ASSESSMENT OF MICROBIAL QUALITY, ADULTERANTS AND PRESERVATIVES IN PASTERIZED MILK

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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

2008

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DECLARATION

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

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Certified that the thesis entitled **"ASSESSMENT OF MICROBIAL QUALITY, ADULTERANTS AND PRESERVATIVES IN PASTEURIZED MILK"** is a record of research work done independently by **BHAGYALAKSHMI P.S** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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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****, Dean, College of Veterinary and Animal Sciences, Mannuthy 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. B. Sunil,*** *Associate Professor and Head, Department of Veterinary Public Health, for the generous encouragement, whole hearted help, inspiration, moral support and personal guidance in the pursuit of this research work.*

I owe my sincere gratitude to ***Dr. G. Krishnan Nair,*** *Professor and Head, Department of Microbiology for his valuable guidance, critical comments and timely help rendered during the entire period of research work.*

I am cordially obliged to ***Dr. P. I. Geevarghese,*** *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 remember with great sense of gratitude ***Dr. M. Mukundan,*** *Associate professor and Head, Deparment of Dairy Science, for his valuable guidance, timely help, moral support and affection rendered during the entire period of research work.*

I would like to place on record my heartfelt thanks to ***Dr. C. Latha,*** *Associate professor and Head, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Pookot, Wynad, for the encouraging advices and inimitable help.*

I am in short of words to express my deep sense of gratitude to ***Dr. Satyanarayana Rao,*** *for the encouraging advices and inimitable help.*

I gratefully acknowledge ***Smt. K. S. Sujatha*** *and* ***Smt. K. A. Mercy*** *for the help rendered in statistical analysis.*

I am thankful for the help rendered for the research by ***Dr. Sisilamma George, Dr. V. Ramnath*** *and* ***Dr. A. D. Joy****.*

*A special thanks to the **Dr. K. A. Mani,** Joint Director and Head, National Salmonella and Escherichia Centre, Central Research Institute, Kasauli for serotyping the Escherichia coli isolates and sending the results on time.*

*Bouquets of special thanks are due to **Dr Vrinda, Dr.Sethulakshmi** and **Dr. Prejit** for their concern and support.*

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

*. No words or deeds are sufficient to express my gratitude to **Dr. Magna, Dr. Asha** and **Dr. Gini** for all the incessant support and guidance they have showered on me.*

*I cherish the spirit of understanding and personal encouragement rendered to me by my friends, **Drs. Siji, Vinod, Vivek, Ambili, Manu, Remya** and **Divya rani**.*

*Words possess no enough power to reflect my thankfulness for the invaluable help, moral support, affection and pleasure rendered by my friends **Drs. Anupama, Elso, Ruby** and **Shijin**.*

*I gratefully acknowledge the help rendered by friends and room mates **Drs. Rajeswari, Jessy** and **Seena** in the progress of my work. I wish to extend my thanks to my friends **Drs**. **Mariyamma**, **Remya, Seena, Dhanya, Manjula, Indu, Reshmi, Rojan** and **Hari**.*

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

*I am also thankful to **Mr. Sundaran, Ms. Bindhu, Mr. Achuthanandhan,** and **Ms. Sheela** 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, Chechi, Chettan, Aniyathi,** and **Grandmother** for being always with me through thick and thin.*

*I am deeply touched by the understanding, love, care, support and constant encouragement by my life partner **Mr. Sudhi** and his family.*

*I express my heartfelt gratitude to the endurance of my little daughter, **Arundathy** and I dedicate this work to her.*

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

Bhagyalakshmi P.S

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Introduction

1. INTRODUCTION

Livestock Sector has been playing an important role in Indian economy and is an important sub sector of Indian agriculture. This sector plays an important and vital role in providing nutritive food rich in animal protein to the general public and in supplementing family incomes and generating gainful employment in the rural sector, particularly among the landless, small, marginal farmers and women. India is ranked as the highest milk producing country of the world. In 2005-06 the milk production in the country was 97.1 million tonnes (Animal Husbandry statistics, 2006). In 2006-2007 the milk production in the country has increased to 100.2 million tonnes.

The process of pasteurization was named after Louis Pasteur who discovered that spoilage organisms could be inactivated in wine by applying heat at temperatures below its boiling point. The process was later applied to milk by Franz von Soxhlet and remains the most important operation in the processing of milk. Pasteurization is the heat treatment that destroys majority of saphrophytic and pathogenic bacteria without reducing the nutritional quality of milk. If carried out correctly, pasteurization will serve the two distinct purposes viz., quality and a longer shelf life.

Milk is a highly nutritious drink preferred by all age groups of people. Besides being a nutritious food it acts as raw material for the preparation of large number of delicacies. Most of the bacteria in fresh milk from a healthy animal are either harmless or beneficial. But rapid changes in the health of an animal, or the milk handler, or contaminants from polluted water, dirt, manure, vermin, air, cuts and wounds can make raw milk potentially dangerous. It provides a very good environment for the growth and survival of microorganisms, both pathogens and spoilage organisms. Illness from contaminated milk and milk products have occurred worldwide since cows have been milked. In the 1900s it was discovered

that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever and Q-fever to humans. Fortunately, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced over the decades due to improved sanitary milk production practices and pasteurization. While pasteurization destroys many microorganisms in milk, improper handling after pasteurization can recontaminate milk.

Lifestyles and food habits of the people are changing. Today the consumers want the assurance that the food they receive is safe and hygienic. People are willing to pay more for quality. Increasing consumer awareness has emphasized the need for microbiologically safe food. There are strict legislations in many countries to monitor the quality of milk available in the market. The rising awareness about hygienic standards and adulteration of loose milk has led consumers in urban areas to switch over to packaged milk. In India about 13 million tones of milk are sold in packed form mostly in pouches.

Kerala being a consumer state depends largely on its neighbouring states for milk and milk products. Large quantities of adulterated and sub-standard milk are being sold in the Kerala market. The Government agencies are not in a position to meet the entire requirement of the state and so people are forced to buy packed milk from other states owing to its easy availability. Creating awareness among the public about the menace is essential to ensure that quality milk is sold in our country. If the consumers are vigilant and united, the vicious practice of adulterating food products can be easily checked.

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

1. Evaluation of the microbiological quality of pasteurized milk retailed in our market by estimating the total viable count, coliform count, faecal

streptococci count, *Escherichia coli* count, psychrotrophic count and yeast and mould count.

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

3. To detect the presence of preservatives like bicarbonates and formaldehyde and adulterants such as starch and cane sugar in pasteurized milk.

Review of literature

2. REVIEW OF LITERATURE

2.1 MICROBIAL COUNTS OF MILK

2.1.1 Total Viable Count

Vijai and Saraswat (1968) studied the bacteriological quality of market milk in Udaipur city. A total of 65 pasteurized milk samples were collected from milk supply scheme and examined for standard plate count. The average SPC of pasteurized milk sample was 32,000 per ml.

Ethiraj *et al.* (1979) collected 10 pasteurized milk samples from a commercial dairy plant and 34 milk samples from four organized dairy farms which were laboratory pasteurized and evaluated the total viable count. The average total viable count of the samples from commercial dairy plant and dairy farm 1, 2, 3 and 4 were 11020000, 18682, 125289, 5882 and 6728 cfu/ml, respectively.

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

Yadava *et al.* (1983) undertook a study to determine the bacteriological quality of milk sold in Ranchi town. They collected 12 samples of pasteurized milk during monsoon and 10 samples during winter, from the town milk supply. The average standard plate count from these sources during monsoon and winter were 1735.83×10^5 and 1881.50×10^5 cfu/ml, respectively. All the 22 samples of pasteurized milk were of poor quality.

Yadava *et al.* (1985) investigated the bacterial flora present in market milk and its public health significance. They examined 22 samples of pasteurized milk from the milk supply scheme in Ranchi town. The total viable count of these samples varied from 45×10^5 to 7500×10^5 cfu/ml with an average count of 1802.04×10^5 cfu/ml.

Arora and Sudarsanam (1986) analyzed the microbiological quality of milk and skim milk powder used as ice cream ingredient in Karnal city. They collected eight samples of pasteurized milk each from the experimental dairy of NDRI, Karnal (source A) and from the market (source B). None of the samples from sources A and B were found to exceed the standard plate count limit of 30,000 cfu/ml for pasteurized milk.

Misra and Kuila (1989) analyzed 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 mean standard plate count of pasteurized milk from dairy plant was 53×10^3 cfu/ml.

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

Mahari and Gashe (1990) enumerated the microorganisms present in pasteurized milk and the sources of contamination in milk after it had reached the processing plant in Addis Abba. Pasteurized milk had aerobic counts of 7×10^5 cfu/ml as it left pasteurizing 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 from Kanpur city. They found that the standard plate count of standardized and

pasteurized milk supplied by milk board varied between 9 x 10^4 and 65 x 10^4/ml with an average count of 33 x 10^4/ml.

Siva *et al.* (1993) investigated the microbial quality of pasteurized and raw milk at its various stages of production. A total of 16 milk samples were collected from students training dairy, Anand. The total viable count in these samples varied from 10,000 to 62,000 cfu/ml with an average count of $3.7 \pm 0.54 \times 10^4$ cfu/ml.

Cerqueira *et al.* (1994) examined 97 samples of type C pasteurized milk collected from a diary plant at Belo Horizonte, Brazil. During the study it was observed that the samples had a mean mesophilic count of 3.7 x 10^4 cfu/ml. Of the samples 14.4 per cent had mesophilic count above the legal limits.

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 raw milk samples were collected from individual suppliers. Analysis of the samples revealed that the samples had a mean bacterial count of four million/ml. They opined that pasteurization reduced the bacterial count to 42,000/ml.

Beuvier *et al.* (1997) evaluated the bacterial quality of raw, pasteurized and micro filtered milk used for the manufacture of Swiss-type cheese. The study revealed that the mean bacterial population of pasteurized milk used for the production of cheese was 5700 cfu/ml.

Cosentino and Palmas (1997) analyzed the sources of contamination and the kind of contaminating microorganisms present in six ewe's milk processing plants in Sardinia, Italy. Samples were collected during production hours three times over a period of six months. The total aerobic count in majority of the heat treated milk samples were less than 10^4 cfu/ml. The mean total aerobic counts of heat treated milk

in these six dairy plants 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 $\log_{10}$ cfu/ml, respectively.

Latha and Nanu (1997) assessed the bacteriological profile of pasteurized milk obtained from five sources namely S_1, S_2, S_3, S_4 and S_5. Twelve samples of pasteurized milk were obtained from each source and the mean aerobic plate counts of the samples belonging to S_1, S_2, S_3, S_4 and S_5 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.

Lopes and Stamford (1997) evaluated 84 samples of milk collected from four points, viz. Storage tank, outlet of pasteurizer unit, pasteurized milk storage tank and packing and filling machine. 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.

Eneroth *et al.* (1998) traced the critical contamination sites in the production line of pasteurized milk in three dairy plants in Sweden or Norway. Samples of raw and pasteurized milk were collected at six sites along the line and repeated 3- 4 times. The initial aerobic counts 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 packages were 8×10^4, 9×10^4, 6×10^2, 1×10^3, 7×10^2 and 6×10^2 cfu/ml, respectively.

John (1999) evaluated 100 pasteurized milk samples from five commercial brands A, B, C, D and E retailed in and around Thrissur and reported that the mean total viable counts of the samples from these sources were 5.68, 7.24, 7.65, 4.47 and 5.77 $\log_{10}$ cfu/ml, respectively. The study revealed that 72 per cent of the samples did not meet the BIS standards.

Gopi *et al.* (2001) evaluated the microbial quality of 12 commercial brands of pasteurized and homogenized milk sold by private vendors in Chennai city and recorded that the average standard plate count of these brands varied between 5.5×10^4 cfu/ml to 175.17×10^4 cfu/ml.

Beloti *et al.* (2002) examined 90 refrigerated pasteurized milk samples consisted of 29 each of grade A and B and 32 of grade C and found that 10.3 per cent of pasteurized milk of grade A, 24.1 per cent of grade B and 9.3 per cent grade C samples did not meet the microbiological specifications prescribed in Brazil. Twenty six samples of grade A had total aerobic microbial counts less than 5×10^2 cfu/ml and 3 had counts greater than 5×10^2 cfu/ml. In grade B samples, 22 had counts less than 4×10^4 cfu/ml and seven had counts greater than 4×10^4 cfu/ml, whereas in grade C samples, 29 had counts less than 1.5×10^5 cfu/ml and 3 samples had counts greater than 1.5×10^5 cfu/ml.

Khalilur *et al.* (2002) examined the microbiological quality of 36 samples of raw and pasteurized milk from Aligarh city. The raw milk samples had a mean total viable count of 98500×10^6 cfu/100 ml and the count in pasteurized milk was 15000×10^6 cfu/100 ml.

Milk samples from 60 randomly selected households in Botshabelo township, South Africa were collected and analyzed for the presence of microorganisms (Lues *et al.*, 2003). In more than 80 per cent of the samples, the total aerobic mesophilic counts exceeded the national standard of 50,000 cfu/ml. Majority of the households samples had a total mesophilic count in the range $10^6 - 10^7$ cfu/ml with a mean count of 8.6×10^8 cfu/ml $\pm$ 1.1×10^9 cfu/ml.

Sethulakshmi *et al.* (2003) assessed the microbial quality of 84 samples of toned pasteurized milk from Thrissur and reported that the samples had a mean total viable count of 2.82 ± 0.14 $\log_{10}$ cfu/ml.

Aaku *et al.* (2004) assessed bottled commercial pasteurized milk from two processing plants A and B in Gaborone, Botswana for the presence of bacterial pathogens. The total mesophilic count of commercial pasteurized milk from the samples of plant A varied from 1×10^2 to 2×10^4 cfu/ml with a mean count of 7×10^3 cfu/ml and in the samples of plant B, the count varied between 4×10^2 and 2×10^4 with a mean count of 1×10^4 cfu/ml.

Prejit (2005) evaluated the quality of 56 samples of pasteurized milk belonging to four brands A, B, C and D. The mean total viable count obtained from the samples belonging to brands A, B, C and D were 4.88 ± 0.122, 5.91 ± 0.01, 5.56 ± 0.13 and 5.45 ± 0.14 $\log_{10}$ cfu/ml, respectively.

A study was conducted by Prejit *et al.* (2006) to assess the effect of pasteurization on the microbial quality of milk. They evaluated 20 samples of pasteurized milk and reported that the samples had a mean total viable count of 4.76 ± 0.15 $\log_{10}$ cfu/ml.

Asha (2007) evaluated a total of 72 pasteurized milk samples belonging to six brands viz., A, B, C D, E and F retailed in and around Thrissur. The mean total viable count of the samples belonging to the brand A, B, C, D, E and F were 5.88 ± 0.18, 5.76 ± 0.16, 5.92 ± 0.02, 5.94 ± 0.09, 5.62 ± 0.12 and 4.89 ± 0.79 $\log_{10}$ cfu/ml, respectively.

2.1.2 Coliform Count

Vijai and Saraswat (1968) compared the bacteriological quality of market milk in Udaipur city. The average coliform count of 65 pasteurized milk samples collected from milk supply scheme was 41/ml.

Kaloianov and Gogov (1977) analyzed 1404 samples of pasteurized milk and found that pasteurization killed 100 per cent of coliform organism**.**

Singh and Ranganathan (1978) examined 30 samples of pasteurized milk. The coliform counts of pasteurized milk ranged from zero to 4500 per ml.

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

Yadava *et al.* (1983) determined the bacterial flora of milk sold in Ranchi town. They analyzed 12 samples of pasteurized milk during monsoon and 10 samples during winter and reported that the samples of monsoon and winter had an average coliform count of 20.39×10^5 and 13.26×10^5 cfu/ml, respectively.

Arora and Sudarsanam (1986) analyzed the microbiological quality of milk and skim milk powder used as ice cream ingredient in Karnal city. They collected eight samples of pasteurized milk each from the experimental dairy of NDRI, Karnal (source A) and from the market (source B). Four samples from source A (50 per cent) and all samples from source B (100 per cent) were within limits of ISI specifications for pasteurized milk (10 coliforms/ml). They obtained an average MPN coliform count of 22.875 cfu/ml for source A and zero for source B.

Raju and Nambudripad (1987) examined the bacterial quality of 75 samples of pasteurized milk collected from the consumer points of Bangalore and NDRI dairies. The mean coliform count in the pasteurized milk samples was 62 cfu/ml.

Misra and Kuila (1989) reported that the pasteurized milk obtained from dairy plant had a mean coliform count of 12 x 10^1 cfu/ml.

Rai and Dwivedi (1990) analyzed 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.

Siva *et al.* (1993) investigated the microbial quality of 10 samples of pasteurized milk from a students training dairy at Anand. The coliform counts in these samples varied from less than 10 to 80 with an average of 22 ± 8.13 cfu/ml. The coliform counts were positively and significantly correlated with total viable count in pasteurized milk.

Cosentino and Palmas (1997) investigated the microbial contamination in six ewe's milk processing plants in Sardinia, Italy. The mean coliform counts of heat treated milk in these plants were 1.0 ± 0.41, 2.1 ± 028, zero, 1.8 ± 0.14, 1.9 ± 0.32 and 2.6 ± 0.45 $\log_{10}$ cfu/ml, respectively.

Latha and Nanu (1997) assessed the bacteriological quality of pasteurized milk samples collected from five sources S_1, S_2, S_3, S_4 and S_5 and reported that the samples had a mean coliforms count 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.

John (1999) evaluated 100 pasteurized milk samples belonging to five brands viz., A, B, C, D and E. The study revealed that the samples belonging to the brands A, B, C, D and E had a mean coliform counts of 3.96, 4.85, 5.38, 1.24 and 3.02 $\log_{10}$ cfu/ml, respectively.

Gopi *et al.* (2001) evaluated the microbial quality of 12 commercial brands of pasteurized and homogenized milk sold by private vendors in Chennai city and the average coliform counts of these brands varied between 0 to 43.33×10^2 cfu/ml.

Khalilur *et al.* (2002) examined the microbiological quality of nine samples of pasteurized milk from the local markets of Aligarh city and reported that the samples had a mean total coliform count of 2.13×10^3 MPN/100 ml.

Lues *et al.* (2003) analyzed the microbial profile of 60 milk samples from randomly selected households of Botshabelo township, South Africa. Eighty per cent of the samples had coliform counts exceeding the national standard of 10 cfu/ml. The counts ranged between 10^2 to 10^9 cfu/ml with an average count of 6.7×10^7 cfu/ml ± 1.7×10^8 cfu/ml.

Sethulakshmi *et al.* (2003) analyzed the bacteriological quality of 84 samples of toned pasteurized milk obtained from Thrissur and reported that the samples had a mean coliform count of 1.8 ± 0.16 $\log_{10}$ cfu/ml.

Prejit (2005) evaluated the mean coliform counts of 56 samples of pasteurized milk belonging to four brands A, B, C and D. Fourteen samples were collected from each brand and the mean coliform count of the samples belonging to the brand A, B, C, and D were 1.15 ± 0.28, 2.30 ± 0.38, 3.08 ± 0.29 and 2.07 ± 0.29 $\log_{10}$ cfu/ml, respectively.

Prejit *et al.* (2006) conducted a study to evaluate the effect of pasteurization on the microbial quality of milk. During the investigation they examined 20 samples of pasteurized milk and recorded that the samples had a mean coliform count of 0.98 ± 0.36 $\log_{10}$ cfu/ml.

