ASSESSMENT OF BACTERIAL QUALITY AND SHELF LIFE OF PASTEURIZED MILK

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

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2007

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DECLARATION

I hereby declare that the thesis entitled **"ASSESSMENT OF BACTERIAL QUALITY AND SHELF LIFE OF PASTEURIZED MILK"** is a record of research work done by me during the course of research and this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or associateship or other similar title, of any other University or Society.

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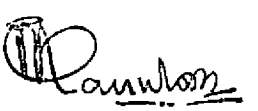
CERTIFICATE

Certified that the thesis entitled **"ASSESSMENT OF BACTERIAL QUALITY AND SHELF LIFE OF PASTEURIZED MILK"** is a record of research work done independently by **ASHA. K** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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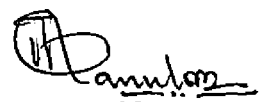

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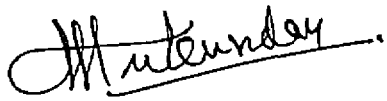

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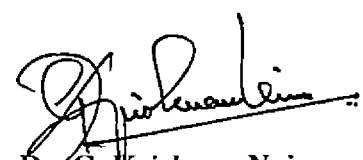

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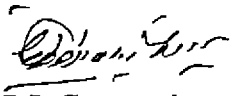

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Asha K.

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Introduction

1. INTRODUCTION

Livestock provides nutritious protein rich balanced food *viz.*, milk and value added products to the population. Milk and milk products are the major protein source to the vegetarian population and are relished by all age groups. The Indian dairy industry is contributing significantly to the country's economy, besides improving the human health standard. In 2001-02 the milk production in the country was 84.6 million tonnes and was ranked as first among the milk producing countries in the world (Animal Husbandry Statistics, 2005). Presently the milk production in the country has increased to 94.5 million tonnes. However the present per capita availability is only 240 g per day which is still below the world average of 285 g per day. The per capita availability in Kerala is 173 g per day which is much less than the national average (Gupta, 2007).

Today's customers of milk, whether they be processors, retailers, exporters or consumers, want assurance that the food they receive is safe and wholesome and produced in a clean environment. In the past, the industry's reputation and verbal assertions on food safety were sufficient to maintain customer trust; however, today buyers want proof that the food they are buying meets clearly defined food safety norms. The components of milk and its physical and chemical properties provide a very favourable milieu for the growth and multiplication of micro organisms which can cause milk spoilage and transmission of pathogenic micro organisms (Chye *et al.*, 2004). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is of considerable significance (Foster, 1990). With the aim of minimizing milk associated health hazards, restrictions and legislations on the marketing of unpasteurized milk have been introduced in most countries. However, this does not necessarily guarantee the safety of milk products. Outbreaks of milk borne diseases have occurred despite pasteurization, caused either by improper pasteurization or by recontamination (Da Silva *et al.*,

1998). Several food-borne disease outbreaks have been linked to pasteurized milk and traced to inadequate pasteurization or post pasteurization contamination.

Production of quality milk is the concern of today's consumers of dairy products, retail distributors, milk and milk product processors, dairy cooperatives, state regulatory departments, veterinarians and dairymen. Apart from microbial quality assurance involved in the production line of freshly pasteurized milk, the concern for keeping quality of pasteurized milk has also gained importance to prevent milk spoilage so as to minimize economic losses to producer and scarcity of milk available to consumer. The main factors affecting the keeping quality of pasteurized milk are raw milk quality, time-temperature combination of heat treatment, post pasteurization contamination, package condition and storage temperature. The determinants of shelf life of pasteurized milk are usually the bacteria that have the ability to grow at refrigerated temperatures (Muir, 1996). Milk being a nutritive food also provides an ideal environment for microbial growth and brings about either spoilage or renders them unsafe due to potential health hazards (Chye *et al.*, 2004). Milk quality deterioration is perceived by the consumer through off-flavours that may be caused by physicochemical or microbiological changes in the product itself (Van Aardt *et al.*, 2001). Pasteurized milk usually spoils when held at refrigeration temperatures because of the effects of psychrotrophic contaminants.

Hence the aim of the present study was to assess the safety and keeping quality of pasteurized milk available in the market. The changes in the organoleptic and physical quality were also evaluated during the storage period.

Considering all the above facts the present study was undertaken with the following objectives

1. Evaluation of keeping quality of pasteurized milk kept under refrigeration (4±1°C) by determining,

   - Bacterial load of samples up to tenth day of storage by estimating total viable count, coliform count, *Escherichia coli* count, psychrotrophic count and faecal streptococcal count.
   - Sensory and physical quality of milk.
   - Isolation and identification of bacteria of public health importance such as *Escherichia coli, Staphylococcus aureus* and bacteria associated with spoilage of milk like *Pseudomonas.*

2. To study the bacterial profile of retail pasteurized milk and isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

3. Identification of *Escherichia coli* isolates obtained from the samples using Polymerase Chain Reaction technique. .

Review of literature

2. REVIEW OF LITERATURE

2.1 MICROBIAL COUNTS OF MILK

2.1.1 Total Viable Count

Jain and Saraswat (1968) analysed 127 raw milk samples obtained from organised dairy farm (50), rural collection centers (40) and local vendors and retailers (37) in Udaipur city. The mean standard plate count of samples belonging to organised dairy farm, rural collection centers and local vendors and retailers was 7,60,000, 1,10,00,000 and 86,00,000/ml, respectively.

Vijai and Saraswat (1968) evaluated the bacteriological quality of 224 samples of raw market milk obtained from organised dairy farm, city market and rural collection centers and 65 pasteurized milk samples collected from milk supply scheme in Udaipur city. The study revealed that the raw milk samples from organised dairy farm had an average standard plate count of 14,00,000/ml. The count in the samples obtained from city market and rural collection center were 30,00,000 and 60,00,000/ml respectively. The count of pasteurized milk sample was 32,000/ml.

Davies (1977) analysed the bacteriological quality of 2090 samples collected from bulk milk supplies in Wales. Frequency distribution of total colony count showed that 19.4 per cent of samples had count less than 10,000/ml, 67 per cent with counts less than 50,000/ml, 77.3 per cent with counts less than 10,00,000/ml and 5.4 per cent with counts of 5,00,000/ml or more. The average count of bulk milk samples for one year was just over 33,000/ml.

Garg *et al.* (1977) evaluated 57 cow milk samples obtained from Hissar city during summer and winter months and reported that the standard plate count of

samples collected during summer were in the range of 4 x 10^5 to 2 x 10^8/ml and that collected during winter was in the range of 5.4 x 10^5 to 4 x 10^7/ml.

Desai and Natarajan (1981) studied the bacterial quality of raw milk collected from societies in three different areas namely A, B and C located around Bangalore city. From each area the samples were collected from three societies and from each society five samples were collected at the time of dispatch. The average standard plate count of the samples collected from the societies in the area A, B, and C were 205 x 10^5; 441 x 10^5 and 92 x 10^5/ml respectively.

Schroder *et al.* (1982) investigated the bacterial quality of raw and commercially pasteurized milk, collected from five dairies *viz.* A, B, C, D and E and reported that the geometric mean of total viable count of the raw milk samples was 1 x 10^5 cfu/ml. Total viable count in commercially pasteurized milk had a geometric mean of 3 x 10^3 cfu/ml.

Reddy *et al.* (1984) studied the bacterial quality of 30 samples of raw milk obtained from Vijayavada milk shed that are used in the manufacture of dried whole milk powder. The standard plate count of the samples ranged from 0.77 to 29.40 million/ml. The count of raw milk was correlated with dried whole milk powder by 0.74. The observation of the study indicated the necessity for good quality raw milk to produce high quality dried whole milk powder.

Yadava *et al.* (1985) studied the bacterial flora of 105 milk samples marketed in Ranchi town. During the study, 42 raw milk samples were collected from organised dairy farm and 22 samples were collected from milk supply scheme. The average total viable count of samples from the former source was 2.23 x 10^5/ml and the count of the latter source was 1802.04 x 10^5/ml.

Arora and Sudarsanam (1986) analysed the microbiological quality of milk used as an ingredient in ice cream. A total of 16 samples, of which eight obtained from experimental dairy NDRI (Source A) and remaining from market (Source B) were analysed. The samples from source A had an average standard plate count of 16.75 x 10^3/ml with a range of 6.2 x 10^3 to 25 x 10^3/ml. The samples from the source B had an average count of 2.212 x 10^3/ml with a range of 0.8 x 10^3 to 4 x 10^3/ml.

Misra and Kuila (1989) analysed 125 samples of raw milk and 25 samples of pasteurized milk to detect the presence of various groups of bacteria and also the quality of milk produced and distributed in Calcutta and its suburbs. During the study they found that the raw milk samples obtained from organised dairy farms, vendors and sweet meat shops had a mean standard plate count of 51 x 10^4, 71.73 x 10^5 and 72.73 x 10^5 cfu/ml, respectively. The mean count of pasteurized milk from dairy plant was 53 x 10^3 cfu/ml.

Rajmany *et al.* (1989) studied the presence of *Staphylococci* in 20 samples each of raw milk, khoa, curd, ice cream, sweetened condensed milk, milk powder and processed cheese, obtained from the local market of Udaipur city. The total bacterial count in raw milk samples varied from 11.6 x 10^6 to 98 x 10^6 cfu/ml, with an average count of 53.4 x 10^6 cfu/ml.

Reddy *et al.* (1989) analysed 30 samples each of raw and pasteurized milk collected from the farm and the distribution center near to the Dairy experimentation station, Tirupati. The mean standard plate counts of raw and pasteurized milk samples were 7.516 ± 1.404 x 10^5 and 9.877 ± 1.058 x 10^5/ml, respectively.

Mahari and Gashe (1990) enumerated the microorganisms present in raw and pasteurized milk and also the sources of contamination of milk after it was received in the processing plant in Addis Ababa. The lowest mesophilic aerobic count of raw

milk samples was 4 x 10^7 cfu/ml while the highest was 1 x 10^9 cfu/ml. Pasteurized milk had mesophilic aerobic count of 7 x 10^5 cfu/ml as it left pasteurizer unit, but the population increased two to four fold as a result of subsequent contamination.

Rai and Dwivedi (1990) studied the bacteriological quality of milk samples collected from four sources. They found that the standard plate count of the standardized and pasteurized milk supplied by milk board varied between 9 x 10^4 and 65 x 10^4/ml with an average count of 33 x 10^4/ml. The count of raw milk supplied by C.S. Azad University of Agriculture and Technology, Kanpur, varied from 120 x 10^4 to 595 x 10^4/ml with a mean count of 295 x 10^4/ml. The count of samples from hawkers varied from 258 x 10^4 to 2570 x 10^4 with an average count of 1142 x 10^4/ml, whereas the samples from ghosies had a mean count of 429.12 x 10^4/ml with a range of 175 x 10^4 to 856 x 10^4/ml.

Sakkarvarthi *et al.* (1990) analysed the bacteriological quality of raw cow and buffalo milk from organised and unorganised sectors. The standard plate count of cow milk samples ranged from 1.38 x 10^6 to 50 x 10^6/ml. The corresponding count for buffalo milk samples obtained from organised sector ranged from 1.2 x 10^6 to 66 x 10^6/ml. Buffalo milk samples from unorganised sector had the count in the range of 1.0 x 10^6 to 197 x 10^6/ml.

Patel *et al.* (1993) collected 21 samples of milk from buffaloes maintained at the reproductive biology research unit, Veterinary College, Anand. Analysis of the samples revealed that the total plate count of the samples varied from less than 1000 to 11,00,000 cfu/ml with an average of 2.1 ± 0.7 x 10000 cfu/ml.

Siva *et al.* (1993) collected 32 samples of raw cow milk from individual producers, collection centers and dairy plant and evaluated the microbiological status of the samples at various stages of collection. Average total plate count of milk

samples collected from individual producers, collection centers and diary plant was 1 $\pm$ 4.15 x 10^6, 9.1 $\pm$ 1.6 x 10^6 and 17 $\pm$ 0.43 x 10^6 cfu/ml, respectively.

Cerqueira *et al.* (1994) examined 96 samples of type C pasteurized milk and also raw milk collected from a diary plant and markets of Belo Horizonte, Brazil. During the study it was observed that pasteurized milk samples had a mean mesophilic count of 3.7 x 10^4 cfu/ml. The mean count in raw milk was 4.5 x 10^6 cfu/ml.

Singh *et al.* (1994) analysed the sanitary quality of 70 samples of raw milk collected from different distribution cans. Standard plate count in the samples ranged from 4.477 to 8.857 $\log_{10}$ cfu/ml with a mean count of 7.30 $\log_{10}$ cfu/ml.

Kapre (1995) studied the bacteriological quality of 21 individual raw milk samples each, collected from three different sources. The study revealed that the average total viable count of samples from S_1, S_2 and S_3 were 7.5 x 10^4, 14 x 10^5 and 2 x 10^5 cfu/ml, respectively. During the study, seven pooled raw milk samples from each source were also tested. The mean total viable count of samples from S_1 source was 4.0 x 10^4/ml. The average counts of the samples belonging to S_2 and S_3 sources were 1.8 x 10^6 and 2.1 x 10^5 cfu/ml, respectively.

Pelezynska and Libett (1995) analysed the hygienic risk factors and CCP in the milking and processing of raw milk for consumption. A total of 100 milk samples were analysed at the collection points and the average bacterial counts in the raw milk was four million/ml. Bacterial counts increased in bulk milk sample and also after transport to the dairy. However, pasteurization significantly reduced the bacterial count to 42,000/ml.

Mutukumira *et al.* (1996) evaluated the quality of 10 samples of raw milk delivered to the Nharira/Lancashire milk collection center, Zimbabwe. The total aerobic counts of the samples ranged between 6.2 x 10^3 and 7.8 x 10^7 cfu/ml. Seven out of the ten samples had a count less than 10^5 cfu/ml, whereas in three samples the count was more than 5.01 x 10^5 cfu/ml.

Cosentino and Palmas (1997) analysed raw and heat-treated ewes milk collected three times over a period of 6 months from six (1,2,3,4,5 and 6) milk-processing plants in Sardina. The mean total microbial counts of raw milk from plant 1, 2, 3, 4, 5 and 6 were 4.9 ± 0.42, 5.9 ± 0.27, 4.1 ± 0.78, 6.0 ± 0.32, 6.9 ± 0.55 and 8.5 ± 0.95 cfu/ml, respectively. The counts of heat-treated milk from above sources were 3.0 ± 0.82, 3.1 ± 0.45, 2.1 ± 0.22, 3.9 ± 0.31, 3.8 ± 0.44 and 6.3 ± 1.07 cfu/ml, respectively.

Garg and Mandokhot (1997) analysed 86 samples of raw milk and found the standard plate count ranged between 7 x 10^4 and 2 x 10^{10}/ml. Sixty-four out of 86 samples (74.4 per cent) had standard plate count even above 5 x 10^6/ml. Only two samples were graded very good. High standard plate count (over 5 x 10^6) in majority of milk sample indicated poor hygienic practice followed at dairy farms in the region.

Latha and Nanu (1997) investigated the bacterial quality of twelve samples each of pasteurized milk obtained from S_1, S_2, S_3, S_4 and S_5 sources. The mean aerobic plate counts of the samples from S_1, S_2, S_3, S_4 and S_5 sources were 5.98 ± 0.05, 5.23 ± 0.06, 5.57 ± 0.06, 4.58 ± 0.03 and 3.55 ± 0.01 $\log_{10}$ cfu/ml, respectively. All samples belonging to S_5 source, 25 per cent of the samples from S_4 source and none of the samples from other three sources met the standards prescribed for aerobic plate count of pasteurized milk by BIS (1977)

Lopes and Stamford (1997) evaluated 84 sample of milk collected from four points, viz. Storage tank, outlet of pasteurizer unit, pasteurized milk storage tank and packing and filling machine. High counts of mesophilic organisms were detected in raw milk production and processing. However, pasteurization reduced the microorganism to acceptable numbers as per Brazilian standards but the statistical analysis showed that number of microorganisms increased significantly ($P<0.01$) in the pasteurized milk storage tank representing an important point of contamination.

Boor *et al.* (1998) studied the microbiological quality of 855 raw milk samples collected by licensed milk haulers from bulk tank. The standard plate count of raw milk obtained from 11 co-operatives or processing plants ranged from 6400 to 22000 cfu/ml with a mean count of 11400 cfu/ml of sample from all farms.

Eneroth *et al.* (1998) studied the critical contamination sites in the production of pasteurized milk from three dairy plants in Sweden and Norway. Samples of raw and pasteurized milk were collected from six points and repeated three to four times. The initial aerobic plate count of samples of milk just before pasteurization, and milk filled in consumer packages were 8×10^4 and 6×10^2 cfu/ml, respectively.

John (1999) studied the bacterial profile of 100 pasteurized milk samples belonging to brand A, B, C, D and E collected from retail outlets in and around Thrissur. The mean count of total viable count of the samples from A, B, C, D and E sources was 5.68 ± 5.28, 7.24 ± 6.72, 7.65 ± 7.22, 4.47 ± 4.23 and 5.77 ± 5.64 $\log_{10}$ cfu/ml, respectively. On comparing the counts obtained with BIS standards revealed that 18 per cent of the samples met the standard.

Jolly *et al.* (2000) evaluated 60 raw market milk collected from three sources viz. A, B and C located in and around Mannuthy. From each source 10 each of

pooled and individual milk samples were collected. The mean total viable count of individual milk samples from A, B and C sources were 5.93 ± 0.05, 6.12 ± 0.23 and 6.2 ± 0.12 $\log_{10}$ cfu/ml, respectively and the mean total viable count of pooled milk samples obtained from the sources were 6.06 ± 0.11, 6.78 ± 0.26 and 6.04 ± 0.10 $\log_{10}$ cfu/ml, respectively.

Gopi *et al.* (2001) evaluated bacteriological quality of 12 private brands of pasteurized and homogenized milk in Chennai city and found that the average standard plate count varied from 5.5 to 175.17 x 10^4 cfu/ml. When compared with the BIS standards for bacterial quality more than 94 per cent of milk tested was graded as poor quality.

Homhual and Jindal (2001) assessed the total plate count of 95 raw milk samples and reported that the count ranged from 6.5 x 10^4 cfu/ml to 1.2 x 10^8 cfu/ml.

Beloti *et al.* (2002) evaluated the bacterial quality of 90 refrigerated pasteurized milk samples (29 of grade A, 29 of grade B and 32 of grade C) purchased from local stores of the city of Londrina, P R, Brazil. It was found that 26 samples of grade A had counts less than 5 x 10^2 cfu/ml and three had counts greater than 5 x 10^2 cfu/ml. The count of grade B pasteurized milk was less than 4 x 10^4 cfu/ml in 22 samples and was greater than 4 x 10^4 cfu/ml in seven samples. The count in grade C pasteurized milk was less than 1.5 x 10^5 cfu/ml in 29 samples and was greater than 1.5 x 10^5 cfu/ml in three samples.

Khalilur *et al.* (2002) studied the microbiological quality of 15 samples of milk consisting of six raw and nine pasteurized milk samples from local markets of Aligarh city. The total viable count of raw milk samples ranged from 15,900 x 10^6 to 2,59,000 x 10^6 cfu/100ml with mean count of 98,500 x 10^6 cfu/100ml. Total viable

count of pasteurized milk samples ranged from 154 x 10^6 to 24,000 x 10^6 cfu/100ml with mean count of 15,000 x 10^6 cfu/100ml.

Sethulakshmi *et al.* (2003) assessed the bacterial quality of 84 samples of toned pasteurized milk retailed in and around Thrissur. The overall mean total viable count of the samples was 2.82 ± 0.14 $\log_{10}$ cfu/ml.

Aaku *et al.* (2004) analysed microbiological quality of 43 samples of pooled raw milk and 86 commercial pasteurized milk samples from two processing plants (A and B) in Gaborone, Botswana. The mean total mesophilic counts of pooled raw milk from the sources A and B were 3 x 10^7 and 1 x 10^6 cfu/ml, respectively. The corresponding counts of pasteurized sample were 7 x 10^3 and 1 x 10^4 cfu/ml.

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. The average total plate counts in samples from the above regions were 14.0 x 10^6, 8.2 x 10^6, 18.0 x 10^6 and 8.6 x 10^6 cfu/ml, respectively.

Jaibi (2006) analysed the microbial quality of a total of 144 raw milk samples, consisting of individual and pooled milk samples collected from three societies, *viz.* S_1, S_2 and S_3. The overall mean total viable count of individual samples was 6.12 ± 0.07 $\log_{10}$ cfu/ml and that of pooled milk samples was 6.52 ± 0.08 $\log_{10}$ cfu/ml.

Prejit *et al.* (2007) conducted a study to assess the microbial quality of milk samples from Kerala Agriculture University dairy processing plant. The average total viable count of pooled raw milk before pasteurization was 5.58 ± 0.14 $\log_{10}$ cfu/ml

and that of pasteurized packaged milk was 4.76 ± 0.15 log_{10} cfu/ml and observed that there is a highly significant reduction in the total viable count of pasteurized milk samples. The mean total viable count in retail samples of milk belonging to brands A, B, C and D were 4.72 ± 0.26, 5.77 ± 0.10, 5.42 ± 0.26 and 5.33 ± 0.17 log_{10} cfu/ml, respectively.

2.1.2 Coliform Count

Vijai and Saraswat (1968) evaluated the bacteriological quality of 224 raw market milk samples collected from organised dairy farm, city market and rural collection center and 65 pasteurized milk samples obtained from milk supply scheme in Udaipur city. The study revealed that milk samples obtained from organised dairy farm, city market and rural collection center had an average Coliform Count of 320, 2200 and 18,000/ml, respectively. The average coliform count of pasteurized milk samples was 41/ml.

Davies (1977) evaluated the bacteriological quality of 2042 samples of milk collected from bulk milk supplies in Wales. Coliform Count of the samples was at the level of 10^2/ml in 57.1 per cent of the samples and at a level of 10^3/ml in 29.1 per cent of samples.

Kaloianov and Gogov (1977) analysed 360 samples of raw and 1404 samples of pasteurized milk collected from three milk centers in Bulgaria and found that pasteurization killed 100 per cent of coli organism.

Singh and Ranganathan (1978) examined 50 samples of raw and 30 samples of pasteurized cow milk collected from villages around karnal. The Coliform Counts of raw and pasteurized milk ranged from 500 to 50000/ml and zero to 4500/ml, respectively.

Desai and Natarajan (1981) assessed the bacterial quality of raw milk collected from three societies in areas *viz.* A, B and C located around Bangalore city. The average Coliform Count of the samples belonging to the areas A, B and C was 1040 x 10^3, 80 x 10^3 and 282 x 10^3/ml, respectively.

Singh and Sinha (1981) studied the presence of coliforms in 104 samples of freshly pasteurized milk collected from experimental dairy of NDRI, Karnal and found that Coliform Count of the samples ranged between zero and 4500/ml. The average Coliform Counts were arbitrarily categorized into four classes namely, less than one, one-10, 10-100 and more than 100/ml. The per cent distribution of coliforms falling in these ranges was 8.6, 38.5, 37.5 and 15.4, respectively.

Yadava *et al.* (1983) evaluated the bacterial flora of 42 raw milk samples from Dairy unit R.V.C and 22 pasteurized milk samples from town milk supply organization (TMS) collected during monsoon and winter. The average coliform count of the samples collected during winter from TMS and R.V.C were 13.26 x 10^5 and 0.33 x 10^5/ml, respectively. The corresponding counts of the samples collected during monsoon from the above sources were 20.39 x 10^5 and 0.778 x 10^5/ml.

Reddy *et al.* (1984) reported that the coliform counts of 30 samples of raw milk collected from Vijayavada milk shed ranged from 4280 to 1,32,000/ml with an average of 28,660/ml.

Arora and Sudarsanam (1986) analysed the bacterial quality of eight milk samples obtained from experimental dairy, NDRI and found that the samples had an average coliform count of 22.875/ml and the count ranged between seven and 40/ml.

Raju and Nambudripad (1987) examined the bacterial quality of 78 raw milk samples collected from organised dairy, private dairy and village pooled milk and 75 samples of pasteurized milk collected from the consumer points of Bangalore and NDRI dairies. The mean coliform count of raw milk from organised dairy, private dairy and pooled milk were 136×10^3, 196×10^3 and 1560×10^3 cfu/ml, respectively. The mean coliform count of pasteurized milk was 62 cfu/ml.

Misra and Kuila (1989) examined 125 raw milk samples collected from organised dairy farm (15), city vendors (60) and sweet meat shops (50) and 25 pasteurised milk samples from dairy plant in Calcutta. They reported that the raw milk samples obtained from organised dairy farms, vendors and sweet meat shops had average coliform counts of 3.96×10^3, 6.54×10^3 and 6.74×10^3 cfu/ml, respectively and pasteurized milk had a mean count of 12×10^1 cfu/ml.

Rai and Dwivedi (1990) analysed the bacteriological quality of milk samples collected from four sources. They found that the coliform counts of standardized and pasteurized milk supplied by milk board varied between zero and 10×10^2/ml with an average count of 5.125×10^2/ml. The count of raw milk supplied by C.S. Azad University of Agriculture and Technology, Kanpur, varied from 10×10^2 to 45×10^2/ml with a mean count of 24.375×10^2/ml. The count of samples from hawkers and ghosies had an average count of 213.375×10^2/ml and 64.125×10^2/ml, respectively.

Rea *et al.* (1992) evaluated raw milk from 70 farms in Ireland and found that coliforms were present in all samples but 65 to 71 per cent of samples had count less than 100/ml.

Patel *et al.* (1993) analysed 21 samples of buffalo milk collected from reproductive biology research unit, Veterinary College Anand. The average coliform count of the samples was 1.9 ± 0.57 x 1000 cfu/ml.

Siva *et al.* (1993) studied the microbiological quality of 32 samples of raw cow milk obtained from individual producers, collection centers and dairy plant and 10 samples of pasteurized milk collected from Students Training Dairy, Anand. Average coliform count of milk samples collected from individual producers, collection centers and dairy plant were 0.63 ± 0.31 x 10^4, 66 ± 38 x 10^4 and 150 ± 0.82 x 10^4 cfu/ml, respectively whereas that of pasteurized milk sample was 22 ± 8.13 cfu/ml.

Kapre (1995) studied the microbial quality of 84 milk samples consisting of 28 each from three sources, S_1, S_2 and S_3. The average coliform count of the individual raw milk samples collected from S_1, S_2 and S_3 sources were 2.4 x 10, 4.8 x 10^4 and 3.8 x 10^3cfu/ml, respectively, and the average counts of pooled raw milk samples collected from S_1, S_2 and S_3 sources were 5.5 x 10, 2.0 x 10^5 and 6.4 x 10^3 cfu/ml, respectively.

Cosentino and Palmas (1997) analysed raw and heat-treated ewes milk collected three times over a period of 6 months from six milk-processing plants in Sardina. The mean coliform counts of raw milk from plant 1, 2, 3, 4, 5 and 6 were 2.0 ± 0.28, 3.6 ± 0.65, 1.8 ± 0.34, 3.1 ± 0.63, 2.9 ± 0.40 and 3.9 ± 0.77 cfu/ml, respectively. The count of heat-treated milk from the above sources were 1.0 ± 0.41, 2.1 ± 0.28, zero, 1.8 ± 0.14, 1.9 ± 0.32 and 2.6 ± 0.45 cfu/ml, respectively.

Latha and Nanu (1997) reported that the pasteurized milk samples collected from S_1, S_2, S_3, S_4 and S_5 sources had mean coliform counts of 3.06 ± 0.06, 2.85 ± 0.06, 2.75 ± 0.07, 1.10 ± 0.05 and 0.77 ± 0.10 $\log_{10}$ cfu/ml, respectively. All samples

from S_5 source and 50 per cent of the samples belonging to S_4 source and none from other sources had coliform count within the limit prescribed for pasteurized milk by Bureau of Indian Standards (1977).

Boor *et al.* (1998) studied the microbiological quality of 855 raw milk samples obtained from 11 co-operatives or processing plants in New York State. The coliform count of the samples ranged from 14 to 290/ml with a mean count of 31/ml.

John (1999) examined 100 pasteurized milk samples belonging to brand A, B, C, D and E and reported that the samples had a mean coliform count of 3.96 ± 3.79, 4.85 ± 4.42, 5.38 ± 5.13, 1.24 ± 1.04 and 3.02 ± 2.62 $\log_{10}$ cfu/ml, respectively.

Jolly *et al.* (2000) evaluated 60 raw market milk collected from three sources viz. A, B and C located in and around Mannuthy. From each source 10 pooled and individual milk samples were collected. The mean coliform count of pooled milk from A, B and C sources were 4.74 ± 0.54, 6.02 ± 0.19 and 5.31 ± 0.12 $\log_{10}$ cfu/ml, respectively. The mean count of individual milk samples of the sources were 5.14 ± 0.15, 5.03 ± 0.58 and 5.34 ± 0.18 $\log_{10}$ cfu/ml, respectively.

Gopi *et al.* (2001) determined the average coliform count of 12 private brands of pasteurized and homogenized milk in Chennai city and found that the count varied from zero to 43.33 x 10^2 cfu/ml.

Khalilur *et al.* (2002) evaluated the microbiological quality of 15 samples of milk consisting of six raw and nine pasteurized milk samples obtained from local markets of Aligarh city. The raw milk samples had mean total coliform count of 2.4 x 10^3 MPN/100ml and pasteurized samples showed a mean count of 2.13 x 10^3 MPN/100ml.

Sethulakshmi *et al.* (2003) assessed the bacterial quality of 84 samples of toned pasteurized milk retailed in and around Thrissur. The mean coliform count was 1.8 ± 0.16 $\log_{10}$ cfu/ml.

Chye *et al.* (2004) determined the bacteriological quality and safety of raw milk in Malaysia. A total of 930 raw milk samples were collected from 360 dairy farmers and reported that the samples had a mean coliform count of 17×10^4 cfu/ml.

Jaibi (2006) assessed the microbial quality of a total of 144 raw milk samples, consisting of individual and pooled milk samples collected from three societies *viz.* S_1, S_2 and S_3. The mean coliform count of individual milk samples of the three sources revealed highly significant ($P<0.01$) difference. The overall mean coliform count of the samples was 3.27 ± 0.04 $\log_{10}$ cfu/ml. Samples from S_2 had the highest mean count (3.50 ± 0.04 $\log_{10}$ cfu/ml) and the lowest count was seen in the samples of S_3 (3.03 ± 0.08 $\log_{10}$ cfu/ml).

Prejit *et al.* (2007) assessed the microbial quality of milk samples collected from Kerala Agricultural University Dairy Plant. Coliforms were found in 100 per cent of milk before pasteurization and the mean was reduced to a highly significant level ($P<0.01$) after pasteurization with 40 per cent of the packaged milk samples found free from the organism. The average coliform count of pooled milk samples before pasteurization was 3.24 ± 0.19 $\log_{10}$ cfu/ml and that of pasteurized packaged milk samples was 0.98 ± 0.36 $\log_{10}$ cfu/ml.

2.1.3 *Escherichia coli* Count

Kapre (1995) evaluated the microbial quality of a total of 84 samples of raw milk collected from three sources *viz.* S_1, S_2 and S_3. From each source, 28 samples consisting of 21 individual milk samples and seven pooled milk samples were

analysed and reported that the individual samples from three sources had an average *Escherichia coli* count of 2.0×10^2, 1.2×10^4 and 1.5×10^3 cfu/ml, respectively. The corresponding count of pooled milk samples from the sources were 2.7×10^2, 8.9×10^4 and 1.9×10^3 cfu/ml.

Cosentino and Palmas (1997) assessed the microbial quality of raw and heat-treated ewes milk collected three times over a period of six months from six milk-processing plants in Sardina. The mean *Escherichia coli* counts from six sources were 1.3 ± 1.07, 1.5 ± 0.95, 1.0 ± 0.48, 2.0 ± 0.87, 1.8 ± 0.93 and 2.9 ± 0.94 cfu/ml, respectively. Heat-treated samples from plant 6 indicated the presence of *Escherichia coli* with a mean count of 1.1 ± 0.32 cfu/ml.

Desmasures *et al.* (1997) analysed raw milk from 27 farms and found that 80 per cent samples had *Escherichia coli* count less than 10 cfu/ml.

Latha and Nanu (1997) studied the bacterial quality of pasteurized milk belonging to S_1, S_2, S_3, S_4 and S_5 sources and found that the samples had mean *Escherichia coli* counts of 2.61 ± 0.08, 2.19 ± 0.09, 2.26 ± 0.12, 0.49 ± 0.13 and 0.19 ± 0.08 $\log_{10}$ cfu/ml, respectively. During the investigation, 82 *Escherichia coli* isolates were identified from 80 per cent of samples tested.

John (1999) assessed the bacterial quality of 100 pasteurized milk samples belonging to brand A, B, C, D and E and reported that the mean *Escherichia coli* counts of five brands were 2.17 ± 1.83, 3.39 ± 2.74, 0.87 ± 0.87, nil and 2.68 ± 2.58 $\log_{10}$ cfu/ml, respectively.

Jolly *et al.* (2000) assessed 60 raw market milk samples collected from three sources viz. A, B and C located in and around Mannuthy. The mean *Escherichia coli* counts of pooled milk from A, B and C sources were 4.02 ± 0.47,

4.97 ± 0.18 and 4.33 ± 0.14 log_{10} cfu/ml, respectively. The mean count of individual milk samples was 4.11 ± 0.20, 3.13 ± 0.7 and 4.08 ± 0.48 log_{10} cfu/ml, respectively.

Khalilur *et al.* (2002) evaluated the microbiological quality of 15 samples of milk consisting of six raw milk samples and nine pasteurized milk samples and reported that the samples had a mean faecal coliform count of 1.9 x 10^3 MPN/ml and 1.5 x 10^3 MPN/ml, respectively.

Gran *et al.* (2003) studied the occurrence of pathogenic bacteria in raw milk produced by small-scale dairies in Zimbabwe. Out of 12 samples examined the samples had a mean *Escherichia coli* count of 4.5 log_{10} cfu/ml.

Sethulakshmi *et al.* (2003) evaluated the bacterial quality of 84 samples of toned pasteurized milk retailed in and around Thrissur and recorded the mean *Escherichia coli* count of the samples as 0.19 ± 0.12 log_{10} cfu/ml.

Chye *et al.* (2004) examined the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. The average *Escherichia coli* counts in samples from the above regions were 15.0 x 10^3, 5.4 x 10^3, 4.8 x 10^3 and 1.9 x 10^3 cfu/ml, respectively. The overall mean *Escherichia coli* count of the samples was 6.8 x 10^3 cfu/ml.

Jaibi (2006) examined a total of 144 milk samples consisting of 108 individual and 36 pooled milk samples obtained from three co-operative societies, *viz.* S_1, S_2 and S_3. The mean *Escherichia coli* count of the individual milk samples was 1.23 ± 0.15 log_{10} cfu/ml. The overall mean count of the pooled milk samples was 1.52 ± 0.27 log_{10} cfu/ml.

Prejit *et al.* (2007) studied the microbial quality of milk samples obtained from Kerala Agricultural University Dairy Plant and reported that the *Escherichia coli* count in raw milk was significantly ($P<0.05$) reduced upon pasteurization. The average *Escherichia coli* count of pooled raw milk samples was 0.63 ± 0.31 $\log_{10}$ cfu/ml and the corresponding count in pasteurized milk samples was 0.31 ± 0.21 $\log_{10}$ cfu/ml.

