THE INFLUENCE OF COLD STORAGE OF MILK ON THE QUALITY OF PROCESSED MILK AND MILK PRODUCTS - PASTEURIZED MILK

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Submitted for the degree of M.Sc. in the Faculty of Science in the University of Glasgow, October, 1982

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ACKNOWLEDGEMENTS

The author wishes to express her gratitude to the Government of the Republic of Iraq for their financial assistance; to Dr. R.J.M. Crawford, Head of the Department of Dairy Technology for his invaluable contributions throughout, and the Milk Marketing Board for England and Wales for their support and help; to Professor J.S. Hall, formerly Principal of The West of Scotland Agricultural College, and the present Principal, Professor J.M.M. Cunningham; to Mr. G. Davies for his help and advice. The author wishes to express her grateful thanks to Mr. D.P. Arnot, Statistician, Advisory and Development Service, for his help in the design of the experiments and carrying out the statistical analysis.

The author would like to thank Mrs. I. Hamilton for her assistance; to Mr. S. Crawford for making the illustrations and graphs of this work.

To my husband, Amir, for his help and my daughter, Deana; to my family and especially my parents for their encouragement and understanding, I express my sincere recognition.

Finally, I wish to thank all members of the Department of Dairy Technology who helped in carrying out this work.

SUMMARY

1. The aim of this work was to investigate the influence of cold storage of raw milk prior to processing and product manufacture on the quality of the pasteurized milk and products derived from it. The work involved a study of the quality of raw milk from a bulk silo purchased from a dairy factory in the West of Scotland. Three trials were undertaken during the period 1979-1980. The raw milks were delivered by road tanker in quantities of around 2,500 litres for each trial. On delivery a portion of the consignment was immediately processed and the remainder was split into two equal amounts, one of which was held at $2^{o}C$ for 2, 4 and 7 days and the other at $6^{o}C$ for the same time.

Pasteurized milks were produced on the day of delivery and after 2, 4 and 7 days of cold storage at each temperature. Samples of pasteurized milk were stored at a refrigerator temperature of $4^{o}C$ for 3, 6 and 9 days.

The microbiological quality of raw and pasteurized milks was assessed using total colony count, coliform count, psychrotrophic count, lipolytic count, proteolytic count and thermoduric count, and tests were made for antibiotic residues in the raw milks. The mean microbial counts of raw milk stored at $6^{o}C$ for 2, 4 and 7 days were significantly higher than the mean microbial counts of raw milk stored at $2^{o}C$, for 2, 4 and 7 days.

The pasteurized milks prepared from raw milk on delivery by road tanker and after storage at $2^{o}C$ for 2 and 4 days did not differ significantly in their microbial count. There was a highly significant increase ($p < 0.001$) in the microbial counts of the pasteurized milk prepared from raw milk which had been stored for more than 4 days at $2^{o}C$, while the pasteurized milks produced from raw milk stored at $6^{o}C$ for more than 2 days showed highly significant increases ($p < 0.001$) in the microbial counts compared with initial levels.

In the case of pasteurized milks produced from raw milk stored at $2^{o}C$ and kept for 3, 6 and 9 days at $4^{o}C$ there was no significant change in the microbial counts compared to the initial value obtained immediately

after processing. With pasteurized milks produced from raw milk stored at $6^{o}C$ the day of processing microbial counts were higher after 4 and 7 days of storage than the initial value and after the raw milk was held for 2 days at $6^{o}C$. Similarly the counts of the pasteurized milks held at $4^{o}C$ had higher ($p < 0.001$) counts than the initial values after 3, 6 and 9 days of storage.

The mean total colony count of samples of raw milk taken on delivery by road tanker was 203×10^{3} per ml (first trial 161×10^{3} per ml, second trial 341×10^{3} per ml, third trial 105×10^{3} per ml), while the processed milk taken from the raw milk above gave a mean count of 48×10^{3}per ml (first trial 106×10^{3} per ml, second trial 30×10^{3} per ml, third trial 85×10^{2} per ml). None of the pasteurized milk samples produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ showed any count of coliforms or psychrotrophs immediately after processing or after storage of the samples for 3, 6 and 9 days at $4^{o}C$.

2. The chemical quality of raw and pasteurized milks was determined by analysis for fat, pH, titratable acidity, cream line, freezing point depression, free sulphydryl groups (F-SH), calcium, total solids, acid degree value (ADV) and phosphatase test (pasteurized samples on day of processing). No significant change was found in the fat content of raw and pasteurized milks during the storage time at different temperatures. The pH value of the raw milk on delivery by road tanker and after storage at $2^{o}C$ for 2 and 4 days showed no significant change. Storage at $6^{o}C$ for 4 days affected the pH value and a significant difference ($p < 0.05$) was found in the pH value of raw milks held at $2^{o}C$ for 7 days. The same significant change was found with raw milk after 4 days storage at $6^{o}C$. The value continued to change for the remaining 3 days of storage of the raw milks at $6^{o}C$. Similar changes were found in pasteurized milks.

No significant difference was found in the titratable acidity of raw milk stored at $2^{o}C$ for 2, 4 and 7 days, while significant changes ($p < 0.001$) were found from the second day of storage at $6^{o}C$. Highly significant differences were found in the titratable acidity of

pasteurized milk produced from raw milk held at $6^{o}C$ after the second day of storage and in the same pasteurized milks after storage for 3, 6 and 9 days at $4^{o}C$, while there was no significant change in the titratable acidity of pasteurized milk produced from raw milk stored at $2^{o}C$ for up to 7 days and kept for 3, 6 and 9 days at $4^{o}C$ after processing.

No significant difference was found in the cream line of raw milk stored at $2^{o}C$ and $6^{o}C$ for up to 7 days. Highly significant differences were found in the cream line of pasteurized milk produced from raw milk stored at $6^{o}C$ for 2 days compared to day of delivery values and these obtained with raw milk held at $2^{o}C$ before processing. Neither the storage period nor the temperature at $2^{o}C$ affected the freezing point of raw milk while in the case of the raw milk stored at $6^{o}C$ a highly significant change had taken place after 7 days. The same significant change was found in the freezing-point for pasteurized milk produced from the raw milk held at $6^{o}C$ for 7 days.

No significant difference was observed in F-SH due to either the length or the temperature of storage of raw milk. On the other hand, pasteurized milk produced from the same milk showed a significant difference ($p < 0.05$) in F-SH levels due to the period of storage at $4^{o}C$.

No significant difference was found in the calcium content or the total solids of both raw and pasteurized milks due to either the length or the temperature of storage before or after processing.

Very highly significant differences ($p < 0.001$) in ADV were associated with the length of storage of the raw milk but the temperature had no effect. However, both the length of storage and temperature of storage caused very highly significant differences ($p < 0.001$) in the ADV of pasteurized milks.

3. Sensory evaluation of raw milks indicated that there were no significant differences in the appearance of the milk due to either duration or temperature of storage.

Very highly significant differences ($p < 0.001$) in the odour of raw milks were found after the second day of storage at $6^{o}C$ while a

significant difference ($p < 0.05$) in odour scores was found after the fourth day of storage at $2^{o}C$.

Flavour (taste) scores showed highly significant change ($p < 0.01$) due to storage at $6^{o}C$ while no significant change in flavour was associated with raw milk stored at $2^{o}C$.

The total scores of raw milks (and corresponding pasteurized milks) showed very highly significant decrease ($p < 0.001$) on storage at $6^{o}C$ and no significant difference where storage was at $2^{o}C$ until the fourth day of storage. Fruity, acid, malty, and bitter flavours were identified in raw and pasteurized milks held at $2^{o}C$ and $6^{o}C$.

There was no significant difference in the total organoleptic scores of pasteurized milks held at refrigerator temperature ($4^{o}C$) for up to 9 days after production from raw milks held at $2^{o}C$ for up to 7 days, whereas highly significant differences in total scores were found with the corresponding pasteurized milks prepared from raw milk held at $6^{o}C$.

4. A highly significant correlation was found between the different microbial counts (total, coliform, psychrotrophic, lipolytic, proteolytic and thermoduric) for raw and pasteurized milks and between all of these counts (except coliform and psychrotrophic which were negative in the pasteurized milks) and the acid degree value.

Free sulphydryl groups correlated negatively with the scores of the appearance, odour, flavour (taste) and total scores of the raw and pasteurized milks.

ABBREVIATIONS

The standard abbreviations, as recommended by the British Standard Institution, B.S. 1991 : Part 1 (1976) are used in this thesis, with the following additions:-

F v rest = The day of delivery compared with the mean value of the other days of storage

RM = Raw milk

PM = Pasteurized milk

DF = Degree of freedom = $n - 1$

MS = Mean of Sum Squares = $\frac{1}{N} \Sigma (x - \bar{x})^2$

REP = Replicates

SE = Standard Error = V Residual MS

SED = Standard Error of Differences of Means = V Residual MS/N (individual variants)

VR = Variance Ratio = MS for individual variants/MS of Residual

PD = The pasteurized milk on the day of delivery

ADV = Acid degree value

F-SH = Free sulphydryl group

SMMB = The Scottish Milk Marketing Board

Fig. = Figure

THE INFLUENCE OF COLD STORAGE OF MILK ON THE QUALITY OF PROCESSED MILK AND MILK PRODUCTS

INTRODUCTION

The effect of raw milk quality on the shelf life of the product

Unlike sterilized and UHT milk, pasteurized milk has a relatively short shelf life or short keeping quality. In the U.S.A. it is generally expected that pasteurized milk can remain in acceptable condition for 18 days or even longer; in some European countries a shelf life of 10-14 days is not uncommon (Storgards, 1961; Langeveld et al., 1973). In some other countries pasteurized milk has a much shorter keeping quality, e.g. 3-5 days or even less. Under ideal circumstances it has been shown possible to produce pasteurized milk with a keeping quality in excess of 170 days (Ashton, 1962) by using strict hygienic precautions in pasteurized milk production and during storage at 2.2°C to 3.3°C (36 to 38°F).

In a general review of the dairy industry, Johnson (1979) suggested that many factors must be considered to assure adequate prolonged shelf life in fluid milk and milk products. He pointed out that for many years, dairy scientists have been exploring mechanisms that affect shelf life and many papers have been published dealing with specific aspects of milk quality. The shelf life of pasteurized milk is affected by many things from raw milk handling through processing, distribution and storage to the consumer's refrigerator. Acceptable shelf life for pasteurized milk was considered by Johnson (1979) to be"processed milk having fresh flavour and aroma for 21 or more days when held at suitable refrigerator temperatures". Cleaning and sanitation are important for producing high quality milk on the farm and through delivery and processing stages.

Effect of the storage temperature on the keeping quality of the milk before and after processing

Many authors found that the shelf life of pasteurized milk as determined by its organoleptic properties - visual appearance, smell and flavour - is related to:-

1. the bacterial flora of the raw milk at the time of processing,
2. bacterial-derived lipases and proteinases which are present in the raw milk,
3. the efficiency of handling and treatment (IDF/FIL, 1980).

It was found that raw, pasteurized and sterilized milk with very high counts were chiefly due to contamination by dirty milking equipment, milk cans and consumers vessels at various stages from producer to consumer (Gernez-Rieux and Buttiaux, 1947).

Chandrasekhar (1951), studied the effect of refrigeration on the bacteriological quality of raw and pasteurized milk after he had incubated one set at 3-5^{o}C for 21 days and the other set at 30^{o}C for the same time. When samples were held for 3 days at 3-5^{o}C, plated and incubated at 3-5^{o}C for 21 days, eleven out of 12 samples plated before refrigeration had colony counts below 100,000 per ml whereas only 6 out of 12 were in the same group after 24 h. at 3-5^{o}C. Similar experiments with pasteurized milk indicated that psychrophilic bacteria present in raw milk do not survive pasteurization temperatures.

Galesloot (1952) observed that the thermoduric count of the milk increased more at the higher temperatures and in summer than in winter milk when milk held for 2 to 3 h. at temperature from 15 to 45^{o}C before laboratory pasteurization. The species responsible for these increased counts were Streptococcus thermophilus, Streptococcus bovis, occasionally Streptococcus durans and in one case Lactobacillus plantarum. The same author suggested that the increase is due not so much to multiplication as to increased heat resistance of these organisms.

It was suggested that the poorer keeping quality of the milk may be due to the adverse effects of heating on bacteriostatic and buffering capacity of milk and to the continuation of enzymic activity of the dead microflora (Kirchner, 1959).

Thakre & Nambudripad (1962) related the keeping quality of raw milk storage at 4^{o} to 37^{o}C and pasteurized milk storage at 5^{o} to 33^{o}C and

reported that pasteurized milk had a better keeping quality than raw milk of a similar bacteriological quality, especially at low temperature.

Hashimoto et al. (1964) studied 88 samples of pasteurized milk in Japan. One had a thermophilic count of > 1,000 per ml, six a mesophilic count of > 50,000 per ml and eight a psychrophilic count of > 50,000 per ml. Of 30 samples of raw milk tested, six had a thermophilic count of > 30 per ml, eight a mesophilic count of > 1,000,000 per ml and five a psychrophilic count of > 1,000,000 per ml. Franklin (1965) concluded that within limits (depending on the general standards of milk hygiene) the composition of the bacterial flora rather than their numbers is the most important factor in determining the keeping quality of the heat-treated milk.

Kim et al. (1966) investigated the effect of storage on bulk raw milk collected in Korea in May, July, October and January cooled to 4^{o}, 20^{o} or 30^{o}C and stored for 17 h at 4^{o}C or for 0-72 h at 4^{o}C was pasteurized for 30 minutes at 63^{o}C. The cooling temperature and the season of the year had little effect on the rate of bacterial development during the first 2 h; initial bacterial counts on the pasteurized milk were < 1,000 per ml, regardless of cooling temperature or season; Escherichia coli was present only in the July milks (maximum 700 per ml in samples cooled at 4^{o}C and 61,000 per ml in samples cooled at 30^{o}C and October milks (maximum 70 per ml).

On Swedish studies (von Bockelmann, 1969) raw milks were stored at 5^{o}C for up to 3 days and examined at daily intervals for bacterial change; the total counts (per ml), were 1.6×10^{6}, 3.8×10^{6} and 17×10^{6} after one, two and three days respectively; the psychrotrophic counts (per ml), were 0.4×10^{3}, 2.1×10^{3} and 11.0×10^{3}; the coliform counts (per ml), were 87×10^{3}, 230×10^{3} and the thermoduric bacteria surviving laboratory pasteurization (per ml), were 45×10^{3}, 45×10^{3} and 55×10^{3}. He concluded that milk containing < 10^{6} bacteria per ml on delivery to the dairy, with < 50 per cent of these being psychrotrophic bacteria, could be satisfactorily cold stored for 2 to 3 days before pasteurization. In their report, Mergl & Cerna (1969) referred to the bacterial flora

of raw milk stored at < 5°C for 0, 25, 30, 49, 54, 78, 102 and 126 h. They reported the following counts (per ml) after 0, 49 and 126 h of storage:- total bacteria, 7,000, 14,500 and 178,000; coliform, 10, 340 and 3,250; psychrophiles, 4,400, 10,000 and 2,100,000; proteolytic organisms 500, 450 and 7,800; lipolytic organisms, 225, 305 and 2,100. Titratable acidity, pH and organoleptic characteristics did not change throughout. Milk pasteurized after low temperature storage was kept for > 48 h without organoleptic changes. Erhola et al. (1970) indicated that increasing the storage time of good quality milk in farm banks from 2 to 4 days did not significantly increase coliform and total bacterial count or the free fatty acid values. On the other hand Aapola & Antila (1970) studied the effect of raw milk storage on the keeping quality of consumption milk when milk at 30°C on production was added daily for 3 days to a farm tank and stored there at 1-2°C. Such a practice did not affect the quality of the milk after pasteurization; similar addition of raw milks with storage at 3-5°C resulted in a poor quality milk after the first day.

Patel & Blankenagel (1972) studied the bacterial flora of 216 raw milk samples which were laboratory pasteurized (62.3°C for 30 min), and stored at 7°C. Their results showed that milks with a count of > 1 million per ml before heating frequently developed objectionable flavours after pasteurization and subsequent storage. Nearly all samples of pasteurized milks which were prepared from raw milk with counts of > 10 million per ml developed objectionable flavours within 2 weeks of processing. In their studies on bulk milk after 24, 48 and 72 h storage in farm tanks Duchateau & Lacrosse (1973) showed that the growth rate of the total flora and of psychrotrophic, caseolytic and pseudomonas organisms increased considerably after 48 h storage at 4-5°C, whereas at 1°C the growth rate was slow throughout storage and the relative proportions of the 3 types of organisms remained fairly constant over 72 h. There was a particularly marked increase in bacterial growth rate after 48 h. It is suggested that milk should not be kept for more than 48 h in farm bulk tanks at < 5°C. A Dutch report indicates that longer storage than 1 day at < 4°C is inadmissible unless the milk is heat-treated on arrival at the dairy, (Nederlands Instituut voor Zuivelonderzoek, 1973).

Hadland & Hoye (1974) reported on experiments conducted with milk stored in bulk for 1-4 days at 2-6^{o}C. The milk stored at 2^{o}C for 1 day gave a satisfactory keeping quality of 14 days after pasteurization, while with the milk kept for 4 days at 2^{o}C the keeping quality of the pasteurized milk was reduced to 6-8 days. Raw milk storage at 6^{o}C for more than 1 day caused a rapid reduction in milk quality before and after pasteurization.

Gritsenko & Surovtseva (1972a) indicated that the total bacterial counts of raw milk, pasteurized milk ready for distribution and pasteurized milk after storage for 1 and 5 days were respectively (per ml) 22 x 10^{6}, 1.6 x 10^{6}, 3 x 10^{6} and 8 x 10^{6}. Coliform bacteria were completely destroyed by pasteurization but enterococci were only reduced tenfold to one hundred fold and were detected in individual cases at levels of 1-10 per ml after pasteurization and 1-100 per ml during storage at 6 to 8^{o}C for 1, 2, 3, 5 and 7 days. Coliforms were present at levels of 10-10,000 per ml in stored milk.

Smith et al. (1978) reported that refrigerated storage caused a 10 per cent ($p < 0.05$) reduction in counts in pasteurized milk and decreased coliform counts by 21 per cent ($p < 0.01$).

Milk contains relatively few bacteria when it leaves the udder of a healthy cow. Probably the two most significant sources of contamination are dairy utensils and milk contact surfaces, including the milk pail or milking machines or the bulk milk cooler and pipelines. Undesirable bacteria from these sources include lactic streptococci, coliform bacteria, psychrotrophic Gram-negative rods, and thermodurics (those which survive pasteurization) e.g. micrococci, bacilli and brevibacteria. When they are cleaned and sanitized properly, utensils and milk contact surfaces add few bacteria to raw milks (Frazier & Westhoff, 1978).

The effect of different heat treatment on the quality of the milk

In the processing of milk and cream, or in their use in making various products, it is desirable to destroy all of the contaminating organisms. Usually, this is accomplished by application of heat. In the development of heat processing methods for milks pasteurization was one of the

earliest procedures used. The objectives of market milk pasteurization are:-

1. to kill all the pathogens that may be present in the milk and be transmitted to people, and
2. to improve the keeping quality of milk.

There are two factors involving the heat treatment, temperature and holding period, and one is as important as the other. After receiving the heat treatment the milk must be cooled immediately to lower than $10^{o}C$ ($50^{o}F$) to prevent growth of the organisms which survive the process. With milk two main types of pasteurization are employed:-

1. the low-temperature, long-time (LTLT) or 'holder' method which has an exposure of at least $61.7^{o}C$ ($143^{o}F$) for at least 30 minutes,
2. the high temperature, short-time (HTST) or continuous or flash pasteurization, with an exposure of at least $71.7^{o}C$ ($161^{o}F$) for at least 15 seconds.

The effects of the heat applied to milk, whenever pasteurization is applied may be considered (Hammer, 1948) under:-

a. efficiency in destroying bacteria,

b. the influence on flavour, and

c. the influence on the cream line.

3. Heat-treatment processes in excess of pasteurization for milk and milk products are referred to as very high-temperature (VHT) systems and ultra heat treated (UHT) systems (Frazier & Westhoff, 1978). UHT treatment is defined as a high temperature process of not less than $132^{o}C$ for not less than 0.5 s applied with the object of destroying all micro-organisms, or at least inhibiting the growth of any residual micro-organisms, (IDF/FIL, 1980).

   The methods of processing used for UHT milk are:-

a. Direct heating method

The milk is heated to temperatures in the range of 135 to 150°C with holding time of a few seconds followed by aseptic filling of the cooled milk (Burton, 1977).

b. Indirect heating method

This method includes heating the milk to 85°C by regeneration, homogenisation, further heating to 138°C with holding for 2 s before cooling either by cold water, regeneration and final cold water to give a filling temperature of approximately 20°C (Ball, 1977).

4. Sterilization is a process by using heat treatment at a temperature exceeding 100°C in order to destroy all micro-organisms, or at least inhibiting the growth of any residual micro-organisms IDF/FIL, 1980).

The methods of processing used for sterilized milk are:-

a. Batch method

In this method the milk is preheated, homogenised, and filled into bottles prior to sterilization by the batch method using a temperature in the range of 104 to 110°C for 30 to 40 min after which the bottles are removed and left to cool (National Dairy Council, 1974).

b. Continuous method

In this method the filled bottles are fed on to a conveyer through hot water tanks into a steam chamber using temperatures in the range of 107 to 110°C for 30 to 40 min (National Dairy Council, 1974).

The effect of storage on the keeping quality of pasteurized milk

Dahlberg (1945) examined pasteurized milk immediately after production and after storage at 1.9° to 4.4°C (35° to 40°F), and at 21.1° to 23.8°C (70° to 75°F) for 6 h to simulate milk left on the doorstep. Other samples were taken on a normal delivery route. Milk samples were tested after 0, 1, 2, 3, 4 and 7 days. Initially the mean plate count

(37°C) of samples stored at 1.9° to 4.4°C (35° to 40°F) was 12,000 per ml; round return samples showed no increase and the increase was only slight with samples stored in a room at 21.1° to 23.8°C (70° to 75°F) for 6 h. Counts of samples stored at 1.9° to 4.4°C (35° to 40°F) were lower on the fourth day than when collected from the plant; those stored at 7.2° to 10°C (45° to 50°F) showed a slight increase on the third day; those stored at 12.7° to 15.5°C (55° to 60°F) showed a definite increase on the second day and a marked increase (from 12,000 per ml to 1,116,000 per ml) on the third day.

Coliform organisms were present in 1 ml at 0 day in 3 of the 18 samples stored at 1.9° to 4.4°C (35° to 40°F), in 2 of the 18 round return samples and in 6 of the 18 doorstep samples. Coliform organisms did not multiply during 4 days storage at 1.9° to 4.4°C (35° to 40°F), but there was an increase in the number of samples where coliform were positive in 1 ml after 2 days storage at 7.2° to 10°C (45° to 50°F) and after 1 day at 12.7° to 15.5°C (55° to 60°F).

In another study, the same author (1946) found that the coliform count increased more rapidly than the total count in samples held at 7.2° to 10°C (45° to 50°F), and at 12.7° to 15.5°C (55° to 60°F). However, these increases were more marked in warm weather. At 1.9° to 4.4°C (35° to 40°F) there was no appreciable increase in total count or coliform counts in October.

Kalkbrenner (1949) found 30 out of 32 pasteurized and irradiated milk samples had plate counts of > 100,000 organisms per ml, and had coliform counts of > 1,000 per ml. No tubercle bacilli or intestinal pathogens were found in any of the samples but 17 contained weakly haemolytic streptococci.

Weese & Henderson (1949) indicated that pasteurized milk of good quality kept well for 3 to 4 days after delivery under reasonably good conditions of home refrigeration where the temperatures varied from 2.7° to 11.1°C (37° to 52°F).

Vieeschauwer *et al.* (1950) studied the results of bacteriological control at 15 dairies during 1948-49 and 19 dairies during 1949-50.

They found that the raw milk was generally of poor quality, with high coliform titre, short dye reduction time, and average bacterial counts of 12,908,000 and 12,525,000 for the two periods. The low count of the pasteurized milk was, however, spoiled in some dairies by post pasteurization contamination from the plant and bottles. On the other hand Egdell & Bird (1950) found there was no significant difference between the flora of samples taken at the pasteurizer and samples taken from the bottles.

Swartling (1953) found no relationship between the bacterial count before and after pasteurization. The keeping quality of pasteurized milk was found to be wholly determined by those thermoduric bacteria capable of multiplying and affecting milk quality during storage, whereas, Piraux et al. (1953) were of the opinion that the keeping quality of pasteurized milk was related to the coliform count rather than the thermoduric count. However, Ashton (1962) stated that spoilage of pasteurized milk appeared to be mainly due to spore-formers.

Types as well as numbers of thermoduric organisms affected keeping quality, the main spoilage organisms being Microbacterium spp. and Bacillus cereus (Mourgues & Auclair, 1970).

Atherton et al. (1954) found that with laboratory pasteurized milk stored at 4.4^{o} or 7.3^{o}C while there was no significant increase in the total plate count during 15 days of storage, the psychrophilic count of the milk held at 4.4^{o}C had increased over this storage period to more than one million per ml. On the other hand Panetsos et al. (1973) stated that pasteurized milk showed no increase in bacterial count (average approximately 50,000 per ml), no off flavours and maintained a low acidity during storage at 4^{o}C for 18 h. Samples of milk from retail shops were compared.

Johannes (1956) stated that the dye reduction and coliform tests were not sufficiently sensitive to predict the shelf life of high quality pasteurized milk and the direct plate count was of doubtful value.

The studies by Kästli & Binz (1956), when they emphasised the importance of the temperature at which pasteurized milk is stored, showed that there

was no appreciable increase in the total bacterial count over a period of 6 days at 2^{o} to 3^{o}C.

Stoyanov (1960) indicated that pasteurized milk retained its quality for 5-6 days at 3-5^{o}C, but no longer than 24 h at 10^{o} to 12^{o}C. He was of the opinion that the total bacterial count, the count of proteolytic bacteria and the coliform titre were suitable criteria for the evaluation of the keeping quality of pasteurized milk.

Ashton (1962) found that pasteurized milk produced from good quality raw milk could be satisfactorily stored in households at ambient temperature ranging from 10^{o} to 27.2^{o}C (50 to 81^{o}F), after 12-15 h refrigerated pre-storage and retail distribution.

A storage temperature of 8 to 9^{o}C appeared to be the maximum for ensuring that the milk remained of good quality for 2-3 days after pasteurization (Vancura, 1967).

Pasteurization at 82^{o}C or 85^{o}C effectively reduced the total bacterial count and eliminated coliform bacteria, the resulting milk being of good organoleptic quality and remaining fresh for several days at 10^{o}C (Gheorghe & Manu, 1968).

In their study, Leali & Quaroni (1968), used 180 samples each of pasteurized whole milk and homogenised whole or partially skimmed milk, packaged in different containers and stored in (i) a refrigerator (5^{o}C), (ii) a refrigerated room (7^{o}C minimum, 15^{o}C maximum), (iii) in a cool environment (shade temperature) and (iv) at normal temperature (22 to 24^{o}C winter, 27 to 28^{o}C summer). They found that the keeping quality of the milks identified above was as follows:- (i) 21 days during all seasons, (ii) 8-12 days, (iii) 56-72 h in winter and 32 h in summer, and (iv) 24 h in winter and 8 h in summer. The conditions of loading and transport had no effect on keeping quality.

Smith (1968) stated that there was an increase in bacterial counts and a decrease in flavour scores with increasing temperature of filling, but the most important factors in prolonging shelf life were the storage temperature which should be 4.4^{o}C (40^{o}F) or below. However,

protection from post-pasteurization contamination was shown by Mourgues & Auclair (1970) to improve keeping quality at 4°C.

Watrous et al. (1971) reported that when samples of commercially pasteurized milk were held at 4.4°C the count increased from 260 per ml to 36 million per ml after 10 days. Where the storage temperature was 7.2°C the counts increased from the original at level of 260 per ml to 7.8 million per ml after 5 days and 770 millions after 10 days.

Further information indicating that the keeping quality of pasteurized milk is related to storage temperature was provided by the studies of Gritsenko & Surovtseva (1972b) where with milk held at room temperature (20 to 28°C) coagulation occurred in 12 to 15 h and at 6 to 8°C the coagulation occurred after 1 to 2 days.

Mourgues & Auclair (1973) showed that the standard plate count of pasteurized milk held at 4°C decreased initially but rose rapidly with the appearance of flavour defects after 30 to 73 days of storage and samples stored at 8°C showed similar changes except that the flavour defects appeared after 10-35 days. This relationship was less evident in samples stored at 4°C. Ademollo et al. (1974) examined 240 samples of commercial homogenized pasteurized milk packaged in cartons after 0-10 days storage at 4°C. Coliform bacteria were detected in 7 samples. The bacterial count averaged 10^5/ml for the first 5 days of storage but rose after the 6th day.

Kim & Lee (1975) studied pasteurized milk (August and early October, 1974) held at 4°, 10° or 35°C. The standard plate counts exceeded the 50,000 per ml legal limit when the milk was stored for 2 or 3 days at 4°C, 1 or 2 days at 10°C, or 3 h at 35°C. The total and psychrophilic counts of samples stored at 10°C for 24 h increased more rapidly than in the samples stored at 4°C. The initial counts of thermophilic bacteria were low due to the High Temperature Short Time and Ultra High Temperature pasteurization. Thermophilic counts of samples decreased at both 4°C and 10°C with increasing storage time. Coliform bacteria developed during storage at 10° and 35°C even though the initial counts were absent in 1 ml. Most of the samples stored at 35°C had coagulated after 9 h.

Gillis (1979) in his study of using different methods for evaluating the

shelf life of pasteurized and re-pasteurized fluid milk, showed in tests using 10 commercial raw milks that samples of double pasteurized milks stored at 7°C for 5-20 days had higher standard plate counts, higher psychrotrophic counts and higher proteolytic counts than the same milks which had only been pasteurized once, but the differences were not significant. The standard plate counts and spore counts obtained during storage correlated with flavour (0.2120 and 0.2066 respectively). Data collected from results of the Hull Test on samples incubated at different temperatures and time showed that this test could be used to predict shelf life of milk.

Bulk collection of the raw milk

Cooling and storage of milk in bulk on the farm for one or two days or longer is becoming increasingly popular. A storage temperature of 4°C is normally used but this fluctuates, especially at milking when warm milk enters the tank and in transport of the raw milk from farm to processing depot in road tankers. Storage of raw milk for 24 h or longer at the dairy factory before heat treatment may also occur. Thus, three days or more can elapse between milk production and processing during which time there is considerable opportunity for psychrotrophic bacteria to grow and produce enzymes, especially lipases and proteases. Though these bacteria are killed by pasteurization the lipases and proteases produced by many of them are not inactivated. These microbial enzymes can affect the quality of many dairy products either by enhancing or causing deterioration of the flavour during storage.

Scope of work

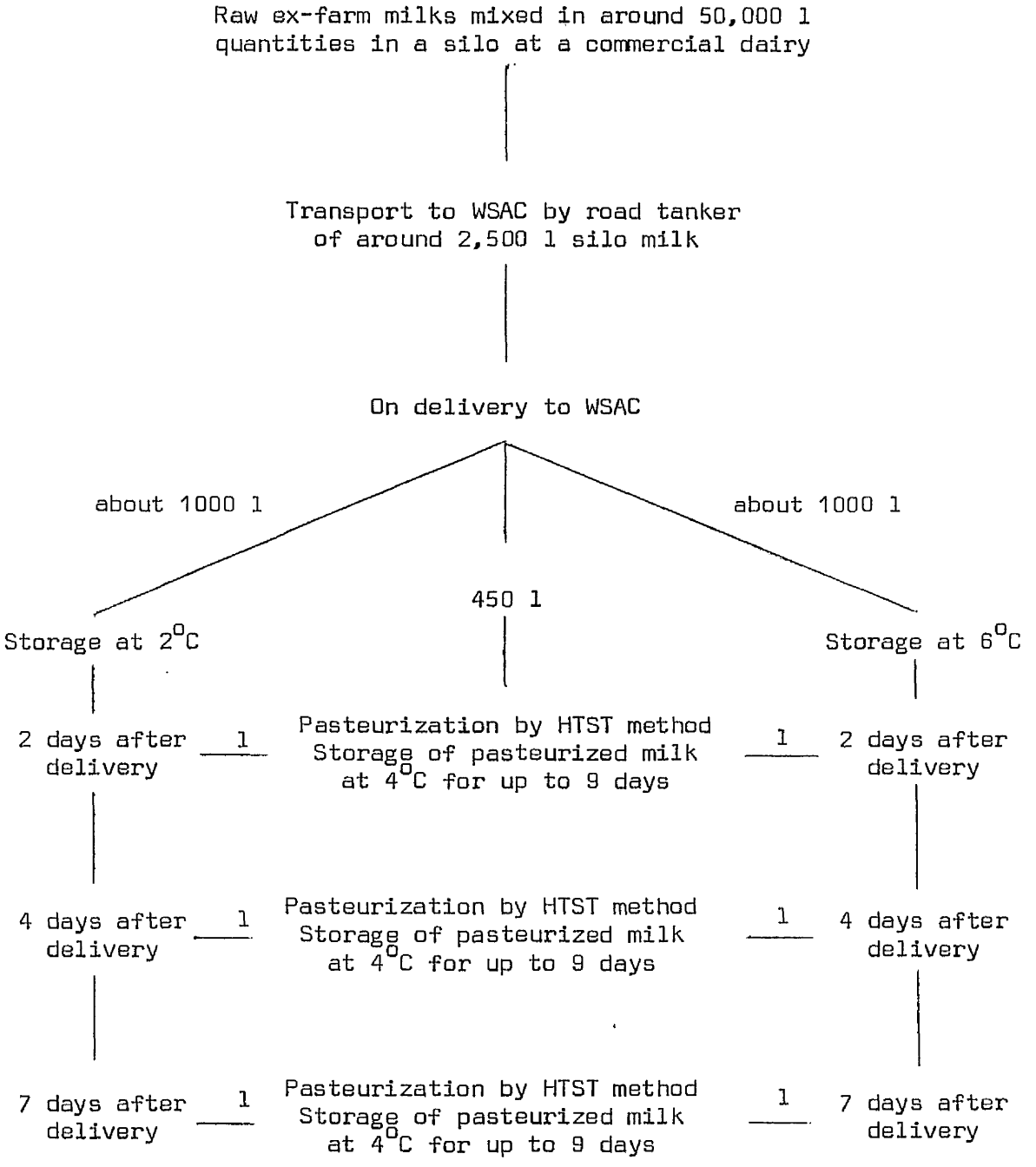
Many investigations have already been carried out on the production of milk and dairy products of superior quality. Processing extends shelf life but freedom from defects requires high quality raw milk. Milk and other ingredients used should be free from offending odours and flavours, abnormal chemical and physical properties, and undesirable micro-organisms or their metabolites.

The increased use of refrigeration to extend the storage life of milk on the farm, in the dairy plant, during distribution and marketing, and in the consumer's home has increased the importance of the micro-

organisms in milk and its products.

This study was intended to provide more information on the effect of the quality of raw milk on the quality of heat processed milks and milk products derived from it.

This study was carried out using bulk silo milk from a dairy factory in the West of Scotland. Pasteurized milk was prepared from the milk on delivery by road tanker and after the milk had been stored at $2^{o}C$ and $6^{o}C$ for up to 7 days. A comparison of the microbiological, chemical and organoleptic quality of both raw and pasteurized milk was made according to the plan below.



Tests made on raw milks on delivery and after 2, 4 and 7 days of storage at 2°C and 6°C. Tests made on pasteurized milks immediately after processing and after 3, 6 and 9 days storage at 4°C.

CHAPTER ONE

MATERIALS AND METHODS

SECTION 1. MICROBIOLOGICAL METHODS

1. Total plate count

During the study the total count of milk was determined according to the IDF standard method 3:1958 (IDF/FIL, 1958).

2. Coliform count

(a) The coliform count of the raw milk was determined according to the IDF standard method 39:1966 (IDF/FIL, 1966).

(b) The coliform count of the pasteurized milk was determined according to the IDF standard method 40:1966 (IDF/FIL, 1966).

3. Psychrotrophic count

Throughout the study the count of psychrotrophic bacteria was determined as described by the American Public Health Association (1978).

4. Lipolytic count

The number of lipolytic bacteria presented in samples was determined according to the IDF standard method 41:1966 (IDF/FIL, 1966).

5. Proteolytic count

Throughout the study these bacteria were detected and enumerated using caseinate agar and the method of Martley et. al. (1970).

6. Thermoduric count

The count of thermoduric bacteria was determined according to British Standard 4285:1968 (British Standards Institution, 1968).

7. Antibiotic residues

The procedure selected was the disc assay method, based on the procedure of Galesloot and Hassing (1962), as used by the Scottish Milk Marketing Board

SECTION 2. CHEMICAL METHODS

1. Fat

During the study the fat content of the milk was determined according to B.S. 696 Part 2; 1969 (British Standards Institution, 1969).

2. pH

The measurement of hydrogen ion concentration (pH) of milk was made with a PYE model 290 pH meter fitted with a combination glass electrode (Activion Glass Ltd., Scotland). Before measurement, the equipment was adjusted using buffers of known pH taking into consideration the temperature of the sample. Duplicate readings were recorded.

3. Titratable acidity

The titratable acidity of milk was determined according to B.S. 1741 (British Standards Institution, 1963) using 10 ml of milk sample with 1 ml of a 0.5 per cent (w/v) solution of phenolpthalein as an indicator. Titration with N/9 NaoH solution was made until the end point, a faint pinkish colour was reached. The volume of N/9 NaOH solution used divided by 10 is recorded as titratable acidity expressed as per cent lactic acid.

4. Cream line

The cream line of the milk was measured by pouring a well mixed sample into a graduated 100 ml measuring cylinder and allowing it to remain unmixed at room temperature for 24 h, when the depth of the cream layer was measured, (Davis, 1951).

5. Entraneous water

Throughout the study the freezing point depression was determined using the Advanced Milk Cryoscope (Model 4L) manufactured by Advanced Instruments Inc., Neddham Heights, Massachusetts. This instrument meets the requirement specified by the Association of Official Agricultural Chemists, (1965).

6. Sulphydryl groups

Sulphydryl groups were determined during the study using the modification

by Beveridge et al. (1974) of the method prepared by Ellman (1959). The method depends on adding 0.5 ml of skim milk to 2.5 ml of 8 M Urea in Tris-glycine buffer and 0.02 ml of Ellman's reagent (5.5'-dithiobis-2-nitrobenzoic acid) to form a yellow compound the reading has been done with absorbance at 412 nm. measurement being made using a PYE Unicam SP 1800 ultraviolet spectrophotometer. The SH groups were expressed as µmol/g dry weight.

7. Calcium

The calcium content of milk was determined according to the method of Pearce (1977). This method depends on the use of a high pH, indicator and direct titration.

8. Total solids

The total solids content of milk was determined according to the IDF standard method 21:1962 (IDF/FIL, 1962).

9. Acid degree value (ADV)

The acid degree value of milk was determined using the modification by Hunter, Wilson and Barclay (1966, 1968) of the method prepared by Thomas et al. (1955). The method depends on the extraction of the fat from the milk with B.D.I reagent, which consists of 30 g Triton x -100 and 7 g of sodium hexametaphosphate made up to one litre. One g of the extracted fat is weighed into a conical flask fitted with glass stopper. The fat is dissolved in 5 ml of fat solvent and titrated against a previously standardised alcoholic solution of KOH immediately before titration after the addition of 5 drops of 1 per cent (w/v) phenolphthalein solution. ADV is defined as the volume in ml of N KOH required to neutralise the free fatty acids present in 100 g of milk fat.

10. Phosphatase test

The phosphatase test was determined according to the IDF standard method 82:1978 (IDF/FIL, 1978). One ml of milk sample was added into 5 ml disodium p-nitrophenyl phosphate buffer solution pH (10.3) in a test tube. The tube was held at 37^{o}C for 2 h. The p-nitrophenol liberated was measured in direct comparison with standard colour glasses in a simple comparator using reflected light. If the reading is more than 10 mg p-nitrophenol per ml the result is recorded as positive.

CHAPTER TWO

THE MICROBIOLOGICAL QUALITY OF RAW AND PASTEURIZED MILK DURING DIFFERENT PERIODS OF STORAGE AT DIFFERENT TEMPERATURES

INTRODUCTION

Bulk milk collection from refrigerated tanks on dairy farms was first produced in 1936 in Oakland, California. The system was introduced to the United Kingdom by the Scottish Milk Marketing Board in Kirkcudbrightshire in 1954, and within the area of the Milk Marketing Board for England and Wales, the largest of the five Milk Marketing Boards in the United Kingdom, in 1955, (Crawford, 1967).

A generation ago milk was delivered to the processing plant, pasteurized, cooled, bottled and delivered to the consumer's doorstep before it was 24 h old and the milk was probably consumed before it was 48 h old. Today the age of milk before consumption is influenced by some or all of the following factors:- daily or alternate day collection of milk from refrigerated tanks at farms, five-day-week plant operations, discontinuance of home delivery, and increased purchase of milk from shops and supermarkets, longer periods of storage of milk in shops and supermarkets in an uncontrolled condition before consumption (Overcast, 1968). Milk which has been stored at low temperature (0-5^{o}C) for 2-3 days differs bacteriologically as well as physiochemically from milk held at a higher temperature or at a low temperature for a short time (Thomas, 1969). The same author stated that the bacterial flora of raw milk cold stored at 3-5^{o}C for 2-3 days is usually dominated by psychrotrophs.

Johns (1970) has suggested that certain compounds produced by micro-organisms in raw milk might be responsible for the development of off-flavour in pasteurized milk. The psychrotrophic bacteria have been associated with the deterioration of flavour in both raw and pasteurized milk (Boyd *et al.*, 1955; Crawford, 1967; Hartley *et al.*, 1969). The raw milk effects keeping quality of refrigerated pasteurized milk but it should be borne in mind that the count of psychrotrophic spore formers in raw milk does not parallel total count (Swartling, 1953). Therefore, low total counts of raw milk are not necessarily guarantees

of good keeping quality of pasteurized milk. The use of refrigeration to prolong the storage life of milk on the farm, in the dairy plant, during distribution and marketing and in the consumer's home has increased the importance of the psychrotrophic group of bacteria (Oliveria and Parmelee, 1976). These micro-organisms, which are able to grow at low temperatures, can cause spoilage of fluid milk and some other dairy products when they are stored at temperatures in the range of 1 to 7°C (Elliker et al., 1964; Ford & Babel, 1969; Lück, 1972; Thomas, 1966; Thomas & Druce, 1969; Thomas et al., 1966).

Bergman et al. (1962) stated that the duration of cold storage on the farm after production can vary to some extent, but in Sweden it is generally between 24 and 72 h at 0-4°C (32-39°F). The milk can be cold stored both at the farm and at the dairy.

The aim of the work described in this chapter was to investigate the microbiological quality of raw milk stored at 2°C and 6°C for up to 7 days and the quality of pasteurized milk produced from the milk after various periods of storage.

EXPERIMENTAL

The milk contained in the silo (around 50,000 l) at the commercial dairy factory was composed of a mixture of at least two milkings collected by tanker from farms in a radius of about 30 km from the factory.

This study was carried out using about 2,500 l quantities of milk from a bulk silo at the Mauchline Creamery of the Scottish Milk Marketing Board in the West of Scotland. The milk was delivered to the Department of Dairy Technology by road tanker and the experiment was carried out on three separate occasions. The actual age of the silo milk was uncertain but was always more than twenty four hours.

1. Collection of the samples

One lot of 1,000 l, of the raw milk was pumped from the receiving tank into a stainless steel refrigerated tank supplied by Desco Dairy Supply Co. Ltd., London. The temperature of the milk was maintained at 2°C. This tank is supplied with a mixer which was timed to mix the milk for

½ h every 6 h. The tank and agitation meets the requirements of B.S. 3976 (British Standards Institution, 1966). The second lot of 1000 l of the milk was stored in a refrigerated room at 6°C in a storage tank fitted with a Rotamilk mixer supplied by the Motor Geer and Emc. Co. Ltd., Essex. The same mixing arrangements were used as for the first batch contained in the Desco tank.

2. Treatment of the samples

Samples of raw milk were taken in sterile containers (568 ml) immediately on delivery by road tanker and after 2, 4 and 7 days of storage. Pasteurized milk was produced from the milk on delivery and from each of the lots of milk after 2, 4 and 7 days of cold storage and examined immediately and after 3, 6 and 9 days of storage at 4°C. The pasteurized samples were taken from the pasteurization plant in a sterile bottle from a sterile valve in the bottom of the pasteurizer and cooled immediately in iced water to less than 7°C.

3. Milk pasteurization

Four hundred and fifty litres (100 gal) of milk were pasteurized at 72°C for 15 secs using a British manufactured pasteurizer supplied by APV Co. Ltd., Crawley, England.

4. Preparation of the samples for testing

Samples of raw and pasteurized milk on each day of testing were shaken thoroughly for:-

a. microbiological analysis

b. chemical analysis.

5. Microbiological analysis

Samples of raw and pasteurized milk were tested on the day of delivery by road tanker and after storage at 2°C and 6°C for 2, 4 and 7 days. Pasteurized milks were tested immediately after production and after 3, 6 and 9 days of storage at 4°C. The following determinations were made:- total colony count, coliform count, psychrotrophic count, lipolytic

count, proteolytic count, thermoduric count and antibiotic test using the methods described in Chapter One (Section 1:1, 2, 3, 4, 5, 6 and 7). All the tests were carried out in duplicate.

RESULTS

The following determinations were made in each of the three different trials.

Total colony count

(i) Raw milk

The results of the colony counts tests made on samples of milk taken on the day of delivery by the road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days are shown in Table 2.1.

A significant difference (at 1 per cent level) was found between the trials*. No significant difference was found in the mean counts of the milk on the day of delivery and after the storage for 2 days at $2^{o}C$.

Storage of the raw milk at $2^{o}C$ for 4 days after delivery resulted in significant differences ($p < 0.01$) in the total colony count compared to the initial value. A high significant ($p < 0.001$) increase in total colony count was found in the 7th day of storage of the raw milk at $2^{o}C$ compared to the initial value.

The mean total colony count of the raw milks stored at $6^{o}C$ for 2 days was significantly different ($p < 0.01$) from the mean initial values. Highly significant increases ($p < 0.001$) were found in the total colony counts of the raw milks stored at $6^{o}C$ for 4 and 7 days compared with the milk on delivery. The mean value of the total colony count for the raw milks on the day of delivery by the road tanker was 203×10^{3} per ml. After 7 days storage of the raw milk at $2^{o}C$ the count had increased to 18×10^{6} per ml. The

*The total bacterial counts in the:-

First trial 161,000 per ml

Second trial 341,000 per ml

Third trial 105,000 per ml

storage of the same milk at $6^{o}C$ for 7 days resulted in a count of 224×10^{6} per ml.

The variations in the total colony count obtained with different time and temperatures are illustrated in Fig. 2.1.

(ii) Pasteurized milk

Table 2.2 shows the total colony counts of samples of pasteurized fresh and cold-stored milks on processing and after storage for 3, 6 and 9 days at $4^{o}C$. A highly significant difference (at 0.1 per cent level) was found in the counts between the different trials*.

The storage of the pasteurized milk for 3, 6 and 9 days resulted in a highly significant difference (at 0.1 per cent level) compared with the day of processing. The mean of the total colony counts of pasteurized milk prepared on the day of delivery of the raw milks and after the storage of the raw milk for 7 days at $2^{o}C$ were not significantly different. On the other hand the total colony counts of pasteurized milks prepared from raw milk stored at $6^{o}C$ for 2, 4 and 7 days showed a highly significant increase ($p < 0.001$) compared to the initial values obtained when pasteurization took place on the day of raw milk delivery.

The mean of the total colony counts of pasteurized milk produced from raw milk on the day of delivery by the road tanker was 48×10^{3} per ml.

