MICROBIAL QUALITY ASSURANCE OF MILK IN ITS PRODUCTION, PROCESSING AND STORAGE

PREJIT

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

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

PREJIT

Mannuthy

19/8/05

CERTIFICATE

Certified that the thesis entitled **"MICROBIAL QUALITY ASSURANCE OF MILK IN ITS PRODUCTION, PROCESSING AND STORAGE"** is a record of research work done independently by **Dr. Prejit,** 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 him.

Mannuthy

Dr. E. Nanu 19/5/05

(Chairman, Advisory Committee)
Professor and Head
Department of Veterinary Public Heath,
College of Veterinary and Animal Sciences
Mannuthy, Thrissur.

CERTIFICATE

We, the undersigned members of the Advisory Committee of Dr. Prejit, a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled **"MICROBIAL QUALITY ASSURANCE OF MILK IN ITS PRODUCTION, PROCESSING AND STORAGE"** may be submitted by Dr. Prejit, in partial fulfillment of the requirement for the degree.

19/8/05
Dr. E. Nanu
(Chairman, Advisory Committee)
Professor and Head
Department of Veterinary Public Health
College of Veterinary and Animal Sciences
Mannuthy

19/8/05
Dr. C. Latha
Assistant Professor
Department of Veterinary Public Health
College of Veterinary and Animal Sciences
Mannuthy
(Member)

19/8/05
Dr. R. Rajendrakumar
Associate Professor & Head
KAU Dairy Plant
Mannuthy
(Member)

Dr. M. Mukundan
Associate Professor and Head
Department of Dairy Science
College of Veterinary and Animal Sciences
Mannuthy
(Member)

External Examiner
Dr. M. Sathyanarayana Rao

ACKNOWLEDGEMENT

I find myself on look out for words as I place on record my sincere and heartfelt gratitude to the Chairperson of the Advisory Committee ***Dr. E. Nanu,*** *Professor and head, Department of Veterinary Public Health, for his meticulous guidance, personal attention, keen interest, affectionate encouragement, persuasion and unstinted help offered to me from the initiation of work to the ship shaping of the manuscript. I reckon it a rare privilege to work under his counsel and indomitable spirit.*

I humbly express my deep sense of gratitude to ***Dr. C. Latha,*** *Assistant Professor, Department of Veterinary Public Health, for the generous encouragement, inspiration and personal guidance in the pursuit of this research work.*

I owe my sincere gratitude to ***M. Mukundan,*** *Associate Professor and Head, Department of Dairy Science for his valuable guidance, critical comments, timely help, moral support and affection rendered during the entire period of research work.*

I am cordially obliged to ***Dr. R. Rajendrakumar,*** *Associate Professor and Head, KAU Dairy Plant, for the supporting attitude, guidance and pleasant co-operation and help rendered to me as a member of my advisory committee.*

I am grateful to ***Dean,*** *College of Veterinary and Animal Sciences, Mannuthy and Kerala Agricultural University for the facilities provided for the conduct of this research work.*

I would like to place on record my heartfelt thanks to ***Dr. Satyanarayana Rao, Dr. Prabhakaran,*** *and* ***Dr. B. Sunil*** *for the encouraging advices and inimitable help.*

I am thankful for the help rendered for statistical analysis of data by ***Smt. K.A. Mercy,*** *Assistant Professor and Smt.* ***Sujatha,*** *Assistant Professor and Head, Department of Statistics.*

I am in short of words to express my deep sense of gratitude to my colleague ***Dr. R Praseeda,*** *whose sisterly support and constant encouragement have helped me to successfully complete the research work.*

No words or deeds are sufficient to express my gratitude to ***Dr. Vrinda Menon, Dr. Ambily, Dr. Siji, Dr. Magna, Dr. Raji, Dr. Sethu, Dr.Deepa Surendran, Dr Shameen and Dr. Binsy*** *for all the incessant support, continuous guidance they have showered on me.*

I cherish the spirit of understanding and personal encouragement rendered to me by my friends, ***Drs. Lekha, Jaibi, Sreeja and Shymaja.***

Words possess no enough power to reflect my thankfulness for the invaluable help, moral support, affection and pleasure rendered by my great friends ***Drs, Jegan, Muthu, Rishi, Balaji, Senthil, Giri, Sivanesan*** *and* ***Raja.***

*I gratefully acknowledge the help rendered by friends and batchmates **Dr. Rajesh, Deepak, Binoy, Jayanth, Laiju, Ratheesh, Jothish, Dileep, Magnus paul, Ajith, Dipu and Philip** in the progress of my work.*

*A special thanks to **Dr. Ajit, Dr. Antony, Dr. Rajaganapati,** and **Dr. Murugan** individually for being of great support to me during the various stages of my studies and research work.*

I treasure the invaluable friendship of My seniors and hostel inmates for all the valuable suggestions and moral support rendered to me during my research

*The generous help, assistance, guidance and support rendered to me by my friends **Drs. Shanmugam, Paulson, Lu, Vikram, Lonkar, Vivek, Rajagopal, Thampan, Hamsa, Ari, Rana,** and others are duly acknowledged.*

*A special thanks to the Librarian Central Institute of Fisheries Technology (CIFT), Cochin, to provide facility to collect reference pertaining to my research work and to **Dr. H.G. Brahmne,** Joint Director and Head, National Salmonella and Escherichia Centre, Central Research Institute, Kasauli for serotyping the E. coli isolates and sending the results on time.*

*I do express my very special and sincere thanks to **Santha auntie, Rekha and Arun** for their cordiality, concern and love.*

*I wish to extend my thanks to **Rajesh, Sreedharan** and other employees of the dairy farm and plant whose assistance has helped me to collect samples.*

*I also thankful to **Mr. Sundaran, Mrs. Suhara, Ms. Priya, Mr. Dhanush, Ms. Sandhya, Ms. Anne, Mr. Anush** for the co-operation rendered to me during my study.*

*No phrase or words in any language can ever express my deep sense of love and gratitude to my beloved **Achan, Amma, Borthers and Sister-in-law** being always with me through thick and thin.*

Above all I bow before the Almighty for all the blessings showered upon me.

Prejit

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
3	MATERIALS AND METHODS	36
4	RESULTS	56
5	DISCUSSION	108
6	SUMMARY	136
	REFERENCES	142
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Grading of milk according to IS 7768 (1975)	50
2	Mean total viable count of raw milk samples	57
3	Distribution of individual, pooled and chilled milk samples based on total viable count	57
4	Mean coliforms count of raw milk samples	58
5	Distribution of individual, pooled and chilled milk samples based on coliform count	59
6	Mean *Escherichia coli* count of raw milk samples	59
7	Distribution of individual, pooled and chilled milk samples based on *Escherichia coli* count	60
8	Mean psychrotrophic count of raw milk samples	60
9	The distribution of individual, pooled and chilled milk samples based on psychrotrophic count	61
10	Mean faecal streptococcal count of raw milk samples	61
11	Distribution of individual, pooled and chilled milk samples based on faecal streptococcal count	62
12	Mean Yeast and mould count of raw milk samples	62
13	Distribution of individual, pooled and chilled milk samples based on Yeast and mould count	63
14	Correlation between microbial counts of raw milk	63
15	Bacteria isolated from pooled raw milk	64
16	Distribution of *E. coli* serotypes from pooled raw milk	64
17	Congo red binding test of *E. coli* isolates from raw milk	65
18	Mean total viable count of milk obtained during various stages of pasteurization	66
19	Distribution of milk obtained during various stages of pasteurization based on total viable count	67

20	Mean Coliform count of milk obtained during various stages of pasteurization	67
21	Distribution of milk obtained during various stages of pasteurization based on coliform count	68
22	Mean *Escherichia coli* count of milk during various stages of pasteurization	68
23	Distribution of milk obtained during various stages of pasteurization based on *Escherichia coli* count	69
24	Mean psychrotrophic count of milk obtained during various stages of Pasteurization	69
25	Distribution of milk obtained during various stages of pasteurization based on psychrotrophic count	70
26	Mean faecal streptococcal count of milk obtained during various stages of pasteurization	70
27	Distribution of milk obtained during various stages of pasteurization based on faecal streptococcal count	71
28	Mean Yeast and mould count of milk obtained during various stages of pasteurization	71
29	Distribution of milk obtained during various stages of pasteurization based on Yeast and mould count	72
30	Correlation between microbial counts in packaged milk sample	72
31	Bacteria isolated from freshly pasteurized and packed milk	73
32	Effect of pasteurization on the microbial quality of milk	74
33	Mean total viable count of retail milk sample	75
34	Distribution of retail milk based on total viable count	76
35	Mean coliform count of retail milk sample	77
36	Distribution of retail milk based on Coliform Count	78
37	Mean *Escherichia coli* Count of retail milk sample	78
38	Distribution of retail milk based on *Escherichia coli* Count	79
39	Mean psychrotrophic Count of retail milk sample	80
40	Distribution of retail milk based on Psychrotrophic Count	80

41	Mean faecal streptococcal count of retail milk sample	81
42	Distribution of retail milk based on faecal streptococcal Count	81
43	Mean Yeast and Mould count of retail milk sample	82
44	Distribution of retail milk based on yeast and mould count	82
45	Bacteria isolated from retail milk	83
46	Mean total viable count refrigerated milk samples	84
47	Distribution of refrigerated milk samples based on TVC	85
48	Mean Coliform count refrigerated milk samples	86
49	Distribution of refrigerated milk samples based on coliform count	86
50	Mean *Escherichia coli* count refrigerated milk samples	87
51	Distribution of refrigerated milk samples based on *Escherichia coli* count	88
52	Mean Psychrotrophic count refrigerated milk samples	88
53	Distribution of refrigerated milk samples based on Psychrotrophic count	89
54	Mean faecal streptococcal count in refrigerated milk samples	90
55	Distribution of refrigerated milk samples based on faecal streptococcal counts	91
56	Mean Yeast and Mould count refrigerated milk samples	91
57	Distribution of refrigerated milk samples based on Yeast and Mould Count	92
58	Correlation coefficient between mean total viable count and other microbial counts of refrigerated milk	93
59	Correlation coefficient between mean coliform count and other microbial counts of refrigerated milk	93
60	Correlation coefficient between mean *E. coli* count and other microbial counts of refrigerated samples	94

61	Correlation coefficient between mean psychrotrophic count and other microbial counts of refrigerated milk	95
62	Correlation coefficient between mean faecal streptococcal count and other microbial counts of refrigerated milk	96
63	Correlation coefficient between mean yeast and mould count and other microbial counts of stored pasteurized milk	96
64	Bacteria isolated from refrigerated milk	97
65	Mean colour and appearance score of milk samples	98
66	Mean odour score of milk samples	99
67	Mean flavour score of milk samples	100
68	Mean Body score of milk samples	100
69	Total score of fresh and refrigerated milk samples	101
70	Mean pH of milk samples	102
71	Result of COB test of milk samples stored under refrigeration	102
72	Mean bacterial count of water samples from dairy farm and dairy plant	103
73	Mean bacterial count of Milk pail/ Milking machine/ Milk can	104
74	Mean bacterial count of Hand wash of milker and dairy farm operators	104
75	Mean bacterial count of Pasteurization and Packaging Equipment	105
76	Mean bacterial count of Package material/ Storage crate/ Strainer	105
77	Mean microbial counts of air samples	106

LIST OF FIGURES

Figure No.	Title	Between pages
1	Effect of Pasteurization on the Microbial Quality of Raw Milk	74&75
2	Comparison of microbial quality of milk brands (A, B, C, D) and "zero day" sample (E)	75&76
3	Effect of Refrigeration on the Microbial Quality of Milk	84&85

LIST OF PLATES

Plate No.	Title	Between pages
1	Congo red binding test of *Escherichia coli* isolates	65&66

LIST OF FLOW CHARTS

Figure No.	Title	Between pages
1	Collection of samples	37&38
2	Isolation and identification of *Escherichia coli*	42&43
3	Isolation and identification of *Staphylococcus aureus*	43&44
4	Isolation and identification of *Listeria monocytogenes*	44&45
5	Critical control points in production of pasteurized milk	107&108

Introduction

1. INTRODUCTION

Milk is the single balanced food available in nature for the maintenance of health and promotion of growth of mammals. Apart from being a nutritious drink, milk also acts as the raw material for the production of various healthy and delicious products. In the year 2001-2002 the milk production in the country was 84.4 million tones and has now increased to 91 million tones (2004-2005). Presently our country is ranked as the highest milk producing country of the world. However the present per capita availability is only 232 grams per day (Animal Husbandry Statistics, 2005).

The concept of hygienic quality and food safety has been rapidly growing to prominence in recent years in our country and consumer awareness of such topic has increased tremendously. Hygiene in all aspects of milk handling, strict maintenance of refrigeration temperature, minimization of storage time of raw milk and a suitable method to kill or remove microorganism, followed by an affective HACCP system are all primary concerns for quality assurance in dairy industry. With the advent quality (ISO 9000) and food safety (HACCP) management system standards, the concept of quality and food safety has taken a tangible form. This presents a good opportunity for Indian dairy industry for upgrading quality and food safety and bringing uniformity and consistency in its supplies in international market.

Milk is an excellent medium for bacterial growth at room temperature. Quality changes occur as milk moves from the animal to can to cooler to tankers to dairy plant and ultimately to consumers. Pasteurization is known to improve the keeping quality of milk apart from destroying pathogens of public health importance. However, the objective of pasteurization of milk will be achieved if such milk is subsequently handled under conditions that would prevent re-entry of spoilage and pathogenic microorganisms. Such contamination can occur at number of points during post pasteurization handling of milk. Hence monitoring of the microbial quality of milk at various points of production process is vital element in ensuring quality care in milk production from dairy farm to consumer.

The two prime requirements to achieve microbiological safety in milk production are knowledge of pathogenic microbial organisms associated with milk and to identify those aspects in production line that can affect fundamentally the microbiological quality of milk and to introduce stringent control over these critical aspects.

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, severity of heat treatment, post pasteurization contamination, package condition and storage temperature.

The question of milk flavor and other sensory attributes is also outstanding importance. Although milk is nutritious, wholesome and attractive to look at, but if its flavor is defective or if there is any color change it is likely to be rejected by the consumer. No matter how valuable the milk is, unless the flavor is agreeable there is no market for it.

Hence it is necessary to have an integrated approach to study the sources of microbial contamination in the production line of pasteurized milk, the quality changes of milk before and after pasteurization, the shelf life and sensorial attributes of refrigerated pasteurized milk and the quality of retail milk.

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

1. Determination of microbial quality of milk involved during its production in the farm and at various stages of pasteurization

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

   - Microbial load of stored sample until spoilage by estimating Total viable count, coliform count, *E. coli* count, yeast and mould count, psychrotrophic count and faecal streptococcal count.

   - Sensory and physico-chemical changes of milk

   - Isolation and identification of bacteria of public health importance such as *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*

3. To study the microbial profile of retailed pasteurized milk and isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*

4. Identification of critical points of microbial contamination involved in the production of pasteurized milk and determining the microbial load of indicator organism from each source.

Review of Literature

2. REVIEW OF LITERATURE

2.1 MICROBIAL COUNTS OF MILK

2.1.1 Total Viable Count

Jain and Saraswat (1968) analysed 50 samples of raw milk collected from organized dairy farm, Udaipur city. The average of Standard Plate Count was 7,60,000 /ml.

Vijai and Saraswat (1968) studied the bacteriological quality of market milk in Udaipur city. A total of 224 raw milk samples obtained from organized dairy farm, city market and rural collection center and 65 pasteurized milk samples collected from milk supply scheme were examined for standard plate counts. The average SPC of milk from organized dairy farm was 14,00,000 counts /ml and from city market and rural collection center was 30,00,000 and 60,00,000 number /ml respectively. However the count of pasteurized milk sample was 32,000 numbers /ml.

Davies (1977) analyzed 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.

Garg *et al.* (1977) evaluated 57 cow milk obtained from Hissar city during summer and winter months and reported that the SPC count of samples collected during summer ranged between $4x10^5$ to $2x10^8$ /ml and the corresponding counts of the samples collected during winter was ranged from $5.4x10^5$ to $4x10^7$ /ml.

Desai and Natarajan (1981) tested 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 samples were collected from three societies and from each society five samples each were collected. The average standard plate count of the samples collected from the societies in the area A, B, and C was $205x10^5$, $441x10^5$ and $92x10^5$/ml respectively.

Schroder *et al.* (1982) investigated the bacterial quality of raw and commercially pasteurized milk, collected from five dairies namely A, B, C, D and E and reported that the geometric mean of total viable count of the raw milk samples was $1x10^5$ cfu/ml. Total viable count in commercially pasteurized milk had a geometric mean of $3x10^3$ cfu/ml.

Yadava *et al.* (1983) examined the bacterial flora of 42 raw milk from Dairy unit R.V.C and 22 pasteurized milk from town milk supply organization (TMS). The average total bacterial count of the samples of pasteurized milk from TMS and raw milk from R.V.C was $1735.83x10^5$ and $4.08x10^5$, respectively. The corresponding count of the samples from above sources during winter was $1881.50x10^5$ and $0.56x10^5$ /ml respectively.

Reddy *et al.* (1984) studied the bacterial quality of 30 samples of raw milk from Vijayavada milk shed that are used in the manufacture of dried whole milk. The SPC of the samples ranged from 0.77 to 29.40 million /ml. It was highlighted that the counts of raw milk was correlated with dried whole milk by 0.74, indicating the necessity for good quality raw milk to obtain a powder of high quality.

Arora and Sudarsanam (1986) analyzed the microbiological quality of milk used as an ingredient in ice cream from 16 samples, eight obtained from experimental dairy NDRI (Source A) and remaining from market (Source B). The samples from source A had an average SPC of $16.75x10^3$ /ml with a range of

6.2×10^3 to 25×10^3 /ml. The samples from the source B had an average count of 2.212×10^3 /ml with a range of 0.8×10^3 to 4×10^3 /ml.

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

Reddy *et al.* (1989) analyzed 30 samples each of raw and pasteurized milk collected from farm and distribution center near to Dairy experimentation station, Tirupati. The mean SPC counts of raw and pasteurized milk samples was $7.516 \pm 1.404 \times 10^5$ and $9.877 \pm 1.058 \times 10^5$ /ml, respectively.

Mahari and Gashe (1990) enumerated the microorganisms present in raw and pasteurized milk and the sources of contamination in milk after it had arrived the processing plant in Addis Abba. The lowest count of raw milk samples was 4×10^7 cfu/ml while the highest was 1×10^9 cfu/ml. Pasteurized milk had aerobic counts of 7×10^5 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 standardized and pasteurized milk supplied by milk board varied between 9×10^4 and 65×10^4/ml with an average count of 33×10^4/ml. The count of raw milk supplied by C.S. Azad University of Agriculture and Technology, Kanpur, varied from 120×10^4 to 595×10^4/ml with a mean count of 295×10^4/ml. The count of samples from hawkers varied from 258×10^4 to 2570×10^4 with an average count of

1142×10^4/ml, whereas the samples from ghosies had a mean count of 429.12×10^4 /ml with a range of 175×10^4 to 856×10^4/ml.

Rahman *et al.* (1992) investigated the bacterial flora in 83 samples of raw milk in Guwahati city and reported that the standard plate count/ml of milk varied from 1.5×10^4 to 3.6×10^7.

Rea *et al.* (1992) analyzed raw milk from 70 farms and obtained a total bacterial count of $\leq$ 30,000 /ml in 63 per cent of the sample.

Patel *et al.* (1993) collected 21 samples of milk from buffaloes maintained at the reproductive biology research unit, Veterinary College, Anand. The total plate count of the samples varied from less than 1000 to 11,00,000 with an average of $2.1 \pm 0.7 \times 10000$ 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 at various stages of collection. Average total plate count of milk samples collected from individual producers, collection centers and diary plant was $1 \pm 4.15 \times 10^6$, $9.1 \pm 1.6 \times 10^6$ and $17 \pm 0.43 \times 10^6$ 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 3.7×10^4 cfu/ml. The mean count in raw milk was 4.5×10^6 cfu/ml.

Singh *et al.* (1994) analyzed 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. They compared the standard plate count of the samples with that of the standard prescribed by Bureau of Indian Standards (BIS, 1969) and graded 28.75 per cent samples were of very good quality, 14.28 per cent good quality, 21.43 per cent fair quality and 37.73 per cent were graded as poor quality.

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 counts of samples from S_1, S_2 and S_3 were $7.5x10^4$, $14x10^5$ and $2x10^5$ /ml, respectively. During the study, seven pooled raw milk samples from each source were also tested. The mean total viable count of samples form S_1 source was $4.0x10^4$ /ml. The average counts in the samples belonging to S_2 and S_3 sources were 1.8×10^6 and $2.1x10^5$ /ml, respectively.

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

A study was conducted to assess the microbial quality of 30 raw milk samples collected from different local dairy sources and reported that the total viable count in the samples ranged between less than $5x10^4$ to $4x10^6$ /ml (Matta and Punj, 1996).

Mutukumira *et al.* (1996) evaluated the quality of 10 samples of raw milk delivered to the milk collection center, Zimbabwe. The total aerobic counts of the samples ranged between $6.2x10^3$ and $7.8x10^7$ 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 $5.01x10^5$ cfu/ml.

Cosentino and Palmas (1997) analyzed raw and heat-treated ewes milk collected three times over a period of 6 months from six (1,2,3,4,5,6) 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 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, respectively.

Garg and Mandokhot (1997) analyzed 86 samples of raw milk and found the SPC ranged between $7x10^4$ and $2x10^{10}$ /ml. Sixty-four out of 86 sample (74.4 per cent) had SPC even above $5x10^6$ /ml. Only two samples were graded very good. High SPC (over $5x10^6$) in majority of milk sample indicated poor hygienic practice followed at dairy farm 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 5.984±0.055, 5.234±0.060, 5.578±0.063, 4.581±0.025 and 3.553±0.012 $\log_{10}$ 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 SPC 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 3-4 times. The initial aerobic plate count of samples collected from silo tank, milk before pasteurization, milk after pasteurization, pasteurized milk from buffer tank, pasteurized milk before filling machine and milk filled in consumer package were $8 x 10^4$, $9 x 10^4$, $6 x 10^2$, $1 x 10^3$, $7 x 10^2$ and $6 x 10^2$ cfu/ml respectively.

John (1999) studied the bacterial profile of 100 pasteurized milk sample belonging to brand A, B, C, D and E collected from retail outlets in and around Thrissur. The mean count of TVC 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 TVC 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 TVC 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 average SPC varied from 5.5 to $175.17 x 10^4$ cfu/ml. When compared with BIS standards for bacterial quality more than 94 per cent of milk tested were graded as poor quality.

Homhual and Jindal (2001) assessed the total plate count of 95 raw milk sample. The counts obtained ranged from $6.5x10^4$ cfu/ml to $1.2x10^8$ cfu/ml.

Beloti *et al.* (2002) evaluated the 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 $5x10^2$ cfu/ml and 3 had counts greater than $5x10^2$cfu/ml. The counts of grade B pasteurized milk was less than $4x10^4$ cfu/ml in 22 samples and greater than $4x10^4$ cfu/ml in 7 and that of grade C pasteurized milk was less than $1.5x10^5$ in in 29 samples and greater than $1.5x10^5$ in 3 samples.

Khalilur *et al.* (2002) evaluated the microbiological quality of 36 samples of milk (raw and pasteurized) from local markets of Aligarh city. The raw milk samples had TVC ranging from $15,900x10^6$ to $2,59,000x10^6$ cfu/100ml with mean count of $98,500x10^6$ cfu/100ml. Pasteurized sample showed a TVC ranging from $154x10^6$ to $24,000x10^6$ cfu/100ml with mean count of $15,000x10^6$ cfu/100ml.

Lues *et al.* (2003) enumerated microbial quality of raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa. The study revealed that the mean count for total aerobic mesophilic organism was $8.6x10^8$ cfu/ml.

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

Aaku *et al.* (2004) analyzed 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 A and B sources were $3x10^7$ and $1x10^6$ cfu/ml, respectively and the counts of pasteurized sample were $7x10^3$ and $1x10^4$, respectively.

Chye *et al.* (2004) studied the bacteriological quality and safety of raw milk in Malaysia. Out of 930 raw milk samples collected from 360 dairy farmers the mean total plate count was $12x10^6$cfu/ml.

Zweifel *et al.* (2004) examined 407 samples of bulk-tank milk, 344 goats milk and 63 ewe's milk from 403 different farms throughout Switzerland. Median SPC from small ruminant bulk milk was 4.68 log_{10} cfu/ml, 4.69 log_{10} cfu/ml for goats milk and 4.79 log_{10} cfu/ml for ewe's milk.

2.1.2 Coliform Count

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

Davies (1977) evaluated the bacteriological quality of 2090 samples 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 and the level of 10^3/ml in 29.1 per cent samples.

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

Singh and Ranganathan (1978) examined 50 samples of raw cow milk and 30 samples of pasteurized milk. The coliform counts of raw and pasteurized milk ranged from 500 to 50000 and zero to 4500 count /ml, respectively.

Desai and Natarajan (1981) assessed the bacterial quality of raw milk collected from three societies in areas A, B and C located around Bangalore city. The average coliform count of the samples collected from societies in the area A, B, and C were 1040 x 10^3, 80 x 10^3 and 282 x 10^3 /ml, respectively.

Singh and Sinha (1981) analyzed the presence of coliform from 104 samples of freshly pasteurized milk collected from experimental dairy of NDRI, Karnal and found that coliform counts of the samples ranged between zero and 4500 /ml.