Asha (2007) examined the bacterial quality of six brands of pasteurized milk viz., A, B, C, D, E and F retailed in and around Thrissur and reported that the mean coliform counts of the samples belonging to the brands were 2.40 ± 0.14, 1.70 ± 0.54, 2.17 ± 0.45, 2.05 ± 0.09, 1.81 ± 0.41 and 1.19 ± 0.42 $\log_{10}$ cfu/ml, respectively.

2.1.3 *Escherichia coli* Count

Cosentino and Palmas (1997) assessed the bacterial flora of milk from six ewe's milk processing plants in Sardinia, Italy. They reported that the samples belonging to one of the plants had a mean *Escherichia coli* count of 1.1 ± 0.32 $\log_{10}$ cfu/ml and the samples from other plants were free from the organisms.

Latha and Nanu (1997) studied the quality of pasteurized milk obtained from five sources viz., S_1, S_2, S_3, S_4 and S_5 and reported that the mean *Escherichia coli* counts of the samples belonging to the sources were 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.

John (1999) evaluated 100 pasteurized milk samples from five commercial brands available in and around Thrissur. The mean *Escherichia coli* counts from these five brands were 2.17, 3.39, 0.87, zero and 2.68 $\log_{10}$ cfu/ml, respectively.

Khalilur *et al.* (2002) assessed the microbiological quality of nine samples of pasteurized milk and reported that the samples had a mean faecal coliform count of 1.5×10^3 MPN/100 ml.

Gran *et al.* (2003) enumerated the presence of pathogenic bacteria in 27 samples of cultured pasteurized milk (CPM) from three small scale dairies in Zimbabwe and reported that the mean level of *Escherichia coli* in CPM was 7.1 $\log_{10}$ cfu/ml.

Lues *et al.* (2003) analyzed 60 milk samples from randomly selected households of Botshabelo township, South Africa. The mean *Escherichia coli* count of the samples was 1.2×10^4 cfu/ml ± 3.1×10^4 cfu/ml.

Sethulakshmi *et al.* (2003) evaluated 84 samples of toned pasteurized milk from Thrissur and recorded that the samples had a mean *Escherichia coli* count of 0.19 ± 0.12 $\log_{10}$ cfu/ml.

Prejit (2005) evaluated the bacterial quality of 56 pasteurized milk samples belonging to four commercial brands viz., A, B, C and D retailed in and around Thrissur. The samples belonging to the brands A, B, C and D had mean *Escherichia coli* count of 0.25 ± 0.17, 1.62 ± 0.28, 2.35 ± 0.38 and 0.45 ± 0.25 $\log_{10}$ cfu/ml, respectively.

Prejit *et al.* (2006) evaluated 20 samples of pasteurized milk from a dairy processing plant and reported that the samples had a mean *Escherichia coli* count of 0.31 ± 0.21 $\log_{10}$ cfu/ml.

Asha (2007) assessed the microbial quality of 72 pasteurized milk samples belonging to the brands A, B, C, D, E and F and reported that the samples had mean *Escherichia coli* count of 0.00 ± 0.58, 0.60 ± 0.60, 3.44 ± 0.72, 0.59 ± 0.59, 0.65 ± 0.65 and 0.59 ± 0.59 $\log_{10}$ cfu/ml, respectively.

2.1.4 Psychrotrophic Count

Arora and Sudarsanam (1986) analyzed the microbiological quality of milk and skim milk powder used as ice cream ingredient in Karnal city. They collected eight samples of pasteurized milk each from the experimental dairy of NDRI, Karnal

(source A) and from the market (source B). The average psychrotrophic count from source A was 143.75/ml and no psychrotrophs were obtained from the samples of source B.

Griffiths and Phillips (1988) studied the bacterial growth in 46 samples of freshly pasteurized milk collected from 18 dairies from Paisley, Scotland. The geometric mean of the initial psychrotrophic count of these samples determined by most probable number (MPN) and was 1.4/ml. 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 25 samples of pasteurized milk from dairy plant as 5.56×10^3 cfu/ml.

Sutherland *et al.* (1993) compared the mean psychrotrophic count of 72 samples of pasteurized whole milk obtained from the supermarkets in Edinburgh city. They reported that the samples had a mean count of 2.95 ± 0.13 $\log_{10}$ cfu/ml by pour plate technique and 3.03 ± 0.12 $\log_{10}$ cfu/ml by spiral plate method.

Beuvier *et al.* (1997) studied the influence of microflora in raw, pasteurized and micro filtered milk on the ripening and quality of Swiss-type cheese. They reported that pasteurized milk used for the production of cheese had a mean psychrotrophic count of less than 10 cfu/ml.

Cosentino and Palmas (1997) investigated the hygienic conditions and microbial contamination in six ewe's milk processing plants in Sardinia, Italy and reported that the mean psychrotrophic count of heat treated milk from the plants 1, 2,

3, 4, 5 and 6 were 1.5 ± 0.63, 1.2 ± 0.14, 1.1 ± 0.32, 2.0 ± 0.27, 1.8 ± 0.48 and 2.2 ± 0.94 $\log_{10}$ cfu/ml, respectively.

John (1999) evaluated 100 pasteurized milk samples belonging to five brands A, B, C, D and E retailed in and around Thrissur. The mean psychrotrophic count of the samples from brands A, B, C, D and E were 5.64, 6.61, 6.95, 3.57 and 4.83 $\log_{10}$ cfu/ml, respectively.

Gopi *et al.* (2001) evaluated the microbial quality of 12 commercial brands of pasteurized and homogenized milk sold by private vendors in Chennai city and the average psychrotrophic count of these brands varied between 12.50 $\times 10^4$ cfu/ml to 99.33 $\times 10^4$ cfu/ml.

Silva *et al.* (2001) evaluated the microbiological quality of 90 samples of pasteurized milk collected from Rio de Janeiro, Brazil. They evaluated 15 samples each of grade B milk belonging to three commercial brands and 15 samples each of grade C milk obtained from three commercial brands and reported that the psychrotrophic count varied between zero to ten per ml in 73.3 per cent, 40 per cent and 46.6 per cent samples of the three brands of grade B milk and also in 73.3 per cent, 33.3 per cent and zero per cent of the three brands of grade C milk.

Aaku *et al.* (2004) assessed the microbial organisms present in bottled commercial pasteurized milk from two processing plants A and B in Gaborone, Botswana. The mean psychrophilic count of milk from source A was 2$\times 10^2$ cfu/ml and that from source B was 6 $\times 10^5$ cfu/ml.

Lopamudra and Kuila (2005) collected 51 pasteurized milk samples from different organized dairies in and around Kolkatha and analyzed for enumerating the

population of different groups of spore forming bacteria. They reported that the average count of psychrotrophic spore forming bacteria in these samples was 88 cfu/ml.

Prejit (2005) enumerated the quality of 56 samples of pasteurized milk belonging to four brands A, B, C and D and reported that the mean psychrotrophic count of the samples were 4.25 ± 0.22, 5.25 ± 0.36, 5.68 ± 0.25 and 4.62 ± 0.21 $\log_{10}$ cfu/ml respectively.

Prejit *et al.* (2006) conducted a study to assess the effect of pasteurization on the microbial quality of milk. They examined 20 samples and recorded that the samples had a mean psychrotrophic count of 4.16 ± 0.09 $\log_{10}$ cfu/ml.

Asha (2007) evaluated the mean psychrotrophic count of 72 pasteurized milk samples belonging to six brands viz., A, B, C, D, E and F and reported that the samples belonging to these brands had a mean count of 4.89 ± 0.09, 4.42 ± 0.08, 4.77 ± 0.13, 5.09 ± 0.16, 4.58 ± 0.07 and 4.44 ± 0.08 $\log_{10}$ cfu/ml, respectively.

2.1.5 Faecal Streptococcal Count

Yadava et al. (1983) investigated the bacterial flora of 22 pasteurized milk samples obtained from the booths of the town milk supply in Ranchi town. Of these, 12 samples were collected during monsoon and 10 samples in winter and reported that the average faecal streptococcal in the samples obtained during monsoon and winter was 14.40×10^5 and 4.34×10^5 cfu/ml, respectively.

Gill *et al.* (1994) reported that five out of 36 cow milk samples and seven out of 40 buffalo milk samples revealed the presence of *Streptococcus* spp.

Latha and Nanu (1997) evaluated pasteurized milk belonging to five sources *viz*. S_1, S_2, S_3, S_4 and S_5 and recorded that the mean faecal streptococcal counts were 2.532 ± 0.096, 1.923 ± 0.116, 1.297 ± 0.131, 1.295 ± 0.203 and 0.820 ± 0.159 $\log_{10}$ cfu/ml, respectively.

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

Prejit (2005) evaluated 56 samples of pasteurized milk belonging to four brands viz., A, B, C and D and reported that the mean faecal streptococcal counts were 0.98 ± 0.22, 1.52 ± 0.22, 1.51 ± 0.30 and 0.78 ± 0.22 $\log_{10}$ cfu/ml, respectively.

Prejit *et al.* (2006) evaluated 20 samples of pasteurized milk obtained from a dairy processing plant. They reported that the samples had a mean faecal streptococcal count of 1.06 ± 0.18 $\log_{10}$ cfu/ml.

Asha (2007) examined the microbial quality of pasteurized milk samples belonging to six retail brands and reported the mean faecal streptococcal counts were 2.45 ± 0.14, 2.05 ± 0.27, 2.10 ± 0.10, 2.87 ± 0.24, 2.03 ± 0.19 and 2.34 ± 0.13 $\log_{10}$ cfu/ml, respectively.

2.1.6 Yeast and mould count

Arora and Sudarsanam (1986) analyzed the microbiological quality of milk used as ice cream ingredient in Karnal city. They collected eight samples of pasteurized milk each from the experimental dairy of NDRI, Karnal (source A) and from the market (source B) and reported that the yeast and mould count of the

samples from source A ranged between 2 and 156 $\times 10^2$ /ml with an average count of 32.5×10^2 per ml.

Cosentino and Palmas (1997) studied the microbial quality of milk from six ewe's milk processing plants in Sardinia, Italy and reported that the yeast was not detected in the heat treated milk from these plants.

Lues *et al.* (2003) investigated the microbial profile of 60 milk samples from the households of Botshabelo township, South Africa. They reported that the samples had an average yeast count of 2.3×10^6 cfu/ml ± 9.7×10^6 cfu/ml and the average mould count was 1.1×10^3 cfu/ml ± 3.8×10^3 cfu/ml.

Prejit (2005) evaluated the quality of four brands of pasteurized milk retailed in and around Thrissur. A total of 56 samples were analyzed and reported that the mean yeast and mould count were 1.21 ± 0.22, 0.65 ± 0.22, 0.88 ± 0.25 and 0.62 ± 0.21 $\log_{10}$ cfu/ml in brands A, B, C and D, respectively.

A study was conducted by Prejit *et al.* (2006) to assess the effect of pasteurization on the microbial quality of milk. During the investigation 20 samples of pasteurized milk were obtained from a dairy processing plant and reported that the samples had a mean yeast and mould count of 0.62 ± 0.13 $\log_{10}$ cfu/ml.

2.2 ISOLATION AND IDENTIFICATION OF BACTERIA FROM MILK

2.2.1 Escherichia coli

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

Yadava *et al.* (1985) assessed the bacterial flora present in 105 milk samples of which 42 was from organized dairy plants, 41 from local vendors and 22 from milk supply schemes. They reported that *Escherichia coli* was isolated from 78 per cent of the samples.

Yadava *et al.* (1987) reported the isolation of 21 *Escherichia coli* isolates from 22 pasteurized milk samples collected from centralized milk supply organizations in Ranchi town.

Gill *et al.* (1994) analyzed the bacteriological quality of 36 cow milk and 40 buffalo milk samples and reported the isolation of *Escherichia coli* from five cow milk and four buffalo milk samples.

Sharma *et al.* (1995) examined 60 samples of milk collected from the local market of Ludhiana and tested for the presence of *Escherichia coli* and reported that only five (8.33 per cent) of the samples contained the organism. The serotypes obtained were O5, O7, O61 and O106.

Cosentino and Palmas (1997) investigated the microbial quality of milk from six ewe's milk processing plants viz., 1, 2, 3, 4, 5 and 6 in Sardinia, Italy. They reported that *Escherichia coli* was isolated only from the heat treated milk belonging to the sixth plant.

Lindberg *et al.* (1998) investigated the presence of *Enterobacteriaceae* in pasteurized milk. They sampled 430 milk packets and reported that *Enterobacteriaceae* were present in high numbers in six per cent of the consumer packets after storage at 7ºC, for 11- 14 days.

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

Silva *et al.* (2001) evaluated 15 samples each of pasteurized milk type B obtained from three different commercial brands and also 15 samples each of pasteurized milk type C received from three different commercial brands purchased from supermarkets and bakeries in Rio de Janeiro, Brazil. The study revealed that 41.1 per cent of the samples had *Escherichia coli.*

Gran *et al.* (2003) enumerated the presence of pathogenic bacteria in 27 samples of cultured pasteurized milk (CPM) from three small scale dairies in Zimbabwe. Enterotoxigenic *Escherichia coli* (ETEC) producing heat stable enterotoxin (ST) was found in 16 of these samples.

John *et al.* (2003) studied the bacteriological quality of 84 samples of pasteurized milk retailed in and around Thrissur recorded the isolation of *Escherichia coli* from six (7.1 per cent) samples.

Aaku *et al.* (2004) analyzed microbiological quality of 86 samples of bottled commercial pasteurized milk from two processing plants, Gaborone, Botswana. The study revealed that none of the samples of pasteurized milk had *Escherichia coli*.

Prejit (2005) evaluated 56 samples of pasteurized milk belonging to four brands viz., A, B, C and D and reported the isolation of *Escherichia coli* from 27 (48.21 per cent) samples.

Asha (2007) examined 72 pasteurized milk samples belonging to six brands viz., A, B, C, D, E and F and reported the isolation of *Escherichia coli* from 15 (20.83 per cent) samples.

2.2.2 Pseudomonas spp

Yadava *et. al* (1985) investigated the bacterial quality of 105 milk samples. Forty two samples were collected from an organized dairy farm, 41 samples from local vendors and 22 from milk supply scheme in Ranchi town. *Pseudomonas aeroginosa* was isolated from five (4.7 per cent) of these samples.

Griffiths and Phillips (1988) evaluated the pasteurized milk samples stored at varying temperature from 1.8 to 21.6 º C and reported the isolation of *Pseudomonas* spp in the samples stored at the above temperatures. The organism was isolated from 92.1 per cent of the pasteurized milk samples stored at 4.4 º C.

A study was conducted by Grover and Srinivasan (1988) at NDRI, Karnal and reported that *Pseudomonas aeroginosa* was isolated from 90.48 per cent of raw milk and five per cent of pasteurized milk samples.

Sutherland *et al.* (1993) assessed the microbial profile of 72 samples of pasteurized whole milk obtained from the supermarkets in Edinburgh. They reported the isolation of 76 psychrotrophic Gram-negative rods and the isolates consisted of *Pseudomonas* spp (50), *Acinetobacter* spp (10), *Achromobacter* spp (10) and *Flavobacteria* spp (6).

Ternstrom *et al.* (1993) studied the spoilage flora of 54 pasteurized milk samples stored at 5°C and 70 samples stored at 7ºC with special reference to *Pseudomonas*. During the investigation *Pseudomonas fluorescens*, *Pseudomonas*

fragi, Pseudomonas lundensis and *Pseudomonas putida* were isolated from samples stored at 5°C and *Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas lundensis and Pseudomonas stutzeri* were isolated from the samples stored at 7°C.

Gill *et al.* (1994) examined 36 cow milk and 40 buffalo milk samples obtained from Ludhiana city for the presence of *Pseudomonas* spp. The organism was isolated from six cow milk and five buffalo milk samples.

Eneroth *et al.* (1998) traced the critical contamination sites in the production line of pasteurized milk in three dairy plants in Sweden or Norway. They collected 87 consumer packages from these three dairies and recorded the isolation of *Pseudomonas* spp from 40 per cent of the total samples.

Dogan and Boor (2003) obtained pasteurized milk samples from four dairy processing plants A, B, C and D in New York City. During the study they isolated 28, 60, 57 and 56 *Pseudomonas* species from the samples collected from dairy plants A, B, C and D, respectively. The *Pseudomonas* strains mostly belonged to the species *Pseudomonas aeruginosa, Pseudomonas putida* and *Pseudomonas flourescens.*

Aaku *et al.* (2004) analyzed 86 samples of bottled commercial pasteurized milk from two processing plants A and B in Gaborone, Botswana for the presence of bacterial organisms and reported the isolation of six *Pseudomonas cichorii* (14 per cent), six *Pseudomonas fragi* (14 per cent) and seven other *Pseudomonas* spp (16 per cent).

Fromm and Boor (2004) collected pasteurized fluid milk from three commercial dairy plants in New York State and were evaluated for microbial,

chemical and sensory attributes throughout the shelf life. The most predominant microorganisms consisted of gram negative rods which made up of 87 per cent of the processed milk microbial count. The gram negative bacteria consisted of *Pseudomonas* (3 per cent) and *Acinetobacter* (1 per cent).

Kumaresan and Villi (2008) examined 80 samples of pasteurized milk samples and isolated 33 psychrotrophic organisms. Of the isolates *Pseudomonas flourescens, Pseudomonas fragi* and *Pseudomonas aeruginosa* consisted of 42.42 per cent, 24.24 per cent and 12.12 per cent, respectively. The study revealed that about 78.78 per cent of the psychrotrophs belonged to *Psuedomonas* species.

2.2.3 Staphylococcus aureus

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

Yadava *et. al* (1985) assessed the bacterial flora present in 22 samples of pasteurized milk from town milk supply in Ranchi town. They isolated *Staphylococcus aureus* from three (13.63 per cent) of the samples. They also reported that the isolation of the organism was higher from sources with low bacterial counts.

Arora and Sudarsanam (1986) analyzed the microbiological quality of eight samples of pasteurized milk from the experimental dairy of NDRI, Karnal and they obtained an average Staphylococcal count of 0.375 per ml.

Gill *et al.* (1994) reported that six out of 36 cow milk and eight out of 40 buffalo milk samples were positive for *Staphylococcus aureus*.

Cosentino and Palmas (1997) analyzed the quality of ewe's milk from six processing plants in Sardinia, Italy. The heat treated milk samples from the plants were found free from *Staphylococcus aureus*.

John (1999) studied the bacteriological quality of 100 samples of pasteurized milk belonging to five commercial brands retailed in and around Thrissur. *Staphylococcus aureus* was not detected from any of the samples examined.

Khalilur *et al.* (2002) studied nine samples of pasteurized milk from Aligarh city and reported that none of the sample had *Staphylococcus*.

Gran *et al.* (2003) examined the presence of pathogenic bacteria in 27 samples of cultured pasteurized milk (CPM) from three small scale dairies in Zimbabwe and reported that 15 of the samples had *Staphylococcus aureus* and the mean count was 7.3 $\log_{10}$ cfu/ml.

John *et al.* (2003) evaluated 84 samples of pasteurized milk collected from Thrissur and reported that only one of the samples revealed the presence of *Staphylococcus aureus*.

Aaku *et al.* (2004) analyzed the microbiological quality of 86 samples of bottled commercial pasteurized milk from two processing plants A and B in Gaborone, Botswana. The study revealed that 10 (11.6 per cent) of the pasteurized milk samples had *Staphylococcus* spp.

Prejit (2005) studied the microbial quality of 56 pasteurized milk samples belonging to four brands viz., A, B, C and D and reported that S*taphylococcus aureus* was obtained from seven (12.5 per cent) out of total 56 samples.

2.2.4 Bacillus cereus

Ethiraj *et al.* (1979) evaluated 44 pooled milk samples consisting of 34 samples from four organized dairy farms and 10 pasteurized milk samples from a commercial dairy plant and reported that *Bacillus cereus* was present in 8.70 per cent of the samples.