2.1.4 Psychrotrophic Count

Jain and Saraswat (1968) examined 50 samples of raw milk collected from organised dairy farm. The average psychrophilic count of milk was 77,000/ml. A highly significant ($P<0.01$) correlation was observed between standard plate count and psychrophilic counts of the samples.

Arora and Sudarsanam (1986) assessed the microbiological quality of eight milk samples obtained from experimental dairy NDRI that was used as an ingredient in ice cream. The study revealed that the samples had average psychrotrophic counts of 143.75/ml with a range of 3 to 720/ml.

Griffiths and Philips (1988) studied the bacterial growth at different temperatures in 46 samples of freshly pasteurized milk collected from 18 dairies from Paisley, Scotland. The study revealed that the geometric mean of the initial psychrotrophic count of these samples determined by most probable number (MPN) was 1.4/ml and the count ranged from approximately one per litre to 2,800/ml. Of the samples, 41.3 per cent had an initial count of below one/ml and 63 per cent had counts below 10/ml.

Misra and Kuila (1989) analysed the microbial quality of 125 samples of raw milk and 25 samples of pasteurized milk collected from Calcutta and its suburbs. The

average psychrophilic count of raw milk samples obtained from organised dairy farms, vendors and sweet meat shops were 30.13 x 10^3, 54.5 x 10^4 and 61.6 x 10^4 cfu/ml, respectively. The average count of pasteurized milk samples from dairy plant was 5.56 x 10^3 cfu/ml.

Saleha (1992) enumerated psychrotrophic bacterial count in 72 samples of pasteurized milk obtained from six retail outlets located in Malaysia. The count ranged from 10 to 53,000/ml.

Singh *et al.* (1994) assessed the microbial quality of 70 samples of raw milk collected from different distribution cans. The psychrophilic count in the samples ranged from 2.579 to 5.113 $\log_{10}$ cfu/ml with a mean count of 3.939 $\log_{10}$ cfu/ml.

John (1999) evaluated the bacterial profile of 100 pasteurized milk samples belonging to brand A, B, C, D and E collected from retail outlets in and around Thrissur. The mean psychrotrophic count of the samples from brand A, B, C, D and E were 5.64 ± 5.51, 6.61 ± 6.16, 6.95 ± 6.73, 3.57 ± 3.23 and 4.83 ± 4.57 $\log_{10}$ cfu/ml, respectively.

Gopi *et al.* (2001) evaluated the bacteriological quality of 12 private brands of pasteurized and homogenized milk in Chennai city and found that the average psychrotrophic count varied from 12.50 to 99.33 x 10^4 cfu/ml.

Silva *et al.* (2001) tested 90 samples of pasteurized milk of grade B and C. Fifteen samples each of grades B and C pasteurized milk of three different commercial brands purchased from supermarkets and bakeries in Rio de Janeiro were tested. The psychrotrophic count varied between zero to 10/ml in 73.4 per cent, 40 per cent and 46.6 per cent samples of the three brands of grade B and also in 73.3 per cent and 33.3 per cent of two brands of grade C milk.

Chye *et al.* (2004) studied the bacteriological quality and safety of raw milk in Malaysia. The mean psychrotrophic count of 930 raw milk samples collected from 360 dairy farmers was 7.5 x 10^3 cfu/ml.

Jaibi (2006) studied the microbial quality of a total of 144 raw milk samples, consisting of 108 individual and 36 pooled milk samples obtained from three co-operative societies, *viz.* S_1, S_2 and S_3. The difference between the psychrotrophic count of the individual milk samples of the three sources was highly significant ($P<0.01$) and the overall mean count of the samples was 3.81 ± 0.06 $\log_{10}$ cfu/ml. The overall psychrotrophic count of the pooled samples was 3.81 ± 0.06 $\log_{10}$ cfu/ml.

Prejit *et al.* (2007) assessed the microbial load of milk samples collected from Kerala Agricultural University Dairy Plant. The average psychrotrophic count of milk samples before pasteurization was 5.42 ± 0.07 $\log_{10}$ cfu/ml and the average count after pasteurization was 4.16 ± 0.09 $\log_{10}$ cfu/ml. The mean reduction in the psychrotrophic count in pasteurized milk with that of raw pooled milk was at the level of 1.26 $\log_{10}$ cfu/ml.

2.1.5 Faecal Streptococcal Count

Davies (1977) evaluated the microbiological quality of bulk milk samples collected from Wales. The frequency distribution of total streptococcal count in 789 samples of milk collected from bulk supplies was determined. The count in 75.2 per cent of the bulk milk samples was less than 10,000/ml and the count in 24.8 per cent of the bulk milk samples was 10,000/ml or more. Count at the level of 50,000/ml or more was observed on 7.6 per cent of bulk supplies.

Yadava *et al.* (1983) analysed the bacterial quality of milk samples consisting of 22 pasteurized milk from town milk supply organization (TMS) and 42 raw milk from Dairy unit of R.V.C from Ranchi town and reported that the samples collected during monsoon had an average faecal streptococcal count of 14.40 x 10^5, and 0.183

x 10^5/ml, respectively. The corresponding counts of the samples collected during winter was at the level of 4.34 x 10^5 and 0.003 x 10^5/ml.

Kapre (1995) evaluated the microbial quality of 84 milk samples consisting of 28 each from three sources, S_1, S_2 and S_3. From each source, 21 individual samples and seven pooled milk samples were collected. The mean faecal streptococcal count of individual samples from S_1, S_2 and S_3 was 1.5 x 10^2, 2.1 x 10^3 and 1.7 x 10^3 cfu/ml, respectively. The mean count of pooled milk samples from the sources S_1, S_2 and S_3 was 2.0 x 10^2, 4.8 x 10^3 and 2.9 x 10^3 cfu/ml, respectively.

Latha and Nanu (1997) examined the bacteriological quality of pasteurized milk belonging to S_1, S_2, S_3, S_4 and S_5 sources. The study revealed that the mean faecal streptococcal counts were 2.53 ± 0.09, 1.92 ± 0.12, 1.29 ± 0.13, 1.29 ± 0.20 and 0.82 ± 0.16 $\log_{10}$ cfu/ml from S_1, S_2, S_3, S_4 and S_5 sources, respectively.

Jolly *et al.* (2000) assessed the microbiological quality of 60 raw market milk samples collected from three sources viz. A, B and C located in and around Mannuthy. The mean faecal streptococcal count of pooled milk samples from the sources A, B and C was 2.90 ± 0.38, 2.00 ± 0.49 and 2.56 ± 0.32 $\log_{10}$ cfu/ml, respectively. The corresponding counts of individual milk samples were 2.55 ± 0.13, 1.44 ± 0.49 and 2.46 ± 0.34 $\log_{10}$ cfu/ml.

Raj *et al.* (2003) analysed a total of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. Samples from A and B were collected from farmers who brought milk to the society. Samples of C and D were collected from individual animals. The overall mean faecal streptococcal count of samples from the sources A, B, C and D were 1.7 ± 0.32, 1.30 ± 0.45, 0.53 ± 0.22 and 0.94 ± 0.28 $\log_{10}$ cfu/ml, respectively.

Sethulakshmi *et al.* (2003) studied the bacteriological quality of 84 samples of toned pasteurized milk retailed in and around Thrissur. The overall mean faecal streptococcal count was 0.89 ± 0.22 $\log_{10}$ cfu/ml.

Jaibi (2006) studied the microbial quality of a total of 144 raw milk samples, consisting of individual and pooled milk samples collected from three societies *viz.* S_1, S_2 and S_3. The overall mean faecal streptococcal count of samples was 3.06 ± 0.05 $\log_{10}$ cfu/ml. The highest mean count of 3.25 ± 0.06 $\log_{10}$ cfu/ml was observed in the samples belonging to S_2 and lowest mean count was seen in samples of S_3 (2.78 ± 0.11 $\log_{10}$ cfu/ml).

Prejit *et al.* (2007) assessed the effect of pasteurization on the microbial quality of 60 milk samples collected from Kerala Agricultural University Dairy Plant and reported that the reduction in faecal streptococcal count after pasteurization was highly significant ($P<0.01$). The average faecal streptococcal count in pooled milk samples before pasteurization was 2.59 ± 0.12 $\log_{10}$ cfu/ml and the count in the samples after pasteurization and packaging was 1.06 ± 0.18 $\log_{10}$ cfu/ml.

2.2 ISOLATION AND IDENTIFICATION OF BACTERIA FROM MILK

2.2.1 *Escherichia coli*

Singh and Ranganathan (1978) evaluated 50 samples of raw cow milk and 30 samples of pasteurized milk and reported the isolation of *Escherichia coli* from 33 samples of raw and 15 samples of pasteurized milk.

Yadava *et al.* (1985) examined the bacterial flora of 105 milk samples collected from town milk supply organization, organised dairy farm and local vendors from Ranchi town and isolated *Escherichia coli* from 82 (78.09 per cent) samples. The isolates belonged to serogroups O1, O17, O22, O11, O84, O55, O125, O86, O36, O45, O18, O2, O76, O9, O58, O30, O82, O34 and O59.

Yadava *et al.* (1987) analysed 105 milk samples collected from an organised dairy farm (42), local vendors (41) and centralised milk supply organizations (22). During the investigation *Escherichia coli* was isolated from 31, 30 and 21 samples obtained from organised dairy farm, local vendors and centralised milk supply organizations, respectively.

Rahman *et al.* (1992) investigated the bacterial flora in 83 raw milk samples obtained from Guwahati city and reported the isolation of *Escherichia coli* from 6.12 per cent of the samples. During the investigation six strains of *Escherichia coli* were isolated and two of these isolates belonged to serogroup O15 and in the remaining isolates; one each belonged to serogroup O9, O60 and O186. One of the isolates was untypable.

Gill *et al.* (1994) isolated *Escherichia coli* from five out of 36 cow milk samples and four out of 40 buffalo milk samples collected from Ludhiana city.

Singh *et al.* (1994) reported the isolation of 49 *Escherichia coli* from 70 raw milk samples collected from milk cans for distribution in Pantnagar.

Kapre (1995) evaluated the microbial quality of 21 individual samples and seven pooled milk samples collected from each of three sources, S_1, S_2 and S_3. *Escherichia coli* were isolated from 16 individual samples (76.19 per cent) belonging to S_1. All individual samples from S_2 and S_3 and all pooled samples revealed the presence of *Escherichia coli.*

Sharma *et al.* (1995) isolated *Escherichia coli* from five out of 60 raw milk samples obtained from local market. The isolates consisted of serotypes O5, O7, O61 (2 samples) and O106.

Singh *et al.* (1996) investigated the prevalence of bacterial pathogens in 50 milk samples collected from individual animals belonging to organised dairy and also 15 pooled samples supplied by the milk vendors. During the investigation *Escherichia coli* was isolated from 7.7 per cent of milk samples

Steel *et al.* (1997) isolated 15 (0.87 per cent) *Verocytotoxigenic Escherichia coli* from 1720 random bulk tank milk samples obtained from Ontario, Canada.

John (1999) studied the bacteriological quality of 100 samples of pasteurized milk collected from Thrissur. The study revealed that *Escherichia coli* were present in 29 samples.

Silva *et al.* (2001) evaluated 45 samples each of type B and C pasteurized milk obtained from three different commercial brands purchased in supermarkets and bakeries in Rio de Janeiro. A total of 208 (41.1 per cent) *Escherichia coli* were isolated out of which 22.1 per cent of the strains were serologically identified as EPEC

Gran *et al.* (2003) isolated six strains of *Escherichia coli* from 12 samples of raw milk obtained from three small-scale societies in Zimbabwe. Two of these isolates were found to produce heat stable enterotoxin.

John *et al.* (2003) studied the bacteriological quality of 84 samples of pasteurized milk obtained from Thrissur. The study revealed that *Escherichia coli* was present in six (7.1 per cent) samples.

Raj *et al.* (2003) analysed a total of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. *Escherichia coli* were isolated from five (12.5 per cent) samples. Two samples (20

per cent) from source A and one sample (10 per cent) each from sources B, C and D revealed the presence of *Escherichia coli.*

Aaku *et al.* (2004) studied the microbiological quality of 43 samples of pooled raw milk from two processing plants *viz.*, A and B in Gaborone, Botswana. *Escherichia coli* were isolated from 10 (23 per cent) samples.

Chye *et al.* (2004) studied the bacteriological quality and safety of raw milk in Malaysia. A total 930 raw milk samples were collected from 360 dairy farmers and reported the isolation of *Escherichia coli* from 600 (64.5 per cent) samples.

Oksuz *et al.* (2004) examined 100 raw milk samples randomly collected from 10 villages in Tekirday and reported the isolation of *Escherichia coli* O157 from one of the samples.

Prejit (2005) studied the microbiological quality of 56 samples of milk belonging to four different brands namely A, B, C and D retailed in and around Thrissur. Isolation of bacterial organisms of public health significance revealed that out of 56 retail samples 48.21 per cent had *Escherichia coli.* The organism was detected in 84.6 per cent of samples each of brand B and C.

Jaibi (2006) studied the microbial quality of a total of 144 raw milk samples, consisting of individual and pooled milk samples collected from three societies *viz.* S_1, S_2 and S_3. *Escherichia coli* was isolated from 42 (38.89 per cent) individual milk samples and 17 (47.22 per cent) pooled milk samples. The serotypes identified were O24 (6), O84 (4), O116 (5), O172 (5), O25 (2), O87 (3), O103 (2), O157 (2), O5 (1) and O68 (1).

Nanu *et al.* (2007) assessed the microbial quality of 240 raw milk samples obtained from the point of production (farmer's level) from Palakkad district in Kerala. The samples were also subjected to isolation of pathogenic and spoilage

causing bacterial pathogens. Out of 240 samples, 76 (31.67 per cent) revealed the presence of *Escherichia coli*. The isolates obtained were serotyped as O24 (9), O116 (9), O172 (8), O84 (7), O157 (3), O103 (3), O87 (3), O25 (2), O125 (2), O145 (2), O5 (1), O68 (1), Rough (16) and Untypable (10).

2.2.2 *Staphylococcus aureus*

Mohan and Misra (1967) assessed 200 samples of raw milk collected from producer, agent, collection center, at dairy from cans, bulk milk and raw cow milk samples supplied to Patna milk supply schemes. During the study 71 strains of Staphylococci were isolated from the sample of which 33 were coagulase positive.

Ghosh and Laxminarayana (1972) analysed 160 raw milk samples and 30 pasteurized milk samples marketed in Karnal and reported that 81 raw milk samples (50.6 per cent) and seven (23.3 per cent) pasteurized milk samples showed the presence of coagulase positive Staphylococci.

Garg *et al.* (1977) isolated 54 strains of *Staphylococcus aureus* from 57-cow milk samples collected from Hissar city during summer and winter months.

Shah *et al.* (1984) studied the bacterial flora of milk samples obtained from 134 healthy cows belonging to the university farm, Anand and isolated *Staphylococcus aureus* from 20 per cent of the samples.

Yadava *et al.* (1985) assessed the bacterial flora of 105 milk samples which consisted of 22 from milk supply scheme, 42 raw milk from organised dairy farm (OD) and 41 from local vendors (LV) from Ranchi town. The number of samples positive for *Staphylococcus aureus* from milk supply scheme, OD and LV sources

were seven (16.66 per cent), 10 (24.39 per cent) and three (13.63 per cent), respectively.

Rajmany *et al.* (1989) studied the occurrence of staphylococci in 20 samples each of raw milk, khoa, curd, ice cream, sweetened condensed milk, milk powder and processed cheese, sold in Udaipur market. Coagulase positive *Staphylococcus aureus* count in the samples varied from 11.2×10^3 to 57×10^3 with an average of 31.5×10^3 cfu/ml. All raw milk samples (100 per cent) were positive for *Staphylococcus aureus*.

Sen *et al.* (1989) analysed 178 milk samples from healthy cows in a large organised farm in West Bengal and isolated *Staphylococcus aureus* from 43 samples (24.1 per cent). Of the isolates, 17 were coagulase positive.

Rahman *et al.* (1992) studied the bacterial flora of 83 raw milk samples obtained from Greater Guwahati city. Out of 83 samples 56.13 per cent constituted *Staphylococcus aureus*. Of the isolates, 21 strains of *Staphylococcus aureus* were subjected to phage typing and found that 16 (76.2 per cent) were typable with international set of phages.

Gill *et al.* (1994) carried out the bacteriological survey of milk and milk products with special reference to *Staphylococcus aureus* and found that six out of 36 cow milk samples and eight out of 40 buffalo milk samples revealed the presence of the organism.

Singh *et al.* (1994) analysed 70 raw milk samples collected from the distribution cans to Pantnagar and reported that 45 *Staphylococcus aureus* were isolated from 110 samples.

Kapre (1995) conducted a study on the bacterial profile of 21 individual samples and seven pooled milk samples collected from each of three sources *viz.* S_1,

S_2 and S_3. *Staphylococcus aureus* was isolated from 14 (66.66 per cent), 13 (61.91 per cent) and 10 (47.61 per cent) individual samples belonging to S_1, S_2 and S_3 sources, respectively. The organism was also isolated from four (57.1 per cent), six (85.71 per cent) and six (85.71 per cent) pooled samples from the sources, S_1, S_2 and S_3, respectively.

Desmasures *et al.* (1997) reported the isolation of *Staphylococcus aureus* from 62 per cent of raw milk samples collected from 27 farms over a period of six months.

Adesiyun *et al.* (1998) studied the prevalence and characteristics of *Staphylococcus aureus* strains from 175 bulk and 287 composite milk samples obtained from eight milking centers in Trinidad. All bulk milk samples and 280 (97.6 per cent) composite milk samples yielded *Staphylococcus aureus.*

John (1999) studied the bacteriological quality of 100 samples of pasteurized milk collected from Thrissur and reported that *Staphylococcus aureus* was not detected in the samples examined.

Jolly *et al.* (2000) studied 60 raw market milk samples obtained from three sources *viz.* A, B and C located in and around Mannuthy. From each source, 10 individual and 10 pooled milk samples were collected. The study revealed that *Staphylococcus aureus* was present in 50 per cent of pooled and 36.67 per cent of individual milk samples.

Gran *et al.* (2003) studied the occurrence of pathogenic bacteria in raw milk produced at small-scale dairies in Zimbabwe and reported that typical *Staphylococcus aureus* were found in seven out of 12 samples examined.

John *et al.* (2003) evaluated 84 samples of pasteurized milk collected from Thrissur and reported the isolation of 22 (26.1 per cent) *Staphylococci.* Only one of the samples was positive for *Staphylococcus aureus.*

Raj *et al.* (2003) assessed the bacterial quality of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and also hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. During the investigation *Staphylococcus aureus* was isolated from 15 (37.5 per cent) samples tested. The organism was isolated from 40, 50 and 60 per cent of samples from source A, C and D, whereas no isolate was obtained from samples of the source B.

Aaku *et al.* (2004) analysed microbiological quality of 43 samples of pooled raw milk obtained from two processing plants (A and B) in Gaborone, Botswana. The study revealed that none of the samples had *Staphylococcus* spp.

Chye *et al.* (2004) studied the bacteriological quality of 930 raw milk samples obtained from Malaysia and reported that *Staphylococcus aureus* was isolated from 61 per cent of the samples.

Prejit (2005) studied the microbiological quality of 56 samples of milk belonging to four different brands namely A, B, C and D retailed in and around Thrissur. During the investigation *Staphylococcus aureus* was isolated from seven (12.5 per cent) samples. The organism was isolated from two samples each from brands B and D and three samples belonging to brand C.

Jaibi (2006) studied the microbial quality of a total of 144 raw milk samples, consisting of individual and pooled milk samples collected from three societies *viz.*

S_1, S_2 and S_3 and reported the isolation of *Staphylococcus aureus* from 31 (28.70 per cent) individual and 10 (27.18 per cent) pooled samples.

Nanu *et al.* (2007) assessed the microbial quality of 240 raw milk samples obtained from the point of production (farmer's level) from Palakkad district in Kerala. The samples were also subjected to isolation of pathogenic and spoilage causing bacterial pathogens. Out of 240 samples, 84 (35 per cent) revealed the presence of *Staphylococcus aureus.*

2.2.3 *Pseudomonas*

Yadava *et al.* (1985) examined the bacterial flora of 105 milk samples collected from Ranchi town and reported the isolation of five (4.7 per cent) strains of *Pseudomonas aeruginosa.*

Griffiths and Phillips (1988) studied the bacterial profile of 46 retail samples of freshly pasteurized milk collected from 18 dairies in Scotland and reported that *Pseudomonas* was isolated from 92.1 per cent of samples.

Grover and Srinivasan (1988) analysed the presence of *Pseudomonas aeruginosa* in 21 raw milk samples and 20 pasteurized milk samples. A high incidence (90.48 per cent) of the organism was observed in raw milk samples but the incidence was only five per cent in case of pasteurized milk samples.

Mahari and Gashe (1990) isolated the microorganisms present in raw and pasteurized milk and also the sources of contamination of milk after it was received in the processing plant in Addis Ababa and reported that the isolates mostly belonged to the genera *Bacillus, Aeromonas and Pseudomonas.*

Sutherland *et al.* (1993) studied the microbial profile of 72 samples of pasteurized whole milk purchased from supermarkets in Edinburgh and isolated 50 strains of *Pseudomonas.*

Ternstrom *et al.* (1993) studied the spoilage flora of raw and pasteurized milk samples with special reference to *Pseudomonas* and reported that 51 per cent of the milk samples stored at 7°C were spoiled by gram negative bacteria which included mainly *Pseudomonas.*

Gill *et al.* (1994) isolated *Pseudomonas* from six out of 36 cow milk samples and five out of 40 buffalo milk samples collected from Ludhiana city.

Desmasures *et al.* (1997) assessed the microbiological quality of raw milk samples from 27 farms over a period of six months and reported that *Pseudomonas* was one among the most numerous groups with a mean count of 2000 cfu/ml.

Dogan and Boor (2003) obtained raw and pasteurized milk samples from four dairy processing plants in New York City and isolated 201 strains from processed milk and 47 strains from raw milk. The strains mostly belonged to the species *Pseudomonas aeruginosa, Pseudomonas putida* and *Pseudomonas flourescens.*

Aaku *et al.* (2004) analysed microbiological quality of 43 samples of pooled raw milk and 86 commercial pasteurized milk samples from two processing plants (A and B) in Gaborone, Botswana and reported the isolation of various species like *Pseudomonas aeruginosa* (10), *Pseudomonas flourescens* (7), *Pseudomonas cichorii* (16), *Pseudomonas fragi* (15) and other *Pseudomonas* (12).

Fromm and Boor (2004) analysed shelf life attributes of pasteurized milk of three processing plants of New York State and reported that among the bacterial isolates obtained from the samples 12 (3 per cent) belonged to *Pseudomonas*.

Nanu *et al.* (2007) assessed the microbial quality of 240 raw milk samples obtained from the point of production (farmer's level) from Palakkad district in Kerala. The samples were also subjected to isolation of pathogenic and spoilage causing bacterial pathogens. Out of 240 samples, 26 (10.80 per cent) revealed the presence of *Pseudomonas aeruginosa*.

2.3 BACTERIAL STANDARDS OF MILK

The standards for microbial quality of milk in India are prescribed by the Bureau of Indian Standards. Bacterial contamination of milk occurs mainly from animals, human beings, environmental and utensils at various stages of production, processing, transport and distribution. Such contamination not only lead to spoilage of milk, but it also result in milk borne infection and intoxication to the consumers.

2.3.1 Raw Milk

The Indian Standards (1977) prescribed the following criteria as a guideline for grading of milk. Raw milk with a plate count of milk not exceeding two lakhs/ml is graded as very good, the counts between two and 10 lakhs/ml is graded as good, the counts between 10 ad 50 lakhs/ml is graded as fair and the counts over 50 lakhs/ml is graded as poor. The coliforms should be absent in 1:100 dilution of satisfactory grade raw milk.

2.3.2 Pasteurized Milk

The criteria prescribed by Indian Standards (1992) stipulated that the plate count of pasteurized milk, at the plant in the final container, should not exceed 30,000/ml and the coliforms should be absent in 1:10 dilution of pasteurized milk.

2.4 BACTERIAL COUNTS OF REFRIGERATED MILK

Sinha *et al.* (1968) studied the keeping quality of 25 samples of pasteurized cow milk obtained from NDRI, Karnal. The study revealed that the samples showed positive Clot on Boiling test after a mean storage period of 35.76 hours at 22°C and the samples had an average total bacterial count of 3.701 $\log_{10}$ cfu/ml.

Schroder *et al.* (1982) evaluated the keeping quality of commercial HTST pasteurized milk and Laboratory pasteurized milk from five dairies. Spoilage occurred when the level of total bacterial count reached around 10^7 cfu/ml. The study revealed that raising the temperature from 5°C to 11°C reduced the shelf life of laboratory-pasteurized milk from 28 to six days and that of commercial pasteurized milk from 13 to five days.

Ledford *et al.* (1983) studied the growth of coliforms from 30 commercially processed milk samples from New York processing plants. The samples were stored at 6.7°C and coliform were found to grow at that temperature. On day one of storage samples showed less than 10 coliform/ml and 385 positive coliform with counts greater than 150/ml were seen on day 13 of storage.

Brown *et al.* (1984) studied the shelf life of pasteurized milk (skimmed, semi skimmed and whole milk) prepared in R and D division of dairy Crest at

Crudgington. It was found that milk stored at 7°C and artificially contaminated with culture of organism had counts which rose from about 5 x 10^3/ml to 1 x 10^7/ml over a period of seven days. Milk sample stored at 4°C had shelf life extended to 10 days indicating that good storage temperature can therefore counter to some extent the effects of poor conditions of production. Findings of the study also revealed that aseptically packaged milk showed increased shelf life of 14 days when stored at 7°C and 35 days on storage at 4°C.

The study conducted by Krasz et al. (1991) revealed that when milk is stored at 15 to 16.5°C there is an increase in total count, coliform count and psychrotrophic count throughout storage and the organoleptic quality of milk deteriorated.

John (1999) evaluated the shelf life of pasteurized milk collected from dairy technology unit and found that in the refrigerated sample the initial bacterial growth rate was very slow and no significant increase in total viable count and psychrotrophic count was noticed up to seven days of storage. The mean total viable count of freshly pasteurized milk increased from 5.05 ± 4.38 $\log_{10}$ cfu/ml to 9.02 ± 8.32 $\log_{10}$ cfu/ml on the 12th day of refrigerated storage and the counts of coliform organism increased from 2.36 ± 2.01 $\log_{10}$ cfu/ml to 3.33 ± 3.81 $\log_{10}$ cfu/ml after 12 days of storage. The study also revealed that sample showed COB test positive from 12th day onwards.

Vassila *et al.* (2002) analysed the changes of mesophilic and psychrophiiic counts of pasteurized milk stored in different packaging material at 4°C. The initial mesophilic count was 4.36 log cfu/ml and after seven days of storage the mesophilic counts were between 6.01 and 6.49 log cfu/ml. The psychrophilic counts on the initial day was 3.48 log cfu/ml and after seven days of storage the counts were between 5.44 and 6.27 log cfu/ml recorded from all package material.

Dogan and Boor (2003) obtained raw and pasteurized milk samples from four dairy processing plants in New York. The mean standard plate count during first visit to the plants was 2 x 10^3/ml of samples on day 7 and 1.9 x 10^8/ml of samples on day 14. The count of the sample during second visit was 2.4 x 10^5 on day 7 and 3.2 x 10^8 on day 14.

Mamani *et al.* (2003) studied the growth and survival of *Escherichia coli* in whole Ultra heat treated milk at 4°C and found that *Escherichia coli* counts were almost similar between the inoculation time (4 log cfu/ml) and four days of storage (4.46 log cfu/ml). Final counts after 68 days of storage at 4°C were about 1 to1.9 log cfu/ml.

Fromm and Boor (2004) analysed shelf life attributes of pasteurized milk of three processing plants of New York State. At seven days of storage at 6°C, 8 per cent of samples tested had bacterial counts greater than 20000 cfu/ml. On day 14 of storage, 58 per cent of the sample had counts higher than 10^6 cfu/ml. The study revealed that a rapid increase in bacterial numbers after 14 days post processing, as illustrated by 92 per cent of the samples with counts greater than 20,000 cfu/ml and 50 per cent of samples had counts greater than 10^6 cfu/ml after 17 days of refrigerated storage.

Moyssiadi *et al.* (2004) studied the keeping quality of low fat pasteurized milk at 4 ± 0.5°C. The initial mesophilic count (day 0) was 4.70 log cfu/ml and after seven days of storage the counts were between 5.82 and 5.98 log cfu/ml. The psychrophilic count on the initial day was 3.59 log cfu/ml and after seven days of storage the psychrophilic counts were between 4.83 and 5.52 log cfu/ml.

Zygora *et al.* (2004) analysed the changes of mesophilic and psychrophilic counts of pasteurized milk stored in different packaging material at 4°C. After seven days of storage the mesophilic counts were between 6.56 and 7.16 log cfu/ml and psychrophilic counts were between 6.11 and 6.69 log cfu/ml, recorded from all package material.

Prejit *et al.* (2006) studied the effect of refrigeration at 4 ± 1°C on microbial quality of pasteurized milk. The mean total viable count of refrigerated milk on 8th day of storage increased from 4.8 ± 0.15 to 6.92 ± 0.18 $\log_{10}$ cfu/ml and showed highly significant ($P<0.01$) difference. Analysis of the data also revealed highly significant ($P<0.01$) increase in mean coliform count, mean psychrotrophic count and mean faecal streptococcal count after one week of storage.

2.5 SENSORY AND PHYSICAL CHANGES IN MILK

Mukundan (1978) studied the keeping quality of 48 samples of boiled milk and found that all the samples had a keeping quality more than 12 days and the maximum keeping quality was found to be 16 days. The number of samples that gave a positive reaction to COB test at the end of 13, 14, 15 and 16 days was 19, 20, six and three, respectively.

Kadan and Bhanumathi (1984) evaluated the organoleptic quality of pasteurized homogenized and unhomogenised cow milk stored in pouches at 5-9°C. The sensory analysis was done as per the method prescribed by ISI. The score for unhomogenised milk after 24 hour was 82 (Grade B) and that of homogenized milk was 75.2 (Grade C). The entire sample remained acceptable organoleptically at the end of 24 hour of storage.

Reinheimer and Suarez (1992) evaluated 70 sample of HTST pasteurized milk stored at different temperatures and revealed a shelf life of 9.1 days for the samples stored at 7°C and 5.3 days for the samples stored at 12°C.

Watson and Mc Ewan (1995) analysed sensory changes of stored liquid skimmed milk over an eight-day storage period at three different storage temperatures 1°C, 5°C and 10°C. It was found that at 10°C stale flavour began to develop after four days and it took six days to go sour.

Gruetzmacher and Bradley (1999) studied the shelf life of 48 samples of pasteurized milk from paperboard cartons and found that shelf life ranged from 4.6 days to 37 days with an average of 15.5 days of milk stored at 7°C. A study was also done to evaluate the shelf life of milk collected after various sections of milk processing systems. The average shelf life (at 7°C) of raw milk, pasteurized surge tank milk, milk after homogenization, milk after pasteurization and milk in cartons were found to be 2.5, 2.5, 9.1, 51.5 and 13.4 days, respectively.

Dogan and Boor (2003) examined the sensory scores of pasteurized milk samples. A decrease in flavour score was observed on seventh day and 14^{th} day, where a decrease was seen from the score 7.8 on day 7 to one on day 14 in dairy B. The flavour score in dairy C and D decreased from 6.4 to one and 8.4 to 7.7, respectively. The sensory analysis milk was scored on a scale from 1 to 10, wherein any rating below 6 was considered as poor, 6 to 7 was fair and 8 to 10 good. Hence the sample showed no serious sensory defects.

Prejit *et al* (2006) assessed the sensory and physicochemical properties of milk samples refrigerated at 4 ± 1°C for one week. The mean colour and appearance score of freshly pasteurized milk decreased from 9.2 ± 0.26 to 7.3 ± 0.42 on eighth

day of storage. The mean scores of odour, flavour and body decreased to highly significant ($P<0.01$) level after one week of refrigeration.

2.6 POLYMERASE CHAIN REACTION.

Allmann *et al.* (1995) studied the sensitiveness of PCR for detection of *Escherichia coli* from dairy products over the conventional microbiological methods. A total of 90 milk samples were analysed and reported the detection of 41 (46 per cent) isolates by PCR method whereas culture method revealed the presence of only 21 (23 per cent) isolates.

Desmarchelier *et al.* (1998) developed a PCR for the detection of *Escherichia coli* O157 based on the *rfbE* O-antigen synthesis genes. A 479-bp product was amplified specifically from *Escherichia coli* O157 in cell lysates containing 2 cfu following crude DNA extraction. The PCR detected <1 cfu of the organism per ml in raw milk following enrichment.

Reid *et al.* (1999) designed a multiplex PCR to detect the *eae* gene and simultaneously identify specific alleles in pathogenic *Escherichia coli*. The method was tested on 87 strains representing the diarrhoeagenic *Escherichia coli* clones. The results showed that the PCR assay accurately detected *eae* gene and as this gene is lineage specific, this multiplex PCR method provides a rapid way to classify suspected pathogens into the major clonal groups of EPEC and EHEC.

Kumar *et al.* (2001) standardized a PCR based assay targeted against 'uidR' gene specific for all *Escherichia coli* biotypes using primers URL-301 and UAR-432 and template DNA from *Escherichia coli*. The assay was fairly sensitive as it could detect as low as 10 cells of the organism in broth cultures and milk spiked with

Escherichia coli ATCC 25922 after 4h enrichment. The total time for detection of the organism by this method was less than 10h.

Daly *et al.* (2002) used PCR-ELISA for detection of *Escherichia coli* in raw and pasteurized milk using unique *alr* primers (Yokoigawa *et al.*, 1999) and reported the detection of 5 *Escherichia coli* colony forming units (cfu) equating to a sensitivity of detection of 100 *Escherichia coli* cfu/ml of pasteurized milk.

Jothikumar and Griffiths (2002) studied the sensitiveness of a SYBR Green Light Cycler PCR assay using a single primer pair which allowed simultaneous detection of *stx1* and/or *stx2* of *Escherichia coli* O157:H7. A distinct sequence of the Shiga-like toxin genes was amplified to yield products of 227 and/or 224 bp, respectively.

Materials and methods

3. MATERIALS AND METHODS

In the present study a total of 254 milk samples were collected which included 14 raw milk samples from two dairy plants *viz.*, Dairy Plant 1 (DP_1) and Dairy Plant 2 (DP_2) just before pasteurization, 84 samples of pasteurized and packaged milk each from Dairy Plant 1 (DP_1) and Dairy Plant 2 (DP_2) and retail samples (72) belonging to six brands (A, B, C, D, E and F). The bacterial quality of the raw milk samples obtained from the DP_1 and DP_2 were examined on day zero and pasteurized milk samples obtained from the plants were analysed on days 0, 2, 4, 6, 8 and 10. The retail milk samples were evaluated on the day of collection. The bacterial quality of all samples were evaluated by estimating the total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC), psychrotrophic count (PC) and faecal streptococcal count (FSC). All samples were also subjected for isolation and identification of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* species. The stored samples of pasteurized milk were also evaluated for sensory qualities (viz. colour and appearance, flavour, odour and body) and physical changes (COB test).