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 monsoon from TMS and R.V.C were 20.39×10^5 and 0.778×10^5 /ml, respectively. The corresponding counts of the samples collected during winter from the above sources were 13.26×10^5 and 0.33×10^5 /ml.

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

Arora and Sudarsanam (1986) evaluated the microbiological quality of eight milk samples obtained from experimental dairy NDRI. They reported that the samples had an average coliform count of 22.875 /ml with a range of 7 to 40 /ml.

Raju and Nambudripad (1987) examined the bacterial quality of 78 raw milk samples collected from organized 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 counts of raw milk from organized dairy, private dairy and village-pooled milk were $136x10^3$, $196x10^3$ and $1560x10^3$ /ml, respectively. The mean count in pasteurized milk was 62 /ml.

Palanniswami *et al.* (1988) collected individual milk samples of 68 animals and 28 pooled samples obtained from three groups consisting of group A where sanitary practices were given second preference to production and dispatch of milk, group B where sanitary practices were moderate and group C where sanitary practices were experimentally imposed during sampling. The mean coliform count /ml of raw milk obtained from the sources A, B and C were 10, 10 and 4, respectively and the count of pooled samples from the above sources were 39000, 27000 and 30 /ml, respectively. The individual milk samples of Group C met standards prescribed by BIS (ISI 1977) while none of the other pooled milk samples met the above standards.

Misra and Kuila (1989) reported that the raw milk samples obtained from organized dairy farms, vendors and sweet meat shops had average coliform counts of $3.96x10^3$, $6.54x10^3$ and $6.74x10^3$ cfu/ml, respectively and pasteurized milk from dairy plant had a mean count of $12x10^1$ 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 $10x10^2$ /ml with an average count of $5.125x10^2$ /ml. The count of raw milk supplied by C.S. Azad University of Agriculture and Technology, Kanpur, varied from $10x10^2$ to $45x10^2$ /ml with a mean count of $24.375x10^2$ /ml. The count of samples from hawkers varied from $50x10^2$ to $408x10^2$ with an average count of $213.375x10^2$ /ml, whereas the samples from ghosies had a mean count of $64.125x10^2$ /ml with a range of $33x10^2$ to $110x10^2$ /ml.

Rea *et al.* (1992) evaluated raw milk from 70 farms and found that coliform were present in all samples but 65 to 75 per cent of samples had less than 100 coliform /ml.

Patel *et al.* (1993) collected 21 samples of milk from buffaloes maintained by reproductive biology research unit, Veterinary College Anand. The average coliform count was $1.9 \pm 0.57x1000$ 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 at various stages of collection. Average coliform count of milk samples collected from individual producers, collection centers and dairy plant were $0.63 \pm 0.31 \times 10^4$, $66 \pm 38x10^4$ and $150 \pm 0.82x10^4$ cfu/ml, respectively.

Singh *et al.* (1994) reported that the coliform count in the raw milk samples ranged between 2.477 and 5.869 $\log_{10}$ cfu/ml with a mean count of 4.477 $\log_{10}$ cfu/ml.

Kapre (1995) reported that the individual raw milk samples collected from S_1, S_2 and S_3 sources had average coliform counts of 2.4x10, $4.8x10^4$ and $3.8x10^3$ cfu/ml, respectively, and the average counts of pooled raw milk samples collected from S_1, S_2 and S_3 sources were 5.5x10, $2.0x10^5$ and $6.4X10^3$ /ml respectively. The study of Mutukumira *et al.* (1996) revealed that the coliform count in the raw milk samples ranged from $3.2x10^2$ to $2.3x10^5$ cfu/ml.

Cosentino and Palmas (1997) analyzed 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 counts of heat-treated milk from the above sources were1.0 ± 0.41, 2.1 ± 0.28, zero, 1.8 ± 0.14, 1.9 ± 0.32 and 2.6 ± 0.45 cfu/ml, respectively.

Desmasures *et al.* (1997) evaluated raw milk from 27 farms sampled over six months and found that coliform was present in most samples but 84 per cent samples had counts less than 100 cfu/ml.

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 3.061 ± 0.061, 2.856 ± 0.058, 2.751 ± 0.069, 1.100 ± 0.049 and 0.771 ± 0.104 $\log_{10}$ 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 coliform counts of 855 raw milk samples obtained from 11 co-operatives or processing plants and reported that the count ranged from 14 to 290 coliforms /ml with a mean count of 31 coliforms /ml.

John (1999) examined 100 pasteurized milk sample belonging to brand A, B, C, D and E and reported that the samples had a the 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 each 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 was 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 43.33×10^2 cfu/ml.

Khalilur *et al.* (2002) evaluated the microbiological quality of 36 samples of milk (raw and pasteurized) from local markets of Aligarh city. The raw milk samples had mean total coliform count of 2.4×10^3 MPN/100ml and pasteurized samples showed a mean count of 2.13×10^3 MPN/100ml.

Lues *et al* (2003) enumerated microbial quality of raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa. The mean coliform count of the samples was 6.7×10^7 cfu/ml.

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 collected from 360 dairy farmers and reported that the samples had a mean coliform count of 17×10^4 cfu/ml.

2.1.3 *Escherichia coli* Count

Kapre (1995) evaluated a total of 63 samples of individual raw milk collected from S_1, S_2 and S_3 sources and reported that the samples had an average E.coli counts of $2.0x10^2$, $1.2x10^4$ and $1.5x10^3$ cfu/ml, respectively. The corresponding counts of 14 pooled milk samples from the sources were $2.7x10^2$, $8.9x10^4$ and $1.9x10^3$ /ml.

Cosentino and Palmas (1997) reported the raw ewes milk from six (1,2,3,4,5,6) milk-processing plants located in Sardina had mean *E.coli* counts of 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 *E.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 *E.coli* count less than 10 cfu/ml.

Latha and Nanu (1997) studied that pasteurized milk belonging to S_1, S_2, S_3, S_4 and S_5 sources and found that the samples had mean *E.coli* counts of 2.611±0.084, 2.188±0.091, 2.258±0.120, 0.493±0.134 and 0.198±0.077 log_{10} cfu/ml, respectively. During the investigation, 82 *E. coli* isolates were identified from 80 per cent of samples tested.

John (1999) obtained the mean *E.coli* counts of 100 pasteurized milk sample belonging to brand A, B, C, D and E as 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 *E.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 nine pasteurized milk sample and reported that the samples had a mean faecal coliform count of $1.5x10^3$ MPN/ml.

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

Lues *et al.* (2003) determined microbial quality of raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa and recorded the mean *E.coli* count of the samples as $1.2x10^4$ 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 *E. coli* count of the samples as 0.19 ± 0.12 log_{10} cfu/ml.

Chye *et al.* (2004) reported that the 930 raw milk samples obtained from Malaysia had a mean *E.coli* count of $6.8x10^3$ cfu/ml.

2.1.4 Psychrotrophic Count

Jain and Saraswat (1968) examined 50 samples of raw milk collected from organized dairy farm. The average of psychrophilic counts of milk was 77,000 /ml. Highly significant correlation was observed between SPC and psychrophilic counts of the samples was observed.

Schroder *et al.* (1982) determined the microbiological quality of raw and commercially pasteurized milk collected from five dairies namely, A, B, C, D and E in United Kingdom. The investigation revealed a mean psychrotrophic count of pasteurized milk samples from the sources A, B, D and E was less than one cfu/ml, and the sample belonging to the source C had a mean count of 10^3 cfu/ml. Raw milk samples from dairies A, B, C, D and E had a mean count of $1x10^4$, $4x10^5$, <1, $8 x10^5$ and $6x10^3$ cfu /ml, respectively.

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) reported the average psychrophilic count of 125 raw milk samples obtained from organized dairy farms, vendors and sweet meat shops were $30.13x10^3$, $54.5x10^4$ and $61.6x10^4$ cfu/ml, respectively and the average count of 25 samples of pasteurized milk from dairy plant was $5.56x10^3$ 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) evaluated 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 sample 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) examined bacteriological quality of 12 private brands of pasteurized and homogenized milk in Chennai city and found that average psychrotrophic count varied from 12.50 to 99.33×10^4 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. The psychrotrophic count varied between zero to ten /ml in 73.7 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 7.5×10^3cfu/ml.

2.1.5 Faecal Streptococcal Count

Yadava *et al.* (1983) evaluated 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 14.40×10^5, and 0.183×10^5 /ml, respectively. The corresponding counts of the samples collected during winter had a count at the level of 4.34×10^5 and 0.003×10^5 /ml.

Latha and Nanu (1997) examined the pasteurized milk belonging to S_1, S_2, S_3, S_4 and S_5 sources. The study revealed that the mean faecal streptococci counts were 2.532 ± 0.096, 1.923 ± 0.116, 1.297 ± 0.131, 1.295 ± 0.203 and 0.820 ± 0.159 from S_1, S_2, S_3, S_4 and S_5 sources, respectively.

Jolly *et al.* (2000) recorded mean faecal streptococci counts of 60 raw market milk samples collected from three sources viz. A, B and C located in and around Mannuthy. The mean 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.

Sethulakshmi *et al.* (2003) studied the bacterial 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.

2.1.6 Yeast and Mould Count

Arora and Sudarsanam (1986) assessed the microbiological quality of eight samples of milk obtained from experimental dairy unit, NDRI. The samples had average yeast and mould counts of 32.5×10^2 /ml and the count ranged from two to 156×10^2 /ml.

Mutukumira *et al.* (1996) evaluated the quality of 10 samples of raw milk obtained from the milk collection center, Zimbabwe. The yeast and mould count in seven out of 10 samples was less than 100 cfu/ml.

Cosentino and Palmas (1997) analyzed raw and heat-treated ewes milk collected three times over a period of 6 months from six (1,2,3,4,5,6) milk-processing plants in Sardina. The mean yeast and mould counts of raw milk from plants 2, 4, 5 and 6 were 1.1 ± 0.32, 1 ± 0 .27, 1.3 ± 0.45, and 1.2 ± 0.63 cfu/ml respectively. The organism could not isolate from the raw milk sample obtained from plant one and three and all the heat-treated milk samples.

Lues *et al.* (2003) enumerated microbial quality of raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa and reported that the samples had an average yeast count of 2.3×10^6 cfu/ml and the average mould count of the sample was 1.1×10^3 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 *E. 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, organized dairy farm and local vendors from Ranchi town and isolated *E. coli* from 82 (78.09 per cent) samples.

Rahman *et al.* (1992) investigated the bacterial flora in 83 raw milk samples obtained from Guwahati city and reported the isolation of *E. coli* from 6.12 per cent of the samples.

Singh *et al.* (1994) reported the isolation of 49 *E. coli* from 70 raw milk samples.

Sharma *et al.* (1995) isolated *E. coli* from five out of 60 raw milk samples obtained from local market. The isolates consisted of serotypes 0:5, 0:7, 0:61 (2 samples) and 0:106.

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

Steel *et al.* (1997) isolated 15 (0.87 per cent) *Verocytotoxigenic E. 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 *E.coli* was 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) *E.coli* were isolated and of this 22.1 per cent strain were serologically identified as EPEC.

Soomro *et al.* (2002) analysed 100 raw milk samples collected from different sources of Tandojam. The results revealed that *E. coli* was found in 57 per cent of samples.

John *et al.* (2003) studied the bacteriological quality of 84 samples of pasteurized milk obtained from Thrissur. The study revealed that *E. coli* was present in six (7.1 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 *E. coli* from 600 samples (64.5 per cent).

2.2.2 Staphylococcus aureus

Mohan and Misra (1967) analysed 200 samples of raw milk obtained 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 Staphylococcus were isolated from the sample of which 33 were coagulase positive.

Ghosh and Laxminarayana (1972) evaluated 160 raw milk samples and 30 pasteurized milk samples marketed in Karnal. The study revealed that 81 raw milk samples (50.6 per cent) and 7(23.3 per cent) pasteurized milk samples showed the presence of coagulase positive Staphylococcus.

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

Shah *et al.* (1984) conducted studies on 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) examined the bacterial flora of 105 milk samples. The samples consisted of 22 from milk supply scheme, 42 raw milk from organized 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 7(16.66 per cent), 10(24.39 per cent) and 3(13.63 per cent), respectively.

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

Singh *et al.* (1994) recovered 45 isolates of *S.aureus* from 110 raw milk samples.

Desmasures *et al.* (1997) detected *S. aureus* from 62 per cent of raw milk from 27 farm samples 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 center in Trinidad. All bulk milk samples and 280 (97.6 per cent) of composite milk sample were positive for *Staphylococcus aureus.*

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

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) Staphylococcus spp. Only one of the samples was positive for *Staphylococcus aureus*

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

2.2.3 Listeria monocytogenes

Yadava *et al.* (1983) examined the bacterial flora of 42 milk samples from dairy farm, Ranchi town and none of the samples revealed the presence of *Listeria monocytogenes*.

Rohrbach *et al.* (1991) studied the prevalence of *Listeria monocytogenes* in 292 samples of milk obtained from bulk tanks and recorded the isolation of *Listeria monocytogenes* from 12 (4.1 per cent) of the samples.

Sharif and Tunail (1991) examined 77 raw milk sample and 22 pasteurized milk samples for the presence of *L. monocytogenes.* The study revealed that 18.2 per cent of raw milk sample had *L. monocytogenes* but none of the pasteurized milk samples revealed the presence of the organism.

Rea *et al.* (1992) evaluated raw milk from 70 farms over the period of 13 months and isolated *Listeria monocytogenes* from 4.9 per cent of the samples.

Bhilegaonkar *et al.* (1997) collected 50 individual cow milk from organized farm and 16 individual cow milk from private dairy farm and examined for the presence of *Listeria monocytogenes*. The study revealed that two samples each from the former and the later group had *Listeria monocytogenes.* Out of 35 pasteurised samples, none revealed the presence of *listeria monocytogenes.*

Desmasures *et al.* (1997) analysed raw milk from 27 farms over the period of six months and reported that four samples were positive for *Listeria monocytogenes.*

Pednekar *et al.* (1997) analysed raw and pasteurized milk samples from local market, Mumbai and could not detect *L. monocytogenes,* from the samples.

Steel *et al.* (1997) isolated 47(2.73 per cent) *Listeria monocytogenes* from 1720 random bulk tank milk samples collected from Ontario.

Carlos *et al.* (2001) analysed 1300 raw milk samples randomly obtained from four stables in South east Mexico City and isolated 300 (23 per cent) Listeria species of which 162 (13 per cent) were positive for *L. monocytogenes.*

Chye *et al.* (2004) studied the bacteriological quality of 930 raw milk samples collected from 360 dairy farmers. *Listeria monocytogenes* was isolated from 18 samples.

Kells and Gilmour (2004) studied the incidence of *Listeria monocytogenes* from two Northern Ireland milk processing plants (dairy A and B) and were able to isolate three Listeria species from dairy A and five Listeria species from dairy B. However only four samples from dairy B were positive for *Listeria monocytogenes.*

Mena *et al.* (2004) studied the incidence of *Listeria monocytogenes* in different food products commercialized in Portugal and were not able to isolate *Listeria monocytogenes* from 28 samples of pasteurized milk but the organism was isolated from one of the six samples of raw milk.

Fyre and Donnelly (2005) conducted a survey on the prevalence of *Listeria monocytogenes* in pasteurized fluid milk. During the survey a total of

5519 sample of pasteurized milk (whole milk, nonfat milk and chocolate milk) were examined and reported the isolation of the organism from only one (0.018 per cent) of the samples.

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 cannot only lead to spoilage of milk, but it can 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 per milliliter of milk not exceeding two lakhs 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 bacterial 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 MICROBIAL COUNTS OF REFRIGERATED MILK

Sinha *et al.* (1968) studied the keeping quality of 25 pasteurized cow milk obtained from NDRI, Karnal. The average keeping quality till samples showed positive for Clot on Boiling were 35.76 hours at 22°C with 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 5×10^3 /ml to 1×10^7 /ml over a space 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 certain extent the effects of poor conditions of production. Finding 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 organoleptic quality of milk deteriorated.

Lindberg *et al.* (1998) stored 430 consumer cartons of pasteurized milk or cream at 7°C and found that storage time was 11 to 14 day for milk and cream samples. Studies also showed that enterobacteriaceae were found in six per cent of stored consumer packs of pasteurized milk or cream.

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 $\pm$4.38 $\log_{10}$ cfu/ml to 9.02 $\pm$8.32 $\log_{10}$ cfu/ml on the 12th day of refrigerated storage and the counts of coliform organism increased from 2.36 $\pm$2.01 $\log_{10}$ cfu/ml to 3.33 $\pm$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) analyzed the changes of mesophilic and psychrophilic counts of pasteurized milk stored in different packaging material at 4°C. The initial mesophillic count was 4.36 log cfu/ml and after seven days of storage the mesophillic counts were between 6.01 and 6.49 log cfu/ml. The psychrophillic 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 the SPC of pasteurized milk samples from dairy during first visit was $2x10^3$/ml of samples on day 7 and $1.9x10^8$/ml of samples on day 14. The count of the sample during second visit was $2.4x10^5$ on day 7 and $3.2x10^8$ on day 14.

Mamani *et al.* (2003) studied the growth and survival of E. coli in whole Ultra heat treated milk at 4°C and found that *E coli* counts were almost similar between the inoculum 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. By day 14, 58 per cent of the sample had counts higher than 10^6cfu/ml. Results also shows 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 sample having 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 mesophillic 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 psychrophillic counts on the initial day was 3.59 log cfu/ml and after seven days of storage the psychrophillic counts were between 4.83 and 5.52 log cfu/ml.

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

2.5 SENSORY AND PHYSICO-CHEMICAL 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, 6 and three, respectively.

Kadan and Bhanumathi (1984) evaluated the organoleptic quality of pasteurized homogenized and unhomogenised cow milk in pouches stored 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-days storage period at three different storage temperature 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 sample 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.

Hsieh and Ren (2001) reported that the samples of skim milk stored at 4°C showed a reduction in pH from 6.78 to 6.16 during a period of 10 days.

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 poor, 6 to 7 was fair and 8 to 10 good. Hence the sample showed no serious sensory defects.

2.6 SOURCES OF MILK CONTAMINATION

Aggarwal and Srinivasan (1980) studied the environmental mould content in well-organized and maintained dairy farm. The fall of mould conidia per plate per minute from hand milking byre, machine milking byre and milk pasteurization unit ranged between zero to 24.5, zero to seven and zero to six per plate per minute.

Palaniswami *et al.* (1988) evaluated coliform counts of water, milk pail, milk can and milkers hand wash from three different sources viz. A, B and C having different sanitary practices. The samples obtained from farms of organized sector where sanitary practices were moderate showed mean coliform count of tap water as 72 MPN /ml. The coliform counts of milk pail and milk can were 450 and 164 per litre capacity each and the count of milkers hand wash was 113 per 100 sq.cm.

McKinnon *et al.* (1990) collected milk at various points during its passage through the milking plant. The total colony count was increased significantly by the milking equipment and the increase in count ranged between 2000 and 3000 organisms/ml. The mean rinse of bacterial count of the milking equipment in summer was $4.4x10^7/m^2$.

Patel *et al.* (1993) obtained the total plate count of ten rinse samples of milk cans (not rinsed with water) as 5.6x1,00,000 to more than 30,000,000 with an average of 1.7 ± 0.44x10 million/can and the coliform count of the sample was 4000 to more than 3,00,000 cfu/can with an average of 0.1 ± 0.04x1 million cfu/can.

Lopes and Stamford (1997) found that 60 per cent samples of water used to clean the milk equipment showed the presence of coliform.

Salustiano *et al.* (2003) evaluated the microbiological quality of air at processing areas in dairy plant by culture settling plate technique (sedimentation technique). The study revealed that for all processing areas in the dairy plant, the number of mesophillic aerobic bacteria and yeast and mould obtained was higher than the standard prescribed by American Public Health Association i.e. 30 $cfu/cm^2/week$.

3. MATERIALS AND METHODS

In the present investigation a total of 296 milk samples consisting of raw milk collected from individual cows (20), pooled samples (20), chilled milk before pasteurization (20) and milk at various stages of pasteurization viz. immediately after heating section (20), pasteurization (20), packaging (20) and 20 samples each during storage at 4 ± 1°C on 2, 4, 6, 8, 10 and 12 days and retail samples (56) belonging to four brands were taken and assessed for the microbial quality. The samples were tested for Total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC), Psychrotrophic count (PC), Faecal Streptococcal count (FSC) and Yeast and Mould count (YMC). All pooled raw milk from the site of production, freshly pasteurized milk, refrigerated stored milk and retail samples were also subjected for isolation and identification of *Escherichia coli, Staphyloccus aureus* and *Listeria monocytogenes.* The stored samples of pasteurized milk were also evaluated for sensory qualities (viz. colour and appearance, flavour, odour and body) and physico-chemical changes (viz. pH and COB test).

In order to assess the critical control points of microbial contamination of milk during milking, samples of air before milking (10), after milking (10), tap water used in milking barn (10), washings of milk pail (20), milkers hand (10), milking machine (20), milk strainer (10) and milk cans (20) were collected and subjected to estimation of various bacterial load. The samples of air before (10) and after (10) pasteurization process, water (10) and washings of workers hands (10) and equipment (10) of the pasteurization area and also samples of air before (10) and after (10) packaging, washings of packaging materials (10), packaging machine (10) and storage crate (10) of the packaging area were also collected and analysed for the bacterial load.

Milk samples were collected from the dairy farm and milk processing plant of College of Veterinary and Animal Sciences, and examined to determine the microbial quality from the point of production till the processed milk is retailed to the consumer. Samples were also taken from various sources to assess the critical points of microbial contamination of milk. Microbial examination of retail samples was done by collecting samples sold in and around Thrissur. The details of collection of samples are shown in flowchart 1.

3.1 MICROBIAL QUALITY OF MILK

In order to get an insight on the microbial quality and the presence of bacterial pathogens in milk, their changes during its production in the farm and in various stages of processing till it reaches the consumer, milk samples were collected from various points of production, processing and distribution and examined for their microbial quality and determination of bacterial pathogens.

3.1.1 Collection of Milk Samples

The samples include raw milk collected from individual animal, Pooled milk, chilled milk and also milk at various stages of pasteurization, collected just after heating section, after pasteurization, after packaging and the retail milk sold in and around Thrissur.

3.1.1.1 Raw Milk of Individual Animal

A total of 20 raw milk samples of individual animals, each consisting of 500ml, were collected during the study. On each day, the samples were collected from two randomly selected animals after complete milking.

3.1.1.2 Pooled Raw Milk

During the study 20 pooled milk samples were collected and examined. The samples were collected aseptically after thorough mixing of milk present in four cans with a plunger and by transferring about 250 ml of milk from each can into a sterile conical flask. After thorough mixing of the milk in the flask, about 500 ml of duplicate samples of pooled milk was transferred to another sterile flask, which finally formed the samples of a batch.

Flowchart 1. Collection of samples

A. COLLECTION OF SAMPLES FROM FARM AND DAIRY PLANT

Raw milk of Individual animal in the milk barn

Milk from individual animal (20)

- Air
  - Before milking (10)
  - After milking (10)
- Water (10)
- Milk pail (20)
- Milkers hand wash (10)
- Milking machine wash (20)

↓

Pooled raw milk

Pooled raw milk (20)

- Washings of Strainer (10)
- Washings of Milk cans (20)

↓

Milk transportation

↓

Raw milk reception

↓

Cooling and Storage of raw milk

Chilled milk before pasteurization(20)

↓

Pasteurization

Milk after heating section (20)

Pasteurized milk (20)

- Air
  - Before Pasteurization (10)
  - After Pasteurization (10)
- Water (10)
- Equipment wash (10)
- Personnel Hand wash (10)

↓

Packaging

Packaged milk (20)

- Air
  - Before Packaging (10)
  - After Packaging (10)
- Washings of Package material (10)
- Washings of Packaging machine (10)
- Washings of Storage crate (10)

↓

Storage

Refrigerated milk 120 (20 each on Days: 2, 4, 6, 8, 10 and 12)

B. COLLECTION OF RETAILED MILK: **A, B, C** AND **D** (56)

3.1.1.3 Chilled Raw Milk

A total of 20 chilled raw milk samples were collected during the investigation just before the process of pasteurization. Each sample consisted of about 500 ml. On the day of collection two cans were selected randomly and the milk in each can was thoroughly mixed by a plunger and collected from each can and immediately transferred to the laboratory.

3.1.1.4 Pasteurization of Milk

1. Immediately after heating section

During the study 20 milk samples just after heating milk at 72°C were collected from the HTST pasteurizer. On each day two samples were collected and each consisted of 500 ml. The samples were collected immediately after heating the milk in the pasteurizer through a sampling cork provided after the heating section. At first the cork is allowed to run freely for few minutes and then the milk sample was collected into a sterile conical flask and transferred immediately to the laboratory.

2. Pasteurized milk

In the present study 20 pasteurized milk samples were collected immediately after pasteurization of milk. On a day two samples were collected and each consisted of 500 ml. The samples were collected through the sampling cork fitted on the line. Before collecting the sample, the cork is opened and allowed to run for a few minutes and samples were collected into a sterile conical flask and transferred to the laboratory in an insulated container.

3. Pasteurized milk after packaging

During the investigation 140 pasteurized and packaged milk samples were collected from the dairy plant belonging to 10 batches. Each sample consisted of 500 ml milk packaged in low-density polyethylene sachets. Each batch consisted of 14 samples. From the samples of a batch, two samples were randomly selected and examined on the day of collection. The remaining 12 samples were stored at refrigeration temperature (4±1°C) and two samples each were selected randomly and examined on day 2, 4, 6, 8, 10 and 12 of storage.

