during processing and packaging, and maintenance of an effective cold chain from production till consumption is desirable for the production of good quality curd.

5.1.1.6 Relationship between the Various Microbial Counts of Fresh and Refrigerated Curd Samples

Relationship between the various microbial counts of fresh and refrigerated curd samples was studied by estimating the correlation coefficient.

Mean total viable count was positively and significantly ($p<0.01$) associated at a high level with faecal streptococcal count. Total viable count and psychrotrophic count was negatively and significantly associated ($p<0.05$) indicating that if the former increased, the latter will decrease. Mean coliform count showed a significant positive correlation with faecal streptococcal and psychrotrophic count indicating that as the coliform count decreased, faecal streptococcal count and psychrotrophic count also decreased. Yeast and mould count was not significantly correlated with coliform count and psychrotrophic count.

5.1.2 Isolation and Identification of Bacteria

Escherichia coli

The organism was not detected in all samples examined. The pH of fresh curd was 3.59 ± 0.01 and was decreased on storage. Simango and Rukure (1992) and Feresu and Nyati (1990) also opined that viable cells of the pathogen were not detected in fermented foods after 24 h of inoculation with an initial inoculum

of 10^6 to 10^7cfu/ml of food. Low pH is detrimental to the growth of this organism. Other mechanisms such as production of bacteriocin, hydrogen peroxides and ethanol by lactic acid bacteria might also contribute to the decline in population of *E. coli.*

Staphylococcus aureus

Staphylococcus aureus was isolated from 35 per cent of fresh curd samples. The organism was isolated on day three and four from 25 and 20 per cent samples, respectively. Ten per cent of the samples revealed presence of the organism on seventh day. The finding is in agreement with that of Tiwari and Singh (1964). Estrada *et al.* (1999) also observed that the organism inoculated at the level of 10^6cfu/ml decreased from the first day of storage at 4°C, and completely inhibited at 9 to 10 days. Although acidic conditions are unfavourable for the growth of Staphylococci, the entry of the organism into the product before addition of lactic culture might have enabled the organism to grow well before the medium was rendered acidic. As the pH got reduced, the organism could not thrive in the product and it was not detected after seven days of storage.

Staphylococci are part of the normal flora of animals and man. *Staphylococcus aureus* has been associated with food poisoning with the consumption of dahi (Saha and Ganguly, 1957). The presence of the organism in the product indicates that the hygienic practices followed during processing are not satisfactory. So attention must be paid to sanitation and personnel hygiene to minimize the contamination of the product with the organism. However Abou-Dhonia *et al.* (1992) reported that *S. aureus* was not recovered in fresh or stored labneh samples stored at 4°C on 0,10 and 20 days. Ashenafai (1992) and Pazakova *et al.* (1997) reported the same in Ethiopian fermented milk and yoghurt samples.

Salmonella

All fresh and refrigerated curd samples were found to be free of Salmonella. The findings of the present study differed from that of Matta *et al.* (1991) who reported that a few organisms survived in dahi even after 48 h storage at 5 to 7°C.

Pseudomonas aeruginosa

All fresh and refrigerated curd samples were found to be free of *Pseudomonas aeruginosa.*

Bacillus species

Bacillus species were present in 70 per cent of the fresh samples tested (Table 18). The organisms were present in 25 per cent each of the samples tested on day 15 and 18. *Bacillus cereus* was not detected in any of the curd samples. *B. subtilis* and *B. coagulans* were the most commonly isolated species. Of the isolates, 55.4 and 25.67 per cent were *B. subtilis* and *B. coagulans*. *B. subtilis and B. coagulans* can thrive in acidic foods. The findings of the present study did not agree with the report of Mohanan *et al.* (1984), who reported that sporeformers were not detected in samples with titratable acidity more than 1.2 per cent. Increase in the number of *Bacillus* species in curd can lead to the production of bitter flavour in the product and thus reduce the shelf life. There is growing concern about the importance of contamination of milk and milk products by aerobic spore formers. Their contamination originates from soil, gains entry into the milk and milk products through dust, feeds, fodder, coat of the animals, utensils used for handling milk and milk products, equipments used for manufacturing milk products, packaging materials and the place of storage. However, if starter cultures produce acid at a fairly faster rate, the growth and multiplication of germinated spores will be prevented resulting in the death of the organisms (Mohanan *et al.*, 1985). Careful sanitary procedures coupled with

proper pasteurisation and low temperature of the finished product would preclude any problem with sporeformers.

5.1.3. Physico-chemical Quality

5.1.3.1 Titratable Acidity

The titratable acidity of fresh samples was 1.85 ± 0.03 % lactic acid (Table19). Titratable acidity increased to 1.88 ± 0.05 % lactic acid on ninth day and then decreased till 21st day of storage. The titratable acidity of fresh samples was very much higher than that reported by Kamruzzaman *et al.* (2002) in dahi. Mathur *et al.* (1999) reported titratable acidity values 0.14 per cent lesser than that of the present study. Cultures grown in buffalo milk produced more acid and acetaldehyde and had higher proteolytic activity than cultures grown in cow or goat milk (Iyengar *et al.*, 1967; Sharma and Jain, 1974; Singh and Kaul, 1982). The curd samples in the present study had higher titratable acidity which might be due to the presence of more acid producing cultures, use of buffalo milk and longer fermentation period. The decrease in acidity after ninth day of storage might be due to increase in yeast and mould which might have utilised the lactic acid produced by the starter bacteria. The initial acidity was very much higher than that prescribed by Indian Standards (1980a) for sour dahi. Shaack and Marth (1988) opined that an optimum level of one per cent acidity is found to be important to prevent the growth of pathogens.

5.1.3.2 pH

The pH of fresh samples was 3.59 ± 0.010. During storage, the pH decreased except on fifth day, and after 15th day, the values increased gradually (Table 20). The mean pH of the fresh samples was very much lower than that reported by Salji and Ismail (1983) in yoghurt and Kamruzzaman *et al.* (2002) in dahi samples. The initial decrease in pH of the curd samples in the present study might be due to more acid producing cultures and longer incubation period, since at six hour of fermentation itself, the pH got reduced to 3.93 ± 0.01. The pH

might have increased later due to reduction in lactic acid bacteria and thereby lowering the lactic acid production. International Dairy Federation, IDF (1992) prescribed a pH of 3.8 to 4.6 for fermented milks. In the present study, both fresh and refrigerated samples did not meet the above standard.