Milk samples were collected from two milk processing plant and also retail samples from shops in and around Thrissur district and examined to determine the bacterial quality of the processed milk retailed to the consumer.

3.1 BACTERIAL QUALITY OF MILK

In order to get an insight on the bacterial quality and the presence of bacterial pathogens in milk before pasteurization, their changes during pasteurization, storage and till it reaches the consumer, milk samples were collected from processing plants and retail distribution system and examined for their bacterial quality and determination of bacterial pathogens.

3.1.1 Collection of Milk Samples

The samples included raw pooled milk collected just before pasteurization, after pasteurization and packaging and the retail milk sold in and around Thrissur. The details of collection of samples are shown in flowchart 1.

3.1.1.1 Pooled Raw Milk

During the study a total of 14 pooled raw milk samples consisting of seven each from DP_1 and DP_2 were collected. On the day of collection of milk from a plant, milk was thoroughly mixed in the can using a plunger and 250 ml was then transferred into a sterile conical flask. Similarly an equal quantity of sample was transferred from another can into the same conical flask. Thus about 500 ml milk collected from both the cans formed a single sample of the plant. Collection of the samples from each plant was repeated seven times.

3.1.1.2 Pasteurized milk after packaging

During the investigation 84 pasteurized and packaged milk samples were collected from each of the two dairy plants belonging to seven batches. Each sample consisted of 500ml milk packaged in low-density polyethylene sachets. Each batch consisted of 12 samples. From the samples of a batch, two samples were randomly selected and examined on the day of collection. The remaining 10 samples were stored at refrigeration temperature (4±1°C) and two samples each were selected randomly and examined on day 2, 4, 6, 8, and 10 of storage.

3.1.1.3 Pasteurized Retail Milk

A total of 72 pasteurized milk samples belonging to six brands *viz.* A, B, C D, E and F were collected from retail outlets in and around Thrissur. Each sample consisted of 500 ml milk packaged in low-density polyethylene sachets. A total of 12 sachets were collected from one brand. Only two samples were collected from a brand on a day and were examined on the day of collection.

3.1.2 Processing of Milk Samples

In order to estimate the bacterial load per milliliter of milk each samples was agitated thoroughly and 25 ml was transferred to 225 ml of 0.1 per cent peptone water (diluent) so as to form one in 10 dilution of the sample. Further 10 fold serial dilutions were prepared by transferring one milliliter of inoculum to nine milliliter of the diluents. Dilutions were made up to 10^{-10} and selected dilutions of each sample were used for the estimation of various microbial counts per ml of sample. All aseptic precautions were taken during collection and processing of milk samples

3.1.3 Bacterial Counts

The selected serial dilutions of each sample were used to estimate the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Psychrotrophic Count (PC) and Faecal Streptococcal Count (FSC). The counts were expressed as $\log_{10}$ cfu/ml.

3.1.3.1 Total Viable Count

Total viable count (TVC) of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected ten fold dilution of each sample, one ml of the inoculum was transferred to duplicate petridishes of uniform size. 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 i.e., clock wise, anticlock wise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37°C for 24 h. At the end of incubation, plates showing between 30 and 300 colonies were selected and counts were taken with the help of a colony counter. The number of colony forming units (cfu) per ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/ml.

3.1.3.2 Coliform Count

Coliforms count (CC) per ml of sample was estimated according to the procedure described by Kornacki and Johnson (2001). From the selected dilution, 0.1 ml of

the inoculum was inoculated onto duplicate plates of violet red bile agar (VRBA) (Hi-media) and was uniformly distributed with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. At the end of incubation, purplish red colonies with diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. The number of organisms per ml of the sample was estimated by multiplying the mean count of duplicate plate samples with dilution factor and expressed as $\log_{10}$ cfu/ml

3.1.3.3 Escherichia coli Count

The number of *Escherichia coli* (ECC) per ml of sample was estimated as prescribed by Indian standards (1980). To estimate the organisms, 0.1 ml of inoculum from the selected dilution was transferred onto duplicate plates of Eosin Methylene Blue (EMB) Agar (Hi-media) and was evenly distributed over the medium with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. After the incubation period, colonies with a greenish black metallic sheen on deflected light were counted as *Escherichia coli*. The number of organism per ml of sample were estimated as described in coliform count and expressed as $\log_{10}$ cfu/ml

3.1.3.4 Psychrotrophic Count

Psychrotrophic count of each sample was assessed by pour plate technique as suggested by Cousin *et al.* (2001). The procedure followed was similar to that of total viable count. The inoculated plates were incubated at 7 ± 1°C for 10 days. At the end of incubation, the colonies were counted with the help of a colony counter. The number of colony forming units per ml of the sample was calculated as described for total viable count and the count was expressed as $\log_{10}$ cfu/ml.

3.1.3.5 Faecal Streptococcal Count

The standard procedure prescribed by Nordic Committee (1968) was followed to estimate the number of faecal streptococci per ml of sample. Accordingly, 0.1 ml of the inoculum from the selected dilution was transferred onto duplicate plates of Karl Friedrich (KF) streptococcal agar (Hi-media). The inoculum was uniformly distributed onto the

plates using an 'L' shaped glass rod. The plates were incubated at 37°C for 48 h. Pink to dark red colonies with a diameter between 0.5 and three millimeter and surrounded with a narrow whitish zone were counted as faecal streptococci. The number of organisms per ml of sample were estimated as described in coliform count and expressed as $\log_{10}$ cfu/ml

3.1.4 Isolation and Identification of Bacteria

All samples of pooled raw milk, fresh pasteurized milk, pasteurized refrigerated milk samples and retail milk samples were subjected for the isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

3.1.4.1 Escherichia coli

For the isolation of *Escherichia coli*, a loopful of inoculum from each sample was inoculated on to duplicate plates of Eosin methylene blue agar and incubated at 37°C for 24 h. (Indian Standards, 1980). 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 selected and transferred on to nutrient agar slants and incubated at 37°C for overnight. These isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions as described by Barrow and Feltham (1993) and are shown in flowchart 2. Isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh.

In-vitro Pathogenicity Studies for *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). Tryptone Soya Agar was supplemented with 0.03 per cent congo red dye (Nesslers) and 0.15 per cent bile salts (Loba Chemie) was used for assay. *Escherichia 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

Flow chart 2. Isolation and identification of *Escherichia coli*

	Characteristics/Reactions
Milk sample	
↓	
EMB agar	
↓	colonies with a dark center and a
Suspected colonies	distinct indelible-ink greenish
↓	black metallic sheen on deflected
	light
Nutrient agar	
↓	
Grams' staining reaction and cell morphology	Gram negative small rods
↓	
Motility test	+
↓	
Growth aerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
Urease	-
↓	
ONPG	+
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate Utilization test	-
↓	
Carbohydrate utilization	
Lactose	+
Gluose	+
Mannitol	+
Inositol	-
Maltose	+

3.1.4.2 Staphylococcus aureus

For the isolation of *Staphylococcus aureus*, a loopful of the sample was inoculated onto Baird-Parker (BP) agar medium (Hi-media) and was incubated at 37°C for 48 h. (Lancette and Bennett, 2001). At the end of incubation, colonies showing characteristics appearance (circular, smooth, convex, moist, 2.3 mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently with outer clear zone) 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. Characterization and identification of the isolates were done following the procedure described by Barrow and Feltham (1993) and are shown in the flowchart 3. The isolates were identified based on the cultural, morphological and biochemical characteristics.

3.1.4.3 Pseudomonas

For the isolation of *Pseudomonas,* a loopful of the sample was streaked on to 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 or non-pigmented smooth circular colonies were transferred to nutrient agar slants and incubated at 30°C overnight and were stored at refrigeration temperature. The isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flowchart 4.

3.1.4.4 Characterization and identification of isolates

The suspected colonies selected as *Escherichia coli, Staphylococcus aureus* and *Pseudomonas* were subjected to various tests and identified based on the cultural, morphological and biochemical characteristics described by Barrow and Feltham (1993) except for the triple sugar test (Edwards and Ewing, 1972).

Flow chart 3. Isolation and identification of *Staphylococcus aureus*

	Characteristics/Reactions
Milk sample	
↓	
Inoculated on to BP agar	
↓	
Suspected colonies on to Nutrient agar slant	Gray black to jet black, frequently with light coloured margin, surrounded by opaque zone
↓	
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	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Mannitol	
Aerobic	+
Anaerobic	+

Flow chart 4. Isolation and identification of *Pseudomonas*

Milk sample	Characteristics/Reactions		
	P. aeruginosa	*P. flourescens*	*P. putida*
Inoculated on to Pseudomonas agar			
Suspected colonies on to Nutrient agar slant			
Gram's staining reaction and Cell morphology	Gram negative rods	Gram negative rods	Gram negative rods
↓			
Motility test	+	+	+
↓			
Growth aerobically	+	+	+
↓			
Catalase	+	+	+
↓			
Oxidase	+	+	+
↓			
OF test	O	O	O
↓			
Citrate utilization	+	+	+
↓			
Arginine hydrolysis	+	+	+
↓			
Lysine hydrolysis	-	-	-
↓			
Ornithine hydrolysis	-	-	-
↓			
Growth in presence of cetrimide	+	+	+
↓			
Urease	+	+	+
↓			
Nitrate reduction	+		-
Carbohydrate utilization			
Glucose	+	+	+
Fructose	+	+	+
Lactose	-	-	-
Maltose	-	+	-
Mannitol	+	+	-
Sucrose	-	+	-

Primary Identification Test

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. Evolution of effervescence with in a few seconds indicates a positive reaction.

b) Tube test

One ml of three percent hydrogen peroxide solution was poured over the slope of a nutrient agar slant on which the isolates was grown. A positive reaction is indicated by the development of effervescence immediately.

Gram staining

The procedure for gram staining is 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 seconds.

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 seconds.

f. Washed the smear and counter stained with dilute carbol fuschin for 30 seconds.

g. Poured off the stain from the slide, washed, dried and examined under oil immersion objective of the microscope.

1) 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 is confirmed to the stab.

2) Oxidase test

A filter paper strip is moistened with a few drops of an aqueous solution of 1 per cent tetramethyl paraphenyline diamine dihydrochloride. 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 indicates a positive reaction.

3) Oxidation – Fermentation test

Each isolate was inoculated into duplicate tubes of Hugh and Liefson'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 one 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 is taken as oxidation whereas a change in colour from green to yellow in both the tubes is regarded as fermentation. Absence of colour change in both tubes indicates no action on carbohydrates.

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 indicates a positive reaction.

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 is indicated by the development of brown colour.

3) Carbohydrate utilization 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 indicates acid production and the production of gas is 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 media.

4) 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 utilize 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.

5) 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 indicates a positive result and delayed clumping is 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.

6) Decarboxylase reaction

Each isolate was heavily inoculated with straight wire into three test tubes containing decarboxylase media. One of the tubes contains lysine and other contains ornithine. The third tubes 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 turns yellow and then becomes purple and the control tubes remain yellow.

7) Eijkman test

Each test organism was inoculated into tubes containing MacConkey 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 indicates a positive reaction.

8) Gelatin hydrolysis/liquefactions

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 is indicated by liquefaction of gelatin.

9) Hippurate hydrolysis

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

10) Indole production

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

11) 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 indicates positive reaction.

12) ONPG (O-nitrophenyl-P-D-glactopyranocide) test

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

13) 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.

14) Phosphatase test

The phenolphthalene 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 reacts with the ammonia and phosphatase positive colonies became bright pink.

15) *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).

16) 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.

17) 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 is indicated by the development of a strong red colour.

3.2 SENSORY AND PHYSICO-CHEMICAL QUALITY OF MILK

3.2.1 Sensory Evaluation

A four member sensory panel evaluated sensory characters viz., colour and appearance, Odour, Flavour and Body of fresh and refrigerated pasteurized milk samples. The scores assigned for each parameter were colour and appearance (10), odour (20), Flavour (40) and Body (30). The grades were assigned to milk according to IS-1975

Table 1. The criteria for grading of milk according to IS 7768 (1975)

GRADE	SCORE	QUALITY
A	90 and above	Excellent
B	80 to 89	Good
C	60 to 79	Fair
D	59 and below	Poor

3.2.2 Clot on Boiling test

Clot on boiling (COB) test was performed following the procedure described by Indian standards, 1981. Five millilitre of milk was taken in a test-tube and the tube was placed in a water bath at boiling temperature for five minutes. The tube was than removed and rotated in an almost horizontal position and examined the side of test tube for any precipated particle. Formation of flakes or clots was taken as a positive test.

3.3 POLYMERASE CHAIN REACTION

Materials

► PCR reaction buffer (10X)

This includes 500mM KCl, 100mM Tris-HCl (pH 9.0) and 15 mM $MgCl_2$.

► Taq DNA polymerase

Taq DNA polymerase enzyme with a concentration of 3U/µl.

► Deoxy ribo Nucleotide Triphosphate (dNTP) mix 10mM (2.5 mM of each dGTP, dCTP, dATP and dTTP in equal volume)

All the above reagents were obtained from Bangalore Genei, India Limited.

Primers for genus specific PCR

Specific primers to detect *Escherichia coli* (Genus specific) designed by Daly *et al.* (2002) were used. The sequences of the primers were as follows:

5'-CTG GAA GAG GCT AGC CTG GAC GAG-3'

5'-AAA ATC GGC ACC GGT GGA GCG ATC-3'

Reconstitution and dilution of primers

Primers obtained in lyophilized form were reconstituted in 100µl of sterile triple distilled water to a concentration of 200 picomoles. The tubes were kept at room temperature with occasional shaking for one hour. They were spun briefly to pellet down the insoluble particles if any and the stock solution was distributed into 10µl aliquots and stored at -7°C. At the time of use the aliquots were thawed and further diluted ten fold to obtain a concentration of 20 picomoles/µl. before using for PCR.

Method

Polymerase Chain Reaction was conducted for the detection of *Escherichia coli* by the method as described by Daly *et al.* (2002).PCR technique was employed using template DNA prepared from the following method.

Preparation of template DNA

Overnight culture of isolates of *Escherichia coli* (37°C for 18h in Trypticase Soya broth or Soybean Casein digest broth) obtained from raw and pasteurized milk is taken in an eppendorf tube (1.5ml) and centrifuged at 3000g for 10 minutes. The supernatant is discarded and the pellet obtained at the bottom of the tube is washed twice with sterile PBS and finally the pellet is resuspended in 100µl of triple distilled water. The mixture is boiled for 10 minutes and then immediately chilled on ice for 30 minutes. The samples were thawed and centrifuged at 3000g for 5 minutes and the supernatant is stored at -20°C for further use as template for PCR.

Setting up of PCR

PCR was performed in a total volume of 25µl reaction mixture. A master mix prepared before setting up the PCR reaction by combining the following reagents in a 20µl volume. The reaction mixture consisted of

Primers	20 picomole of each primer
10X PCR buffer	50mMKCl, 10mM Tris-HCl and 1.5 mM $MgCl_2$
Taq DNA polymerase	1.0 unit
dNTP mix	200µl of each dNTP

Preparation of 200µl master mix for 10 reactions

PCR reaction buffers	25µl
Forward primer	10µl
Reverse primer	10µl
dNTPmix	20µl
Taq DNA polymerase	3.3µl
Triple distilled water	200µl

To each PCR tube 20µl of master mix and 5µl of template DNA were added. One negative control without DNA was also added. The PCR amplification was carried out in an automated thermal cycler (Eppendorf Master Cycler, Germany) according to the following programme.

Initial denaturation at 95°C for one minute followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 72°C for 90 seconds and extension at 72°C for 5 minutes and a final extension at 72°C for 6 minutes. The whole reaction was conducted under the heated lid. The product was analyzed by submarine agarose gel electrophoresis.

Submarine Agarose Gel Electrophoresis

Materials

A. (0.5 M) EDTA (pH 8.0)

Dissolved 18.61g of EDTA (disodium, dihydrate) in 70ml of triple distilled water. The pH was adjusted to 8.0 with 1N NaOH. The volume was made upto 100ml, filtered, autoclaved at 121°C for 15 minutes at 15 lbs pressure and stored at room temperature.

B. TAE (Tris-Acetate EDTA) buffer (50X) pH 8.0

Tris base	48.40 g
Glacial acetic acid	11.42 ml
0.5 M EDTA pH 8.0	20.00 ml
Distilled water to	1000 ml

Autoclaved at 121°C for 15 minutes at 15 lbs pressure and stored at room temperature.

C. TAE (1X)

TAE 50X	2 ml
Distilled water	98 ml

D. Agarose Gel (1.5 per cent)

Agarose low EEO (Genei)	1.5 g
TAE buffer (1X)	100 ml

E. Gel loading buffer (6X)

Bromophenol blue	0.25 g
Xylene cyanol	0.25 g
Sucrose	40.00 g
Distilled water to	100 ml

Stored at 4°C.

F. Ethidium bromide

Ethidium bromide	100 mg
Distilled water	10 ml

Stored at 4°C in amber coloured bottles.

G. DNA molecular size marker

pUC19DNA/*Msp*I digest with fragments 501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34 and 26 bp.

The molecular size markers were obtained from Bangalore Genei (India).

Method

The PCR product was detected by electrophoresis in 1.5 per cent agarose gel in TAE buffer (1X). Agarose was dissolved in TAE buffer (1X) by heating. When the mixture was cooled to around 50°C, Ethidium bromide was added to a final concentration of 0.5 µg/ml. Melted agarose was then poured into clean, dry, gel platform, the edges of which were sealed with adhesive tape and the comb was kept in proper position. Once the gel was set, the comb and adhesive tape were removed gently and the tray containing the gel was completely covered

Amplified PCR product (5 µl) was mixed with one µl of 6X gel loading buffer and the samples were loaded in the wells. The pUC19DNA/*Msp*I DNA molecular size

digest was used as marker. Electrophoresis was carried at 5V/cm for one hour (or) until the bromophenol blue dye migrated to more than two-third of the length of the gel.

The gel was visualized under UV transilluminator (Hoefer, USA) and the images were documented in a gel documentation system (Bio-Rad Laboratories, USA).

3.4 STATISTICAL ANALYSIS

The data obtained from the various studies were subjected to statistical analysis following procedure described by Rangaswamy (1995).

Results

4. RESULTS

In the present study the bacterial quality of milk was evaluated by determining the bacterial load of milk collected from two dairy plants and retail outlets. The effect of pasteurization on the bacterial quality of raw milk and the changes in bacterial load of the pasteurized milk samples during refrigeration were also analysed. During the study, the changes in sensory and physical quality of fresh and refrigerated samples were evaluated and the shelf life of pasteurized milk under refrigerated conditions was also determined. The samples of raw pooled, freshly pasteurized, refrigerated and retail milk were subjected to the isolation and identification of *Escherichia coli, Staphylococcus aureus* and *pseudomonas*. The isolates of *Escherichia coli* were subjected to serotyping and Polymerase Chain Reaction for further confirmation of the strains.

4.1 BACTERIAL QUALITY OF MILK

The bacterial quality of all milk samples collected from two dairy plants *viz.* DP_1 and DP_2 were evaluated by estimating the various bacterial counts.

4.1.1 Bacterial Counts of Raw Milk

The raw milk samples collected from the two dairy plants (DP_1, DP_2) were tested to determine the quality by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Psychrotrophic Count (PC) and Faecal Streptococcal Count (FSC).

4.1.1.1 Total Viable Count

The mean total viable count (TVC) of raw pooled milk from DP_1 and DP_2 are given in table 2. The mean total viable count of the samples belonging to DP_1 was 7.11 ± 0.02 $\log_{10}$ cfu/ml and the count of the samples belonging to DP_2 was 7.18 ± 0.03 $\log_{10}$ cfu/ml. Cent per cent of samples from DP_1 had total viable

count at the level of 10^7 cfu/ml (table 3) and the count of samples belonging to DP_2 was also at that level (table 4).

4.1.1.2 Coliform Count

The mean coliform count (CC) of pooled raw milk from DP_1 and DP_2 are given in table 2. Analysis of the data using simple t test revealed highly significant ($P<0.01$) difference between mean counts of milk samples from two sources *viz.* DP_1 and DP_2. The samples of pooled milk from DP_1 had a mean coliform count of 3.34 ± 0.05 $\log_{10}$ cfu/ml and the sample from DP_2 had a count of 2.96 ± 0.13 $\log_{10}$ cfu/ml.

Cent per cent of samples from DP_1 had coliform count at the level of 10^3 cfu/ml (table 3). The samples belonging to DP_2 had the count at the level of 10^2 cfu/ml in 57 per cent and 10^3 cfu/ml in 43 per cent samples (table 4).

Table 2. Mean bacterial counts of pooled raw milk samples

Bacterial count	Mean bacterial count of milk samples ($\log_{10}$cfu/ml)	
	DP_1	DP_2
TVC	7.11 ± 0.02	7.18 ± 0.03
CC	$3.34^a \pm 0.05$	$2.96^b \pm 0.13$
ECC	$1.79^a \pm 0.65$	$2.22^b \pm 0.41$
PC	7.07 ± 0.02	7.06 ± 0.03
FSC	3.15 ± 0.03	3.12 ± 0.06

Figures bearing different superscript between columns of a row differ significantly; N=7 in each group

4.1.1.3 Escherichia coli Count

The mean *Escherichia coli* count (ECC) of pooled raw milk from DP_1 and DP_2 are given in table 2. The mean count of the organism in the samples from DP_1 was 1.79 ± 0.65 $\log_{10}$ cfu/ml and the count of samples from DP_2 was 2.22 ± 0.41 $\log_{10}$ cfu/ml.

The distribution of pooled milk samples from DP_1 based on *Escherichia coli* counts revealed that 28.5 per cent samples each had count at the level of 10^2 and 10^3 cfu/ml, respectively (table 3). Of the seven samples from DP_2, 43 per cent had count at the level of 10^3 cfu/ml and 14 per cent had count at the level of 10^2 cfu/ml. The organism was absent in 43 per cent of the samples of DP_1 and DP_2.

4.1.1.4 Psychrotrophic Count

The mean psychrotrophic count (PC) of pooled raw milk samples from DP_1 and DP_2 was 7.07 ± 0.02 $\log_{10}$ cfu/ml and 7.06 ± 0.03 $\log_{10}$ cfu/ml, respectively (table 2). The psychrotrophic count in cent per cent of samples belonging to DP_1 was at the level of 10^7 cfu/ml (table 3). The count in 86 per cent of samples belonging to DP_2 were at the level of 10^7 cfu/ml and 14 per cent had count at the level of 10^6 cfu/ml.

Table 3. Frequency distribution of pooled milk samples from DP_1 based on bacterial counts

Bacterial count	Level of bacterial count in the samples (cfu/ml)							
	ND	10^1	10^2	10^3	10^4	10^5	10^6	10^7
TVC								7(100)
CC				7(100)				
ECC	3 (43)	ND	2 (28.5)	2(28.5)				
PC								7(100)
FSC				7(100)				

Figures in parenthesis indicate percent; N=7 samples in each group

4.1.1.5 Faecal streptococcal Count

The mean faecal streptococcal counts of pooled raw milk from DP_1 and DP_2 are shown in table 2. The samples from the former source had a mean count of 3.15 ± 0.03 $\log_{10}$ cfu/ml and the samples belonging to the latter source had a mean count of 3.12 ± 0.06 $\log_{10}$ cfu/ml. Count at the level of 10^3 cfu/ml was seen in cent per cent of samples from DP_1 (table 3). Of the samples from DP_2, 86 per

cent of samples had count at the level of 10^2 cfu/ml and 14 per cent of samples had a count at the level of 10^3cfu/ml (table 4).

Table 4. Frequency distribution of pooled milk samples from DP_2 based on bacterial counts

Bacterial count	Level of bacterial count in the samples (cfu/ml)							
	ND	10^1	10^2	10^3	10^4	10^5	10^6	10^7
TVC								7(100)
CC			4(57)	3(43)				
ECC	3(43)	ND	1(14)	3(43)				
PC							1(14)	6(86)
FSC			6(86)	1(14)				

Figures in parenthesis indicate percent; N=7 samples in each group

4.1.1.6 Correlation between bacterial counts of Raw Milk

The correlation coefficient between various bacterial counts of raw milk samples from DP_1 showed a highly significant ($P<0.01$) and positive association between the total viable count and psychrotrophic count and also a positive and significant ($P<0.05$) correlation between the psychrotrophic and coliform counts (table 5).

Table 5. Correlation between bacterial counts of raw pooled milk from DP_1

Bacterial Counts	Correlation coefficient			
	CC	FSC	PC	ECC
TVC	0.716	0.513	0.920**	-0.511
CC		0.306	0.532*	-0.184
FSC			0.332	-0.703
PC				-0.397

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, ** =$P<0.01$, * = $P<0.05$

The correlation coefficient between various bacterial counts of raw milk samples from DP_2 revealed a significant and positive association between the total viable count and psychrotrophic count (table 6).

Table 6. Correlation between bacterial counts of raw pooled milk from DP_2

Bacterial Counts	Correlation coefficient			
	CC	FSC	PC	ECC
TVC	0.158	0.409	0.300*	-0.096
CC		0.663	0.299	-0.005
FSC			0.458	-0.330
PC				-0.282

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = P<0.05

4.1.2 Isolation and Identification of Bacteria

The bacteria isolated from pooled raw milk are given in table 7. A total of 14 samples from both the sources were tested for the isolation of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

Table 7. Bacteria isolated from pooled raw milk

Bacteria	No. of samples from DP_1		No. of samples from DP_2	
	Positive	Per cent	Positive	Per cent
Escherichia coli	4	57	4	57
Staphylococcus aureus	4	57	2	29
Pseudomonas	3	43	5	71

N= 7 samples from each source

4.1.2.1 Escherichia coli

Pooled raw milk samples were tested for the isolation and identification of *Escherichia coli.* The isolates collected were identified by cultural, morphological and biochemical test. The isolates were also subjected to Eijkman test. The

organism was isolated from 57 per cent of samples from DP_1 and DP_2 (table 7). Of the four isolates belonging to the samples of DP_1, three showed Eijkmann positive reaction and all isolates from the samples of DP_2 revealed a positive Eijkmann test.

Table 8. Distribution of *Escherichia coli* serotypes from pooled raw milk

Isolate No.	Serotype
1	O116
2	R
3	O68
4	R
5	UT
6	O116
7	O116
8	UT

UT-Untypable R- Rough strains

The isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. A total of four isolates were obtained from pooled raw milk samples of DP_1, of which 25 per cent isolates each belonged to the serotype O116 and O68 and the remaining 50 per cent of isolates belonged to rough strains. Of the four isolates obtained from DP_2, 50 per cent belonged to serotype O116 and the rest were Untypable.

Escherichia coli isolated from pooled raw milk were subjected to Congo red binding test and the results are given in table 9. Five of these isolates showed a positive reaction, which indicates the property of invasiveness of the isolates.

Table 9. Congo red binding test of *E. coli* isolates from raw milk samples

Isolate No.	Congored binding test
1	+
2	-
3	+
4	+
5	+
6	-
7	+
8	-

+ Positive, - Negative

4.1.2.2 Staphylococcus aureus

All pooled milk samples were tested for the isolation of *Staphylococcus aureus*. All isolates were identified by the cultural, morphological and biochemical characteristics. The organism was isolated from four (57 per cent) pooled raw milk samples from DP_1 and two (29 per cent) samples from DP_2 (table 7).

4.1.2.3 Pseudomonas

All pooled milk samples were tested for the isolation and identification of *Pseudomonas*. The isolates obtained were identified by cultural, morphological and biochemical tests. The organism was detected in 42.85 per cent of the samples collected from DP_1 and 71.4 per cent of samples collected from DP_2 (Table 7).

Table 10. *Pseudomonas* isolates from pooled raw milk samples of DP_1 and DP_2

Organism isolated	No. of isolates from DP_1		No. of isolates from DP_2	
	Positive	Per cent	Positive	Per cent
Pseudomonas aeruginosa	ND	0	1	14.28
Pseudomonas flourescens	2	28.57	3	42.84
Pseudomonas putida	1	14.28	1	14.28
Total	3	42.85	5	71.40

4.1.3 Effect of Pasteurization on the Bacterial Quality of Raw Milk

The effect of pasteurization on the bacterial quality of milk samples from DP_1 is given in the table 11 and that of samples from DP_2 is given in table 12 and illustrated in fig. 1 and 2, respectively. The mean TVC, CC, ECC, PC and FSC of raw milk from DP_2 have reduced to a highly significant ($P<0.01$) level upon

Table 11. Effect of pasteurization on the bacterial quality of milk from DP_1

Bacterial Count	Bacterial load of milk (log_{10} cfu/ml)	
	Before pasteurization	Pasteurized packaged milk
TVC	$7.11^a \pm 0.02$	$5.04^b \pm 0.03$
CC	$3.37^a \pm 0.05$	$2.53^b \pm 0.10$
ECC	$1.79^a \pm 0.65$	$0.37^b \pm 0.24$
PC	$7.07^a \pm 0.02$	$4.45^b \pm 0.06$
FSC	$3.16^a \pm 0.04$	$2.21^b \pm 0.17$

Figures bearing the same superscript between columns of a row do not differ significantly

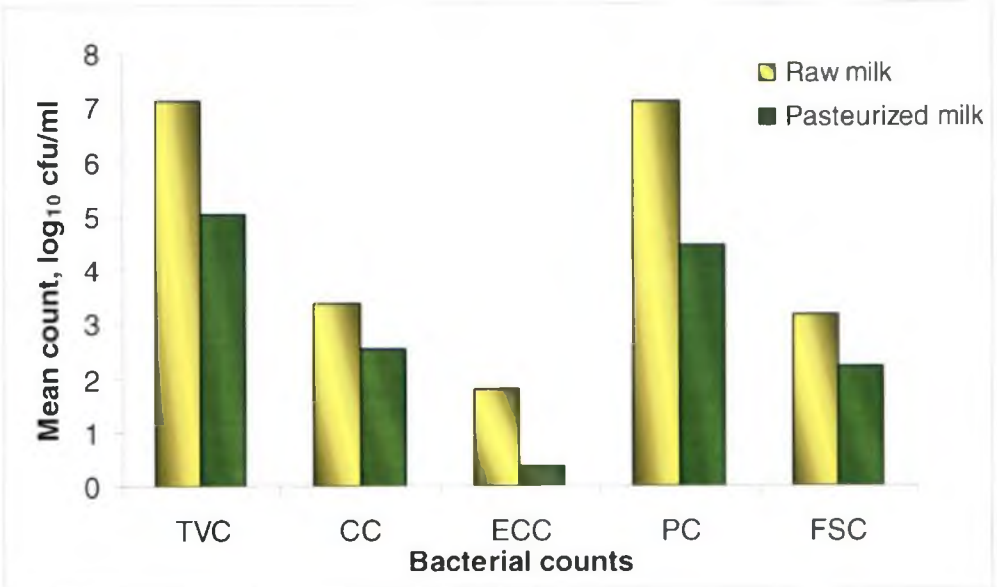

Fig. 1 Effect of pasteurization on bacterial quality of milk (DP_1)

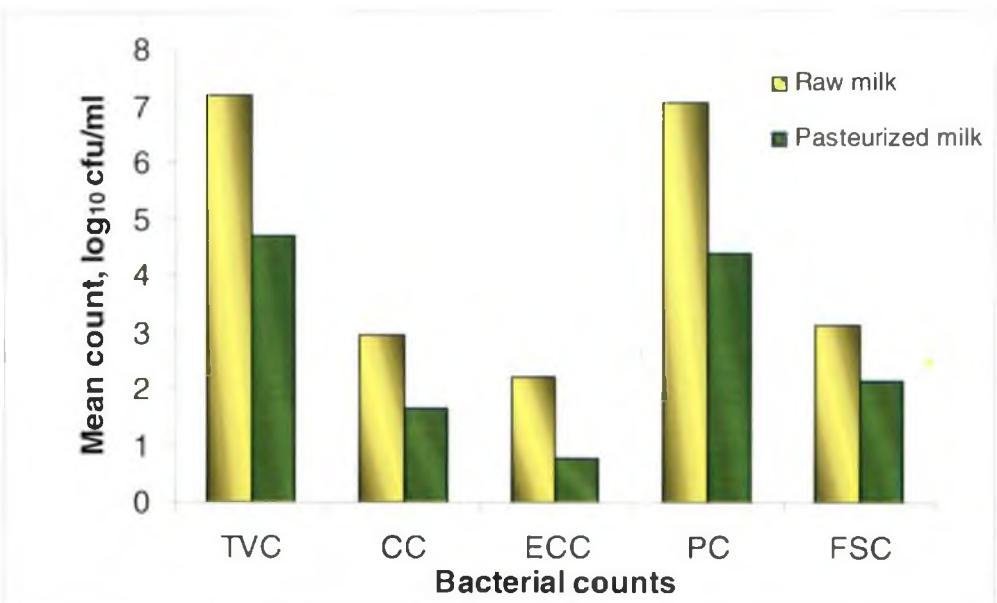

Fig. 2 Effect of pasteurization on bacterial quality of milk (DP_2)

pasteurization. The mean TVC, CC, PC and FSC of raw milk from DP_1 were reduced to a highly significant ($P<0.01$) level while *Escherichia coli* count was significantly ($P<0.05$) reduced upon pasteurization.

The mean reduction in TVC, CC, ECC, PC and FSC in pasteurized milk with that of pooled milk from DP_1 and DP_2 were at the level of 2.07, 0.836, 1.42, 2.62 and 0.94 log_{10} cfu/ml and 2.46, 1.31, 1.44, 2.66 and 0.98 log_{10} cfu/ml, respectively.

Table 12. Effect of pasteurization on the bacterial quality of milk from DP_2

Bacterial Count	Bacterial load of milk (log_{10} cfu/ml)	
	Before pasteurization	Pasteurized packaged milk
TVC	$7.18^a \pm 0.03$	$4.72^b \pm 0.09$
CC	$2.96^a \pm 0.13$	$1.65^b \pm 0.21$
ECC	$2.22^a \pm 0.41$	$0.78^b \pm 0.28$
PC	$7.06^a \pm 0.03$	$4.40^b \pm 0.08$
FSC	$3.12^a \pm 0.07$	$2.15^b \pm 0.15$

Figures bearing the same superscript between columns of a row do not differ significantly

4.1.4 Bacterial Counts of samples under storage study

Bacterial quality of freshly pasteurized milk samples was evaluated on day zero and refrigerated milk samples was evaluated on day two, four, six, eight and 10 of storage at 4 ± 1 °C.

Dairy Plant 1

A total of 84 pasteurized milk samples were collected from Dairy Plant 1 (DP_1) and estimated the bacterial counts of fresh samples (14) on day zero and samples under storage (70) on day 2, 4, 6, 8 and 10.

4.1.4.1 Total Viable Count

The mean total viable count of fresh and refrigerated milk samples is given in table 13. During 10 days of storage the mean count has increased from

5.04 ± 0.03 to 8.27 ± 0.11 $\log_{10}$ cfu/ml, as illustrated in fig.3. Analysis of the data was done using the paired t test. The mean count of fresh sample had highly significant (P<0.01) difference with the mean count of the samples stored on day two, four, six, eight and 10. The mean count of samples on second day of storage had highly significant (P<0.01) difference with the mean count of samples stored

Table 13. Mean total viable count of fresh and refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	5.04^{a} ± 0.03
2	5.69^{b} ± 0.04
4	7.13^{c} ± 0.04
6	7.62^{d} ± 0.10
8	8.15^{e} ± 0.04
10	8.27^{f} ± 0.11

Figures bearing the same superscript do not differ significantly N= 14 samples on each day of storage.

on day four, six, eight and 10. Similarly the mean count of samples on fourth day of storage differed highly significantly (P<0.01) from the mean count of samples stored on day six, eight and 10. Highly significant (P<0.01) difference was also noticed between samples stored on day six and eight, six and 10 and eight and 10.