The mean total colony count of the pasteurized milk produced from raw milk on delivery by road tanker and after the storage for up to 9 days was 71×10^{3} per ml. The mean total colony count of the pasteurized milks produced from raw milks stored at $2^{o}C$ for 7 days was 88×10^{3} per ml, while the mean total colony count for the pasteurized milks produced from raw milks stored at $6^{o}C$ for 7 days was 667×10^{3} per ml. The mean total colony count for pasteurized

*The total bacterial counts in the:-

First trial 106,000 per ml
Second trial 30,000 per ml
Third trial 8,500 per ml

TABLE 2.1

The total colony count/ml (30°C for 3 days), expressed as $\log_e$, of raw milk (RM) on delivery by road tanker, and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	11.99	12.29	12.66	14.73	13.21	13.02	18.99
2	12.74	12.81	15.26	16.33	16.38	16.71	19.40
3	11.56	12.46	15.38	17.50	14.73	17.66	19.25
Mean	12.10	12.52	14.43	16.19	14.77	15.79	19.21

	DF	MS	VR
Trial	2	7.141	6.953**
Treatment (1 – 4 below)	6	17.373	16.915***
1. F v rest	1	29.550	35.6928***
2. Storage	2	25.290	30.5470***
3. Temperature	1	22.038	26.6198***
4. Storage. Temperature	2	1.034	1.2496
Residual	12	1.027	
Total	20	6.542	

Table	Trial	Treatment	Storage	Temperature	Storage. Temperature
REP	7	3	Unequal	Unequal	3
SED	0.524	0.827	0.679	0.640	0.784

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 2.2

The overall three-trial total colony count/ml (30°C for 3 days), expressed as $\log_e$ of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The total colony counts of the pasteurized milks were made on the day of processing and after storage of the milks for 3, 6 and 9 days at 4°C

PM storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	10.31	10.62	10.59	10.88	11.01	11.55	11.69
3 days	10.50	10.79	10.85	10.91	11.31	11.89	11.73
6 days	10.74	10.85	10.92	10.96	11.36	11.94	12.18
9 days	10.83	11.11	11.23	11.17	12.97	12.82	13.14
Mean	10.61	10.84	10.91	10.98	11.66	12.05	12.18

	DF	MS	VR
Trial	2	81.109	188.975***
PM storage	3	3.547	8.263***
Treatment (1 - 4 below)	6	4.883	11.378***
1. F v rest	1	7.266	8.861***
2. Storage	2	0.687	0.838
3. Temperature	1	20.135	24.541***
4. Storage. Temperature	2	0.262	0.319
Trial. PM storage	6	0.090	0.210
Trial. Treatment	12	2.760	6.431***
PM storage. Treatment	18	0.273	0.637
Residual	36		
Total	83		

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage Temperature
REP	12	3	Unequal	Unequal	12
SED	0.268	0.535	0.2723	0.2576	0.3144

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.1 Changes in the total colony count of raw milk (RM) after various periods of storage at 2°C and 6°C

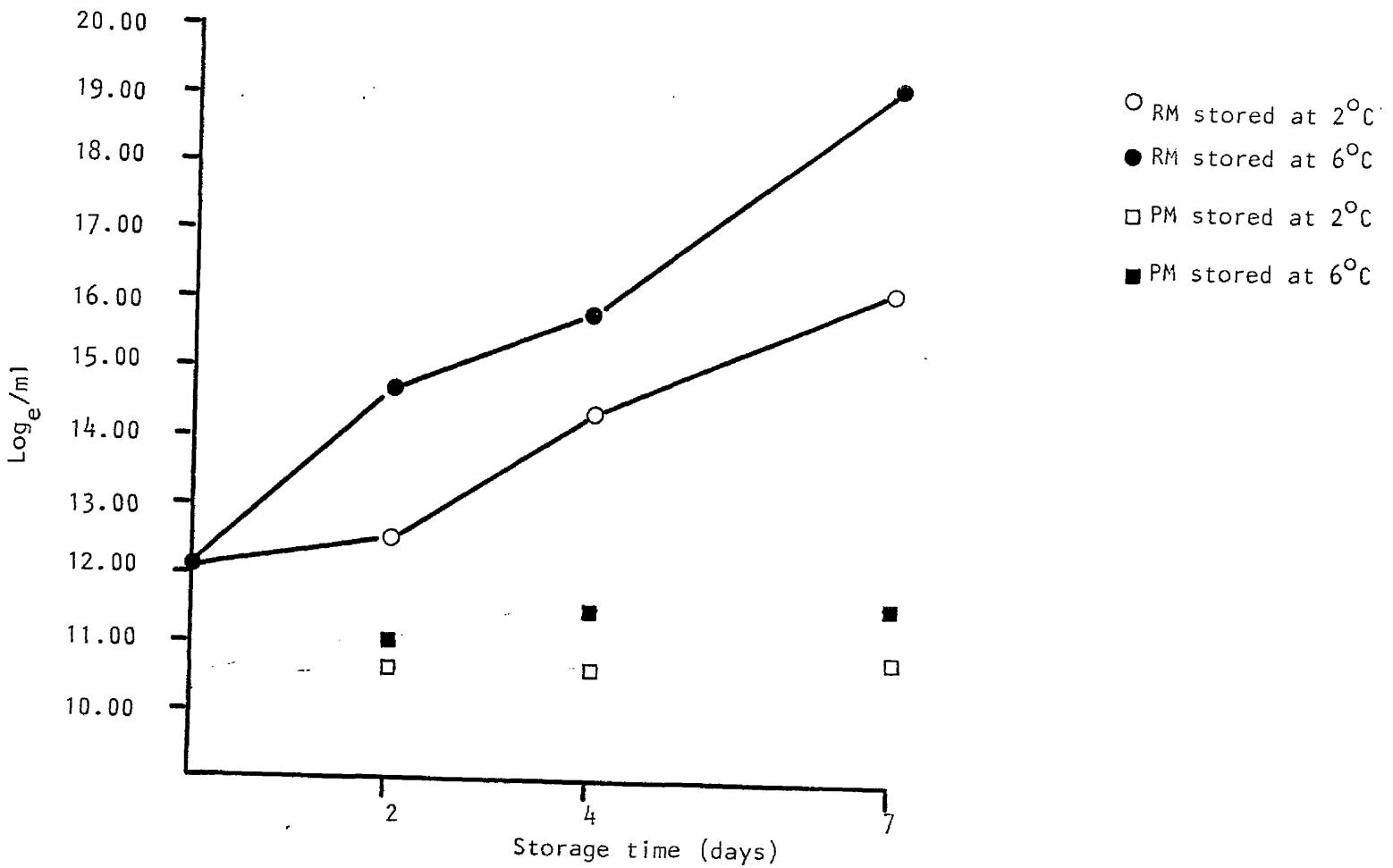


Fig. 2.2 Changes in the overall three-trial total colony count after various periods of storage at 4°C of the pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for 2, 4 and 7 days

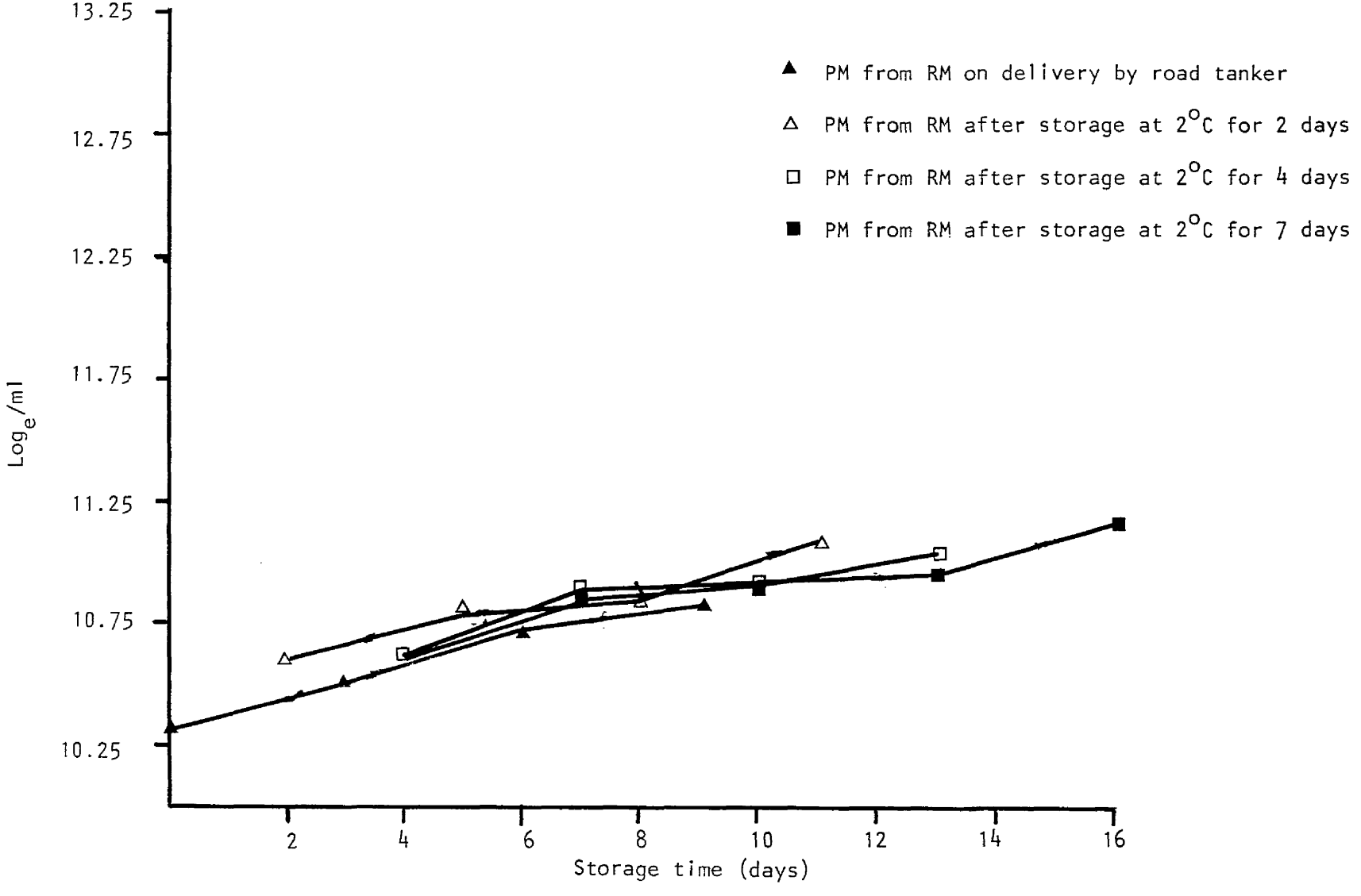
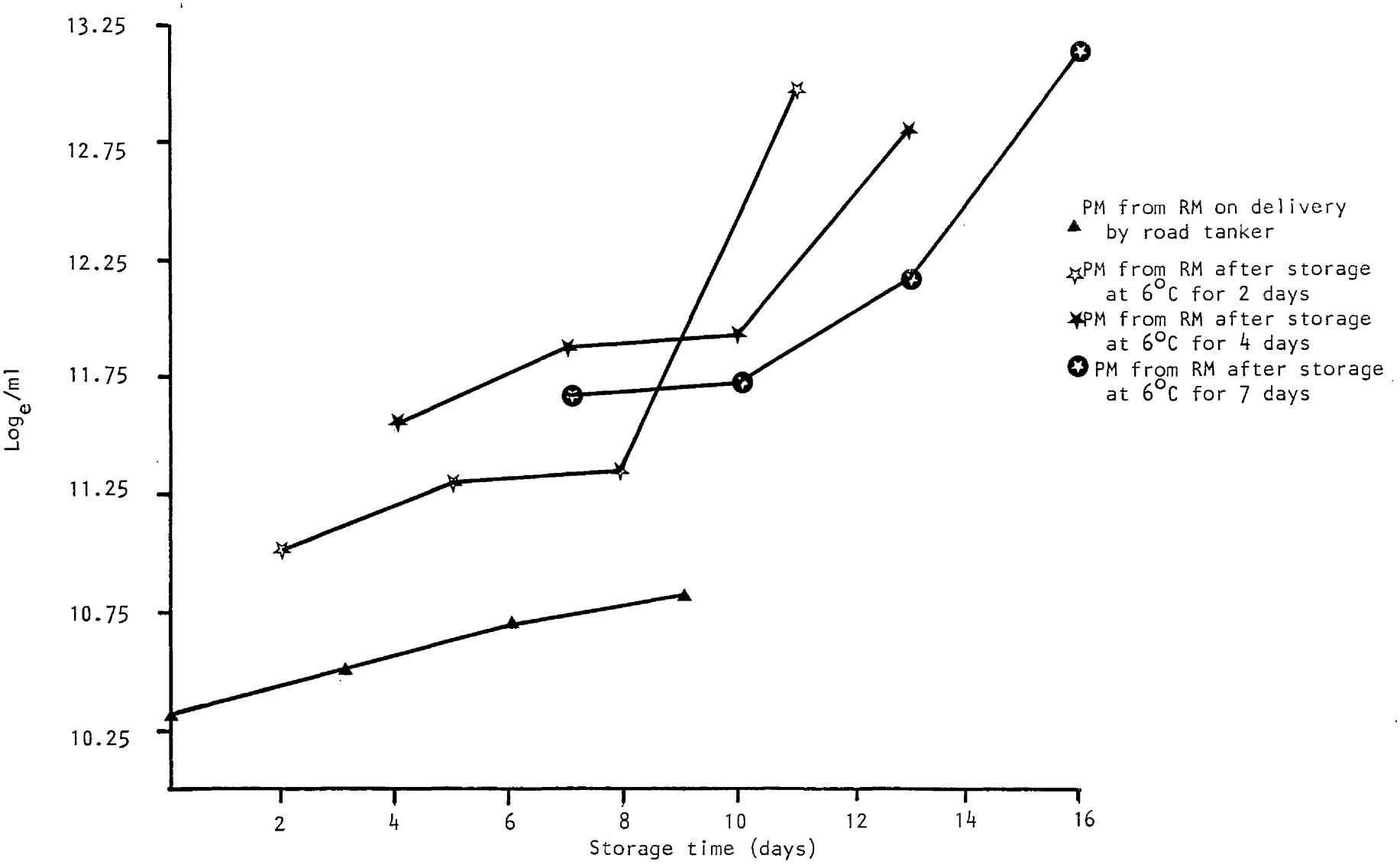


Fig. 2.3 Changes in the overall three-trial total colony count after various periods of storage at 4°C of pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for 2, 4 and 7 days



milk after the storage for 16 days was 121 x 10^3 per ml (from raw milk stored at 2^{o}C for 7 days), while the mean of the total colony count for pasteurized milk after the storage for 16 days was 872 x 10^3 per ml (from raw milk stored at 6^{o}C for 7 days). The variation in the total colony count obtained from different treatments with the storage time are illustrated in Figs 2.2 and 2.3.

Coliform count

(i) Raw milk

The coliform counts of raw milk on the day of delivery by a road tanker and after the storage at 2^{o}C and 6^{o}C for 2, 4 and 7 days are presented in Table 2.3.

A highly significant difference (at 0.1 per cent level) was found in the coliform count between different trials*. The storage of the raw milk for 2, 4 and 7 days showed a highly significant difference (at 0.1 per cent level) in the coliform count from the day of delivery.

The mean of the coliform counts of raw milk samples showed no significant difference between milks tested on the day of delivery and after 2 days stored at 2^{o}C. There was however a significant difference ($p < 0.05$) after 4 days and a highly significant difference ($p < 0.001$) after 7 days of storage of the same milk at 2^{o}C. The coliform count of the raw milk stored at 6^{o}C was significantly increased ($p < 0.01$) by 2 days of storage. Storage for 4 days resulted in an increase in count ($p < 0.001$) and the highest significant increase after 7 days of storage. The count of coliform organisms increased more in the samples of raw milk stored at 6^{o}C than in the samples of raw milk stored at 2^{o}C, (Fig 2.4). The mean count of the coliform for the raw milk on the day of delivery from the road ranker is 3 x 10^3 per ml. After 7 days of storage

*The coliform count in the:-

First trial 1,775 per ml
Second trial 5,745 per ml
Third trial 2,385 per ml

TABLE 2.3

The coliform count/ml (30°C for 1 day), expressed as $\log_e$, of samples of raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

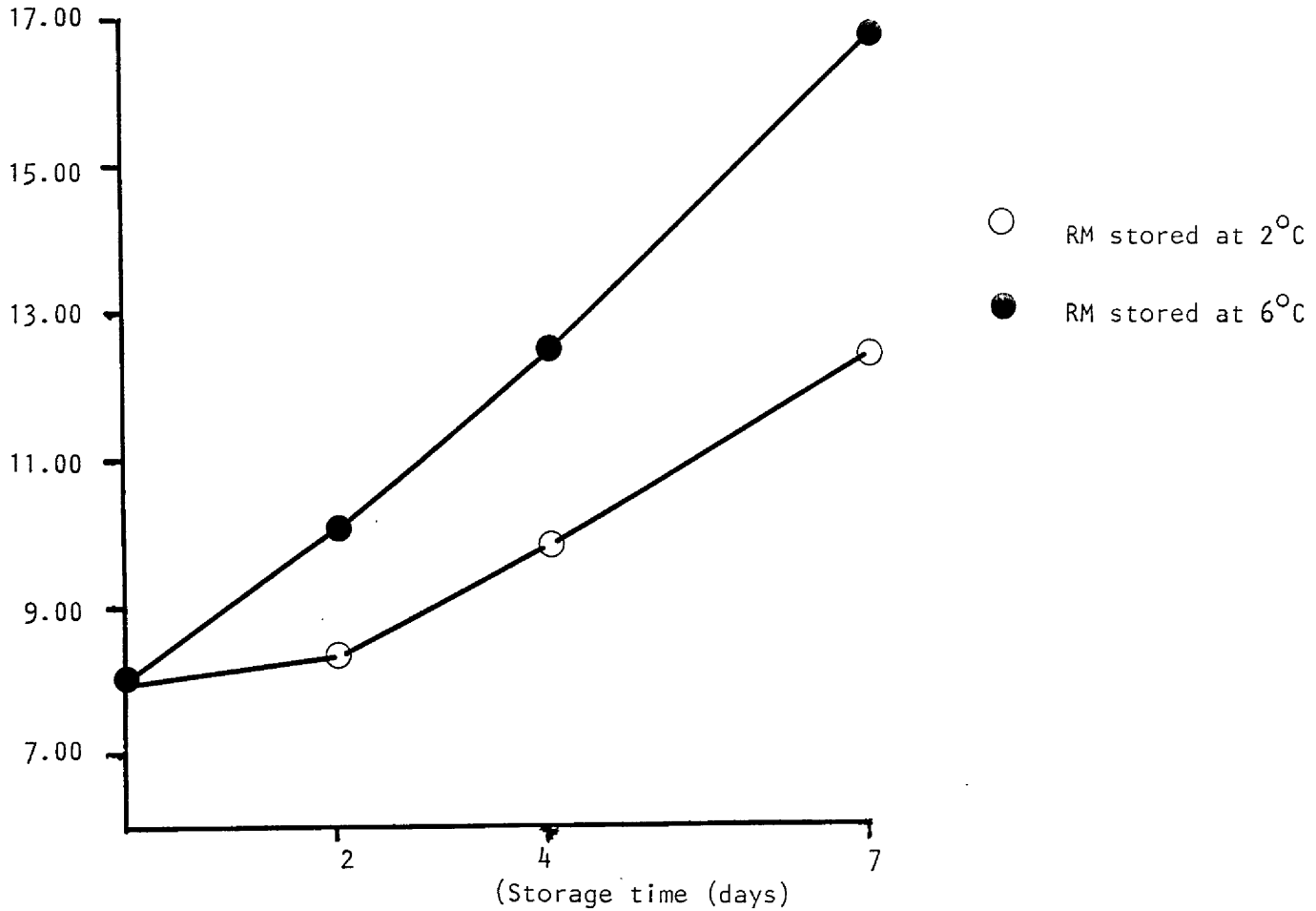
Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	7.48	7.99	8.97	11.41	9.07	9.99	14.99
2	8.66	8.63	10.31	13.06	10.69	12.79	17.62
3	7.78	8.29	10.13	12.65	10.28	14.78	17.64
Mean	7.97	8.30	9.80	12.37	10.02	12.52	16.74

	DF	MS	VR
Trial	2	6.5579	22.148***
F v rest	1	34.3692	116.074***
Trial. F v rest	2	0.5569	1.881
F v rest. Storage	2	44.6948	150.947***
F v rest. Temperature	1	38.7450	130.853***
Trial. F v rest. Storage	4	0.6686	2.258
Trial F v rest. Temperature	2	1.4801	4.999
F v rest. Storage. Temperature	2	2.7037	9.131*
Residual	4	0.2961	
Total	20	9.4480	

Table	Storage. Temperature	Storage	Temperature	Trial
REP	3	Unequal	Unequal	7
SED	0.444	0.385	0.363	0.291

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.4 Change in the coliform count of raw milk (RM) after various periods of storage at 2°C and 6°C



at $2^{o}C$ the raw milk coliform count was 291 x 10^{3} per ml, while it was 3 x 10^{6} per ml for the same raw milk stored at $6^{o}C$ after 7 days.

The coliform count showed a highly significant correlation coefficient ($r = 0.9521$, $p < 0.001$) with the total colony count. The correlation coefficient with psychrotrophic count was highly significant ($r = 0.8305$, $p < 0.001$) Table 2.11.

(ii) Pasteurized milk

In all the experiments, non of the samples of pasteurized milk produced from raw milk on delivery by road tanker or after storage at $2^{o}C$ and $6^{o}C$ for up to 7 days gave positive results for coliform in 1 ml.

Storage of the pasteurized milk for 3, 6 and 9 days after processing did not result in the presence of coliform in samples of the pasteurized milk.

Psychrotrophic count

(i) Raw milk

Table 2.4 shows the psychrotrophic counts of raw milk on the day of delivery by a road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days. The mean psychrotrophic count of raw milk samples stored at $6^{o}C$ was higher than the count of the same milk stored at $2^{o}C$. A significant difference (at the 5 per cent level) in the psychrotrophic count was found between the individual trials*. The storage of the raw milk for 2, 4 and 7 days was associated with significant difference (at 1 per cent level) in the psychrotrophic count. The temperature of storage had a significant effect (at 5 per cent level) on the psychrotrophic count of milk on the day of delivery by the road tanker and after storage for 7 days. No significant difference was found between the mean psychrotrophic count for raw milk on the day of

*The psychrotrophic count in the:-

First trial 5,195 per ml

Second trial 484,000 per ml

Third trial 34,500 per ml

TABLE 2.4

The psychrotrophic count/ml (7°C for 10 days), expressed as $\log_e$, of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

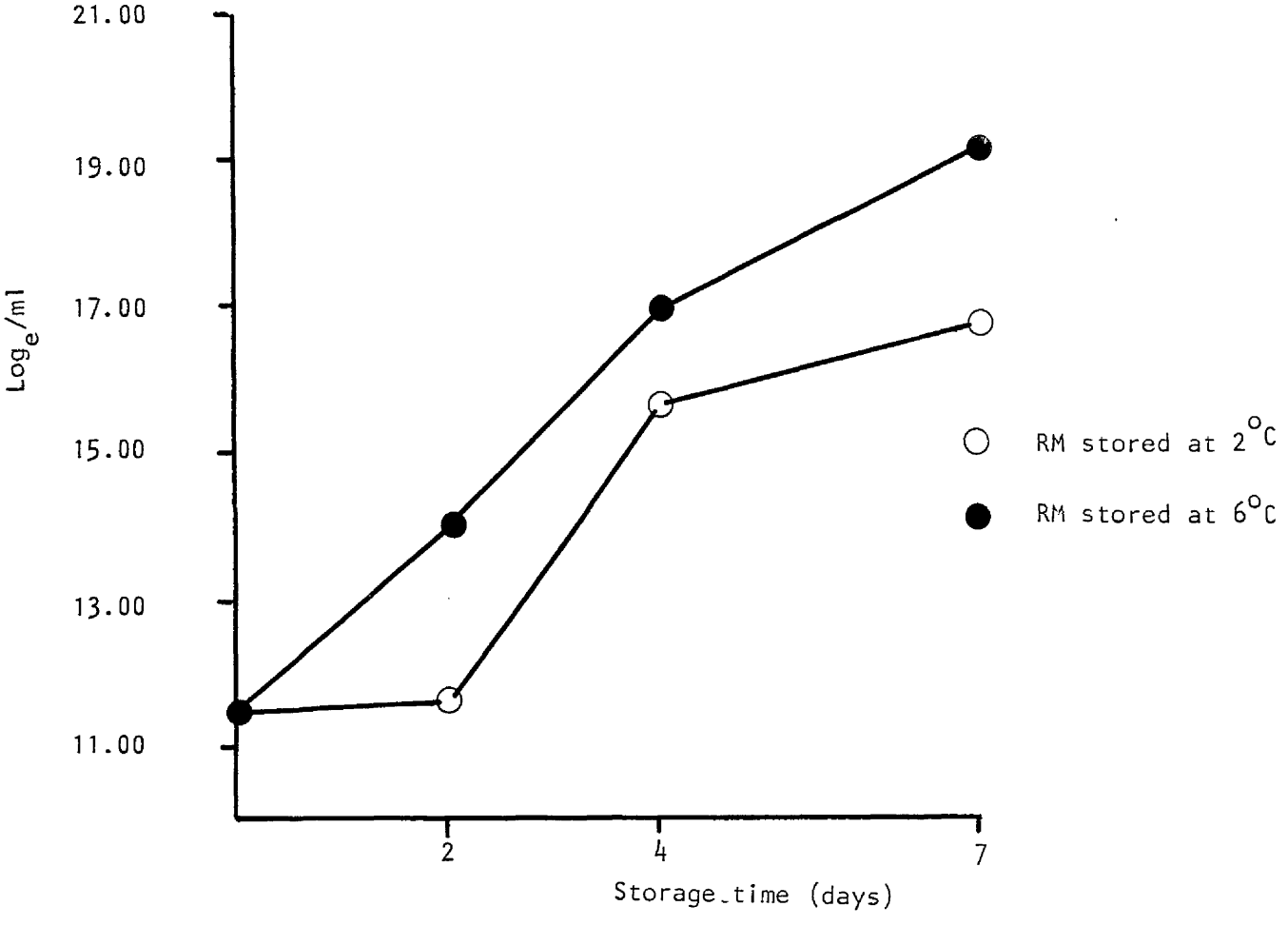
Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	8.56	9.08	16.03	16.64	9.26	13.83	18.44
2	13.09	13.44	16.08	17.13	15.98	17.37	19.54
3	10.45	12.33	14.95	16.91	14.60	17.71	19.11
Mean	11.47	11.62	15.69	16.89	14.05	17.07	19.20

	DF	MS	VR
Trial	2	19.067	16.547*
F v rest	1	47.243	41.000**
Trial. F v rest	2	1.388	1.204
F v rest. Storage	2	42.507	36.889**
F v rest. Temperature	1	18.705	16.233*
Trial. F v rest. Storage	4	4.646	4.032
Trial. F v rest. Temperature	2	5.421	4.704
F v rest. Storage. Temperature	2	0.491	0.426
Residual	4	1.152	
Total	20	11.344	

Table	Storage Temperature	Storage	Temperature	Trial
REP	3	Unequal	Unequal	7
SED	0.876	0.759	0.716	0.574

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.5 Changes in the psychrotrophic count of raw milks (RM) after various periods of storage at 2°C and 6°C



delivery and after its storage for 2 days at $2^{o}C$ and a significant difference ($p < 0.01$) after 4 days at $2^{o}C$, and ($p < 0.001$) after 7 days at $2^{o}C$, while the raw milk stored at $6^{o}C$ showed a significant difference ($p < 0.05$) after 2 days and ($p < 0.001$) after 4 days and highest significant after 7 days in the psychrotrophic count, Table 2.11.

The variations in the counts of psychrotrophic organisms in the raw milk during the storage time at $2^{o}C$ and $6^{o}C$ are illustrated in Fig 2.5. The mean of the psychrotrophic counts of raw milks on the day of delivery by a road tanker was 175×10^{3} per ml. After storage of the raw milk samples at $2^{o}C$ for 7 days the mean count was 22×10^{6} per ml while the mean count of the raw milk samples at $6^{o}C$ for 7 days was 246×10^{6} per ml.

The psychrotrophic count showed a highly significant correlation coefficient ($r = 0.8668$, $p < 0.001$) with the total colony count, Table 2.11.

(ii) Pasteurized milk

All samples of pasteurized milk on processing and after storage at $4^{o}C$ for up to 9 days gave no count when plated out at 1 ml in the psychrotrophic test.

Lipolytic count

(i) Raw milk

Table 2.5 presents the lipolytic counts of raw milks on the day of delivery by a road tanker and after the storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days. A significant difference (at 1 per cent level) was found in the lipolytic counts of the raw milks obtained in different trials*. The mean of the lipolytic counts showed no

*The lipolytic count in the:-

First trial 33,000 per ml
Second trial 55,000 per ml
Third trial 33,000 per ml

significant alteration after 2 days storage at $2^{o}C$, while there was significant ($p < 0.01$) increase in the lipolytic count after storage of the milk for 4 and 7 days at $2^{o}C$. A significant increase ($p < 0.05$) in the lipolytic count was found after the storage of the raw milk at $6^{o}C$ for 2 days, while after 4 days of storage of the same milk at $6^{o}C$ a further increase in numbers ($p < 0.01$) was observed. A highly significant ($p < 0.001$) difference was found in the lipolytic count after 7 days storage of the raw milk at $6^{o}C$.

The variations in the lipolytic count due to the storage time at different treatments are shown in Fig 2.6. The mean of the lipolytic count of raw milk on the day of delivery by the road tanker was 40×10^{3} per ml. After the storage of the raw milk at $2^{o}C$ for 7 days the count was 87×10^{6} per ml, while the storage of the same milk at $6^{o}C$ for 7 days resulted in a count of 501×10^{6} per ml.

The lipolytic count showed a highly significant correlation coefficient ($r = 0.9102$, $p < 0.001$) with the total colony count. The correlation coefficient with psychrotrophic count was highly significant ($r = 0.8770$, $p < 0.001$). The correlation coefficient with coliform count was highly significant ($r = 0.8761$, $p < 0.001$). Table 2.11.

(ii) Pasteurized milk

The lipolytic count of pasteurized milks produced from raw milk on the day of delivery by a road tanker and after the storage of the raw milk at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days and of the corresponding processed milk stored for 3, 6 and 9 days at $4^{o}C$ are given in Table 2.6. A highly significant difference (at 0.1 per cent level) was found between the trials* in relation to lipolytic counts.

No significant difference was found in the lipolytic count of milk pasteurized on delivery by road tanker and that obtained for

*The lipolytic count in the:-

First trial 3,400 per ml
Second trial 32,000 per ml
Third trial 1,000 per ml

TABLE 2.5

The lipolytic count/ml (30°C for 3 days), expressed as $\log_e$, of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	10.40	11.23	12.77	15.07	12.55	12.47	16.42
2	10.92	13.08	17.50	19.25	15.92	19.30	20.99
3	10.40	12.10	15.42	17.15	14.65	17.25	19.06
Mean	10.57	12.14	15.23	17.16	14.37	16.34	18.82

	DF	MS	VR
Trial	2	24.456	21.132**
Treatment (1 - 4 below)	6	24.632	21.285**
1. F v rest	1	66.9269	5505.0795***
2. Storage	2	33.6807	328.5687***
3. Temperature	1	12.5464	1032.0042***
4. Storage. Temperature	2	0.4797	39.4605**
Residual	4	1.157	
Total	20	10.530	

Table	Treatment	Trial	Storage	Temperature	Storage. Temperature
REP	3	7	Unequal	Unequal	3
SED	0.878	0.575	0.2435	0.2296	0.2812

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 2.6

The overall three trial lipolytic count/ml (30°C for 3 days), expressed as $\log_e$, of pasteurized milk (PM) produced from raw milk (RM) on delivery by a road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The counts for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	8.47	8.66	9.25	9.27	9.77	10.80	10.97
3 days	9.14	9.01	9.61	9.82	10.07	10.91	10.27
6 days	9.25	9.74	99.74	9.98	10.24	10.95	10.50
9 days	9.49	9.90	10.42	10.49	10.45	12.21	12.38
Mean	9.09	9.33	9.75	10.00	10.14	11.22	11.03

	DF	MS	VR
Trial	2	167.788	592.941***
PM Storage	3	4.915	17.369***
Treatment (1 – 4 below)	6	7.741	27.354***
1. F v rest	1	13.763	20.696***
2. Storage	2	4.733	7.117**
3. Temperature	1	21.867	32.884***
4. Storage. Temperature	2	0.673	1.013
Trial. PM Storage	6	1.085	3.835**
Trial. Treatment	12	2.098	7.412***
PM Storage. Treatment	18	0.339	1.198
Residual	36		
Total	83		

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage Temperature
REP	12	3	Unequal	Unequal	12
SED	0.2172	0.4343	0.2328	0.2195	0.2688

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.6 Changes in the lipolytic count of raw milk (RM) after various periods of storage at 2°C and 6°C

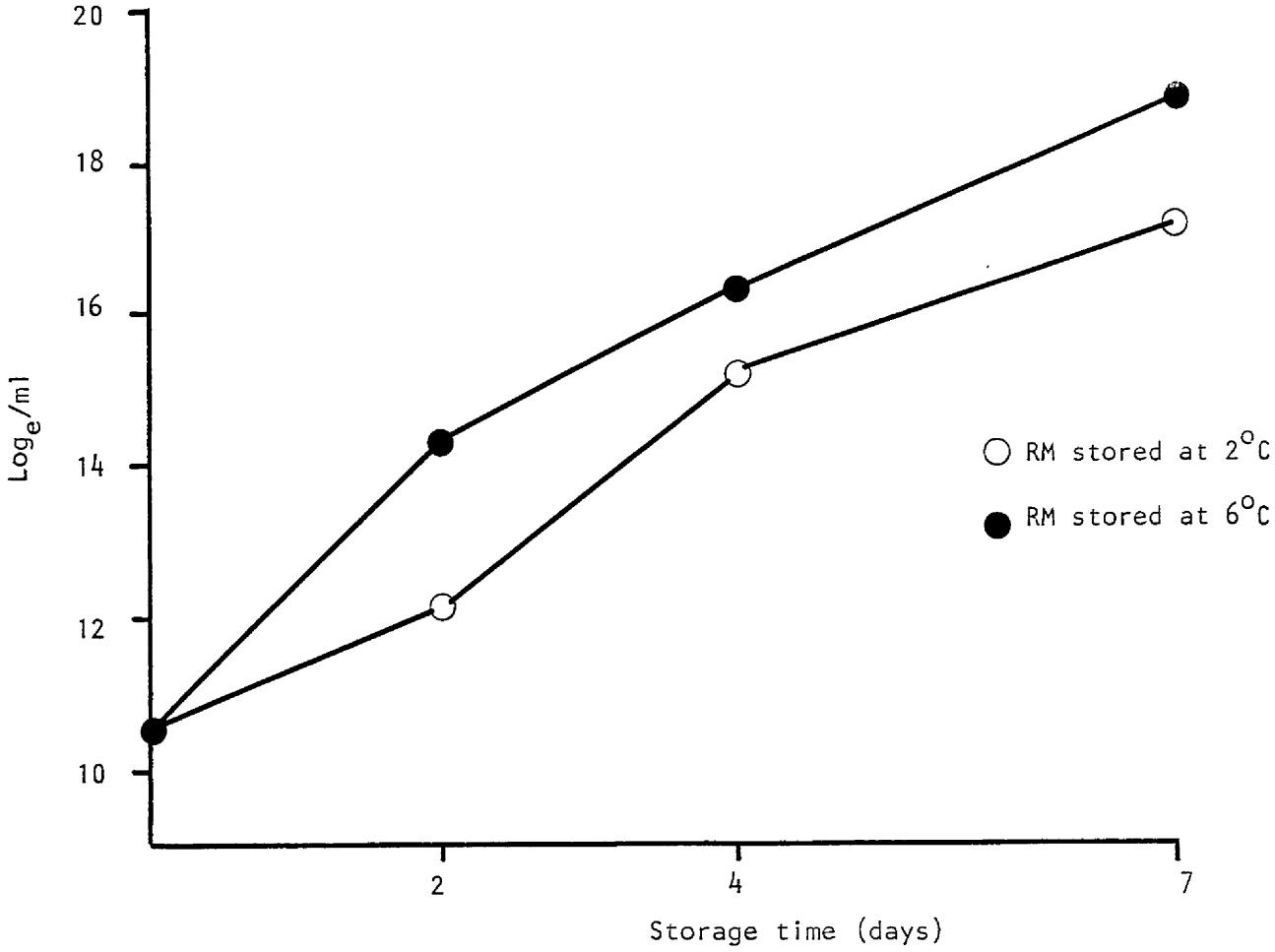


Fig. 2.7 Changes in the overall three-trial lipolytic counts after various periods of storage at 4°C of the pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for 2, 4 and 7 days

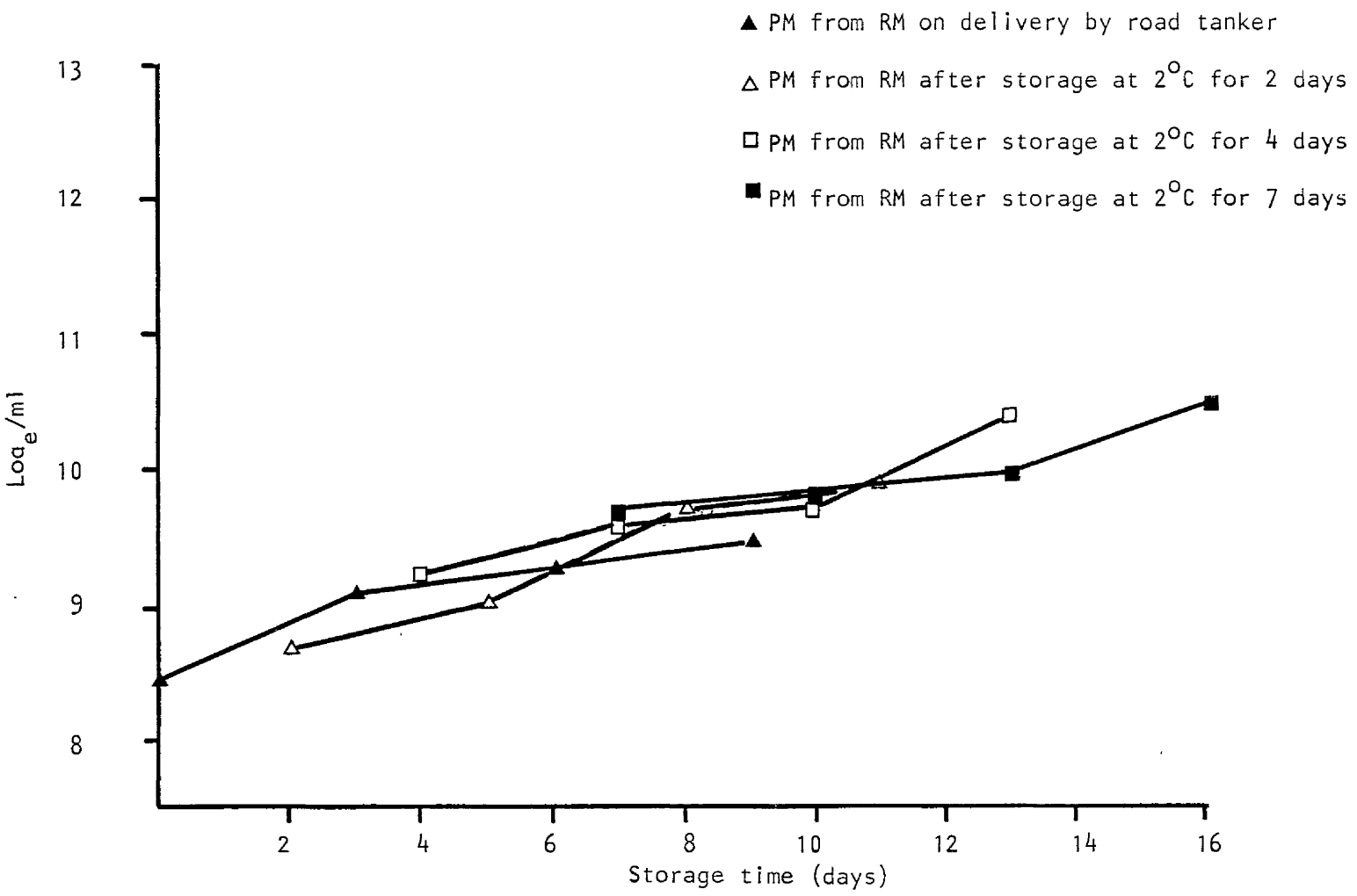
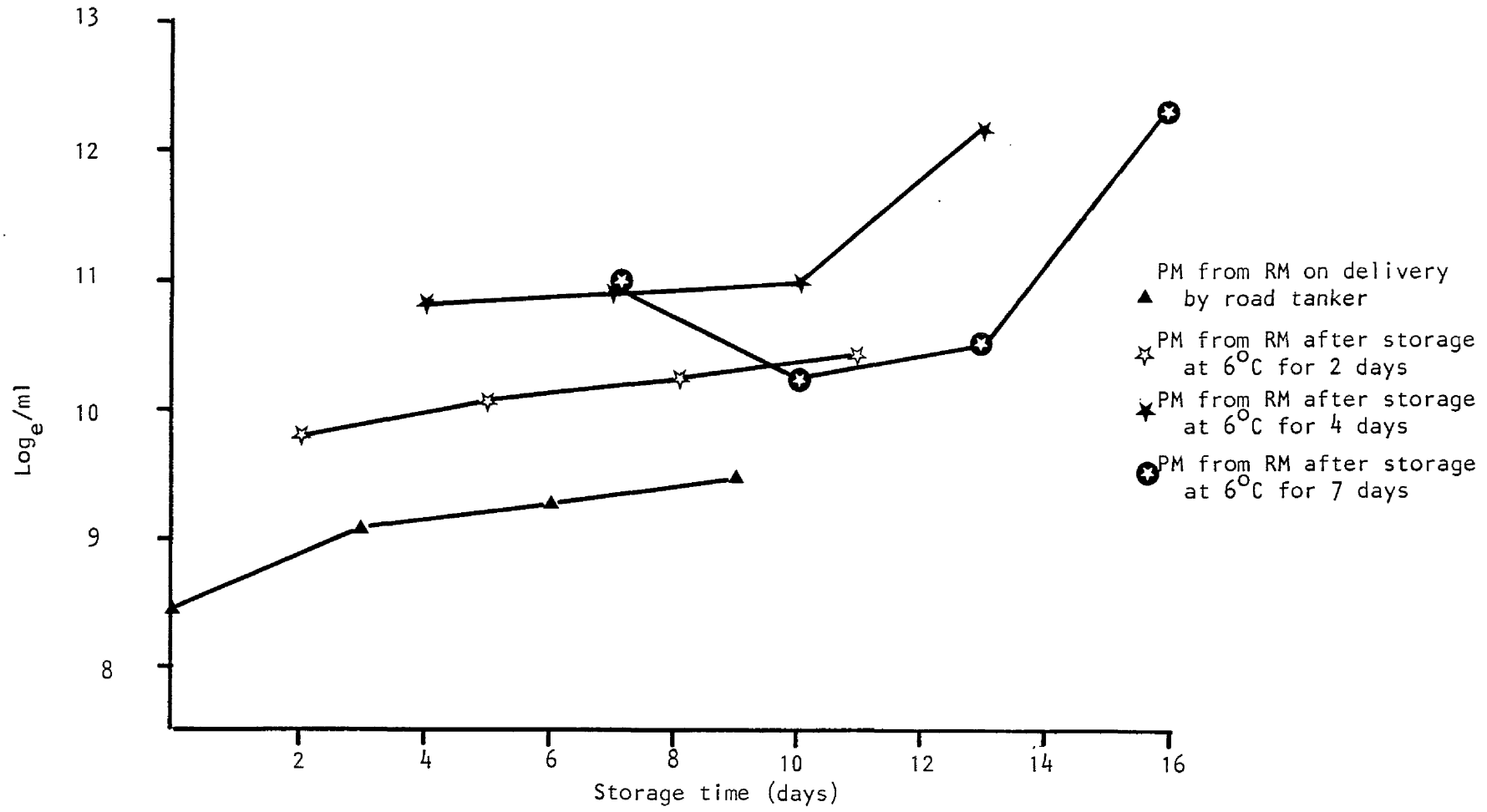


Fig. 2.8 Changes in the overall three-trial lipolytic count after various periods of storage at 4°C of the pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for 2, 4 and 7 days.



pasteurized milk derived from the same milk after storage for 2 days at $2^{o}C$. A highly significant ($p < 0.001$) increase in lipolytic organisms was found in pasteurized milk produced from raw milk stored at $2^{o}C$ for 4 and 7 days, while the pasteurized milk produced from raw milk stored at $6^{o}C$ for 2, 4 and 7 days gave the greatest increase in numbers ($p < 0.001$). A significant difference (at 1 per cent level) was found between the individual trials and the storage of the pasteurized milk for 3, 6 and 9 days.

The storage of the pasteurized milk for 3, 6 and 9 days after processing resulted in a highly significant increase (at 0.1 per cent level) in the lipolytic count. The variations in the lipolytic count due to the storage time at different temperatures are illustrated in Figs 2.7 and 2.8. The mean of the lipolytic counts for the pasteurized milks on the day of delivery by a road tanker was 12×10^{3} per ml. After storage of the raw milk at $2^{o}C$ for 7 days the mean count of lipolytic organisms in the pasteurized milk produced from the same milk was 45×10^{3} per ml. The count of lipolytic organisms of the pasteurized milk produced from raw milk stored at $6^{o}C$ for 7 days was 107×10^{3} per ml. The lipolytic count of the pasteurized milk produced from raw milk stored at $2^{o}C$ for 7 days and held after heat treatment for 9 days was 81×10^{3} per ml, while the count for the corresponding pasteurized milk produced from raw milk stored at $6^{o}C$ for 7 days and held in cold storage at $4^{o}C$ for 9 days was 368×10^{3}.

The lipolytic count showed a high significant correlation coefficient ($r = 0.7332$, $p < 0.001$) with the total colony count, Table 2.12.

Proteolytic count

(i) Raw milk

The proteolytic counts of raw milks are presented in Table 2.7. A significant difference (at 1 per cent level) was found between different trials* in the proteolytic count. No significant

*The proteolytic count in the:-

First trial 127,500 per ml
Second trial 400,000 per ml
Third trial 73,750 per ml

difference was found in the mean proteolytic count of milks on the day of delivery by a road tanker and the count of the same milk after the storage at $2^{o}C$ for 2 days. A highly significant difference ($p < 0.001$) was found between the initial proteolytic count and that obtained after 4 and 7 days of storage at $2^{o}C$. The raw milk stored for 2 days at $6^{o}C$ had a proteolytic count which showed a highly significant increase ($p < 0.001$) compared with the initial values. Higher increases after 4 days of storage on the same temperature and highest increase after 7 days storage of the same milk at $6^{o}C$ on the proteolytic count. The variation in the proteolytic count due to the storage time at different temperatures is illustrated in Fig. 2.9.

The mean proteolytic count of the raw milks on the day of delivery by a road tanker was 200×10^{3} per ml. After 7 days storage of the raw milks at $2^{o}C$ the mean count was 47×10^{6} per ml, while after 7 days storage of the same milks at $6^{o}C$ the mean count was 258×10^{6} per ml.

The proteolytic count showed a highly significant correlation coefficient ($r = 0.9580$, $p < 0.001$) with the total colony count. The correlation coefficient with the psychrotrophic count was highly significant ($r = 0.8957$, $p < 0.001$). The correlation coefficient with the coliform count was highly significant ($r = 0.9154$, $p < 0.001$). The correlation coefficient with the lipolytic count was highly significant ($r = 0.9363$, $p < 0.001$). Table 2.11.

(ii) Pasteurized milk

The proteolytic counts are presented in Table 2.8. There was a highly significant difference (at 0.1 per cent level) in the proteolytic counts of the milks produced in the different trials*. Storage of raw milk at $2^{o}C$ before processing had no significant effect on the proteolytic count of the pasteurized milk compared

*The proteolytic count in the:-

First trial 15,900 per ml

Second trial 118,000 per ml

Third trial 2,100 per ml

TABLE 2.7

The proteolytic count/ml (30°C for 3 days), expressed as $\log_e$, of samples of raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	11.79	13.07	12.62	16.24	13.84	15.65	18.92
2	12.90	13.51	16.06	17.75	17.22	18.68	19.78
3	11.21	12.58	15.88	18.20	15.18	17.45	19.21
Mean	11.95	13.05	15.19	17.39	15.41	17.26	19.30

	DF	MS	VR
Trial	2	5.8594	10.277**
Treatment (1 – 4 below)	6	19.8378	34.796***
1. F v rest	1	47.8376	93.3525***
2. Storage	2	25.4564	49.67708***
3. Temperature	1	20.1218	39.2666***
4. Storage. Temperature	2	0.0773	0.15072
Residual	12	0.5701	
Total	20	6.8794	

Table	Treatment	Trial	Storage	Temperature	Storage. Temperature
REP	3	7	Unequal	Unequal	3
SED	0.617	0.404	0.520	0.490	0.600

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 2.8

The overall three trial proteolytic count/ml (30°C for 3 days), e expressed as $\log_e$, of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The count for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	9.67	10.12	10.27	10.36	10.25	10.46	10.56
3 days	10.31	10.60	10.51	10.37	11.01	10.61	10.91
6 days	10.72	10.69	10.32	10.71	11.07	10.59	11.39
9 days	10.81	10.73	11.17	10.87	11.25	13.38	12.96
Mean	10.37	10.53	10.57	10.58	10.89	11.26	11.45

	DF	MS	VR
Trial	2	138.041	316.836***
PM Storage	3	6.838	15.694***
Treatment (1 – 4 below)	6	1.998	4.586**
1. F v rest	1	2.633	6.972*
2. Storage	2	0.557	1.476
3. Temperature	1	7.421	19.653***
4. Storage. Temperature	2	0.411	1.088
Trial. PM Storage	6	1.118	2.565*
Trial. Treatment	12	0.815	2.454*
PM Storage. Treatment	18	0.436	1.871*
Residual	36		
Total	83		

Table	Treatment	Treatment PM Storage	Storage	Temperature	Storage Temperature
REP	12	3	Unequal	Unequal	12
SED	0.2695	0.5389	0.2251	0.2123	0.2600

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.9 Changes in the proteolytic count of raw milk (RM) after various periods of storage at 2°C and 6°C

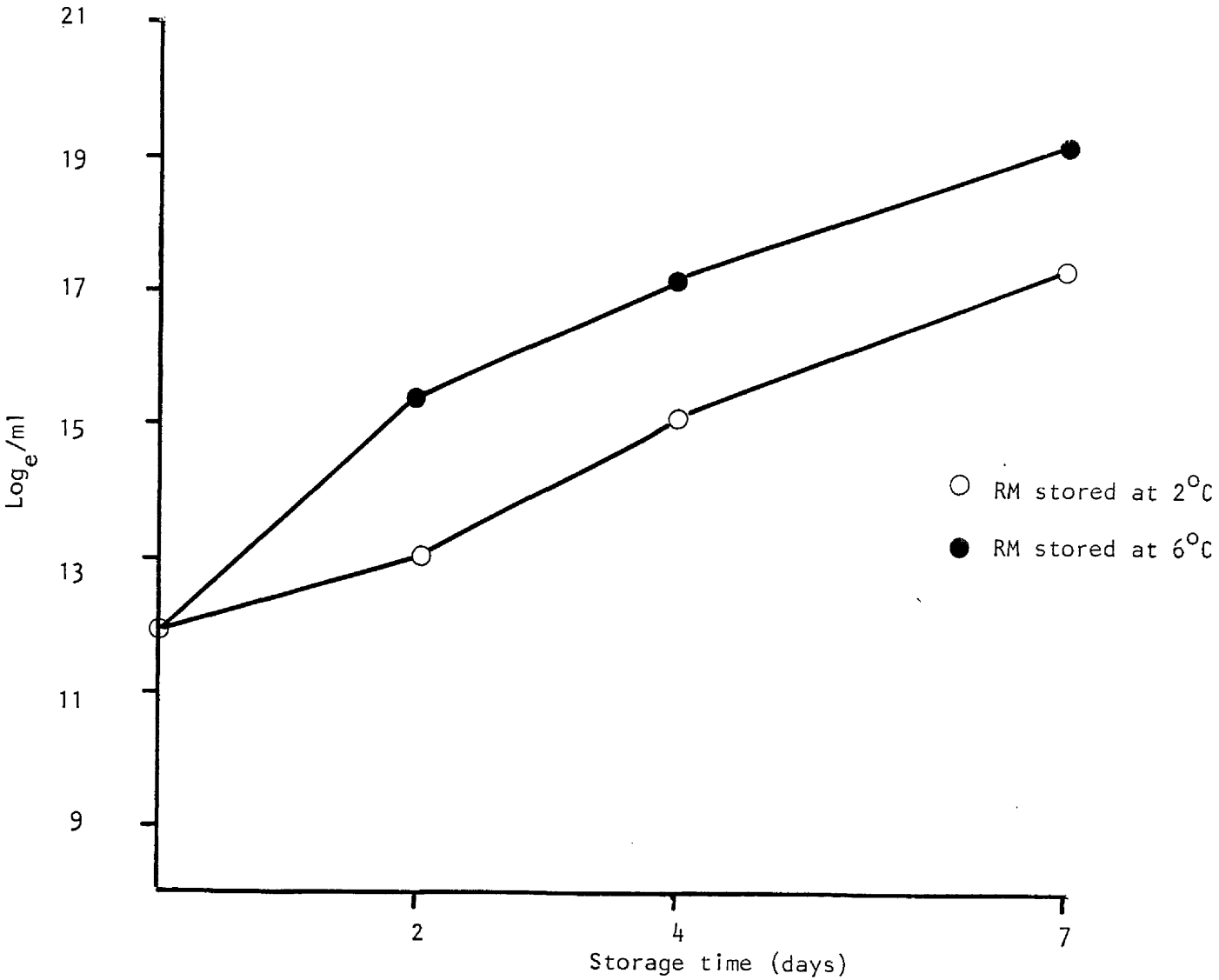


Fig. 2.10 Changes in the overall three-trial proteolytic count after various periods of storage at 4°C of the pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for 2, 4 and 7 days

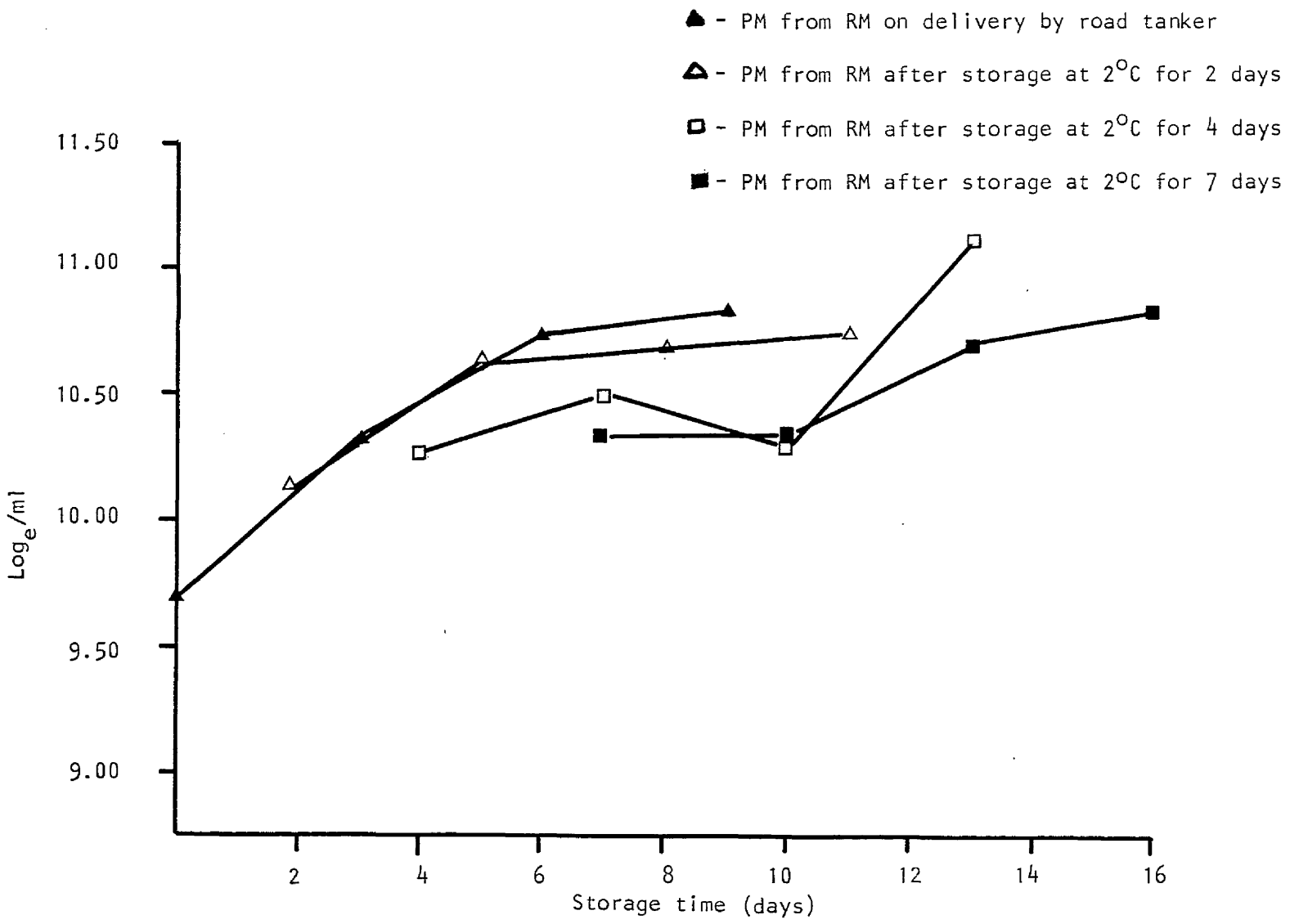
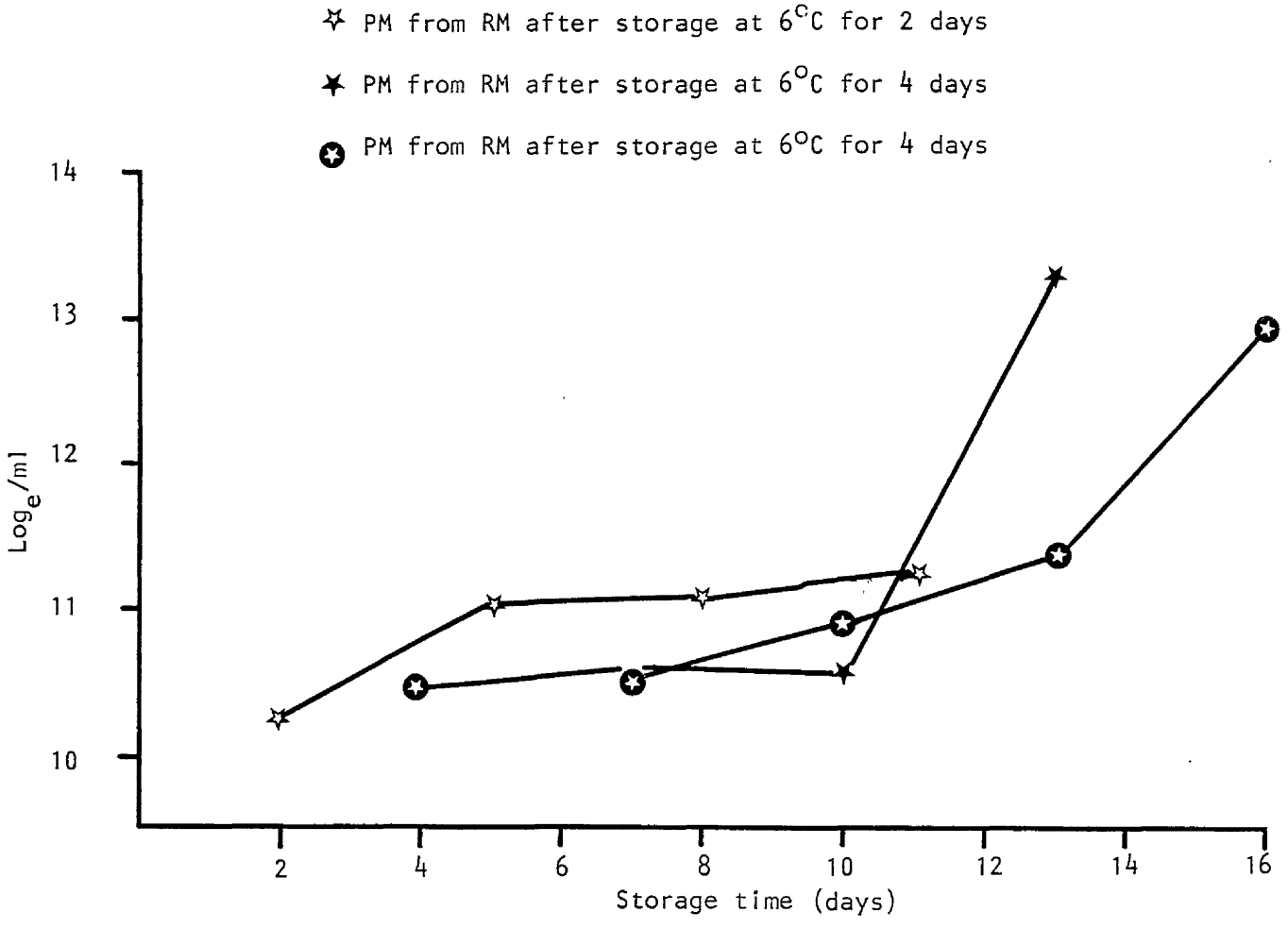


Fig. 2.11 Changes in the overall three-trial proteolytic count after various periods of storage at 4°C of the pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for 2, 4 and 7 days



with the initial values for each processed on the day of delivery but further storage to 4 and 7 days from delivery resulted in significant ($p < 0.05$) increases in the proteolytic count of corresponding pasteurized milks. The same significant increase in proteolytic count was found where the raw milk was stored at $6^{o}C$ for 2 days before pasteurization. A significant increase ($p < 0.01$) was found in the proteolytic count of pasteurized milks produced from raw milks stored at $6^{o}C$ for 4 days. The variations in the proteolytic count of pasteurized milk on storage at $4^{o}C$ are presented in Figs 2.10 and 2.11.