Rajarathinam *et al.* (1986) examined a total of 180 raw and pasteurized milk samples obtained from farms and retail markets in and around Bangalore city and reported the isolation of 12 strains of *Bacillus cereus*.

Wong *et al.* (1988) investigated various dairy products to determine the incidence of *Bacillus cereus* in Taiwan and observed that two per cent of the pasteurized milk samples were contaminated with the organism.

Sutherland *et al.* (1993) examined 72 samples of pasteurized whole milk obtained from the supermarkets in Edinburgh and isolated 53 strains of *Bacillus* spp.

Gill *et al.* (1994) examined 36 cow milk samples and 40 buffalo milk samples from Ludhiana city and reported the isolation of one and four isolates of *Bacillus cereus* from cow milk and buffalo milk, respectively.

Cosentino *et al.* (1997) analyzed 90 pasteurized milk samples from the supermarkets of Sardinia, Italy and reported the isolation of *Bacillus cereus* from nine samples.

Giffel *et al.* (1997) collected 334 samples of pasteurized low fat milk from the household refrigerators in Netherlands and reported the isolation of *Bacillus cereus* without pre-incubation from 133 samples (40 per cent).

Larsen and Jorgensen (1997) examined 458 samples of pasteurized full fat milk, pasteurized low fat milk and pasteurized double cream from three Danish dairies A, B and C over a period of one year. *Bacillus cereus* was isolated from 257 (56 per cent) of the 458 samples.

Zhou *et al.* (2008) investigated the microbial quality of 54 samples of pasteurized full fat milk packaged in cartons during spring and autumn from the supermarkets in Wuhan, China. They reported that the occurrences of *Bacillus cereus* were 71.4 and 33.3 per cent during spring and autumn, respectively.

2.3 BACTERIAL STANDARDS OF MILK

In India, the Bureau of Indian Standards (BIS, 1992) prescribes the bacterial count limit for raw and pasteurized milk.

2.3.1 Pasteurized Milk

The criteria prescribed by Bureau of Indian Standards (BIS, 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 DETECTION OF ADULTERANTS AND PRESERVATIVES IN MILK

Garg and Mandokhot (1997) examined 80 milk samples and reported that 41 samples were adulterated with carbonates/bicarbonates.

Rao *et al.* (2002) analyzed chemical quality of 20 samples each of full cream milk obtained from three sources viz., Andra Pradesh Dairy Development Cooperative Federation (APDDCF), private dairies and local vendors. The study revealed that none of the samples from APDDCF contained any added adulterant, neutralizer or preservative. The samples obtained from private dairies had water and bicarbonate in 30 and 40 per cent, respectively, but sugar, formalin and hydrogen peroxide were absent in the samples. Of the samples from local venders 60, 95 and 10 per cent samples were positive for added bicarbonates, water and sugar, respectively, and were free from formalin and hydrogen peroxide.

Saxena and Agrawal (2004) assessed quality of 81 milk samples collected from three sources, viz., Government dairy (8), private dairies (37) and vendors (36). During the investigation sugar, salt, neutralizer and formaldehyde was found in 51.35, 32.40, 62.10 and 35.10 per cent of samples belonging to private dairies and 33.30, 8.30, 56.60 and 27.80 per cent samples belonging to vendors. All the samples from Government dairy were free from any kind of adulteration. None of the samples from the three sources showed the presence of starch.

2.5 MISCELLANEOUS

Walker and Harmon (1966) assessed the thermal resistance of four strains of coagulase positive *Staphylococcus aureus* in phosphate buffer, whole milk, skim milk and cheddar cheese whey and reported that the heat resistance of the organism

was more in skim milk and cheddar cheese whey than in phosphate buffer and whole milk.

Kulshrestha (1990) examined 169 samples of milk and milk products obtained from Bareilly and reported the isolation of *Escherichia coli* belonging to18 serotypes including O4. Majority of the isolates possessed multiple drug resistance.

De Buyser *et al.* (2001) estimated the proportion of food borne diseases due to milk and milk products in France and in other countries. The French surveillance system revealed that during 1992-1997, 69 documented outbreaks was occurred from milk and its products. The food vehicle consisted of milk (10 per cent), cheese (87 per cent) and others (3 per cent). The *Staphylococcus aureus* was responsible for 85.5 per cent of the outbreaks.

Burdova *et al.* (2002) studied the influence of storage temperature on the shelf life of the pasteurized milk and the enzymatic activities of psychrotrophic bacteria in whole and skimmed pasteurized milk. The study revealed that the storage temperature of 10° C reduced the shelf life of pasteurized milk to one third in comparison with storage at 4° C. The study also revealed that the lipolytic and proteolytic enzyme activity of psychrotrophic organisms increased by storage at 10° for two to three days.

Ikeda *et al.* (2005) analyzed 11 batches of skim milk powder which were the raw material for the massive food poisoning outbreak in Osaka (Japan) in 2000. The study revealed that the outbreak was due to multiple staphylococcal enterotoxins mainly A and H.

McAuley *et al.* (2005) determined the heat resistance of twenty eight isolates of *Enterococci durans* and *Enterococci hirae* in the laboratory pasteurized milk in

Australia. It was found that pasteurization temperature in excess of 78° C would be required to inactivate these bacteria by more than one $\log_{10}$ cfu/ml under 30 seconds.

Saito *et al.* (2005) investigated the cause of food borne outbreak in 103 individuals in Miyagi prefecture (Japan) in August 2004. They examined the food samples and stool samples and reported that the outbreak was due to *Escherichia coli* O115:H19 strain.

Dooley and Roberts (2006) reviewed the microorganisms associated with food including the pathogens and spoilage organisms. They opined that most of the *Escherichia coli* strains are harmless but some strains were responsible for diarrhea and strains bearing virulence properties had emerged as a serious public health hazard.

Elmagli and El Zubeir (2006) conducted an investigation on the effects of storage condition on the hygienic quality and shelf life of pasteurized milk and about the possible sources and causes of contamination of milk in Khartoum state, Sudan. They examined a total of 60 pasteurized milk samples and reported the isolation of *Escherichia coli* in 80 per cent of the bottled pasteurized milk.

Majhenic (2006) reviewed the technological and therapeutic significance of enterococci (faecal streptococci), their positive and negative effects, pathogenic nature and antibiotic resistance. He opined that faecal streptococci were the natural inhabitants in soil, food, water and gastro intestinal tract of humans and animals and faecal contamination was the important route for spreading.

Materials and methods

3. MATERIALS AND METHODS

In the present study a total of 200 pasteurized and packaged milk samples were collected which consisted of 100 samples each from Thrissur and Palakkad districts. The samples from five different brands were selected from each district. During the investigation 20 samples each were collected from a brand and each consisted of 500 ml of pasteurized milk. All samples collected were brought to the laboratory in a thermocool container and were examined on the day of collection. Only two samples were collected from a brand on the day of examination. All samples collected were tested for its microbial quality, isolation of bacterial pathogens and the presence of preservatives and adulterants.

3.1 MICROBIAL QUALITY OF MILK

In order to assess the overall quality of the retailed pasteurized milk in the market and to identify the health hazards on the consumers, different brands of pasteurized milk from the retail shops were collected and subjected to microbial analyses and detection of bacterial pathogens.

3.1.1 Collection of Pasteurized Retail Milk

A total of 100 pasteurized and packaged milk samples were obtained from five different brands retailed in and around Thrissur and equal number of the samples were collected from another five different brands of milk marketed in and around Palakkad. From each brand 20 samples were collected. Each sample consisted of 500 ml milk packaged in low-density polyethylene sachets. Only two samples were collected from a brand at a time.

3.1.2 Processing of Milk Samples

The samples were brought to laboratory in thermocool containers. The contents of the packs were homogenized by manual shaking. The milk sachets were cleaned with a wet cotton ball and then disinfected with 70 per cent ethanol. The sachets were punctured with a sterile scissor. In order to estimate the

microbial load per milliliter of milk, 25 ml was transferred from each sachet 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^{-6} 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 Petri dishes 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). The plates were incubated at 37°C for 24 h. After the incubation period, colonies with a greenish black metallic sheen on deflected light were counted as *Escherichia coli*. The number of organism per ml of sample were estimated as described in coliform count and expressed as $\log_{10}$ cfu/ml.

3.1.3.4 Psychrotrophic Count

Psychrotrophic count of each sample was assessed by pour plate technique as suggested by Cousin *et al.* (2001). The procedure followed was similar to that of 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 Streptococcal Count

The standard procedure prescribed by Nordic Committee on Food Analysis (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 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 Cousin (2001) was followed for estimation of Yeast and Mould Count per ml of milk sample. Potato dextrose agar (Hi-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 incubation the colonies in duplicate plates were counted with the help of a colony counter and the mean count was multiplied by the dilution factor and expressed as $\log_{10}$ cfu/ml.

3.1.4 Isolation and Identification of Bacteria

All the samples of retail milk were subjected for the isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas.*

3.1.4.1 Escherichia coli

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

Congo red Binding Assay of *Escherichia coli*

Congo red binding assay of the *Escherichia coli* isolates were carried out by the method given by Rajil *et al.* (2003). Tryptone Soya Agar supplemented with 0.03 per cent congo red dye (Nesslers) and 0.15 per cent bile salts (Loba Chemie) was used for assay. *Escherichia coli* isolates were cultured on duplicate plates of the congo red medium and incubated at 37°C for 24 h. After incubation, the cultures were left at room temperature for 48 h to facilitate annotation of results. Invasive *Escherichia coli* were identified by their ability to take up congo red dye and production of characteristic brick red colonies.

3.1.4.2 Staphylococcus aureus

For the isolation of *Staphylococcus aureus*, a loopful of the sample was inoculated onto Baird-Parker (BP) agar medium (Hi-media) and was incubated at 37°C for 48 h. (Lancette and Bennett, 2001). At the end of incubation, colonies showing characteristics appearance (circular, smooth, convex, moist, 2.3 mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently with outer clear zone) on BP agar medium were selected and transferred to nutrient agar slants

Flow chart 1. Isolation and identification of *Escherichia coli*

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

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 2. The isolates were identified based on the cultural, morphological and biochemical characteristics.

3.1.4.3 Pseudomonas

For the isolation of *Pseudomonas*, a loopful of the sample was streaked on to duplicate plates of *Pseudomonas* Agar Base (Hi-media) supplemented with Cetrinix (FD 029, Hi-media) and plates were incubated at 30^{o}C for 24 h (Cousin *et al*., 2001). At the end of incubation, pigmented or non-pigmented smooth circular colonies were transferred to nutrient agar slants and incubated at 30^{o}C overnight and were stored at refrigeration temperature. The isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flowchart 3.

3.1.4.4 Bacillus cereus

For the isolation of *Bacillus cereus*, a loopful of milk sample was inoculated into duplicate plates of *Bacillus cereus* Agar Base (Hi-media) supplemented with Polymyxin B and egg yolk and incubated at 30 to 32 ^{o}C for 24 h (Bennett and Belay, 2001). At the end of incubation period, peacock blue colonies surrounded by an egg yolk precipitate of similar colour were transferred to nutrient agar slants and incubated at 30^{o}C overnight. The isolates were subjected to further characterization and identified by the cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flow chart 4.

For the isolation of aerobic spore formers, the samples were tested using standard plate count agar after heat treatment of samples at 80^{o}C for 10 minutes, followed by immediate cooling (Pillai *et al*., 1993). The plates were incubated at

Flow chart 2. Isolation and identification of *Staphylococcus aureus*

Milk sample	Characteristics/Reactions
↓	
Inoculated on to BP agar	
↓	
Suspected colonies On to Nutrient agar slant	Gray black to jet black, frequently with light coloured margin, surrounded by opaque zone
↓	
Gram's staining reaction and cell Morphology	Gram positive cocci in singles, pairs, cluster or bunch of grapes appearance
↓	
Motility test	-
↓	
Growth aerobically	+
↓	
Growth anaerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
VP	+
↓	
Arginine hydrolysis	+
↓	
Phosphatase	+
↓	
Gelatin liquefaction	+
↓	
Urease	+
↓	
Coagulase test	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Mannitol	
Aerobic	+
Anaerobic	+

Flow chart 4. Isolation and identification of *Pseudomonas*

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

Flow chart 4. Isolation and identification of *Bacillus cereus*

Milk sample	Characteristics/Reactions
↓	
A loopful to B cereus agar base + polymyxin B supplement + egg yolk emulsion	
↓	
Select suspected colonies	Peacock blue colonies surrounded by an egg yolk precipitate of similar colour
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram positive rods
↓	
Motility	+
↓	
Growth aerobic	+
↓	
Growth anaerobic	-
↓	
Catalase	+
↓	
OF	F
↓	
VP	+
↓	
Casein hydrolysis	+
↓	
Citrate utilisation	+
↓	
Starch hydrolysis	+
↓	
Urease	D
↓	
Nitrate	+
Carbohydrate utilization	
Glucose	-
Arabinose	-
Xylose	-
Mannitol	-

37^{o}C for 24 hours. Representative colonies that had developed on the plates after incubation were selected and inoculated on nutrient agar. The isolates were identified by the cultural, morphological and biochemical reactions described by Barrow and Feltham (1993).

3.1.5 Characterization and identification of isolates

The suspected colonies selected as *Escherichia coli, Staphylococcus aureus, Pseudomonas* and *Bacillus cereus* 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. Evolution of effervescence with in a few seconds indicates a positive reaction.

b) Tube test

One millilitre 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.

2. 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.

3. Motility test

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

4. Oxidase test

A filter paper strip is moistened with a few drops of an aqueous solution of one 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.

5. Oxidation – Fermentation test

Each isolate was inoculated into duplicate tubes of Hugh and Liefson's media by stabbing with a straight wire. One of the tubes was sealed with a layer of melted soft paraffin to a depth of about one cm above the medium. The tubes were incubated at 37°C for up to 14 days. A change in colour of the medium

from green to yellow in the open tubes alone is taken as oxidation whereas a change in colour from green to yellow in both the tubes is regarded as fermentation. Absence of colour change in both tubes indicates no action on carbohydrates.

Secondary Tests

1. Aesculin hydrolysis

The organism was inoculated into aesculin broth and was incubated at 37°C and examined daily for five days. Blackening of the broth due to hydrolysis of aesculin indicates a positive reaction.

2. Arginine hydrolysis

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

3. Carbohydrate utilization test

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

4. Citrate utilisation test

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

5. Coagulase test

a) Slide test

A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. Macroscopic clumping with in few seconds indicates a positive result and delayed clumping is considered as a negative reaction.

b) Tube test

Mixed 0.5 ml undiluted rabbit plasma with an equal volume of an 18 to 24 hour 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 tube is taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a positive reaction, the medium first turns yellow and then becomes purple and the control tubes remain yellow.

7. Eijkman test

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

8. Gelatin hydrolysis/liquefactions

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

9. Hippurate hydrolysis

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

10. Indole production

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

11. Methyl red (MR) reaction

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

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

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

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

15. Triple sugar iron agar test

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

16. Urease activity

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

and daily for 5 days. Development of a red colour in the medium indicated a positive reaction.

17. Voges-Proskauer reaction

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

3.2 TESTS FOR DETECTION OF PRESERVATIVES AND ADULTERANTS

All pasteurized milk samples were tested to detect the presence of adulterants *viz*., starch and cane sugar and preservatives *viz*., formaldehyde and carbonates as prescribed by Indian standards (1981).

3.2.1 Tests for Preservatives

1. Formaldehyde

To about 10 ml of milk in a wide mouthed test tube add about half the volume of concentrated sulphuric acid pouring the acid carefully down the side of test tube so that it forms a layer at the bottom without mixing with the milk. A violet or blue colour at the junction of the two liquids indicates the presence of formaldehyde.

2. Carbonates

To about 5 ml of milk in a test tube, add 5 ml of alcohol, a few drops of 1 per cent (*w/v*) alcoholic solution of rosalic acid, and mix. Rose red colour shows the presence of carbonates and bicarbonate and a brownish colour shows the absence of carbonates.

3.2.2 Tests for adulterants

1. Cane Sugar

Take 15 ml of milk in a test tube, add 1 ml of concentrated hydrochloric acid and 0.1g of resorcinol powder and mix thoroughly. Place the tube on a boiling water bath for 5 minutes and observe the colour. The development of red colour indicates presence of sugar, whereas, colourless solution, shows the absence of sugar.

2. *Starch*

Take about 3ml of milk sample in a test tube. Boil the milk over a flame. Allow to cool the sample to room temperature. Add one drop of 10 per cent iodine solution. Presence of blue colour indicates presence of starch, whereas colourless solution shows the absence of starch in milk.

3.3 BACTERIAL STANDARDS OF MILK

3.3.1 RAW MILK

The Bureau of Indian Standards (IS, 1977) prescribed the following criteria as a guideline for grading of milk based on total viable count.

Grade	**Total viable count (Lakh/ml)**
Very good	Less than 2 lakh
Good	2-10 lakh
Fair	10-50 lakh
Poor	More than 50 lakh

Coliforms should be absent in 1:100 dilutions of satisfactory grade raw milk.

3.3.2 PASTEURIZED MILK

The bacterial criteria prescribed by the Bureau of Indian Standards (BIS 1992) stipulated that the plate count count of pasteurized milk, at the plant in the

final container, should not exceed 30,000 per ml and the coliform should be absent in 1:10 dilution of pasteurized milk.

3.4 STATISTICAL ANALYSIS

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

Results

4. RESULTS

In the present investigation, the microbial quality of pasteurized milk was evaluated by determining the microbial load of the pasteurized milk marketed in Thrissur and Palakkad districts. Hundred samples of pasteurized milk belonging to five different brands were selected from each district. The samples were also subjected to the isolation and identification of *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Pseudomonas*. The isolates of *Escherichia coli* were subjected to serotyping at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. The samples were also tested to detect the adulterants like starch and cane sugar and preservatives like formaldehyde and carbonates added in the milk.

4.1 MILK FROM THRISSUR DISTRICT

Pasteurized milk from five different commercial brands (A, B, C, D and E) available in and around Thrissur was evaluated by estimating various microbial counts. Twenty samples were analyzed from each brand and were also subjected to isolation and identification of bacteria. The presence of adulterants and preservatives were also examined.

4.1.1 Microbial quality of pasteurized milk

All pasteurized milk samples were tested to determine the microbial quality by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) Psychrotrophic Count (PC) and Yeast and Mould Count (YMC).

4.1.1.1 Total Viable Count

The mean total viable count (TVC) of milk samples belonging to the brand A, B, C, D and E are shown in table 2 as illustrated in fig 1. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean total viable count of the samples of the brands. The samples belonging to brand E had the lowest mean count and the highest mean count was seen in the samples belonging to brand A.

Table 2. Mean Total Viable Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
A	$5.39^{c} \pm 0.04$
B	$5.09^{b} \pm 0.08$
C	$5.22^{bc} \pm 0.06$
D	$5.21^{bc} \pm 0.06$
E	$4.51^{a} \pm 0.08$

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

The mean count of samples of brand A differed highly significantly ($P<0.01$) with the count of samples of brands B and E. Similarly the mean count of samples of brand B differed highly significantly ($P<0.01$) with the mean count of samples of brands A and E and the mean count of samples of brand E differed highly significantly ($P<0.01$) from the mean count of samples of brands A, B, C and D.

