EFFECTS OF LOW TEMPERATURE STORAGE AND THERMISATION ON THE QUALITY OF RAW AND HEAT TREATED MILK

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Submitted for the degree of Ph.D. in the Faculty of Science in the University of Glasgow.
December 1988

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ACKNOWLEDGEMENTS

The author wishes to express his gratitude to the Jabatan Perkhidmatan Awam for financial support, to the University Pertanian Malaysia for granting the leave of abscence for the duration of the study and to Professor J.M.M. Cunningham, former Principal and Professor P.C. Thomas, the present Principal of the West of Scotland College for their support and encouragement.

The author is especially grateful to Dr. R.J.M. Crawford, former Head of the Department of Dairy Technology who contributed invaluable assistance, advice and supervision throughout. To Dr. V.N. Wade, the present Head of the Department of Food Technology, the author expresses thanks for the continuing support and encouragement.

Many thanks are also due to Mr. G. Davies for advice and help in the free fatty acids analysis, to Mr. C. Kelly, Mr. R. Dunlop and Mr. A. Allan for their assistance throughout the work; and to all the staff of the Computing and Information Technology Department, especially to Mr. D. P. Arnot for the computing and statistical assistance.

To all staff and fellow post-graduate students of the Department of Dairy Technology, the author expresses his gratitude for their friendship and companionship that have made his stay in Auchincruive an extremely wonderful experience.

To his parents and siblings, the author expresses his sincere recognition and gratitude.

SUMMARY

ON-FARM MILK QUALITY STUDIES

1.0 Three surveys were conducted approximately at three months apart to evaluate the quality of raw milk produced from dairy farms in the south-west of Scotland. The farms assinged to the study were within the scheduled route of the three road tankers supplied by the local bulk milk haulage contractor of the Scottish Milk Marketing Board. The sequence of milk collection by each road tanker during the first survey was noted and the sequence was repeated in the subsequent surveys. The number of farms taken to fill each road tanker to full capacity, limits the number of farms investigated. A total of 24 farms were included in the investigation.

1.1 The results of the investigations showed that the bacteriological quality of raw milk sampled from farms in the present study gave little cause for concern. The means of total plate count (9.8×10^3 cfu/ml), psychrotrophic bacteria count (2.2×10^3 cfu/ml) and thermoduric bacteria count (22 cfu/ml) compared favourably with earlier studies (Smillie *et al.*, 1958). No seasonal variations in bacterial counts were noted, but slight variations between farms were observed.

1.2 The temperature of milk taken from the farm bulk tank immediately before pick-up varied highly between the farms and seasons studied. Farms milk temperature of more than 6 ^{o}C was not uncommon and were frequently noted especially from farms with higher milk production.

1.3 The variations between farms and seasons in freezing point depression (-0.541 ^{o}C) and pH (6.72) of raw milk were slight, however, large seasonal variation in the percentage lactic acid content was observed. The percentage lactic acid was higher in December (0.154) than in March (0.137) or June (0.129). The mean free fatty acids content of milk sampled from the farms studied were 0.64 mEq/100 ml milk. No significant variations between farms and season were observed.

SIMULATED BULK MILK SILO STUDIES

2.0 A simulated study was conducted to measure the effects of blending and subsequent storage of raw milk at low temperatures on the quality of pasteurised milk made from it. Raw milk collected by the three road tankers from farms in the south-west of Scotland were delivered to the West of Scotland College and a proportion from each tanker was blended in a 1200 litres storage tank. The percentage of milk used for blending from each road tanker (44, 28 and

28 per cent) , was calculated based on the capacity of the road tanker and the final volume of the blended milk. The blended milk was then divided into 2 lots. Each lot was stored for 9 days either at 2 ^{o}C or 5 ^{o}C. The milks were subsequently pasteurised after 2, 4 and 7 days of raw storage.

2.1 The mean bacterial counts of milk sampled from the road tanker was slightly higher than the mean bacterial counts of the corresponding milk sampled on the farms. This was expected, the increase in handling, increased the surface area (potential source of contamination) that comes in contact with the milk. In addition, higher milk temperature during transport ($\geq$ 8 ^{o}C), the possible break-up of bacterial clumps through agitation during handling and the mixing of raw milks from various sources, all contributed to the higher counts of milk sampled from the road tanker. No seasonal variation was observed.

2.2 The mean total plate count and psychrotrophic bacteria count of the blended raw milk following storage at 2 ^{o}C was consistently lower than that stored at 5 ^{o}C. The differences in the bacterial counts were significant 2 days following storage and continued throughout the storage period. Taking the total plate count or psychrotrophic bacteria count of more than 10^{6} cfu/ml as the standard for deterioration of raw milk quality to occur, storage of raw milk at 2 ^{o}C was found to increase its *keeping*

quality by one day as compared to raw storage at 5 ^{o}C (4 days vs 3 days). The thermoduric bacteria count was unchanged throughout both storage temperatures.

2.3 The means of titratable acidity and free fatty acids content of raw milk stored at 2 ^{o}C were consistently lower than the raw milk stored at 5 ^{o}C. The changes in the titratable acidity value did not correlate with the increase in total plate count and the psychrotrophic bacterial count, for raw milks stored at both storage temperatures. However, the correlation between the changes in free fatty acids and the total plate count ($r = 0.92$), and between the free fatty acids and the psychrotrophic bacterial count ($r = 0.86$) were significant ($p < 0.01$) for milks stored at both storage temperatures. The correlation was stronger in milk stored at 5 ^{o}C than at 2 ^{o}C.

2.4 In the present study, the free fatty acids content of the raw milk stored at 2 ^{o}C remained unchanged initially but increased after 4 days and the free fatty acids of raw milk stored at 5 ^{o}C increased immediately following storage. The increase in the free fatty acids content of the raw milk during storage was observed to be dependent on the storage temperature. This was expected. The free fatty acids content of raw milks stored at 2 ^{o}C and 5 ^{o}C when measured after 4 days of storage were 0.58 mEq/100 ml

and 0.72 mEq/100 ml, respectively.

2.5 The length of raw storage before pasteurisation had no influence on the bacterial counts of the pasteurised milk. The total plate count was reduced to a common level in all the pasteurised milks regardless of the length of storage and bacterial load before pasteurisation. In addition, the psychrotrophic bacteria was effectively eliminated by pasteurisation. However, the raw milk storage temperature before pasteurisation was found to influence the subsequent increase in the bacterial counts. The increases in total plate count during storage of pasteurised milk prepared from raw milk stored at 2 ^{o}C was significantly lower than the raw milk stored at 5 ^{o}C before pasteurisation.

2.6 In the present study pasteurisation was found to cause a slight depression in the level of free fatty acids and tyrosine value, when the pasteurised milk was tested one day after pasteurisation. The reason for the depression could not be explained. The free fatty acids content and the tyrosine value of the pasteurised milk was observed to increase during subsequent storage. The hydrolysis of milk lipids was expected to continue throughout storage after pasteurisation due to the presence of heat-resistant endogenous as well as extracellular lipases. The level of free fatty acids before pasteurisation (0.64 mEq/100 ml) was reached after 5 days of storage when

the pasteurised milk was prepared from raw milk stored at 2 ^{o}C, and after 3 days when prepared from raw milk stored at 5 ^{o}C.

2.7 Storage of raw milk at low temperature causes the migration of calcium and phosphorous from the colloidal to the soluble phase. In the present study, the soluble calcium and soluble phosphorous contents of raw milk stored at 2 ^{o}C was slightly lower than that stored at 5 ^{o}C. In addition, thermisation of milk was found to reduce the level of soluble calcium and soluble phosphorous (during the subsequent storage) more than the storage at low temperatures (2 and 5 ^{o}C). Thermisation probably induces the readsorption of the soluble calcium and phosphorous back into the casein micelle.

2.8 In the present study the level of proteolysis in raw milk during storage, as measured by the level of TCA-N in milk, was found to be influenced by the storage temperature and the length of storage. The level of proteolysis for raw milk stored at 2 ^{o}C was consistently lower than the raw milk stored at 5 ^{o}C. The increase in the level of TCA-N was significant 3 days after storage. The TCA-N content of milk after 3 days of storage at 2 ^{o}C was 0.26 per cent and 0.23 per cent when stored at 5 ^{o}C. The TCA-N level of thermised milk during storage was lower than the raw milks stored either at 2 ^{o}C and 5 ^{o}C. The influence

of thermisation on the nitrogen distribution in milk during storage was similar to that of pasteurised milk. The TCA-N content of both the thermised and the pasteurised milks was 0.22 per cent

EFFECTS OF THERMISATION ON MILK QUALITY

3.0 Two preliminary trials were conducted to compare the effects of a range of thermisation heat treatments on bacterial counts and alkaline phosphatase content of milk. The heat treatments investigated were 55, 60, 63, 65 and 68 ^{o}C with 16 seconds holding time. Batches of milk were heated using a pilot scale HTST milk pasteuriser, at the rate of 500 litres/hour, in the order according to the severity of the heat treatments. The heated milk samples were taken using 200 ml sterile sample bottles and analysed on the same day, and subsequently, after 1, 2, 3, 4, 7, and 15 days of storage at 5 ^{o}C.

3.1 The results of bacteriological analysis of the heated milks indicated that all heat treatments investigated significantly ($p < 0.01$) reduced the level of total plate count, psychrotrophic bacteria count, the proteolytic and lipolytic bacterial counts, and completely eradicated the coliform bacteria. The effectiveness of the heat treatments showed distinct differences between the heating temperatures. Heat treatment at 65 ^{o}C for 16 seconds was found to be the

most effective of the heat treatments investigated and was recommended as the heat treatment of choice for thermisation and would be used in the subsequent studies because:-

3.1.1 it was the lowest of the heat treatments investigated which resulted in the effective eradication of coliform bacteria and keeping quality extension of 4 days of storage at 6 ^{o}C without the total plate count and the psychrotrophic bacteria count exceeding 10^{6} cfu/ml, and

3.1.2 it was the highest of the heat treatments investigated that retained the detectable level of alkaline phosphatase activity, as such, if thermisation was followed by pasteurisation, the second and more severe heat treatment does not constitute double pasteurisation.

THE EFFECTS OF EXTENDED RAW MILK STORAGE ON THE EFFECTIVENESS OF THERMISATION AND THE EFFECTS OF DOUBLE HEAT TREATMENTS ON PASTEURISED MILK QUALITY

4.0 Three trials were conducted to measure the effects of extended storage of raw milk for 2, 4 and 7 days at 5 ^{o}C on effectiveness of thermisation and the quality of pasteurised milk made from thermised milks stored for 2 and 4 days of storage at 6 ^{o}C were studied. The results showed that thermisation reduces the

total plate count and the psychrotrophic bacterial count to a common level regardless of the initial counts before thermisation. Significant differences in total plate count ($p < 0.01$) and psychrotrophic bacterial count ($p < 0.001$) of milk thermised after 2, 4 and 7 days of raw storage at 5 ^{o}C was observed. It showed that thermisation heat treatment was more effective when applied to raw milks after prolonged storage period (up to 7 days).

4.1 Thermisation significantly ($p < 0.01$) delayed the initiation of lipolysis and proteolysis of milk during storage. The increases in free fatty acids and tyrosine values of milk after thermisation were gradual. The level of free fatty acids (0.64 mEq/100 ml) of milk before thermisation was reached again after 3 days of storage and the initial tyrosine value (0.12 mg/ml) was reached after more than 7 days of storage.

4.2 In terms of total plate count and psychrotrophic bacteria count, there are no benefits to be gained when the thermised milks were pasteurised. The results of bacterial counts showed that pasteurisation alone was capable of reducing the total plate count and the psychrotrophic bacterial count of the pasteurised milk to a common level regardless of the initial counts before the heat treatments (thermisation and pasteurisation). However, thermisation was found to be effective in

reducing the lipolytic activity in the corresponding pasteurised milk during the subsequent storage.

FREE FATTY ACIDS PROFILE STUDIES

5.0 An attempt was made to establish the relationships between the bacterial counts and the quantitative changes in the free fatty acids profile of raw milks stored at 2 ^{o}C and 5 ^{o}C and thermised milk stored at 6 ^{o}C. The results showed that the relationships between total plate count and psychrotrophic bacteria count with the free fatty acids studied (C:4 - C18:2) were dependent on the milk storage treatments (temperature and the length of storage period).

5.1 The increase in the total plate count and the growth of the psychrotrophic bacteria during storage were both strongly correlated with the long chain fatty acids (C:14 - C18:2) when the raw milk was stored at 2 ^{o}C. No correlations between the bacterial counts with the short chain fatty acids (C:4 - C:12) were observed.

5.2 Storage of raw milk at 5 ^{o}C strengthened the correlations between the total plate count and the psychrotrophic bacteria count with all the fatty acids studied. In addition, the correlations were especially stronger with the short chain fatty acids (C:6 - C:12) as compared to the correlations with the

long chain fatty acids (C:14 - C18:2). The growth of psychrotrophic bacteria in milk stored at 5 ^{o}C correlated strongly with the increase in short chain free fatty acids (C:6 - C:12). These fatty acids (C:4 - C:12) have been implicated to be mainly responsible for the lipolytic off-flavour in milk.

5.3 The increase in total plate count of milk stored at 6 ^{o}C after thermisation correlated strongly with the short chain fatty acids (C:4 - C:10). However, these correlations were less strongly, as compared to that of raw milks stored at 2 ^{o}C and 5 ^{o}C. No correlation was observed between the fatty acids studied and the growth of psychrotrophic bacteria.

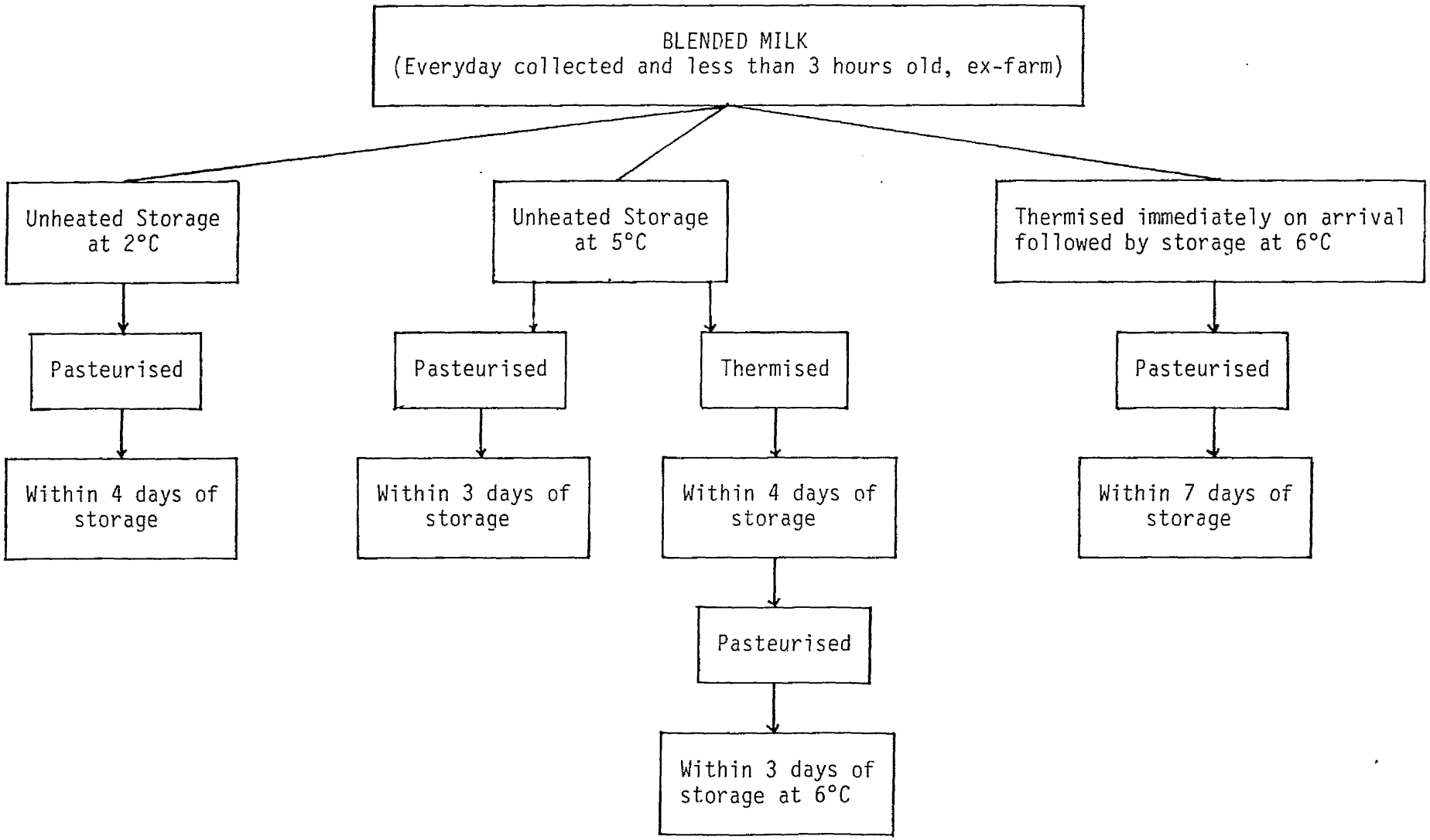
5.4 In the present study, slight depression in the total free fatty acids content of the pasteurised milk was observed due to the pasteurisation heat treatment. In addition, the storage temperature and the length of storage before pasteurisation were found to influence the changes in free fatty acids profile of the corresponding pasteurised milk during storage. The reason could not be explained. The short chain fatty acids (C:4 - C:12) of pasteurised milk prepared from raw milk stored for 2 and 4 days at 2 ^{o}C, remained unchanged during storage (at 5 ^{o}C), 15 days after pasteurisation. However, the long chain fatty acids (C:14 - C18:2) increased significantly ($p < 0.01$) following pasteurisation, regardless of the length of the raw storage period before pasteurisation. Further

investigations are recommended.

5.5 The free fatty acids content of pasteurised milk prepared from raw milk stored at 5 ^{o}C were found to be higher than that prepared from milk stored at 2 ^{o}C, irrespective of the length of raw storage period before pasteurisation. Nevertheless, the fatty acids profile of the pasteurised milk remained unchanged throughout storage after pasteurisation.

5.6 The fatty acids content of the pasteurised milk prepared from thermised milk were lower than those prepared from raw milks stored at 2 ^{o}C and 5 ^{o}C. The free fatty acids content of pasteurised milks prepared from thermised milk remained unaltered throughout storage for 21 days. Further investigations are recommended on the effects of pasteurisation following thermisation on the free fatty acids profile of the pasteurised milk and their influence on flavour.

RECOMMENDATIONS



LIST OF ABBREVIATIONS

The standard abbreviations recommended by the British Standards Institution BS: 1991 Part 1 (1976) are used throughout the thesis, in addition to the following:-

AOAC	=	Association of Analytical Chemists
AP	=	Alkaline phosphatase
C:4	=	*n*-Butyric acid
C:6	=	*n*-Hexanoic (-caproic) acid
C:8	=	*n*-Octanoic (-caprylic) acid
C:9	=	*n*-Nonanoic acid
C:10	=	*n*-Decanoic (-capric) acid
C:12	=	Lauric acid
C:14	=	Myristic acid
C:16	=	Palmitic acid
C18:0	=	Stearic acid
C18:1	=	Oleic acid
C18:2	=	Linoleic acid
cfu	=	Colony forming unit
CS	=	Cold storage at 5 ^{o}C
CST	=	Thermisation immediately on arrival
CS_T	=	Thermisation after CS storage (day)
CS2TCS4T	=	Thermisation 2 days after CS and thermisation 4 days after CS
DC	=	Deep cooling at 2 ^{o}C
DCP_	=	Pasteurised after DC storage (day)
df	=	Degree of freedom
EC	=	Enzyme Commission of the International Union of Biochemistry
F	=	Variance ratio
FFA	=	Free fatty acids
FPD	=	Freezing point depression
HTST	=	High temperature short time
IDF/FIL	=	International Dairy Federation
LA	=	Lipolytic activity
LPS	=	Lactoperoxidase system
mEq	=	milliequivalent

MLBC	=	Mesophilic lipolytic bacterial count
MMB	=	Milk Marketing Board for England and Wales
MPBC	=	Mesophilic proteolytic bacterial count
MS	=	Treatment mean square
MSE	=	Error mean square
NCN	=	Non-casein nitrogen
NPN	=	Non-protein nitrogen
PN	=	Protein nitrogen
PBC	=	Psychrotrophic bacterial count
PLBC	=	Psychrotrophic lipolytic bacterial count
PPBC	=	Psychrotrophic proteolytic bacterial count
SED	=	Standard error of differences of means
SMCA	=	Standard methods caseinate agar
SMMB	=	Scottish Milk Marketing Board
SNF	=	Solids-not-fat
SS	=	Treatment sum of squares
TA	=	Titratable acidity
TCA	=	Trichloroacetic acid
TCA-N	=	Trichloroacetic acid soluble nitrogen
TBC	=	Thermoduric bacterial count
TN	=	Total nitrogen
TPC	=	Total plate count
TS	=	Total solids
TV	=	Tyrosine value
UHT	=	Ultra heat treated

INTRODUCTION

A trend is developing within the dairy industry in which raw milk is held longer on the farm and at the factory, before it is processed. The widespread acceptance and use of the farm bulk storage tanks by the milk producers (Crawford, 1967), the collection and delivery of milk by large road tankers (Cox, 1975) and the changing frequency of collection from everyday collection practices to alternate day collection (Thomas and Thomas, 1973a) especially on the longer more costly routes, have significantly extended the storage of the raw milk on the farm. The gross over suppply during the period of the spring flush may result in milk not being processed for some time after receipt. Alternatively, the daily supply of milk to the individual processing centre may be inadequate for economic operation of the processing plant and has resulted in problems of stock milk rotation in the SMMB area (SMMB, 1986) and probably in most of the MMBs in the United Kingdom. Consequently, raw milks are transported over long distances or stored for a few days to accumulate a sufficiency for processing. In addition, the changed working practices at the processing plant, including the introduction of a five day working week (Zall and Chen, 1983) exacerbate the difficulties faced by the dairy industry. Consequently, raw milks may be held for extended periods in the factory.

The extended storage of raw milk at refrigeration temperature is selective to the growth of psychrotrophic

microorganisms (Witter, 1961). These microorganisms are present in most milk supplies (Stadhouders, 1982) and are capable of growth at low temperature (Morita, 1975). They can cause direct deterioration of milk quality when present in large numbers (Cousin, 1982).

While the majority of these microorganisms may be eliminated by the conventional methods of milk pasteurisation, their extracellular enzymes such as proteases (Adams *et al.*, 1975; Griffiths *et al.*, 1981 and Fairbairn and Law, 1986) and lipases (Driessen and Stadhouders, 1974; Andersson *et al.*, 1981; and Bozoglu *et al.*, 1984) are not only capable of hydrolysing the milk lipids and protein, but are also thermostable (Cogan, 1977) thus surviving heat treatments such as pasteurisation (Elliker *et al.*, 1964) or the more severe heat treatment such as UHT heat treatment (Adams *et al.*, 1976; Corradini and Pecis, 1979 and Andersson *et al.*, 1981). During the extended raw milk storage, these enzymes may accumulate in the milk and survive the heat treatments at levels which can deleteriously affect the quality and shelf life of the final products.

In addition, some of the psychrotrophs are themselves heat resistant (Collins, 1981) and some are sporeformers, capable of resisting pasteurisation. The spores would subsequently germinate and grow (Coghill, 1982); hence, affecting the shelf life of the final products.

The dominance of psychrotrophic microorganisms in the milk supply has posed a new technological problem to the

dairy industry. To eliminate their presence in the raw milk supply is unattainable and an expensive proposition (Thomas and Thomas, 1973a). Notwithstanding, several methods have been proposed to inhibit their growth in the raw milk supply, to such a degree that minimised their deleterious effects on milk and products quality.

Juffs and Babel (1975) suggested the addition of lactic starter cultures to raw milk immediately after milking followed by cold storage. However, the addition of bacteria to milk would result in increased counts and subsequently, lead to flavour defects in certain milk products (Stadhouders, 1982).

Bjorck *et al.*, (1975) and in a subsequent study, Bjorck (1978) suggested the activation of the natural lactoperoxidase system in milk, but with mixed results due to the variability in the lactoperoxidase system's components.

The use of food additives and preservatives such as sorbates, propionate and benzoates (Moustaffa and Collins, 1969 and Robach, 1978) have been shown to control the growth of psychrotrophic bacteria. However its application is selective only to some dairy products (Mistry and Kosikowski, 1985). In addition, the presence of additives in liguid milk are unlikely to be acceptable to the consumer.

Perhaps, the most effective method of reducing the bacterial load of milk is by heat treatment (Griffith *et al.*, 1986). Sub-pasteurisation heat treatment has been applied to milk on receipt at the creamery (Stadhouders,

1982 and Johnston *et al.*, 1981) and at the farm (Zall, 1980; Zall and Chen, 1984). The heat treatment has been termed thermisation (Casalis, 1958 and van den Berg, 1984) and has been used in the manufacture of skim milk (West *et al.*, 1986), Cheddar cheese (Karlikanova, 1977 and Banks *et al.*, 1986) and Cottage cheese (Dzurec and Zall, 1982).

The purpose of the present study was to evaluate the effects of low temperature storage (2 ^{o}C and 5 ^{o}C), and thermisation on milk quality.

CHAPTER ONE

SECTION 1.1 PRESENCE AND ACTIVITIES OF PSYCHROTROPHIC MICROORGANISMS IN MILK

Milk processing operations are becoming increasingly centralised and raw milk is held longer before processing. Control of bacterial growth during the storage periods depends primarily on good hygiene and refrigeration. However, psychrotrophic bacteria which are present in most milk supplies (Cousin, 1982) can grow readily at the refrigeration temperature. With longer storage, psychrotrophic bacteria are becoming even more important than in the past to the quality of fluid and other dairy products.

They are now the most commonly encountered spoilage organisms in the dairy industry (Cousin, 1982). From a typical initial population in milk of 10^4 cfu/ml, the psychrotrophic count can exceed 10^6 cfu/ml after 2 or 3 days of storage at 5 ^{o}C (Law, 1979) and subsequently becomes dominant. Unfortunately, storage of raw milk for 4 days or longer before pasteurisation was not uncommon due to social, economics and other reasons (Muir *et al.*, 1978).

1.1.0 Terminology

The definition for organisms that can grow at temperatures close to 0 ^{o}C have confused microbiologists. Since the discovery by Forster (1887 and 1892) (cited by Cousin, 1982), of organisms that grow at low temperatures, various terminologies have been used to describe them. Unlike the terms mesophile and thermophile which defined microorganisms according to their optimum temperatures for growth (Eddy, 1960), psychrophiles have been defined, in addition to their optimum temperature for growth, according to their ability to grow at low temperatures (Ingraham and Stokes, 1959), methods of enumeration (Stokes, 1963) and according to other criteria unrelated to temperature (Witter, 1961).

To define these organisms as psychrophile (derived from Greek *psychros*, meaning cold and *philos*, meaning loving) (Schmidt-Nielson, 1902, as cited by Thomas and Thomas, 1973b) was misleading; because their optimum temperature for growth was higher. Morita (1975) observed an optimum growth temperature of between 15-25 ^{o}C even though the minimum growth temperature was lower.

Stokes (1963) suggested the term psychrophile should only refer to those microorganisms that showed formation of colonies within one week at 0 ^{o}C on solid media. In an earlier study (Ingraham and Stokes, 1959) it was recognised that some bacteria grow well at 0 ^{o}C and at temperatures above 20 ^{o}C. They termed these microorganisms as facultative psychrophiles, and reserved the term

obligative psychrophiles to those that not only grow rapidly at 0 ^{o}C but also grow most rapidly at or lower than 20 ^{o}C. The definition proposed by Stokes (1963) encompasses all microorganisms that cause food spoilage at low temperatures and seems the most convenient to use. The term obligative psychrophiles and facultative psychrophiles have a reasonably precise meaning and distinguished between psychrophiles with low and high minimum temperatures of growth. This subdivision is useful and is familiar to most microbiologists.

Morita (1975), extended the definition for psychrophiles to cover all organisms having an optimum growth temperatrure of 15 ^{o}C, a maximum growth temperature of 20 ^{o}C and a minimum growth temperature of 0 ^{o}C or lower.

Other terms that described the ability of the psychrophiles to grow at lower temperature have also been proposed. According to Kruse (1910) (cited by Cousin, 1982) used the term psychro-cartericus (derived from Greek *carterícus*, meaning cold conquering); but, Horowitz-Wlassowa and Grinsberg (1933) (cited by Cousin, 1982) suggested the term psychrotolerant or cold tolerant and Zobell (1934) (cited by Cousin, 1982) used the term eurythermic or capable of growing over a wide range of temperatures. Nevertheless, these descriptions having proven unrealistic, therefore, gained little support (Cousin, 1982).

Eddy (1960) proposed the term psychrotrophic (derived from Greek *trophos*, meaning to flourish) as first used in

1902 by Schmidt-Neilsson (cited by Cousin, 1982), to describe organisms that grow significantly at low temperatures. Morita (1975) and Cousin (1982) supported the definition and Morita (1975) contended that those mesophiles that could grow at 0 ^{o}C or lower were correctly termed psychrotrophic and were capable of growth on solid media at 7 ^{o}C or below, regardless of their optimum temperature for growth.

In the dairy industry, the term psychrophile was used to describe those microorganisms having an optimum temperature for growth at 15 ^{o}C, maximum temperature for growth of 20 ^{o}C and a minimum temperature for growth of 0 ^{o}C or lower as used by Morita (1975). Psychrotrophic microorganisms were defined as those microorganisms that grow at 7 ^{o}C or lower in a solid media regardless of their optimum temperature for growth (Thomas and Thomas 1973b). These definitions were used throughout the present study.

1.1.1 **Types of psychrotrophic bacteria**

Psychrotrophic microorganisms were found in many genera; most frequently they were Gram-negative, rod-shaped bacteria of the genus *Pseudomonas* (Witter, 1961 and Cousin, 1982). *Pseudomonas geniculata* was the most frequently isolated psychrophilic organisms (Thomas and Thomas, 1973a) together with *Pseudomonas putrefaciens*, *Pseudomonas fragi* and *Pseudomonas fluorescens* (Witter, 1961). Also fairly common were psychrophilic strains of

Flavobacterium, *Achromobacter*, *Alcaligenes* and *Arthrobacter* (Cousin, 1982). Psychrophilic strains of *Escherichia*, *Proteus* and *Serratia* (Witter, 1961) had also been described.

The psychrotrophs found in refrigerated bulk milk were reported to be mainly Gram-negative rod-shaped bacteria of the genera *Pseudomonas*, *Achromobacter*, *Alcaligenes* and *Enterobacter* (Thomas *et al.*, 1973a and Shelley *et al.*, 1986). However, Gram-positive psychrotrophs of the genus *Bacillus* have also been isolated (Grosskopf and Harper, 1969; and Collins, 1981).

Thomas and Thomas, (1973b) studied the distribution of psychrotrophic microflora of refrigerated raw milk. They reported, that most common were Gram-negative rods which did not survive pasteurisation. *Pseudomonas* was the most dominant genus present with *Acinetobacter* (*Achromobacter/ Alcalienes*), *Flavobacterium* and *Enterobacter* consistently present but in less frequency. The dominance of the *Pseudomonas* species in milk, increased during cold storage at 5-7 ^{o}C. The increases were marked in bulk collected milk held at higher temperatures.

Nakae (1970) made similar observation. From the total psychrotrophs isolated from raw milk, the majority were identified as *Pseudomonas* strains which grow well both at 5 ^{o}C and 20 ^{o}C and the psychrotrophic strains of *Enterobacter*, *Achromobacter* and *Alcaligenes* were not infrequently present.

1.1.2 Sources and incidence of psychrotrophic bacteria in milk

Psychrotrophic microorganisms are ubiquitous in nature. They were found in temperate and polar regions (Cousin, 1982), on land, in water, and in a large variety of plants and animals (Morita, 1975), and in milk (Thomas and Thomas, 1973a). They exist as bacteria (Witter, 1961), yeast and moulds (Cousin, 1982) and were taxonomically similar to mesophiles (Shelley *et al.*, 1987). Although a large proportion of the psychrophilic bacteria were Gram-negative rods, Gram-positives had been isolated,

According to Thomas and Druce, (1971), the incidence of psychrotrophic microorganisms in raw milk varied depending on the type and the initial load of the microorganisms present. It reflected the conditions under which the milk was produced and the temperature and duration of storage and handling before processing. Milk produced under sanitary conditions normally does not show rapid increases in psychrotrophs when held at 4 ^{o}C or less. In contrast, milk produced under unsanitary conditions, has a rapid increase in psychrotrophic microorganisms. The increase, however, was not the result of the initial number of psychrotrophs but rather the presence of actively multiplying psychrotrophs (Thomas and Thomas, 1976).

Durr (1975) studied the development of psychrotrophic bacteria in refrigerated raw milk from dairy farms in France and reported that the initial load of psychrotrophic bacteria greatly influenced the keeping

quality of raw milk during storage. He observed that from an initial psychrotrophic bacteria population of 2.5×10^4 cfu/ml (obtained directly after milking), the number of psychrotrophic bacteria increased significantly to more than 10^6 after 4 days of storage at 2 ^{o}C and after 2 days of storage at 4 ^{o}C. Milk samples containing 1.0×10^5 to 5.0×10^5 cfu/ml of psychrotrophic bacteria could not be stored for more than 2 days at 2 ^{o}C without the count increasing to more than 10^6 cfu/ml.

Muir *et al.* (1978) investigated the quality of blended raw milk in the south-west of scotland and reported that safe storage of raw milk under refrigeration was about 72 hours at less than 8 ^{o}C. They observed that after 96 hours of storage at 8 ^{o}C, 93.8 per cent of milk samples studied had more than 5.0×10^6 cfu/ml of psychrotrophic bacteria as compared to 12.2 per cent for milk samples held at 4 ^{o}C.

Storage of raw milk at 3-7 ^{o}C for periods of more than 24 hours is selective for psychrotrophs, so that stored milks will, in time, have a bacterial flora dominated by them.

1.1.3 Growth and temperature relationship

Microbiologists were well aware that bacteria ceased to grow at a certain low temperature. When serious microbial activities were examined, it was usually found that there were reasonably well-defined optimum, maximum and minimum temperatures for each activity. The critical temperature

for one of these activities did not necessarily coincide with the critical temperature for the other activities.

A useful way of expressing quantitatively the effect of temperature on a microbial activity is in the form of the temperature coefficients. The disadvantage in using temperature coefficients in detailed studies of temperature effect on biological processes is, however, that the values for any one process may vary considerably over the temperature span tested.

Arrhenius (1908) (cited by Cousin, 1982) who studied the laws that relate temperature to chemical reaction rates, found that the rate of chemical reaction is a logarithmic function of the reciprocal of absolute temperature:

$$K = Ae^{-U/RT}$$

where, K is the velocity constant for the particular reaction and T the temperature in degrees absolute, R is the gas constant, A is called the 'frequency factor', which includes the collision number of the reacting molecules and a probability factor, and u refers to the temperature characteristic of the process. Upon taking logarithms of the equation:

$$\text{Log } K = \text{Log } A - \frac{U}{2.303\ RT}$$

This postulated that for one particular reaction, the plot of log K against 1/T would be linear and the slope being equal to -U/2.303R. The constant U, which is

characteristic of the reaction and determined the influence of temperature on the reaction rate, can be calculated from the slope.

Arrhenius' equation, therefore, predicts that a chemical reaction does not stop at a certain low temperature; it merely proceeds more slowly as the temperature was reduced (Cousin, 1982).

At a defined low temperature, most reactions that are enzymatically catalysed give a linear 'Arrhenius plot' over a wide temperature range, but for bacterial growth this is usually true only in a limited interval between 20 ^{o}C and 35 ^{o}C. Below 20 ^{o}C the temperature characteristic (U) increases progressively, and this has also been found to be true for many biological processes (Morita, 1975). The existence of a minimum temperature of growth in bacteria therefore exemplified a general biological phenomenon.

1.1.3.1 Species and strains variation

Bacterial growth has been found to occur at temperatures ranging from -20 ^{o}C (Morita, 1975) to 90 ^{o}C (Cousin, 1982), with different species and strains having different maximum and minimum growth temperature within this range. Microorganisms have been divided into psychrophiles, mesophiles and thermophiles according to the range of temperatures over which they will grow. Although rigid differentiation has not been possible, owing to the occurrence of borderline cases and the

influence of the environment; nevertheless examination of the characteristics of the member of the three groups may give some indication of the way in which they were able to cope with their environments.

An Arrhenius' plot of log bacterial growth rate versus the reciprocal of incubation temperatures, when linear, gave a value analogous to the activation energy and is the temperature characteristic of growth (U). Studies by Morita (1975), indicates that U values may be the property of a particular species or of the growth medium, but not of the temperature range of growth.