The increase in the proteolytic count during the storage period of up to 9 days was greater in the pasteurized milks, prepared from raw milks held at $6^{o}C$ for 7 days before processing. Storage of the pasteurized milks for 3, 6 and 9 days resulted in highly significant differences ($p < 0.001$) in the proteolytic count of the processed milks prepared from raw milk held at $2^{o}C$ and $6^{o}C$.

The proteolytic count showed a highly significant correlation with the total colony count ($r = 0.9444$, $p < 0.001$). The correlation coefficient with lipolytic count was highly significant ($r = 0.8679$, $p < 0.001$). Table 2.12.

Thermoduric count

(i) Raw milk

Thermoduric counts of samples of raw milks on the day of delivery by a road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days are presented in Table 2.9.

A highly significant difference (at 0.1 per cent level) was found in the thermoduric count of the milks received in the different trials*. The length of storage of the raw milks (2, 4 and 7 days) had no effect on the thermoduric count, while the temperature of the storage

*The thermoduric count in the:-

First trial 87,00 per ml
Second trial 66,00 per ml
Third trial 99,00 per ml

showed a significant effect (at 5 per cent level) on the counts when comparing the VR with F value in the statistical tables.

No significant change was found in the mean value of the thermoduric count for raw milk samples stored at $2^{o}C$ for 2, 4 and 7 days, and when storage at $6^{o}C$ was extended to 4 and 7 days there were significant increases, ($p < 0.05$) and ($p < 0.01$) respectively, by comparison with the initial values.

The variation in the thermoduric count of the raw milks stored at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days is shown in Fig. 2.12. The mean thermoduric count on the day of delivery by a road tanker was 84×10^{2} per ml. After 7 days storage of the raw milk at $2^{o}C$ the count had increased to 86×10^{2} per ml, while in the case of raw milk held at $6^{o}C$ for 7 days the count was 89×10^{2} per ml.

(ii) Pasteurized milk

Thermoduric counts of pasteurized milk produced from raw milk on the day of delivery by a road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days and the effect of post-pasteurization storage of the pasteurized milk for 3, 6 and 9 days are presented in Table 2.10.

The storage of the raw milks at $2^{o}C$ for 7 days resulted in a highly significant difference ($p < 0.001$) in the thermoduric count of the pasteurized milk produced from it.

The level of significance was higher in the thermoduric count for pasteurized milk produced from raw milk stored at $6^{o}C$.

The storage of the pasteurized milk for 3, 6 and 9 days showed highly significant differences (at 0.1 per cent level) in the thermoduric count*.

*The thermoduric count in the:-

First trial 8,000 per ml
Second trial 7,000 per ml
Third trial 5,400 per ml

TABLE 2.9

The thermoduric count/ml (30°C for 3 days), expressed as $\log_e$, of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	9.071	9.071	9.083	9.094	9.083	9.105	9.083
2	8.795	8.811	8.825	8.854	8.825	8.825	8.839
3	9.200	9.190	9.210	9.220	9.251	9.278	9.315
Mean	9.022	9.024	9.039	9.056	9.052	9.069	9.079

	DF	MS	VR
Trial	2	0.3051836	1522.504***
F v rest	1	0.0024897	12.421*
Trial. F v rest	2	0.0001808	0.902
F v rest. Storage	2	0.0013015	6.493
F v rest. Temperature	1	0.0033217	16.571*
Trial. F v rest. Storage	4	0.0002219	1.107
Trial. F v rest. Temperature	2	0.0024700	12.322*
F v rest. Storage. Temperature	2	0.0000213	0.106
Residual	4	0.0002004	
Total	20	0.0312907	

Table	Trial	Storage	Temperature	Storage. Temperature
REP	7	Unequal	Unequal	3
SED	0.00757	0.01001	0.00944	0.01156

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 2.10

The overall three-trial thermoduric count/ml (30°C for 3 days), expressed as $\log_e$, of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The counts for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	8.825	9.923	8.983	8.966	8.991	9.008	9.061
3 days	8.831	8.927	8.958	8.970	8.995	8.975	9.086
6 days	8.834	8.979	8.958	8.987	9.041	9.012	9.071
9 days	8.882	9.002	8.987	8.995	9.086	9.024	9.090
Mean	8.843	8.947	8.966	8.971	9.021	8.993	9.061

	DF	MS	VR
Trial	2	0.530835	380.094***
PM storage	3	0.009397	6.728***
F v rest	1	0.266799	101.036***
Trial. PM storage	6	0.003169	2.269
Trial. F v rest	2	0.241876	173.191***
PM Storage. F v rest	3	0.000268	0.192
F v rest. Storage	2	0.009217	6.599**
F v rest. Temperature	1	0.073790	52.836***
Trial. PM Storage. F v rest	6	0.000963	0.689
Trial. F v rest. Storage	4	0.018783	13.449***
PM Storage. F v rest. Storage	6	0.002339	1.675
Trial. F v rest. Temperature	2	0.002844	2.037
PM Storage. F v rest. Temperature	3	0.000059	0.042
F v rest. Storage. Temperature	2	0.006726	4.816
Residual	40	0.001397	
Total	83	0.025573	

Table	PM Storage	Storage	Temperature	Storage. Temperature
REP	21	Unequal	Unequal	12
SED	0.01153	0.01321	0.01246	0.01526

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 2.12 Changes in the thermoduric count of the raw milk (RM) after various periods of storage at 2°C and 6°C

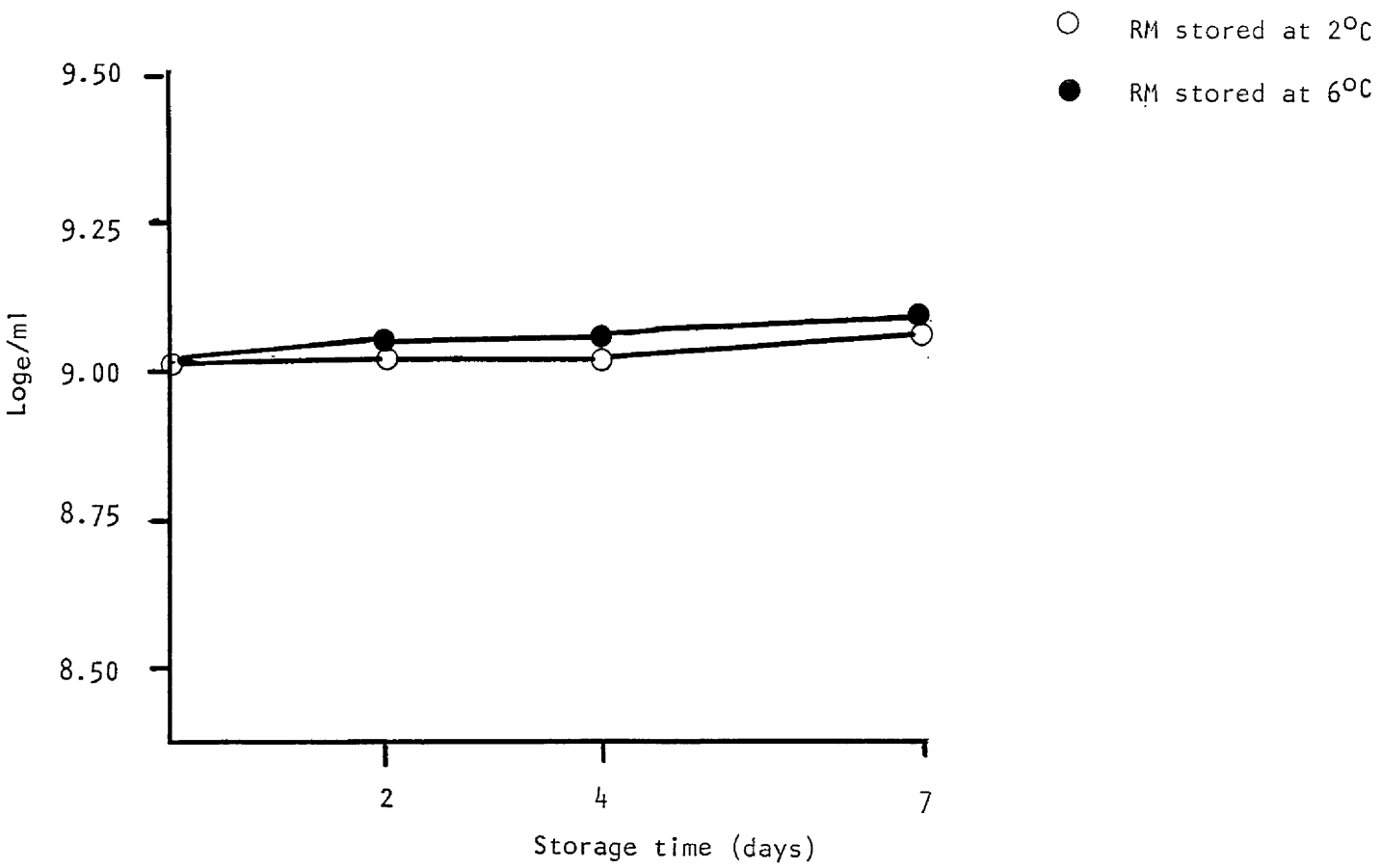


Fig. 2.13 Changes in the overall three-trial thermoduric count of pasteurized milks (PM) produced from raw milks (RM) stored at 2°C for 2, 4 and 7 days. The counts of the pasteurized milks were made on production and after 3, 6 and 9 days of storage at 4°C

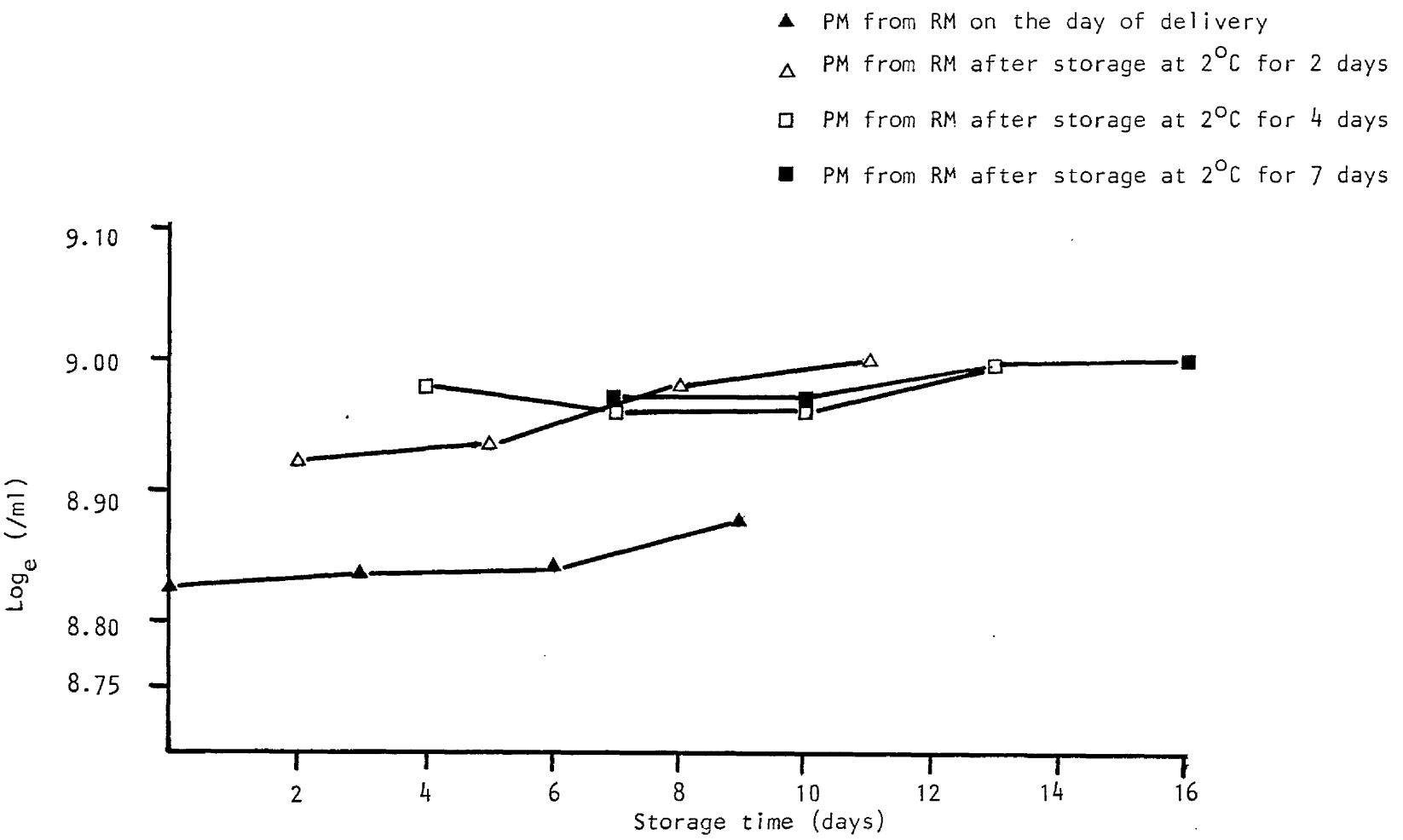


Fig. 2.14 Changes in the overall three-trial thermoduric count of pasteurized milks (PM) produced from raw milks (RM) stored at 6°C for 2, 4 and 7 days. The counts of the pasteurized milks were made on production and after 3, 6 and 9 days of storage at 4°C.

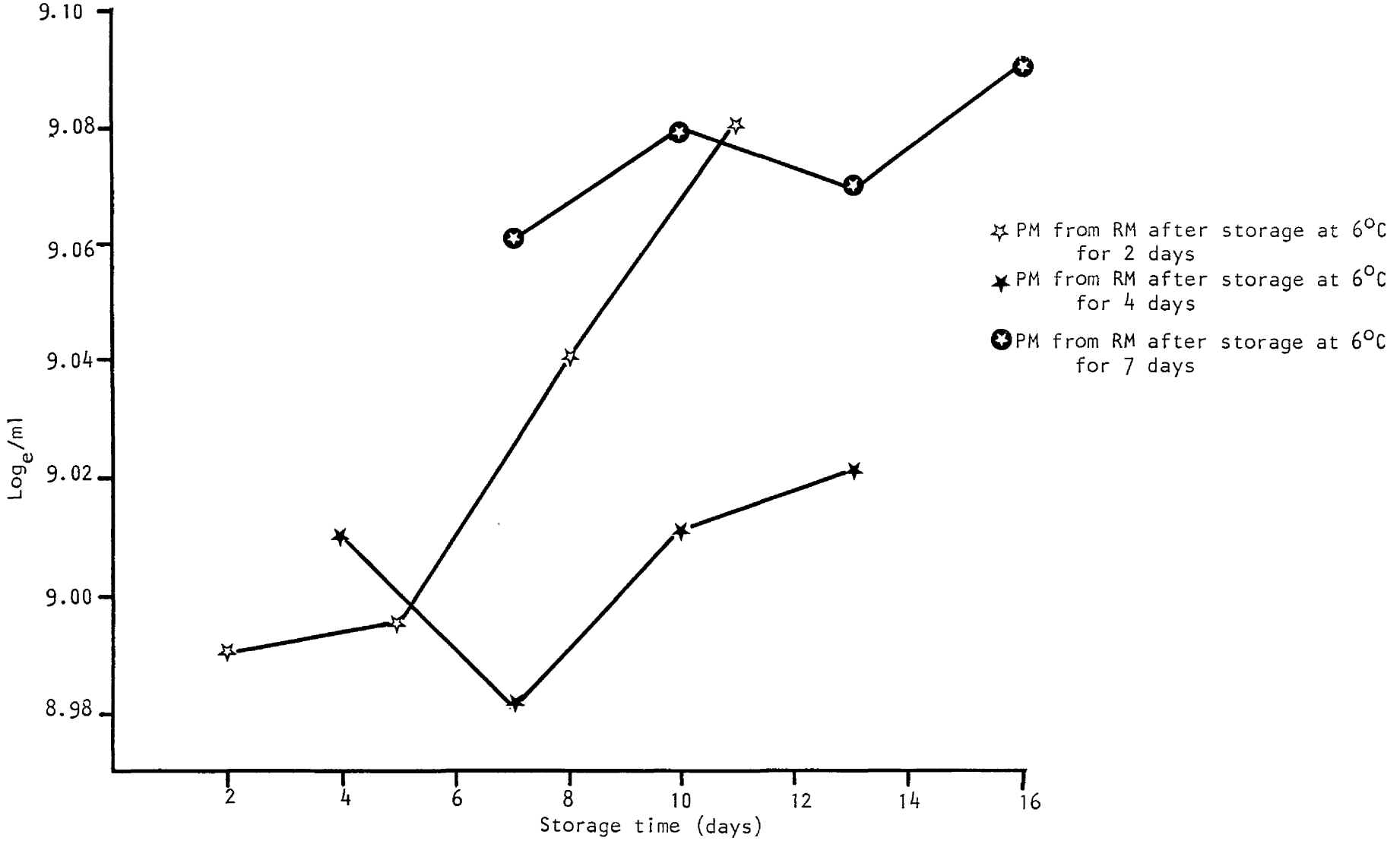


TABLE 2.11

The correlation coefficient matrix between the different microbiological tests used to assess the microbiological quality of the raw milk on delivery and after storage at $2^{o}C$ and $6^{o}C$ for up to 7 days

1.	Total colony count	1.0000					
2.	Psychrotrophic count	0.8668***	1.0000				
3.	Coliform count	0.9521***	0.8305***	1.0000			
4.	Lipolytic count	0.9102***	0.8770***	0.8761***	1.0000		
5.	Proteolytic count	0.9580***	0.8957***	0.9154***	0.9363***	1.0000	
6.	Thermoduric count	0.0230	-0.1489	0.0715	-0.1653	-0.0745	1.0000
		1	2	3	4	5	6

DF = 19

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 2.12

The correlation coefficient matrix between the different microbiological test to assess the microbiological quality of the pasteurized milk produced from raw milk after different periods of storage at $2^{o}C$ and $6^{o}C$

1.	Total colony count	1.0000			
2.	Lipolytic count	0.7332***	1.0000		
3.	Proteolytic count	0.9444***	0.8679***	1.0000	
4.	Thermoduric count	0.1170	-0.0801	0.0506	1.0000
		1	2	3	4

DF = 103

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Coliform count and psychrotrophic count bacteria were absent in 1 ml of milk samples in all three trials.

The variations in the thermoduric count for pasteurized milk are presented in Figs 2.13 and 2.14.

The mean thermoduric count of pasteurized milk on processing on the day of delivery of the raw milk by road tanker was 68 x 10^2 per ml. The thermoduric count of pasteurized milk produced from the raw milk stored at $2^{o}C$ for 7 days was 78 x 10^2 per ml, while the thermoduric count for pasteurized milk produced from raw milk at $6^{o}C$ after 7 days was 86 x 10^2 per ml.

Antibiotic residue

The milk should pass the test for antibiotic residues before using for any purpose. The Disc Assay method based on the procedure of Galesloot & Hassing (1962) as used by the Scottish Milk Marketing Board was selected for use in these studies.

This test detects antibiotics or other inhibitory substances at levels of 0.01 to 0.02 international units of penicillin per ml of milk. The antibiotic test was done immediately on delivery of the silo milk by road tanker. The sample was tested on the day of delivery of the milk and the results indicated as follows:-

1. The first trial gave a -ve result.
2. The second trial gave a +ve result at the level of 0.02 international units of penicillin per ml of milk.
3. The third trial gave a +ve result at the level of 0.02 international units of penicillin per ml of milk.

DISCUSSION

1. Total colony count

On 1st April 1981 the Scottish Milk Marketing Board introduced a quality scheme based on the centralised testing of the total bacteria count of ex-farm raw milk. Each milk producer's supply is sampled and tested four times per month. At the end of each month the counts are averaged and if the average count exceeds a bacterial count level set by the SMMB then the milk producer is penalised by an amount determined from time to

time by the SMMB (United Kingdom Federation of Milk Marketing Boards, 1981). The results of this study showed that total colony count for raw milk on delivery by road tanker in the first and third trial were 161×10^3 per ml and 105×10^3 per ml respectively while in the second trial the higher total colony count of 341×10^3 per ml was found. In all three cases the SMMB guideline standard of 100,000 per ml was exceeded. The mean total colony count for raw milk mentioned above on delivery by road tanker was 202×10^3 per ml and then increased after the storage for 7 days at $2^{o}C$ and $6^{o}C$ to 18×10^6 per ml and 224×10^6 per ml respectively. These results are in agreement with Randolph et al. (1965); Crawford (1967); Fryer (1972); Patel & Blankenagel (1972) and Muir et al. (1978) all of whom have stated that there was an increase in the total colony count of the raw milk during the storage time.

The Scottish Milk Marketing Board, and the 'trade' sector of the dairy industries in Scotland have recently agreed on a Code of Practice concerning the quality standards for tanker loads of milk delivered to buyer's premises. This Code of Practice came into operation on 1st February 1982 and concerns failure or rejection standards for temperature, total bacteria count, thermoduric count, sediment, antibiotics and entraneous water. In relation to total bacteria count a failure is recognised where a count of 100,000 per ml is exceeded on four or more occasions in any 14 day period. In respect of the thermoduric count a failure is recognised where a count of 5,000 per ml is exceeded on three or more occasions in any four-week period with the further conditions that there must be an interval of at least two weeks between the first and last failures.

The results obtained in this study show a very distinct difference in the increase in bacterial count at $2^{o}C$ and $6^{o}C$, the latter temperature resulting in a ten-fold higher count at $6^{o}C$ after 7 days compared to the same time at $2^{o}C$. The total colony counts of the processed milks which were prepared from the corresponding raw milks on the day of delivery were 106×10^3 per ml in the first trial, 30×10^3 per ml in the second trial and 85×10^2 per ml in the third trial. The pasteurized milks produced from raw milks held at $2^{o}C$ and $6^{o}C$ for 7 days had mean total viable counts of 88×10^3 per ml and 7×10^5 per ml respectively. Finekher (1981) stated that the standard in the U.S.A.

for grade A pasteurized milk was not more than 30,000 bacteria per ml. In these trials the increase in the total colony count is much lower in the processed milks produced from raw milks stored at $2^{o}C$ for up to 7 days than where the higher temperature of $6^{o}C$ was used. (Figs 2.2 and 2.3.)

The coliform count of the raw milks on Violet Red Bile Agar (Table 2.3) showed an increase during the storage periods of the different treatments. A highly significant increase ($p < 0.001$) in the coliform count of the raw milk was found between the day of delivery and the 7th day of storage at $2^{o}C$, while the highest significant increase in coliform numbers was found in the raw milk stored at $6^{o}C$ for 7 days (Fig. 2.4)

None of the samples of pasteurized milk tested on the day of production gave a positive result when 1 ml quantities were added to brilliant green broth. This result means there was no contamination by these organisms after pasteurization. Confirmation of the absence of coliforms in the pasteurized milks was obtained by the fact that none of the samples even after 9 days at $4^{o}C$, gave positive results when 1 ml samples were added to brilliant green broth. The psychrotrophic count increased in the raw milks stored at $6^{o}C$ more rapidly than in the corresponding raw milks stored at $2^{o}C$ for up to 7 days.

These results are in agreement with Fluckiger (1976); Overcast (1968); Thomas (1958); Thomas (1971) and Oliveria & Parmelee (1976) who found that storage of milks at higher temperature resulted in higher increase in the number of psychrotrophic bacteria. The pasteurized milk produced from raw milk showed no psychrotrophic count in 1 ml samples on production and after storage for up to 9 days at $4^{o}C$. This result confirmed the fact that with few exceptions psychrotrophic bacteria do not withstand HTST pasteurization. The storage time of the raw milks at $2^{o}C$ and $6^{o}C$ was associated with an increase in the lipolytic and the proteolytic count and the increase was significantly higher in the raw milks stored at $6^{o}C$.

Pasteurized milks produced from these raw milks showed similar variations in bacterial numbers. In other words, pasteurized milks produced

from milks held at $2^{o}C$ were found to contain lower bacterial counts than pasteurized milks produced from the same raw milks stored at $6^{o}C$. These variations were also observed on further storage of the pasteurized milks.

The results of the thermoduric count for the raw milk indicated that over the period of storage at $2^{o}C$ there was no significant increase in heat-resistant bacteria, and in the case of the raw milk stored at $6^{o}C$ there was no significant increase over a 2-day period but thereafter significant increases ($p < 0.05, p < 0.01$) in count were obtained after 4 and 7 days storage.

Highly significant differences were found in the thermoduric count between the raw milk samples obtained in different trials. Highly significant increases ($p < 0.001$) in the thermoduric counts of the processed milks were found after various periods of storage of the raw milk. Storage of the pasteurized milks for 3, 6 and 9 days at $4^{o}C$ did not alter the thermoduric counts.

CONCLUSION

1. The storage of the raw milk at $6^{o}C$ for 2, 4 and 7 days resulted in higher increases in the total colony count, and the coliform, psychrotrophic, lipolytic and proteolytic count than storage at $2^{o}C$ for the same periods of time.

2. The pasteurized milks produced from raw milk stored at $2^{o}C$ for 2, 4 and 7 days showed little change in bacterial numbers compared with the initial level obtained on processing the milk on the day of delivery. On the other hand, the pasteurized milks produced from the same milks stored for 2, 4 and 7 days at $6^{o}C$ showed considerable increase in bacterial numbers compared to the initial value. Further storage of the pasteurized milks (from raw milk stored at $2^{o}C$) for 3, 6 and 9 days did not result in significant increases in the bacterial numbers in the pasteurized milk, while the bacterial number of pasteurized milk produced from raw milk stored at $6^{o}C$ increased after further storage at $4^{o}C$.

3. Coliform bacteria and psychrotrophs were absent in 1 ml quantities

of all pasteurized milks, on production from raw milks held at $2^{o}C$ and $6^{o}C$ for up to 7 days and after storage at $4^{o}C$ for up to 9 days.

4. The thermoduric counts of raw milks showed negligible increases during the storage time of 7 days at $2^{o}C$ and $6^{o}C$. Similarly the thermoduric counts of pasteurized milks produced from these raw milks showed little change on storage for 9 days at $4^{o}C$.

5. According to these experiments, the author does not recommend the storage of raw milks for more than 2 days at $6^{o}C$ prior to pasteurization. On the other hand storage of raw milk at $2^{o}C$ for up to 7 days prior to processing did not affect the microbiological quality of the milk and so milk processing companies may consider using a storage temperature at $2^{o}C$ as a means of preventing deterioration in raw milk quality prior to pasteurization.

CHAPTER THREE

THE CHEMICAL QUALITY OF RAW AND PASTEURIZED MILK DURING DIFFERENT PERIODS OF STORAGE AT DIFFERENT TEMPERATURES

INTRODUCTION

Previous to 1850, milk had been found to contain fat, sugar, protein and minerals. These constituents are present in the three physical states of solution, colloidal dispersion and emulsion.

It is required by law that certain dairy products receive specific heat treatments. The purposes of heat processing may be summarized as follows:- to meet public health requirements, that is, pasteurization and sterilization, to destroy pathogens and avoid disease and food poisoning, to destroy enzymes, to facilitate mixing and blending processes, to achieve incubation temperatures, that is, cheese and cultured dairy products, and to impart desirable properties. Keeping quality from a microbiological standpoint is the overriding consideration (Jenness & Patton, 1959).

Davis (1951) mentioned that the most constant physical property of milk is its freezing point. This point is dependent upon the concentration of salts and lactose in milk.

The freezing point test is now regarded in this country as the best test for assessing the amount of extraneous water in a sample of milk. Pasteurization affects the freezing point of milk only as far as it affects the concentration of the constituents in solution.

The average titratable acidity of normal fresh milk may be expected to be from 0.14 to 0.17 per cent calculated as the equivalent of lactic acid. This acidity is not necessarily the result of the presence of lactic acid, but is due primarily to the acid-reacting salts in the milk along with the acidity registered from carbonic acid (0.01 to 0.02 per cent). Pasteurization reduces the titratable acidity by about 0.01 per cent.

Normal raw milk yields from 15 to 20 per cent cream volume depending upon many factors such as fat content, stage of lactation, presence of colostrum, mastitis, rate of cooling etc. (Hall & Trout, 1968). The consumer

tends to judge the richness of the milk by the depth of the cream layer and also by the colour.

The volume of cream layer is defined as the distance from the top of the milk down to that point at which the demarcation between cream and milk is apparent to the eye (Davis, 1951).

Milk, cream and other dairy products sometimes develop unpleasant flavours. These flavours may be caused by the chemical breakdown of the milkfat to produce free fatty acids. This breakdown of fat is called lipolysis and is caused by the enzyme lipase. Milk lipase is relatively unstable and can be inactivated by salt, acid, light, oxidation and heat.

Treatments which cause activation include agitation and foaming, homogenization, certain temperature changes, and freezing and thawing (Deeth & Fitz-Gerald, 1976). Rapid cooling of the milk after milking tends to inhibit lipolysis (IDF-FIL-1975). During the cold storage of milk for 48 to 72 h period, the milk is the centre of phenomena of a biological and bacteriological nature often still badly defined which modify some of its qualities or its properties. Lipolysis is perhaps the best known of these phenomena because its effects are detected by the senses: the hydrolysis of the fat causes the formation of free fatty acids (FFA) which are responsible for off-flavours (IDF-FIL-1975).

Taylor & Richardson (1980) stated that sufficient heat treatment of milk forms 'reactive' sulphydryl groups that are believed to be responsible for increased oxidative stability and the cooked flavour of heated milk. However a wide variation in the sulphydryl content of raw and heated milk has been reported.

The purpose of this work was to study the effect of cold storage at $2^{o}C$ and $6^{o}C$ on the raw milk and the pasteurized milk produced from it and also the effect of further storage of the pasteurized milks at $4^{o}C$ for 3, 6 and 9 days. Aspects investigated were:-

1. The fat content.
2. The change in the pH and the titratable acidity of the milk during the storage period.

3. The cream layer for both milks.

4. The change in the freezing point.

5. The free sulphydryl groups (F-SH).

6. The calcium content.

7. The total solid content.

8. The change in the acid degree value (ADV) for raw milk and pasteurized milk during the storage time.

The phosphatase test was used as a control test in this experiment to establish that heat treatment had been properly carried out.

EXPERIMENTAL

1. Treatment of the samples

The remainder of the milk from each sample described in Chapter Two (Experimental, 4b) was used for the chemical analysis.

2. Chemical analysis

Duplicate samples of raw milk, as described in Chapter Two (Experimental, 1, 4), were tested on the day of delivery by the road tanker and after 2, 4 and 7 days storage at $2^{o}C$ and $6^{o}C$. The pasteurized milks were tested immediately after heat treatment and after storage at $4^{o}C$ for 3, 6 and 9 days. Fat, pH, titratable acidity, cream line and freezing point, sulphydryl groups, total solids, and acid degree value (ADV) were determined using the methods described in Chapter One (Section 2:1, 2:2, 2:3, 2:4, 2:5, 2:6, 2:8, 2:9). The calcium determination was made for both raw and pasteurized milk on the day of delivery by the road tanker and after 2, 4 and 7 days storage of the raw milk, using the method described in Chapter One (Section 2:7). The phosphatase test was made on the samples of pasteurized milk on the day of processing using the method described in Chapter One (Section 2:10).

RESULTS

1. Fat

The results of measurements of the fat content of raw milks are presented

TABLE 3.1

The fat content (per cent) of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	3.85	3.85	3.80	3.85	3.85	3.85	3.83
2	3.80	3.80	3.80	3.80	3.80	3.80	3.85
3	3.55	3.60	3.60	3.60	3.55	3.60	3.60
Mean	3.73	3.75	3.73	3.75	3.73	3.75	3.76

	DF	MS	VR
Trials	2	0.2668	240.159***
F v rest	1	0.0008	0.723
Trial. F v rest	2	0.0012	1.152
F v rest. Storage	2	0.0006	0.563
F v rest. Temperature	1	0.00006	0.063
Trial. F v rest. Storage	4	0.0008	0.750
Trial. F v rest. Temperature	2	0.0009	0.813
F v rest. Storage. Temperature	2	0.0009	0.813
Residual	4	0.0011	18.667
Total	20	0.0275	461.796

Table	Trial	Storage	Temperature	Storage Temperature
REP	14	Unequal	Unequal	6
SED	0.01260	0.01667	0.01571	0.0193

* significant at 5 per cent
** " " 1 " "
*** " " 0.1 " "

TABLE 3.2

The overall three-trial mean fat content (per cent) of samples of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	3.73	3.75	3.73	3.74	3.78	3.75	3.73
3 days	3.75	3.73	3.73	3.73	3.75	3.73	3.73
6 days	3.73	3.73	3.73	3.73	3.74	2.73	3.75
9 days	3.73	3.73	3.73	3.73	3.73	3.73	3.73
Mean	3.74	3.74	3.73	3.73	3.75	3.74	3.74

	DF	MS	VR
Trial	2	0.7879	1422.480***
PM Storage	3	0.00128	2.328
Treatment (1-4 below)	6	0.0007	1.307
1. F v rest	1	0.00030	0.750
2. Storage	2	0.00111	2.777
3. Temperature	1	0.001406	3.514
4. Storage. Temperature	2	0.00020	0.521
Trial. PM Storage	6	0.00105	1.899
Trial. Treatment	12	0.0007	1.442
PM Storage. Treatment	18	0.00061	1.116
Residual	36	0.0005	3.722
Total	83	0.01965	132.053

Table	Treatment	Treatments. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.00679	0.01358	0.005001	0.004715	0.005775

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

in Table 3.1.

A very highly significant difference (at 0.1 per cent level) was found in the fat content between the trials.

No significant differences were found in the fat content due to the storage time, the temperature of storage or the interaction between them in the raw milk samples.

The results of measurements of the fat content of the pasteurized milk samples are presented in Table 3.2. A very highly significant difference (at 0.1 per cent level) was found between the trials, while no significant difference was found in the fat content due to different treatments during storage.

2. pH

The results for the pH values of the raw milk samples are presented in Table 3.3.

A significant difference (at 5 per cent level) was found between the trials in the pH values of the raw milks.

The storage time and the temperature of storage gave a very highly significant difference (at 0.1 per cent level) in the pH value. There was also a very highly significant interaction (at 0.1 per cent level) in the pH value between the storage time and the temperature of storage.

The mean of the pH value for the raw milk showed no significant change after storage at $2^{o}C$ for 2 and 4 days, while storage of the same milk after 7 days gave significant decrease ($p < 0.05$) in the pH value. The storage of the raw milk at $6^{o}C$ showed no significant difference in the pH value after 2 days, but a significant decrease ($p < 0.05$) after 4 days of storage, while the significance was very high ($p < 0.01$) after 7 days storage of the raw milk at $6^{o}C$.

The results for the pH value of the pasteurized milks are presented in Table 3.4.

TABLE 3.3

The pH value of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$.

Trial	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	6.75	6.75	6.73	6.66	6.75	6.67	5.84
2	6.71	6.67	6.61	6.51	6.67	6.53	5.41
3	6.74	6.72	6.62	6.55	6.70	6.48	5.53
Mean	6.73	6.71	6.65	6.57	6.70	6.56	5.59

	DF	MS	VR
Trial	2	0.00857	11.699*
F v rest	1	0.0369	50.357**
Trial. F v rest	2	0.00090	1.229
F v rest. Storage	2	1.364	186.207***
F v rest. Temperature	1	1.168	159.424***
Trial. F v rest. Storage	4	0.00148	2.019
Trial. F v rest. Temperature	2	0.0010	1.378
F v rest. Storage. Temperature	2	0.08730	119.247***
Residual	4	0.000732	1064.882
Total	20	0.03156	45863.332

Table	Trial	Storage	Temperature	Storage. Temperature
REP	14	Unequal	Unequal	6
SED	0.032354	0.042801	0.040353	0.049422

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.4

The overall three-trial mean pH value of samples of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) after storage for 2, 4 and 7 days at 2°C and 6°C. The values for pasteurized milk were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM at 2°C			PM from RM at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	6.73	6.71	6.65	6.59	6.71	6.60	6.04
3 days	6.68	6.68	6.64	6.58	6.68	6.60	6.02
6 days	6.66	6.67	6.62	6.57	6.66	6.61	5.89
9 days	6.65	6.65	6.61	6.53	6.64	6.61	5.79
Mean	6.68	6.68	6.63	6.57	6.67	6.60	5.93

	DF	MS	VR
Trial	2	0.01779	70.959***
PM Storage	3	0.05384	21.481***
Treatment (1-4 below)	6	1.752	698.952***
1. F v rest	1	0.05748	130.651***
2. Storage	2	2.550E	579.741***
3. Temperature	1	1.803E	409.886***
4. Storage. Temperature	2	1.516E	344.717***
Trial. PM Storage	6	0.006338	2.529*
Trial. Treatment	12	0.02661	10.615***
PM Storage. Treatment	18	0.008102	3.232***
Residual	36	0.002507	531.023
Total	83	0.1400	29665.629

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.01445	0.02890	0.016582	0.015633	0.019147

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

A very highly significant difference (at 0.1 per cent level) was found between the trials in the pH value of the pasteurized milk.

No significant difference was found in the pH values in the pasteurized milk on the day of delivery and after storage of the milk at $2^{o}C$ and $6^{o}C$ for 2 days.

There was a ver highly significant difference ($p < 0.001$) in the pH value of the pasteurized milk prepared on the day of delivery and that prepared from the raw milk stored for 4 days at $2^{o}C$ and $6^{o}C$.

Storage for a further three days at $2^{o}C$ and $6^{o}C$ resulted in greater changes compared with the original (on delivery) values.

The storage of the pasteurized milk for 3, 6 and 9 days had little effect on the pH values except in the samples produced from raw milk which had been stored for 7 days at $6^{o}C$. In these samples there was a further very highly significant decrease in the pH values.

3. Titratable acidity

The results of the titratable acidity determinations on the raw milk samples are presented in Table 3.5.

No significant difference was found between the trials in the titratable acidity.

The storage time of the raw milk showed a highly significant effect (at 1 per cent level) on the titratable acidity, as did the temperature of storage. A highly significant interaction (at 1 per cent level) was found between the storage time and temperature in their effect on the titratable acidity of the raw milk samples.

The variation in the titratable acidity of the raw milk samples during the storage time is illustrated in Fig. 3.1.

The mean titratable acidity of the raw milks during the storage time at $2^{o}C$ showed no significant change. At $6^{o}C$ there was no significant change after 2 and 4 days storage but a very highly significant change

TABLE 3.5

The titratable acidity (per cent lactic acid) of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Trial	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	0.150	0.155	0.155	0.165	0.155	0.165	0.280
2	0.155	0.165	0.168	0.175	0.165	0.175	0.370
3	0.155	0.160	0.165	0.170	0.165	0.170	0.375
Mean	0.153	0.160	0.163	0.170	0.162	0.170	0.342

	DF	MS	VR
Trial	2	0.0002	2.848
F v rest	1	0.00082	11.274*
Trial. F v rest	2	0.000020	0.277
F v rest. Storage	2	0.00346	47.458**
F v rest. Temperature	1	0.00305	41.823**
Trial. F v rest. Storage	4	0.00006	0.910
Trial. F v rest. Temperature	2	0.000093	1.273
F v rest. Storage. Temperature	2	0.0028	39.002**
Residual	4	0.000073	1227.846
Total	20	0.0008860	14884.543

Table	Trial	Storage	Temperature	Storage. Temperature
REP	14	Unequal	Unequal	6
SED	0.0102	0.01351	0.01274	0.01560

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.6

The overall three-trial mean titratable acidity (per cent lactic acid) of samples of pasteurized milk (PM produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	0.153	0.160	0.162	0.168	0.160	0.168	0.276
3 days	0.158	0.163	0.164	0.175	0.165	0.170	0.287
6 days	0.161	0.163	0.170	0.178	0.168	=.175	0.298
9 days	0.165	0.166	0.174	0.180	0.171	0.180	0.306
Mean	0.159	0.163	0.168	0.175	0.166	0.173	0.292

	DF	MS	VR
Trial	2	0.00125	40.155***
PM Storage	3	0.00147	46.991***
Treatment (1-4 below)	6	0.0538	1719.355***
1. F v rest	1	0.001857	236.176***
2. Storage	2	0.007013	892.102***
3. Temperature	1	0.006258	796.126***
4. Storage. Temperature	2	0.005072	645.196***
Trial. PM Storage	6	0.000013	0.441
Trial. Treatment	12	0.00033	10.843***
PM Storage. Treatment	18	0.00070	2.238*
Residual	36	0.000031	1314.419
Total	83	0.0040	170184.875

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.001614	0.0032298	0.0022165	0.0020898	0.0025595

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig 3.1 The variations in the titratable acidity (per cent lactic acid) after storage of raw milks (RM) at 2°C and 6°C

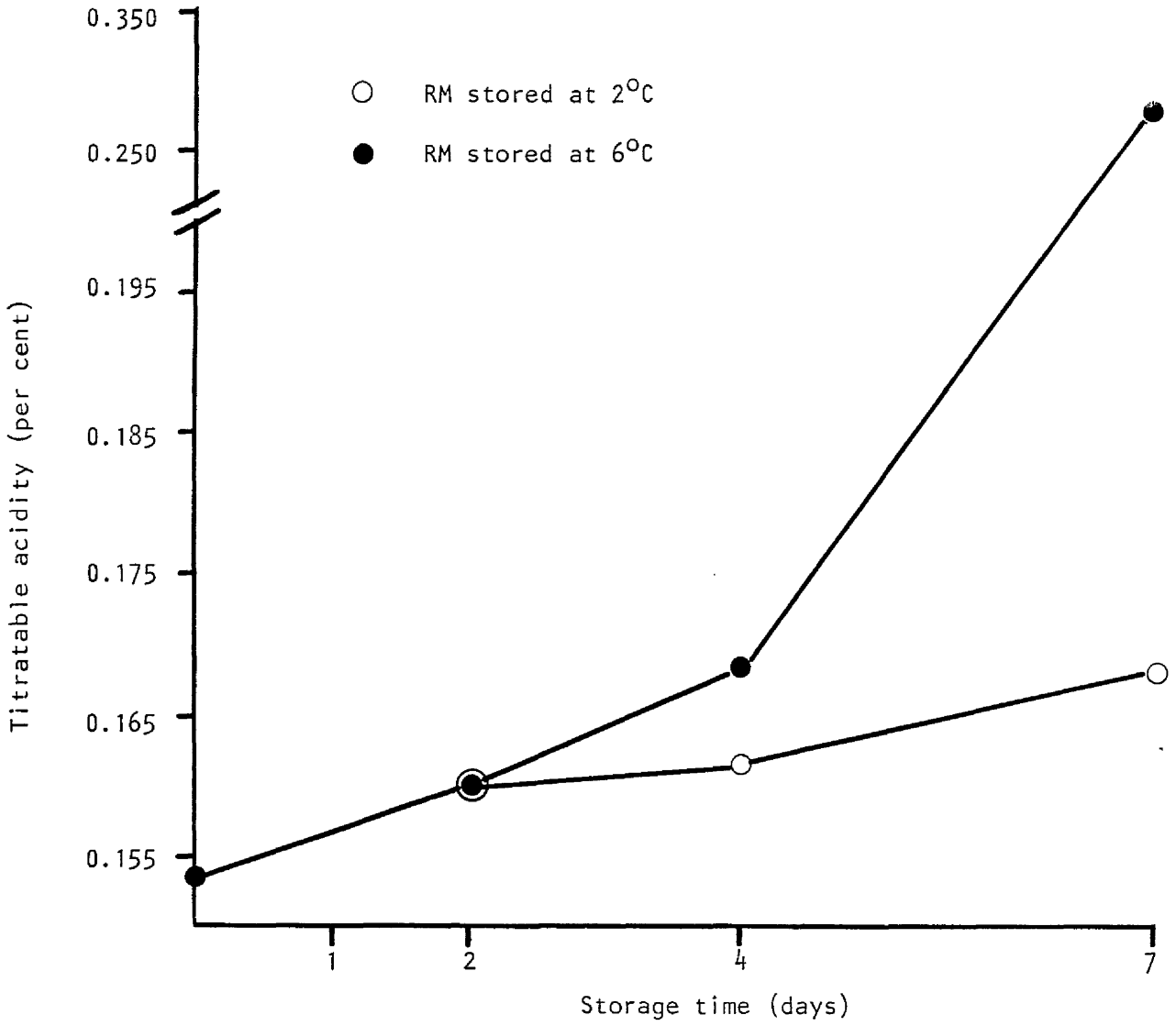


Fig. 3.2 Changes in the overall three-trial mean titratable acidity (per cent lactic acid) during storage at 4°C for 3, 6 and 9 days of pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for 2, 4 and 7 days prior to processing

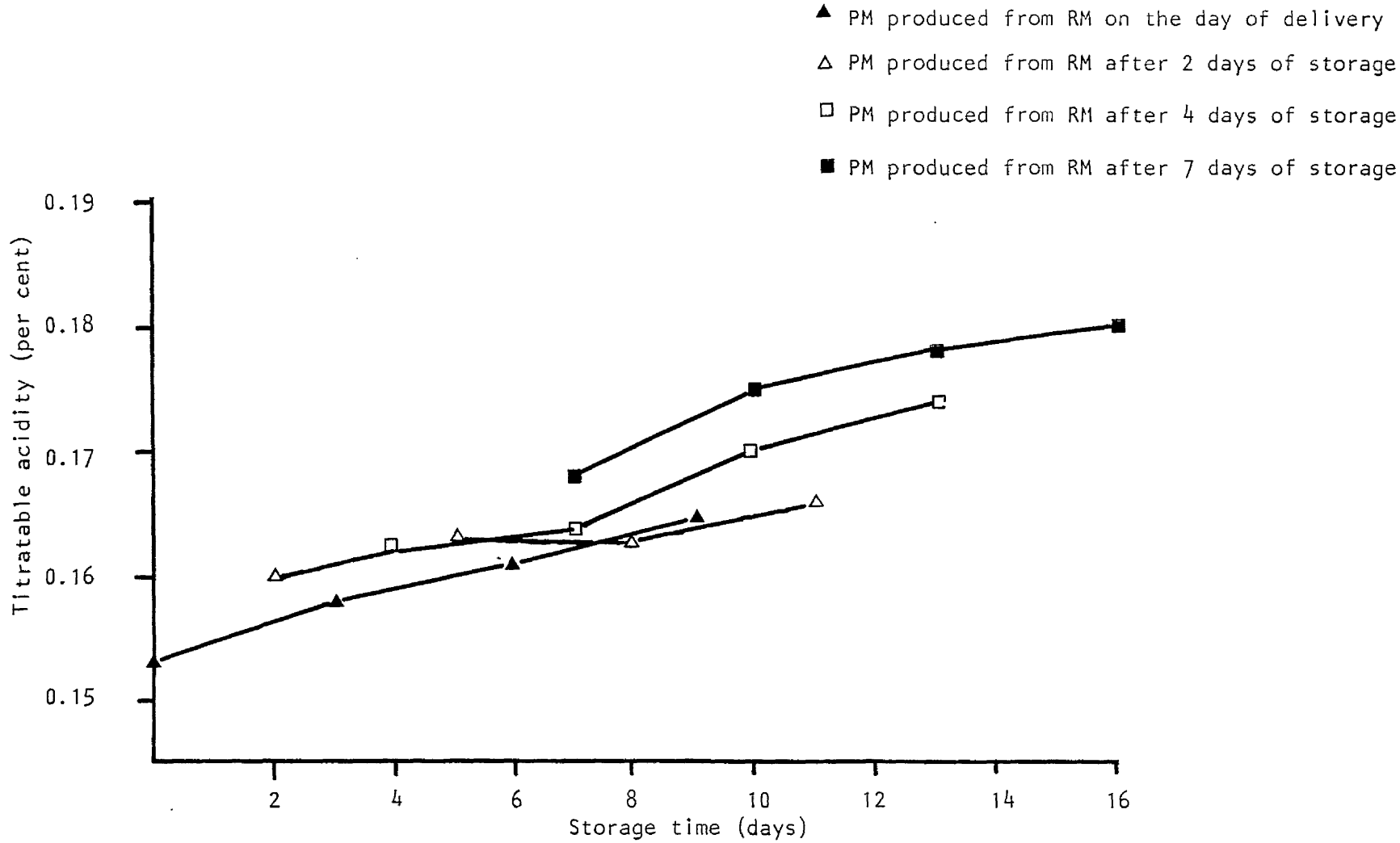
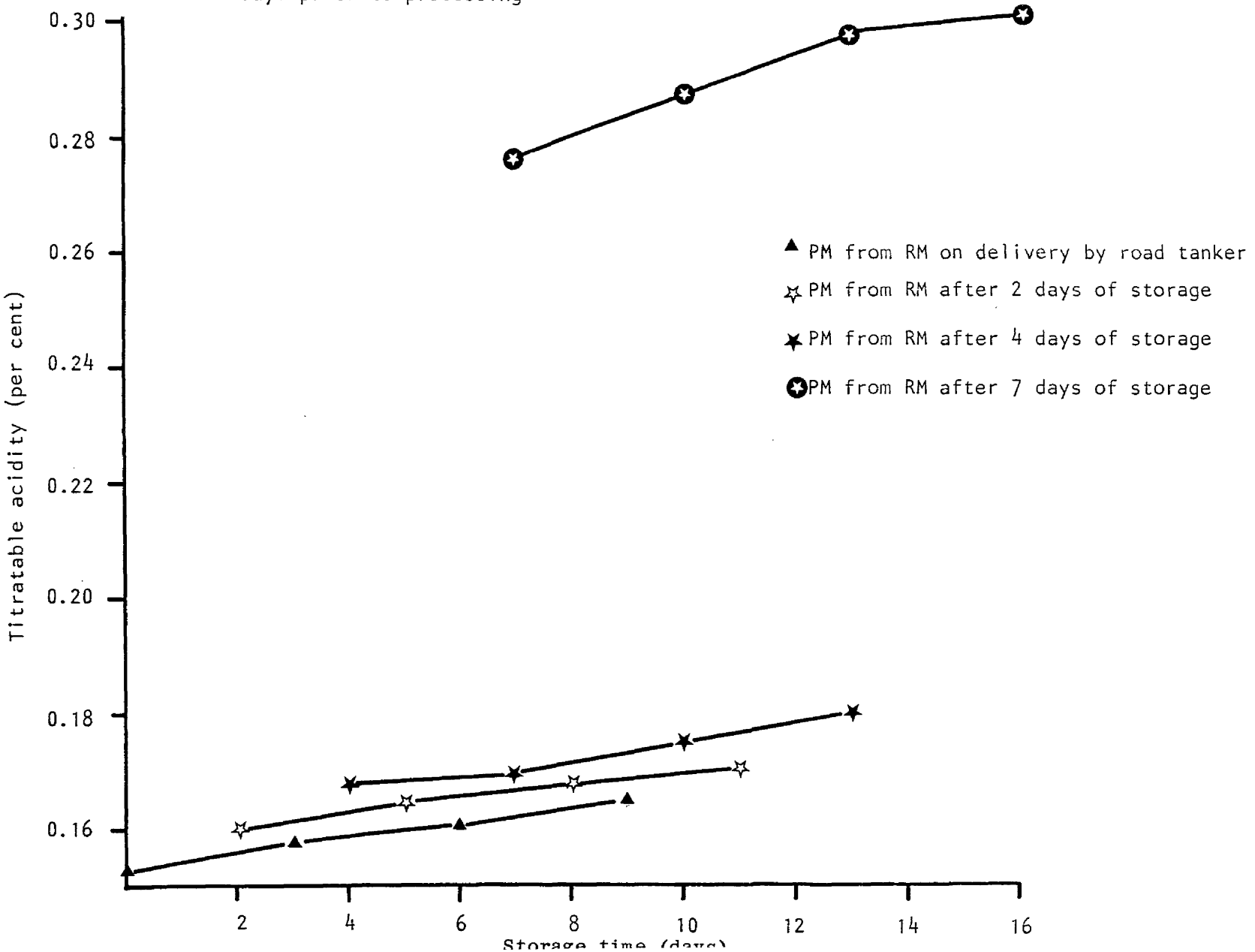


Fig. 3.3 Changes in the overall three-trial mean titratable acidity (per cent lactic acid) during storage at 4°C for 3, 6 and 9 days of pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for 2, 4 and 7 days prior to processing



($p < 0.001$) was found after 7 days.

The results for the titratable acidity of the pasteurized samples are presented in Table 3.6.

A significant difference ($p < 0.01$) was found between the titratable acidity of the pasteurized samples of milk processed on the day of delivery and after 2 days storage at $2^{o}C$ and $6^{o}C$, while the difference in the titratable acidity is high ($p < 0.001$) after 4 days storage at $2^{o}C$ and $6^{o}C$. The pasteurized milk produced from raw milk after 7 days storage at $2^{o}C$ gave a more significant difference ($p < 0.001$), while the most highly significant difference was found in the pasteurized milk produced from raw milk after 7 days storage at $6^{o}C$. The storage of the pasteurized milk for 3, 6 and 9 days at $4^{o}C$ gave a highly significant difference (at 0.1 per cent level) in the titratable acidity. The same significance was found between the trials. The interaction between the trials and the treatments was significant (at 0.1 per cent level), while the storage time for the pasteurized milk and the treatment also showed a significant interaction (at 5 per cent level).

The variations in the titratable acidity of the pasteurized milks are illustrated in Figs 3.2, 3.3.

The maximum titratable acidity found throughout the experiment was 0.375 per cent lactic acid and the minimum was 0.150 per cent.

4. The cream line

The results of the cream line measurement of the raw milk samples are presented in Table 3.7.

A significant difference (at 5 per cent level) was found in the cream line between the samples from different trials. No significant difference was found between the mean values of the cream line for the raw milk on the day of delivery from the road tanker and that of the milk after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$.

The results of the cream line values for the pasteurized milk are presented in Table 3.8.