Distribution of fresh and refrigerated samples based on level of total viable count is given in table 14. The count in cent per cent of the samples on day two and the count in 57 per cent of fresh samples was at the level of 10^{5} cfu/ml and 43 per cent of fresh samples had count at the level of 10^{4} cfu/ml. Cent per cent of samples on day four and six of storage had count at the level of 10^{7}cfu/ml. On day 8 of storage 71 per cent samples had count at the level of 10^{8} cfu/ml and on day 10 of storage the count in 86 per cent samples was at the level of 10^{8} cfu/ml.

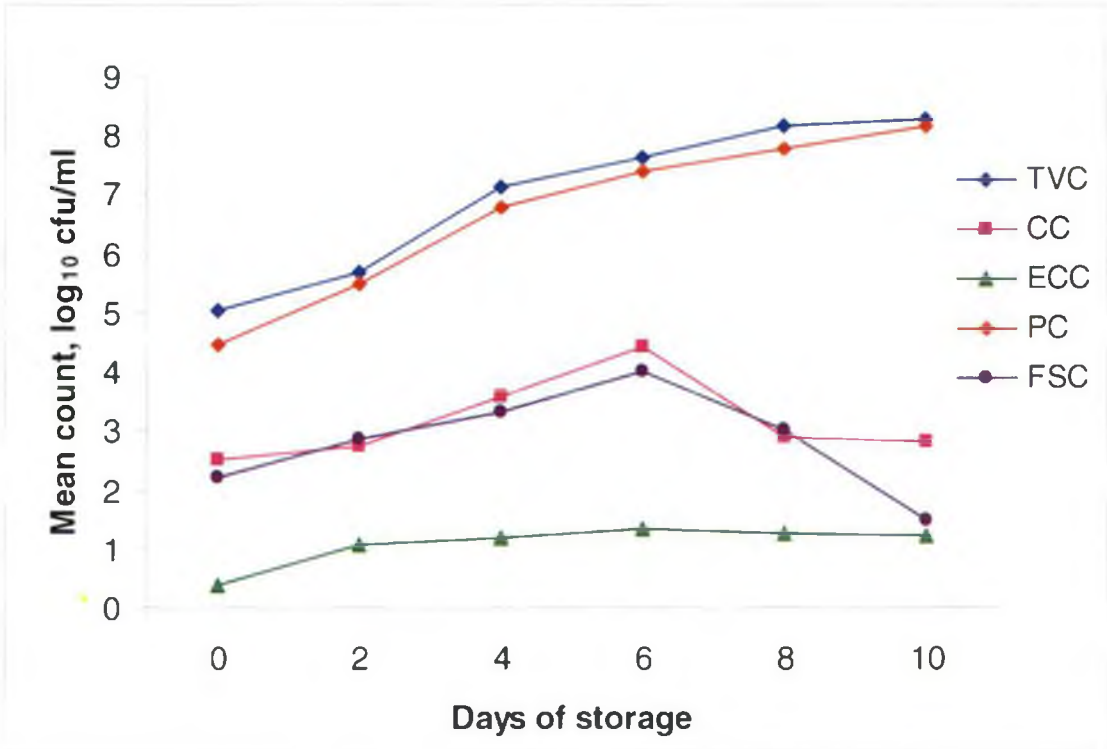

Fig. 3 Effect of refrigeration on bacterial quality of milk (DP_1)

Table 14. Distribution of fresh and refrigerated milk samples based on total viable count

Days of storage	Total Viable Count (cfu/ml)				
	10^4	10^5	10^6	10^7	10^8
0	6 (43)	8 (57)			
2		14 (100)			
4				14 (100)	
6				14 (100)	
8				4 (29)	10 (71)
10				2 (14)	12 (86)

Figures in parenthesis indicate percent; N=14 samples on each day of storage

4.1.4.2 Coliform Count

The mean coliform count (CC) of fresh and refrigerated milk samples is given in table 15. Analysis of the data using paired t test revealed a gradual increase in coliform count up to sixth day of storage after which the milk started getting spoiled and thereafter a reduction in count was observed. The mean coliform count of freshly

Table 15. Mean coliform count of fresh and refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	$2.53^{a} \pm 0.10$
2	$2.73^{a} \pm 0.08$
4	$3.60^{b} \pm 0.12$
6	$4.44^{c} \pm 0.17$
8	$2.88^{abc} \pm 0.56$
10	$2.83^{ad} \pm 0.86$

Figures bearing the same superscript do not differ significantly. N=14 samples on each day of storage

pasteurized milk was 2.53 ± 0.10 log_{10} cfu/ml. On the sixth day of storage the count reached a level of 4.44 ± 0.17 log_{10} cfu/ml and then reduced to 2.83 ± 0.86 log_{10}

cfu/ml on 10^{th} day, as illustrated in fig. 3. The mean counts of the samples stored on zero and second day were highly significantly ($P<0.01$) different from that of the count of the samples stored on day four and six of storage. Highly significant ($P<0.01$) difference was also observed between the mean count of samples stored on day four and six, four and 10 and six and 10.

Distribution of fresh and refrigerated samples based on level of coliform count is given in table 16. The count at the level of 10^2 cfu/ml was observed in 57 per cent of samples on day zero of storage and 43 per cent had count at the level of 10^1 cfu/ml. On day two of storage 86 per cent of samples had count at the level 10^2 cfu/ml and on day four of storage cent per cent of samples had count at the level of 10^3 cfu/ml. The count in 86 per cent of the samples was at the level of 10^4 cfu/ml, on day six of storage. The organism could not be detected in 43 per cent of samples on day eight of storage but an equal per cent of samples had count at the level of 10^3 cfu/ml. On day 10 of storage 86 per cent of samples did not reveal the presence of the organism and 14 per cent samples had count at the level of 10^4 cfu/ml.

Table 16. Distribution of fresh and refrigerated milk samples based on coliform count

Days of storage	Coliform count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
0		6 (43)	8(57)		
2			12 (86)	2 (14)	
4				14 (100)	
6				2 (14)	12(86)
8	6 (43)	2 (14)		6 (43)	
10	12 (86)				2 (14)

Figures in parenthesis indicate percent; N=14 samples on each day of storage

4.1.4.3 Escherichia coli Count

The mean *Escherichia coli* count of fresh and refrigerated milk samples is given in table 17. The count showed an increasing trend till sixth day but was not significant; as illustrated in fig. 3.

Table 17. Mean *Escherichia coli* count of fresh and refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	0.37 ± 0.65
2	1.07 ± 0.51
4	1.18 ± 0.57
6	1.32 ± 0.66
8	1.25 ± 0.74
10	1.21 ± 0.78

N=14 samples on each day of storage

Distribution of fresh and refrigerated samples based on level of *Escherichia coli* count is given in table 18. The organism could not be detected in 71.43 per cent of the

Table 18. Distribution of fresh and refrigerated milk samples based on *Escherichia coli* count

Days of storage	*Escherichia coli* Count (cfu/ml)				
	ND	10	10^2	10^3	10^4
0	10 (71.43)	4(28.57)			
2	10 (71.43)	ND	4(28.57)		
4	10 (71.43)	ND	4(28.57)		
6	10 (71.43)	ND		4(28.57)	
8	10 (71.43)	ND		4(28.57)	
10	10 (71.43)	ND		4(28.57)	

Figures in parenthesis indicate percent; N=14 samples on each day of storage; ND-Not detected

samples tested during the period of study. The count in 28.57 per cent of the samples examined on day zero and two was at a level of 10^1 cfu/ml and 10^2 cfu/ml, respectively.

An equal per cent of samples stored on day four and six had count at the level of 10^2 cfu/ml and 10^3 cfu/ml, respectively. The count at the level of 10^3 cfu/ml was seen in 28.57 per cent of samples examined on day eight and 10 of storage.

4.1.4.4 Psychrotrophic Count

The mean psychrotrophic count of the fresh and refrigerated samples is given in table 19. An increase in mean psychrotrophic count was observed throughout the period of storage. The mean psychrotrophic count of samples on zero day of storage was 4.45 ± 0.06 and was found to increase to 8.17± 0.04 $\log_{10}$ cfu/ml on the 10^{th} day, as illustrated in fig. 3. Analysis of the data using paired t test showed highly significant ($P<0.01$) increase in the mean counts of the samples stored between day zero and day second, fourth, sixth, eighth and 10^{th} of storage. Highly significant ($P<0.01$) increase was observed between the mean count of samples tested on day two and day four, six, eight and 10 of storage. Similarly highly significant ($P<0.01$) increase in mean counts was observed between mean counts of day four and six, eight and 10.

Table 19. Mean psychrotrophic count of fresh and refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	4.45^{a} ± 0.06
2	5.49^{b} ± 0.09
4	6.80^{c} ± 0.06
6	7.40^{d} ± 0.07
8	7.79^{e} ± 0.06
10	8.17^{f} ± 0.04

Figures bearing the same superscript do not differ significantly. N=14 samples on each day of storage

Distribution of fresh and refrigerated milk samples based on level of psychrotrophic count is given in table 20. The count in cent per cent of the samples on day zero and two of storage was at the level of 10^4 cfu/ml and 10^5 cfu/ml, respectively. The count in cent per cent of samples on day four and 10 of storage was at the level of

10^6 cfu/ml and 10^8 cfu/ml, respectively. On day eight of storage 86 per cent of samples had count at the level of 10^7 cfu/ml and 71 per cent of samples had count at the same level, on day six of storage.

Table 20. Distribution of fresh and refrigerated milk samples based on Psychrotrophic count

Days of storage	Psychrotrophic count (cfu/ml)					
	10^3	10^4	10^5	10^6	10^7	10^8
0		14(100)				
2			14(100)			
4				14(100)		
6				4 (29)	10 (71)	
8				2 (14)	12 (86)	
10						14 (100)

Figures in parenthesis indicate percent; N=14 samples on each day of storage

4.1.4.5 Faecal Streptococcal Count

The faecal streptococcal count of fresh and refrigerated milk samples is given in table 21. Analysis of the data using paired t test revealed that the mean count of fresh

Table 21. Mean faecal streptococcal Count in fresh and refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
Fresh sample	$2.21^{a} \pm 0.17$
2	$2.85^{b} \pm 0.05$
4	$3.32^{c} \pm 0.07$
6	$4.01^{d} \pm 0.05$
8	$3.02^{abcde} \pm 0.50$
10	$1.48^{abce} \pm 0.70$

Figures bearing the same superscript do not differ significantly. N=14 samples on each day of storage

sample had a highly significant (P<0.01) difference with the mean count of the samples on the day two, four and six of storage. Highly significant (P<0.01) difference was also seen between the mean count of the sample stored on day two and four, two and six and four and six as illustrated in fig 3. However, the mean count on day six and 10 showed only significant (P<0.05) difference.

Distribution of fresh and refrigerated samples based on level of faecal streptococcal count is given in table 22. The count in 71 per cent of the samples stored on day zero, two and four was at the level of 10^2 cfu/ml, 10^2 cfu/ml and 10^3 cfu/ml, respectively. On sixth day of storage the count in 57 per cent of samples reached the level of 10^4 cfu/ml. But the count in 86 per cent of samples on eighth day of storage reduced to 10^3 cfu/ml. In 43 per cent of samples stored on 10^{th} day also the count was at the level of 10^3 cfu/ml. However, the count was not detected in 14 and 57 per cent of samples on eighth and 10^{th} day of storage, respectively.

Table 22. Distribution of fresh and refrigerated milk samples based on faecal streptococcal counts

Days of storage	Faecal streptococcal count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
0	4 (29)		10 (71)		
2		4 (29)	10 (71)		
4			4 (29)	10 (71)	
6				6 (43)	8 (57)
8	2 (14)			12 (86)	
10	8 (57)			6 (43)	

Figures in parenthesis indicate percent; N=14 samples on each day of storage; ND-Not detected

4.1.4.6 Correlation between Bacterial Counts of Fresh and Refrigerated Milk

Total Viable Count

Correlation between mean total viable count and the other bacterial counts of milk samples under storage study are depicted in table 23. A significant ($P<0.05$) and positive association was observed between total viable count and coliform count of samples on day zero of refrigerated storage. A similar relationship was observed between the mean total viable count and psychrotrophic counts on eighth and 10^{th} day of storage.

Table 23. Correlation coefficient between mean total viable count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between TVC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
CC	0.759*	-0.333	-0.426	0.248	-0.026	-0.286
FSC	-0.109	-0.502	0.281	0.116	0.495	-0.044
PC	0.310	-0.003	0.054	-0.207	0.428*	0.427*
ECC	0.216	-0.104	0.148	0.090	-0.523	0.519

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = $P<0.05$

Coliform count

Correlation between mean coliform count and other bacterial counts of pasteurized milk stored at refrigeration temperature is given in table 24. Association between the mean coliform count and total viable count was positive and significant ($P<0.05$) on day zero of refrigerated storage of samples. A highly significant ($P<0.01$) association was observed between mean coliform count and Faecal Streptococcal count on day 10 of storage. A significant ($P<0.05$) and

positive association was also observed between mean coliform count and *Escherichia coli* count on day six of storage.

Table 24. Correlation coefficient between mean Coliform count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between CC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	0.759*	-0.333	-0.426	0.248	-0.026	-0.286
FSC	-0.291	-0.039	0.195	-0.114	-0.017	0.906**
PC	0.459	0.063	0.748	-0.458	-0.600	0.586
ECC	0.019	-0.112	0.216	0.391*	-0.027	-0.258

TVC: Total Viable Count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = P<0.05, ** = P<0.01.

***Escherichia coli* count**

Correlation between mean *Escherichia coli* count and other bacterial counts fresh and refrigerated samples is given in table 25. The mean *Escherichia*

Table 25. Correlation coefficient between mean *Escherichia coli* count and other bacterial counts of fresh and refrigerated samples

Bacterial counts	Correlation coefficient values between ECC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	0.216	-0.104	0.148	0.090	-0.523	0.519
CC	0.019	-0.112	0.216	0.391*	-0.027	-0.258
FSC	-0.118	0.248	-0.489	-0.624	0.146	0.175
PC	-0.408	0.420	0.230	-0.597	-0.302	0.000

TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = P<0.05

coli count and coliform count of the samples stored on day six had shown a positive and significant (P<0.05) relationship.

Psychrotrophic count

Correlation between psychrotrophic count and other bacterial counts of fresh and refrigerated samples are given in table 26. Relationship between total viable count and psychrotrophic counts of the samples on eighth and 10^{th} day of storage was positive and significant ($P<0.05$).

Table 26. Correlation coefficient between mean psychrotrophic count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between PC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	0.310	-0.003	0.054	-0.207	0.428*	0.427*
CC	0.459	0.063	0.748	-0.458	-0.600	0.586
FSC	-0.644	0.626	0.577	0.285	0.500	0.616
ECC	-0.408	0.420	0.230	-0.597	-0.302	0.000

TVC: Total Viable Count, CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, * = $p<0.05$

Faecal streptococcal count

Correlation between mean faecal streptococcal count and other microbial

Table 27. Correlation coefficient between mean faecal streptococcal count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Faecal streptococcal count					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.109	-0.502	0.281	0.116	0.495	-0.044
CC	-0.291	-0.039	0.195	-0.114	-0.017	0.906**
PC	-0.644	0.626	0.577	0.285	0.500	0.616
ECC	-0.118	0.248	-0.489	-0.624	0.100	0.175

TVC: Total viable count, CC: Coliform count, ECC: *Escherichia coli* count, PC Psychrotrophic count, ** = $P<0.01$.

counts of refrigerated milk is given in table 27. A highly significant ($P<0.01$) association was observed between mean faecal streptococcal count and coliform count on day 10 of storage.

4.1.5 Isolation and Identification of Bacteria

The bacteria isolated from fresh and refrigerated milk samples from DP_I are given in table 28. A total of 84 samples were tested for the isolation of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

Table 28. Bacteria isolated from fresh and refrigerated milk samples

Bacteria	Number of positive samples					
	Days of storage					
	0	2	4	6	8	10
Escherichia coli	1	1	1	1	ND	ND
Staphylococcus aureus	1	1	1	2	ND	ND
Pseudomonas	4	1	2	2	5	5

ND: Not detected

4.1.5.1 Escherichia coli

Fresh and refrigerated milk samples from DP_I were tested for the isolation and identification of *Escherichia coli*. The isolates were identified by cultural, morphological and bio-chemical characteristics. All isolates were Eijkman test positive. The isolates were serotyped at National *Salmonella* and *Escherichia* Centre and were also subjected to congored binding test and are shown in plate 1. Two of the isolates belonged to serotype O116 and these were positive for congored binding test indicating their property of invasiveness. The other two belonged to the rough variety.

Plate 1 *Escherichia coli* showing congo red binding peoperty

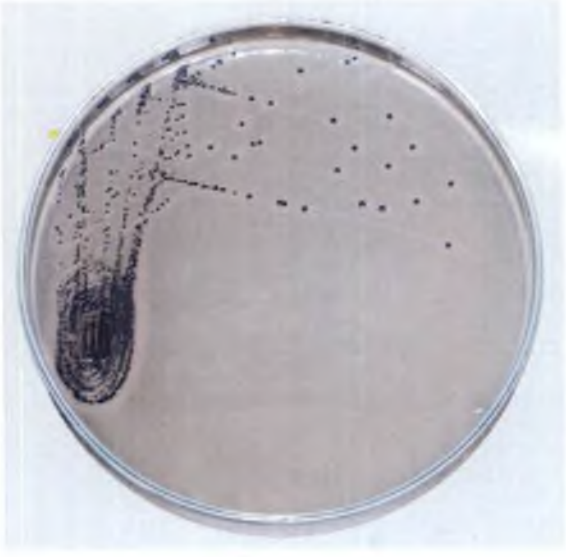

Plate 2 *Staphylococcus aureus* in Baird-Parker Agar

4.1.5.2 Staphylococcus aureus

All fresh and refrigerated samples were tested for the isolation of *Staphylococcus aureus* and isolates obtained were identified by cultural, morphological and biochemical characteristics. Of the five organism isolated, two of the isolates were from the samples stored on sixth day and the other three were isolated from the samples stored on zero, second and fourth day, respectively (plate 2).

4.1.5.3 Pseudomonas

All the fresh and refrigerated samples from DP_1 were tested for the isolation of *Pseudomonas* and the isolates obtained were identified by cultural, morphological and biochemical characteristics. From the 84 samples of DP_1, 19

Table 29. *Pseudomonas* isolates from fresh and refrigerated samples of DP_1

Organism isolated	Number of isolates
Pseudomonas aeruginosa	6 (31.58)
Pseudomonas flourescens	6 (31.58)
Pseudomonas putida	7 (36.84)
Total	19

Figures in parenthesis indicate per cent.

(22.62 per cent) isolates were obtained. The isolates were identified as *Pseudomonas putida* (7), *Pseudomonas flourescens* (6) and *Pseudomonas aeruginosa* (6).

4.1.6 ORGANOLEPTIC AND PHYSICAL QUALITY OF MILK

Organoleptic qualities are important parameters controlling the shelf life of the product.

4.1.6.1 Organoleptic Quality

The changes in the sensory quality of milk was evaluated by a panel of four judges who assessed the changes in colour, odour, flavor and body and the grades were assigned according to IS 7768 (1975).

4.1.6.2 Colour and Appearance

The mean score of colour and appearance of fresh and refrigerated milk samples are shown in table 30. The total score allotted was 10. The score of samples on day zero and two of storage differed highly significantly ($p<0.01$) from the score of the samples on fourth day and thereafter up to 10^{th} day of storage the difference was highly significant ($p<0.01$). The mean score showed a gradual

Table 30. Mean colour and appearance score of fresh and refrigerated milk samples

Days of storage	Colour and Appearance Score (Mean ± SE)
0	$9.67^{a} \pm 0.24$
2	$9.21^{a} \pm 0.10$
4	$8.50^{b} \pm 0.22$
6	$7.50^{c} \pm 0.15$
8	$6.93^{d} \pm 0.13$
10	$6.43^{d} \pm 0.07$

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

decrease throughout the period of storage. The mean score of freshly pasteurized milk decreased from 9.67 ± 0.24 to 6.43 ± 0.07 on the 10^{th} day of refrigerated storage. The mean score of samples on fourth day of storage also differed highly significantly ($P<0.01$) from the mean scores of samples on sixth, eighth and 10^{th}

day of storage. Similarly the mean score of samples on sixth day differed highly significantly (P<0.01) from the samples of eighth and 10th day of storage.

4.1.6.3 Odour

The mean score of odour in fresh and refrigerated milk samples is given in table 31. The total score allotted for odour was 20. Analysis of the data using Mann- Whitney test revealed that the score of fresh sample differed highly

Table 31. Mean Odour score of fresh and refrigerated milk samples

Days of storage	Odour Score (Mean ± SE)
0	$19.14^{a} \pm 0.26$
2	$18.14^{b} \pm 0.26$
4	$16.14^{c} \pm 0.22$
6	$13.71^{d} \pm 0.42$
8	$11.43^{e} \pm 0.48$
10	$9.86^{f} \pm 0.26$

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

significantly (P<0.01) with the mean scores of the samples stored on second, fourth, sixth, eighth and 10th day of storage. The mean score of samples stored on second day also differed highly significantly (P<0.01) from the scores of samples stored on day four, six, eight and 10. Similarly the mean score of samples on fourth day of storage also differed highly significantly (P<0.01) from the scores of samples stored on sixth, eighth and 10th days of storage. Highly significant (P<0.01) reduction in the score was also seen in the samples stored between six and eight, six and 10 and eight and 10 days.

4.1.6.4 Flavour

The mean flavour score of fresh and refrigerated samples is given in table 32. The total score for flavour allotted was 40. Analysis of the data using Mann-Whitney test revealed that the samples stored on zero day had highly significant ($P<0.01$) differences with the mean flavour score of second, fourth, sixth, eighth and 10^{th} day of storage. The mean score of samples stored on second day differed

Table 32. Mean Flavour score of fresh and refrigerated milk samples

Days of storage	Flavour Score (Mean ± SE)
0	$38.71^{a} \pm 0.28$
2	$37.28^{b} \pm 0.22$
4	$35.14^{c} \pm 0.26$
6	$32.00^{d} \pm 0.31$
8	$29.57^{e} \pm 0.37$
10	$25.00^{f} \pm 0.31$

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

highly significantly ($P<0.01$) from the scores of samples stored on fourth, sixth, eighth and 10^{th} day of storage. Similarly the mean scores of samples tested on fourth and sixth day was highly significantly ($P<0.01$) different from the scores of samples tested on the following days till the end of the storage period. The mean score of samples tested on eighth day of storage also differed highly significantly ($P<0.01$) from the mean score of samples stored on 10^{th} day.

4.1.6.5 Body

The mean body score of fresh and refrigerated milk samples is given in table 33. The total score allotted for body was 30. The mean body score of the sample decreased throughout the period of storage. Analysis of the data using Mann-Whitney test

revealed that the mean score of fresh sample differed highly significantly (P<0.01) from the mean score of samples stored on two, four, six, eight and 10. The mean score of samples stored on day two also differed highly significantly (P<0.01) from the mean

Table 33. Mean Body score of fresh and refrigerated milk samples

Days of storage	Body Score (Mean ± SE)
0	$28.43^{a} \pm 0.20$
2	$27.00^{b} \pm 0.31$
4	$25.00^{c} \pm 0.31$
6	$21.00^{d} \pm 0.31$
8	$18.29^{e} \pm 0.36$
10	$15.14^{f} \pm 0.26$

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

score of the samples stored on day four, six, eight and 10 of storage. Similar difference in scores was noticed in the samples stored on day four with samples stored on day six, eight and 10. Also the mean score of samples stored on eighth day differed highly significantly (P<0.01) from the score of samples stored on 10^{th} day of storage.

4.1.6.6 Total Score

The mean total scores obtained by the samples during the period of storage and qualities scored by the samples are shown in table 34. The mean total score of samples on zero day was 96.29 ± 0.36 and it reduced throughout the storage period and on 10^{th} day it was 56.43 ± 0.69. The mean total scores revealed that the samples had excellent quality for up to second day of storage. The sensory quality of the sample stored on fourth day was good and thereafter the quality of milk remained as fair till eighth day and on 10^{th} day the quality was poor.

Table 34. Total score of fresh and refrigerated milk samples

Days of storage	Total score Mean ± SE	Grade	Quality
0	96.29 ± 0.36	A	Excellent
2	91.64 ± 0.46	A	Excellent
4	84.79 ± 0.54	B	Good
6	74.21 ± 0.54	C	Fair
8	66.21 ± 0.23	C	Fair
10	56.43 ± 0.69	D	Poor

Figures bearing the same superscript in the column do not differ significantly, N=14 samples on each day of storage

4.1.7 Physical Quality

4.1.7.1 Clot on Boiling test

All samples were subjected to Clot on Boiling (COB) test during storage. The result of the test is given in table 35. All samples stored on 10^{th} day revealed a positive test. Six samples stored on day eight were found COB test positive and only one sample stored on day six was found to be COB test positive

Table 35. Result of COB test of milk samples stored under refrigeration

Batches	Clot On Boiling					
	Days of storage					
	0	2	4	6	8	10
I	-	-	-	+	+	+
II	-	-	-	-	+	+
III	-	-	-	-	+	+
IV	-	-	-	-	+	+
V	-	-	-	-	-	+
VI	-	-	-	-	+	+
VII	-	-	-	-	+	+

+ COB positive, -COB negative; N=14 samples on each day of storage.

4.1.8 Dairy Plant 2

A total of 84 pasteurized milk samples were collected from Dairy Plant 2 (DP_2) and estimated the bacterial counts of fresh samples (14) on day zero and samples under storage (70) on day 2, 4, 6, 8 and 10.

4.1.8.1 Total Viable Count

The mean total viable count (TVC) of fresh and refrigerated milk samples is given in table 36. Analysis of the data was done using the paired t test. The mean count of samples during storage period increased from 4.72 ± 0.09 to 9.50 ± 0.10 $\log_{10}$ cfu/ml, as illustrated in fig. 4. The mean count of fresh sample had highly significant ($P<0.01$) difference with the mean count of the samples stored on day two, four, six, eight and 10. The mean count of samples on second day of storage also had highly significant ($P<0.01$) difference with mean count of samples stored on day four, six, eight and 10. Similarly the mean count of samples on fourth day of storage differed highly significantly ($P<0.01$) from the mean count of samples stored on day six, eight and 10. Highly significant ($P<0.01$) difference was also noticed between samples stored on day six and eight, six and 10 and eight and 10.

Table 36. Mean total viable count of fresh and refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	$4.72^{a} \pm 0.09$
2	$5.44^{b} \pm 0.07$
4	$6.76^{c} \pm 0.08$
6	$7.81^{d} \pm 0.07$
8	$8.66^{e} \pm 0.08$
10	$9.50^{f} \pm 0.10$

Figures bearing the same superscript do not differ significantly N= 14 samples on each day of storage.

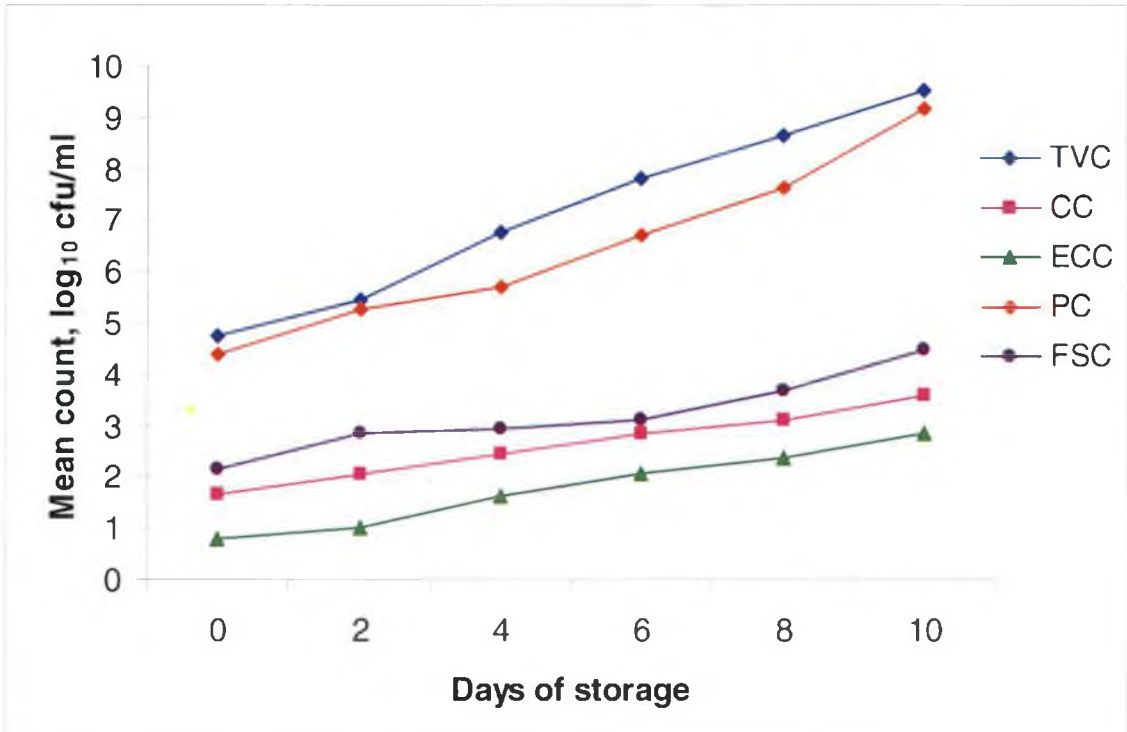

Fig. 4 Effect of refrigeration on bacterial quality of milk (DP_2)

Distribution of fresh and refrigerated samples based on level of total viable count is given in table 37. Cent per cent of the samples on day zero, eight and 10 had count at the level of 10^4, 10^8 and 10^9 cfu/ml, respectively. The count in 85.71 per cent of samples on day two, four and six had count at the level of 10^5, 10^6 and 10^7 cfu/ml, respectively.

Table 37. Distribution of fresh and refrigerated milk samples based on total viable count

Days of storage	Total viable count (cfu/ml)					
	10^4	10^5	10^6	10^7	10^8	10^9
0	14(100)					
2	2(14.29)	12(85.71)				
4			12(85.71)	2(14.29)		
6				12(85.71)	2(14.29)	
8					14(100)	
10						14(100)

Figures in parenthesis indicate percent; N=14 samples on each day of storage

4.1.8.2 Coliform Count

The mean coliform count (CC) of fresh and refrigerated milk samples is given in table 38. Analysis of the data using paired t test revealed a gradual increase in coliform count up to tenth day of storage. The mean coliform count of freshly pasteurized milk was 1.65 ± 0.21 $\log_{10}$ cfu/ml and at the end of storage period it reached a level of 3.60 ± 0.94 $\log_{10}$ cfu/ml, as illustrated in fig. 4. The mean counts of the samples stored on zero day was significantly ($P<0.05$) different from the count of the samples on day two, four, six, eight and 10 of storage. A significant ($P<0.05$) difference was also observed between the mean count of samples stored on day four and six, four and eight and four and 10. Significant ($P<0.05$) difference was also observed between the mean counts of samples stored on day six and eight and six and 10.

Table 38. Mean coliform count of fresh and refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	$1.65^{a} \pm 0.21$
2	$2.08^{b} \pm 0.17$
4	$2.44^{bc} \pm 0.56$
6	$2.85^{bd} \pm 0.75$
8	$3.13^{be} \pm 0.81$
10	$3.60^{bcf} \pm 0.94$

Figures bearing the same superscript do not differ significantly. N=14 samples on each day of storage

Distribution of fresh and refrigerated samples based on level of coliform count is given in table 39. On day zero of storage, the count at the level of 10^2 cfu/ml was observed in 28.57 per cent of samples and 71.43 per cent samples had count at the level of 10^1 cfu/ml. On day two of storage, 85.71 per cent of samples

Table 39. Distribution of fresh and refrigerated milk samples based on coliform count

Days of storage	Coliform count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
0		10(71.43)	4(28.57)		
2			12(85.71)	2(14.29)	
4	4(28.57)		2(14.29)	8(57.14)	
6	4(28.57)			4(28.57)	6(42.86)
8	4(28.57)			4(28.57)	6(42.86)
10	4(28.57)				10(71.43)

Figures in parenthesis indicate per cent; N=14 samples on each day of storage

had count at the level 10^2 cfu/ml and 14.29 per cent samples had count at the level of 10^3 cfu/ml. The count of 57.14 per cent of samples on day four of storage was also at the level of 10^3 cfu/ml. The count in 42.86 per cent of the samples was at the level of 10^4 cfu/ml on day six and eight of storage. On day 10 of storage 71.43 per cent of samples had count at the level of 10^4 cfu/ml. The organism was not detected in 28.57 per cent of samples on day four, six, eight and 10 of storage.

4.1.8.3 Escherichia coli Count

The mean *Escherichia coli* count (ECC) of refrigerated milk samples is given in table 40. The count showed an increasing trend as illustrated in fig. 4. The mean count of samples stored on day zero and two showed highly significant ($P<0.01$) difference with the mean count of samples stored on day eight and 10 and significant ($P<0.05$) difference with the mean count of samples stored on day four and six. A highly significant ($P<0.01$) difference was also observed between the mean count of samples stored on day four and eight, four and 10 and eight and 10. However, only significant ($P<0.05$) difference was observed between the mean count of samples stored on days four and six and six and 10.

Table 40. Mean *Escherichia coli* count of fresh and refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	$0.78^{a} \pm 0.28$
2	$1.01^{a} \pm 0.37$
4	$1.61^{b} \pm 0.46$
6	$2.06^{c} \pm 0.54$
8	$2.39^{cd} \pm 0.46$
10	$2.86^{e} \pm 0.52$

Figures bearing the same superscript do not differ significantly. N=14 samples on each day of storage

Distribution of fresh and refrigerated samples based on level of *Escherichia coli* count is given in table 41. The organism was not detected from 57.14 per cent of samples stored on day zero and two. The count in 42.86 per cent of the samples examined on day zero and two was at the level of 10^1 cfu/ml. Out of 14 samples stored on day four, eight (57.14 per cent) had counts at the level of 10^1 cfu/ml. The count at

Table 41. Distribution of fresh and refrigerated milk samples based on *Escherichia coli* count

Days of storage	*Escherichia coli* Count (cfu/ml)			
	ND	10	10^2	10^3
0	8 (57.14)	6 (42.86)		
2	8 (57.14)	6 (42.86)		
4	6 (42.86)	8 (57.14)		
6	4 (28.57)	4 (28.57)	6 (42.86)	
8	2 (14.29)	4 (28.57)	8 (57.14)	
10	2 (14.29)		10 (71.42)	2 (14.29)

Figures in parenthesis indicate percent; N=14 samples on each day of storage; ND-Not detected

the level of 10^2 cfu/ml was seen in 42.86 per cent of samples examined on day six, 57.14 per cent of samples examined on day eight and 71.42 per cent of samples examined on day 10 of storage. Two (14.29 per cent) out of 14 samples stored on day 10 had a count at the level of 10^3 cfu/ml.