Distribution of retail milk samples based on Total Viable Count

The distribution of retail milk based on total viable count is shown in table 3. Overall, 62 per cent samples had count the level of 10^5 cfu/ml and 38 per cent

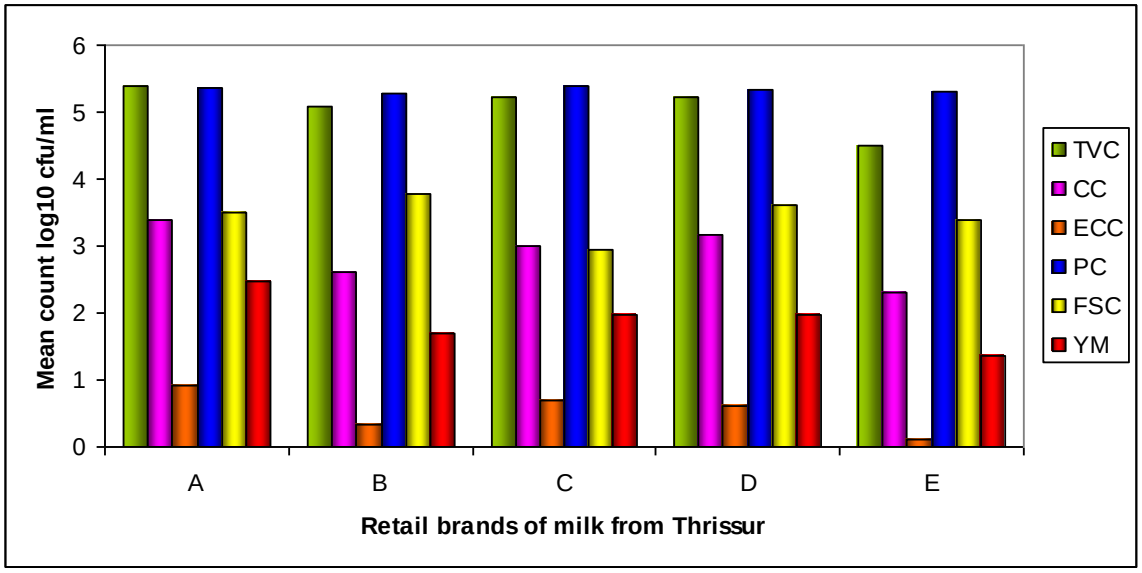

Fig 1 Comparision of the microbiological quality of the retail brands of pasteurized milk of Thrissur district

samples had count at the level of 10^4 cfu/ml. Among the 100 retail samples from Thrissur, highest count was seen in the samples of the brand A, of which cent per cent samples had count at level of 10^5 cfu/ml. Lowest count was seen in the samples of the brand E, of which cent per cent of the samples had count at the level of 10^4 cfu/ml. The counts in 50 per cent samples from brand B and 80 per cent of samples from brands C and D were at the level of 10^5 cfu/ml.

Table 3. Distribution of retail milk samples based on Total Viable Count

Brands	Total viable count (cfu/ml)	
	10^4	10^5
A	-	20 (100)
B	10 (50)	10 (50)
C	4(20)	16 (80)
D	4(20)	16 (80)
E	20 (100)	-
Overall	38 (38)	62 (62)

Figures in the parenthesis indicate per cent; N=20 from each brand

None of the samples from brands A, B, C and D were graded as satisfactory according to the total viable count limits prescribed by BIS (1992). Fifty per cent of samples from brand E were graded as satisfactory.

4.1.1.2 Coliform Count

The mean coliform counts (CC) of milk samples belonging to the brands A, B, C, D and E are shown in table 4 and illustrated in fig 1. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count

of samples from different brands. The highest mean count was observed in the samples from brand A (3.40 ± 0.17 $\log_{10}$ cfu/ml) and the lowest mean count was observed in the samples from brand E (2.30 ± 0.09 $\log_{10}$ cfu/ml). The mean count of samples of

Table 4. Mean Coliform Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
A	$3.40^{c} \pm 0.17$
B	$2.60^{ab} \pm 0.20$
C	$2.99^{bc} \pm 0.20$
D	$3.16^{c} \pm 0.16$
E	$2.30^{a} \pm 0.09$

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

The brands A and D differed highly significantly ($P<0.01$) with the count of samples of brands B and E. Similarly the mean count of samples of brand E differed highly significantly ($P<0.01$) with the count of samples of brands A, C and D.

Distribution of retail milk samples based on Coliform Count

The distribution of retail milk samples based on coliform counts is shown in table 5. Of the 100 samples, 54 and 46 per cent had count at the level of 10^2 cfu/ml and 10^3 cfu/ml, respectively. The highest count at the level of 10^3 cfu/ml was seen in 18 (90 per cent) samples of the brand A. Lowest count was seen in the samples of the brand E of which cent per cent of the samples had count at the level of 10^2 cfu/ml. Seventy per cent of the samples of brand B and 60 per cent of samples of brand C had counts at the level of 10^2 cfu/ml. Seventy per cent of samples of brand D had count at the level of 10^3 cfu/ml. Out of 100 samples, none of the samples were

graded as satisfactory based on the BIS standards (1992) and all the samples were graded as unsatisfactory.

Table 5. Distribution of retail milk samples based on Coliform Count

Brands	Coliform counts (cfu/ml)	
	10^2	10^3
A	2(10)	18(90)
B	14(70)	6(30)
C	12(60)	8(40)
D	6(30)	14(70)
E	20(100)	-
Overall	54 (54)	46 (46)

Figures in the parenthesis indicate per cent; N=20 from each brand

4.1.1.3 Escherichia coli Count

The mean *Escherichia coli* count (ECC) of milk samples belonging to the

Table 6. Mean *Escherichia coli* Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ ml)
A	0.92 ± 0.31
B	0.34 ± 0.17
C	0.69 ± 0.29
D	0.60 ± 0.31
E	0.10 ± 0.10

N=20 from each brand

brands A, B, C, D and E are shown in table 6 and illustrated in fig 1. Analysis of variance test of the data revealed that no significant difference exists between the mean counts of samples of the brands. The lowest mean count was seen in samples belonging to brand E (0.10 ± 0.10 $\log_{10}$ cfu/ml) and the highest count in the samples of the brand A (0.92 ± 0.31 $\log_{10}$ cfu/ml).

Distribution of retail milk samples based on *Escherichia coli* Count

The distribution of retail milk based on *Escherichia coli* count is shown in table 7. The organism was not detected in 68 per cent of the total samples. The count in 20 and 12 per cent of the samples were at the level of 10^1cfu/ml and 10^2 cfu/ml, respectively. The highest count at the level of 10^2 cfu/ml was seen in four (20 per cent) samples belonging to the brand A. The organism was detected from 50 per cent of the samples of brand A but not detected from 12 (60 per cent) samples of brand C and 70 per cent of the samples of brands B and D, respectively.

Table 7. Distribution of retail milk samples based on *Escherichia coli* Count

Brands	*Escherichia coli* count (cfu/ml)		
	ND	10^1	10^2
A	10 (50)	6 (30)	4 (20)
B	14 (70)	6 (30)	-
C	12 (60)	4 (20)	4 (20)
D	14 (70)	2 (10)	4 (20)
E	18 (90)	2 (10)	-
Overall	68 (68)	20 (20)	12 (12)

Figures in the parenthesis indicate per cent; N=20 from each brand; ND: Not Detected

Thirty per cent samples from brands A and B, 20 per cent samples of brands C, and 10 per cent samples of brand D and E had counts at the level of 10^1cfu/ml. None of the samples from brands B and E had counts at the range of 10^2 cfu/ml. The lowest count was seen in the samples belonging to brand E and the organism could not detect in 90 per cent of the samples of the brand. The count in 10 per cent samples of the brand was at the level of 10^1 cfu/ml.

4.1.1.4 Psychrotrophic Count

The mean psychrotrophic counts (PC) of milk samples belonging to the brand A, B, C, D and E are shown in table 8 and illustrated in fig 1. Analysis of variance test of the data revealed that no significant difference between the mean counts of samples of the brands. The samples belonging to brand B had the lowest mean count and the highest count was seen in the samples belonging to brand C.

Table 8. Mean Psychrotrophic Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
A	5.36 ± 0.06
B	5.29 ± 0.05
C	5.39 ± 0.02
D	5.32 ± 0.02
E	5.30 ± 0.01

N=20 from each brand

Distribution of retail milk samples based on Psychrotrophic Count

The distribution of retailed milk samples based on psychrotrophic count is shown in table 9. Overall, 96 per cent of samples had count at the level of 10^5 cfu/ml and only four per cent of the samples had count at the level of 10^4 cfu/ml. Cent per cent of the samples belonging to brands C, D and E had count at the level of 10^5 cfu/ml whereas 90 per cent of the samples belonging to brands A and B had count at the above level.

Table 9. Distribution of retail milk samples based on Psychrotrophic Count

Brands	Psychrotrophic count (cfu/ml)	
	10^4	10^5
A	2 (10)	18 (90)
B	2 (10)	18 (90)
C	-	20 (100)
D	-	20 (100)
E	-	20 (100)
Overall	4 (4)	96 (96)

Figures in the parenthesis indicate per cent; N=20 from each brand

4.1.1.5 Faecal Streptococcal Count

The mean faecal streptococcal count (FSC) of milk samples belonging to the brand A, B, C, D and E are shown in table 10 and illustrated in fig 1. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of samples from different brands. The lowest mean count was seen in samples belonging to brand C and the highest count was seen in the samples of the

brand B. The mean count of samples of brand C had highly significant (P<0.01) difference with the mean count of samples of brands A, B, D and E.

Table 10. Mean Faecal Streptococcal Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
A	$3.51^{b} \pm 0.06$
B	$3.78^{b} \pm 0.16$
C	$2.95^{a} \pm 0.17$
D	$3.61^{b} \pm 0.10$
E	$3.40^{b} \pm 0.14$

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

Distribution of retail milk based on Faecal Streptococcal Count

The distribution of retail milk based on faecal streptococcal count is shown in

Table 11. Distribution of retail milk based on Faecal Streptococcal Count

Brands	Faecal streptococcal count (cfu/ml)		
	10^2	10^3	10^4
A	-	20 (100)	-
B	-	12(60)	8 (40)
C	10 (50)	10 (50)	-
D	-	20 (100)	-
E	4 (20)	16 (80)	-
Overall	14 (14)	78 (78)	8 (8)

Figures in the parenthesis indicate per cent; N=20 from each brand

table 11. Of 100 samples, the count at the level of 10^4, 10^3 and 10^2 cfu/ml was seen in 8, 78 and 14 per cent of the samples, respectively. Cent per cent of samples of brands A and D and 80 per cent samples of brand E had count at the level of 10^3 cfu/ml. The count at that level was also seen in 50 per cent samples of brand C and 60 per cent samples of brand B. The count at the level of 10^4 cfu/ml was seen in 40 per cent samples of the brand B. The count in 50 and 20 per cent samples of brands C and E was at the level of 10^2 cfu/ml.

4.1.1.6 Yeast and Mould Count

The mean Yeast and mould count (YMC) of milk samples belonging to the brands A, B, C, D and E are shown in table 12 and illustrated in fig 1. Analysis of

Table 12. Mean Yeast and Mould Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
A	$2.46^{c} \pm 0.17$
B	$1.69^{ab} \pm 0.12$
C	$1.98^{b} \pm 0.14$
D	$1.98^{b} \pm 0.20$
E	$1.35^{a} \pm 0.08$

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

variance test of the data revealed highly significant ($p<0.01$) difference between the mean count of samples of different brands. The mean count of samples of brand A differed highly significantly ($P<0.01$) with the count of samples of brands B, C, D and E. Similarly the mean count of samples of brand E differed highly significantly ($P<0.01$) from the mean count of samples of brands A, C and D. The samples belonging to brand A had the highest mean count (2.46 ± 0.17 $\log_{10}$ cfu/ml) and the

lowest mean count (1.35 ± 0.08 $\log_{10}$ cfu/ml) was observed in the samples of the brand E.

Distribution of retail milk based on Yeast and Mould Count

The distribution of retail milk samples based on yeast and mould count is shown in table 13. Among 100 samples, 62, 36 and 2 per cent had counts at the level of 10^1, 10^2 and 10^3 cfu/ml, respectively. Cent per cent of the samples belonging to brand E had counts at the level of 10^1 cfu/ml. The count at this level was also seen in 90, 50 and 50 per cent of samples of the brands B, C and D, respectively. The count at the level of 10^2 cfu/ml was seen in 14 samples (70 per cent) of brand A. Ten per cent of samples of brand A had count at the level of 10^3 cfu/ml. None of the samples of the brands B, C, D and E had count at the level of 10^3 cfu/ml.

Table 13. Distribution of retail milk based on Yeast and Mould Count

Brands	Yeast and Mould count (cfu/ml)		
	10^1	10^2	10^3
A	4 (20)	14 (70)	2 (10)
B	18 (90)	2 (10)	-
C	10 (50)	10 (50)	-
D	10 (50)	10 (50)	-
E	20 (100)	-	-
Overall	62 (62)	36 (36)	2 (2)

Figures in the parenthesis indicate per cent; N=20 from each brand

4.1.2 Grading of Pasteurized Milk

The pasteurized milk samples were graded according to the level of bacterial load prescribed by the Bureau of Indian Standards (BIS, 1992).

4.1.2.1 Grading of pasteurized milk based on total viable count

According to the criteria prescribed by the Bureau of Indian Standards (BIS, 1992) the total viable count of satisfactory grade of pasteurized milk samples should be less than 30,000/ml. None of the samples from brands A, B, C and D were graded as satisfactory according to the total viable count limits prescribed by BIS (1992). Only 50 per cent of samples from brand E was graded as satisfactory.

4.1.2.2 Grading of pasteurized milk based on coliform count

The Bureau of Indian Standards (BIS 1992) prescribed that coliform should be absent in 1:10 dilution of satisfactory grade of pasteurized milk. Out of 100 samples, none of the samples were graded as satisfactory based on the BIS standards (1992).

4.1.3 Isolation and Identification of Bacteria

In the present investigation 100 samples of pasteurized milk belonging to five brands retailed in and around Thrissur was subjected to isolation and identification of

Table 14. Bacteria isolated from retail milk samples

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

ND: Not detected; N=20 from each brand

organisms of public health significance like *Escherichia coli, Staphylococcus aureus, Pseudomonas* spp and *Bacillus cereus*. The organisms were isolated and identified and are given in table 14.

4.1.3.1 Escherichia coli

Pasteurized milk samples (100) obtained from five different commercial brands was subjected to isolation and identification of *Escherichia coli.* The suspected colonies of the organism were selected from the selective media and transferred to nutrient agar slants and incubated at 37^0 C for overnight. At the end of the incubation period the isolates were stored under refrigeration and were further characterized by cultural, morphological and biochemical reactions. A total of 10 isolates were identified as *Escherichia coli* (Table 14).

Table 15. Distribution of *Escherichia coli* serotypes in retail milk samples

Brands	*Escherichia coli* Serotypes			Overall
	O 4	R	UT	
A	1 (10)	1 (10)	3 (30)	5 (50)
B				
C		1 (10)	1 (10)	2 (20)
D			3 (30)	3 (30)
E				
Overall	1 (10)	2 (20)	7 (70)	10 (100)

UT- Untypable., R- Rough., figures in parenthesis indicate per cent of total isolates

All the 10 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. *Escherichia coli* was isolated from brands A, C and D. Two isolates were found to show congo red binding ability which indicated the property of invasiveness of the isolates.

All isolates obtained from the pasteurized milk samples of brands A, B, C, D and E were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. Only one (10 per cent) out of 10 isolates were serotyped (Table 15). The isolate belonged to serotype O4. Out of the 10 isolates seven were untypable and two were rough.

Distribution of *Escherichia coli* serotypes from brand A

Distribution of *Escherichia coli* serotypes obtained from the samples of brand A is shown in table 15. From the 20 samples of brand A, five (25 per cent) samples yielded the organism (Table14). Among the isolates only one was serotyped, three were untypable and one was rough. The serotype belonged to O4.

Distribution of *Escherichia coli* serotypes from brand C

Different serotypes of *Escherichia coli* obtained from individual samples of brand C is depicted in table 15. Two (20 per cent) *Escherichia coli* isolates were obtained from 20 samples of brand C (Table14) and none were serotyped. One of the isolates was untypable and the other was rough.

Distribution of *Escherichia coli* serotypes from brand D

The distribution of different serotypes of *Escherichia coli* obtained from the samples of the brand D is depicted in table 15. A total of three (15 per cent) *Escherichia coli* were isolated from the samples of brand D (Table14). All the isolates were untypable.

Congo red binding test of *Escherichia coli* isolates

A total of 10 *Escherichia coli* isolates were subjected to congo red binding test and the results are given in table 16. The serotype O4, two rough isolates and five untypable isolates did not revealed congo red binding property. Only two untypable isolates from brand D showed positive congo red activity (Plate 1).

Table 16. Congo red binding test of *Escherichia coli* isolates

Serotype	Congo red binding test	
	No. Positive	No. Negative
O4	-	1
Rough	-	2
Untypable	2	5
Total	2	8

4.1.3.2 Staphylococcus aureus

All samples of pasteurized milk (100) obtained from five different commercial brands were subjected to isolation and identification of *Staphylococcus aureus.* The suspected colonies on Baird Parker agar medium were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature for further characterization. None of the samples from these five brands revealed the presence of the organism.

4.1.3.3 Pseudomonas

All retail milk samples were subjected to the isolation and identification of *Pseudomonas* spp. The organism was isolated from four (4 per cent) samples. The isolates were identified by the cultural, morphological and biochemical characteristics. The isolates were identified as *Pseudomonas aeruginosa* (2) and *Pseudomonas flourescens* (2) and are depicted in table 17.