3.1.1.5 Pasteurized Retail Milk

A total of 56 pasteurized milk samples belonging to four brands viz. A, B, C and D 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 14 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 microbial 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 loads per ml of sample. All aseptic precautions were taken during collection and processing of milk samples

3.1.3 Microbial 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), Faecal streptococcal count (FSC) and Yeast and mould count (YMC). 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 on 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 Consin *et al.* (2001). The procedure followed was similar to that of TVC. 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 Streptococci 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 a '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

3.1.3.6 Yeast and Mould Count

Method described by Beuchat and Consin (2001) was followed for estimation of Yeast and Mould Count per ml of milk sample. Potato dextrose agar (H-media) was used for the estimation of yeast and mould count by spread plate technique. From the selected dilution of each sample, 0.1 ml of inoculum was transferred on to duplicate plates containing the media and the inoculum was evenly distributed on the media with a sterile 'L' shaped glass rod. The plates were incubated at 25°C for 3 to 5 days. After the period of incubation the colonies in duplicate plates were counted with the help of a colony counter and the mean count was multiplied with the dilution factor 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 *Listeria monocytogenes.*

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.

Flowchart 2. Isolation and identification of *Escherichia coli*

	Characteristics/Reactions
Milk sample	
↓	
EMB agar	
↓	
Suspected colonies	
↓	
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	+

F-fermentation, + = positive, - =negative

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

3.1.4.2 Staphylococcus aureus

For the isolation of *S. 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 Listeria monocytogenes

The procedure used for the isolation and identification of *L. monocytogenes* was similar to that described by Ryser and Donnelly (2001). Milk sample (25 ml) was mixed with 225ml of Listeria enrichment broth (LEB, Hi-media) and incubated at 30°C for four hours. After four hours of enrichment

Flowchart 3. Isolation and identification of *Staphylococcus aureus*

	Characteristics/Reactions
Milk sample ↓	
Inoculated on to BP agar ↓	
suspected colonies On to Nutrient agar slant ↓	
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	+

F = fermentation, + = positive, - = negative selective agents viz. acriflavin 10mg/ml, nalidixic acid 40mg/ml and cyclohexamide 50mg/ml was added. The samples were incubated for 48 hours. After 24 and 48 hour a loop full of the inoculum was streaked on to oxford medium (Hi-media) plates. The plates were incubated at 35°C for 28-48 h. Listeria colonies develop a black halo. The suspected colonies were transferred into slant of Trypticase soy agar-yeast extract (TSA-YE) and broth of Trypticase soy bile-yeast extract (TSB-YE) incubated at 35°C for 18-24 hours and kept under refrigeration. The isolates were characterized for the cultural, morphological and biochemical characters as 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 *E. coli, S. aureus,* and *L.monocytogenes* 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).

Primary Identification Test

1. Catalase test

a) Slide test

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

Flowchart 4. Isolation and identification of *Listeria monocytogenes*

Test	Characteristics/Reactions
Milk sample ↓	
Enriched with LEB ↓	
Inoculated into Oxford medium ↓	
Suspected colonies ↓	
TSA-YE agar and TSB-YE broth ↓	
Gram's reaction and cell morphology ↓	Gram positive short rods
Catalase ↓	+
Oxidase ↓	-
OF ↓	F
Motility ↓	Motile at 20-25°C
MR ↓	+
VP ↓	+
Citrate ↓	-
Urease ↓	-
Phosphatase ↓	+
Aesculin hydrolysis ↓ ↓	+
Carbohydrate utilization	
Mannitol	-
Rhamnose	+
Xylose	-

F =**fermentation, + = positive, - = negative**

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 3 cm above the medium. The tubes were incubated at 37°C for up to 14 days. A change in colour of the medium from green to yellow in the open tubes alone 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 liquefactions 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 deaminaion

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 react 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 á- 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 assigned to milk according to IS-1975

Table 1. 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 Physico-chemical quality

Measurement of pH

To measure the pH of milk the method described by Scott *et al.* (2001) was followed. The pH was recorded using a digital pH meter (LI 612 "ELICO"). Before measuring the pH the temperature of the milk was brought to room temperature. The electrodes were rinsed and blot dried and immersed into the sample and the pH was read after one minute for the meter to stabilize. The electrodes were rinsed and blot dried and repeated on a fresh portion of sample. The readings were taken and values have reported to two decimal places.

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 CRITICAL CONTROL POINTS OF MICROBIAL CONTAMINATION OF MILK

In order to assess the critical control points of microbial contamination of milk during various stages of production, processing and distribution, the samples of air, water, utensils, equipment, hand wash, strainer and package material were collected and their bacterial load were determined

3.3.1. Collection of Samples

3.3.1.1. Water Sample

The samples of tap water used in farm and dairy plant were collected following the procedures described by Indian Standards (1978). Allowed the water from the tap to run to waste for about two minutes in order to flush the interior of the nozzle and discharge the stagnant water. A sterile bottle of 250 ml capacity was used to collect the water. The bottle was held near the base with one hand and filled from a gentle stream of water from the tap, avoiding splashing and brought to the laboratory in an insulated container.

3.3.1.2. Milk Pail

Rinse method (Evancho *et al.*, 2001) was followed for the collection of samples from milk pail. One hundred milliliter of sterile 0.1 per cent peptone water was poured into the milk pail and mixed thoroughly by agitating. The sample was transferred into a sterile conical flask and brought to the laboratory in an insulated container.

3.3.1.3. Milk Can Sample / Milking Machine Sample/ Storage Crate

Swab contact method (Evancho *et al.*, 2001) was followed for the collection of samples from milk can, milking machine and storage crate. For sampling sterile cotton swab was moistened with 0.1 per cent peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial. The swab head was rubbed slowly and thoroughly over the interior surface of 100-cm^2 areas. The swab was rubbed three times, reversing the direction between strokes. After swabbing the cotton swab was cut with sterile scissors, transferred into sterile flask containing 100 ml of 0.1 per cent peptone water and brought to the laboratory in a an insulated container. The flask was thoroughly agitated with the help of a cyclomixer at 8000 rpm for three minutes, so as to extricate the bacteria attached to the cotton swab into the diluent.

3.3.1.4. Milker and Dairy Personnel's Hand Wash

On each visit the hand washing of a randomly selected individual milker in the farm and a person involved in processing operations in the dairy plant were collected. The individual's hand was washed in 100 ml of 0.1 percent sterile peptone water and washing was collected in sterile conical flask and brought to the laboratory in a an insulated container.

3.3.1.5 Strainer Sample

Washings of the strainer sample were collected before the milk is strained. Wash sample of the strainer was taken by allowing 100 ml of 0.1 per cent sterile peptone water to flow through the strainer and the sample was collected in a polythene bag sterilized by ultra violet light.

3.3.1.6. Pasteurization Equipment Wash Sample

On every day the HTST pasteurizer is thoroughly cleaned after pasteurization of milk by alkali treatment and thereafter with hot water circulation. Every day before pasteurization the pasteurizer is again cleaned with hot water circulation and then by chilled water. A sample of one litre of chilled water washing is collected in a sterile conical flask and was brought to the laboratory in an insulated container and processed immediately.

3.3.1.7. Packaging Equipment Wash Sample

On every day after packaging is over the equipment is thoroughly cleaned by alkali treatment and hot water circulation. It is again cleaned with water before the machine is used for packaging. From this water washing about one litre of samples was taken in a sterile conical flask and brought to the laboratory in an insulated container.

3.3.1.8. Package Material

In order to assess the microbiological quality of low density polyethylene sachets (500 ml capacity), 20 ml of sterile peptone water was poured into the bag and was agitated vigorously by holding in both horizontal and vertical position (Evancho *et al.*, 2001). The polyethylene bag with its content was brought to the laboratory in an insulated container by keeping it in an upright position.

3.3.2 Processing of Samples

Samples brought to the laboratory were agitated vigorously for about 25 times. In order to estimate the bacterial load per milliliter of water sample, 10 ml was transferred to 90 ml of 0.1 per cent peptone water so as to form one in 10 dilution of the sample. Further 10 fold serial dilutions were prepared by transferring one ml of inoculum to 9ml of the diluent. Dilutions were made up to 10^{-4}.

3.3.3 Bacterial Counts

The selected serial dilutions of each sample was used to estimate the Total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC) and Faecal streptococcal count (FSC) as described earlier.

3.3.4 Collection and Estimation of Microbial Load in Air

Air sample was collected from livestock farm before and after milking process. Samples were also collected from dairy plant before and after pasteurization and packaging of milk.

Total viable count

Direct exposure method described by Evancho *et al.* (2001) was employed for the estimation of total viable count in the air samples of milking barn, pasteurization and packaging unit before and after processing. In order to estimate the count, duplicate Petridishes (90mm diameter) containing sterile nutrient agar medium were exposed in the rooms for 15 min. The plates were brought to the laboratory in thermocool container and incubated at 37°C for 24 h. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as cfu/ft^2/min.

Yeast and Mould Count

In order to estimate the yeast and mould count, procedure described by Evancho *et al.* (2001) was followed. Duplicate plates of Potato Dextrose Agar (Hi-media) medium were exposed for 15 minutes in the rooms of milking barn, pasteurization and packaging unit before and after processing and the plates were brought to the laboratory in a insulated container and incubated at 25°C for five days. At the end of incubation the number of colonies developed in duplicate plates were counted and the mean count was expressed as $cfu/ft^2/min$.

3.4 STATISTICAL ANALYSIS

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

4. RESULTS

In the present investigation the microbial quality of milk was studied by determining the microbial load of milk produced in the livestock farm, the dairy plant and in retail outlets. The effect of pasteurization on the microbial quality of raw milk and the changes in microbial load of the pasteurized milk sample during refrigeration were also analyzed. During the study, the changes in sensory and physicochemical quality of fresh and refrigerated samples were evaluated and the shelf life of pasteurized milk under refrigerated conditions was determined. The samples of pooled raw, freshly pasteurized, refrigerated and retail milk was subjected to the isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes.* The various critical control points of microbial contamination of milk during the production and processing were identified and their microbial load was also determined.

4.1 MICROBIAL QUALITY OF MILK

4.1.1 Microbial Counts of Raw Milk

All raw milk samples collected from individual animals, pooled milk and chilled milk were tested to determine the quality by estimating Total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC), Psychrotrophic count (PC), Faecal Streptococcal count (FSC) and Yeast and Mould count (YMC).

4.1.1.1 Total Viable Count (TVC)

The mean TVC of raw milk from individual animal and also pooled and chilled milk samples are given in table 2. Analysis of the data by students 't' test revealed highly significant ($p<0.01$) difference between mean counts of the samples from individual animals and pooled milk and also between the samples of individual animals milk and Chilled milk. The samples of chilled milk before pasteurization had highest mean count (5.70 ± 0.13 $\log_{10}$ cfu/ml) and the lowest count was seen in the milk of individual animals in the milking barn (5.14 ± 0.13 log_{10} cfu/ml).

Table 2. Mean total viable count of raw milk samples

Milk samples	Mean ± SE (log_{10} cfu/ml)
Milk from individual animal	5.14 ± 0.13^{a}
Pooled milk	5.58 ± 0.14^{b}
Chilled milk	5.70 ± 0.13^{b}

Figures bearing the same superscript do not differ significantly; N=20 sample in each group

The distribution of individual, pooled and chilled milk samples based on total viable count are given in table 3. Of the 60 samples, 46 (76.67 per cent) had count at the level of 10^5 cfu/ml. The count at the level of 10^6 cfu/ml was seen in 10 sample (16.67 per cent) of samples. The lowest count at the level of 10^4 cfu/ml was seen in only four samples of individual animal milk and the highest count.

Table 3. Distribution of individual, pooled and chilled milk samples based on total viable count

Sample	Total viable count (cfu/ml)		
	10^4	10^5	10^6
Individual animal milk	4 (20)	16 (80)	
Pooled milk		16 (80)	4 (20)
Chilled milk		14 (70)	6 (30)

Figures in parenthesis indicate per cent; N=20 was seen at the level of 10^6 cfu/ml, in 30 per cent of chilled milk and 20 per cent of pooled milk. However none of the samples from individual animals had count at the level of 10^6 cfu/ml.

4.1.1.2 Coliform Count (CC)

The mean CC of raw milk from individual animal, pooled and chilled milk samples are given in table 4. Analysis of the data revealed highly significant ($p<0.01$) difference between mean counts of milk from individual animal and pooled milk and between individual animal milk and chilled milk. The samples of pooled milk had a highest mean coliform count and the individual animal sample

Table 4. Mean coliforms count of raw milk samples

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk from individual animal	1.83 ± 0.22^{a}
Pooled milk	3.24 ± 0.19^{b}
Chilled milk	3.10 ± 0.17^{b}

Figures bearing the same superscript do not differ significantly; N=20 in each group

had the lowest mean count. The distribution of individual, pooled and chilled milk samples based on coliform count are given in table 5. Of the 60 samples, 40 per cent had a count at the level of 10^3 cfu/ml and 33.33 per cent of the samples had count at the level of 10^2 cfu/ml. The count at the level of 10^4 cfu/ml was seen in only one of the sample of chilled milk. Coliforms were not detected in 45 per cent of the Individual animal milk.

Table 5. Distribution of individual, pooled and chilled milk samples based on coliform count

Sample	Coliform count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
Individual animal milk	9(45)	6 (30)	3 (15)	2(10)	
Pooled milk			8(40)	11(55)	1(5)
Chilled milk			9(45)	11(55)	

Figures in parenthesis indicate per cent; ND-Not Detected; N=20 in each group

4.1.1.3 Escherichia coli Count (ECC)

The mean *Escherichia coli* count of raw milk from individual animal, pooled and chilled milk samples are given in table 6. The highest level of the organisms was seen in chilled milk samples. Pooled milk samples had the lowest mean count.

Table 6. Mean *Escherichia coli* count of raw milk samples

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk from individual animal	1.01 ± 0.21
Pooled milk	0.63 ± 0.31
Chilled milk	1.05 ± 0.29

N=20 in each group

The distribution of individual, pooled and chilled milk samples based *Escherichia coli* count are given in table 7. Of the 60 samples, *E.coli* was not detected in 60 per cent of the samples. The count at the level of 10 cfu/ml was seen in 16.66 per cent of samples.

Table 7. Distribution of individual, pooled and chilled milk samples based on *Escherichia coli* count

Sample	*Escherichia coli* count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
Individual animal milk	8(40)	7(35)	5(25)		
Pooled milk	16(80)	1(5)	ND	2(10)	1(5)
Chilled milk	12(60)	2(10)	3(15)	3(15)	

Figures in parenthesis indicate per cent, ND-Not Detected N=20 in each group

4.1.1.4 Psychrotrophic Count (PC)

The mean psychrotrophic count of raw milk from individual, pooled and chilled milk samples are given in table 8. Analysis of the data revealed that significant ($p<0.05$) difference between mean counts of milk samples from individual animal and pooled milk and also between mean counts of chilled and individual animals milk. The samples of chilled milk had a highest mean count.

Table 8. Mean psychrotrophic **count of raw milk samples**

Milk samples	Mean ± SE (log_{10} cfu/ml)
Milk from individual animal	4.69 ± 0.24^a
Pooled milk	5.42 ± 0.07^b
Chilled milk	5.56 ± 0.14^b

Figures bearing the same superscript do not differ significantly; N=20 in each group

The distribution of individual, pooled and chilled milk samples based on psychrotrophic count are given in table 9. In 40 per cent of raw milk samples

Table 9. The distribution of individual, pooled and chilled milk samples based on psychrotrophic count

Sample	Psychrotrophic count (cfu/ml)			
	10^3	10^4	10^5	10^6
Individual animal milk	2(10)	10 (50)	8(40)	
Pooled milk			18 (90)	2(10)
Chilled milk		2(10)	16 (80))	2(10)

Figures in parenthesis indicate per cent; N=20 in each group

from individual animals had count at the level of 10^5 cfu/ml. The count at the level was seen in 90 per cent and 80 per cent of pooled and chilled milk, respectively. The count at the level of 10^6 cfu/ml was seen in 10 per cent each of pooled and chilled milk. The lowest count was at the level of 10^3 cfu/ml and was seen in 10 per cent of milk from individual animal.

4.1.1.5 Faecal streptococcal Count

The mean faecal streptococcal count of raw milk from individual animal, pooled and chilled milk samples are given in table 10. Analysis of the data revealed a significant ($p<0.05$) difference between mean counts of milk samples from individual animal and pooled milk and also between the counts of individual animal and chilled milk.

Table 10. Mean faecal streptococcal count of raw milk samples

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk from individual animal	1.89 ± 0.08^a
Pooled milk	2.59 ± 0.11^b
Chilled milk	2.57 ± 0.12^b

Figures bearing the same superscript do not differ significantly. N=20 in each group

The distribution of individual, pooled and chilled milk samples based on faecal streptococcal count are given in table 11. From the 60 samples, 56.66 per cent had a count at the level of 10^2 cfu/ml and 20 per cent had count at the level

Table 11. Distribution of individual, pooled and chilled milk samples based on faecal streptococcal count

Sample	Faecal streptococcal count (cfu/ml)		
	10^1	10^2	10^3
Individual animal milk	12(60)	8 (40)	
Pooled milk	2(10)	12(60)	6(30)
Chilled milk		14(70)	6 (30)

Figures in parenthesis indicate per cent. N=20 in each group

of 10^3 cfu/ml. The count at the level of 10 cfu/ml was seen in 60 per cent of individual animal milk and 10 per cent of pooled milk.

4.1.1.6 Yeast and Mould Count

The mean Yeast and mould count of raw milk from individual animal, pooled and chilled milk samples are given in table 12. Pooled milk samples had the highest mean count. The mean count was lowest for the milk of individual animals.

Table 12. Mean yeast and mould count of raw milk samples

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk from individual animal	1.58 ± 0.27
Pooled milk	1.86 ± 0.19
Chilled milk	1.84 ± 0.24

N=20 in each group

The distributions of individual, pooled and chilled milk samples based on yeast and mould count are given in table 13. The count at the level of 10^2 cfu/ml was seen in 60 per cent each of pooled and chilled milk samples. The organism could not detect in 23.33 per cent of the samples but the count at the level of 10^3cfu/ml was seen in 10 per cent each of individual, pooled and chilled milk samples.

Table 13. Distribution of individual, pooled and chilled milk samples based on Yeast and mould count

Sample	Yeast and mould count (cfu/ml)			
	ND	10^1	10^2	10^3
Individual animal milk	6(30)	4(20)	8(40)	2(10)
Pooled milk	4(20)	2(10)	12(60)	2(10)
Chilled milk	4(20)	2(10)	12(60)	2(10)

Figures in parenthesis indicate per cent. N=20 in each group

4.1.1.7 Correlation between microbial counts of Raw Milk

The correlation coefficient between various microbial counts of raw milk samples is given in the table 14. A highly significant ($p<0.01$) and positive

Table 14. Correlation between microbial counts of raw milk

Counts	CC	ECC	PC	FSC	YMC
TVC	0.118	0.009	0.465**	0.574**	-0.013
CC		0.25	0.447*	0.183	-0.029
ECC			0.009	-0.144	0.15
PC				0.314	0.168
FSC					0.133

association between the total viable count and psychrotrophic count and also between the total viable count and faecal streptococcal count was observed. However, a positive and significant ($p<0.05$) correlation between the psychrotrophic and coliform counts was also observed.

4.1.2 Isolation and Identification of Bacteria

The bacteria isolated from pooled raw milk are given in table 15.

Table 15. Bacteria isolated from pooled raw milk

Bacteria	Number of samples tested	Isolates	
		Positive	Per cent
Escherichia coli	20	4	20
Staphylococcus aureus	20	12	60
Listeria monocytogenes	20	ND	-

ND: Not detected

4.1.2.1 Escherichia coli

Pooled raw milk samples were tested for the isolation and identification of *Escherichia coli.* The organism was detected in 20 per cent of the samples. The isolates collected were identified by cultural, morphological and biochemical test. Three of the isolates showed an Eijkman positive reaction. The identified isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh and were also subjected to congo red binding tests. The distribution of *E. coli* serotypes isolated from pooled raw milk is given in Table 16. A total of four isolates were obtained from pooled raw milk

Table 16. Distribution of *E. coli* serotypes from pooled raw milk

Isolate No.	Serotype
1	O5
2	O141
3	O5
4	O172

samples, of which 50 per cent isolates belonged to the serotype 05 and the remaining 25 per cent of isolates each belonging to serotype 0141 and 0172, respectively. *E. coli* isolated from pooled raw milk were subjected to Congo red binding test and the results are given in table 17. Three of these isolates had Congo red binding characteristics, which indicates the property of invasiveness of the isolates.

Table 17. Congo red binding test of *E. coli* isolates from milk

Isolate No.	Congored binding test
1	+
2	-
3	+
4	+

+ 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 only from 12 (60 per cent) of the pooled raw milk sample.

4.1.2.3 Listeria monocytogenes

All pooled milk samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples revealed the presence of this organism

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

4.1.3 Microbial Count of Milk at Various Stages of Pasteurization

4.1.3.1 Total Viable Count

The mean TVC of milk after heating, pasteurization and packaged samples are shown in table 18. Analysis of the data by students 't' test revealed highly significant ($p<0.01$) difference in the mean count of pasteurized milk

Table 18. Mean total viable count of milk obtained during various stages of pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after Heating section	3.00 ± 0.15^{a}
Pasteurized milk	3.64 ± 0.15^{b}
Packaged milk	4.76 ± 0.15^{c}

Figures bearing the same superscript do not differ significantly; N=20 in each group

and packaged milk, heated and pasteurized milk and also heated and packaged milk. Packaged milk samples had highest mean count of 4.76 ± 0.15 $\log_{10}$ cfu/ml and heated milk had the lowest count of 3.00 ± 0.15 $\log_{10}$ cfu/ml.

The distribution of milk at different stages of pasteurization based on TVC is given in table 19. The count at the level of 10^5 cfu/ml was found in 30 per cent of packaged milk sample. The count was at the level of 10^3 cfu/ml was seen in 55 per cent of milk collected immediately after heating and in 70 per cent of pasteurized milk but none of the pasteurized milk samples had count at the level of 10^4 cfu/ml.

Table 19. Distribution of milk obtained during various stages of pasteurization based on total viable count

Sample	Total viable count (cfu/ml)			
	10^2	10^3	10^4	10^5
Milk after Heating	9 (45)	11 (55)		
Pasteurized milk		14(70)	6 (30)	
Packaged milk			14 (70)	6 (30)

Figures in parenthesis indicate percent; N=20 in each group

4.1.3.2 Coliform Count

The mean coliform count of milk collected immediately after heating, pasteurized milk and packaged milk samples are shown in table 20.

Table 20. Mean Coliform count of milk obtained during various stages of pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after Heating section	ND
Pasteurized milk	0.25 ± 0.19^a
Packaged milk	0.98 ± 0.36^b

Figures bearing the same superscript do not differ significantly: N=20 in each group

Statistical analysis of the mean count revealed highly significant ($p<0.01$) difference between the mean counts of pasteurized milk and packaged milk. Coliforms were not detected from heated milk.

The distribution of milk samples based on coliform count is given in table 21. The count was not detected in 100 per cent of milk collected immediately after

Table 21. Distribution of milk obtained during various stages of pasteurization based on coliform count

Sample	Coliform count (cfu/ml)		
	ND	10^1	10^2
Milk after Heating section	20(100)		
Pasteurized milk	16(80)	4(20)	
Packaged milk	8(40)	8(40)	4 (20)

Figures in parenthesis indicate percent; ND-Not Detected; N=20 in each group

heating. In 80 and 40 per cent of pasteurized and packaged milk sample also showed no presence of the organism.The count in 20 per cent of the packaged milk samples was at the level of 10^2 cfu/ml. The count at the level of 10 cfu/ml was seen in 40 per cent of packaged milk samples.

4.1.3.3 Escherichia coli count

The mean *Escherichia coli* count of milk collected immediately after heating, pasteurization and packaged milk samples are shown in table 22.

Table 22. Mean *Escherichia coli* count of milk during various stages of pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after heating section	ND
Pasteurized milk	ND
Packaged milk	0.31 ± 0.21

ND-Not Detected, N=20 in each group

The distributions of milk samples during various stages of pasteurization based on *Escherichia coli* counts are shown in table 23. The organisms could not detect in 100 per cent of heated and pasteurized milk and in 90 per cent of packaged milk. However in one sample each of packaged milk had count at the level of 10^2 and 10^3 cfu/ml.

Table 23. Distribution of milk obtained during various stages of pasteurization based on *Escherichia coli* count

Sample	*Escherichia coli* count (cfu/ml)			
	ND	10^1	10^2	10^3
Milk after Heating section	20(100)	ND		
Pasteurized milk	20(100)	ND		
Packaged milk	18(90)	ND	1 (5)	1 (5)

Figures in parenthesis indicate per cent; ND-Not Detected; N=20 in each group

4.1.3.4 Psychrotrophic Count

The mean psychrotrophic count of milk collected immediately after heating, pasteurized milk and packaged milk samples are shown in table 24. Analysis of the data by students 't' test revealed a highly significant ($p<0.01$)

Table 24. Mean psychrotrophic count of milk obtained during various stages of Pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after heating section	1.20 ± 0.22^a
Pasteurized milk	2.15 ± 0.14^b
Packaged milk	4.16 ± 0.09^c

Figures bearing the same superscript do not differ significantly; N=20 in each group

difference between the mean counts of milk collected immediately after heating and pasteurization, packaged milk and milk after heating section and also between pasteurized and packaged milk. The lowest mean count was seen in the milk collected immediately after heating, (1.20 ± 0.22 $\log_{10}$ cfu/ml) and the count in the packaged milk was at the level of 4.16 ± 0.09 $\log_{10}$ cfu/ml.