4.1.8.4 Psychrotrophic Count

The mean psychrotrophic count of the fresh and refrigerated samples is given in table 42. An increase in mean psychrotrophic count was observed throughout the period of storage. The mean psychrotrophic count of samples on zero day of storage was 4.40 ± 0.08 and was found to increase to 9.18 ± 0.04 $\log_{10}$ cfu/ml on the 10^{th} day, as illustrated in fig. 4. Analysis of the data using paired t test showed highly significant ($P<0.01$) increase in the mean counts of the samples

stored on days zero, two, four, six, eight and 10 of storage. The mean count of samples stored on day two differed highly significantly ($P<0.01$) from the mean count of samples stored on day four, six, eight and 10. Similarly the mean count of samples stored on day four was highly significantly ($P<0.01$) different from the mean count of samples examined on day six, eight and 10. Highly significant

Table 42. Mean psychrotrophic count of fresh and refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	$4.40^{a} \pm 0.08$
2	$5.28^{b} \pm 0.07$
4	$5.72^{c} \pm 0.07$
6	$6.73^{d} \pm 0.08$
8	$7.63^{e} \pm 0.06$
10	$9.18^{f} \pm 0.04$

Figures bearing the same superscript in the same column do not differ significantly. N=14 samples on each day of storage

($P<0.01$) difference was also observed between the mean count of samples examined on day six and eight, six and 10 and eight and 10 of storage.

Distribution of fresh and refrigerated milk samples based on level of psychrotrophic count is given in table 43. Cent per cent of the samples on day zero, two and six of storage had mean count at the level of 10^4, 10^5 and 10^6cfu/ml, respectively. The count in 50 per cent of samples each on day four of storage was at the level of 10^6 and 10^5 cfu/ml, respectively. On day eight of storage, 85.71 per cent of samples had count at the level of 10^7 cfu/ml and 14.29 per cent of samples had count at the level of 10^8 cfu/ml. Out of 14 samples

Table 43. Distribution of fresh and refrigerated milk samples based on psychrotrophic count

Days of storage	Psychrotrophic count (cfu/ml)					
	10^4	10^5	10^6	10^7	10^8	10^9
0	14(100)					
2		14 (100)				
4		7 (50)	7 (50)			
6			14 (100)			
8				12 (85.71)	2 (14.29)	
10					4 (28.57)	10 (71.43)

Figures in parenthesis indicate percent; N=14 samples on each day of storage

examined at the end of storage period, 10 (71.43 per cent) samples had counts at the level of 10^9 cfu/ml and four (28.57) samples had counts at the level of 10^8 cfu/ml.

4.1.8.5 Faecal Streptococcal Count

The faecal streptococcal count of fresh and refrigerated milk samples is given in table 44. Analysis of the data using paired t test revealed that the mean count of fresh sample had a highly significant ($P<0.01$) difference with the mean count of the samples on the day two of storage and a significant ($P<0.05$) difference with the mean count of samples examined on day four, six, eight and 10. Highly significant ($P<0.01$) difference was also seen between the mean count of the sample stored on day four and 10 and eight and 10, as illustrated in fig. 4. However, the mean count of samples on days four and eight showed significant ($P<0.05$) difference and was also seen between the mean count of samples on day four and eight of storage.

Table 44. Mean faecal streptococcal count in fresh and refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	$2.15^{a} \pm 0.15$
2	$2.83^{b} \pm 0.04$
4	$2.96^{bc} \pm 0.52$
6	$3.10^{bcd} \pm 0.77$
8	$3.70^{bdc} \pm 0.62$
10	$4.47^{bdf} \pm 0.75$

Figures bearing the same superscript in the same column do not differ significantly. N=14 samples on each day of storage

Distribution of fresh and refrigerated samples based on level of faecal streptococcal count is given in table 45. Cent per cent of samples examined on day two and 71.43 per cent of the samples examined on day zero had counts at the level

Table 45. Distribution of fresh and refrigerated milk samples based on faecal streptococcal counts

Days of storage	Faecal streptococcal count (cfu/ml)				
	ND	10^{1}	10^{2}	10^{3}	10^{4}
0		4 (28.57)	10 (71.43)		
2			14 (100)		
4	2 (14.29)		10 (71.43)	2 (14.29)	
6	4 (28.57)			10 (71.43)	
8	2 (14.29)			12 (85.71)	
10	2 (14.29)				12 (85.71)

Figures in parenthesis indicate percent; N=14 samples on each day of storage; ND-Not detected

of 10^{2} cfu/ml and in 28.57 per cent samples examined on day zero had the counts at the level of 10^{1} cfu/ml. On day four, out of 14 samples examined, 10 (71.43 per cent) had count at the level of 10^{2} cfu/ml, while two (14.29 per cent) had counts at

the level of 10^3 cfu/ml. In 71.43 per cent of samples stored on sixth day and 85.71 per cent of samples stored on eighth day, the count was at the level of 10^3 cfu/ml. On tenth day of storage, the count in 85.71 per cent of samples was at the level of 10^4 cfu/ml. However, the organism was not detected in 14.29 per cent of samples examined on day four, eight and 10 and also in 28.57 per cent of samples examined on day six of storage.

4.1.8.6 Correlation between Bacterial Counts of Fresh and Refrigerated Milk

Total Viable Count

Correlation between mean total viable count and all other bacterial counts of milk samples during the storage at 4 ± 1°C are shown in table 46. A highly significant ($P<0.01$) and positive relation was found between the mean total viable count and psychrotrophic count of samples on 10^{th} day of storage. A significant ($P<0.05$) and positive association was observed between mean total viable count and coliform count on day two of refrigerated storage. A similar relationship was observed between the mean total viable count and faecal streptococcal count on 10^{th} day of storage.

Table 46. Correlation coefficient between mean total viable count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between TVC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
CC	0.208	0.809*	0.184	0.154	-0.102	-0.071
FSC	-0.507	0.088	0.653	-0.820*	0.549	0.868*
PC	-0.133	-0.088	0.385	0.154	-0.029	0.130**
ECC	-0.437	-0.541	-0.075	-0.754	-0.270	0.021

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, ** = P<0.01, * = P<0.05

Coliform count

Correlation between mean coliform count and other bacterial counts of fresh and refrigerated pasteurized milk are shown in table 47. A highly significant (P<0.01) and positive association was observed between mean coliform count and faecal streptococcal count on day four of storage. Association between the mean coliform count and total viable count was positive and significant (P<0.05) on day

Table 47. Correlation coefficient between mean coliform count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between CC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	0.208	0.809*	0.184	0.154	-0.102	-0.071
FSC	-0.260	-0.247	0.528**	-0.228	-0.199	0.135
PC	-0.532	0.304	-0.352	-0.591	0.486	0.137
ECC	0.198	-0.121	-0.565	0.082	0.039	0.850*

TVC: Total Viable Count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = P<0.05, ** = P<0.01.

two of refrigerated storage of samples. A significant (P<0.05) and positive association was also observed between mean coliform count and *Escherichia coli* count on day 10 of storage.

***Escherichia coli* count**

Correlation between mean *Escherichia coli* count and other bacterial counts of fresh and refrigerated samples is given in table 48. The mean *Escherichia coli* count and coliform count of the samples stored on day 10 had shown a positive and significant (P<0.05) relationship. A positive and significant (P<0.05) correlation was also observed between the mean *Escherichia coli* count and psychrotrophic count of samples on day 10 of storage.

Table 48. Correlation coefficient between mean *Escherichia coli* count and other bacterial counts of fresh and refrigerated samples

Bacterial counts	Correlation coefficient values between ECC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.437	-0.541	-0.075	-0.754	-0.270	0.021
CC	0.198	-0.121	-0.565	0.082	0.039	0.850*
FSC	-0.137	-0.207	0.075	0.728	-0.077	0.197
PC	-0.321	0.195	0.312	-0.222	-0.440	0.624*

TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, * = P<0.05

Psychrotrophic count

Correlation between mean psychrotrophic count and other bacterial counts of fresh and refrigerated samples are given in table 49. Relationship between mean total viable count and psychrotrophic counts of the samples on 10^{th}

Table 49. Correlation coefficient between mean psychrotrophic count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between PC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.133	0.088	0.385	0.154	-0.029	0.130**
CC	-0.532	0.304	-0.352	-0.591	0.486	0.137
FSC	0.724	-0.388	0.123	0.037	-0.228	0.154
ECC	-0.321	0.195	0.312	-0.222	-0.440	0.624*

TVC: Total Viable Count, CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, **= P<0.01, *=P<0.05

day of storage was positive and highly significant (P<0.01). Similarly a positive and significant (P<0.05) correlation was observed between the mean

psychrotrophic count and *Escherichia coli* count of samples on 10th day of storage.

Faecal Streptococcal Count

Correlation between mean faecal streptococcal count and other microbial counts of refrigerated milk is given in table 50. A highly significant (P<0.01) and

Table 50. Correlation coefficient between mean faecal streptococcal count and other bacterial counts of fresh and refrigerated milk samples

Bacterial counts	Correlation coefficient values between FSC and other bacterial counts					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.507	0.088	0.653	-0.820*	0.549	0.868*
CC	-0.260	-0.247	0.528**	-0.228	-0.199	0.135
PC	0.724	-0.388	0.123	0.037	-0.228	0.154
ECC	-0.137	-0.207	0.075	0.728	-0.077	0.197

TVC: Total viable count, CC: Coliform count, ECC: *Escherichia coli* count, PC: Psychrotrophic count, ** = P<0.01, *=P<0.05

positive relation was observed between mean faecal streptococcal count and coliform count in the samples of day four of storage. A significant (P<0.05) association was also observed between mean faecal streptococcal count and total viable count on day six and 10 of storage.

4.1.9 Isolation and Identification of Bacteria

The bacteria isolated from fresh and refrigerated milk samples from DP_2 are given in table 51. A total of 84 samples were tested for the isolation of bacteria *viz. Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

Table 51. Bacteria isolated from fresh and refrigerated milk samples

Bacteria	Number of positive samples					
	Days of storage					
	0	2	4	6	8	10
Escherichia coli	1	ND	ND	2	2	1
Staphylococcus aureus	1	ND	ND	ND	ND	3
Pseudomonas	3	1	2	2	4	5

ND: Not detected

4.1.9.1 Escherichia coli

Fresh and refrigerated milk samples from DP_2 were tested for the isolation and identification of *Escherichia coli*. The isolates were identified by cultural, morphological and bio-chemical characteristics. Four out of six isolates obtained, were Eijkman test positive. The isolates were serotyped at National *Salmonella* and *Escherichia* Centre and were also subjected to congored binding test. The serotypes obtained were O22, O46, O95, O116 and O65 (2). Three of the isolates were positive for congored binding test indicating their property of invasiveness.

4.1.9.2 Staphylococcus aureus

All fresh and refrigerated samples were tested for the isolation of *Staphylococcus aureus* and isolates obtained were identified by cultural, morphological and biochemical characteristics. Of the four organism isolated, three of the isolates were from the samples stored on 10^{th} day and the other one was isolated from the samples stored on zero day.

4.1.9.3 Pseudomonas

All fresh and refrigerated samples from DP_2 were tested for the isolation of *Pseudomonas* and the isolates obtained were identified by cultural, morphological and biochemical characteristics. From the 84 samples of DP_2, 17

Table 52. *Pseudomonas* isolates from fresh and refrigerated samples of DP_2

Organism isolated	Number of isolates
Pseudomonas aeruginosa	7 (41.18)
Pseudomonas flourescens	2 (11.76)
Pseudomonas putida	8 (47.06)
Total	17

Figures in parenthesis indicate percent.

(20.24 per cent) samples yielded positive colonies. The isolates were identified as *Pseudomonas putida (8), Pseudomonas aeruginosa (7) and Pseudomonas flourescens* (2) (table52).

4.1.10. ORGANOLEPTIC AND PHYSICAL QUALITY OF MILK

4.1.10.1 Organoleptic Quality

The changes in the sensory quality of milk was evaluated by a panel of four judges who assessed the changes in colour, odour, flavor and body and the grades were assigned according to IS 7768 (1975).

4.1.10.2 Colour and Appearance

The mean score of colour and appearance of fresh and refrigerated milk samples is given in table 53. The total score allotted was 10. The mean score showed a gradual decrease throughout the period of storage. The mean score of freshly pasteurized milk decreased from 9.75 ± 0.26 to 7.64 ± 0.14 on the 10^{th} day

of refrigerated storage. The mean score of samples on day zero and two of storage differed highly significantly (P<0.01) from the score of the samples on day four, six, eight and 10. The mean score of samples on fourth day of storage also differed highly significantly (P<0.01) from the mean scores of samples on eighth and 10th day of storage. Similarly the mean score of samples on sixth day differed highly significantly (P<0.01) from the samples of eighth and 10th day of storage. There was also highly significant (P<0.01) difference between the mean scores of samples on day eight and 10 of storage.

Table 53. Mean colour and appearance score of fresh and refrigerated milk samples

Days of storage	Colour and Appearance Score (Mean ± SE)
0	$9.75^{a} \pm 0.26$
2	$9.36^{a} \pm 0.14$
4	$8.93^{b} \pm 0.13$
6	$8.43^{b} \pm 0.13$
8	$8.07^{c} \pm 0.16$
10	$7.64^{d} \pm 0.14$

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

4.1.10.3 Odour

The mean score of odour of fresh and refrigerated milk samples is given in table 54. The total score allotted for odour was 20. Analysis of the data using Mann-Whitney test revealed that the score of fresh sample differed highly significantly (P<0.01) with the mean scores of sample stored on second, fourth, sixth, eighth and 10th day of storage. The mean score of samples stored on second day also differed highly significantly (P<0.01) from the scores of samples stored on day four, six, eight and 10. Similarly the mean score of samples on fourth day of storage differed highly significantly (P<0.01) from the scores of samples

tested on sixth, eighth and 10th day of storage. Highly significant (P<0.01) reduction in the score was also seen between the samples examined on day six and eight, six and ten and eight and 10 of storage.

Table 54. Mean Odour Score of Fresh and Refrigerated Milk Samples

Days of storage	Odour Score (Mean ± SE)
0	$19.71^{a} \pm 0.18$
2	$18.71^{b} \pm 0.36$
4	$17.43^{c} \pm 0.37$
6	$15.86^{d} \pm 0.40$
8	$14.14^{e} \pm 0.63$
10	$11.86^{f} \pm 0.63$

Figures bearing the same superscript in the same column do not differ significantly N=14 samples on each day of storage

4.1.10.4 Flavour

The mean flavor score of fresh and refrigerated samples is given in table 55. The total score allotted for flavour was 40. Analysis of the data was done using Mann-Whitney test and it was observed that the samples stored on zero and second day had highly significant (P<0.01) differences with the mean flavour score of samples examined on fourth, sixth, eighth and 10th days of storage. Similarly the mean scores of samples tested on fourth and sixth day differed highly significantly (P<0.01) from the scores of samples tested on the following days till the end of the storage period. The mean score of samples tested on eighth day of storage also differed highly significantly (P<0.01) from the mean score of samples stored on 10th day.

Table 55. Mean Flavour score of fresh and refrigerated milk samples

Days of storage	Flavour Score (Mean ± SE)
0	$38.71^{a} \pm 0.28$
2	$38.14^{a} \pm 0.34$
4	$36.14^{b} \pm 0.35$
6	$33.57^{c} \pm 0.20$
8	$31.43^{d} \pm 0.28$
10	$29.43^{e} \pm 0.37$

Figures bearing the same superscript in the same column do not differ significantly
N=14 samples on each day of storage

4.1.10.5 Body

The mean body score of fresh and refrigerated milk samples is given in table 56. The total score allotted for body was 30. The mean body score of the sample decreased throughout the period of storage. Analysis of the data using Mann-Whitney test revealed that the mean score of fresh samples differed highly

Table 56. Mean Body score of fresh and refrigerated milk samples

Days of storage	Body Score (Mean ± SE)
0	$28.14^{a} \pm 0.14$
2	$27.00^{b} \pm 0.31$
4	$25.71^{c} \pm 0.29$
6	$23.57^{d} \pm 0.57$
8	$21.00^{e} \pm 0.54$
10	$18.71^{f} \pm 0.61$

Figures bearing the same superscript in the same column do not differ significantly
N=14 samples on each day of storage

significantly (P<0.01)from the mean score of samples stored on two, four, six eight and 10. The mean score of samples stored on day two also differed highly significantly (P<0.01) from the mean score of the samples stored on day four, six, eight and 10 of storage. Similar difference in scores was noticed in the samples stored on day four with samples stored on day six, eight and 10. Also the mean score of samples stored on eighth day differed highly significantly (P<0.01) from the score of samples examined on 10^{th} day of storage.

4.1.10.6 Total Score

The mean total scores obtained by the samples during the period of storage and qualities scored by the samples are shown in table 57. The mean total

Table 57. Total score of fresh and refrigerated milk samples

Days of storage	Total score Mean ± SE	Grade	Quality
0	96.57 ± 0.30	A	Excellent
2	93.21 ± 0.50	A	Excellent
4	88.21 ± 0.57	B	Good
6	81.43 ± 0.89	B	Good
8	74.64 ± 0.27	C	Fair
10	67.64 ± 0.51	C	Fair

Figures bearing the same superscript in the column do not differ significantly
N=14 samples on each day of storage

score of samples on zero day was 96.57 ± 0.30 and it reduced throughout the storage period and on tenth day it was 67.64 ± 0.51. The mean total scores revealed that the samples had excellent quality for up to second day of storage. The sensory quality of the sample stored up to sixth day was good and thereafter the quality of milk remained as fair till the end of storage period.

4.1.11 Physical Quality

4.1.11.1 Clot on Boiling test

All samples were subjected to Clot on Boiling (COB) test during storage. And the result of the test is given in table 58. Three samples stored on 10^{th} day and one sample stored on eighth day revealed a positive test.

Table 58. Result of COB test of milk samples stored under refrigeration

Batches	Clot On Boiling				
	Days of storage				
	2	4	6	8	10
I	-	-	-	+	+
II	-	-	-	-	+
III	-	-	-	-	-
IV	-	-	-	-	-
V	-	-	-	-	-
VI	-	-	-	-	+
VII	-	-	-	-	-

+: COB positive, -: COB negative; N=14 samples on each day of storage.

4.1.12. Comparison of Quality of Milk Samples from the Two Dairy Plants

Bacterial quality

4.1.12.1 Total Viable Count

The mean total viable count (TVC) of samples from the two dairy plants *viz.* DP_1 and DP_2 was analysed using simple t test and the results are depicted in table 59. Analysis of the data revealed a highly significant ($P<0.01$) difference between the mean counts of the samples from the two sources on day zero, two, four, eight and 10. However, only a significant ($P<0.05$) difference was observed between the mean counts on day six of storage.

Table 59. Mean total viable count of fresh and refrigerated milk samples

Days of storage	Mean Count ± SE (log_{10} cfu/ml)	
	DP_1	DP_2
0	$5.04^{a} \pm 0.03$	$4.72^{b} \pm 0.09$
2	$5.69^{a} \pm 0.04$	$5.44^{b} \pm 0.07$
4	$7.13^{a} \pm 0.04$	$6.76^{b} \pm 0.08$
6	$7.62^{c} \pm 0.10$	$7.81^{d} \pm 0.07$
8	$8.15^{a} \pm 0.04$	$8.66^{b} \pm 0.08$
10	$8.27^{a} \pm 0.11$	$9.50^{b} \pm 0.10$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.12.2 Coliform Count

The mean coliform count (CC) of samples from the two sources was compared using simple t test and is shown in table 60. The data showed that the

Table 60. Mean coliform count of fresh and refrigerated milk samples

Days of storage	Mean Count ± SE (log_{10} cfu/ml)	
	DP_1	DP_2
0	$2.53^{a} \pm 0.10$	$1.65^{b} \pm 0.21$
2	$2.73^{a} \pm 0.08$	$2.08^{b} \pm 0.17$
4	$3.60^{a} \pm 0.12$	$2.44^{b} \pm 0.56$
6	$4.44^{c} \pm 0.17$	$2.85^{d} \pm 0.75$
8	$2.88^{a} \pm 0.56$	$3.13^{a} \pm 0.81$
10	$2.83^{c} \pm 0.86$	$3.60^{d} \pm 0.94$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

mean count of samples from DP_1 and DP_2 varied highly significantly ($P<0.01$) on day six and 10 whereas the counts differed significantly ($P<0.05$) from each other on day zero, two and four.

4.1.12.3 Escherichia coli Count

The mean *Escherichia coli* count (ECC) of samples from the two sources was analysed using simple t test (table 61) and the result revealed that the mean count of samples from the two plant did not differ significantly during the storage period except on day six, eight and 10 where the counts differed highly significantly ($P<0.01$).

Table 61. Mean *Escherichia coli* count of fresh and refrigerated milk samples

Days of storage	Mean Count ± SE (log_{10} cfu/ml)	
	DP_1	DP_2
0	$0.37^a \pm 0.65$	$0.78^a \pm 0.28$
2	$1.07^a \pm 0.51$	$1.01^a \pm 0.37$
4	$1.18^a \pm 0.57$	$1.61^a \pm 0.46$
6	$1.32^a \pm 0.66$	$2.06^b \pm 0.54$
8	$1.25^a \pm 0.74$	$2.39^b \pm 0.46$
10	$1.21^a \pm 0.78$	$2.86^b \pm 0.52$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.12.4 Psychrotrophic Count

The mean psychrotrophic count (PC) of the samples from the two sources

Table 62. Mean psychrotrophic count of fresh and refrigerated milk samples

Days of storage	Mean Count ± SE (log_{10} cfu/ml)	
	DP_1	DP_2
0	$4.45^a \pm 0.06$	$4.40^a \pm 0.08$
2	$5.49^a \pm 0.09$	$5.28^a \pm 0.07$
4	$6.80^a \pm 0.06$	$5.72^b \pm 0.07$
6	$7.40^a \pm 0.07$	$6.73^b \pm 0.08$
8	$7.79^a \pm 0.06$	$7.63^a \pm 0.06$
10	$8.17^a \pm 0.04$	$9.18^b \pm 0.04$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

(DP_1 and DP_2) was compared using simple t test and is shown in table 62. The analysis of the data revealed that the mean count of samples differed highly significantly ($P<0.01$) on day four, six and 10.

4.1.12.5 Faecal Streptococcal Count

The mean faecal streptococcal count (FSC) of the samples was compared using simple t test (table 63) and the result showed that mean counts of samples from the two sources varied highly significantly ($P<0.01$) on day six, eight and 10 of storage.

Table 63. Mean faecal streptococcal count of fresh and refrigerated milk samples

Days of storage	Mean Count ± SE (log_{10} cfu/ml)	
	DP_1	DP_2
0	$2.21^a \pm 0.17$	$2.15^a \pm 0.15$
2	$2.85^a \pm 0.05$	$2.83^a \pm 0.04$
4	$3.32^a \pm 0.07$	$2.96^a \pm 0.52$
6	$4.01^a \pm 0.05$	$3.10^b \pm 0.77$
8	$3.02^a \pm 0.50$	$3.70^b \pm 0.62$
10	$1.48^a \pm 0.70$	$4.47^b \pm 0.75$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

Organoleptic quality

4.1.12.6 Colour and Appearance

The mean score of colour and appearance of fresh and refrigerated milk samples from the two sources were compared using Mann-Whitney test and the results are shown in table 64. The analysis of the data showed that the mean score of samples from the two sources stored on day six, eight and 10 differed highly significantly ($P<0.01$).

Table 64. Mean colour and appearance score of fresh and refrigerated milk samples

Days of storage	Colour and Appearance Score (Mean ± SE)	
	DP_1	DP_2
0	$9.67^a \pm 0.24$	$9.75^a \pm 0.26$
2	$9.21^a \pm 0.10$	$9.36^a \pm 0.14$
4	$8.50^a \pm 0.22$	$8.93^a \pm 0.13$
6	$7.50^a \pm 0.15$	$8.43^b \pm 0.13$
8	$6.93^a \pm 0.13$	$8.07^b \pm 0.16$
10	$6.43^a \pm 0.07$	$7.64^b \pm 0.14$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.12.7 Odour

The mean score of odour in fresh and refrigerated milk samples collected from the two sources were compared using Mann-Whitney test and the result is shown in table 65. The mean score of odour in the samples from two sources differed highly significantly ($P<0.01$) on day four, six, eight and 10 of storage.

Table 65. Mean odour score of fresh and refrigerated milk samples

Days of storage	Odour Score (Mean ± SE)	
	DP_1	DP_2
0	$19.14^a \pm 0.26$	$19.71^a \pm 0.18$
2	$18.14^a \pm 0.26$	$18.71^a \pm 0.36$
4	$16.14^a \pm 0.22$	$17.43^b \pm 0.37$
6	$13.71^a \pm 0.42$	$15.86^b \pm 0.40$
8	$11.43^a \pm 0.48$	$14.14^b \pm 0.63$
10	$9.86^a \pm 0.26$	$11.86^b \pm 0.63$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.12.8 Flavour

The mean flavour score of fresh and refrigerated samples of milk from the two sources were compared using Mann-Whitney test (table 66). The mean score of samples from DP_1 and DP_2 differed highly significantly ($P<0.01$) on day two, four, six, eight and 10 of storage.

Table 66. Mean flavour score of fresh and refrigerated milk samples

Days of storage	Flavour Score (Mean ± SE)	
	DP_1	DP_2
0	$38.71^a \pm 0.28$	$38.71^a \pm 0.28$
2	$37.28^a \pm 0.22$	$38.14^b \pm 0.34$
4	$35.14^a \pm 0.26$	$36.14^b \pm 0.35$
6	$32.00^a \pm 0.31$	$33.57^b \pm 0.20$
8	$29.57^a \pm 0.37$	$31.43^b \pm 0.28$
10	$25.00^a \pm 0.31$	$29.43^b \pm 0.37$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.12.9 Body

Table 67. Mean body score of fresh and refrigerated milk samples

Days of storage	Body Score (Mean ± SE)	
	DP_1	DP_2
0	$28.43^a \pm 0.20$	$28.14^a \pm 0.14$
2	$27.00^a \pm 0.31$	$27.00^a \pm 0.31$
4	$25.00^a \pm 0.31$	$25.71^a \pm 0.29$
6	$21.00^a \pm 0.31$	$23.57^b \pm 0.57$
8	$18.29^a \pm 0.36$	$21.00^b \pm 0.54$
10	$15.14^a \pm 0.26$	$18.71^b \pm 0.61$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

The mean body score of fresh and refrigerated milk samples from the two sources were analysed using Mann-Whitney test (table 67) and the results revealed that the mean score of samples from DP_1 and DP_2 differed highly significantly ($P<0.01$) on day six, eight and 10 of storage.

4.1.12.10 Total Score

The mean total scores obtained by the samples from the two sources during the period of storage were compared using Mann-Whitney test (table 68). The results showed that the mean total score of the samples from the sources had a highly significant ($P<0.01$) difference on day two, four, six, eight and 10 of storage.

Table 68. Mean total score of fresh and refrigerated milk samples

Days of storage	Total Score (Mean ± SE)	
	DP_1	DP_2
0	$96.29^a \pm 0.36$	$96.57^a \pm 0.30$
2	$91.64^a \pm 0.46$	$93.21^b \pm 0.50$
4	$84.79^a \pm 0.54$	$88.21^b \pm 0.57$
6	$74.21^a \pm 0.54$	$81.43^b \pm 0.89$
8	$66.21^a \pm 0.23$	$74.64^b \pm 0.27$
10	$56.43^a \pm 0.69$	$67.64^b \pm 0.51$

Figures bearing the same superscript in the same row of different columns do not differ significantly. N=14 samples on each day of storage

4.1.13 Grading of Milk Samples Based on Total Viable Count

Raw milk

Pooled raw milk samples collected from the two processing plants were graded as very good, good, fair and poor. The distribution of the samples of different grades is given in table 69. None of the pooled samples was graded as very good. Of the samples from DP_2, 14.28 per cent were graded as good, whereas

Table 69. Distribution of pooled raw milk samples from two dairy plants belonging to different grades

Sources	Number of samples			
	Very good	Good	Fair	Poor
DP_1	0	0	4 (57.14)	3 (42.86)
DP_2	0	1 (14.28)	3 (42.86)	3 (42.86)
Overall	0	1 (7.14)	7 (50.00)	6 (42.86)

N=7 from each source; figures in parenthesis indicate per cent

no sample from the other plant belonged to that grade. Samples belonging to fair grade accounted for 57.14 and 42.86 per cent of the samples from DP_1 and DP_2, respectively. In the samples of DP_1 and DP_2, 42.86 per cent were graded as poor (fig. 5).

Pasteurized milk

Freshly pasteurized milk samples collected from the two processing plants were graded as satisfactory according to the total viable count limits prescribed by BIS (1992). None of the samples from DP_1 were graded as satisfactory but 42.86 per cent samples from DP_2 were graded as satisfactory (table 70 and fig.6).

Table 70. Distribution of fresh pasteurized milk samples from two dairy plants

Sources	Number of samples belonging to satisfactory quality
DP_1	0
DP_2	6 (42.86)
Overall	6 (21.43)

N=14 from each source; figures in parenthesis indicate per cent

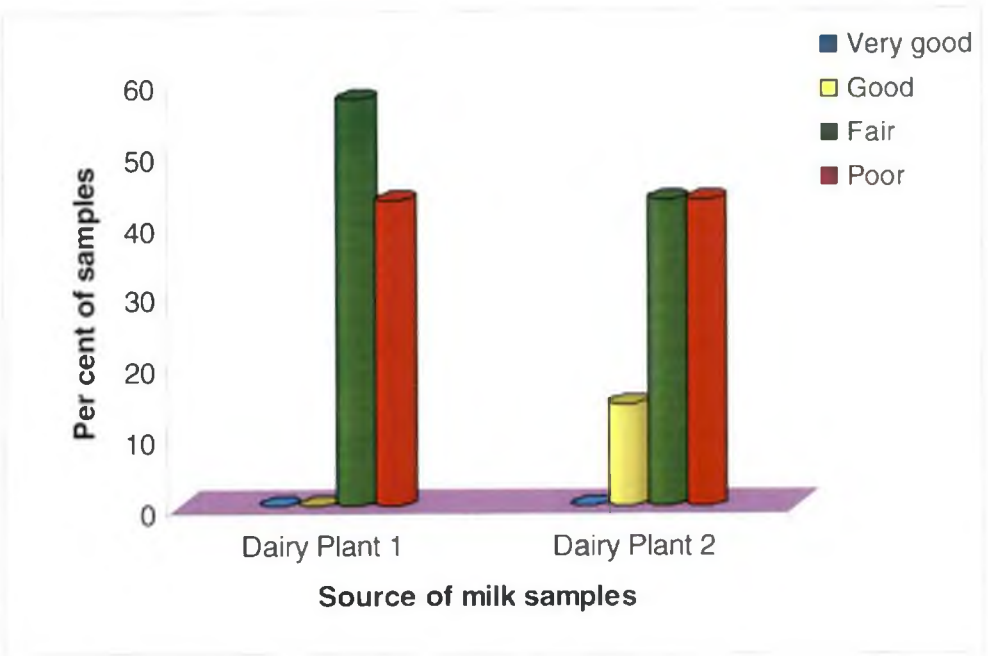

Fig. 5 Frequency distribution of pooled raw milk samples of DP_1 and DP_2 based on total viable count

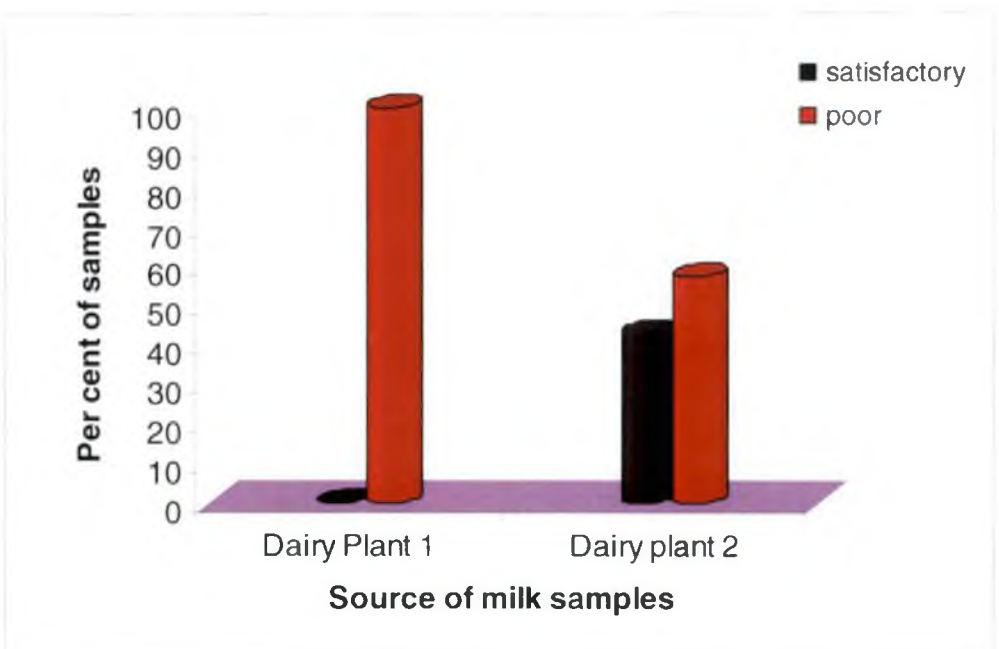

Fig. 6 Frequency distribution of pasteurized milk samples of DP_1 and DP_2 based on total viable count

4.1.14 Grading of Milk Samples Based on Coliform Count

Raw milk

Pooled raw milk samples collected from the two processing plants were graded based on coliform count. The distribution of the samples of different grades is given in table 71. Of the samples from DP_2, 28.57 per cent was graded as good. Cent per cent of the samples from DP_1 was graded as poor whereas 71.43 per cent of samples belonging to DP_2 were graded as poor (fig. 7).

Table 71. Distribution of raw pooled milk samples from two dairy plants

Sources	Number of samples	
	Very Good/Good/Fair	Poor
DP_1	0	7 (100)
DP_2	2 (28.57)	5 (71.43)
Overall	2 (14.29)	12 (85.71)

N=7 from each source; figures in parenthesis indicate per cent

Pasteurized milk

Fresh pasteurized milk samples collected from the two processing plants were graded based on coliform count. The distribution of the samples of different grades is given in table 72. Out of 14 samples from DP_2, four (28.57 per cent) samples were graded as satisfactory and cent per cent of samples from DP_1 were graded as poor (fig. 8).