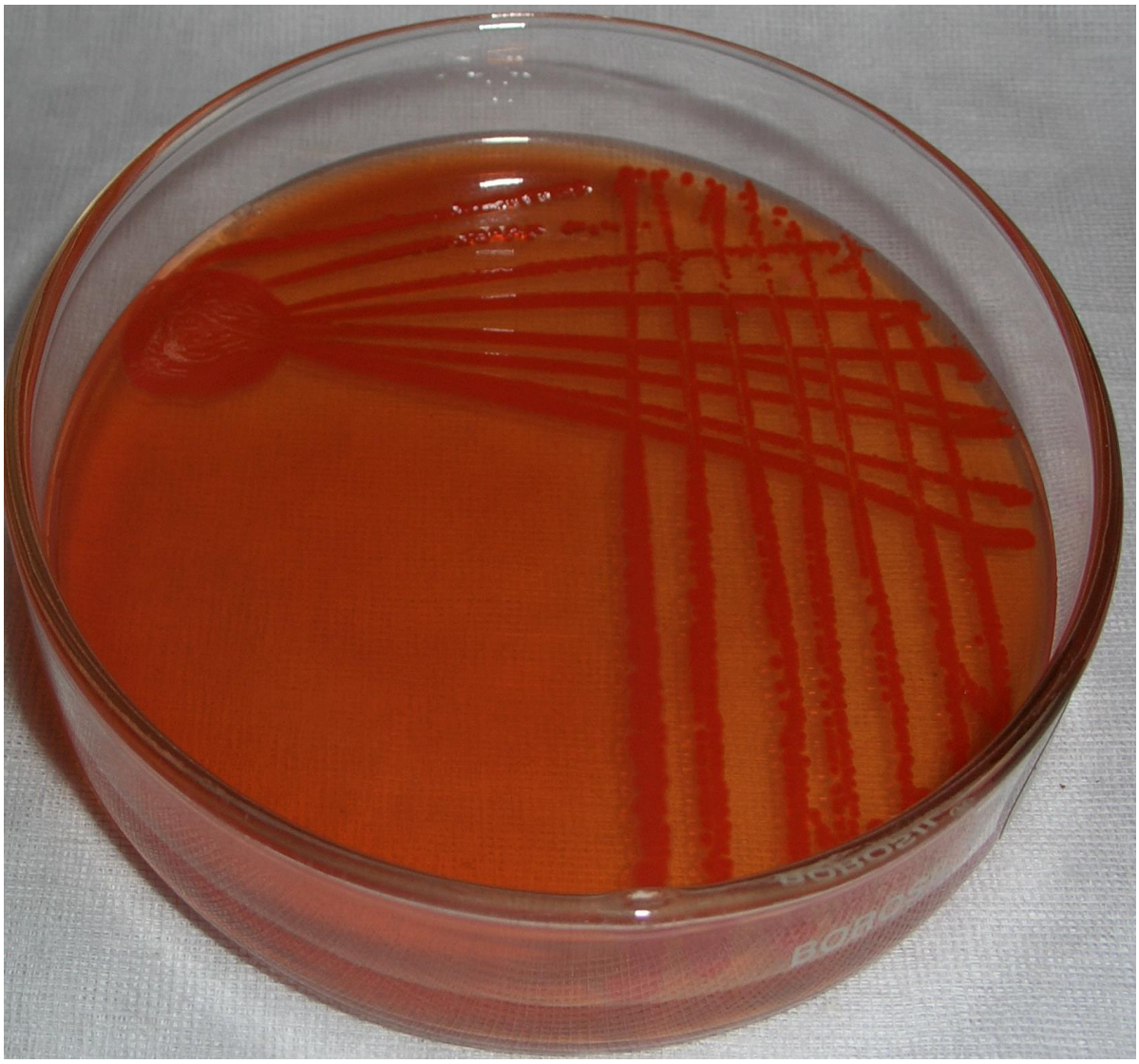

Fig. 1 Congo Red binding property of *Escherichia coli*

Table 17. *Pseudomonas* isolates from milk samples

Organism isolated	Number of isolates					Total
	A	B	C	D	E	
Pseudomonas aeruginosa	1	0	1	0	0	2
Pseudomonas flourescens	1	0	0	1	0	2
Total	2	0	1	1	0	4

4.1.3.4 Bacillus cereus

All samples of pasteurized milk (100) obtained from five different commercial brands were subjected to isolation and identification of *Bacillus cereus* and the results are shown in table 14. The suspected colonies from *Bacillus cereus* agar supplemented with polymyxin B and egg yolk were transferred to nutrient agar slants and incubated at 30^0C for overnight. The isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions. Two isolates belonging to brand A and one isolate from brand D was identified as *Bacillus cereus*.

4.1.4 Adulterants and Preservatives in the milk

All the 100 retail milk samples were tested to determine the presence of adulterants *viz.*, starch and cane sugar and preservatives *viz.*, formaldehyde and carbonates. None of the samples from these five brands was positive for starch, cane sugar and carbonates. Only samples belonging to brand A and E revealed the presence of formaldehyde (Table 18).

Table 18. Presence of Formaldehyde in retail milk samples

Brands	No of samples	
	Tested	Positive
A	20	3 (15)
B	20	0
C	20	0
D	20	0
E	20	16 (80)
Overall	100	19 (19)

Figures in the parenthesis indicate per cent

Distribution of retail milk samples based on the presence of Formaldehyde

Distribution of retail milk samples containing formaldehyde is shown in table 18. Eighty per cent of the samples from brand E were positive for formaldehyde. Fifteen per cent of the samples belonging to brand A also had formaldehyde as preservative.

4.2 MILK FROM PALAKKAD DISTRICT

Pasteurized milk belonging to five different commercial brands (F, G, H, I and J) available in and around Palakkad was evaluated by estimating various microbial counts. Twenty samples were selected from each brand and were subjected to the isolation and identification of various bacteria. These samples were also examined for the presence of adulterants and preservatives.

4.2.1 Microbial quality of pasteurized milk

All the 100 pasteurized milk samples were tested to determine the microbial quality by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) Psychrotrophic Count (PC) and Yeast and Mould Count (YMC).

4.2.1.1 Total Viable Count

The mean total viable count (TVC) of milk samples belonging to the brand F, G, H, I and J are shown in table 19 and illustrated in fig 2. Analysis of variance test of the data revealed that there was no significant difference between the mean total viable count of the samples of the brands. The samples belonging to brand J had the lowest mean count (5.15 ± 0.09 $\log_{10}$ cfu/ml) and the highest count (5.35 ± 0.05 $\log_{10}$ cfu/ml) was seen in the samples belonging to brand I. The mean total viable count of the samples of brand F, G and H were 5.23 ± 0.12, 5.19 ± 0.09 and 5.30 ± 0.07 $\log_{10}$ cfu/ml, respectively.

Table 19. Mean Total Viable Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
F	5.23 ± 0.12
G	5.19 ± 0.09
H	5.30 ± 0.07
I	5.35 ± 0.05
J	5.15 ± 0.09

N=20 from each brand

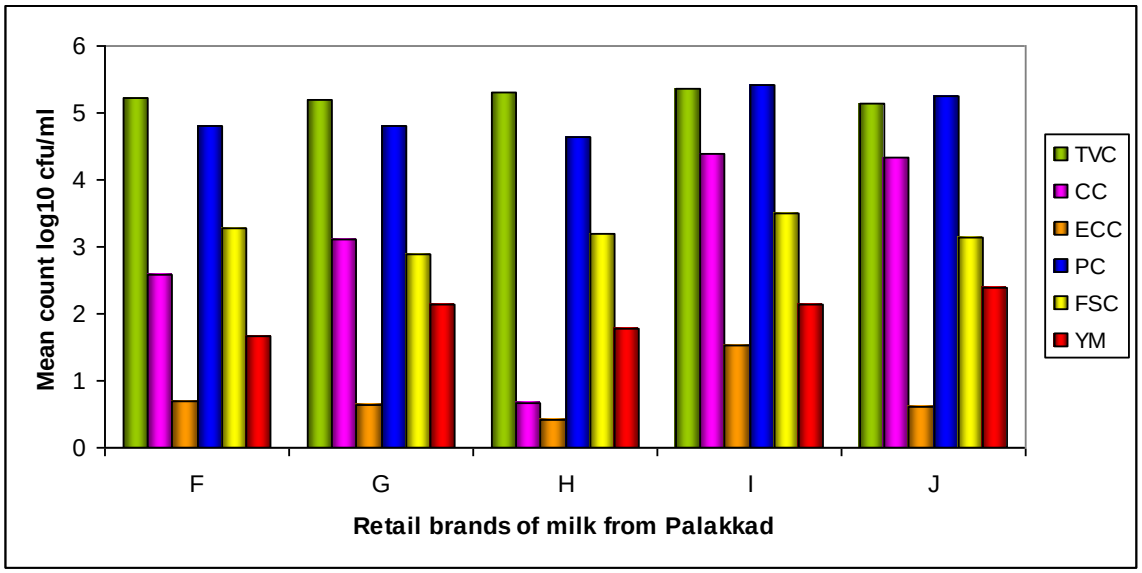

Fig 2 Comparision of the microbiological quality of the retail brands of pasteurized milk of Palakkad district

Distribution of retail milk samples based on Total Viable Count

The distribution of retail milk based on total viable count is shown in table 20. Among the 100 retail samples, 80 and 20 per cent had count at the level of 10^5

Table 20. Distribution of retail milk samples based on Total Viable Count

Brands	Total viable count (cfu/ml)	
	10^4	10^5
F	6 (30)	14 (70)
G	4 (20)	16 (80)
H	2 (10)	18 (90)
I	2 (10)	18 (90)
J	6 (30)	14 (70)
Overall	20 (20)	80 (80)

Figures in the parenthesis indicate per cent; N=20 from each brand

cfu/ml and 10^4 cfu/ml, respectively. The counts in 90 per cent of the samples of the brands H and I were at the level of 10^5 cfu/ml. The count at the later level was observed in 80 per cent of samples of the brand G and also in 70 per cent of the samples of the brands F and J. Only 10 per cent of the samples of the brands H and I had count at the level of 10^4 cfu/ml. According to the total viable count limits prescribed by BIS (1992), none of the samples belonging to the brands F, G, H, I and J were graded as satisfactory quality.

4.2.1.2 Coliform Count

The mean coliform count (CC) of milk samples belonging to the brands F, G, H, I and J are shown in table 21 and illustrated in fig 2. Analysis of variance test of

the data revealed highly significant ($P < 0.01$) difference between the mean counts of samples from different brands.

Table 21. Mean Coliform Count of retail milk samples

Brands	Mean ± SE (log_{10} cfu/ml)
F	$2.57^{b} \pm 0.15$
G	$3.12^{c} \pm 0.09$
H	$0.67^{a} \pm 0.34$
I	$4.39^{d} \pm 0.02$
J	$4.32^{d} \pm 0.05$

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

The mean count of samples of brand F differed highly significantly ($P<0.01$) with the count of samples of brands G, H, I and J. Similarly the mean count of samples of brand G differed highly significantly ($P<0.01$) with the count of samples of brands F, H, I and J. The mean count of samples of brand H differed highly significantly ($P<0.01$) with the count of samples of brands F, G, I and J. The highest mean count (4.39 ± 0.02 log_{10} cfu/ml) was observed in the samples of the brand I and the lowest mean count (0.67 ± 0.34 log_{10} cfu/ml) was observed in samples of the brand H.

Distribution of retail milk samples based on Coliform Count

The distribution of retailed milk samples based on coliform counts is shown in table 22. Of the 100 samples, 40 per cent had count at the level of 10^4 cfu/ml and 14 per cent samples did not reveal the presence of the organism. The organism was seen in cent per cent of the samples belonging to the brands F, G, I and J. The samples of the brand H had count at the highest level of 10^2 cfu/ml and was observed

only in 30 per cent of the samples. The count in 90 per cent of the samples of the brand G was at the level of 10^3 cfu/ml and in 80 per cent of the samples of the brand F was at the level of 10^2 cfu/ml. Out of 100 samples, except 14 samples of brand H was graded as unsatisfactory based on the BIS standards (1992).

Table 22. Distribution of retail milk samples based on Coliform Count

Brands	Coliform counts (cfu/ml)				
	ND	10^1	10^2	10^3	10^4
F	-	-	16 (80)	4 (20)	-
G	-	-	2 (10)	18 (90)	-
H	14 (70)	-	6 (30)	-	-
I	-	-	-	-	20 (100)
J	-	-	-	-	20 (100)
Overall	14 (14)	-	24 (24)	22 (22)	40 (40)

Figures in the parenthesis indicate percent; N=20 from each brand; ND: Not Detected

4.2.1.3 Escherichia coli Count

The mean *Escherichia coli* count (ECC) of milk samples belonging to the brands F, G, H, I and J are shown in table 23 and illustrated in fig 2. Analysis of variance test of the data revealed that significant difference ($P<0.05$) exists between the mean counts of samples of the brands. The mean count of the samples of brand I varied significantly ($P<0.05$) with the mean count of samples of brands F, G, H and J. The lowest mean count (0.43 ± 0.23 $\log_{10}$ cfu/ml) was seen in samples belonging to brand H and the highest count (1.54 ± 0.11 $\log_{10}$ cfu/ml) was seen in the samples of the brand I.

Table 23. Mean *Escherichia coli* Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
F	$0.69^{a} \pm 0.35$
G	$0.63^{a} \pm 0.26$
H	$0.43^{a} \pm 0.23$
I	$1.54^{b} \pm 0.11$
J	$0.61^{a} \pm 0.27$

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

Distribution of retail milk samples based on *Escherichia coli* Count

The distribution of retail milk samples of the brands F, G, H, I and J based on *Escherichia coli* count are shown in table 24. Of the 100 samples, the organism could not detect in 52 per cent of the samples but was detected in 38 and 10 per cent

Table 24. Distribution of retail milk samples based on *Escherichia coli* Count

Brands	*Escherichia coli* count (cfu/ml)		
	ND	10^1	10^2
F	14 (70)	-	6 (30)
G	12 (60)	6 (30)	2 (10)
H	14 (70)	6 (30)	-
I	-	20 (100)	-
J	12 (60)	6 (30)	2 (10)
Overall	52 (52)	38 (38)	10 (10)

Figures in the parenthesis indicate per cent; N=20 from each brand; ND: Not Detected

of the samples at the level of 10 and 10^2 cfu/ml, respectively. All samples belonging to the brand I had count at the level of 10 cfu/ml. The organism was not detected from 70 per cent each of the samples of brand F and H and 60 per cent each of the samples of brand G and J. The organism was detected at the level of 10^2 cfu/ml in 30 and 10 per cent of the samples belonging to the brands F and G, respectively.

4.2.1.4 Psychrotrophic Count

The mean psychrotrophic counts (PC) of milk samples belonging to the brand F, G, H, I and J are shown in table 25 and illustrated in fig 2. Analysis of variance test of the data revealed highly significant difference ($P<0.01$) between the mean count of samples of the brands. The counts in the samples of brand I varied highly significantly ($P<0.01$) with the mean count of samples of brands F, G and H. Similarly the mean counts in the samples of brand J varied highly significantly ($P<0.01$) with the mean count of the brands F, G and H. The samples belonging to brand H had the lowest mean count and the highest count was seen in the samples of the brand I.

Table 25. Mean Psychrotrophic Count of retail milk samples

Brands	Mean ± SE (log_{10} cfu/ml)
F	$4.81^a \pm 0.12$
G	$4.81^a \pm 0.08$
H	$4.63^a \pm 0.10$
I	$5.43^b \pm 0.01$
J	$5.26^b \pm 0.03$

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

Distribution of retail milk samples based on Psychrotrophic Count

The distribution of retail milk samples based on psychrotrophic count is shown in table 26. Of the 100 samples, 52 and 48 per cent had count at the level of

Table 26. Distribution of retail milk samples based on Psychrotrophic Count

Brands	Psychrotrophic count (cfu/ml)	
	10^4	10^5
F	16 (80)	4 (20)
G	18 (90)	2 (10)
H	18 (90)	2 (10)
I	-	20 (100)
J	-	20 (100)
Overall	52 (52)	48 (48)

Figures in the parenthesis indicate percent; N=20 from each brand

10^4 and 10^5 cfu/ml, respectively. All samples belonging to brand I and J had count at the level of 10^5 cfu/ml. The count in 90 per cent samples of the brands G and H and 80 per cent of the samples of the brand F was at the level of 10^4 cfu/ml.

4.2.1.5 Faecal Streptococcal Count

The mean faecal streptococcal count (FSC) of the samples belonging to the brand F, G, H, I and J are shown in table 27 and illustrated in fig 2. The lowest mean count (2.88 ± 0.12 $\log_{10}$ cfu/ml) was seen in samples belonging to brand G and the highest count (3.51 ± 0.21 $\log_{10}$ cfu/ml) was seen in the samples of the brand I.

Table 27. Mean Faecal Streptococcal Count of retail milk samples

Brands	Mean ± SE (log_{10} cfu/ml)
F	3.28 ± 0.22
G	2.88 ± 0.12
H	3.20 ± 0.07
I	3.51 ± 0.21
J	3.15 ± 0.17

N=20 from each brand

Distribution of retail milk based on Faecal Streptococcal Count

The distribution of retail milk samples based on faecal streptococcal count is shown in table 28. Only 10 per cent of the samples belonging to brand I had counts at the level of 10^4 cfu/ml, 70 per cent had count at the level of 10^3 cfu/ml and 20 per

Table 28. Distribution of retail milk based on Faecal Streptococcal Count

Brands	Faecal streptococcal count (cfu/ml)		
	10^2	10^3	10^4
F	6 (30)	14 (70)	-
G	10 (50)	10 (50)	-
H	4 (20)	16 (80)	-
I	4 (20)	4 (70)	2 (10)
J	4 (20)	16 (80)	-
Overall	28 (28)	60 (60)	2 (2)

Figures in the parenthesis indicate per cent; N=20 from each brand

cent had count at the level of 10^2 cfu/ml. The count in 50 per cent samples each of the brand G had count at the level of 10^3 cfu/ml and 10^2 cfu/ml, respectively. The count at the level of 10^3 cfu/ml was also seen in 70 per cent samples of brand F, and 80 per cent of samples of brands H and J.

4.2.1.6 Yeast and Mould Count

The mean Yeast and mould count (YMC) of milk samples belonging to the brands F, G, H, I and J are shown in table 29 and illustrated in fig 2. The lowest mean count (1.66 ± 0.09 $\log_{10}$ cfu/ml) was seen in samples belonging to brand F and the highest mean count (2.40 ± 0.24 $\log_{10}$ cfu/ml) was seen in samples belonging to brand J.

Table 29. Mean Yeast and Mould Count of retail milk samples

Brands	Mean ± SE ($\log_{10}$ cfu/ml)
F	1.66 ± 0.09
G	2.15 ± 0.23
H	1.79 ± 0.20
I	2.14 ± 0.17
J	2.40 ± 0.24

N=20 from each brand

Distribution of retail milk based on Yeast and Mould Count

The distribution of retail milk samples based on yeast and mould count is shown in table 30. Of the 100 samples from the various brands, 48, 42 and 10 per cent had count at the level of 10, 10^2 and 10^3 cfu/ml, respectively. The count at the level of 10^3 cfu/ml was observed in 20 and 30 per cent of samples of the brands G

and J, respectively. In 80 and 60 per cent samples of the brands F and H had counts at the level of 10 cfu/ml. The counts at the level of 10^2 cfu/ml was observed in 70 per cent of the samples of the brand I and 40 per cent samples each of the brands G, H and J.

Table 30. Distribution of retail milk based on Yeast and Mould Count

Brands	Yeast and Mould count (cfu/ml)		
	10^1	10^2	10^3
F	16 (80)	4 (20)	-
G	8 (40)	8 (40)	4 (20)
H	12 (60)	8 (40)	-
I	6 (30)	14 (70)	-
J	6 (30)	8 (40)	6 (30)
Overall	48 (48)	42 (42)	10 (10)

Figures in the parenthesis indicate percent; N=20 from each brand

4.2.2 Grading of Pasteurized Milk

4.2.2.1 Grading of pasteurized milk based on total viable count

The pasteurized milk samples were graded according to the standards prescribed by the Bureau of Indian Standards (BIS 1992). None of the samples from brands F, G, H, I and J were graded as satisfactory according to the total viable count limits prescribed by BIS (1992).

4.2.2.2 Grading of pasteurized milk based on coliform count

Out of 100 samples, 70 per cent of the samples of brand H was graded as satisfactory based on the coliform count limit prescribed by BIS standards (1992).

4.2.3 Isolation and Identification of Bacteria

In the present investigation 20 samples each of pasteurized milk belonging to five retail brands from Palakkad district was examined for the presence of *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Pseudomonas* spp. The organisms isolated and identified from these samples are given in table 31.

Table 31. Bacteria isolated from retail milk samples of Palakkad

Bacteria	Number of positive samples				
	Brands of milk				
	F	G	H	I	J
Escherichia coli	2	4	2	1	2
Staphylococcus aureus	ND	ND	ND	ND	ND
Pseudomonas	2	ND	1	1	2
Bacillus cereus	ND	ND	1	1	ND

ND: Not detected; N=20 from each brand

4.2.3.1 Escherichia coli

Pasteurized milk samples obtained from five different retail brands were subjected for the isolation and identification of *Escherichia coli.* The suspected colonies of the organism were selected from the media are transferred to nutrient agar slants and incubated at 37^0 C for overnight. At the end of the incubation period the isolates were stored under refrigeration and were further characterized by

cultural, morphological and biochemical reactions. Eleven isolates were identified as *Escherichia coli* (Table 31). All the 11 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

Table 32. Distribution of *Escherichia coli* serotypes in pasteurized milk samples

Brands	*Escherichia coli* Serotypes				Overall
	O 4	O 60	R	UT	
F		1 (9.09)		1 (9.09)	2 (18.18)
G		1 (9.09)	1 (9.09)	2 (18.18)	4 (36.36)
H	1 (9.09)			1 (9.09)	2 (18.18)
I				1 (9.09)	1 (9.09)
J		1 (9.09)		1 (9.09)	2 (18.18)
Overall	1 (9.09)	3 (27.27)	1 (9.09)	6 (54.54)	11 (100)

UT- Untypable., R- Rough., figures in parenthesis indicate per cent of total isolates

sugars. The organism was isolated from two (18.18 per cent) samples each of the brands F, H and J, four (36.36 per cent) samples obtained from brand G and only one (9 per cent) sample from brand I. Two isolates showed congo red binding ability which indicated the invasive property of the isolates.