Table 3.7

The cream line value (per cent) of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	5.00	5.00	7.00	6.50	5.00	6.00	6.50
2	5.00	7.00	6.00	6.00	7.00	6.00	6.00
3	6.00	5.00	5.00	5.00	5.00	6.00	5.00
Mean	5.33	5.67	6.00	5.83	5.67	6.00	5.83

	DF	MS	VR
Trial	2	1.333	8.00*
F v rest	1	0.6429	3.857
Trial. F v rest	2	1.1667	7.000*
F v rest. Storage	2	0.1667	1.000
F v rest. Temperature	1	0.000	0.000
Trial. F v rest. Storage	4	1.0833	6.500*
Trial. F v rest. Temperature	2	0.1667	1.000
F v rest. Storage. Temperature	2	0.000	0.000
Residual	4	0.1667	
Total	20	0.5655	

Table	Trial	Storage	Temperature	Storage. Temperature
REP	7	Unequal	Unequal	3
SED	0.2182	0.2887	0.2722	0.3333

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.8

The overall three-trial mean cream line value (per cent) of samples of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{o}C$)

PM Storage	PD	PM from RM stored at $2^{o}C$			PM from RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	3.71	5.33	3.71	4.33	5.00	4.71	2.71
3 days	5.00	5.33	4.00	4.71	5.33	4.71	2.71
6 days	5.00	5.33	4.33	4.33	5.33	5.00	2.71
9 days	5.00	5.00	4.00	4.33	5.33	4.71	2.71
Mean	4.667	5.25	4.00	4.42	5.25	4.75	2.71

	DF	MS	VR
Trial	2	3.000	18.900***
PM Storage	3	0.6032	3.800*
Treatment (1 - 4 below)	6	9.5952	60.450***
1 F v rest	1	0.7937	2.002
2. Storage	2	17.5138	44.168***
3. Temperature	1	2.0000	5.044*
4. Storage. Temperature	2	9.8749	24.904***
Trial. PM Storage	6	0.2698	1.700
Trial. Treatment	12	1.6667	10.500***
PM Storage. Treatment	18	0.2143	1.350
Residual	36	0.1587	
Total	83	1.1635	

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	12	3	Unequal	Unequal	12
SED	0.1626	0.3253	0.2226	0.2099	0.2571

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

A very highly significant difference (at 0.1 per cent level) was found in the cream line between the samples from different trials.

The pasteurized samples gave smaller cream line value than the corresponding raw milk samples. Storage of raw milk at $2^{o}C$ for 2, 4 and 7 days had no significant effect on the cream line of samples of pasteurized milk derived from it.

There were very highly significant differences (at 0.1 per cent level) in the cream line of the pasteurized milk produced from raw milk stored at $6^{o}C$ for 2, 4 and 7 days. The temperature of the storage brought about a decrease in the cream line of the pasteurized milk (significant at 5 per cent level).

A very highly significant interaction (at 0.1 per cent level) was found between storage and temperature in their effect on cream line.

The storage of the pasteurized milk for 3, 6 and 9 days had a significant effect (at 5 per cent level) on the cream line.

5. Freezing Point

The results of tests for the freezing point value of raw milk samples are presented in Table 3.9.

A highly significant difference (at 1 per cent level) was found between the trials in the freezing point value of the raw milk samples.

A very highly significant difference (at 0.1 per cent level) was found in the freezing point values due to the storage time and the temperature of storage of the raw milk samples. There was a significant interaction between storage time and temperature in relation to freezing point value where the milk had been stored for 7 days at $6^{o}C$. This sample showed a marked decrease in its freezing point (or increase in the freezing point depression).

The results of freezing point tests for the pasteurized milks are presented in Table 3.10.

Table 3.9

The freezing-point value (in ^{o}C) of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Trial	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	-0.529	-0.532	-0.528	-0.529	-0.531	-0.533	-0.582
2	-0.531	-0.533	-0.530	-0.532	-0.531	-0.532	-0.592
3	-0.537	-0.538	-0.537	-0.536	-0.538	-0.541	-0.589
Mean	-0.532	-0.534	-0.532	-0.532	-0.533	-0.535	-0.588

	DF	MS	VR
Trial	2	0.00001903	19.858**
F v rest	1	0.00005229	54.562**
Trial. F v rest	2	0.000000144	0.150
F v rest. Storage	2	0.000273	284.893***
F v rest. Temperature	1	0.0003306	344.996***
Trial. F v rest. Storage	4	0.000001933	2.017
Trial. F v rest. Temperature	2	0.00000005833	0.061
F v rest. Storage. Temperature	2	0.0002904	303.049***
Residual	4	0.0000009583	50.314
Total	20	0.00007799	4094.641

Table	Trial	Storage	Temperature	Storage. Temperature
REP	14	Unequal	Unequal	6
SED	0.0011701	0.0015479	0.0014593	0.0017873

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.10

The overall three-trial mean freezing-point values (in ^{o}C) of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{o}C$

PM Storage	PD	PM from RM at $2^{o}C$			PM from RM at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	-0.524	-0.531	-0.531	-0.532	-0.532	-0.532	-0.571
3 days	-0.528	-0.531	-0.531	-0.532	-0.532	-0.532	-0.571
6 days	-0.528	-0.531	-0.532	-0.533	-0.532	-0.533	-0.575
9 days	-0.528	-0.533	-0.533	-0.536	-0.535	-0.535	-0.574
Mean	-0.527	-0.532	-0.532	-0.534	-0.533	-0.534	-0.573

	DF	MS	VR
Trial	2	0.0004938	68.339***
PM Storage	3	0.00007134	9.874***
Treatment (1-4 below)	6	0.005947	823.028***
1. F v rest	1	0.0003186	525.096***
2. Storage	2	0.0007007	1154.966***
3. Temperature	1	0.0006889	1135.480***
4. Storage. Temperature	2	0.0005795	955.171***
Trial. PM Storage	6	0.00001204	1.666
Trial. Treatment	12	0.00002443	3.381*
PM Storage. Treatment	18	0.000005341	0.739
Residual	36	0.000007225	86.702
Total	83	0.0004530	5436.500

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.0007760	0.0015519	0.0006158	0.0005806	0.0007110

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

A very highly significant difference (at 0.1 per cent level) was found between the trials in the freezing point.

The pasteurized milks gave a lower value for the freezing point depression than the corresponding raw milk due to incorporation of small amounts of water from the pasteurizing plant and pipelines etc.

The pasteurized milks which were produced from raw milk stored at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days showed a very highly significant difference ($p < 0.001$) in the freezing point and highest value was found in the pasteurized milk produced from raw milk after 7 days storage at $6^{o}C$.

The storage of the pasteurized milk for 3, 6 and 9 days gave a very highly significant difference (at 0.1 per cent level) in the freezing point between the samples. The highest values were found in the processed milks prepared from raw milk stored at $6^{o}C$ for 7 days.

6. The free sulphydryl groups

The results of the determinations of free sulphydryl groups of the raw milk samples are presented in Table 3.11.

No significant difference was found in the amounts of free sulphydryl groups in the samples from different trials.

No significant difference was found in the free sulphydryl groups due to the storage time or to the temperature of storage or the interaction between them.

The variations in the free sulphydryl groups for the raw milk stored at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days are illustrated in Fig. 3.4.

The results of determinations of free sulphydryl groups for the pasteurized milks are presented in Table 3.12.

A very highly significant difference (at 0.1 per cent level) was found in the free sulphydryl groups (F-SH) of milks in different trials. Storage of the milk for 2, 4 and 7 days led to significant differences

TABLE 3.11

The free sulphydryl groups (expressed as μmpl/g dry weight) of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Trial	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	5.15	4.83	5.02	8.85	4.39	5.02	6.41
2	3.59	4.15	4.94	4.02	3.15	2.32	3.88
3	3.33	6.44	6.28	3.96	4.15	3.85	4.30
Mean	4.02	5.14	5.41	5.61	3.91	3.73	4.91

	DF	MS	VR
Trial	2	13.2646	5.253
F v rest	1	2.9322	1.161
Trial. F v rest	2	0.8104	0.321
F v rest. Storage		1.92598	0.763
F v rest. Temperature	1	13.4358	5.321
Trial. F v rest. Storage	4	5.2368	2.074
Trial. F v rest. Temperature	2	0.18397	0.073
F v rest. Storage. Temperature	2	0.6608	0.262
Residual	4	2.5252	825.806
Total	20	4.0553	1326.211

Table	Trial	Storage	Temperature	Storage. Temperature
REP	14	Unequal	Unequal	6
SED	0.60062	0.79455	0.74911	0.91746

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.12

The overall three-trial mean free sulphydryl groups (expressed as μmol/g dry weight) of samples of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	3.95	3.58	3.61	3.29	3.98	3.56	3.64
3 days	6.15	6.11	3.58	4.30	7.26	3.73	5.75
6 days	6.51	5.31	4.61	4.47	4.60	5.23	5.28
9 days	5.11	3.96	4.37	5.08	4.30	4.19	5.88
Mean	5.43	4.74	4.04	4.28	5.04	4.18	5.14

	DF	MS	VR
Trial	2	100.3880	25.661***
PM Storage	3	22.4622	5.742**
Treatment (1-4 below)	6	6.8780	1.758
1. F v rest	1	15.184399	5.161*
2. Storage	2	8.038078	2.732*
3. Temperature	1	6.619881	2.250
4. Storage. Temperature	2	1.693881	0.576
Trial. PM Storage	6	10.1483	2.594
Trial. Treatment	12	4.7223	1.207
PM Storage. Treatment	18	3.6818	0.941
Residual	36	3.9120	1494.745
Total	83	7.6397	2919.042

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.57097	1.14193	0.42882	0.40430	0.49516

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 3.4 The variations in the free sulphydryl groups value after storage at 2°C and 6°C of raw milk (RM) and the pasteurized milk (PM) produced from the same raw milk.

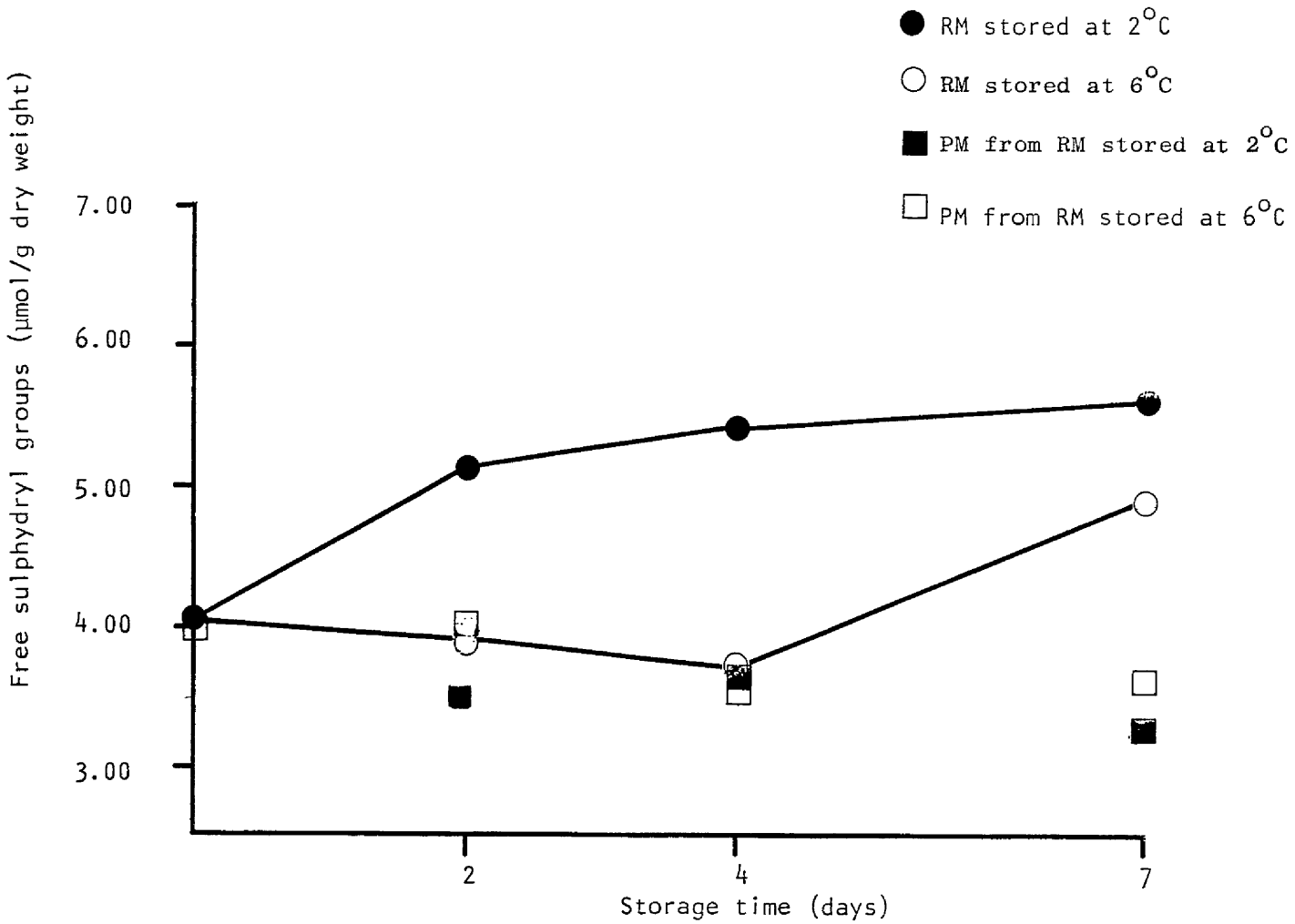


Fig. 3.5 The variations in the overall three-trial mean free sulpydryl groups value after storage at 4°C of pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for up to 7 days

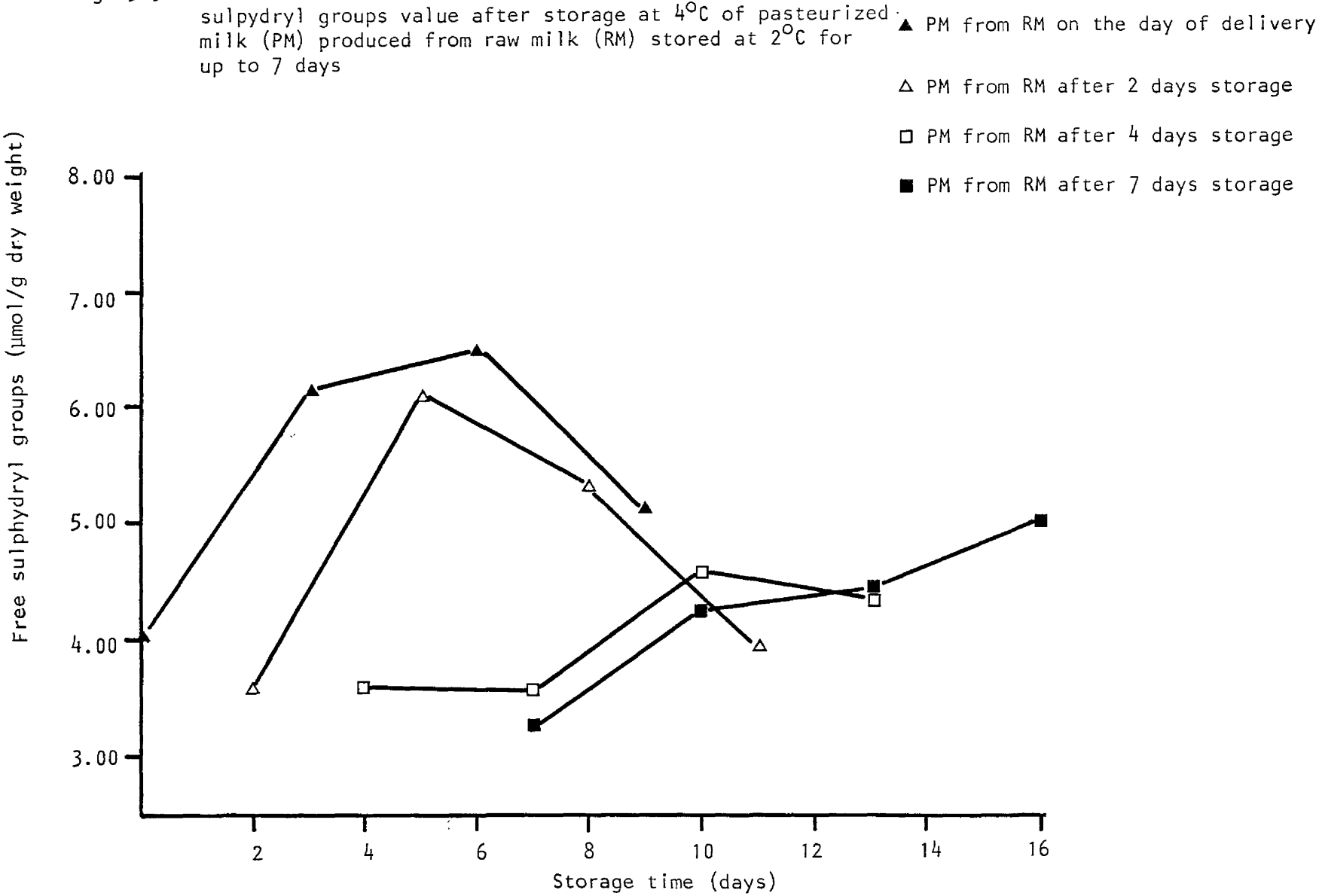
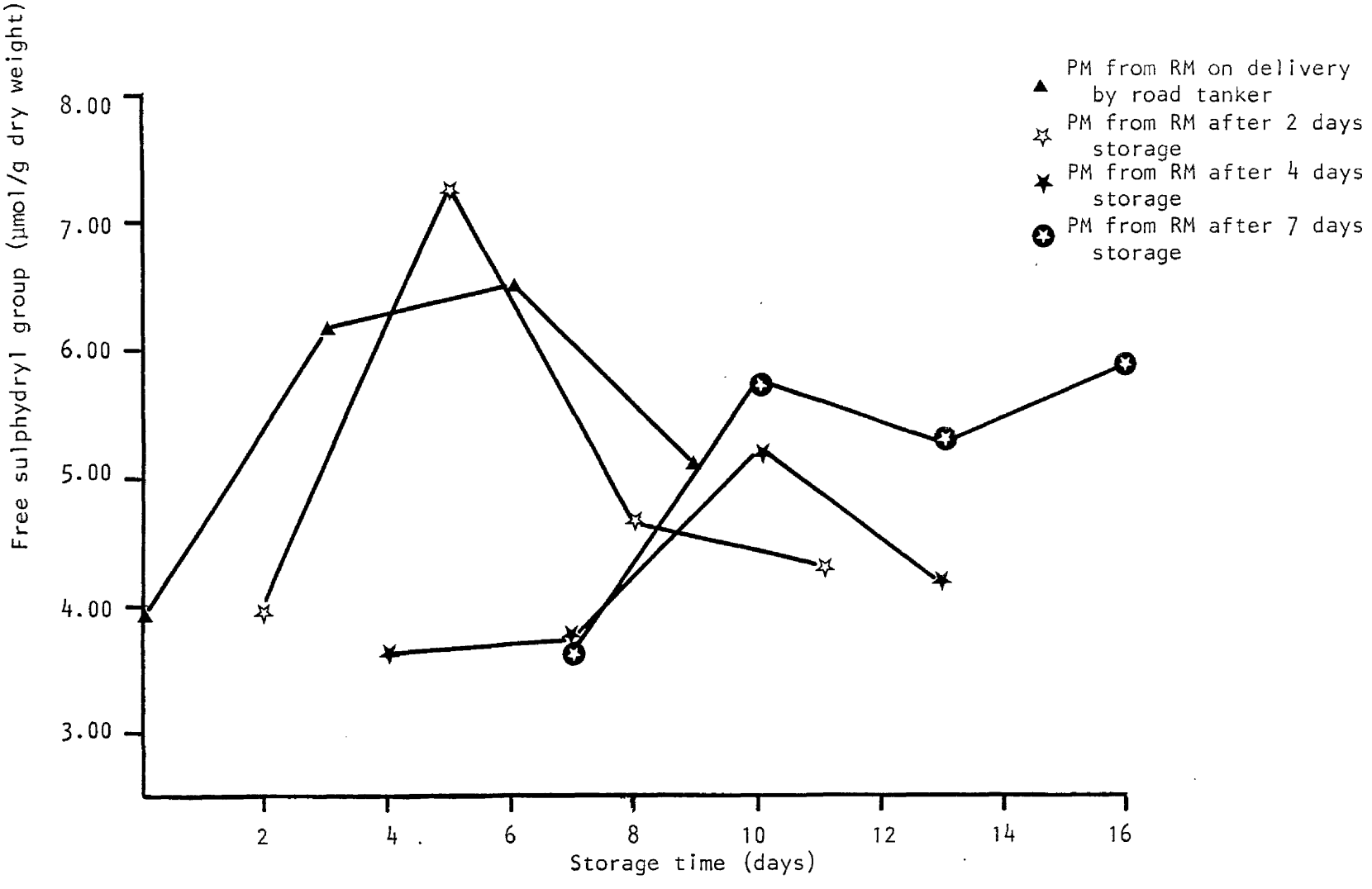


Fig. 3.6 The variations in the overall three-trial mean free sulphydryl groups value after storage at 4°C of pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for up to 7 days



(at 5 per cent level) in the content of F-SH groups but the temperature of storage did not have a significant effect.

The variations in the F-SH groups for the pasteurized milk produced from raw milk stored at $2^{o}C$ and $6^{o}C$ are shown in Figs 3.5 and 3.6.

The storage of the pasteurized milk for 3, 6 and 9 days showed a highly significant difference (at 1 per cent level) in the free sulphydryl groups.

7. Calcium determination

The results of the determinations of calcium in raw and corresponding pasteurized milks are presented in Table 3.13.

A very highly significant difference (at 0.1 per cent level) was found in the calcium level of the samples between trials.

The only difference found in the calcium levels during the storage period was an unusually high result after two days in the third trial. This may have been due to analytical error.

8. Total solids

The results of determinations of the total solids of the raw milks are presented in Table 3.14.

A significant difference (at 5 per cent level) was found between the total solids level of milk in different trials.

No significant difference was found in the total solids content of raw milk samples due to the effect of the storage time or the effect of the temperature of storage and similarly there was no significant interaction between the temperature and the storage time.

The results of the toal solids values for the pasteurized milks are presented in Table 3.15.

A very highly significant difference (at 0.1 per cent level) was found

TABLE 3.13

The calcium determination expressed as µmol/kg of raw milk (RM) and pasteurized milk (PM) on the day of delivery from the road tanker and after the storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Trial	Type	Day of delivery	Storage at $2^{o}C$			Storage at $6^{o}C$		
			2 days	4 days	7 days	2 days	4 days	7 days
1	RM	28.38	28.37	28.38	28.36	28.34	28.37	28.38
	PM	28.37	28.35	28.37	28.37	28.36	28.38	28.39
2	RM	28.38	28.38	28.38	28.38	28.38	28.38	28.38
	PM	28.38	28.38	28.38	28.38	28.38	28.38	28.38
3	RM	28.77	28.95	28.71	28.61	29.01	28.84	28.68
	PM	28.72	29.01	28.63	28.66	29.01	28.71	28.53
Mean		28.50	28.57	28.47	28.46	28.58	28.51	28.46

	DF	MS	VR
Trial	2	1.4807148	588.460***
Type	1	0.003419	1.359
Treatment	6	0.03184	12.656***
Trial. Type	2	0.004662	1.853
Trial. Treatment	12	0.03778	15.017***
Type. Treatment	6	0.002209	0.878
Residual	12	0.002516	5.374
Total	41	0.08932	190.749

Table	Trial	Treatment	Treatment.Trial	Type	Type. Treatment
REP	28	12	4	42	6
SED	0.01341	0.02048	0.03547	0.01095	0.02896

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.14

The total solids content (expressed as per cent) of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Trial	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	12.272	12.121	12.270	12.243	12.322	12.309	12.271
2	12.222	12.258	12.271	12.226	12.250	12.197	12.122
3	12.116	12.199	12.111	12.076	12.112	12.151	12.077
Mean	12.203	12.193	12.228	12.157	12.228	12.219	12.200

	DF	MS	VR
Trial	2	0.0711093	10.739*
F v rest	1	0.0000823	0.012
Trial. F v rest	2	0.0001946	0.029
F v rest. Storage	2	0.008325	1.257
F v rest. Temperature	1	0.0001361	0.021
Trial. F v rest. Storage	4	0.0048951	0.739
Trial. F v rest. Temperature	2	0.017979	2.715
F v rest. Storage. Temperature	2	0.002758	0.417
Residual	4	0.0066215	6.694
Total	20	0.012350	12.485

Table	Trial	Storage	Temperature	Storage. Temperature
REP	14	Unequal	Unequal	6
SED	0.03076	0.04069	0.03836	0.04698

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.15

The overall three-trial mean total solids content (expressed as per cent) of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The value for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	12.075	12.060	12.166	12.166	12.229	12.243	12.161
3 days	12.145	12.197	12.160	12.128	12.184	12.174	12.144
6 days	12.178	12.169	12.157	12.201	12.165	12.175	12.189
9 days	12.131	12.177	12.197	12.218	12.175	12.204	12.133
Mean	12.132	12.151	12.170	12.178	12.189	12.199	12.157

	DF	MS	VR
Trial	2	0.32084	32.288***
PM Storage	3	0.004181	0.421
Treatment (1-4 below)	6	0.012746	1.283
1. F v rest	1	0.0358454	4.236
2. Storage	2	0.0041017	0.485
3. Temperature	1	0.0083570	0.988
4. Storage. Temperature	2	0.0120363	1.422
Trial. PM Storage	6	0.0040765	0.410
Trial. Treatment	12	0.017299	1.741
PM Storage. Treatment	18	0.009306	0.936
Residual	36	0.0099372	45.004
Total	83	0.017928	81.194

Table	Treatment	Treatment. PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.02878	0.05755	0.02300	0.02168	0.02656

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

in the total solids values of the pasteurized milk in different trials.

Neither storage time nor temperature had any significant effect on the total solids level of pasteurized milks, and storage of the pasteurized milk for 3, 6 and 9 days at $4^{o}C$ had no influence on the level of total solids.

9. Acid degree value

The results of the acid degree value tests of the raw milk are presented in Table 3.16.

A very highly significant difference (at 0.1 per cent level) was found in the acid degree value between the milks in the different trials, and with the storage time of the raw milk samples.

The mean values of the acid degree value showed a highly significant ($p < 0.001$) and progressive increase over the storage period at both $2^{o}C$ and $6^{o}C$.

The variations in the acid degree value during the storage time at $2^{o}C$ and $6^{o}C$ are illustrated in Fig. 3.7 for the raw milk samples. There was no significant difference in acid degree values due to the temperature of storage of the raw milk samples.

The results of the acid degree value for the pasteurized milks are presented in Table 3.17.

A very highly significant difference (at 0.1 per cent level) was found in the acid degree value between the trials.

A very highly significant difference (at 0.1 per cent level) was found between the initial acid degree value of the pasteurized milk and samples produced from raw milk after storage for 2, 4 and 7 days. The temperature of storage of the raw milks prior to processing had a very highly significant effect (at 0.1 per cent level) on the acid degree value of the pasteurized milk. The interaction between the storage time and temperature showed a highly significant effect (at 1 per cent level) on the acid degree values. There was an increase

TABLE 3.16

The acid degree value (ADV) expressed as (mEq/100 g fat) of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Trial	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	1.145	1.756	1.793	2.096	1.689	1.970	2.131
2	1.423	1.782	1.762	2.745	1.753	1.884	2.570
3	1.051	1.487	1.734	1.587	1.381	1.814	1.615
Mean	1.207	1.675	1.763	2.143	1.608	1.890	2.106

	DF	MS	VR
Trial	2	0.76247	111.479***
F v rest	1	2.223102	325.032***
Trial. F v rest	2	0.018592	2.718
F v rest. Storage	2	0.711708	104.056***
F v rest. Temperature	1	0.000477	0.070
Trial. F v rest. Storage	4	0.276261	40.391**
Trial. F v rest. Temperature	2	0.00444	0.649
F v rest. Storage. Temperature	2	0.032656	4.775
Residual	4	0.006840	5.699
Total	20	0.320786	267.295

Total	Trials	Storage	Temperature	Temperature. Storage
REP	14	Unequal	Unequal	6
SED	0.03126	0.04135	0.03899	0.04775

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 3.17

The overall three-trial mean acid degree value (ADV) expressed as (mEq/100 g fat) of samples of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker (PD) and after storage for 2, 4 and 7 days at 2°C and 6°C. The values for pasteurized milks were obtained on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM at 2°C			PM from RM at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	1.289	1.599	1.843	1.782	1.665	1.903	1.993
3 days	1.413	1.556	1.671	1.741	1.711	1.723	2.287
6 days	1.586	1.774	1.750	1.884	1.847	1.837	2.247
9 days	1.583	1.716	1.727	1.822	1.882	1.704	2.473
Mean	1.468	1.662	1.748	1.807	1.777	1.792	2.249

	DF	MS	VR
Trial	2	2.18795	26.323***
PM Storage	3	0.196198	2.360
Treatment (1-4 below)	6	1.335040	16.062***
1. F v rest	1	2.835288	31.713***
2. Storage	2	1.318867	14.752***
3. Temperature	1	1.450015	16.219***
4. Storage. Temperature	2	0.543601	6.080**
Trial. PM Storage	6	0.103926	1.250
Trial. Treatment	12	0.262313	3.156**
PM Storage. Treatment	18	0.066108	0.795
Residual	36	0.083118	62.826
Total	83	0.252147	190.588

Table	Treatment	Treatment PM Storage	Storage	Temperature	Storage. Temperature
REP	24	6	Unequal	Unequal	24
SED	0.08323	0.16645	0.07475	0.07048	0.08632

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 3.7 The variations in the acid degree values after storage at 2°C and 6°C for 2, 4 and 7 days of raw milk (RM) and pasteurized milk (PM) produced from the same raw milk

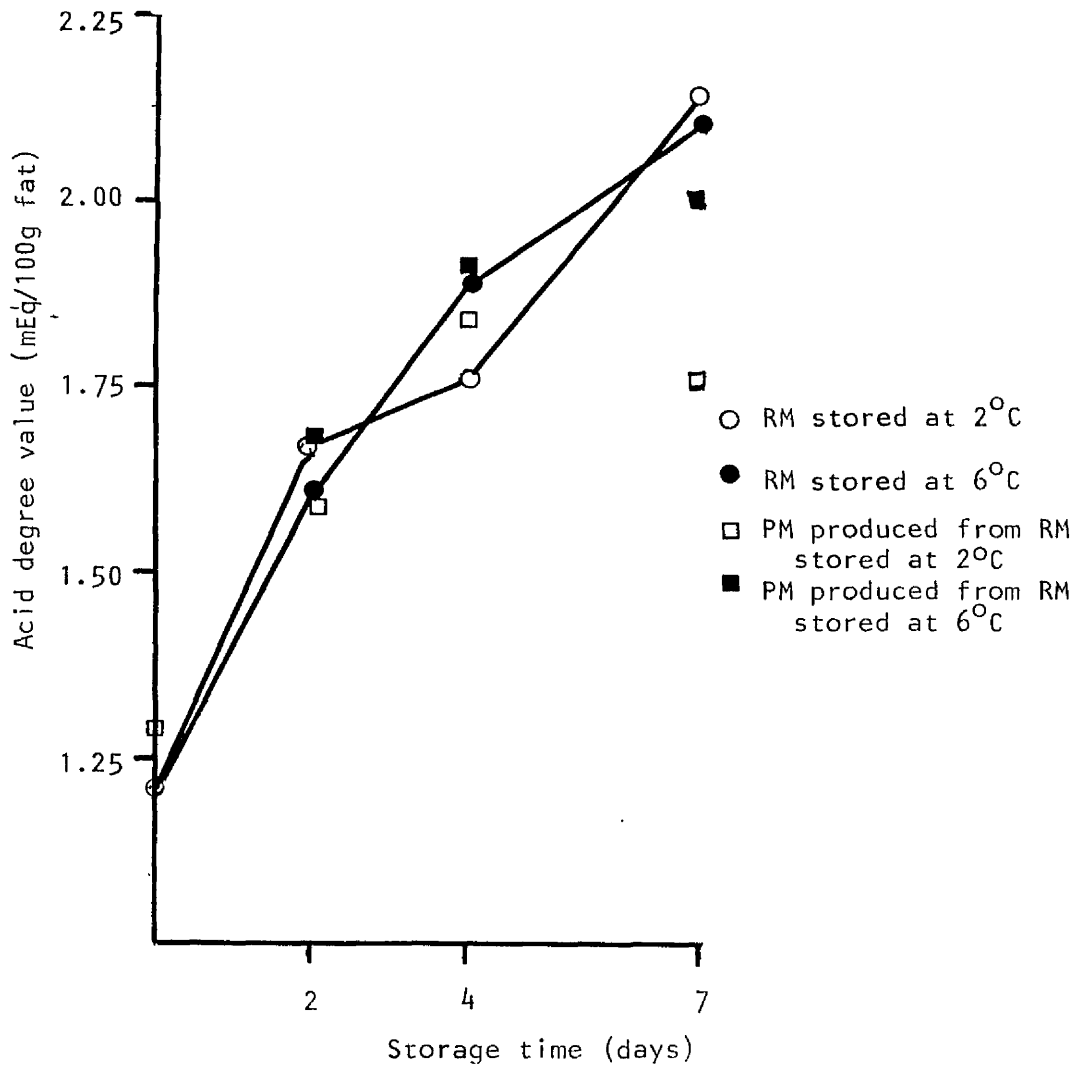


Fig. 3.8 The variations in the overall three-trial mean acid degree value after storage at 4°C for 3, 6 and 9 days of pasteurized milk (PM) produced from raw milk (RM) stored at 2°C for up to 7 days prior to processing

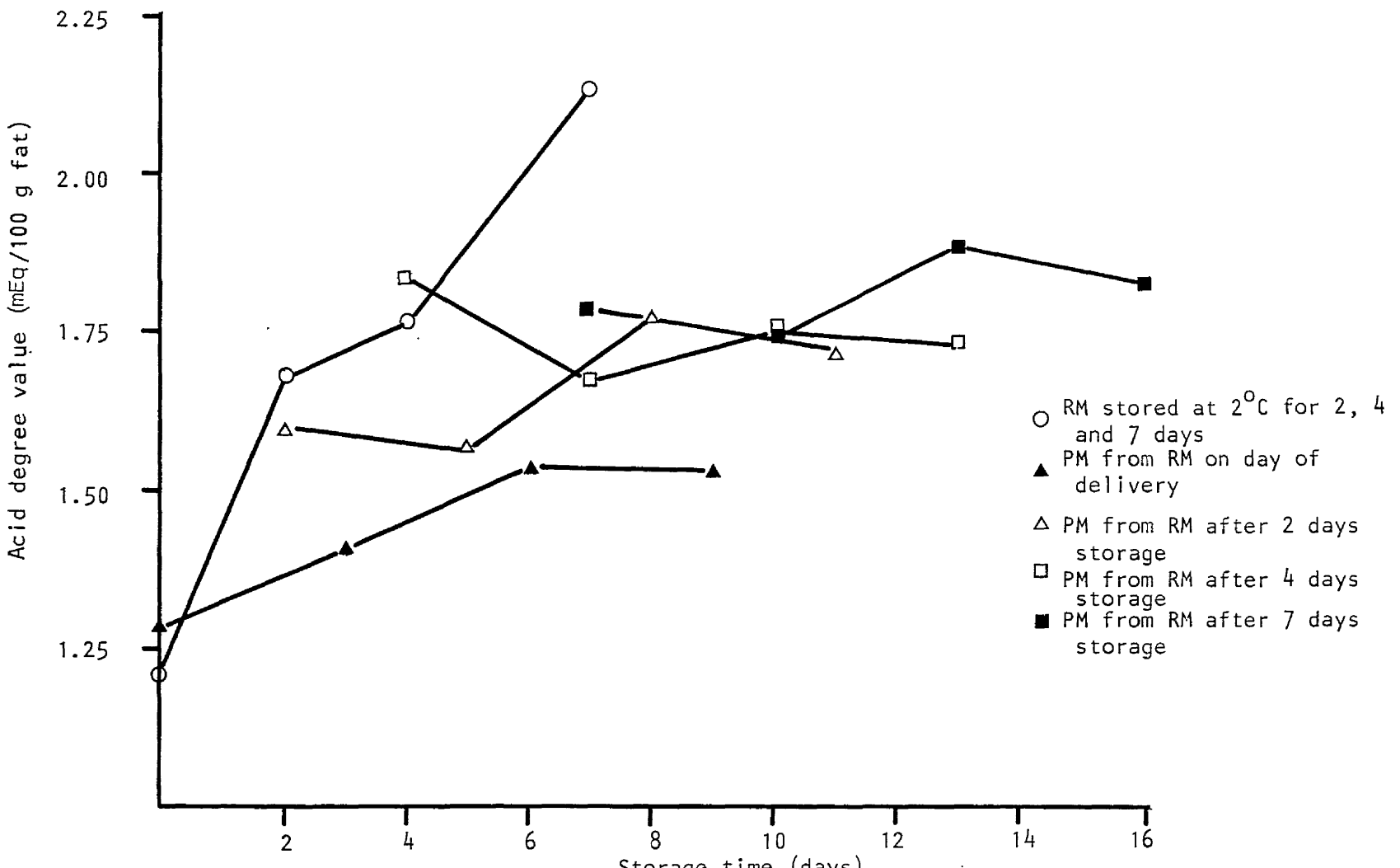


Fig. 3.9 The variations in the overall three trial mean acid degree value after storage at 4°C for 3, 6 and 9 days of pasteurized milk (PM) produced from raw milk (RM) stored at 6°C for up to 7 days prior to processing

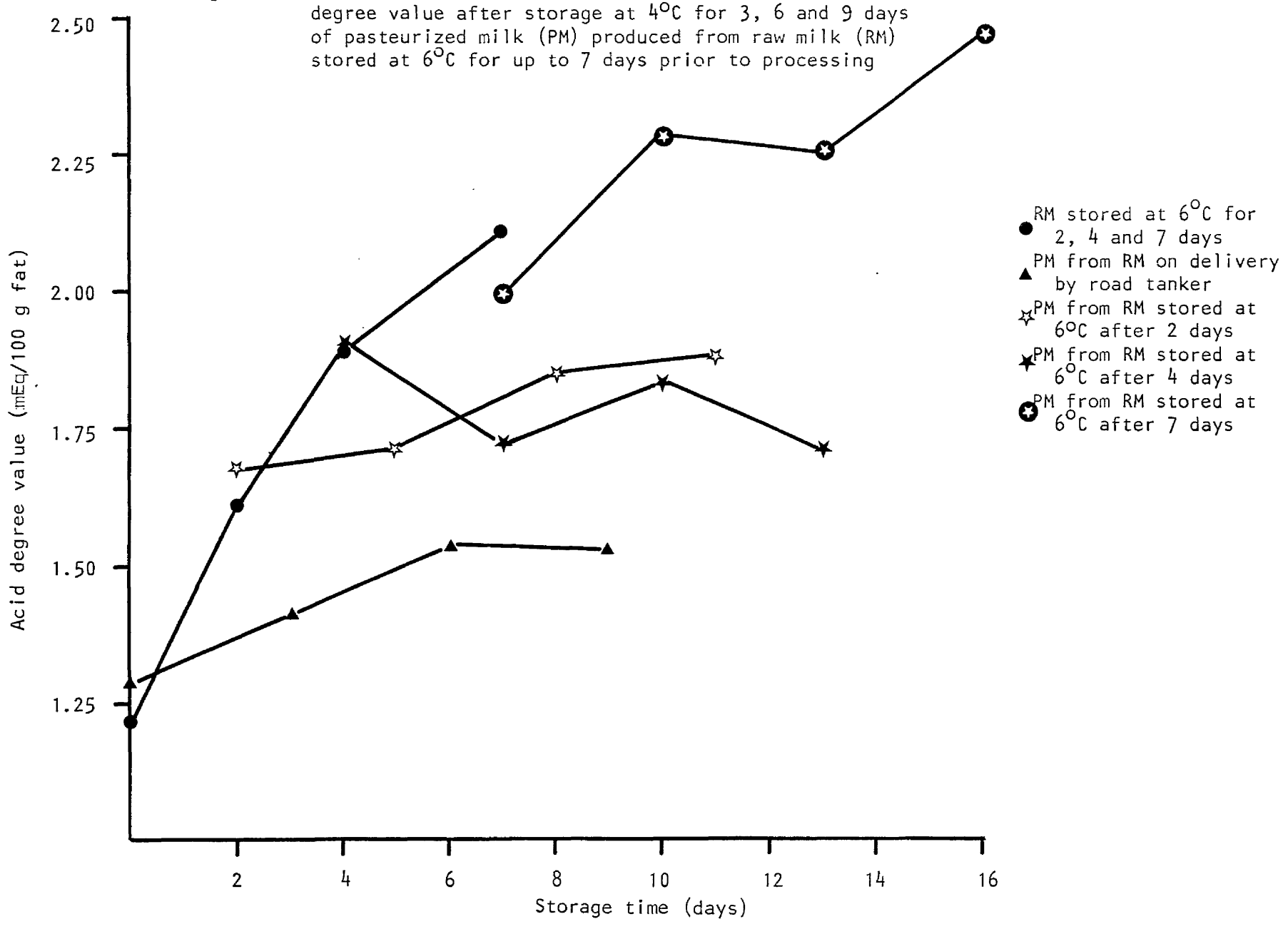


TABLE 3.18

The phosphatase test (expressed as mg p-nitrophenal/ml) of samples of pasteurized milk produced from raw milk on the day of delivery by road tanker and after storage at $2^{o}C$ and for up to 7 days

Trial	Day of delivery	$^{+}P2$			$^{x}P6$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	-ve	-ve	>10 +ve	>10+ve	>10 +ve	>10 +ve	>10 +ve
3	-ve	-ve	-ve	-ve	-ve	-ve	-ve

$^{+}P2$ - is the pasteurized milk produced from raw milk stored at $2^{o}C$

$^{x}P6$ - is the pasteurized milk produced from raw milk stored at $6^{o}C$

in the acid degree value during the storage of raw milks for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$.

The variations in the acid degree value of the pasteurized milks are illustrated in Figs. 3.8 and 3.9.

Storage of the pasteurized milk for 3, 6 and 9 days after processing did not result in a significant change in the acid degree value of the samples.

10. Phosphatase test

The results of the phosphatase tests are presented in Table 3.18.

This test was done to ensure that the time and temperature of pasteurization had been sufficient to destroy the phosphatase enzymes so indicating proper pasteurization, also to indicate whether or not recontamination with raw milk had occurred.

In the first and third trials all processed milks gave negative results. In the second trial negative results were obtained in tests made on samples of pasteurized milk prepared from raw milk on delivery by road tanker and after storage at $2^{o}C$ for 2 days. However, the samples of pasteurized milks prepared from the raw milks held at $2^{o}C$ for 4 and 7 days and at $6^{o}C$ for 2, 4 and 7 days gave positive results. The reasons for this are unclear. Coliform tests on all samples of pasteurized milk were negative in 1 ml as were for test psychrotrophis.

DISCUSSION

1. Fat

From the results shown in Tables 3.1 and 3.2 it is clear that there was no change in the fat content of the samples from the day of delivery of the raw milk by the road tanker over the seven day period of storage at $2^{o}C$ and $6^{o}C$ (Table 3.1). Similarly no changes occurred in the fat content of the pasteurized milk which were produced from these raw milks either on the day of delivery of the raw milk or after a period of storage, or on further storage of the resulting pasteurized milks for 3, 6 and 9 days at $4^{o}C$ (Table 3.2).

The differences in fat content of the milks between the trials were highly significant ($p < 0.001$), and this is due to the seasonal variation in fat content of the milk which is higher in the winter months than in the summer months. This is in agreement with Muir et al.(1978). So the storage of the raw milk for up to 7 days at $2^{o}C$ and $6^{o}C$ before pasteurization has no effect on the fat content of the milk before or after processing.

2. pH

The results of the pH values for the raw milk samples which are presented in Table 3.3, show a significant difference in the pH value between the samples (at 5 per cent level) due to the difference between the trials.

No significant change was found in the mean value of the pH of the raw milk samples after storage for 2 days at $2^{o}C$ and $6^{o}C$ or after 4 days at $2^{o}C$, while it was significant (at $p < 0.5$) after 7 days storage at $2^{o}C$ and after 4 days storage at $6^{o}C$ and highly significant ($p < 0.001$) after 7 days storage at $6^{o}C$.

From these results we may see a decrease in the pH value during the storage time at $2^{o}C$ and $6^{o}C$, and the decrease is more in raw milk samples stored at $6^{o}C$ than the raw milk samples stored at $2^{o}C$. Of the raw milk samples lowest pH value was 5.41 and the highest was 6.75.

This decrease in the pH value is a result of acid production which is more active at $6^{o}C$ than at $2^{o}C$. The pH of normal fresh milk is between 6.5-6.7 (Jenness & Patton, 1959).

The results for the pH value of the pasteurized milk produced from the raw milk referred to above are shown in Table 3.4. The changes occurring during storage of the raw milk are reflected in similar differences in the pH value of the pasteurized milk produced from these raw milks.

There was a further decrease in the pH value for samples of pasteurized milk stored for 3, 6 and 9 days due to the enzyme activity in the milk during storage. The storage of pasteurized milk produced from milks stored for 4 days at $2^{o}C$ caused a decrease of 0.04 pH unit while the

corresponding decrease in pH values of pasteurized milk from raw milk stored at $2^{o}C$ for 7 days was 0.06. While the pH of pasteurized milks produced from raw milk held for 4 days at $6^{o}C$ did not change on further storage up to 9 days the pH of milks produced from raw milks held for 7 days at $6^{o}C$ showed dramatic decrease in the pH to 5.79.

3. Titratable acidity

The results given in Table 3.5 for the titratable acidity of the raw milk show no significant differences between the trials.

No significant difference was found in the titratable acidity between the samples during the storage time at $2^{o}C$ for 2, 4 and 7 days, and at $6^{o}C$ for 2 and 4 days. There was however a highly significant ($p < 0.001$) increase after storage for 7 days at $6^{o}C$.

The variation in the titratable acidity illustrated in Fig. 3.1 for the raw milk during the storage time at $2^{o}C$ and $6^{o}C$ shows the increase in the titratable acidity in the raw milk stored at $6^{o}C$ on the fourth day of storage compared with the raw milk stored at $2^{o}C$.

The acidity of the raw milk during the storage time at $2^{o}C$ is normal and it is between 0.15 to 0.17 per cent and these results are in agreement with Hall & Trout, (1968) who stated that the average titratable acidity of normal fresh milk may be expected to be from 0.14 to 0.17 per cent calculated as the equivalent of lactic acid. The increase in the titratable acidity of raw milk during the storage time at $6^{o}C$ is due to the effect of the microbial enzyme which produces lactic acid and causes off-flavour in the milk, (Hammer, 1948). A significant difference (at 5 per cent level) was found in the titratable acidity between the raw milk samples on the day of delivery and after storage. The results of the titratable acidity of pasteurized milk produced from the raw milks above are shown in Table 3.6. There is a highly significant difference ($p < 0.001$) in the titratable acidity of the pasteurized milk produced from raw milk on the day of delivery and after 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ and the difference is greater at $6^{o}C$ than at $2^{o}C$.

The increase in the titratable acidity of the pasteurized milk during storage for 3, 6 and 9 days at $4^{o}C$ is shown in Figs 3.2, 3.3. The increase was less than in the case of the raw milk and that means the pasteurization temperature destroyed some of the lactic acid organisms. Other bacteria which survive pasteurization temperature such as some species of thermoduric streptococci may grow very slowly during the storage of pasteurized milk at $4^{o}C$ (Finekher, 1980).

A highly significant difference (at 0.1 per cent level) was found in the acidity of the pasteurized milk samples in different trials.

The increase in the titratable acidity of the pasteurized milk produced from raw milk stored at $6^{o}C$ was more than the increase in the pasteurized milk produced from raw milk stored at $2^{o}C$ (Figs 3.3 and 3.2).

4. Cream line

The results of the measurement of cream line formation for the raw milks during the storage time at $2^{o}C$ and $6^{o}C$ are presented in Table 3.7. No significant differences were found in the cream line formed in the raw milk samples due to the storage time or the temperature of storage.

A significant difference (at 5 per cent level) was found in the cream line between the raw milk samples in different trials.

Davis (1951) stated that the depth of the cream layer depended upon a number of factors, especially:-

(1) the percentage of fat in the milk;

(2) the time during which the milk has remained undistrubed;

(3) the size of the fat globules;

(4) the previous treatment to which it has been subjected, especially temperature and the degree of agitation.

Cream line production in pasteurized milks produced from raw milks held at $2^{o}C$ and $6^{o}C$ are presented in Table 3.8.

Highly significant differences (at 0.1 per cent level) were found in the

cream line produced in the pasteurized samples from different trials. A very highly significant difference (at 0.1 per cent level) was found in the cream line production in pasteurized milk produced from raw milk stored at $6^{o}C$ for 2, 4 and 7 days.

It was indicated (Milk Industry Foundation, 1967) that the cream volume of milk decreases as the temperature and time of storage increases. Temperatures between $1.6^{o}C$ ($35^{o}F$) and $4.4^{o}C$ ($40^{o}F$) are best for the storage of milk. Storage at $10^{o}C$ ($50^{o}F$) may cause a 10% reduction in cream volume. This decrease is due to the fact that the clusters of fat globules pack closer together as time goes on and cream layer tends to shrink.

Hall & Trout (1968) stated that there is a slight reduction in cream volume when milk was pasteurized at $71.7^{o}C$ for 15 s. The same authors explain that the effects of heat on the cream volume include:-

(a) the electric charge on the fat globules becomes neutralised;

(b) the protein associated with the fat globule surface are dehydrated, thereby losing their stickiness;

(c) certain salts of milk favourable to fat clumping are rendered ineffective;

(d) factors affecting the viscosity of the milk may be involved;

(e) the natural "fat agglutinin" (euglobulin) undergoes denaturation.

5. Freeze-point depression (FPD)

The values for the freezing-point depression (FPD) of the raw milks held at $2^{o}C$ and $6^{o}C$ for up to 7 days (Table 3.9) show a significant difference (at 1 per cent level) between the raw milk samples from different trials. No significant difference was found in the FPD between raw milk samples during the storage at $2^{o}C$ for 2, 4 and 7 days and at $6^{o}C$ for 2 and 4 days, while it was significantly greater ($p < 0.001$) in the raw milk samples stored at $6^{o}C$ for 7 days. This is due to the effect of microbial activity in the samples (Hall & Trout, 1968).

The freezing-point depression of the raw milk stored at 6°C is high and that is due to the souring which involves increase in the number of molecules in solution as lactose (and sometimes citrate) breakdown (Jenness & Patton, 1959).

The freezing-point depression values for the pasteurized milk produced from raw milks held in cold storage for varying periods at 2°C and 6°C are presented in Table 3.10. A highly significant difference (at 0.1 per cent level) was found between the FPD values for the pasteurized milks in different trials. The same significance was found in the difference between FPD values of pasteurized milks produced from raw milk during the storage time at 2°C and 6°C but the difference was significantly higher in the pasteurized milk prepared from raw milk after 7 days storage at 6°C.

Hall & Trout (1968) reported that pasteurization affects the freezing-point of milk only as far as it effects the concentration of the constituents in solution. Theoretically no significant effects should occur if the milk has been properly pasteurized.

The storage of the pasteurized milk for 3, 6 and 9 days showed a very highly significant difference (at 0.1 per cent level) on the FPD values.

There was no difference in the FPD values between the raw and the processed milks which were prepared from it.

6. Free sulphydryl groups

The results of analysis of the free sulphydryl groups in the raw milk during the storage time at 2°C and 6°C presented in Table 3.11 shows no significant difference between the level of free sulphydryl groups in raw milk samples obtained in different trials.

Neither the storage time nor the temperature of the storage had any significant effect on the sulphydryl levels in the raw milk samples. The variations in the free sulphydryl groups (F-SH) of raw milk stored at 2°C and 6°C for 2, 4 and 7 days and the pasteurized milk produced

from the same raw milk (Fig. 3.4) showed an increase in the F-SH in raw milk stored at $2^{o}C$ compared to the raw milk stored at $6^{o}C$.

The F-SH groups of pasteurized milk showed a decrease after pasteurization. These changes in the F-SH groups have been discussed in Chapter Five.

The values for the F-SH groups of the pasteurized milks on production and after storage for up to 9 days at $4^{o}C$ produced from raw milk after storage at $2^{o}C$ and $6^{o}C$ are presented in Table 3.12. A highly significant difference (at 0.1 per cent level) was found in the F-SH values between the pasteurized samples in the different trials.

The storage of the pasteurized milk for 2, 6 and 9 days at $4^{o}C$ led to a significant change (at 2 per cent level) in the F-SH groups. An increase in the level of F-SH groups took place during the storage of the pasteurized milk for 3 and 6 days. Further storage of these milks for 3 (i.e. a total of 9 days from processing) days resulted in a decrease in the levels of the F-SH groups in the milk, but these levels are still higher than the initial values.

The variations in the F-SH groups of the pasteurized milk produced from raw milk stored before processing at $2^{o}C$ and $6^{o}C$ are presented in Figs 3.5 and 3.6, which shows a fluctuation in the F-SH groups during the storage of pasteurized milk for 3, 6 and 9 days. The possible reasons for this is discussed in Chapter Five.

7. Calcium

Highly significant difference ($p < 0.001$) was found in the calcium content of the milk samples in different trials. This is due to the seasonal variation in the composition of the milk (Jenness & Patton, 1959). No significant difference was found in the total calcium content of milk during the storage time at both $2^{o}C$ and $6^{o}C$. This indicates that the storage conditions did not cause evaporation of the water of milk so that the total calcium content would be changed. There was no effect for the pasteurization on the level of calcium in milk.

8. Total solids

The total solids of the raw milks during the storage time at 2°C and 6°C are presented in Table 3.14 and show that there was a significant difference (at 5 per cent level) in the total solids content of the raw samples in different trials. This is due to seasonal variation in the composition of milk (Jenness & Patton, 1959; Muir et al., 1978). Neither the temperatures nor duration of storage had any effect on the total solids content of the raw milk samples.

The total solids levels of the pasteurized milks are presented in Table 3.15. As was found for the corresponding raw milks there was a highly significant difference (at 0.1 per cent level) in the total solids content of milks in different trials. There was no significant difference in the total solids content of the pasteurized milk produced from raw milk stored at 2°C and 6°C and the total solids level of pasteurized milk did not alter on storage after processing for 3, 6 and 9 days.

9. Acid degree value

The acid degree values (ADV) of the raw milks during the storage time at 2°C and 6°C are presented in Table 3.16. A highly significant difference (at 0.1 per cent level) was found in the acid degree value of the raw milk samples in different trials.

Deeth & Fitz-Gerald, (1976) indicated that if fresh raw milk which has been cooled to 5°C or lower (refrigeration temperatures) is warmed to 25-30°C and then recooled and stored, lipolysis may occur. They stated that the longer milk is stored, the greater is the possibility that bacterial growth will proceed to such an extent that it too contributes significantly to the amount of lipolysis.

The mean values of ADV obtained in this study for the raw milk stored at 2°C and 6°C showed significant increase ($p < 0.001$). These results are in agreement with those of Muir et al. (1978). There is a range for the amount of ADV of milk which corresponds with the acceptability of dairy products. If the ADV was ranged between 1.5 to 2.0 an off-

flavour would be detected by some people. But if it was more than 2.0 the flavour would be unacceptable to most people (Deeth & Fitz-Gerald, 1976). They stated that many bacterial lipases differ from milk lipase in that they are not inactivated by pasteurization even though the organisms which produce them are destroyed. Among the treatments which increase lipolysis in milk are agitation, aeration and cooling the warm milk (and vice versa) cause much higher effects than freezing and thawing of milk (Willart & Sjöström, 1966)

The variation in the ADV between the raw milk samples are shown in Fig. 3.7 which shows an increase in the ADV during the storage time (from 1.207 to 2.143 mEq/100 g fat).

