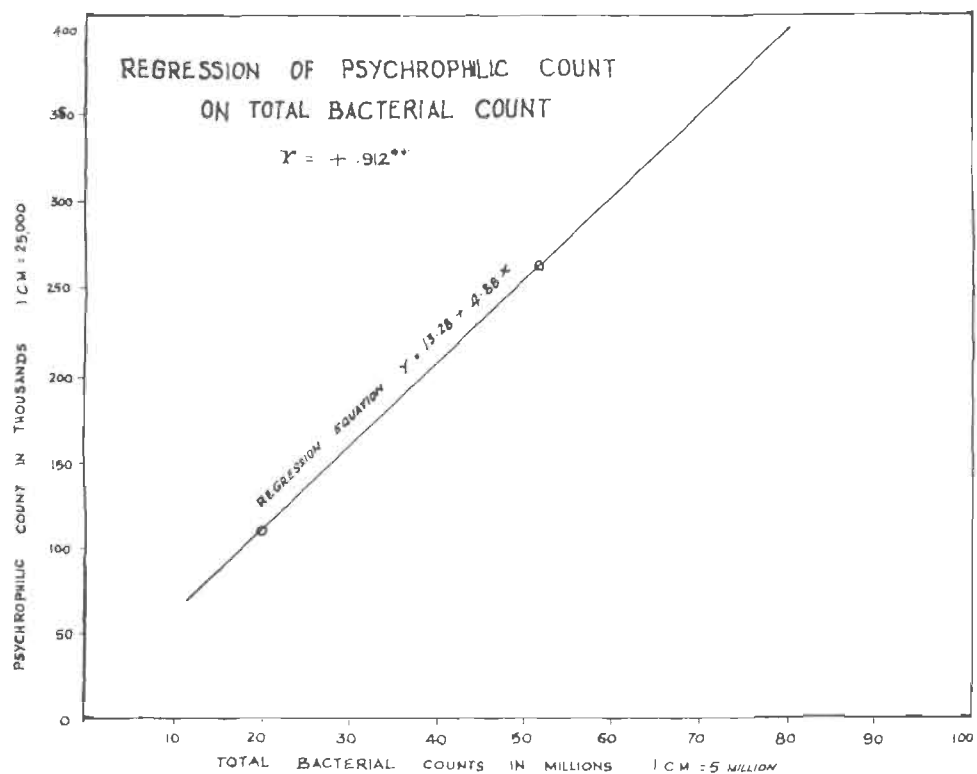


GRAPH - 1

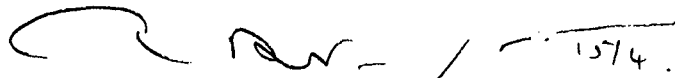


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Certificate.

I certify that this dissertation has been prepared under my supervision by Mr. R. Nagarajan, a candidate for the M.V.Sc. Degree (Dairy Science - Main) 1967, and that it incorporates the results of his independent study.

  
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BACTERIOLOGICAL STUDIES ON THE CHILLING OF MILK  
FOR BULK STORAGE AND TRANSPORT

A Dissertation

Submitted to the University of Madras  
in partial fulfilment of the requirements  
for the Degree of  
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## INTRODUCTION

Refrigeration has long been recognised as one of the methods of increasing the keeping quality of milk. But when milk of poor hygienic quality is stored at refrigeration temperatures for long periods it has been found that psychrophilic organisms increase in number and cause undesirable flavours which affect the quality of market milk.

In India the pattern of dairying is changing. The demand for milk in urban areas has increased considerably. It is estimated that three million tons of milk or 50 per cent of the total fluid milk marketed in India are consumed by the urban population. The bulk of the milk (over 90 per cent) is produced in villages (Iya, 1962). The demand for milk from urban areas has necessitated the pooling and transport of milk from long distances. From the time milk is produced till it reaches the processing plant considerable time lag occurs. Indian Union is situated in the tropical and subtropical zones and is generally characterised by high temperatures during major part of the year which is most favourable for bacterial proliferation. In order to combat these adversities of time lag and temperature chilling centres have been established for collection and transport of milk.

The knowledge regarding the keeping quality of milk at low temperatures has become important due to the activity of psychrophilic organisms at that temperature in which milk is stored for considerable periods before processing.

## REVIEW OF LITERATURE

Under the present system of dairying with regard to market milk the time lag between production and consumption is very long. Even though chilling helps to a great extent to increase the keeping quality of the milk it has been found that the number of psychrophilic bacteria determines the quality of milk.

### 1. Total bacterial count.

Park (1901) examined the bacteriological quality of ordinary commercial milk and good quality milk kept at 5°C for one, two, four and seven days and reported that appreciable growth occurred in the ordinary milk in two days and it took ten days for an equivalent amount of growth in the good quality milk. He further stated that holding at 10°C or above resulted in rapid growth of bacteria in both types of milk. Ravenel et al (1910) stated that marked increase in bacterial content was noticed in barn milk as well as dairy milk kept at 0°C and examined at varying intervals upto 203 days. Babel (1953) stated that high quality raw milk showed a significant increase in total bacteria after two to four days storage at 4.4°C. Marth et al (1954) reported that milk of good quality could be held in farm bulk cooling tanks for E.O.D. collection. At the same time milk with low initial count need not necessarily remain in good bacteriological condition during the storage period in the bulk cooling tanks. Atherton and Bredfield (1957) evaluated the bacteriological quality of milk under bulk collection system and found all samples regardless of original bacterial content gave colony counts at 5°C and 32°C over  $10^8$  per ml after five days at 5°C. Marth and

Frazier (1957) studied the effect of numbers of bacteria in milk on bacterial counts subsequently noticed after storage at 2.2, 3.3, and 7.2°C for a period of four days. They found that more samples of high count milk showed appreciable bacterial growth within one, two, three, or four days than the low count milk. Foster et al (1957) and Hammer and Babel (1957) showed that bacteria found in milk produced under good hygienic conditions did not multiply actively for a reasonable period at 4.4°C though they exceeded  $10^6$  per ml at 10°C. The total bacterial count of milk samples from two collecting routes was reported by Hesselberg (1960) as 2.5 to 3 times higher during periods of every other day collection than during daily collection. The increase was attributed to the admixture of warm milk to the cooled milk after each milking and failure to cool the milk immediately after milking. Huskey, Edmondson and Smith (1960) tested bacteriological quality of samples of homogenised milk kept at 36°F, 41°F, 46°F and 51°F upto ten days. The authors did not observe any change in bacterial content in the milk held at 36°F during the entire duration of storage. Increase in bacterial counts were noted as the storage temperature increased. Lacrosse and Pirauxe (1962) studied the bacteriological quality of milk stored at 4° and 7°C under bulk collection system and found that the multiplication rates of total numbers of bacteria were low except under conditions when the initial count was high. Thom Valerie (1962) stated that the bacterial development in tank milk during storage was markedly influenced by degree and type of contamination and rapid multiplication of organisms occurred at 4°C when gram negative rods were predominant.

Fossum and Kummenegje(1964) observed very slight increase in total count in milk of very high hygienic quality while making a study on the prolonged storage of milk in farm bulk tanks. Orr et al (1964) found the bacteriological quality of milk samples taken on alternate days from a bulk tank before collection to be satisfactory on the basis of bacteriological quality of samples taken after first days storage. On further investigations on the same subject Orr et al (1965) reported increased counts in both alternate day collected milk and in milk held for a further day at 5°C under bulk collection system. Ross (1965) collected raw milk from three machine milkings at 12 hour intervals added to a farm bulk milk tank and stored at 4.5°C. The milk was sampled before the addition of next milking. In six trials the milk after 12 hours at 4.5°C contained an average of 30,220 bacteria per ml. Average values 24 hours later after three milkings had accumulated were 83,533 per ml. Bockelmann (1965) while studying the bacterial development in tank milk reported that changes in the total count during storage for less than two days were generally small. Hadland, Bo and Solberg (1965) stated that the microbiological quality of milk stored in bulk tanks for four days was good except during the latter period. Jackson and Clegg (1966) observed that colony counts of samples of raw bulk tanks from 141 farms ranged from less than  $5 \times 10^8$  to more than  $2 \times 10^5$  per ml. Kruger (1966) reported that raw milk intended for more than 24 hours storage must not contain more than a million bacteria and be kept at a constant temperature of 3 to 4°C. Abo Elanga (1966) studied changes in the bacterial content of raw milk stored at 15°C, 10°C, 4°C and 2.5°C for a period of 25 days and reported an increase in the total count during storage.