5.1.4 Organoleptic Qualities

Fresh and refrigerated samples of curd were subjected to sensory evaluation. Statistical analysis revealed that flavour scores decreased during storage. Body and texture score remained almost the same throughout the storage except a slight decrease in score on the third day of storage. Colour and appearance score decreased significantly during the storage period. The flavour and taste of the product depends on the type of the starter culture used (Gupta *et al.*, 2000). The finding of the present study is in accordance with that of Kamruzzaman *et al.* (2002) in dahi samples, who reported that colour score was acceptable for 14 days under refrigeration and also opined that at refrigeration temperature, plain dahi is suitable for consumption up to 12 days. In case of dahi, uncontrolled incubation and post-production handling and storage cause increase in acidity during summer and subsequent decrease during winter season (Younus *et al.*, 2002). However, in the present study, the product acidity score decreased significantly ($p<0.01$) till 12 days of storage and then increased from 15^{th} day onwards. Findings of the present study indicate that the product has a shelf life of 9 days, because thereafter a decrease in the flavour scores, product acidity and an increase in the psychrotrophic count was observed. The growth and multiplication of the psychrotrophs can lead to spoilage conditions and reduce the shelf life. Moreover South Indians prefer dahi with a slight acidic taste (Laxminarayana *et al.*, 1952). The results of the present study are in accordance with that of Patidar and Prajapati (1998). The above researchers opined that flavour changes could be considered as a failure criterion to determine the shelf life of dahi and that milk heated in a vat had little variation in the body and texture and syneresis was less. The high acidity and sour taste of the curd samples in the present study might be attributed to the role of high acid producing

organisms. Changing of starter culture that is contaminated with yeasts and high acid producing organisms will be a remedy in extending the shelf life of the product.

5.1.5 Shelf Life

Findings of the present study indicated that the product has a shelf life of nine days. The presence of the starter organisms decreased considerably after nine days of storage and there was an increase in the psychrotrophic count also. The flavour score also decreased during storage where as the body and texture remained the same almost throughout the storage period. Kamruzzaman *et al.* (2002) also reported similar findings and opined that dahi was suitable for consumption up to 12 days under refrigerated conditions. At refrigeration temperature, acid production of the bacteria will be slow and thus resulting in an increased shelf life as compared to room temperature, wherein acid production will be rapid and shelf life will be reduced. The high acidity in the curd samples of the present study might be due to the use of buffalo milk as reported by Iyengar *et al.* (1967) and Mathur *et al.* (1999). Ramaswamy *et al.* (1999) reported that the shelf life of sweetened dahi is one week at a storage temperature of 5°C.

5.2 MICROBIAL, PHYSICO-CHEMICAL AND ORGANOLEPTIC QUALITIES OF MARKET CURD SAMPLES

5.2.1 Microbial Counts

5.2.1.1 Total Viable Count

The highest count was observed in the samples belonging to brand C (8.93 ± 0.2 $\log_{10}$ cfu/g) and lowest in the samples of brand A (8.40 ± 0.24 $\log_{10}$ cfu/g), as shown in table 25. The mean count in the present study was one log higher than that of the count reported by Jayaram and Gandhi (1987), Rajmany *et al.* (1989), Misra *et al.* (1993) and Younus *et al.* (2002). However, Mohanan *et al.* (1984) has reported counts similar to that of the samples from brand A (8.4 ±

0.24 $\log_{10}$ cfu/g). The high bacterial count of the samples of the present study might be attributed to the low bacteriological quality of the milk used in the preparation of curd or contamination of the curd after its production or both. The high count of the samples may reduce its keeping quality and the chance for the presence of bacterial pathogens is also high.

5.2.1.2 Coliform Count

Analysis of variance test revealed a highly significant ($p<0.01$) difference in coliform count of the samples of curd from different brands as shown in table 27. The highest mean count was observed in the samples of the brand A (1.258 ± 0.32 $\log_{10}$ cfu/g). The organism was not detected in all samples of brand B. Coliforms were absent in 80, 70 and 10 per cent of samples of the brands C, D and A, respectively. Jayaram and Gandhi (1987) reported the presence of the organism in 80 per cent of dahi samples procured from hotels. Gupta *et al.* (2000) reported that Mishti Doi samples had count 0.5 log higher that of the samples of brand A. However, the coliform count in samples of brand A and D were almost 2 and 3 log lower than that of the findings of Younus *et al.* (2002), in case of dahi samples.

Out of the 80 samples examined, 50 per cent samples from brand A, cent per cent of samples from brand B, 80 per cent of brand C and 90 per cent of the samples from brand D met the standards for coliform prescribed by Indian Standards (1980a). Presence of coliforms indicates contamination from environment, utensils and hands of persons during the preparation and handling of dahi. Coliform count is used to assess the overall quality and hygienic condition prevailing during processing of food (Kornacki and Johnson, 2001). Coliforms are suitable hygienic indications as long as they are determined in the first days after production. They are highly sensitive to the acidic environment in cultured milk products (Birollo *et al.*, 2001), which could be one of the reasons of lower count in the samples of the present study.

5.2.1.3 Faecal Streptococcal Count

Analysis of the variance test of faecal streptococcal count revealed a highly significant ($p<0.01$) difference between the mean count of samples of the four brands as shown in table 29.

Critical difference test of the data revealed highly significant ($p<0.01$) difference between the mean counts of samples of the brand A and B, A and C and A and D. The highest mean count was observed in the samples of the brand A (2.518 ± 0.40 $\log_{10}$ cfu/g) and lowest count in the samples of the brand B (0.709 ± 0.33 $\log_{10}$ cfu/g). The organisms were found in all the samples belonging to the four brands which indicated that the milk used for the production had the organism or post-processing contamination of the curd might have occurred.

Presence of faecal streptococci in large numbers indicates direct or indirect faecal contamination of curd and thus it may be inferred that the hygienic practices followed during the production is unsatisfactory. Enterococci were isolated from 37.5 per cent of the samples from the four brands. Tzanetakes *et al.* (1981) detected the organism in 30 per cent of Greek yoghurt samples. Khalaf and Shareef (1985) had observed counts in yoghurt, 0.5 log higher than that of samples from brand A.

5.2.1.4 Psychrotrophic Count

The highest psychrotrophic count, 4.23 ± 0.26 $\log_{10}$ cfu/g, was found in samples belonging to brand C and the lowest count, 3.60 ± 0.28 $\log_{10}$ cfu/g in the samples of the brand A as shown in table 31. Importance of psychrotrophs has increased, on account of the fact that the organisms can cause flavour defects, ropiness, colour changes and other deteriorative changes that affect the acceptability of the product kept under refrigeration. A comparison of the findings of the present study could not be made due to paucity of similar reports.

However Medina *et al.* (1993) reported that commercial cultured milk had count less than 10cfu/g.