In the same figure it is shown that pasteurization resulted in lowered ADVs in the milks. The results of the ADVs for the pasteurized milk produced from raw milk are presented in Table 3.17. A highly significant difference (at 0.1 per cent level) was found between the ADVs of the pasteurized milks in different trials.

A highly significant effect (at 0.1 per cent level) was found in the ADV between the pasteurized milk produced from raw milk stored at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days.

No significant difference was found in the ADV on storage of the pasteurized milk for 3, 6 and 9 days.

The variation in the ADV between the pasteurized milk produced from raw milk at $2^{o}C$ and $6^{o}C$ are shown in Figs 3.8 and 3.9 respectively.

CONCLUSIONS

1. It is concluded from determinations of the fat content of raw milk and corresponding pasteurized milk derived from it that storage of raw milk at $2^{o}C$ and $6^{o}C$ and storage of the pasteurized milk at $4^{o}C$ did not affect the level of fat present in the milks. Differences detected in the fat level of milks in different trials were due to the seasonal variation in milk composition.

2. The results for the titratable acidity and the pH value of the milk

showed an increase in the titratable acidity of the raw milk samples over the storage time at 2°C and 6°C. The increase was highly significant with raw milk storage at 6°C for 7 days while there was no significant change in the titratable acidity of the same milk held at 2°Cfor the same period of time.

No significant change was found in the mean value for the pH in the raw milk stored for 2 and 4 days at 2°C and 2 days at 6°C while it was significant after 7 days at 2°C and 4 days at 6°C and highly significant after 7 days at 6°C. There was a decrease in the pH value for the pasteurized milk produced from the same raw milk above, but the decrease in the pH value during storage of the pasteurized milk for 3, 6 and 9 days is very small.

3. The results for cream line formation indicated that neither the storage time nor the temperature of storage of the raw milk affected the extent of cream line formation. The cream line was significantly affected by pasteurization but there was no significant change due to the storage of the pasteurized milk for up to 9 days at refrigerator temperature (4°C).

4. The freezing-point depression values showed no significant change during the storage time of the raw milk at 2°C for 2, 4 and 7 days and at 6°C for 2 and 4 days. The FPD was significantly changed after the fourth day of storage at 6°C due to acid production through bacterial activity. Storage of pasteurized milk for up to 6 days did not affect the FPD but further storage to 9 days resulted in an increase in the FPD.

5. Neither the period nor temperature of storage affected the level of F-SH groups in the raw milks. Similarly the F-SH levels of corresponding pasteurized milks produced from the raw milks were unaffected by storage time and temperature before processing. Storage at 4°C of the pasteurized milks for 3, 6 and 9 days resulted in a significant difference in the F-SH groups between samples. Further work has been done to give more information about the effect of storage on the F-SH groups and is reported in Chapter Five.

6. The calcium levels in raw milks held at $2^{o}C$ and $6^{o}C$ for up to 7 days prior to pasteurization were not affected by the pre-processing treatment and the calcium levels in the corresponding pasteurized milks were unaffected by the processing conditions or subsequent storage at $4^{o}C$. Variations in the levels of raw milks in different trials were attributed to seasonal variations in the composition of milk.

7. As expected, no change was observed in the total solid content during the storage of both raw (up to 7 days at $2^{o}C$ and $6^{o}C$) and pasteurized milk (for up to 9 days at $4^{o}C$).

8. Increasing the period of storage of raw milk at both $2^{o}C$ and $6^{o}C$ resulted in increase in the level of ADV. The corresponding pasteurized milks have lower ADV values. Further storage of these pasteurized milks for 3, 6 and 9 days did not affect the level of ADV.

CHAPTER FOUR

THE ORGANOLEPTIC QUALITY OF RAW AND PASTEURIZED MILK DURING STORAGE

INTRODUCTION

Many consumers relate the keeping quality of perishable foods to lack of deterioration in flavour, odour and palatability on storage. Colour change may also play a role in the consumer's assessment of quality. What the consumer wants, therefore, is assurance that the product was prepared in a hygienic manner, and that it has a good flavour and will retain its good quality for a reasonable time.

The shelf life of pasteurized milk is affected by many factors, in the chain of stages starting with raw milk handling through processing, packaging, distribution and storage to the consumer's refrigerator. Johnson (1979) defined satisfactory shelf life of pasteurized milk as being 21 days or more when held at suitable refrigerator temperatures and the milk having fresh flavour and aroma.

Serious flavour defects in both raw and pasteurized milk may result from an accumulation of the products of bacterial metabolism (Morgan, 1970a; Morgan, 1970b). These are produced by the action of the complex enzyme systems of contaminating organisms on the constituents of milk.

Pasteurized milk may be recontaminated by subsequent contact with unsanitary equipment. Such contamination often includes psychrotrophic bacteria (Boyd *et al.*, 1955; Hosono *et al.*, 1974; Punch *et al.*, 1965; Shehata & Collins, 1971; Shehata *et al.*, 1971), which are commonly responsible for flavour defects in pasteurized milk. These organisms multiply slowly at 4.4^{o}C or lower and, unless the contamination is appreciable, flavour defects may not be evident before 10 to 14 days storage. However, in milk which has not been cooled to below 4.4^{o}C immediately after pasteurization or which is stored at 7.2 to 15.6^{o}C, the psychrotrophic organisms may multiply rapidly and cause flavour defects in a few days (Shehata *et al.*, 1971).

Johnson (1979) stated that many types of flavour defects are attributable to bacterial growth, including acid flavour which is caused by the growth of lactic cultures in milk. *Streptococcus lactis*, an acid-

producing organism which is also used for starter cultures, is universally distributed in the dairy environment. It is extremely important to cool raw milk immediately after milking so that this organism cannot multiply and thereafter, produce lactic acid and so develop a sour off-flavour and other defects including maltiness. Fruity flavours may develop in milk as a result of the growth of psychrotrophic gram-negative contaminants producing off-flavour such as apple, ester-like, or fruity. However, normal pasteurization will effectively control these organisms. Johnson (1979) summarized the microbiologically induced off-flavours and physical defects in milks in the following scheme:-

Flavour	Organism	Mechanism
Acid flavour	Strep. lactis	Acetic, propionic acids
Malty	Strep. lactis var. maltigenes	Aldehydes, alcohols and mechanisms of production from amino acids.
Fruit, bitter putrid	Ps. fragi	Hydrolyze milk fat & esterify lower fat with ethanol.
Ropiness	Alcaligenes viscosus Strep. cremoris var. hollandicus (acid development) Micrococcus spp. Lactobacillus bulgaricus Escherichia-enterobacter group	Capsular material (mucins, galactans)

Unclean, bitter, and putrid flavours result from the growth of psychrotrophic organisms in pasteurized milk. Bacteria from this group are capable of growth as low as $0^{o}C$ ($32^{o}F$). Off-flavours developed from these organisms generally occur due to protein break-down in the milk, which produce bitter peptides and a putrid flavour due to the decomposition of amino acids.

There are four kinds of heat-induced flavour alterations:- cooked or sulphurous flavour, heat or rich, caramelized and scorched. Light-

induced off-flavours are caused when milk is exposed to sunlight, fluorescent light, or diffused day-light. Plastic milk containers have increased the occurrence of light-induced off-flavours significantly, and homogenization of milk products increases the incidence of light induced flavours due to the breakdown of milk fat in the product (Johnson, 1979).

Lipolyzed flavours are caused by the breakdown of milk fat by the enzyme lipase, which catalyzes the breakdown into free fatty acids, causing a characteristic off-flavour.

Flavour scores of pasteurized milk are very important, firstly as a guide to its expected shelf life, and secondly to help identify the type of any spoilage present in the milk so facilitating the search for organisms or other factors that may be involved in off-flavour development.

The purpose of this experiment was to investigate the effect of cold storage of raw milk at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days on its organoleptic quality and on the organoleptic quality of pasteurized milk produced from it immediately after heat treatment and after storage at refrigerator temperature ($4^{o}C$) for up to 9 days.

EXPERIMENTAL

1. The flavour scores for the milk

The method which was used for the organoleptic evaluation of the raw and pasteurized milks is that described by Horner et al. (1980) using a hedonic scale. This scale contained eight categories of response defined as follows:-

Like extremely;	8
Like very much;	7
Like moderately;	6
Like slightly;	5
Dislike slightly;	4
Dislike moderately;	3
Dislike very much;	2
Dislike extremely;	1

The intensity of any flavour defect which the panel could find was assessed using a four point scale:-

Very slight;	1
Slight;	2
Distinct;	3
Pronounced.	4

2. Collecting the samples for the organoleptic evaluation

Raw milk samples were collected on the day of delivery from the road tanker and after storage of the split supply for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$. Samples were kept in a frozen condition.

Pasteurized milks were prepared from milk immediately on delivery by the road tanker and after the split consignment had been held for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$. Samples were taken on the day of processing and also after 3, 6 and 9 days storage at $4^{o}C$. These samples were also kept in a frozen condition.

3. The taste panel

The taste panel was composed of members of the staff of the Department of Dairy Technology, The West of Scotland Agricultural College, Ayr, with experience in quality assessment of milk and milk products.

4. Presentation of the samples for testing

The samples of raw and pasteurized milk were taken from the deep freeze cabinet and warmed slowly to $19^{o}C$ and filled into 50 ml beakers using the same procedure described by Schonborn et al. (1975). Each sample was offered to five judges for the organoleptic test. The samples were presented in random order.

5. The tea test - effect of milk addition on the odour and flavour (taste) of hot tea

A considerable proportion of the milk sold in the United Kingdom and elsewhere is added to tea by consumers. It is important therefore, that in evaluating milk quality some attempt should be made to determine how the milks under test affect the appearance, odour and flavour

(taste) of tea to which they are added.

In all trials freshly made tea was provided for the grader and tea bags were used to standardise the strength of the beverage. The same strength of tea was provided for each grader. Initially it was considered desirable to standardize the quantity of milk added but it became apparent that the panel members preferred a wide range of milk addition, from the small quantity, a few drops required to change the colour of the tea to much larger quantities, around one fifth of the total volume, which gave the tea a distinctly milky appearance and taste. Because of this range of preferences of the panelists it was decided not to standardise the quantities of milk used but to allow the panelists to use their own chosen mixture of tea and milk.

RESULTS

1. Appearance

(i) Raw milk

The means of the scores given by the taste panel for the appearance of the raw milks examined on the day of delivery and after 2, 4 and 7 days storage at two different temperatures ($2^{o}C$ and $6^{o}C$) are presented in Table 4.1.

The difference between the judges in their grade scores for appearance was significant (at the 5 per cent level).

Milks graded on the day of delivery showed lower scores for the appearance of the raw milk comparing with the other days of storage, and statistically this was highly significant (at the 1 per cent level). On the other hand the means of the scores for the appearance of the raw milk show that there was no significant effect on the scores during the storage time at two different temperatures.

The variations in the scores given for the appearance of the milk on storage at $2^{o}C$ and $6^{o}C$ are shown in Fig. 4.1. There was a slight increase in the scores given for the appearance of the

raw milk during the storage at $2^{o}C$ on the second and the fourth day of storage and a decrease on the seventh day, while the raw milk stored at $6^{o}C$ shows a decrease from the second day of storage.

(ii) Pasteurized milk

The means of the scores given by the taste panel (5 judges) for the appearance of pasteurized milks which were produced from the raw milk on the day of delivery by the road tanker and after 2, 4 and 7 days of storage of the raw milk at $2^{o}C$ and $6^{o}C$ and sampled on the day of production and also after further storage of the pasteurized milk at $4^{o}C$ for 3, 6 and 9 days are presented in Table 4.2.

The differences between the judges in their grade scores for appearance were very highly significant (at 0.1 per cent level).

The samples of pasteurized milk produced at 2, 4 and 7 days storage of the raw milk at $2^{o}C$ showed no significant difference in the scores for the appearance.

There was a very highly significant difference (at 0.1 per cent level) in the appearance scores between the pasteurized milk produced from raw milk stored at $6^{o}C$ for 2, 4 and 7 days.

Storage of the pasteurized milk for 3, 6 and 9 days had a very highly significant effect (at 0.1 per cent level) on the appearance scores.

The scores for the appearance of pasteurized milk produced from raw milk on the day of delivery by the road tanker were higher (significant difference at 0.1 per cent level) than those of milks processed after storage.

In the case of pasteurized milks prepared from raw milk stored at $2^{o}C$ for 2, 4 and 7 days the appearance scores after 3 and 6 days were higher than the initial levels or the levels after 9 days of

TABLE 4.1

Means of the scores[+] given by a panel (5 judges) for the appearance of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

Judges	Day of delivery	RM stored at 2°C			RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
1	5.50	7.00	7.00	6.00	7.00	6.00	7.00
2	5.50	5.00	5.00	6.00	6.00	3.00	6.00
3	6.00	7.00	8.00	6.00	7.00	8.00	6.00
4	5.00	5.00	7.00	6.00	6.00	7.00	5.00
5	5.50	6.00	7.00	6.00	6.00	7.00	5.00
Mean	5.50	6.00	6.80	6.00	6.40	6.20	5.80

[+]Scale of 1 = dislike extremely to 8 = like extremely

	DF	MS	VR
Judges	4	2.6500	6.235*
F v rest	1	3.675	8.647**
Judges. F v res	4	0.550	1.294
F v rest. Storage	2	0.900	2.118
F v rest. Temperature	1	0.133	0.314
Judges. F v rest. Storage	8	1.525	3.588*
Judges. F v rest. Temperature	4	0.050	0.188
F v rest. Storage. Temperature	2	0.633	1.490
Residual	8	0.425	0.224
Total	34	1.043	0.549

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	0.326	0.292	0.266	0.357

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

RM - Raw Milk

F v rest - The day of delivery compared with the means of the other days of storage at 2°C and 6°C

TABLE 4.2

Means of the scores given by a panel (5 judges) for the appearance of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigeratore temperature ($4^{o}C$)

PM Storage	PD	PM from RM stored at $2^{o}C$			PM from RM stored at $6^{o}C$			Mean
		2 days	4 days	7 days	2 days	4 days	7 days	
Initial	4.60	3.60	3.80	4.40	3.80	3.00	3.60	3.93
3 days	6.10	3.80	5.60	5.80	5.20	4.60	4.00	5.15
6 days	5.70	6.20	6.00	5.40	5.00	4.80	4.80	5.45
9 days	4.70	5.20	4.40	4.60	5.00	2.80	4.40	4.48
Mean	5.27	4.70	4.95	5.05	4.75	3.80	4.20	

	DF	MS	VR
Judges	4	14.7813	17.619***
PM Storage	3	18.750	22.349***
F v rest	1	14.7000	17.522***
Judges. PM Storage	12	1.4896	1.776
Judges. F v rest	4	1.0021	1.194
PM Storage. F v rest	3	1.6389	1.953
F v rest. Storage	2	1.3000	1.550
F v rest. Temperature	1	12.675	15.108***
Judges. PM Storage. F v rest	12	0.5937	0.708
Judges. F v rest. Storage	8	1.6646	1.984
PM Storage. F v rest. Storage	6	2.2889	2.728*
Judges. F v rest. Temperature	4	1.7167	2.046
PM Storage. F v rest. Temperature	3	0.4750	0.566
F v rest. Storage. Temperature	2	3.900	4.649*
Residual	74	0.8390	1.766
Total	139	2.0468	4.309

Table	Temperature	Storage. Temperature	PM Storage. Temperature	PM Storage. Storage	PM Storage
REP	Unequal	Unequal	Unequal	10	40
SED	0.1870	0.2508	0.3739	0.4096	0.2048

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

PD - Pasteurized milk produced from raw milk on delivery by road tanker and after 3, 6 and 9 days of storage at $4^{o}C$

Fig. 4.1 Means of appearance scores awarded by a panel (5 judges) for raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

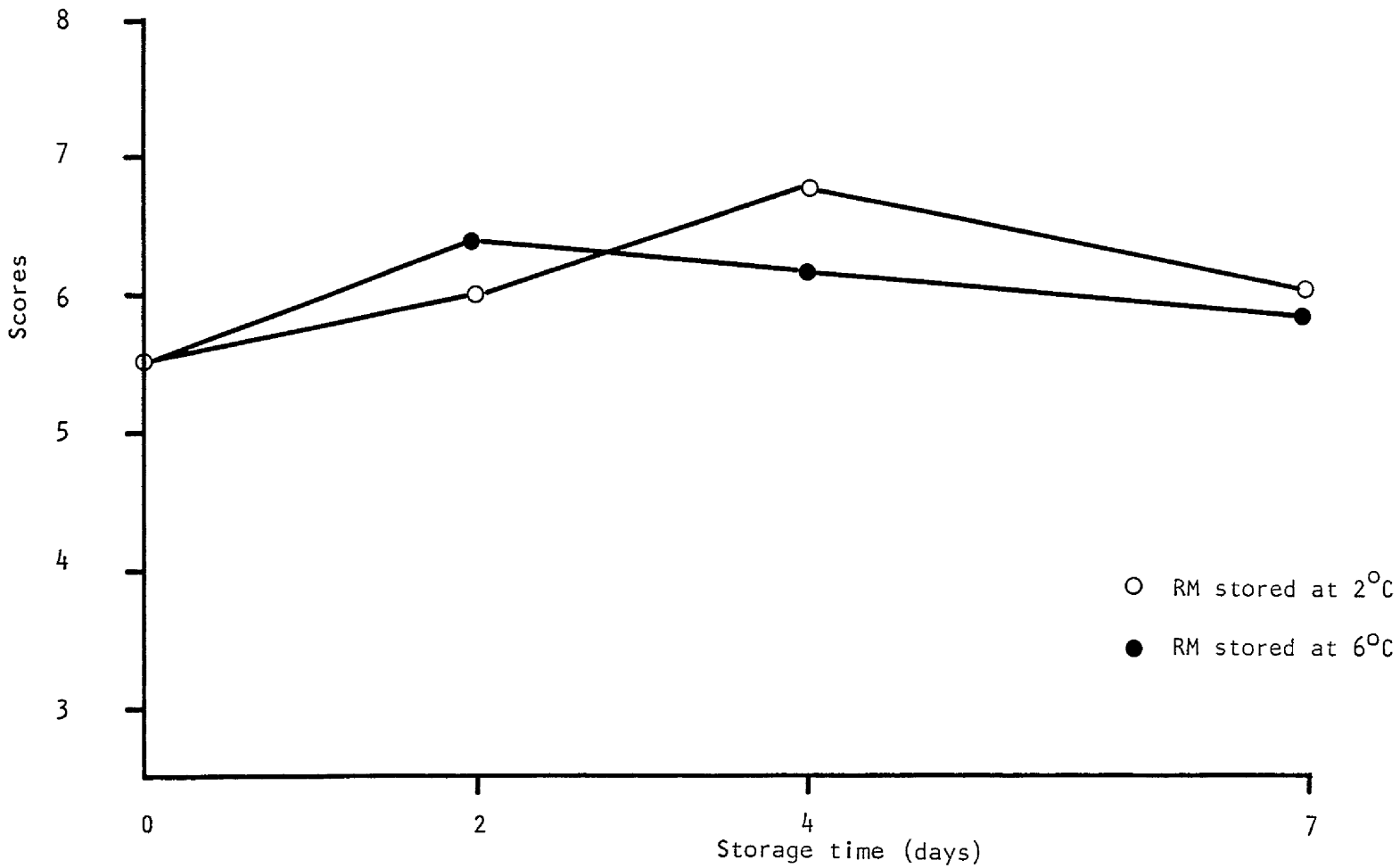


Fig. 4.2 Means of appearance scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

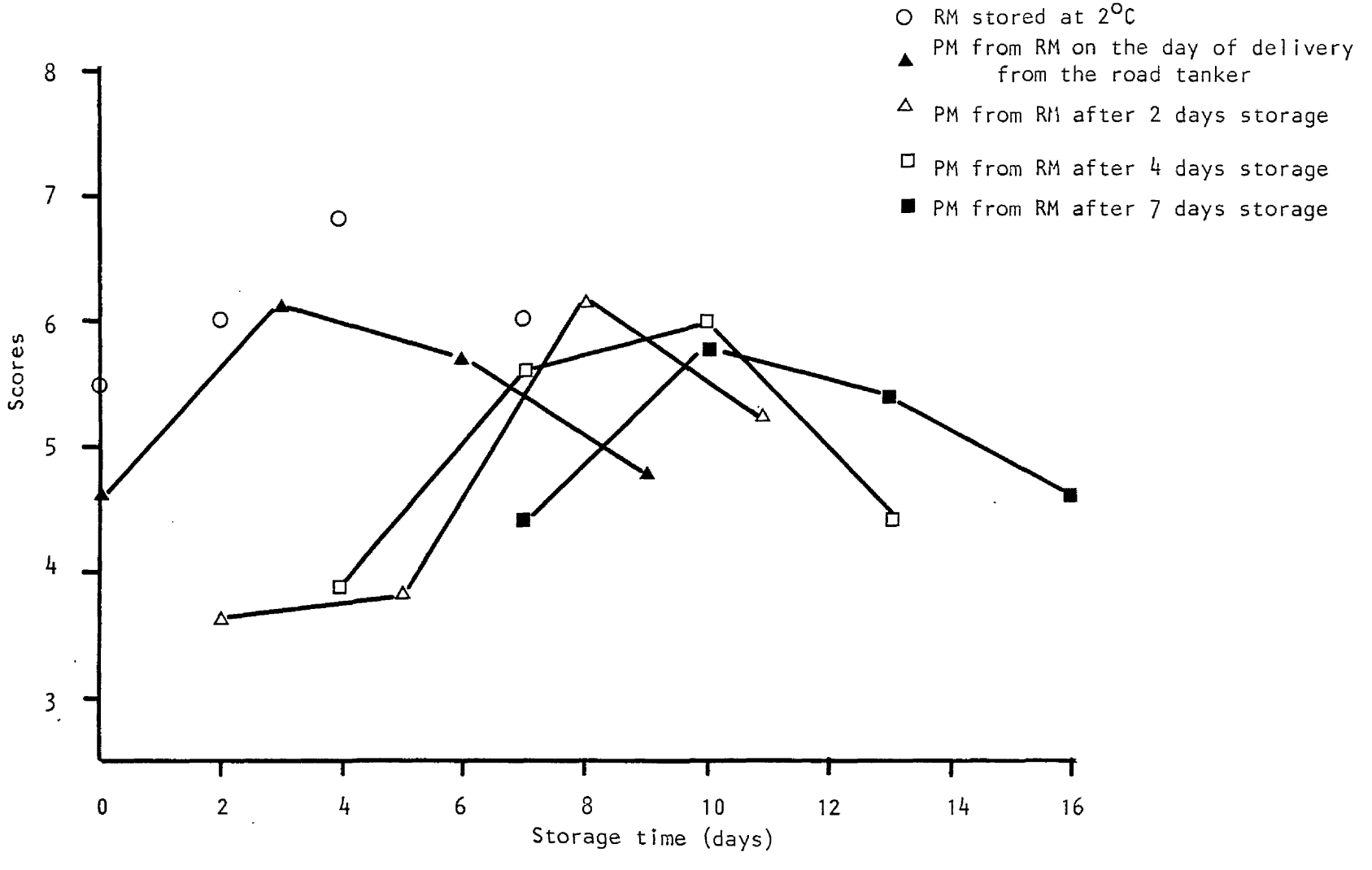
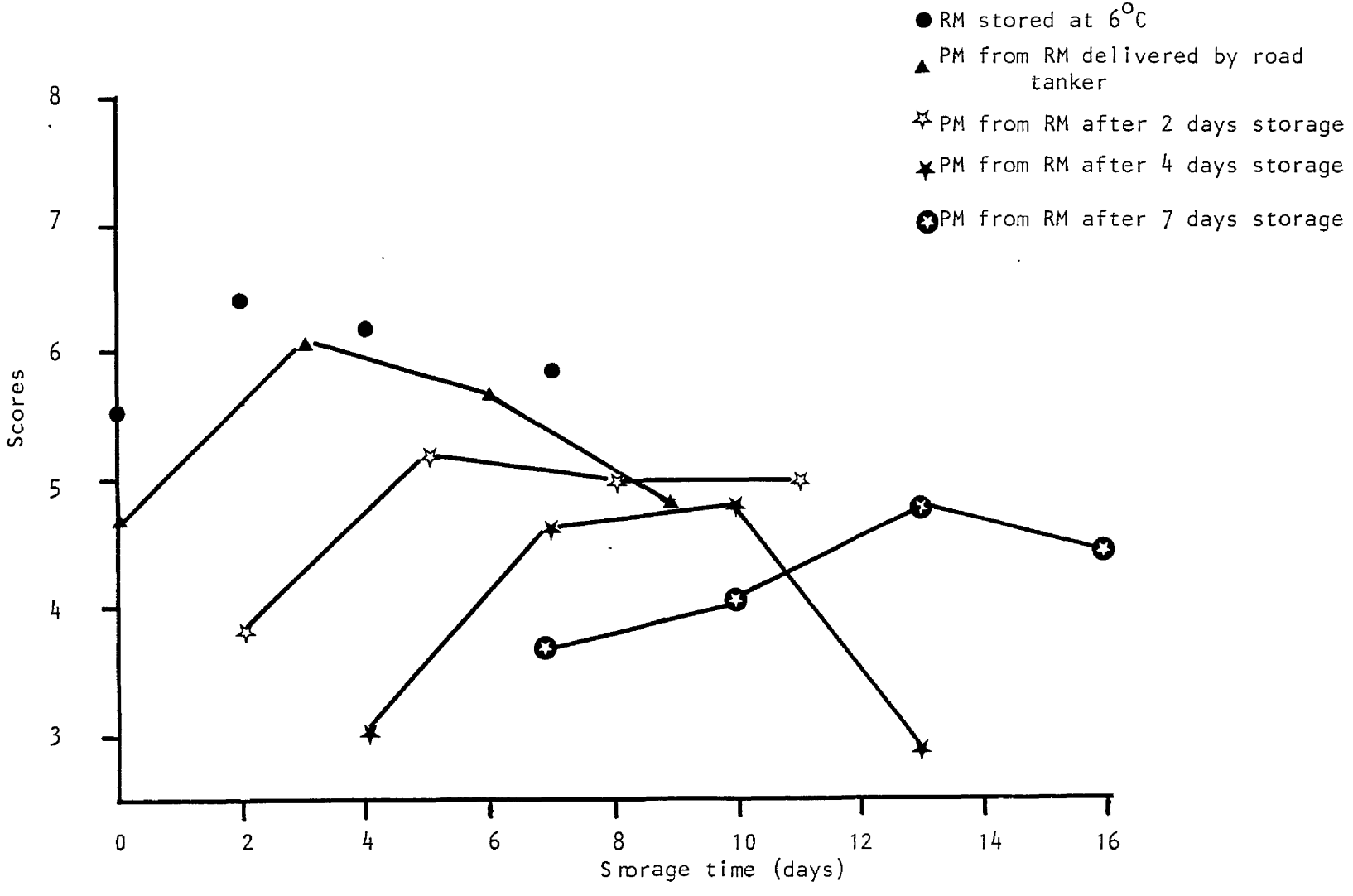


Fig. 4.3 Means of appearance scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)



storage of the pasteurized milks (Fig. 4.2).

In Fig. 4.3 which shows the mean scores for the appearance of pasteurized milk prepared from raw milk stored at $6^{o}C$, there is an increase between the third and sixth day of storage and a decrease on the ninth day of storage.

The means of the scores given by the taste panel for the appearance of pasteurized milks were all lower than the scores for the corresponding raw milks.

2. Odour

(i) Raw milk

The means of the scores given by the taste panel for the odour of raw milk on the day of delivery by the road tanker and after 2, 4 and 7 days storage at two different temperatures $2^{o}C$ and $6^{o}C$, are given in Table 4.3.

No significant difference was found between the scores awarded by individual members of the taste panel.

There was a significant difference (at 5 per cent level) between the odour scores of the raw milk comparing the milks on delivery with samples taken after various periods of storage.

The storage of raw milk for 2, 4 and 7 days at $6^{o}C$ resulted in a highly significant difference (at 1 per cent level) in the odour scores of the milks.

The temperature of storage of the raw milk was related to significant difference (at 5 per cent level) in the odour scores of the milk.

The variation in the scores given by the panel for the odour of the raw milks at $2^{o}C$ and $6^{o}C$ is shown in Fig. 4.4. The odour scores of the raw milks stored at $6^{o}C$ for 2, 4 and 7 days were lower than the initial (day of delivery) scores. There was a slight decrease in the scores given for the raw milk stored at

$2^{o}C$ compared with the initial values.

(ii) Pasteurized milk

The mean of the scores given by the taste panel for the odour of freshly processed and refrigerated (3, 6 and 9 days at $4^{o}C$) pasteurized milk produced from the raw milk on the day of delivery by the road tanker and after 2, 4 and 7 days of storage at two different temperatures ($2^{o}C$ and $6^{o}C$) are presented in Table 4.4.

Very highly significant differences (at 0.1 per cent level) were found in the odour scores awarded by the different members of the panel. No significant difference was found in the odour scores due to the storage of pasteurized milk for 3, 6 and 9 days at $4^{o}C$. A significant difference (at 5 per cent level) was found in the mean scores of the pasteurized milk produced from raw milk on delivery by the road tanker and after storage for 2, 4 and 7 days.

The pasteurized milk produced from raw milk after 2, 4 and 7 days storage showed very highly significant differences (at 0.1 per cent level) in the odour scores. Very highly significant differences (at 0.1 per cent level) in the odour scores were observed between the pasteurized milks produced from raw milk stored at $2^{o}C$ and that stored at $6^{o}C$.

A highly significant effect (at 1 per cent level) in the odour scores of the pasteurized milks was found due to the interaction between the storage time and temperature.

The scores for the odour of pasteurized milk produced from raw milk stored at $6^{o}C$ were lower after storage for 2 and 4 days compared to the initial values.

The variation in the scores given by the panel for the odour of the pasteurized milks are shown in Figs 4.5 and 4.6.

The odour scores given by the panel for the pasteurized milk produced from raw milk stored at $2^{o}C$ are less than the scores

TABLE 4.3

Means of the scores[+] given by a panel (5 judges) for the odour of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	6.00	6.00	6.00	2.00	6.00	4.00	2.00
2	4.50	5.00	5.00	5.00	4.00	2.00	1.00
3	6.50	6.00	7.00	4.00	6.00	2.00	2.00
4	7.00	5.00	7.00	5.00	5.00	7.00	1.00
5	5.00	5.00	5.00	5.00	6.00	6.00	3.00
Mean	5.80	5.40	6.00	4.20	5.40	4.20	1.80

[+]Scale of 1 = dislike extremely to 8 = like extremely

	DF	MS	VR
Judges	4	2.8500	2.165
F v rest	1	12.6750	9.627*
Judges. F v rest	4	1.1333	0.861
F v rest. Storage	2	17.1000	12.987**
F v rest. Temperature	1	14.7000	11.165*
Judges. F v rest. Storage	8	1.8833	1.278
Judges. F v rest. Temperature	4	1.8667	1.418
F v rest. Storage. Temperature	2	3.9000	2.962
Residual	8	1.3167	2.194
Total	34	3.4346	5.724

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	0.574	0.513	0.468	0.628

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.4

Means of scores given by a panel (5 judges) for the odour of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C			Mean
		2 days	4 days	7 days	2 days	4 days	7 days	
Initial	5.80	5.00	5.40	4.60	4.80	3.60	3.60	4.82
3 days	4.80	4.60	5.60	5.20	5.60	3.20	2.60	4.55
6 days	5.00	5.20	5.00	5.20	5.40	3.60	4.00	4.80
9 days	4.70	5.40	4.80	5.20	5.80	1.80	3.40	4.48
Mean	5.07	5.05	5.20	5,05	5.40	3.05	3.40	

	DF	MS	VR
Judges	4	11.0531	8.314***
PM Storage	3	1.2417	0.934
F v rest	1	9.075	6.826*
Judges. PM Storage	12	2.6323	1.980
Judges. F v rest	4	0.3302	0.248
PM Storage. F v rest	3	1.8806	1.414
F v rest. Storage	2	14.800	11.132***
F v rest. Temperature	1	39.6749	29.842***
Judges. PM Storage. F v rest	12	0.8788	0.661
Judges. F v rest. Storage	8	0.7271	0.547
PM Storage. F v rest. Storage	6	2.122	1.596
Judges. F v rest. Temperature	4	1.4667	1.103
PM Storage. F v rest. Temperature	3	0.6972	0.524
F v rest. Storage. Temperature	2	17.499	13.163**
Residual	74	1.3295	3.128
Total	139	2.4120	5.675

Table	Temperature	Storage Temperature	PM Storage. Temperature	PM Storage. Storage	PM Storage
REP	Unequal	Unequal	Unequal	10	40
SED	0.2354	0.3158	0.4707	0.5157	0.2578

PD - pasteurized milk produced from raw milk on delivery by road tanker

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.4 Means of odour scores awarded by a panel (5 judges) for raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

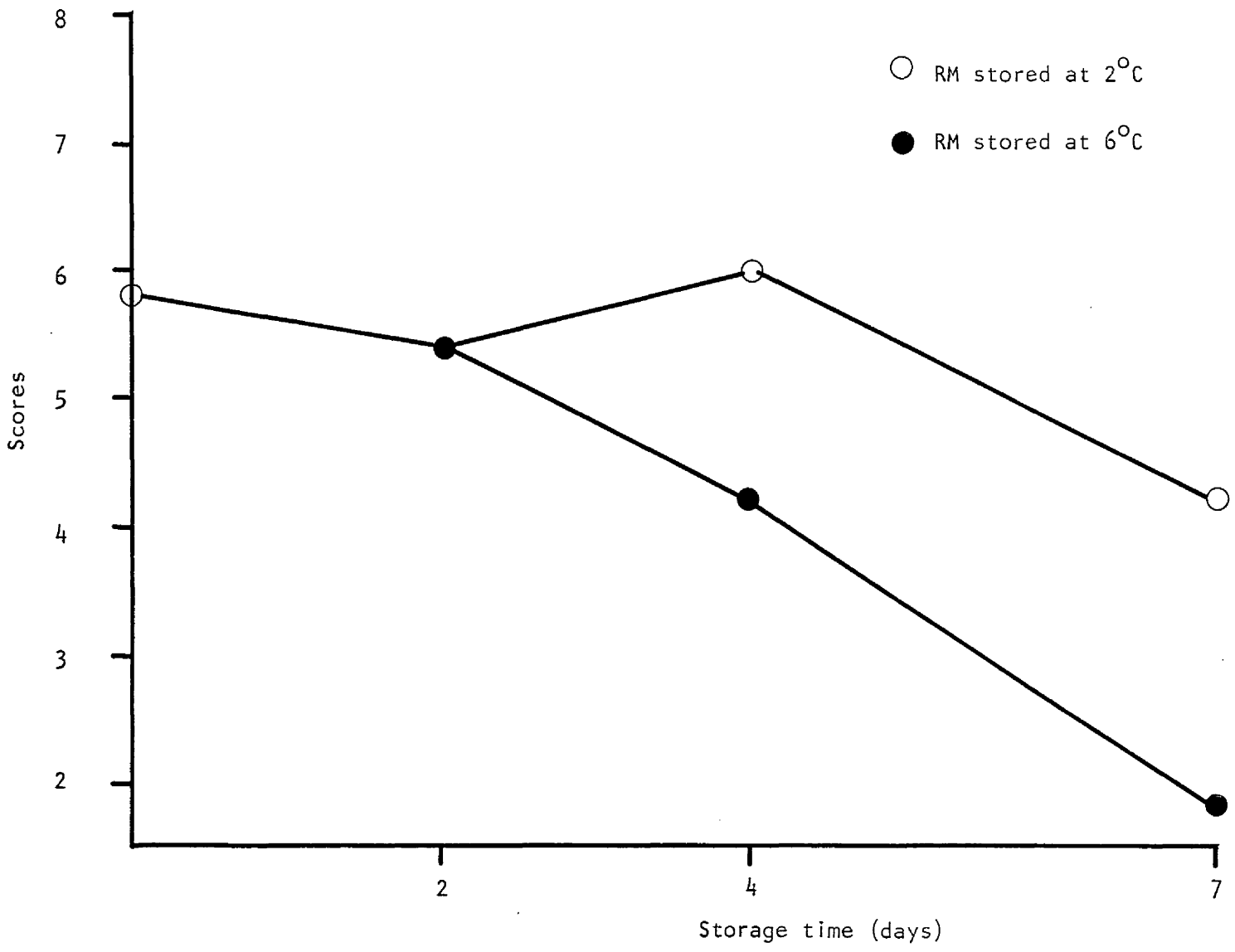


Fig. 4.5 Means of odour scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigeratore temperature (4°C)

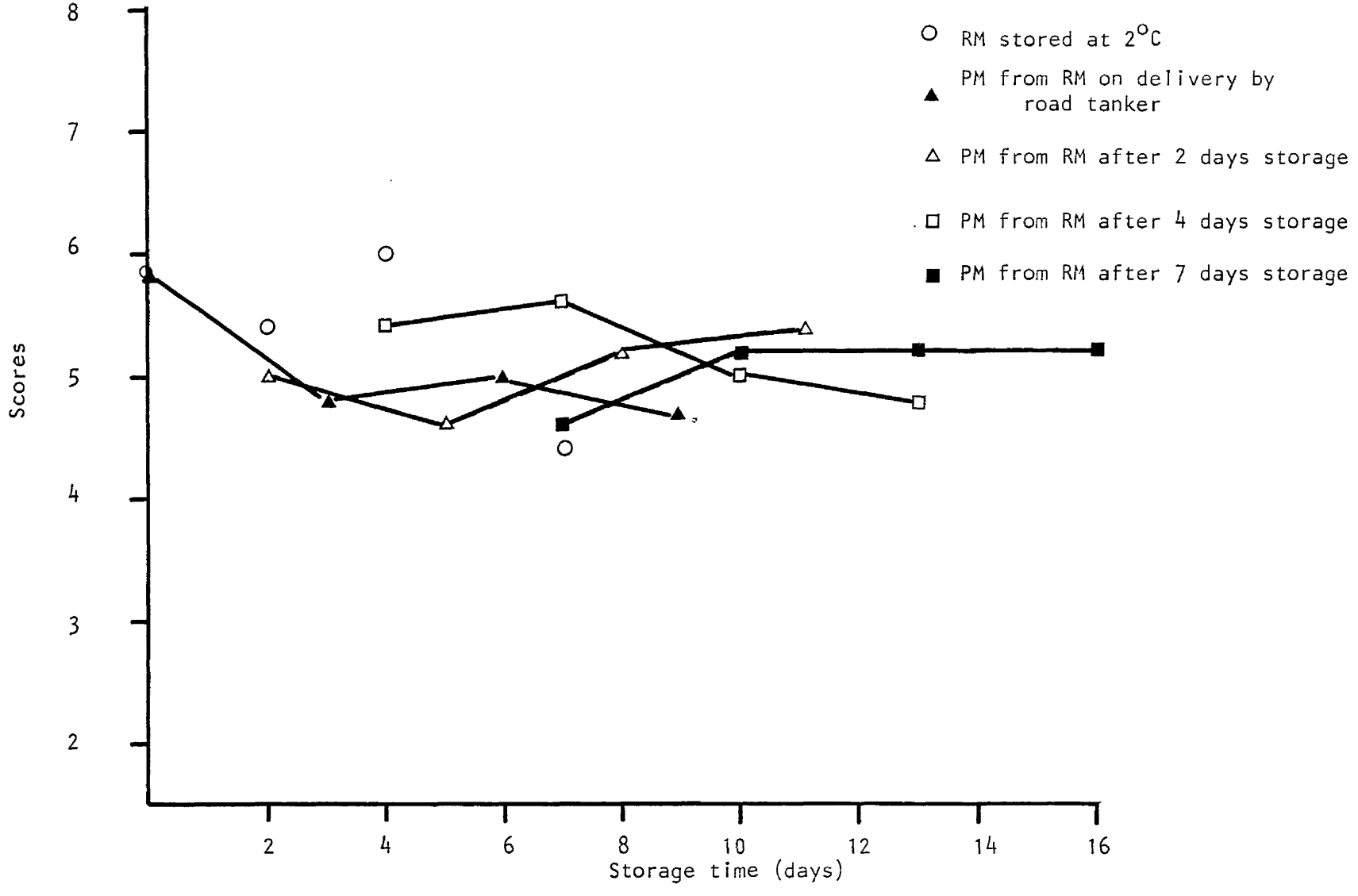
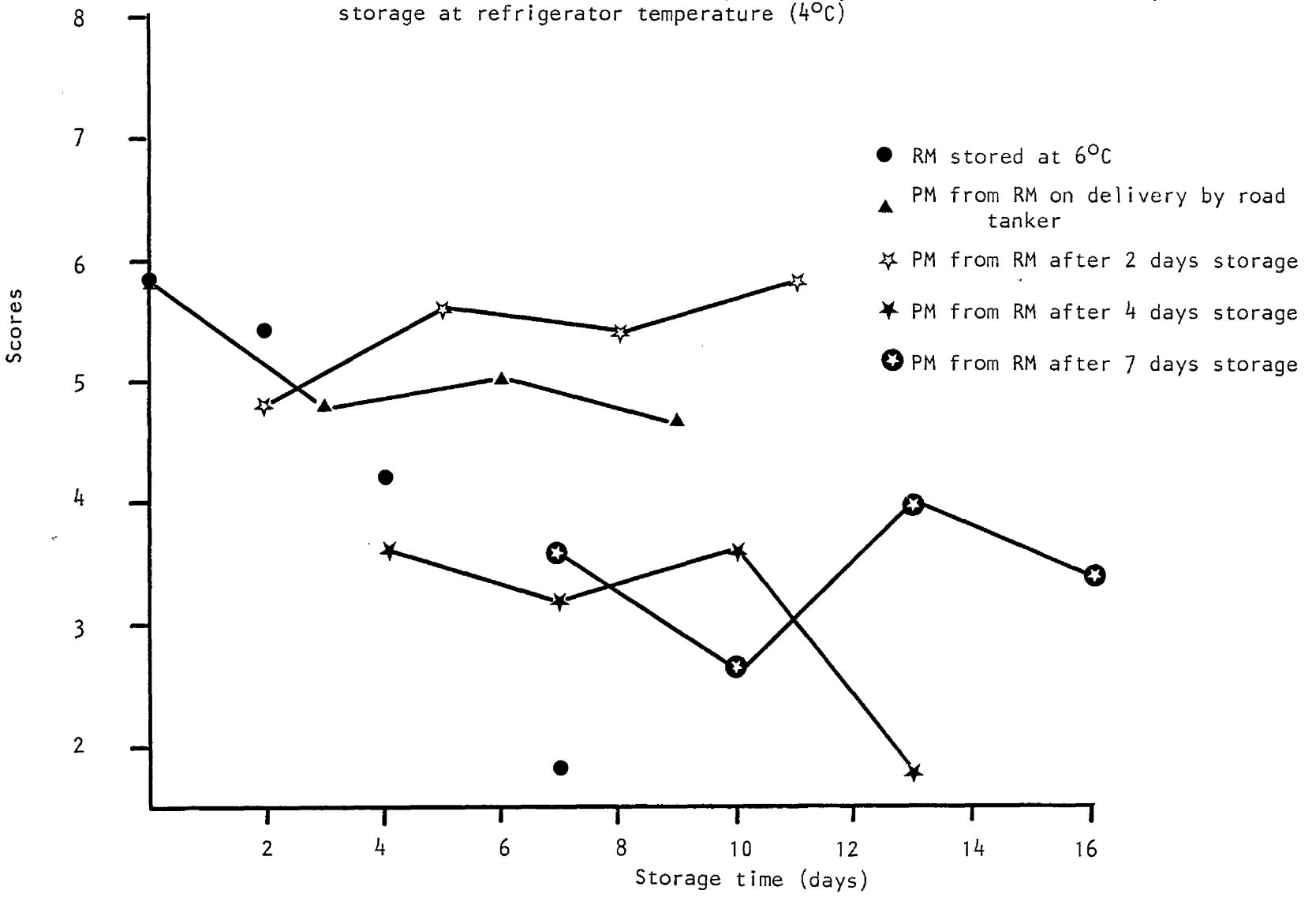


Fig. 4.6 Means of odour scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)



given for the raw milk except where the pasteurized milk was produced from raw milk stored for 7 days prior to processing.

There was very little difference in the odour scores for the pasteurized milk produced from raw milk stored at $2^{o}C$ for up to 7 days. Storage of the pasteurized milks after processing for 3, 6 and 9 days did not materially affect the scores. On the other hand there was a big decrease in the scores given for the pasteurized milk produced from raw milk stored at $6^{o}C$ for 4 and 7 days prior to pasteurization. Storage of the pasteurized milk after processing for 3, 6 and 9 days at $4^{o}C$ brought about further falls in grade scores for odour where the pasteurized milk was produced from milk which had been stored for 4 and 7 days.

3. Flavour (taste)

(i) Raw milk

The scores given by the panel for the flavour (taste) of raw milk on the day of delivery by road tanker and after it had been stored for 2, 4 and 7 days at two different temperatures, $2^{o}C$ and $6^{o}C$, are given in Table 4.5.

A significant difference (at 5 per cent level) was found between the judges in their scoring of the flavour of the raw milks.

After 2, 4 and 7 days storage of the raw milk a significant difference (at 5 per cent level) was found in the flavour scores. The temperature of storage of the raw milk had a highly significant effect (at 1 per cent level) on the flavour score.

There was a decrease in the flavour scores of the raw milk which had been stored for 7 days, the scores having dropped from the fourth day of storage Fig. 4.7.

While the decrease in flavour scores for the raw milk stored at $2^{o}C$ was slight, there was a substantial decrease in the scores for the flavour of the raw milk stored at $6^{o}C$ over the 7 day period.

(ii) Pasteurized milk

The scores given by the panel for the flavour (taste) of

pasteurized milk produced from raw milk on the day of delivery by the road tanker and after the storage of the raw milk for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$, and scores for the flavour of the pasteurized milks on storage at $4^{o}C$ for 3, 6 and 9 days after production, are presented in Table 4.6.

A very highly significant difference (at 0.1 per cent level) in the flavour scores was found between the judges.

The storage of the pasteurized milks for 3, 6 and 9 days at $4^{o}C$ had a highly significant effect (at 1 per cent level) on the flavour scores of the different samples.

The pasteurized milks produced from raw milk on the day of delivery and after 2, 4 and 7 days showed a highly significant difference (at 1 per cent level) in the flavour scores between samples.

Very highly significant differences (at 0.1 per cent level) were found in the flavour scores for the pasteurized milks produced from raw milk stored at $2^{o}C$ and $6^{o}C$.

A very highly significant effect (at 0.1 per cent level) on the flavour scores was found due to the interaction between the storage time and temperature.

Fig. 4.8 shows the variation in the scores for the pasteurized milk produced from raw milk stored at $2^{o}C$ for 2, 4 and 7 days and further storage of the pasteurized milk for 3, 6 and 9 days at $4^{o}C$.

There was a decrease in the scores given for the flavour of pasteurized milk produced from raw milk stored at $6^{o}C$ after the fourth and seventh day.

The effect of storage of the pasteurized milks for 3, 6 and 9 days at $4^{o}C$ is shown in Fig. 4.9. The flavour scores given for the pasteurized milk produced from milk stored at $2^{o}C$ are higher than the flavour scores for the pasteurized milk produced from raw milk stored at $6^{o}C$.

TABLE 4.5

Means of the scores[+] given by a panel (5 judges) for the flavour (taste) of samples of raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	5.00	6.00	5.00	1.00	6.00	3.00	1.00
2	3.50	5.00	4.00	5.00	4.00	1.00	1.00
3	6.50	6.00	7.00	4.00	7.00	2.00	2.00
4	2.50	3.00	2.00	6.00	4.00	1.00	2.00
5	4.50	4.00	5.00	6.00	5.00	3.00	2.00
Mean	4.40	4.80	4.60	4.40	5.20	2.00	1.60

[+]Scale of 1 = dislike extremely to 8 = like extremely

	DF	MS	VR
Judges	4	7.063	4.216*
F v rest	1	3.675	2.194
Judges. F v rest	4	1.071	0.639
F v rest. Storage	2	13.300	7.940*
F v rest. Temperature	1	24.300	14.507**
Judges. F v rest. Storage	8	2.508	1.498
Judges. F v rest. Temperature	4	0.800	0.478
F v rest. Storage. Temperature	2	9.300	5.552*
Residual	8	1.675	1.396
Total	34	4.187	3.490

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	0.647	0.579	0.528	0.709

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.6

Means of the scores given by a panel (5 judges) for the flavour (taste) of pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

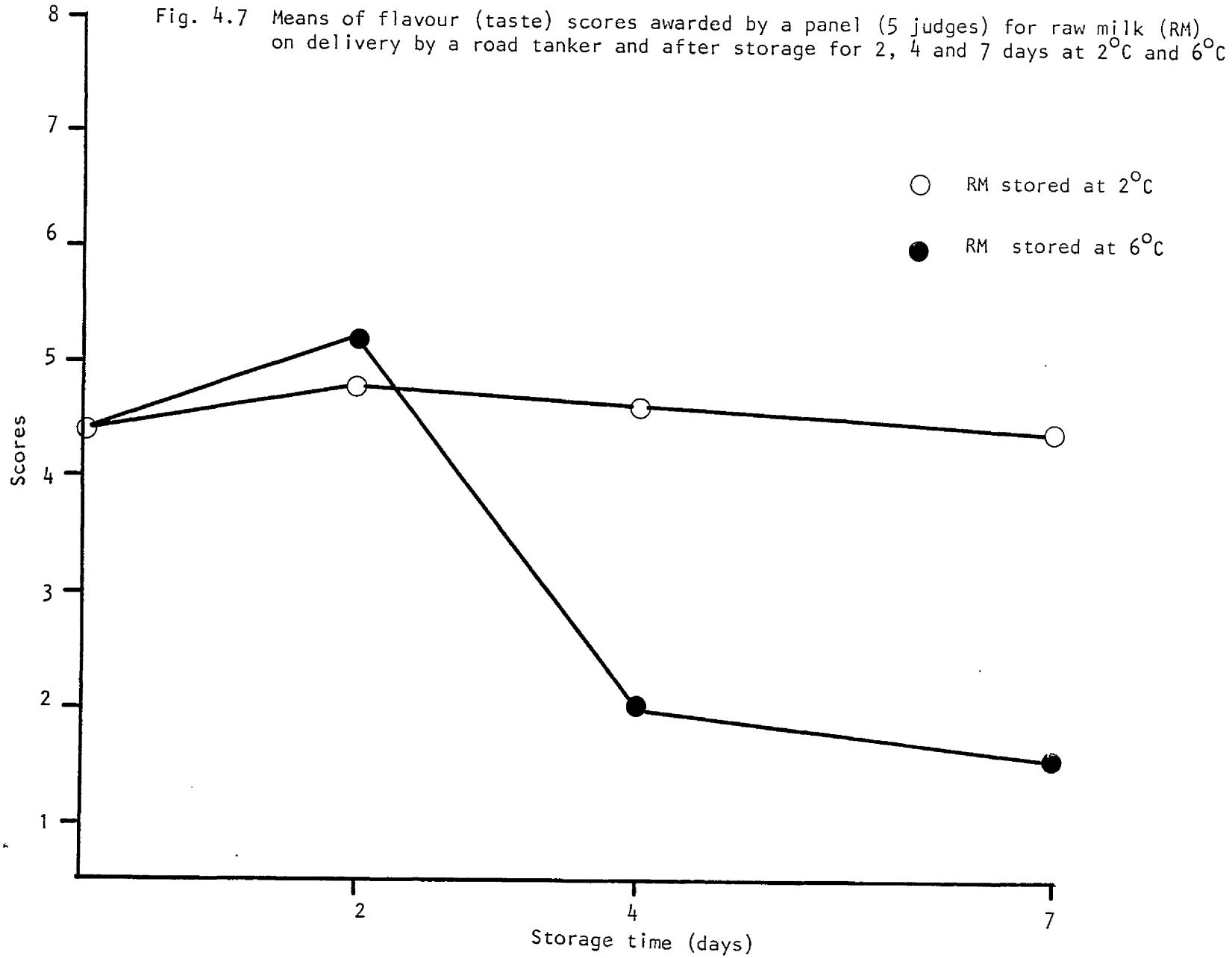
PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C			Mean
		2 days	4 days	7 days	2 days	4 days	7 days	
Initial	5.30	4.40	4.80	4.20	4.00	2.80	3.00	4.18
3 days	4.20	3.00	6.00	5.00	2.80	2.80	1.60	3.70
6 days	3.50	2.80	4.80	3.60	4.00	2.60	2.00	3.35
9 days	4.00	5.00	2.80	3.40	5.20	1.20	1.60	3.40
Mean	4.25	3.70	4.60	4.05	4.00	2.35	2.05	

	DF	MS	VR
Judges	4	38.9444	33.880***
PM Storage	3	5.7396	4.988**
F v rest. PM Storage	1	18.8021	16.340***
Judges. PM Storage	12	2.526	2.195*
Judges. F v rest	4	1.099	0.955
PM Storage. F v rest	3	2.168	1.885
F v rest. Storage	2	6.408	5.569**
F v rest. Temperature	1	52.008	45.198***
Judges. PM Storage. F v rest	12	1.396	1.213
Judges. F v rest. Storage	8	2.627	2.283*
PM Storage. F v rest. Storage	6	9.9416	8.640***
Judges. F v rest. Temperature	4	1.9458	1.691
PM Storage. F v rest. Temperature	3	3.0750	2.672**
F v rest. Storage. Temperature	2	19.7582	17.171***
Residual	74	1.1507	1.534
Total	139	3.864	5.152

Table	Temperature	Storage. Temperature	PM Storage. Temperature	PM Storage. Storage	PM Storage
REP	10	Unequal	Unequal	10	40
SED	0.4797	0.2938	0.4379	0.4797	0.2399

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.7 Means of flavour (taste) scores awarded by a panel (5 judges) for raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$



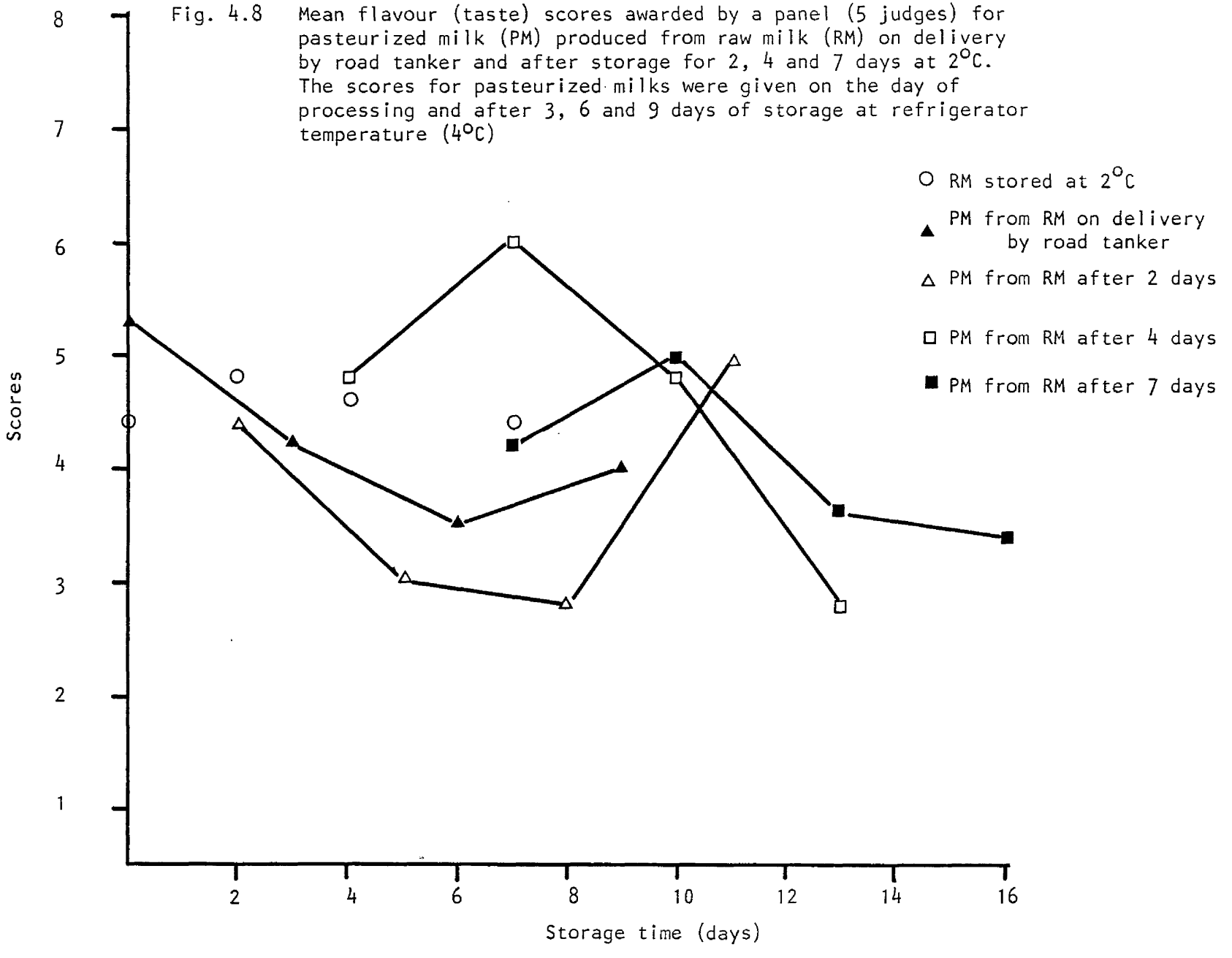


Fig. 4.8 Mean flavour (taste) scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)
RM stored at 2°C
PM from RM on delivery by road tanker
PM from RM after 2 days
PM from RM after 4 days
PM from RM after 7 days
Scores
8
7
6
5
4
3
2
1
2
4
6
8
10
12
14
16
Storage time (days)

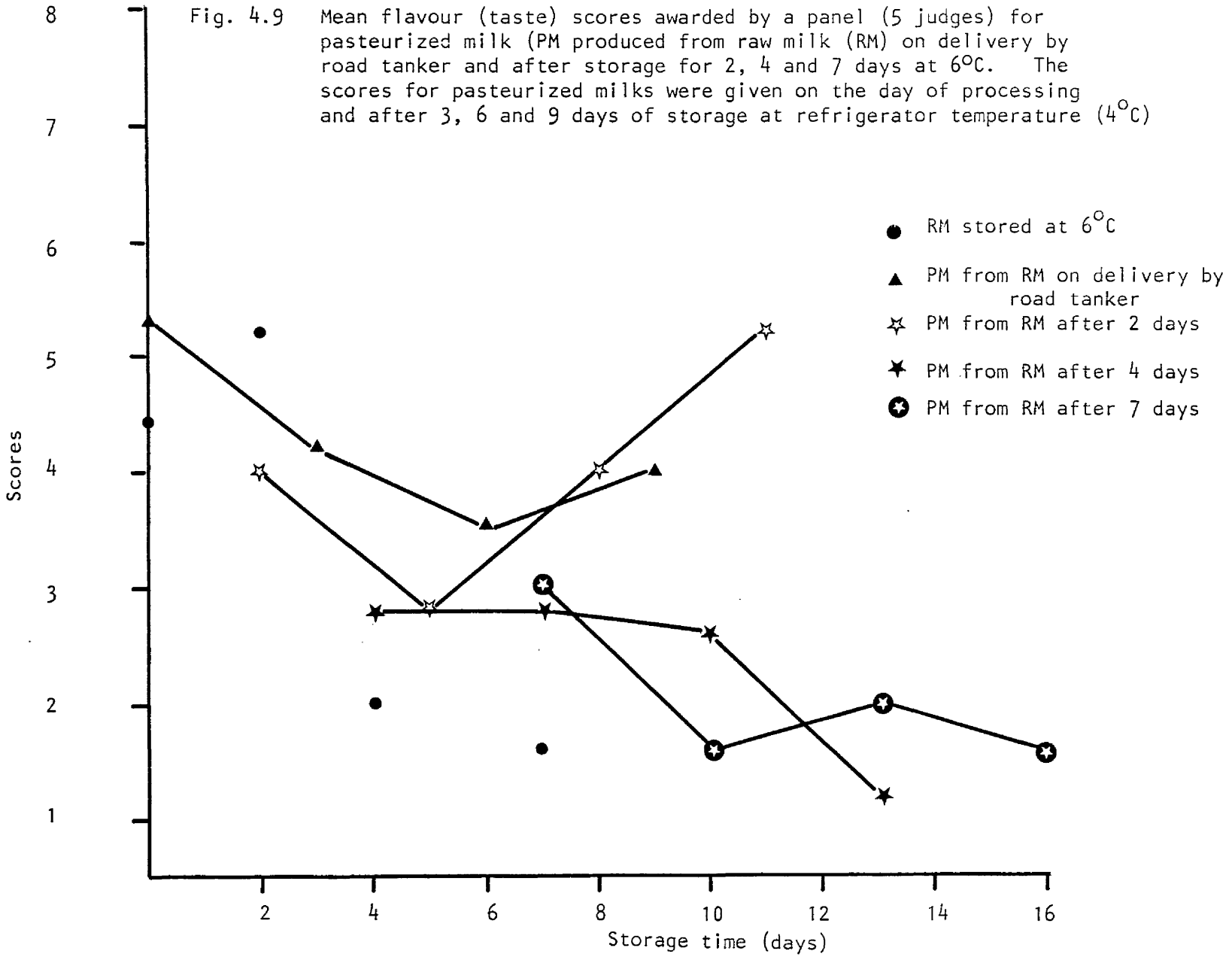


Fig. 4.9 Mean flavour (taste) scores awarded by a panel (5 judges) for pasteurized milk (PM produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

4. Total scores

(i) Raw milk

Total scores are the sum of the scores for the three characteristics:- appearance, odour, and flavour, of the milk.