The effect of blend temperatures in bulk tanks on the bacteriological quality of milk was investigated by Vandemark and March (1957). Total counts did not increase at blend temperatures of 45, 50 or 60°F. A significant difference was noticed between types and numbers of bacteria at a blend temperature of 70°F with a slight but significant increase in total counts.

## 2. Psychrophilic count.

Kennedy and Weiser (1950) defined psychrophils as those organisms having an optimum temperature range of 5 to 25°C for growth. Olson, Parker and Mueller (1955) used the term psychrophils to indicate bacterial species capable of growing at temperatures ranging from 35 to 45°F. Psychrophils or cold loving organisms are those which are able to grow at temperatures below 5°C and do not survive heating in milk at 63°C for 30 minutes (Thomas, 1958). Their temperature relationships are as follows:

|         |            |
|---------|------------|
| Minimum | 0 to 5°C   |
| Optimum | 10 to 20°C |
| Maximum | 25 to 35°C |

Foster et al (1957) defined psychrophils as those which are able to develop comparatively rapidly at temperatures below 15°C.

Lloyd Witter (1961) stated that psychrophils could be defined in four major different ways based on

- a) optimum growth temperature: as those having an optimum growth temperature below some specified temperature (20°C), but occasionally below 15 or 20°C.
  - b) ability to grow at low temperature: as those bacteria which grow at a relatively rapid rate at refrigeration temperature.
-

c) the method of enumeration: as those organisms capable of forming visible colonies on plates incubated at 5 to 7°C for seven to ten days.

d) criteria which are independent of temperature: as those which are predominantly gram negative rods, catalase positive, asporogenous, resistant to penicillin, non-acid forming, resistant to the toxicity of basic dyes.

#### Sources of Psychrophils.

(a) Water supplies: Castell and Garrard (1941) analysed well water samples in Canada and found that 85 per cent of them contained lipolytic and proteolytic organisms that were able to grow at 4 to 5°C. Jezeski and Macy (1946) examined 12 water samples obtained from Minnesota and Wisconsin creameries and found psychrophilic count exceeding  $10^3$  per ml in three instances. Thomas and Thomas (1947) made a study of psychrophilic microflora by examining 126 farm water supplies in Wales. They found that 3 per cent of the samples gave psychrophilic counts under 10 per ml. Thomas et al (1949), Hammer and Babel (1957) obtained from water many of the strongly lipolytic and caseolytic bacteria that affected the keeping quality of milk held at low temperatures. Parker et al (1953) stated that soil and water were the original sources of psychrophils in milk. Harmon (1957) investigated water supplies at 14 butter and 12 cottage cheese plants in U.S.A. and found that most of the supplies contained psychrophilic strains of lipolytic and proteolytic bacteria.

(b) Dairy equipment: Unsterile dairy equipment constituted the main source of contamination of milk with psychrophils. Morris (1941) reported that milk cans rinsed with contaminated water on return to the farm introduced psychrophils in large numbers. Marth et al (1954) reported that in bulk cooling tanks the valves

contributed large number of psychrophils to the milk passing through them. Thomas and Rowland (1957) examined rinses of washed milk cans at 5 creameries within half an hour of passage through can washing machines and observed that psychrophilic counts were less than 500 per ml in three instances and 150,000 and 60,000 in two instances. They further stated that milking machine clusters played a significant part as a psychrophilic source. Thomas et al (1963) examined farm dairy equipment in 100 farms for psychrophilic bacteria and reported that majority of the rinses did not contain large numbers of bacteria. They further reported that psychrophilic counts were higher in pipe line milking plants than in milking machine clusters, buckets, or cooling units.

(c) Other habitats: Thomas et al (1949) stated that psychrophilic colony counts at 3 to 5° C ranged from  $10^3$  to  $10^6$  per square foot, of sterile milk surfaces exposed for ten minutes in a fairly clean cow house during milking time. Swabbings of flanks and udder of the cows on examination gave  $10^5$  to  $10^6$  psychrophilic colonies per square foot. Cattle feed on examination gave a wide range of psychrophilic count. Cattle cake gave psychrophilic count as little as 73 per gm while counts over  $10^7$  per gram were recorded for oat grains.

Psychrophilic organisms in raw and chilled milk.

Thomas and Sekhar (1946) and Sekhar (1950), (1951) reported that psychrophilic colony count for a series of 137 farm milk samples examined after holding for 24 hours at atmospheric shade temperature ranged from less than 10 to more than  $10^8$  per ml. They stated that further contamination took place during cooling and bulking at country creameries and psychrophilic count exceeded  $10^8$  per ml.

Sekhar (1947) demonstrated the rapid increase in psychrophilic counts of raw milk held at 3 to 5°C as follows:

| Holding time at 3 to 5°C<br>(hours) | Mean colony count per ml<br>(21 days at 3 to 5°C) |
|-------------------------------------|---------------------------------------------------|
| 2 (fresh)                           | 6,400                                             |
| 24                                  | 7,500                                             |
| 48                                  | 1,192,000                                         |
| 72                                  | 14,700,000                                        |

Chandrasekhar (1950) while studying the effect of refrigeration (3 to 5°C) on the microbial population of raw milk observed that there was not even an initial drop in bacterial numbers after refrigeration for 21 days. Babel (1953) reported that high quality raw milk when held at 4.4°C for two to four days showed a significant increase in bacterial growth majority of which were psychrophils. Wartrous (1953) collected 75 raw milk samples from milk receiving platforms and kept them at 5°C for 21 days. The average psychrophilic count increased from 680 to 84 million during storage. Prouty (1955) examined 628 raw milk samples from three farm tanks for psychrophilic counts and standard plate count and observed that psychrophilic counts increased more rapidly than did the standard plate count but were not excessive after 24 hours of storage. After 48 hours one quarter of the samples had psychrophils exceeding  $10^5$  per ml. Thomas and Rowland (1957) stated that milk produced under good conditions with few psychrophils may show appreciable growth after holding for a few days at refrigeration temperature. The authors obtained the following results while examining the milk.

| Farm | Colony counts within<br>2 hours of milking<br>(7 days at 3 to 5°C) | Colony counts 1000 s/ml<br>after holding for 3 days<br>at 3 to 5°C |                     |
|------|--------------------------------------------------------------------|--------------------------------------------------------------------|---------------------|
|      |                                                                    | (7 days at<br>3 to 5°C)                                            | (7 days at<br>22°C) |
| 1    | 0                                                                  | .1                                                                 | 18.6                |
| 2    | 0                                                                  | .1                                                                 | 10.2                |
| 3    | 0                                                                  | .4                                                                 | 4.7                 |
| 4    | 580                                                                | 4,640                                                              | 6,750               |
| 5    | 1,540                                                              | 7,290                                                              | 4,580               |
| 6    | 9,600                                                              | 28,700                                                             | 11,700              |

Hammer and Babel (1957) illustrated the differences in the growth of psychrophils in two samples of low count milk held at 4.4°C. Sample one had the following counts per ml when fresh and after two, three and four days of storage, as 3,100, 4,200, 4,500 and 8,000. Sample two had corresponding counts as 5,000, 103,000, 420,000 and 1,700,000. Marth and Frazier (1957) stated that in raw milk samples stored at 2.2, 3.3 and 7.2°C for four days psychrophils increased in number more rapidly at 3.3 and 7.2°C in summer milk than in winter milk. They also found out that psychrophils grew faster especially in two days in low count milk. Foster et al (1957) and Hammer and Babel (1957) showed that milk contaminated by psychrophils derived from unsterile milk equipment, ~~rinse~~ water and manure in 24 hours at 10°C; had a colony count at 30°C exceeding  $10^6$  per ml. Atherton and Bredford (1957) observed that every other day pick up system was comparable to fresh milk held below 40°F. The psychrophilic count was identical in both cases. Lagrange and Nelson (1959) studied the extent of psychrophilic growth at 5°C for seven days in bulk tank milk samples collected during various seasons of the year. The psychrophilic and standard plate count of the samples ranged from less than 30,000 to