Table 72. Distribution of fresh pasteurized milk samples from two dairy plants

Sources	Number of samples	
	Satisfactory	Poor
DP_1	0	14 (100)
DP_2	4 (28.57)	10 (71.43)
Overall	4 (14.29)	24 (85.71)

N=14 from each source; figures in parenthesis indicate per cent

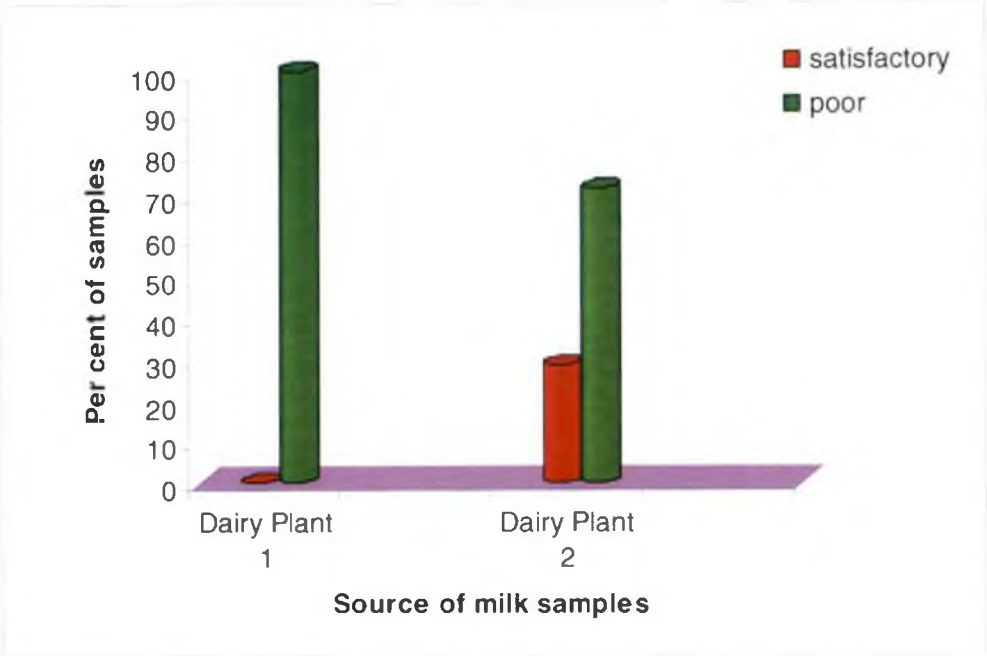

Fig. 7 Frequency distribution of pooled raw milk samples of DP_1 and DP_2 based on coliform count

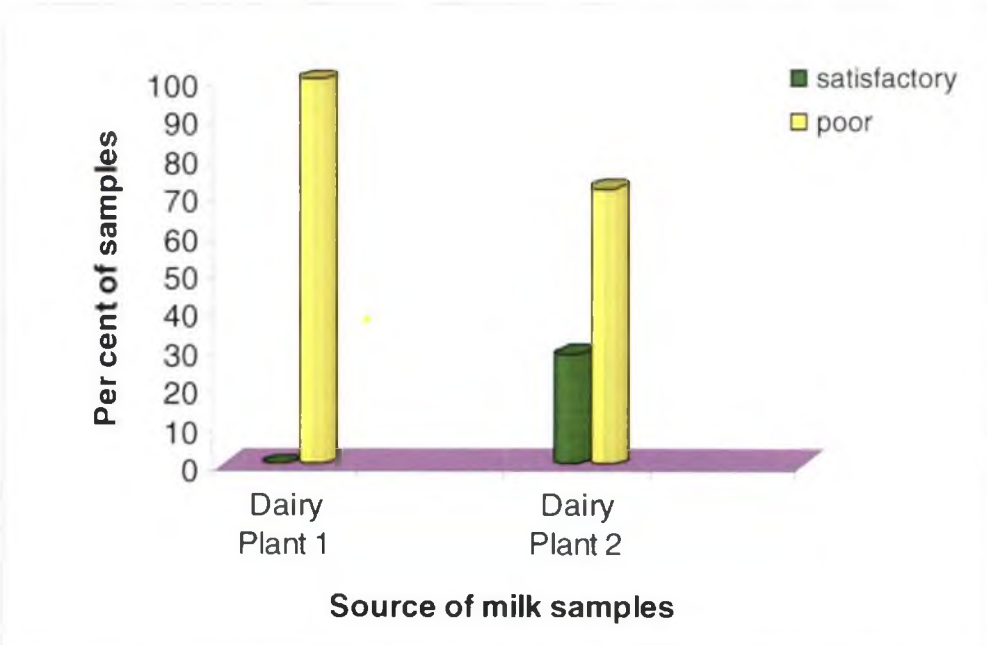

Fig. 8 Frequency distribution of pasteurized milk samples of DP_1 and DP_2 based on coliform count

4.1.15 Bacterial Counts of Retail Milk Samples

Bacterial quality of a total of 72 retail milk samples obtained from six different brands (A, B, C, D, E and F) available in and around Thrissur was evaluated by estimating various parameters like total viable count, coliform count, *Escherichia coli* count, psychrotrophic count and faecal streptococcal count.

4.1.15.1. Total Viable Count

The mean total viable count (TVC) of milk samples belonging to the brand A, B, C, D, E and F are shown in table 73 and illustrated in fig. 9. Analysis of variance test of the data revealed that the mean total viable count of brand F differed highly significantly ($P<0.01$) from the mean counts of the samples of the

Table 73. Mean total viable count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	$5.88^{a} \pm 0.18$
B	$5.76^{a} \pm 0.16$
C	$5.92^{a} \pm 0.02$
D	$5.94^{a} \pm 0.09$
E	$5.62^{a} \pm 0.12$
F	$4.89^{b} \pm 0.79$

Critical Difference 0.4357; Figures bearing the same superscript do not differ significantly; N=12 from each retail brand

other five brands. The highest mean count (5.94 ± 0.09) was seen in the samples belonging to brand D and the lowest (4.89 ± 0.79) from samples belonging to brand F.

Distribution of milk based on total viable count

The distribution of retail milk based on total viable count is shown in table 74. Of the 72 retail samples, highest count was seen in the samples of the brand D of which 91.67 per cent samples had count at level of 10^5 cfu/ml. Lowest count was seen in the samples of the brand F, of which 83.33 per cent of the samples had count at the level of 10^4 cfu/ml. The counts in 83.33 per cent of the samples from brand C, 50 per cent samples each of brand B and E and 66.67 per cent of samples from brand A were at the level of 10^5 cfu/ml.

Out of 72 samples, 22 (30.56 per cent) samples were graded as satisfactory according to the total viable count limits prescribed by BIS (1992). All the samples of the brands C and D were graded as poor.

Table 74. Distribution of retail milk samples based on total viable count

Brands	Total viable count (cfu/ml)	
	10^4	10^5
A	4 (33.33)	8 (66.67)
B	6 (50.00)	6 (50.00)
C	2 (16.67)	10 (83.33)
D	1 (8.33)	11 (91.67)
E	6 (50.00)	6 (50.00)
F	10 (83.33)	2 (16.67)

Figures in the parenthesis indicate per cent; N=12 from each retail brand

4.1.15.2 Coliform Count

The mean coliform counts of milk samples belonging to the brands A, B, C, D, E and F are shown in table 75 and illustrated in fig. 9. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of samples from brand F with the count of samples from other brands.

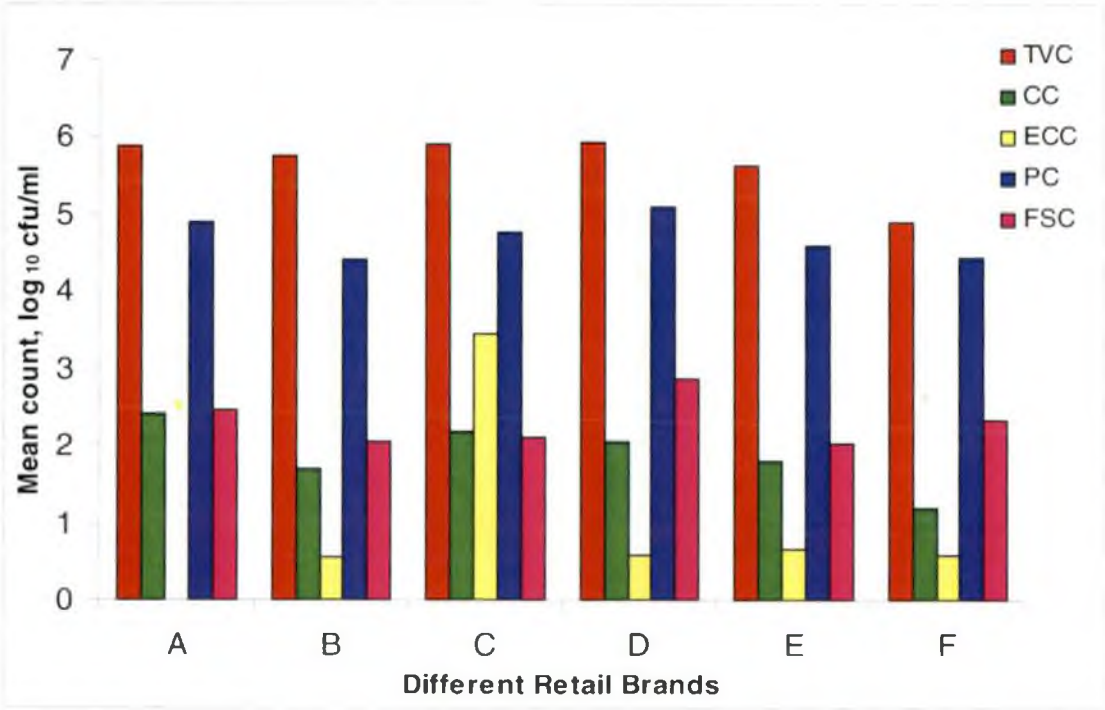

Fig. 9 Comparison of bacterial quality of retail milk brands

Table 75. Mean coliform count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	$2.40^{a} \pm 0.14$
B	$1.70^{a} \pm 0.54$
C	$2.17^{a} \pm 0.45$
D	$2.05^{a} \pm 0.09$
E	$1.81^{a} \pm 0.41$
F	$1.19^{b} \pm 0.42$

Critical Difference 0.5687; Figures bearing the same superscript do not differ significantly; N=12 from each retail brand

The distribution of retailed milk samples based on coliform counts is shown in table 76. Of the 72 samples, highest count at the level of 10^4 cfu/ml was seen in one (8.33 per cent) of the samples of the brand A. Lowest mean count was seen in the samples of brand F of which the organism was not detected in 33.33 per cent of the samples. The count at level of 10^2 cfu/ml was seen in 75 per cent of the sample

Table 76. Distribution of retail milk samples based on coliform count

Brands	coliform counts (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
A		2 (16.67)	9 (75.00)		1 (8.33)
B	3 (25.00)		9 (75.00)		
C	2 (16.67)	2 (16.67)	8 (66.66)		
D		6 (50.00)	6 (50.00)		
E	2 (16.67)	2 (16.67)	8 (66.66)		
F	4 (33.33)	4 (33.33)	4 (33.33)		

Figures in the parenthesis indicate percent; N=12 from each retail brand

of brands A and B whereas, 66.66 per cent of samples of brands C and E had count at the above level. In 50 per cent of samples of brand D and 33.33 per cent of

samples of brand F had count at the level of 10^2 cfu/ml. The count was not detected in 16.67 per cent of samples of brands C and E, and 25 per cent of samples of brand B also.

Out of 72 samples, 11 (15.2 per cent) were graded as satisfactory based on the BIS standards (1992) and all the samples of the brands A and D were graded as poor.

4.1.15.3 *Escherichia coli* Count

The mean *Escherichia coli* count (ECC) of milk samples belonging to the brands A, B, C, D, E and F are shown in table 77 and illustrated in fig. 9. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of samples of the brands. The lowest mean count was seen in samples belonging to brand A and the highest count in the samples of the brand C.

Table 77. Mean *Escherichia coli* Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	$0.00^b \pm 0.58$
B	$0.60^b \pm 0.60$
C	$3.44^a \pm 0.72$
D	$0.59^b \pm 0.59$
E	$0.65^b \pm 0.65$
F	$0.59^b \pm 0.59$

Critical Difference: 0.674; Figures bearing the same superscript do not differ significantly: N=12 from each retail brand

The distribution of retail milk based on *Escherichia coli* count is shown in table 78. Of the 72 samples, the highest count was seen in the samples belonging to the brand C of which 4 (33.33 per cent) samples had a count at the level of 10^4 cfu/ml. The organism was not detected in 83.33 per cent of samples of brands B,

Table 78. Distribution of retail milk samples based on *Escherichia coli* count

Brands	*Escherichia coli* count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
A	11 (91.67)	1 (8.33)			
B	10 (83.33)	ND	2 (16.67)		
C	2 (16.67)	ND	6 (50.00)		4 (33.33)
D	10 (83.33)	ND	2 (16.67)		
E	10 (83.33)	ND	2 (16.67)		
F	10 (83.33)	ND	2 (16.67)		

Figures in the parenthesis indicate per cent; N=12 from each retail brand; ND: Not Detected

D, E and F. The organism was also not detected in 91.67 per cent of samples of brand A and 16.67 per cent of samples of brand C. The count at the level of 10^2 cfu/ml was seen in 50 per cent of samples of brand C and 16.67 per cent of samples of brands B, D, E and F, respectively. Only one sample (8.33 per cent) of brand A had count at the level of 10^1 cfu/ml.

4.1.15.4 Psychrotrophic Count

The mean psychrotrophic counts (PC) of milk samples belonging to the brand A, B, C, D, E and F are shown in table 79 and illustrated in fig. 9. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of samples of the brands. The samples belonging to brand B had the lowest mean count and the highest count was seen in the samples belonging to brand D. The mean count of samples of brand D differed highly significantly ($P<0.01$) with the count of samples of brands B, C, E and F. Similarly the mean count of samples of brand C differed highly significantly ($P<0.01$) from the mean count of samples of brands B and F.

Table 79. Mean psychrotrophic count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	$4.89^{ab} \pm 0.09$
B	$4.42^{c} \pm 0.08$
C	$4.77^{b} \pm 0.13$
D	$5.09^{a} \pm 0.16$
E	$4.58^{bc} \pm 0.07$
F	$4.44^{c} \pm 0.08$

Critical Difference: 0.3052; Figures bearing the same superscript do not differ significantly: N=12 from each retail brand

The distribution of retailed milk samples based on psychrotrophic count is shown in table 80. Of the 72 samples, the highest mean count was seen in the samples belonging to brand D of which 10 (83.33 per cent) samples had a count

Table 80. Distribution of retail milk samples based on Psychrotrophic Count

Brands	Psychrotrophic Count (cfu/ml)		
	10^3	10^4	10^5
A		8 (66.67)	4 (33.33)
B	3 (25.00)	9 (75.00)	
C		9 (75.00)	3 (25.00)
D		2 (16.67)	10 (83.33)
E		12 (100)	
F	4 (33.33)	8 (66.67)	

Figures in the parenthesis indicate percent; N=12 from each retail brand

at the level of 10^5 cfu/ml. The count at that level was also seen in four (33.33 per cent) samples of brand A and in three (25 per cent) samples of brand C. Cent per

cent of samples of brand E, 75 per cent of samples of brands B and C and 66.67 per cent of samples of brands A and F had count at the level of 10^4 cfu/ml. The count in 33.33 per cent of the samples of the brand F and 25 per cent of samples of brand B was at the level of 10^3 cfu/ml.

4.1.15.5 Faecal Streptococcal Count

The mean faecal streptococcal count (FSC) of milk samples belonging to the brand A, B, C, D, E and F are shown in table 81 and illustrated in fig. 9. The lowest mean count was seen in samples belonging to brand E and the highest count was seen in the samples of the brand D. The mean count of samples of brand D had highly significant ($P<0.01$) difference with the mean count of samples of brands B, C and E.

Table 81. Mean Faecal Streptococcal count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	$2.45^{ab} \pm 0.14$
B	$2.05^{b} \pm 0.27$
C	$2.10^{b} \pm 0.10$
D	$2.87^{a} \pm 0.24$
E	$2.03^{b} \pm 0.19$
F	$2.34^{ab} \pm 0.13$

Critical Difference: 0.548; Figures bearing the same superscript do not differ significantly: N=12 from each retail brand

The distribution of retail milk based on faecal streptococcal count is shown in table 82. Of the 72 samples, only six samples of brand D had a count at the level of 10^3 cfu/ml. Cent per cent of samples of brand F and 83.34 per cent samples of brand A had count at the level of 10^2 cfu/ml. The count at that level was also seen in 50 per cent samples each of brands D and E, 33.33 per cent samples of brand B and 41.67 per cent samples of brand C. The count at the level

Table 82. Distribution of retail milk based on faecal streptococcal Count

Brands	Faecal streptococcal count (cfu/ml)			
	ND	10^1	10^2	10^3
A		2 (16.66)	10 (83.34)	
B		8 (66.67)	4 (33.33)	
C	2 (16.66)	5 (41.67)	5 (41.67)	
D			6 (50.00)	6 (50.00)
E		6 (50.00)	6 (50.00)	
F			12 (100)	

Figures in the parenthesis indicate percent; ND-Not detected; N=12 from each retail brand

of 10^1 cfu/ml was seen in 66.67 per cent samples of brand B. The count in 50 per cent samples of brand E and 41.67 per cent samples of brand C was also at the above level. The count at the above level was also seen in 16.66 per cent the samples belonging to the brand A. The organism was not detected in 16.66 per cent samples of brand C.

4.1.16 Isolation and Identification of Bacteria

The bacteria isolated from pasteurized retail milk samples are given

Table 83. Bacteria isolated from retail milk samples

Bacteria	Number of positive samples					
	Brands of milk					
	A	B	C	D	E	F
Escherichia coli	ND	3	4	1	5	2
Staphylococcus aureus	ND	1	3	1	1	ND
Pseudomonas	2	3	4	2	3	2

ND: Not detected N=12 from each brand of milk

in table 83. A total of 72 samples were tested for the isolation of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas.*

4.1.16.1 Escherichia coli

Out of 72 samples, 15 (20.83 per cent) had colonies with characteristics of *E. coli*. These colonies were selected and subjected to the characterization of the organism by primary and secondary tests. From this isolates eight showed Ejikman positive reaction and six revealed congo red binding ability. The isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. The isolates belonged to the serotypes O46, O116 (3), O65 (3), O95 (2), O166, O171, UT (2) and Rough (2).

4.1.16.2 Staphylococcus aureus

All retail milk samples were tested for the isolation of *Staphylococcus aureus* and the isolates were identified by the cultural, morphological and biochemical characteristics. The organism was isolated only from one sample each of the brands B, D and E and from three samples of the brand C.

4.1.16.3 *Pseudomonas*

All retail milk samples were tested for the isolation and identification of *Pseudomonas spp.* A total of 16 samples (22.22 per cent) yielded positive colonies

Table 84. *Pseudomonas* isolates from retail milk samples

Organism isolated	Number of isolates
Pseudomonas aeruginosa	6 (37.50)
Pseudomonas flourescens	3 (18.75)
Pseudomonas putida	7 (43.75)
Total	16

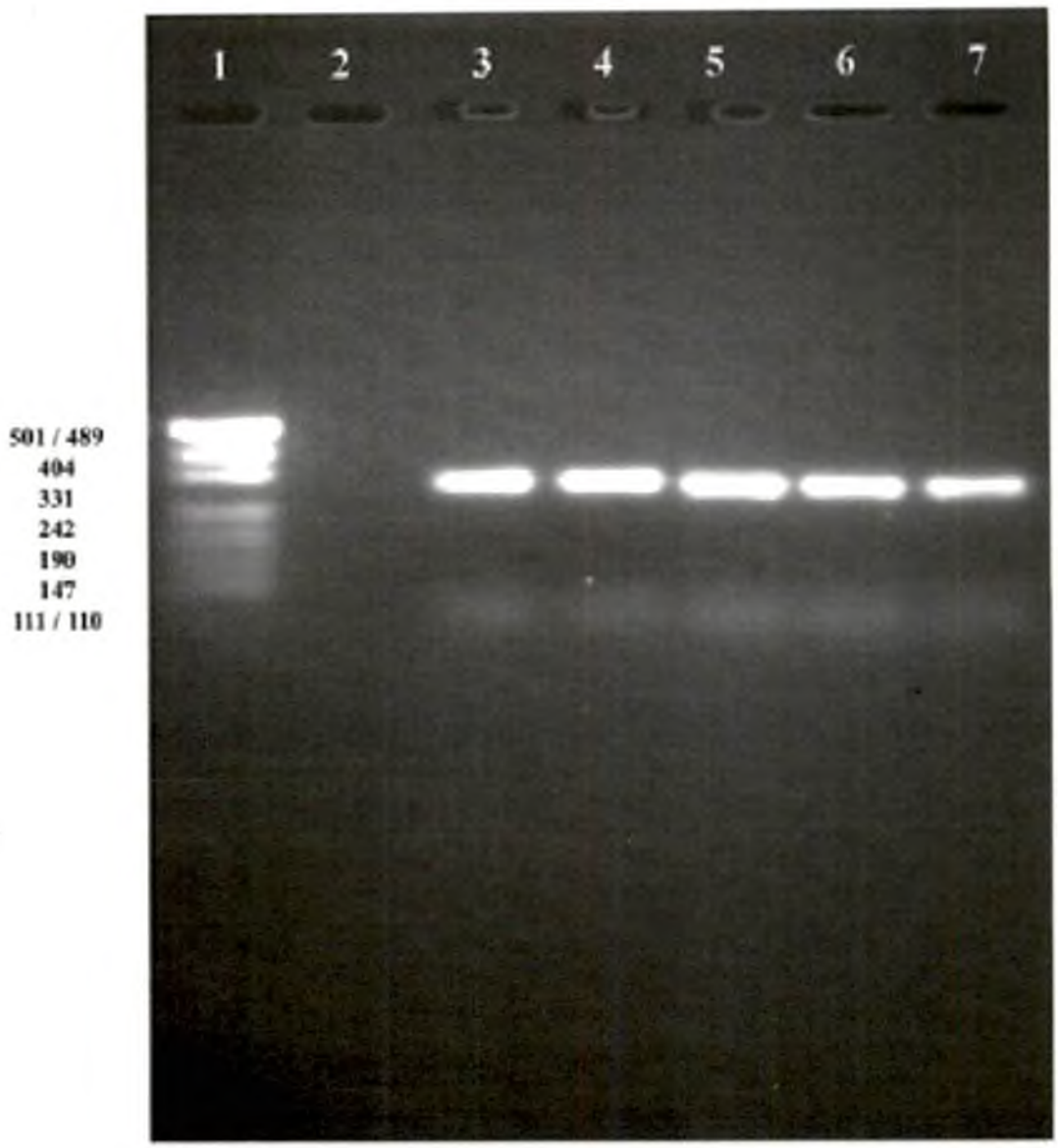

Fig. 10. ***E.coli*** **genus specific** ***alr*** **PCR II**

Lane 1 pUC 19 DNA/ ***Msp*** **I digest marker**

Lane 2 Negative control

Lane 3 - 7 ***E.coli*** **isolates**

and the isolates were identified by the cultural, morphological and biochemical characteristics. The isolates were identified as *Pseudomonas putida* (7), *Pseudomonas aeruginosa* (6) and *Pseudomonas flourescens* (3) (table 84).

4.1.17 Polymerase Chain Reaction

The isolates of *Escherichia coli* obtained throughout the study were subjected to polymerase chain reaction using the method as described by Daly *et al.*, (2002). The specific primer for the *alr* gene was used along with an $MgCl_2$ concentration of 1µl at an annealing temperature of 55°C and it successfully primed the synthesis of an expected 366 bp fragment of all isolates of *Escherichia coli*. Also a single 366 bp band was obtained when the PCR products were detected by gel electrophoresis (fig.10).

Discussion

5. DISCUSSION

Milk is a highly perishable food of animal origin and it has significant role in human nutrition. It gets contaminated with microorganism at various stages of its production. It serves as an ideal medium for the growth and multiplication of saprophytic and pathogenic microorganism. The growth and multiplication of former group of organism may result in spoilage of milk and the latter group may cause food poisoning or food borne infection. Therefore, hygienic handling of milk during the various stages of its production, transportation and subsequent pasteurization and storage at refrigeration play a significant role in quality assurance of fluid milk so as to safeguard consumer health and extent its shelf life. During the present investigation milk samples were subjected to evaluate its bacterial quality, to detect the presence of bacterial pathogens and also evaluated the organoleptic quality.

5.1 BACTERIAL QUALITY OF MILK

5.1.1 Bacterial Counts of Pooled Raw Milk

5.1.1.1 Total Viable Count

Total viable count (TVC) of food serves as an important index of its bacterial quality, and also the degree of freshness of food. The mean total viable count of pooled milk samples obtained from DP_1 (7.11± 0.02 $\log_{10}$ cfu/ml) and DP_2 (7.18 ± 0.03 $\log_{10}$ cfu/ml) (table 2) was in agreement with the counts reported by Jain and Saraswat (1968) Siva *et al.* (1993) and Singh *et al.* (1994). The counts of the samples from the plants were two log higher than that observed in the pooled samples of dairy plant (Prejit *et al.* 2007). The disparity in the mean count might be due to the difference in hygienic measures taken during production, transport and handling of milk in the processing plants. However, the count in the samples of the current study

was four log lower than that observed in the milk samples of local milk supply scheme of Aligarh city (Khalilur *et al.*, 2002).

In the present study, cent per cent of the samples from DP_1 and DP_2 had count at the level of 10^7 cfu/ml (table 3 and 4). This indicated that the bacterial quality of pooled raw milk samples collected from both the plants were same. However, Prejit (2005) reported that 80 per cent of the samples had count below 10^5 cfu/ml. The high levels of counts in the samples of the current study might be attributed to the lack of proper hygienic measures observed in these plants.

5.1.1.2 Coliform Count

Highly significant ($P<0.01$) difference was observed between the mean coliform count of pooled milk samples belonging to DP_1 and DP_2 (table 2). The count of samples of the former plant was 3.34 ± 0.05 and the latter plant was 2.96 ± 0.13 $\log_{10}$ cfu/ml. The count in the samples of the current study was at a level of 3 $\log_{10}$ cfu/ml and was similar to that reported Khalilur *et al.* (2002), Jaibi (2006) and Prejit *et al.* (2007). The count observed in the present study was one log lower than that recorded by Chye *et al.* (2004) and two log lower than the counts reported by Jolly *et al.* (2000). The count in cent per cent of samples of DP_1 and 43 per cent of samples of DP_2 was at the level of 10^3/ml. However, Davies (1977) reported that only 29.1 per cent sample had count at that level.

Coliform organism can gain entry into milk through environmental and faecal contamination, and also the organism can rapidly build up in moist milk residues in the milking equipment and become a major source for contamination of milk (Robinson, 2002).

5.1.1.3 Escherichia coli Count

The mean *Escherichia coli* count of pooled raw milk samples from DP_1 and DP_2 were 1.79 ± 0.65 and 2.22 ± 0.41 log_{10} cfu/ml (table 2) and the counts differed significantly ($P<0.05$) with each other. The difference in the counts of two plants might be due to the lack of proper hygienic measures followed in the latter plant. The mean count of the samples of the former source was similar to that reported by Cosentino and Palmas (1997) but was much lower than that recorded by Kapre (1995), Jolly (2000) and Khalilur *et al.* (2002). The count at the level of 10^2 cfu/ml in the samples belonging to DP_2 was in agreement with the counts reported in the samples belonging to one of the three sources examined by Kapre (1995) and also one of the six processing plants studied by Cosentino and Palmas (1997).

Escherichia coli was not detected in 43 per cent of raw milk samples belonging to DP_1 and DP_2 while Prejit (2005) reported that the organism was not detected in 60.00 per cent raw milk samples. However, the per cent of samples yielded the organism in the current study was much greater than that reported as 12.5 per cent by Raj *et al.* (2003). The presence of this organism in milk is a clear indication of the poor hygienic practices followed during the production and handling of milk, since the organism is of intestinal origin in man and animal.

5.1.1.4 Psychrotrophic Count

The presence of psychrotrophic bacteria in milk are of special importance when milk is stored at relatively low temperatures. The present study revealed that mean psychrotrophic count obtained from pooled milk samples from DP_1 and DP_2 were 7.07 ± 0.02 and 7.06 ± 0.03 log_{10} cfu/ml (table 2). Psychrotrophs thrive and grow under refrigerated temperatures. The psychrotrophic count observed from both the plants in this study were two log higher than those reported by Prejit *et al* (2007).

The source of psychrotrophic bacteria in raw milk can be from the non-sterile farm utensils and equipments, coats of cows and water supplies. Thus hygienic practices at the producers' level can minimize psychrotrophic counts in milk.

5.1.1.5 Faecal Streptococcal Count

The faecal streptococcal count of pooled milk samples from DP_1 and DP_2 were 3.15 ± 0.03 and 3.12 ± 0.06 $\log_{10}$ cfu/ml (table 2). The count in pooled milk sample was in agreement with the count reported by Kapre (1995) and Jaibi (2006) but the count was one log higher as compared to the count recorded by Prejit *et al.* (2007).

An improvement in the sanitary practices followed in the farm will minimize the contamination of milk and thereby reduce the count, as the organism is an inhabitant of the intestine of man and animals.

5.1.1.6 Correlation of Bacterial Counts of Raw Milk

The study revealed a highly significant ($P<0.01$) and positive association between total viable count and psychrotrophic count (table 5) of samples from DP_1, whereas only significant association was observed between the two counts in the samples from DP_2. Jain and Saraswat (1968) also found highly significant correlation (0.922) between counts of psychrotrophs and total viable count in raw milk. Robinson (2002) stated that on an average the psychrotrophs comprises between 10 to 50 per cent of initial microflora. Similar significant and positive correlation was also seen between the total viable count and coliform count and was in agreement with the reports of Vijai and Saraswat (1968), Patel *et al.* (1993) and Siva *et al.* (1993).

5.1.2 Isolation and Identification of Organisms in Raw Milk

Milk serves as an excellent medium for the growth of certain microorganisms, particularly bacterial pathogens, whose multiplication is mainly dependent on temperature and on competing microorganisms and their metabolic products. Some of the pathogens having major public health significance include *Escherichia coli* and *Staphylococcus aureus*. *Pseudomonas* plays a significant role in the spoilage of milk.

5.1.2.1 Escherichia coli

Pooled raw milk samples were tested for the isolation and identification of *Escherichia coli*. The organism was isolated from 57 per cent of the samples of DP_1 and DP_2 (table 7). The per cent of organism isolated from the samples of both plants were almost similar to that recorded by Gran *et al.* (2003) and Jaibi (2006) and were lower than that recorded by Singh *et al.* (1994) and Chye *et al.* (2004). However, the per cent of the organism isolated from the samples of DP_1 and DP_2 were more than that reported by Rahman *et al.* (1992), Sharma *et al.* (1995) and Singh *et al.* (1996).

The isolates were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli. Of the four isolates from DP_1, one isolate each belonged to serotypes O68 and O116 (table 8) and two of the isolates were rough strains. Of the four isolates from DP_2, two isolates belonged to serotype O116 and the other two were untypable. The serotype 0116 belongs to Enterohaemorrhagic group. Infection with the organism belonging to the group causes diarrhea, haemorrhagic colitis, and haemolytic ureamic syndrome in man. The serotype O68 belongs to EAEC group associated with persistent diarrhea in infants and children (Jay, 2005).

Among the *Escherichia coli,* three isolates from DP_1 and two from DP_2 revealed congo red binding property which indicated their property of invasiveness. A good correlation between pathogenic potential and congo red binding property had

been reported by Abhilasha *et al.* (2001). The characteristics of congo red binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker (Rajil *et al.*, 2003).

Escherichia coli are the commensal organism found in the intestine of humans and animals. The organism is associated with various disease conditions in human beings and animals. The presence of the organisms in milk indicates contamination of milk with faecal matter and also from water contaminated with faeces.

5.1.2.2 Staphylococcus aureus

Staphylococcus aureus was isolated from four (57 per cent) pooled raw milk samples from DP_1 and two (29 per cent) samples from DP_2 (table 7). The per cent of isolate obtained from the samples of DP_1 in the study was in accordance with the findings of Rahman *et al.* (1992), Kapre (1995) and Jolly *et al.* (2000). The per cent of isolation of the organism from the samples of both the plants were lower than that reported by Desmasures *et al.* (1997), Adesiyun *et al.* (1998) and Chye *et al.* (2004). However, the per cent of isolation of the organism from the samples of DP_1 and DP_2 was greater than that reported by Yadava *et al.* (1985), Gill *et al.* (1994) and Jaibi (2006). The per cent of isolates obtained from the samples of DP_2 was in agreement with the findings of John *et al.* (2003) and Jaibi (2006) but was lower than that reported by Kapre (1995), Adesiyun *et al.* (1998) and Nanu *et al.* (2007).

Staphylococci form a part of the normal flora of animals and man. *Staphylococcus aureus* has been associated with food poisoning outbreaks with the consumption of raw milk (Carmo *et al.*, 2002). The presence of the organism in milk indicates the poor hygienic practices and health conditions of animal. Therefore, attention must be paid to sanitation and personnel hygiene to minimize the contamination of the product with the organism.

5.1.2.3. Pseudomonas

The organism was detected in 42.85 per cent of the samples collected from DP_1 and 71.4 per cent of samples collected from DP_2 (table 7). The per cent of isolation of organism from DP_1 and DP_2 in the present study were higher than that reported by Gill *et al.* (1994), Fromm and Boor (2004) and Nanu *et al.* (2007). However the per cent of isolation from both the plants was lower than that reported by Griffiths and Phillips (1988), Grover and Srinivasan (1988) and Aaku *et al.* (2004).

Pseudomonas can cause degradation of milk components through various enzymatic activities and reduce the shelf life of milk and its products. Most of the species are heat sensitive and hence their presence is considered to be due to post pasteurization contamination and/or improper time temperature relationship during the pasteurization of milk.

5.1.3 Effect of Pasteurization on Bacterial Quality of Milk

Highly significant ($P<0.01$) reduction in the Total Viable Count, coliform count, psychrotrophic count and faecal streptococcal count was observed during the pasteurization of milk samples belonging to DP_1 (table 11). A significant ($P<0.05$) reduction in *Escherichia coli count* of the samples was also observed. The bacterial counts of the samples belonging to DP_2 had a highly significant ($P<0.01$) reduction (table 12).

The total viable count and psychrotrophic count of raw milk had reduced to three $\log_{10}$ cfu/ml after pasteurization of samples of DP_2 and in samples of DP_1 the total viable count reduced to two and psychrotrophic count reduced to three $\log_{10}$ cfu/ml after pasteurization. The findings of the present study was in agreement with the results of Mahari and Gashe (1990) who reported a two to four log reduction in the aerobic count after pasteurization. Pelezynska and Libett (1995) also reported a

reduction of two log in the bacterial counts after pasteurization. Prejit (2005) reported that the total viable count and psychrotrophic count of raw milk reduced to one to two log_{10} cfu/ml after pasteurization.

The present study revealed that pasteurization reduced coliform count by one log in the samples belonging to both the plants. But, contrary to the findings of the current study, Kaloianov and Gogov (1977) reported that pasteurization killed 100 per cent of coliforms in the samples. Thus the presence of the organism in pasteurized milk samples of the dairy plants in the present study might be due to lack of adequate thermal processing since the organism cannot survive pasteurization or due to post pasteurization contamination.

The reduction in the *Escherichia coli* count was highly significant ($P<0.01$) in samples of DP_2 and significant ($P<0.05$) in samples of DP_1. However 71.43 per cent of fresh pasteurized milk from DP_1 and 57.14 per cent from DP_2 revealed absence of the *Escherichia coli* organism. The presence of *Escherichia coli* in pasteurized milk indicate either process failure or, more commonly, post processing contamination. At present the organism is the most widely accepted indicator of fecal contamination and the presence of the organism in milk imply a risk that other enteric pathogens may also be present.

The reduction in faecal streptococcal count from raw milk to pasteurized packaged milk was highly significant ($P<0.01$). However, the organism was present in cent per cent of samples from DP_2 and 71 per cent of samples from DP_1, indicating post pasteurization contamination or insufficient thermal processing.