The total scores given for raw milk on the day of delivery by the road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ are given in Table 4.7.

No significant difference was found between panel members in the scores awarded by them for milks on delivery or after varying periods of storage.

A significant difference (at 5 per cent level) was found in the total scores due to storage of raw milk samples for 2, 4 and 7 days. The temperature of storage of the milk, $2^{o}C$ or $6^{o}C$, also had a significant effect (at 5 per cent level) on the total scores.

The reduction in the total scores for the raw milk stored at $6^{o}C$ for 2, 4 and 7 days can be seen in the same Table.

Fig. 4.10 shows the variation in the total scores for the raw milk stored at $2^{o}C$ and $6^{o}C$. There was a slight decrease after seven days in the total scores for the raw milk stored at $2^{o}C$, while there was a big decrease in the total scores for the raw milk stored at $6^{o}C$.

(ii) Pasteurized milk

The total scores given for pasteurized milk produced from raw milk on the day of delivery by road tanker and after 2, 4 and 7 days storage at $2^{o}C$ and $6^{o}C$, and the total scores for the pasteurized milks on production and after storage at $4^{o}C$ for 3, 6 and 9 days are given in Table 4.8.

There was a very highly significant difference (at 0.1 per cent level) between judges in the total scores given to the various samples.

TABLE 4.7

The mean total scores given by individual judges of a panel for appearance, odour and flavour (taste) of raw milk (RM) on delivery by road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	16.50	19.00	18.00	9.00	19.00	13.00	10.00
2	13.50	15.00	14.00	16.00	14.00	6.00	8.00
3	19.00	19.00	22.00	14.00	20.00	12.00	10.00
4	14.00	13.00	16.00	17.00	15.00	15.00	6.00
5	15.00	15.00	17.00	17.00	17.00	16.00	10.00
Mean	15.70	16.20	17.40	14.60	17.00	12.40	8.80

	DF	MS	VR
Judges	4	21.462	2.887
F v rest	1	12.675	1.705
Judges, F v rest	4	2.071	0.279
F v rest. Storage	2	61.900	8.327*
F v rest. Temperature	1	83.333	11.211*
Judges. F v rest. Storage	8	9.233	1.242
Judges. F v rest. Temperature	4	4.583	0.617
F v rest. Storage. Temperature	2	32.433	4.363
Residual	8	7.433	0.853
Total	34	15.602	1.793

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	1.363	1.219	1.113	1.493

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.8

The mean total of the scores for appearance, odour and flavour (taste) given by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on the day of delivery by road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days. The pasteurized milks were graded on the day of production and after storage at $4^{o}C$ for 3, 6 and 9 days

PM Storage	PD	PM from RM stored at $2^{o}C$			PM from RM stored at $6^{o}C$			Mean
		2 days	4 days	7 days	2 days	4 days	7 days	
Initial	15.70	12.60	14.00	13.20	12.60	9.40	10.20	12.92
3 days	15.10	11.40	17.00	16.00	13.60	10.60	8.20	13.40
6 days	14.20	14.20	15.80	14.20	14.40	11.00	10.80	13.60
9 days	13.40	15.60	12.00	13.20	16.00	5.80	9.40	12.35
Mean	14.60	13.45	14.75	14.15	14.15	9.20	9.65	

	DF	MS	VR
Judges	4	134.928	25.275***
PM Storage	3	12.390	2.321
F v rest	1	125.052	23.425***
Judges. PM Storage	12	8.478	1.588
Judges. F v rest	4	3.297	0.618
PM Storage. F v rest	3	11.885	2.226
F v rest. Storage	2	46.308	8.674***
F v rest. Temperature	1	291.408	54.586***
Judges. PM Storage. F v rest	12	7.075	1.325
Judges. F v rest. Storage	8	7.756	1.453
PM Storage. F v rest. Storage	6	32.042	6.002
Judges. F v rest. Temperature	4	9.637	1.805
PM Storage. F v rest. Temperature	3	3.631	0.680
F v rest. Storage. Temperature	2	112.008	20.981***
Residual	74	5.339	2.053
Total	139	16.146	6.210

Table	Temperature	Storage. Temperature	PM Storage. Temperature	PM Storage. Storage	PM Storage
REP	Unequal	Unequal	Unequal	10	40
SED	0.472	0.633	0.943	1.033	0.517

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.10 Means of total scores awarded by a panel (5 judges) for raw milk (RM) on delivery by a road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

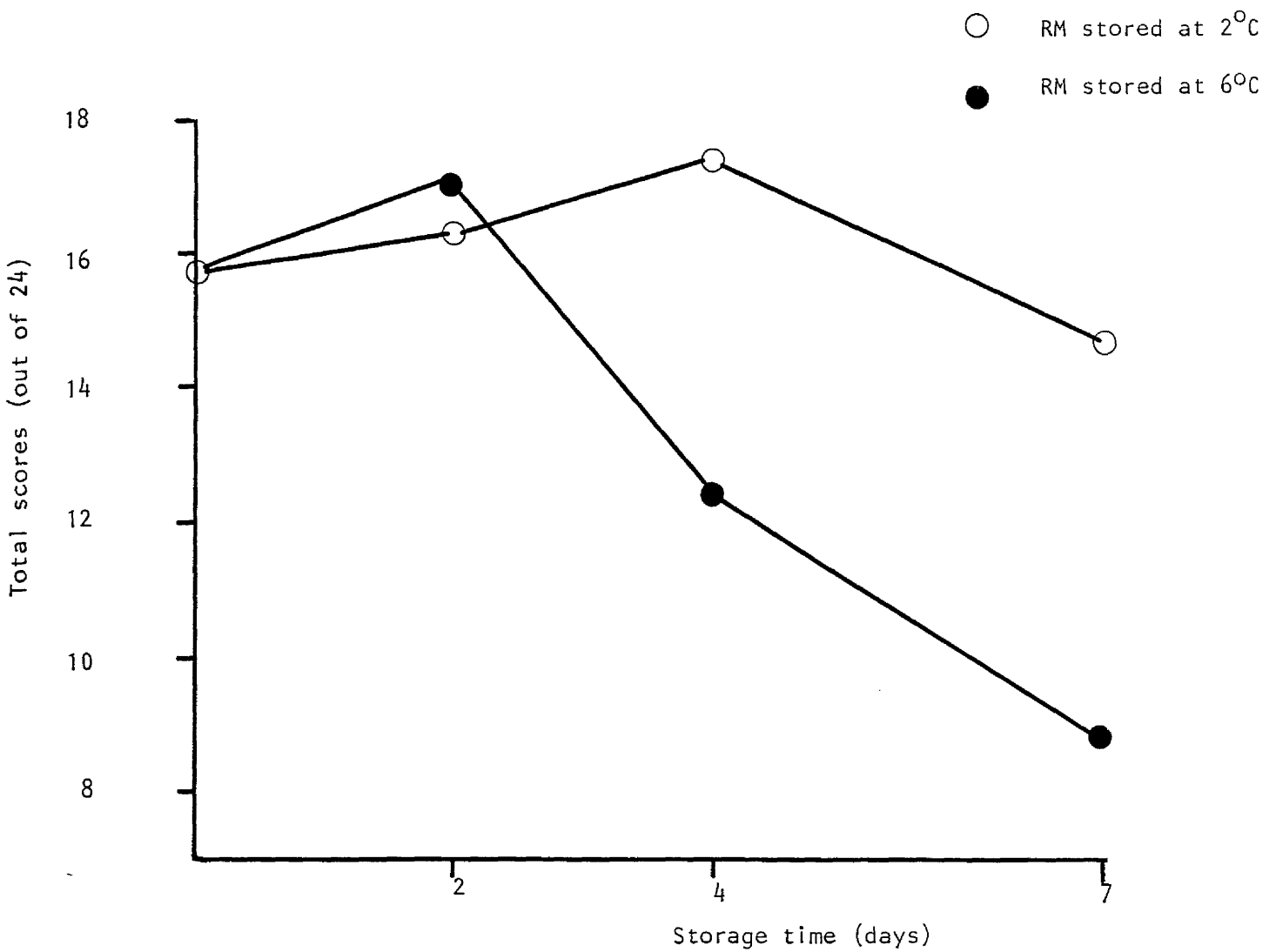


Fig. 4.11 Means of total scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores for pasteurized milks were given on the day of processing and after 3,6 and 9 days of storage at refrigeratore temperature (4°C)

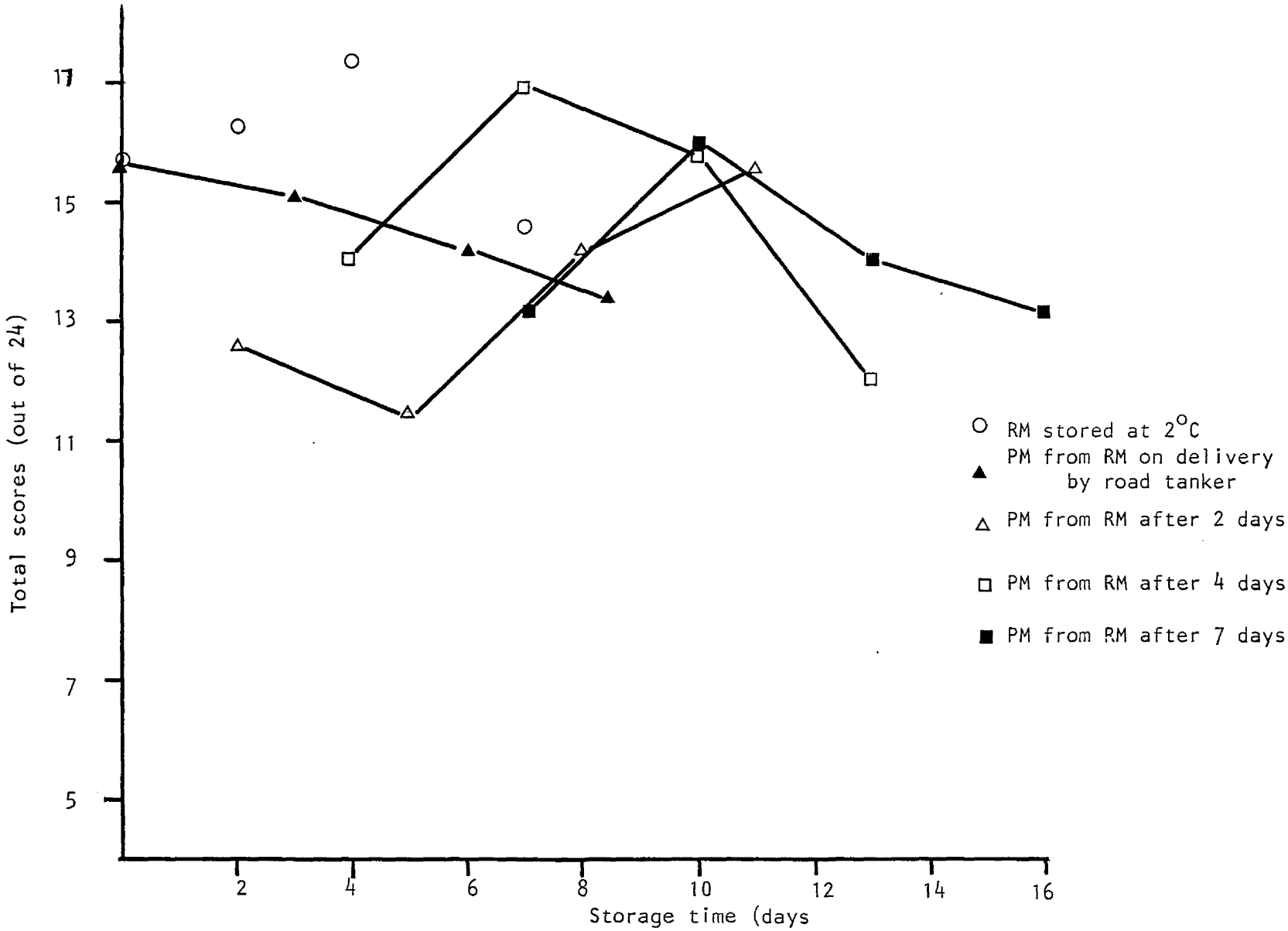
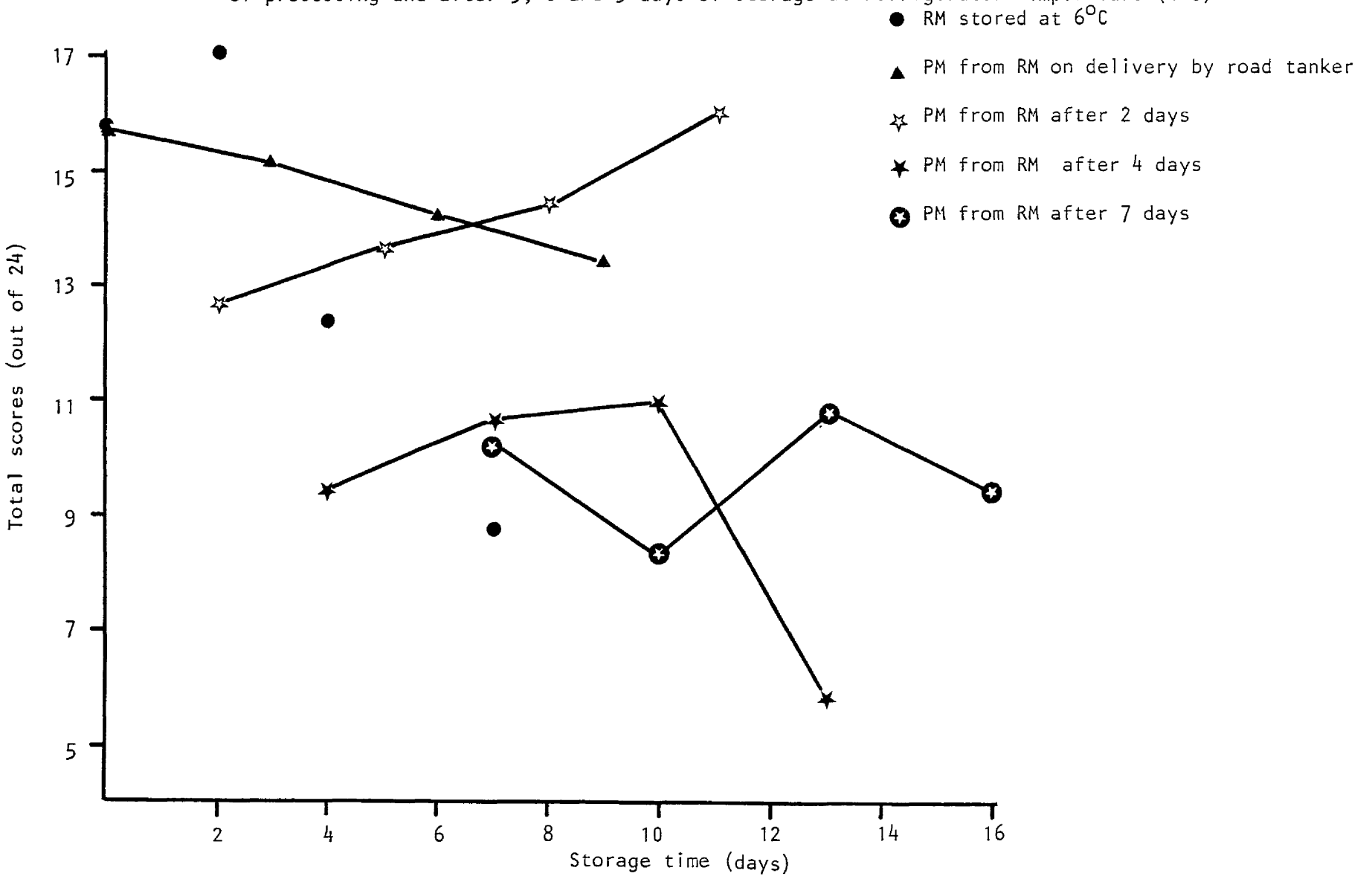


Fig. 4.12 Means of total scores awarded by a panel (5 judges) for pasteurized milk (PM) produced from raw milk (RM) on delivery by road tanker and after storage for 2, 4 and 7 days at 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)



The total scores for pasteurized milks produced from milks on the day of delivery by road tanker showed highly significant differences (at the 0.1 per cent level) compared to the scores awarded to pasteurized milks produced from milk stored at $2^{o}C$ and $6^{o}C$ which in both cases decreased with duration of storage.

There was no significant alteration in the total scores on storage of pasteurized milks for 3, 6 and 9 days at $4^{o}C$. From Fig. 4.11 it can be seen that there was a slight decrease in the total scores for the samples of pasteurized milk produced from raw milk stored at $2^{o}C$.

Further storage at $4^{o}C$ for 3, 6 and 9 days led to variable results. In Fig. 4.12 it may be seen there were decreases in the total scores of the pasteurized milks which were prepared from raw milks stored at $6^{o}C$, the scores began to fall between the second and the fourth day of storage.

The tea test - effect of milk addition on the odour and flavour (taste) of hot tea

Odour

The results of the odour scores for tea to which raw milk was added are presented in Table 4.9.

A highly significant difference (at 0.1 per cent level) was found in the odour scores between the taste panel members. Very highly significant differences (at 0.1 per cent level) were found in the odour scores using raw milks stored for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$.

A significant effect (at 5 per cent level) was found in the odour scores due to the effect of the temperature of storage $2^{o}C$ or $6^{o}C$.

The odour scores for tea with milk decreased on the addition of milk stored for 4 and 7 days at both $2^{o}C$ and $6^{o}C$ and the decrease was greater where the raw milk samples had been stored at $6^{o}C$. The variations in the odour scores are shown in Fig. 4.13. The results of the odour scores for tea to which pasteurized milks produced from the various treatments of raw milks are given in Table 4.10.

TABLE 4.9

The tea test means of the scores[+] given by a panel (5 judges) for the odour of tea containing raw milk (RM) samples on the delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$.

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	6.00	7.00	5.00	3.00	8.00	2.00	3.00
2	4.00	5.00	4.00	1.00	4.00	1.00	3.00
3	2.00	5.00	2.00	2.00	4.00	1.00	1.00
4	3.00	2.00	1.00	2.00	1.00	1.00	1.00
5	3.00	5.00	3.00	2.00	4.00	1.00	1.00
Mean	3.50	4.80	3.00	2.00	4.20	1.20	1.80

[+]Scale of 1 = dislike extremely to 8 = like extremely

	DF	MS	VR
Judges	4	12.0625	12.267**
F v rest	1	3.3333	3.390
Judges. F v rest	4	1.1042	1.123
F v rest. Storage	2	20.9333	21.288***
F v rest. Temperature	1	5.6333	5.729*
Judges. F v rest. Storage	8	1.5167	1.542
Judges. F v rest. Temperature	4	0.1333	0.136
F v rest. Storage. Temperature	2	1.7333	1.763
Residual	8	0.9833	1.093
Total	34	3.7500	4.167

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	0.496	0.443	0.405	0.543

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.10

The tea test - means of the scores given by a panel (5 judges) for the odour of tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigeratore temperature (4°C)

PM Storage	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	5.50	4.80	4.00	4.20	4.80	3.60	3.80
3 days	4.40	2.60	5.00	4.80	3.60	4.80	3.40
6 days	3.70	4.80	5.40	4.40	2.40	4.80	3.40
9 days	4.60	5.00	5.60	3.60	4.60	3.80	1.80
Mean	4.55	4.30	5.00	4.25	3.85	4.25	3.10

	DF	MS	VR
Judges	4	27.8687	18.253***
PM Storage	3	1.6396	1.074
F v rest	1	5.4187	3.549
Judges. PM Storage	12	2.2229	1.456
Judges. F v rest	4	2.7521	1.803
PM Storage. F v rest	3	4.0910	2.679
F v rest. Storage	2	9.1000	4.960**
F v rest. Temperature	1	18.4083	12.057***
Judges. PM Storage. F v rest	12	2.0701	1.356
Judges. F v rest. Storage	8	1.1833	0.775
PM Storage. F v rest. Storage	6	7.3889	4.839***
Judges. F v rest. Temperature	4	1.7208	1.127
PM Storage. F v rest. Temperature	3	3.0305	1.985
F v rest. Storage Temperature	2	1.2333	0.808
Residual	74	1.5268	2.545
Total	139	3.0104	5.017

Table	Storage. Temperature	PM Storage. Storage	PM Storage. Temperature
REP	Unequal	10	Unequal
SED	0.3384	0.5526	0.5044

* significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.13 The tea test - means of odour scores awarded by a panel (5 judges) for tea containing raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

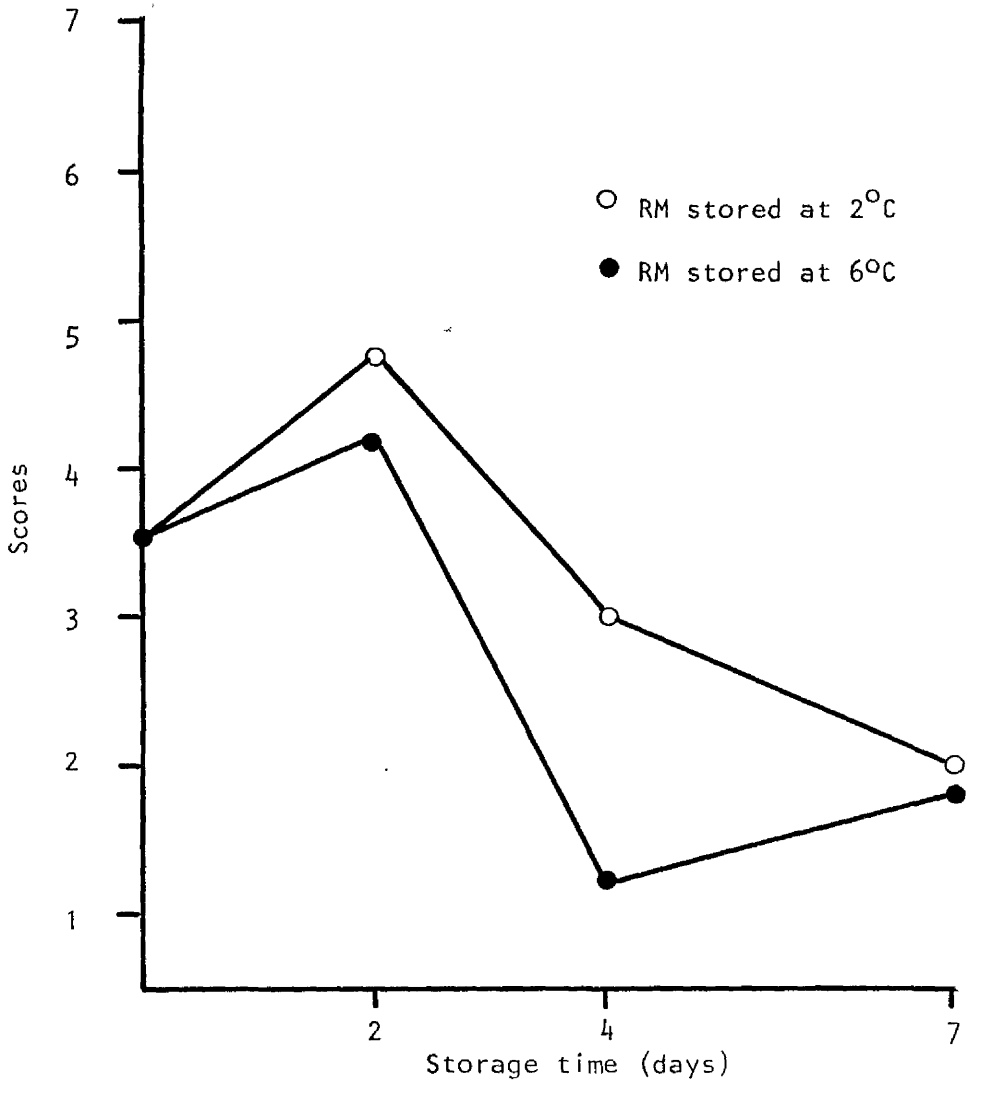


Fig. 4.14 The tea test - means of odour scores awarded by a panel (5 judges) for tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery and after storage at 2°C for 2, 4 and 7 days storage of the raw and processed milk stored for 3, 6 and 9 days at 4°C

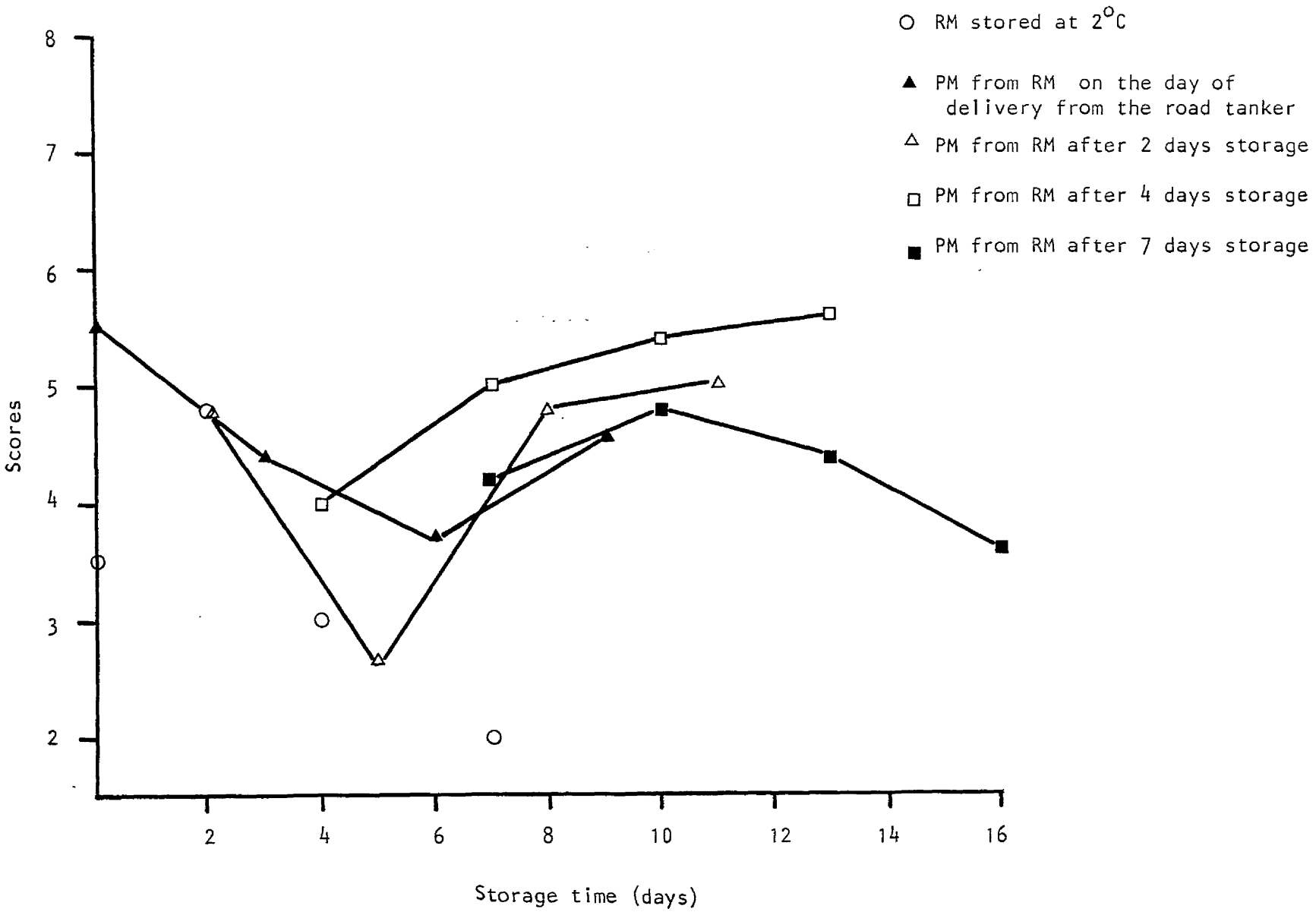
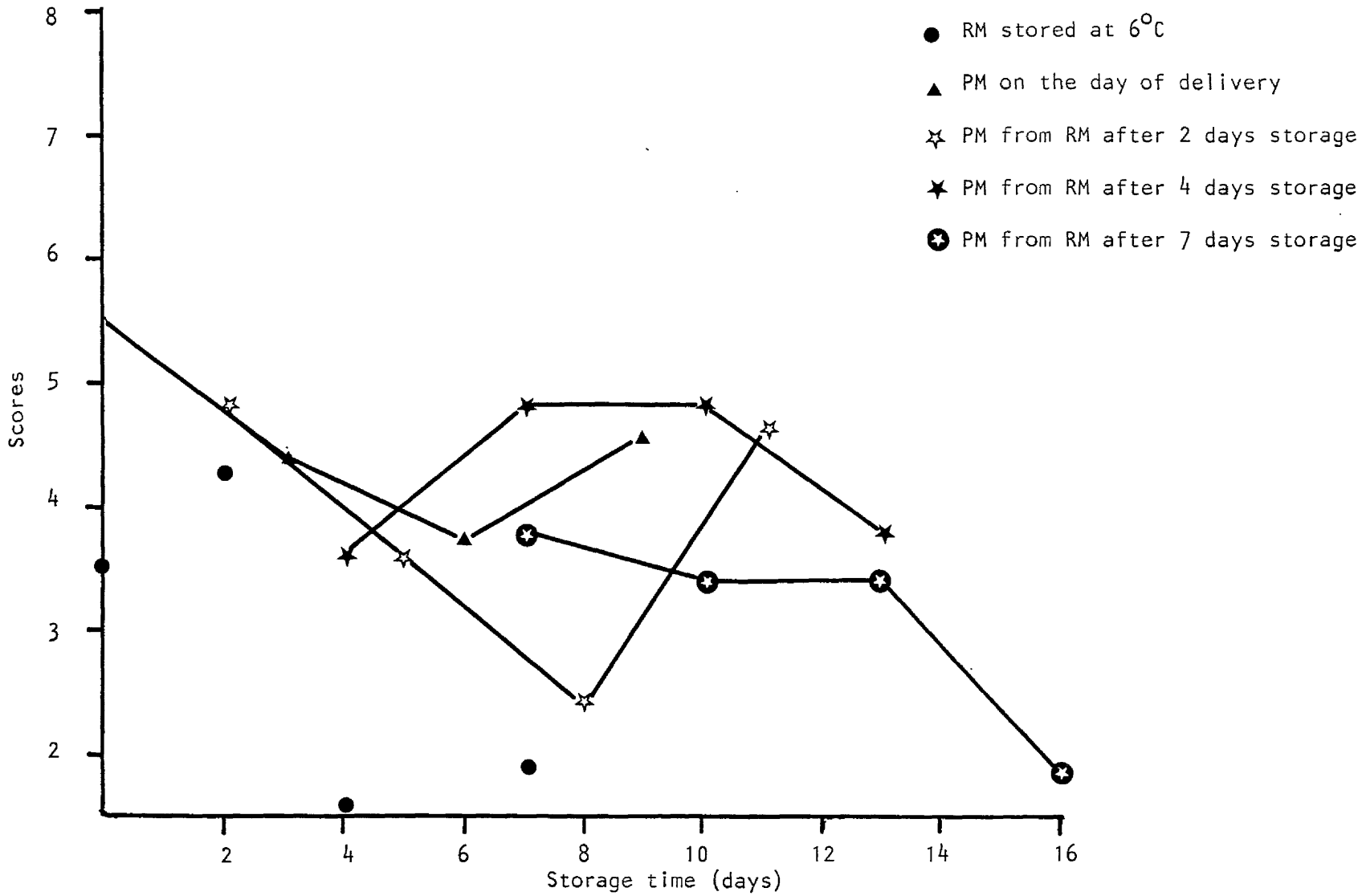


Fig 4.15 The tea test - means of odour scores awarded by a panel (5 judges) for tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery and after storage at 6°C for 2, 4 and 7 days storage of the raw and processed milk stored for 3, 6 and 9 days at 4°C.



A very highly significant effect (at 0.1 per cent level) was found between the judges in the odour scores using this technique.

The pasteurized milk produced from raw milk over a storage time of 2, 4 and 7 days had a highly significant effect (at 1 per cent level) on the odour scores when added to tea. The pasteurized milk produced from raw milk stored at $2^{o}C$ and $6^{o}C$ had a very highly significant effect (at 0.1 per cent level) on the odour scores of tea to which they were added.

The storage of the pasteurized milk for 3, 6 and 9 days at $4^{o}C$ after processing had no significant effect on the odour scores. A very highly significant difference (at 0.1 per cent level) was found in the odour scores due to the interaction between the storage of the pasteurized samples for 3, 6 and 9 days and the storage of the raw milk from which the pasteurized milks were produced.

The variation in the odour scores for pasteurized milk are presented in Figs. 4.14 and 4.15.

Flavour (taste)

The results for the flavour of tea to which raw milks stored for various periods of time at $2^{o}C$ and $6^{o}C$ are presented in Table 4.11. A highly significant difference (at 1 per cent level) was found in the flavour scores due to the difference between the taste panel members.

The storage of the raw milk for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ produced a significant effect (at 5 per cent level) on the flavour scores.

The temperature of the storage had no significant effect on the flavour scores. The variation in the flavour scores for raw milk stored at $2^{o}C$ and $6^{o}C$ are illustrated in Fig. 4.16. There was a decrease in the flavour scores given to raw milks on the fourth and seventh days of storage at both temperatures.

The results for the flavour scores of tea to which pasteurized milk

TABLE 4.11

The tea test - means of the scores[+] given by a panel (5 judges) for the flavour (taste) of tea containing raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	7.00	8.00	7.00	1.00	8.00	1.00	2.00
2	4.00	5.00	3.00	1.00	2.00	1.00	1.00
3	2.00	3.00	1.00	2.00	4.00	1.00	1.00
4	3.00	2.00	1.00	2.00	1.00	1.00	1.00
5	3.00	2.00	1.00	1.00	2.00	1.00	1.00
Mean	3.60	4.00	2.60	1.40	3.40	1.00	1.20

[+]Scale of 1 = dislike extremely to 8 = like extremely

	DF	MS	VR
Judges	4	16.712	8.682**
F v rest	1	13.333	6.926*
Judges. F v rest	4	1.854	0.963
F v rest. Storage	2	16.033	8.329*
F v rest. Temperature	1	4.800	2.494
Judges. F v rest. Storage	8	3.242	1.684
Judges. F v rest. Temperature	4	1.050	0.545
F v rest. Storage. Temperature	2	1.300	0.675
Residual	8	1.925	1.375
Total	34	5.076	3.626

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	0.694	0.620	0.566	0.760

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.12

The tea test - means of the scores given by a panel (5 judges) for the flavour (taste) of tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature 4°C

Test date	PD	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	5.40	3.80	4.00	4.00	4.40	3.00	2.60
3 days	4.00	2.40	4.60	4.60	3.80	4.80	2.40
6 days	3.70	4.20	6.00	4.00	1.20	4.00	2.40
9 days	4.70	4.80	4.80	4.20	4.20	3.40	1.40
Mean	4.45	3.80	4.85	4.20	3.40	3.80	2.20

	DF	MS	VR
Judges	4	38.4156	18.737***
PM Storage	3	1.5229	0.743
F v rest	1	16.5021	8.049**
Judges. PM Storage	12	0.9865	0.481
Judges. F v rest	4	4.0594	1.980
PM Storage. F v rest	3	4.4743	2.182
F v rest. Storage	2	13.0083	6.345**
F v rest. Temperature	1	39.6749	19.352***
Judges. PM Storage. F v rest	12	2.2330	1.089
Judges. F v rest. Storage	8	1.3312	0.649
PM Storage. F v rest. Storage	6	6.0639	2.958*
Judges. F v rest. Temperature	4	1.4875	0.726
PM Storage. F v rest. Temperature	3	6.2750	3.061*
F v rest. Storage. Temperature	2	6.4750	3.158*
Residual	74	2.0502	5.126
Total	139	3.9222	9.806

Table	Storage. Temperature	PM Storage. Storage	PM Storage. Temperature
REP	Unequal	10	Unequal
SED	0.3921	0.6403	0.5846

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.16 The tea test - means of flavour (taste) scores awarded by a panel (5 judges) for tea containing raw milk (RM) sampled on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

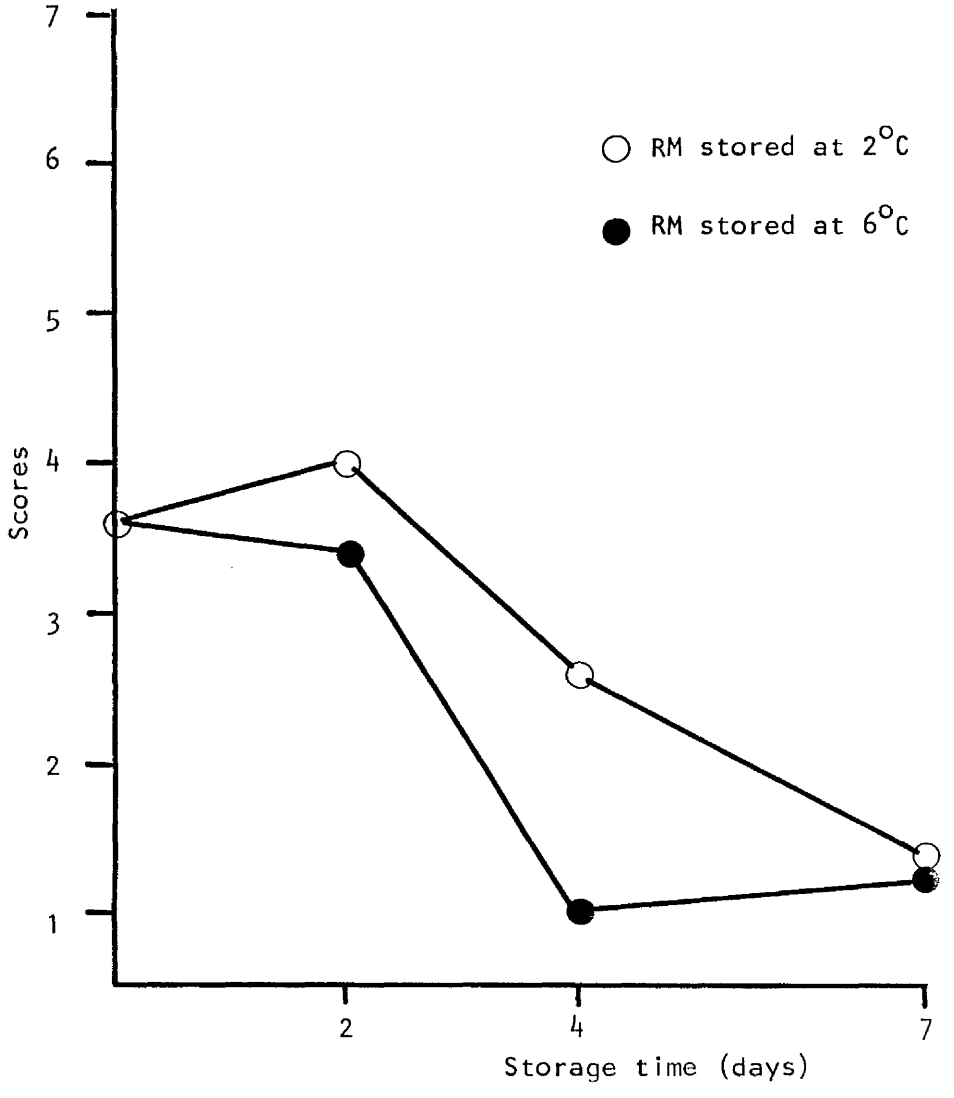


Fig. 4.17 The tea test - means of flavour (taste) scores awarded by a panel (5 judges) for tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and processed milk stored for 3, 6 and 9 days at refrigerator temperature 4°C

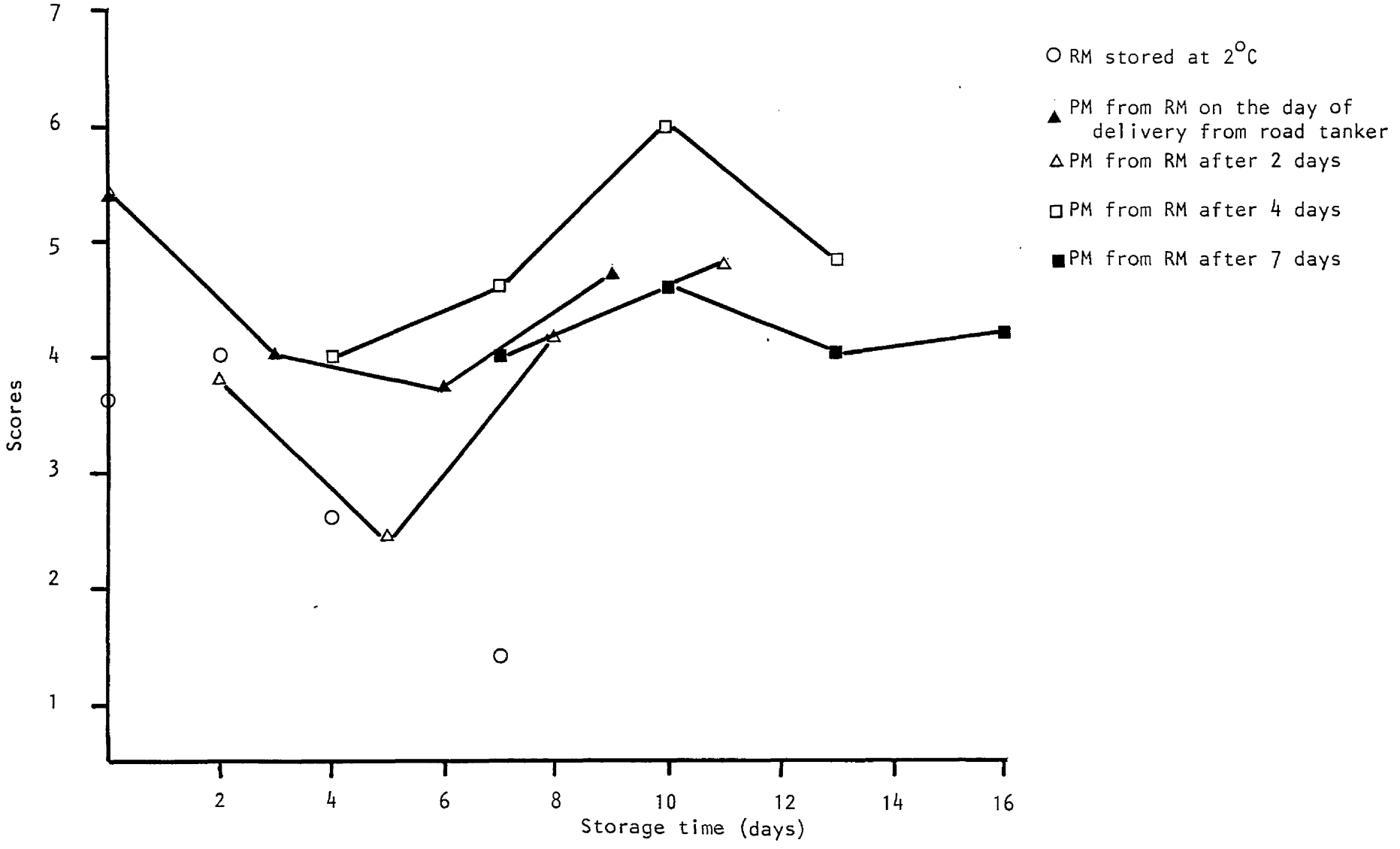
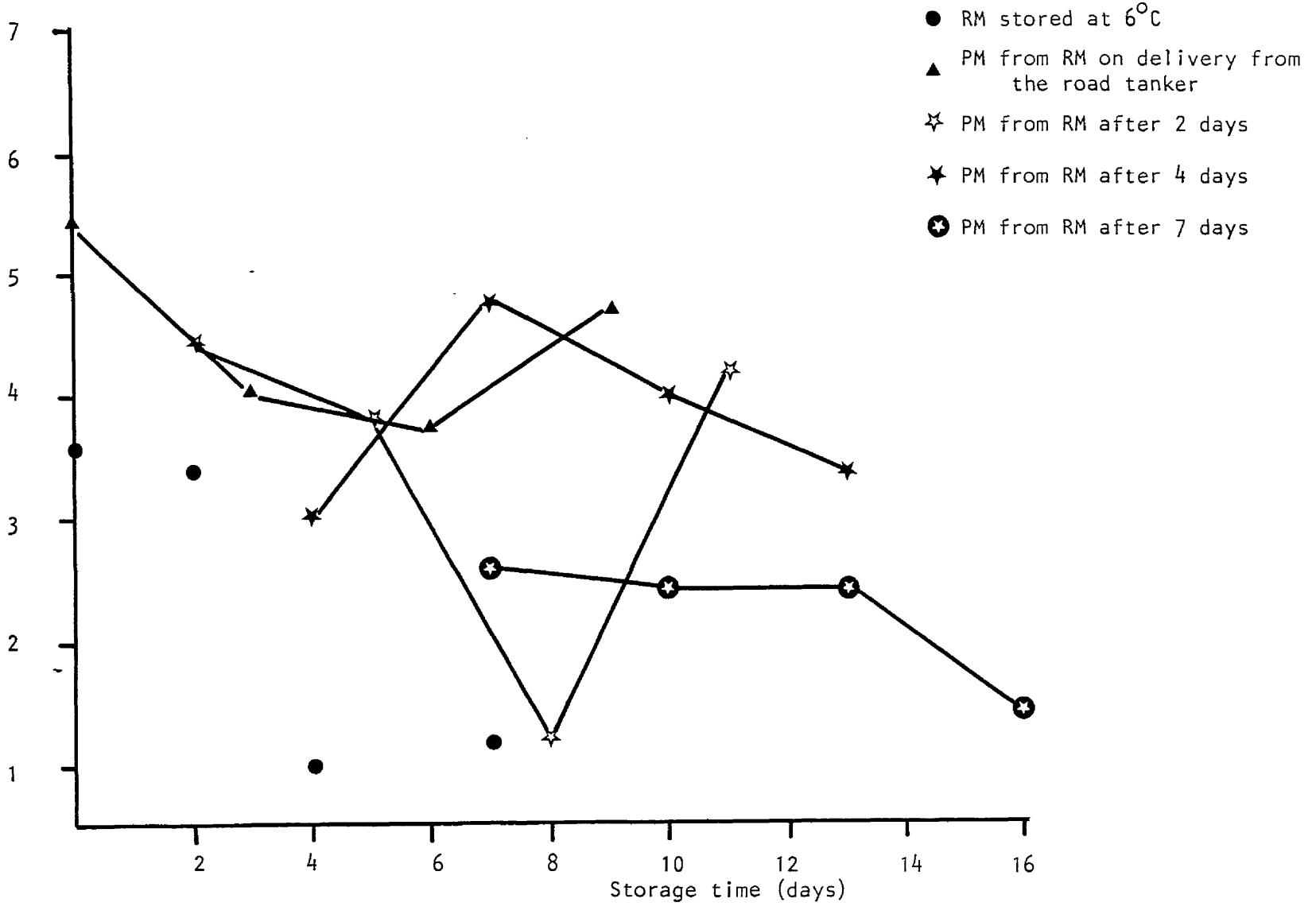


Fig. 4.18 The tea test - means of flavour (taste) scores awarded by a panel (5 judges) for tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 6°C and the processed milk stored for 3, 6 and 9 days at refrigerator temperature 4°C



was added are given in Table 4.12. These showed a very highly significant difference (at 0.1 per cent level) in the flavour scores awarded by different judges.

The pasteurized milk produced from raw milk stored for 2, 4 and 7 days showed a highly significant difference (at 1 per cent level) in the flavour scores. A very highly significant difference (at 0.1 per cent level) was found in the flavour scores of the pasteurized milk produced from raw milk stored at $2^{o}C$ and $6^{o}C$.

No significant effect was found in the flavour scores of pasteurized milk stored for 3, 6 and 9 days at $4^{o}C$ compared with the corresponding processing day value.

The variation in the flavour scores of pasteurized milk are given in Figs. 4.17 and 4.18.

There was a decrease in the flavour scores of pasteurized milk produced from raw milk stored at $6^{o}C$ for 7 days. The flavour scores of these pasteurized milks fell further on storage for up to 9 days at $4^{o}C$.