200,000. In many instances psychrophilic counts exceeded or was in the same range as standard plate count. Jayshankar (1962) reported that psychrophilic count was very low for raw and pasteurised fresh farm milk and village milk. Lacrosse and Pirauxe (1962) reported that multiplication rates of psychrophilic bacteria in raw milk stored in bulk tank at 4 and 7°C was low unless there was a high initial count of psychrophilic bacteria. Orr et al (1964) reported that the bacteriological quality of bulk cooled milk samples taken from farm tanks on alternate days was satisfactory as compared with the quality of samples taken after the first day's storage. However preincubation of samples at 13°C for 20 hours lead to an increased psychrophilic count, especially in second day samples. While making further investigation on alternate day collected milk and that held for a further day Orr et al (1965) reported that lipolytic and proteolytic organisms increased with storage at 5°C. Mourges and Auclair (1965) reported that psychrophilic proliferation took place in milk held at refrigeration temperature. Ross (1965) observed that in farm bulk tanks psychrophilic bacteria increased in number within 36 hours of storage.

Vandemark and March (1957) studied the influence of maximum temperature rise in bulk tanks on bacterial counts. They observed that psychrophilic counts did not increase at blend temperatures of 45, 50 and 60°F.

3. Keeping quality of milk at refrigeration temperature.

(a) Organoleptic quality: Flavour is an important quality of any food. In spite of a food being wholesome and nutritious, it will be rejected by the consumer if it does not have good flavour. The taste, smell and touch of the food perceived by the sensory receptors in the nose and mouth constitutes flavour. It is needless to say that flavour is of primary importance in market milk.

Sherman et al (1941) reported spoilage of milk held at low temperatures was characterised principally by proteolysis and bitter flavour. Sekhar (1947) found that most of the milk samples held at 3 to 5°C developed a slight fruity odour within five days. Davis (1951) stated that bitterness was a fault, characteristic of low temperatures brought about by butter fat degradation and formation of peptones. Pearson (1955) showed that psychrophils caused off flavours due to protein breakdown. Magill and Stein (1957) found that milk held in refrigerated farm bulk tank for more than five days was not suitable for manufacturing purposes since products made from such milks were characterised by undesirable flavour not removed by condensation of milk under vacuum. Atherton and Bredford (1957) stated that the flavour of milk cooled in farm bulk tanks and collected by E.O.D. system was good as compared to the daily collection system. Thomas (1958) reported that proliferation of psychrophilic organisms in milk held at low temperatures may cause spoilage of milk by development of fruity, rancid and putrid defects. Huskey, Edmondson and Smith (1960) studied the effect of temperature on the organoleptic quality of milk in

market channels by keeping samples of homogenised milk at four temperatures, 36, 41, 46 and 51°F upto ten days. They observed little or no change in flavour in milk held at 36°F during the entire duration of storage. Deterioration in flavour was noticed as the storage temperature increased and samples stored at 46 and 51°F remained acceptable for only about four days. Punch, Olson and Thomas (1961) made observations on population levels of pure cultures of psychophilic bacteria necessary to induce flavour or physical changes in pasteurised milk.

|                |                      |
|----------------|----------------------|
| Alcaligenes    | 2.5 to 14 millions.  |
| Flavobacterium | 8.3 to 120 millions. |
| Coliforms      | 2.7 to 150 millions. |
| Yeasts         | 2.5 to 16 millions.  |

Ellingsen (1962) stated that market milk collected from two Oslo dairy plants had good flavour for one week at 4°C and one day at 17°F. Rehe (1962) stated that organoleptic examination was a reliable means of detecting changes in the milk and neither total count nor count of mesophilic caseolytes gave reliable indication of quality of milk. Ellingsen (1962) reported that samples of bottled whole milk held at atmospheric temperature for 4½ hours in Oslo during June and July subsequently remained satisfactory with good flavour after 7 days storage at 4°C. Milk stored at 17°C remained acceptable for one to three days. Olson (1963) observed that four types of thermoduric psychophilic organisms could cause spoilage in milk stored at 45°F with fruity unclean bitter and sweet curdling defects. Fossum and Kummeneje (1964) stated that farm milk kept in refrigerated farm tanks for four to five days was satisfactory throughout with good flavour. Al Shabibi (1964) studied the effect of fatty acids on

the flavour of milk and concluded that the rancid flavour of milk was caused by several fatty acids the most prominent being capric and lauric acids. Schultze et al (1965) observed no trends in organoleptic scores in most of farm bulk tanks even under slow cooling conditions.

Vandemark and March (1957) observed rancid flavour in three out of 15 samples of bulk tank milk when blend temperature was raised from 60 to 70°F.

(b) Free fatty acids: The multiplication of psychrophilic organisms in milk is followed by the liberation of free fatty acids in milk due to the hydrolysis of fat by the enzymes liberated by the organisms. The free fatty acids are responsible for the rancid flavour noticed in milk.

Overcast and Skean (1959) studied the growth of certain lipolytic organisms and their influence on free fat acidity. Freshly pasteurised whole milk was inoculated with 25 pure cultures of lipolytic organisms mostly obtained from market milk and kept at 4 ± 1°C for 12 days. Growth of cultures was accompanied by increase in free fat acidity. Harper and Gould (1959) stated that the free fatty acid content of homogenised milk kept at 40°F increased during storage. Hadland, Bo and Solberg (1965) stated that the free fatty acid content was a useful indicator of chemical quality of milk while studying the milk quality following collection and storage of milk.

Thomas, Harper and Gould (1954) stated that the first drawn milk had the highest and the last drawn milk the lowest acid degree value. El Hagarawy (1963) stated that freshly drawn milk contained measurable amounts of free fatty acids.

Hunter and Deane (1964) observed that the average acid degree value of fresh milk increased from first month to the fifth month and varied during the remainder of the lactation period. They stated that the storage of milk at  $35 \pm 2^{\circ}\text{F}$  for 48 hours slightly increased the acid degree value.

Vandemark and March (1957) reported that an increase of blend temperature from 60 to  $70^{\circ}\text{F}$  caused an increase in acid degree value by 0.2. Results obtained from experiments simulating every other day pickup showed a significant increase in acid degree value at blend temperature of  $70^{\circ}\text{F}$  but not at 40 or  $60^{\circ}\text{F}$ . The increase in acid degree value between 24 to 48 hours was less than the samples picked up daily. This was attributed to the dilution of rancidity by addition of fresh milk supplies.

(c) Titration acidity: Babel (1953) reported that the acidity of milk increased slowly during storage at  $4.4^{\circ}\text{C}$ . Ravenel et al (1910) noticed increases in acidity in barn milk held at  $0^{\circ}\text{C}$  and examined at various intervals.

### SCOPE OF WORK

On account of the starting of large number of processing plants in urban areas, chilling centres are being established for collection, storage and transport of milk to the plant.

From the review of literature it could be seen that the keeping quality of chilled milk depended upon the bacterial load at the time of chilling. It is a well known fact that the hygienic quality of milk produced under the present conditions in India is poor. Hence the knowledge of the keeping quality of milk stored at  $4.4 \pm 0.5^{\circ}\text{C}$  has become very important.

The present investigation was taken up to study the following:

#### 1. Laboratory study.

- a) Effect of storage at  $4.4 \pm 0.5^{\circ}\text{C}$  on the total bacterial count and the keeping quality of milk.
- b) Effect of storage at  $4.4 \pm 0.5^{\circ}\text{C}$  on the psychrophilic count and the keeping quality of milk.

#### 2. Field study.

- a) Effect of chilling and storage on the total bacterial count, psychrophilic count and the keeping quality of milk.
- b) Effect of transport on the bacterial and keeping quality of chilled milk.