5.1.4 Effect of Refrigeration on Bacterial Quality of Milk

Dairy Plant 1

5.1.4.1 Total Viable Count

Fresh milk had a mean total viable count of 5.04 ± 0.03 log_{10} cfu/ml (table 13 and fig. 3). The count did not differ up to two days of storage but on day four of storage at 4 ± 1°C, the count increased by two log. The high lag phase taken by the organism may be attributed to the effect of temperature coefficient and sub lethal injury of the organism during pasteurization. Hence some time would have taken by the organisms to get rejuvenated. The findings of the present study were in accordance with the observations of Prejit (2005). An increase in the count on day four, six, eight and 10 of storage was observed and the count was highly significantly ($P<0.01$) different between the days of storage. The mean count on the 10^{th} day was 8.27 ± 0.11 log_{10} cfu/ml and the count was almost similar to that reported by Prejit (2005). Similar increase in counts during storage was reported by Brown *et al.* (1984) and Krasz *et al.* (1993).

5.1.4.2 Coliform Count

Highly significant ($P<0.01$) difference was noticed between the mean coliform count of samples examined on zero, fourth and sixth days (table 15). A gradual increase in count was noticed till the sixth day and on eighth day the count was reduced to 2.88 ± 0.56 log_{10} cfu/ml and remained the same till the end of the storage period (illustrated in fig.3). The mean coliform count of freshly pasteurized milk increased from 2.53 ± 0.10 to 4.44 ± 0.17 log_{10} cfu/ml on the 6^{th} day of refrigerated storage. However, contrary to the findings of present study, Prejit (2005) reported an increase in count throughout storage period. A significant ($P<0.05$) and

positive association was observed between total viable count and coliform count on day zero of refrigerated storage.

Coliforms are generally accepted as an index of environmental contamination and/or faecal pollution. The organisms are recommended over *Escherichia coli* because coliforms are often present in higher numbers than the latter one. The count is used to assess the overall quality and hygienic condition prevailing in the plant during processing of food.

5.1.4.3 Escherichia coli Count

Escherichia coli count was seen in only four batches of samples examined and a gradual increase in count was observed upto sixth day of storage and then the count remained almost the same (table 17). However no significant difference was seen between mean counts of fresh and refrigerated sample. The findings of the present study was in agreement with Mamani *et al.* (2003) who reported that *Escherichia coli* counts were almost similar between the inoculum time (4 log cfu/ml) and four days of storage (4.46 log cfu/ml) in whole Ultra heat-treated milk stored at 4°C. The mean *Escherichia coli* and coliform count of the samples stored on day six had a positive and significant ($P<0.05$) relationship.

The isolation of *Escherichia coli* from milk, which is associated with various diseases in man and animals, is of great significance. Since the organism is a commensal of intestinal tract of man and animals, and a pathogen, the presence of the organisms in milk is of great public health importance.

5.1.4.4 Psychrotrophic Count

The mean psychrotrophic count of fresh milk samples was 4.45 ± 0.06 $\log_{10}$ cfu/ml (table 19). The count increased throughout the period of storage, and the count on 10^{th} day was 8.17 ± 0.04 $\log_{10}$ cfu/ml. The increase in count as observed in

the current study was similar to that reported by Krasz *et al.* (1993) and Prejit (2005). The present investigation revealed a highly significant ($P<0.01$) increase in counts during 10 days of storage.

Psychrotrophic bacteria are important because many of them produce extracellular proteolytic and lipolytic enzymes. These enzymes are proven to hydrolyze milk proteins and lipids and produce off-flavour and also reduce the shelf life of milk by causing spoilage of milk and its products.

5.1.4.5 Faecal Streptococcal Count

The mean faecal streptococcal count of fresh milk samples was 2.21 ± 0.17 $\log_{10}$ cfu/ml (table 21). Out of 14 samples of milk examined, 29 per cent were free of the organism on the zero day. But all the samples revealed the presence of the organism on second, fourth and sixth day of storage (table 22). Highly significant ($P<0.01$) increase in count was seen up to sixth day and thereafter it reduced.

The presence of faecal streptococci in the samples indicates the survival of the organism during pasteurization of milk or post pasteurization contamination.

5.1.5 Isolation and Identification of Organism from Refrigerated Milk

5.1.5.1 Escherichia coli

A total of four isolates were obtained from fresh and refrigerated milk samples (table 28). Two of the isolates belonged to the serotype O116 and they were positive for congo red binding indicating their invasive ability. The serotype is characterized as Enterohaemorrhagic *Escherichia coli* (EHEC) and is associated with diarrhea, colitis and haemolytic ureamic syndrome in man. Ingestion of the organism at the level of 10^6-10^8 viable cells per gram causes colonization in small intestine and produces toxin thus leading to development of gastroenteritis in the consumer. The

count in the present study was highest at the level of 10^5 cfu/ml in one of the sample stored on day 10 (Montville and Matthews, 2005).

5.1.5.2 Staphylococcus aureus

Staphylococcus aureus was isolated from one of the samples each examined on zero, second and fourth day and from two samples examined on sixth day of storage (table 28). *Staphylococcus aureus* constitutes a part of the normal microflora of animal and human body, being found on skin and hair, nose, mouth and throat and hence the presence of the organism in pasteurized milk mostly can be due to post pasteurization contamination. The toxins produced by the organism are thermo stable hence can survive pasteurization.

5.1.5.3 Pseudomonas

Pseudomonas was isolated from the samples throughout the storage period. Out of 84 samples, 22.62 per cent revealed the presence of the organism (table 28). The result obtained in the present study was much lower than that reported as 92.1 per cent by Griffiths and Phillips (1988) and much higher than that reported by Fromm and Boor (2004).

Pseudomonas is an important group of psychrotrophic bacteria that grow and multiply in low temperatures and cause spoilage. Hence is an important organism to be screened for determining the shelf life of milk and its products.

5.1.6 Effect of Refrigeration on the Sensory and Physical Quality of Milk

5.1.6.1 Sensory Evaluation

The sensory evaluation of fresh and refrigerated milk sample was done and comparison was made using Mann-Whitney test.

a. Colour and appearance

The mean colour and appearance score of freshly pasteurized milk decreased from 9.67 ± 0.24 to 6.43 ± 0.07 on the 10^{th} day of refrigerated storage (table 30) which was in agreement with the results obtained by Prejit (2005). The mean score of fresh sample differed significantly ($P<0.05$) with the mean score on fourth, sixth, eighth and 10^{th} day. Fresh sample had the maximum score and appeared white.

Singh and Patil (1987) found that after pasteurization or UHT treatment, milk developed whitening due to an increase in size of casein micelles, which is related to serum protein denaturation and complex formation.

b. Flavour score

Flavour is one of the characteristics of milk that is perceived by common consumer instantly on opening the packet. The mean score differed highly significantly ($P<0.01$) from second day onwards and the difference was maximum between fourth and sixth day (table 32). The findings of the current study were in accordance with the observation of Dogan and Boor (2003) and Prejit (2005) who reported a decrease in score as the storage period progressed. In the present study, slight stale flavour began to develop on sixth day and on eighth day of storage sourness developed. Similar to the findings of present study, Watson and Mc Ewan (1995) reported that at 10°C stale flavour began to develop after four days and sourness developed after six days.

c. Odour score

Odour score was found to decrease throughout the ten day storage period and the scores differed highly significantly ($P<0.01$). The odour score was reduced from 19.14 ± 0.26 to 9.86 ± 0.26 after a period of 10 days of storage (table 31). Off odour was first detected on sixth day and was marked on 10^{th} day. But contrary to the results of current study, John (1999) reported development of off-odour on the tenth

day and Prejit (2005) reported off-odour development on eighth day in refrigerated milk samples.

d. Body

Body had high score on day zero and two of storage of milk. Highly significant ($P<0.01$) difference in the body score was observed throughout the period of storage. The score on day zero was 28.43 ± 0.20 and was reduced to 15.14 ± 0.26 on tenth day of storage (table 33). The common defect noticed was presence of clotted particles and was observed first on sixth day and all the samples except two were curdled on eighth day of storage.

e. Total score

The mean of the total score revealed that samples were of excellent quality for up to second day and on day four the quality was good. Thereafter the sensory quality decreased and from sixth day onwards the sample was graded as fair and on tenth day the sample was graded as poor (table 34). Sensory analysis revealed the samples had a shelf life of six days. As per the Indian Standards (1975), the samples in the present study showed the qualities of grade B milk only after 96 h. (four days) of storage at $4 \pm 1°C$ but Kadan and Bhanumathi (1984) reported that pasteurized and homogenized milk was graded as B after storage at 24 h. at 5-9°C.

5.1.6.2 Clot on Boiling test

All samples were COB test positive by the end of storage period. Samples of one batch showed COB test positive on the sixth day of storage and one batch showed COB positive reaction on 10^{th} day but the rest batches were positive on eighth day (table 35). But the findings of present study did not corroborate with the findings of Prejit (2005) who reported that out of 10 batches only two batches showed a positive test on eighth day. The Analysis of the data revealed that a

positive COB test is dependent upon the microbial load of the samples. The observation in the present study confirmed the observation of Mukundan (1978).

5.1.7 Shelf Life of Pasteurized Milk

The present study revealed that pasteurized milk kept under refrigeration (4±1°C) had a maximum shelf life of six days. The sensory analysis showed that milk started to develop stale flavour and off-odour after six days of storage and curdy appearance was seen on eighth day. The mean sensory score of colour and appearance, flavour and body also showed excellent quality up to second day, good quality on fourth day, fair from sixth day onwards and poor quality on 10^{th} day. Bacterial analysis revealed gradual increase in total viable count, coliform count, *Escherichia coli* count, psychrotrophic count and faecal streptococcal count till sixth day. The counts on eighth and 10^{th} day of storage showed a reduction which might be due to the spoilage and subsequent acid production to which these organisms are sensitive. When quality of refrigerated milk turned into fair on sixth day of storage the sample had mean total viable count of 7.62 ± 0.10 $\log_{10}$ cfu/ml and the mean psychrotrophic count was at the level of 7.40 ± 0.07 $\log_{10}$ cfu/ml. Therefore, it may be inferred that the milk becomes organoleptically unacceptable when the total viable count and psychrotrophic count are greater than 7 $\log_{10}$ cfu/ml. The findings of the current study were similar to the observation of Schroder *et al.* (1982) who reported that spoilage of milk occurred when the total count reached around 10^7 cfu/ml, and the milk also developed off flavour. The shelf life of milk in the present study was lesser than that reported by Reinheimer *et al.* (1994) and Lindberg *et al.* (1998) who reported a shelf life of 9.1 days and 11 days, respectively for the HTST pasteurized milk samples stored at 7°C.

Refrigerated samples showed sensory deterioration earlier to the development of positive COB test. Although, the COB test is an important criterion to determine shelf life of milk, it was not useful to determine the sensory shelf life

which is very important for public acceptability since the samples become organoleptically unacceptable before the samples showed positive COB test.

5.1.8 Dairy Plant 2

5.1.8.1 Total Viable Count

The mean count of samples during storage period increased from 4.72 ± 0.09 to 9.50 ± 0.10 $\log_{10}$ cfu/ml (table 36 and fig. 4). There was a gradual and highly significant ($P<0.01$) increase in the count during the entire storage period. The time taken for the count to increase by one log was only two days. But contrary to the findings of the current study, John (1999) reported that for the count to increase by one log it took four days. This low generation time or short lag phase in the present study can be attributed to the faster acclimatization of organisms to the reduced temperature. The mean count on 10^{th} day was 9.50 ± 0.10 $\log_{10}$ cfu/ml and was two log higher than that reported by Prejit (2005).

5.1.8.2 Coliform Count

Fresh milk had a mean coliform count of 1.65 ± 0.21 $\log_{10}$ cfu/ml (table 38) and the count differed significantly ($P<0.05$) from the count on day two, four, six, eight and 10 of storage. A gradual increase in the count was observed till the end of storage period and the mean count on 10^{th} day was 3.60 ± 0.94 $\log_{10}$ cfu/ml. The findings of the present study were in agreement with the findings of Krasz *et al.* (1991) who reported an increase in coliform count throughout the storage period. The count increased by two log by eight days of storage which indicate the rate of growth of the organism was slow. However, contrary to the findings of the present study, Prejit (2005) reported that the count increased by two log after 10 days of storage.

Coliforms are primarily indicators of faecal contamination of milk. Most of these are destroyed during pasteurization hence their presence in pasteurized milk can be due to post pasteurization contamination or insufficient thermal processing. However, the presence can also be due to the thermal resistance of some strains and rejuvenation of heat injured cells after pasteurization.

5.1.8.3 Escherichia coli Count

The organism was absent in 57.14 per cent of samples on the day of pasteurization. The mean count of organism on zero day was 0.78 ± 0.28 $\log_{10}$ cfu/ml and at the end of storage it increased to 2.86 ± 0.52 $\log_{10}$ cfu/ml (table 40). The count changed significantly by two log at the end of storage period. The finding of the present study did not corroborate with that of Prejit (2005) who reported no significant increase in count during the 12 day storage period. A positive and significant correlation was observed between *Escherichia coli* count and coliform count on 10^{th} day of storage.

Escherichia coli are food borne pathogens which produce severe illness even with a small infectious dose (<100 cells for *E. coli* O157: H7). Presence of the organism in heat processed milk indicates inadequate time temperature treatment or post pasteurization contamination.

5.1.8.4 Psychrotrophic count

The mean psychrotrophic count of fresh milk samples was 4.40 ± 0.08 $\log_{10}$ cfu/ml (table 42). The count increased subsequently throughout storage till 10^{th} day of storage and similar increase was also reported by Prejit (2005). The present investigation revealed a highly significant ($P<0.01$) increase in counts during 10 days of storage. The count on eighth day of storage was 7.63 ± 0.06 $\log_{10}$ cfu/ml. But contrary to the findings of the present study, Zygora *et al.* (2004) reported that after

seven days of storage of the samples at 4°C psychrophilic counts ranged between 6.11 and 6.69 log cfu/ml. However, the psychrotrophic counts increased at a higher rate after eighth day and a difference of two log was seen within a period of 48 h. The count at the end of 10 day storage period was 9.18 ± 0.04 log_{10} cfu/ml and was higher than that reported by Vassila *et al.* (2002) and Moyssiadi *et al.* (2004).

Defects of fluid milk are associated with the extracellular enzymes produced during the growth of psychrotrophic bacteria. However, most psychrotrophs in milk grow slowly and causes spoilage of refrigerated milk in 10 to 20 days.

5.1.8.5 Faecal Streptococcal Count

The mean faecal streptococcal count of fresh milk samples was 2.15 ± 0.15 log_{10} cfu/ml (table 44). The count showed an increase throughout the storage period. The count on zero day was significantly ($P<0.05$) different from the counts on day eight and 10 of storage. The findings of the present study corroborate with the observation of Prejit (2005) who reported significant increase in the count on day 10 of storage. However, in the present study, 14.29 per cent of samples were free of the organism on 10th day of storage which differs with the findings of Prejit (2005) who reported the organism was present in cent per cent samples on 10th day of storage. The presence of the organism indicated post pasteurization contamination of milk from contaminated atmosphere or filling machine.

5.1.9 Isolation and Identification of Organism from Refrigerated Milk

5.1.9.1 Escherichia coli

A total of six isolates were obtained from fresh and refrigerated milk samples (table 51). Two of the isolates belonged to the serotype O65 and the other serotypes were O22, O46, O116 and O95. The serotype O22, O46 and O116 is characterized as Enterohaemorrhagic *Escherichia coli* (EHEC) and is associated with diarrhea, colitis and haemolytic ureamic syndrome in man. Ingestion of the organism at the level of

10^6-10^8 viable cells per gram causes colonization in small intestine and produces toxin thus leading to development of gastroenteritis in the consumer. The serotypes O65 and O95 are known to produce diarrhea in man. The result of congo red binding test of *Escherichia coli* revealed that three isolates belonging to serotypes O116, O46 and O22 showed a positive reaction indicating their property of invasiveness.

5.1.9.2 Staphylococcus aureus

Staphylococcus aureus was isolated from 4.76 per cent of samples examined during storage. The organism was isolated from one of the samples examined on zero day and from three samples examined on 10^{th} day of storage (table 51). Personal hygiene and sanitation are important to minimize the contamination of milk with the organism. Large number of organism if present in raw milk can produce enterotoxins which are heat stable and can survive pasteurization and produce food borne intoxication to the consumers.

5.1.9.3 Pseudomonas

Pseudomonas was isolated from the samples throughout the storage period. Out of 84 samples, 20.24 per cent revealed the presence of the organism (table 51). Pseudomonas species can produce heat stable enzymes when stored at low temperatures. These enzymes are not inactivated during heat treatment and may be responsible for spoilage of milk.

5.1.10 Effect of Refrigeration on the Sensory and Physical Quality of Milk

5.1.10.1 Sensory Evaluation

The sensory evaluation of fresh and refrigerated milk sample was done and comparison was made using Mann-Whitney test.

a. Colour and appearance

The mean colour and appearance score of freshly pasteurized milk was 9.75 ± 0.26 (table 53) and decreased significantly ($P<0.05$) at the end of storage period. The score on 10^{th} day of storage was 7.64 ± 0.14. The mean score of fresh sample differed significantly ($P<0.05$) with the mean score on fourth, sixth, eighth and 10^{th} day.

b. Flavour score

The mean flavour score on day zero was 38.71 ± 0.28 and the score decreased highly significantly ($P<0.01$) from second day onwards (table 55). The findings of the current study were in accordance with Prejit (2005) who also reported a decrease in score as the storage period progressed. In the present study, stale flavour was noticed only on tenth day in all batches except one in which the stale flavour was noticed on eighth day of storage which corroborated with the findings of Prejit (2005).

c. Odour score

The mean odour score of freshly pasteurized samples was 19.71 ± 0.18 and the score was found to decrease throughout the ten day storage period. The score on each day differed highly significantly ($P<0.01$) with the scores on subsequent days. The odour score at the end of storage period was 11.86 ± 0.63 (table 54). Off odour was first detected on eighth day and was marked on 10^{th} day which concurred with the findings of Prejit (2005). But contrary to the results of current study, John (1999) reported the development of off-odour in the samples examined on the 10^{th} day.

d. Body

Body had high score on day zero and two of storage of milk. Highly significant ($P<0.01$) decrease in the body score was observed throughout the period

of storage. The score on day zero was 28.14 ± 0.14 and was reduced to 18.71 ± 0.61 on tenth day of storage (table 56). The body of the milk samples did not show any gross changes except in one batch where clotted particles were seen on tenth day of storage.

e. Total score

The mean of the total score revealed that samples were of excellent quality up to second day and till sixth day the quality was good. Thereafter the sensory quality decreased and from eighth day onwards the sample was graded as fair till the end of storage period (table 57). Sensory analysis revealed that the samples had a shelf life of eight days. As per the Indian Standards, 1975, the samples in the present study showed the qualities of grade B milk only after 144 h. (six days) of storage at 4 ± 1°C and the findings of the current study was in agreement with that of Prejit (2005).

5.1.10.2 Clot on Boiling test

Out of seven batches only three batches were COB test positive by the end of storage period. Samples of one batch showed COB test positive on the eighth day and two batches on 10^{th} day of storage (table 58). In the present study 57.14 per cent of samples were positive by 10^{th} day of storage while Prejit (2005) reported that 70 per cent of samples were positive on that day of storage.

5.1.11 Shelf Life of Pasteurized Milk

The present study revealed that pasteurized milk kept under refrigeration (4±1°C) revealed a maximum shelf life of eight days. The sensory analysis showed that milk started to develop stale flavour and off-odour after eight days of storage. The mean sensory score of colour and appearance, flavour, odour and body also showed excellent quality up to second day, good quality up to sixth day, fair from eighth day onwards till the end of storage period. Bacterial analysis revealed gradual

increase in total viable count, coliform count, *Escherichia coli* count, psychrotrophic count and faecal streptococcal count throughout the storage. When quality of refrigerated milk turned into fair on eighth day of storage the sample had mean psychrotrophic count at the level of 7.63 ± 0.06 $\log_{10}$ cfu/ml. Therefore it may be inferred that the milk becomes organoleptically unacceptable when the psychrotrophic count is greater than 7 $\log_{10}$ cfu/ml. The shelf life of milk in the present study was similar to that found by Prejit (2005) who reported a shelf life of eight days for pasteurized milk samples stored at 4 ± 1°C.

The COB test was positive in majority of samples only on tenth day but the samples were organoleptically poor even before that indicating the unsuitability of this test for assessing the sensory shelf life of refrigerated milk samples.

5.1.12. Comparison of Quality of Milk Samples from the Two Dairy Plants

5.1.12.1 Bacterial Quality

The total viable count in the samples of DP_1 on day zero was at a level of 5 log which was very high as compared to the standards prescribed by Indian Standards (1992) while the count in samples of DP_2 was at a level of 4 log which met the standards. The high count in samples of DP_1 may be due to inadequate temperature exposure during pasteurization or unhygienic handling of pasteurized milk. The total viable count of the samples of DP_1 was highly significantly ($P<0.01$) higher than that of the count in samples of DP_2 on days zero, two, four, eight and 10 and significantly ($P<0.05$) higher on day six (table 59). This clearly indicates the lack of proper hygienic measures followed in the plant 1 compared to plant 2. The count of samples of DP_1 was higher than the count of samples of DP_2 till sixth day of storage and then the count of samples of DP_1 reduced than that of the counts of samples of DP_2 since the commencement of spoilage of milk.

Highly significantly ($P<0.01$) difference was observed between the mean coliform count of the samples of DP_1 and DP_2 on day six and 10 of storage and also revealed significant ($P<0.05$) difference between the mean count of samples on day zero, two and four (table 60). The count of samples of DP_1 was 2 log on day zero of storage and was increased to 4 log on sixth day, and then reduced to 2 log on eighth and 10^{th} day as the milk started curdling. The reduction in the count might be due to the acidic pH of the spoiled milk to which the organism is sensitive. The highest count obtained in samples of DP_2 was at the level of 3 $\log_{10}$ cfu/ml only whereas in DP_1 it was at the level of 4 $\log_{10}$ cfu/ml. This difference clearly points out the inadequacy of hygienic measures implemented in DP_1. However, the count in both plants was high as compared to the standards prescribed by Indian Standards (1992).

The *Escherichia coli* count of both the plants were almost the same on day zero, two and four of storage (table 61). The count in the samples of DP_1 remained almost the same throughout the period of storage whereas, the count in samples of DP_2 increased throughout the period and showed highly significant ($P<0.01$) difference between the mean count of samples of DP_1 on day six, eight and 10. The highest count observed in samples of DP_1 was at the level of 1 log and in samples of DP_2 it was at the level of 2 $\log_{10}$ cfu/ml. The organism was absent in 71.43 per cent samples of DP_1 and 57.14 per cent samples of DP_2. The presence of the organism indicates unhygienic practices followed in the pasteurization plant and also post pasteurization contamination.

The psychrotrophic count in both the plants showed an increasing trend throughout the storage period. The count in the samples of DP_1 increased even when the milk samples showed signs of spoilage indicating that the psychrotrophic flora is able to thrive even in spoiled milk. The count in the samples of both plants was at the level of 7 log when it started showing signs of spoilage. The finding of the current

study corroborate with the finding of Schroder *et al.* (1982) who observed that spoilage of milk occurred when the total count reached around 10^7 cfu/ml.

The mean faecal streptococcal count of samples of DP_1 varied highly significantly ($P<0.01$) from the count of samples of DP_2 on day six, eight and 10. The count of samples of both plants reached at the level of 4 log when it started spoilage. In the samples of DP_1 the count increased up to 4 log on sixth day and then decreased up to 1 log on day 10. This can be attributed to the inability of the organism to multiply in the acidic pH.

5.1.12.2 Organoleptic quality

The organoleptic parameters like colour and appearance, odour, flavour and body were found to be decreasing throughout the storage period in the samples of both DP_1 and DP_2. However, the rate of decrease in the quality of milk was much higher in the samples of DP_1. The presence of a stale flavour was noticed in samples of DP_1 from sixth day onwards while in samples of DP_2, it was noticed on 10^{th} day only. The presence of an off-odour was observed in samples of DP_1 from sixth day onwards and in samples of DP_2, from eighth day onwards. The presence of clotted particles were seen in samples of DP_1 from sixth day onwards and in samples of DP_2, no gross changes in the body of milk were observed except in one batch. The total score of the samples of DP_1 differed highly significantly ($P<0.01$) from the score of samples of DP_2 and the samples of former plant were graded fair from sixth day onwards and poor on 10^{th} day whereas the samples of latter plant were graded fair till the end of storage period. The findings of the current study clearly indicate that the quality of milk produced from DP_2 is much better than that of samples from DP_1.

5.1.13 Interpretation of Quality of Milk Based on Grades of Milk

5.1.13.1. Raw milk

In India milk is graded based on total viable count limit prescribed by Indian Standards (IS: 1977). According to the criteria, 57.14 per cent samples from DP_1 and 42.86 per cent samples from DP_2 were graded as fair and 42.86 per cent of samples each from DP_1 and DP_2 were graded as poor. The findings of the present study was much lower than that reported by Jain and Saraswat (1968), who reported that 61.42 per cent sample was graded as poor. From the samples of DP_2, 14.28 per cent were graded as good. However, none of the samples from either of the plants were graded as very good. The findings of the current study did not corroborate with the observations of Singh *et al.* (1994), who observed that 28.75 per cent of the milk samples were graded very good and 37.73 per cent as poor quality. None of the samples of the current study were graded as very good but Garg and Mandokhot (1997) reported that 2.35 per cent samples were graded as very good.

According to Indian Standards (IS: 1977), coliform count in satisfactory grade raw milk should be absent in 1:100 dilution. The present study revealed that 28.57 per cent of pooled raw milk samples from DP_2 confirmed with the standard. However none of the samples from DP_1 met the standards. The high levels of coliforms in milk could be attributed to the contamination of milk from the environmental sources, particularly from contaminated water. The organisms are associated with spoilage of milk and thereby shortening of shelf life of milk.

5.1.13.2 Pasteurized milk

According to the criteria prescribed by Indian Standards (1992), the total viable count in satisfactory grade pasteurized milk should not exceed 30,000 organism/ml. In the current study, 42.86 per cent of the freshly pasteurized samples

from DP_2 met the criterion but none of the samples from DP_1 met the criteria prescribed by BIS.

As per the Indian Standards (1992), coliform count of satisfactory grade pasteurized milk should be absent in 1:10 dilution. The findings of the present study showed that only 42.86 per cent of samples from DP_2 met the standards while none of the samples from DP_1 confirmed with the standard.

5.1.14 Bacterial counts of Pasteurized Milk from Retail Market

5.1.14.1 Total Viable Count

The highest total viable count was observed in the samples of the brand D (5.94 ± 0.09 $\log_{10}$ cfu/ml) and lowest count in the samples of the brand F (4.89 ± 0.79 $\log_{10}$ cfu/ml). A highly significant ($P<0.01$) difference of mean count of the samples of brand F with the mean count of other brands was observed (table 73). This indicates the quality of pasteurized milk of brand F varied greatly with the others. The reason for variation in counts could be attributed to unhygienic conditions prevailing in the processing plants or post pasteurization contamination of milk or as a result of time lag from retailing and storage of milk under refrigeration. The mean count of the brand A, B, C D and E at the level of 5 $\log_{10}$ cfu/ml was similar to that recorded by Latha and Nanu (1997) and John (1999). The count of the samples from brand F (4.89 ± 0.79 $\log_{10}$ cfu/ml) was similar to that of Cerqùeira *et al.* (1994) and Prejit (2005).

Of the samples of the brand D, 91.67 per cent samples had count at level of 10^5 cfu/ml whereas, in the samples of the brand F, only 16.67 per cent of the samples had count at that level. This clearly indicates the variation in the maintenance of hygienic practices in different processing plants.

5.1.14.2 Coliform Count

Among the retail brands coliform count was highest in the samples of the brand A which had mean count of 2.40 ± 0.14 log $_{10}$ cfu/ml and lowest count of 1.19 ± 0.42 $\log_{10}$ cfu/ml was seen in the samples of the brand F (table 75). A highly significant ($P<0.01$) difference was observed in the mean count of the samples of brand F with the counts of the samples of all other brands. Hence the quality of pasteurized milk belonging to brand F was good as compared to the quality of milk belonging to the other brands. Difference in the coliform counts of various brands marketed in this area was also seen by Latha and Nanu (1997), John (1999) and Prejit (2005). The mean count of brand F was similar to that recorded by Arora and Sudarsanam (1986) and Raju and Nambudripad (1987). Samples from the brands A, C and D showed the coliform count at the level of 10^2 cfu/ml level and were similar to the mean count obtained by Misra and Kuila (1989) and Rai and Dwivedi (1990). Coliforms if present in large numbers can produce spoilage of milk stored at low temperatures and also imply the risk of the presence of other enteric pathogens in the milk.

5.1.14.3 Escherichia coli Count

Escherichia coli are considered as a part of the normal flora of the intestinal tract of humans and animals and are used primarily as an indicator of faecal contamination. Pasteurized milk sample has to be free of the organism. The organisms was absent in 91.67 per cent samples of brands A, in 83.33 per cent samples of brands B, D, E and F and 16.67 per cent samples of brands C, respectively. The count obtained in the samples of the brands B, D, E and F was almost similar to that reported by Latha and Nanu (1997) who observed an overall incidence of 80 per cent from the brands examined in the area. Hence the quality of market milk samples had not improved much in the area. The count observed was highest in brand C as 3.44 ± 0.72 $\log_{10}$ cfu/ml (table 77) which was similar to that

reported by John (1999) but higher than that recorded by Latha and Nanu (1997) and Prejit (2005). The presence of *Escherichia coli* in pasteurized milk is of great public health significance since the organisms are responsible for a variety of illness from mild diarrhea to severe hemorrhagic colitis.

5.1.14.4 Psychrotrophic Count

The highest count (5.09 ± 0.16 $\log_{10}$ cfu/ml) was found in the samples belonging to brand D and the lowest count (4.42 ± 0.08 $\log_{10}$ cfu/ml) was observed in the samples of brand B (table 79). A highly significant ($P<0.01$) difference was observed in the mean count of the samples from six brands. Gopi *et al.* (2001) also found variation in psychrotrophic counts in retail milk brands available in Chennai and the count ranged between 12.50 to 99.33×10^4 cfu/ml. In comparison to the present study the psychrotrophic organism had a lower count as observed by Saleha (1992). High psychrotrophic count will reduce the shelf life of refrigerated milk considerably.

5.1.14.5 Faecal streptococcal Count

The faecal streptococcal count was highest in the samples of brand D and the samples had mean count of 2.87 ± 0.24 $\log_{10}$ cfu/ml (table 81). The lowest mean count was observed in the samples of brand E which was 2.03 ± 0.19 $\log_{10}$ cfu/ml. In comparison to the findings of the current study, observations made by Latha and Nanu (1997) and Sethulakshmi *et al.* (2003) from the retail brands of the area were much lower.

5.1.15 Isolation and Identification of Organism in Retail Milk

5.1.15.1 Escherichia coli

Market brands of milk were tested for the isolation and identification of *Escherichia coli*. Out of 72 samples examined, 15 (20.83 per cent) revealed the

presence of the organism (table 83). The per cent of isolates obtained from the samples was lower than that recorded by John (1999) and Silva *et al.* (2001) and was much higher than that reported by John *et al.* (2003).

The isolates consisted of the serotypes O46, O65 (3), O116 (3), O95 (2), O166 and O171. Two of the isolates were rough strains and two were untypable. The serotypes O46 and O116 belong to Enterohaemorrhagic *Escherichia coli* group and are associated with diarrhea, colitis and haemolytic ureamic syndrome in man. The serotypes O65 and O95 are associated with diarrhea in man while serotype O166 is associated with enterocolitis and diarrhea in man. The serotype O171 belongs to Enteroinvasive *Escherichia coli* group and is associated with nonbloody diarrhea and dysentery. Six of the isolates belonging to serotypes O65 (2), O116 (3) and O171 showed a positive congo red binding reaction indicating their property of invasiveness.

5.1.15.2 Staphylococcus aureus

Staphylococcus aureus was isolated from six (8.3 per cent) samples of retail milk. The organism was isolated from three samples of the brand C and one sample each of B, D and E (table 83). But contrary to the findings of the present study, John (1999) could not detect the organism from 100 samples of retail milk and John *et al.* (2003) reported the isolation of the organism from one of the 84 samples. However, the per cent of isolation of the organism in the current study was much lower than that reported by Ghosh and Laxminarayana (1972) and Prejit (2005).

5.1.15.3 Pseudomonas

Pseudomonas was isolated from 22.22 per cent of retail samples (table 83). A total of 16 samples yielded the organism and the isolates were identified as *Pseudomonas putida* (7), *Pseudomonas aeruginosa* (6) and *Pseudomonas*

flourescens (3) (Table 84). The result of the current study was much lower than the findings of Griffiths and Phillips (1988) and Sutherland *et al.* (1993) and higher than the findings of Grover and Srinivasan (1988). The presence of *Pseudomonas putida, Pseudomonas aeruginosa* and *Pseudomonas flourescens* in pasteurized milk samples were also reported by Dogan and Boor (2003).

5.1.16. Polymerase Chain Reaction

Polymerase Chain Reaction offers a sensitive and specific method to confirm bacteria isolated from milk samples. The results demonstrate that the PCR process could be a useful tool for the rapid identification of *Escherichia coli*. The expected 366 bp level amplification specific for *Escherichia coli* alanine racemase gene (alr) were obtained when DNA extracted from the organism were subjected to Polymerase chain reaction. Agarose gel electrophoresis of the amplified polymerase chain reaction product was carried out along with a negative control and a molecular size marker in 1X TAE buffer. Analysis of the electrophoresed gel under UV transilluminator revealed the presence of a 366 bp band in the isolates and is shown in fig. 10. In the negative control no amplification product was detected.

Summary

6. SUMMARY

In the present study the bacterial quality of a total of 254 milk samples was evaluated by determining the bacterial load of milk before and after pasteurization and also during storage at 4 ± 1°C from samples collected from two dairy plants viz., DP_1 and DP_2. The changes in sensory and physical quality of fresh and refrigerated samples were evaluated and the shelf life of pasteurized milk under refrigerated conditions was also determined. An assessment of the bacterial quality of 72 samples of retail pasteurized milk belonging to brands A, B, C, D, E and F was also done.

Results on bacterial quality of raw milk revealed that pooled raw milk from both the plants had a total viable count (TVC) at the level of 7 $\log_{10}$ cfu/ml and an overall of 50 per cent samples were graded fair.

Coliform count (CC) was more in samples obtained from DP_1 (3.34 ± 0.05 $\log_{10}$ cfu/ml) with highly significant ($P<0.01$) difference when compared to DP_2. Also an overall of 85.7 per cent samples from both sources were graded poor.

Escherichia coli count (ECC) from DP_1 and DP_2 was at a level of 1.79 and 2.22 $\log_{10}$ cfu/ml. The psychrotrophic count (PC) and faecal streptococcal count (FSC) was at the level of 7 and 3 $\log_{10}$ cfu/ml, respectively from both the sources. Correlation studies revealed a highly significant ($P<0.01$) and positive association between the total viable count and psychrotrophic count (from DP_1 and DP_2) and a positive and significant ($P<0.05$) correlation between the psychrotrophic count and coliform count (DP_1).