5.2.1.5 Yeast and Mould Count

The highest mean yeast and mould count was 5.500 ± 0.14 log_{10}cfu/g for samples from the brand C, while the lowest count, 5.148 ± 0.24 log_{10}cfu/g was for samples from brand A as shown in table 33. The lowest count in the samples of the present study was two log higher than that of the count reported by Mohanan *et al.* (1984) and Sharma *et al.* (1993). None of the samples from the four brands met the standards prescribed by Indian Standards (1980a). The present study revealed that dahi purchased at retail level had poor keeping quality.

The most spoilage flora in fermented milks is yeasts and moulds, which are highly tolerant to low pH and can grow under refrigeration temperatures. They are responsible for off flavours, loss of texture quality due to gas production, and package swelling and eventual blowing of the product container (Mohanan *et al.*, 1985; Moreira *et al.*, 2001; Richter and Vedamuthu, 2001). The high yeast and mould count could be attributed to contamination from air, utensils, packaging materials and also from the contaminated starter culture, during repeated transfers. The use of yeast free dahi starter cultures, application of strict hygienic measures during processing and packaging and maintenance of an effective cold chain from the period of production to consumption could help control yeast.

5.2.2 Isolation and Identification of Bacteria

Escherichia coli

E. coli was not detected in the samples of brands B and D. The organism was isolated from 5 and 15 per cent of samples of brands A and C, respectively (Table 36). Of the 80 samples, the organism was detected in 5 per cent of the

samples. The rate of isolation of the organism from the samples of the present study was too low as compared to the isolation of the organism from 28 per cent (Misra *et al.*, 1993) and 55 per cent (Soomro *et al.*, 2002) samples. The low rate of isolation in the present study might be due to the high acidity of the samples, since the survival of the organism is less in fermented milk products with pH less than 4.5 (Jay, 1996). However, presence of the commensal organism of the intestine of human and animals, in curd indicates the contamination of the product or raw material used in the production of curd and the presence of the organism is of serious concern since the organism is associated with various diseases in human and animals.

The isolates were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli. The serotype O5 and O157 were belonging to Enterohaemorrhagic group. The serotype O157 was isolated from one of the samples of the brand A. These organism produce verotoxins, VT_1 and VT_2, out of which VT_1 is a relatively heat stable high molecular weight, shiga like toxin, whereas VT_2 is immunologically distinct. Infection with VTEC in man causes diarrhoea, haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Smith and Cheasty, 1998). Morgan *et al.* (1993) reported verotoxin producing *E. coli* O157 infection associated with the consumption of yoghurt, and five of the affected children developed haemolytic uraemic syndrome.

The serotypes O5 is one of the most important, recently emerged groups of pathogens in food chain, transmitted to human through direct or indirect contamination of food, by faecal material.

The serotype O148 was categorised as enterotoxigenic *E. coli* (ETEC) which is associated with traveller's diarrhoea and cholera like disease in children under five years of age.

All the isolates obtained from curd samples were Congo red positive which indicated the invasive property of the organism. The characteristics of

Congo red binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker (Rajil *et al.*, 2003). A good correlation between pathogenic potential and Congo red binding property was reported by Soni *et al.* (2002). The isolates were Eijkman's test positive. The isolation of *Escherichia coli* from curd, which are associated with various diseases in man and animals, is of great significance and indicate potential risk of infection to man. Since the organism is of intestinal origin of man and animals, and is considered as an indicator organism, the possibility of the presence of other intestinal bacterial pathogens cannot be ruled out. Recovery of *E. coli* from heat processed foods is indicative of either inadequate processing or subsequent contamination. Since the product is consumed as such, adequate measures have to be taken to control the contamination of curd with such bacterial pathogens.

Staphylococcus aureus

Staphylococci were obtained from 10 (12.50 %) samples among the four brands, but *S. aureus* was detected in 2.5 per cent of the samples, one (5 %) sample, each from brand A and C (Table 36). The organism was not recovered from samples of the brand B. The rate of isolation of the organism in the present study is less than that reported by Rajmany *et al.* (1989), Misra *et al.* (1993) and Kahlon and Grover (1984) in dahi.

Staphylococci are ubiquitous in nature and considered as opportunistic pathogens. *Staphylococcus aureus* constitutes a normal part of the microflora of animal and human body, being found on skin and hair, nose, mouth and throat (Martin and Myers, 1994). Foods subjected to contamination with *S. aureus* pose a significant hazard due to elimination of competitive organisms that normally restrict the growth of *S. aureus* and the production of enterotoxins. Contamination of food occurs mainly by process line workers with hand or arm lesions caused by the organism coming in contact with the food. So also, contaminated processing surfaces are an important source of the organism (Bergdoll, 1990; Lancette and Bennett, 2001). *Staphylococcus aureus* has been

associated with food poisoning with the consumption of dahi (Saha and Ganguly, 1957). The presence of the organism in the product indicates that the hygienic practices followed during processing are not satisfactory.

Salmonella

None of the samples from the four brands revealed the presence of *Salmonella* (Table 36). Isolation of the organism from dahi and fermented milk was reported by Kulshreshta (1976) and Savadogo *et al.* (2004a), respectively. However, many workers had reported that the organism could not be isolated from yoghurt (Rubin *et al.*, 1982), cultured milk (Medina *et al.*, 1993), soy cheese slurries (Kumari and Singh, 1996) and South African traditional fermented milk (Beukes *et al.*, 2001).

Pseudomonas aeruginosa

Pseudomonas aeruginosa was present in 2.5 per cent of the samples from the four brands (Table 36). Among the brands, the organism was detected only in two samples of brand C. However, the presence of the organism in fermented milk product has been reported by Khalaf and Shareef (1985) and Tayar and Sen (1993). The organism is of great public health significance since it causes spoilage in milk and its products under refrigeration, thus reducing the shelf life of the products.