3. Statistical analysis of the results to evolve a tentative bacterial standard in milk for chilling.

## MATERIALS AND METHODS

### Laboratory study.

Ten samples of raw milk from individual producers and ten samples of pooled raw milk from various parts of the city (each 2 litres) were collected under sterile conditions and brought to the laboratory in an ice chest. The samples were divided into four equal portions in four sterile half litre bottles. One portion was analysed immediately for

Total bacterial count,  
Psychrophilic count,  
Organoleptic quality,  
Titrable acidity,  
Free fatty acid content.

The other three portions were placed in the frigidaire at  $4.4 \pm 0.5^{\circ}\text{C}$  for examination at 24 hour intervals. The total bacterial count, psychrophilic count and titrable acidity were determined as per the methods recommended by the standard methods for the examination of dairy products (A.P.H.A., 1960).

The organoleptic quality was determined by smelling the samples immediately after opening it and by placing a drop of the milk on the back of the tongue.

The sample of milk was centrifuged in Babcock's cream test bottles and the cream was pipetted out and converted into ghee by direct heating. The free fatty acid content in ghee was determined as per the methods recommended in I.C.A.R. Bulletin No. 70 (1951).

Field study.

A total of 16 samples were analysed for field study. From chilling centres half litre each of pooled raw milk, chilled milk just before shipment to the tanker, and chilled milk from the tanker immediately on arrival at the plant, were collected under sterile conditions in 500 ml sterile sample bottles and brought to the laboratory in an ice box (packed with salt and ice). They were examined separately for the above mentioned tests.

## RESULTS AND DISCUSSION

### Laboratory study.

- (a) Effect of storage at  $4.4 \pm 0.5^{\circ}\text{C}$  on the total bacterial count and the keeping quality of milk.

The total bacterial count of raw milk samples from individual animals ranges from a minimum of 80,000 per ml to a maximum of 1,600,000 per ml on initial examination as represented in Table No.1. It is also seen from the table that during storage at  $4.4 \pm 0.5^{\circ}\text{C}$  for 72 hours the total bacterial count increases and reaches 5,000,000 per ml in the case of minimum count and 7,000,000 per ml in the case of maximum count. The average initial count of the ten samples is 684,600 and the average total bacterial count after 72 hours storage is 8,640,000. Table 2 represents the total bacterial counts of pooled raw milk samples collected from various parts of the city during storage at  $4.4 \pm 0.5^{\circ}\text{C}$ . The minimum and the maximum initial total bacterial counts are 9,000,000 and 94,000,000 per ml and the corresponding counts after 72 hours storage are 27,000,000 and 224,000,000. The average initial total bacterial count of the ten samples of pooled raw milk is 39,000,000 and the average count after 72 hours storage is 102,700,000. It is quite clear from Tables 1 and 2 that significant increases in total bacteria occur in milk during storage at  $4.4 \pm 0.5^{\circ}\text{C}$ . This is in accordance with the findings of Park (1901) and Babel (1953).

The effect of total bacterial count on the keeping quality of milk stored at  $4.4 \pm 0.5^{\circ}\text{C}$  is dealt with in Table 3. From the table it is seen that samples with initial total bacterial counts ranging from 80,000 per ml to 11,000,000 per ml remain in good condition for 48 hours and develop off-flavour between 48 and

72 hours of storage. Milk samples with counts ranging from 12,000,000 per ml to 94,000,000 per ml remain acceptable for only 24 hours and develop off-flavour between 24 and 48 hours of storage. This clearly indicates that the keeping quality of milk reduces as the total bacterial count increases.

The titrable acidity as seen from the table increases during storage in all samples. But there is no correlation between the initial total bacterial count and the titrable acidity since the average titrable acidity of samples with low and high counts are the same. The changes noticed in the titrable acidity during storage also does not have any correlation with the total bacterial count. Besides the organoleptic quality also cannot be judged from the titrable acidity since it is seen from the table that samples with acidity in the normal range develop off-flavour during storage.

(b) Effect of storage at  $4.4 \pm 0.5^{\circ}\text{C}$  on the psychrophilic count and the keeping quality of milk.

The psychrophilic count of samples of milk from individual animals during storage at  $4.4 \pm 0.5^{\circ}\text{C}$  is presented in Table 4. The minimum and maximum initial psychrophilic counts are 350 and 8,000 per ml and the corresponding counts after 72 hours of storage are 25,000 and 90,000. The average initial psychrophilic count is 2,657 and the average count after 72 hours of storage is 86,740. Table 5 represents the psychrophilic counts of samples of pooled raw milk during storage at  $4.4 \pm 0.5^{\circ}\text{C}$ . The minimum and maximum initial psychrophilic counts are 30,000 and 650,000, and they increase during 72 hours of storage to 250,000 and 2,600,000. The average initial psychrophilic count is 225,000 and the average psychrophilic count after storage is 1,583,000. An observation

of Tables 4 and 5 reveals that rates of multiplication of psychrophilic bacteria is a little greater in samples with low counts than in samples with high counts. This concurs with Marth and Frazier's (1957) findings. A comparison of Tables 4 and 5 with 1 and 2 shows that psychrophils grow at a faster rate than total bacteria.

The relationship between the initial psychrophilic count and the keeping quality of milk stored at  $4.4 \pm 0.5^{\circ}\text{C}$  is represented in Table 6. As in Table 3 it is seen that the keeping quality of milk decreases as the psychrophilic count increases. Samples with counts ranging from 350 to 100,000 keep well for 48 hours and develop off-flavour during 48 and 72 hours of storage. Samples with counts ranging from 150,000 to 650,000 exhibit off-flavour in 48 hours of storage itself. It is also seen from Tables 3 and 6 that initial psychrophilic count increases commensurate with the increase in initial total bacterial counts.

In all samples there is an increase in free fatty acid content during storage at  $4.4 \pm 0.5^{\circ}\text{C}$ . The increase observed in free fatty acid content in samples with high initial psychrophilic counts is higher than in samples with low initial psychrophilic counts. The slight increase in the initial free fatty acid content of pooled raw milk samples with high psychrophilic counts may be due to the mixture of milk from animals in various stages of lactation. From the table it is seen that in samples with low psychrophilic counts the free fatty acid content at the time of off-flavour development is between .19 and .28. In samples with high counts the off flavour development occurs when free fatty acid content ranges from .34 to .39. This shows that off-flavour development in low count samples may be due to protein breakdown

which is also caused by psychrophilic proliferation (Pearson, 1955).

Field study.

- (a) Effect of chilling and storage on the total bacterial count, psychrophilic count and keeping quality of pooled raw milk.

The effect of chilling and storage on the total bacterial count of samples of pooled raw milk during storage for less than 24 hours is presented in Table 7. The initial total bacterial count of raw milk given is the average of the total bacterial counts of morning and evening milk. The minimum and maximum initial total bacterial counts recorded are 5.6 million and 46 million, and the corresponding counts after storage at 4.4°C are 7,800,000 and 56,000,000. The average initial total bacterial count is 23,000,000 and increased count after storage is 29,000,000. In Table 8, the changes noticed in total bacterial count during storage for 24 hours is recorded. The minimum and maximum total bacterial counts are 5,000,000 and 56,000,000 and the corresponding counts after 24 hours storage are 7.6 million and 86,000,000. The average initial total bacterial count of raw milk is 37,500,000 and the average count after 24 hours storage at 4.4°C is 51,600,000. The increase observed in the total bacterial counts during storage for less than 24 hours and 24 hours is less when compared to the results obtained in the laboratory study. This may be due to the admixture of fresh chilled milk of the evening in the stored morning milk.

The effect of chilling and storage on the psychrophilic count of pooled raw milk during storage at 4.4°C for less than 24 hours is presented in Table 9. Here again the initial

psychrophilic count given is the average psychrophilic count of the morning and evening milk. The minimum and maximum initial psychrophilic counts are 60,000 and 220,000 and after storage for less than 24 hours the corresponding counts are 96,000 and 350,000. The average initial psychrophilic count is 135,000 and after storage it increases to 179,800. In Table 10 the initial psychrophilic counts of raw milk and chilled raw milk stored for 24 hours is given. The minimum and maximum psychrophilic counts in raw milk is 40,000 and 230,000, and after storage at 4.4°C for 24 hours the corresponding counts are 62,000 and 287,000. The average initial psychrophilic count is 170,000 and the average psychrophilic count after storage is 287,000.