1.1.3.2 Biochemical basis of temperature relationship

In the progression from psychrophilic to thermophilic bacteria, various biochemical differences have been identified between organisms in the different groups, while similarities appear to exist between organisms in the same group. Considerable variations have occurred in the enviromental conditions and in the species and strains studied, but there appears to be a relationship between certain biochemical characteristics of bacteria and their ability to grow at different temperatures.

Since most, if not all, pathogenic bacteria are mesophiles, this group of organisms first attracted attention and the psychrophilic and thermophilic bacteria have been examined for characteristics which differentiate them from mesophiles. A more useful way of looking at the

problem may be to examine these groups of bacteria for properties which alter along the temperature scale rather than diverge from the centre.

The observations of Baxter and Gibbons (1962) on the ability of psychrophilic and mesophilic strains of yeast to oxidise exogenous glucose led them to suggest that at low temperatures only the psychrophile was able to transport substrate into the cell.

Senyk *et al.* (1982) supported this conclusion on their finding that the minimum growth temperatures of mesophilic strains of *Arthrobacter* and *Candida utilis* were approximately the same as those at which uptake of glucosamine and respiration of exogenous glucose by these organisms ceased. Uptake of the purines, uric acid and xanthine by *Candida utilis* was also reported to be prevented at 4 ^{o}C.

Such differences in uptake of exogenous materials may be related to the finding of Marr and Ingraham (1962) that psychrophilic organisms contained a higher proportion of unsaturated fatty acids than mesophiles. Although, as they emphasised, the composition of organisms grown at any one temperature was dependent upon the growth medium composition.

The unsaturated fatty acids of the psychrophiles would have a lower melting point than those in the mesophiles (Senyk *et al.*, 1982). In fact it was suggested by Goughran (1947) that bacteria cannot grow at temperatures below the solidification point of their lipids and this may be related to uptake of substrates via lipid containing

membranes.

Perhaps the most widely studied class of bacterial constituents have been the enzymes. Coghill (1982) suggested that thermophiles may possess a capacity for rapid resynthesis of proteins which were, in fact, denatured at growth temperatures. However, in earlier reports, Stokes (1963) pointed out that maximum growth temperature of bacteria may be related to the heat stability of their essential components.

Stokes (1963) reported that studies on psychrophilic organisms had indicated that heat denaturation of respiratory enzymes seems to be involved in determining the maximum growth temperature of the organisms. Additional support for this involvement was provided by observations on the exceptional heat stability of enzymes from thermophilic bacteria. Among these were ATP-ase, malate dehydrogenase and inorganic pyrophosphatases.

Akagi and Campbell (1962) provided strong evidence when they compared the heat stability of hydrogenase and sulphate adenylyl transferase in *Clostridium nigrificans* organism (thermophilic) and the mesophilic, *Desulphovibrio desulphuricans*. The enzymes were more heat stable in the case of the thermophile. These experiments were performed on impure enzymes preparations, thus allowing the possibility that heat stability may be due to some protective factors rather than an inherent property of the enzyme. However, a purified alpha-amylase of *Bacillus stearothermophilus* also exhibited the property of heat

stability.

Not all enzymes of mesophilic bacteria show an equal degree of heat sensitivity and indeed a formate dehydrogenase of *Escherichia coli* has been shown to have maximum activity at 80 ^{o}C, which was well above maximum growth temperature of this organism. It appears, therefore that the activity of certain enzymes may be rate limiting.

Another class of compounds which are vital to the functioning of the bacterial cell were the nucleic acids. Comparison of melting temperature of DNA from thermophilic and mesophilic microorganisms revealed little or no difference (Akagi and Campbell, 1962). No apparent relationship between the thermal stability of DNA and optimum growth temperature of microorganisms can be concluded.

In contrast, Cousin (1982) reported that the RNA of thermophilic organisms was more heat stable than RNA from mesophiles.

Griffiths *et al.* (1981) studied the thermostability of proteases and lipases from psychrotrophic bacteria of dairy origin and reported that micromolecules of thermophilic bacteria were relatively heat stable and this probably has a key role in thermophily. The roles of characteristic lipid solidification point and rapid resynthesis have not been vindicated experimentally and were likely to be minor. The increased stability may be an intrinsic property of the micromolecules or may be due to the presence of protective substances in the cells. .pa

1.1.3.3 Growth of Psychrotrophic bacteria

Normally, growth at a low temperature is characterised by a long lag phase and a slow logarithmic phase (Ingraham and Stokes 1959). The lag phase was shortest at the optimum temperature and became increasingly longer as the temperature was lowered. The optimum growth temperature was defined as the temperature at which the generation time was shortest.

Though psychrotrophs can grow at temperatures close to 0 ^{o}C, their optimum temperature is much higher. Elliott and Michener, (1965) reported that the optimum temperature for growth of most microorganisms are 20 to 30 ^{o}C with some having optima of 30 to 45 ^{o}C and very few of 15 ^{o}C or below. The minimum growth temperature for psychrotrophic bacteria has been reported as -10 ^{o}C (Ingraham and Stokes 1959). The maximum growth temperature for psychrotrophy was stated as 30 ^{o}C but some have maxima of 37 to 45 ^{o}C (Ingraham and Stokes, 1959).

Yano and Kembo (1974) studied the growth characteristics of psychrotrophic bacteria in refrigerated raw milk. They reported that the generation time for psychrotrophic bacteria in refrigerated raw milk was in the range of 6.6 to 12.7 h at 5 ^{o}C and 12.2 to 26.1 hours at 0 ^{o}C. Table 1.1 shows the generation times of various bacteria commonly found in milk stored at 3 temperature ranges.

Table 1.1.1

Generation times (h) of some selected psychrotrophs

Microorganism	Generation time (h), at 0-2 °C	4-6 °C	10 °C
Bacillus coagulums	24-30	-	-
Bacillus spp. (Gp A)	30	6-9	4-4.5
Bacillus spp. (Gp B)	-	24-36	8-14.5
Clostridium hastiforme	-	73	25.5
Enterobacter aerugenes	37.7	12.2	4.1
Pseudomonas spp	26.6	11.7	5.43
Pseudomonas spp	29.1	14.7	6.52
Pseudomonas fragi	-	5.5	-
Pseudomonas fluorescens	-	7.2	-
Pseudomonas fluorescens	30.2	6.7	-
Pseudomonas spp	20	-	3
Pseudomonas spp (92)	11.1	-	-

Note: Adapted from: Cousin, (1982) and Yano and Kembo, (1974).

1.1.4 Quality Standard for Psychrotrophic Counts

Even though psychrotrophic bacteria and their thermostable enzymes posed a serious technological problem in the dairy industry, there are no quality standards established in the United Kingdom (Thomas and Thomas, (1973a).

Tatini and Olson, (1965) had suggested that an initial psychrotrophic colony count of less than 10^4 cfu/ml, determined on solid media and incubated at 7 ^oC for 10 days could be considered as a satisfactory guide standard for on-farm raw milk quality. From their observations, initial psychrotrophic counts between 10^4-10^5 cfu/ml was an indication of the need for improvement in milking hygiene and/or the cleaning and sterilisation of farm dairy equipment. Colony counts of more than 10^5/ml were considered as definite indication of unsatisfactory production conditions or of inefficient cooling and refrigeration of the milk at the farm.

Punch *et al.* (1965) studied the population level of psychrotrophs associated with flavour and physical changes in milk. They observed that flavour changes were detected when the population was more than 10^6 cfu/ml. However, acid degree values which measure the degree of lipolysis remained insignificant until after the change in flavour was noted. A similar relationship between flavour changes and protein hydrolysis was noted. Little, if any increase in free tyrosine was noted when drastic flavour impairment occurred.

SECTION 1.2 ENZYMES SYSTEM IN MILK

Of all the hydrolytic enzymes present in milk only those described as lipolytic and proteolytic will be discussed. These groups of enzymes are to a large extent responsible for the rancid (Andersson *et al.*, 1981) and bitter flavours, developed during storage of milk and milk products (Law, 1979). These enzymes are either indigenous and secreted with the milk or extracellular, introduced by the contaminating bacteria. The indigenous lipolytic and proteolytic enzymes are highly heat labile and destroyed by pasteurisation Cogan, (1977). However, those associated with the growing psychrotrophic bacteria are highly thermostable and therefore a major concern during storage of heat processed, commercially sterile foods (Cogan, 1977 and Mottar, 1981).

1.2.0 Terminology

The Enzyme Commission of the International Union of Biochemistry (IUB, 1978) introduced the numeric system of enzyme classification (EC). Enzymes were divided into six main groups on the basis of the reactions which they catalysed. They were (1) oxidoreductases, (2) transferases, (3) hydrolases, (4) lyases, (5) isomerases, and (6) ligases (synthetases).

The enzymes capable of degrading food lipids and proteins were listed under group 3 (hydrolases), sub-class 1 (acting on ester bonds) and sub-class 4 (acting on peptides bonds).

The esterases (EC 3.1) were further sub-divided into those acting on carboxylic esters (EC 3.1.1), thioesterases (EC 3.1.2), phosphoric monoesters hydrolases, the phosphatases (EC 3.1.3), phosphodiesters hydrolases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), sulphatases (EC 3.1.6) and diphosphoric monoesterases (EC 3.1.7).

The peptide hydrolases (EC 3.4) were further divided into 2 sets of sub-groups; the peptidases or exopeptidases (EC 3.4.11-17) and the proteinases (EC 3.4.21-24). In the first set the peptidases were classified according to their specificity into those hydrolysing single amino acids from the N-terminus of the peptide chain (EC 3.4.11), those hydrolysing single residues from the C-terminus (EC 3.4.16-17). In the second set were those specific for dipeptide substrate (EC 3.4.13) and those splitting off peptides units either from N-terminus (EC 3.4.14) or the C-terminus (EC 3.4.15).

The proteinases (proteolytic enzymes, endopeptidases, peptidyl-peptide hydrolases) were divided into sub-groups on the basis of their catalytic mechanisms and not according to specificty.

The proteolytic enzyme preparation from microorganisms are described as proteases containing mixtures of proteinases and peptidases (Frazier and Westhoff, 1977). Therefore, they were included in the classification in category EC 3.4.11-17 and EC 3.4.21-24, respectively.

1.2.1 Endogenous milk enzymes

Bovine milk contained a large number of enzymes which originated from the mammary gland tissues, from the blood plasma and the blood leucocytes (Jenness and Patton, 1959). They represented a minor fraction of the total milk proteins but are of technological significance to the dairy industry.

Milk lipases or acylglycerol acylhydrolases (EC 3.1.1.3) are defined as enzymes which hydrolyse esters of long-chain aliphatic acids from glycerol at oil/water interfaces. Phospholipids and diolesteryl esters are not included as substrates, although there are lipases which will hydrolyse acylglycerols and phospholipids or cholesteryl esters. Emulsion globules, fat bodies or lipoprotein particles usually provide the interface and these have been termed as supersubstrate (Walstra and Jenness, 1984).

The presence of proteolytic activity in milk was well documented (Humbert and Alais, 1979). This activity was attributed to one of its constituents and many research works have been done to isolate and purify this milk constituent.

The origin of proteolytic activity in milk has long been the subject of discussion. While some attribute it to bacterial source, many deem it native in the milk. Kitchen (1976) detected some proteolytic activity in milk and in enzyme preparations extracted from the mammary gland tissues but no similar activity in blood under the same

experimental conditions. Nevertheless, other workers (Halpaap *et al.*, 1977) have concluded that the milk proteases originated in the blood plasma with a subsequent transfer to the mammary gland.

Accordingly, the alkaline protease was thought to be nothing more than plasmin or its zymogen plasminogen, and similar activity was observed by urokinase treatment (Tomich *et al.*, 1976). The latter reported other analogies between plasmin and milk protease, namely the same optimum pH for activity, the same stability pattern with respect to pH and temperature; the same molecular weight. They were found to be equally sensitive to the same inhibitors and to have similar proteolytic activities on beta caseins, this having been observed when examined by polyacrylamide gel electrophoresis.

Tomich *et al.* (1976) did not succeed in isolating plasminogen from skim milk by affinity chromatography, but were not succesful either with control samples to which plasminogen has been added. Halpaap *et al.* (1977) isolated plasminogen from bovine blood and the protease in milk by the same affinity chromatography process on lysine sepharose 4B. They obtained identical elution diagrams and concluded that this protease was effectively plasmin. All recent works lead to the same conclusion: native protease in milk originates from the blood.

Native milk proteases have been implicated in gelation of UHT milk and the formation of amino acids during Cheddar cheese manufacture and ripening (Noomen, 1975) and the production of bitter flavours in milk and dairy

products (Law, 1979).

However, having studied the phenomena of gelation in UHT milks, other authors attributed the enzyme action to protease of bacterial origin (Snoren and Both, 1981).

1.2.2 Extracellular enzymes of bacterial origin

For many years, numbers of microorganisms in milk have been used as one index of quality; however microbial numbers do not indicate activities of enzymatic origin. Many psychrotrophs are active producers of extracellular enzymes such as lipases and proteases (Cogan, 1977). When large numbers of such bacteria are present in raw milk, in time, they will secrete sufficient levels of enzymes to have a noticeable affect on the quality of milk or products made from the milk.

Some of these enzymes are more heat resistant than the bacteria from which they originated. Pasteurisation kills the Gram-negative psychrotrophs but does not inactivate their enzymes.

Adams *et al.* (1975) studied the heat resistant proteases produced in milk by psychrotrophic bacteria of dairy origin. They reported that most of the raw milk supplies studied contain heat-stable microbial proteases which causes bitterness and gelation of UHT milk. Other workers (Richter, 1979) reported bitterness in whipping cream and decreases in nitrogen content of curd and cheese yield (Cousin and Marth, 1977a) were due to the proteolytic activities of microbial origin.

1.2.3.1 Lipases of psychrotrophic bacteria

Extracellular lipases produced by psychrotrophic bacteria have considerable potential for causing hydrolytic rancidity in milk and milk products. The bacteria responsible for these lipases are predominantly *Pseudomonads*, particularly *Pseudomonas fluorescens*, *Enterobacteriaceae* such as *Serratia* and *Acinetobacter* species. Other significant organisms include, *Achromobacter*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus* and *Moraxella*.

Pseudomonas species normally constitute the largest proportion of lipolytic psychrotrophs in raw milk and cream, hence their lipases have been most studied. Lipases from *Pseudomonas fluorescens* and *Pseudomonas fragi* have been purified into homogeneity (Senyk *et al.*, 1981).

The production of heat-resistant extracellular lipases by Gram-negative rods has been described (Law *et al.*, 1979). Lipolytic activities varied according to the type of bacteria. Even within species, considerable variations have been reported. Therefore, it is quite difficult to decide from the knowledge of the numbers and species present in raw milk, the presence and the extent of activity of the bacterial lipases. However, it is highly likely that with higher psychrotroph count, for example in the order of 1.0×10^6 cfu/ml in milk as suggested by Punch *et al.* (1965), active microbial lipases would be present.

1.2.3.2 Isolation and characterisation of lipases of bacterial origin

Sugiura *et al.* (1977) purified the lipolytic enzymes from *Pseudomonas fluorescens* species isolated from milk and found it to be a single polypeptide chain with no lipid, carbohydrate or disulphide linkage. In general, they concluded that, the molecular weight of microbial lipases were in the region of 35,000-50,000 and unlike the bacterial proteases, the bacterial lipases studied do not appear to contain metal ions.

However, Fitz-Gerald *et al.* (1982) contended that microbial lipases do require metal ions such as Ca^{2+} or Mg^{2+} for activity. They observed that excess EDTA causes complete inhibition of most bacterial lipases, which can be reversed by Ca or Mg. In addition they observed that some heavy metals are inhibitory, in particular Zn, Fe, Hg, Ni, Cu and Co. A similar observation was made by Sugiura *et al.* (1977).

Sugiura *et al.* (1977) reported that low levels of NaCl (10 mM) may cause activation of the the bacterial lipases, while at higher concentrations, the NaCl was inhibitive to the activity of the lipases studied. However, Woo and Lindsay, (1984) observed that, more than half of the lipase activity remained in the presence of 2 M NaCl, a level similar to that in the aqueous phase of salted butter.

Bozoglu (1984) purified the extracellular, heat-stable lipase produced by *Pseudomonas fluorescens* MC50 by

successive gel chromatography on Sephacryl S-500 and Sephadex G-200. They observed that the purified enzyme was homogenous as judged by gel electrophoretic analyses and exhibited characteristics of a true lipase. They are, the substrates required emulsification to obtain activity; trioctanoin appeared to be the best synthetic triacylglycerol and coconut oil was the best natural substrate. The activity of the lipase studied shows an alkaline pH for optimum activity and was inhibited by tratement with EDTA and partially inhibited by reagents specific for reaction with sulfhydryl groups. Comparison of the amino acid composition with that of pancreatic lipase indicated a a strong correlation (r = 0.90) of the two enzymes, suggesting possible similar functioning. A sub-unit weight of 55,000 MW was estimated by gel electrophoresis in sodium dodecyl sulfate.

1.2.3.3 **Thermostability studies**

The optimum pH for avtivity for the lipases of the bacterial origin are usually in the alkaline region between 7 and 9 but some have considerable or even optimal activity at the pH of milk. They generally show highest activity at 40-50 ^{o}C although there are reports of higher or lower temperature optima (Sugiura *et al.*, 1977).

The psychrotrophic bacterial lipases are heat stable. But the stability varies with the species and strain of organism and also with the medium in which they are heated. Their stability decreases with increasing purity.

Many are sufficiently stable to retain at least some activity after HTST pasteurisation, and even after UHT treatment. Some workers have reported a two-stage inactivation on heating, an initial rapid loss of activity followed by a slow or even negligible decline (Driessen and Stadhouders, 1974).

A number of the lipases are less stable at temperatures below 70 ^{o}C than at higher temperatures (Stepaniak and Fox 1983). This suggested that like the proteases of some psychrotrophic bacteria, the lipases would be susceptible to low temperature heat inactivation (55 ^{o}C for 1 hour) (Barach *et al.*, 1976).

However, Fitz-Gerald *et al.* (1982) reported that in fat-containing media, considerable lipolysis can occur during prolonged heating at 55 ^{o}C. Thus, such treatment was considered to be of little value for eliminating these lipases from milk products

The extent to which lipolytic activity remained after pasteurisation was studied by Cousin and Marth (1977b). After heating at 63 ^{o}C for 30 minutes the average lipolytic activity retained by *Pseudomonas* species was 80 per cent, 98 percent for *Acinetobacters* and 65 per cent by *Aeromonas* species.

Law, (1979) reported that when the lipases of *Pseudomonas fluorescens* and *Pseudomonas fragi* was subjected to heating at 100 ^{o}C for 10 nmin, some lipase activity was found to survive. They observed that heat treatment of 120 ^{o}C for 15 min was necessary for complete inactivation of the enzyme activities.

1.2.4.1 Proteases of psychrotrophic bacteria

The most common bacteria found in milk that produce proteolytic enzymes are of the genus *Pseudomonas*, especially *Pseudomonas fluorescens* (Cousin and Marth, 1977b). However, other Gram-negative rod-shaped species had also been isolated, such as *Flavobacterium*, *Acinetobacter* (DeBeukelar *et al.*, 1977), *Proteus*, *Enterobacter*, *Escherichia* and *Alcaligenes* (Law, 1979). Gram-positive bacteria of the genera *Micrococcus* and *Bacillus* have been less frequently reported (Collins, 1981)

Adam *et al.* (1975) studied the presence of heat resistance proteases produced in milk by psychrotrotrophic bacteria of dairy origin. They reported that 70-90 per cent of milks examined contained Gram-negative bacteria capable of producing extracellular heat-resistant proteases.

Bacterial proteases are very heat resistant. Kishonti (1975) found that pasteurisation did not destroy the extracellular proteases of 60 strains of psychrotrophic bacteria examined. Adams *et al.* (1975) observed similar results when milk was UHT treated at 149 ^{o}C for 10 seconds.

Cousin and Marth (1977a) studied the changes in stability of milk to coagulation by heating. They observed that the protease from a strain of *Pseudomonas fluorescens* was able to resist heating at 140 ^{o}C for 3.5 seconds.

Law *et al.* (1977) studied the gelation phenomenon in commecially UHT-sterilised milk by proteases from

Pseudomonas fluorescens species isolated from raw milk. They reported that the proteases survived the UHT treatment with the development of gelation, bitterness and clearing on long term storage of the UHT milk studied.

The bacterial proteases from *Pseudomonas fluorescens* investigated by Cousin and Marth (1977b) was able to break down both β^- and κ-caseins. They reported that the action of the proteases studied on κ-casein with the formation of κ_{s1}^- casein was very similar to that of rennet.

Most studies on the degradation of milk proteins by psychrotrophic bacteria have involved *Pseudomonas* species and casein protein. Limited reports are available dealing with the degradation of whey protein.

Cousin and Marth (1977b) reported the changes in milk protein after innoculation with one of 5 psychrotrophic *Pseudomonas* species studied. They observed species preferential attack on the α^- and β-caseins. After incubation at 7 ^oC for 3 days, one species was observed to split α-caseins into 2 fractions and caused a slight decrease in β-casein; two species split β-casein and the remaining 2 species studied caused α^- and β-caseins to decrease continually with almost complete disappearance of the fractions after 12 days of storage at 7 ^oC.

Law *et al.* (1977) supported the observation and reported that *Pseudomonas putida* caused κ^- and β-caseins degradation after 3 days of storage at 7.5 ^oC with counts exceeding 10^7 cfu/ml. In contrast, at a concentration of 10^7 cfu/ml *Pseudomonas flurescens* caused only slight degradation of κ^- and β-caseins after 3 days of storage

at 7.5 ^{o}C.

Richardson and TeWhaiti (1978) characterised the heat stable extracellular Proteases from psychrotrophic bacteria in raw milk. They observed that *Pseudomonas* species decreased κ-casein during storage at 5 ^{o}C and followed subsequently by the reduction of γ^{-}, β^{-} and α_{s1}^{-} casein. However, they reported that the hydrolysis of β-casein was more rapid than that of α_{s1}^{-} casein at 37 ^{o}C after 24 hours incubation period.

Adams *et al.* (1976) studied the effects of psychrotrophic bacteria counts in raw milk on milk protein. They reported that *ca* 10^{5} cfu/ml of *Pseudomonas* species degraded 10-20 per cent κ-casein during 2 days of storage at 5 ^{o}C

In addition, they observed that whey proteins were also slightly degraded, α-lactalbumin was degraded by *ca* 9 per cent when the count reached 1.3 10^{8} cfu/ml after 6 days at 5 ^{o}C, but *B*-lactoglobulin was degraded *ca* 29 per cent after 2 days at 5 ^{o}C and a count 7.3 x 10^{6} cfu/ml in the medium.

1.2.4.2 **Isolation and characterisation**

Richardson (1981) has described purification of a heat-stable protease from *Pseudomonas fluorescens* B52 isolated from refrigerated raw milk. The protease was found to be active over a wide pH range (pH 6.0-10.5) and had an optimum activity at 45-50 ^{o}C. It contained a single Zn

atom and eight Ca atoms, and had a molecular weight of 46.9 kD (kilo Dalton). When the purified enzyme was heated in milk at 150 ^{o}C, it had a half-life of 37.5 seconds.

Barach *et al.* (1978) isolated and characterised the extracellular protease of *Pseudomonas* species MC60 (a psychrotroph). They observed that the enzyme contained Zn and Ca atoms and was quite strongly thermostable.

Patel *et al.* (1986) reported that a heat-stable extracellular protease from *Pseudomonas fluorescens* T16 purified by affinity chromatography on carbobenzoxy-D-phenylalanine-triethylene-tetramine sepharose 4B column, had a molecular weight of 38.9 *k*D and preferred *X* casein amongst many protein substrates tested. The half-life values of the enzyme during inactivation at 50, 90 and 120 ^{o}C were 7.1, 30.8 and 7.6 minutes, respectively.

Patel *et al.* (1986) studied the physicochemical properties of heat-stable proteases from psychrotrophic *Pseudomonas* spp. and concluded that the proteases consisted of single polypeptides with varied molecular weight, the smallest in size was from *Pseudomonas fluorescens* P26 (23 *k*D) while the protease enzymes from *Pseudomonas fluorescens* B52 and *Pseudomonas fluorescens* MC60 are large with molecular weights of 47 kD and 48.5 kD, respectively.

In addition, they observed that the catalytic role of the metal ions in the proteases studied was evident from the inhibitory effects of EDTA. The reactivation of the apoenzymes (T13, T16, T20 and T25) by Mg and Mn separates

them from protease B52 and MC60. They observed that the latter two were reactivated by Zn.

It was not uncommon to find heat-stable proteases which have different divalent metal ions requirements, like Mn and Mg by the protease from *Pseudomonas putrefaciens*; Ca, Mg, 2n, Mn and Co by the protease from *Pseudomonas fragi* Ca and Zn by the enzyme from *Pseudomonas* species MC60 (Stepaniak *et al.*, 1982). Thus, Stepaniak *et al.* (1982) contended that the common feature of most psychrotrophic proteases was their Ca^{2+} ion requirements. They showed that the Ca^{+2} ion content of the protease was high and varied considerably between the bacteria proteases studied.

The stabilising effects of Ca on heat-treated bacterial protease has been observed with other bacterial proteases (Barach *et al.*, 1978 and Richardson and TeWhaiti, 1978).

Stepaniak and Fox (1983) described that the most interesting characteristics of the extracellular proteases from psychrotrophic bacteria were their remarkable heat stability and their instability at 50 ^{o}C. They reported that all psychrotrophic proteases are metalloproteases requiring Ca, Zn, Mn, Mg, Co or Fe for activation. The ability of these metalloenzymes to survive extreme temperature most likely reflects their structural flexibility and the interplay of divalent cations in allowing rapid and precise enzyme renaturation. They reiterated that the hypothesis was true by the finding that heat-treated protease regains considerable activity

after storage from 0-4 ^{o}C.

Stepaniak *et al.* (1982) isolated to homogeneity and characterised a heat-stable proteinase from *Pseudomonas fluorescens* AFT 36 by chromatography on DEAE-cellulose and Sephadec G-150. They observed that the protease was optimally active at pH 6.5 and 45 ^{o}C. Its activity declined rapidly at higher temperatures but significant activity persisted down to 4 ^{o}C. Its activity was stongly inhibited by 0.001 M EDTA and was partially restored by the addtion of Zn^{+2}, Ca^{2+} or Co^{+2}. The enzyme was very heat labile in phosphate buffer and in a milk salt buffer at 55 ^{o}C but was very stable in the latter at temperature of more than 80 ^{o}C. The molecular weight was estimated to be 46,200 by gel filtrations.

1.2.4.3 **Thermostability studies**

Griffiths *et al.* (1981) studied the thermostability of proteases and lipases from a number of species of psychrotrophic bacteria of dairy origin and observed that the proteases and lipase synthesised by a wide variety of psychrotrophs isolated from dairy products can survive standard pasteurisation and sterilization procedures. About 86 per cent of the psychrotrophs studied were shown to synthesise either protease or lipase or both.

Kishonti (1975) observed that 24 of 60 strains of psychrotrophic bacteria isolated from milk and including species of *Pseudomonas*, *Alcaligenes* and *Aerobacter* produced extracellular enzymes capable of retaining at

least 75 per cent of their activity after exposure to pasteurisation at 63 ^{o}C for 30 min.

Stadhouders *et al.* (1980) reported that strains of *Achromobacter* and *Serratia* species produced lipases which could withstand 72 ^{o}C for 4 seconds, but lipases from certain strains of *Alcaligenes* and *Flavobacterium* species were not able to withstand the treatment.

Barach *et al.* (1976) obtained greater than 90 per cent inactivation of proteases synthesized by eight strains of psychrotrophic *Pseudomonads* after treatment at 55 ^{o}C for 10 minutes.

Griffiths *et al.* (1981) studied the thermostability of psychrotrophic bacteria of dairy origin. When partially purified protease from a number of strains of psychrotrophic bacteria were tested for their ability to withstand heating at 55 ^{o}C for 1 hour, there was a considerable variation in their resistance to the process. However, in most cases they observed that there was a decrease in residual activity after heating at 55 ^{o}C for 1 hour compared with that after 77 ^{o}C for 17 seconds.

Barach *et al.* (1976) studied the effects of low temperature inactivation of heat resistant proteases from psychrotrophic bacteria. They reported that at temperatures between 55 and 60 ^{o}C, the observed rate of inactivation of a number of proteases was greater than the heat treatment at 73 ^{o}C.

Barach *et al.* (1978) investigated the mechanism of low temperature inactivation of heat resistant proteases in

milk. They suggested that the low temperature inactivation of protease in milk was a two-step process. Firstly the protease molecule undergoes a conformational transition at 55 ^{o}C resulting in a reversible loss of catalytic activity and susceptibility of the protease to autolysis. The second stage involves aggregation of the altered proteases with casein micelles to form an enzyme-casein complex which was the result of altered hydrophobic interactions.

Griffiths *et al.* (1981) conceded that in their studies it was observed, that heat treatment of protease at 77 ^{o}C for 17 seconds followed by 55 ^{o}C for 1 hour resulted in retention of more activity than when the low temperature treatment was carried out before the HTST steps. They speculated that heating at temperatures above 70 ^{o}C, an irreversible denaturation process may occur, which prevented the enzyme undergoing the conformational transition involved in the first stage of the low temperature inactivation process. Alternatively, they suggested that a reactivation process, which was a reversal of the first stage of the low temperature inactivation mechanism, might occur when the protease was subjected to temperatures in excess of 70 ^{o}C after prior heat treatment at 55 ^{o}C.

Stepaniak and Fox (1983) studied the thermal stability of extracellular proteinase from *Pseudomonas fluorescens* AFT36. They observed that the protease secreted was very similar in most respects to that secreted by *Pseudomonas fluorescens* MC60 which have been investigated by other workers (Barach *et al.*, 1978). They reported that the

proteinase from *Pseudomonas fluorescens* AFT36 was quite heat labile in phosphate buffer, pH 6.6, being completely denatured at 70 ^{o}C in 1 minute, and significantly denatured at much lower temperatures, even as low as 50 ^{o}C. The stability of protease from *Pseudomonas fluorescens* AFT36 was also relatively low in a milk salt buffers below 60 ^{o}C. However, they were very heat labile at approximately 55 ^{o}C in buffer systems with or without Ca.

Richardson (1981) characterised the heat stable protease from *Pseudomonas fluorescens* B52 at various heating temperatures. They suggested that inactivation at 55 ^{o}C was due to autolysis.

The studies by Barach *et al.* (1978) supported the hypothesis put forward by Stepaniak and Fox (1983) that at approximately 55 ^{o}C the protease from *Pseudomonas fluorescens* MC60 undergoes a conformational change which opens its structure, rendering departed molecules suceptible to proteolysis by enzyme molecules not yet denatured.

The fact that protease from *Pseudomonas fluorescens* AFT36 does not continue to autolyse on cooling to 50 or 45 ^{o}C following brief exposure to 55 ^{o}C appeared to suggest intermolecular proteolysis (Stepaniak and Fox, 1983). In addition, Stepaniak and Fox, (1983) suggested that on heating to 55 ^{o}C the enzyme molecules unfold sufficiently to be susceptible to autolysis while still retaining sufficient structure to be proteolytically active, and on

heating to higher temperatures unfolding continues, but the enzyme by then has lost its proteolytic (and autolytic) activity and was relatively stable, although inactive. According to them, the slower heating rate would lead to more extensive inactivation.

Stepaniak and Fox (1983) observed an important difference between the heat stability characteristics of proteinases *Pseudomonas fluorescens* AFT36 and MC60 in that the latter enzyme inactivated itself at 55 ^{o}C regardless of whether it attains this temperature by heating or during cooling, while protease from *Pseudomonas fluorescens* AFT36 underwent autolysis only if it was heated to 55 ^{o}C.

The same authors reported that after heating to higher temperatures (80 ^{o}C and above), the enzyme was quite stable at 55 ^{o}C, but its autolytic properties at 55 ^{o}C could be restored following a short holding period at temperatures below 50 ^{o}C and especially below 25 ^{o}C. They hypothesised that the natural conformation was restored after heating, only if the solution was cooled, preferably to below 25 ^{o}C.

The instability of proteinase from *Pseudomonas fluorescens* MC60 at 55 ^{o}C, led West *et al.* (1978) to propose low temperature treatment (at 55 ^{o}C for 1 hour) of milk, before or after UHT processing (150 ^{o}C for 5 seconds), as a means of reducing the activity of psychrotrophic proteases and thus prolonging its shelf-life. However, Griffith *et al.* (1981) showed that low temperature inactivation was effective on proteases from

only some psychrotrophs. The observation by Stepaniak and Fox (1983) supported this conclusion.

SECTION 1.3 INHIBITION OF GROWTH OF PSYCHROTROPHIC BACTERIA IN MILK

Proper cleaning and sanitizing practices on the farm, during transport and at the processing plant are critical steps in eliminating the undesirable bacteria from contaminating the milk after milking. Steam sterilisation and detergent-hypochlorite sanitizing of all equipments that came in contact with the milk, if applied properly can effectively reduced and possibly eliminated to a certain extent, the initial load of psychrotrophic bacteria (Thomas and Thomas, 1976). However, the complete elimination of their presence in milk is impossible to achieve

Since the enzymes of psychrotrophic bacteria cannot be destroyed by heat treatment alone, the only means of preventing their production is to reduce or inhibit the growth of the psychrotrophs themselves. Several approaches have been suggested to inhibit or reduce the growth of psychrotrophic bacteria in the milk supply once contamination has occurred.

The growth rate of psychrotrophic bacteria in raw milk supplies can be reduced by maintaining strict refrigeration at 4 ^{o}C or lower during storage (Witter, 1961), addition of lactic starter cultures (Juffs and

Babel, 1975), activation of the lactoperoxidase system (LPS) of milk (Reiter *et al.*, 1964 and Bjorck *et al.*, 1975) and the addition of selected inhibitory agents such as, food additives (Moustafa and Collins, 1969 and Robach, 1978) and preservatives (Kosikowski, 1978) and by a subpasteurisation heat treatment called thermisation (Casalis, 1958) and followed by cooling to 6 ^{o}C (van den Berg, 1984). Thermisation heat treatment has been applied to milk when received at the creamery (Banks *et al.*, 1986) and has also been applied on the farm (Zall and Chen, 1980) or applied in conjunction with the natural inhibitory systems (Zall *et al.*, 1983).

1.3.1 Storage at low temperatures

Refrigeration on the farm and in the dairy plant is important in delaying the multiplication of psychrotrophic bacteria, many of which may have an optimum growth temperature in range of 8-14 ^{o}C (Witter, 1961).

Tompkins (1973) reported that the generation time for bacteria in milk increases from less than 8 hours at 7 ^{o}C to 12 hours at 5 ^{o}C and to 16 hours at 2 ^{o}C. He reported that although psychrotrophs may increase by 100 or 1000 fold during storage at 3-5 ^{o}C for 3 days, the psychrotrophs studied do not become a major problem in milk with low initial bacterial counts until the storage temperature exceeds 7 ^{o}C.

Thus, it was essential to hold milk at less than 5 ^{o}C

(Witter, 1961). Unfortunately, this storage temperature could not be maintained consistently.

1.3.2 Addition of lactic starter cultures

Lactic acid-producing or citric acid-fermenting bacteria had been shown to inhibit the growth of psychrotrophic bacteria in milk (Juffs and Babel, 1975). Provided the temperature of the milk was maintained at 4 ^{o}C or less, the lactic cultures should not grow sufficiently to affect the pH of the raw milk. They can readily be destroyed by pasteurisation heat treatment during further processing (Law and Mabbit, 1983).

Reiter and Marshall, 1979) reported that the production of H_2O_2 by the microorganisms present was responsible for controlling growth of the psychrotrophs. Inhibition was due to reduction in growth rate of the bacteria due to the presence of hydrogen peroxide.

Reiter *et al.* (1964) reported that during low temperature storage of milk, *Lactobacillus* species were the organisms that were actively producing H_2O_2. They observed a direct relationship between acid production and H_2O_2 accumulation in milk during storage.

Juffs and Babel (1975) reported that the formation of H_2O_2 by lactic *Streptococci* was favoured by low temperature incubation. They suggested the addition of 5 per cent lactic cultures to raw milk to delay the outgrowth of many of the Gram-negative non-sporeforming psychrotrophs. They observed that the rate of H_2O_2

produced by the *Streptococci* was constant, regardless of the number of psychrotrophs.

However, the amount of lactic acid and its effects on subsequent processing was an important consideration. It could be advantages if the milk was to be used for cheese manufacuture, since the slightly lower pH would hasten the action of milk coagulators (Juff and Babel, 1975). With other products, acid formation could be a disadvantage.