All isolates obtained from the samples of brands F, G, H, I and J were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. Only 4 (36.36 per cent) out of 11 isolates were serotyped. One of these isolates belonging to serotype O4 and three isolates belonging to serotypes O60. Out of the 11 isolates six were untypable and one was rough.

Distribution of *Escherichia coli* serotypes from brand F

Distribution of *Escherichia coli* serotypes obtained from the samples of brand F is shown in table 32. Two (18.18 per cent) isolates were obtained from the samples of the brand F. Only one of the isolates was serotyped as O4 and the other was untypable.

Distribution of *Escherichia coli* serotypes from brand G

The *Escherichia coli* serotypes obtained from individual samples of brand G are given in table 32. Among the four (36.36 per cent) isolates, one of the isolate was serotyped as O60, one was rough and two isolates were untypable.

Distribution of *Escherichia coli* serotypes from brand H

The distribution of different *Escherichia coli* serotypes isolated from the samples of the brand H is depicted in table 32. Only two (18.18 per cent) isolates was obtained from the brand H. One of the isolates belong to serotype O4 and the other isolate was untypable.

Distribution of *Escherichia coli* serotypes from brand I

The *Escherichia coli* serotypes obtained from individual samples of the brand I is depicted in table 32. Only one (9.09 per cent) isolate was obtained from the samples of brand I and the organism was untypable.

Distribution of *Escherichia coli* serotypes from brand J

The distribution of different serotypes of *Escherichia coli* obtained from samples of brand J are shown in table 32. Of the two (18.8 per cent) isolates, one of the isolates was serotyped as O60 and the other was untypable.

Congo red binding test of *Escherichia coli* isolates

A total of 11 *Escherichia coli* isolates obtained from the samples of Palakkad district were subjected to congo red binding test and the results are given in table 33. Three isolates belonging to serotype O60, one isolate belonging to serotype O4 and two untypable isolates did not reveal congo red binding property. Two untypable and a rough *Escherichia coli* isolated from the samples of the brand G revealed congo red binding charecteristics. The untypable isolates isolated from the samples of the brands H and J also showed congo red property.

Table 33. Congo red binding test of *Escherichia coli* isolates

Serotype	Congo red binding test	
	No. Positive	No. Negative
O4	-	1
O60	-	3
Rough	1	-
Untypable	4	2
Total	5	6

4.2.3.2 Staphylococcus aureus

All samples of pasteurized milk (100) obtained from five different retail brands were subjected to isolation and identification of *Staphylococcus aureus*, but none of the samples from these five brands revealed the presence of the organism.

4.2.3.3 Pseudomonas

All retail milk samples were subjected to isolation and identification of *Pseudomonas* spp.

Table 34. *Pseudomonas* isolates from milk samples

Organism isolated	Number of isolates					Total
	F	G	H	I	J	
Pseudomonas aeruginosa	1	0	0	0	1	2
Pseudomonas flourescens	1	0	0	1	0	2
Pseudomonas cepacia	0	0	0	0	1	1
Pseudomonas putida	0	0	1	0	0	1
Total	2	0	1	1	2	6

The suspected colonies selected from the media were identified by the cultural, morphological and biochemical characteristics. The isolates were identified as *Pseudomonas aeruginosa* (2), *Pseudomonas flourescens* (2), *Pseudomonas cepacia* (1) and *Pseudomonas putida* (1) and are depicted in table 34.

4.2.3.4 Bacillus cereus

All the samples of pasteurized milk (100) obtained from five different commercial brands were subjected to isolation and identification of *Bacillus cereus* and the results are shown in table 31. The suspected colonies form *Bacillus cereus* agar supplemented with polymyxin B and egg yolk were transferred to nutrient agar slants and incubated at 30^0C for overnight. The isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions and only one isolate each from brands H and I was identified as *Bacillus cereus*.

4.2.4 Adulterants and Preservatives in the milk

All the 100 retail milk samples were tested to determine the presence of adulterants *viz.*, starch and cane sugar and preservatives *viz.*, formaldehyde and carbonates. None of the samples from these five brands was positive for starch, cane sugar and carbonates. Samples belonging to brands G, I and J had the presence of formaldehyde (Table35). All the samples of brand G were positive for formaldehyde. Seventy per cent of samples from brand J and 65 per cent of samples of brand I also had formaldehyde.

Table 35. Presence of Formaldehyde in milk samples

Brands	No of samples	
	Tested	Positive
F	20	0
G	20	20 (100)
H	20	0
I	20	13 (65)
J	20	14 (70)
Overall	100	47 (47)

Figures in the parenthesis indicate per cent

4.3 COMPARISION OF MILK BETWEEN THRISSUR AND PALAKKAD

A total of 200 pasteurized milk samples consisted of 100 samples each from Thrissur and Palakkad districts were collected during the investigation. The samples were collected from five brands of each district were examined to assess the microbial quality of milk, isolation of various microorganisms of public health significance and to detect the presence of preservatives and adulterants.

4.3.1 Microbial quality of pasteurized milk

The microbial quality of the pasteurized milk samples were analyzed by estimating the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) Psychrotrophic Count (PC) and Yeast and Mould Count (YMC).

The overall mean total viable count, coliform count, *Escherichia coli* count, psychrotrophic count, faecal streptococcal count and yeast and mould count of the samples collected from Thrissur and Palakkad districts and are given in table 36.

Table 36. The overall mean microbial counts of the samples from Thrissur and Palakkad

Microbial counts	Mean microbial count (Mean ± SE, $\log_{10}$ cfu/ml)		Z value
	Thrissur	Palakkad	
Total viable count	5.08 ± 0.05	5.24 ± 0.04	1.73^{NS}
Coliform count	2.89 ± 0.09	3.01 ± 0.20	3.97**
Escherichia coli count	0.53 ± 0.11	0.78 ± 0.12	0.71^{NS}
Psychrotrophic count	5.30 ± 0.01	4.99 ± 0.05	3.77**
Faecal streptococcal count	3.40 ± 0.14	3.20 ± 0.07	1.17^{NS}
Yeast and mould count	1.89 ± 0.08	2.03 ± 0.09	0.91^{NS}

N= 100 from each district; NS-Non significant; **- Significant at 1 per cent level (P<0.01)

Each of these mean count of the samples belonging to Thrissur and Palakkad districts were compared using Z test revealed that coliform count of the samples belonging to Thrissur and Palakkad and psychrotrophic count the districts differed highly significantly (Z>1.96). The overall mean total viable count, *Escherichia coli* count

and yeast and mould count of the samples of Palakkad district was higher than that of the count of the samples obtained from Thrissur district. The overall mean faecal streptococcal count was higher in the samples of Thrissur.

4.3.2 Correlation between microbial counts of pasteurized milk from Thrissur and Palakkad districts

Total Viable Count

Correlation between mean total viable count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 37. A highly significant ($P<0.01$) and positive correlation was found between the mean total viable count and coliform count of samples of pasteurized milk from Thrissur district. Association between the mean total viable count and yeast and mould count of pasteurized milk samples from Thrissur district was also positive and significant ($P<0.05$).

Table 37. Correlation coefficient between mean total viable count and other microbial counts

District	Correlation coefficient values of microbial counts				
	PC	CC	FSC	YM	ECC
Thrissur	0.200	0.430**	-0.157	0.340*	0.120
Palakkad	0.099	0.031	0.220	0.147	0.171

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count, ** = $P<0.01$, * = $P<0.05$

Coliform Count

Correlation coefficient between the mean coliform count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 38. A highly significant (P<0.01) and positive correlation was found between

Table 38. Correlation coefficient between mean coliform count and other microbial counts

District	Correlation coefficient values of microbial counts				
	TVC	PC	FSC	YM	ECC
Thrissur	0.430**	0.091	-0.004	0.473 **	-0.023
Palakkad	0.031	0.714**	0.218	0.259	0.022

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count, ** = P<0.01

the mean coliform count and total viable count and also between the mean coliform count and yeast and mould count of samples from Thrissur district. A similar association was observed between the mean coliform count and psychrotrophic count of samples of pasteurized milk from Palakkad district.

Escherichia coli Count

Correlation coefficient between mean *Escherichia coli* count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 39. The mean *Escherichia coli* count did not reveal correlation with other microbial counts of the samples from Thrissur and Palakkad districts.

Table 39. Correlation coefficient between mean *Escherichia coli* count and other microbial counts

District	Correlation coefficient values of microbial counts				
	TVC	PC	CC	FSC	YM
Thrissur	0.120	0.183	-0.023	-0.132	0.194
Palakkad	0.171	-0.023	0.022	0.001	-0.026

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count

Psychrotrophic Count

Correlation between mean psychrotrophic count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 40. A highly significant (P<0.01) but negative association was observed between the mean psychrotrophic count and faecal streptococcal count of the samples from Thrissur district. The assosciation between the mean psychrotrophic count and yeast and mould count of the samples from Thrissur district was positive and significant

Table 40. Correlation coefficient between mean psychrotrophic count and other microbial counts

District	Correlation coefficient values of microbial counts				
	TVC	CC	FSC	YM	ECC
Thrissur	0.200	0.091	-0.369**	0.297*	0.183
Palakkad	0.099	0.714**	0.247	0.269	-0.023

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count, ** = P<0.01, * = P<0.05

(P<0.05). Similarly a positive and highly significant (P<0.01) correlation was observed between the mean psychrotrophic count and coliform count of the samples of Palakkad district.

Faecal Streptococcal Count

Correlation between mean faecal streptococcal count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 41. A highly significant (P<0.01) and negative correlation was found between the mean faecal streptococcal count and psychrotrophic count of the samples from Thrissur district.

Table 41. Correlation coefficient between mean faecal streptococcal count and other microbial counts

District	Correlation coefficient values of microbial counts				
	TVC	PC	CC	YM	ECC
Thrissur	-0.157	-0.369 **	-0.004	-0.140	-0.132
Palakkad	0.220	0.247	0.218	-0.073	0.001

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count, ** = P<0.01

Yeast and Mould Count

Correlation between mean yeast and mould count and other microbial counts of pasteurized milk samples from Thrissur and Palakkad districts are shown in table 42. A highly significant (P<0.01) and positive association was observed between the mean yeast and mould count and coliform count of the samples from Thrissur district. The association between the mean yeast and mould count and total viable

count and also between the mean yeast and mould and psychrotrophic count of the samples belonging to Thrissur district was positive and significant (P<0.05).

Table 42. Correlation coefficient between mean yeast and mould count and other microbial counts

District	Correlation coefficient values of microbial counts				
	TVC	PC	CC	FSC	ECC
Thrissur	0.340 *	0.297 *	0.473 **	-0.140	0.194
Palakkad	0.147	0.269	0.259	-0.073	-0.026

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, TVC: Total viable count, PC: Psychrotrophic count, YM: Yeast and Mould count, ** = P<0.01, * = P<0.05

4.3.3 Grading of Pasteurized Milk

4.3.3.1 Grading of pasteurized milk samples based on total viable count

Pasteurized milk samples collected from Thrissur and Palakkad districts were graded as satisfactory and unsatisfactory based on the total viable count limits

Table 43. Distribution of pasteurized milk samples from Thrissur and Palakkad districts based on total viable count

District	Number of samples	
	Satisfactory	unsatisfactory
Thrissur	10 (10)	90 (90)
Palakkad	0	100 (100)
Overall	10 (5)	190 (95)

N=100 from each district; figures in parenthesis indicate per cent

prescribed by BIS (1992) and are shown in table 43 and illustrated in fig 3. Out of the 200 samples collected from Thrissur and Palakkad district, only 10 samples (5 per cent) were graded as satisfactory and 95 per cent of the samples were graded as unsatisfactory. None of the samples from Palakkad district was graded as satisfactory but 10 per cent of the samples belonging to Thrissur district was graded as satisfactory.

4.3.3.2 Grading of pasteurized milk samples based on coliform count

The pasteurized milk samples collected from Thrissur and Palakkad districts were graded as satisfactory or unsatisfactory based on coliform count limit prescribed by BIS (1992) and the distribution of samples is given in table 44 and illustrated in fig 4. Cent per cent of the samples collected from Thrissur district was graded as unsatisfactory whereas, 14 per cent of the samples belonging to Palakkad district was graded as satisfactory. Overall 93 per cent of the samples procured from Thrissur and Palakkad districts were graded as unsatisfactory.

Table 44. Distribution of pasteurized milk samples from Thrissur and Palakkad districts based on coliform count

District	Number of samples	
	Satisfactory	Poor
Thrissur	0	100 (100)
Palakkad	14 (14)	86 (86)
Overall	14 (7)	186 (93)

N=100 from each district; figures in parenthesis indicate per cent

4.3.4. Presence of Adulterants and Preservatives in the milk

The pasteurized milk samples from Thrissur and Palakkad districts were assessed for the presence of adulterants viz., starch and cane sugar and also

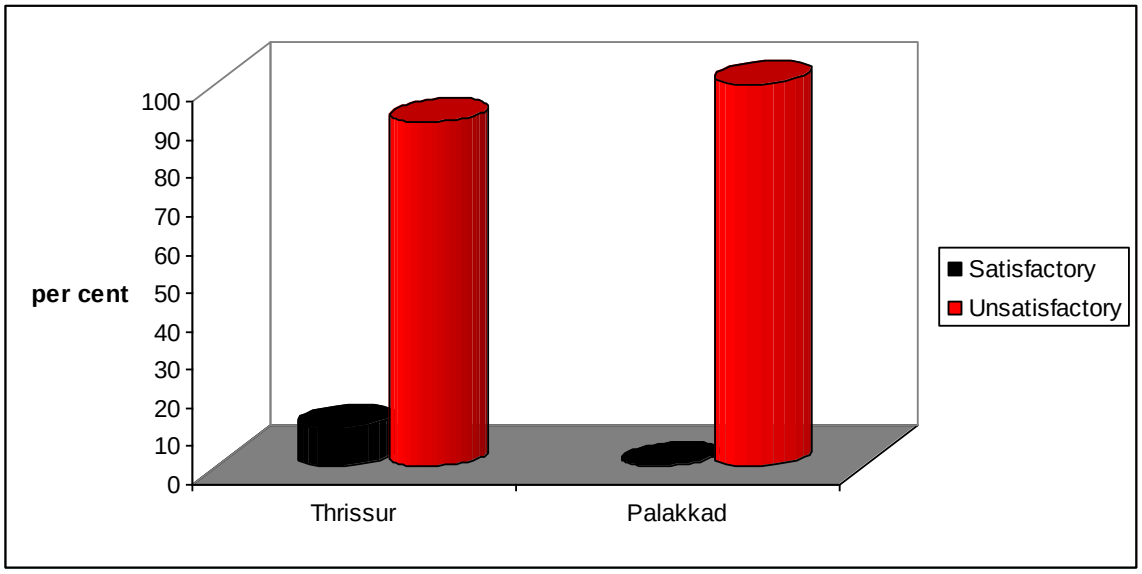

Fig 3 Frequency distribution of pasteurized milk samples based on Total viable count

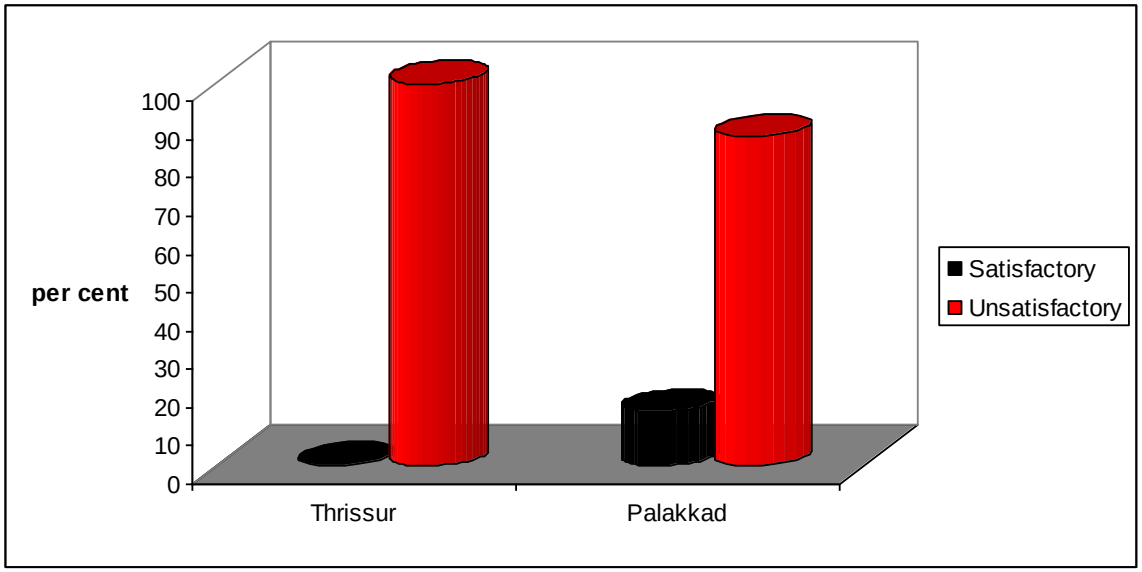

Fig 4 Frequency distribution of pasteurized milk samples based on Coliform count

preservatives viz., formaldehyde and carbonates. Only formaldehyde was detected from the samples and distribution of the samples with formaldehyde is given in table 45. Formaldehyde was detected in 33 per cent of the samples collected from Thrissur and Palakkad districts. Out of the 100 samples from Palakkad district, 47 per cent had formaldehyde whereas, only 19 per cent of samples belonging to Thrissur district had formaldehyde.

Table 45. Presence of Formaldehyde in the Pasteurized milk samples from Thrissur and Palakkad districts

District	No of samples	
	Tested	Positive
Thrissur	100	19 (19)
Palakkad	100	47 (47)
Overall	200	66 (33)

Figures in the parenthesis indicate per cent

Discussion

5. DISCUSSION

Milk is a highly nutritious food which forms a major constituent of diet among all age groups of people and hence, quality control is considered essential for the health and welfare of the society. It's a balanced diet having a high protein bioavailability. Milk is an excellent growth media and when stored improperly will allow rapid proliferation of pathogenic and spoilage organisms. The pathogens can cause diseases or episodes of food poisoning whereas, the spoilage organisms can produce off-flavours, coagulation, ropiness etc. which adversely affects the keeping quality of milk. Generally pasteurization destroys saprophytic and pathogenic bacteria without reducing the nutritional quality of milk thereby, ensures quality and a longer shelf life. Cheaper commodities, preservatives or neutralizers are sometimes added to improve the aesthetic appearance and to delay or prevent the spoilage. Increasing consumer awareness has emphasized the need for microbiologically and chemically safe food. In the present study the quality of pasteurized milk available in the market was analyzed by estimating the microbial load and the presence of potential pathogens and spoilage organisms. The presence of some adulterants and preservatives was also examined. This gives an overall idea about the quality of pasteurized milk available to the consumers.