Bacillus species

A total of 99 *Bacillus* species were isolated from 50 (62.5 %) out of 80 curd samples obtained from four brands (Table 37). *B. subtlis* was the highest (52.52%) followed by *B. cereus* (14.14%), *B licheniformis* (12.12%), *B. megaterium* (10.11%), *B. pumilus* (8.08%) and *B. coagulans* (3.03%). Isolation of *Bacillus* species from curd samples has been reported by Jayaram and Gandhi (1987), Mohanan *et al.* (1984) and Misra *et al.* (1993). The percentage of

isolation of the organism in the present study is much higher than that of the findings of Misra *et al.* (1993) who reported that the organism was detected in only four per cent of the samples. Kroger (1975) reported the presence of *B. subtilis* in fermented milk. *Bacillus subtilis* and *B.cereus* can cause bitter flavours in fermented milks if large numbers survive pasteurisation (Richter and Vedamuthu, 2001). *Bacillus cereus* has been implicated in food poisonings owing to the production of various types of toxins including haemolysins, proteases, phospholipase, cytotoxins and a heat labile diarrhoeal enterotoxin and heat stable emetic toxin. Most outbreak of *B. cereus* poisoning has been traced to foods containing at least 10^6organism/g. The organism is a psychrotroph and is able to grow at temperature of 50°C to as low as 4°C and over a pH range of 4.4 to 9.3 (Ryser, 1998). The increase in numbers of *Bacillus* species in curd result in the production of bitter flavours and is of public health concern as it may lead to spoilage of the product thereby causing economic loss to the traders. The presence of the organisms in the product may be due to contamination from the environment, utensils and packaging materials.

5.2.3 Physico-chemical Quality

5.2.3.1 Titratable acidity

Analysis of variance test revealed a highly significant ($p<0.01$) difference in the titratable acidity of the samples among the various brands (Table 38). The highest acidity was observed in samples of brand A (1.643 ± 0.10) and lowest in the samples of brand D (1.123 ± 1.10). Dahi from South India were of poor or watery texture, sour taste, alcoholic flavour and contained lactobacilli and yeast, while streptococci were present only in samples collected from North (Laxminarayana *et al.*, 1952). Repeated subculturing of the starter culture will cause elimination of streptococci and there will be predominance of lactobacilli and yeast which result in increased acid production and thereby reduce the shelf life of the product. (Mohanan *et al.*, 1985). The higher acidity content in market

curd could be due to the use of different types of cultures, varied processing techniques and storage of dahi at high temperatures.

The acidity of the samples from brand D is in accordance with the findings of Rao *et al.* (2002) and Mohanan *et al.* (1984) in dahi. The samples in the present study had much higher acidity than that reported in dahi (Soomro *et al.*, 2003; Younus *et al.*, 2002) and in misti dahi (Sarkar *et al*; 1996). All the samples from brands A, B and C did not meet the standards prescribed by Indian Standards (1980a) for sour dahi, but 20 per cent of the samples from brand D met the standards.

5.2.3.2 pH

Analysis of variance test revealed a highly significant ($p<0.01$) difference in the mean pH of curd from the four different brands (Table 39). The samples of brand D had the highest mean pH (3.840 ± 0.06) and the lowest mean pH was observed in the samples of brand B (3.621 ± 0.01). However, the pH of curd samples of the present study was much lower than that reported by Soomro *et al.* (2002) and Younus *et al.* (2002). The low pH values might be due to presence of acid-producing organism in large numbers. However, 50, 40 and 10 per cent of the samples of brand D, C and A met the standards prescribed for fermented milks by IDF (1992). The samples having lower pH values had yeasty flavour and alcoholic aroma which reduced the shelf life of the product.

5.2.4 Organoleptic Evaluation of Market Curd

The organoleptic score of market curd was analysed by Kruskal-Wallis test and no significant variation was found in score among the brands for flavour, body and texture and colour and appearance. But the product acidity of the market curds varied at a highly significant ($p<0.01$) level.

5.2.4.1 Flavour Score

Flavour had the highest score for samples of brand C (28.56 ± 0.65) and lowest for samples of brand B (27.15 ± 1.2) (Table 40). High acid taste was reported among two samples of brand A, and four samples of brand B. One of the samples from brand C had yeasty and another had abnormal flavour. Bitter flavour was noticed in two of the samples of brand B. Gupta *et al.* (2000) also has reported yeasty flavour in misti dahi samples procured from market. Mohanan *et al.* (1984) observed that yeast can cause reduction in flavour producing organism in dahi starter cultures. High acid taste of curd may be due to excessive amount of inoculum used in the production or storage of dahi at high temperatures.

5.2.4.2 Body and Texture Score

Body and texture score was highest in the samples of brand A (27.3 ± 0.96) and lowest in the samples of brand C (24.25 ± 0.704) as shown in table 41. Wheying off was noticed in four samples of brand A, and two samples of brand D, which might be associated with higher acidity, higher temperature of incubation and prolonged storage of the product. Thin body was mainly noticed in samples of brand C which could be due to low total solid content or insufficient acid production in the curd. One of the samples of the brand produced gassiness, which is a serious defect noticed in dahi due to growth of contaminated yeast or with coliforms, which can reduce the shelf life of the product. Grainy texture was noticed in two of the samples of brand A. Rangappa and Acharya (1973) also reported that milk stored for too long, before seeding often gives rise to broken curd with poor taste.

5.2.4.3 Colour and Appearance Score

Colour and appearance of the samples of various brands did not differ significantly. The highest score was observed in the samples of brand B and D and was lowest in the samples of brand A (Table 42). All the curd samples were found to be free of surface discolouration and visible foreign matter.

5.2.4.4 Product Acidity Score

Perusal of data presented in table 43 revealed that the samples of brand C had the highest mean product acidity score (11.25 ± 0.417) and the lowest in the samples of brand A (9.5 ± 0.269). Highly significant ($p<0.01$) variation between the mean product acidity of the samples of the four brands was observed. Excessive acidity gives curd, a sour, biting taste.

5.3 ASSESSMENT OF CRITICAL CONTROL POINTS IN THE PRODUCTION LINE OF CURD

Microbial quality assurance of curd during its production was assessed by examining the samples at various stages in the production line. In order to identify various critical control points of microbial contamination during preparation of curd samples, samples of air, water, equipment and raw ingredients were analysed for their microbial quality.

5.3.1 Changes in the Mean Microbial Counts during the Various Stages of Production of Curd

Raw milk collected for the production of curd had a mean total viable count of 5.96 ± 0.22 $\log_{10}$cfu/ml and on heat treatment the count of skim milk was reduced to 2.55 ± 0.34 $\log_{10}$cfu/ml (Table 44). Starter inoculated skim milk after one and six hour of incubation had mean count at the level of 5.97 ± 0.27 $\log_{10}$cfu/ml and 7.19 ± 0.05 $\log_{10}$cfu/ml, respectively.

Coliforms were present in raw, pasteurised and skim milk. However, in heat treated skim milk, the organisms could not be detected. But fresh curd samples had a coliform count at the level of 0.12 ± 0.2 $\log_{10}$cfu/g. Starter culture inoculated milk samples, at one and six hour of incubation had coliforms at the level of 0.98 ± 0.23 $\log_{10}$cfu/ml and 0.75 ± 0.31 $\log_{10}$cfu/g, respectively. The presence of coliforms in the inoculated milk after one hour of incubation suggested that the contamination of milk with the organism could have occurred

from the storage cans, since the heat treated skim milk and starter culture were found to be free from coliforms.