The distribution of milk sample based on psychrotrophic count is given in table 25. The organism could not detect in 30 per cent of sample collected immediately after heating. In packaged milk 80 per cent samples had count at the level of 10^4 cfu/ml. All samples of pasteurized milk had psychrotrophic organism and 50 per cent of the samples had count at the level of 10^2 cfu/ml.

Table 25. Distribution of milk obtained during various stages of pasteurization based on psychrotrophic count

Sample	Psychrotrophic count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
Milk after heating section	6(30)	8 (40)	5(25)		
Pasteurized milk		6(30)	10(50)	4 (20)	
Packaged milk				4 (20)	16(80)

Figures in parenthesis indicate per cent; ND- Not detected; N=20 in each group

4.1.3.5 Faecal Streptococcal Count

The mean faecal streptococcal count of milk collected immediately after heating, pasteurized milk and packaged milk samples are shown in table 26. Analysis of the data revealed a highly significant ($p<0.01$) difference in the mean counts of milk collected immediately after heating and packaged milk.

Table 26. Mean faecal streptococcal count milk obtained during various stages of pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after heating section	0.23 ± 0.10^a
Pasteurized milk	0.70 ± 0.16^b
Packaged milk	1.06 ± 0.18^b

Figures bearing the same superscript do not differ significantly

The distribution of milk sample based on faecal streptococcal count at different stages of pasteurization is given in table 27. The organism could not detect in 48.33 per cent of samples but 51.66 per cent samples had the organism at the level of 10 cfu/ml of milk collected at different stages of pasteurization. However, 85 per cent packaged milk and 50 per cent pasteurized milk had faecal streptococcal count at the level of 10 cfu/ml.

Table 27. Distribution of milk obtained during various stages of pasteurization based on faecal streptococcal count

Sample	Faecal streptococcal count (cfu/ml)	
	ND	10^1
Milk after heating section	16(80)	4(20)
Pasteurized milk	10(50)	10(50)
Packaged milk	3(15)	17(85)

Figures in parenthesis indicate percent; N=20 in each group

4.1.3.6 Yeast and Mould Count

The mean yeast and mould count of milk collected immediately after heating, pasteurized milk and packaged milk samples are shown in table 28. Analysis of the mean count revealed a significant ($p<0.05$) difference between the counts of pasteurized milk and packaged milk.

Table 28. Mean Yeast and mould count of milk obtained during various stages of pasteurization

Milk samples	Mean ± SE ($\log_{10}$ cfu/ml)
Milk after heating section	0.11 ± 0.07^a
Pasteurized milk	0.23 ± 0.11^a
Packaged milk	0.62 ± 0.13^b

Figures bearing the same superscript do not differ significantly; N=20

The distributions of samples collected at different stages of pasteurization based on YMC per ml are given in table 29. Yeast and mould could not detect in 90 per cent of heated milk, 80 and 35 per cent of pasteurized milk and packaged milk, respectively. In packaged milk 65 per cent had the count at the level of 10 cfu/ml.

Table 29. Distribution of milk obtained during various stages of pasteurization based on Yeast and mould count

Sample	Yeast and mould count (cfu/ml)	
	ND	10^1
Milk after heating section	18(90)	2(10)
Pasteurized milk	16(80)	4(20)
Packaged milk	7(35)	13(65)

Figures in parenthesis indicate per cent; N=20 in each group

4.1.3.7 Correlation between Microbial Counts in Fresh Milk

The correlation of microbial counts of freshly packaged milk sample is given in table 30. Analysis of the data revealed a highly significant ($p<0.01$) and

Table 30. Correlation between microbial counts in packaged milk sample

Counts	CC	ECC	FSC	PC	YMC
TVC	0	0.54	-0.1	0.06	0.13
CC		0.778**	0.46	-0.1	0.048
ECC			0.27	0.28	0.41
FSC				-0.66*	-0.61
PC					0.133

positive correlation between mean coliforms and *E. coli* counts of packaged milksample and a significant ($p<0.05$) and negative association was observed between the counts of psychrotrophic and faecal streptococcal count of the samples.

4.1.4 Isolation and Identification of Organism in Pasteurized Milk

All freshly pasteurized milk samples were tested for the isolation and identification of *E. coli, S. aureus and L. monocytogenes.* The numbers of isolates obtained from the samples are given in table 31.

Table 31. Bacteria isolated from freshly pasteurized and packed milk

Bacterial isolate	Number of freshly pasteurized and packed milk	
	Tested	Positive
Escherichia coli	20	2
Staphylococcus aureus	20	1
Listeria monocytogenes	20	ND

ND: Not detected

4.1.4.1 Escherichia coli

Escherichia coli was isolated from 10 per cent of freshly pasteurized milk. The isolate were identified by cultural, morphological and bio-chemical characteristics and were subjected to serotyping at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh and congo red binding test was also performed from the isolates. The two isolate obtained from the samples belonged to the serotype 0148 and are congo red binding test positive and also showed positive reaction for Eijkman test.

4.1.4.2 Staphylococcus aureus

Staphylococcus aureus was isolated only from one (5 per cent) of the freshly pasteurized milk.

4.1.4.3 ***Listeria monocytogenes***

All pasteurized packaged milk samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples revealed the presence of the organism

4.1.5 Grading of Pasteurized Milk

According to IS standards (1992) the Standard Plate Count of pasteurized milk should be less than 30,000 per ml and coliform count should be absent in 1:10 dilution are considered satisfactory. In the present investigation based on SPC, 55 per cent of the sample was considered as satisfactory. The grading of milk based on Coliform count revealed 40 per cent samples as satisfactory.

4.1.6 Effect of Pasteurization on the Microbial Quality of Raw Milk

The effect of pasteurization on the microbial quality of milk is given in the table 32 and illustrated in fig 1. The mean TVC, CC, PC, FSC and YMC of raw milk have reduced to a highly significant ($p<0.01$) level upon pasteurization.

Table 32. Effect of pasteurization on the microbial quality of milk

Microbial Count	Microbial load of milk ($\log_{10}$ cfu/ml)	
	Before pasteurization	Pasteurized packaged milk
TVC	5.70 ± 0.13^a	4.76 ± 0.15^b
CC	3.10 ± 0.17^a	0.98 ± 0.36^b
ECC	1.01 ± 0.29^a	0.31 ± 0.21^b
PC	5.56 ± 0.14^a	4.16 ± 0.09^b
FSC	2.57 ± 0.12^a	1.06 ± 0.18^b
YMC	1.84 ± 0.24^a	0.62 ± 0.13^b

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

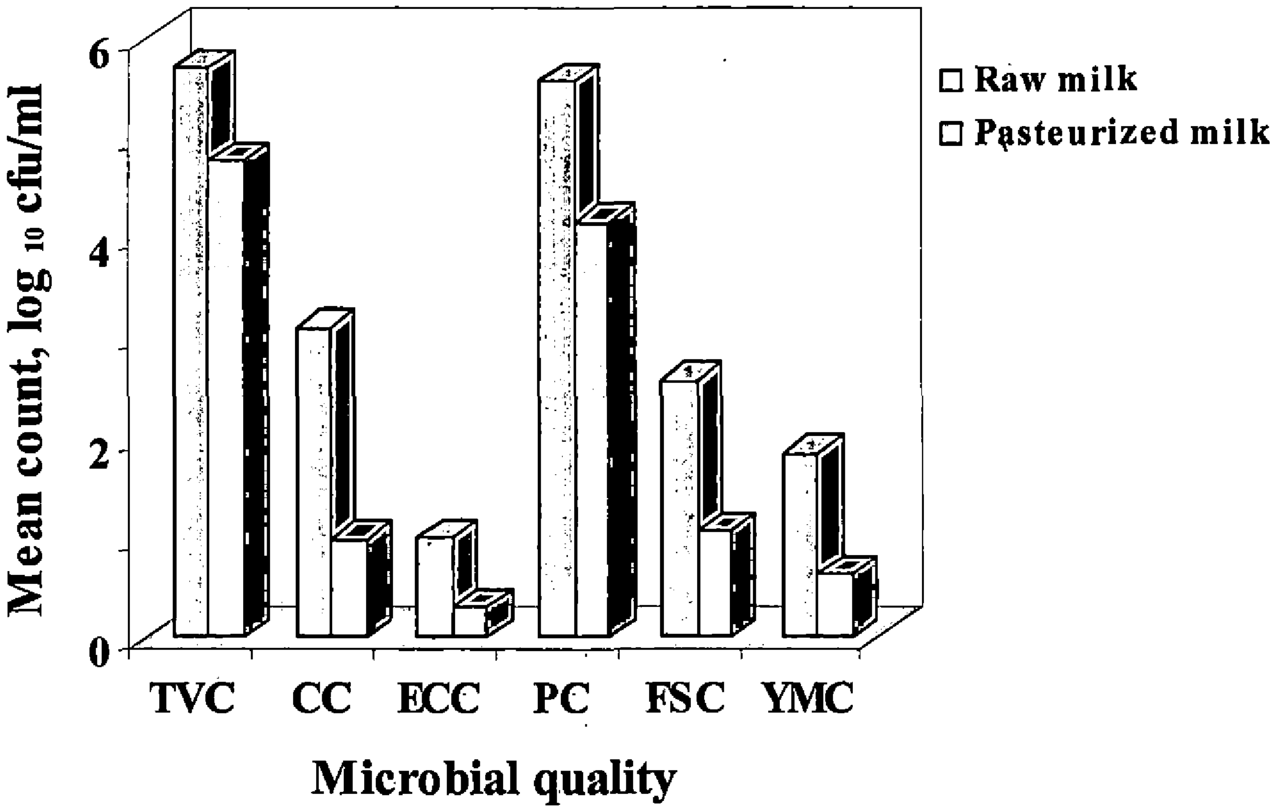

Fig ; Effect of Pasteurization on the Microbial Quality of Raw Milk pasteurization. The mean reduction in TVC, CC, PC and FSC in pasteurized milk with that of chilled milk was at the level of 0.94, 2.12, 1.40 and 1.51 log_{10} cfu/ml. Coliform was found in 100 per cent of milk before pasteurization and the mean count was reduced to a highly significant ($p<0.01$) level with 40 per cent of the final product (packaged milk) found free from the organism. *E. coli* count in raw milk was significantly ($p<0.05$) reduced upon pasteurization.

4.1.7 Microbial Counts of Retailed Milk Samples

Microbial counts of retail milk samples obtained from different brands available in and around Thrissur were compared with the count of zero day samples (E) of pasteurized milk obtained from dairy plant. The retail samples were evaluated for TVC, CC, ECC, PC, FSC and YMC.

4.1.7.1. Total Viable Count

The mean TVC of milk samples belonging to the brand A, B, C, D and zero day samples 'E' are shown in table 33 and illustrated in fig 2.

Table 33. Mean total viable count of retail milk sample

Brands	Mean ± SE (log_{10} cfu / ml)
A	4.88 ± 0.12^{b}
B	5.91 ± 0.01^{a}
C	5.56 ± 0.13^{a}
D	5.45 ± 0.14^{a}
E	4.76 ± 0.15^{b}

Critical Difference 0.4657; Figures bearing the same superscript do not differ significantly; N=14 in retail brands

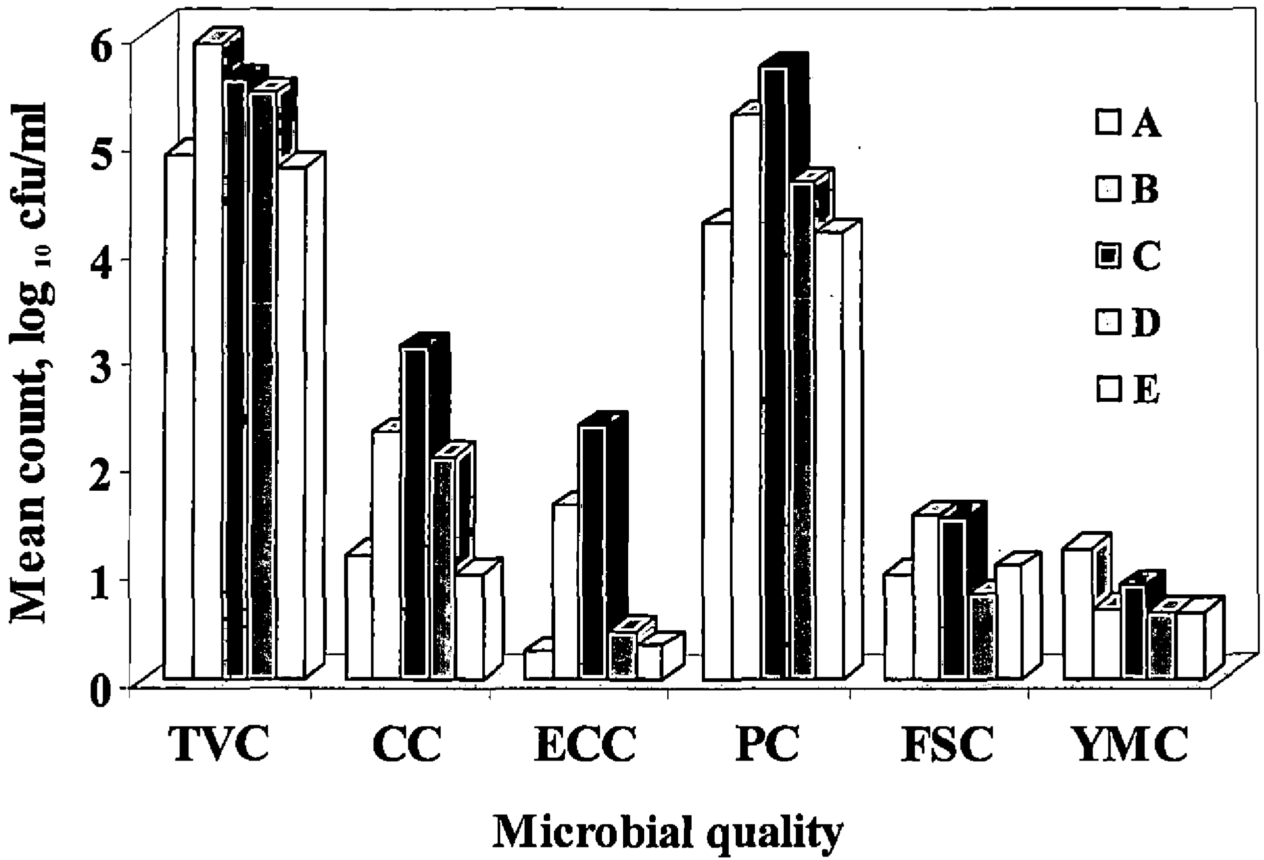

Fig 2. Comparison of microbial quality of milk brands (A, B, C, D) and "zero day" sample (E)

Analysis of variance test of the data revealed highly significant ($p<0.01$) difference between the mean count of samples from the brands.

The highest mean count was seen in the samples belonging to brand B and the lowest from samples belonging to Brand A. The critical difference test revealed highly significant difference between the mean counts of brand A and B, A and C, D and A, and also between the mean counts of sample E with the counts of brands B, C, and D.

Distribution of milk based on total viable count

The distribution of retail milk based on total viable count is shown in table 34. Of the 56 retail samples, highest counts was seen in the samples of the brand B of which one sample had a count at the level of 10^7 cfu/ml and 50 per cent samples of the brand had count at level of 10^6 cfu/ml. Only two samples of the brand D (14.29 per cent) had count at that level of 10^6 cfu/ml. Lowest count was seen in the samples of the brand A, of which 64.28 per cent of the samples had count at the level of 10^4 cfu/ml. The counts in 50 per cent of the sample of brand C and 71.42 per cent of the samples from brand D were at the level of 10^6 and 10^5, respectively.

Table 34. Distribution of retail milk based on total viable count

Brands	Total viable count (cfu/ml)			
	10^4	10^5	10^6	10^7
A	9(64.28)	5(35.72)		
B	1(7.14)	5(35.72)	7(50)	1(7.14)
C	3(21.44)	4(28.57)	7(50)	
D	2(14.29)	10(71.42)	2(14.29)	

Figures in the parenthesis indicate percent; N=14

4.1.7.2 Coliform Count

The mean Coliform counts of milk samples belonging to the brand A, B, C and D and the count of the source E are shown in table 35 and illustrated in fig 2. Analysis of variance test of the data revealed highly significant ($p<0.01$) difference between the mean count of samples between the brands.

Table 35. Mean coliform count of retail milk sample

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	1.15 ± 0.28^{c}
B	2.30 ± 0.38^{ab}
C	3.08 ± 0.29^{a}
D	2.07 ± 0.29^{b}
E	0.98 ± 0.36^{c}

Critical Difference 0.93; Figures bearing the same superscript do not differ significantly; N=14 in retail brands

The critical difference test revealed high significant ($p<0.01$) difference between the mean counts of the samples from A and B, A and C, A and D, C and D and also from the mean counts of sample E with the counts of brands B, C, and D.

The distribution of retailed milk based on coliform counts is shown in table 36. Of the 56 samples, highest count at the level of 10^4 cfu/ml was seen in one (7.14 per cent) of the samples of the brand C. Lowest mean count was seen in the samples of brand A of which the count was not detected in 64.28 per cent of the samples. The count at level of 10^2 cfu/ml was seen in 64.28 per cent of the sample of brand C whereas 57.14 per cent of sample of brand B had count at the above level. In 64.28 per cent of samples of the samples of brand D had count at the level of 10 cfu/ml.

Table 36. Distribution of retail milk based on Coliform Count

Brands	coliform counts (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
A	9(64.28)	5(35.72)			
B	2(14.29)	1(7.14)	8(57.14)	3(21.43)	
C	1(7.14)	1(7.14)	9(64.28)	2(14.29)	1(7.14)
D	3(21.44)	9(64.28)	2(14.29)		

Figures in the parenthesis indicate percent; N=14

4.1.7.3 Escherichia coli Count

The mean *Escherichia coli* count of milk samples belonging to the brand A, B, C and D and the source E are shown in table 37 and illustrated in fig 2. 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. The critical difference test revealed highly significant ($p<0.01$) difference between the mean counts of the brand A and B, A and C and B and D, B and E and C and E.

Table 37. Mean *Escherichia coli* Count of retail milk sample

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	0.25 ± 0.17^b
B	1.62 ± 0.28^a
C	2.35 ± 0.38^a
D	0.45 ± 0.25^b
E	0.31 ± 0.22^b

Critical Difference 0.78; Figures bearing the same superscript do not differ significantly: N=14 in retail brands

The distribution of retail milk based on *Escherichia coli* count is shown in table 38. Of the 56 samples, the highest count was seen in the samples belonging to the brand C of which 2 (14.29 per cent) samples had a count at the level of 10^3 cfu/ml. The count was not detected in 85.72 per cent and 78.57 per cent of samples of brand A and D, respectively. The count at level of 10^2 cfu/ml was seen in 57.14 per cent of the sample of brand B and C.

Table 38. Distribution of retail milk based on *Escherichia coli* count

Brands	*Escherichia coli* count (cfu/ml)			
	ND	10^1	10^2	10^3
A	12(85.72)	1(7.14)	1(7.14)	
B	3(21.44)	3(21.44)	8(57.14)	
C	3(21.44)	1(7.14)	8(57.14)	2(14.29)
D	11(78.57)	1(7.14)	2(14.29)	

Figures in the parenthesis indicate per cent; N=14

4.1.7.4 Psychrotrophic Count

The mean Psychrotrophic counts of milk samples belonging to the brand A, B, C and D and the source E are shown in table 39 and illustrated in fig 2. 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 A had the lowest mean count and the highest count was seen in the samples belonging to brand C. The critical difference test revealed highly significant ($p<0.01$) difference between the mean counts of the samples from A and B, A and C, A and D, B and E, C and D, C and E and also D and E.

Table 39. Mean psychrotrophic Count of retail milk sample

Brands	Mean ± SE (log_{10} cfu / ml)
A	4.25 ± 0.22^{c}
B	5.25 ± 0.36^{ab}
C	5.68 ± 0.25^{a}
D	4.62 ± 0.21^{b}
E	4.16 ± 0.09^{c}

Figures bearing the same superscript do not differ significantly; N=14 in retail brands

The distribution of retailed milk based on Psychrotrophic count is shown in table 40. Of the 56 samples, the highest mean counts was seen in the samples belonging to brand C of which five (35.71 per cent) samples had a count at the level of cfu/ml10^6. In three (21.43 per cent) samples of brand B had count at that level. The count at the level of 10^4 cfu/ml was seen in 64.28 per cent of the samples of brand A and 57.14 per cent of the samples of brand D. The count in 58 per cent of the samples each of the brands B and C was at the level of 10^5 cfu/ml.

Table 40. Distribution of retail milk based on Psychrotrophic Count

Brands	Psychrotrophic Count (cfu/ml)			
	10^3	10^4	10^5	10^6
A	3(21.43)	9(64.28)	2(14.29)	
B		4(28.57)	7(50)	3(21.43)
C		2(14.29)	7(50)	5(35.71)
D	2(14.29)	8(57.14)	4(28.57)	

Figures in the parenthesis indicate percent; N=14

4.1.7.5 Faecal Streptococcal Count

The mean Faecal Streptococcal counts of milk samples belonging to the brand A, B, C and D and the source E is shown in table 41 and illustrated in fig 2. The lowest mean count was seen in samples belonging to brand D and the highest count was seen in the samples of the brand B.

Table 41. Mean faecal streptococcal count of retail milk sample

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	0.98 ± 0.22
B	1.52 ± 0.22
C	1.51 ± 0.30
D	0.78 ± 0.22
E	1.06 ± 0.18

N=14 in retail brands

Table 42. Distribution of retail milk based on faecal streptococcal Count

Brands	Faecal streptococcal count (cfu/ml)			
	ND	10^1	10^2	10^3
A	5(35.72)	9(64.28)		
B	1(7.14)	9(64.28)	4(28.57)	
C	1(7.14)	10(71.43)	2(14.29)	1(7.14)
D	5(35.72)	9(64.28)		

Figures in the parenthesis indicate percent; ND-Not detected; N=14

The distribution of retail milk based on faecal streptococcal count is shown in table 42. Of the 56 samples, only one sample of brand C had a count at the level of 10^3 cfu/ml. The count at that level of 10^2 cfu/ml was seen in 28.57 per cent of samples of brand B and 14.29 per cent the sample of the brand C.

The count at the level of 10 cfu/ml was seen in 64.28 per cent samples of brand A, B and D. The count at the above level was seen in 71.43 per cent of the samples belonging to the brand C.

4.1.7.6 Yeast and Mould Count

The mean Yeast and Mould counts of milk samples belonging to the brand A, B, C and D and the source E is shown in table 43 and illustrated in fig 2. The lowest mean count was seen in samples belonging to brand D and the highest count in the samples of brand A.

Table 43. Mean Yeast and Mould count of retail milk sample

Brands	Mean ± SE ($\log_{10}$ cfu / ml)
A	1.21 ± 0.22
B	0.65 ± 0.22
C	0.88 ± 0.25
D	0.62 ± 0.21
E	0.62 ± 0.13

N=14 in retail brands

The distribution of retail milk based on yeast and mould count is shown in table 44.

Table 44. Distribution of retail milk based on Yeast and Mould count

Brands	Yeast and Mould count (cfu/ml)		
	ND	10^1	10^2
A	3(21.44)	8(57.14)	3(21.44)
B	7(50)	7(50)	
C	5(35.72)	8(57.14)	1(7.14)
D	8(57.14)	6(42.86)	

Figures in the parenthesis indicate per cent; ND-Not detected; N=14

The organism could not detect in 57.14, 50.00, 35.72 and 21.44 per cent samples belonging to brands D, B, C and A, respectively. The count at level of

10^2 cfu/ml was seen in 21.44 per cent of the samples of the brand A and in 7.14 per cent of sample of the brand C.

4.1.8 Isolation and Identification of Bacteria

The bacterial organisms of public health importance are isolated from retail milk and are given in table 45

Table 45. Bacteria isolated from retail milk

Bacteria	No. of samples tested	Number of positive samples			
		Brands of milk			
		A	B	C	D
Escherichia coli	56	2	11	11	3
Staphylococcus aureus	56	ND	2	3	2
Listeria monoctogenes	56	ND	ND	ND	ND

ND: Not detected

4.1.8.1 Escherichia coli

Out of 56 samples, 27 (48.21 per cent) had colonies with characteristics of *E. coli*. From this samples 38 colonies were selected and subjected to the characterization of the organism by primary and secondary tests. All the 38 isolates were Gram negative, small coccoid rods, which were catalase positive, oxidase negative and motile. The Isolates were also urease negative and showed characteristic IMViC reaction (Indole+, MR+, VP-, and Citrate+). All the isolates also utilized glucose, lactose, mannitol and maltose sugars. From this isolates 34 showed Ejikman positive reaction and 29 were found to show congo red binding ability.

4.1.8.2 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 two (14.28 per cent) samples each of the brands B and D and from three (21.43 per cent) samples of the brand C.

4.1.8.2 Listeria monocytogenes

All retail milk samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples revealed the presence of this organism

4.1.9 Microbial Counts of Refrigerated Milk

Microbial quality of refrigerated milk samples was evaluated on day two, four, six, eight, 10 and 12 of storage at 4 ± 1 °C.

4.1.9.1 Total Viable Count

The mean total viable count of refrigerated milk samples is given in table 46. During 12 days of storage the mean count has increased from 4.76 ± 0.15 to 8.72 ± 0.15 $\log_{10}$ cfu/ml, as illustrated in fig 3. The mean count of fresh sample had highly significant ($p<0.01$) difference with the mean count of the samples stored on day four, six, eight, ten and 12.