The results obtained for psychrophilic counts during storage is comparable to the findings in the laboratory study though slight decrease in the counts are noted which may be due to the addition of fresh chilled milk of the evening to the stored morning milk. The increase noted in psychrophilic counts during 24 hours storage is more than <sup>that</sup> during less than 24 hours of storage.

Table 11 deals with the effect of total bacterial count on the keeping quality of chilled and stored milk for 24 hours and less than 24 hours at 4.4°C. The organoleptic quality of milk is good for samples with bacterial counts ranging from 5,000,000 per ml to 56,000,000 per ml. The titrable acidity increases in several cases during storage. In Table 12 the effect of psychrophilic count on the keeping quality of chilled and stored milk for 24 hours and less than 24 hours is presented. The milk with initial counts ranging from 60,000 to 230,000 does not develop off-flavour during 24 hours or less than 24 hours storage at

4.4°C. A slight but significant increase is observed in free fatty acid content during storage. The increase in free fatty acid content is less when compared to the increase in laboratory study and this may be due to the dilution of rancidity with the addition of fresh chilled milk in the evening to the chilled and stored milk of the morning.

(b) Effect of transport on the bacterial and keeping quality of chilled milk.

The effect of transport on the bacterial and keeping quality of chilled milk is tabulated in Table 13. It is seen from the table that there is no change in the keeping quality during transport. The titrable acidity does not increase. A very slight increase is observed in free fatty acid content which may be due to activity of lipase caused by agitation or due to the activity of psychrophils itself during transport time of 4.5 hours. Considering all the facts together it can be said that there is no appreciable change in the bacterial and keeping quality during transport. The reason for this may be the minor rise in temperature to the extent of 1°C and shorter duration of transport i.e., 4.5 hours.

Statistical analysis of the results to evolve a tentative bacterial standard in milk for chilling.

To arrive at a tentative bacterial standard in milk for chilling, statistical analysis of Tables 3, 6, 11 and 12 was done. The correlation coefficient between total bacterial count and psychrophilic count was worked out. It was found that a highly significant positive correlation exist between the initial total bacterial count and psychrophilic count in raw milk. The

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regression line in Graph 1 indicates the direct relationship between the total bacterial count and psychrophilic count.

Correlation coefficient and regression coefficient  
between total bacterial count and psychrophilic count

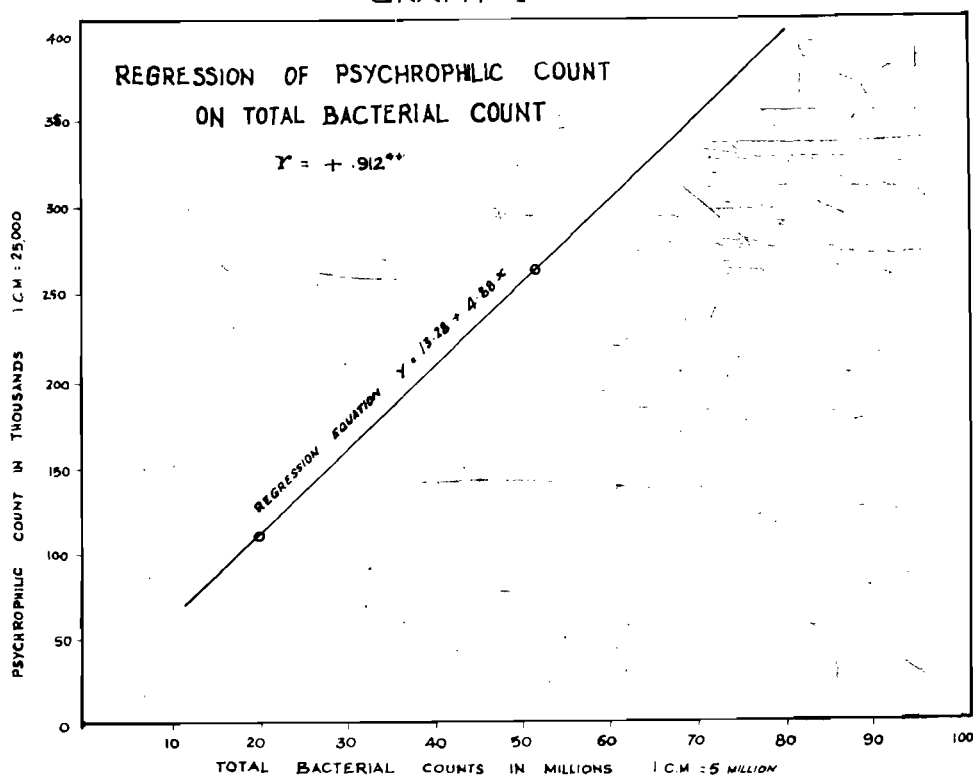
| No. of<br>samples        | Sum of squares and products<br>of deviation from mean. |             |              | Correla-<br>tion co-<br>efficient | Regression<br>coefficient |
|--------------------------|--------------------------------------------------------|-------------|--------------|-----------------------------------|---------------------------|
|                          | $\Sigma x^2$                                           | $\Sigma xy$ | $\Sigma y^2$ |                                   |                           |
| 36                       | 20,134.6218                                            | 98,288.8619 | 576,658.8111 | +0.912                            | 4.88                      |
| r for 34 d.f. = +0.912** |                                                        |             |              |                                   |                           |

From the review of literature it could be seen that psychrophils are responsible for the spoilage of milk held at low temperatures by causing fruity, rancid and putrid defects (Sherman, 1941; Thomas, 1958). To increase the keeping quality of milk held at low temperature it is essential to reduce the number of psychrophils in milk. Since there is a highly significant positive correlation between total bacterial count and psychrophilic count in milk the total bacterial count will indirectly reflect the psychrophilic count.

A perusal of Tables 3 and 6 reveals that milk samples with total bacterial counts upto 11 million per ml and corresponding psychrophilic counts of 100,000 per ml remain with good flavour for 48 hours at  $4.4 \pm 0.5^\circ\text{C}$ . Off-flavour develops between 24 and 48 hours in milk samples with bacterial counts exceeding 11 millions per ml.

Hence a tentative bacterial standard of 11 millions per ml is suggested in milk intended for chilling and storage for 48 hours.

GRAPH - 1



### SUMMARY AND CONCLUSIONS

A total of 20 samples of raw milk (ten samples of pooled raw milk, ten samples from individual animals) were stored at  $4.4 \pm 0.5^{\circ}\text{C}$  for 72 hours and the total bacterial count, psychrophilic count, organoleptic quality, titrable acidity and free fatty acid content were estimated initially and at intervals of 24 hours.

The total bacterial count, psychrophilic count, titrable acidity and free fatty acid content increased during storage. A highly significant positive correlation was found between the initial total bacterial count and psychrophilic count. The shelf life of the milk samples reduced as the initial total bacterial counts increased. This may be attributed to the increase in psychrophilic counts consequent to the increase of total bacterial counts. The organoleptic quality of milk samples with initial total bacterial counts upto 11 million per ml remained good till 48 hours of storage. In milk samples with initial total bacterial counts exceeding 11 millions per ml, off-flavour developed during 24 and 48 hours of storage. Hence tentative bacterial standard of 11 million per ml was suggested in milk for chilling.

The titrable acidity increased during storage gradually in all samples stored at  $4.4 \pm 0.5^{\circ}\text{C}$ . There was no correlation between initial total bacterial count and initial titrable acidity. Correlation was not also found between initial total bacterial count and increases in titrable acidity noticed during storage.

The free fatty acid content increased during storage in all samples. A non-significant positive correlation was found between initial psychrophilic count and free fatty acid increase during storage. The off-flavour development was noticed in several samples when <sup>(A)</sup>the free fatty acid content was 0.33 per cent and above. In some samples the off-flavour developed when the free fatty acid content was as low as 0.19 or 0.20 per cent. This may be attributed to the protein degradation following the growth of psychrophilic organisms (Pearson, 1955; Sekhar, 1947).