The mean faecal streptococcal count of heat treated skim milk was significantly ($p<0.01$) lower than that of inoculated milk after one and six hour of incubation. In the present study, it was observed that the faecal streptococcal count increased during the incubation of inoculated milk and in fresh curd samples. Therefore enterococci can be considered as hygienic indicators during the production of curd.

Yeast and mould could not be detected in heat treated skim milk, but the organisms were found in starter inoculated milk at one and six hour of incubation. Fresh curd samples had yeast and mould at the level of 10^3cfu/g. Starter culture inoculated samples had yeast and mould which indicated that the organisms might be present in the starter culture. Growth of yeast cells in dahi is one of the major problems of maintaining starter cultures in pure form under household conditions of preparation of dahi. Due to their ability to tolerate acidic environment, yeasts can multiply in dahi producing gassiness and flavour defects associated with lipolysis of milk fat, if the product is held too long at warm ambient temperatures. However, they grow slowly in dahi and their numbers will be low, unless the inoculum is transferred from old and yeasty dahi (Yadav *et al.*, 1993).

5.3.2 Changes in the Mean pH during the Production of Curd

The change in the mean pH during the production of curd was assessed (Table 45). The pH reduced from 6.83 ± 0.001 in heat treated milk to 3.59 ± 0.01 in fresh curd samples. The findings of the present study are in agreement with the report of Birollo *et al.* (2001) and Samolada *et al.* (1998).

5.3.3 Starter Culture

The total viable count of starter culture was 6.43 ± 0.21 log_{10}cfu/g (Table 46). Aneja *et al.* (2002) stated that yeasts and moulds in starter culture should be <10 cfu/g and a pH of 4.2 is desirable. The yeast and mould count of the starter culture in the present study was 3.70 ± 0.21 log_{10}cfu/g which might have led to rapid fermentation and lowering of the pH of the product at a faster rate and high acidity of the curd can lead to development of wheying off in the curd. The presence of yeasts can also lead to elimination of flavour producing organisms in curd. Coliforms and faecal streptococci were not detected in the starter culture.

5.3.4 Air

The mean total viable count of air samples from the area of pasteurisation, cream separation and thermal treatment, before and after processing were 27.6 ± 1.52, 20.4 ± 2.84 and 70.5 ± 2.3 and 69 ± 1.75, 45.2 ± 4.5 and 96.3 ± 1.65 cfu/ft^2/min, respectively (Table 47). The mean total viable count of air samples in the cold room was 10 ± 1.3 cfu/ft^2/min.The counts obtained in the present study from various processing areas were well above the standards prescribed by APHA (Hickley *et al.*, 1992). Salustiano *et al.* (2003) also reported that there was considerable increase in the number of indicative organisms during the processing of various dairy products.

The mean yeast and mould count of air samples from the area of pasteurisation, cream separation and thermal treatment, before and after processing were 12.7 ± 1.2, 4.8 ± 1.2 and 4 ± 0.35 and 13.5 ± 0.93, 7.83 ± 1.5 and 12.3 ± 0.92 cfu/ft^2/min, respectively. The mean yeast and mould count of air samples was maximum in the cold room (19 ± 1.64 cfu/ft^2/min). The findings of the present study differed from that of Rajarajan and Nareshkumar (2003) who recorded higher yeast and mould count in the vat section of the dairy plant. Yeast and mould are ubiquitous in the environment and many are psychrotrophic in

nature and they are the main spoilage organisms in the production of fermented milk products like curd.

Air samples from the thermal treatment room had the highest mean total viable count whereas yeast and mould count was highest in the cold room. The study revealed that microflora of air may contribute to the contamination of product in the processing room.

5.3.5 Water

The mean total viable count of water samples from storage tank, tap and hand washings was 3.50 ± 0.10, 4.01 ± 0.25 and 4.53 ± 0.24 $\log_{10}$cfu/ml as shown in table 48. Water samples from the storage tank did not reveal the presence of coliforms. The samples from the tap water and hand washings had coliforms and the mean counts were 1.03 ± 0.06 and 1.19 ± 0.38 $\log_{10}$cfu/ml, respectively. *Escherichia coli* were detected only in samples of hand washings. Faecal streptococcal count was detected in both tap water and hand washings and the corresponding counts were 1.02 ± 0.19 and 0.49 ± 0.23 $\log_{10}$cfu/ml. The faecal streptococcal count of tap water was higher than that prescribed by the Government of British Columbia (2001).The high bacterial count in the water samples indicates its unwholesomeness and its role in the contamination of the product.

Hand washings of personnel involved in the production of curd had coliforms as well as *E. coli*. The detection of *E. coli* and faecal streptococci in the hand wash of the workers indicated the poor personnel hygiene. Therefore, it may be inferred that these employees might also contribute to the contamination of the curd samples with spoilage and pathogenic organism particularly of intestinal origin. The findings of the present study point to the importance of maintaining personnel hygiene by the plant workers.

5.3.6 Processing Equipments

The bacterial counts of samples collected from the equipments were estimated to assess the extent of involvement in contamination of the curd as shown in table 49. The mean total viable count of processing equipments such as pasteuriser, cream separator, double jacketed vat, storage can and packaging material ranged from 3.20 ± 0.18 to 4.95 ± 0.07 log_{10}cfu/cm^2 or per ml. The coliform count varied between 0.31 ± 0.26 and 1.16 ± 0.16 log_{10}cfu/cm^2 or per ml. Faecal streptococci were also detected in all the samples tested.

Among the equipments involved in the production of curd, the cream separator had a significant role in contributing to total viable count of the product, followed by the double jacketed vat. But the mean total viable count of the storage can is important because thermal treatment at the temperature of 90°C for 15 min destroys most of the organisms and the important source of contamination is the storage can on to which milk is transferred for cooling, addition of starter culture and incubation. Packaging material was free of coliforms, but faecal streptococci were present at the level of 0.10 ± 0.10 log_{10}cfu/ml. Singh (1978) opined that the total viable count in 23 per cent samples examined had counts similar to the present study and coliforms were also present in dahi containers. Counts of pasteuriser, cream separator and storage can were very much higher than that reported by Yadav *et al.* (1993).