In addition, the use of lactic cultures had other limitations. The addition of lactic organisms to raw milk was not permitted in the United Kingdom (Reiter and Marshall, 1979). Furthermore, the addition of these organisms may increase the bacterial counts beyond that permitted for raw milk by the regulatory agencies. Temperature abuse during storage could result in a sour or heat-instable product having no commercial value.

The production of hydrogen peroxide by the starter cultures was a variable phenomenon (Juff and Babel, 1975). Care had to be taken for proper selection of the culture. They reported that citrate fermenters were found not to be an effective inhibitor of psychrotrophs.

Lactic acid bacteria also produce other bacterial inhibitors. For example *Lactobacillus acidophilus* secretes an inhibitor of *Escherichia coli* (Hosano *et al.*, 1977) and *Streptrococcus thermophilus* produces a heat stable low molecular weight amine that inhibits *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and

Bacillus subtilis (Pulusani *et al.*, 1979)

The gaseous environment can also affect growth of psychrotrophs. Brandt and Ledford, (1982) reported that the reduction of O_2 level in milk from 9-12 to 1-3 ppm at 3 ^{o}C slowed the growth of *Pseudomonas fluorescens* by 63 per cent and also inhibited the growth of *Pseudomonas putida* and *Pseudomonas aeruginosa*.

Eklund and Jarmund (1983) studied the effects of various gas atmospheres on microbial growth at different storage temperatures of milk. They reported that carbon dioxide and nitrogen are strong inhibitors of psychrotrophic bacteria and they were particularly effective in milk stored at the range of 4-7 ^{o}C. Their use extended the safe storage time of poor quality milk by about 3 days at 4 ^{o}C and 2 days at 7^{o}C. Considerably longer conservation was observed with good quality milk.

Mabbit, (1981) reported that the advantages for the use of nitrogen and carbon dioxide were that, they were relatively cheap, readily available and readily removed from milk under reduced pressure. And no adverse effects on the production of the fermented products from the treated milk.

1.3.3 Activation of natural lactoperoxidase system

Of the several natural inhibitory systems in raw milk (immuno-globulin, lysozyme, lactoferrin, etc) the most effective, particularly against Gram-negative bacteria has been the lactoperoxidase system (LPS) (Reiter *et al.*,

1964).

The complete LPS consisting of lactoperoxidase (LP), thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2) was first demonstrated to inhibit the multiplication and acid production of some lactic acid *Streptococci* (Reiter *et al.*, 1964). Of the 3 components, LP was always present in bovine milk at sufficient concentrations (up to 30 mg ml^{-1}) but the SCN^- content depends on the feeding regime. It is derived from the metabolism of S-containing amino acids, by detoxification of HCN (e.g. from Clover) and by enzyme hydrolysis of glucosides. The level of SCN^- can be as low as 0.01 mM or as high as 0.25 mM when the cows were on natural pasture (Jenness and Patton, 1959). The limiting factor was H_2O_2 but lactic acid bacteria produce sufficient H_2O_2 under aerobic conditions to activate the LPS system.

Catalase-positive organisms (e.g. Coliforms, *Pseudomonads*, *Salmonellae* and *Shigellae*) can only be inhibited by the LP system in the presence of an exogenous source of H_2O_2, supplied either by addition of H_2O_2 itself or may be generated *in situ* by the addition of glucose and glucose oxidase to milk (Reiter and Marshall, 1979).

Reiter and Marshall (1979) inoculated aseptically drawn milk with *Pseudomonas fluorescens* and then treated after two days of storage at 5 ^oC with glucose, glucose oxidase and thiocyanate. They observed bacterial counts in the raw milk was maintained at lower level than the untreated milk and the cheese made from it was normal as compared to

the untreated milk. And after 4 months of storage the FFA concentration in the cheese was elevated and the complete development of subsequent rancidity.

Bjorck, (1978) and Bjorck *et al.* (1975) have suggested that the LPS of milk could be actived with thiocyanate and hydrogen peroxide to inhibit Gram-negative organisms. They presented convincing evidence that the activated LPS in milk will have both a bactericidal and bacteriostatic effect on the psychrotrophic flora.

H_2O_2 has been used as an antimicrobial agent either as direct additive to milk or on coating of packaging materials. Kosikowski (1978) reported that in the United States the application of bacteriostatic H_2O_2 to cheese milk followed by catalase was legal for Cheddar and Swiss cheese under the United States Federal Standards of Identity.

1.3.4 Addition of Food Additives and preservatives

Food additives such as propionate, sorbates and benzoates has been used extensively for the control of yeast and moulds in food products (Robach, 1978). In the dairy industry these preservatives were used especially in acid products (Kosikowski, 1978). They were most effective as microbial inhibitors when in the undissociated state, that is, at pH 3.0 rather than at pH 6.0. Their effects on psychrotrophic bacteria in milk were not clearly understood. However, benzoates and sorbates had been reported to be effective against *Pseudomonads* in milk

products with low pH (Robach, 1978).

Sorbic acid and its potassium salts were widely used as antimicrobial and antifungal agents in foods such as yogurt and cottage cheese (Kosikowski, 1978). Sorbic acid is a straight-chain β- unsaturated *trans* 2, 4-hexadecanoic monocarboxylic aliphatic acid. Its potassium salt is highly soluble in water (Robach, 1978). It is non toxic, and can be metabolised similarly to naturally occurring fatty acids.

Mistry and Kosikowski (1985) studied the influence of potassium sorbate at two level of concentrations on the growth of psychrotrophic bacteria in pasteurised milk. At 0.075 per cent and 0.1 per cent sorbate concentration, the growth rate of bacteria was greatly retarded. They noted that the presence of 0.075 per cent sorbate in pasteurised milk, doubled the shelf life of the product. However, the initial bacterial load in the raw milk influenced its effectiveness. At low initial bacterial numbers of less than 30 cfu/ml, 0.075 per cent sorbate was almost totally inhibitory, but, at higher initials count (4.0×10^4 cfu/ml) some growth was observed, although greatly suppressed.

Hydrogen peroxide would enhance the antimicrobial activity of potassium sorbate. Mistry and Kosikowski (1985) studied the effects of different levels of H_2O_2 and potassium sorbate on the shelf life of pasteurised milk. They observed the shelf life of the pasteurised milk when stored at 6.8 ^oC was longer when 0.075 per cent sorbate

was added in combination with 0.005 per cent H_2O_2 to pasteurised milk than when sorbate was used alone. They deduced that their effects were additive.

However, even though food preservatives could reduce bacterial growth, they should not be used to mask microbiological problems resulting from poor milk quality, post-pasteurisation contamination, low grade fruit products and/or improper cooling, handling, and manufacturing practices.

1.3.5 Sub-lethal heat shock

The difficulties caused by psychrotrophic bacteria in milk were increased by the psychrotrophic *Bacillus* species capable of producing spores (Collins, 1981) which resist heat treatment such as pasteurisation. However, such bacterial spores can germinate and complete the transition from spore to vegetative state with the loss of their heat resistance (Shehata *et al.*, 1984). Induction of this transition would be a simple way to solve the problem due to the presence of spores in dairy products.

The transition and the consequent loss of heat resistance in much of the bacterial spores in dairy products can be stimulated by the addition of certain amino acids (Shehata *et al.*, 1984), especially nisin (Mikolajcik and Simon, 1978). In addition, sublethal heating has been reported to have stimulatory effects on spore germination (Collins, 1981).

It has been suggested that heat shock made the enzyme

which triggered germination more accessible to L-alanine (Shehata *et al.*, 1984). Nisin acts on the germinated spore to prevent outgrowth. As surviving spores germinate, the nisin would act to prevent vegetative cell proliferation.

Shehata *et al.* (1984) studied the cumulative effects of heat-shocking and germinant stimulants, namely, *L*-nisin and β-alanine, on the germination of spores of psychrotrophic strains of *Bacillus cereus*, *Bacillus pumilus*, and *Bacillus licheniformis*. They observed that percentage germination was highest with heat shock at 85 ^{o}C (without holding time) in combination with *L*-alanine plus nisin.

1.3.6 Thermisation

Heat treatment, is probably the most effective method of reducing the bacterial load of milk after contamination has occurred. One technique which has been advocated was thermisation (Casalis, 1958; Zall, 1980; van den Berg, 1984; and Banks *et al.*, 1986).

Thermisation was defined as a heat treatment, less severe than pasteurisation (Bender, 1968) followed by cooling (van dan Berg, 1984). These conditions were such that the bacterial count remained acceptable after 3 days of storage at 7 ^{o}C (Johnston, *et al.*, 1981).

The expression thermisation was first used by Casalis (1958), as a mild form of heat treatment to destroy the initial bacterial flora and to give a suitable environment for the multiplication of selected starter culture in cheese processing. Today, the process has been advocated for the inhibition and control of the growth of psychrotrophic bacteria in milk during cold storage prior to processing (Gilmour *et al.*, 1981). It was widely used in the continent of Europe (van den Berg, 1984) especially in the Netherlands, West Germany and Sweden, but as yet, has not been adopted in the UK (Banks *et al.*, 1986).

Thermisation has been applied to milk prior to the production of Cheddar cheese (Casalis, 1958; Sebela, 1978 and Banks *et al.*, 1986), dried skim milk (West *et al.*, 1986), tvaroh (Sabela 1978), and quarg (Dender *et al.*, 1985), with promising results.

Various time/temperature combinations have been used

for thermisation. These are usually between 50-68 ^{o}C with a holding period of 10-20 seconds. It can be applied at any stage of milk handling. However, it was more advantages when applied to milk on receipt at the creamery (Johnston *et al.*, 1981) than when applied on the farm (Zall, 1980 and Zall and Chen, 1980).

Heating of milk at temperatures between 60-65 ^{o}C for 15-20 seconds reduces the number of psychrotrophic bacteria and prevents total increase in total plate count during 3 days storage at 5-7 ^{o}C (van den Berg, 1984). Stadhouders (1982) made similar observations at 68 ^{o}C but recommended 64 ^{o}C heating with 10 seconds holding as the lower limit for thermisation.

According to van den Berg, (1984) the Royal Dutch Dairy Association reported in 1978 that adequate effects of thermisation can be obtained by heating at 62 ^{o}C for 15 seconds but for practical use heating at 63-65 ^{o}C for 15-20 seconds was recommended for milk with an initial psychrotrophic bacteria load of less than 10^{6} cfu/ml. Gilmour *et al.* (1981) recommended thermisation temperature of 65 ^{o}C for 15 seconds followed by storage at 7 ^{o}C. Fonden (1982) use the same heat treatment but recommended storage at 5 ^{o}C.

Senyk *et al.* (1982) heated milk at 57.2 and 65.6 ^{o}C for 10 seconds, and reported no growth of psychrotrophic bacteria during subsequent storage at 4.4-6.7 ^{o}C. Banks *et al.* (1986) investigated the effects of thermisation treatment at 55 ^{o}C for 60 seconds and 65 ^{o}C for 15 seconds on the extension of the storage life of milk and yield of

milk quality and recommended thermisation at 65 ^{o}C for 15 seconds.

Bjorgum *et al.* (1978) recommended heating at 66 ^{o}C for 15 seconds. Some authors recommended temperatures higher than 65 ^{o}C for milk high total count. Bjorgum *et al.* (1978) suggested that the temperature had to be raised to 69 or even 72 ^{o}C to get a suitable reduction of the bacterial counts. Stadhouders (1982) investigated the effects of thermisation at 68 ^{o}C on bacterial count and recommended 64 ^{o}C as a lower limit for an optimum effect.

van den Berg (1984) reported that a higher temperature (66 ^{o}C, 15 seconds) was advocated in Norway and (68 ^{o}C, 15-20 seconds) in West Germany.

Barach *et al.* (1976) used the term sub-pasteurisation on heat treatment for heat treatment of milk at 55 ^{o}C for 30 minutes for inactivation of heat-resistant proteases of psychrotrophic bacteria.

1.3.6.1 Effects of thermisation on enzyme and natural inhibitory agents in milk

The activity of alkaline phosphatase content in milk drops by almost half after heat treatment at 65 ^{o}C with holding at 15 seconds (Walstra and Jenness, 1984).

Natural lipase and proteinase were less heat-sensitive and inactivated to a lesser extent by thermisation heat treatment (Driessen, 1983).

The natural inhibitory factors, the lactoperoxidase

system and the immunoglobulins were almost unaffected by temperature up to 65 ^{o}C for 10-20 seconds (Walstra and Jenness, 1984).

Lipases and proteinases of bacterial origin were heat resistant. The inactivation of bacterial enzyme during thermisation was slight. The highest inactivation was obtained from lipase producing *Pseudomonas* spp. Heating at 65 ^{o}C for 10 minutes resulted in approximately 50 per cent inactivation (Fitz-Gerald *et al.*, 1982) but this heat treatment was more severe than thermisation as defined in the present study.

CHAPTER TWO

MATERIALS AND METHODS

SECTION 2.1 HEATING OF MILK

A continuous high-temperature short-time pasteuriser (APV Paraflow pasteuriser type HX, APV Co. Ltd., Crawley, Sussex, England) was used throughout the studies for thermisation and pasteurisation heat treatment of milk. The holding time was fixed at 16 seconds, the volume flow rate was kept constant at 300 litres per hour and the heating temperature was varied by adjusting the control panel. The holding tube was insulated to minimise heat loss by conduction.

The residence time was determined by injecting through a port located just after the heating section, 5 ml of 40 per cent (w/v) nickel chloride solution (BDH Chemicals Ltd., Poole, England) and detected from a sampling port at the end of the holding tube visually, by reaction with dimethylglyoxime solution (1 g dimethylglyoxime (BDH Chemicals Ltd., Poole, England) dissolved in 100 ml ethyl alcohol (BDH Chemicals Ltd., Poole, England) containing 1 ml of 33 per cent (w/w) ammonia solution (May and Baker Ltd., Dagenham, England). The reaction of the nickel chloride salt with the organic reagent gives a yellow-pink precipitate.

During the trial, milk lots were heated in batches, continuously in the descending order of severity of

heating. In between treatment lots, the flow of processing was diverted by the flow diversion valve, thus a continuous flow was retained while the heating stabilised to the required temperature for the next treatment. The plant was cleaned and sterilized immediately before and after processing.

2.1.1 Preparation of skim milk ultrafiltrate

The skim milk was prepared by centrifuging 50 ml of whole milk at 1500 **g** for 20 min and pipetting the skim portion from below the fat layer. The Amicon Ultrafiltration cell, model 202 (Amicon Corporation, Lexington, Massachussets, USA) fitted with YM2 membrane was used. The membrane has a molecular weight cut off $\geq$ 1000. The filtration was performed at 4^{o}C overnight, using pressure at 3.5 kg/cm^{2} supplied by compressed nitrogen.

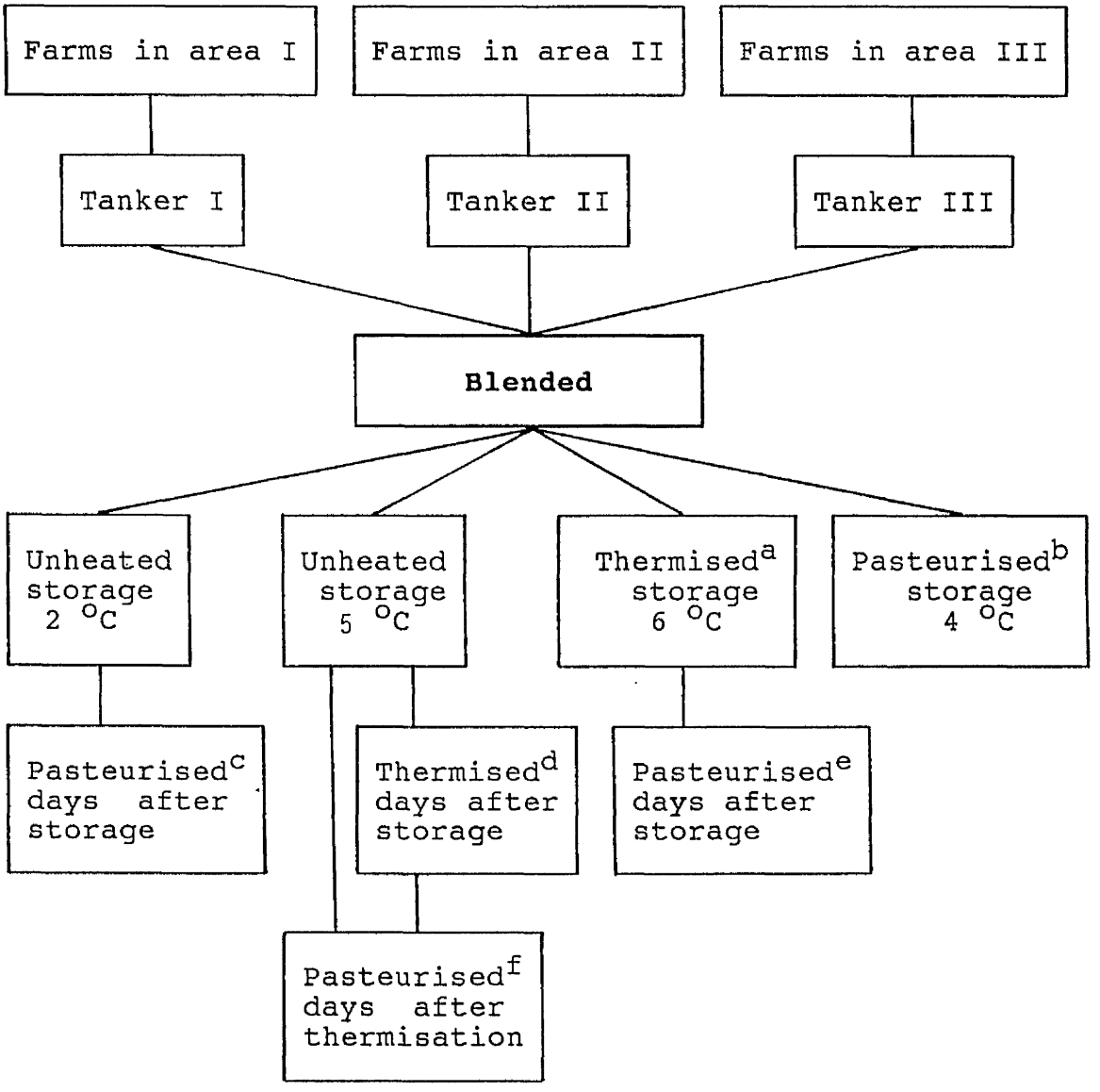
The ultrafiltrate was stored in 30 ml universal glass bottles at -20 ^{o}C until analysed. The ultrafiltrate was tested for soluble calcium and phosphorous content using the method as described in sections 2.2.10 and 2.2.11, respectively.

2.1.2 Main trial

Schemetic representation of the main trial is given in figure 2.1. The main trial was carried out continuously. Three trials were carried out, beginning on the following dates:- 2/12/86, 9/3/87 and 15/6/88.

Figure 2.1

Schemetic representation of the experimental procedures



Note: (a) = Thermised immediately on arrival and pasteurised (e) 2, 4 and 7 days after storage at 6 °C, (b) = Pasteurised immediately on arrival, (c) = Pasteurised 2, 4 and 7 days after raw storage at 2 °C, (d) = Thermised 2, 4 and 7 days after cold storage at 5 °C and then (f) pasteurised 2 and 4 days after thermisation and storage at 6 °C or pasteurised directly after 2, 4 and 7 days of cold storage at 5 °C. All pasteurised milk was stored at 4 °C and analysed 1, 3, 5, 7, 15 and 21 days after storage.

SECTION 2.2 CHEMICAL METHOD

2.2.1 Fat

Throughout the trial, the fat content was determined according to B.S. 696 part 2 (British Standards Institution, 1969) which is based on the Gerber method.

2.2.2 Lactose

The IDF standard method 28 A (IDF/FIL, 1974) which is based on the chloramine T method was used to determine the lactose content of milk. The procedure was as follows: After deproteinization of the milk, the lactose content was determined indirectly by titrimetric estimation of the amount of halogen reduced in the reaction between lactose and chloramine T - potassium iodide. Results were expressed as per cent lactose monohydrate.

2.2.3 Total nitrogen (TN)

The improved micro-Kjeldahl method of the Association of Official Agricultural Chemists (AOAC) (1965) was used to determined the total nitrogen content of milk with the use of Kjeldahl copper catalyst tablets (BDH Chemicals Ltd., Poole, England) instead of mercuric oxide. Standard HCl was used as the receiver solution during distillation. The excess of the acid was titrated with standard sodium hydroxide solution using a Kjeltec Auto 1030 Analyzer (Tecator AB, Box 70, Hoganas, Sweden). 1 ml of milk sample

with 3 ml of concentrated HCl and 2 tablets of copper catalyst was digested at 540 ^{o}C in a Kjeldahl digestion tube. Digestion was completed approximately within one hour when a clear blue solution appeared. 10 ml of distilled water was added and then titrated. Per cent nitrogen was calculated using the formula:

$$N_2 \text{ (per cent)} = \frac{(14.01 \times m \times f)}{(\text{sample wt. mg})(\text{ml titrant})}$$

Where 14.01 is the atomic weight of nitrogen; m is the molarity of receiver solution (mol/litre) and f is the standard Kjeldahl factor.

2.2.4 Non-casein nitrogen (NCN)

The IDF standard method 29 (IDF/FIL, 1964) was used to precipitate the casein content of milk. The nitrogen content of the filtrate was determined according to the improved micro-Kjeldahl method as previously described (section 2.2.3). NCN was expressed as a percentage and per cent casein was calculated using the formula:

$$\text{Casein content (per cent)} = 6.38 \text{ (TN - NCN)}$$

2.2.5 Non-protein nitrogen (NPN).

Trichloroacetic acid soluble nitrogen (TCA-N) was prepared using 10 ml milk and 40 ml of 15 per cent (w/v) trichloroacetic acid (TCA) in a 50 ml Erhlenmeyer flask. The mixture was filtered through number 42 Whatman filter

paper and the nitrogen content of the filtrate, described as NPN was determined according to the micro-Kjeldahl method as described (section 2.2.3). The protein nitrogen was calculated using the formula:

$$\text{Protein nitrogen} = 6.38\ (TN - NPN)$$

2.2.6 Rapid method for the determination of fat, protein and lactose in milk

The Milko-Scan 130 series (Foss Electric, Hillerod, Denmark), a semi automatic, microprocessor controlled infrared instrument was used for rapid determination of fat, protein and lactose in milk. The instrument was calibrated by the corresponding standard analysis; fat by Gerber method (section 2.2.1), protein by micro-Kjeldahl method (section 2.2.3) and lactose by the chloramine T method (section 2.2.3). Pilot samples with known composition were used to ensure the calibration remained stable throughout the analysis.

2.2.7 Total solid (TS)

Throughout the study the total solids (TS) content of milk was determined by drying in a hot air oven (Gallenkamp, Loughborough, Leicestershire, England) at 102 ^{o}C for 4 hours, according to the IDF standard method 21 (IDF/FIL, 1962).

2.2.8 Solids-not-fat content (SNF)

The solids-not-fat content of the milk was computed by deduction using the formula:

SNF = Total solids - fat content

2.2.9 Ash content

Throughout the study ash content was determined by the AOAC (1965) method for milk. Approximately 5.0 g of sample was weighed into ashing crucibles and evaporated to dryness in a drying oven (Gallenkamp, Loughborough, Leicestershire, England) at 102 ^{o}C for 4 hours. The dried sample was ignited in a muffle furnace (Baird and Tetlock, London, England) at 540 ^{o}C overnight until ash is carbon free.

2.2.10 Calcium content

Throughout the study the total calcium content of milk was determined by the complexometric method as modified by Pearce (1977). The method was based on titration with ethylenediaminetetra-acetic acid (EDTA) using Patton and Reader's (P & R) reagent [2-Hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid] (Fison Scientific, Loughbourough, England) as an indicator specific for calcium when used at high pH ($\geq$ 13). The addition of a little less than the expected titre of EDTA (found by a preliminary titration) before the addition of alkali minimised the problems caused by precipitation of calcium

phosphate and the co-precipitation of calcium and magnesium hydroxides.

2.2.11 Phosphorous

The IDF standard method 42 (IDF/FIL, 1967) was used. Phosphorous content was determined calorimetrically by the reduction of ammonium phosphomolybdate by diaminophenol (amidol) (BDH Chemical Ltd. Poole, England). The absorbance was measured at 750 *nm* using a Spectronic 20 spectrophotometer (Bausch and Lomb Inc. Rochester NY., USA). Ash obtained from ashing (section 2.2.4) was used for the analysis.

2.2.12 Titratable acidity (TA)

The titratable acidity of milk was determined according to B.S. 1741 (British Standards Institution, 1963) using 10 ml of milk and 1 ml of 0.5 per cent (w/v) solution of phenolpthalein as indicator. Titrant used was N/9 NaOH solution. The volume of NaOH solution used divided by 10 to give the figure which expresses the acidity as percentage lactic acid.

2.2.13 Hydrogen ion concentration (pH)

The hydrogen ion concentration (pH) of milk was measured by a Pye 290 pH meter fitted with a combined glass electrode (Activion Glass Ltd., Fife, Scotland). Buffer solutions of pH 4 and pH 7 were used for calibration.

2.2.14 Extraneous water

The freezing point depression (FPD) of milk was determined using the Advanced Milk Cryoscope model 41 (Advanced Instrument Inc., Massachussetts, USA). The instrument met the requirements specified by the AOAC (1965).

2.2.15 Antibiotic residues in milk

The disc assay method, based on the method of Galesloot and Hassing (1962) and as used by the SMMB for the control of antibiotic residues in ex-farm milk was used throughout the study as a routine test.

2.2.16 Total and free sulphydryl (SH^-) and disulfide (SS) groups

The method of Beveridge *et al.* (1974) was used throughout the study for the determination of SH^- and SS groups. 0.5 ml of skim milk which was prepared by centrifugation at 1,500 g was added to 2.5 ml of 8 M urea in tris-glycine buffer (10.4 g tris, 6.9 g glycine and 2.2 g EDTA per litre, pH 8.0; denoted as tris-gly buffer) and 0.02 ml of Ellmen's reagent (BDH Chemicals Ltd., Poole, England) in tris-gly buffer (4 mg/ml)

For SS groups, 0.2 ml of skim milk, 1 ml of 10 M urea in tris-gly buffer and 0.02 ml of 2-mercaptoethanol were incubated at 25 ^{o}C for 1 hour. After an additional 1 hour incubation with 10 ml of 12 per cent TCA, the tubes were

centrifuged at 4000 g for 10 min using a BTL Bench centrifuge. The precipitate were twice resuspended in 5 ml of 12 per cent TCA and centrifuged to remove 2-mercaptoethanol. The precipitate was dissolved in 3 ml of 8 M urea in tris-glycine buffer and 0.03 ml of Ellman's reagent was added for color development. Absorbance was measured at 412 *nm* using a Pye Unicam SP 1800 UV spectrophotometer.

The total solids of skim milk were determined by drying at 102 ^{o}C for 4 hours (section 2.2.7). The concentration of SH^{-} groups was calculated using the formula:

$$uM\ SH/g = (73.53 \times \text{absorbance} \times \text{dilution factor})/C$$

Where C is the sample concentration in mg solids/ml; the dilution factor is 6.04 for SH and 15 for total SH^{-} (SH^{-} + reduced SS) in milk.

2.2.17 Free fatty acids (FFA)

2.2.17.1 Copper soap method (quantitative)

Throughout the study, the copper soap method of Koops and Klomp (1977) as modified by Shipe *et al.* (1980) was used to determine the free fatty acids concentration. The method involved the conversion of FFA to copper soaps, then extracted, and the copper reacted with the color reagent.

The procedures were as follows: 0.1 ml of 0.7 N HCl was

mixed with 0.5 ml of sample in a 10 ml glass stoppered tube, using a vortex mixer (Whirlimixer, Fisons Scientific, Loughbourough, England) for one minute. Then 2 ml of copper reagent (prepared by mixing 5 ml triethanolamine, 10 ml of 1.0 M aqueous Cu $(NO_3)2.3H_2O$ and 85 ml saturated NaCl solution). The pH was adjusted to 8.3 with 1.0 M NaOH and 6 ml of solvent added (49:49:2 mixture of chloroform, heptane and methanol, respectively). The mixture was shaken continuously for 30 minutes using Julabo paramix 3 rotary shaker (Julabo Lab. GmBH., Seelbach, W. Germany) at 240 rpm, followed by centrifugation at 2000 *g* for 10 minutes. 3.5 ml of the solvent layer then transferred to an acid washed tube containing 0.1 ml of colour reagent (0.5 per cent (v/v) sodium diethyl dithiocarbamate solution in N-butanol). The absorbance was measured at 440 *nm* within one hour using a Spectronic 20 spectrophotometer (Bausch and Lomb Incorporation, Rochester, NY, USA). To convert absorbance to mEq/100 ml milk, a standard curve was prepared with palmitic acid as specified by Koops and Klomp (1977). The regression equation (section 2.4.1) for this curve is y = 0.0073X - 0.0704 where y = absorbance at 440 *nm* and x = mEq/100 ml milk.

2.2.17.2 Gas chromatographic method (qualitative)

Throughout the study, the method proposed by Deeth *et al.* (1983) was used. FFAs were extracted using a mixture of hexane - diethyl ether in the presence of anhydrous

sodium sulphate and then passed through a column of neutral, deactivated alumina. The FFAs retained on the alumina was eluted by diisopropyl ether containing formic acid. An aliquot of this solution is chromatographed using a stationary phase SP-216-PS gas liguid chromatography.

The hexane-diethyl ether extract of milk was obtained according to the method of Salih *et al.* (1977), described as follows: 3.3 ml of concentrated HCl was added to 10 ml milk in a 50 ml screw cap polypropylene sorval centrifuge tube (Dupont, Connecticut, USA) , shaken gently, followed by 20 ml of ice-cold (approximately 4 ^{o}C) diethyl ether containing 5 *u*g/ml *n*-nonanoic acid as internal standard (described as C:9 in the subsequent text). The mixture was shaken very gently by circular motion of the wrist for 2 minutes and centrifuged at 2000 **g** for 5 minutes. The mixture was shaken gently for a second time by a circular motion of the wrist for another minute, then again centrifuged at 2000 **g** for 10 minutes. All centrifugations were performed at 4 ^{o}C using a MSE coolspin centrifuge (MSE Scientific Instruments, Sussex, England). The sample was kept at 4 ^{o}C throughout the extraction process.

15 ml of the ether layer of the extract was added to 15 ml hexane containing 1 g of anhydrous sodium sulphate in a 50 ml Erhlenmayer flask. The total volume was passed through the chromatography column containing 1 g alumina which has been deactivated with 4 per cent (w/w) added water for 2 hours (as recommended by Deeth *et al.* 1980). The solution was passed through the column twice at the

rate of approximately 3 ml/min. It was followed by 2 x 5 ml of 1:1 (v/v) hexane-diethyl ether to remove any traces of triglycerides.

The alumina with adsorbed fatty acids was vacuum dried and transferred into a glass-stoppered glass tube and stored at 4 ^{o}C until chromatographed. Two ml of di-isopropyl ether containing 6 per cent (v/v) formic acid were added, mixed thoroughly using a vortex mixer and then centrifuged at 2000 **g** for 15 minutes. One ml of the supernatant was transferred into a 2 ml vial, which was capped and then placed on the auto-injecter tray ready for chromatography.

Gas liguid chromatography was performed using a United Technologies Packard system equipped with a dual 1 metre x 3 mm i.d. packed glass columns with flame ionization detector and an integrator (Spectra Physics). Samples were injected by an automatic liguid auto sampler (model 911) fitted with a 0.5 *ul* syringe. A data storing and processing system (LABNET, Spectra Physics) was linked interactively for data management. The temperature programme was as follows : initial temperature 110 ^{o}C, heating rate 8 ^{o}C/min to 195 ^{o}C, holding time at final temperature (195 ^{o}C) 22 minutes. The injector/detector temperature was 230 ^{o}C. The carrier gas (N_2) flow rate was 55 ml/min.

The chromatograph was calibrated on a set of fatty acids standard prepared in diisopropyl ether containing 4 per cent formic acid (BDH Chemicals Ltd., Poole, England).

The concentrations (mg/100 ml) of the individual fatty acids in the standard solution were C4:0, 12.4; C6:0, 10.4; C8:0, 8.7; C9:0, 10.8; C10:0, 13.7; C12:0, 20.8; C14:0, 13.1; C16:0, 31.3; C17:0, 10.8; C18:0, 32.6; C18:1, 60; C18:2, 59. Response factors were automatically determined by data processor with respect to C9:0.

All chromatograms were quantified on the data processor by relating the corrected peak areas to the peak area of the C9:0 internal standard. As a precaution, C17:0 internal standard of the same concentration as C9:0 was included as a check on the recovery of the higher carbon acids.

2.2.18 Lipolytic activity (LA)

The improved pH-stat technique (Parry *et al.*, 1966) was used. The method was based on the principle of maintaining a designated pH by automatic titration. In the lipase assay, released free fatty acids caused a slight depression in pH, which is automatically corrected by the addition of standard alkali. The amount of alkali added over time is recorded simultaneously thereby giving a continuous record of lipase activity. The substrate consisted of an emulsion prepared by dispersing 10 per cent (v/v) salt-free butter oil in a 10 per cent aqueous solution of gum arabic (Sigma Chemicals Ltd., Poole, England). The mixture was warmed to 50 ^{o}C and mixed to homogeneity using an Ultrasonics Rapid Disintegrator (Ultrasonics Ltd., York, England) at maximum power for 30

minutes.

The assay mixture consisted of 5 ml of the butteroil emulsion, 0.2 ml of 2.85 M NaCl, and 0.5 ml of milk sample as the enzyme source. Prior to mixing of the assay mixture the pH of the emulsion was adjusted to 8.8. A constant temperature of 37 ^{o}C was maintained throughout.

The titration was done using Metrohms Combititrator 3D (Metrohms Ltd., Herisau, Switzerland). The system consisted of model E 512 pH/mV meter with the accuracy of 0.01 pH, model E 473 Impulsomat with a minimum response of 0.01 pH and model E 425-1 Micro-Dosigraph for microtitration with minimum delivery of 0.001 ml.

Titration was done at 37 ^{o}C with continuous stirring. Prior to mixing the assay mixture, the pH of the emulsion was adjusted to 8.8. The assay was carried out for 10 minutes. The amount of titrant used (0.025 N NaOH) was recorded. The initial slope of the titration curve was taken as a measure of lipolytic activity and expressed as microEquivalent of alkali required per minute to titrate the free fatty acids released at pH 8.8 and temperature of 37 ^{o}C (uM/min/ml of milk). Assays were performed in duplicate.

2.2.20 Alkaline phosphatase activity

The IDF standard method 63 (IDF/FIL, 1971) was used to measure the quantity of active alkaline phosphatase present in milk. 1 ml of the milk sample was diluted with barium borate-hydroxide buffer at pH 10.6 and incubated at a temperature of 37 ^{o}C for 1 hour. The presence of alkaline phosphatase in the sample will liberate phenol from added phenyldisodium-orthophosphate ($NA_2C_6H_5PO_4.2H_2O$) (BDH Chemicals Ltd., Poole, England). The phenol liberated is measured photometrically after reaction with Gibb's reagent (BQC (2.6-dibromoquinonechloroimide) solution).

The optical density was converted to *ug* of phenol by refrence to a standard curve. Phosphatase activity is expressed as *ug* of phenol by the formula:

Phosphatase activity = 2.4 x *ug* phenol

2.2.21 Tyrosine value

The method proposed by Hull, (1947) as modified by Citti *et al.* (1963) was used throughout the study for the determination of protein hydrolysis in milk. The procedure was as follows: Five ml milk sample were added to a clean dry test tube; 1 ml of distilled water was added, followed by 10 ml of 0.72 N TCA while agitating the test tube to mix the milk. The tube was then stoppered and shaken vigorously and allowed to stand for 10 minutes before filtering the content through number 2 Whatman filter

paper. Five ml of the filtrate were added to a 50 ml Erlenmeyer flask and 10 ml of sodium carbonate reagent were added and mixed thoroughly before 3 ml of Folin and Ciocalteau reagent (BDH Chemicals Ltd., Poole, England) were added. About 5 minutes were allowed for the blue colour to develop. The sodium carbonate reagent was prepared by dissolving 75 g of anhydrous sodium carbonate (BDH Chemicals Ltd., Poole, England) and 10 g of sodium hexametaphosphate (May and Baker, Dagenham, England) in 500 ml distilled water.

The absorbance was measured at 650 *n*m after 5 minutes using a Spectronic 20 spectrophotometer (Bausch and Lomb Incorporation, Rochester, NY, USA). To convert absorbance to mg tyrosine/ml milk, a standard curve was prepared using tyrosine as specified by Hull, (1947). The regression equation (section 2.4.1) for this curve is $y = 0.0217 + 3.8544X$ where y = absorbance at 650 *n*m and x = mg tyrosine/ml milk.