Total scores

The total scores for odour and flavour (taste) of tea to which raw milks were added are presented in Table 4.13.

A highly significant difference (at 1 per cent level) was found in the total scores awarded by different taste panel members.

The storage of the raw milk for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ resulted in a highly significant difference (at 1 per cent level) in the total scores awarded. The temperature of storage had no significant effect on the total socres.

The variations in the total scores for tea with added raw milk are presented in Fig. 4.19.

The total scores for the odour and flavour of tea to which pasteurized milk was added are presented in Table 4.14.

A very highly significant difference (at 0.1 per cent level) was

TABLE 4.13

The tea test - means of total scores of odour and flavour given by a panel (5 judges) for tea containing raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

Judges	Day of delivery	RM stored at $2^{o}C$			RM stored at $6^{o}C$		
		2 days	4 days	7 days	2 days	4 days	7 days
1	13.00	15.00	12.00	4.00	16.00	3.00	5.00
2	7.00	10.00	7.00	2.00	6.00	2.00	4.00
3	3.50	8.00	3.00	4.00	8.00	2.00	2.00
4	6.00	4.00	2.00	4.00	2.00	2.00	2.00
5	6.00	7.00	4.00	3.00	6.00	2.00	2.00
Mean	7.10	8.80	5.60	3.40	7.60	2.20	3.00

	DF	MS	VR
Judges	4	55.837	11.673**
F v rest	1	30.000	6.272*
Judges. F v rest	4	5.312	1.111
F v rest. Storage	2	73.300	15.324**
F v rest. Temperature	1	20.833	4.355
Judges. F v rest. Storage	8	8.300	1.735
Judges. F v rest. Temperature	4	0.583	0.122
F v rest. Storage. Temperature	2	6.033	1.261
Residual	8	4.783	1.167
Total	34	16.503	4.025

Table	Judges	Storage	Temperature	Storage. Temperature
REP	8	10	Unequal	Unequal
SED	1.094	0.978	0.893	1.198

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 4.14

The tea test - means of total scores for odour and flavour (taste) given by a panel (5 judges) of tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

PM Storage	Day of delivery	PM from RM stored at 2°C			PM from RM stored at 6°C		
		2 days	4 days	7 days	2 days	4 days	7 days
Initial	10.90	8.60	8.00	8.20	9.20	6.60	6.40
3 days	8.40	5.00	9.60	9.40	7.40	9.60	5.80
6 days	7.40	9.00	11.40	8.40	3.60	8.80	5.80
9 days	9.30	9.80	10.40	7.80	8.80	7.20	3.20
Mean	9.00	8.10	9.85	8.45	7.25	8.05	5.30

	DF	MS	VR
Judges	4	130.422	21.048***
PM Storage	3	5.683	0.917
F v rest	1	40.833	6.590*
Judges. PM Storage	12	5.105	0.824
Judges. F v rest	4	10.036	1.620
PM Storage. F v rest	3	16.406	2.648
F v rest. Storage	2	43.808	7.070**
F v rest. Temperature	1	112.133	18.097***
Judges. PM Storage. F v rest	12	7.220	1.165
Judges. F v rest. Storage	8	4.121	0.665
PM Storage. F v rest. Storage	6	25.564	4.126
Judges. F v rest. Temperature	4	5.612	0.906
PM Storage. F v rest. Temperature	3	17.622	2.844*
F v rest. Storage. Temperature	2	13.358	2.156
Residual	74	6.196	4.131
Total	139	12.687	8.458

Table	Storage. Temperature	PM Storage. Storage	PM Storage. Temperature
REP	Unequal	10	Unequal
SED	0.6817	1.1132	1.0162

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 4.19 The tea test means of total scores for odour and flavour (taste) awarded by a panel (5 judges) for tea containing raw milk (RM) sampled on delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

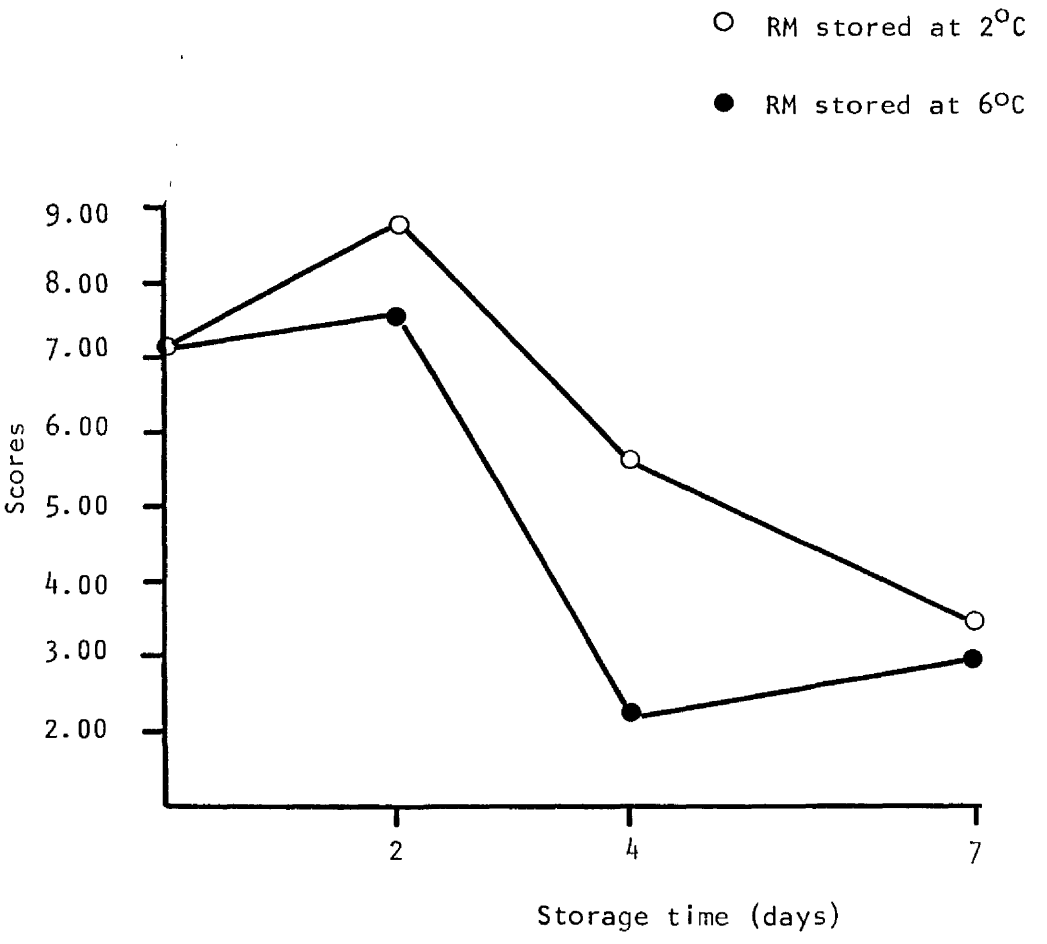


Fig. 4.20 The tea test means of total scores for odour and flavour of tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 2°C. The scores were given for pasteurized milk on the day of processing and after storage for 3, 6 and 9 days at refrigerator temperature 4°C.

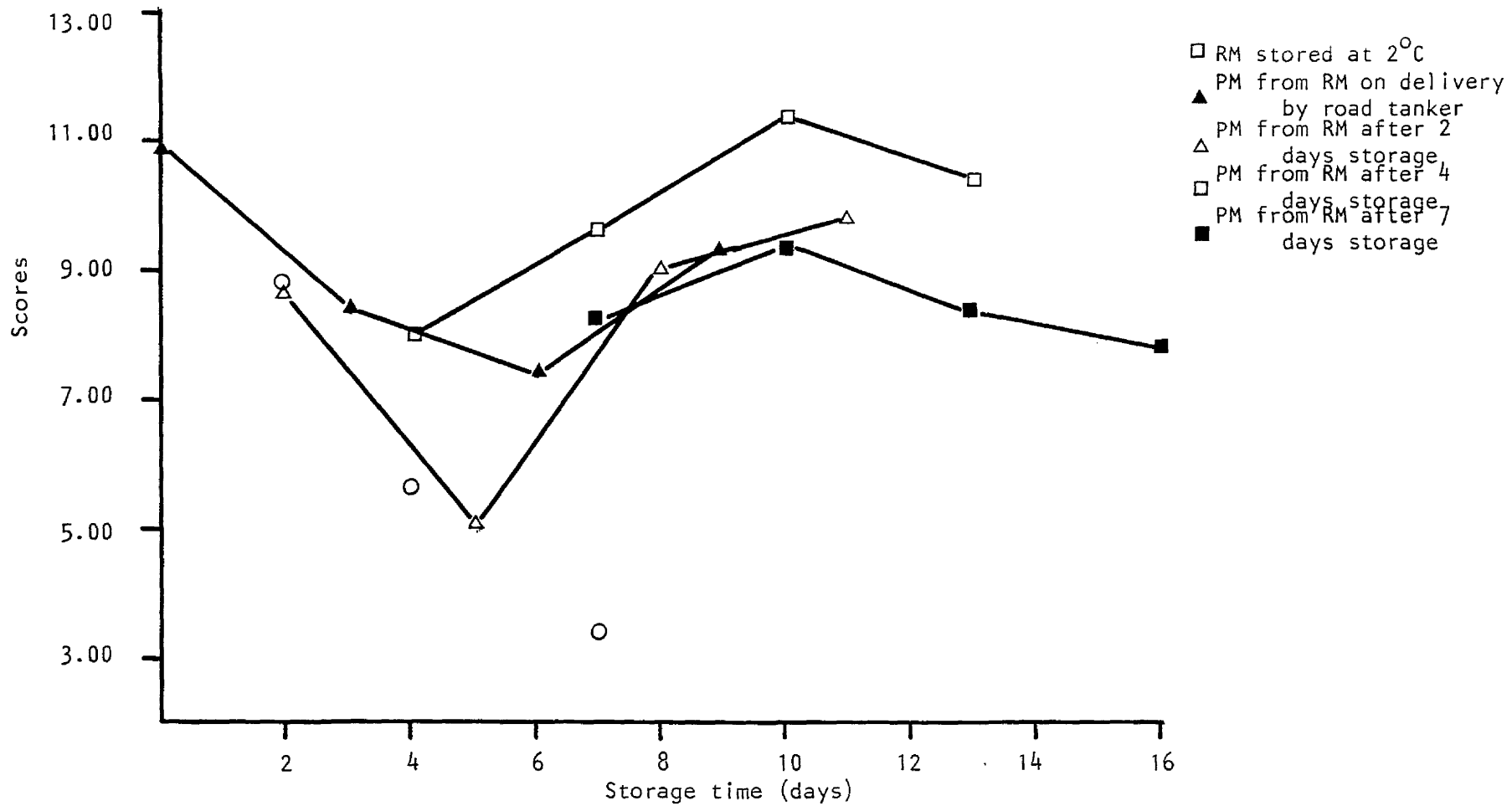
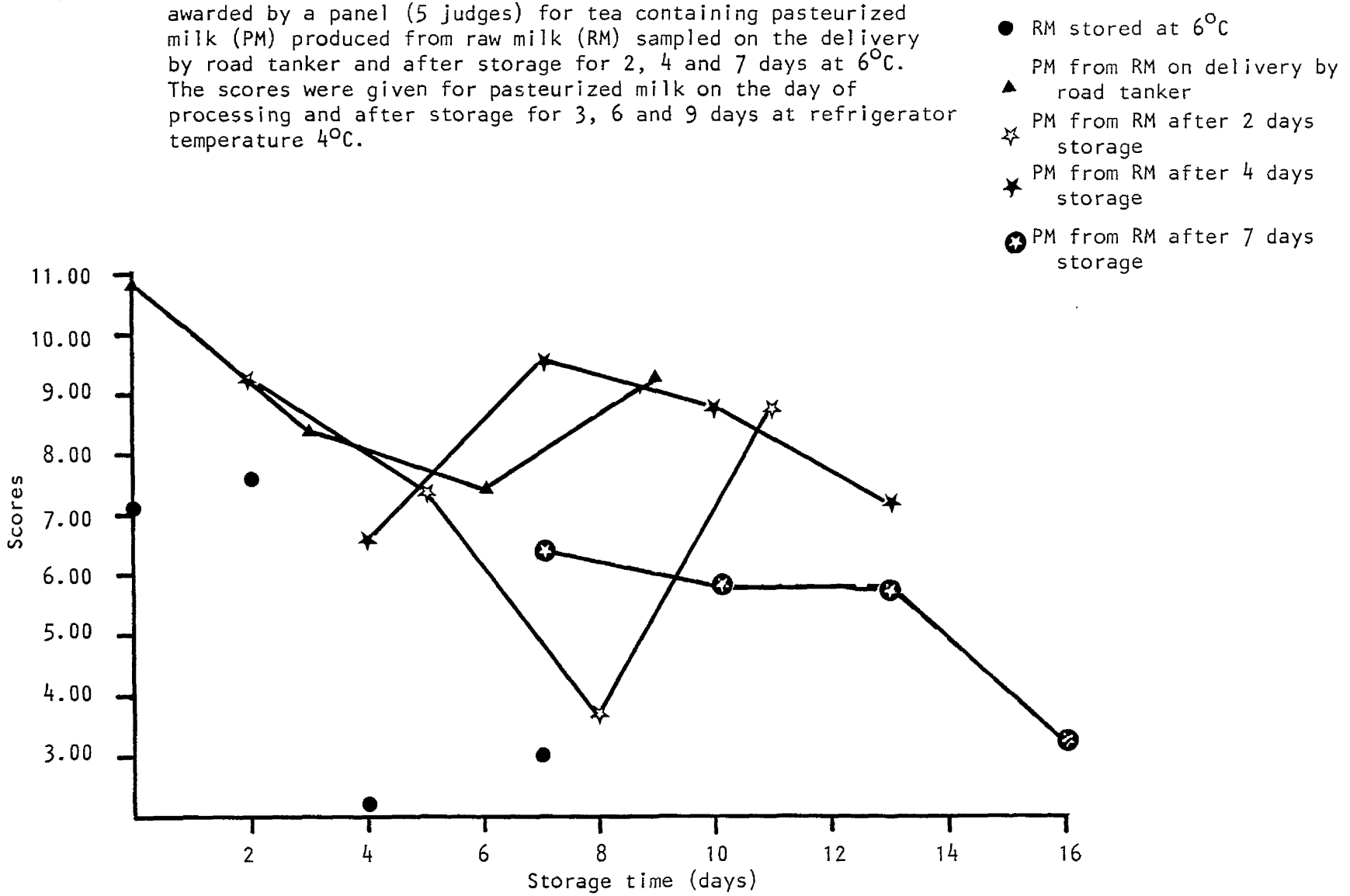


Fig 4.21 The tea test means total scores for odour and flavour (taste) awarded by a panel (5 judges) for tea containing pasteurized milk (PM) produced from raw milk (RM) sampled on the delivery by road tanker and after storage for 2, 4 and 7 days at 6°C. The scores were given for pasteurized milk on the day of processing and after storage for 3, 6 and 9 days at refrigerator temperature 4°C.



found in the total scores awarded by different judges. The pasteurized milk produced from raw milk stored for 2, 4 and 7 days at 6°C showed a highly significant difference (at 1 per cent level) in the total scores. The temperature of storage of the raw milk from which the pasteurized milk was produced had a very highly significant effect (at 0.1 per cent level) on the total scores. The storage of the pasteurized milk for 3, 6 and 9 days after processing had no significant effect on the total scores compared to the initial day of processing value.

The variations in the total scores for pasteurized milks are illustrated in Figs 4.20 and 4.21.

DISCUSSION

The scores for the appearance of the raw milks showed no significant alteration due to the duration of storage of up to 7 days and the temperature of the storage (2°C and 6°C) had no effect on the appearance scores of the same raw milks (Table 4.1). The scores awarded for the appearance of the tea to which the milks mentioned above were added have been ignored because the samples were kept in frozen condition and even freshly frozen milk gives an abnormal appearance to tea to which it has been added. It was found to be necessary to conduct the sensory panel investigation over a period of time because of the large number of samples, which if examined at the same session would have been too great a task for the panel and might have led to inaccuracies in the panelists judgement.

The means of the appearance scores showed that the taste panel liked the appearance of the milk 'only moderately'. The difference between the scores given for appearance of the raw milk on the day of delivery and after 2, 4 and 7 days of storage at 2°C and 6°C was less than 2 points on the grading scale.

No significant effect was found in the appearance scores of pasteurized milks which were produced from the raw milks mentioned above during the storage time up to 7 days while the temperature of storage (2°C and 6°C) had highly significant effect on the appearance scores of

the corresponding pasteurized milks.

The results awarded by the departmental taste panel for the appearance scores of pasteurized milks are in disagreement with those of Schönborn et al. (1975) who reported that there was no differences in the appearance of pasteurized milk one day after delivery and no statistically significant difference in the appearance of the milks when examined five and eight days after delivery. On the studies under discussion the storage of pasteurized milks for 3, 6 and 9 days after processing resulted in a highly significant effect ($p < 0.001$) on the appearance scores compared to the initial value but here again the effect may have been caused by the freezing and thawing procedure.

The storage of raw milk for up to 7 days at $2^{o}C$ and the storage of the corresponding pasteurized milk for up to 9 days did not cause defects in the odour of either milks. As a matter of fact these raw (7 days at $2^{o}C$) and pasteurized milks produced from these milks were given higher scores for odour than the initial raw and pasteurized. On the other hand the storage of raw milk for 4 and 7 days at $6^{o}C$ caused a decrease in the scores awarded for these raw milks and the corresponding pasteurized milks. These variations between different milks can be easily explained on effect of higher rates of microbiological and enzymatic activity in the milk stored at higher temperature ($6^{o}C$) for longer periods of time (4 and 7 days) for the raw milk and 9 days for the pasteurized milk.

The scores awarded for the raw milks were higher than the corresponding pasteurized milk. These results might be due to the panelists showing some dislike for the odour changes brought about by heat treatments, on the other hand it was found that the pasteurization had no effect on the odour of the milk (Nelson & Trout, 1964). Surprisingly the use of pasteurized milks which earned lower scores compared to the corresponding raw milks resulted in higher scores for the tea to which they had been added compared to the raw milks. The author would suggest further studies concerning the effect of adding milk to tea as a means of determining milk quality and detecting milk defects. It should be born in mind that percentage of milk used in England and

Wales, in the consumption of tea, 1980/81 is 1390 million litres (Kennedy, 1982). The results for the odour scores of pasteurized milk are in agreement with Schönborn et al. (1975) who stated that there was practically no difference in the odour scores one and five days after delivery but further storage until eight days after delivery resulted in significantly poorer odour scores.

There was no decrease in the flavour scores of raw milks stored at $2^{o}C$ for up to 7 days and the pasteurized milks produced from these milks when held at $4^{o}C$ for up to 9 days. There was, however, a decrease in the flavour scores of the raw milks stored at $6^{o}C$ after the second day of storage and this poorer flavour quality was also seen in the corresponding pasteurized milks.

During the storage of raw and pasteurized milk the following off-flavours could be detected by the taste panel members: acid, malty, fruity and very slight bitter flavours. These off-flavours were more distinct in the raw milk stored at $6^{o}C$ than at $2^{o}C$. Storage at refrigeration temperatures of the pasteurized milks after processing caused further development of these off-flavours where the original milk had been held at $6^{o}C$.

Patel & Blankenagel (1971) suggested that one or more of the following may be the reasons for the development of flavour defects in pasteurized milks:-

(a) end products of microbiol metabolism in the raw milk may become apparent in the pasteurized product;

(b) constituents of large numbers of heat inactivated and lysed bacterial cells may impart off-flavours to the pasteurized milk;

(c) heat-stable microbiol enzymes produced in raw milk may remain active after pasteurization and then cause changes in some constituents of the pasteurized milk during storage;

(d) the presence of thermoduric psychrotrophs and their growth in pasteurized milk during prolonged storage.

The following categories were reported by Shipe et al. (1978) who

identified the off-flavours in spoiled milk products as a key to determining their cause.

Categories of off-flavours in milk

Cause	Descriptive or associative terms
Heated	Cooked, caramelized, scorched
Light induced	Light, sunlight, activated
Lipolysed	Rancid, butyric, bitter, goaty
Microbial	Acid, bitter, fruity, malty, putrid, unclean
Oxidised	Papery, cardboard, metallic, oily, fishy
Transmitted	Feed, weedy, cowy, barny
Miscellaneous	Absorbed, astringent, bitter, chalky, chemical, flat, foreign, lacks freshness, salty.

Johnson (1979) stated that the microbiologically induced off-flavours are caused by the metabolism of milk proteins and carbohydrates by micro-organisms that contaminate milk products. These defects are found in both raw and pasteurized milk, and can normally be controlled by rigid sanitation. Milk is an ideal growth medium for bacteria; consequently a low bacterial contamination can produce detectable off-flavours in milk in a very short time. In addition, off-flavours that develop as a result of bacterial growth in raw milk are not effectively removed by pasteurization or by vacuum heat treatment.

CONCLUSION

1. The results obtained in this study showed highly significant differences between the scores given for the quality of the milk by different judges. These differences may be related to differences in judging standards of the panel members.

2. It is concluded that storage of the raw milk at $2^{o}C$ for up to 7 days did not affect its quality and some of the scores of milks stored at $2^{o}C$ were higher than on delivery. On the other hand the raw milk stored at $6^{o}C$ was unsatisfactory some time

between the second day of storage and the fourth day of storage at $6^{o}C$.

3. The results obtained in this study showed that the taste panel preferred the raw milk to the corresponding pasteurized milk, and they gave higher scores to the raw milk than to the pasteurized milk, but when the samples were tested by addition to tea, higher scores were given to the pasteurized milk than to the raw milk.

4. It is concluded from the scores for the appearance, odour and flavour that the pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ was of good quality, while the odour and flavour of the pasteurized milk produced from raw milk stored at $6^{o}C$ was good only up to the second day of storage and started to deteriorate after 4 and 7 days of storage.

5. The results obtained in this study for appearance, odour and flavour of the pasteurized milk produced from raw milk stored at $2^{o}C$ for up to 7 days, and the samples of pasteurized milk graded after 3, 6 and 9 days at $4^{o}C$ showed that the pasteurized milk was still good in quality. While the scores for the pasteurized milk produced from raw milk stored at $6^{o}C$ for up to 7 days, and the samples of pasteurized milk graded after 3, 6 and 9 days at $4^{o}C$ started to deteriorate after the second day of storage. This indicates that pasteurized milk can be produced from raw milk stored at $2^{o}C$ for up to 7 days, while it is not possible to produce good pasteurized milk from raw milk stored at $6^{o}C$ for more than 3 days.

6. The total scores (appearance, odour and flavour) of the raw milk stored at $2^{o}C$ for up to 7 days showed no significant variations, while storage of raw milk at $6^{o}C$ more than 2 days cause significant deterioration in the quality of milk as determined organoleptically. The total scores awarded for the corresponding pasteurized milk gave the same result.

7. Acid, malty and fruity off-flavours were found to be the most common defects detected in both raw and pasteurized milks.

CHAPTER FIVE

THE EFFECT OF COLD STORAGE OF RAW AND PASTEURIZED MILK ON FREE SULPHYDRYL GROUPS

INTRODUCTION

There has been a growing interest in the processing of ultra-heat treated (UHT) sterilized milk. Two major defects associated with UHT sterilized milk are identified as:-

1. cooked flavour followed by the development of other flavour defects;
2. destabilization of milk proteins resulting in the formation of deposits in the heat treatment equipment, the formation of fine precipitates in the package or gelation of the milk on storage (Ashton, 1965; Burton, 1968; Burton, 1969).

Instability of whey proteins during heat treatment also appears to be associated with changes in the sulphydryl/ disulphide groups of these proteins (Townend & Gyuricsek, 1974).

(1) The origin of sulphydryl groups in milk protein

The sylphydryl groups (-SH) in skim-milk are confined to the whey protein and among them almost entirely to the β-lactoglobulin, (Hutton & Patton, 1952; Zweig & Block, 1953). They are related to the characteristics of pasteurized milk especially the cooked flavour (Crowe *et al.*, 1948; Gould & Sommer, 1939; Harland & Ashworth, 1945; Jackson, 1936; Josephson & Doan, 1939; Larson *et al.*, 1949; Larson & Jenness, 1950a; Larson & Jenness, 1950b; Patton & Josephson, 1949; Townley & Gould, 1943a; Townley & Gould, 1943b).

Hutton & Patton (1952) added whey protein fraction to skim milk. After heat treatment, the greatest development of cooked flavour was in the samples, which before heat treatment had given the highest value for -SH group.

(2) The effect of heat treatment on sulphydryl groups

Heating skim milk above a critical temperature at 50 to 60°C caused an initial slight rise in -SH groups, followed by rapid fall. The -SH content of dried skim milk was affected by both the temperature and the duration of pre-heating (Zweig & Block, 1953).

Maximum -SH contents were found in milk heated for 30 min at 80°C to 100°C. On storage after heating the SH content declined; this decline was faster in milk exposed to sunlight and was found to parallel the oxidation of ascorbic acid (Burton, 1959; Kiermeier & Hamed, 1962).

Rao & Gould (1960) reported that the concentration of -SH groups fluctuated during the storage of milk at 4.4°C (40°F) for up to 48 h.

Dill et al. (1961) explained that the -SH content increased with the severity of the heat treatment but with vacuum cooling the maximum value was obtained with less than the maximum heat treatment. The -SH content decreased during storage in all samples.

Kiermeier & Hamed (1961) showed that the formation of free -SH groups as measured by cysteine content, first becomes evident in milk when heated to about 75°C and increases rapidly with increase in heating temperature until a maximum is reached between 95°C and 110°C. Beyond this it declines due to oxidation of -SH groups.

Skim milk was heated for either 2 or 150 s at temperatures from 87.7° to 149.9°C (190° to 300°F) and the heat activated sulphydryl groups were estimated amperometrically and the volatile sulphur compounds were identified. Volatile sulphur compounds, mainly H_2S, were found with the more severe heat treatments that caused a loss in titratable sulphydryl groups (Dill et al., 1962).

Kiermeier & Hamed (1962) stated that the sulphydryl content of milk decreased more in milk that was heated to 80° or 90°C than in milk heated to 85°C. The loss of -SH groups was slightly less in whole milk than in skim milk. Loss of -SH groups in heated

whole milk was influenced by temperature of heating as well as the storage temperature. Losses were higher during storage at 20°C than at 3°C. The loss of -SH groups during storage of dried whole milk was greatly influenced by relative humidity.

Yoshino et al. (1962) found that the sterilization of concentrated milk decreased the titratable -SH and increased -SS-. On the other hand Blankenagel & Humbert (1963) reported that sterile skim milk, processed at 140.5°C (285°F) and held at room temperature, contained no -SH groups after 1 week of storage, and they indicated that β-lactoglobulin which was thought to be the main source of -SH groups was almost entirely denatured at 129.4°C (265°F).

Demott & Gibbs (1965) found that the addition of Ca to raw milk caused a reduction in the intensity of cooked flavour on heating. Exchanging cations in the milk for calcium by ion-exchange was more effective in reducing cooked flavour than direct addition of extra calcium. It was found that the -SH content of the raw milk and the extent of liberation of -SH groups by heating varied significantly with the individuality of the cow, but not its age. The -SH content was not affected by the change in feeding regime. Summer milks were more resistant than winter milks to a reduction in free -SH content by heating (Thanh et al., 1968). Some authors found that β-lactoglobulin contained 1 -SH and 2 —SS groups per 18,300 g α-lactalbumin 4.55 per 14,400 g and K-casein 1 -SS per 20,000 g (Manning et al., 1969). Riel & Thanh (1970) found that the change of the feed given to cows and the age of the cow had no influence on the sulphydryl group except that for the older cows the sulphydryl content was higher in the winter than in the summer milk. In 1970 it was found that the susceptible milks contained a higher proportion of -SH groups than did the resistant milks and these were reduced to a greater extent on heating. The susceptible milks also had a higher proportion of non-protein N compounds. Age of the cow did not appear to influence the milk susceptibility (Thanh et al., 1970).

During pasteurization an insignificant reduction in the quantity of

S-S groups takes place as a result of oxidation and destruction (Seitov & Seitkaliev, 1970; Klostermeyer & Reimerdes, 1976) while studies by Patrick & Swaisgood (1975) of sterilized skim milk stored at refrigeration or room temperature suggested that the "reactive" sulphydryl groups oxidized more rapidly and also that a larger fraction was oxidized during storage at room temperature. These concentrations of "reactive" sulphydryl groups in sterilized milk have been correlated with undesirable "cooked" flavours and possibly could contribute to instability of milk protein through disulphide interchange reactions.

Görner et al. (1978) studied Ultra High Temperature (UHT) milk stored at 2 to 3°C and 20 to 23°C. The most intense cooked flavour occurred on the third day in UHT milk stored at 20 to 23°C which coincided with the highest total sulphydryls content, whereas at the lower storage temperature the sulphydryls reached their maximum on the third day but cooked flavour was most pronounced on the eighth day. Similar results were obtained for disulphides.

Bürki & Blanc (1978) noticed that during storage there were decreases in SH-groups and also in volatile S compounds. The characteristic early flavour of UHT milk appeared linked to the latter.

Because there is not a lot of information about the effect of cold storage of the raw and pasteurized milk on the free SH groups the following experiment was undertaken.

EXPERIMENTAL

1. Collection of milks samples

This experiment was carried out in two trials with two kins of milk, (i) raw, and (ii) pasteurized. Raw milks were taken from bulk milk in a road tanker (i.e. consisting of a mixture from several farms) from a commercial dairy in West Scotland. Triplicate samples A1, A2 and A3 were collected from each tanker. Samples were kept in an insulated box provided with ice bags and transferred cold to the laboratory. The samples were taken from six tankers on two occasions, one in February 1980, and one in May 1980. The samples of pasteurized milk were taken

from six different local shops each supplied by a different dairy. These samples were also obtained on two occasions.

2. Storage of the raw and pasteurized milks

In the first trial, one sample from each tanker was held at 6°C with agitation, these were coded A1, B1, C1, D1, E1, F1. One from each tanker was held at the same temperature without agitation, these were coded A2, B2, C2, D2, E2 and F2, and one from each tanker was held at 2°C without agitation, these were coded A3, B3, C3, D3, E3 and F3.

The second trial was carried out using the same procedure with group one having agitation at 2°C only, the second group being held at 2°C without agitation while the third group was held at 6°C without agitation. Throughout this study a MK Reciprocal Shaker (L.H. Engineering Co. Ltd., Stoke Poges, Bucks, England) was used which in conjunction was timed to shake the samples for ½ h every 6 h. The samples of pasteurized milk were stored at 4°C without agitation.

3. Determination of free sulphydryl groups

Free sulphydryl groups in the raw milks were determined initially and after 2, 4 and 7 days of storage at 2°C and 6°C. The samples of pasteurized milks were analysed after retail purchase and then after 3, 6 and 9 days of storage at 4°C. Skim milks were prepared by centrifugation of about 20 ml samples at 1,000 g using a BTL Bench centrifuge and the F-SH were determined in these using the method described in Chapter One (Section 2.6). The analysis was mde in duplicate on each sample.

RESULTS

1. Raw milk

The contents of free sulphydryl groups in samples of raw milk are presented in Tables 5.1 and 5.2.

The variations in F-SH levels were not the same in the two trials, the milks in trials 1 and 2 gave 2.835, 3.251 (µmol/g dry weight) free sulphydryl groups respectively. There was a significant difference

TABLE 5.1

The free sulphydryl groups (expressed as µmol/g dry weight) of the raw milk samples from different road tankers determined on the day of delivery

	Road tanker						Mean
Trial	A	B	C	D	E	F	
1	2.870	2.805	2.876	2.690	4.076	1.786	2.851
2	2.664	2.843	2.822	3.274	2.637	2.974	2.870
Mean	2.767	2.823	2.849	2.982	3.357	2.379	

	DF	MS	VR
Trial	1	0.0041	0.058
Tankers	5	0.8034	11.238***
Trial Tankers	5	1.5475	21.645***
Residual	12	0.0714	76.130
Total	23	0.5485	584.110

Table	Tankers	Trial Tankers	Trial
REP	8	4	24
SED	0.1337	0.1890	0.07719

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 5.2

The free sulphydryl groups (expressed as µmol/g dry weight) of raw milk samples from different road tankers determined after 2, 4 and 7 days of storage without agitation at 2°C and 6°C

Road Tanker	Storage at 2°C			Storage at 6°C		
	2 days	4 days	7 days	2 days	4 days	7 days
A	3.072	2.580	2.883	2.534	3.477	3.157
B	2.611	2.671	3.122	2.505	3.085	2.984
C	2.669	2.927	4.447	2.126	2.745	3.417
D	2.389	2.412	5.667	2.413	2.545	4.883
E	2.094	2.105	5.111	2.250	2.871	4.819
F	2.206	2.592	2.729	3.413	3.198	2.730
Mean	2.507	2.549	3.993	2.540	2.987	3.665

	DF	MS	VR
Trial	1	6.087	13.063***
Tankers	5	1.209	2.594
Storage	2	23.13	49.645***
Temperature	1	0.0083	0.178
Trial Tankers	5	0.0747	1.604
Trial Storage	2	0.0747	1.549
Tanker Storage	10	4.544	9.752***
Trial Temperature	1	0.0514	1.104
Tanker Temperature	5	0.0964	2.138
Storage Temperature	2	1.767	3.793
Trial Tanker Storage	10	2.149	4.612*
Trial Tanker Temperature	5	0.0586	1.259
Trial Storage Temperature	2	2.8000	6.010*
Tanker Storage Temperature	10	0.0322	0.692
Residual	10	0.0465	1926.458
Total	71	2.198	9086.410

Table	Temperature	Storage Temperature	Tanker Storage Temperature
REP	72	24	4
SED	0.1137	0.1970	0.4826

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 5.1 The effect of storage at 2°C and 6°C of the samples of raw milks (RM) on the level of free sulphydryl groups (µmol/g dry weight)

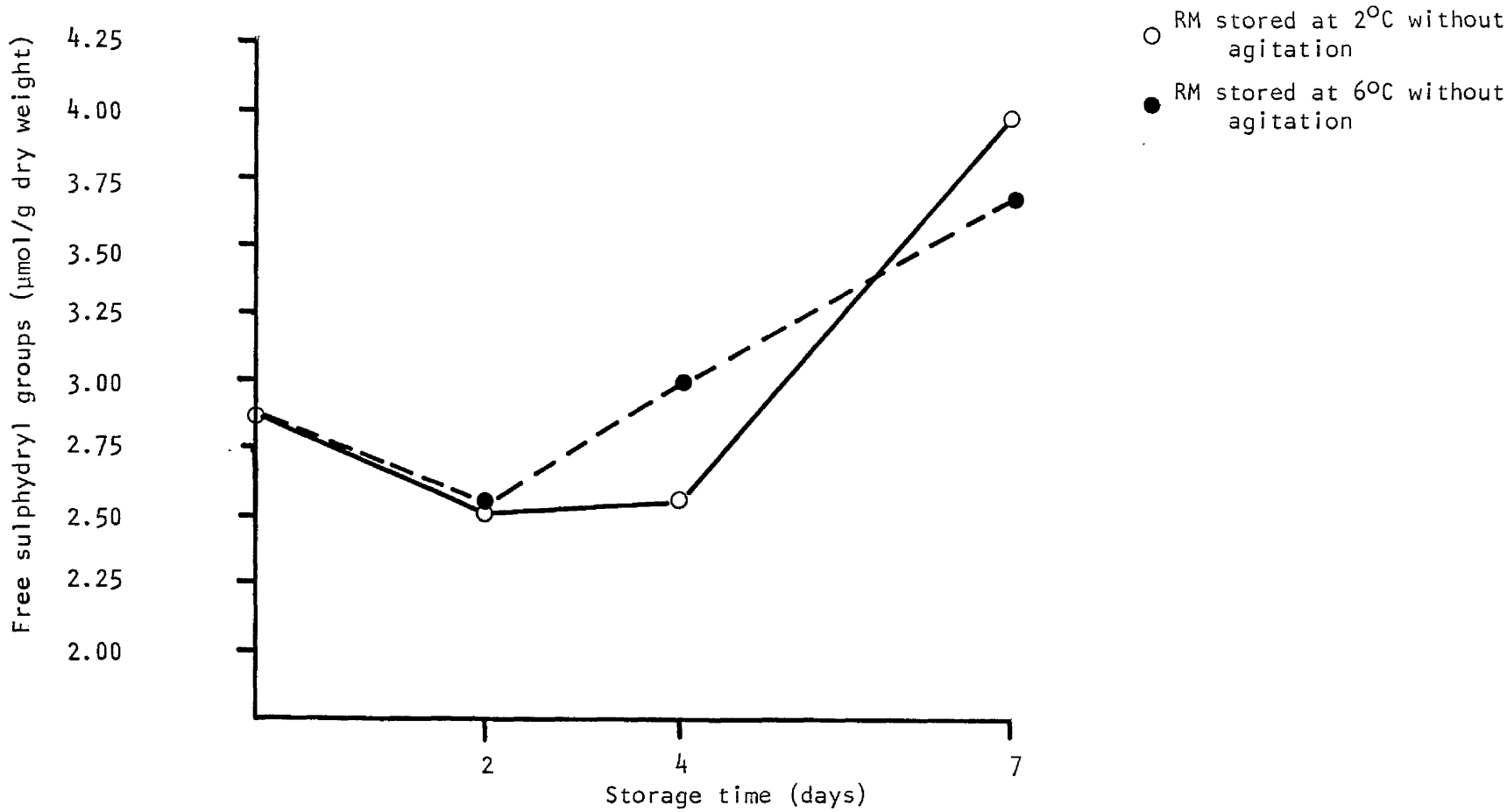


TABLE 5.3

The free sulphydryl groups (expressed as µmol/g dry weight) of raw milk (RM) samples taken from different road tankers determined on delivery (initial) and after 2, 4 and 7 days of storage with and without agitation at $2^{o}C$.

Storage	RM Storage at $2^{o}C$ without agitation	RM Storage at $2^{o}C$ with agitation
Initial	2.860	
2 days	3.023	2.994
4 days	2.665	3.326
7 days	3.798	4.054
Mean	3.162	3.458

	DF	MS	VR
Tankers	5	0.07420	2.214
Storage	2	6.837	20.397***
Agitation	1	1.579	4.711
Tankers Storage	10	0.03526	1.052
Tankers Agitation	5	0.03241	0.967
Storage Agitation	2	0.07228	2.157
Residual	10	0.0352	10682.773
Total	35	0.08259	26323.027

Table	Agitation	Agitation Storage
REP	36	12
SED	0.136458	0.236352

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 5.4

The free sulphydryl groups (expressed as µmol/g dry weight) of raw milk (RM) samples taken from different road tankers determined on delivery (initial) and after 2, 4 and 7 days of storage with and without agitation at 6°C

Storage	RM Storage at 6°C without agitation	RM Storage at 6°C with agitation
Initial	2.850	
2 days	2.365	2.052
4 days	2.794	2.581
7 days	3.237	3.175
Mean	2.799	2.603

	DF	MS	VR
Tankers	5	0.7150	1.782
Storage	2	5.9736	14.887***
Agitation	1	0.68894	1.717
Tanker Storage	10	3.7986	9.467***
Tankers Agitation	5	0.93450	2.329
Storage Agitation	2	0.09526	0.237
Residual	10	0.40125	698.570
Total	35	1.80210	3137.390

Table	Agitation	Agitation Storage
REP	36	12
SED	0.14931	0.25860

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 5.2 The effect of cold storage at 2°C and 6°C of raw milk (RM) (with and without agitation) on the level of free sulphydryl groups (µmol/g dry weight)

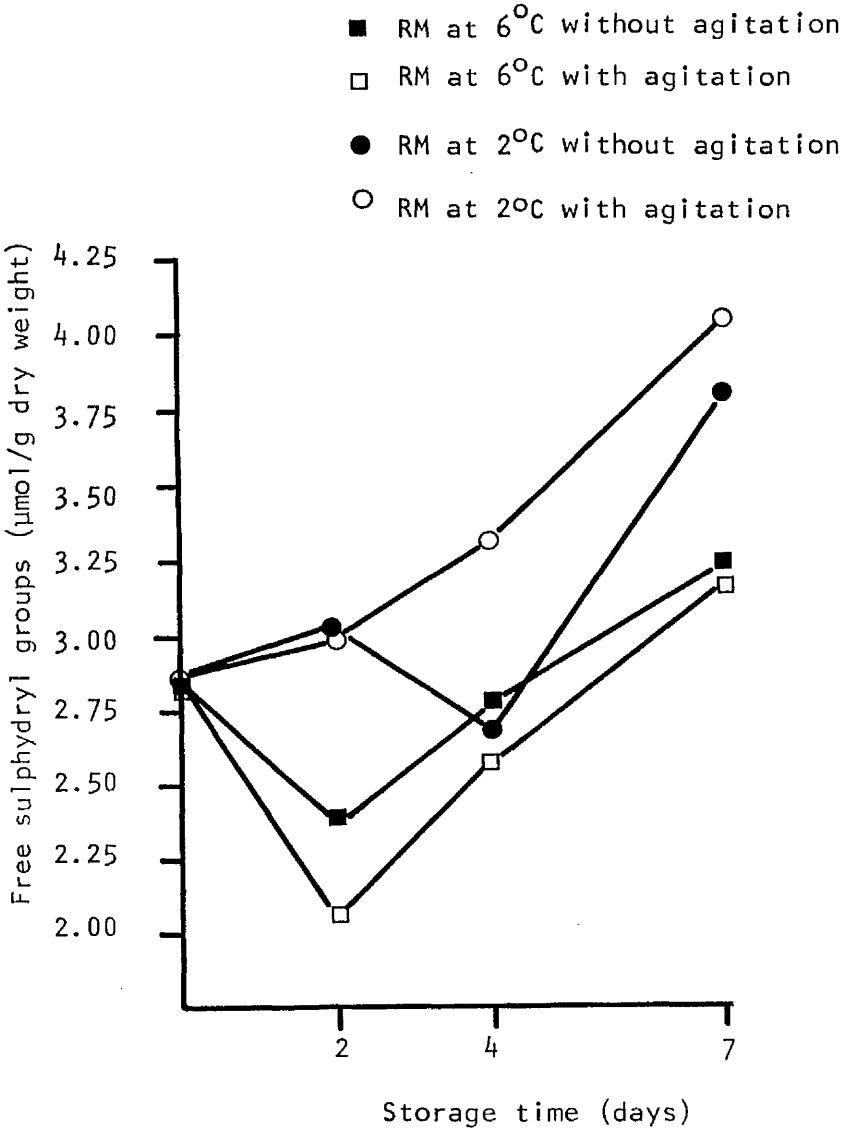


TABLE 5.5

The free sulphydryl groups (expressed as µmol/g dry weight) of pasteurized milk samples from different sources taken on the day of purchase (initial) and after 3, 6 and 9 days of storage at 4°C. The figures represent the mean of 6 samples.

Trial	Storage			
	Initial	3 days	6 days	9 days
1	2.689	1.840	3.741	2.761
2	3.070	3.859	3.061	4.018
Mean	2.881	2.851	3.398	3.389

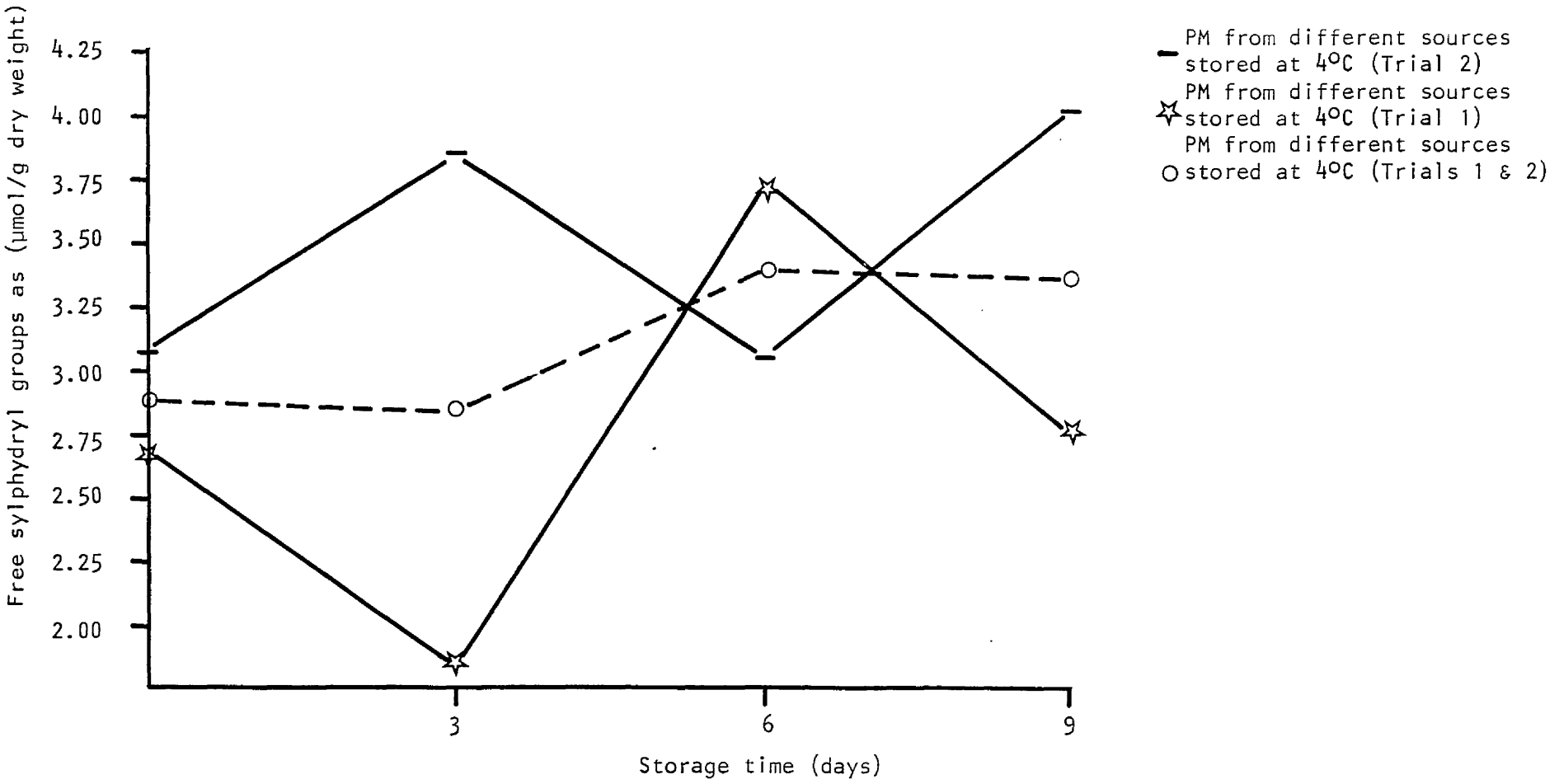
Sources	Trial 1	Trial 2	Mean
P1	2.681	3.224	2.951
P2	2.698	4.015	3.356
P3	2.599	3.594	3.096
P4	2.565	3.966	3.266
P5	3.062	3.124	3.093
P6	2.944	3.084	3.014
Mean	2.757	3.501	

	DF	MS	VR
Trial	1	13.2766	60.029***
Sources	5	0.3779	1.709
Storage	3	2.2445	10.149***
Trial Sources	5	1.3536	6.120**
Trial Storage	3	8.1058	36.650***
Sources Storage	15	0.2636	1.193
Residual	15	0.2211	786.819
Total	47	1.2821	4561.277

Table	Storage	Trial	Trial Storage	Trial Sources	Sources
REP	24	48	12	4	16
SED	0.13576	0.09600	0.19199	0.33254	0.16627

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Fig. 5.3 The effect of storage at 4°C on the free sulphydryl groups of the samples of pasteurized milk (PM) stored for 3, 6 and 9 days (µmol/g dry weight)



(at the 1 per cent level) in the free sulphydryl groups in the milks obtained on the separate days. A very highly significant difference (0.1 per cent level) was found in the level of free sulphydryl groups due to the storage of the milk. The same level of significance was found in the level of F-SH due to the interaction between the tankers and the storage. Agitation had no significant effect on the level of free sulphydryl groups at $2^{o}C$ and at $6^{o}C$ (Tables 5.3 and 5.4 respectively).

The variations in the levels of free sulphydryl groups of samples of raw milk stored at two different temperatures are shown in Fig. 5.1.

The effect of agitation on the free sulphydryl groups in samples of raw milk stored at two different temperatures are illustrated in Fig. 5.2.

2. Pasteurized milk

The results of the free sulphydryl group determination on samples of pasteurized milk are presented in Table 5.5. Differences between trials in the levels of free sulphydryl groups were found in the various milks on the day of sampling. These differences in the level of free sulphydryl groups were still apparent after 3, 6 and 9 days of storage at $4^{o}C$. The level of free sulphydryl groups increased in the pasteurized milk during the storage time at $4^{o}C$ (Fig. 5.3). This difference was very highly significant (0.1 per cent level).

DISCUSSION

Very little information is available on the effect of cold storage on the free sulphydryl groups in raw and pasteurized milk and in view of the increasing importance of cold storage on milk on the farm and in the stages of processing and distribution it was considered to be of value to examine what changes took place in the level of free sulphydryls. The results of this study showed that the quantity of -SH groups in raw and pasteurized milks varies from batch to batch of milk even when obtained from the same farm. This finding is in agreement with the reports by Zweig & Block (1953) and Jenness (1970).

1. Raw milk stored without agitation

The statistical analysis of results of F-SH determination made on the

raw milk samples (Table 5.1) showed a significant difference (at 0.1 per cent level) between the free sulphydryl groups in the milks from different sources on the day of delivery, but the differences were not consistent between trials.

After the storage of the raw milk samples at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days, significant differences (at 1 per cent level) were found in the F-SH groups between the trials (Table 5.2). The length of storage had a highly significant effect (at 0.1 per cent level) on the level of F-SH groups.

The level of F-SH increased during storage but the extent of this increase varied with different milk samples.

No significant difference was found due to the storage temperature. There are increases in the F-SH groups (Fig. 5.1) during the storage time of the raw milk at $2^{o}C$ and $6^{o}C$.

2. Raw milk stored with agitation

There was very high significant difference (at 0.1 per cent level) in the level of free sulphydryl groups due to the storage of the milk at $2^{o}C$ (Table 5.3).

There was a highly significant difference ($p < 0.01$) in the level of free sulphydryl groups due to the storage time of the milk samples at $2^{o}C$ without agitation, while the significance was very high ($p < 0.001$) in the level of free sulphydryl groups due to the storage time of the milk samples at $2^{o}C$ with agitation. No significant difference was found in the mean value for the level of free sulphydryl groups between the samples with and without agitation.

There was very high significant difference (at 0.1 per cent level) in the level of free sulphydryl groups due to the storage time of the milk samples at $6^{o}C$ (Table 5.4). While there was no significant difference due to the effect of agitation at $6^{o}C$. A very highly significant difference (at 0.1 per cent level) was found due to the interaction between the tankers and the storage at $6^{o}C$.

The variations in the free sulphydryl groups were shown in Fig. 5.2.

There was an increase in the level of free sulphydryl groups in the samples stored at $2^{o}C$ with agitation. While there was no effect of the agitation on samples stored at $6^{o}C$.

3. Pasteurized milk

The statistical analysis of results for pasteurized milks (Table 5.5) show that there were no significant differences in the level of free sulphydryl groups in milks from different sources. On the other hand the storage time was related to very highly significant differences (at 0.1 per cent level) in the free sulphydryl levels.

The changes during storage differed in the two trials. The mean results showed an increase in F-SH (Fig. 5.3) between the third and sixth day but in each trial the levels fluctuated considerably. The reason for these changes is not known.

The means of the free sulphydryl groups ranged from 2.05 - 4.05 µmol/g dry weight. This was in agreement with most authors {Yo shino et al. (1962), Pofahl & Vakaleris (1968), Sasago et al. (1963), Beveridge et al. (1974)} whose results fall within the range of 1.78 to 5.8 µmol/g dry weight.

There is not a lot of information about the effect of different storage temperature on the free sulphydryl groups but various authors suggest that these groups may participate in a variety of reactions, e.g.:-

(a) they may be lost by volatilization, or

(b) they may oxidize to disulphides, or

(c) they may be reburied in protein structures (Patrick & Swaisgood, 1975)

(d) the possibility that some denatured β-lactoglobulin had reverted to the native state (Lyster, 1964).

F-SH levels will be affected by the balance of these reactions.

Variations in the free sulphydryl groups values found by different workers may be due to differences in the sensitivities among the various methods, Pofahl & Vakaleris (1968).

CONCLUSION

1. It is concluded that there was a highly significant difference in the level of free sulphydryl groups from different sources of the raw milk. The storage time at $2^{o}C$ and $6^{o}C$ had a significant effect on F-SH levels (at 1 per cent level), the level increasing with storage time.

   No significant change was found in the free sulphydryl group due to the temperature of the storage of the raw milk samples from different sources.

2. The study showed that there was a significant difference in the free sulphydryl groups in the samples of pasteurized milk from several commercial sources stored for 3, 6 and 9 days at $4^{o}C$, the values increasing during the storage time. No differences were found between the sources. A highly significant difference was found between the trials.

3. It is concluded that there was a very highly significant difference in the level of free sulphydryl groups due to the storage of the samples of raw milk at $2^{o}C$ and $6^{o}C$ with and without agitation. Agitation had no effect on the level of free sulphydryl groups in samples stored at $2^{o}C$ and $6^{o}C$, but there was an increase in the level of F-SH groups during the storage time.

4. The results obtained in this study showed a highly significant difference in the free sulphydryl groups values from different sources of raw milk and in the runs. These differences may be related to differences in the milk because of composition, age of cow, a season variation and handling.

CHAPTER SIX

THE RELATIONSHIP BETWEEN THE MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC TESTS FOR RAW AND PASTEURIZED MILKS DURING DIFFERENT PERIODS OF STORAGE

INTRODUCTION

Randolph *et al.* (1965) indicated that there were highly significant correlations between the initial standard plate count of pasteurized milks, the standard plate count after 4, 7, 10 and 14 days storage at 4.4°C 0 5.5°C (40-42°F), the age of samples when purchased, and the keeping quality. The correlations were as follows:- initial standard plate count (SPC) and age, 0.43; initial SPC and SPC after storage, 0.25; initial SPC and keeping quality, -0.38; SPC after storage and age on date purchased, 0.24; and SPC after storage and keeping quality, -0.55.

Mikolajcik (1979) stated that psychrotrophic micro-organisms may have an indirect as well as a direct effect on the quality of dairy products. Indirectly, psychrotrophs produce off-flavours and odours during growth in stored refrigerated raw milk which may carry over into the finished product even though the organisms fail to survive pasteurization. Directly, organisms surviving pasteurization or resulting from post-pasteurization contamination may multiply in sufficient numbers during manufacture and storage of dairy products so as to reduce the shelf life and the quality of the finished product.

The purpose of this study was to find the relation between the different microbiological tests and the quality of the milks, and between the microbiological tests, the chemical tests and the quality assessment of the milks.