4.1 COMPARISION OF MILK FROM THRISSUR AND PALAKKAD

4.1.1 Microbial quality of pasteurized milk

All the pasteurized milk samples were tested to determine the microbial quality by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) Psychrotrophic Count (PC) and Yeast and Mould Count (YMC).

4.1.1.1 Total Viable Count

The mean total viable count of the samples belonging to the brands A, B, C, D and E collected from Thrissur were 5.39 ± 0.04, 5.09 ± 0.08, 5.22 ± 0.06, 5.21 ± 0.06 and 4.51 ± 0.08 $\log_{10}$ cfu/ml, respectively and is shown in table 2. A highly significant ($P<0.01$) difference of mean count of the samples of the brand E with the mean count of the samples of the brands A, B, C and D was observed. This indicated that the quality of the samples belonging to brand E was superior to other brands. The mean count of the samples of the brands F, G, H, I and J procured from Palakkad were 5.23 ± 0.12, 5.19 ± 0.09, 5.30 ± 0.07, 5.35 ± 0.05 and 5.15 ± 0.09 $\log_{10}$ cfu/ml, respectively (Table 19). The milk samples collected from Palakkad district were of similar quality. The overall mean count of the samples collected from Thrissur and Palakkad districts were 5.08 ± 0.05 and 5.24 ± 0.04 $\log_{10}$ cfu/ml, respectively (Table 36).

The mean total viable count at the level of 5 $\log_{10}$ cfu/ml was similar to that recorded by Reddy *et al.* (1989), Mahari and Gashe (1990), Rai and Dwivedi (1990), Latha and Nanu (1997), John (1999) Prejit (2005) and Asha (2007). The mean count of the samples from brand E (4.51 ± 0.08 $\log_{10}$ cfu/ml) was similar to that reported by Vijai and Saraswat (1968), Misra and Kuila (1989) Cerqueira *et al.* (1994) and Prejit *et al.* (2006). The mean count of the samples belonging to various brands of Thrissur and Palakkad districts was very high when compared to the count of 2.82 ± 0.14 $\log_{10}$ cfu/ml reported by Sethulakshmi *et al.* (2003). Total viable count gives an idea about the overall quality of milk and the sanitary and hygienic conditions adopted during production, processing, post production handling and storage. When the total number of microorganisms in the milk increases, the shelf life of the milk decreases and gets spoiled easily.

The high total viable count in the samples may be due to poor bacterial quality of raw milk, post pasteurization contamination, high storage temperature of milk after pasteurization, milk handling, storage equipments and the filling machine as stated by Elmagli and El Zubeir (2006) and also due to contaminated packaging material (Eneroth *et al.*, 1998). Initial quality of raw milk is an important factor affecting the quality of pasteurized milk (Beloti *et al.*, 2002) and hence emphasis has to be given for clean milk production. The holding of milk at collection centers at atmospheric temperature can also cause a substantial increase in the bacterial load (Vijai and Saraswat, 1968). Pasteurization do not destroy all the microorganisms present in the milk, but only one to two log reduction of bacterial load can be made possible (Eneroth *et al.*, 1998; Aaku *et al.*, 2004; Prejit *et al.*, 2006). The storage temperature is another important factor affecting the generation time of natural flora of pasteurized milk. There is a sharp decline in the average generation time with increase in temperature up to 15°C (Griffiths and Philips, 1988). Lopes and Stamford (1997) suggested that storage tank represents an important point of contamination for pasteurized milk. During the investigation it was observed that most of the retailers are not storing milk in refrigeration temperature either because of lack of facility, shooting electricity bills or due to unawareness. The use of skim milk powder for the production of reconstituted and toned pasteurized milk can also result in high total viable count (Ethiraj *et al.*, 1979). The relatively lower count in the samples of some brands may be attributed to the presence of formaldehyde (Table 18, 35).

4.1.1.2 Coliform Count

The mean coliform count of the samples of the brands A, B, C, D and E belonging to Thrissur district were 3.40 ± 0.17, 2.60 ± 0.20, 2.99 ± 0.20, 3.16 ± 0.16 and 2.30 ± 0.09 $\log_{10}$ cfu/ml, respectively (Table 4). A highly significant ($P<0.01$) difference was observed in the mean count of the samples of brand E with the mean count of samples of the brands A, C and D. This indicated that the quality of

pasteurized milk of the brand E varied greatly with that of others. The mean count of samples of the brands A and D differed highly significantly (P<0.01) with the count of samples of the brands B and E. The samples of the brands F, G, H, I and J collected from Palakkad had mean coliform count of 2.57 ± 0.15, 3.12 ± 0.09, 0.67 ± 0.34, 4.39 ± 0.02 and 4.32 ± 0.05 $\log_{10}$ cfu/ml, respectively (Table 21). Analysis of variance test of the data revealed highly significant (P < 0.01) difference between the mean coliform count of different brands of pasteurized milk from Palakkad. The overall mean coliform count of the samples from Thrissur was 2.89 ± 0.09 $\log_{10}$ cfu/ml and that from Palakkad was 3.01 ± 0.20 $\log_{10}$ cfu/ml (Table 36). Analysis of data by Z test revealed a highly significant difference (Z>1.96) between the mean coliform count of the samples from both the districts.

Difference in the coliform count of the samples belonging to various brands marketed in Thrissur was also reported by Latha and Nanu (1997), John (1999) Sethulakshmi *et al.* (2003), Prejit (2005) and Asha (2007). The mean count in the samples of the present study did not concur with the reports of Kaloianov and Gogov (1977). The later authors reported that coliform organism could not isolate from 1404 samples of pasteurized milk. The mean count observed in the present study disagreed with the observations of Yadava *et al.* (1983) and Lues *et al.* (2003), who recorded the counts at the level of 10^6 and 10^7 cfu/ml, respectively. The mean count observed in the present study at the level of three log was similar to that of the findings of Latha and Nanu (1997), John (1999), Gopi *et al.* (2001) and Khalilur *et al.* (2002). The mean count observed in the current study, at the level of 10^2 cfu/ml, was similar to that of the mean count reported by Misra and Kuila (1989), Rai and Dwivedi (1990), Cosentino and Palmas (1997) and Asha (2007). The count in the samples of the brand H was in accordance with the result of Prejit *et al.* (2006). Cent per cent of samples of brands I and J had count at the level of 10^4 cfu/ml which was three log greater than that of the findings of Vijai and Saraswat (1968), Arora and Sudarsanam (1986), Raju and Nambudripad (1987) and Siva *et al.* (1993).

Coliforms are indicators of faecal contamination of milk and if present in large numbers can result in the spoilage of milk stored at low temperatures. The high coliform count in the samples of pasteurized milk can be due to recontamination at the point of processing, storage or packaging (Singh and Ranganathan, 1978). Most of the coliforms are destroyed during pasteurization and hence their presence in pasteurized milk can be due to insufficient pasteurization or post pasteurization contamination. Prejit *et al.* (2006) reported that pasteurization killed coliform organism in 40 per cent of the samples. However, the presence of the thermal resistant strains and rejuvenation of heat injured cells after pasteurization (Raju and Nambudripad, 1987) also contribute to the presence of organism in pasteurized milk.

4.1.1.3 Escherichia coli Count

The mean *Escherichia coli* count of the samples of the brands A, B, C, D and E were 0.92 ± 0.31, 0.34 ± 0.17, 0.69 ± 0.29, 0.60 ± 0.31 and 0.10 ± 0.10 $\log_{10}$ cfu/ml, respectively (Table 6). The mean count of the samples of the brands F, G, H, I and J were 0.69 ± 0.35, 0.63 ± 0.26, 0.43 ± 0.23, 1.54 ± 0.11 and 0.61 ± 0.27 $\log_{10}$ cfu/ml, respectively (Table 23). Analysis of variance test of the data revealed that the mean count of the samples of the brand I varied significantly ($P<0.05$) with the count of the samples of other brands. The overall mean count of the samples from Thrissur and Palakkad districts were 0.53 ± 0.11 and 0.78 ± 0.12 $\log_{10}$ cfu/ml, respectively (Table 36). The count of the organism in the present study was much less than that reported by Latha and Nanu (1997), John (1999), Gran *et al.* (2003) and Lues *et al.* (2003). But the mean count was in accordance with the count reported by Sethulakshmi *et al.* (2003), Prejit *et al.* (2006) and Asha (2007) in the same area. Therefore, it may be inferred that the quality of pasteurized milk in the area was not improved.

Escherichia coli are considered as the normal flora of the intestinal tract of humans and animals. Pasteurized milk sample has to be free of the organism.

Generally the number of this organism in the sample increases with the number of coliforms (Singh and Ranganathan, 1978). The presence of *Escherichia coli* in pasteurized milk is of great public health significance since the organisms are indicators of faceal contamination and it also suggests the presence of other microorganisms, including pathogens (Khalilur *et al.*, 2002). The presence of the organism in pasteurized milk indicated insufficient pasteurization or post pasteurization contamination of milk. The lack of maintaining proper temperature during transportation and storage at the retailers also enhances the multiplication of organism. The lack of refrigerated transportation, intermittent electrical supply and poor quality of water are also responsible for the presence of this organism in pasteurized milk samples (Elmagli and El Zubeir, 2006). Commercial pasteurization can destroy *Escherichia coli* associated with milk (Aaku *et al.*, 2004), but the isolation of heat resistant strain, B23 was cited by Raju and Nambudripad (1987).

4.1.1.4 Psychrotrophic Count

The mean psychrotrophic count of the samples of the brands A, B, C, D and E from Thrissur were 5.36 ± 0.06, 5.29 ± 0.05, 5.39 ± 0.02, 5.32 ± 0.02 and 5.30 ± 0.01 $\log_{10}$ cfu/ml, respectively (Table 8). The mean psychrotrophic counts of samples belonging to the brands F, G, H, I and J were 4.81 ± 0.12, 4.81 ± 0.08, 4.63 ± 0.10, 5.43 ± 0.01 and 5.26 ± 0.03 $\log_{10}$ cfu/ml, respectively (Table 25). Analysis of variance test of the data revealed highly significant difference ($P<0.01$) between the mean count of samples of the brands. The overall mean psychrotrophic count of the samples from Thrissur district was 5.30 ± 0.01 $\log_{10}$ cfu/ml and that from Palakkad district was 4.99 ± 0.05 $\log_{10}$ cfu/ml (Table 36). Analysis of the count using Z test revealed that the mean count of the samples from both the districts differed highly significantly ($Z>1.96$). The result of the present study was in agreement with the reports of John (1999), Gopi *et al.* (2001), Aaku *et al.* (2004), Prejit (2005) and Asha (2007). However, the count observed in the current study was greater than that

reported by Arora and Sudarsanam (1986), Misra and Kuila (1989), Sutherland *et al.* (1993), Beuvier *et al.* (1997), Cosentino and Palmas (1997) and Lopamudra and Kuila (2005).

Psychrotrophs are gram negative rods which do not survive pasteurization temperature and hence its presence in pasteurized milk indicates post pasteurization contamination (Mahari and Gashe, 1990). The use of unpotable water and unclean utensils during the processing of milk can act as sources of psychrotrophs (Arora and Sudarsanam, 1986). Griffiths and Philips (1988) cited that the shelf life of pasteurized milk was dependent on initial psychrotrophic count. Psychrotrophs in milk adversely affects the keeping quality of milk by the production of proteolytic and lipolytic enzymes that results in spoilage conditions like bitter taste and undesirable flavour (Aaku *et al.*, 2004). Most of the psychrotrophs are thermo labile gram negative cocci which are inactivated at pasteurization temperature, but the enzymes produced by these organisms, are heat resistant and, can degrade important components of milk and can result in coagulation and bitterness of milk (Burdova *et al.*, 2002).

4.1.1.5 Faecal Streptococcal Count

The mean faecal streptococcal count of milk samples belonging to the brand A, B, C, D and E were 3.51 ± 0.06, 3.78 ± 0.16, 2.95 ± 0.17, 3.61 ± 0.10 and 3.40 ± 0.14 $\log_{10}$ cfu/ml, respectively (Table 10). The mean count of samples of brand C had highly significant ($P<0.01$) difference with mean the count of samples of the brands A, B, D and E. The mean faecal streptococcal count of brands F, G, H, I and J were 3.28 ± 0.22, 2.88 ± 0.12, 3.20 ± 0.07, 3.51 ± 0.21 and 3.15 ± 0.17 $\log_{10}$ cfu/ml, respectively (Table 27). The overall mean count of samples from Thrissur and Palakkad districts were 3.40 ± 0.14 and 3.20 ± 0.07 $\log_{10}$ cfu/ml, respectively (Table 36).

In the present study, the mean count of the samples of the brands C and G were almost in conformity with the count recorded by Asha (2007). The count in the present study was higher than that reported by Latha and Nanu (1997), Sethulakshmi *et al.* (2003) and Prejit (2006) who also examined various brands of pasteurized milk from the same area. The level of the organism observed in the samples of the current study was less than that observed by Yadava *et al.* (1983), who reported mean counts at the level of 10^5 cfu/ml and 10^6 cfu/ml. Faecal streptococci are natural inhabitants in soil, food, water and gastro intestinal tract of humans and animals and faecal contamination seems to be the important route for spreading (Majhenic, 2006). They are the common contaminants in milk and are frequently responsible for producing bitterness, proteolysis and other defects in milk (Yadava *et al.*, 1983). The heat resistance of various species of this organism has been reported by McAuley *et al.* (2005). The presence of the organism in pasteurized milk indicated post pasteurization contamination.

4.1.1.6 Yeast and Mould Count

The mean Yeast and mould count of milk samples belonging to the brands A, B, C, D and E were 2.46 ± 0.17, 1.69 ± 0.12, 1.98 ± 0.14, 1.98 ± 0.20 and 1.35 ± 0.08 $\log_{10}$ cfu/ml, respectively (Table 12). The mean count of samples of brands A and E differed highly significantly ($P<0.01$) with the count of samples of other brands from Thrissur. The mean Yeast and mould count of milk samples belonging to the brands from Palakkad were 1.66 ± 0.09, 2.15 ± 0.23, 1.79 ± 0.20, 2.14 ± 0.17 and 2.40 ± 0.24 $\log_{10}$ cfu/ml, respectively (Table 29). The overall mean count of the samples from Thrissur and Palakkad districts were 1.89 ± 0.08 and 2.03 ± 0.09 $\log_{10}$ cfu/ml, respectively and is depicted in table 36.

The count in the present study was lower than that recorded by Arora and Sudarsanam (1986) and Lues *et al.* (2003) but greater than that reported by Prejit (2005). Cosentino and Palmas (1997) had not detected any yeast from heat-treated

milk. Yeast and mould has significance in causing spoilage of milk and some of them are capable of producing toxins which are heat stable and hence a matter of public health concern. High yeast and mould count in milk indicates unsanitary conditions of handling and contamination from air (Arora and Sudarsanam, 1986). The contamination of air in the processing area with yeast and mould was also reported by Cosentino and Palmas (1997). Yeast and mould are active at the low pH of milk produced as a result of storage and thus increase in the count may be due to the physio-chemical changes of milk during spoilage (Prejit *et al.*, 2006). Yeast and mould has significant role in spoilage of milk and some of them are capable of producing toxins which are heat stable and hence a matter of public health concern.

4.1.2 Interpretation on the Quality of Pasteurized Milk

4.1.2.1 Total Viable Count

Only 50 per cent of the samples from brand E were graded as satisfactory. None of the samples of other brands from Thrissur and Palakkad districts were graded as satisfactory according to the total viable count limits prescribed by BIS (1992). The quality of milk obtained from that brand is almost comparable with the findings of Prejit *et al.* (2006) who opined that 55 per cent of the samples examined by them were satisfactory grade whereas, Misra and Kuila (1989) reported that 40 per cent of samples from three dairy plants in Culcutta were within the prescribed standards. But Arora and Sudarsanam (1986) reported that none of the samples from sources A and B at Karnal were found to exceed the standard plate count limit of 30,000/ml for pasteurized milk as prescribed by ISI specifications. Out of the 100 samples from Thrissur district only 10 samples (10 per cent) were graded as satisfactory. None of the samples from Palakkad district were graded as satisfactory (Table 43). Similar observation was also recorded by Gopi *et al.* (2001) who reported that more than 94 per cent of the pasteurized milk tested in Chennai was of

unsatisfactory quality. Nanu and Latha (1997) cited that three out of five sources did not meet the standards.

4.1.2.2 Coliform Count

None of the samples collected from Thrissur met the coliform count criteria prescribed by Bureau of Indian Standards (1992). Seventy per cent of the samples belonging to the brand H was graded as satisfactory. Only 14 per cent of the total milk samples from Palakkad district were graded as satisfactory (Table 44). The observation of the current study was not comparable with the findings of Arora and Sudarsanam (1986) who reported that 50 per cent samples from source A and cent per cent of samples from source B were within limits of ISI specifications. Gopi *et al.* (2001) reported that 55 per cent of the pasteurized milk in Chennai possessed poor coliform standards when compared to BIS specifications and similar observation was also reported by Prejit *et al.* (2006) who reported that 40 per cent samples were satisfactory.

4.1.3 Isolation and Identification of Bacteria

4.1.3.1 Escherichia coli

All the brands of pasteurized milk were subjected to isolation and identification of *Escherichia coli.* Out of 100 samples collected from Thrissur, only 10 (10 per cent) revealed the presence of the organism (Table 14). Five, three and two isolates were obtained from the individual milk samples belonging to the retail brands A, D and C, respectively. The isolates consisted of serotype of O4 (1), rough strains (2) and untypable(7) (Table 15). Two of these isolates had congo red binding ability (Table 16) which indicates the property of invasiveness of the isolates. Out of 100 samples obtained from Palakkad, only 11 (11 per cent) revealed the presence of the organism (Table 31). Four isolates were obtained from the samples belonging to

the brand G, two isolates each were isolated from the samples of the brands F, H and J, and one isolate was obtained from the samples of the brand I. Among the isolates, one isolate was serotyped as O4 and the other three organisms were serotyped as O60. One of the isolates was typed as rough and six isolates were untypable (Table 32). Two of these isolates revealed congo red binding charecteristics (Table 33).

The per cent of *Escherichia coli* isolates obtained from the samples of various brands was lower than that recorded by John (1999), Silva *et al.* (2001), Prejit (2005) and Asha (2007) and was much higher than that reported by Sharma *et al.* (1995) and John *et al.* (2003). The findings of the present study disagrees with the reports of Aaku *et al.* (2004) who reported that the organism was not detected from any of the 84 samples of pasteurized milk. *Escherichia coli* is a commensal organism found in the intestine of humans and animals. Most of the strains are harmless but some strains bearing virulence properties have emerged as a serious public health hazard (Dooley and Roberts, 2006). Reports of food poisoning outbreaks caused by enteropathogenic strains (Saito *et al.*, 2005) had been reported from Japan. Presence of the organism in pasteurized milk indicates inadequate time temperature treatment of milk during pasteurization or post pasteurization contamination. There are chances that they have escaped detection owing to their small number (Sharma *et al.*, 1995). The isolation of *Escherichia coli* serotype O4 from milk is of great public health significance since the organism is associated with urinary tract infection.

4.1.3.2 Pseudomonas

Pseudomonas was isolated from four per cent of the retail samples from Thrissur (Table 14). The isolates were identified as *Pseudomonas aeruginosa* (2) and *Pseudomonas flourescens* (2) (Table 17). *Pseudomonas* was isolated from six per cent of the samples from Palakkad (Table 31) and the isolates were identified as *Pseudomonas aeruginosa* (2) and *Pseudomonas flourescens* (2) *Pseudomonas cepacia* (1) and *Pseudomonas putida* (1) (Table 34). The result was in agreement

with the findings of Grover and Srinivasan (1988), but was much lower than the findings of Griffiths and Phillips (1988) and Ternstrom *et al.* (1993). The occurrence of *Pseudomonas aeruginosa, Pseudomonas putida* and *Pseudomonas flourescens* in pasteurized milk samples was also reported by Dogan and Boor (2003).