The present study highlights the effect of refrigeration on microbial, physico-chemical and organoleptic qualities of curd as well as the quality of curd procured from retail outlets. Shelf life under refrigerated storage was found to be nine days. Spoilage under refrigerated storage might be attributed to the high initial microbial load of milk, contamination of the starter culture and contamination from the processing environment. Unhygienic handling during the various steps in the production line can also cause contamination of the final product. Curd, being a product consumed as such, the study emphasises the importance of the implementation of strict hygienic measures throughout the

production line. Therefore, an overall improvement in the production, packaging, storage and distribution of the product is of great significance to safeguard consumer health. Identification of the critical control points will help the manufacturers to take appropriate remedial measures to curtail the chances of microbial contamination of curd and will help the retailers in preventing economic loss due to product spoilage. The study also reflects the importance of implementing principles of Hazard Analysis and Critical Control Programme, which helps to prioritise process steps focusing on hazards that can be reduced or eliminated.

Summary

6. SUMMARY

The consumer safety and shelf-life of milk and its products are influenced by their microbial quality. The quality assurance of these products is important as the survival of microbes can render these foods hazardous and can reduce shelf-life. In the present investigation, a total of 180 freshly prepared curd samples belonging to 10 batches were collected on the day of production. Two samples from each batch were selected randomly and examined on zero day and the remaining samples were stored under refrigeration and duplicate samples examined on day 3, 5, 7, 9, 12, 15, 18 and 21 of storage. A total of 80 curd samples belonging to four brands *viz.* A, B, C and D were also collected from retail outlets in and around Thrissur Corporation. All samples were evaluated for their microbial, physico-chemical and organoleptic quality by estimating the total viable count (TVC), coliform count (CC), faecal streptococcal count (FSC), psychrotrophic count (PC) and yeast and mould count (YMC). All samples were subjected to isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, Salmonellae, *Pseudomonas aeruginosa* and *Bacillus* species. The organoleptic qualities of curd such as flavour, body and texture, colour and appearance and product acidity were also evaluated. During the study, the critical control points of microbial contamination of curd in the production line were also evaluated

Paired't' test of the data revealed that mean total viable count increased significantly ($p<0.01$) from zero day (8.28 ± 0.16 $\log_{10}$cfu/g) to fifth day of storage (9.20 ± 0.19 $\log_{10}$cfu/g) and thereafter the count decreased gradually till the end of the storage period.

Of the 180 samples, 90 per cent of the samples examined on zero day and 95 per cent of the samples evaluated on day 9 and 12 did not reveal the presence of coliforms. The mean coliform count of fresh curd samples was 0.12 ± 0.12 $\log_{10}$ cfu/g. The counts showed a gradual decreasing trend till the 12^{th} day of

storage. The samples stored on day 15, 18 and 21did not reveal the presence of organism. The mean faecal streptococcal count of fresh curd samples was 2.01 ± 0.40 log_{10}cfu/g. The count decreased at a highly significant ($p<0.01$) level on 21st day of storage. On the day of production, 30 per cent samples had count at the level of 10^3cfu/g as against 10 per cent on the third day of storage. On 18th and 21st day, 70 per cent samples did not reveal the presence of the organism.

The mean psychrotrophic count of fresh curd samples was 3.20 ± 0.24 log_{10} cfu/g. The count decreased subsequently till 12th day of storage and after which a slight increase in the count was observed. Highly significant ($p<0.01$) difference was noticed between the mean count of fresh curd and that stored on day 9 and 12. On zero day, 55 and 15 % samples had count at level of 10^3 and 10^4 cfu/g, respectively.

The mean yeast and mould count of fresh curd was 3.91 ± 0.11 log_{10}cfu/g. The count gradually increased from third day of storage and reached 1.5 log higher than that of the count observed on the 12th day of storage. The mean count of the fresh curd samples revealed a highly significant ($p<0.01$) difference than that of the mean counts observed during the period of storage. In fresh curd samples, 75 per cent had count at the level of 10^3cfu/g. The count in 80 per cent of the samples stored on 21st day was at the level of 10^5cfu/g.

The correlation coefficient between the various counts was determined by Spearman's rank correlation test and a highly significant ($p<0.01$) and positive association was observed between the mean TVC and FSC on 21st day of storage. A significant ($p<0.05$) and negative association was observed between the mean TVC and PC on day nine of storage. A similar association was observed between mean CC and PC of samples on 12th day of storage, and mean YMC and PC of samples on 15th day of storage.

Escherichia coli was not detected from any of the fresh and refrigerated samples of curd. *Staphylococcus aureus* was isolated from 35 per cent of fresh

curd samples. On day 3, 5 and 7 of storage, 25, 20 and 10 per cent of the samples revealed the presence of the organism and all isolates were coagulase and TNase positive. All fresh and refrigerated curd samples were found to be free of Salmonellae and *Pseudomonas aeruginosa*. *Bacillus* species were present in 70 per cent of the fresh samples and 25 per cent each of the samples tested on day 15 and 18. *Bacillus cereus* was not detected in any of the curd samples. Among the isolates, 55.4 and 25.67 per cent were *B. subtilis* and *B. coagulans*.

The mean titratable acidity of fresh curd samples was 1.85 ± 0.03 % lactic acid and it increased to 1.88 ± 0.05 on ninth day and then decreased till 21st day of storage. The reduction in the mean titratable acidity of the samples stored for 21 days was highly significant ($p<0.01$) than that of fresh curd samples.

The mean pH of fresh curd samples was 3.59 ± 0.010 and during storage, it decreased initially and increased gradually after 15th day of storage. The pH increased by 0.05 units on 12th day of storage compared to the fresh curd samples. A highly significant ($p<0.01$) difference between the mean pH values of the samples on day zero and three were observed.

Analysis of data on organoleptic qualities revealed that flavour scores decreased during storage. Body and texture score remained almost the same throughout the storage except a slight decrease in score on the third day. Colour and appearance score decreased significantly during the storage period and product acidity score also got reduced during storage. However, the product acidity score later increased from 15th day onwards. Findings of the present study indicated that the product has a shelf life of nine days under refrigerated storage.

The highest mean TVC was observed in the samples of the brand C (8.93 ± 0.2 $\log_{10}$ cfu/g). The samples of the brand A had the lowest count (8.40 ± 0.24 $\log_{10}$ cfu/g). Of the 80 samples, 42.5, 37.5 and 20 per cent had a count at the level of 10^9, 10^8 and 10^7 cfu/g, respectively.

Analysis of the variance test of the data revealed highly significant ($p<0.01$) difference between the mean coliform count of the samples of curd from the four brands. Critical difference test of the data revealed significant difference between the mean counts of samples of brand A and B, A and C and A and D. None of the samples of brand B had coliforms. The organism was detected in 80 and 70 per cent samples of brands C and D. Of the 80 samples, 50, 80 and 90 per cent of the samples of brands A, B and C met the standards for coliforms prescribed by BIS.