SECTION 2.3 MICROBIOLOGICAL METHODS

2.3.0 General procedure

The guide to general procedures part 1 section 1.1, 1.2, and 1.3 of B.S. 4285 (British Standards Institution, 1984) was followed for sampling; apparatus, media preparation and sterilisation; and procedures for obtaining incubation conditions, respectively. The number of organisms per millilitre was calculated as follow :

$$N = (C / 0.1 (n_1 + 0.1 (n_2)) d$$

Where N is the number of organisms per millilitre, C is the sum of colonies on all plates counted, n_1 is the number of plates in the first dilution counted, n_2 is the number of plates in the second dilution counted, d is the first dilution from which the first count was obtained.

In reporting, the counts were rounded off and transformed into natural logarithm. When the test portion failed to produce colonies the count was reported as log 0

2.3.1 Total plate count

The B.S. 4285 (British Standards Institution, 1968) as modified by B.S. 4285, section 2.2.1 (British Standards Institution, 1984) was used throughout the studies. The medium used was plate count agar CM 325 (Oxoid Ltd., Basingstoke. Hants., England). Incubation conditions were 30 ^{o}C for 3 days.

2.3.2 Psychrotrophic count

Psychrotrophic microorganisms were defined as those psychrotrophs able to grow at 7 ^{o}C or less regardless of their optimum growth temperatures (Thomas, 1969). The B.S. 4285 (British Standards Institution, 1968) as modified by B.S. 4285 (British Standards Institution, 1984) section 2.3.1 was used throughout the studies. The medium used was plate count agar CM 325 (Oxoid Ltd., Basingstoke, Hants, England). To each plate 14 ml of growth medium was used. Plates were incubated at 7 ^{o}C for 7 days.

2.3.3 Thermoduric count

Thermoduric organisms were defined according to the B.S. 4285 section 3.2 (British Standards Institution, 1984) as those that survived heat treatment at 63.5 $\pm$0.5 ^{o}C for 30 minutes. was used throughout the studies. The medium used was plate count agar CM 325 (Oxoid Ltd., Basingstoke, Hants, England). To each plate 14 ml of the growth medium was used. Plates were incubated at 30 $\pm$1 ^{o}C for 72 hours.

2.3.4 Proteolytic counts

Proteolytic microorganisms were defined as those organisms that split peptides thus releasing the amino acids. The growth medium was prepared according Martley *et al*. (1970). Standard plate count agar CM 463 (Oxoid Ltd., Basingstoke, Hants., England) was used as the general purpose basal medium. Sodium caseinate (Difco Laboratories, Detroit, USA) was added as the substrate for proteolysis, sodium citrate used as a buffer to prevent acid forming organisms from precipitating casein at pH 5.0 and Ca^{++} to ensure the precipitation of the insoluble para casein resulting from proteolysis.

The composition of the final medium per litre were as follows; standard plate count agar 23.5 g, sodium caseinate 10 g, hydrated trisodium citrate 4.41 g and calcium chloride hexahydrate ($CaCl_2.6H_2O$) 4.38 g. The sodium caseinate was dispersed in half (500 ml) of the 0.015 M trisodium citrate solution using the Silverson

mixer (London, England) and added to the standard plate count agar which has been rehydrated in the balance of the trisodium citrate solution before autoclaving at 121 ^{o}C for 15 minutes.

20 ml of sterile 1 M $CaCl_2$ solution was added to the molten agar and mixed gently using a magnetic stirrer. The Standard Method Caseinate agar (SMCA) was then dispensed into petri dishes. 12 ml were dispensed into each petri dish placed on a flat surface bench and allowed to cool and solidified overnight. The plates were stored at 4 ^{o}C until used or discarded after 30 days.

0.1 ml of a 10-fold dilution sample was surface innoculated on the the SMCA medium using a sterile glass rod. Incubatation conditions were 30 ^{o}C for 72 hours for mesophilic proteolytic microorganisms and at 7 ^{o}C for 7 days for psychrotrophic proteolytic microorganisms.

Those colonies surrounded by a clear ring were considered as proteolytic and were counted.

2.3.5 Lipolytic count

The B.S. 4285 (British Standards Institution, 1968) was used throughout the trial. The growth medium used was Tributyrin agar PM 4 (Oxoid Ltd., Basingstoke, Hents, England). According to the manufacturer, the medium consisted of stable homogenate of nutrient agar and tributyrin (glyceryl tributyrate) as recommended in B.S. 895 (British Standards Institution, 1968) and replaced by B.S. 4285 (British Standards Institution, 1968). Apparent

separation of the homogeinate was checked and care taken to mix the medium gently before pouring.

The inoculated plates were incubated at 30 ^{o}C for 72 hours for mesophilic lipolytic microorganisms and at 7 ^{o}C for 7 days for psychrotrophic lipolytic microorganisms. Colonies sorrounded with a clear ring were considered to be lipolytic and were counted.

2.3.6 Coliforms count

The IDF standard method 73 (IDF/FIL, 1974) routine method B (using solid medium) was used throughout the studies. Coliforms were defined as rod shaped, Gram-negative, aerobic and and facultatively anaerobic non-spore forming bacteria which ferment lactose, with the formation of gas and acid. Using the medium and method prescribed, they formed red colonies.

Violet red bile agar, product code CM 107 (Oxoid Ltd., Basingstoke, Hents, England) was prepared 3 hours before used as recommended by the manufacturer. Incubation was at 30 ^{o}C for 24 hours. The number of the characteristic red colonies of at least 0.5 mm in diameter, typical for coliform organisms, were counted.

2.4. Statistical analysis

Analysis of variance was performed according to the procedure described by Neter and Wesseman, (1974) using Genstat V Mark 4.03 (Lawes Agricultural Trust, Rothamsted Experimental Station, England). The variance ratios (F = MSTR/MSE) were used for the testing of significance. Multiple comparison of the differences of means was performed using the procedure of the standard error of differences (SED) of the means as described by Steel and Torrie (1980). Student *t* test was used for significance testing between means:

$$t = (U_1 - U_2)/\text{SED}$$

Linear regression analysis was performed according to the procedures described by Neter and Wesserman (1974), using MINITAB version 5.1.1 (Minitab Inc., State College, PA 16801, U.S.A.).

CHAPTER THREE

ON-FARM MILK QUALITY STUDIES

Introduction

The introduction of bulk refrigerated storage on the farm enhanced quality. Smillie *et al*. (1958) surveyed the bacterial quality of raw milk on the farm during the first year of the introduction of bulk refrigerated storage tank in the south-west of Scotland. They observed significant improvement in bacteriological quality of milk. Crawford, (1967) made similar observations and reported an increase in the usage of refrigerated bulk storage tanks on the farms.

The transition from churn bulk milk storage to on-farm refrigerated bulk storage tank in SMMB was completed in 1984 (Federation of United Kingdom MMB, 1987). The refrigerated raw storage facilities available on the farms have been used not only for safe storage of milk before collection, but also as a management tool by the MMB for milk stock rotation. This also has enabled the MMB to reduce the soaring cost of milk transportation by the introduction of alternate day collection. This is especially true in the longer and more costly routes. Consequently, raw milk may be subjected to extended storage on the the farm. Crawford (1967) reported that milk could be 36 hours old when it reached the creamery and Cox (1975) suggested that it might even be older on receipt at creameries located in large cities.

The widespread used of refrigerated storage has changed the bacterial profile of raw milk. Extended refrigerated raw storage has been selective to psychrotrophic bacteria (Thomas *et al.*, 1975b). Therefore, the purpose of the present short survey was to evaluate the quality of raw milk produced in that part of south-west of Scotland from which milk for the main trials would be received.

Materials and methods

Three insulated road tankers from the local haulage contracter of the SMMB were assigned to the study, two with a capacity of 9,000 litres and one with 14,000 litres capacity. The collection area of these tankers was within 15 kilometres radius of the West of Scotland College (WSC). Milk from the first collection, which was a mixture of the previous day's evening milk (PM) and the morning (AM) milk of the day of sampling was studied. The number of farms taken to fill each tanker to the full capacity limit the number of farms investigated. The collection route taken by each tanker was that usually scheduled by the SMMB and without any alteration.

Three surveys were carried out. Each approximately at three months apart. The dates were as follows: 5th December 1986, 15th March 1987 and 14th June 1987. These dates were selected to account for season and for the ease of operation due to limited recources.

The farm sequence of collection from the first survey was noted and the sequence repeated in the subsequent

surveys. If the tanker was full to capacity before the sequence was completed, the remaining farms were eliminated from the survey.

Each tanker driver was accompanied by a WSC technician to sample milk from the farm tank prior to pick-up and to complete a questionaire (appendix I). Milk was sampled according to IDF Standard Method 50A (IDF/FIL, 1980). Two 200 ml samples were taken using a sterile (by autoclaving at 121 ^{o}C for 15 minutes) 100 ml stainless steel dipper. The samples were kept cool in an insulated box during the overall collection period and transit to the laboratory.

On arrival at the WSC, one set of the samples was immediately transferred and kept in a refrigerator at 4 ^{o}C and analysed on the same day and the other set was stored in a deep freeze cabinet at -20 ^{o}C for later analysis.

The milk samples were analysed for chemical composition and bacteriological quality. The chemical analysis were:- fat, lactose and protein according to the method described in section 2.2.6 and total solids (TS), solids-not-fat (SNF), ash, titratable acidity (TA), hydrogen ion concentration (pH), extraneous water (FPD), antibiotic residues and total free fatty acids (FFA) concentration according to the methods described sections 2.2.7, 2.2.8, 2.2.9, 2.2.12, 2.2.13, 2.2.14, 2.2.15 and 2.2.17.1, respectively.

The bacteriological tests performed consist were, total plate count (TPC), psychrotrophic bacterial count (PBC), thermoduric bacterial count (TBC), psychrotrophic and

mesophilic lipolytic bacterial count (PLBC and MLBC, respectively), and coliforms count according to the methods described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.5 and 2.3.6 respectively.

All data were subjected to the analysis of variance as described in section 2.4., using farms, tankers and seasons as main effects.

RESULTS

SECTION 3.1 ON-FARM MILK QUALITY STUDIES

The total number of farms surveyed is shown in table 3.1. A total of 77 sets of milk samples were analysed. The total number of farms investigated varied from 24 to 27 in each survey. The mean milk collected by the tankers varied between the surveys (Table 3.2). Milk production was higher in March than in June or December.

Table 3.3, illustrates the time of collection, and the time interval the tankers spent on each farm. Milk collection began at approximately 7:20 a.m. The time taken to collect the milk from each farm ranged from 9.7 to 15.9 minutes and the time taken to complete the collection run ranged from 1 hour 42 minutes to 2 hours 54 minutes. The distance covered by each tanker was approximately 16 km.

The temperature of milk taken from the farm tank immediately before pick up are tabulated in table 3.4. The temperature ranges within and between surveys were large. The farm milk temperature was higher in June (1.1 to 9.4 ^{o}C) than in December (2.8 to 7.0 ^{o}C) or March (2.2 to 6.1 ^{o}C). However, the majority of the farm tank milk temperature, in all the surveys, were observed to be between 3.7 and 5.5 ^{o}C (Figure 3.4) and within the temperature of the SMMB acceptance limits (SMMB, 1986). It was noted that higher milk temperature ($\geq$ 6 ^{o}C) were from farms with higher milk production. Consequently, the temperature of the commingled milks were greatly affected (Table 3.4).

Results of the platform test for antibiotic residues performed on all farm milk samples, indicated no positive results.

Table 3.1

On-farm milk quality survey: The number of farms investigated and the tankers used

Survey[c]	Tanker			Survey total[d]
	1[a]	2[b]	3[a]	
1. December	8(9)*	8(9)	8	24(26)
2. March	8(9)	8(10)	8	24(27)
3. June	8	8	8	24
Tanker total[e]	24(26)	24(27)	24	72(77)

Note: (a) = Tanker capacity 9000 litres, (b) = tanker capacity 14,000 litres, (c) = the consecutive dates were, 2.12.1986, 9.3.1987 and 15.6.1987, approximately three months apart, (d) = total number of farms investigated in each survey, (e) = total number of farms within each tanker collection area, * = actual number of farms surveyed.

Table 3.2

On-farm milk quality survey : The range of daily farm milk production (PM and AM milks) per farm in the south-west of Scotland, during the survey period.

Survey[a]	Area[b]	volume (litres)			Std dev
		Low	Median	High	
December	1	321.0	965.0	2327.0	658.5
	2	764.0	1492.0	3120.0	721.4
	3	824.0	1395.5	3985.0	1028.3
March	1	260.0	1328.0	2977.0	976.3
	2	800.0	1384.0	2788.0	618.3
	3	333.0	1263.0	3307.0	680.2
June	1	571.0	1114.5	1843.0	514.4
	2	598.0	1729.0	2584.0	720.2
	3	976.0	1657.0	2228.0	501.7

Note: (a) = The dates were as follows, 2.12.1986, 9.3.1987 and 15.7.1987, (b) = Three areas, each serviced by one tanker.

Table 3.3

On-farm milk quality survey : The time taken by each road tanker from first collection at first farm to the last collection at the last farm, the time of arrival at WSAC, the average time the tanker spent on the farm and the interval taken from the first loading to the time the tanker arrived with the full load at the WSAC.

Survey	Area	N	Time (hrs:min)			Interval		Tanker load (litres)
			First (A)	Last (B)	Arrival (C)	X time at each farm (minutes)	(hrs:min) (C-A)	
December	1	8	7:14	9:20	9:38	15.8	2:24	9,565
	2	9	7:20	9:35	10:14	15.0	2:54	10,448
	3	9	7:18	8:45	9:00	9.7	1:42	13,469
March	1	8	7:08	8:36	9:00	12.6	1:52	9,631
	2	10	7:20	9:30	10:10	13.0	2:50	9,348
	3	9	7:20	9:00	9:15	11.1	1:55	14,495
June	1	8	7:21	9:28	10:10	15.9	2:49	13,362
	2	8	7:15	8:49	9:09	15.7	1:54	9,533
	3	8	7:18	9:23	9:38	15.6	2:20	14,829

Note : N = Number of farms surveyed.

Table 3.4

On-farm milk quality survey : The temperature (^{o}C) of milk in the farm storage tank immediately prior to pick up.

Tanker	Survey								
	December			March			June		
	1	2	3	1	2	3	1	2	3
1[b]	4.4	4.4	2.8	4.4	4.0	6.1	1.1	6.7	5.0
2	4.5	3.5	5.0	4.0	5.0	3.9	4.0	3.9	4.0
3	5.6	4.4	4.0	4.0	5.0	5.0	4.4	5.6	5.0
4	6.0	4.5	2.8	4.0	2.8	2.2	5.0	9.4	4.0
5	4.4	7.0	5.0	4.0	4.4	5.0	4.0	3.9	4.6
6	4.0	5.6	4.4	4.0	5.0	3.9	7.8	5.5	4.0
7	4.4	4.0	2.8	4.0	4.4	4.5	4.4	4.4	4.0
8	4.0	5.0	3.3	4.0	4.0	4.6	4.4	4.7	4.0
9	3.3	5.0		4.0	5.0				
10					3.3				
Minimum	3.3	3.5	2.8	4.0	2.8	2.2	1.1	3.9	4.0
Median	4.4	4.5	4.4	4.0	3.7	4.5	4.4	5.5	4.0
Maximum	6.4	7.0	5.0	4.4	5.0	6.1	7.8	9.4	5.0
Std dev	0.8	1.0	0.8	0.5	1.0	1.3	1.8	2.0	0.1

Note: (a) = Farm bulk tank milk temperature, ^{o}C, (b) = number of farms surveyed.

SECTION 3.2 CHEMICAL PROPERTIES OF MILK

The distribution of FPD of milk for farms in the survey was illustrated in figure 3.4. The mean FPD was given in table 3.5. The means FPD were -0.542, -0.543 and -0.539 ^{o}C for the surveys in December, March and June, respectively. Analysis of the data indicated that the differences between surveys were significant ($p < 0.05$). The mean FPD of milk surveyed in June was higher than in December or in March. No significant difference ($p > 0.05$) was found between farms within the same tanker. However, milks collected by tanker 2 and 3 showed consistently higher values in June than in December or March. Two farms within these collection areas showed values of -0.535 ^{o}C or higher during the survey (Figure 3.4a).

The means TA and pH values are given in tables 3.6 and 3.7, respectively. Their frequency distributions are shown in figures 3.4c and 3.4b, respectively. The mean TA values of the surveys were 0.154, 0.137 and 0.129 for December, March and June survey, respectively. The mean pH values were 6.73, 6.73 and 6.70 for December, March and June, respectively. Analysis of the data indicated large and highly significant ($p < 0.001$) variation in TA values between surveys as illustrated in figure 3.4c. The TA in December was higher than in March and June. The interaction between Tanker and Survey was highly significant ($p < 0.001$). The interaction indicated that the variations in TA between surveys varied greatly between the tankers. In contrast, the differences in pH of

milk between surveys was not significant ($p > 0.05$), but the mean difference in pH of milk of farms between the tanker routes was significant ($p < 0.05$). However, the differences between farms in tanker 1, 2 and 3 were not significant ($p > 0.05$). The interaction between Tanker and Survey was significant ($p < 0.05$).

The mean FFA concentration of milk as measured by the copper soap method of Shipe *et al*. (1980) were, 0.65, 0.64 and 0.63 mEq/100 ml milk for survey in December, March and June, respectively (table 3.14). The grand mean of the survey was 0.64 mEq/100 ml. The FFA content of the majority of the farm milks surveyed were within 0.68 mEq/100 ml as illustrated in the figure 3.4d. No significant differences ($p > 0.05$) were observed either due to the differences between surveys, tankers and farms within the tanker routes.

Table 3.5

Means of freezing point depression (°C) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	-0.541	0.0010	-0.542	0.0009	-0.545	0.0015	-0.542	0.0007
March	-0.541	0.0025	-0.543	0.0013	-0.544	0.0024	-0.543	0.0012
June	-0.541	0.0025	-0.539	0.0021	-0.537	0.0009	-0.539	0.0012
Wt. means[c]	-0.541	0.0012	-0.542	0.0009	-0.542	0.0012	-0.541[d]	0.0006

Note: (a) = Bulk tankers used (b) = weighted means between tankers
(c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.00023	0.00012	3.764*
Tankers	2	0.00001	0.00001	0.230
Between farms within tanker 1	7	0.00004	0.00001	0.206
Between farms within tanker 2	7	0.00016	0.00002	0.728
Between farms within tanker 3	7	0.00012	0.00002	0.537
Tanker.Survey interaction	4	0.00018	0.00005	1.472
Error	42	0.00130	0.00003	
Total	71	0.00205	0.00003	

Statistical significance : * Significant, $p < 0.05$

Table 3.6

Means of titratable acidity (expressed as percentage lactic acid) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	mean[b]	SE
December	0.152	0.0032	0.151	0.0020	0.159	0.0013	0.154	0.0015
March	0.133	0.0037	0.138	0.0025	0.142	0.0031	0.137	0.0019
June	0.134	0.0038	0.134	0.0037	0.119	0.0023	0.129	0.0024
Wt. means[c]	0.140	0.0026	0.141	0.0020	0.140	0.0040	0.140[d]	0.0016

Note: (a) = Bulk tankers used (b) = weighted means between tankers
(c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.00837	0.00419	86.760**
Tankers	2	0.00000	0.00000	0.005
Between farms within tanker 1	7	0.00170	0.00023	4.725*
Between farms within tanker 2	7	0.00057	0.00007	1.398
Between farms within tanker 3	7	0.00031	0.00004	0.925
Tanker.Survey interaction	4	0.00187	0.00044	9.183**
Error	42	0.00203	0.00005	
Total	71	0.01455	0.00020	

Statistical significance : * Significant, $p < 0.05$,
** significant, $p < 0.001$.

Table 3.7

Means of hydrogen ion concentration (pH) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	6.68	0.010	6.74	0.020	6.75	0.013	6.72	0.011
March	6.73	0.010	6.72	0.008	6.73	0.005	6.73	0.005
June	6.70	0.000	6.69	0.026	6.71	0.013	6.70	0.009
Wt. means[c]	6.70	0.007	6.72	0.011	6.73	0.007	6.72[d]	0.005

Note: (a) = Bulk tankers used (b) = weighted means between tankers
(c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.00821	0.00410	2.814
Tankers	2	0.01114	0.00557	3.817*
Between farms within tanker 1	7	0.00360	0.00051	0.352
Between farms within tanker 2	7	0.01907	0.00272	1.867
Between farms within tanker 3	7	0.00800	0.00114	0.783
Tanker.Survey interaction	4	0.02212	0.00553	3.791*
Error	42	0.61273	0.00146	
Total	71	0.13340	0.00188	

Statistical significance : * Significant, $p < 0.05$

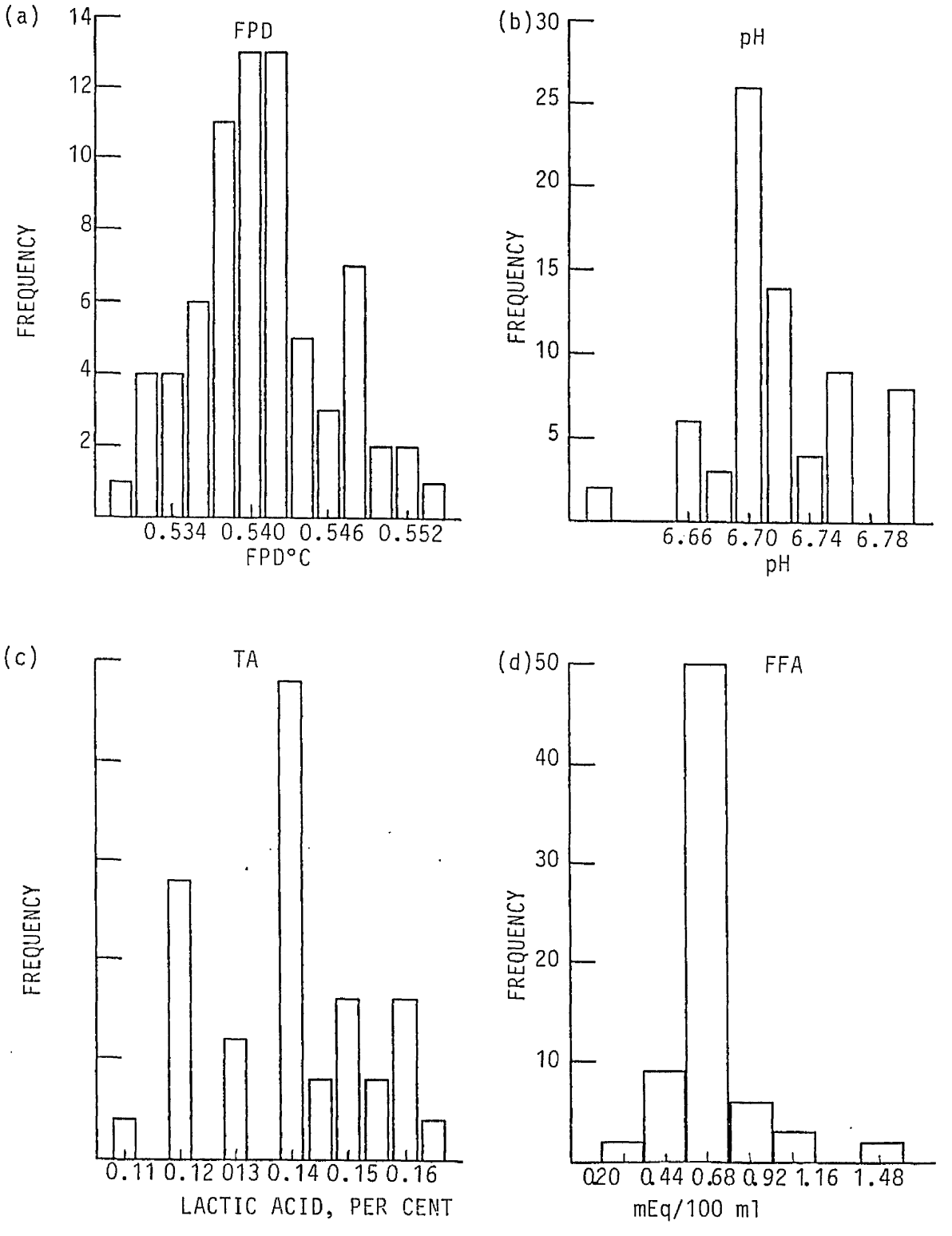


Figure 3.4: On-farm milk quality survey: Frequency distribution of freezing point depression (FPD), pH, titratable acidity (TA) and free fatty acids content (FFA) of milk sampled from the farm tank.

SECTION 3.3 CHEMICAL COMPOSITION OF MILK

The mean gross chemical composition of milks sampled from the farm tanks are given in tables 3.8, 3.9, 3.10 and 3.13 and the their frequency distribution were illustrated in figures 3.5a, 3.5b, 3.5c and 3.5d for the per cent composition of fat, protein,lactose and ash, respectively. The mean percentage TS and SNF of milk are shown in tables 3.11 and 3.12, and illustrated in figures 3.6a and 3.6b, respectively.

The mean fat content of milk sampled on the farms were 3.61, 3.80 and 3.81 per cent for the December, March and June surveys, respectively. The grand mean for the survey was 3.74 per cent. The differences of means between surveys were highly significant ($p < 0.001$). The percentage fat content of farm milk surveyed in December was significantly ($p < 0.01$) lower than in March or June. However, the mean differences between March and June surveys were not significant ($p > 0.05$). The frequency distribution of fat content of milk in the survey was normal with majority of the farms were within the 3.8 per cent range as illustrated in figure 3.5a.

A contrasting observation was made for the protein content of the farms milk surveyed. The mean protein content of milk for the survey in December was 3.88 per cent, higher than in March (3.66 per cent) or the June (3.38 per cent) survey. The grand mean of the survey was 3.65 per cent. The variations of mean protein content of milk between surveys was very highly significant ($p <$

0.001). The mean difference between tankers was significant ($p < 0.01$). The protein content of milk collected from farms in tanker route 1 was higher in December and the March survey when compared to the means of farms in tanker route 2 or 3. No significant ($p > 0.05$) interaction was observed. Frequency distribution of the protein content of the milk surveyed shown in figure 3.5b. The majority of milk from the farms surveyed were within the range of 3.2 and 4.2 percent protein.

The variations in lactose content of milk surveyed was observed to follow similar trend as to the protein content. The differences between surveys were significant ($p < 0.01$). The mean lactose content in December was 4.71 percent and significantly ($p < 0.01$) higher than in March (4.64 per cent) or in the June (4.66 per cent) survey. The differences between tankers were very highly significant ($p < 0.001$). The mean lactose content of milks from farms in tanker 3 was consistently higher than those in tanker 1 or 2 in all the surveys. The interaction between Tanker and Survey was significant ($p < 0.01$). The variations in lactose content of farm milk between tankers were not the same for all the surveys. The frequency distribution of lactose content of all the milks surveyed (Figure 3.5c) was normal and the majority of the milk from the farms surveyed contained more than 4.6 per cent lactose.

The grand mean percentage ash content of the farm milk surveyed was 7.28 per cent. No significant differences ($p > 0.05$) between the means of survey and farms between the tanker routes were observed. No significant ($p > 0.05$)

interaction was observed. Analysis of the frequency distribution of the ash content of the farm milk surveyed as illustrated in figure 3.5c indicated a normal distribution with most of the farms milk with > 0.72 per cent ash content.

The mean TS content of the farm milks surveyed were 12.62, 12.51 and 12.45 per cent for the December, March and June survey, respectively. The grand mean of the survey was 12.53. The mean SNF content of milk were 8.59, 8.91 and 8.65 per cent for the December, March and June surveys, respectively. The grand mean of the survey was 8.72. The differences in TS and SNF content of milks between the surveys and farms in the tanker route within the survey were not significant ($p > 0.05$). Significant differences between means of farms in tanker route 3 were observed. The frequency distribution as illustrated in figures 3.6a and 3.6b showed a normal distribution curve. The TS and SNF content of the majority of the farms surveyed were > 8.4 and > 12.2 percent, respectively.

Table 3.8

Means of fat content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	mean[b]	SE
December	3.52	0.082	3.55	0.037	3.79	0.032	3.61	0.039
March	3.87	0.067	3.77	0.048	3.76	0.063	3.80	0.034
June	3.85	0.069	3.79	0.065	3.78	0.062	3.81	0.036
Wt. means[c]	3.74	0.052	3.70	0.035	3.78	0.029	3.74[d]	0.024

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.58576	0.29288	9.452**
Tankers	2	0.05676	0.02838	0.916
Between farms within tanker 1	7	0.24763	0.03538	1.142
Between farms within tanker 2	7	0.22852	0.03265	1.054
Between farms within tanker 3	7	0.11670	0.01667	0.538
Tanker.Survey interaction	4	0.36021	0.09005	2.906*
Error	42	1.30143	0.03099	
Total	71	2.89700	0.04080	

Statistical significance : * Significant, $p < 0.05$
** Significant, $p < 0.001$

Table 3.9

Means of protein content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	4.02	0.134	3.82	0.079	3.80	0.051	3.88	0.058
March	4.05	0.135	3.46	0.130	3.42	0.323	3.66	0.116
June	3.42	0.036	3.38	0.032	3.34	0.034	3.38	0.020
Wt. means[c]	3.85	0.086	3.56	0.067	3.53	0.096	3.65[d]	0.050

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	3.2491	1.6246	13.429**
Tankers	2	1.3175	0.6587	5.445*
Between farms within tanker 1	7	0.8452	0.1207	0.998
Between farms within tanker 2	7	0.4444	0.0635	0.525
Between farms within tanker 3	7	1.1839	0.1691	1.398
Tanker.Survey interaction	4	1.9475	0.2369	1.958
Error	42	5.0811	0.1210	
Total	71	13.0687	0.1841	

Statistical significance : * Significant, $p < 0.01$
** Significant, $p < 0.001$

Table 3.10

Means of lactose content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	4.61	0.022	4.70	0.062	4.82	0.020	4.71	0.029
March	4.61	0.017	4.64	0.021	4.68	0.036	4.64	0.014
June	4.63	0.017	4.67	0.010	4.68	0.025	4.66	0.012
Wt. means[c]	4.62	0.011	4.67	0.023	4.73	0.021	4.67[d]	0.012

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.07154	0.03577	6.463*
Tankers	2	0.15098	0.07549	13.639**
Between farms within tanker 1	7	0.04630	0.00661	1.195
Between farms within tanker 2	7	0.08673	0.01239	2.239
Between farms within tanker 3	7	0.01513	0.00216	0.390
Tanker.Survey interaction	4	0.07861	0.01965	3.551*
Error	42	0.23246	0.00554	
Total	71	0.68173	0.00960	

Statistical significance : * Significant, $p < 0.01$
** Significant, $p < 0.001$

Table 3.11

Means of total solids content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	12.52	0.022	12.77	0.059	12.57	0.082	12.62	0.043
March	12.57	0.109	12.40	0.065	12.61	0.357	12.51	0.094
June	12.51	0.041	12.44	0.076	12.41	0.085	12.45	0.040
Wt. means[c]	12.53	0.043	12.54	0.050	12.52	0.101	12.53[d]	0.037

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.3344	0.1672	1.654
Tankers	2	0.0007	0.0003	0.003
Between farms within tanker 1	7	0.2857	0.0408	0.404
Between farms within tanker 2	7	0.4063	0.0580	0.574
Between farms within tanker 3	7	1.4024	0.2003	1.982
Tanker.Survey interaction	4	0.5282	0.1321	1.306
Error	42	4.2463	0.1011	
Total	71	7.2041	0.1015	

Table 3.12

Means of solids not fat content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	8.63	0.123	8.52	0.130	8.63	0.066	8.59	0.064
March	9.15	0.480	8.63	0.081	9.02	0.476	8.91	0.206
June	8.66	0.051	8.66	0.038	8.62	0.038	8.65	0.024
Wt. means[c]	8.82	0.172	8.60	0.055	8.73	0.130	8.72[d]	0.074

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.1413	0.0707	0.452
Tankers	2	0.1492	0.0746	0.477
Between farms within tanker 1	7	0.6952	0.0993	0.635
Between farms within tanker 2	7	0.2397	0.0342	0.219
Between farms within tanker 3	7	2.4484	0.3498	2.237*
Tanker.Survey interaction	4	0.4459	0.1115	0.713
Error	42	6.5668	0.1564	
Total	71	10.6864	0.1505	

Statistical significance : * Significant, $p < 0.05$

Table 3.13

Means of ash content (per cent) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	0.741	0.008	0.742	0.009	0.742	0.008	0.742	0.005
March	0.739	0.008	0.732	0.009	0.728	0.005	0.733	0.005
June	0.744	0.009	0.747	0.008	0.725	0.006	0.738	0.005
Wt. means[c]	0.741	0.005	0.739	0.005	0.732	0.004	0.738[d]	0.003

Note: (a) = Bulk tankers used (b) = weighted means between tankers
(c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.00151	0.00075	1.138
Tankers	2	0.00151	0.00076	1.140
Between farms within tanker 1	7	0.00303	0.00043	0.652
Between farms within tanker 2	7	0.00505	0.00072	1.089
Between farms within tanker 3	7	0.00218	0.00031	0.469
Tanker.Survey interaction	4	0.00153	0.00038	0.578
Error	42	0.02786	0.00066	
Total	71	10.04267	0.00060	

Table 3.14

Means of free fatty acid concentration (mEq /100 ml milk) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	0.64	0.06	0.46	0.08	0.87	0.23	0.65	0.08
March	0.68	0.05	0.59	0.02	0.65	0.02	0.64	0.02
June	0.65	0.02	0.63	0.20	0.61	0.02	0.63	0.01
Wt. means[c]	0.65	0.03	0.56	0.03	0.72	0.08	0.64	0.03

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.25	0.12	0.020
Tankers	2	23.72	11.86	1.895
Between farms within tanker 1	7	17.11	2.45	0.390
Between farms within tanker 2	7	29.35	4.19	0.670
Between farms within tanker 3	7	87.24	12.46	1.991
Tanker.Survey interaction	4	48.85	12.21	1.951
Error	42	262.94	6.26	
Total	71	469.46	6.61	

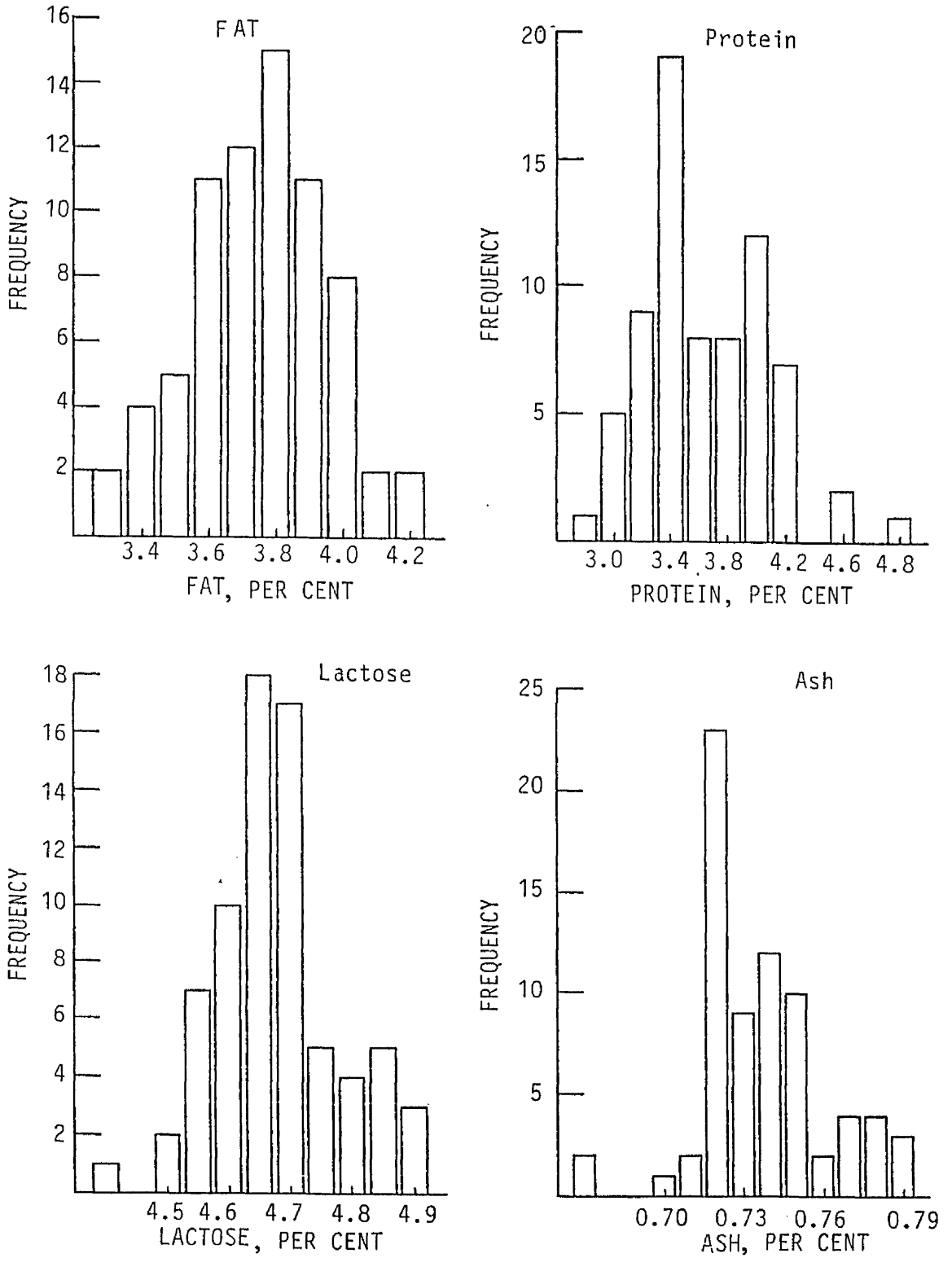


Figure 3.5: On-farm milk quality survey. Frequency distribution of percentage fat, protein, lactose and ash of milk sampled from the farm.