Pseudomonas are the dominating spoilage microflora in refrigerated storage milk (Eneroth *et al.*, 1998). It has been reported that the proportion of the organism isolated from the samples decreases with increase in storage temperature (Griffiths and Philips, 1988). These species are capable of producing proteases and lipases and produces degradative changes in milk which ultimately results in the spoilage of milk (Kumaresan and Villi, 2008). Burdova *et al.*, (2002) cited that the lipases of *Pseudomonas flourescens* are not only heat resistant but also resistant to chemical denaturants. The presence of these organisms in pasteurized milk may be due to lack of maintenance of proper time temperature relationship during pasteurization of milk or due to post pasteurization contamination since the organisms are killed at pasteurization temperature of milk (Grover and Srinivasan, 1988).

4.1.3.3 Bacillus cereus

Of the 100 samples collected from Thrissur district, *Bacillus cereus* was isolated from two samples belonging to brand A and one sample belonging to brand D (Table 14). Among the samples collected from Palakkad district, the organism was isolated from one samples each belonging to the brands H and I. The findings of the present study was in agreement with that reported by Wong *et al.* (1988) that two per cent of the pasteurized milk samples in Taiwan were contaminated with the organism. The per cent of isolation of the organism in the present study was much lower than that reported by Giffel *et al.* (1997), who recorded the isolation of organism from 40 per cent of the samples and Larsen and Jorgensen (1997), who isolated the organism from 56 per cent of the samples.

Bacillus cereus causes food poisoning, and also produces emetic and diarrheal syndrome. The organism is an aerobic spore former and its presence in milk indicates the contamination of milk from the environment. Bacillus spores survive the heat treatment, germinate and multiply in the refrigerated milk and hence it is difficult to know whether recontamination has occurred along the processing line (Eneroth *et al.,* 1998). The practice of using skim milk powder for the reconstitution of milk is yet another source of spore formers in pasteurized milk (Ethiraj *et al.,* 1979).

4.1.4 Adulterants and Preservatives in the milk

None of the pasteurized milk samples from Thrissur and Palakkad districts had revealed the presence of adulterants like starch and cane sugar and preservatives like bicarbonates. Formaldehyde was detected from 19 per cent of the milk samples from Thrissur. Fifteen per cent of pasteurized milk samples from brand A and 80 per cent of samples from brand E had formaldehyde (Table 18). All the samples of brand G, 70 per cent of samples from brand J and 65 per cent of samples from brand I were positive for formaldehyde (Table 35). Nearly half of the (47 per cent) pasteurized milk samples from Palakkad had formaldehyde. The result of the present study was not in accordance with the findings of Rao *et al.* (2002), who could not detect formalin in the samples from private dairies. The relative lower counts in some of the samples may be attributed to the presence of formaldehyde in them.

4.3.2 Correlation between microbial counts of pasteurized milk from Thrissur and Palakkad districts

The study revealed a highly significant ($P<0.01$) and positive assosciation between the mean total viable count and coliform count of samples from Thrissur district. Siva *et al.* (1993) stated that coliform counts were positively and

significantly correlated with total viable counts of pasteurized milk samples collected from Anand. A positive and significant ($P<0.05$) association was seen between total viable count and yeast and mould count of samples from Thrissur district. A highly significant ($P<0.01$) and positive association was observed between the mean coliform count and yeast and mould count of the samples of Thrissur district. A similar association was observed between the mean coliform count and psychrotrophic count of samples of pasteurized milk from Palakkad district. A weak negative correlation was observed between the mean coliform count and *Escherichia coli* count of the samples of Thrissur district and was in agreement with the report of Lues *et al.* (2003). A highly significant ($P<0.01$) and negative correlation was found between the mean psychrotrophic count and faecal streptococcal count of pasteurized milk samples from Thrissur district. Also a positive and significant ($P<0.05$) correlation was observed between the mean yeast and mould count and psychrotrophic count of pasteurized milk collected from Thrissur district.

The present study will help in understanding the overall quality of pasteurized milk retailed in the market and the malpractices employed by the manufactures. This study will make the consumers aware of hygienic production and the need for microbiologically and chemically safe food. The present study emphasis the need for clean milk production and also highlights the significance of hygienic and sanitary practices required during processing, handling and storage of milk. The sources of contamination in the production of pasteurized milk have to be identified and continuous quality monitoring programs are to be launched to ensure the quality of milk and consumer safety. The study will give an insight into the overall quality of the milk in the market and will help the traders, retailers and societies to take necessary steps to improve the quality of milk so as to safeguard the consumer health.

Summary

6. SUMMARY

In the present study the microbiological quality of 200 pasteurized milk samples consisted of 100 samples each from Thrissur and Palakkad districts, were collected and evaluated to assess the microbial quality, detection of bacteria of public health significance and to detect the presence of adulterants and preservatives. Milk samples belonging to five different brands viz., A, B, C, D and E were selected from Thrissur district and the samples from five brands viz., F, G, H, I and J were collected from Palakkad district. From each brand, two samples were collected at a time and the collection of the samples from each brand was repeated ten times. The microbial quality of the samples was tested by estimating the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC), Psychrotrophic Count (PC) and Yeast and Mould Count (YMC). All samples were examined to detect the presence of bacteria of public health significance such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* and *Bacillus cereus*. The samples were also evaluated for the presence of adulterants and preservatives.

A highly significant ($P<0.01$) difference was observed between the mean total viable count (TVC) of brands of pasteurized milk samples of Thrissur district. The highest mean count was seen in samples of the brand A (5.39 ± 0.04 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of the brand E (4.51 ± 0.08 $\log_{10}$ cfu/ml). A highly significant ($P<0.01$) and positive association was observed between the mean total viable count and coliform count of samples from Thrissur district. Among the brands from Palakkad district, the highest mean count was seen in samples of the brand I (5.35 ± 0.05 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of brand G (5.19 ± 0.09 $\log_{10}$ cfu/ml). The overall mean total viable count of the samples from both the districts was 5 $\log_{10}$ cfu/ml.

According to the total viable count limit prescribed by BIS (1992), 10 per cent of samples belong to Thrissur district and none of the samples belong to Palakkad district was graded as satisfactory.

Among the samples from Thrissur district, coliform count (CC) was more in the samples obtained from brand A (3.40 ± 0.17 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of brand E (2.30 ± 0.09 $\log_{10}$ cfu/ml). The count in the samples of the later brand was highly significantly ($P<0.01$) different with the count of the samples of other brands. The association of the count with yeast and mould count of samples was highly significant ($P<0.01$) and positive. A highly significant ($P<0.01$) difference was observed between the mean coliform count of the samples of different brands belonged to Palakkad district. The highest mean count was observed in samples of the brand I (4.39 ± 0.02 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of the brand H (0.67 ± 0.34 $\log_{10}$ cfu/ml). Only 14 per cent of samples from Palakkad district were graded as satisfactory based on coliform count limit prescribed by BIS (1992) and none of the samples from Thrissur district was graded as satisfactory.

The samples belonging to brand A of Thrissur district had the highest mean *Escherichia coli* count (0.92 ± 0.31 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of the brand E (0.10 ± 0.10 $\log_{10}$ cfu/ml). Among the samples of the brands belonging to Palakkad district, the highest mean count (1.54 ± 0.11 $\log_{10}$ cfu/ml) was observed in the samples of brand I and the lowest count (0.43 ± 0.23 $\log_{10}$ cfu/ml) was seen in the samples of the brand H. Analysis of variance test revealed significant difference ($P<0.05$) between the mean counts of the samples from Palakkad district.

Among the samples from Thrissur, the highest mean psychrotrophic count (5.39 ± 0.02 $\log_{10}$ cfu/ml) was found in the samples of the brand C and the lowest

count (5.29 ± 0.05 $\log_{10}$ cfu/ml) was observed in the samples of brand B. A highly significant (P<0.01) and negative association of psychrotrophic count with faecal streptococcal count and a positive and significant (P<0.05) association with yeast and mould count was observed among the samples of the brands of Thrissur district. Among the samples from Palakkad district, the psychrotrophic count was maximum (5.43 ± 0.01 $\log_{10}$ cfu/ml) in the samples of the brand I and minimum (4.63 ± 0.10 $\log_{10}$ cfu/ml) in the samples of the brand H. The difference between the mean count of the samples obtained from various brands of Palakkad district was highly significant (P<0.01). A positive and highly significant (P<0.01) correlation was observed between psychrotrophic count and coliform count of the samples of the brands from Palakkad district.

The samples belonging to brand C of Thrissur district had the lowest mean faecal streptococcal count (2.95 ± 0.17 $\log_{10}$ cfu/ml) and the highest count was seen in the samples of the brand B (3.78 ± 0.16 $\log_{10}$ cfu/ml). A highly significant (p<0.01) difference was observed between the mean count of the samples of brand C with the mean count of all other brands from Thrissur district. Among the samples of the brands from Palakkad district, the lowest mean count was seen in the samples belonging to brand G (2.88 ± 0.12 $\log_{10}$ cfu/ml) and the highest count was seen in the samples of the brand I (3.51 ± 0.21 $\log_{10}$ cfu/ml).

Among the samples collected from Thrissur district, the highest mean yeast and mould count (2.46 ± 0.17 $\log_{10}$ cfu/ml) was seen in the samples of the brand A and lowest count (1.35 ± 0.08 $\log_{10}$ cfu/ml) was observed in the samples of the brand E. The mean count of samples of brand A differed highly significantly (P<0.01) with the count of samples of brands B, C, D and E. Correlation between the mean yeast and mould count and coliform count of the samples were highly significant (P<0.01) and positive. Among the samples from Palakkad, the highest

mean count was seen in the samples of the brand J (2.40 ± 0.24 $\log_{10}$ cfu/ml) and lowest count was in the samples of the brand F (1.66 ± 0.09 $\log_{10}$ cfu/ml).

During the study attempts were made to isolate and identify *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* and *Bacillus cereus* from all the samples collected from various brands belonging to Thrissur and Palakkad districts. *Escherichia coli* was isolated from 10 (10 per cent) samples collected from Thrissur. Five, three and two isolates were obtained from the samples of the brands A, D and C, respectively and the isolates consisted of serotype of O4 (1), rough strains (2) and untypable (7). From the samples of Palakkad 11 (11 per cent) samples revealed the presence of *Escherichia coli*. Four, two, two, one and two isolates were obtained from the samples of the brands G, F, H, I and J, respectively. The isolates consisted of serotypes O4 (1), O60 (3), rough (1) and untypable (6). Two isolates each from both the districts were positive for congo red binding ability.

Staphylococcus aureus was not isolated from any one of the samples belonging to the various brands from Thrissur and Palakkad districts. *Pseudomonas* was isolated from four per cent of retail samples from Thrissur and the isolates were identified as *Pseudomonas aeruginosa* and *Pseudomonas flourescens*. One isolate each of *Pseudomonas aeruginosa* was obtained from the samples of the brands A and C and one isolate each of *Pseudomonas flourescens* was obtained from the samples of the brands A and D. *Pseudomonas* was also isolated from six per cent of the samples from Palakkad and the isolates were identified as *Pseudomonas aeruginosa* (2) and *Pseudomonas flourescens* (2) *Pseudomonas cepacia* (1) and *Pseudomonas putida* (1). *Pseudomonas aeruginosa* was isolated from the samples of the brands F and J and *Pseudomonas flourescens* from the samples of the brands F and I. *Pseudomonas cepacia* was isolated from the samples of the brand J and *Pseudomonas putida* was isolated from the samples of the brand H.

Bacillus cereus was isolated from the samples belonging to both districts. Two isolates were obtained from the samples of the brand A and one isolate was obtained from the samples of the brand D. Among the samples of Palakkad district, one isolate each was obtained from the samples of the brands H and I.

All samples of pasteurized milk collected from both the districts were tested for the presence of starch, cane sugar, bicarbonates and formaldehyde. None of the samples from Thrissur and Palakkad districts had revealed the presence of the adulterants viz., starch and cane sugar and preservative like bicarbonates. But formaldehyde was detected from 19 per cent of the samples obtained from Thrissur. Fifteen per cent of the samples from brand A and 80 per cent of samples from brand E had formaldehyde. Of the samples collected from Palakkad, cent per cent of the samples belonging to brand G, 70 per cent of the samples from brand J and 65 per cent of the samples from brand I were positive for formaldehyde. Nearly half of the (47 per cent) samples from Palakkad was adulterated with formaldehyde.

The quality of pasteurized milk retailed in the two districts was far from satisfactory. The microbial counts were high compared to the standards. The presence of indicators of faecal contamination like coliform, faecal streptococci and *Escherichia coli* in milk indicated the unhygienic practices employed either during production or processing of milk. Some of the brands were adulterated with harmful substances like formaldehyde. The present study emphasis the need for clean milk production and also highlights the significance of hygienic and sanitary practices during processing, handling and storage. The identification of critical control points in the production of pasteurized milk and the implementation of suitable quality monitoring programs are essential to ensure the quality of milk produced and to safeguard consumer health.

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ASSESSMENT OF MICROBIAL QUALITY, ADULTERANTS AND PRESERVATIVES IN PASTEURIZED MILK

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

Master of Veterinary Science

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2008

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ABSTRACT

In the present study 200 pasteurized milk samples were collected from the retail shops of Thrissur and Palakkad districts. From Thrissur district, 20 samples each were collected from five brands viz., A, B, C, D and E, and an equal number of samples were obtained from the brands F, G, H, I and J of Palakkad district. All the samples were analyzed for the microbial quality by estimating various bacterial counts and yeast and mould count and also assessed the presence of certain pathogenic and spoilage bacteria. The milk samples were also tested to detect the presence of adulterants viz., starch and cane sugar and preservatives like carbonates and formaldehyde.

The study revealed that the samples belonging to Thrissur district had an overall mean total viable count, coliform count, *Escherichia coli* count, psychrotrophic count, faecal streptococcal count and yeast and mould count of 5.08 ± 0.05, 2.89 ± 0.09, 0.53 ± 0.11, 5.30 ± 0.01, 3.40 ± 0.14 and 1.89 ± 0.08 $\log_{10}$ cfu/ml, respectively. The corresponding count in the samples of Palakkad district was 5.24 ± 0.04, 3.01 ± 0.20, 0.78 ± 0.12, 4.99 ± 0.05, 3.20 ± 0.07 and 2.03 ± 0.09 $\log_{10}$ cfu/ml. According to the total viable count limit prescribed by BIS (1992) 50 per cent samples from brand E were graded as satisfactory and the samples from all other brands were graded as unsatisfactory. The highest mean total viable count was seen in the samples of brand A (5.39 ± 0.04 $\log_{10}$ cfu/ml). Of the samples collected from Thrissur district, the lowest count (4.51 ± 0.08 $\log_{10}$ cfu/ml) was recorded from the samples of brand E. The samples collected from I brand of Palakkad district had the highest mean total viable count (5.35 ± 0.05 $\log_{10}$ cfu/ml) and the lowest count (5.19 ± 0.09 $\log_{10}$ cfu/ml) was observed in the samples of brand G.

Of the 100 samples collected from Thrissur district, the samples belonging to brand A had the highest mean coliform count (3.40 ± 0.17 $\log_{10}$ cfu/ml). An equal number of samples collected from Palakkad district revealed

that the highest mean count (4.39 ± 0.02 $\log_{10}$ cfu/ml) was observed in the samples belonging to brand I. According to the bacterial count limit prescribed by BIS (1992) 70 per cent of the samples from brand H were graded as satisfactory and the samples belonging to all other brands were graded as unsatisfactory. The overall mean coliform count of the samples belonging to various brands from Thrissur and Palakkad districts were at the level of two and three $\log_{10}$ cfu/ml, respectively. The samples belonging to brand E of Thrissur and brand H of Palakkad had the lowest mean count.

The samples collected from brand A of Thrissur district had the highest mean *Escherichia coli* count (0.92 ± 0.31 $\log_{10}$ cfu/ml) and the lowest count (0.10 ± 0.10 $\log_{10}$ cfu/ml) was observed in the samples belonging to the brand E. Among the samples collected from Palakkad district, the highest mean count (1.54 ± 0.11 $\log_{10}$ cfu/ml) was observed in the samples of the brand I and the lowest count (0.43 ± 0.23 $\log_{10}$ cfu/ml) was seen in the samples belonging to the brand H.

The highest mean psychrotrophic count (5.39 ± 0.02 $\log_{10}$ cfu/ml) was seen in the samples belonging to brand C of Thrissur district and the lowest count (5.29 ± 0.05 $\log_{10}$ cfu/ml) was observed in the samples of the brand B. Among the samples from Palakkad district, the highest mean count (5.43 ± 0.01 $\log_{10}$ cfu/ml) was seen in the samples of the brand I and the lowest count (4.63 ± 0.10 $\log_{10}$ cfu/ml) was observed in the samples of the brand H.

Of the samples collected from Thrissur district, the lowest mean faecal streptococcal count (2.95 ± 0.17 $\log_{10}$ cfu/ml) was seen in samples belonging to brand C and the highest count (3.78 ± 0.16 $\log_{10}$ cfu/ml) was observed in the samples of the brand B. Among the samples belonging to various brands of Palakkad district, the lowest mean count (2.88 ± 0.12 $\log_{10}$ cfu/ml) was seen in samples of the brand G and the highest count (3.51 ± 0.21 $\log_{10}$ cfu/ml) was observed in the samples of the brand I.

Among the samples collected from the five brands of Thrissur district, the highest mean yeast and mould count (2.46 ± 0.17 log $_{10}$ cfu/ml) was seen in the samples of the brand A and the lowest count (1.35 ± 0.08 log $_{10}$ cfu/ml) was observed in the samples of the brand E. Of the samples belonging to the five brands of Palakkad, the highest mean count was seen in the samples of the brand J (2.40 ± 0.24 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of the brand F (1.66 ± 0.09 $\log_{10}$ cfu/ml).

A highly significant ($P<0.01$) difference was noticed among the mean total viable count, coliform count, faecal streptoccal count and yeast and mould count of various brands of pasteurized milk from Thrissur district. Similarly a highly significant ($P<0.01$) difference was noticed among the mean coliform count, *Escherichia coli* count and psychrotrophic count of the samples belong to the five brands of pasteurized milk from Palakkad district.

Escherichia coli was isolated from 10 per cent of the samples belonging to Thrissur and the isolates consisted of serotype of O4 (1), rough strains (2) and untypable strains (7). The organism was isolated from 11 per cent of the samples collected from Palakkad. One of the isolates was serotyped as O4 and three isolates were serotyped as O60. One isolate fell in the class rough and six isolates were untypable. Two isolates each from Thrissur and Palakkad districts revealed congo red binding charecteristics.

Staphylococcus aureus could not isolate from the samples obtained from Thrissur and Palakkad districts.

Pseudomonas organism was isolated from four and six per cent of the samples from Thrissur and Palakkad. The isolates were identified as *Pseudomonas aeruginosa*, *Pseudomonas flourescens*, *Pseudomonas cepacia* and *Pseudomonas putida.*

Bacillus cereus was isolated from three samples obtained from Thrissur district and two samples belonging to Palakkad district.

None of the samples from Thrissur and Palakkad districts revealed the presence of the adulterants like starch and cane sugar and preservative like bicarbonates. But formaldehyde was detected from 19 per cent of the samples from Thrissur and 47 per cent of the samples from Palakkad.