The samples of the brand A had the highest mean faecal streptococcal count (2.518 ± 0.40 $\log_{10}$ cfu/g) and lowest in the samples of the brand B (0.709 ± 0.40 $\log_{10}$ cfu/g). A highly significant ($p<0.01$) difference was observed between the mean count of samples from the four brands. Critical difference test revealed significant difference between the mean counts of the samples of the brand A and B, A and C and A and D. Of the samples, 40 per cent did not reveal the presence of the organism. The count in 50 per cent samples from brand A was at the level of 10^3cfu/g. None of the samples of the brands B and C had the count greater than 10^2cfu/g.

The samples of brand C had the highest mean psychrotrophic count ($4.23 \pm 0.26\log_{10}$ cfu/g) and the lowest was observed in the samples of the brand A ($3.60 \pm 0.28\log_{10}$ cfu/g). Of the 80 samples, 55 per cent had count at the level of 10^4 cfu/g. The count in 10 and 35 per cent samples was at the level of 10^2 and 10^3cfu/g, respectively.

The mean yeast and mould count was highest in the samples of brand C (5.500 ± 0.14 $\log_{10}$cfu/g), while the lowest count (5.148 ± 0.24 $\log_{10}$cfu/g) was seen in the samples of the brand A. The yeast and mould count of the samples varied from 10^3 to 10^6cfu/g, respectively. The count in 55 and 15 per cent samples was at the level of 10^5 and 10^6cfu/g. None of the samples of brand B had counts at the level of 10^6cfu/g.

A highly significant ($p<0.01$) positive association was observed between mean TVC and PC.

E. coli was not detected in the samples of brands B and D. The organisms were isolated from 5 and 15 per cent samples of the brands A and C. The four isolates belonged to the serotype O157, O5, O148 and rough type. The serotypes O5 and O157 belonged to enterohaemorrhagic (EHEC) group and the serotype O148 was categorised as enterotoxigenic *E. coli* (ETEC). All the isolates were Congo red positive, which indicated their invasive ability.

None of the samples from the four brands revealed the presence of Salmonellae. *Pseudomonas aeruginosa* was present in 10 per cent of the samples from the brand C. *Staphylococcus aureus* was detected in two of the curd samples, one (5%) each from brand A and C. All the isolates were coagulase positive. Out of the 99 isolates of *Bacillus* species obtained from 50 samples of four brands, *B. subtlis* was the highest (52.52%) followed by *B. cereus* (14.14%), *B licheniformis* (12.12%), *B. megaterium* (10.11%), *B. pumilus* (8.08%) and *B. coagulans* (3.03%).

Analysis of variance test revealed a highly significant ($p<0.01$) difference in the titratable acidity of the samples among the various brands. The highest acidity was observed in the samples of the brand A (1.643 ± 0.10) and lowest in the samples of the brand D (1.123 ± 1.10).

Analysis of variance test revealed a highly significant ($p<0.01$) difference in the mean pH of samples from the four brands. The highest mean pH was observed in the samples of brand D (3.840 ± 0.06) and lowest in the samples of the brand B (3.621 ± 0.01).

Analysis of the organoleptic score by Kruskal-Wallis test revealed that the product acidity score of the market curds varied significantly ($p<0.01$). The samples of the brand A had lowest product acidity score (9.5 ± 0.269) and highest score was observed in the samples of brand C (11.25 ± 0.417).

Evaluation of curd samples from the four brands revealed that the samples of the brand B was superior to the samples of the other brands in respect of the microbial counts and the presence of the bacterial pathogens, but had poor organoleptic quality.

Air samples were collected from the various areas of the production line of curd before and after processing and the latter samples had high mean TVC and YMC.

Among the water samples collected, hand washings of personnel involved in processing had the highest mean total viable count and coliform count. *E. coli* was detected only in hand washings at the level of 0.05 ± 0.05 $\log_{10}$cfu/ml. Faecal streptococci were detected both in hand washings and tap water samples.

The hygienic status of the pasteuriser, cream separator, double jacketed vat, milk storage can and packaging material were also assessed. Among the equipment washings, cream separator had the highest total viable count and lowest count was observed for the packaging material. Faecal streptococci were detected in all the samples tested and highest count was observed in washings from storage can. Double jacketed vat had the highest coliform count. Coliforms were not detected in the washings of the packaging material. *E. coli* was not detected in any of the samples collected from the equipments.

The changes in the microbial counts in milk during the production of curd were assessed. Coliforms were present in raw, pasteurised and skim milk. However, in heat treated skim milk, coliforms were not detected. But fresh curd samples had a coliform count of 0.12 ± 0.2 $\log_{10}$cfu/g. Milk samples collected at one and six h after starter inoculation and incubation had coliforms at the level of 0.98 ± 0.23 $\log_{10}$cfu/ml and 0.75 ± 0.31 $\log_{10}$cfu/g, respectively. The mean faecal streptococcal count of heat treated skim milk was significantly lower than that of starter inoculated milk after one and six h. Yeast and moulds were not detected in heat treated skim milk, but was observed in starter culture inoculated milk at one

and six h of incubation. Fresh curd samples had yeast and mould at the level of 10^3cfu/g. The change in the mean pH during the production of curd was assessed and observed that it reduced from 6.83 ± 0.001 in heat treated skim milk to 3.59 ± 0.01 in fresh curd samples.

The total viable count of starter culture was 6.43 ± 0.21 $\log_{10}$cfu/g. Coliforms and faecal streptococci were not detected in the starter culture. The mean pH was 3.75 ± 0.01.

In the present study, it was observed that fresh curd samples did not meet standards prescribed by Indian Standards with respect to the yeast and mould count and titratable acidity; whereas the coliform count in 90 per cent samples met the standard. Shelf life of curd was found to be nine days under refrigerated storage. The count in 50, 80 and 90 per cent samples from brands A, C and D met the standards for coliform prescribed by Indian Standards. Only 20 per cent of the samples from the brand D satisfied the standards for titratable acidity. The samples with high acidity were found to be free of coliforms and other pathogenic bacteria. The study points to the necessity of preparation of curd with standard acidity and free from pathogens. This could be achieved by maintaining pure starter cultures, use of potable water, strict adherence to recommended time-temperature combination for pasteurisation and refrigerated storage of the product. The study gives an insight into the microbial contamination of the product during its production and also physico-chemical and organoleptic changes during storage. Hence improvement in the quality of milk, strict observance of hygienic practices at each and every step in the preparation of curd will help in the production of a safe product and thus protect consumer health.

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Appendix

SCORE CARD FOR SENSORY EVALUATION OF CURD

Date :

Code No.