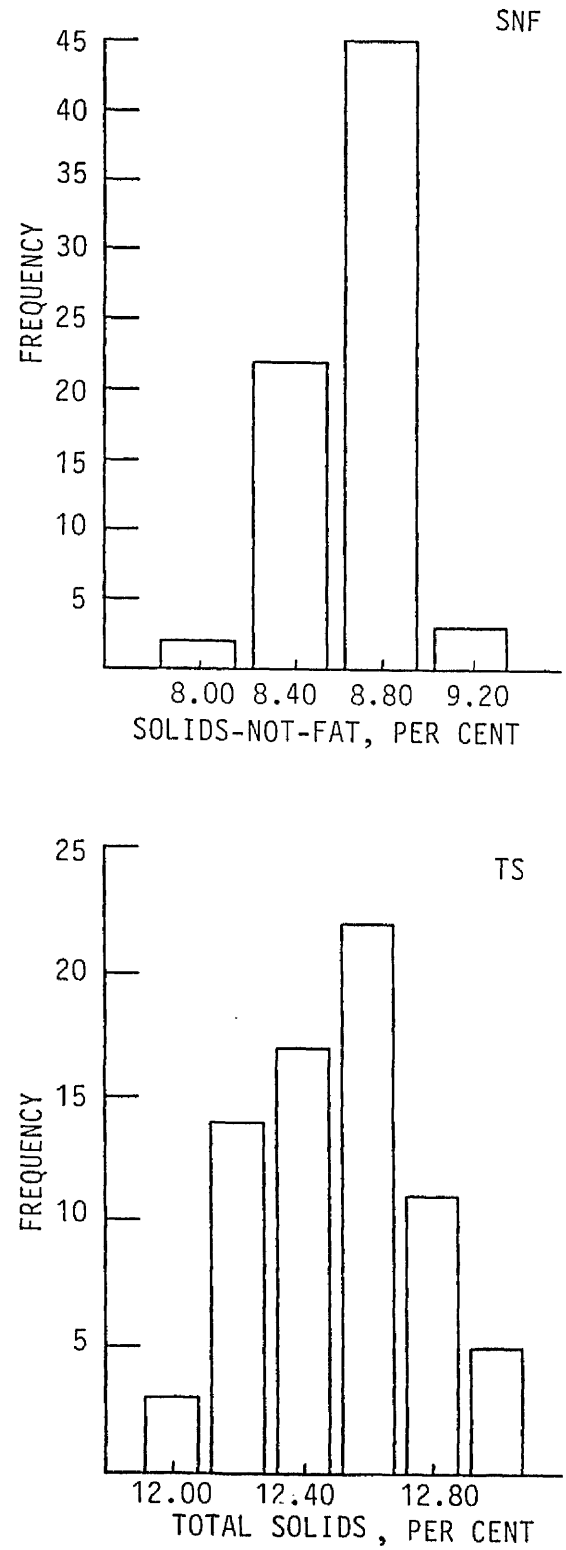


Figure 3.6: On-farm milk quality survey. Frequency distribution of percentage, solids-not-fat (SNF) and total solids (TS) of milk sampled from the farm.

SECTION 3.4 MICROBIOLOGICAL QUALITY OF MILK

The mean bacterial counts of milk sampled from the farm tanks are shown in tables 3.15, 3.16, 3.17, 3.18, 3.19 and 3.20 and their frequency distributions as illustrated in figures 3.7b, 3.7a, 3.7c, 3.7d, 3.8a and 3.8b for coliforms count, TPC, PBC, PLBC and MLBC, respectively.

The grand mean coliforms count of the survey was 5.6 x 10^2 cfu/ml. The differences between the means of surveys and tanker effects were not significant ($p < 0.05$). The frequency distribution of the coliforms count of milk in the survey was normal and only a few farms with coliforms count > 10^3 cfu/ml, as illustrated in figure 3.7b.

The grand mean TPC of farm milk surveyed was 9.8 x 10^3 cfu/ml. The mean TPC of the farm milks were 1.1 x 10^4, 9.8 x 10^3 and 8.3 x 10^3 cfu/ml for the December, March and the June surveys, respectively. The differences between the mean TPC of farms between the surveys and the tanker routes were not significant ($p > 0.05$), although the mean TPC of farm milk surveyed in December was slightly higher. The frequency distribution of the TPC of milk in the survey indicated (figure 3.7a) very few farms milk supplies with TPC > 2.5 x 10^4 cfu/ml.

The grand mean PBC of the farm milks surveyed was 2.2 x 10^3 cfu/ml. The PBCs were 2.6 x 10^3, 1.7 x 10^3 and 2.3 x 10^3 cfu/ml for the December, March and the June surveys, respectively. The differences between the means of survey and tanker routes were not significant ($p > 0.05$). The

frequency distribution of the PBC of milks in the survey indicated less than 5 farm milk with PBC of $> 3.2 \times 10^4$ cfu/ml.

The grand mean TBC of the farm milk surveyed was < 22 cfu/ml. The differences between the means of survey and the effects of tanker were not significant ($p > 0.05$). Analysis of the frequency distribution as illustrated in figure 3.7d indicates a tailed distribution.

The mean PLBC of the farm milks surveyed were 1.9×10^3, 4.5×10^2 and 3.5×10^2 cfu/ml for the December, March and June surveys, respectively. The grand mean of the farm milks surveyed was 6.8×10^2 cfu/ml. The difference between surveys was significant ($p < 0.01$). However, the PLBC was slightly higher in the December than in March or in the June survey. The differences due to the effects of tankers were not significant ($p < 0.05$). No significant interaction ($p > 0.05$) was observed. Analysis of the frequency distribution as illustrated in figure 3.8a indicated a normal distribution with very few farms with PLBC $> 1.0 \times 10^4$ cfu/ml.

The mean MLBC of the farm milks surveyed were 3.7×10^3, 5.6×10^3 and 1.9×10^3 cfu/ml for the December, March and the June surveys, respectively. The grand mean of the survey was 3.5×10^3 cfu/ml. The difference between means of surveys was significant ($p < 0.05$). The MLBC was significantly ($p < 0.05$) higher in March than in December or in the June survey. The difference between tankers effect was not significant ($p > 0.05$). No significant ($p >$

Table 3.15

Means of coliforms count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	2.81	0.241	2.40	0.071	2.35	0.198	2.53	0.110
March	2.89	0.250	2.56	0.171	3.12	0.337	2.83	0.139
June	2.59	0.217	3.30	0.373	2.91	0.251	2.92	0.164
Wt. means[c]	2.77	0.133	2.71	0.137	2.76	0.157	2.75[d]	0.081

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	2.8733	1.4366	3.007
Tankers	2	0.1522	0.0761	0.159
Between farms within tanker 1	7	2.2697	0.3242	0.697
Between farms within tanker 2	7	3.3931	0.4847	1.015
Between farms within tanker 3	7	2.3358	0.3337	0.698
Tanker.Survey interaction	4	2.1628	0.5407	1.132
Error	42	19.5875	0.4777	
Total	71	32.7744	0.4682	

Table 3.16

Means of total plate count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	4.09	0.075	3.97	0.161	4.13	0.185	4.06	0.082
March	4.09	0.184	3.87	0.114	4.04	0.157	3.99	0.087
June	4.05	0.096	3.97	0.110	3.76	0.128	3.92	0.068
Wt. means[c]	4.08	0.072	3.93	0.075	3.97	0.095	3.99[d]	0.046

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.1341	0.0671	0.401
Tankers	2	0.0609	0.0305	0.182
Between farms within tanker 1	7	1.6846	0.2407	1.438
Between farms within tanker 2	7	1.1599	0.1657	0.990
Between farms within tanker 3	7	0.7039	0.1005	0.601
Tanker.Survey interaction	4	0.6686	0.1671	0.998
Error	42	7.0313	0.1674	
Total	71	11.4432	0.1612	

Table 3.17

Means of psychrotrophic bacterial count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	3.31	0.136	3.49	0.166	3.44	0.280	3.41	0.110
March	3.27	0.298	3.10	0.123	3.36	0.292	3.22	0.133
June	3.45	0.202	3.64	0.207	3.07	0.294	3.37	0.142
Wt. means[c]	3.34	0.125	3.38	0.100	3.28	0.164	3.34[d]	0.073

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	0.1994	0.0997	0.213
Tankers	2	0.2472	0.1236	0.264
Between farms within tanker 1	7	3.8133	0.5448	1.164
Between farms within tanker 2	7	0.3133	0.0448	0.096
Between farms within tanker 3	7	2.0033	0.2862	0.612
Tanker.Survey interaction	4	1.9030	0.4758	1.017
Error	42	19.6552	0.4680	
Total	71	28.1346	0.3963	

Table 3.18

Means of thermoduric bacterial count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	0.98	0.195	1.41	0.067	1.47	0.164	1.28	0.095
March	1.77	0.186	1.65	0.094	1.61	0.110	1.69	0.079
June	1.08	0.049	1.13	0.061	1.04	0.038	1.08	0.028
Wt. means[c]	1.28	0.116	1.42	0.060	1.35	0.084	1.35[d]	0.052

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	4.2865	2.1433	17.954*
Tankers	2	0.2765	0.1383	1.158
Between farms within tanker 1	7	0.3173	0.0453	0.380
Between farms within tanker 2	7	0.2661	0.3808	0.318
Between farms within tanker 3	7	0.6452	0.0922	0.772
Tanker.Survey interaction	4	0.4737	0.1184	0.992
Error	42	4.8945	0.1194	
Total	71	11.1600	0.1594	

Statistical significance : * Significant, $p < 0.001$

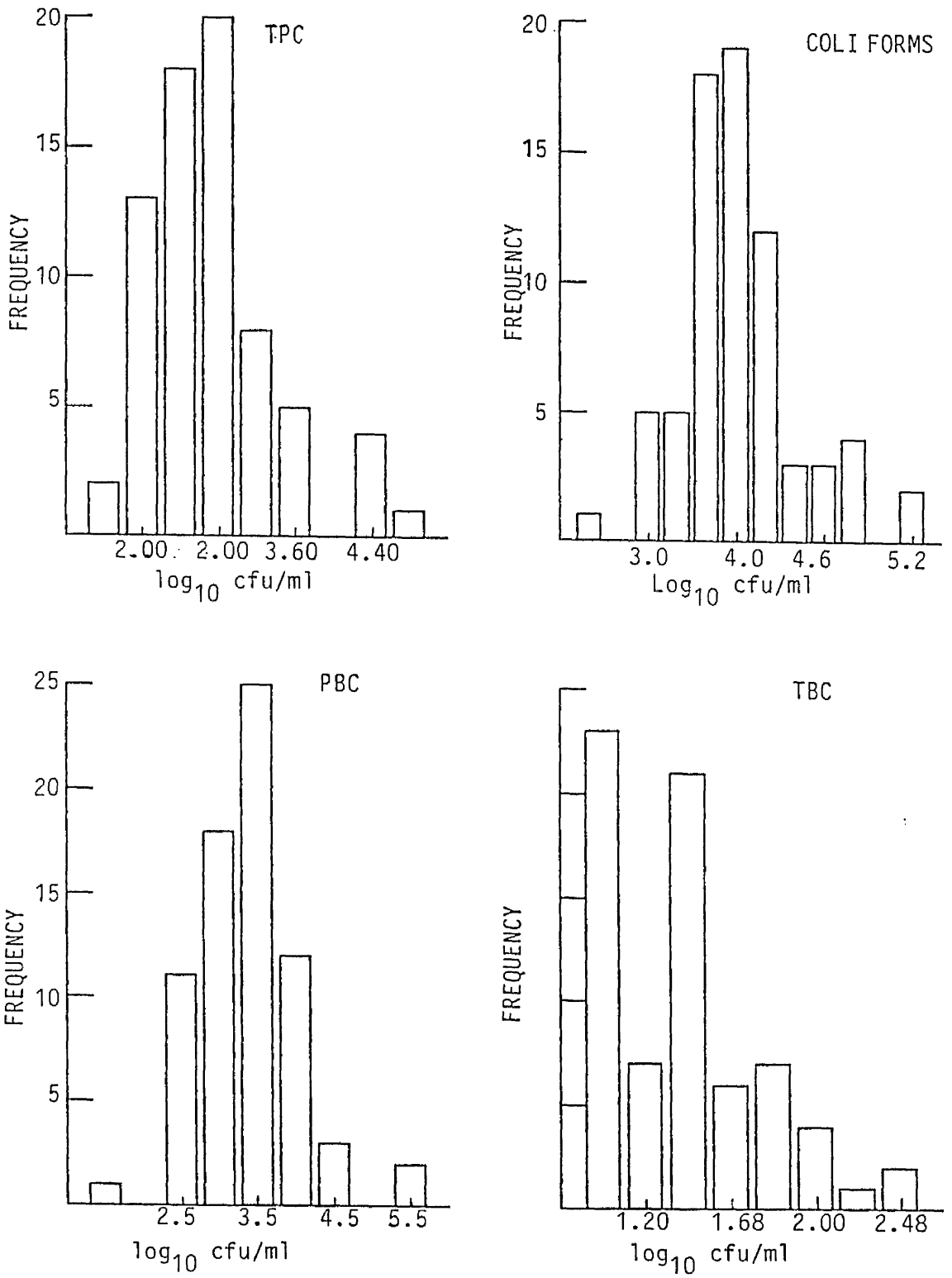


Figure 3.7: On-farm milk quality survey. Frequency distribution of total plate count (TPC), Coliforms count (COLIFORMS), psychrotrophic bacteria count (PBC) and thermoduric bacteria count (TBC) of milk sampled from the farm.

Table 3.19

Means of psychrotrophic lipolytic bacterial count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	3.23	0.141	3.13	0.212	3.49	0.274	3.27	0.121
March	2.97	0.221	2.44	0.119	2.52	0.226	2.65	0.114
June	2.73	0.170	2.33	0.076	2.57	0.204	2.55	0.097
Wt. means[c]	2.98	0.109	2.65	0.111	2.89	0.165	2.83[d]	0.074

Note: (a) = Bulk tankers used (b) = weighted means between tankers
(c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	7.0747	3.5374	13.227*
Tankers	2	0.5978	0.2989	1.118
Between farms within tanker 1	7	4.8022	0.6860	2.565
Between farms within tanker 2	7	2.1190	0.3027	1.132
Between farms within tanker 3	7	1.2908	0.1844	0.690
Tanker.Survey interaction	4	0.7761	0.1940	0.725
Error	42	11.2320	0.2674	
Total	71	27.8924	0.3929	

Statistical significance : * Significant, $p < 0.01$

Table 3.20

Means of mesophilic lipolytic bacterial count (log 10 cfu/ml) of fresh raw milk sampled from the farm tank prior to pick-up by the road tanker.

Tanker[a]	1		2		3		Wt.	
Survey	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	means[b]	SE
December	3.40	0.268	3.54	0.231	3.79	0.229	3.57	0.139
March	3.78	0.165	3.63	0.134	3.91	0.192	3.75	0.091
June	3.35	0.028	3.28	0.071	3.22	0.095	3.28	0.041
Wt. means[c]	3.51	0.112	3.51	0.098	3.61	0.119	3.54[d]	0.062

Note: (a) = Bulk tankers used (b) = weighted means between tankers (c) = weighted means between trials (d) = grand mean

Analysis of variance

Source of variation	df	SS	MS	F
Surveys	2	2.9060	1.4530	6.376*
Tankers	2	0.5265	0.2633	1.155
Between farms within tanker 1	7	2.3080	0.3297	1.447
Between farms within tanker 2	7	1.7355	0.2479	1.088
Between farms within tanker 3	7	2.3237	0.3320	1.457
Tanker.Survey interaction	4	0.8080	0.2020	0.886
Error	42	9.5712	0.2279	
Total	71	20.1790	0.2842	

Statistical significance : * Significant, $p < 0.05$

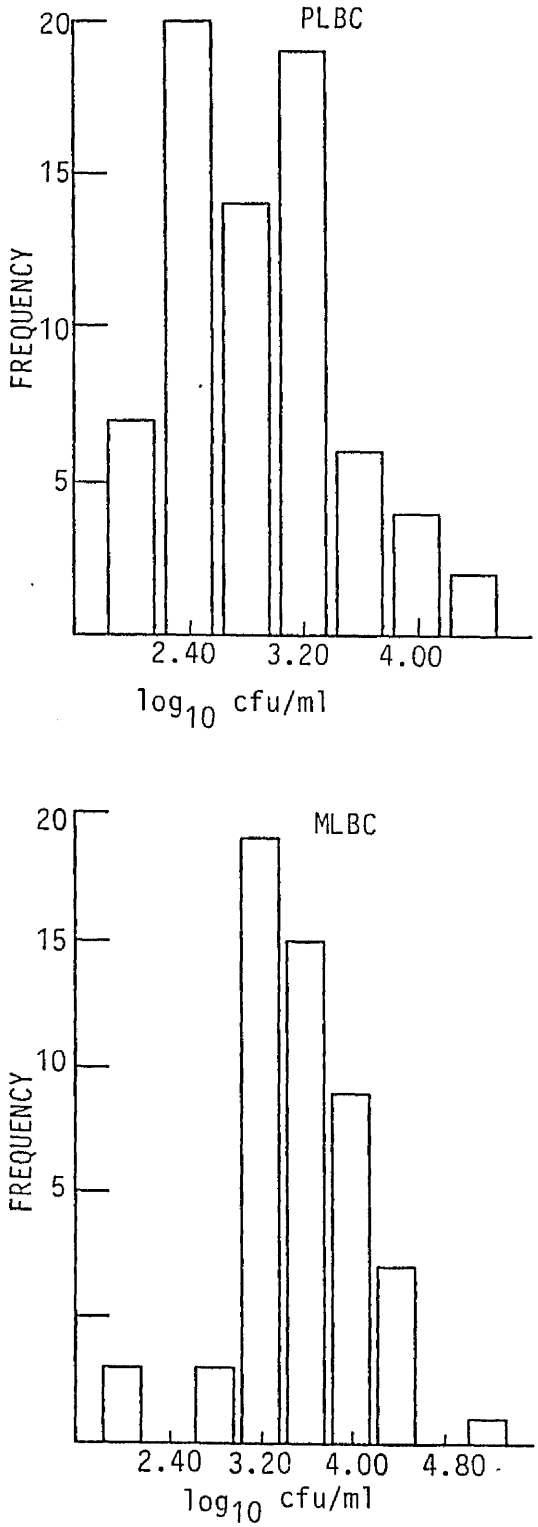


Figure 3.8: On-farm milk quality survey. Frequency distribution of psychrotrophic lipolytic bacterial count (PLBC) and mesophilic lipolytic bacterial count (MLBC) of milk sampled from the farm tank.

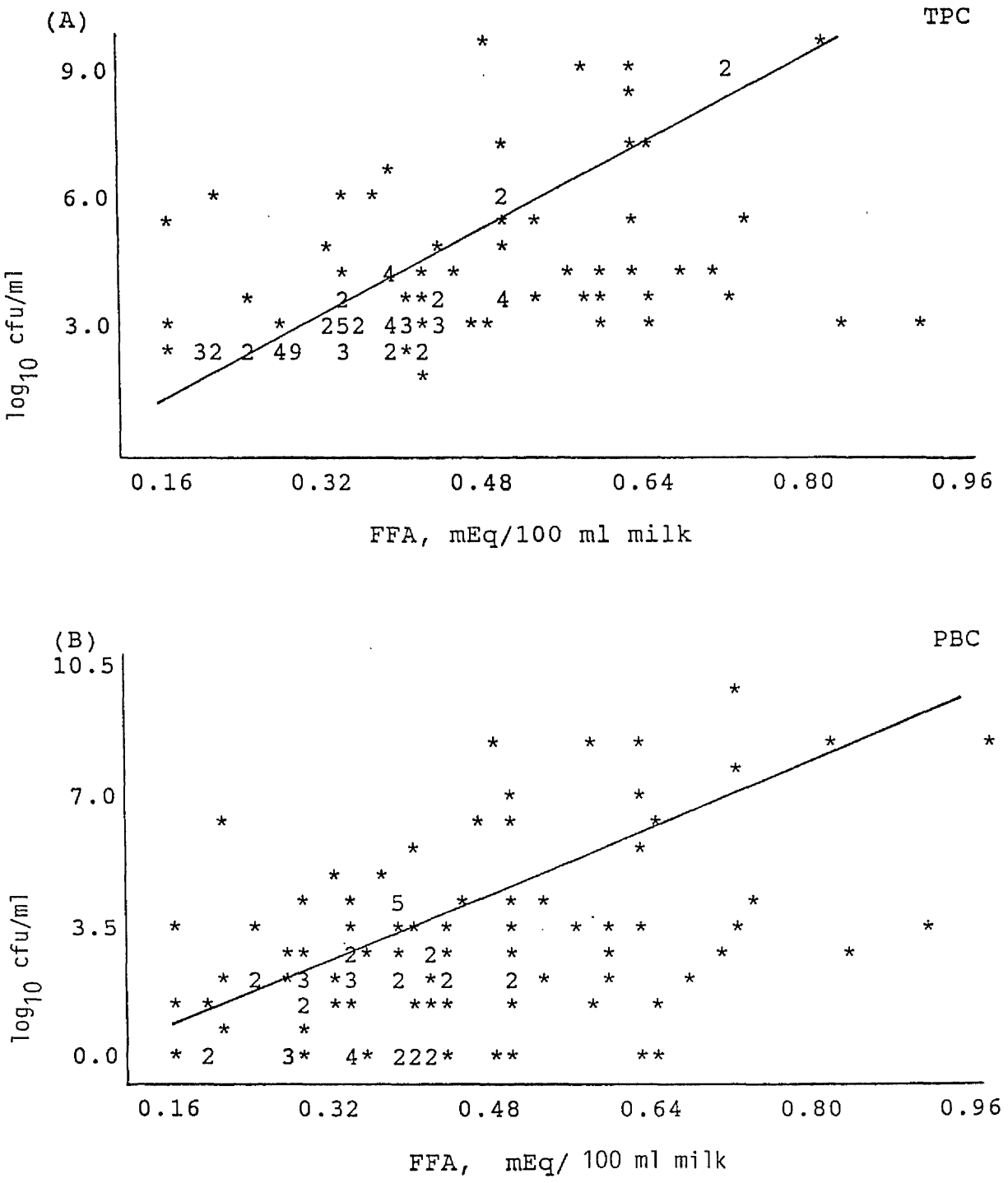


Figure 3.9 The relationships between (a) total plate count (TPC, r = 0.92) and (b) psychrotrophic bacterial count (PBC, r = 0.86) with free fatty acids (FFA) concentration as measured by the copper soap method (Shipe *et al.*,1978). n=120 observations.

0.05) interaction was observed. Analysis of the frequency distribution of the MLPC as illustrated in figure 3.8b indicated a normal distribution with the majority of the farms were within the $< 1.0 \times 10^4$ cfu/ml.

Figure 3.9 illustrates the relationships between TPC and FFA and PBC and FFA. A strong correlation between the variables were observed. The correlation between TPC and FFA was higher ($r = 0.92$) than the correlation between PBC and FFA ($r = 0.86$).

Discussion

The results of the present survey give little cause for concern over the quality of the milk supply in the south west of Scotland. The milk composition compares favourably with the quality standards laid down by the SMMB although the slightly higher on-farm milk temperature could be overcome by longer storage tank residence time of the last milking (AM milk) or with the introduction of direct expansion tanks or plate cooling systems in some of the larger farms to reduce the cooling time.

The bacterial counts of milk sampled on the farm suggest that the general standard of hygiene during production and handling on the farm was *acceptable* although some improvement would be necessary if the farm milk supply was required to meet a standard of fewer than 20,000 cfu/ml. The milk investigated in the present survey was a mixture of the previous day's evening milking and the morning of collection milking. Therefore, half of the

mixture would be less than 6 hours old and half would be 12 hours old when it was analysed. The length of storage at low temperature was insufficient for significant bacterial multiplication. A long lag phase is a characteristic of the growth of psychrotrophic bacteria (van den Berg, 1981). Therefore the bacterial counts in the present survey represented the initial bacterial load before significant multiplication has taken place.

The variations in farm milk temperature were large in June (1.1-9.4 ^{o}C) as compared to December (2.8-7.0 ^{o}C) or in March (2.2-6.1 ^{o}C). Farms with high milk production was observed frequently to have higher milk temperature at collection. Higher ambient temperature in addition to the limited cooling capacity of the refrigeration system to cope with the higher milk production during the spring flush could be responsible.

The temperature rise due to mixing adequately and inadequately cooled milks is always a potential risk. It is critical for the tanker operator to measure the temperature before pumping the milk into the tanker. This is especially true for milk collected in the first trip when the tank residence time of the last milking was less than the minimum 2 hours suggested to achieve sufficient cooling to < 4 ^{o}C (Crawford, 1967).

Slight seasonal variation was observed in the FPD of the milk. But, the variation was within the usual range of -0.530 to -0.570 and close to the mean value -0.540 ^{o}C. The FPD of milk, like that of any aqueous system, depends

on the concentration of water-soluble constituents. The objective of FPD determination in the present study was restricted to the determination of the water content of the product in order to detect the adventitious or illegal addition of water.

The pH and the TA of milk in the present study was comparable to the previous investigations (Smillie *et al.*, 1958).

The fat content of the milk was slightly higher in June and March than in December. There are various factors which influenced the fat content of milk. Seasonal variation is just one of them.

There appeared to be an indirect relationship between the fat and the protein content of the milk. The protein content of milk in the present survey was observed to be lower when the fat content was higher. Similar to fat content, protein content of milk can vary according to various factors (Webb and Johnson, 1965).

The lactose content of milk in the present study changes slighly between the surveys. The lactose content was higher in December than in March or June. Lactose is the least variable of the gross composition on a percentage basis; but because of its osmotic relationship in milk (Webb and Johnson, 1965), it increases slightly as the other solids decrease and decreases as the other solids increase.

The percentage TS and SNF of milk in milk in the present study did not show a definitive variation between the surveys

There was no seasonal variation or variation between farms in each of the tanker collection area in the bacterial counts of the milk. The grand mean bacterial counts of the milk were summarised as follows:

i.	Coliforms	5.6×10^2	cfu/ml
ii.	PBC	2.2×10^3	cfu/ml
iii.	TPC	9.8×10^3	cfu/ml
iv.	TBC	22	cfu/ml
v.	PLBC	6.8×10^2	cfu/ml
vi.	MLBC	3.5×10^3	cfu/ml

The control of the bacteriological quality of milk supplies is important in maintaining the overall quality of milk received at the creamery. The addition of poor quality milk has an adverse effects on bacterial growth (van den Berg, 1981).

The coliforms count of milk in the present study was comparable to the earlier studies (Muir *et al.*, 1978). Thomas and Thomas (1972), indicated that heavy coliform contamination in milk ($> 10^3$ cfu/ml) is infrequent in bulk collected milk.

The presumptive test using bile salt lactose broth at 30 ^{o}C is widely used but the violet red bile agar method of coliform detection was more practical. It is quicker and has the possibility of requiring only one dilution (10^{-1} ml) of milk, to detect coliform colony count ranging from < 10 to $> 2.0 \times 10^2$ cfu/ml (Thomas and Thomas, 1972).

As the optimum temperature for growth of psychrotrophic bacteria lies between 22-30 ^{o}C (Morita, 1975) it may be

logical to assume that the psychrotrophic contamination of farm bulk tanks from poorly cleaned milking plant will be higher in the summer than in the winter. The results of the investigation indicated that there is no such seasonal variation in the survey area. This is probably due to the generally lower summer temperature in Scotland. However other workers have found such variation exists (Thomas *et al*, 1966).

The incidence of psychrotrophic bacteria in raw milk have been shown to vary in a wide range, depending on the amount and the type of initial contamination as well as the temperature and duration of cold storage prior to processing (Thomas and Thomas , 1975b).

Thomas and Thomas, (1973a) reported that farm milk supplies produced and handled under reasonably good hygiene conditions may often contain very few psychrotrophs. They reported that 55 per cent of a series of milks studied, had psychrotrophic counts of $< 10^2$ cfu/ml and 90 per cent were $< 10^3$ cfu/ml when examined within 2-4 hours of milking.

Smillie *et al.* (1958) reported a high proportion of farms investigated in the south-west of Scotland had low psychrotrophic counts of $< 10^3$ cfu/ml and only 8 percent exceeded 10^4 cfu/ml. They attributed these excellent result to steam sterilisation of milking equipment and the careful cleaning and disinfection of the farm tanks. However they recorded a very wide range of psychrotrophic counts for some individual farms, varying from < 10 cfu/ml

to > 10^6 cfu/ml. Thomas and Thomas, (1973b) conclude that since psychrotrophic bacteria can multiply in refrigerated milk, they are primarily responsible for limiting the keeping quality of raw milk held at temperatures less than 7 ^oC, in which they may produce a variety of defects.

The TBC in the present study is low. According to Thomas and Thomas, (1975b) the significance of high thermoduric count (> 1.0 x 10^4 cfu/ml) in bulk collected milk is dependent on four factors;

i. they are rarely derived from the bovine udder,

ii. it requires 2-3 weeks of persistent neglect of milking plant for a build-up on the plant,

iii. these organisms cannot multiply at low temperatures, hence they do not attain large numbers even in poorly cleaned farm bulk tanks, and

iv. thermoduric bacteria do not increase in bulk collected milk during storage at < 5 ^oC even after 4 days of storage.

A high thermoduric colony count for bulk collected milk is therefore a fairly conclusive indication of persistent neglect of cleaning of milk plant, although it is probable that the organisms were originally derived from dust and manure during an unhygienic milking procedure.

Shelley *et al.* (1986) studied the lipolytic activity of

4 *Pseudomonas* species isolated from raw milk and observed that *Pseudomonas fragi* were found to be potentially more lipolytic than *Pseudomonas fluorescens*. They grew faster and produced higher lipase activities in both mixed and in pure cultures in cold stored milk. In addition, they observed that the lipases produced by *Pseudomonas fragi* retained a greater proportion of their activity after pasteurisation.

Thomas and Thomas (1973b) summarised the results of investigations by different groups of workers and showed that 68-97 per cent of the strains of psychrotrophs and 77 per cent of the *Pseudomonas* species isolated from refrigerated and bulk collected milk, were strongly lipolytic. Tributyrin agar was reported the simplest and the most widely used selective medium for the estimation of the numbers of lipolytic micro-organisms in dairy products. The tributyrin used in the present work was dispersed in fine globules in the agar medium and they disappear around lipolytic colonies, since the product of hydrolysis were completely soluble. However, this method of estimation has been criticised due to the presence of bacteria that produce clear zones on the tributyrin agar but failed to hydrolyse butter fat (Cousin, 1982). But, the simplicity of the method has been used as a presumptive test in milk both for PLBC and MLBC.

Conclusion

1. The result of the present study give little cause for concern over the quality of the milk supply in the south-west of Scotland. The chemical composition and the bacteriological counts compare favourably with the quality standards laid down in the SMMB monthly report.

2. As expected, seasonal variation in percentage fat, protein and lactose content of milk was observed. However, no significant variation in TS was observed.

3. In the present study, large variation in farm bulk milk temperature immediately before collection was observed. Higher farm bulk milk temperature ($\geq$ 6.6 ^{o}C) was observed in farms with high milk production.

4. The result of the survey indicated that an initial psychrotrophic bacterial count of $< 10^4$ cfu/ml could be considered a satisfactory guide standard for farm bulk-tank milk.

5. No significant seasonal variation in bacterial counts was observed.

CHAPTER FOUR

SECTION 4.1 SIMULATED BULK MILK SILO STUDY

Introduction

Bulk collection of milk from the farm by road tanker is the quickest and the most hygienic method of milk transport from the farms to the creamery. It is also the most cost effective (Crawford, 1967). Today, bulk milk collection is centralised and highly regulated. Milk is processed in larger volume (Cox, 1975) to minimise the escalating cost of processing. Hence, it is not uncommon for raw milk to be transported over long distances from the producer's farm to the processing plant. On arrival at the processing plant, milk from various sources and origin are blended and stored, until used, for a few days in large insulated silo tanks at temperature more than 4 ^{o}C before processing (Cox, 1975). Extended storage and mixing of raw milk of various sources at the creamery affect quality (Thomas *et al.*, 1975b and Oz and Farnsworth, 1986).

It has been suggested that tanker milk arriving at the processing plants should satisfy the bacteriological and chemical requirements as listed in table 4.1.1 (SMMB, 1982). However, it was difficult to implement, especially the bacteriological requirements due to the long incubation periods necessary before tests results can be obtained. Therefore, it was probably assumed that

temperature lower than 6.6 ^{o}C would suggest high milk quality, for processing. Temperature measurement came to be used as the critical platform test before the tanker load of milk was accepted by the creamery (Table 4.1.1).

Storage of milk at lower temperature has been known to be selective for psychrotrophic bacteria which would in time dominate the bacterial flora of the milk (Cousin, 1982). It has been shown that these bacteria produced extra-cellular enzymes such as lipases and proteases that were detrimental to milk quality (Speck and Adams, 1976).

Therefore, the purpose of this study was to investigate the effects of extended storage of raw milk at 2 and 5 ^{o}C on bacteriological and selected aspects of milk quality.

Table 4.1.1

The quality standards for tanker load of milk delivered to buyers premises (from SMMB 1982)

1)	Temperature[a]	6.6 ^{o}C
2)	Total plate counts	1.5×10^{5} cfu/ml
3)	Thermoduric counts	7.5×10^{3} cfu/ml
4)	Sediments	2.0 mg/litre
5)	Anti-biotic	0.02 iu/ml
6)	Extraneous water (FPD)[b]	- 0.534 ^{o}C

Note: (a) = Platform test. All other tests are laboratory test.

(b) = Freezing point depression

Materials and methods

Commingled milk (defined as milk delivered by road tanker and from more than one farm) used in this study was supplied by the local area office of the SMMB. The milk was from the first morning collection run and delivered to the WSC by 3 three road tankers. On arrival at the WSC a portion of the the commingled milk from each tanker was blended in a storage tank at the WSC according to the calculated proportion as shown in table 4.1.2. The total volume of blended milk (defined as a mixture of commingled milks) used in each trial was 1200 litres.

Once the blending was completed, the milk was divided into 800 and 300 litres lots and stored at 5 ± 1 ^{o}C and 2 $\pm 1^{o}$C respectively. The milks were stored continuously with intermittent automatic mechanical agitation every 2 hours for 2 minutes. The milks were sampled fresh and after 2, 3, 4, 7 and 9 days of storage and analysed for changes in bacteriological and chemical composition.

The milk was analysed for total plate count (TPC), psychrotrophic bacterial count (PBC), thermoduric bacterial count (TBC), psychtrophic lipolytic bacterial count (PLBC) and coliform counts using the methods described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.5 and 2.3.6, respectively.

The chemical analysis of milk were as follows: fat, protein and lactose according to the methods as previously described in section 2.2.6; total solids (TS), solids-not-fat (SNF) and ash content as in sections 2.2.7, 2.2.8,

and 2.2.9; and the titratable acidity (TA), pH, extraneous water (FPD) and total free fatty acids (FFA) as described in sections 2.2.12, 2.2.13, 2.2.14 and 2.2.17.1, respectively.

Three trials were carried out. Each trial concurrent with the on-farm milk quality studies (Chapter 3) began on each of the following dates; 5th December 1986, 15th March 1987 and 14th June 1987.

Data were subjected to a three way analysis of variance as described in section 2.4. using trial, storage time and treatments as main effects

Results

The proportion of milk used to simulate the effects of blending during bulk silo storage is shown in table 4.1.2. The total volume of blended milk used in each trial was 1200 litres with percentage contribution of 44, 28 and 28 per cent from tanker 1, 2 and 3, respectively.