Table 46. Mean total viable count of refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	4.76 ± 0.15^{a}
2	5.12 ± 0.16^{a}
4	5.65 ± 0.14^{b}
6	6.38 ± 0.14^{c}
8	6.92 ± 0.18^{d}
10	7.97 ± 0.16^{e}
12	8.72 ± 0.15^{f}

Figures bearing the same superscript do not differ significantly.

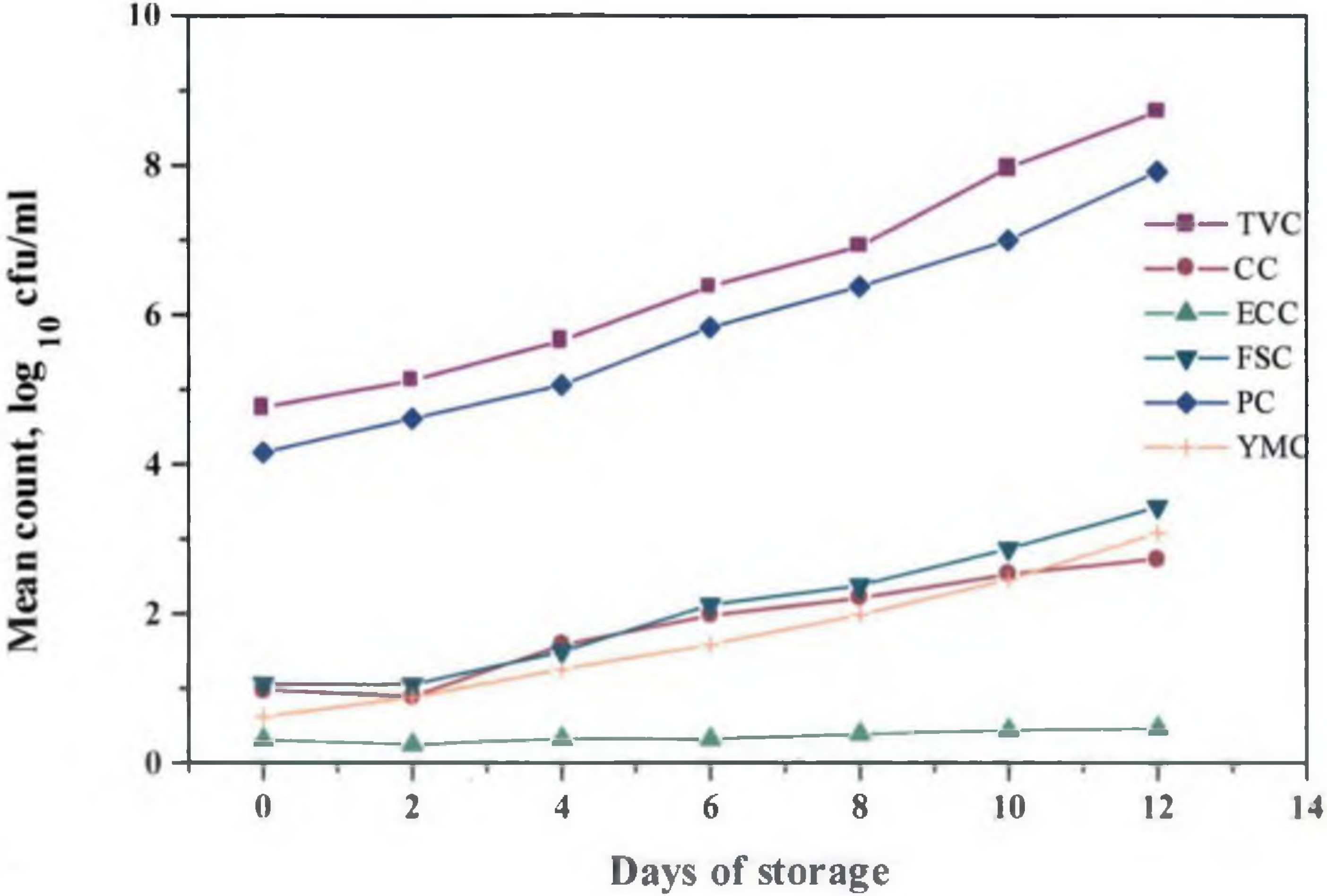

Fig 3 Effect of Refrigeration on the Microbial Quality of Milk

Distribution of refrigerated samples based on level of total viable count is given in table 47. The count was at the level of 10^5cfu/ml in 60 per cent of the samples on day two of storage and on day four of storage, 80 per cent samples had count at the above level. On day six of storage 70 per cent samples had count at the level of 10^6 cfu/ml and on day eight the count in 50 per cent samples each had at the level of 10^6 cfu/ml and 10^7 cfu/ml, respectively. On day 10 the count in 60 per cent samples was at the level of 10^8 cfu/ml whereas on day 12 the count in 70 per cent samples was at the above level. However, on the later day of storage 30 per cent samples had count at the level of 10^9cfu/ml.

Table 47. Distribution of refrigerated milk samples based on TVC

Days of storage	Total viable count (cfu/ml)					
	10^4	10^5	10^6	10^7	10^8	10^9
0	14 (70)	6 (30)				
2	8 (40)	12 (60)				
4		16 (80)	4 (20)			
6		4 (20)	14 (70)	2 (10)		
8			10 (50)	10 (50)		
10				8 (40)	12 (60)	
12					14 (70)	6 (30)

Figures in parenthesis indicate percent; N=20 in each group

4.1.9.2 Coliform Count

The mean coliform count of refrigerated milk samples is given in table 48. Analysis of the data revealed a gradual increase in coliform count throughout the period of storage. The mean coliform count of freshly pasteurized milk increased from 0.98 ± 0.36 to 2.74 ± 0.37 $\log_{10}$ cfu/ml on the 12th day of storage, as illustrated in fig 3. The mean count of zero day differed highly significantly ($p<0.01$) from the count of fourth day onwards up to 12th day and also highly significant ($p<0.01$) difference was seen between fourth and eight day and also between the counts of sixth and tenth day of storage.

Table 48. Mean Coliform count of refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	0.98 ± 0.36^{a}
2	0.89 ± 0.38^{ab}
4	1.58 ± 0.34^{bc}
6	1.98 ± 0.28^{cd}
8	2.21 ± 0.32^{de}
10	2.53 ± 0.32^{f}
12	2.74 ± 0.37^{f}

Figures bearing the same superscript do not differ significantly. N=20 in each group

Distribution of refrigerated samples based on level of coliform count is given in table 49. The count could not be detected in 60 per cent of the sample on

Table 49. Distribution of refrigerated milk samples based on coliform count

Days of storage	Coliform count (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
0	8(40)	8(40)	4 (20)		
2	12 (60)	4(20)	2(10)	2(10)	
4	6(30)	6(30)	6(30)	2(10)	
6	2(10)	8(40)	7(35)	3(15)	
8	2(10)	2(10)	12 (60)	4(20)	
10	2(10)		8(40)	10 (50)	
12	2(10)		7(35)	7(35)	4(20)

Figures in parenthesis indicate percent; N=20 in each group day two of storage. However in 10 per cent of the sample stored on day two had count at the level of 10^3 cfu/ml. On day 12 of storage 20 per cent of samples had count at the level 10^4 cfu/ml but on all the other days of storage the sample do not have count more than 10^3 cfu/ml. In 40, 60 and 50 per cent samples stored on day six, eight, and 10 had count at the level of 10, 10^2 and 10^3 cfu/ml, respectively.

4.1.9.3 Escherichia coli Count

The Mean *Escherichia coli* count of refrigerated milk samples is given in table 50. A reduction in mean count was observed in the samples stored on day two. However, there after, except on day six of storage, the count showed an increase in trend but was not significant, as illustrated in fig 3.

Table 50. Mean *Escherichia coli* count of refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
0	0.31 ± 0.21
2	0.25 ± 0.17
4	0.33 ± 0.22
6	0.32 ± 0.21
8	0.39 ± 0.27
10	0.44 ± 0.30
12	0.47 ± 0.32

N=20 in each group

Distribution of refrigerated samples based on level of *Escherichia coli* count is given in table 51. The organism could not be detected in 90 per cent of the samples stored during the period of study. The count at the level of 10^3cfu/ml was seen in 10 per cent of the samples examined on day four. However, none of the samples examined on day 10 and 12 revealed count at the above level. The count at the level of 10^5 cfu/ml was seen form only one samples examined on day 12.

Table 51. Distribution of refrigerated milk samples based on *Escherichia coli* count

Days of storage	*Escherichia coli* Count (cfu/ml)					
	ND	10	10^2	10^3	10^4	10^5
2	18 (90)	ND	2(10)			
4	18 (90)	ND		2(10)		
6	18 (90)	ND	1(5)	1(5)		
8	18 (90)	ND		1(5)	1(5)	
10	18 (90)	ND			2(10)	
12	18 (90)	ND			1(5)	1(5)

Figures in parenthesis indicate percent; N=20 in each group; ND-Not detected

4.1.9.4 Psychrotrophic Count

The mean psychrotrophic count of the samples is given in table 52

Table 52. Mean Psychrotrophic Count of refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	4.16 ± 0.09^a
2	4.61 ± 0.18^{ab}
4	5.06 ± 0.22^c
6	5.83 ± 0.16^d
8	6.38 ± 0.24^e
10	7.00 ± 0.24^f
12	7.92 ± 0.25^g

Figures bearing the same superscript do not differ significantly. N=20 in each group

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 4.16 ± 0.09 and was increased to 7.92 ± 0.25 $\log_{10}$ cfu/ml on the 12th day, as illustrated in fig 3. Analysis of the data showed highly significant ($p<0.01$) increase in the mean counts of the samples stored between day fourth and day sixth of storage. A similar difference in count was observed between the mean count of the samples stored on day six and eight and between day 10 and 12.

Distribution of milk samples based on level of Psychrotrophic count is given in table 53. The count in 70 per cent of the samples was at the level of 10^4cfu/ml on day two but on day eight none of the samples had count at that level. The count on the eighth day of storage reached at the level of 10^7 cfu/ml but in 50 per cent samples had count at the level of 10^6 cfu/m. At the end of 12 day of storage 50 per cent of the sample had count at the level of 10^8cfu/ml and in 40 per cent sample the count was at the level 10^7 cfu/ml.

Table 53. Distribution of refrigerated milk samples based on Psychrotrophic count

Days of	Psychrotrophic count (cfu/ml)					
storage	10^3	10^4	10^5	10^6	10^7	10^8
0	4 (20)	16(80)				
2	2 (10)	14 (70)	4 (20)			
4		10 (50)	8 (40)	2 (10)		
6		2 (10)	10 (50)	8 (40)		
8			6(30)	10 (50)	4 (20)	
10			2 (10)	8 (40)	8 (40)	2 (10)
12				2(10)	8 (40)	10 (50)

Figures in parenthesis indicate percent; N=20 in each group

4.1.9.5 Faecal Streptococcal Count

The faecal streptococcal count of refrigerated milk samples is given in table 54. Analysis of the data 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 six to day 12 of storage. Highly significant ($p<0.01$) difference was also seen between the mean count of the sample stored on day two and six, six and ten and ten and 12, as illustrated in fig 3.

Table 54. Mean faecal streptococcal Count in refrigerated milk samples

Days of storage	Count (log_{10} cfu/ml) Mean ± SE
Fresh sample	1.06 ± 0.18^{a}
2	1.05 ± 0.15^{ab}
4	1.49 ± 0.30^{abc}
6	2.12 ± 0.30^{cd}
8	2.38 ± 0.35^{de}
10	2.87 ± 0.28^{ef}
12	3.43 ± 0.29^{g}

Figures bearing the same superscript do not differ significantly. N=20 in each group

Distribution of refrigerated samples based on level of faecal streptococcal count is given in table 55. The count on day two was at the level of 10 cfu/ml in 70 per cent of the samples. On sixth day of storage the count reached to the level of 10^3 cfu/ml and was maintained at the above level on 10^{th} day of storage. In 60 per cent samples stored on 10 and 12^{th} day had count at the level of 10^3 cfu/ml. However, the count in 20 per cent samples stored on 10 and 12 day had count at the level of 10^3 cfu/ml. The count in 20 per cent samples stored on day 12 had count at the level of 10^4 cfu/ml

Table 55. Distribution of 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	3(15)	17(85)			
2	4(20)	14(70)	2(10)		
4	4(20)	8(40)	8(40)		
6	2(10)	4(20)	10(50)	4(20)	
8	3(15)	1(5)	10(50)	6(30)	
10		2(10)	6(30)	12(60)	
12		2(10)	2(10)	12(60)	4(20)

Figures in parenthesis indicate percent; N=20 in each group; ND-Not detected

4.1.9.6 Yeast and Mould Count

The mean yeast and mould count of samples is given in table 56. A gradual increase in yeast and mould count was observed throughout the period of storage.

Table 56. Mean Yeast and Mould Count of refrigerated milk samples

Days of storage	Count ($\log_{10}$ cfu/ml) Mean ± SE
0	0.62 ± 0.13^{a}
2	0.88 ± 0.16^{ab}
4	1.26 ± 0.16^{c}
6	1.59 ± 0.17^{cd}
8	1.99 ± 0.27^{e}
10	2.46 ± 0.16^{ef}
12	3.08 ± 0.17^{g}

Figures bearing the same superscript do not differ significantly. N=20

The count of freshly pasteurized milk was 0.62 ± 0.13 $\log_{10}$ cfu/ml and was increased to 3.08 ± 0.17 $\log_{10}$ cfu/ml on the 12^{th} day of storage, as illustrated in fig 3. A significant increase ($p<0.05$) between the mean count of the samples stored on day two and four was observed and highly significant ($p<0.01$) increase in the mean count of samples stored between day six and eight and also on day ten and 12 was observed.

Distribution of refrigerated samples based on level of *Yeast and Mould* count is given in table 57. The count in 70 per cent of the samples was at the level of 10 cfu/ml on day two of storage. In 80 per cent of samples on day four and 70 per cent of

Table 57. Distribution of refrigerated milk samples based on Yeast and Mould Count

Days of storage	Yeast and Mould Count ($\log_{10}$ cfu/ml)			
	ND	10^1	10^2	10^3
2	6(30)	14(70)		
4	2(10)	16(80)	2(10)	
6		14(70)	6(30)	
8		4 (20)	14(70)	
10			16 (80)	4 (20)
12			6(30)	14(70)

Figures in parenthesis indicate percent

samples on day six had count at the above level. On day eight of storage 70 per cent of the samples had count at the level of 10^4 cfu/ml. On the 10^{th} day of storage the count reached at the level of 10^3 cfu/ml but 80 per cent samples had count at the level of 10^2 cfu/ml. In 70 per cent of sample on the 12^{th} day of storage had count at the level of 10^3 cfu/ml.

4.1.9.7 Correlation Between Microbial Counts of Fresh and Refrigerated Milk

Total viable count

Correlation between mean total viable count and all other counts of milk samples during the storage at 4 ± 1°C is given in table 58. A significant ($P<0.05$) and positive association was observed between total viable count and coliform count on fourth day of refrigerated storage. A similar relationship was observed between the mean total viable count and faecal streptococcal count on sixth day of refrigerated storage and between total viable count and psychrotrophic counts on fourth day and sixth day.

Table 58. Correlation coefficient between mean total viable count and other Microbial counts of refrigerated milk

Microbial counts	Total viable count Days of storage					
	2	4	6	8	10	12
CC	0.14	0.67*	0.22	0.10	0.20	0.30
ECC	0.46	0.44	-0.22	0.20	0.16	0.22
FSC	0.15	0.08	0.66*	0.58	0.47	-0.14
PC	0.37	0.64*	0.71*	0.32	0.24	0.45
YMC	0.17	-0.05	-0.44	-0.33	-0.05	-0.29

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mould count * = $p<0.05$

Coliform count

Correlation between mean coliform count and other microbial counts of pasteurized milk stored at refrigeration is given in table 59.

Table 59. Correlation coefficient between mean coliform count and other microbial counts of refrigerated milk

Microbial counts	Coliform count Days of storage					
	2	4	6	8	10	12
TVC	0.14	0.67*	0.22	0.10	0.20	0.30
ECC	0.48	0.72*	0.41	0.57*	0.63*	0.59*
FSC	-0.49	-0.08	0.015	-0.36	-0.46	-0.24
PC	-0.25	0.07	0.59	0.28	0.07	-0.17
YMC	-0.19	-0.34	-0.18	-0.03	-0.01	0.22

TVC: Total viable count, ECC: *Escherichia coli* count,FSC: Faecal streptococcal count, PC Psychrotrophic count,YMC: Yeast and mould count,* = $P<0.05$

Association between the mean coliform count and TVC was positive and significant (P<0.05) on forth day of refrigerated storage of samples. Such association was also observed between CC and ECC on day four, eight, ten and 12 of storage.

***Escherichia coli* count**

Correlation between mean *Escherichia coli* count and other microbial counts of samples stored under refrigeration is given in table 60. The mean *E. coli* and coliform count of the samples stored on day four, eight, ten and12 had

Table 60. Correlation coefficient between mean *E. coli* count and other microbial counts of refrigerated samples

Microbial counts	*Escherichia coli* count					
	Days of storage					
	2	4	6	8	10	12
TVC	0.46	0.44	-0.22	0.20	0.16	0.22
CC	0.48	0.72*	0.41	0.57*	0.63*	0.59*
FSC	0.18	0.29	-0.22	0.16	-0.46	0.06
PC	0.26	0.27	0.04	0.66*	0.534	0.41
YMC	-0.11	-0.09	0.40	0.51	0.17	0.30

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

shown a positive and significant (p<0.05) relationship. *E. coli* count and psychrotrophic count of the samples stored on day eight also revealed similar association.

Psychrotrophic count

Correlation between Psychrotrophic count and other microbial counts of refrigerated sample are given in table 61. Relationship between total viable count and psychrotrophic counts of the samples on fourth and sixth day was positive and significant ($p<0.05$). Similar relationship was observed between the mean ECC and PC on eight day of storage.

Table 61. Correlation coefficient between mean psychrotrophic count and other microbial counts of refrigerated milk samples

Microbial counts	Psychrotrophic count Days of storage					
	2	4	6	8	10	12
TVC	0.37	0.64*	0.71*	0.32	0.24	0.45
CC	-0.25	0.07	0.59	0.28	0.07	-0.17
ECC	0.26	0.27	0.04	0.66*	0.534	0.41
FSC	0.32	0.35	0.34	0.32	0.46	-0.18
YMC	-0.19	-0.34	-0.18	-0.03	-0.006	0.22

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

Faecal streptococcal count

Correlation between mean faecal streptococcal count and other microbial counts of refrigerated milk is given in table 62.

Relationship between faecal streptococcal count and yeast and mould count of the samples was highly significant ($p<0.01$) and positive. However, the relationship between the TVC and faecal streptococcal counts of the samples stored on day six had revealed a positive and significant ($p<0.05$) association.

Table 62. Correlation coefficient between mean faecal streptococcal count and other microbial counts of refrigerated milk samples

Microbial counts	Faecal streptococcal count Days of storage					
	2	4	6	8	10	12
TVC	0.15	0.08	0.66*	0.58	0.47	0.13
CC	-0.49	-0.08	0.015	-0.36	-0.46	-0.24
ECC	0.18	0.29	-0.22	0.16	-0.46	0.06
PC	0.32	0.35	0.34	0.32	0.46	-0.18
YMC	0.34	-0.21	0.15	0.85**	0.46	-0.41

TVC: Total viable count, CC: Coliform count,ECC: *Escherichia coli* count,PC Psychrotrophic count,YMC: Yeast and mould count,** = $p<0.01$

Yeast and mould count

Correlation between mean yeast and mould count and other microbial counts of samples stored under refrigeration are given in table 63.

Table 63. Correlation coefficient between mean yeast and mould count and other microbial counts of stored pasteurized milk

Microbial counts	Yeast and mould count Days of storage					
	2	4	6	8	10	12
TVC	0.17	-0.05	-0.44	-0.33	-0.05	-0.29
CC	-0.19	-0.34	-0.18	-0.03	-0.01	0.22
ECC	-0.11	-0.09	0.40	0.51	0.17	0.30
FSC	0.34	-0.21	0.15	0.85**	0.46	-0.41
PC	-0.19	-0.34	-0.18	-0.03	-0.006	0.22

TVC: Total viable count, CC: Coliform count, ECC:*Escherichia coli* count, FSC: Faecal streptococcal count, PC Psychrotrophic count, ** = $p<0.01$.

A positive and highly significant ($P<0.01$) association was observed between the mean faecal streptococcal count and yeast and mould count on eight day of storage

4.1.10 Isolation and Identification of Bacteria

The bacteria of public health importance are isolated from refrigerated milk samples and are given in table 64.

Table 64. Bacteria isolated from refrigerated milk

Bacteria	Samples tested on each day	Number of positive samples					
		Days of storage					
		2	4	6	8	10	12
E. coli	20	1	1	1	1	1	1
S. aureus	20	ND	ND	1	2	ND	ND
L. monocytogenes	20	ND	ND	ND	ND	ND	ND

ND: Not detected

4.1.10.1 Escherichia coli

Refrigerated milk samples were tested for the isolation and identification of *Escherichia coli*. The isolates were identified by cultural, morphological and bio-chemical characteristics. All samples showed positive for Eijkman test. The isolates were serotyped at National *Salmonella* and *Escherichia* Centre and were subjected to congored binding test and are shown in plate 1. All the isolates belonged to serotype 0148 and are positive for congo red binding test indicating their invasive ability.

4.1.10.2 Staphylococcus aureus

All samples were tested for the isolation of *Staphylococcus aureus* and isolates were identified by cultural, morphological and biochemical characteristics. Of the three organism isolated, one of the isolate was from the samples stored on sixth day and the other two were isolated from the sample stored on eight day.

4.1.10.3 Listeria monocytogenes

All stored samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples revealed the presence of this organism

4.2. ORGANOLEPTIC AND PHYSICO-CHEMICAL QUALITY OF MILK

4.2.1 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)

4.2.1.1. Colour and Appearance

The mean score of colour and appearance of fresh and refrigerated milk samples is given in table 66. The total score allotted was 10. The mean score of

Table 65. Mean colour and appearance score of milk samples

Days of storage	COLOUR AND APPEARANCE SCORE (Mean ± SE)
0	9.25 ± 0.26^{a}
2	8.95 ± 0.24^{ab}
4	7.80 ± 0.41^{bc}
6	7.65 ± 0.40^{cd}
8	7.30 ± 0.42^{cde}
10	7.25 ± 0.26^{cdef}
12	6.90 ± 0.35^{def}

Figures bearing the same superscript in the column do not differ significantly.

fresh sample differed significantly ($p<0.05$) with the score of the samples on sixth day and from then onwards up to 12th day the difference was highly significant ($p<0.01$). The mean score showed a gradual decrease throughout the period of storage. The mean score of freshly pasteurized milk decreased from 9.25 ± 0.26 to 6.90 ± 0.35 on the 12th day of refrigerated storage.

4.2.1.2 Odour

The mean score of odour in fresh and refrigerated milk samples is given in table 66. The total score allotted for odour was 20. Analysis of the data revealed that the count of fresh sample differed highly significantly ($p<0.01$) only after four days of storage. Highly significant ($p<0.01$) reduction in the score was also seen in the samples stored between six and 10 days and between eight and ten days.

Table 66. Mean Odour score of milk samples

Days of storage	ODOUR SCORE (Mean ± SE)
0	19.10 ± 0.35[a]
2	18.40 ± 0.34[ab]
4	18.60 ± 0.37[abc]
6	17.20 ± 0.25[d]
8	15.30 ± 0.76[e]
10	12.80 ± 0.44[f]
12	11.40 ± 0.39[f]

Figures bearing the same superscript in the column do not differ significantly.

4.2.1.3 Flavour

The mean flavor score of fresh and refrigerated samples is given in table 67. The total score for flavour allotted was 40. Analysis of the data revealed highly significant ($p<0.01$) differences between the mean flavour score of second and fourth, fourth and sixth, and sixth and eight days of storage.

Table 67. Mean Flavor score of milk samples

Days of storage	FLAVOR SCORE (Mean ± SE)
0	39.10 ± 0.28^{a}
2	39.40 ± 0.22^{ab}
4	36.80 ± 0.55^{c}
6	34.70 ± 0.58^{d}
8	31.50 ± 0.68^{e}
10	30.30 ± 0.42^{ef}
12	29.50 ± 0.79^{efg}

Figures bearing the same superscript in the column do not differ significantly.

4.2.1.3 Body

The mean body score of fresh and refrigerated samples is given in table 68. The total score for body allotted was 30. The mean body score of the sample

Table 68. Mean Body score of milk samples

Days of storage	BODY SCORE (Mean ± SE)
0	29.40 ± 0.26^{a}
2	29.10 ± 0.31^{ab}
4	27.90 ± 0.38^{c}
6	25.30 ± 0.76^{d}
8	19.90 ± 0.69^{e}
10	18.30 ± 0.52^{ef}
12	16.50 ± 0.43

Figures bearing the same superscript in the column do not differ significantly.

decreased throughout period of storage. Analysis of the data revealed highly significant ($p<0.01$) difference between zero and four, four and six, six and eight. Significant ($p<0.05$) difference was also noticed between the mean score of the samples stored on day two and day five.

4.2.1.5 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 69. The mean total scores revealed that the samples had excellent quality for up to fourth day of storage. The sensory quality of the sample stored on sixth day was good and thereafter the quality of milk remained as fair.