In chilling centres, the total bacterial count, psychrophilic count, organoleptic quality, titrable acidity and free fatty acid content of raw milk and chilled milk stored for 24 hours and less than 24 hours were studied. The increase obtained in total bacterial count, psychrophilic count and free fatty acid content were comparatively low when compared with the results of the laboratory study. This may be attributed to the admixture of fresh chilled milk to the chilled and stored morning milk.

The effect of transport of chilled milk from chilling centres to the dairy plant on the bacterial and keeping quality was studied. Appreciable changes did not occur in bacterial and keeping quality after transport. The shorter duration of transport of 4½ hours and rise in temperature to the extent of only 1°C may be the cause for such minor variation.

# BIBLIOGRAPHY

- Abo Elanga 1966 Archlebensmittelhyg. 17(6):125-128\*
- American Public Health Association 1960 Standard Methods for the Examination of Dairy products.
- Atherton, H.V. and Bradfield, A. 1957 J.Milk.Food.Tech., 20:223.
- Atherton, M.V. and Bredford, A. 1957 J.Milk Food Tech., 20:223.
- Babel, F.J. 1953 J.Dairy Sci., 36:562.
- Bockelmann. 1965 Svenska Mejeritdn, 57(36):425-430\*
- Castell, G.H. and Garrard, E.H. 1941 Canad.Dairy Ice.Cr.J. 20(3):18.
- Chandrasekhar, C.V. 1950 Indian J.Dairy Sci., 3(4):173-178.
- Davis, J.G. 1951 Proc.Soc.Appl.Bact., 14:216.
- Ellingsen, J.K. 1962 Meieriposten 51(10):196-201\*  
51(34):699-702\*
- Fossum, K. and Kummeneje, K. 1964 Meieriposten 53(30):723-728\*
- Foster, R.M., Nelson, F.R., Speck, M.L., Doetsch, R.N. and Olson, J.C. Jr. 1957 Dairy Microbiology. Prentice Hall Inc.
- Hadland, G., Bo, S. and Solberg, P. 1965 Meieriposten, 54(20):409-416\*  
(21):451-460\*  
(22):473-479\*
- Hagarawy, I.S. El. 1963 Indian J.Dairy Sci., 16:86-91.
- Hammer, B.W. and Babel, F.J. 1957 Dairy Bacteriology, 4th ed. New York. Wiley & Sons, Inc.
- Harmon, L.G. 1957 J.Milk Food Tech., 20:196.
- Harper, W.J. and Gould, I.A. 1959 J.Dairy Sci., 60:86-91.
- Hesselberg, S. 1960 Svenska Mejeritdn, 52(8):101-102\*
- Hunter and Deane, D.D. 1964 J.Dairy Sci., 47:110.
- Huskey, J., Edmondson, J.E. and Smith, K. 1960 J.Dairy Sci., 43(6):843.

- Indian Council of Agri- 1951 Bulletin No.70. Manager of  
cultural Research. Publications, Delhi.
- Iya, K.K. 1962 Milk Hygiene. W.H.O.Geneva. p.613  
& 614
- Jackson, M. and 1966 Canad.J.Microbiol. 12(3):429-432.  
Clegg, L.F.L.
- Jayshankar, S.R. 1962 Indian J.Dairy Sci., 15(3):127.
- Jezeski, J.J. and 1946 J.Dairy Sci., 29:439.  
Macy, H.
- Kennedy, L. and 1950 J.Milk Food Tech., 13:353.  
Weiser, H.H.
- Kolar, C.W. Jr. and 1962 J.Dairy Sci., 45:645.  
Mickle, J.B.
- Kruger, K.E. 1966 Archlebensmittelhyg, 17(6):128-131\*
- Lacrosse, R. and 1962 Int. Dairy Cong.App. 473-478.  
Pirauze, E.
- Lagrange, W.S. and 1959 J.Dairy Sci., 42:905.  
Nelson, F.E.
- Lloyd D.Witter. 1961 J.Dairy Sci., 44:983.
- Magill, B.F. and 1957 Proc.W.Div.Amer.Dairy Sci.Asso.\*  
Stein, R.W.
- Marth, E.H. and 1957a, b. J.Milk Food Tech. 20:72 and 79.  
Frazier, W.C.
- Morris, C.S. 1941 Abstr.Proc.Soc.Agric.Bact., p.33.
- Mourges, R. and 1965 Industrie.bact.Paris. 220:192-198\*  
Auclair, J.
- Olson, H.C. 1963 J.Dairy Sci. 46:362.
- Olson, J.C., Parker, 1955 J.Milk Food Tech., 18(8):200.  
H.B. and Mueller, W.S.
- Orr, M.J., Mc Larty, 1964 Dairy Ind. 29(3):169-173.  
R.M., Mc Cance, M.E. and  
Bainess, S.
- Orr, M.J., Margaret, J., 1965 Dairy Ind. 30(4):278-284.  
Mc Larty, R.M. and  
Bainess, S.

- |                                                                                    |                                       |
|------------------------------------------------------------------------------------|---------------------------------------|
| Overcast, W.W. and<br>Skean, J.D.                                                  | 1959 J.Dairy Sci., 42:1479-1485.      |
| Park, W.H.                                                                         | 1901 J.Hyg.Camb., 1:391.              |
| Pearson.                                                                           | 1955 Canad.Dairy Ice.Cr.J., 34(5):35. |
| Prouty, C.C.                                                                       | 1955 J.Milk Food Tech., 18:250.       |
| Punch, J.D., Olson,<br>J.C.Jr. and Thomas El.                                      | 1961 J.Dairy Sci., 44(6):1160-1161.   |
| Ravenel, M.P., Hastings<br>E.G., and Hammer, B.W.                                  | 1910 J.Infect.Dis., 7:38.             |
| Rehe, H.D.                                                                         | 1961 Inaug.Diss.Doct.Med.Vet.*        |
| Ross, T.J.                                                                         | 1965 Riv.Latti. 21(1):17-26*          |
| Schultze, E., Johnson,<br>J.W., Smith, L.E. and<br>Campbell.                       | 1965 J.Dairy Sci. 48:771.             |
| Sekhar, C.V.                                                                       | 1947 University of Wales. Thesis*     |
| Sekhar, C.V.                                                                       | 1950 Indian J.Dairy Sci. 3:173.       |
| Sekhar, C.V.                                                                       | 1951 J.Soc.Dairy Tech., 4:253.        |
| Shabibi, M.M.A., Al.,<br>Langner, E.H., Toblas,<br>J. and Tuckey, S.L.             | 1964 J.Dairy Sci., 47:295.            |
| Sherman, J.M., Cameron,<br>G.M. and White, J.C.                                    | 1941 J.Dairy Sci., 24:526.            |
| Thomas, S.B.                                                                       | 1958 D.S.A. Vol.20. p.357.            |
| Thomas, S.B, Hobson,<br>Phyllis, M., King.,<br>Kay, P., Griffiths and<br>Janet, M. | 1963 Dairy Engng. 80(8):290-292.      |
| Thomas, S.B. and<br>Rowland, A.M.                                                  | 1957 D.S.A. Vo.20. p.364 & 366.       |
| Thomas, S.B. and<br>Sekhar, C.V.                                                   | 1946 Proc.Soc.appl.Bact. p.47.        |
| Thomas, S.B., and<br>Thomas, B.F.                                                  | 1947 Proc.Soc.Appl.Bact. p.65.        |
| Thomas, S.B., Thomas,<br>B.F. and Ellison, D.                                      | 1949 Dairy Ind. 14:921.               |

- Thomas, W.H., Harper,  
W.J. and Gould, I.A. 1954 J.Dairy Sci., 37:717.
- Thom Valerie, M. 1962 Int.Dairy Cong.App. 409-416.
- Vandemark, P.J. and  
March, R.P. 1957 J.Milk Food Tech., 20:317-322;  
348-351.
- Wartrous, G.H. Jr. 1953 Southern Dairy Products J.54(3):162.