The milk used in each trial was collected from 24 farms (8 farms/tanker) with a combined total production of 33,420; 45,474 and 38,624 litres in the December, March and in June surveys, respectively (Table 4.1.3). The temperature of the commingled milk was higher than would have been expected.

Table 4.1.2

Simulated bulk milk silo studies: The proportion of the raw commingled milk blended in the WSAC storage tank

Tanker	Tanker holding capacity (litres)	Percentage of total	Proportion for blending (litres)
1	14,400	44	528
2	9,000	28	336
3	9,000	28	336
Total	32,400	100	1200

Table 4.1.3

Time taken by the milk tankers to complete the collection run, total volume of milk picked up, and the temperature of commingled milk on arrival at the WSAC.

Survey	Tanker	Time taken to complete run (hours:minutes)	Total volume picked up (litres)	Temperature on arrival (^{o}C)
December	1	2:54	10,448	8.0
	2	1:55	13,407	8.0
	3	2.20	9,565	4.5
	Total		33,420	
March	1	1:42	14,495	7.6
	2	2:50	21,348	8.0
	3	1:54	9,631	7.2
	Total		45,474	
June	1	2:24	14,829	6.7
	2	1:52	10,433	7.3
	3	2:49	13,362	7.2
	Total		38,624	
Trials total			117,518	

The time taken to complete the collection run and deliver the milk to the WSC (table 4.1.3) was approximately 2 hours and 23 minutes, 2 hours and 8 minutes and 2 hours and 22 minutes in December, March and June, respectively. The temperature of the commingled milks when received at the WSC range from 4.5-8.0 ^{o}C in December and 7.2-8.9 and 6.7-7.3 ^{o}C in March and June, respectively.

The mean bacteriological counts of commingled milk received at the WSC are shown in table 4.1.4. The bacteriological quality of the tanker load of milk namely the TPC and TBC were within the maximum acceptance limit of the SMMB (1982) as shown in table 4.1.1. The mean TPC was 1.3×10^{4}, No trial differences were observed but significant differences ($p < 0.05$) were detected between tankers within and between the trials. These differences could be explained by variations in quality of milk on the farms as picked up by the tankers as shown in tables 3.1.15 to 3.1.20. Even though no statistical differences in counts between tankers were detected the small differences that were present, could account for the subsequent differences in the bacterial counts after the milk was blended at the WSC. Breaking up of bacterial clumps during handling and the type and species of bacteria present in the initial count are known to influence bacterial count during subsequent storage (Thomas and Thomas. (1975b).

Table 4.1.4

Ex-farm milk quality studies. Total coliforms count (CC) (log_{10} cfu/ml); total plate count (TPC), psychrotrophic bacterial count (PBC), thermoduric bacterial count (TBC), psychrotrophic and mesophilic lipolytic bacterial counts (PLBC and MLBC, respectively) (log_{10} cfu/ml) and free fatty acid (FFA) concentration (mEq/100 ml) and of commingled milk sampled from the road tanker.

Survey	December				March				June				
Tanker	1	2	3	$\bar{x}^a$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	$\bar{\bar{x}}^b$
CC	2.70	2.83	2.64	2.72	4.48	2.78	4.66	3.97	4.11	4.20	3.60	3.97	3.56
TPC	4.00	3.88	4.53	4.14	4.06	4.03	4.08	4.06	4.11	4.21	4.11	4.14	4.11
PBC	3.30	3.30	4.46	3.69	3.78	3.43	3.68	3.63	3.60	3.05	3.54	3.40	3.57
TBC	1.45	1.60	1.60	1.55	1.48	1.66	1.78	1.64	1.00	1.00	1.00	1.00	1.40
PLBC	3.20	3.00	4.00	3.40	3.32	3.46	2.76	3.18	3.32	3.10	2.30	2.91	3.16
MLBC	4.00	3.72	4.00	3.91	3.80	3.40	3.20	3.47	3.45	3.40	3.08	3.31	3.56
FFA	0.37	0.58	0.53	0.49	0.38	0.45	0.59	0.48	0.64	0.67	0.67	0.66	0.54

Note: (a) = Mean of survey, (b) = grand mean of surveys

Table 4.1.5

Ex-farm milk quality studies: Freezing point depression (FPD) (^{o}C), titrable acidity (TA) (percentage lactic acid), pH; percentage (w/w) of fat, protein, lactose, ash, solids-not-fat (SNF) and the total solids (TS) content of commingled milks sampled from the road tanker.

Survey	December				March				June				
Tanker	1	2	3	$\bar{x}^a$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	$\bar{x}^b$
FPD	-0.540	-0.536	-0.543	-0.540	-0.544	-0.545	-0.540	-0.543	-0.537	-0.538	-0.537	-0.537	-0.540
TA	0.150	0.160	0.150	0.153	0.140	0.150	0.160	0.150	0.120	0.140	0.100	0.120	0.141
pH	6.690	7.000	6.720	6.803	6.720	6.750	6.730	6.730	6.700	6.700	6.700	6.700	6.750
FAT	3.650	3.600	3.620	3.623	3.750	3.990	3.780	3.840	3.790	3.730	3.820	3.780	3.748
PROTEIN	3.870	3.700	3.400	3.657	3.860	3.030	3.210	3.367	3.440	3.360	3.330	3.377	3.467
LACTOSE	4.650	4.700	4.700	4.683	4.630	4.720	4.620	4.657	4.640	4.680	4.680	4.667	4.669
ASH	0.717	0.734	0.746	0.732	0.730	0.757	0.705	0.731	0.757	0.730	0.705	0.731	0.731
SNF	8.430	8.400	8.100	8.310	8.490	8.450	8.530	8.490	8.680	8.640	8.620	8.647	8.482
TS	12.520	12.850	12.700	12.690	12.240	12.440	12.310	12.330	12.470	12.370	12.440	12.437	12.482

Note: (a) = Mean of survey, (b) = grand mean of surveys.

Table 4.1.6

The grand mean of selected bacteriological counts (log_{10} cfu/ml) of milk sampled fresh from the farm tank, from the delivery tanker and immediately after blending.

Count	Onfarm	Comingled	Blended
CC[a]	2.75	3.56	3.66
TPC[b]	3.99	4.11	4.47
PBC[c]	3.34	3.57	4.20
TBC[d]	1.35	1.40	1.07
PLBC[e]	2.83	3.16	3.21
MLBC[f]	3.54	3.56	*

Note: * = Not determined, (a) = coliforms count, (b) = total plate counts, (c) = psychrotrophic bacterial counts, (d) = thermoduric bacterial counts, (e) = psychrotrophic lipolytic bacterial counts and (f) = mesophilic lipolytic bacterial counts.

Table 4.1.7 a

Means of lipolytic activity (LA) of raw unheated milk after storage at 2 ^{o}C (DC) and 5 (CS) ^{o}C.

Storage (days)	uml 0.025 N NaOH/min/ml milk	
	DC (2 ^{o}C)	CS (5 ^{o}C)
Fresh[a]	0.0253	0.0253
2	0.0195	0.0220
3	0.0193	0.0200
4	0.0090	0.0140
7	0.0068	0.0110
9	0.0034	0.0009

Note: a = Blended milk at the initiation of the trial.

Table 4.1.7 b

Means of lipolytic activity (LA) of thermised and pasteurised milk stored at 6 ^{o}C and 4 ^{o}C, respectively

Storage (days)	uml 0.025 N NaOH/min/ml milk	
	Thermised	Pasteurised
Fresh[a]	0.0120	0.0120
2	0.0100	0.0092
3	0.0093	0.0070
4	0.0070	0.0040
7	0.0008	0.0005
9	0.0003	0.0000

Note: (a) = Analysed at the initiation of the experiment.

The mean FFA concentration was higher in June than in December or the March trials. The seasonal response in the level of FFA is difficult to explain, except that, the higher ambient temperature during the summer months could influence the level of lipolysis. The correlations between TPC and FFA and PBC and FFA in the present study were not significant ($P > 0.05$). However, other factors could also influence lipolysis in milk (Shipe and Senyk, 1982).

The chemical composition of the tanker load of milk is given in table 4.1.5. The commingled milks used in the study were of *acceptable* quality. The mean FPD of all the milk sampled from the tanker were more tha - 0.534 ^{o}C, which was less than the lower limit set by the SMMB (1982) (table 4.1.1) for tanker load of milk at the buyer's premises. The TA, pH, and the percentages of fat, protein, lactose, ash, SNF and TS were comparable to earlier studies (Smillie, 1958 and Muir *et al.*, 1978).

The mean LA of raw milks stored at 2 ^{o}C and 5 ^{o}C are given in table 4.1.7. The lipolytic activity of raw milk store at 5 ^{o}C was higher than that stored at 2 ^{o}C.

The means TPC of the blended raw milk following storage at 2 and 5 ^{o}C are given in table 4.1.8. The milk used was of *acceptable* quality; the initial TPC was 3.0×10^4 cfu/ml. As expected, the TPC increased highly significantly ($p < 0.001$) through storage. The TPC of the milk stored at 5 ^{o}C was observed to increase very significantly ($p < 0.01$) faster than that of milk stored at 2 ^{o}C. The difference was almost 10-fold and was constant, from the second day of storage onwards. However,

the increase in TPC was not significant for milk stored at 2 ^{o}C, in the first 3 days of storage as compared to storage at 5 ^{o}C. The TPC of milk stored at 2 ^{o}C remained less than 10^{6} cfu/ml up to 4 days of storage whereas milk stored at 5 ^{o}C attained that level after 3 days of storage. It has been reported that deterioration of raw milk quality begins to occur when the TPC reach 10^{6} cfu/ml (Punch *et al.*, 1965).

The initial PBC of the milk used in the trial was 1.6 x 10^{4} cfu/ml (table 4.1.9). The result was better than earlier reported (Smillie, 1958 and Mikolajcik, 1979) perhaps because the sources of milk used in the present trial were restricted to milk from the first tanker collection. Therefore the milk used was not more than 15 hours old. Analysis of the data revealed that the response of PBC to storage temperature was similar to TPC. The increase in PBC was very highly significantly faster in milk stored at 5 ^{o}C than at 2 ^{o}C. The PBC remained less than 10^{6} cfu/ml up to 4 and 3 days of storage at 2 and 5 ^{o}C, respectively. Trial differences in the increase of psychrotrophic bacteria were also observed. Interaction between trial and treatment was highly significant ($p < 0.01$). An indication that the response to count due to storage treatments was related to trials. Since very large differences ($p < 0.001$) between trials were detected, seasonal variation in the bacterial species present (Thomas *et al.*, 1971) could have exerted greater influence than the effectiveness of storage temperature or other

factors in the control of growth of psychtrophic bacteria. The presence of significant interactions ($p < 0.05$) between factors; trial and storage time; and, storage time and treatment, reinforced this observation.

The growth of thermoduric bacteria was not significant following storage at the 2 temperatures and no significant differences between trials was also observed (table 4.1.10). However, differences due to treatments were significant ($p < 0.001$).

The total coliforms count are shown in table 4.1.11. The increase in the coliforms count with storage time for both storage treatments were very highly significant ($p < 0.001$). The increase was higher ($p < 0.001$) for milk stored at 5 ^{o}C than at 2 ^{o}C. The coliforms count remained at 1.5×10^4 cfu/ml after 4 days storage at 2 ^{o}C compared to storage at 5 ^{o}C in which case the count reached 1.3×10^4 cfu/ml 2 days after storage. The difference was very highly significant ($p < 0.001$). The response in growth of coliforms due to the storage treatments varied significantly ($p < 0.001$) with trial. It indicated that growth response due to storage treatments was seasonal. Initial coliforms count, as shown in table 3.15 were significantly higher ($p < 0.05$) in June than in the December or the March survey.

The difference in PLBC between storage treatments through the storage time were not significant (table 4.1.12). The PLBC remained unchanged, after 3 days at 2 ^{o}C but increased significantly ($p < 0.05$) from 1.6×10^3 to 1.7×10^5 cfu/ml on the 4^{th} day of storage. However,

the PLBC count began to increase significantly ($p < 0.05$) immediately on storage. Large significant ($p < 0.001$) differences due to trial were noted.

Changes in TA during storage are given in table 4.1.13. The increases in TA were significant between the storage treatments ($p < 0.05$). As expected, the mean TA of milk stored at 2 ^{o}C were lower than milk stored at 5 ^{o}C.

The means FFA of milks during storage are given in table 4.1.14. Changes in FFA through storage and between the treatments were highly significant ($p < 0.001$). The FFA remained level at < 0.60 mEq / 100 ml, up to 4 days of storage at 2 ^{o}C, but increased significantly on the 7th day of storage. Comparable observations but at significantly ($p < 0.05$) higher concentrations of FFA were noted in milk stored at 5 ^{o}C. Highly significant interaction between storage time and treatments was also observed, an indication that changes in FFA due to treatments were not comparable in every trial.

The changes in TV through storage time were very highly significant ($p < 0.001$) for both treatments (Table 4.1.15). However, the TV of milk stored at 5 ^{o}C was consistently higher than that milk stored at 2 ^{o}C and the difference was very highly significant ($p < 0.001$). The response was significantly different ($p < 0.05$) between trials and was not comparable in every trial, the interaction between trial and treatment was very highly significantly different ($p < 0.001$).

Table 4.1.8

Mean[a] total plate count (TPC) (log_{10} cfu/ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	log_{10} cfu/ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	4.47	4.47	4.47
2	4.65	5.22	4.94
3	5.19	6.55	5.87
4	6.59	7.08	6.84
7	8.48	8.79	8.64
9	9.01	10.36	9.68
Mean[d]	6.40	7.08	6.74[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.24	0.34	0.21	0.34	0.59	0.48

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	5.90	2.95	8.510**
Day	5	128.41	25.68	74.133***
Treatment	1	4.15	4.15	11.984**
Trial.Treatment	2	2.76	1.38	3.988*
Trial.Day	10	4.85	0.48	1.399
Day.Treatment	5	2.36	0.47	1.365
Mean square error	9	3.12	0.35	
Total	34	151.55	4.46	

Note: *Significant $p < 0.05$, **Significant < 0.01, ***Significant $p < 0.001$

Table 4.1.9

Mean[a] psychrotrophic bacterial count (PBC) (log_{10} cfu/ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	log_{10} cfu/ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	4.20	4.20	4.20
2	4.53	4.99	4.76
3	5.15	6.26	5.70
4	6.64	7.12	6.80
7	8.08	7.98	8.03
9	8.28	9.91	9.09
Mean[d]	6.15	6.74	6.44[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.14	0.19	0.11	0.19	0.33	0.27

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	12.23	6.11	54.647***
Day	5	108.83	21.77	194.560***
Treatment	1	3.17	3.17	28.359***
Trial.Treatment	2	2.28	1.14	10.210**
Trial.Day	10	6.66	0.67	5.957*
Day.Treatment	5	3.25	0.65	5.815*
Mean square error	9	1.01	0.11	
Total	34	137.44	4.04	

Note: *Significant $p < 0.05$, **Significant < 0.01, ***Significant $p < 0.001$

Table 4.1.10

Mean[a] thermoduric bacterial count (TBC) (log_{10} cfu/ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	log_{10} cfu/ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	1.07	1.07	1.07
2	1.57	1.20	1.38
3	2.60	1.82	2.21
4	3.54	1.93	2.74
7	4.11	0.87	2.49
9	4.97	1.07	3.02
Mean[d]	2.98	1.33	2.15[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.59	0.83	0.48	0.83	1.44	1.17

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	0.11	0.056	0.027
Day	5	17.87	3.574	1.737
Treatment	1	24.51	24.519	11.914*
Trial.Treatment	2	46.41	23.206	11.275*
Trial.Day	10	11.94	1.194	0.580
Day.Treatment	5	19.09	3.818	1.855
Mean square error	10	20.58	2.058	
Total	35	140.52	4.015	

Note: * Significant $p < 0.001$

Table 4.1.11

Mean[a] coliforms count (log_{10} cfu/ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	log_{10} cfu/ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	3.66	3.66	3.66
2	2.27	4.10	3.18
3	3.33	4.40	4.09
4	4.17	5.84	5.00
7	5.80	7.22	6.51
9	7.20	8.62	7.91
Mean[d]	4.40	5.71	5.06[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.25	0.36	0.21	0.36	0.62	0.51

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	8.41	4.2	10.842**
Day	5	99.90	19.98	51.503***
Treatment	1	15.43	15.43	39.780***
Trial.Treatment	2	3.67	1.83	4.730*
Trial.Day	10	5.71	0.57	1.473
Day.Treatment	5	3.28	0.66	1.691
Mean square error	10	3.88	0.39	
Total	35	140.28	4.01	

Note: *Significant $p < 0.05$, **Significant < 0.01, ***Significant $p < 0.001$

Table 4.1.12

Mean[a] psychrotrophic lipolytic bacterial count (PLBC) (log_{10} cfu/ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	log_{10} cfu/ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	3.21	3.21	3.21
2	3.32	4.43	3.88
3	3.64	5.10	4.37
4	5.22	5.35	5.29
7	7.02	6.57	6.79
9	7.91	7.01	7.46
Mean[d]	5.05	5.28	5.17[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.40	0.57	0.33	0.57	0.99	0.81

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	40.00	20.00	20.500*
Day	5	84.25	16.85	17.271*
Treatment	1	0.46	0.46	0.474
Trial.Treatment	2	2.73	1.37	1.400
Trial.Day	10	8.29	0.83	0.850
Day.Treatment	5	6.12	1.22	1.255
Mean square error	9	8.78	0.98	
Total	34	150.64	4.43	

Note: * Significant $p < 0.001$

Table 4.1.13

Mean[a] titratable acidity (TA) (percentage lactic acid) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	Percentage lactic acid		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	0.133	0.133	0.133
2	0.143	0.146	0.145
3	0.147	0.147	0.147
4	0.147	0.150	0.148
7	0.153	0.180	0.167
9	0.160	0.213	0.187
Mean[d]	0.147	0.162	0.154[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.011	0.015	0.009	0.149	0.026	0.021

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	0.0035	0.0018	2.618*
Day	5	0.0109	0.0021	3.263*
Treatment	1	0.0019	0.0019	1.805*
Trial.Treatment	2	0.0022	0.0011	1.672
Trial.Day	10	0.0051	0.0005	0.771
Day.Treatment	5	0.0035	0.0007	1.042
Mean square error	10	0.0067	0.0007	
Total	35	0.0339	0.0010	

Note: * Significant $p < 0.05$

Table 4.1.14

Mean[a] free fatty acids (FFA) concentration (mEq/100 ml) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	mEq/100 ml		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	0.603	0.603	0.603
2	0.599	0.626	0.612
3	0.498	0.617	0.558
4	0.580	0.717	0.649
7	0.800	1.096	0.948
9	0.946	1.923	1.434
Mean[d]	0.671	0.931	0.801[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.076	0.107	0.062	0.107	0.185	0.151

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	10.34	5.17	1.504
Day	5	347.84	69.57	20.237**
Treatment	1	60.61	60.61	17.632**
Trial.Treatment	2	7.68	3.84	1.116
Trial.Day	10	30.18	3.02	0.878
Day.Treatment	5	100.87	20.17	5.869*
Mean square error	10	34.38	3.44	
Total	35	591.91	16.91	

Note: * Significant < 0.01, ** Significant p < 0.001

Table 4.1.15

Mean[a] tyrosine value (TV) (mg of tyrosine/ml milk) of raw milk tested fresh[b], 2, 3, 4, 7 and 9 days after storage at 2 °C (DC) and 5 °C (CS).

Storage (days)	ug/ml milk		
	DC (2 °C)	CS (5 °C)	Mean[c]
Fresh[b]	0.120	0.120	0.120
2	0.108	0.137	0.122
3	0.099	0.160	0.129
4	0.140	0.165	0.152
7	0.155	0.185	0.170
9	0.154	0.245	0.200
Mean[d]	0.121	0.169	0.145[e]

Note: (a) Means of 3 trials, (b) tested on day of delivery, (c) mean by day, (d) mean by treatment and (e) grand mean.

SED of mean:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.008	0.012	0.007	0.012	0.021	0.017

Analysis of variance

Source of variance	df	SS	MS	F
Trial	2	0.0042	0.0021	4.990*
Day	5	0.0409	0.0082	19.395**
Treatment	1	0.0201	0.0201	47.667**
Trial.Treatment	2	0.0155	0.0077	18.357**
Trial.Day	10	0.0100	0.0010	2.358*
Day.Treatment	5	0.0048	0.0010	2.293
Mean square error	10	0.0042	0.0004	
Total	35	0.1000	0.0029	

Note: * Significant $p < 0.05$, ** Significant $p < 0.001$

Discussion

The milk used in the present bulk silo storage simulated trial was collected from 24 farms. The milk received was representative of the total combined milk of three tanker loads of 33,420, 45,474 and 38,624 litres in December, March and June, respectively. The milk was delivered to the WSC in a little less than two and a half hours from the time of the first collection. The time was much less than that of earlier reportes (Crawford, 1967 and Cox, 1975). The temperature of the commingled milk when received at the WSC ranged from 4.5-8.0, 7.2-8.0 and 6.7-7.3 ^{o}C for the December, March and June survey, respectively. Therefore, these temperatures were higher than were expected for tanker load of milk (Table 4.1.1).

Cox (1975) reported that raw milk received at a creamery could be up to 36 hours old (ex-farm) and depending on the location, the duration could be longer. It is speculated that similar situation continued to the present time, due mainly to the changing practices of bulk milk collection, from the everyday collection to an alternate day milk collection schedule. In addition it was reported that the raw milk would be stored at 4.4 ^{o}C for another 12 hours before it was pasteurised.

4.1.1 The quality of commingled milk

The bacterial quality of the commingled milk was within the acceptance limit of the SMMB (Table 4.1.1). The

bacterial counts of the tanker loads of milk delivered to the WSC were higher than the mean counts of the milk tested from the farm bulk tank. It was observed that the TPC, PBC and the coliforms count of milk from road tankers was significantly ($p < 0.05$) higher than that from the farm bulk tanks. These differences (in bacterial counts) between tankers were significantly different ($p < 0.05$) in all the trials.

Milk collected by road tanker is a cold-stored milk consisting of a mixture of different number of milkings and from a number of different producers. The first milking determines the age of the milk and the milk with the highest active psychrotrophs influences the storage life of the milk.

Thomas and Thomas (1976) reported that the bacterial count of milk from road tankers was usually higher than of milk collected from the farm tanks. This unwanted increase was believed to be caused by contaminats from the tanker and the filling equipment or by the intake of milk which has not been properly cooled or by the intake of poor quality milk. In addition, milk is a good medium for bacteriological growth. The age of the milk, the temperature variations during transport, increases in the surfaces the milk comes in contact with and the constant agitation during transport all contributed to the higher counts.

The bacterial content of tanker milk can also be influenced by the frequencies of milk collection. Cousin (1982) reported that the difference in the bacterial

counts between the first collection and the second collection of the same tanker were significantly lower. The introduction of bulk storage on the farm has enabled milk to be collected after a period of storage on the farm. Everyday collected milk normally had lower bacterial counts than milk collected on an alternate day basis (Thomas and Druce, 1971)

The mixing of milk from many farms with varying bacteriological quality has been reported to contribute to the increase in the bacterial content of the commingled milk and the subsequent high count of the milk received at the creamery (Thomas and Druce, 1971) .

Dommett *et al.* (1980) studied the effects tanker cleaning procedures on the quality of raw milk supplies. They emphasised that thorough cleaning of all milk contacting surfaces must be given higher priority over cooling. They recommended daily cleaning and disinfection of tankers before the first trip and a short rinse between trips. They observed that the increase in bacterial counts on the first trip after through cleaning was identical to that found on the second trip after a simple intermediate rinse.

The FFA content of the tanker load of milk was significantly different between surveys. The FFA content was significantly higher in June than in March or December. The influence of higher ambient temperature in addition to the mechanical agitation during transport has been reported (Walstra and Jenness, 1984).

4.1.2 The effects of raw storage at 2 °C and 5 °C on milk quality

The bacterial count of milk stored at 2 and 5 °C to simulate the effects of extended low temperature storage of raw milk, increased significantly over the storage time. The increases in TPC of milk stored at 5 °C were significantly greater than that of milk stored at 2 °C. The milk stored at 5 °C reached the level of TPC of more than 10^6 cfu/ml after 3 days of storage. The same milk stored at 2 °C reached the same level of TPC after 4 days of storage.

The changes in PBC in milks stored at 2 and 5 °C in the present study, followed a similar trend as that of TPC. The PBC increased much faster in milk stored at 5 °C than milk stored at 2 °C and reached counts of more than 10^6 cfu/ml after storage for 4 and 3 days at 2 and 5 °C, respectively.

It has been quite clearly established that psychrotrophs were responsible for the bacterial multiplication which occured in milk held at temperatures of 5 °C or below (Cousin, 1982) and consequently the microflora of bulk collected milk is largely composed of psychrotrophs after 3-4 days of cold storage.

Cousin (1982) reported that the maximum period of storage of milk prior to processing is dependent on the initial number and type of psychrotrophs present and the and the temperature of milk storage. It was observed that when the milk was stored at 10 °C the rate of bacterial

multiplication was such that only one day storage was possible, while for 2-3 days safe storage required cooling to less than 4 ^{o}C.

The changes in the coliforms count were significant over the storage time. The mean coliforms count of milk stored at 5 ^{o}C was significantly higher than that of the same original milk stored at 2 ^{o}C. The coliforms count reached count of > 10^4 cfu/ml after 4 days of storage at 2 ^{o}C as compared to 2 days when stored at 5 ^{o}C.

The changes in PLBC during storage were significant but no significant difference was observed between the PLBC of milks stored at 2 and 5 ^{o}C.

The results of the surveys and investigations reviewed by Thomas and Druce (1971) showed quite clearly that for AD collected bulk tank milk to be suitable for processing, the following essential conditions must be complied with:-

i. strictly hygienic methods of milk production,

ii. effective cleaning and sterilisation of farm and creamery equipment,

iii. immediate cooling of milk to at least 4 ^{o}C within 2 hours of milking and control of blended temperature to less than 10 ^{o}C, and

iv. maintainance of storage temperatures at less than 4 ^{o}C until processing, which should be carried out within 24 hours of the arrival of milk at the creamery.

According to Mikolajcik (1979), psychrotrophic microorganisms may have a direct as well as indirect

effects 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 products even though the organisms failed to survive pasteurisation.

Directly, organisms surviving pasteurisation or resulting from post-pasteurisation contamination may multiply in sufficient numbers during manufacture and storage of dairy products so as to reduce the shelf-life and the quality and quantity of the finished product. Furthermore, live organisms need not be present to produce defects. The organisms produce heat resistant lipolytic and proteolytic enzymes.

In the present study the differences in TA and FFA of raw milks stored at 2 ^{o}C and 5 ^{o}C were significant. The TA and FFA contents of milk stored at 5 ^{o}C was consistently higher than that of milk stored at 2 ^{o}C. The increase in TA and FFA had been attributed to enzymic hydrolysis that continued throughout the storage period (Deeth and Fitz-Gerald 1976).

Lipolysis in milk due to the accumulation of FFA resulting from hydrolysis of milk triglycerides by lipase is well established (Deeth and Fitz-Gerald, 1976) Lipolysis in raw milk is promoted by the physical disruption of the protective milk fat globule membrane thereby exposing the fat to lipase action.

Conclusions

1. The microbiological counts (TPC, PBC and coliforms count) of tanker milks received at the WSC, were higher than the mean count of milks sampled from the individual farms although the time difference from first collection to delivery was a little less than 2 hours and 30 minutes. The increased in the microbiological counts could be attributed to contamination by the road tanker.

2. Deep cooled storage of raw milk, proved to reduce the rate of bacterial growth. The difference in TPC and PBC between raw milks stored at 2 ^{o}C and 5 ^{o}C throughout the storage periods was almost always 10-fold.

3. The growth of psychrotrophic bacteria was characterised by a long lag phase. In the present study the, the lag phase was observed longer in raw milk stored at 2 ^{o}C as compared to storage at 5 ^{o}C.

4. Deep cooled storage (2 ^{o}C) was observed to extend the keeping quality of raw milk. In terms of TPC and PBC (count $\leq 10^{6}$ cfu/ml), the keeping quality of the raw milk in the present study was extended by one day when stored 2 ^{o}C as compared to milk stored at 5 ^{o}C (4 days vs 3 days).

5. In terms of FFA, the level of lipolysis was lower in raw milk stored at 2 ^{o}C than that stored at 5 ^{o}C. The differences increased with storage time.

6. In terms of TV, the partial hydrolysis of milk proteins was lower in raw milk stored at 2 ^{o}C than that stored at 5 ^{o}C. The differences increased with storage time.

SECTION 4.2 EFFECTS OF EXTENDED RAW STORAGE AT 2 AND 5 °C ON THE QUALITY OF PASTEURISED MILK

Introduction

There is continuing interest among processors to extend the shelf life of milk and milk products to meet the demand of the consumer for longer shelf-life products. Among the many factors which account for the wide variations in keeping quality of pasteurised milk, post-pasteurisation recontamination was the predominant cause of early spoilage. In addition storage temperature of the milk is also of importance. In an adequately refrigerated pasteurised milk, shelf-life could be determined by the numbers and activities of psychrotrophic bacteria (Grosskopf and Harper, 1969).

Langeveld and Cuperus (1980) studied the relationships between temperature and the growth rate of different types of bacteria in pasteurised milk. They reported that, in milks virtually free from post-pasteurisation contamination, spoilage of pasteurised milks were linked to the presence of psychrophilic thermodurics from the initial raw milk.

Changes in the on-farm procedures of milking with the tendency towards longer refrigerated storage of raw milk at the farm, plus additional storage periods in bulk silos prior to processing, may contribute to problems of shortened life of pasteurised milk. Extended refrigerated storage has lead to the predominance of psychrotrophic

bacteria in raw milk prior to processing.

Psychrotrophic bacteria are difficult to exclude from raw milk and may grow and produce proteolytic as well as lipolytic enzymes. Many of these enzymes are heat stable and survived pasteurisation temperatures (Fairbairn and Law, 1986). Subsequently these enzymes would influence the keeping quality of the pasteurised milk.

Because of the trend towards extended holding times for raw milk prior to pasteurisation, the objective of the present study was to determine the effect of age of raw milk stored at 2 ^{o}C and 5 ^{o}C on the shelf-life of the pasteurised milk.

Materials and methods

The milks stored at 2 and 5 ^{o}C in section 4.1 were pasteurised after 2, 4 and 7 days of storage according to the procedures as described in section 2.1. In addition, the blended milk was also pasteurised fresh, i.e. milk pasteurised on the day of delivery. The pasteurised milks were stored at 4$\pm$1 ^{o}C and analysed 1, 3, 7, 15 and 21 days after storage for TPC, PBC, TBC, PLBC, TA, FFA and TV; using the methods as described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.5, 2.2.12, 2.2.17.1 and 2.2.20, respectively.

All data were subjected to the three way analysis of variance as described in section 2.4., using trial, the extended raw storage time at 2 and 5 ^{o}C and the storage period after pasteurisation as main effects of the analysis.

Results

The response of TPC, PBC, TBC, PLBC, FFA and TV of the pasteurised milks to the storage treatments varied in general, varied highly significantly between trials ($p < 0.01$).

The effects of the various raw milk storage treatments on the TPC of milk after pasteurisation are shown in table 4.2.1. Pasteurisation was effective in reducing the TPC to a common level, regardless of the TPC before pasteurisation.

The TPC remain unchanged but increase significant 15 days after storage. No significant differences ($p > 0.05$) were observed when the TPC of the milk pasteurised fresh were compared with the milks pasteurised after 2, 4 and 7 days of storage. However, significant differences ($p < 0.001$) were observed when the TPC of milks pasteurised after 2, 4 and 7 days were compared with each other. The TPC of raw milks stored at 2 ^{o}C were significantly lower ($p < 0.001$) than milk stored at 5 ^{o}C.

The three-way interaction, Storage.Temperature.Time was significant ($p < 0.01$). The presence of this interaction was explained by the significant interactions between Storage.Temperature ($p < 0.001$), Temperature.Time ($p < 0.001$) and Storage.Time ($p < 0.05$).

Table 4.2.2 illustrate the effects of various raw storage treatments on the PBC of milk after pasteurisation. Pasteurisation was effective in eliminating psychrotrophic bacteria in milk regardless of

the initial PBC.

The PBC was observed to increase slightly after pasteurisation but was significant ($p < 0.01$) after 7 days of storage. However, the differences in PBC of the pasteurised milks due to storage temperature before pasteurisation was not significant ($p > 0.05$).

No significant ($p > 0.05$) interactions were observed.

The difference between fresh pasteurised and milks pasteurised after the storage time was not significant ($p > 0.05$). In addition, no significant differences ($p > 0.05$) were observed when milks pasteurised after 2, 4 and 7 days of storage at 2 and 5 ^{o}C were compared with each other.

The TBC of milk pasteurised fresh, 2, 4 and 7 days after storage at 2 and 5 ^{o}C are given in table 4.2.3. Pasteurisation has no effect on TBC. The TBC of the raw milk remain unchanged after pasteurisation.

Table 4.2.1

Mean[a] total plate count (TPC) (log_{10} cfu/ml) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milk was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b] days	Fresh[c]	Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
		2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	2.95	3.05	3.10	3.22	2.82	2.90	3.33	3.06	3.08
3	3.10	3.01	2.94	3.03	2.90	3.29	3.22	3.11	3.02
7	2.96	3.65	3.68	3.69	3.49	3.94	4.37	3.76	3.85
15	4.75	4.67	4.62	5.22	4.73	4.52	4.78	4.80	4.71
21	6.38	6.16	6.50	5.08	5.80	4.96	5.91	5.40	6.07
Mean	4.03	4.11	4.17	4.05	3.95	3.92	4.32	4.03	4.15

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.11	0.13	0.15	0.10	0.12	0.25	0.17	0.21

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.16	0.22	0.27	0.17	0.39

(Contd...)

(...contd)

Analysis of variance

Source of variation	df	F
Trial	2	164.71***
Fresh[a]	1	2.09
Storage	4	167.91***
Temperature	1	14.97***
Time[b]	2	9.97***
Trial.Storage	8	5.22***
Trial.Temperature	4	5.89***
Trial.Time	6	0.66
Storage.Fresh	4	2.16
Storage.Temperature	4	6.94***
Storage.Time	8	2.11*
Temperature.Time	2	16.66***
Storage.Temperature.Time	8	3.22**
Mean square error	50	0.23

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, ** highly significant $p < 0.01$, *** very highly significant $p < 0.001$.

Table 4.2.2

Mean[a] psychrotrophic bacterial count (PBC) (log_{10} cfu/ml) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pateurised milk was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b]		Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
days	Fresh[c]	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	0.00	1.29	0.00	0.00	0.00	1.63	0.50	0.97	0.17
3	0.97	0.43	1.46	1.00	2.06	1.92	1.56	1.12	1.69
7	0.67	3.13	2.32	2.36	2.55	0.85	1.93	2.11	2.27
15	3.53	2.99	2.13	2.39	1.87	2.07	4.47	2.48	2.82
21	2.80	4.04	3.27	4.50	3.20	4.02	5.76	4.08	4.74
Mean	1.59	2.38	1.84	2.05	1.94	2.10	2.84	2.15	2.34

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.35	0.41	0.45	0.31	0.38	0.78	0.53	0.65

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.49	0.69	0.84	0.53	1.19

(Contd...)

(...contd

Analysis of variance

Source of variation	df	F
Trial	2	16.16***
Fresh[a]	1	2.67
Storage	4	20.16***
Temperature	1	0.28
Time[b]	2	1.86
Trial.Storage	8	1.66
Trial.Temperature	4	1.55
Trial.Time	6	4.11**
Storage.Fresh	4	1.27
Storage.Temperature	4	0.69
Storage.Time	8	1.53
Temperature.Time	2	2.72
Storage.Temperature.Time	8	1.61
Mean square error	50	2.12

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, ** highly significant $p < 0.01$, *** very highly significant $p < 0.001$.

Table 4.2.3

Mean[a] thermoduric bacterial count (TBC) (log_{10} cfu/ml) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milk was fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b] days	Fresh[c]	Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
		2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	1.30	1.73	1.07	1.45	0.93	0.80	0.83	1.33	0.94
3	0.73	1.40	1.50	1.33	1.69	1.17	0.80	1.30	1.33
7	1.80	1.70	0.40	1.23	1.37	1.27	1.92	1.40	1.23
15	2.67	1.95	1.62	2.23	1.20	1.50	1.59	1.89	1.47
21	2.13	2.69	2.67	3.33	2.39	2.00	4.66	2.68	3.24
Mean	1.73	1.89	1.45	1.92	1.52	1.35	1.96	1.72	1.64

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.21	0.24	0.27	0.18	0.22	0.46	0.32	0.39

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.29	0.41	0.50	0.32	0.71

(Contd...)