Table 69. Total score of fresh and refrigerated milk samples

Days of storage	Total score Mean ± SE	Grade	Quality
0	96.85 ± 0.56	A	Excellent
2	95.85 ± 0.50	A	Excellent
4	91.05 ± 0.84	A	Excellent
6	84.85 ± 1.04	B	Good
8	74.00 ± 1.23	C	Fair
10	68.65 ± 0.47	C	Fair
12	64.30 ± 0.77	C	Fair

4.2.2 Physico-chemical Quality

4.2.2.1 pH

The Mean pH of fresh and refrigerated samples is given in table 70. A gradual decrease in the pH value was observed throughout the period of storage. The mean pH of freshly pasteurized milk decreased from 6.60 ± 0.01 to 6.25 ± 0.06 on the 12th day of storage. Significant ($p<0.05$) difference was seen in the reduction of mean pH of the samples stored between day two and six, four and eight, and eight and 12.

Table 70. Mean pH of milk samples

Days of storage	pH (Mean ± SE)
0	6.60 ± 0.01[a]
2	6.57 ± 0.01[ab]
4	6.55 ± 0.02[bc]
6	6.52 ± 0.02[cd]
8	6.44 ± 0.02[e]
10	6.38 ± 0.03[ef]
12	6.25 ± 0.06[g]

Figures bearing the same superscript do not differ significantly.

4.2.2.2 Clot on Boiling test

All the samples were subjected to Clot On Boiling (COB) test during storage. And the result of the test is given in table 71. All samples stored on 12[th] day revealed a positive test. Only two samples stored on day eight and seven samples stored on day 10 were also found COB test positive.

Table 71. Result of COB test of milk samples stored under refrigeration

Batches	Clot On Boiling					
	Days of storage					
	2	4	6	8	10	12
I	-	-	-	-	+	+
II	-	-	-	-	+	+
III	-	-	-	-	+	+
IV	-	-	-	-	-	+
V	-	-	-	-	-	+
VI	-	-	-	+	+	+
VII	-	-	-	-	+	+
VIII	-	-	-	+	+	+
IX	-	-	-	-	-	+
X	-	-	-	-	+	+
Total	Nil	Nil	Nil	2	7	10

+ COB positive, -COB negative; N=20 samples per batch

4.3 ASSESSMENT OF CRITICAL CONTROL POINTS OF MICROBIAL CONTAMINATION OF MILK

4.3.1 Microbial load of contamination sources of milk

The presence and extent of microbes in environmental samples, utensils used in the farm, hand wash of milker and workers in the dairy plant, the wash sample of equipments and other sources like strainer, storage crate and package material and their role in the contamination of milk was determined.

4.3.1.1 Water

The mean total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) of water samples collected from dairy farm and dairy plant are given in table 72. Among the samples, water used in dairy plant had the highest mean total viable count whereas water from dairy farm samples had the highest mean coliform count. *E. coli* was not detected in the water samples of dairy plant. Faecal streptococci count at the level of 2.15±0.27 $\log_{10}$ cfu/ml and 2.10±0.21 was detected in the water samples of dairy farm and dairy plant, respectively.

Table 72. Mean bacterial count of water samples from dairy farm and dairy plant

Tap water	Bacterial count ($\log_{10}$ cfu/ml)			
	TVC	CC	ECC	FSC
Dairy farm	3.60±0.17	1.45±0.59	0.80±0.27	2.15±0.27
Dairy plant	3.84±0.26	1.12±0.23	ND	2.10±0.21

ND-Not Detected

4.3.1.2 Utensils

The hygienic status of the utensils/Equipment used in the milking parlor was evaluated. Bacterial counts of milk pail, milking machine and milk can sample were estimated and the mean count is given in table 73. Milk pail samples revealed the highest mean total viable count, whereas milk can had the lowest count. Coliforms were present at the level of one log cfu/ml in the samples of milk pail. *E. coli* was not detected in samples of milk can. Faecal streptococcal count was highest in samples of milk pail, whereas milk can had the lowest number of the organism.

Table 73. Mean bacterial count of Milk pail/ Milking machine/ Milk can

Utensil	Bacterial count (log_{10} cfu/cm^2 or per ml)			
	TVC	CC	ECC	FSC
Milk pail	3.81±0.27	1.10±0.25	0.73±0.35	1.42±0.16
Milking machine	3.37±0.33	0.55±0.24	0.41±0.26	1.36±0.16
Milk can	2.29±0.30	0.74±0.35	ND	0.47±0.31

4.3.1.3 Hand Wash

The mean TVC, CC, ECC and FSC of hand wash of individual milker in the farm and persons involved in processing operations in the dairy plant are given in table 74. The hand wash of milker revealed a higher TVC, CC, ECC and FSC when compared to hand wash obtained from person involved in processing operations in the dairy plant.

Table 74. Mean bacterial count of Hand wash of milker and dairy farm operators

Hand wash	Bacterial count (log_{10} cfu/ml)			
	TVC	CC	ECC	FSC
Individual milker	5.05±0.29	1.92±0.37	0.72±0.23	2.38±0.30
Working personnel	4.13±0.31	0.80±0.27	0.51±0.33	1.92±0.17

4.3.1.4 Equipment Wash Sample

The mean TVC, CC, ECC and FSC of Pasteurization equipment and Packaging equipment wash sample are given in table 75. The packaging machine wash sample had the highest TVC, CC and FSC compared to the wash of pasteurization equipment. None of the samples revealed the presence of *E. coli*

Table 75. Mean bacterial count of Pasteurization and Packaging Equipment

Equipment wash	Bacterial count (log_{10} cfu/ml)			
	TVC	CC	ECC	FSC
Pasteurization equipment	3.03±0.34	0.68±0.32	ND	0.79±0.37
Packaging equipment	5.12±0.24	1.70±0.44	ND	2.11±0.27

4.3.1.5 Package Material/ Storage Crate/Strainer Sample

The mean TVC, CC, ECC and FSC of Package material/ storage crate/Strainer sample are given in table 76. Storage crate revealed the highest mean total viable count and lowest was detected in Strainer sample. Coliforms were present in samples of Package material and Storage crate whereas the organism could not detect in Strainer samples. *E. coli* was absent in all samples and faecal streptococci was present in all the three samples analysed. The samples of storage crate had the highest level of the later organism.

Table 76. Mean bacterial count of Package material/ Storage crate/ Strainer

Sample	Bacterial count (log_{10} cfu/ml)			
	TVC	CC	ECC	FSC
Package material	2.82±0.18	0.32±0.31	ND	0.43±0.28
Storage crate	3.70±0.17	0.73±0.34	ND	1.00±0.38
Strainer sample	2.26±0.18	ND	ND	0.58±0.37

4.3.1.6 Air

The mean microbial count of air samples obtained from the milking barn before and after the milking process and from dairy plant before and after pasteurization and packaging of milk is given in table 77. Considerable increase in the mean total viable count and yeast and mould count of air samples collected from the milking barn, pasteurization room and packaging room was observed

Table 77. Mean microbial counts of air samples

Samples		Mean ± SE ($cfu/ft^2/min$)	
Process	Collection	TVC	YMC
Milking of animals	Before	98± 3.58	16.67± 0.84
	After	127.83± 3.90	23.17± 1.49
Pasteurization	Before After	34± 2.2 56.3± 3.21	9.5± 0.76 12.16± 1.37
Packaging	Before After	41.66± 2.74 73.66± 2.23	13.17± 1.07 18.66± 2.44

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

after the process of milking, pasteurization and packaging. Highest mean TVC and YMC was seen from air samples collected from dairy farm after the process of milking.

4.3.2 Assessment of CCP of Pasteurized Milk

The critical control points of bacterial contamination during production of pasteurized milk are given in Flow Chart 5. The assessment of critical control points revealed that maximum contamination was seen when the milk samples were pooled and packaged. The contamination of pooled samples was due to the contribution of microbes by handling of milk by different milkers, where the personnel hygiene and cleanliness varied and also by transferring milk from different containers, air and water acting as source of contamination as detailed above. The hand wash showed the presence of *E.coli*, faecal streptococcus, coliform and had high TVC. The water used in the cleaning of equipments also revealed the presence of coliform and a mean count of 1.45±0.59 log_{10} cfu/ml. Utensils had also contributed to increase in the microbial count and the milk pail used for transferring the individual animal milk revealed highest contamination.

The process of packaging of pasteurized milk revealed next major sources of contamination and package machine wash sample had higher TVC, CC and FSC than the samples obtained from HTST pasteurizer. Also the air sample of package room showed more TVC and YMC than pasteurization area. The package material also had contamination with coliform and faecal streptococcus, which may be contributed from improperly cleaned package equipment and the quality of water used for washing.

Flowchart 5. Critical control points in production of pasteurized milk

Milk from Individual animal — o CCP_1

↓

Pooled raw milk — • CCP_2

↓

Milk transportation

↓

Chilled milk — o CCP_3

↓

Pasteurisation

- Milk after heating — o CCP_4
- Pasteurized milk — o CCP_5

↓

Packaged milk — • CCP_6

↓

Chilled storage — o CCP_7

↓

Marketing

• Point of major contamination
o Point of minor contamination

CCP_1=Milkers personal hygiene, Hygienic milking process and Animal hygiene.

CCP_2=Periodic cleaning and sanitation of utensils, Environment sanitation

CCP_3=Good quality pooled milk, Maintenance of chilled temperature

CCP_4=Proper temperature of heating, Equipment hygiene

CCP_5=Periodic cleaning and sanitation of HTST pasteurizer

CCP_6=Aseptic package material, Equipment hygiene, Environment hygiene

CCP_7=Maintenance of chilled temperature, Minimizing post-pasteurization contamination

5. DISCUSSION

The assessment of microbial quality of milk is very essential as the microorganisms not only affect the shelf life of milk but also have implication in public health as they may include potential pathogens. Hygiene of milk handling during various stages of production and transport followed by efficient pasteurization and refrigeration are the major concerns for quality assurance of fluid milk. In the present study the microbial quality assurance of milk is assessed by determining the microbial load of milk at its production in the farm and also during various stages of pasteurization and storage. An evaluation of the microbial quality of retail milk was done to understand quality of milk supplied and consumed by the public. The quality assurance will be complete only if source of contamination of milk in the farm and in processing plants are identified and their bacterial loads are determined. From the quality point of view the knowledge about sensory or organoleptic changes is also very essential to fetch good market and consumer acceptability.

5.1 MICROBIAL QUALITY OF MILK

5.1.1 Microbial Counts of Raw Milk

5.1.1.1 Total Viable Count

Total viable count (TVC) serves as an important criteria for evaluating the microbial quality of various foods, and also degree of freshness of food. The mean TVC obtained from individual animal milk samples (5.14 ± 0.13 $\log_{10}$ cfu/ml) was found to be lower than that of the pooled (5.58 ± 0.14 $\log_{10}$ cfu/ml) and chilled (5.70 ± 0.13 $\log_{10}$ cfu/ml) milk and this difference was highly significant ($p<0.01$) (Table 2). The mean TVC of the individual milk and pooled samples in the present study was about one log lower than that reported by Jolly *et al.* (2000). The author reported more mean count in pooled milk when compared to individual animal milk samples from the same source. The difference in count of the individual and pooled milk samples might be due to contamination of the milk during handling, from the contaminated utensils, from milking process and from the environmental sources. The mean count of milk from individual animal at the level of 10^5 cfu/ml was in agreement with the counts obtained by Kapre (1995). The count from pooled milk samples was similar to those obtained by Jain and Saraswat (1968), Schroder *et al.* (1982), Misra and Kuila (1989) and Boor *et al.* (1998). However, the count was lower than the findings reported by Lues *et al.* (2003), Aaku *et al.* (2004) and Chye *et al.* (2004)

The process of chilling of pooled milk in the dairy plant and storage at 7-10°C for 17 h. did not significantly increase the total viable count due to the presence of antimicrobial substance in milk and the effect of low temperature. Fresh raw milk is known to possess bactericidal properties due to the presence of antimicrobial substances like immunoglobulin, leucocytes, lactoferrin, lactanin, lysozyme and lactoperoxidase. Raw milk kept under cold storage with initial counts of 11,500 bacteria /ml had counts decreased to 7,800 at 12 h. and then increased to 62,000 after 24 h. (Yadav *et al.*, 1992).

5.1.1.2 Coliform Count

The mean coliform count of pooled milk (3.24 ± 0.19 $\log_{10}$ cfu/ml) samples was highest (Table 4) and had highly significant difference ($p<0.01$) with individual animal milk sample (1.83 ± 0.22 $\log_{10}$ cfu/ml), but the count had no significant difference with chilled milk (3.10 ± 0.17 $\log_{10}$ cfu/ml). Similar variation in the count of individual and pooled milk samples was made by Palanniswami *et al.* (1988) who evaluated milk samples from farm where sanitary practices were moderate and reported mean coliform of 10/ml from samples of individual animal and a mean count of 2.7×10^4 /ml in pooled milk sample. The coliform count of pooled milk at a level of 3-$\log_{10}$ cfu/ml was in accordance to the findings made by Rai and Dwivedi (1990), Misra and Kuila

(1989) and Mutukumira *et al.* (1996). The coliform count at the level of 10^3 /ml was seen from 40 per cent of the samples of raw milk examined in the study but Davies (1977) obtained only 29.1 per cent sample at that level.

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

5.1.1.3 Escherichia Count

The mean *E.coli* count of raw milk samples from individual, pooled and chilled milk did not differ however mean count was more in chilled milk (1.05 ± 0.29) $\log_{10}$ cfu/ml (Table 6). *E. coli* not detected in 60 per cent of raw milk samples examined while Desmasures (1997) obtained 80 per cent of raw milk with *E.coli* counts less than 10 cfu/ml. The mean count at the level of one $\log_{10}$ cfu/ml was similar to those obtained by Cosentino and Palmas (1997) but was much lower than the counts obtained by Kapre (1995), Jolly (2000), Khalilur *et al.* (2002), Lues *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 this organism is of intestinal origin in man and animal.

5.1.1.3 Psychrotrophic Count

In connection with the holding of milk at relatively low temperatures, psychrotrophic bacteria are of special importance. Psychrotrophs are widely distributed in raw milk (Hammer and Babel, 1957). The present study revealed that mean psychrotrophic count obtained from chilled milk was highest (5.56 ± 0.14 $\log_{10}$ cfu/ml) but there was no significant difference with pooled milk (5.42 ± 0.07 $\log_{10}$ cfu/ml) (Table 8). Samples of milk from individual animal had mean count of 4.69±0.24 $\log_{10}$ cfu/ml. Psychrotrophs thrive and grow under refrigerated temperatures. Thus chilled milk held at 7°C for 17 h. had highest count. The psychrotrophic count at 5 $\log_{10}$ cfu/ml was also observed in the findings of Jain and Saraswat (1968), Misra and Kuila (1989) but Singh *et al.* (1994) observed a lesser mean count at the level of 3.94 $\log_{10}$cfu/ml. Hammer and Babel (1957) suggested the source of psychrotrophic bacteria in raw milk as non-sterile farm utensils and equipments, coats of cows and water supplies. Thus hygiene in farm minimizes psychrotrophic counts.

5.1.1.4 Faecal Streptococcal Count

The faecal streptococcal count was at the level of 2.5 $\log_{10}$ cfu/ml in pooled and chilled milk. The individual milk showed the presence of the organism with mean count of 1.89 ± 0.08 $\log_{10}$ cfu/ml (Table 10). The count had significant ($p<0.05$) difference from that of pooled and chilled milk sample. The count in pooled milk sample was in agreement with the count reported by Jolly *et al.* (2000) but the count was very less when compared to count recorded by Yadava *et al.* (1983). If better sanitary practices are followed in the farm with minimal faecal contamination lesser will be the count, as these organisms are the inhabitants of the intestines of man and animals.

5.1.1.5 Yeast and Mould Count

The mean count of pooled milk was 1.86 ± 0.19 $\log_{10}$ cfu/ml. The mean count of yeast and mould in individual, pooled and chilled sample did not show significant difference (Table 12). Similar counts were obtained by Cosentino and Palmas (1997). The count obtained in the present study was lesser than that observed by Lues *et al.* (2003). In the present investigation 36.67 per cent of milk sample examined had count lesser than 100 cfu/ml (Table 13) but, Mutukumira *et al.* (1996) reported 70 per cent of the sample had count less than 100 cfu/ml. High yeast and mould count indicates unsanitary conditions of handling and contamination from environment.

5.1.1.6 Correlation of Microbial Counts of Raw Milk

The study revealed a highly significant ($p<0.01$) positive association of total viable count and psychrotrophic count (Table 14). Jain and Saraswat (1968) also found highly significant correlation (0.922) between counts of psychrotrophs and TVC in raw milk. Robinson (2002) stated that on an average the psychrotrophs comprises between 10 to 50 per cent of initial microflora. Similar positive correlation was also seen between the TVC and FSC, which was in agreement with the observation of Jolly *et al.* (2000).

A significant correlation was also observed between the coliform and Psychrotrophs. Robinson (2002) opined that some species of genera making up the coliform group of bacteria are psychrotrophs and constitute 10 to 30 per cent of milk microflora.

5.1.2 Interpretation on the Quality of Raw Milk

In India milk is graded by Indian Standards (IS: 1977). According to the criteria prescribed by IS: 1479 (1977) 46.7 per cent samples were graded as very good, 36.6 per cent as good and 16.7 per cent as fair quality. None of the samples were graded poor. The study conducted by Jain and Saraswat (1968) reported that 61.42 per cent sample was graded as poor. Singh *et al.* (1994) observed that 28.75 per cent of the milk samples were graded very good and 37.73 per cent as poor quality. Garg and Mandokhot (1997) observed two out of 86 sample graded as very good grade. Thus on comparing the bacteriological quality of milk in the present study with the quality of milk reported from various parts of India revealed that the quality of raw milk produced in the farm was superior.

According to Indian standards the coliform organisms absent in 1:100 dilution of raw milk is considered to be satisfactory. The present study revealed 25 per cent of raw milk confirmed with the standard. However none of the pooled and chilled milk sample examined met the standards. The findings of the present study confirmed with Palanniswami *et al.* (1988).

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 shorten the shelf life of milk.

5.1.3 Isolation and Identification of Organisms in Raw Milk

Milk serves as an excellent culture and protective medium for certain microorganisms, particularly bacterial pathogens, whose multiplication is mainly dependent on temperature and on competing microorganism and their metabolic products. Some of the pathogens having major public health significance include, *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes.*

5.1.3.1 Escherichia coli

Pooled raw milk samples were tested for the isolation and identification of *Escherichia coli* and was detected in 20 per cent of the samples (Table 15). The percentage of organism isolated from the samples were less than that recorded by Singh and Ranganathan (1978), Yadava *et al.* (1985) Singh *et al.* (1994), Soomro *et al.* (2002) and Chye *et al.* (2004). However the percentage of the organism isolated from the samples 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, two isolates belonged to serotype 05 (Table 16) and one each of the isolate belonged to the serotype 0141 and 0172 respectively. The serotype O5 and 0172 belong to Enterohaemorrhagic group. Similarly Sharma *et al.* (1995) also reported the isolation of serotype 05 from raw milk. Infection with Enterohaemorrhagic group of *E.coli* in man causes diarrhea, haemorrhagic colitis, and haemolytic ureamic syndrome (HUS). The serotype 0141 belonged to Enterotoxigenic serotype which is associated with traveller's diarrhea and cholera-like disease in man.

The isolates of *E. coli* were subjected to congo red binding test and three (Table 17) of the isolates had Congo red binding property which indicated their property of invasiveness. The characteristics of congo red binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker (Rajil *et al.*, 2003). A good correlation between pathogenic potential and congo red binding property had been reported by Abhilasha *et al.* (2001). The congo red binding of the serotype 05 was also reported by the above author. From the milk samples three samples showed a positive for Eijkman test.

Escherichia coli is 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 faecal contamination.

5.1.3.2 Staphylococcus aureus

S. aureus was isolated from 12 (60 per cent) of the pooled raw milk sample(Table 15). The percentage of isolate obtained in the study was lower than that reported by Garg *et al.* (1977), Desmasures *et al.* (1997), Adesiyun *et al.* (1998) and Chye *et al.* (2004). However, the percentage of isolation of the organism was greater than that reported by Mohan and Misra (1967), Ghosh and Laxminarayana (1972), Singh *et al.* (1994) and Gran *et al.* (2003)

Staphylococci form part of the normal flora of animals and man. *S. 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. So attention must be paid to sanitation and personnel hygiene to minimize the contamination of the product with the organism.

5.1.3.3 Listeria monocytogenes

All the pooled milk samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples (Table 15) revealed the presence of this organism. Yadava *et al.* (1983) obtained no isolate from 42 milk samples examined from Dairy unit R.V.C. Such low incidence of *Listeria monocytogenes* in milk was also reported by Rohrbach *et al.* (1991), Rea *et al.* (1992), Bhilegaonkar *et al.* (1997), Desmasures *et al.* (1997), Pednekar *et al.* (1997), Steel *et al.* (1997), Chye *et al.* (2004) and Kells and Gilmour (2004).

5.1.4 Microbial Counts of Milk at Various Stages of Pasteurization

5.1.4.1 Total Viable Count

The mean TVC obtained from milk samples after heating section was 3.00 ± 0.15 $\log_{10}$ cfu/ml as shown in table 18 and the mean count in pasteurized milk was 3.64 ± 0.15 $\log_{10}$ cfu/ml but the mean count in packaged milk was 4.76 $\pm$ 0.15 $\log_{10}$ cfu/ml. The difference between the mean count of milk was highly significant ($p<0.01$). The counts obtained in the study was higher than that reported by Eneroth *et al.* (1998) who recorded the initial aerobic plate count of milk after the pasteurization was 6×10^2 milk and in milk filled in consumer package also had the count of 6×10^2 cfu/ml. The count of heated milk was similar to that reported by Cosentino and Palmas (1997) who found that the majority of heat-treated milk had count less than 10^4 cfu/ml. The advantage of heat treatment is to eliminate the risk associated with disease producing organisms and to reduce the saprophytes or organisms associated with spoilage of milk and to extend the keeping quality. The highly significant ($p<0.01$) difference in count of milk after pasteurization and packaged milk samples may be attributed to post pasteurization contamination which includes improperly cleaned pasteurizer equipment, storage tank and packaging units, package materials and working personnel. The presence of milk residues on inadequately cleaned milk contact surfaces can support the growth of microorganism and contaminate milk. Mahari and Gashe (1990) observed that pasteurized milk had aerobic counts of 7×10^5 cfu/ml as it left pasteurizer unit, but the population increased 2 to 4 fold as a result of subsequent contamination. Milk samples after packaging had highest count but 55 per cent of this freshly pasteurized and packaged samples were within 30,000 /ml, the standards prescribed by Indian standards (1992). The mean count of packaged milk at the level of 10^4 cfu/ml was in agreement with the counts obtained by Arora and Sudarsanam (1986), Misra and Kuila (1989), Rai and Dwivedi (1990) Cerqueira *et al.* (1994) and Aaku *et al.* (2004). However the mean count was lower to that reported by Reddy *et al.* (1989), John (1999), Gopi *et al.* (2001) and Khalilur *et al.* (2002).

5.1.4.2 Coliform Count

The coliform organism was absent in cent per cent of heated milk. However 20 per cent of milk after pasteurization and 60 per cent of milk sample after packaging revealed the presence of the organism (Table 21). Birollo *et al.* (2001) also reported that heat treatment killed 100 per cent of coliform organism. However, Cosentino and Palmas (1997) revealed the presence of coliforms in heat-treated milk in five processing plants and the organism was absent in one plant. The mean count of coliform in packaged milk (0.98 ± 0.36 $\log_{10}$ cfu/ml) samples was highest and the count showed highly significant ($p<0.01$) difference with mean count of milk samples immediately after pasteurization (0.25 ± 0.19 $\log_{10}$ cfu/ml) (Table 20). The presence of coliforms in pasteurized milk may be due to the post pasteurization contamination or due to heat resistance of some coliform organism or due to large number of organism in raw milk (Hammer and Babel, 1957).

The bacterial standards prescribed by IS in 1992 stipulate that coliform should be absent in 1:10 dilution of pasteurized milk. Among the milk samples collected at various stages of pasteurization, 73.33 per cent sample met IS standards but only 40 per cent of packaged milk sample met the standards. The mean count of packaged milk was in agreement with the counts obtained by Vijai and Saraswat (1968), Raju and Nambudripad (1987) Cosentino and Palmas (1997) and Latha and Nanu (1997).

5.1.4.3 Escherichia Count

E.coli was not detected from heated and pasteurized milk. However, the organism was found in 10 per cent of the packaged milk samples and the mean count was 0.31 ± 0.21 $\log_{10}$ cfu/ml (Table 22). The count in packaged milk may be due to post pasteurization contamination from storage tank or packaging machine. Cosentino and Palmas (1997) reported the absence of *E.coli* in heat-treated milk in five processing plants but count at the level of 1.10 ± 0.32 $\log_{10}$ cfu/ml was seen in the sample obtained from one of the plants. The mean count at the level of less than one $\log_{10}$ cfu/ml was similar to that reported by Latha and Nanu (1997) of the samples from two sources. The association between coliforms and *E. coli* in freshly packaged milk samples was highly significant ($p<0.01$) and positive (Table 30).

The presence of this organism in pasteurized milk is of great concern as it is associated with various diseases in man and animals. The presence of the organism in pasteurized milk indicates insufficient pasteurization or post pasteurization contamination of milk.