ATTRIBUTES	MAXIMUM SCORE	SAMPLES		
		A	B	C
FLAVOUR	40			
BODY AND TEXTURE	35			
COLOUR & APPEARANCE	10			
PRODUCT ACIDITY	15			
TOTAL	100			

DESIRABLE CHARACTERISTICS

Curd (dahi) is a cultured milk product. It shall have a pleasing bouquet flavour resulting from the blend of a clean delicate somewhat aromatic odour and a pronounced though clean acid taste. It should be free from the following undesirable flavours like bitter, coarse due to over-ripening, flat (lack of flavour), off odour, metallic and yeasty. Body and texture should be firm, solid and uniform with negligible whey separation. (Indian Standards, 1980a)

Taster

Signature

MICROBIAL QUALITY ASSURANCE OF CURD DURING PRODUCTION AND STORAGE

PRASEEDA R.

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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ABSTRACT

In the present investigation, a total of 180 freshly prepared curd samples belonging to 10 batches were collected on the day of production. Two samples from each batch were selected at random and examined on zero day and the remaining samples were stored under refrigeration and duplicate samples examined on day 3, 5, 7, 9, 12, 15, 18 and 21 of storage. A total of 80 curd samples belonging to four brands *viz.* A, B, C and D were also collected from retail outlets in and around Thrissur Corporation. All samples of curd were tested to evaluate the microbial quality by estimating the total viable count (TVC), coliform count (CC), faecal streptococcal count (FSC), psychrotrophic count (PC) and yeast and mould count (YMC). Isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, Salmonellae, *Pseudomonas aeruginosa* and *Bacillus* species were also carried out. The organoleptic qualities of curd such as colour and appearance, flavour, body and texture and product acidity and physico-chemical parameters such as pH and titratable acidity were also assessed. Microbial quality assurance of curd during its production and critical control points of microbial contamination in the production line were also evaluated during the study.

Paired't' test of the data revealed that mean TVC of the samples increased at a highly significant ($p<0.01$) level from zero day to fifth day of storage, after which it decreased gradually. The mean CC of curd samples showed a gradual decreasing trend till the 12^{th} day of storage. Coliforms were not detected in 90 per cent of the fresh curd samples. The mean FSC of curd samples also decreased significantly through out the storage period. On 18^{th} and 21^{st} day, 70 per cent of the samples did not reveal the presence of faecal streptococci. The mean PC decreased subsequently during storage till 12^{th} day, after which it slightly increased. The mean YMC of fresh curd was 3.91 ± 0.11 $\log_{10}$cfu/g and it increased gradually from third day of storage compared to the count of the fresh

sample. The count increased by 1.5$\log_{10}$cfu/g on the 12th day of storage and the increase was highly significant ($p<0.01$). A highly significant ($p<0.01$) and positive association was observed between the mean TVC and FSC on 21st day of storage. A significant ($p<0.05$) and negative association was observed between the mean TVC and PC on day nine of storage. A similar association was observed between mean CC and PC of samples on 12th day of storage and mean YMC and PC of samples on 15th day of storage.

E. coli was not detected from any of the fresh and refrigerated samples of curd. *Staphylococcus aureus* was isolated from 35 per cent of fresh curd samples. On day 3, 5 and 7 of storage, 25, 20 and 10 per cent of the samples revealed the presence of the organism and all isolates were coagulase and TNase positive. None of the curd samples revealed the presence of Salmonellae and *Pseudomonas aeruginosa. Bacillus* species were present in 70 per cent of the fresh samples and 20 per cent each of the samples tested on day 15 and 18. *Bacillus cereus* was not detected in any of the curd samples and among the isolates, 55.4 and 25.67 per cent were *B. subtilis* and *B. coagulans*.

The mean titratable acidity showed an increase till ninth day of storage and then decreased throughout the storage period. The pH of fresh curd samples decreased on storage and after 15th day, it increased gradually. Analysis revealed that flavour scores decreased during storage. Body and texture score remained almost the same throughout the storage period except a slight decrease on the third day. Colour and appearance score decreased significantly during the storage period and product acidity score also got reduced during storage.

Of the 80 samples collected from the four brands, 42.5, 37.5 and 20 per cent had mean TVC at the level of 10^9, 10^8 and 10^7 cfu/g, respectively. Highly significant ($p<0.01$) difference was observed in mean CC of the samples of the different brands. Coliforms were not detected in the samples of brand B and 80 and 70 per cent samples of the brands C and D, respectively. A highly significant ($p<0.01$) difference was observed between the mean FSC count of samples from

the four brands. Of the samples, 40 per cent did not reveal the presence of the organism and the count in 50 per cent samples from brand A was at the level of 10^3cfu/g.

E. coli was not detected in the samples of brands B and D. The organism was isolated from 5 and 15 per cent of samples of brand A and C and the four isolates belonged to the serotype O157, O5, O148 and rough type. None of the samples from the four brands revealed the presence of Salmonellae. *Pseudomonas aeruginosa* was present in 2.5 per cent of the samples from the four brands. *Staphylococcus aureus* was detected in two of the curd samples, one (5%) each from brand A and C. *Bacillus* species was isolated from 62.5 per cent of the samples and among the isolates, 14.14 per cent was *B. cereus.*

Analysis of variance test revealed a highly significant ($p<0.01$) difference in the mean titratable acidity and pH of the samples from the various brands. Organoleptic score analysed by Kruskal-Wallis test revealed that the product acidity score of the market curds varied significantly ($p<0.01$).

Air samples collected from the various areas of curd production revealed highest bacterial count at the area of thermal treatment and lowest at the area of cream separation. The mean YMC was higher at the pasteurisation room. Hand washings of the personnel had the highest mean TVC and CC. *E. coli* was detected only in hand washings of personnel. Samples collected from the cream separator had the highest mean TVC and least in packaging material. Faecal streptococci were detected in all the samples tested and highest count was observed in the case of storage can. Samples from the double jacketed vat had the highest CC. Coliforms were not detected in the washings of the packaging material.

Heat treated skim milk was free of coliforms and the mean FSC was significantly ($p<0.05$) lower than that of starter inoculated milk after one and six h of incubation. Yeast and moulds were not detected in heat treated skim milk,

but present in starter inoculated one and six h milk samples. The mean pH of fresh curd samples was significantly ($p<0.01$) lower than that of heat treated skim milk. The starter culture was free of coliforms and faecal streptococci.

The present study reflects the importance of quality assurance during every step of production and storage of curd to avoid early spoilage and to safeguard consumer health. Presence of pathogenic organisms in curd is of great public health significance as it is consumed as such paving way to food poisoning.