(...contd

Analysis of variance

Source of variation	df	F
Trial	2	7.83***
Fresh[a]	1	0.04
Storage	4	13.38***
Temperature	1	0.17
Time[b]	2	0.41
Trial.Storage	8	1.88
Trial.Temperature	4	0.60
Trial.Time	6	0.64
Storage.Fresh	4	1.91
Storage.Temperature	4	0.96
Storage.Time	8	0.73
Temperature.Time	2	3.57*
Storage.Temperature.Time	8	1.66
Mean square error	50	0.75

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, *** very highly significant $p < 0.001$.

Table 4.2.4

Mean[a] psychrotrophic lipolytic bacterial count (PLBC) (log_{10} cfu/ml) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milk was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b]		Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
days	Fresh[c]	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	0.00	0.53	0.67	0.79	0.78	0.54	0.67	0.62	0.70
3	0.00	1.00	1.10	0.83	1.00	0.70	0.87	0.84	0.99
7	1.00	1.59	2.12	1.75	1.62	1.30	3.25	1.55	2.33
15	2.73	3.17	2.90	1.88	2.30	1.73	3.68	2.26	2.96
21	3.67	3.32	4.19	2.63	2.85	3.23	4.13	3.76	3.72
Mean	1.48	1.92	2.20	1.58	1.71	1.50	2.52	1.67	2.14

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.23	0.27	0.30	0.20	0.25	0.51	0.35	0.43

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.32	0.45	0.55	0.35	0.78

(Contd...)

(...contd)

Analysis of variance

Source of variation	df	F
Trial	2	129.06***
Fresh[a]	1	2.36
Storage	4	32.78***
Temperature	1	4.95*
Time[b]	2	2.76
Trial.Storage	8	3.74***
Trial.Temperature	4	1.72
Trial.Time	6	2.05
Storage.Fresh	4	1.02
Storage.Temperature	4	0.50
Storage.Time	8	2.16*
Temperature.Time	2	6.02**
Storage.Temperature.Time	8	1.52
Mean square error	50	0.91

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, ** highly significant $p < 0.01$, *** very highly significant $p < 0.001$

The TBC of milk pasteurised fresh as compared to that of milk pasteurised after 2, 4 and 7 days of storage was not significant ($p > 0.05$). The TBC increased significantly ($p < 0.001$) during post pasteurisation storage and especially greater after 15 days of storage ($p < 0.01$). No significant differences ($p > 0.05$) in response due to raw storage temperatures or time of pasteurisation after the extended storage were observed.

The PLBC increase significantly ($p < 0.001$) during storage for all the pasteurised milks. The increase was greater in December than the trial in March or June, respectively. However, the differences between fresh pasteurised milk and milks pasteurised after 2, 4 and 7 days of storage were not significant ($p > 0.05$).

The differences in PLBC between milk pasteurised after 2, 4 and 7 days of storage were not significant ($p > 0.05$) when they were compared with each other. But the difference between milks stored at 2 and 5 ^{o}C was significant ($p < 0.05$).

The interaction Temperature.Time was significant ($p < 0.01$). The differences in PLBC between milk stored at 2 ^{o}C and 5 ^{o}C were not always the same when the milk was pasteurised 2, 4 or after 7 days of storage. The differences in PLBC between milk stored at 2 and 5 ^{o}C was largest when the milk was pasteurised 7 days after storage as compared to pasteurisation on 4 or 2 days of storage, respectively.

The TA increase significantly ($p < 0.001$) through storage and the differences between fresh pasteurised and

milks pasteurised after 2, 4 and 7 days of storage were significant ($p < 0.01$). The TA of milk pasteurised fresh was consistently lower than milk pasteurised after the storage time.

The differences in TA between milk pasteurised after 2, 4 and 7 days of storage were significant ($p < 0.001$). The TA was higher in milk stored at 5 ^{o}C than at 2 ^{o}C in all the pasteurised milks. The difference increased with longer storage time before pasteurisation.

The interactions Trial.Temperature and Trial.Time were significant ($p < 0.01$). They signified the variability of responses of raw storage temperatures and the duration of raw storage between trials.

In addition, significant interaction ($p < 0.01$) Temperature.Time was observed. The differences in the increament of TA due to the extended raw storage time before pasteurisation were not the same for raw milk stored at 2 ^{o}C as for storage at 5 ^{o}C. The mean differences were not significant when the milk was pasteurised 2 and 4 days after storage but was significant ($p < 0.01$) when the milk was pasteurised after 7 days.

The means of FFA concentration of milk during pasteurised storage are shown in table 4.2.6. The percentage reduction in FFA of milk due to pasteurisation (from table 4.1.13) was 20.4 per cent when the milk was pasteurised fresh and 38.2, 42.4 and 31.5 per cent when the milk was pasteurised 2, 4 and 7 days after storage at 2 ^{o}C, and 52.2, 33.8 and 29.1 per cent after storage at 5

^{o}C, respectively. The percentage reduction of FFA appeared indirectly proportional to the initial concentration before pasteurisation.

The difference between trials were highly significant ($p < 0.001$). The mean FFA concentration was 0.71, 0.74 and 0.52 mEq/100 ml for the trial in December, March and June, respectively. The difference between March and the June trial was significant ($p < 0.05$). The mean FFA of milk pasteurised fresh was always lower than milk pasteurised after the storage time. The difference was significant ($p < 0.05$).

The FFA increased highly significantly ($p < 0.001$) through pasteurised storage. The differences between the storage temperatures and the extended raw storage time were significant ($p < 0.001$). The mean FFA concentration of milk pasteurised after extended storage at 2 ^{o}C was consistently lower than milk stored at 5 ^{o}C. The differences increase with longer raw storage.

The interaction between temperature and storage time was significant ($p < 0.001$).

The means of TV of milk during storage after pasteurisation are shown in table 4.2.7. The percentage reduction in TV of milk due to pasteurisation (from table 4.14) was 35.8 per cent when the milk was pasteurised

Table 4.2.5

Mean[a] titrable acidity (TA) (percentage lactic acid) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milk was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 and 5 °C.

Storage[b]		Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
days	Fresh[c]	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	0.133	0.140	0.143	0.133	0.140	0.143	0.163	0.139	0.149
3	0.133	0.137	0.143	0.137	0.150	0.147	0.187	0.140	0.160
7	0.150	0.127	0.150	0.153	0.153	0.143	0.170	0.141	0.158
15	0.143	0.163	0.173	0.150	0.170	0.163	0.243	0.159	0.196
21	0.160	0.190	0.187	0.180	0.193	0.207	0.279	0.192	0.220
Mean	0.144	0.151	0.159	0.151	0.161	0.161	0.208	0.155	0.176

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.006	0.007	0.008	0.005	0.006	0.013	0.009	0.011

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.008	0.012	0.014	0.009	0.020

(Contd...)

(...contd)

Analysis of variance

Source of variation	df	F
Trial	2	3.81*
Fresh[a]	1	9.70**
Storage	4	4.02**
Temperature	1	18.35***
Time[b]	2	13.87***
Trial.Storage	8	4.16***
Trial.Temperature	4	2.15
Trial.Time	6	3.44**
Storage.Fresh	4	1.47
Storage.Temperature	4	0.79
Storage.Time	8	1.33
Temperature.Time	2	6.16**
Storage.Temperature.Time	8	0.71
Mean square error	50	0.0006

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, ** highly significant $p < 0.01$, *** very highly significant $p < 0.001$.

Table 4.2.6

Mean[a] free fatty acids (FFA) concentration (mEq/100 ml) of pasteurised (73 °C/16 seconds) milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milk was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b] days	Fresh[c]	Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
		2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	0.48	0.37	0.28	0.33	0.48	0.55	0.78	0.42	0.51
3	0.39	0.33	0.33	0.47	0.54	0.62	1.21	0.48	0.69
7	0.45	0.33	0.34	0.49	0.65	0.60	1.39	0.47	0.79
15	0.63	0.48	0.43	0.54	0.70	0.72	1.51	0.58	0.88
21	0.63	0.55	0.58	0.64	0.81	0.91	2.36	0.70	1.25
Mean	0.52	0.41	0.39	0.49	0.63	0.68	1.45	0.53	0.83

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.06	0.08	0.08	0.06	0.07	0.14	0.10	0.12

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.09	0.13	0.16	0.10	0.22

(Contd...)

(...contd)

Analysis of variance

Source of variation	df	F
Trial	2	6.97***
Fresh[a]	1	4.98*
Storage	4	8.96***
Temperature	1	17.14***
Time[b]	2	47.58***
Trial.Storage	8	1.39
Trial.Temperature	4	5.73***
Trial.Time	6	6.75***
Storage.Fresh	4	0.65
Storage.Temperature	4	0.98
Storage.Time	8	1.92
Temperature.Time	2	22.71***
Storage.Temperature.Time	8	1.78
Mean square error	50	7.20

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, *** very highly significant $p < 0.001$.

Table 4.2.7

Mean[a] tyrosine value (TV) (mg/ml) of pasteurised (73 °C/16 seconds)milk tested 1, 3, 7, 15 and 21 days after storage at 5 °C. The pasteurised milks was prepared fresh[c], 2, 4, and 7 days after raw storage at 2 °C and 5 °C.

Storage[b]		Pasteurised days after cold storage						Mean by storage temperature	
		2 days		4 days		7 days			
days	Fresh[c]	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C	2 °C	5 °C
1	0.077	0.096	0.088	0.093	0.093	0.091	0.094	0.093	0.092
3	0.083	0.099	0.098	0.091	0.083	0.100	0.100	0.100	0.094
7	0.091	0.094	0.084	0.112	0.091	0.110	0.098	0.102	0.091
15	0.129	0.093	0.102	0.117	0.097	0.133	0.108	0.114	0.102
21	0.145	0.115	0.112	0.138	0.119	0.152	0.131	0.135	0.121
Mean	0.105	0.099	0.097	0.110	0.096	0.117	0.106	0.109	0.100

Note: (a) Means of 3 trials, (b) stored at 4 °C, (c) pasteurised on the initial day of each trial, (d) mean after storage by temperature.

SED of means:

Table	Trial	Fresh	Storage	Temperature	Time	Trial Storage	Trial Temperature	Trial Time
SED	0.004	0.004	0.005	0.003	0.004	0.009	0.006	0.007

Table	Storage Fresh	Storage Temperature	Storage Time	Temperature Time	Storage/Temperature Time
SED	0.005	0.007	0.009	0.006	0.013

(Contd...)

(...contd)

Analysis of variance

Source of variation	df	F
Trial	2	12.12***
Fresh[a]	1	0.12
Storage	4	24.49***
Temperature	1	10.18***
Time[b]	2	3.69*
Trial.Storage	8	0.50
Trial.Temperature	4	1.19
Trial.Time	6	4.50***
Storage.Fresh	4	2.71
Storage.Temperature	4	0.15
Storage.Time	8	2.03
Temperature.Time	2	1.50
Storage.Temperature.Time	8	0.60
Mean square error	50	0.00026

Note: (a) = Pasteurised fresh vs pasteurised after storage, (b) = pasteurised days after storage, * Significant $p < 0.05$, *** very highly significant $p < 0.001$.

fresh and 11.1, 33.6 and 41.3 per cent when the milk was pasteurised after 2, 4 and 7 days of storage at 2 ^{o}C, respectively. The reductions after storage at 5 ^{o}C, were 35.8, 43.6 and 49.2 per cent when pasteurised 2, 4 and 7 days of storage at 5 ^{o}.

The responses of the various preprocessing treatments to the increase in TV were very highly significantly different ($p < 0.001$) between trials. The mean TV was 0.114, 0.101 and 0.96 mg/ml for trial in December, March and June, respectively.

The changes in TV during storage after pasteurisation was very highly significant ($p < 0.001$). The difference between fresh pasteurised and milks pasteurised 2, 4 and 7 days after extended raw storage at 2 and 5 ^{o}C was not significant ($p > 0.05$). However, significant difference was observed when milk pasteurised after 2, 4 and 7 days of raw storage at 2 and 5 ^{o}C were compared with each other. The TV of milk pasteurised after the extended storage at 2 ^{o}C was consistently lower than milk pasteurised after storage at 5 ^{o}C. The difference increased with the extended raw storage time.

The interaction between Trial and the extended storage time was significant ($P < 0.001$). However, it was not of practical importance.

Discussion

According to Baker (1983) the period between processing and the time at which milk becomes unacceptable to consumers has been referred to as the *shelf-life* or *consumable life* of milk. Although it reflects the *keeping quality* of milk, there was no adequate working definition of shelf-life. Similarly, there is no adequate objective measure of shelf-life, much less a way of predicting the shelf-life or keeping quality of milk.

Solberg, (1981) reported that in the USA it was normal that grade A pasteurised milk stored at a maximum temperature of 7 ^{o}C remained *acceptable* for 18 days or even longer. In some continental west-European countries a shelf life of 10-14 days was not uncommon (Langeveld and Cuperus, 1980). In the UK, pasteurised milk with longer shelf-life has been thought unnecessary because over 80 per cent of the pasteurised milk was delivered to the door step daily (Blake, 1979 as cited by Schroder *et al.*, 1982)

The production of pasteurised milk with longer keeping quality is possible. Baker (1983) demonstrated that under optimum conditions a maximum shelf life of 170 days was achievable.

There are various factors that could influence the keeping quality of pasteurised milk. Allan and Joseph (1985) reported that these factors can be divided into two main categories:-

i. factors inherent in the milk itself, such as ascorbic acid content, FFA levels and native metal

content, and

ii. external and processing factors, including handling, agitation, temperature, exposure to light, and contamination by metals or microorganisms.

In addition, because of its reactivity with many milk components and its support of aerobic growth of microorganisms, dissolved O_2 content has also been implicated. These factors were interlinked with each other, therefore deterioration may be due to a combination of several of them.

It was not the purpose of the present study to discuss all the factors mentioned. However, the factors affecting microbiologically induced deterioration will be reported.

Allen and Joseph (1985) reported that deterioration of pasteurised milk on storage caused by microorganisms could originate from:-

i. organisms present in raw milk which either produce heat-resistant hydrolases or which can themselves survive pasteurisation, or

ii. from post-pasteurisation contamination.

4.2.1 **The effects of pasteurisation on bacterial counts**

The results of bacterial counts in the present study were in general agreement with other workers (Maxy, 1967 and Yan *et al.*, 1983). The TPC of milk after pasteurisation was reduced to $\leq 10^3$ cfu/ml when tested 1 day after pasteurisation.

The TPC increased slightly during cold storage after pasteurisation and significantly after 15 days of storage. However, the increase was not significantly different between milk pasteurised fresh and the milks pasteurised after the extended raw storage.

In the present study it was observed that pasteurisation was effective in reducing the TPC to as common level, regardless of the TPC before pasteurisation. The increase in TPC

Population levels in excess of 10^6 cfu/ml were usually required before any organoleptic changes were detected (Punch *et al.*, 1965).

Mikolajcik (1979) observed the effects of increasing bacterial counts in milk on the organoleptic properties of pasteurised milk. It was reported that although the number of bacteria was important in inducing flavour changes in raw and pasteurised milks, the initial count and the net increase in the number of bacterial counts were more important than total number present at the time of analysis or when the off-flavours were detected.

The PBC content of the pasteurised milks in the present study were in general agreement with an earlier investigation (Yan *et al.*, 1983). Pasteurisation completely inactivated the psychrotrophic bacteria irrespective of the initial PBC load before pasteurisation. However, the psychrotrophic bacteria were detected 7 days after storage in all the pasteurised milks. The presence of psychrotrophic bacteria during storage after pasteurisation, suggests the presence of

spore-forming psychrotrophic bacteria in the milk which survived pasteurisation (Collins, 1981).

Raw milk storage temperature did not affect the increase in PBC in the pasteurised milk. It was assumed that raw storage at 2 and 5 ^{o}C were selective for the growth of psychrotrophic bacteria that resulted in their complete inactivation after pasteurisation.

Thermoduric organisms are defined as those those that survived pasteurisation. Schroder *et al.* (1982) examined milk immediately after laboratory HTST pasteurisation and observed that the TBC of the freshly pasteurised milk was similar to the initial count of the corresponding raw milk. Similar observations were made in the present study.

In addition, the slight inconsistency in TBC of the pasteurised milks relative to the initial TBC before pasteurisation was due to the procedure of the analysis. The organisms counted as thermodurics had to survive a second heat treatment af 63 ^{o}C for 30 minutes, and it is possible that the second heat treatment i.e laboratory pasteurisation accounted for the inconsistency observed, as suggested by Schroder *et al.* (1982).

In the present study it was observed that the TBC increased only after 15 days of pasteurised milk storage. The extended low temperature storage of the raw milk before pasteurisation had no affect on the TBC. A similar observation was made by Brown *et al.* (1984). They suggested that TBC had little relevance to the quality of pasteurised milk because of the low temperature storage

maintained after pasteurisation.

Thomas *et al.* (1963) studied the effects of temperature and time of plate incubation on the enumeration of pasteurisation resistant bacteria in milk. They reported that the TBC of milk after pasteurisation increased with longer refrigerated raw storage time before pasteurisation. The reason for the increase was not explained.

The effect of pasteurisation on PLBC was similar to that of the PBC. The extended storage time has no significant effect on the PLBC during storage after pasteurisation. However, it was observed that the PLBC of milk stored at 2 ^{o}C was consistently lower than milk stored at 5 ^{o}C. The difference due to the storage temperature increased with increasing storage time.

4.2.2 The effects of pasteurisation on chemical properties of milk

The TA decreased slightly in all the pasteurised milks in the present study, as compared to the corresponding raw milks. The decrease in TA was caused by the thermal decomposition of carbohydrates to organic acids, thus included in the TA components of the milk analysis (Webb and Johnston, 1965).

The TA of milk pasteurised fresh and milks pasteurised after the extended cold storage time was significantly different. The TA of all the pasteurised milks increased with increasing raw milk storage time and storage

temperature before pasteurisation. However, no significant difference was observed when milk pasteurised after 2 and 4 days of cold storage was compared with each other, but significant differences were found when they were compared to the value for milk pasteurised after 7 days of cold storage at both storage temperatures.

The development of lipolytic flavour in pasteurised milk has been used as a measure of *shelf life* (Shipe *et al.*, 1978). Lipolysis is an enzymic hydrolysis of milk lipids to FFA which may give rise to off-flavours which are often described as rancidity. The enzyme responsible include both endogenous type as well as those of microbial origin (Deeth and Fitz-Gerald, 1976).

The FFA concentration of pasteurised milks in the present study was comparable to earlier observation (Shipe *et al*, 1980). Pasteurisation reduced the the FFA content of all the pasteurised milks slightly, when tested 1 day after pasteurisation but increased significantly with increasing storage period although the level was relatively lower than the corresponding raw milks before pasteurisation. In addition, the FFA content of the pasteurised milks was observed to increase with increasing raw milk storage time and temperature.

HTST pasteurisation was generally believed to inactivate the endogenous milk lipase completely (Antila and Kankara, 1983) although earlier reports indicated that some activity remained after pasteurisation (Cousin, 1982).

Kuzdzal-Savoie (1980) reported that the increase in FFA

after pasteurisation did not involve hydrolysis by lipases of bacterial origin. The observation was based on the assumption that the TPC in their study was $\leq 10^4$ cfu/ml which was much less than 10^6 cfu/ml as suggested by Punch *et al.* (1965) and Suhren *et al.* (1976), the level of bacterial counts when off-flavours could be detected, organoleptically.

Shipe and Senyk, (1982) reported that the type of bacteria and their specific hydrolytic activities were more important than numbers in predicting off-flavour defects. In addition, Suhren *et al.* (1976) reported that the degree of lipolysis varied with different microbial species.

Shipe *et al.* (1978) reported that unclean, bitter and putrid flavours had been attributed to the growth of psychrotrophic organisms. The mechanism involved in the development of these flavour defects involved limited hydrolysis of milk proteins and the production of bitter peptides and decomposition products of amino acids.

Mikolajcik (1979) reported that off-flavours, principally bitterness, were detected in pasteurised milk that had high bacterial counts before pasteurisation, desapite low numbers of bacteria in the pasteurised products.

The TV of milks in the present study were comparable to that reported in earlier investigations (Juffs, 1975b and Yan *et al.* 1983). Pasteurisation reduced the TV of milk (tested 1 day after pasteurisation) by between 11 to 50

per cent depending on the initial level before pasteurisation.

The TV was observed to increase during storage in all the pasteurised milks and increased with an extension of raw milk storage time and with decreased raw storage temperature. However, the differences between milk pasteurised after 2 and 4 days of raw storage were not significant but were significant when they were compared to the values of milk pasteurised after 7 days of storage.

Yan *et al.* (1983) studied the effects of raw milk storage on changes during storage of pasteurised milk. They reported that the increase in TE (tyrosine equivalent) in pasteurised milk depended directly on the duration of its storage as raw milk before pasteurisation. They linked the increase in proteolysis, as measured by TE during storage of pasteurised milk, to bacterial growth before and after pasteurisation. They observed that marked increased in the TV were always associated with higher counts of the corresponding raw milk and increasing bacterial count of the pasteurised milk.

Juffs (1975) studied the relationship between bacterial populations, TV and organoleptic quality during storage extended cold storage of milk and cream. He reported that the variations of TV in milk were related to storage temperature, amount of aeration, amount and type of bacterial contaminations before pasteurisation and the inherent natural variations in the NPN fraction of the milk.

The slight increases in TV after pasteurisation might

have been caused by the release of amino acids or small peptides from milk proteins during heating (Juffs, 1975).

Conclusions

1. The effectiveness of pasteurisation when measured in terms of bacterial counts one day after the heat treatment was dependent on counts before pasteurisation. In the present study, the TPC of the pasteurised milks when tested one day after pasteurisation were observed to be less than 10^3 cfu/ml, irrespective of the initial count before pasteurisation. In addition, pasteurisation effectively eliminated the psychrotrophic bacteria but has no effect on the TBC.

2. As expected, the TPC of the pasteurised milks increased during storage. However, in the present study, the increase was directly affected by the raw storage temperature and the length of the raw storage time before pasteurisation. The TPC and PBC of milk pasteurised after raw storage at 2 ^{o}C was lower than that pasteurised after storage at 5 ^{o}C.

3. In terms of bacterial counts ($\leq 10^6$ cfu/ml), the pasteurised milks in the present study remained *acceptable* 21 days after pasteurisation regardless of the duration and temperature of raw storage. However, in terms of TA (≤ 0.16), FFA (≤ 0.64 mEq/100 ml) and

TV ($\leq$ 0.12 mg/ml) shorter *shelf life* was observed.

4. Pasteurisation reduced the level of FFA when when the milk was tested one day after pasteurisation. The FFA of the pasteurised milk increase during cold storage. The increase in FFA was greater in milk pasteurised after longer raw storage time and higher raw milk storage temperature before pasteurisation

5. Similar observations were made of TV of the pasteurised milks but lower TV were found when the raw milk was stored at 5 ^{o}C rather at 2 ^{o}C. No significant difference was observed between the TV values of milk pasteurised after 2 and 4 days of raw milk storage but significant differences were found between the TV of pasteurised milk produced from raw milk stored at low temperature for 2 and 4 days and pasteurised milk prepared from milk held cold for 7 days before processing.

6. Further investigations into the influence of extended raw milk storage at 2 ^{o}C in relation to organoleptic flavour changes during storage of pasteurised milk are recommended.

CHAPTER FIVE

THE EFFECTS OF THERMISATION ON MILK AND ITS PROPERTIES

Introduction

Thermisation is a milder form of heat treatment less severe than pasteurisation (Bender, 1968). It is a relatively new process to the dairy industry (Coghill *et al.*, 1982). One of the first reports of the process appeared when Casalis (1958) discussed a heat treatment suitable for cheese making. Since that time other investigators have studied the effect of this heat treatment on raw milk destined for the manufacture of various dairy products, such as yogurt (Sebela, 1978), Cheddar cheese (Banks *et al.*, 1986), UHT milk (Griffiths *et al.*, 1986), dried milk products (West *et al.*, 1986), cottage cheese (Dzurec and Zall, 1982) and various other fermented dairy products (Karlikanova, 1977).

Thermisation has also been used as a post-pasteurisation heat treatment of dairy product such as yoghurt and Bryndza cheese (Sebela, 1978), tvaroh and various dairy products (Karlikanova, 1977). The purpose was to destroy contaminants and extend product shelf life.

Quite recently, van den Berg (1984) described thermisation as a process to improve the keeping quality of raw milk during refrigerated storage by means of a mild heat treatment and subsequent cooling in such a way that

the raw milk properties remained almost unchanged after 4 days of storage.

Various heat treatments have been investigated in the laboratory. It has been applied both on farms (Zall and Chen, 1980) as well as at the creamery (van den Berg, 1984). The thermisation temperature usually ranges from 55 to 68 ^{o}C with varied holding time from 10 to 120 seconds (Griffiths *et al.*, 1986). Although higher temperature has been investigated (Zall and Chen, 1984), heating temperature from 63-68 ^{o}C holding for 10-20 seconds have been widely recommended (van den Berg, 1984). Gilmour *et al.* (1981) recommended that thermisation heat treatment should result in effective eradication of coliform organisms and in providing the desired shelf life extension of 4 days at 6 ^{o}C. In addition, van den Berg (1984) recommended that thermisation heat treatment should result in a detectable level of alkaline phosphatase.

The purpose of the present investigation was to evaluate the effectiveness of various heat treatments in prolonging the storage life of raw milk (section 5.1). The most effective thermisation treatment was selected and applied in subsequent experiments to study the effects of extended raw milk storage on thermisation (section 5.2) and the quality of pasteurised milk made from the stored thermised milk (section 5.3).

SECTION 5.1 PRELIMINARY STUDY

Materials and methods

Raw milk was obtained from the local SMMB and delivered by road tanker to the WSC. On receipt, the milk was processed in an APV plate heat exchanger as described in section 2.1 in sequence of descending order of severity of the heat treatment. Two trials were conducted. The thermisation temperatures investigated were 55, 60, 63, 65, 68 and 73 ^{o}C with holding time of 16 seconds. Samples of milk were collected prior to heating (unheated control) and after each heat treatment. Analyses were carried out immediately (0 day) and after storage periods of 1, 2, 3, 4, 7 and 15 days at 5 ^{o}C.

All samples were examined for total plate count (TPC), psychrotrophic bacterial count (PBC), thermoduric bacterial count (TBC), psychrotrophic and mesophilic proteolytic bacterial counts (PPBC and MPBC), psychrotrophic and mesophilic lipolytic bacterial count (PLBC and MLBC), and Coliforms count using the methods described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.4, 2.3.5 and 2.3.6, respectively. Alkaline phosphatase content (AP) was determined on the unheated control and the heated milk immediately after storage, according to the procedure as described in section 2.2.19.

All data were subjected to the analysis of variance as described in section 2.4, using trial, heat treatment and storage period after thermisation as main effects of the analysis.

Results

The differences in all the measured parameters between trials 1 and 2 due to thermisation treatments, were highly significant ($p < 0.001$). The responses in trial 2 were higher than those in trial 1 and consistent after all storage times.

The initial count of raw milk used in the study and the bacterial counts after the various heat treatments are shown in table 5.1.1. It was apparent from the present study that all thermisation heat treatments resulted in pronounced reductions, both in bacterial counts and the alkaline phosphatase concentration. The effectiveness of thermisation increased with increased severity of heat treatments. Thermisation at 63 ^{o}C was observed to be the lowest heat treatment that resulted in the complete eradication of coliform organisms and 50 percent reduction of AP content. The reduction of TPC ranges from 10.4 per cent at 55 ^{o}C to 42.4 per cent at 73 ^{o}C and the difference was distinct between each temperature.

However, it was observed that the TPC remained unchanged when the heating temperature was increased from 63 to 65 ^{o}C. The reductions of PBC following thermisation were greater than TPC at all the temperatures investigated. The PBC was reduced from 13.2 per cent at 55 ^{o}C to > 99.9 percent at 73 ^{o}C. When the heating was increased from 63 to 65 ^{o}C the PBC was reduced by 8.4 percentage units as compared to 6.5 percentage units when the temperature was raised to 68 ^{o}C from 65 ^{o}C.

The reductions in PPBC, MPBC, PLBC, and MLBC appeared to follow similar patterns according to their growth temperature classification. The correlation between heating temperature and the percentage reductions of AP content of milk was high ($r = 0.94$) as shown in figure 5.1.1.

The unheated controls exihibited a definite increase in TPC. The time taken for the TPC to reach $> 1 \times 10^6$ cfu/ml was 4 days. The TPC increased significantly ($p < 0.001$) during storage following thermisation (table 5.1.2) and the mean counts over the storage periods were higher for the lower heating temperatures and little change was observed when the milk had been heated at 73 ^{o}C. The significant ($p < 0.05$) interaction between factors: Storage and Treatment described this relationship. During the first 4 days of storage little change in count was noticed. This was in aggrement with previous studies where thermised milk was stored satisfactorily for 4 days (Gilmour et al., 1981; Coghill *et al.*, 1982 and Griffiths *et al.*, 1986).

Similar results were obtained when the milk samples were examined for PBC (table 5.1.3). The PBC increased significantly during storage and the increase was higher at lower heating temperatures and no growth was observed when the milk had been heated at 73 ^{o}C. The increase was not significant during the first 4 days of storage for all heat treatments but significant after 7 days when the milk was heated at 55 ^{o}C.

The difference in TBC during storage was very highly

significant ($p < 0.001$) when the milk was heated at 55, 60 and 63 ^{o}C; but was not significant when the milk was heated at higher temperatures as evidenced by the significant ($p < 0.001$) interaction between factors: Storage and Treatments. However, the increase in TBC during storage at 5 ^{o}C in the first 7 days was very small and not significant for all heat treatments.

The PPBC, MPBC, PLBC and MLBC during storage are shown in tables 5.1.5 to 5.1.8, respectively. It appeared that their growth sequence emulate the patterns of TPC and PBC for the mesophilic and psychrotrophic type bacteria, respectively.

Table 5.1.1

Preliminary trial. The percentage reduction in total plate count (TPC), psychrotrophic bacterial count (PBC), thermoduric bacterial count (TBC), total direct coliform count (DCC), psychrotrophic and mesophilic proteolytic counts (PPBC and MPBC), psychrotrophic and mesophilic lipolytic counts (PLBC and MLBC) and alkaline phosphatase (AP) concentration due to the heat treatments.

Counts[a]	Unheated	Heat treatment (°C for 16 seconds)											
		55	% redn[d]	60	% redn	63	% redn	65	% redn	68	% redn	73	% redn
TPC	4.34	3.89	10.4	3.50	19.4	3.32	23.5	3.23	25.6	2.93	32.5	2.50	42.4
PBC	3.95	3.43	13.2	2.91	26.3	2.69	31.9	2.36	40.3	2.10	46.8	+	++
TBC	2.51	2.57	2.4	2.41	4.0	2.29	8.8	2.31	8.0	+	++	+	++
CC[b]	2.53	2.15	15.0	1.65	38.8	+	++	+	++	+	++	+	++
PPBC	2.66	1.50	77.3	2.26	15.0	1.70	36.1	1.60	39.9	*	*	+	++
MPBC	3.42	2.05	40.1	2.05	40.1	+	70.8	2.05	40.1	1.60	53.2	+	++
PLBC	3.60	3.37	1.7	2.38	33.3	2.35	3.3	1.47	59.2	+	72.2	+	72.2
MLBC	5.07	3.62	28.6	3.22	36.5	3.37	33.5	2.94	42.0	1.90	62.5	1.65	66.9
AP[c]	55.9	50.0	10.4	33.7	39.7	27.9	50.0	26.9	51.7	4.8	91.4	0.00	100

Note : * = Missing value, + = < 1/ml, ++ = > 99 per cent, (a) = $\log_{10}$ cfu/ml milk (b) = coliforms count, (c) = ul phenol/ml milk, (d) = reduction.

Table 5.1.2

The mean[a] of total plate counts (log_{10} cfu / ml) of raw and heated milk after cold storage at 5 ^{o}C.

Storage (days)	Raw	Heating (^{o}C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	4.34[c]	3.89	3.50	3.32	3.23	2.93	2.50	3.23
1	4.85	3.90	3.50	3.38	3.39	3.01	2.37	3.26
2	5.28	4.11	3.73	3.65	3.33	3.26	2.58	3.44
3	6.70	5.15	4.23	4.12	3.90	3.07	2.53	3.83
4	7.85	5.02	5.84	5.49	4.67	3.56	1.98	4.43
7	10.20	7.21	6.73	6.54	6.38	4.82	2.25	5.66
15	ND	8.55	7.00	7.96	7.16	4.90	2.98	6.42
Mean[d]	6.49	5.40	4.93	4.92	4.58	3.65	2.55	4.32

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined.

SED of means:	Trial	Treatment	Storage	Storage Treatment
	0.14	0.25	0.27	0.66

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	17.70	17.70	40.68**
Storage	6	110.59	18.43	42.36**
Treatment	5	73.92	14.79	33.98**
Trial.Storage	6	4.76	0.79	1.82
Trial.Treatment	5	5.12	1.02	2.36
Storage.Treatment	30	27.69	0.92	2.12*
Error	30	13.05	0.44	

Note : * significant $p < 0.05$, ** significant $p < 0.001$

Table 5.1.3

The mean[a] of the total psychrotrophic bacterial counts (log_{10} cfu/ml) of raw and heated milk after storage at 5 °C .