5.1.4.4 Psychrotrophic Count

Psychrotrophic organism was found in heated milk at the level of 1.20 ± 0.22 $\log_{10}$ cfu/ml and this shows highly significant ($p<0.01$) variation from the count of pasteurized and packaged milk (Table 24). The mean psychrotrophic count obtained from packaged milk was 4.16 ± 0.09 $\log_{10}$ cfu/ml. This indicates post pasteurization contamination of milk. Similar findings was reported by Eneroth *et al.* (1998) where maximum recontamination by gram-negative psychrotrophs occured due to post pasteurization contamination by the filling procedure of the pasteurized milk. In the present study the count obtained for packaged milk was similar to that reported by Saleha (1995). The study conducted by John (1999) revealed a higher psychrotrophic count in freshly pasteurized milk.

Psychrotrophic contamination of milk is the key factor in causing milk spoilage under refrigeration as this organism thrive and grow in cold temperature. To minimize psychrotrophic contamination of milk, hygiene should be followed in all aspects of raw milk handling and proper cleaning and sanitation of pasteurization and packaging equipment must be practiced.

5.1.4.5 Faecal Streptococcal Count

The faecal streptococcal count was minimum (0.23 ± 0.10 $\log_{10}$ cfu/ml) in heated milk and maximum in packaged milk (1.06 ± 0.18 $\log_{10}$ cfu/ml) and the difference between the mean count of the samples was highly significant ($p<0.01$) (Table 26). However the presence of the organism in heated milk indicates the survival of the organism after heating. Hammer and Babel, 1957 also reviewed the thermal resistance of *Streptococcus faecalis* organism in milk. The count of packaged milk sample was similar to that observed by Latha and Nanu (1997) and Sethulakshmi *et al.* (2003). However the count obtained by Yadava *et al.* (1983) was very high with an average count of 1.440×10^6 /ml. Sanitary practices followed in the plant minimises contamination by this organism, as they are the inhabitants of the intestines of man and animals.

5.1.4.6 Yeast and Mould Count

Analysis of the mean count revealed a significant ($p<0.05$) difference in the Yeast and Mould counts of pasteurized milk and packaged milk and also between pasteurized and heated milk. The mean count of packaged milk was 0.62 ± 0.13 $\log_{10}$ cfu/ml (Table 28). The count was much lower to that obtained by Arora and Sudarsanam (1986). Heating process killed 90 per cent of yeast and mould organism. Cosentino and Palmas (1997) and Birollo *et al.* (2001) did not detect yeast from heat-treated milk. High yeast and mould indicates unsanitary conditions of handling and contamination from environment.

5.1.5 Isolation and Identification of Organism in Freshly Pasteurized Milk

5.1.5.1 Escherichia coli

E. coli was detected in 2 (10 per cent) of freshly pasteurized milk samples (Table 31) and was comparatively less than that reported by Singh and Ranganathan (1978), John *et al.* (1999), Silva *et al.* (2001). However the per cent of isolation was more than that recorded by John. (1999) and John *et al.* (2003). Both the isolate belonged to the serotype 0148. The serotype is categorized as enterotoxigenic *E. coli* (ETEC) that is associated with traveller's diarrhoea and cholera like disease in children under five years of age. The congo red binding property of the isolates indicates the property of invasiveness. Both the isolates showed positive Eijkman test.

5.1.5.2 Staphylococcus aureus

S. aureus was isolated from one (5 per cent) of the freshly pasteurized milk (Table 31). Ghosh and Laxminarayana (1972) obtained 7 (23.3 per cent) pasteurized milk samples showing the presence of coagulase positive staphylococcus wheras none of the freshly pasteurized milk samples showed the organism (John, 1999). The presence of the organism may be as a result of more number of organisms in raw milk and due to improper hygiene in plant operation.

5.1.5.3 Listeria monocytogenes

All the freshly pasteurized milk tested for the isolation and identification of *Listeria monocytogenes* revealed the absence of this organism (Table 31). This is in consonance with the findings of Sharif and Tunail (1990), Bhilegaonkar *et al.* (1997), Pednekar *et al.* (1997) and Mena *et al.* (2004)

5.1.6 Effect of Pasteurization on Microbial Quality of Milk

Pasteurization was effective in reducing the microbial population of raw milk. There was highly significant ($p<0.01$) reduction in the counts of TVC, coliform, psychrotrophic, faecal streptococcal and Yeast and mould count and a significant ($p<0.05$) reduction in *Escherichia coli count* as shown in Table 32 and illustrated in Fig 1. The TVC and psychrotrophic organism of raw milk has reduced to one to two $\log_{10}$ cfu/ml after the process of pasteurization. Mahari and Gashe (1990) observed count of raw milk samples was between $4x10^7$ to $1x10^9$cfu/ml and after milk had left pasteurizer unit the aerobic count reduced to $7x10^5$ cfu/ml. Eneroth *et al.* (1998) reported that the aerobic count of milk before pasteurization at the level of $9x10^4$ cfu/ml and the count of pasteurized milk from buffer tank was $1x10^3$ cfu/ml. Pelezynska and Libett (1995) found the average bacterial counts in the raw milk to be four million /ml and after pasteurization the bacterial count was significantly reduced to 42,000/ml

The present study revealed that pasteurization process killed coliform organisms in 40 per cent of samples. Kaloianov and Gogov (1977) found that pasteurization killed 100 per cent of coliforms. However 90 per cent of pasteurized milk revealed absence of the *E. coli* organism. The reduction in faecal streptococcal count was highly significant ($p<0.01$) from the raw milk before pasteurization. However, the organism was present in 85 per cent of packaged sample, indicating post pasteurization contamination or thermal resistance of the organism. The yeast and mould count was detected in 80 per cent raw milk samples but after pasteurization only 65 per cent samples revealed the presence of the organism.

5.1.7 *Microbial* counts of Pasteurized Milk from Retail Market

5.1.7.1 Total Viable Count

The highest TVC was observed in the samples of the brand B (5.91 ± 0.01 $\log_{10}$ cfu/ml) and lowest count in the samples of the brand A (4.88 ± 0.12 $\log_{10}$

cfu/ml). A highly significant ($p<0.01$) difference between the mean counts of the brands was observed (Table 33, fig 2). This indicates the quality of different brands of pasteurized milk varied greatly. On comparison of the counts of the four brands with freshly pasteurized milk from dairy plant revealed that mean count of the sample was less and highly significantly ($p<0.01$) different from that of brand B, C and D. The reason for variation in counts could be attributed to unhygienic condition 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 B, C and D at the level of 5 $\log_{10}$ cfu/ml was similar to that recorded by Reddy *et al.* (1989) and Rai and Dwivedi (1990) John (1999), Latha and Nanu (1997). The count of the samples from brand A ($4.88 \pm 0.12 \log_{10}$ cfu/ml) was similar to that of Vijai and Saraswat (1968), Misra and Kuila (1989) and Cerqueira *et al.* (1994)

5.1.7.2 Coliform Count

Among the retail brands coliform count was highest in the samples of the brand C which had mean count of $3.08 \pm 0.29 \log_{10}$ cfu / ml and lowest was seen in the samples of the brand A ($1.15 \pm 0.28 \log_{10}$ cfu / ml) (Table 35, fig 2). A highly significant ($p<0.01$) difference was observed between the coliform counts of the brands. Hence the quality of different brands of pasteurized milk varied greatly. Comparison of the counts of the four brands with freshly pasteurized milk from dairy plant revealed that the mean counts of brand B, C and D were more and highly significantly ($p<0.01$) different. Difference in the coliform counts of various brands marketed in this area was also seen by Latha and Nanu (1997), John (1999) and Sethulakshmi *et al.* (2003). The mean count of brand A was similar to that recorded by Vijai and Saraswat (1968), Arora and Sudarsanam (1986) and Raju and Nambudripad (1987). Samples from the brands B and D showed the coliform count at 10^2 cfu/ml level and was similar to the mean count obtained by Misra and Kuila (1989) and Rai and Dwivedi (1990). The high coliform count in the samples can be due to contamination from the environment and contaminated equipments used in the plant.

5.1.7.3 Escherichia coli Count

E.coli is considered as a part of the normal flora of the intestinal tract of humans and animals and is used as a definite indicator of faecal contamination. Pasteurized milk sample has to be free of *E. coli.* The organisms was absent in 85.72, 78.57, 21.44 and 21.44 per cent samples from brands A, D, B and C, respectively. In 1997, Latha and Nanu observed an overall incidence of 80 per cent from the brands examined in the area. Hence the quality of market milk samples showed an improvement. Freshly pasteurized milk from dairy plant revealed similar mean count to that of brands A and D. The count observed was highest in brand C (2.35 ± 0.38 $\log_{10}$ cfu/ml). Similarly Latha and Nanu (1997) also obtained highest mean count of 2.611±0.084 $\log_{10}$ cfu/ml from S_1 source out of five retail brands examined in this area. However, John (1999) obtained a highest mean count of 3.39 ± 2.74 $\log_{10}$ cfu/ml from brand B retailed in the area. The presence of *E. coli* in pasteurized milk is of great public health significance since the organisms are responsible for a variety of illness from mild diarrhoea to severe hemorrhagic colitis.

5.1.7.4 Psychrotrophic Count

The importance of psychrotrophs is understood after the use of refrigeration for preservation of food items has become more common. The highest count (5.68 ± 0.25 $\log_{10}$ cfu/ml) was found in the samples belonging to brand C and the lowest count (4.25 ± 0.22 $\log_{10}$ cfu/ml) in the samples of brand A (Table 39, fig 2). A highly significant ($p<0.01$) difference was observed in the mean count of the samples from four brands. The mean count of the samples from the dairy plant showed highly significant ($p<0.01$) difference from that of the milk from brands B, C and D. Gopi *et al.* (2001) also found variation in psychrotrophic counts from retail brands available in Chennai and the count ranged between 12.50 to 99.33x10^4 cfu/ml. In comparison to the present study the psychrotrophic organism had a lower count as observed by Schroder *et al.* (1982)

and Saleha (1992). High psychrotrophic count will reduce the shelf life of refrigerated milk considerably.

5.1.7.5 Faecal streptococcal Count

The FSC count was highest in samples of brand B and the samples had mean count of 1.52 ± 0.22 $\log_{10}$ cfu / ml (Table 41, fig 2). The lowest mean count was observed in the samples of brand D which was 0.78 ± 0.22$\log_{10}$ cfu /ml. Similar observations were made by Latha and Nanu (1997) and Sethulakshmi *et al.* (2003) from the retail brands of the area.

5.1.7.6 Yeast and Mould Count

The highest count was seen in samples from brand A which had mean count of 1.21 ± 0.22 $\log_{10}$ cfu / ml and lowest count in the samples of the brand D and the mean count of the brand was 0.62 ± 0.21$\log_{10}$ cfu / ml (Table 43, fig 2). The count was much lower to that obtained by Arora and Sudarsanam (1986). The count was not detected from 57.14, 50, 35.72 and 21.44 per cent of the samples of Brand D, B, C and A, respectively. Cosentino and Palmas (1997) and Birollo *et al.* (2001) have not detected yeast from heat-treated milk. High count of yeast and mould has significance in causing spoilage and mould contamination can also cause toxicity.

5.1.8. Isolation and Identification of Organism in Retailed Milk

5.1.8.1 Escherichia coli

Market brands of milk were tested for the isolation and identification of *E.coli.* Out of 56 samples examined *E. coli* was detected in 48.21 per cent of the samples (Table 45). From this 38 colonies were isolated and identified as *E. coli* based on the cultural, morphological and biochemical characteristics of the organism. The percentage of isolate obtained were almost similar to that obtained by Singh and Ranganathan (1978) and Silva *et al.* (2001).

5.1.8.2 Staphylococcus aureus

S. aureus were isolated from seven (12.5 per cent) samples of retail milk. The organism was isolated from two samples each from brand B and D and three samples of brand C (Table 45). Ghosh and Laxminarayana (1972) had isolated the organism from 23.3 per cent pasteurized milk. However, 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.

5.1.8.3 Listeria monocytogenes

All retail milk tested revealed the absence of *L. monocytogenes*. (Table 45). The results are in agreement with the findings of Sharif and Tunail (1990), Bhilegaonkar *et al.* (1997), Pednekar *et al.* (1997), Mena *et al.* (2004). Low incidence of the organism was observed by Kells and Gilmour (2004), Fyre and Donnelly (2005)

5.1.9 Interpretation on the Quality of Pasteurized Milk

5.1.9.1. Total Viable Count

According to the criteria prescribed by Indian Standards (1992), 76.8 per cent of the total brands could not meet the criterion. None of the sample from brand B met the standards of <30,000 organisms /ml. Only eight, three and two samples from brands A, C and D respectively, met the standards prescribed. Latha and Nanu (1997) recorded that the samples from three out of five sources did not meet the Indian standards. In the present study 23.2 per cent samples examined met the standard while 18 per cent of the samples examined by John (1999) and 6 per cent of pasteurized milk examined by Gopi *et al.* (2001) met the above standard. From these it could be inferred that the bacterial quality of pasteurized milk vary from place to place and it was observed that a high percentage of milk available in the market are of unsatisfactory quality.

5.1.9.2 Coliform Count

None of the samples from the brands had 100 per cent acceptability on the basis of coliform count criteria prescribed by Indian Standards (1992). Of the samples 64.28, 21.44, 14.29 and 7.14 per cent of samples from brand A, D, B and C, respectively met the standard. Latha and Nanu (1997) reported that 100 per cent of samples from one source and only 50 per cent of samples from another source met the above criteria whereas samples from other three sources examined did not meet the criteria at all.

5.1.10 Effect of Refrigeration on Microbial Quality of Milk

5.1.10.1 Total Viable Count

Fresh milk had a mean total viable count of 4.76 ± 0.15 $\log_{10}$cfu/ml. The count did not differ up to two days of storage as shown in table 46 and illustrated in fig 3. The initial growth of bacteria was very slow. For a change in the initial count by one log at 4 ± 1°C storage it took four days. The initial generation time taken may be attributed to the effect of temperature coefficient and sub lethal injury during pasteurization. Hence some time would have taken for the organisms to get rejuvenated. An increase in count during the storage period was observed. The count in the 12^{th} day was 8.72 ± 0.15 $\log_{10}$cfu/ml. John (1999) also observed no variation in total viable count up to four days of storage under refrigeration however 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 12^{th} day of refrigerated storage. Similar increase in counts during storage was reported by Brown *et al.* (1984) and Krasz *et al.* (1993) and Fromm and Boor (2004). The TVC of refrigerated sample on the eighth day was 6.92 ± 0.18 $\log_{10}$cfu/ml and almost similar count was obtained by Zygora *et al.* (2004), Vassila *et al.* (2002) and Moyssiadi *et al.* (2004).

5.1.10.2 Coliform Count

The present study revealed that mean count of the samples from the zero day and 4, 8, 10, and 12 day had high significant ($p<0.01$) difference. A gradual increase in coliform count throughout the period of storage was noticed (illustrated in fig 3). The mean coliform count of freshly pasteurized milk increased from 0.98 ± 0.36 to 2.74 ± 0.37 $\log_{10}$ cfu/ml on the 12th day of refrigerated storage (Table 48). Ledford *et al.* (1983) and Krasz *et al.* (1993) revealed an increase in coliform count throughout storage period. A positive and significant assocition was found between TVC and CC on the fourth day of storage.

Coliforms may be faecal or non-faecal in origin. Non- faecal may enter into milk from milky residues (biofilms) on the equipment (Robinson, 2002). The organisms are generally accepted as an index of environmental contamination and/or faecal pollution. Coliform count is used to assess the overall quality and hygienic condition prevailing during processing of food.

5.1.10.3 Escherichia coli Count

E. coli count was seen in only one batch of samples examined and a gradual increase in count was observed (Table 50). However no significant difference was seen between mean counts of fresh and refrigerated sample. Mamani *et al.* (2003) found that *E 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 *E. coli* and coliform count of the samples stored on day four, eight, ten and12 had a positive and significant ($p<0.05$) relationship.

The isolation of *E. 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 as a pathogen, the presence of the organisms in milk is of great public health significance.

5.1.10.4 Psychrotrophic Count

The mean psychrotrophic count of fresh milk samples was 4.16 ± 0.09 log_{10} cfu/ml. The count increased subsequently throughout storage till 12^{th} day of storage, and the count on the 12^{th} day was 7.92 ± 0.25 log_{10} cfu/ml (Table 52). Krasz *et al.* (1993) also found an increase in psychrotrophic bacterial count throughout storage. The present investigation revealed a highly significant ($P<0.01$) increase in counts during 12 days of storage. The count on eighth day of storage was 6.38 ± 0.24 log_{10} cfu/ml. Similar observation was seen by Zygora *et al.* (2004). The later investigation observed that after seven days of storage of the samples at 4°C psychrophillic counts ranged between 6.11 and 6.69 log cfu/ml. However the psychrotrophic counts after eight days was more than that obtained by Vassila *et al.* (2002) and Moyssiadi *et al.* (2004).

5.1.10.5 Faecal Streptococcal Count

The mean faecal streptococcal count of fresh milk samples was 1.06 ± 0.18 log_{10}cfu/ml. Their presence indicates the survival during pasteurization or due to post pasteurization contamination (Hammer and Babel, 1957). However, 20 per cent of the samples were free of the organism on the zero day and second day of storage. But all the samples revealed the presence of the organism on 10^{th} day of storage (Table 54). Highly significant increase ($p<0.01$) in count was seen on day 10 and day 12. Jay (1997) reported enterococci to grow at from 0 to over 50°C and are better indicators of food sanitary quality than coliforms.

5.1.10.6 Yeast and mould Count

The mean yeast and mould count of fresh pasteurized milk was 0.62 ± 0.13 log_{10}cfu/ml (Table 56). About 35 per cent of the freshly pasteurized milk revealed absence of the organism. Cosentino and Palmas (1997) and Birollo *et al.* (2001) did not detect yeast and mould in heat-treated milk. The count was seen at the level of 10 cfu/ml in 80 per cent of the samples in second day of storage. The count at the above level was seen in 70 per cent samples stored on day 6 and in 20 per cent of the samples stored on the day eight (Table 57). The count increased gradually from fourth day of storage and reached one log higher than the initial count on sixth day of storage. Yeasts and moulds are active at low pH and can grow under refrigeration temperature and thus increase in count may be attributable to the physico-chemical changes of milk during storage.

5.1.11 Isolation and Identification of Organism from Refrigerated Milk

5.1.11.1 Escherichia coli

A total of six isolates of *E.coli* was obtained from refrigerated milk samples (Table 64). All the isolates belonged to the serotype 0148 and are positive for congo red binding indicating their invasive ability. The serotype is characterized as Enterotoxigenic *E.coli* (ETEC) and is associated with traveller's diarrhea and cholera like disease in humans. Ingestion of the organism at the level of 10^6-10^8 viable cells per gram colonise in small intestine and produces toxin thus lead to development of gastroenteritis in the consumer. The syndrome is characterized by non-bloody diarrhea without inflammatory exudates in the stool. The count in the present study was maximum at the level of 10^5 cfu/ml in one of the sample stored on day 12.

5.1.11.2 Staphylococcus aureus

S. aureus was isolated from one (5 per cent) of the samples stored on sixth day and from two (10 per cent) samples stored on the eight day (Table 64). *S. 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 indicates post pasteurization contamination.

5.1.11.3 Listeria monocytogenes

All refrigerated milk samples were tested for the isolation and identification of *L. monocytogenes*, but none of the samples revealed the presence of this organism (Table 64). Low incidence of the organism was observed by Kells and Gilmour (2004) and Fyre and Donnelly (2005)

5.2 EFFECT OF REFRIGERATION ON THE SENSORY AND PHYSICO-CHEMICAL QUALITY OF MILK

5.2.1 Sensory Evaluation

The sensory evaluation of fresh and refrigerated milk sample was done and comparison was made by students 't' test.

a. Colour and appearance

The mean colour and appearance score of freshly pasteurized milk decreased from 9.25 ± 0.26 to 6.90 ± 0.35 on the 12th day of refrigerated storage. The mean score of fresh sample differed significantly ($p<0.05$) with the mean score on fourth day (Table 65). Fresh sample had the maximum score and appeared white. Singh and Patel. (1987) found that after pasteurization or UHT treated milk developed a 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 score increased on the second day of storage indicating that flavour improved after two days of refrigeration of milk (Table 67). Fresh pasteurized milk showed cooked flavour in some batches, which is due to heating of milk during pasteurization as a result of volatile sulphides, particularly hydrogen sulphide from activated SH-group of B-lactoglobulin (Mishra and Nayak, 2002). The mean score differed highly significantly ($p<0.01$) from second day onwards and the difference was maximum between six and eight day. Dogan and Boor (2003) noticed a decrease in flavour score observed on seventh day and 14th day, where a decrease was seen from 7.8 on day 7 to one on day 14 in dairy B. In the present study, slight stale flavour began to develop on eight day and on tenth day of storage the staleness was pronounced. Watson and Mc Ewan (1995) found that at 10°C stale flavour began to develop after four days and sourness developed after six days. Salty flavour was seen in most of the sample as the storage period increased. The other flavour defects noticed in fresh and refrigerated milk was oxidized, cowy, rancid, and sweet flavour.

c. Odour score

Odour score up to fourth day of storage did not differed significantly. However after fourth day there was highly significant ($p<0.01$) reduction in the score and between eight to ten days the reduction was maximum (Table 68). Off odour (stale odour) was first detected on eigth day and was marked on 12th day. The odour score was reduced from 19.10 ± 0.35 to 11.40 ± 0.39 after period of 12 days of storage. John (1999) reported that off-odour in refrigerated milk was developed on the tenth day.

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 beween zero and four, four and six, six and eight days of storage. The score on day two was 29.10 ± 0.31 and was reduced to 18.30 ± 0.52 on tenth day of storage (Table 68). The common defect noticed is presence of clotted particles and was observed on tenth day and all the samples were curdled after 12 days of storage. The other defects noticed was watery or ropy which was found during later period of refrigeration.

Total score

The mean of the total score revealed that samples were of excellent quality for up to fourth day of storage thereafter the sensory quality was decreased and from eight day onwards the sample was graded as fair (Table 69). Sensory analysis revealed 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 but Kadan and Bhanumathi (1984) reported that pasteurized and homogenized milk was graded as B after storage at 24 h. at 5-9°C.

5.2.2 pH

A gradual decrease in the pH of the samples was observed throughout the period of storage. The mean pH of freshly pasteurized milk was 6.60 ± 0.01 and was decreased to 6.25 ± 0.06 on the 12th day of refrigerated storage. Significant ($p<0.05$) difference in the reduction of pH was seen in the samples stored between day two and six, four and eight, and eight and 12 of storage (Table 70). Almost similar findings was observed by Hsieh and Ren (2001) where samples of skim milk stored at 4°C which showed a reduction in pH from 6.78 to 6.16 during a period of 10 days. The drop in pH may be related with the interaction between lactose and milk proteins with the hydrolytic dephosphorization of casein and with changes in calcium phosphorus equilibrium (Singh and Patel, 1987)

5.2.3 Clot on Boiling test

All the samples showed COB test positive on the twelfth day of storage. Similar results of COB test were observed by John (1999). The samples from two batches showed positive test on the eight-day of storage and samples from seven batches revealed a positive test on the tenth day of storage (Table 71). Analysis of the data revealed that a positive COB test is dependent upon the initial microbial load of the samples. The observation in the present study confirmed the observation of Mukundan (1978).

5.3 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 and curdy appearance was seen on tenth day. The mean sensory score of colour and appearance, flavour and body also showed excellent quality up to four days, good quality on sixth day and fair from eighth day onwards. Microbial analysis revealed gradual increase in TVC, CC, ECC, PC, FSC and YMC throughout storage period. When quality of refrigerated milk turned into fair on eight day of storage the sample had mean TVC of 6.92 ± 0.18 log_{10} cfu/ml and the mean PC was at the level of 6.38 ± 0.24 log_{10} cfu/ml. Therefore it may be inferred that the milk becomes organoleptically unacceptable when the TVC and CC are greater than 6 log_{10} cfu/ml. The findings were almost similar to the observation of Schroder *et al.* (1982) who observed 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 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 sample showed sensory deterioration earlier to the development of positive COB test. Although, the COB test is an important criteria 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.

5.4 ASSESSMENT OF CRITICAL CONTROL POINTS IN THE PRODUCTION OF PASTEURIZED MILK

5.4.1 Water

Water used in dairy plant had the highest mean total viable count whereas water from dairy farm samples had the highest mean coliform count. The coliform count of water samples from dairy farm was 1.45±0.59 (Table 72). Palaniswami *et al.* (1988) obtained the mean coliform count of tap water in the dairy farm, as 72 MPN /ml. *E. coli* was not detected in the water samples of the dairy plant. In the study only 30 per cent of water used in the plant revealed the presence of the coliforms however, Lopes and Stamford (1997) found 60 per cent samples of water used to clean the milk equipment revealed the presence of coliform. Faecal streptococci count at the level of 2 $\log_{10}$ cfu/ml was detected in the samples of water obtained from the dairy plant and also from the taps of the farm supplies.

5.4.2 Utensils

Milk pail samples revealed the highest mean total viable count, whereas milk can had the lowest count. The TVC of milking machine sample was 3.81±0.27 (Table 73). McKinnon *et al.* (1990) reported that the mean bacterial count of the milking equipment rinse was at the level of $4.4x10^7$ per m^2. Patel *et al.* (1993) obtained the total plate count of ten rinse samples of milk cans as $5.6x10^5$ to more than $3x10^7$ with an average of 1.7 ± $0.44x10^6$/can and the coliform count of the sample was $4x10^3$ to more than $3x10^5$ cfu/can with an average of 0.1 ± $0.04x10^6$ cfu/can. Coliforms were present at the level of one log cfu/ml level in samples of milk pail. The coliforms count was more in milk pail in comparison with the count of milk can and milking machine. Similar observation was reported by Palaniswami *et al.* (1988). They found the coliform count in milk pail was highest with count of 450/litre capacity and in milk can was lesser with the count of 164 per litre capacity. *E. coli* was not detected in samples of milk can.