\* Dairy Science Abstracts.

TABLE - 1

Total bacterial count per ml of samples from individual animals during storage at  $4.4 \pm 5^{\circ}\text{C}$

| Particulars | 0-hours   | 24-hours  | 48-hours  | 72-hours  |
|-------------|-----------|-----------|-----------|-----------|
| Minimum     | 80,000    | 600,000   | 3,000,000 | 5,000,000 |
| Maximum     | 1,600,000 | 5,400,000 | 6,600,000 | 7,000,000 |
| Average     | 684,600   | 1,884,000 | 4,106,000 | 8,640,000 |

TABLE - 2

Total bacterial count per ml of pooled raw milk samples during storage at  $4.4 \pm 5^{\circ}\text{C}$

| Particulars | 0-hours    | 24-hours    | 48-hours    | 72-hours    |
|-------------|------------|-------------|-------------|-------------|
| Minimum     | 9,000,000  | 14,000,000  | 20,000,000  | 27,000,000  |
| Maximum     | 94,000,000 | 134,000,000 | 174,000,000 | 224,000,000 |
| Average     | 39,400,000 | 62,400,000  | 80,800,000  | 102,700,000 |

TABLE - 3

Total bacterial count on the keeping quality  
of milk stored at  $4.4 \pm 5^{\circ}\text{C}$

| Initial total<br>bacterial<br>count/ml. | Organoleptic quality and titrable acidity |      |          |      |          |      |          |      |
|-----------------------------------------|-------------------------------------------|------|----------|------|----------|------|----------|------|
|                                         | 0-hours                                   |      | 24-hours |      | 48-hours |      | 72-hours |      |
|                                         | O.Q.                                      | T.A. | O.Q.     | T.A. | O.Q.     | T.A. | O.Q.     | T.A. |
| 80,000                                  | N                                         | .16  | N        | .17  | N        | .19  | 0        | .21  |
| 186,000                                 | N                                         | .17  | N        | .17  | N        | .19  | 0        | .20  |
| 200,000                                 | N                                         | .16  | N        | .17  | N        | .18  | 0        | .20  |
| 350,000                                 | N                                         | .17  | N        | .18  | N        | .19  | 0        | .20  |
| 630,000                                 | N                                         | .17  | N        | .17  | N        | .19  | 0        | .20  |
| 650,000                                 | N                                         | .16  | N        | .17  | N        | .17  | 0        | .19  |
| 700,000                                 | N                                         | .17  | N        | .17  | N        | .19  | 0        | .20  |
| 950,000                                 | N                                         | .16  | N        | .17  | N        | .19  | 0        | .21  |
| 1,500,000                               | N                                         | .18  | N        | .18  | N        | .20  | 0        | .21  |
| 1,600,000                               | N                                         | .17  | N        | .18  | N        | .20  | 0        | .21  |
| 9,000,000                               | N                                         | .17  | N        | .18  | N        | .19  | 0        | .20  |
| 11,000,000                              | N                                         | .15  | N        | .16  | N        | .17  | 0        | .19  |
| 12,000,000                              | N                                         | .15  | N        | .16  | 0        | .17  | 0        | .19  |
| 17,000,000                              | N                                         | .15  | N        | .15  | 0        | .16  | 0        | .17  |
| 33,000,000                              | N                                         | .15  | N        | .15  | 0        | .16  | 0        | .17  |
| 43,000,000                              | N                                         | .15  | N        | .16  | 0        | .17  | 0        | .21  |
| 47,000,000                              | N                                         | .17  | N        | .17  | 0        | .18  | 0        | .19  |
| 54,000,000                              | N                                         | .15  | N        | .16  | 0        | .17  | 0        | .18  |
| 74,000,000                              | N                                         | .15  | N        | .15  | 0        | .17  | 0        | .19  |
| 94,000,000                              | N                                         | .16  | N        | .17  | 0        | .19  | 0        | .20  |

N - Normal; 0 - Off flavour; T.A. - Titrable acidity;  
O.Q. - Organoleptic quality.

TABLE - 4

Psychrophilic counts per ml of samples of milk from  
individual animals during storage at  
 $4.4 \pm 5^{\circ}\text{C}$

| Particulars | 0-hours | 24-hours | 48-hours | 72-hours |
|-------------|---------|----------|----------|----------|
| Minimum     | 350     | 1,600    | 4,800    | 25,000   |
| Maximum     | 8,000   | 16,000   | 40,000   | 90,000   |
| Average     | 2,657   | 6,940    | 40,600   | 86,740   |

TABLE - 5

Psychrophilic count per ml of samples of pooled raw  
milk during storage at  $4.4 \pm 5^{\circ}\text{C}$

| Particulars | 0-hours | 24-hours  | 48-hours  | 72-hours  |
|-------------|---------|-----------|-----------|-----------|
| Minimum     | 30,000  | 60,000    | 170,000   | 250,000   |
| Maximum     | 650,000 | 1,360,000 | 1,850,000 | 2,600,000 |
| Average     | 225,500 | 542,100   | 1,013,000 | 1,583,000 |

TABLE - 6

Psychrophilic count on the keeping quality of  
milk stored at  $4.4 \pm 5^{\circ}\text{C}$

| Initial<br>Psychro-<br>philic<br>count/ml. | Organoleptic quality and free fatty acids |        |          |        |          |        |          |        |
|--------------------------------------------|-------------------------------------------|--------|----------|--------|----------|--------|----------|--------|
|                                            | 0.hours                                   |        | 24-hours |        | 48-hours |        | 72-hours |        |
|                                            | O.Q.                                      | F.F.A. | O.Q.     | F.F.A. | O.Q.     | F.F.A. | O.Q.     | F.F.A. |
| 520                                        | N                                         | .16    | N        | .19    | N        | .22    | 0        | .24    |
| 350                                        | N                                         | .16    | N        | .18    | N        | .23    | 0        | .28    |
| 500                                        | N                                         | .13    | N        | .17    | N        | .20    | 0        | .23    |
| 1,400                                      | N                                         | .12    | N        | .17    | N        | .22    | 0        | .25    |
| 1,800                                      | N                                         | .12    | N        | .16    | N        | .19    | 0        | .21    |
| 600                                        | N                                         | .15    | N        | .20    | N        | .24    | 0        | .26    |
| 2,400                                      | N                                         | .13    | N        | .16    | N        | .17    | 0        | .19    |
| 3,500                                      | N                                         | .14    | N        | .15    | N        | .16    | 0        | .20    |
| 7,500                                      | N                                         | .20    | N        | .23    | N        | .25    | 0        | .27    |
| 8,000                                      | N                                         | .16    | N        | .20    | N        | .23    | 0        | .26    |
| 30,000                                     | N                                         | .18    | N        | .22    | N        | .27    | 0        | .30    |
| 100,000                                    | N                                         | .15    | N        | .25    | N        | .31    | 0        | .42    |
| 150,000                                    | N                                         | .21    | N        | .24    | 0        | .34    | 0        | .38    |
| 160,000                                    | N                                         | .23    | N        | .30    | 0        | .39    | 0        | .44    |
| 170,000                                    | N                                         | .24    | N        | .30    | 0        | .34    | 0        | .37    |
| 150,000                                    | N                                         | .23    | N        | .31    | 0        | .39    | 0        | .47    |
| 225,000                                    | N                                         | .23    | N        | .29    | 0        | .35    | 0        | .40    |
| 300,000                                    | N                                         | .23    | N        | .30    | 0        | .34    | 0        | .39    |
| 320,000                                    | N                                         | .20    | N        | .26    | 0        | .33    | 0        | .38    |
| 650,000                                    | N                                         | .24    | N        | .29    | 0        | .34    | 0        | .38    |

N - Normal; 0 - Off flavour; F.F.A. - Free fatty acids

TABLE - 7

Effect of chilling and storage at 4.4°C of pooled raw milk on the total bacterial count (during storage for less than 24 hours)

| Particulars | Total bacterial count/ml of pooled raw milk (initial) | Total bacterial count per ml after storage |
|-------------|-------------------------------------------------------|--------------------------------------------|
| Minimum     | 5,600,000                                             | 7,800,000                                  |
| Maximum     | 46,000,000                                            | 56,000,000                                 |
| Average     | 23,000,000                                            | 29,000,000                                 |