Bacterial pathogens having public health significance like *Escherichia coli* and *Staphylococcus aureus* were isolated and the bacteria causing milk spoilage like *Pseudomonas* was also isolated. *Escherichia coli* was isolated from four samples each belonging to dairy plant 1 and dairy plant 2. Of the isolates, two from samples of DP_2 and one from DP_1 belonged to serotype O116. One of the isolates from samples of DP_1 belonged to serotype O68.

Pasteurization was very effective in reducing total viable count, coliform count, psychrotrophic count and faecal streptococcal count to a highly significant ($P<0.01$) level as observed from both the dairies. Pasteurization had reduced the total viable count and psychrotrophic count by even more than 2 $\log_{10}$ cfu/ml of the samples obtained from both dairies.

The mean total viable count of the samples during 10 days of storage has increased from 5.04 ± 0.03 to 8.27 ± 0.11 $\log_{10}$ cfu/ml in DP_1 and 4.72 ± 0.09 to 9.50 ± 0.10 $\log_{10}$ cfu/ml in DP_2. Every alternate day recorded one log increase in counts as observed in samples of DP_2. Comparison of total viable count between two dairies revealed a highly significant ($P<0.01$) difference between the mean counts on day zero, two, four, eight and 10. Correlation between total viable count and psychrotrophic count of samples on eighth and 10^{th} day of storage was positive and significant ($P<0.05$) in the samples of DP_1.

A gradual increase in the coliform count of the samples from DP_1 was observed upto six days and then the counts decreased. However, the counts of samples from DP_2 showed a gradual increase throughout the storage period. On comparison of coliform count between two dairies using simple t test, the mean count of samples from DP_1 and DP_2 varied highly significantly ($P<0.01$) on day six and 10, whereas the counts differed significantly ($P<0.05$) from each other on day

zero, two and four. *Escherichia coli* count of DP_1 showed an increasing trend till sixth day but was not significant and samples from DP_2 showed an increase of two log after 10 days of storage.

An increase in psychrotrophic count in the samples from DP_1 and DP_2 was observed throughout the period of storage. The mean psychrotrophic count of samples on zero day of storage from DP_1 was 4.45 ± 0.06 and was found to increase to 8.17 ± 0.04 $\log_{10}$ cfu/ml on 10^{th} day of storage. A similar observation and more than 4 log increase in counts was observed in DP_2.

Faecal streptococcal count of DP_1 showed highly significant ($P<0.01$) association (0.906) with mean coliform count on day 10 of storage. A highly significant ($P<0.01$) difference of the mean faecal streptococcal count of samples of DP_1 was observed on the day two, four, and six of storage. However, the counts decreased after six days of storage. Similarly, highly significant ($P<0.01$) difference was observed between the mean count of samples on day zero and two from DP_2. However, the count in samples from DP_2 showed a gradual increase throughout the storage period.

During the study attempts were made to isolate and identify *Escherichia coli, Staphylococcus aureus* and *Pseudomonas. Escherichia coli* was isolated from the samples of DP_1 on all days except eighth and 10^{th} days of storage and a total of six isolates were obtained from DP_2. The isolates were serotyped and their invasive property was also found out by congo red binding assay. Out of four isolates from DP_1, two belonged to the serotype O116 and two belonged to rough variety. From the six isolates obtained from DP_2, two belonged to the serotype O65 and the rest were O22, O46, O95 and O116. Two out of four isolates and three out of six isolates

from DP_1 and DP_2 respectively, gave a positive congo red binding reaction indicating their property of invasiveness.

Staphylococcus aureus was also isolated from refrigerated samples and in the samples of DP_1, two isolates were from the samples stored on sixth day and three from the samples stored on zero, second and fourth day, respectively. From DP_2, three strains were isolated from the samples examined on 10^{th} day and one from fresh samples. From the 84 samples of DP_1, 19 (22.62 per cent) yielded *Pseudomonas*. The isolates were identified as *Pseudomonas putida* (7), *Pseudomonas flourescens* (6) and *Pseudomonas aeruginosa* (6). From DP_2, 17 (20.24 per cent) samples yielded the organism. The isolates were identified as *Pseudomonas putida* (8), *Pseudomonas aeruginosa* (7) and *Pseudomonas flourescens* (2).

Sensory quality of milk was evaluated by a panel of four judges who assessed the changes in colour, odour, flavour and body and the grades were assigned according to IS 7768 (1975). Sensory analysis of refrigerated milk samples from DP_1 showed a reduction in the score of colour and appearance, odour and body as the mean total score of samples on day zero was 96.29 ± 0.36 and it reduced throughout the storage period and on 10^{th} day it was 56.43 ± 0.69. The sensory quality of the samples stored on fourth day was good and thereafter the quality of milk remained as fair till eighth day and on 10^{th} day the quality was poor. Observations from DP_2 showed mean total score of samples on day zero was 96.57 ± 0.30 and it reduced throughout the storage period and on 10^{th} day it was 67.64 ± 0.51. The total scores revealed that the samples had excellent quality for upto second day of storage. From eighth day onwards the milk samples had fair quality and remained the same till the end of storage period.

All samples were subjected to Clot on Boiling (COB) test during storage. Analyses of the samples from DP_1 showed that all samples stored on 10^{th} day revealed a positive test. Seven samples stored on day eight were found COB test positive and only one sample stored on day six was found to be COB test positive. Findings from DP_2 showed that three samples stored on 10^{th} day and one sample stored on eighth day gave a positive COB test.

The retail milk samples belonging to the brands A, B, C, D, E and F were subjected for bacterial evaluation and the results revealed that the samples of brand D had the highest mean total viable count (5.94 ± 0.09 $\log_{10}$ cfu/ml), psychrotrophic count (5.09 ± 0.16 $\log_{10}$ cfu/ml) and faecal streptococcal count (2.87 ± 0.24 $\log_{10}$ cfu/ml). Highest count of coliform was seen in the samples of the brand A (2.40 ± 0.14 $\log_{10}$ cfu/ml) and *Escherichia coli* count in the samples of the brand C. Low counts especially total viable count (4.89 ± 0.79 $\log_{10}$ cfu/ml) and *Escherichia coli* count (3.44 ± 0.72 $\log_{10}$ cfu/ml) were seen in the samples belonging to the brand F. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between various bacterial counts *viz.*, total viable count, coliform count, *Escherichia coli* count and psychrotrophic count of samples from six brands which indicate that the quality of different brands of pasteurized milk retailed in the area varied greatly.

Isolation of bacterial organisms of public health significance revealed that out of 72 retail samples, 15 (20.83 per cent) had *Escherichia coli*. The isolates belonged to the serotypes O46 (3), O116 (4), O65 (2), O95 (1), UT (2) and Rough (3). Out of 15 isolates, six were positive for congo red binding test indicating their property of invasiveness.

Staphylococcus aureus was present in six samples (8.3 per cent). The organism was isolated from three samples of brand C and one sample each of brand

B, D and E. A total of 16 samples (22.22 per cent) yielded *Pseudomonas* and the isolates were identified as *Pseudomonas putida* (7), *Pseudomonas aeruginosa* (6) and *Pseudomonas flourescens* (3).

Polymerase chain reaction process was employed to detect the sensitiveness of the technique in identifying the isolates obtained from milk. The isolates of *Escherichia coli* were subjected to polymerase chain reaction and a specific PCR product with 366 bp fragment was obtained.

The hygienic practices followed during the production of milk and pasteurization, storage and retailing needs an improvement with regard to reduction in bacterial count and overcoming the impact of harmful pathogens like *Escherichia coli* and *Staphylococcus aureus*. The presence of coliform, faecal streptococci and *Escherichia coli* in milk indicated contamination of milk from the environmental sources and also from human and animal sources. Hence strict hygiene and health education needs to be implemented to minimize contamination occurring in the processing plants and retailing chains. The concept of quality and food safety (HACCP) management system needs to be strictly adopted.

Reference

REFERENCES

Aaku, E.N., Collison, E.K., Gashe, B.A. and Mapuchane, S. 2004. Microbiological quality of milk from two processing plants in Gaborone Botswana. *Fd. Control.* 15: 181-186

Abhilasha, Singh, S.P. and Gupta, R.S. 2001. Pathogenicity of *Escherichia coli* strains by congo red binding test. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 22: 172-173

Adesiyun, A.A., Webb, L.A. and Romain, H.T. 1998. Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. *J. Fd. Prot.* 61: 629-632

Allmann, M., Hofelein, C., Koppel, E., Luthy, J., Meyer, R., Niederhauser, C., Wegmuller, B. and Candrian, U. 1995. Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. *Res. Microbiol.* 146: 85-97

Animal Husbandry Statistics. 2005. Current topics. *Processed Food Industry.* 8: 38

Arora, A. and Sudarsanam, T.S. 1986. Microbiological quality of milk and spray dried skim milk powder used as ice cream ingredients in Karnal. *J. Fd. Sci. Technol.* 23: 170-172

Barrow, C.J. and Feltham, R.K.A. 1993. *Cowan and Steel's manual for the Identification of Medical Bacteria.* Third edition. Cambridge Press, London, p.238

Beloti, V., Barros, M.A.F., Nero, L.A., Pachemshy, H.A.S., Santana, E.H.W. and Franco, B.D.G.M. 2002. Quality of pasteurized milk influences the performance of ready to use systems for enumeration of aerobic microorganisms. *Int. Dairy J.* 12: 413-418

Boor, K.J., Brown, D.B., Murphy, S.C., Kozlowski, S.M. and Bandler, D.K. 1998. Microbiological and chemical quality of raw milk in Newyork state. *J. Dairy Sci.* 81: 1743-1748

Brown, J.V., Ranjith, H.M.P. and Prentice, G.A. 1984. Comparative shelf lives of skimmed, semi-skimmed and whole milks. *J. Soc. Dairy Technol.* 37: 132-135

Carmo, L.S., Dias, R., Linardi, V.R., Sena, M.J., Santos, D.A.D., Faria, M.E., Pena, E.C., Jett, M. and Heneine, L. 2002. Food poisoning due to enterotoxigenic strains of *Staphyloccus* present in Minas Cheese and Raw milk in Brazil. *Fd. Microbiol.* 19: 9-14

*Cerqueira, M.M.O.P., Souza, M.R., Rodriguez, R., Fonseca, L.M., Rubinich, J. and Quintaes, I.A.S. 1994. Microbiological characteristics of raw and pasteurized milk from Belo Horizonte, Brazil. *Arq. Braz. Med. Vet. Zootec.* 46: 713-721

Chye, F. Y., Abdullah, A. and Ayob, M.K. 2004. Bacteriological quality and safety of raw milk in Malaysia. *Fd. Microbiol.* 21: 535-541

Cousin, M.A., Jay, J.M. and Vasavada, P.C. 2001. Psychrotrophic microorganism. *Compendium of methods for the microbial examination of foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington D.C, pp. 159-164

Cosentino, S. and Palmas, F. 1997. Hygienic conditions and microbial contamination in six ewe's milk processing plants in Sardina, Italy. *J. Fd. Prot.* 3: 283-287

Da Silva, M.C.D., Hofer, E. and Tibana, A. 1998. Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil. *J. Fd Prot.* 61:354-356

Daly, P., Collier, T. and Doyle, S. 2002. PCR-ELISA detection of *Escherichia coli* in milk. *Lett. Appl. Microbiol.* 34: 222-226

Davies, D.G. 1977. The bacteriological quality of bulk and churn collected milk supplies in Wales. *J. Soc. Dairy Technol.* 30: 52-59

Desai, S.C. and Natarajan, A.M. 1981. Bacteriological quality of raw milk collected from societies for transportation to chilling centers. *Cheiron.*10: 146-150

Desmarchelier, P.M., Bilge, S.S., Fegan, N., Mills, L., Vary, J.C. Jr. and Tarr, P.I. 1998. A PCR specific for *Escherichia coli* O157 based on the *rfb* locus encoding O157 lipopolysaccharide. *J. clin. Microbial.* 36: 1801-1804

*Desmasures, N., Bazin, F. and Gueguen, M. 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy. *J. Appl. Microbiol.* 83: 53-58

Dogan, B. and Boor, K. J. 2003. Genetic diversity and spoilage potential among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. *Appl. Environ. Microbiol.* 69: 130-138

Edwards, P.R. and Ewing, W.H. 1972. Identification of Enterobacteriaceae. Third edition. Burgess Publishing Company, Georgia, p.362

Eneroth, A., Christiansson, A., Brendehaug, J. and Molin, G. 1998. Critical contamination sites in the production line of pasteurized milk, with

special reference to the psychrotrophic spoilage flora. *Int. Dairy J.* 8: 829-831

Foster, E.M. 1990. *Perennial issues in food safety.* In: Cliver, D.O (Ed.), Food-borne Diseases. Academic Press, San Diego, pp. 369-381

Fromm, H.I. and Boor, K.J. 2004. Characterization of pasteurized fluid milk shelf life attributes. *J. Fd. Sci.* 69: 207-213

Garg, D.N., Bhargava, D.N. and Narayan, K.G. 1977. Pathogenic bacterial flora of raw market milk. *Indian J. Dairy Sci.* 30: 36-39

Garg, S. R. and Mandokhot, U.V. 1997. Reliability of rapid and routine quality control tests for grading raw milk under Indian conditions. *J. Fd. Sci. Technol.* 34: 357-359

Ghosh, S.S. and Laxminarayana, H. 1972. Enterotoxigenic *Staphylococci* in milk and milk products. *Indian J. Anim. Sci.* 42: 492-498

Gill, J.P.S., Joshi, D.V. and Kwatra, M.S. 1994. Qualitative bacteriological survey of milk and milk products with special reference to *Staphylococcus aureus*. *Indian J. Dairy Sci.* 47: 680-682

Gopi, H., Parthiban, M. and Dhanalakshmi, B. 2001. Bacteriological quality of private brands of milk in Chennai city. *Cheiron.* 30:106-107

Gran, H.M., Wetlesen, A., Mutukumira, A.N., Rukure, G. and Narvhus, J.A. 2003. Occurrence of pathogenic bacteria in raw milk; cultured pasteurized milk and naturally soured milk produced at small-scale dairies in Zimbabwe. *Fd. Control.* 14: 539-544

Griffiths, M.W. and Philips, J.D. 1988. Modelling the relation between bacterial growth and storage temperature in pasteurized milk of varying hygienic quality. *J. Soc. Dairy Technol.* 41: 96-102

Grover, S. and Srinivasan, R.A. 1988. Isolation and characterization of *Pseudomonas aeruginosa* from milk and milk products. *Indian J. Dairy Sci.* 41: 326-329

Gruetzmacher, T.J. and Bradley, R.L. 1999. Identification and control of processing variables that affect the quality and safety of fluid milk. *J. Fd. Prot.* 6: 625-631

Gupta, S. 2007. *Dairy Industry Profile 2005*. Sixth edition. Thomson Press India Limited. p.836

Homhual, S. and Jindal, V.K. 2001. Simple tests for rapid assessment of the quality of raw milk. *J. Fd. Prot.* 64: 1996-2000

Indian standard. 1975. IS: 7768. *Method for Sensory Evaluation of Milk.* Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi – 1.p.8

Indian standard. 1977. IS: 1479. *Methods for text for dairy industry.* Part III. Bacteriological analysis of milk (First revision). Bureau of Indian Standards, Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi – 1.p.25

Indian Standards. 1980. SP: 18. *ISI Hand Book of Food Analysis.* Part I. Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi- 1. p. 85

Indian Standards. 1981. SP: 18. *Handbook of food analysis -Part XI. Dairy products*. Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi-1. p.171

Indian Standards. 1992. IS: 13688. *Pasteurized milk-specification* (First revision). Bureau of Indian Standards, Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi – 1. p. 4

Jaibi, K. 2006. Bacterial quality of raw milk at the co-operative society level with special reference to quality assurance programme. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.178

Jain, P.C. and Saraswat, D.S. 1968. Studies on bacteriological quality of market milk in Udaipur city -2. Enumeration of thermoduric and psychrophillic bacteria in raw milk. *Indian J. Dairy Sci.* 21: 238-243

Jay, J.M. 2005. *Modern Food Microbiology*. seventh edition. Chapman and Hall, New York, p.790

John, R. 1999. Bacterial profile of pasteurized milk and its public health importance. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.85

John, S., Jacob, R.R., Nanu, E. and Sunil, B. 2003. Isolation and identification of bacteria of public health significance from retail pasteurized milk. Poster abstracts of fifth international food convention, 5-8 December 2003, Mysore, India. Abstract: 57

Jolly, D., Nanu, E. and Sunil, B. 2000. Evaluation of certain quality characteristics of raw market milk. *Smallholder Livestock Production System in Developing Countries*. p. 451-458

Jothikumar, N. and Griffiths, M.W. 2002. Rapid detection of *Escherichia coli* O157: H7 with multiplex real-time PCR assays. *Appl. Environ. Microbiol.* 68: 3169-3171

Kadan, P.S. and Bhanumathi, J.L. 1984. Comparative studies on the packaging of milk in glass bottles and polyethelene pouches. *J. Fd. Sci. Technol.* 21: 353-355

*Kaloianov, I. and Gogov, I. 1977. Coliform bacteria in raw and pasteurized milk. *Vet. Med. Nauki.* 14: 46-52

Kapre, A.R. 1995. Assessment of bacteriological quality of raw milk in Trichur and its public health importance. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.107

Khalilur, M., Khan, R. and Malik, A. 2002. Microbiological quality of milk, vegetables and fruit juices. *J. Fd. Sci. Technol.* 39: 120-123

Kornacki, J.L. and Johnson, J.L. 2001. *Enterobacteriaceae*, coliform and *Escherichia coli* as quality and safety indicators. *Compendium of Methods for the Microbial Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American public health association. Washington, DC, pp. 75-92

*Krasz, A., Zapletal, Z. and Boka, E. 1991. Examination of 1) Lesion of milk fat 2) Changes during storage of pasteurized milk. *Tejipar.* 41: 2-4

Kumar, S.S., Batish, V.K. and Grover, S. 2001. Rapid detection of *Escherichia coli* in milk by 'uidR' targeted PCR assay. *Indian J. Dairy Sci.* 54: 129-137

Lancette, G.A. and Bennett, R.W. 2001. *Staphylococcus aureus* and staphylococcal enterotoxins. *Compendium of methods for the microbiological examination of foods* (eds. Downes, F.P. and Ito, K.) Fourth edition. American public health association, Washington DC, pp. 387-403

Latha, C. and Nanu, E. 1997. Bacteriological quality of pasteurized milk and its public health significance. Proceedings of the ninth Kerala Science Congress, Kerala, pp. 115-117.

Ledford, R.A., Senyk, G.F., Kotsides, E. and Wolff, E.T. 1983. Preliminary incubation method for detection of coliform in commercially processed milk samples. *Indian J. Dairy Sci.* 66: 1607-1610

Lindberg, A.M., Ljungh, A., Ahrne, S. and Molin, G. 1998. Enterobacteriaceae found in high numbers in fish, minced meat and pasteurized milk or cream and the presence of toxin encoding genes. *Int. J. Fd. Microbiol.* 39: 11-17

*Lopes, A.C. and Stamford, T.L. 1997. Critical control points in the pasteurized milk processing fluxogram. *Latinoam Nutr.* 47: 367-371

*Mahari, T. and Gashe, B.A. 1990. A survey of the microflora of raw and pasteurized milk and the sources of contamination in a milk processing plant in Addis Ababa, Ethiopia. *J. Dairy Res.* 57: 233-238

Mamani, Y., Quinto, E.J., Simalgandara, J. and Mora, M.T. 2003. Growth and survival of *Escherichia coli* 0157:H7 in different types of milk stored at 4°C or 20°C. *J. Fd. Sci.* 68: 2558-2563

Misra, A.K. and Kuila, R.K. 1989. Bacteriological quality of market milk. *Indian Dairyman.* 101: 487-491

Mohan, K. and Misra, S.K. 1967. *Staphylococcus aureus* in milk supplied to Patna milk supply scheme. *Indian J. Dairy Sci.* 20:178-180

Montville, T.J. and Matthews, K.R. 2005. *Food Microbiology: An Introduction.* ASM Press, Washington, DC. p. 380

Mortan, D.R. 2001. Aerobic plate count. *Compendium of methods for the microbial examination of foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington DC, pp. 63-68

Moyssiadi, T., Badeka, A., Kondyli, E., Vakirtzi, T., Savvaidis, I. and Kontominas, M.G. 2004. Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensory aspects. *Int. Dairy J.* 14: 429-436

Muir, D. 1996. The shelf life of dairy products: 1. Factors influencing raw milk and fresh products. *J. Soc. Dairy Tech.* 49: 24-32

Mukundan, M. 1978. Studies on microflora in boiled milk. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.144

Mutukumira, A.N., Feresu, S.B., Narvhus, J.A. and Abrahamsen, R.K. 1996. Chemical and Microbiological quality of raw milk produced by smallholder farmers in Zimbabwe. *J. Fd. Prot.* 59: 984-987.

Nanu, E., Latha, C., Sunil, B., Prejit, Thomas, M. and Menon, K.V. 2007. Quality assurance and public health safety of raw milk at the production point. *Am. J. Fd. Technol.* 2: 145-152

Nordic committee on Food Analysis. 1968. *Determination of faecal streptococci in foods.* 68. UDC. 576, 851, 21

Oksuz, O., Arici, M., Kurultay, S. and Gumus, T. 2004. Incidence of *Escherichia coli* 0157 in raw milk and white pickled cheese manufactured from raw milk in Turkey. *Fd. Control* 15: 453-456

Patel, A.D., Siva, C.V. and Sannabhadti, S.S. 1993. Sources of microbial contamination of raw milk. *Indian J. Dairy Sci.* 46: 65-70

*Pelezynska, E. and Libett, K.1995. Analyses of hygienic risk factors and CCP in milking and processing of raw milk for consumption. *Medycyna Weterynaryjna.* 51: 396-399

Prejit. 2005. Microbial quality assurance of milk in its production, processing and storage. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.157

Prejit, Nanu, E. and Latha, C. 2006. Effect of pasteurization and refrigeration storage (1 week) on microbial quality of milk. *J.Vet. Pub. Hlth.* 4: 37-42

Prejit, Nanu, E. and Latha, C. 2007. Microbial quality assurance of milk during production, processing and Marketing. *Am. J. Fd. Technol.* 2: 136-144

Rahman, H., Nath, N.C. and Boro, B.R. 1992. Bacterial flora and insecticidal residue in raw milk marketed in Guwahati city, Assam. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 13: 105-108

Rai, C.K. and Dwivedi, H.B. 1990. Study of the bacterial quality of milk supplied in Kanpur city by different sources. *Indian Dairyman.* 102: 520-523

Raj, B.R., Nanu, E., Sunil, B. and Sujatha, K.S. 2003. Evaluation of bacterial quality and detection of bacterial pathogens from raw milk. *Proceedings of fifteenth Kerala Science Congress. 29-31 January 2003.* Thiruvanthapuram, pp. 319-325

Rajmany, L. Garg, S. K., Yadav, R.K. 1989. Incidence of Staphylococci in milk and milk products. *Indian J. Dairy Sci.* 42: 136-138

Rajil, M.A., Adekeyel, J.O., Kwaga, J.K.P. and Bale, J.O.O. 2003. In vitro and in vivo pathogenicity studies of *Escherichia coli* isolated from poultry in Nigeria. *Israel J. Vet. Med.* 58:81-87

Raju, V.V.R. and Nambudripad, V.K.N. 1987. Incidence and growth of heat resistant coliforms bacteria in milk and other media. *Indian J. Dairy Sci.* 40 : 354-358

Rangaswamy, R. 1995. *A Textbook of Agricultural Statistics*. New Age International Publishers Ltd, New Delhi, p. 495

*Rca, M. C., Cogan, T.M. and Tobin, S. 1992. Incidence of pathogenic bacteria in raw milk in Ireland. *J. Appl. Bact.* 73: 331-336

Reddy, N.S., Rao, V.J. and Venkayya, D. 1984. Studies on bacteriological quality of dried whole milk in relation to the initial quality of raw milk. *J. Fd. Sci. Technol.* 21: 415-417

Reddy, I.S., Reddy, R.R.K.K., Jairam, A. and Venkayya, D. 1989. Bacteriological quality of cow milk. *Indian J. Dairy Sci.* 42: 650-652

Reid, S.D., Betting, D.J. and Whittam, T.S. 1999. Molecular detection and identification of intimin alleles in pathogenic *Escherichia coli* by multiplex PCR. *J. Clin. Microbiol.* 37: 2719-2722

*Reinheimer, J.A. and Suarez, V.B. 1992. Influence of storage temperature on the keeping quality of pasteurized (HTST) milk. *Revista Argentina de Lactologia.* 4: 47-65

Robinson, R.K. 2002. *Dairy Microbiology Hand book; The microbiology of milk and milk products.* Third edition. John Wiley and sons, Newyork, p. 765

Sakkarvarthi, S., Mathur, D.K. and Malik, R.K. 1990. Bacterollogical quality of buffalo milk in relation to processing at high temperatures. *Indian J. Dairy Sci.* 43:207-212

*Saleha, A.A. 1992. Psychrotrophic bacteria in pasteurized milk in Malaysia. Proceedings of the third world congress on food borne infection and intoxications, Berlin, pp. 190-192

Schroder, M.J.A., Cousins, C.M. and Mckinnon, C.H. 1982. Effect of psychrotrophic post-pasteurization contamination on the keeping quality at 11 and 5°C of HTST – pasteurized milk in the U.K. *J. Dairy Res.* 49: 619-630

Sen, G.P., Haldar, S.K. and Aich, A.C. 1989. Healthy bovine udder as a source of enterotoxigenic Staphylococcus. *Indian J. Al. Hlth.* 28: 163-165

Sethulakshmi, C., Jacob, R.R., Nanu, E. and Sunil, B. 2003. Bacterial quality of toned pasteurized milk retailed in and around Thrissur. Poster abstracts of fifth international food convention, 5-8 December 2003, Mysore, India, *Abstract*: 57

Shah, N.M., Rajput, H.A., Dholakia, P.M. and Simaria, M.B. 1984. Studies on bacterial flora from milk secretion of healthy cows. *Indian Dairyman.* 37: 323-324

Sharma, D.K., Singh, N. and Joshi, D.V. 1995. *Escherichia coli* in milk, meat and meat products: Isolation, characterization, Antibiogram and Zoonotic significance. *J. Fd. Sci. Technol.* 32: 409-412

Silva, Z.N., Cunha, A.S., Lins, M.C., Carneiro, L.A.M., Almeida, A.C.F. and Queiroz, M.L.P. 2001. Isolation and serological identification of

enteropathogenic *Escherichia coli* in pasteurized milk in Brazil. *Rev. Saude Publs.* 35:375-379

Singh, R.S. and Ranganathan, B. 1978. Incidence and distribution of *Escherichia coli* in dairy products. *Indian J. Dairy Sci.* 31: 82-84

Singh, K. and Sinha, R.N. 1981. Rapid detection of coliforms in pasteurized milk. *Indian J. Dairy Sci.* 34: 305-309.

Singh, R.R.B. and Patil, G.R.1987. Physico-chemical and sensory changes during processing and storage of UHT milk. *Indian Dairyman.* 39: 209-214

Singh, Y., Kumar, A. and Sharma, V.D. 1994. Sanitary analysis of milk, cream and burfi. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 15: 12-14

Singh, B.R., Kapoor, K.N., Kumar, A., Agarwal, R.K. and Bhilegaonkar, K.R. 1996. Prevalence of Enteropathogens of Zoonotic significance in meat, milk and their products. *J. Fd. Sci. Technol.* 33: 251-254

Sinha, R.N., Singh, I.P. and Nambudripad, V.K.N. 1968. Studies on keeping quality of pasteurized milk. *Indian J. Dairy Sci.* 21: 1-5

Siva, C.V., Patel, D.A. and Sannabhadti, S.S. 1993. Microbiological status of raw and pasteurized milk. *Indian J. Dairy Sci.* 46: 62-66

Steel, M.L., McNab, W.B., Poppe, C., Griffiths, M.W., Chen, S., Degrandis, S.A., Fruhner, L.C., Larkin, C.A., lynch, J.A. and Odumeru, J.A. 1997. Survey of Ontario bulk tank raw milk for food-borne pathogens. *J. Fd. Prot.* 60: 1341-1346

Sutherland, A.D., Limond, A.M., MacDonald, F. and Hirst, D. 1993. Evaluation of the incubated plate count test for pasteurized milk. *J. Soc. Dairy Technol.* 46: 107-113

Ternstrom, A., Lindberg, A.M. and Molin, G. 1993. Classification of the spoilage flora of raw and pasteurized bovine milk, with special reference to *Pseudomonas* and *Bacillus*. *J. Appl.bact.* 75:25-34

Van Aardt, M., Duncan, S.E., Marcy, J.E., Long, T.E. and Hackey, C.R. 2001. Effectiveness of polyethylene terephthalate and high-density polyethylene in protection of milk flavour. *J. Dairy Sci.* 84: 1341-1347

Vassila, E., Badeka, A., Kondyli, E., Savvaidis, I. and Kontominas, M.G. 2002. Chemical and microbiological changes in fluid milk as affected by packaging conditions. *Int. Dairy J.* 12: 715-722

Vijai, R.G. and Saraswat, D.S. 1968. Studies on bacteriological quality of market milk in Udaipur city-1. Enumeration of standard plate and coliform counts in raw and pasteurized milk. *Indian J. Dairy Sci.* 21: 233-237

Watson, P.M. and Mc Ewan, J.A. 1995. Sensory changes in liquid milk during storage and the effect on consumer acceptance. *J. Soc. Dairy Technol.* 48: 1-8

Yadav, J.S., Grover, S. and Batish, V.K. 1992. *A Comprehensive Dairy Microbiology.* Metropolitan, New Delhi, p.764

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1983. Bacteriological quality of market milk at Ranchi. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 4: 215-219

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1985. Bacterial flora of market milk and its public health importance. *Indian J. Dairy Sci.* 38: 235-237

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1987. Serotypes of *Escherichia coli* in market milk. *Indian J. Dairy Sci.* 23: 466-467

Yokoigawa, K., Inoue, K., Okubo, Y. and Kawai, H. 1999. Primers for amplifying an alanine racemase gene fragment to detect *Escherichia coli* strains in foods. *J. Fd. Sci.* 64: 571-575

Zygora, P., Moyssiadi, T., Badeka, A., Kondyli, E., Savvaidis, I. and Kontominas, M.G. 2004. Shelf life of whole pasteurized milk in Greece: Effect of packaging material. *Fd. Chem.* 87: 1-9

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ASSESSMENT OF BACTERIAL QUALITY AND SHELF LIFE OF PASTEURIZED MILK

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ABSTRACT

In the present study raw and pasteurized milk samples were collected from two processing plants *viz.*, DP_1 and DP_2 and pasteurized milk from retail shops. A total of 254 samples were analyzed for the bacterial quality by estimating various bacterial counts and also assessed the presence of certain bacteria of public health importance. The bacterial, physical and organoleptic qualities of pasteurized milk samples from two dairies stored under refrigeration (4 ± 1°C) were evaluated.

Raw milk revealed an inferior bacterial quality with 50 per cent samples graded as fair (based on total viable count) and 85.7 per cent as poor quality (based on coliform count). The total viable count from both dairies was obtained at the level of 7 log_{10} cfu/ml but coliform count was high in the samples obtained from DP_1 (3.34 ± 0.05 log10 cfu/ml). The psychrotrophic count and faecal streptococcal count in the samples belonging to both sources were at the level of 7 and 3 log_{10} cfu/ml, respectively. Bacteria of public health significance like *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* was detected from a few samples. Pasteurization reduced the level of total viable count, coliform count, psychrotrophic count and faecal streptococcal count to a highly significant ($P<0.01$) level.

Pasteurized milk under refrigeration (4 ± 1°C) showed an increase in total viable count and psychrotrophic count throughout the storage period with a difference of more than 3 log with that of fresh sample. However, coliform count, *Escherichia coli* count, and faecal streptococcal count of samples belonging to DP_1 initially showed increasing tendency up to six days and thereafter the counts decreased. The increase in total viable count, coliform count, *Escherichia coli* count, psychrotrophic count and faecal streptococcal count between zero and 10^{th} day from DP_2 was 4.8, 1.95, 2.08, 4.78 and 2.32 log_{10} cfu/ml, respectively. The increase in

the counts during storage may lead to the reduction in shelf life due to bacterial deterioration of milk. Isolates of *Escherichia coli* was obtained from DP_1 on all days except eighth and 10^{th} day. A total of six isolates were obtained from DP_2. The isolates belonged to O116 (3), O22, O46, O65 (2), O95 and the rest were rough variety. *Staphylococcus aureus* was also isolated from two samples stored on sixth day and three from the samples stored on zero, second and fourth day, respectively (DP_1). From DP_2, three isolates were obtained from the samples stored on 10^{th} day and one from fresh samples. A total of 22.62 and 20.24 per cent *Pseudomonas* were isolated from DP_1 and DP_2, respectively and the isolates were identified as *Pseudomonas putida*, *Pseudomonas aeruginosa* and *Pseudomonas flourescens*.

Sensory and physico-chemical (COB test) analyses of refrigerated milk samples showed an overall reduction in the score of colour and appearance, flavour, odour and body as the storage period increased. The mean total scores from DP_1 revealed that the samples were of excellent quality for up to second day of storage. The sensory quality of the samples stored on fourth day was good and then the quality of milk remained fair till eighth day and on 10^{th} day the quality became poor. In DP_2 samples had excellent quality for upto second day of storage. The sensory quality of the sample stored up to sixth day was good and thereafter the quality of milk remained as fair till the end of storage period. COB test of samples from DP_1 showed positive test on all samples stored on 10^{th} day. However, one sample stored on day six was COB test positive. The samples belonging to DP_2 showed that three samples stored on 10^{th} day and one sample stored on eighth day was COB positive.

The bacterial profile of the retail milk samples of the brands A, B, C, D, E and F was assessed and the samples belonging to the brand D had highest mean total viable count (5.94 ± 0.09 log10 cfu/ml), psychrotrophic count (5.09 ± 0.16 $\log_{10}$ cfu/ml) and faecal streptococcal count (2.87 ± 0.24 $\log_{10}$ cfu/ml). Highest coliform

count was seen in the samples of brand A (2.40 ± 0.14 $\log_{10}$ cfu/ml) and *Escherichia coli* count (3.44 ± 0.72 $\log_{10}$ cfu/ml) in samples of the brand C. Low counts especially total viable count (4.89 ± 0.79 $\log_{10}$ cfu/ml) and coliform count (1.19 ± 0.42 $\log_{10}$ cfu/ml) were seen in the samples of the brand F.

Escherichia coli were detected from 20.8 per cent samples and the isolates consisted of the serotypes O46, O65, O95, O116, O166 and O171. Out of 15 isolates obtained six showed a positive congo red reaction indicating their property of invasiveness. *Staphylococcus aureus* was isolated from only six samples (6.94 per cent). All retail milk samples were also tested for the isolation and identification of *Pseudomonas* and the organism was isolated from 16 (22.22 per cent) samples. The isolates were identified as *Pseudomonas putida* (7), *Pseudomonas aeruginosa* (6) and *Pseudomonas flourescens* (3). Polymerase Chain reaction was employed to identify and confirm the *Escherichia coli* isolates obtained from the milk samples and a 366 bp product was obtained.

Flowchart 1. Collection of samples