5.4.3 Hand Wash

Hand washings of personnel involved had CC, ECC and also FSC. Highest mean total count was obtained from milkers hand wash (Table 74). The detection of *E. coli* and faecal streptococci in the hand wash of the workers indicated the poor hygienic practices of those personnels. Therefore, it may infer that these employees might also contribute to the contamination of the milk samples with microorganism. The finding points to the importance of maintaining personnel hygiene by the plant workers. Palaniswami *et al.* (1988) found the coliforms counts of milkers hand wash as 113 per 100 sq.cm.

5.4.4 Equipment Wash Sample

Among the equipment wash sample examined, Packaging machine had the highest total viable count (4.91±0.24 $\log_{10}$ cfu/ml) when compared to the count of Pasteurization equipment (3.03 ± 0.34 $\log_{10}$ cfu/ml) (Table 75). *E. coli* were not detected in any of the samples. The mean faecal streptococcal and coliform count was more (at the level of 1 $\log_{10}$ cfu/ml) in washings collected from Packaging machine.

5.4.5 Package Material/ Storage Crate/Strainer Sample

Storage crate revealed the highest mean total viable count and lowest was detected in strainer sample. Coliforms were present in samples of package material and storage crate whereas the organism was not detected in strainer samples (Table 76). *E. coli* was absent and faecal streptococci were present in all the three samples analysed. Thus all the sources act as an important source of contamination of milk.

5.4.6 Air

Mean total viable count of air samples collected from the milking barn, pasteurization room and packaging room was higher after the process of milking, pasteurization and packaging. Mean fungal counts were also found to be increased considerably after the above process. Aggarwal and Srinivasan (1978) found that fall of mould conidia per plate per minute from hand milking byre, machine milking byre and milk pasteurization unit ranged between zero to 24.5, zero to seven and zero to six per plate per minute.

Highest count of mean TVC and YMC was seen from air samples collected from dairy farm. The counts obtained in the present study from various processing areas were well above the standards prescribed by APHA (Hickley *et al.,* 1992). Similar findings was seen by Salustiano *et al.* (2003) where all the processing areas in the dairy plant, the number of mesophillic aerobic bacteria and yeast and mould obtained was higher than the standard prescribed by APHA.

The assessment of the critical control points revealed that maximum contamination was seen from sample take from milker, milk pail and environment in the dairy farm thereby resulting in an increased microbial count in pooled milk. However, pasteurization process reduced the microbial count to a highly significant level. The contamination subsequent to pasteurization was evident and packaged milk sample revealed high microbial count with only 55 per cent samples meeting the Indian Standards. This was as a result of improperly cleaned packaging equipment, packaging material and environmental contamination of the area.

Milk besides being an important nutritive drink consumed by the public, is also an important raw ingredient for the manufacture of various products. Thus hygienic milk production usually is the most important key factor in deciding the final product quality and shelf life. The study will provide the basis for hygienic milk production and how and where control measures needs to be adopted so as to minimize microbial contamination of milk. The comparison of retail brands will throw light on the pasteurized milk manufacturer about the milk microbial quality, sources of contamination and the control measures that can be adopted to minimize the contamination. Further the study will also serve as the indicator for estimating the average shelf life of milk by knowing the changes with regards to microbial, sensory and physico chemical qualities of milk.

Summary

6. SUMMARY

A total of 296 milk samples were collected from dairy farm, dairy plant and retail shops. Ten batches of milk, consisting of raw milk (60) collected from individual cows, pooled samples, chilled milk before pasteurization and milk at various stages of pasteurization (60) viz. immediately after heating section, pasteurization, packaging and samples (120) stored at 4±1°C were collected in duplicates on 2, 4, 6, 8, 10 and 12 days, were evaluated. Retailed samples (56) belonging to four brands (A, B, C, D) were also collected and compared with freshly pasteurized milk.

The mean total viable count of individual animal milk was 5.14±0.13 $\log_{10}$ cfu/ml, and that of pooled milk was 5.58±0.14 $\log_{10}$ cfu/ml. Highly significant ($p<0.01$) increase in the total viable count was noticed between the individual animals milk and pooled milk.

Highest mean coliform count was recorded for pooled milk (3.24±0.19 $\log_{10}$ cfu/ml). Coliforms were not detected in 45 per cent of the individual animal milk samples. *E. coli* was not detected in 60 per cent of the samples. The mean *E. coli* count of the pooled milk samples was 0.63±0.31 $\log_{10}$ cfu/ml.

Mean psychrotrophic count obtained from chilled milk was highest (5.56±0.14 $\log_{10}$ cfu/ml) and the count differed highly significantly ($p<0.01$) from that of Individual animal milk samples (4.69±0.24 $\log_{10}$ cfu/ml). Psychrotrophic count showed a highly significant ($p<0.01$) and positive association between the Total viable count.

The mean faecal streptococcal count was at the level of 2.5 $\log_{10}$ cfu/ml in pooled and chilled milk samples, respectively. The mean count of the organism in individual milk sample was 1.89±0.08 $\log_{10}$ cfu/ml. The mean yeast and mould count of pooled milk was 1.86±0.19 $\log_{10}$ cfu/ml, and the organism could not be detected in 23.33 per cent of the total raw milk samples. .

Escherichia coli contaminates milk through unhygienic milk handling and 20 per cent of the samples of raw pooled milk showed its presence. The isolates were serotyped at National *salmonella* and *Escherichia* center, Central Research Institute, Kasauli. It was found that two isolates belonged to 05 and the remaining two consisted of serotype 0141 and 0172.

Staphylococcus aureus was isolated from 12 (60 per cent) of the pooled raw milk samples, however none of the samples tested showed the presence of *Listeria monocytogenes*.

The total viable count of milk at various stages of pasteurization, revealed highly significant ($p<0.01$) difference between the mean TVC count of the samples collected after heating section and the samples after packaging. The packaged milk sample had an increase of 1.12 $\log_{10}$ cfu/ml when compared to milk immediately after pasteurization. Thus post pasteurization contamination is evident.

The coliform organisms were absent in cent per cent of heated milk however the organisms were present in 20 per cent of pasteurized milk and 60 per cent of packaged milk. *E. coli* was not detected from milk samples after heating and after pasteurization. However the organism was found in 10 per cent of the packaged samples. The mean coliform and *E.coli* count of freshly packaged milk samples had highly significant ($p<0.01$) positive association. The contamination of packaged milk with the organism indicates that contamination occur in the milk storage tank or packaging section of milk. However, 40 per cent of packaged milk samples were graded satisfactory according to the coliform standards prescribed by Indian standards (1992).

The mean psychrotrophic organism in heated milk was highly significantly ($p<0.01$) different from that of the count of pasteurized and packaged milk. The mean faecal streptococcal count of heated milk was 0.23 ± 0.10 $\log_{10}$ cfu/ml and in packaged milk the count was 1.06 ± 0.18 $\log_{10}$

cfu/ml. The mean count of the samples revealed highly significant ($p<0.01$) difference. Analysis of the mean yeast and mould count revealed significant ($p<0.05$) difference between the counts of pasteurized and packaged milk and also between pasteurized and heat treated milk. The mean count of packaged milk was $0.62 \pm 0.13\log_{10}$cfu/ml.

Escherichia coli was isolated from 2 (10 per cent) of freshly pasteurized milk. The two isolates belonged to the serotype 0148 and both had congo red binding property and were Eijkman positive. *S. aureus* was isolated from one (5 per cent) of the freshly pasteurized milk whereas none of the sample had *L. monocytogenes.*

Pasteurization reduced the microbial population of raw milk and a highly significant ($p<0.01$) reduction was observed in the counts of TVC, coliform, psychrotrophic, yeast and mould and faecal streptococcal count and significant ($p<0.05$) reduction in *E. coli count.*

The retail milk samples of brands A, B, C and D which were subjected for microbial evaluation revealed highest mean TVC and faecal streptococcal count in the samples of the brand B. Highest count of coliform, *E. coli* and psychrotrophs were observed in the samples of brand C. Highest level of yeast and mould count was seen in the samples obtained from brand A. Analysis of variance test of the data revealed highly significant ($p<0.01$) difference between the mean TVC, CC, ECC and PC of samples from four brands which indicate that the quality of different brands of pasteurized milk retailed in the area varied greatly. On comparison of freshly pasteurized milk with the retail brands revealed that the TVC, CC, ECC and PC was more in brands B, C and D and differed highly significantly ($p<0.01$).

Isolation of bacterial organisms of public health significance revealed that out of 56 retail samples 48.21 per cent had *E. coli.* The organism was detected in 84.6 per cent of samples each of brand B and C. *S. aureus* was present in seven

(12.5 per cent) samples. The organism was isolated from two samples each from brands B and D and three samples of brand C. However, none of the retail milk revealed the presence of *L. monocytogenes.*

The mean TVC of pasteurized milk was increased at a highly significant ($p<0.01$) level from the fourth day of storage. On eighth day of storage the count was 6.92 ± 0.18 $\log_{10}$cfu/ml and on 12th day mean count reached 8.72± 0.15 $\log_{10}$cfu/ml

A gradual increase in the coliforms count of the samples was observed throughout the period of storage. The mean count of the freshly pasteurized milk was 0.98 ± 0.36 $\log_{10}$ cfu/ml and was increased to 2.74 ± 0.37 $\log_{10}$ cfu/ml on the 12th day of storage. Association between the mean coliform count and TVC was positive and significant ($P<0.05$) on fourth day of refrigerated storage.

Ten per cent of freshly pasteurized milk had *Escherichia coli* and a gradual increase in the count was observed throughout the period of storage. *E. coli* and coliform count of the samples stored on day 4, 8, 10 and 12 showed a positive and significant ($p<0.05$) relationship. Psychrotrophic count was present in all the samples and the mean count on zero day was 4.16 ± 0.09 $\log_{10}$ cfu/ml and the count on 12th day was 7.92 ± 0.25 $\log_{10}$ cfu/ml. Highly significant ($p<0.01$) increase in the mean counts of the samples stored between day fourth and sixth and on day six and eight and day 10 and 12 was observed. The increase in count during storage may lead to deterioration in milk quality and reduction in shelf-life.

Faecal streptococcal count also increased throughout the period of storage. Analysis of the data 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 6 to day 12 of storage. The mean yeast and mould count of fresh pasteurized milk was 0.62 ± 0.13$\log_{10}$cfu/ml. About 35 per cent of the freshly pasteurized milk revealed absence of the organism. A positive and highly significant ($p<0.01$)

association was observed between the mean faecal streptococcal count and yeast and mould count on eighth day of storage

E. coli was isolated from five per cent samples on each day of storage. Isolates belonged to serotype O148 and are positive for congo red binding test indicating their invasive ability. *S. aureus* was isolated only from one (5 per cent) of the samples on the sixth day and from two (10 per cent) samples on the eight day of storage. None of the samples revealed the presence of *L. monocytogenes.*

Sensory analysis of refrigerated milk samples showed a reduction in the score of colour and appearance, odour and body as the storage period increased. However mean flavour scored increased after two days. Colour and appearance of fresh sample differed significantly ($p<0.05$) with the score of the samples stored on fourth day. Slight stale flavour began on eighth day of storage and on tenth day the staleness was pronounced. Off odour (stale odour) was also detected on eighth day and was marked on 12^{th} day. Development of clots or curdy appearance of the body of milk indicates that sample was spoiled and it was seen on tenth day of storage. An overall reduction in pH was seen over the storage period and all the samples showed COB test positive on 12^{th} day of storage.

The microbial loads of various source of contamination of milk in the farm and in processing plants are identified and their bacterial loads are determined. The water used in dairy plant had the highest mean total viable count (3.84 ± 0.26 $\log_{10}$ cfu/ml) whereas water from dairy farm samples had the highest mean coliform count of 1.45 ± 0.59 $\log_{10}$ cfu/ml. *E. coli* was not detected in the water samples of the dairy plant however the organism was detected in the farm water supplies. The utensils used in the dairy farm revealed bacterial contamination and the rinse samples of milk pail revealed high TVC, CC, ECC and FSC. Milkers hand wash revealed higher TVC, CC and ECC than that of persons working in the dairy plants. Among the equipment wash sample, Packaging machine had the highest total viable count (4.91 ± 0.24) compared to the count of Pasteurization equipment (3.03 ± 0.34). *E. coli* was not detected in any of the equipment wash samples. The samples of storage crate had high TVC, CC and FSC than that of the samples of packaging material and strainer. The TVC and YMC of the air sample were lower in packaging unit in comparison with that of the pasteurization unit. Mean TVC and YMC of air samples collected from the milking barn, pasteurization room and packaging room was higher after the process of milking, pasteurization and packaging.

The hygienic practices followed during the production of milk at the farm and also during pasteurization, packaging, storage and retailing needs an improvement with regard to reduction in microbial count and overcoming the impact of the harmful pathogens like *E.coli* and *S. aureus*. The presence of coliform, faecal streptococci and *E.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 farm. Based on TVC, 55 per cent of freshly pasteurized samples were within the limits prescribed by Indian Standards. However post pasteurization contamination of milk was evident and packaging machine was found to be the important contaminating source. Hence timely cleaning and sanitation of equipment is necessary. The microbial quality of pasteurized milk brands retailed in the area varied and overall 76.8 per cent samples was considered unsatisfactory based on the TVC limits prescribed by Indian Standards. None of the samples from the brands had 100 per cent acceptability on the basis of coliform count criteria prescribed by Indian Standards. Thus the concept of quality and food safety (HACCP) management system needs to be strictly adopted. The shelf life of milk under refrigeration was 8 days with acceptable sensory characteristics. An increase in microbial counts was noticed on storage, however significant increase occurred only after two days. Thus microbial quality deteriorated with increase in storage days. Thus under ideal refrigerated condition in India pasteurized milk can be kept up to eight days however the keeping quality varies based on the initial microbial load and the type of microorganism present.

References

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

Aggarwal, P.K. and Srinivasan, R.A. 1980. Mould contamination from dairy and farm environment. *Indian J. Dairy Sci.*33: 117-118

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

Beuchat, L.R. and Consin, L.R. 2001. Yeasts and Molds. *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. 209-213

Bhilegaonkar, K.N., Kulshrestha, S.B., Kapoor, K.N., Kumar, A., Agarwal, R.K. and Singh, B.R. 1997. Isolation of *Listeria monocytogenes* from milk. *J. Fd. Sci. Technol.* 34: 248-250

Birollo, G.A., Reinheimer, J.A. and Vinderola, C.G. 2001. Enterococci vs non-lactic acid microflora as hygiene indicators for sweetened yoghurt. *Fd. Microbiol.* 18: 597-904

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

Carlos, V.S., Oscar, R.S. and Irma, Q.R.E. 2001. Occurrence of *Listeria* species in raw milk in farms on the outskirts of Mexico City. *Fd. Microbiol.* 18: 177-181

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

Consin, 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

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

*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

Evancho, G.M., Sveum, W.H., Moberg, L.J. and Frank, J.F. 2001. Microbiological monitoring of the food-processing environment. *Compendium of methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington D.C, pp. 25-35

Fromm, H.I. and Boor, K.J. 2004. Characterization of pasteurized fluid milk shelf life attributes. *J. Fd. Sci.* 69: 207-213

*Fyre, C. and Donnelly, C.W. 2005. Comprehensive survey of pasteurized fluid milk produced in the United States reveals a low prevalence of *Listeria monocytogenes*. *J. Fd. Prot.* 68: 973-979

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

Gil, 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

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

Hammer, B.W. and Babel, F.J. 1957. *Dairy Bacteriology*. Fourth edition. John Wiley and Sons, Newyork, p.621

Hickley, P.J., Beckelheimer, C.E. and Parrow, T. 1992. Microbiological tests for equipment, containers, water and air. *Standard Methods for the examination of Dairy Products.* (ed. Marshal, T.). Sixteenth edition. American Public Health Association, Washington D.C, pp. 397-412

Homhual, S. and Jindal, V.K. 2001. Simple tests for rapid assessment of the quality of raw milk. *J. Fd. Prot.* 64: 1996-2000

*Hsieh, Y.H.P. and Ren, Z. 2001. Determination of microbial quality in pasteurized milk using a non-instrumental microrespirometer. *Fd. Microbiol.* 88: 24

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. 1978. IS: 1622-1964. *Methods of sampling and test for microbiological examination of water used in industry.* Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi-1.p.39

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

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. 1997. *Modern Food Microbiology*. Fifth edition. Chapman and Hall, New York, p.661

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*. 451-458

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

Kells, J. and Gilmour, A. 2004. Incidence of *Listeria monocytogenes* in two milk processing environments, and assessment of *Listeria monocytogenes* in blood agar for isolation. *Int. J. Fd. Microbiol.* 91: 167-174

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

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

Lues, J.F.R., Venter, P. and Westhuizen, H.V. 2003. Enumeration of potential microbiological hazard in milk from a marginal urban settlement in central South Africa. *Fd. Microbiol.* 20: 321-326

*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

Matta, H. and Punj, V. 1996. Isolation and identification of proteolytic psychrotrophic spore forming bacteria from milk. *Indian J. Dairy Sci.* 49: 695-699

*McKinnon, C.H., Rowlands, G.J. and Bramley, A.J. 1990. The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds. *J. Dairy Res.* 57: 307-318

Mena, C., Almeida, G., Carneiro, L., Teixeira, P., Hogg, T. and Gibbs, P.A. 2004. Incidence of *Listeria monocytogenes* in different food products commercialized in Portugal. *Fd. Microbiol.* 21: 213-216

Mishra, S. and Nayak, S.K. 2002. Milk flavour-A marketing attribute. *Indian Dairyman.* 54: 65-68

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

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

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.

Nordic committee on Food Analysis. 1968. *Determination of faecal streptococci in foods.* 68. UDC. 576, 851, 21

Palanniswami, K.S., Venugopalan, A.T. and Masillamony, P.R. 1988. Studies of coliform of farm milk and its environment. *Indian J. Dairy Sci.* 41: 149-153

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

Pednekar, M.D., Kamat, A.S. and Adhikari, H.R. 1997. Incidence of *Listeria* species in milk and milk products. *Indian J. Dairy Sci.* 50: 143-151

*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

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

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

*Rea, 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

*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

Rohrbach, B.W., Draughton, F.A., Davidson, P.M. and Oliver, S.P.1991. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in Bulk tank milk: Risk factors and Risk of human exposures. *J. Fd. Prot.* 55: 93-97

Ryser, E.T. and Donnelly, C.W. 2001. *Listeria. 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.343-353

*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

Salustiano, V.C., Andrade, N.J., Brando, S.C.C., Azeredo, R.M.C. and Lima, S.A.K. 2003. Microbiological air quality of processing areas in a dairy plant as evaluated by the sedimentation technique and a one-stage air sampler. *Brazilian. J. Microbiol.* 34: 1-8

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

Scott, V.N., Clavero, R.S. and Troller, J.A. 2001. Measurement of water activity (a_w), acidity and brix. *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.649-658

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

*Sharif, A. and Tunail, N. 1991. *Listeria monocytogenes* contamination of raw milk from different regions of Anatolia and pasteurized milk sold in Ankara. *Microbiol. Bull.* 25: 15-20

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

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

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

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

Soomro, A.H., Araim, M.A., Khaskheli, M. and Bhutto, B. 2002. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market condition at Tandojam. *Pakist. J. Nutr.* 1: 151-152

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

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

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

Yadav, J.S., Grover, S. and Batish, V.K. 1992. *A Comprehensive Dairy Microbiology*. Metropolitan, New Delhi, p.764

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

Zweifel, C., Muehlherr, J.E., Ring, M. and Stephan R. 2004. Influence of different factors in milk production on Standard Plate Count of raw small ruminant's bulk-tank milk in Switzerland. *Small Rumin. Res*. 30:1-8

* Originals not consulted.

MICROBIAL QUALITY ASSURANCE OF MILK IN ITS PRODUCTION, PROCESSING AND STORAGE

PREJIT

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

In the present study 296 milk samples were collected from dairy farm, processing plant and retail shops and analysed for the microbial quality by estimating various microbial counts and assessing the presence of certain bacteria of public health importance. The microbial, physico-chemical and organoleptic qualities of pasteurized milk samples stored under refrigeration were evaluated. The critical points of bacterial contamination of milk at various stages of production, pasteurization, packaging and storage were also assessed during the investigation.

Out of 60 samples of raw milk from individual animal, pooled milk and chilled milk analysed, 46.7 and 36.6 per cent samples were graded as very good and good based on BIS (IS 1977). However only 25 per cent samples were only considered satisfactory based on coliform count with none of the pooled milk samples meeting the standards. Pooled milk samples revealed maximum contamination with highly significant ($p\leq0.01$) difference between mean TVC and CC and significant ($p\leq0.05$) difference between mean PC and FSC of milk from individual animal. However mean *E.coli* count was more in individual animal milk sample when compared with pooled milk. The pooled milk sample was kept under chilled condition for 17 hours. The chilled milk had higher mean TVC, ECC and PC however the increase was not statistically significant.

Pasteurization of milk was effective in reducing the microbial population of raw milk and there was highly significant ($p\leq0.01$) reduction in TVC, CC, PC, FSC and YMC. From the sample of milk collected immediately after heating, after pasteurization and after packaging the mean TVC was high in packaged milk sample (4.76 ± 0.15 $\log_{10}$ cfu/ml). Coliform was absent in cent percent of heated milk. However, 60 per cent of packaged milk had the organism with the mean count of 0.98 ± 0.36 $\log_{10}$ cfu/ml. Only 40 per cent of packaged

milk was graded satisfactory based on coliform standards prescribed by BIS (Indian Standards, 1992). *E.coli* could not be detected from milk collected after heating section and immediately after pasteurization. Maximum microbial contamination was seen in packaged milk. This indicates that contamination of milk occur in the storage tank or packaging section. Hence proper cleaning and sanitation of storage tank and packaging machine will reduce the microbial contamination to a considerable extent.

The microbial analysis of the retail milk sample (56) of the brands A, B, C and D revealed that the samples of the brand B had highest mean TVC (5.91 ± 0.01 log_{10} cfu/ml) and FSC (1.52 ± 0.22 log_{10} cfu/ml). The highest mean coliform (3.08 ± 0.29 log_{10} cfu/ml), *E. coli*, (2.35 ± 0.38 log_{10} cfu/ml), psychrotrophic counts (5.68 ± 0.25 log_{10} cfu/ml) were seen in the samples of brand C. Brand A had highest mean YMC (1.21 ± 0.22 log_{10} cfu/ml). Only 23.2 and 26.8 per cent retail samples met the TVC and CC standards prescribed by BIS (IS-1992). However, 57.14 per cent of the samples of the brand A met the standard. On comparison of freshly packed milk obtained from dairy plant with the retail brands revealed that freshly packed milk sample had highly significant ($p \leq 0.01$) difference and lesser TVC, CC and PC in comparison with the brands B, C and D. Thus the microbial quality of retail milk available in the locality varied among different brands hence strict hygienic measures should be adopted to minimize microbial contamination.

Pasteurized milk stored under refrigeration (4 ± 1 °C) showed an increase in TVC, CC, ECC, PC, FSC and YMC throughout the storage period. However, the initial growth rate of microorganism was slow due to sub lethal injury of microorganism during pasteurization and storage of milk under refrigerated condition. The increase in TVC, CC, ECC, PC, FSC and YMC between zero day and 12^{th} day was 3.96, 1.76, 0.16, 3.76 and 2.37 log_{10} cfu/ml, respectively. The increase in the count of organism during storage may present the problem of shelf life deterioration of milk.

The public health impact on the consumers was assessed by isolation and identification of *E.coli, S. aureus* and *L. monocytogenes*, the organisms of public health importance. *Escherichia coli* contamination of milk occurs through unhygienic handling of milk. The organism was detected in four samples (20 per cent) of the samples of raw pooled milk and was of serotypes 05 (2 samples), 0141 and 0172. Ten per cent of freshly packaged and refrigerated milk samples revealed the presence of the organism and all isolates were of serotypes 0148. A total of 38 *E.coli* isolates were obtained from retail milk samples. *S. aureus* was isolated from 60, 5, 12.5 per cent of the samples of raw, freshly pasteurized and retail milk, respectively. The organism was isolated from 5 per cent of the samples stored on sixth day and from 10 per cent samples stored on the eight day. *L. monocytogenes* was not isolated from milk samples.

Sensory analysis of refrigerated milk samples showed an overall reduction in the score of colour and appearance, flavour, odour and body as the storage period increased. Development of off odour, salty or stale flavour and presence of clotted particles indicated the sensory spoilage and the maximum shelf life obtained was eight days. There was reduction in mean pH value throughout the storage period. All the samples showed positive to clot on boiling test on the day 12 of storage but the sample showed sensory unacceptability earlier.

The various critical points of bacterial contamination of milk was evaluated by collecting samples of air, water, rinse samples from utensils, equipments, hand washing of milker/personnel in the processing line, strainer and packaging material and were subjected to estimation of various bacterial counts. The mean total viable count and yeast and mold count of air samples were found to increase after the milking process or processing. Among the water samples, coliform and *E. coli* was detected more in the samples obtained from dairy farm. High microbial count was recorded for milk pail, milkers hand washings and package machine wash indicating an important sources of contamination. Strict hygienic practices followed by health education will minimize the microbial contamination to a considerable extent.