TABLE - 8

Effect of chilling and storage at 4.4°C of pooled raw milk on the total bacterial count (during storage for 24 hours)

| Particulars | Total bacterial count per ml of pooled raw milk (initial) | Total bacterial count/ml after storage |
|-------------|-----------------------------------------------------------|----------------------------------------|
| Minimum     | 5,000,000                                                 | 7,600,000                              |
| Maximum     | 56,000,000                                                | 86,000,000                             |
| Average     | 37,500,000                                                | 51,600,000                             |

TABLE - 9

Effect of chilling and storage at 4.4°C of pooled raw milk on the psychrophilic count (during storage for less than 24 hours).

| Particulars | Initial psychrophilic count per ml of pooled raw milk | Psychrophilic count per ml after storage |
|-------------|-------------------------------------------------------|------------------------------------------|
| Minimum     | 60,000                                                | 96,000                                   |
| Maximum     | 220,000                                               | 350,000                                  |
| Average     | 135,000                                               | 179,800                                  |

TABLE - 10

Effect of chilling and storage at 4.4°C of pooled raw milk on the psychrophilic count (during storage for 24 hours)

| Particulars | Initial psychrophilic count per ml of pooled raw milk | Psychrophilic count per ml after storage |
|-------------|-------------------------------------------------------|------------------------------------------|
| Minimum     | 40,000                                                | 62,000                                   |
| Maximum     | 230,000                                               | 420,000                                  |
| Average     | 170,000                                               | 287,000                                  |

TABLE - 11

Total bacterial count on the keeping quality of chilled and stored milk for 24 hours and less than 24 hours at 4.4°C.

| Total bacterial<br>count per ml of<br>pooled raw milk. | Organoleptic quality and titrable acidity |      |                       |      |          |      |
|--------------------------------------------------------|-------------------------------------------|------|-----------------------|------|----------|------|
|                                                        | 0 hours                                   |      | Less than<br>24 hours |      | 24 hours |      |
|                                                        | O.Q.                                      | T.A. | O.Q.                  | T.A. | O.Q.     | T.A. |
| 5,600,000                                              | N                                         | .14  | N                     | .14  |          |      |
| 10,000,000                                             | N                                         | .16  | N                     | .17  |          |      |
| 12,000,000                                             | N                                         | .15  | N                     | .16  |          |      |
| 12,600,000                                             | N                                         | .15  | N                     | .16  |          |      |
| 14,800,000                                             | N                                         | .14  | N                     | .15  |          |      |
| 20,000,000                                             | N                                         | .16  | N                     | .17  |          |      |
| 31,000,000                                             | N                                         | .18  | N                     | .19  |          |      |
| 36,000,000                                             | N                                         | .15  | N                     | .15  |          |      |
| 42,000,000                                             | N                                         | .16  | N                     | .17  |          |      |
| 46,000,000                                             | N                                         | .15  | N                     | .16  |          |      |
| =                                                      |                                           |      |                       |      |          |      |
| 5,000,000                                              | N                                         | .16  |                       |      | N        | .18  |
| 28,000,000                                             | N                                         | .16  |                       |      | N        | .16  |
| 36,000,000                                             | N                                         | .15  |                       |      | N        | .16  |
| 46,000,000                                             | N                                         | .17  |                       |      | N        | .17  |
| 54,000,000                                             | N                                         | .16  |                       |      | N        | .18  |
| 56,000,000                                             | N                                         | .15  |                       |      | N        | .15  |

N - Normal; T.A. - Titrable acidity; O.Q. Organoleptic quality.

TABLE - 12

Psychrophilic count on the keeping quality of chilled and stored milk for 24 hours and less than 24 hours at 4.4°C

| Initial psychro-<br>philic count<br>per ml of pooled<br>raw milk. | Organoleptic quality and free fatty acids |        |                       |        |          |        |
|-------------------------------------------------------------------|-------------------------------------------|--------|-----------------------|--------|----------|--------|
|                                                                   | 0 hours                                   |        | Less than<br>24 hours |        | 24 hours |        |
|                                                                   | O.Q.                                      | F.F.A. | O.Q.                  | F.F.A. | O.Q.     | F.F.A. |
| 88,000                                                            | N                                         | .203   | N                     | .217   |          |        |
| 60,000                                                            | N                                         | .176   | N                     | .208   |          |        |
| 120,000                                                           | N                                         | .138   | N                     | .160   |          |        |
| 200,000                                                           | N                                         | .180   | N                     | .202   |          |        |
| 98,000                                                            | N                                         | .156   | N                     | .170   |          |        |
| 120,000                                                           | N                                         | .150   | N                     | .170   |          |        |
| 130,000                                                           | N                                         | .216   | N                     | .244   |          |        |
| 150,000                                                           | N                                         | .163   | N                     | .190   |          |        |
| 164,000                                                           | N                                         | .170   | N                     | .189   |          |        |
| 220,000                                                           | N                                         | .230   | N                     | .280   |          |        |
| =                                                                 |                                           |        |                       |        |          |        |
| 40,000                                                            | N                                         | .138   |                       |        | N        | .157   |
| 196,000                                                           | N                                         | .190   |                       |        | N        | .220   |
| 156,000                                                           | N                                         | .155   |                       |        | N        | .180   |
| 210,000                                                           | N                                         | .209   |                       |        | N        | .240   |
| 188,000                                                           | N                                         | .193   |                       |        | N        | .234   |
| 230,000                                                           | N                                         | .180   |                       |        | N        | .209   |

N - Normal; O.Q. - Organoleptic quality; F.F.A- Free fatty acids

TABLE - 13

Effect of transport on the bacterial and organoleptic quality of chilled milk.

| Chilled milk just before transport |                            | Chilled milk immediately after transport        |                              |
|------------------------------------|----------------------------|-------------------------------------------------|------------------------------|
| Total bacterial count per ml       | Psychrophilic count per ml | O.Q. T.A. F.F.A.                                | Total bacterial count per ml |
|                                    |                            |                                                 | Psychrophilic count per ml   |
|                                    |                            |                                                 | O.Q. T.A. F.F.A.             |
| 7,800,000                          | 120,000                    | N .14 .217                                      | 8,000,000                    |
| 13,800,000                         | 96,000                     | N .17 .208                                      | 14,000,000                   |
| 15,000,000                         | 136,000                    | N .16 .160                                      | 15,400,000                   |
| 16,400,000                         | 260,000                    | N .16 .202                                      | 18,000,000                   |
| 19,000,000                         | 136,000                    | N .15 .170                                      | 19,600,000                   |
| 25,000,000                         | 150,000                    | N .17 .170                                      | 26,000,000                   |
| 36,000,000                         | 180,000                    | N .19 .244                                      | 36,500,000                   |
| 45,000,000                         | 180,000                    | N .15 .190                                      | 46,000,000                   |
| 56,000,000                         | 190,000                    | N .17 .220                                      | 58,000,000                   |
| 56,000,000                         | 350,000                    | N .16 .280                                      | 57,000,000                   |
| =                                  |                            |                                                 |                              |
| 7,600,000                          | 62,000                     | N .18 .157                                      | 7,800,000                    |
| 39,000,000                         | 350,000                    | N .16 .220                                      | 40,000,000                   |
| 39,000,000                         | 226,000                    | N .16 .180                                      | 40,000,000                   |
| 70,000,000                         | 380,000                    | N .17 .240                                      | 74,000,000                   |
| 68,000,000                         | 284,000                    | N .18 .234                                      | 70,000,000                   |
| 86,000,000                         | 420,000                    | N .15 .209                                      | 90,000,000                   |
| Transport time - 4.5 hours         |                            | Increase of temperature during transport - 1°C  |                              |
| O.Q. Organoleptic quality;         |                            | T.A. Titrable acidity. F.F.A. Free fatty acids. |                              |