RESULTS

The relations between the psychrotrophic count and total colony count, coliform count and total colony count, lipolytic count and total colony count, proteolytic count and total colony count for raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C are

presented in Tables 6.1, 6.2, 6.3 and 6.4 respectively. The correlation coefficient for these relations were highly significant being respectively $r = 0.8756$, $p < 0.001$; $r = 0.9521$, $p < 0.001$; $r = 0.9102$, $p < 0.001$; $r = 0.9580$, $p < 0.001$. The standard curves for these relations are shown in Figs 6.1, 6.2 6.3 and 6.4 respectively.

The relation between the lipolytic count and total colony count, proteolytic count and total colony count for pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$ are presented in Tables 6.5 and 6.6, the value for pasteurized milk being given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{o}C$).

The correlation coefficient for these relations were highly significant $r = 0.7332$, $p < 0.001$; $r = 0.9444$, $p < 0.001$ respectively. The standard curves for these relations are shown in Figs 6.5 and 6.6.

The results of the relation between the odour and proteolytic count for the raw and the pasteurized milk, between the flavour and acid degree value, flavour and proteolytic count and odour and acid degree value for pasteurized milk only are presented in Tables 6.7, 6.11, 6.8, 6.9 and 6.10 respectively. There was no significant correlation of these characteristics with the quality of raw milks. These tables show the following correlation coefficient which were significant, $r = 0.8403$, $p < 0.05$; $r = 5504$, $p < 0.01$; $r = 5375$, $p < 0.01$; $r = 0.6277$, $p < 0.001$ and $r = 0.4552$, $p < 0.05$ respectively. The standard curves for these relations are shown in Figs 6.7, 6.8 and 6.9.

The correlation coefficient found between the organoleptic tests, the microbiological tests, acid degree values and free sulphydryl groups for raw milks, pasteurized milks and all the milks are presented in Tables 6.12, 6.13 and 6.14 respectively.

TABLE 6.1

The regression analysis of $\log_e$ psy[1] on $\log_e$ ($\log_e$ tcc[2])

	$\log_e$ Psy			
	Regression			
	Estimate	SE	T	Correlation coefficient
Y-intercept of $\log_e$ psy	-32.029	5.980	-5.36	0.8756***
Slope of $\log_e$ tcc	17.505	2.215	7.90	

The regression equation is:

$$\log_e \text{Psy} = -32.029 + 17.505 \times \log_e \{\text{Log (tcc)}\}$$

Analysis of variance:-

	DF	SS	MS
Regression	1	173.96	173.965
Residual	19	52.93	2.786
Total	20	226.89	11.345
Change	0	-3.48	0

% variance accounted for 75.4

[1] = $\log_e$ of psychrotrophic count for the raw milk during different periods of storage at 2°C and 6°C

[2] = $\log_e$ of total colony count for the raw milk during different periods of storage at 2°C and 6°C

***significant at 0.1 per cent level

Fig. 6.1 The standard curve of the relation between the psychrotrophic count and total colony count for raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

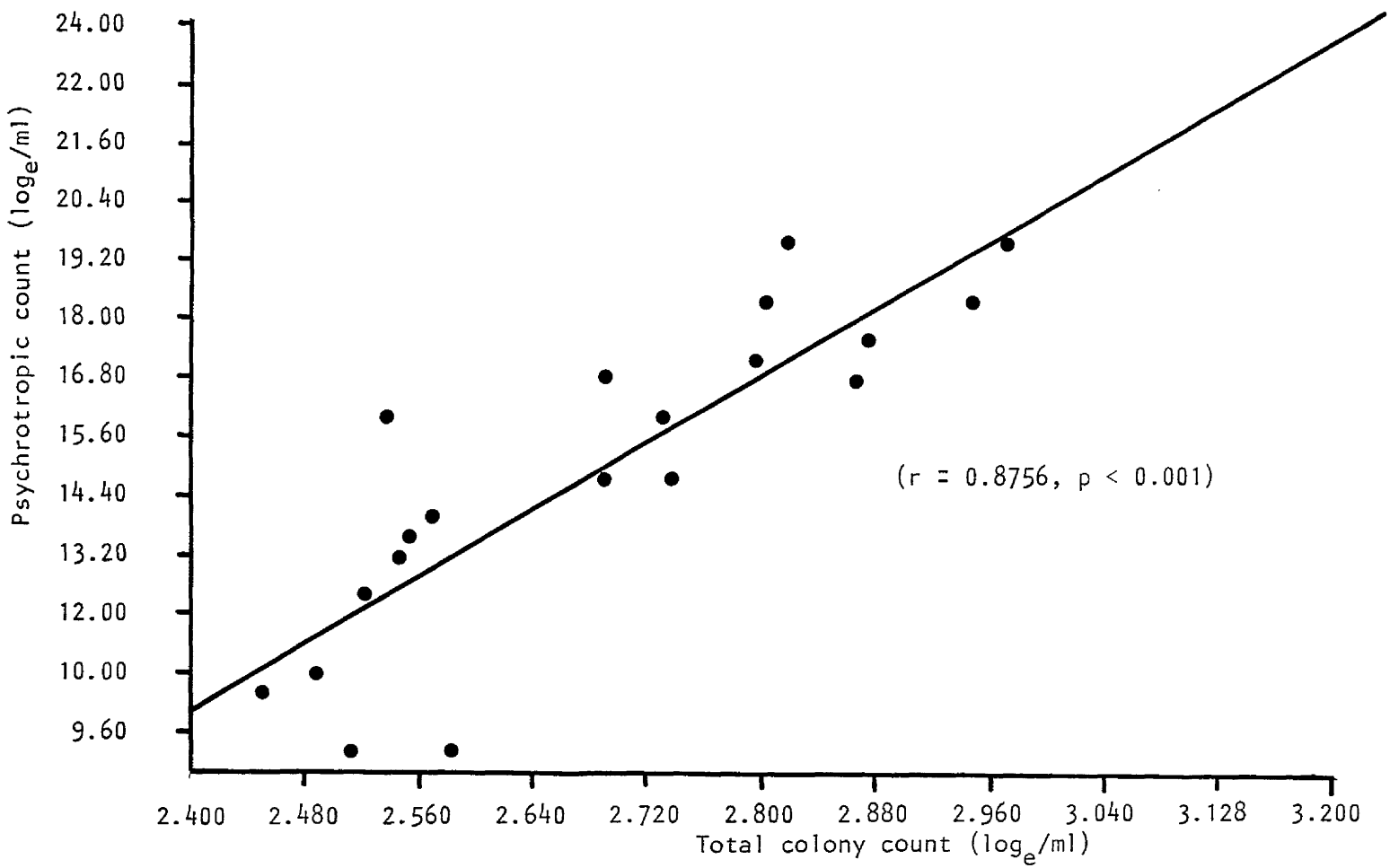


TABLE 6.2

Regression analysis of $\log_e$ col[3] on $\log_e$ tcc[4]

	$\log_e$ psy			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of $\log_e$ col	-6.0609	1.2819	-4.73	0.9521***
Slope of $\log_e$ tcc	1.1442	0.0843	13.58	

The regression equation is:- -6.060

$$\log_e \text{col} = -6.0609 + 1.1442 \times \log_e (\text{tcc})$$

Analysis of variance:-

	DF	SS	MS
Regression	1	171.31	171.3108
Residual	19	17.66	0.9296
Total	20	188.97	9.4487
Change	-1	-171.31	171.3108

% variance accounted for 90.2

[3] = $\log_e$ coliform count for the raw milk during different periods of storage at 2^oC and 6^oC

[4] = $\log_e$ of total colony count for the raw milk during different periods of storage at 2^oC and 6^oC

***significant at 0.1 per cent level

Fig. 6.2 The standard curve of the relation between the coliform count and total colony count for raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

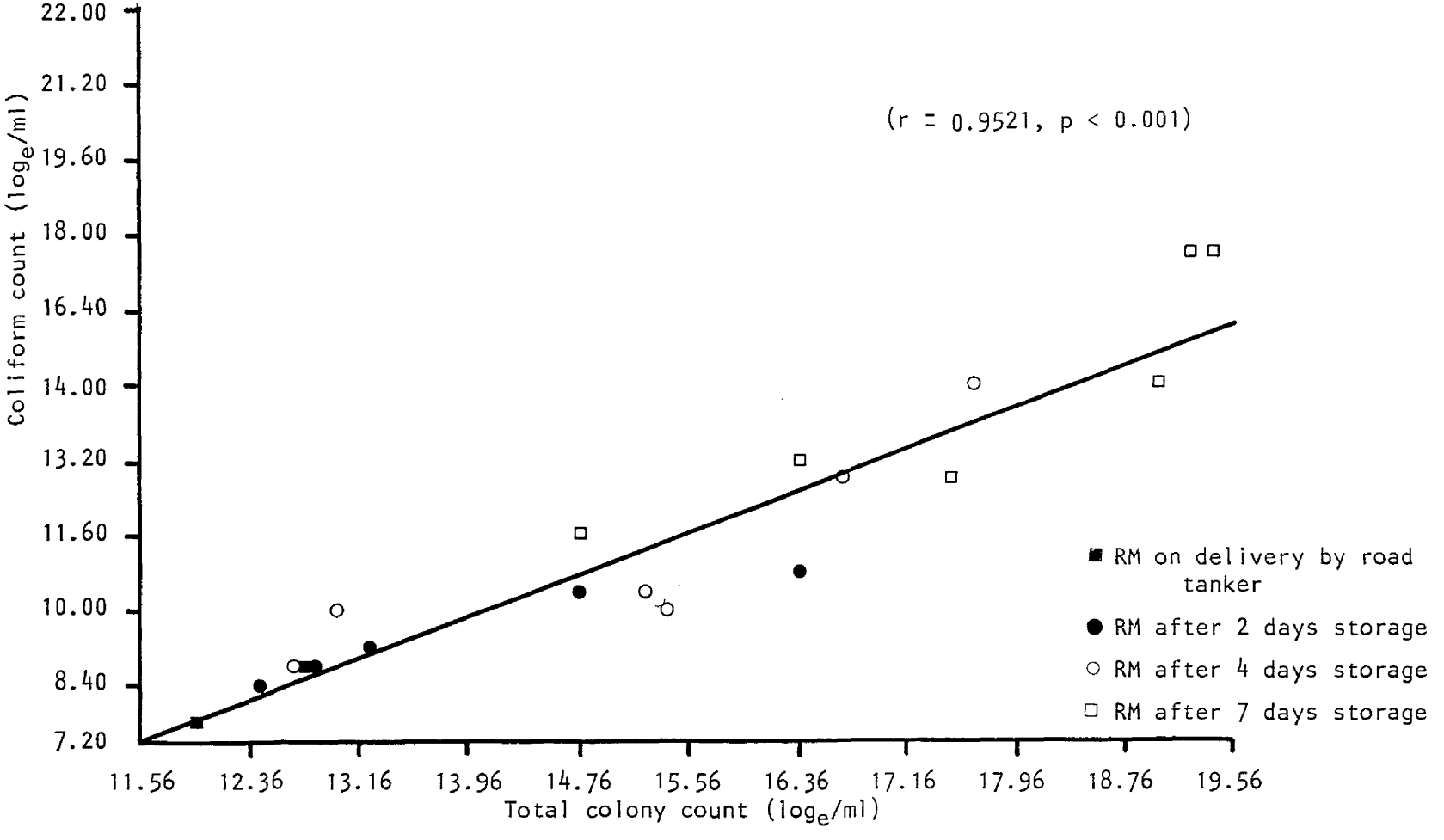


TABLE 6.3

Regression analysis of $\log_e$ lip[5] on $\log_e$ tcc[6]

	$\log_e$ lip			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of $\log_e$ lip	-2.3770	1.8330	-1.30	0.9102***
Slope of $\log_e$ tcc	1.1548	0.1205	9.58	

The regression equation:-

$$\text{Log}_e = -2.3770 + 1.1548 \times \log_e \text{tcc}$$

Analysis of variance

	DF	SS	MS
Regression	1	174.48	174.480
Residual	19	36.11	1.901
Total	20	210.59	10.530
Change	-1	-174.48	174.480

% variance accounted for 81.9

[5] = Log_e of lipolytic count for the raw milk during different period of storage at $2^{\circ}C$ and $6^{\circ}C$

[6] = Log_e of total colony count for the raw milk during different periods of storage at $2^{\circ}C$ and $6^{\circ}C$

***significant at 0.1 per cent level

Fig. 6.3 The standard curve of the relation between the lipolytic count and total colony count for raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

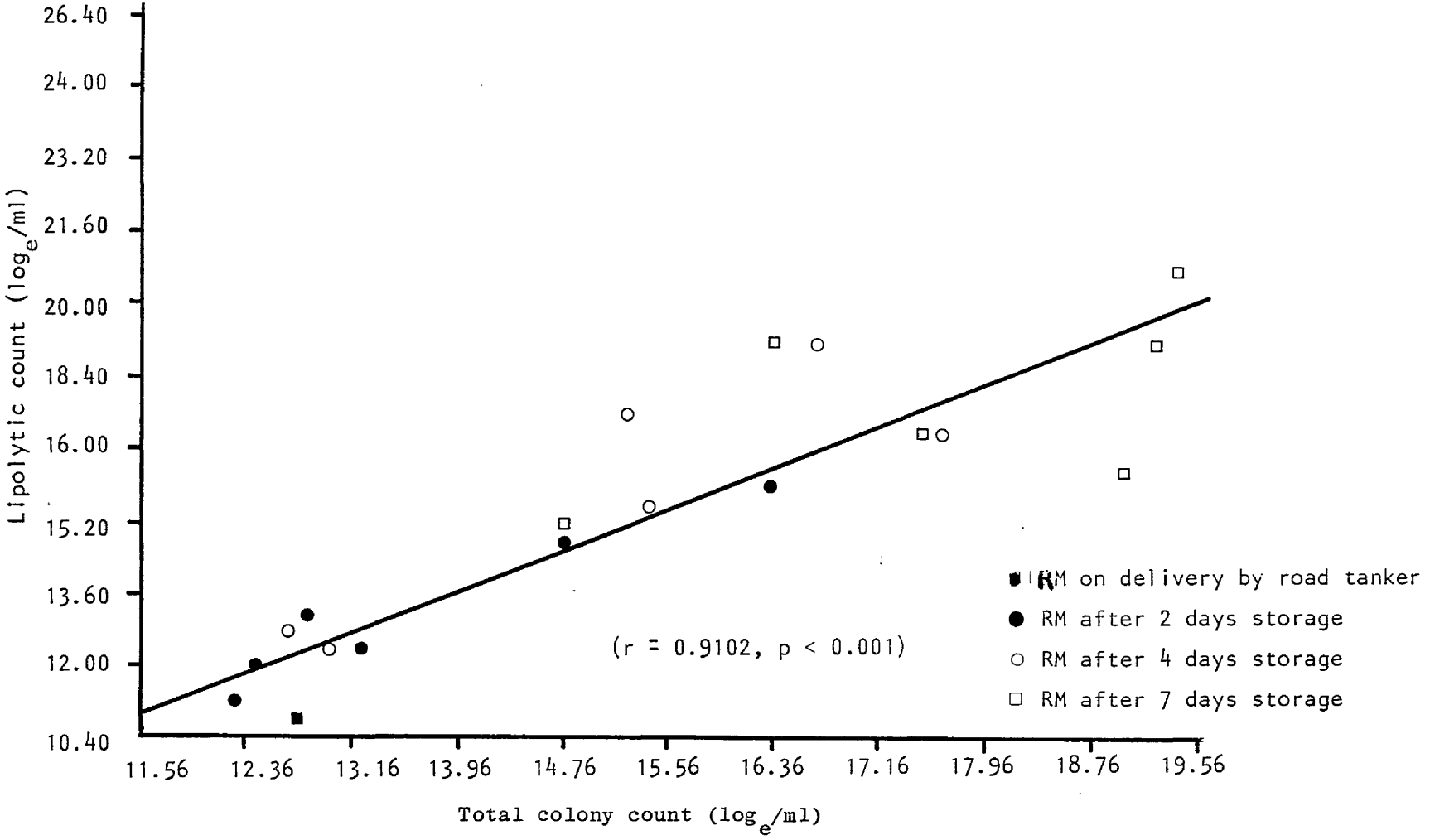


TABLE 6.4

Regression analysis of $\log_e$ prot[7] on $\log_e$ tcc[8]

	$\log_e$ Prot			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of $\log_e$ prot	0.9131	1.0257	0.89	0.9580***
Slope of $\log_e$ tcc	0.9824	0.0674	14.57	

The regression equation is

$$\log_e \text{ prot} = 0.9131 + 0.9824 \times \log_e \text{ tcc}$$

Analysis of variance

	DF	SS	MS
Regression	1	126.28	126.2795
Residual	19	11.31	0.5951
Total	20	137.59	6.8794
Change	-1	-126.28	126.2795

% variance accounted for 91.3

[7] = $\log_e$ of proteolytic count for the raw milk during different periods of storage at 2^oC and 6^oC

[8] = $\log_e$ of total colony count for the raw milk during different periods of storage at 2^oC and 6^oC

***significant at 0.1 per cent level

Fig. 6.4 The standard curve of the relation between the proteolytic count and total colony count for raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C

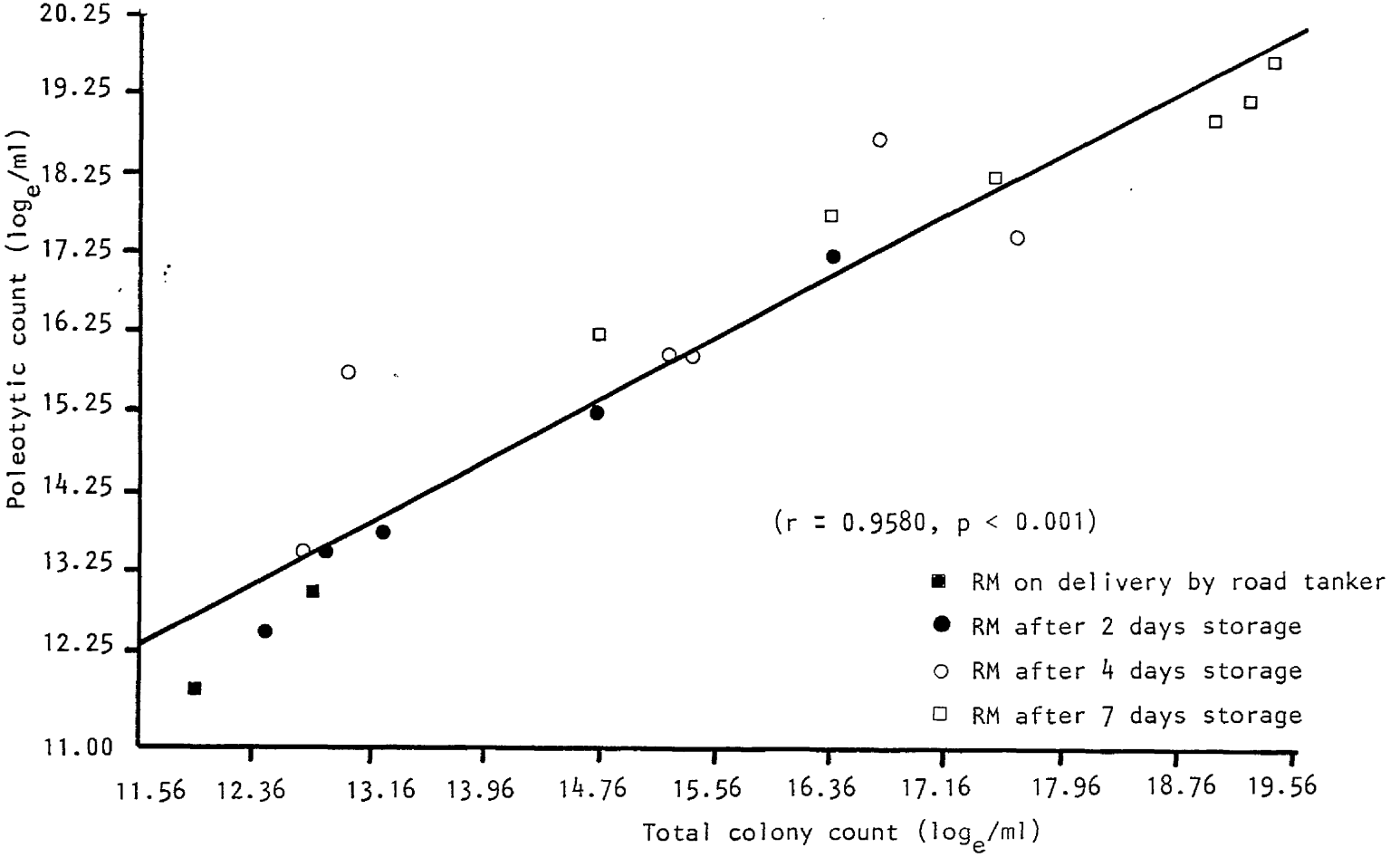


TABLE 6.5

Regression analysis of $\log_e$ lip[9] on $\log_e$ tcc[10]

	$\log_e$ lip			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of $\log_e$ lip	-2.6086	0.8749	-2.98	0.7332***
Slope of $\log_e$ tcc	1.1212	0.0764	14.67	

The regression equation is

$$\log_e \text{ lip} = -2.6086 + 1.1212 \times \log_e \text{ tcc}$$

Analysis of variance

	DF	SS	MS
REgression	1	322.1	322.062
Residual	82	122.7	1.496
Total	83	444.7	5.358
Change	-1	-322.1	322.062

% variance accounted for 72.1

[9] = $\log_e$ of lipolytic count for the pasteurized milk produced from raw milk on delivery and after different periods of storage at 2°C and 6°C and after further storage of the pasteurized milk for 3, 6 and 9 days at 4°C

[10] = $\log_e$ of total colony count for the pasteurized milk produced from raw milk during different periods of storage at 2°C and 6°C and after further storage of the pasteurized milk for 3, 6 and 9 days at 4°C

***significant at 0.1 per cent level

Fig. 6.5 The standard curve of the relation between the lipolytic count of pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The pasteurized samples were stored for 3, 6 and 9 days at 4°C

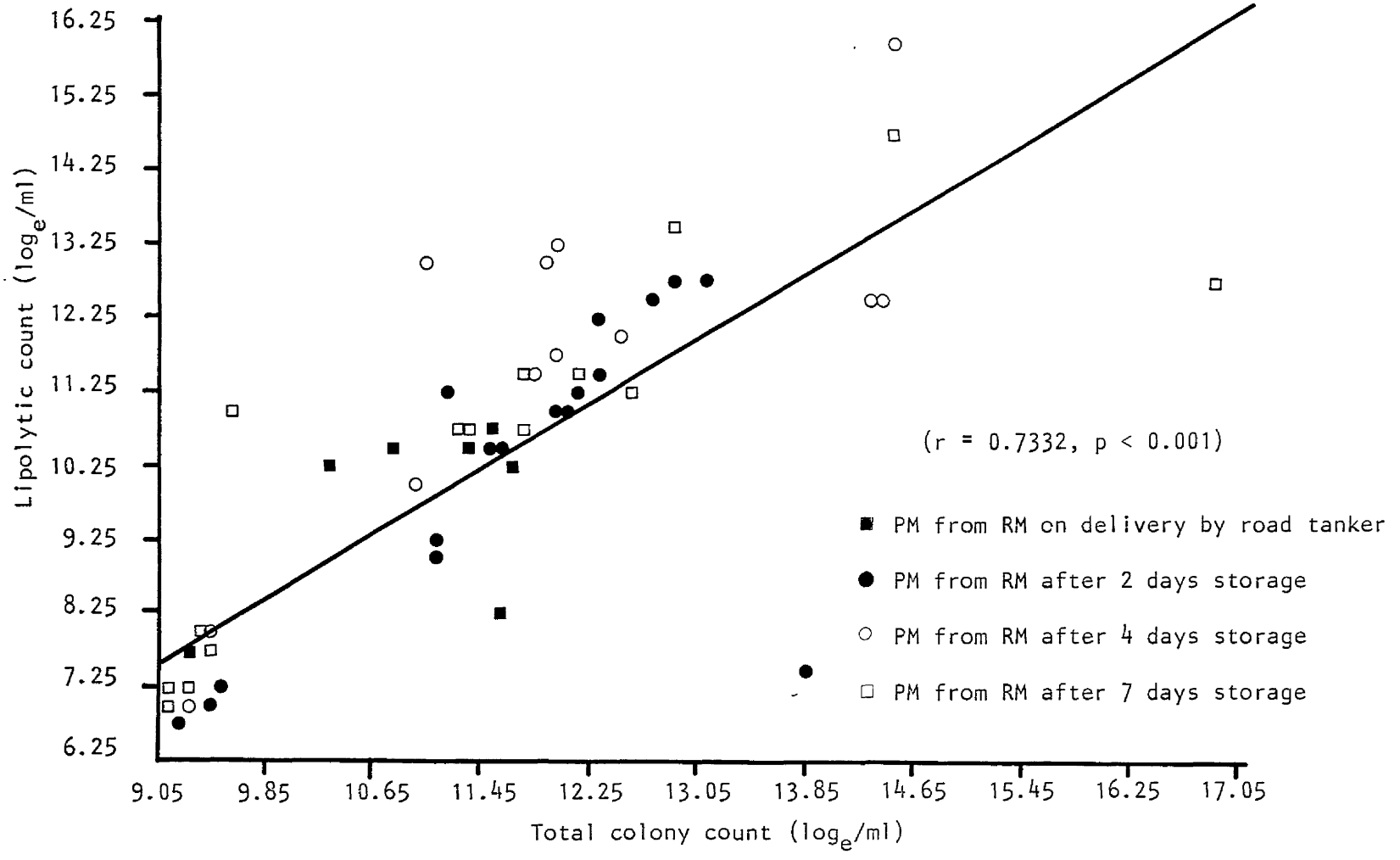


TABLE 6.6

Regression analysis of $\log_e$ prot[11] on $\log_e$ tcc[12]

	$\log_e$ Prot			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of $\log_e$ prot	-0.2932	0.8355	-0.35	0.9444***
Slope of $\log_e$ tcc	0.9810	0.0729	13.44	

The regression equation is

$$\text{Log}_e \text{ prot} = -0.2932 + 0.9810 \times \log_e \text{tcc}$$

Analysis of variance

	DF	SS	MS
Regression	1	246.6	246.594
Residual	82	111.9	1.364
Total	83	358.5	4.319
Change	-1	-246.6	246.594

% variance accounted for 68.4

[11]= Log_e of proteolytic count for the pasteurized milk produced from raw milk on delivery and after different periods of storage at 2^oC and 6^oC and after further storage of the pasteurized milk for 3, 6 and 9 days at 4^oC

[12]= Log_e of total colony count for the pasteurized milk produced from raw milk during different periods of storage at 2^oC and 6^oC and after further storage of the pasteurized milk for 3, 6 and 9 days at 4^oC

***significant at 0.1 per cent level

Fig. 6.6 The standard curve of the relation between the proteolytic count and total colony count of pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The pasteurized samples were stored for 3, 6 and 9 days at 4°C.

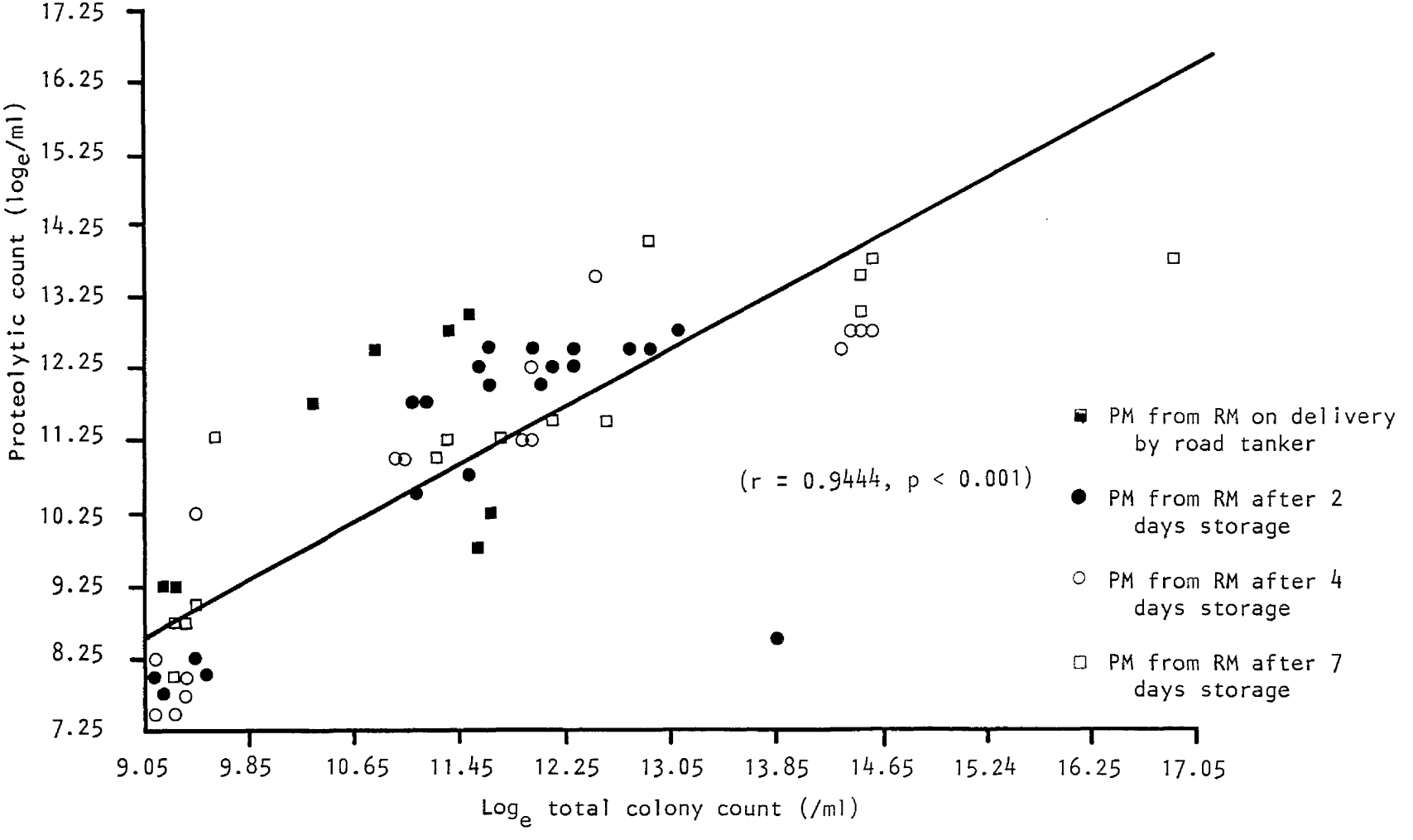


TABLE 6.7

Regression analysis of odour scores[13] on $\log_e$ prot[14]

	Odour Scores			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of odour scores	12.1528	2.1789	5.58	-0.8403*
Slope of $\log_e$ prot	-0.4770	0.1376	-3.47	

The regression equation is

Odour Scores = 12.1528 + (-0.4770) x $\log_e$ prot

Analysis of variance

	DF	SS	MS
Regression	1	9.031	9.0308
Residual	5	3.758	0.7516
Total	6	12.789	2.1314
Change	0	-2.384	0

% variance accounted for 64.7

[13] = The odour scores of raw milk on delivery and after different periods of storage at $2^{o}C$ and $6^{o}C$ and the pasteurized milk produced from these raw milks and pasteurized milk after storage for 3, 6 and 9 days

[14] = Log_e of proteolytic count of raw milk during different periods of storage at $2^{o}C$ and $6^{o}C$ and the pasteurized milk produced from these raw milk and pasteurized milk after storage for 3, 5 and 9 days

*significant at 5 per cent level

Fig. 6.7 The standard curve of the relation between the odour scores and the proteolytic count of raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for the pasteurized milks produced from corresponding raw milk were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

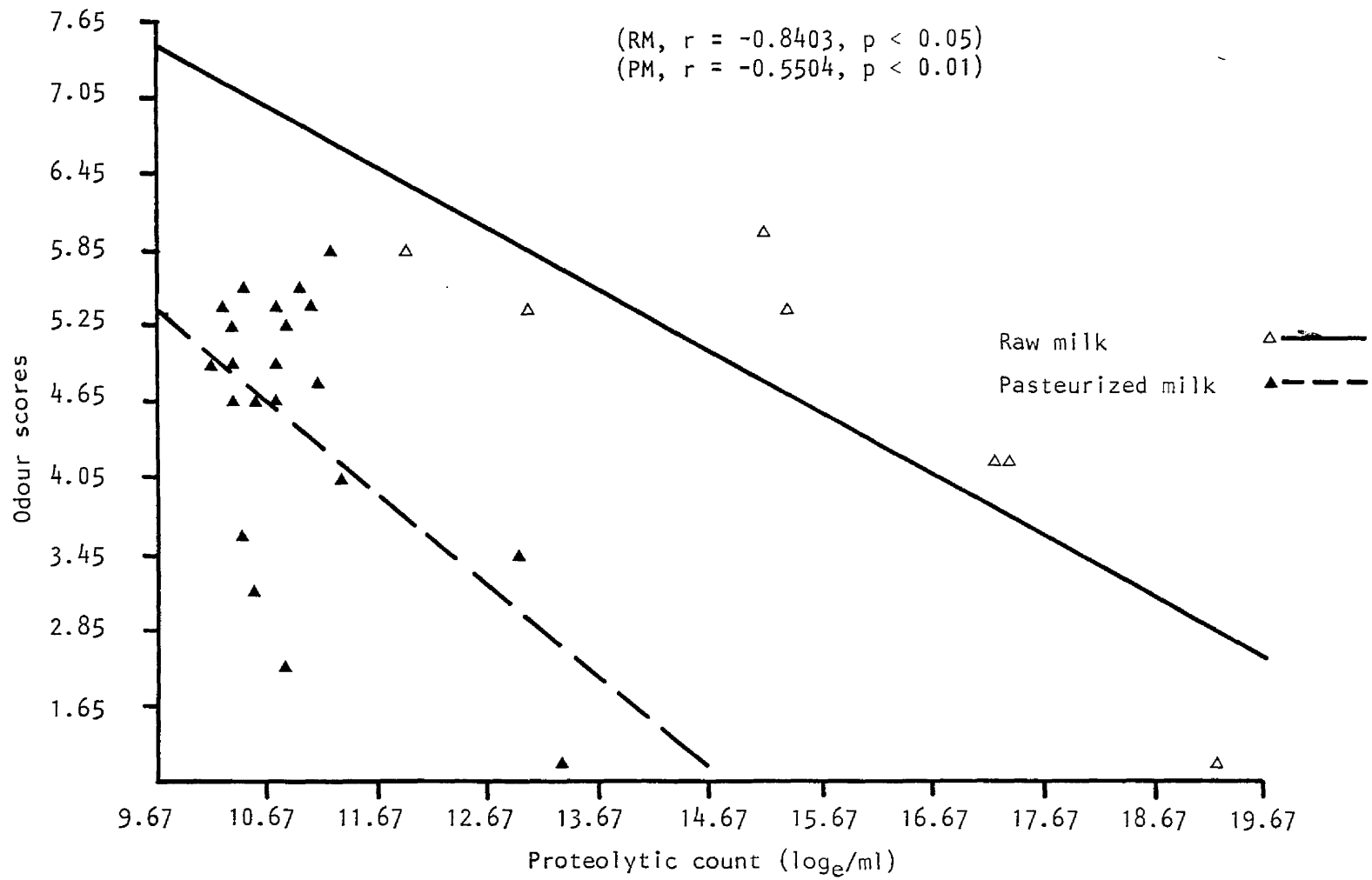


TABLE 6.8

Regression analysis of flavour[15] scores on ADV[16]

	Flavour scores			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of flavour scores	8.36306	1.48769	5.62	-0.5375**
Slope of ADV	-0.00268	0.00082	-3.25	

The regression equation is

Flavour scores = 8.36306 + (-0.00268) x ADV

Analysis of variance

	DF	SS	MS
Regression	1	11.74	11.740
Residual	26	28.90	1.111
Total	27	40.64	1.505
Change	-1	-11.74	11.740

% variance accounted for 26.2

[15] = The flavour scores of pasteurized milk produced from raw milk on delivery by road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{o}C$)

[16] = The acid degree value of the corresponding milks referred to above.

**significant at 1 per cent level

Fig. 6.8 The standard curve of the relation between the flavour scores and the acid degree value of pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

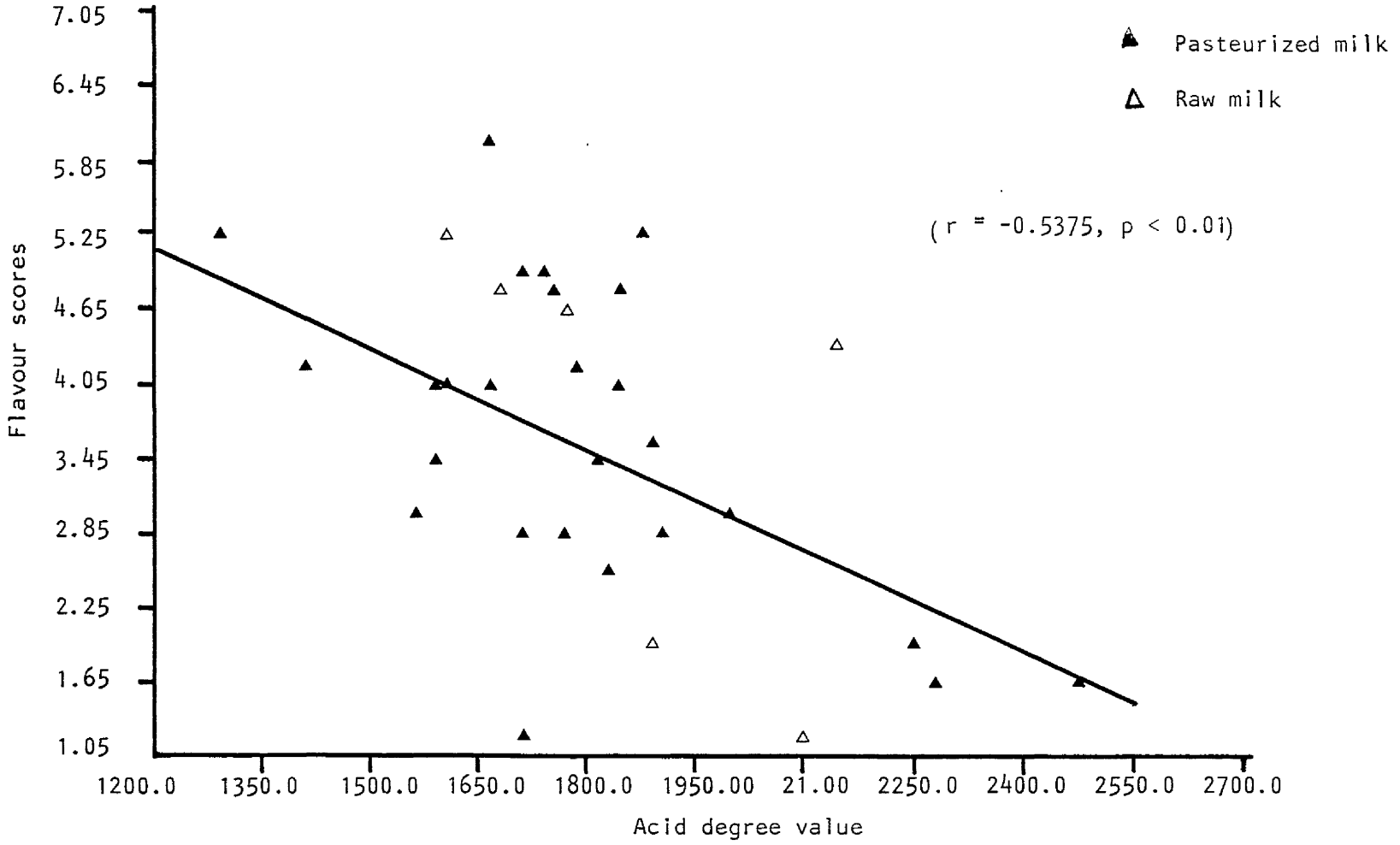


TABLE 6.9

Regression analysis of flavour[17] on $\log_e$ prot[18]

	Flavour scores			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of flavour scores	14.4772	2.6589	5.44	-0.6277***
Slope of $\log_e$ prot	-1.0091	0.2454	-4.11	

The regression equation is

$$\text{Flavour scores} = 14.4772 + (-1.0091) \times \log_e \text{prot}$$

Analysis of variance

	DF	SS	MS
Regression	1	16.01	16.0105
Residual	26	24.63	0.9472
Total	27	40.64	1.5051
Change	0	-4.27	0

% variance accounted for 37.1

[17] = The flavour scores of pasteurized milk produced from raw milk on delivery by road tanker and after storage at $2^{\circ}C$ and $6^{\circ}C$ for 2, 4 and 7 days. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{\circ}C$)

[18] = Log_e of proteolytic count of the corresponding milks referred to above.

***significant at 0.1 per cent level

Fig. 6.9 The standard curve of the relation between the flavour scores and the proteolytic count of pasteurized milk produced from raw milk on delivery by road tanker and after storage for 2, 4 and 7 days at 2°C and 6°C. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

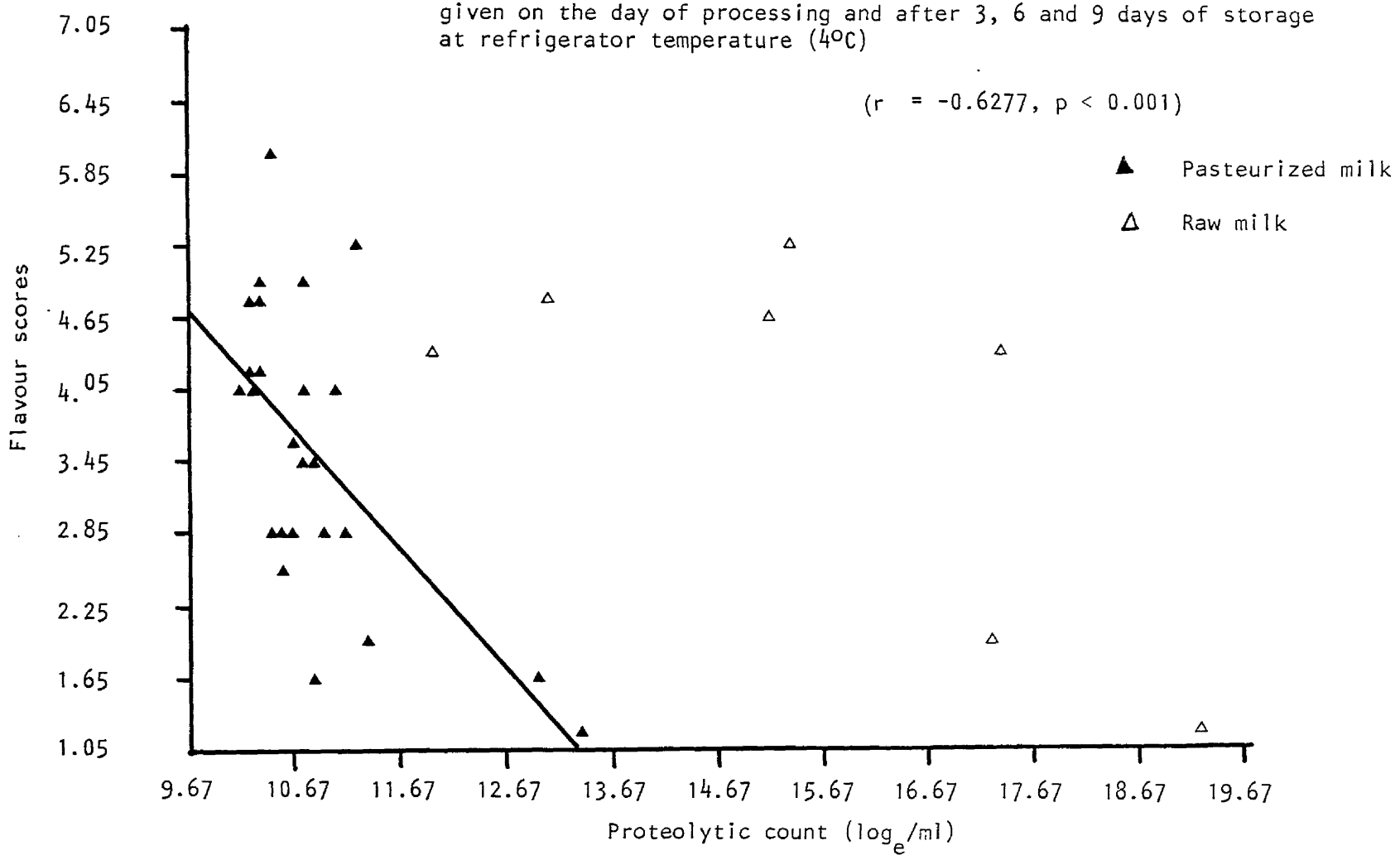


TABLE 6.10

Regression analysis of odour scores[19] on ADV[20]

	Odour scores			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of odour scores	7.93902	1.29099	6.15	-0.4552*
Slope of ADV	-0.00186	0.000716	-2.61	

The regression equation is

Odour scores = 7.93902 + (-0.00186) x acid degree value

Analysis of variance

	DF	SS	MS
Regression	1	5.69	5.6888
Residual	26	21.76	0.8370
Total	27	27.45	1.0167
Change	-1	-5.69	5.6888

% variance accounted for 17.7

[19] = The odour scores of pasteurized milk produced from raw milk on delivery by road tanker and after storage at $2^{o}C$ and $6^{o}C$ for 2, 4 and 7 days. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature ($4^{o}C$)

[20] = The acid degree value of the corresponding milks referred to above.

*significant at 5 per cent level

TABLE 6.11

Refression analysis of odour scores[21] on $\log_e$ prot[22]

	Odour scores			
	Regression			
	Estimate	SE	T	Correlation Coefficient
Y - intercept of odour scores	12.46340	2.34371	5.32	-0.5504**
Slope of $\log_e$ prot	-0.72723	0.21633	-3.36	

The regression equation is

Odour scores = 12.46340 x (-0.72723) x $\log_e$ proteolytic count

Analysis of variance

	DF	SS	MS
Regression	1	8.32	8.3161
Residual	26	19.13	0.7359
Total	27	27.45	1.0167
Change	0	-2.63	0

% variance accounted for 27.6

[21] = The odour scores of pasteurized milk produced from raw milk on delivery by road tanker and after storage at 2°C and 6°C for 2, 4 and 7 days. The scores for pasteurized milks were given on the day of processing and after 3, 6 and 9 days of storage at refrigerator temperature (4°C)

[22] = Log_e of the proteolytic count of the corresponding milk referred to above.

**significant at 1 per cent level

TABLE 6.12

Correlation coefficients between the organoleptic tests and the microbiological tests, the acid degree values and the free sulphydryl groups obtained from raw milk stored at 2°C and 6°C for 2, 4 and 7 days

Appearance	1	1.0000										
Odour	2	0.3422	1.0000									
Flavour	3	0.2721	0.8513*	1.000								
Total scores	4	0.4393	0.9567***	0.9512***	1.0000							
Log_e of total colony count	5	0.0366	-0.9029***	-0.7469	-0.8052*	1.0000						
Log_e of coliform count	6	-0.0980	-0.9554***	-0.8372*	-0.8946**	0.9846***	1.0000					
Log_e of lipolytic count	7	0.2335	-0.7907*	-0.6500	-0.6753	0.9594***	0.9228**	1.0000				
Log_e of proteolytic count	8	0.1498	-0.8403*	-0.7081	-0.7400	0.9761***	0.9513***	0.9890***	1.0000			
Log_e of thermoduric count	9	0.0946	-0.8318*	-0.7693*	-0.7746*	0.9451**	0.9329**	0.9211**	0.9650***	1.0000		
Acid Degree Value	10	0.2075	-0.7209	-0.5138	-0.5766	0.8333	0.8075	0.9259	0.8961	0.7692	1.0000	
Free Sulphydryl Groups	11	0.1965	-0.0784	0.1860	0.0878	0.1496	0.1101	0.2783	0.1560	-0.1077	0.4999	1.0000
		1	2	3	4	5	6	7	8	9	10	11

DF = 5

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 6.13

Correlation coefficients between the organoleptic tests, the microbiological tests, acid degree value and total sulphydryl groups of the pasteurized milk obtained from raw milk stored at $2^{o}C$ and $6^{o}C$, the pasteurized samples were stored at $4^{o}C$ for 3, 6 and 9 days after processing

Appearance	1	1.000									
Odour	2	0.5612**	1.0000								
Flavour	3	0.4209*	0.7929***	1.0000							
Total scores	4	0.7378	0.9235***	0.8931***	1.0000						
Log_e of total colony count	5	-0.3081	-0.5914**	-0.5607**	-0.5800**	1.0000					
Log_e of lipolytic count	6	-0.3216	-0.6896***	-0.6545***	-0.6639***	0.8830***	1.0000				
Log_e of proteolytic count	7	-0.2542	-0.5504**	-0.6277***	-0.5779**	0.7950***	0.8025***	1.0000			
Log_e of thermoduric count	8	-0.2986	-0.3654	-0.4159*	-0.4264*	0.7483***	0.7160***	0.5091**	1.0000		
Acid Degree Value	9	-0.1879	-0.4552*	-0.5375**	-0.4780*	0.6752***	0.6648***	0.5069**	0.8375***	1.0000	
Free Sulphydryl Groups	10	0.3579	-0.0113	-0.4058*	-0.0684	0.0651	0.0121	0.2459	-0.1120	0.0984	1.0000
		1	2	3	4	5	6	7	8	9	11

DF = 26

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

TABLE 6.14

Correlation coefficients between the organoleptic tests and the microbiological tests, acid degree value and the free sulphydryl groups of all the milks (raw and pasteurized) used in this trial

Appearance	1	1.0000										
Odour	2	0.4223**	1.0000									
Flavour	3	0.3550**	0.8078***	1.0000								
Total scores	4	0.6832***	0.9046***	0.8932***	1.0000							
Log_e of total colony count	5	0.3638*	-0.4118*	-0.3090	-0.1688	1.0000						
Log_e of coliform count	6	0.4007*	-0.2157	-0.2443	-0.0503	0.7236***	1.0000					
Log_e of lipolytic count	7	0.3963*	-0.3826*	-0.2897	-0.1371	0.9751***	0.7351***	1.0000				
Log_e of proteolytic count	8	0.4334**	-0.3244	-0.2613	-0.0884	0.9683***	0.7988***	0.9767***	1.0000			
Log_e of thermoduric count	9	0.0432	-0.3134	-0.3389*	-0.2571	0.6360***	0.2375	0.6243	0.5585***	1.0000		
Acid Degree Value	10	-0.1235	-0.5336***	-0.5313***	-0.4916**	0.4080*	0.0196	0.4053*	0.3205	0.6727***	1.0000	
Free Sulphydryl Groups	11	0.2764	-0.0237	-0.2977	-0.0448	0.0398	0.3155	0.0414	0.0832	-0.1025	0.1632	1.0000
		1	2	3	4	5	6	7	8	9	10	11

DF = 33

*significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

DISCUSSION

The results of this study showed that the total colony count was positively correlated with the psychrotrophic count, coliform count, lipolytic count, proteolytic count, thermoduric count, acid degree values and free sulphydryl groups. All the counts stated above and the acid degree values and free sulphydryl groups correlated negatively with the scores for the appearance, odour, flavour and total scores of the raw milk and pasteurized milk. This means that during the storage time at different temperatures it is likely that these organisms may be responsible for deterioration of flavour scores and for causing flavour defects and affecting the keeping quality of the milk.

Storgards (1961) in his study on the relationship between quality of producer's milk and market milk found a significant correlation (-0.63) between the occurrence of coliforms on delivery from the dairy and the keeping quality, by organoleptic determination of milk kept after delivery for 2 days at $5^{o}C$, and then for 1 day at $17^{o}C$.

The results of the present study agree with the results of Patel & Blankenagel (1972), who found that when raw milk was held at $7^{o}C$ for up to four additional days from the initial day before pasteurization, the bacterial population increased significantly. Although at the time of processing no objectionable flavours were detected in such milk, the pasteurized product developed flavour defects during subsequent storage at $7^{o}C$. Standard plate counts (SPC) of > 1 million per ml in raw milk frequently resulted in off-flavours of various types and when the SPC exceeded 10 million per ml, bitterness accounted for nearly all of the flavour defects.

The lipolytic count of the pasteurized milks (Table 6.13) showed a highly significant correlation with the odour, flavour and the total scores of the milk being respectively $r = -0.6896$, $p < 0.001$; $r = -0.6545$, $p < 0.001$; $r = -0.6639$, $p < 0.001$. That means during the storage time the lipolytic bacteria increased in numbers and caused a flavour defect in the product. This result is in agreement with Deeth & Fitz-Gerald (1976) who stated that many of the micro-organisms which contaminate dairy products are lipolytic, i.e., they produce

lipase, and can contribute to the development of rancid flavours. The most common sources of these lipases are the psychrotrophic bacteria, those which grow at refrigeration temperatures. The numbers of these bacteria in milk increase during storage and may produce significant amounts of lipase after about three days. The lipases are not inactivated by pasteurization even though the organisms which produce them are destroyed. They can therefore be carried through in an active form into manufactured products and cause fat break-down during storage of these products.

Hankin et al. (1977) reported that pasteurized milk stored at 1.7, 5.6 and 10.0°C remained organoleptically acceptable on the average for 17.5, 12.1 and 6.9 days respectively. They found a significant correlation between keeping quality at 10.0°C storage and the other two storage temperatures. When samples were arranged by flavour defect, certain microbial tests were significantly related to keeping quality.

The results in Table 6.13 show that the free sulphydryl groups gave a significant correlation with the flavour of the pasteurized milk ($r = -0.4058$, $p < 0.05$). However, there was no significant correlation between the SH contents and the quality of raw milk. There appears to be a lack of information on the effect of SH-level on the flavour and odour of pasteurized milk. In relation to UHT milk Shipe et al. (1978) stated that milk which has been exposed to 135 to 150°C for several seconds exhibits a strong sulfurous or cooked odour immediately after heat treatment, but after several days of refrigerated storage, the sulphurous odour dissipates and a rich or heated odour is perceptible.

CONCLUSIONS

1. The results obtained in this study showed that the microbiological counts (total colony count, coliform, psychrotrophic, lipolytic, proteolytic, thermoduric) correlated positively with each other and negatively with the appearance, odour, flavour and total scores of the milk.

2. It is concluded that the correlation coefficient between the quality assessments of raw and pasteurized milk, the microbiological counts

and the chemical analysis showed different degrees of significance ($p < 0.05$, $p < 0.01$, $p < 0.001$). The effect of different organisms on flavour defects was discussed in Chapter Four.

3. No significant correlation was found in the free sulphydryl groups of the raw milks stored at $2^{o}C$ and $6^{o}C$ for up to 7 days with the odour and the flavour of the raw milk. The free sulphydryl groups for pasteurized milks produced from the corresponding raw milk gave a significant correlation ($p < 0.05$) with the flavour of the milks.

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