Storage (days)	Raw	Heating (°C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	3.95[c]	3.42	2.91	2.60	2.35	2.09	*	2.24
1	4.20	3.50	2.51	2.65	2.30	*	*	1.83
2	4.98	4.08	2.97	3.54	1.80	*	*	1.98
3	5.65	4.86	3.57	3.99	3.67	2.03	*	2.94
4	6.85	5.51	4.84	5.42	4.55	2.78	*	3.77
7	8.25	7.23	5.83	6.66	6.29	3.93	*	4.91
15	ND	7.80	6.86	7.30	7.41	3.75	*	5.52
Mean[d]	5.65	5.20	4.22	4.61	3.98	1.87	*	3.31

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:	Trial	Treatment	Storage	Storage Treatment
	0.21	0.36	0.39	0.96

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	19.32	19.32	21.14**
Storage	6	154.63	25.77	28.20**
Treatment	5	273.87	54.77	59.93**
Trial.Storage	6	9.80	1.63	1.79
Trial.Treatment	5	16.79	3.39	3.71*
Storage.Treatment	30	44.65	1.48	1.63
Error	30	27.42	0.91	

Statistical significance: * highly significant $p < 0.01$, ** very highly significant $p < 0.001$

Table 5.1.4

The mean[a] of the total thermoduric bacterial counts (log_{10} cfu/ml) of raw and heated milk after storage at 5 °C

Storage (days)	Raw	Heating (°C for 16 seconds)						Means[b]
		55	60	63	65	68	73	
0	2.51[c]	2.57	2.40	2.28	2.31	*	*	1.60
1	2.40	2.17	2.42	2.10	2.32	2.25	*	1.88
2	2.45	2.42	2.69	2.42	2.34	2.23	*	2.02
3	2.58	2.29	2.50	2.20	1.52	2.39	*	1.73
4	2.72	2.28	2.11	2.20	2.30	2.32	*	1.87
7	2.90	3.24	1.90	2.42	2.15	2.16	2.00	2.31
15	ND	3.80	2.40	3.29	2.80	2.15	1.55	2.58
Mean[d]	2.59	2.68	2.35	2.42	2.18	1.93	0.44	2.00

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:	Trial	Treatment	Storage	Storage Treatment
	0.08	0.14	0.14	0.35

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	0.96	0.96	8.00*
Storage	6	8.44	1.41	11.66**
Treatment	5	45.43	9.09	75.34**
Trial.Storage	6	1.33	0.22	1.84
Trial.Treatment	5	0.55	0.11	0.91
Storage.Treatment	30	18.69	0.62	5.17**
Error	30	3.62	0.12	

Statistical significance: * highly significant $p < 0.01$
** very highly significant $p < 0.001$

Table 5.1.5

The mean[a] of the total psychrotrophic proteolytic bacterial counts (log_{10} cfu/ml) of raw and heated milk after storage at 5 °C

Storage (days)	Raw	Heating (°C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	2.66[c]	1.50	2.26	1.70	1.60	2.06	*	1.27
1	2.85	2.81	2.72	2.03	1.95	1.50	*	1.59
2	3.20	2.60	2.78	2.24	1.50	*	*	1.35
3	4.70	3.91	3.67	4.05	3.55	3.39	*	3.09
4	6.20	4.80	3.20	3.30	2.80	2.12	*	2.70
7	8.15	5.29	4.13	4.01	3.79	2.10	*	3.22
15	ND	6.56	5.56	4.10	4.60	2.60	*	3.90
Mean[d]	4.63	3.86	3.48	2.85	2.61	1.90	*	2.45

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:

Trial	Treatment	Storage	Storage Treatment
0.12	0.21	0.23	0.56

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	31.00	31.00	98.84**
Storage	6	78.32	13.05	41.62**
Treatment	5	133.27	26.65	84.98**
Trial.Storage	6	5.28	0.88	2.80*
Trial.Treatment	5	23.47	4.69	14.96**
Storage.Treatment	30	38.67	1.29	4.11**
Error	30	9.41	0.31	

Statistical significance: * significant $p < 0.05$, ** very highly significant $p < 0.001$

Table 5.1.6

The mean[a] of the total mesophilic proteolytic bacterial counts (log_{10} cfu/ml) of raw and heated milk after storage at 5 °C

Storage (days)	Raw	Heating (°C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	3.42[c]	2.05	2.05	*	2.05	1.60	*	0.96
1	4.72	3.34	2.33	2.26	2.84	2.79	1.78	2.31
2	5.20	3.76	3.45	3.53	3.11	2.88	*	2.79
3	6.25	4.17	2.82	2.98	2.77	2.40	2.22	2.48
4	7.01	4.19	2.77	4.08	3.07	3.40	*	2.75
7	9.20	3.70	2.70	3.82	4.80	3.85	1.10	3.16
15	ND	3.30	3.50	5.30	5.40	4.60	1.82	3.99
Mean[d]	5.97	3.43	2.45	3.00	3.22	2.93	0.78	2.64

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:

Trial	Treatment	Storage	Storage Treatment
0.19	0.33	0.35	0.87

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	94.11	94.11	125.66**
Storage	6	61.04	10.17	13.59**
Treatment	5	65.75	13.15	17.56**
Trial.Storage	6	21.50	3.58	4.79*
Trial.Treatment	5	21.66	4.33	5.78**
Storage.Treatment	30	39.71	1.32	1.77
Error	30	22.47	0.75	

Statistical significance: * significant $p < 0.05$, ** very highly significant $p < 0.001$

Table 5.1.7

The mean[a] of the total psychrotrophic lipolytic bacterial counts (log_{10} cfu / ml) of raw and heated milk after storage at 5 °C

Storage (days)	Raw	Heating (°C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	3.60[c]	3.36	2.37	2.35	1.46	*	*	1.51
1	4.00	2.95	2.31	1.78	*	*	*	1.09
2	4.50	3.36	2.74	2.30	2.03	1.50	*	1.74
3	5.50	4.28	3.42	3.16	2.97	2.66	*	2.75
4	6.20	4.74	3.59	4.38	3.78	3.61	*	3.35
7	8.20	6.69	4.80	5.00	4.70	5.71	*	4.48
15	ND	6.55	4.72	4.90	5.50	5.40	*	4.51
Mean[d]	5.33	4.57	3.42	3.27	2.78	2.63	*	2.78

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:	Trial	Treatment	Storage	Storage Treatment
	0.22	0.39	0.42	1.03

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	53.76	53.76	51.21***
Storage	6	141.39	23.56	22.45***
Treatment	5	162.29	32.46	30.92***
Trial.Storage	6	18.52	3.09	2.94*
Trial.Treatment	5	25.47	5.09	4.85**
Storage.Treatment	30	45.94	1.53	1.46
Error	30	31.50	1.05	

Statistical significance: * significant $p < 0.05$, ** highly significant $p < 0.01$, *** very highly significant $p < 0.001$

Table 5.1.8

The mean[a] of the total mesophilic lipolytic bacterial counts (log_{10} cfu / ml) of raw and heated milk after storage at 5 °C

Storage (days)	Raw	Heating (°C for 16 seconds)						Mean[b]
		55	60	63	65	68	73	
0	5.07[c]	3.62	3.22	3.37	2.94	1.90	1.68	2.62
1	4.40	3.47	3.14	3.33	3.16	2.58	2.32	3.00
2	5.10	3.89	3.37	3.71	3.38	2.92	1.68	3.08
3	5.95	4.89	3.75	4.29	3.86	2.87	1.76	3.57
4	6.75	5.05	3.80	5.28	4.77	3.39	1.20	4.01
7	9.25	6.72	5.15	5.89	5.76	4.73	1.92	5.03
15	ND	7.20	4.30	4.40	6.85	5.21	1.30	5.04
Mean[d]	6.09	4.98	3.82	4.33	4.39	3.30	1.78	3.77

Note: (a) = Means of two trials (b) = all treatments means, (c) = fresh sample (d) = treatment means and ND = not determined, * = count < 1/ml.

SED of means:	Trial	Treatment	Storage	Storage Treatment
	0.16	0.27	0.29	0.72

Analysis of variance

Source of variation	df	SS	MS	F
Trial	1	34.27	34.27	65.74*
Storage	6	68.37	11.40	21.86*
Treatment	5	88.95	17.79	34.13*
Trial.Storage	6	7.35	1.23	2.35
Trial.Treatment	5	4.95	0.99	1.90
Storage.Treatment	30	24.52	0.82	1.57
Error	30	15.64	0.52	

Statistical significance: * very highly significant $p < 0.001$

Table 5.1.9

Effects of heat treatments on the alkaline phosphatase concentration of milk. Means of 2 trials.

Heat Treatment ^{o}C	*U*g phenol/ml milk	% inhibition
Unheated	55.9	-
55	50.0	10.4
60	33.7	39.7
63	27.9	50.0
65	26.9	51.7
68	4.8	91.4
73	0	100.0

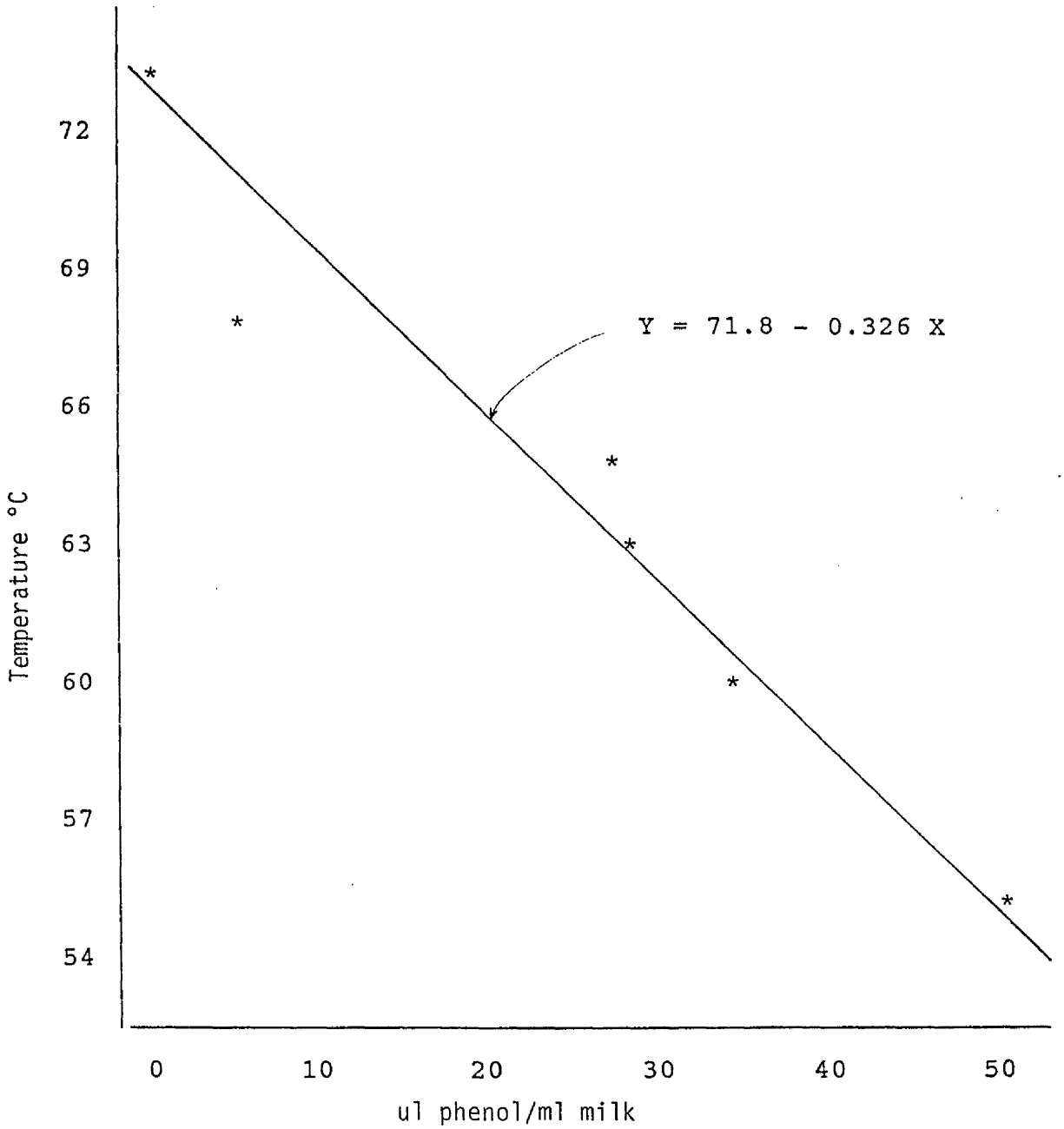


Figure 5.1.1 :- The relationship between thermisation temperature and the reduction of alkaline phosphatase content of milk. r = 0.94.

Discussion

The differences between trials in all the parameters measured were very highly significant ($p < 0.001$). The bacteriological counts of raw milk used in trial 2 were consistently higher than trial 1. The variation seemed to influence the effectiveness of the thermisation process. The total bacterial count of the thermised milks remained less than 1×10^6 after 4 days of storage. However, thermisation treatment at 65 ^{o}C for 16 seconds appeared to be the most effective and this confirms results of other workers (Stadhouders, 1982 and van den Berg, 1984; Johnston *et al.* 1983; Gebre-Egziabher *et al.*, 1985 and Banks *et al.*, 1986).

Banks *et al.* (1986), measured the storage life of milk thermised at 55 ^{o}C for 60 seconds and 65 ^{o}C for 15 seconds and reported that the storage life were 2.9 and 6.3 days, respectively. The storage life was longer when the heat treatment was more severe.

Banks *et al.* (1986), studied the effects of thermisation at 55 ^{o}C for 60 seconds and 65 ^{o}C for 15 seconds on the extension of the storage life of milk and reported that thermisation at 55 ^{o}C for 60 seconds produced a ten-fold reduction in psychrotrophs count as compared to thermisation at 65 ^{o}C for 15 seconds which produced a 1000-fold reduction in count.

In addition, they observed that the storage life of the raw milk as measured by the time for the psychrotrophs count to reach 10^6 cfu/ml, was increased from 1.9 days for

the unthermised milk to 2.9 and 6.3 days for milk thermised at 55 and 65 ^{o}C, respectively.

Griffiths *et al.* (1986) study the effects of sub-pasteurisation heat treatments on the shelf life of UHT milk. They observed that initial total count of raw milk was strongly correlated ($r = 0.87$; $p < 0.001$) to the count of thermised milk during subsequent storage at 6 ^{o}C. When raw milk with 1.8×10^{4} cfu/ml and 7.2×10^{6} cfu/ml were thermised, the times taken to reach 1×10^{6} cfu/ml were 5.3 and 4.4 days respectively.

van den Berg (1984) speculated that a considerable increase in count during storage of thermised milk could be an indicative of high initial counts. Kimenai (1978) made a similar observation. Bjorgum *et al.* (1978) suggested that higher thermisation temperatures were required for effective thermisation of milks with high initial counts.

West *et al.* (1986) study the effects of thermisation on the production of dried skim milk. They observed that thermisation treatment at 65 ^{o}C for 15 seconds reduced psychrotrophs count to less than 100 cfu/ml after thermisation but had little effect on thermoduric or spore counts. This is to be expected. During subsequent storage, they observed a slow proliferation of psychrotrophic bacteria and little change in other counts. Johnston *et al.* (1987), applied similar heat treatments and reported comparable results.

Stadhouders (1982) thermised milk at 68 ^{o}C for 10 seconds followed by varied storage conditions from 4 to 7

^{o}C. It was observed that the thermised milk could be stored for up to 3 days without any appreciable increase in TPC and PBC and products made from it equalled in quality to those made from fresh raw milk. It was suggested that thermisation may not be less than 64 ^{o}C for 10 seconds followed by storage temperature of less than 7 ^{o}C.

Driessen and Stadhouders (1978) (cited by Stadhouders, 1982), observed that thermisation at 60, 65 and 70 ^{o}C for 20 seconds, inactivate milk lipase to the extent of 40, 85 and 90 per cent respectively, and inactivate alkaline phosphatase partially.

Dzurec and Zall (1982) *thermalized* milk on the farm at 74 ^{o}C for 10 seconds and followed this treatment by cooling to 3 ^{o}C. They observed that after 7 days of storage, the bacterial count of *thermalized* milk complied with microbial standards prescribed by the US Pasteurised Milk Ordinance for grade A milk (U.S Public Health Service, 1978). The standard plate count of the 7 days old pasteurised milk was less than 10^5 cfu/ml.

However, the on-farm heat treatment of milk is not in compliance with the U.S. Pasteurised Milk Ordinance (U.S Public Health Service, 1978), which forbids heating of milk above 52 ^{o}C except for pasteurisation.

Thermisation reduces the bacterial content of milk and enhance the quality and yield of products made from it. Zall (1985) reported a 100-fold reduction of TPC after thermisation treatment on the farm and increases the yield

of Cheddar cheese made from it. Banks *et al.* (1986) reported a reduction of 1000-fold in TBC following thermisation at 65 ^{o}C for 10-20 seconds holding time and the milk remain in *acceptable* quality after 3 days of storage.

Coghill, (1982) thermised milk at 62, 65 and 67 ^{o}C for 10-20 seconds. They observed that the reduction in psychrotrophic bacteria was dependent on temperature and holding times and greater destruction of psychrotrophs than of total bacteria. This was expected since most psychrotrophs are heat labile (Senyk *et al.*, 1982). The increase of PBC during storage was negligible.

Thermisation treatments at temperatures above 65 ^{o}C were the most effective and this confirms the results of other workers. Bjorgum *et al.* (1978) and Gilmour *et al.* (1981)

Conclusions

1. Thermisation is a relatively new process to the dairy industry. It usually applies to a heat treatments of 63 to 65 ^{o}C for 15 to 20 seconds. The process is being used widely in the continent, particularly in the Netherlands (Foley and Buckley, 1978), but as yet to be practiced in the UK (Griffiths *et al.*, 1986).

2. Thermisation is an effective means of extending the shelf life of raw milk. Results from the investigation suggested that the most practical heat treatment to

accomplish the objectives of thermisation would be 65 ^{o}C for 16 seconds. This recommendation was based on the premise that it was the heat treatment which eradicated coliform bacteria completely and reduced both TPC and PBC to a level that allowed milk to be stored at 5 ^{o}C for up to 4 days while still retaining (in terms of TPC and PBC of 10^{6}) an *acceptable* quality level

3. This heat treatment (65 ^{o}C for 16 seconds) reduced the alkaline phosphatase content of the heated milk by approximately 50 per cent. Thus, if the thermisation treatment was followed by commercial pasteurisation, the combination of pretreatment and full pasteurisation would not constitute double pasteurisation.

4. Therefore, thermisation heat treatment at 65 ^{o}C for 16 seconds will be employed in the ensuing studies.

SECTION 5.2 THE EFFECTS OF THERMISATION ON MILK QUALITY

Materials and methods

The milk used in the experiment was from the earlier trial (chapter 4, section 4.1). After 2, 4 and 7 days of storage at 5 ^{o}C, 50 litres of raw milk were drawn and thermised at 65 ^{o}C for 16 seconds as previously described (section 2.1). Samples of milk were taken immediately after thermisation and analysed after 1, 3, 7, 15 and 21 days of storage at 5 ^{o}C. The samples were analysed for TPC, PBC, TBC, PLBC, TA, FFA and TV using the procedures as described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.2, 2.2.12, 2.2.17.1 and 2.2.20, respectively.

The data were subjected to the analysis of variance as described in section 2.4, using trials, storage temperatures and storage after thermisation as the main effects.

Results

Thermisation was effective in reducing the initial TPC and controlling the increase in milk during subsequent storage. The TPC was reduced 10, 100, 10,000 and 100,000-fold from the initial count of 3.0×10^4, 1.7×10^5, 1.2×10^7 and 6.2×10^8 immediately before the heat treatment (fresh, 2, 4 and 7 days after storage at 5 ^{o}C) to 2.0×10^3, 2.5×10^3, 5.9×10^3 and 2.3×10^3, respectively, 1 day after the heat treatment and storage at 5 ^{o}C.

The effectiveness of thermisation was observed to be directly related to the initial bacterial count. Thermisation reduced the TPC to a common level, regardless of the TPC before thermisation. The TPC after treatment was observed to be similar for all treatments at less than 10^4 after 3 days of storage at 6 ^oC, except the milk that was thermised 7 days after cold storage. The count was observed significantly higher ($p < 0.05$) when compared to the other treatments. However, based on the TPC data, milk under all treatment conditions investigated remained *acceptable* 3 days after thermisation treatment and storage at 6^oC.

After 3 days of storage the TPC increased more than 2 log cycles for all treatments. The differences between treatment were significant ($p < 0.05$) and the differences in response due to treatments were related to the storage time ($p < 0.05$). There was a highly significant difference ($p < 0.001$) in response between trials. This was expected.

The means of PBC after thermisation following storage were given in table 5.2.2. The reduction in PBC due to thermisation was substantial. From the initial count of 1.6×10^4, 9.8×10^4, 1.3×10^7 and 9.5×10^7 cfu/ml immediately before the heat treatment (Table 4.1.8), the PBC was reduced to 1.7×10^2, 6.3×10, 2.5×10^2 and to less than 10 cfu/ml, respectively, 1 day after the heat treatment. The PBC remained unchanged up to 3 days of storage. Significant increase in PBC was observed in all treatments from the 7^{th} day of storage onwards. No significant differences between treatments were noted. In

addition, no significant interaction was observed.

The trial differences and differences due to treatment in TBC were highly significant ($p < 0.01$). However, the differences were not important because the counts were too low to affect the quality of the milk adversely. Low temperature storage was not conducive to the growth of the thermoduric bacteria.

The reduction in PLBC due to thermisation followed the same trend as that for the PBC. Thermisation was most effective against psychrotrophic lipolytic bacteria in milk with the highest count. From the initial counts of 1.6×10^3, 2.1×10^3, 1.7×10^7 and 1.0×10^7 immediately before thermisation (table 4.1.11) the PLBC was reduced to < 100 cfu/ml in all treatments. The mean differences due to trials and treatments were significant ($p < 0.001$). The interaction between storage time and treatments was also significant ($p < 0.05$), however, the PLBC remained lower and not significantly different between treatments up to 3 days of storage.

Highly significant increases were observed after the 7^{th} day of storage. The increase was greatest in milk thermised fresh as compared to the milk thermised after 4, 7 and 2 days storage at 5 ^{o}C, respectively. The differences in PLBC between milk thermised after 2, 4 and 7 days after storage was significant. Milk that was thermised after 4 days storage had a higher PLBC than milk that was thermised 2 or 7 days after storage. No

Table 5.2.1

Mean[a] total plate counts (TPC) (log_{10} cfu/ml) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	3.30	3.40	3.77	3.36	3.46
3	3.46	3.85	3.63	4.61	3.89
7	7.18	4.96	6.79	6.77	6.42
15	9.34	7.67	9.02	8.33	8.59
21	9.25	9.41	8.25	9.20	9.03
Mean[d]	6.51	5.86	6.29	6.45	6.28[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.23	0.30	0.27	0.46	0.52	0.60

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	16.65	8.32	15.571**
Day	4	319.05	79.76	149.215**
Treatment	3	3.87	1.29	2.413*
Trial.Treatment	6	2.07	0.35	0.647
Trial.Day	6	14.80	2.47	4.614*
Day.Treatment	10	15.19	1.52	2.842*
Mean square error	15	8.02	0.53	

Note: * significant $p < 0.05$, ** significant $p < 0.001$

Table 5.2.2

Mean[a] psychrotrophic bacterial count (PBC) (log_{10} cfu/ml) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	2.22	1.80	2.40	0.73	1.79
3	2.04	2.58	0.83	3.26	2.18
7	7.61	3.04	5.72	5.81	5.54
15	8.21	7.32	8.02	7.67	7.80
21	9.32	9.68	6.87	8.66	8.63
Mean[d]	5.88	4.88	4.77	5.23	5.19[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.45	0.59	0.52	0.91	1.02	1.17

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	15.79	7.90	3.833*
Day	4	473.30	118.32	57.427**
Treatment	3	11.24	3.75	1.819
Trial.Treatment	6	7.45	1.24	0.603
Trial.Day	6	12.31	2.05	0.996
Day.Treatment	10	50.69	5.07	1.460
Mean square error	15	30.91	2.06	

Note: * significant $p < 0.05$, ** significant $p < 0.001$

Table 5.2.3

Mean[a] thermoduric bacterial count (TBC) (log_{10} cfu/ml) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Pasteurised after thermisation and storage at 6 °C				
	Fresh[b]	2	4	7	Mean[c]
1	1.30	1.33	1.27	1.16	1.27
3	0.73	1.07	1.77	1.44	1.25
7	1.80	1.70	1.47	1.41	1.59
15	2.67	2.67	2.33	1.97	2.41
21	2.13	3.02	2.80	1.32	2.32
Mean[d]	1.73	1.96	1.93	1.46	1.77[e]

Note: (a) Means of 3 trials, (b) Pasteurised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.20	0.30	0.28	0.45	0.50	0.58

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	5.22	2.61	5.191**
Day	4	85.15	21.29	42.347***
Treatment	3	7.08	2.36	4.695**
Trial.Treatment	6	27.26	4.54	9.038***
Trial.Day	8	21.97	2.75	5.463*
Day.Treatment	12	12.56	1.05	2.082
Mean square error	21	10.56	0.50	

Note: * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$

Table 5.2.4

Mean[a] psychrotrophic lipolytic bacterial count (PLBC) (log_{10} cfu/ml) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	1.92	1.07	1.37	1.80	1.54
3	1.70	1.93	1.04	1.30	1.49
7	5.68	2.40	3.13	2.65	3.47
15	7.67	5.50	5.37	4.70	5.81
21	8.71	7.31	6.61	7.54	7.54
Mean[d]	5.14	3.64	3.50	3.60	3.97[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.25	0.32	0.29	0.50	0.56	0.65

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	91.64	45.82	72.601***
Day	4	341.59	85.40	135.303***
Treatment	3	27.39	9.13	14.465***
Trial.Treatment	6	15.14	2.52	3.998*
Trial.Day	7	4.78	0.68	1.082
Day.Treatment	11	17.68	1.61	2.547*
Mean square error	17	10.73	0.63	

Note: * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$

differences was observed between milk that was thermised 2 and 7 days after storage.

The mean TA of the thermised milk are shown in table 5.2.5. Thermisation reduced the total percentage lactic acid in milk slightly. The reduction was observed to be directly related to the level of the initial TA of the milk before treatment. The higher the initial acidity the higher the proportion of the reduction.

The mean TA of the thermised milk varied significantly ($p < 0.001$) with trial. A highly significant ($p < 0.001$) increase was observed during the storage time. The changes in TA during storage following thermisation was noted to be highly significantly dependent ($p < 0.01$) on trial changes. After 3 days of storage negative changes was observed in milk that was thermised 4 and 7 days after storage. No change was observed with the milk thermised 2 days after storage. But a significant increase was observed in milk that was thermised fresh on the day of arrival. Eventhough a highly significant changes was observed in the TA of milk during storage, no differences due to treatments were observed. Based on the TA data, the milk should be considered as of *poor* quality from the 7^{th} day of storage onwards.

The means of FFA concentration as measured by the copper soap method of Koops and Klomp (1977) as modified by Shipe *et al.* (1980) are shown in table 5.2.6. The procedure was sensitive and rapid for the determination of FFA content and the related lipolysed flavour as compared

to the Bureau of Dairy Industry (BDI) method developed by Thomas *et al.* (1949) (as cited by Shipe *et al.*, 1980).

Thermisation affects the FFA concentration of milk. The effectiveness was noted to be directly related to the FFA concentration before treatment. From the level before thermisation of 0.60, 0.63, 0.72 and 1.10 mEq/100 ml, on the day of delivery (fresh) and after 2, 4 and 7 days of cold storage at 5 ^{o}C (Table 4.1.13) the FFA was decreased to 0.48, 0.56, 0.50, and 0.49 mEq/100 ml of milk, respectively. The FFA increase significantly ($p < 0.001$) 3 days after storage. The increase was significantly different ($p < 0.05$) between the milks thermised fresh or milks thermised after storage for 2, 4 or 7 days.

The means of TV of milk thermised milk following cold storage are given in table 5.2.7. Tyrosine value measures the partial hydrolysis of milk protein. The tyrosine content of milk increased when proteolysis occurred. Tyrosine is heat labile and thermisation lowered the tyrosine value of milk. From the concentration of 0.120, 0.137, 0.165 and 0.185 ug/ml immediately before treatment (table 4.14) the TV was reduced to 0.085. 0.077, 0.098 and 0.095 ug/ml milk respectively 1 day after storage following thermisation.

TV value of the thermised milk increased very highly significantly ($p < 0.001$) through the cold storage time. However, the increase was not significant in the first 3 days of storage for milk thermised fresh and after 2 and 4 days storage but was significant ($p < 0.05$) for milk

Table 5.2.5

Mean[a] titratable acidity (TA) (percentage lactic acid) of milk thermised fresh[b] (65 ^{o}C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 ^{o}C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	0.140	0.140	0.147	0.147	0.143
3	0.153	0.140	0.147	0.143	0.146
7	0.157	0.167	0.167	0.167	0.165
15	0.163	0.183	0.180	0.187	0.178
21	0.217	0.183	0.220	0.232	0.213
Mean[d]	0.166	0.163	0.172	0.175	0.169[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.005	0.007	0.006	0.010	0.011	0.013

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	0.0129	0.0065	24.994**
Day	4	0.0417	0.0104	40.423**
Treatment	3	0.0012	0.0004	1.579
Trial.Treatment	6	0.0020	0.0003	1.270
Trial.Day	8	0.0140	0.0018	6.794*
Day.Treatment	12	0.0053	0.0004	1.734
Mean square error	22	0.0057	0.0003	

Note: * significant $p < 0.01$, ** significant $p < 0.001$

Table 5.2.6

Mean[a] FFA (mEq/100 ml) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	0.480	0.508	0.498	0.489	0.496
3	0.507	0.576	0.439	0.507	0.507
7	0.681	0.678	0.652	0.617	0.651
15	0.836	0.759	0.695	0.644	0.733
21	0.973	0.873	0.713	0.844	0.851
Mean[d]	0.696	0.678	0.594	0.620	0.594[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.048	0.062	0.055	0.095	0.107	0.123

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	55.25	27.62	12.170**
Day	4	108.39	27.09	11.938**
Treatment	3	27.06	9.02	2.974*
Trial.Treatment	6	16.28	2.71	1.196
Trial.Day	8	38.42	4.80	2.116
Day.Treatment	12	27.83	2.31	1.022
Mean square error	22	49.93	2.27	

Note: * significant $p < 0.05$, ** significant $p < 0.001$

Table 5.2.7

Mean[a] tyrosine value (TV) (mg tyrosine/ml milk) of milk thermised fresh[b] (65 °C/16 seconds) and after 2, 4 and 7 days of cold storage at 5 °C.

Storage (days)	Thermised after raw storage				
	Fresh[b]	2	4	7	Mean[c]
1	0.085	0.077	0.098	0.095	0.089
3	0.087	0.077	0.093	0.102	0.090
7	0.096	0.088	0.102	0.129	0.104
15	0.140	0.100	0.110	0.146	0.124
21	0.302	0.124	0.153	0.228	0.202
Mean[d]	0.142	0.094	0.111	0.140	0.122[e]

Note: (a) Means of 3 trials, (b) thermised on day of delivery, (c) means by day, (d) means by treatment and (e) grand mean.

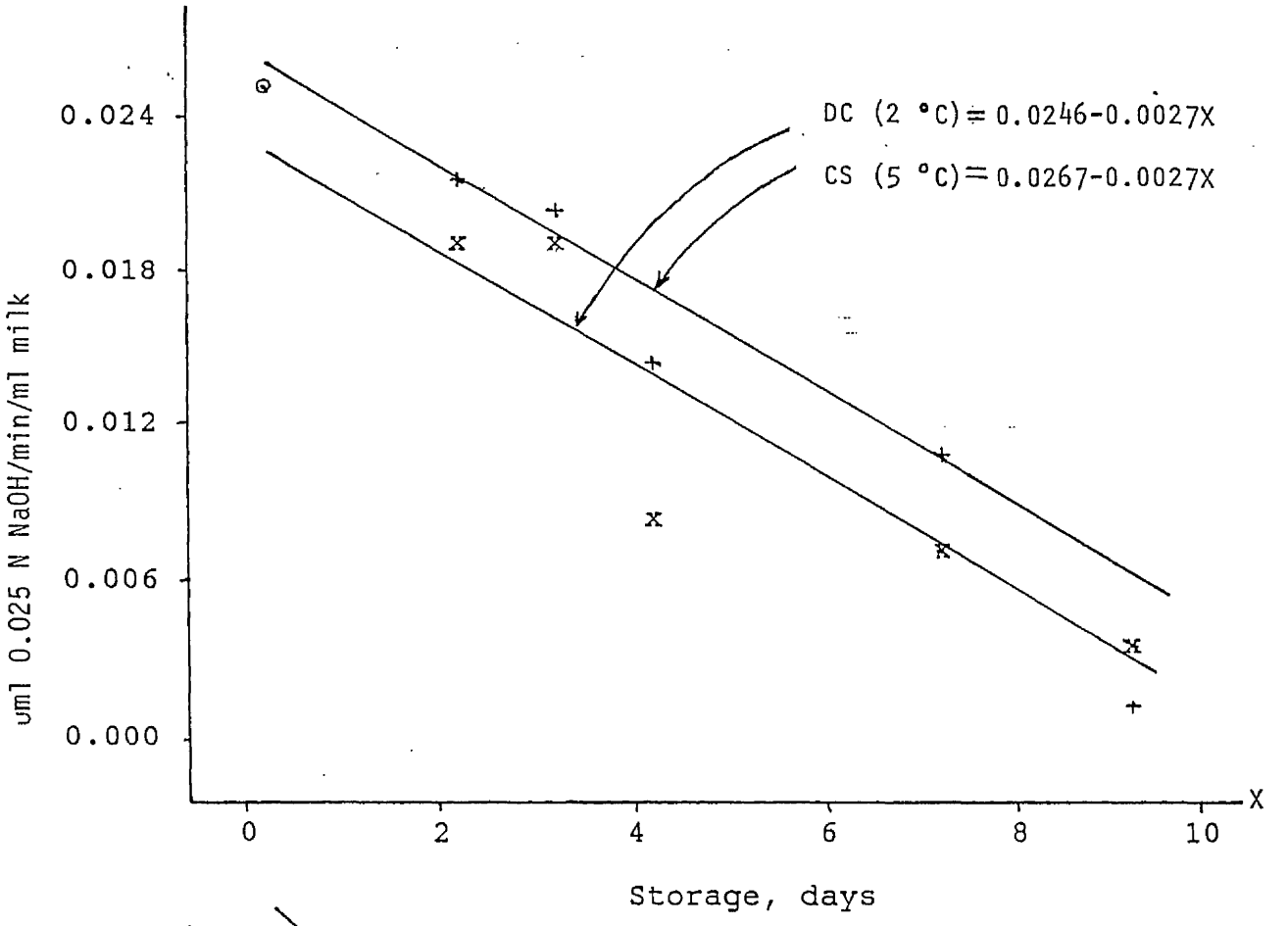
SED of means:

Trial	Day	Treatment	Trial Treatment	Trial Day	Day Treatment
0.016	0.020	0.018	0.031	0.035	0.040

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	0.028	0.014	5.791**
Day	4	0.106	0.026	10.962***
Treatment	3	0.025	0.008	3.454*
Trial.Treatment	6	0.018	0.003	1.212
Trial.Day	8	0.029	0.004	1.508
Day.Treatment	12	0.042	0.003	1.439
Mean square error	22	0.053	0.002	

Note: * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$



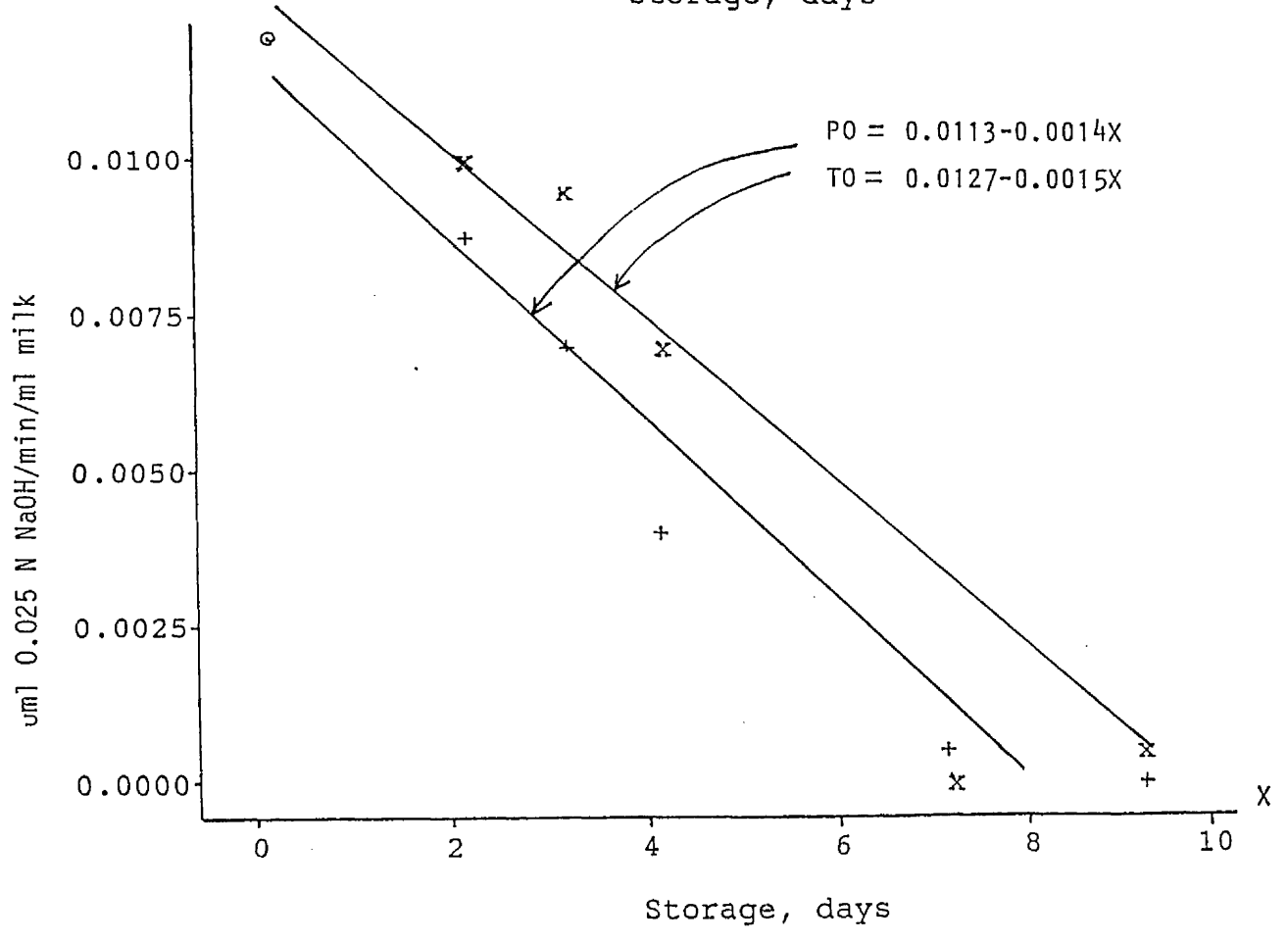


Figure 5.2.1 Lipolytic activity of raw milk stored at 2°C and 5°C, thermised milk stored at 6°C and pasteurised milk stored at 5°C, measured during storage.

thermised after 7 days cold storage. The TV of milk thermised fresh, and after 7 days were observed to be higher when compared to the milk thermised 2 or 4 days after storage. Milk thermised 2 days after storage had the lowest values.

Discussion

The initial TPC of the raw milk used in this study was 3.0×10^4 cfu/ml. The mean value was comparable to the quality of bulk silo milk in the South West of Scotland as reported in the earlier studies (Smillie *et al.* 1958 and Crawford, 1967).

The TPC of the raw unheated milk exhibited obvious increases during storage at 5 ^{o}C. After 2, 4 and 7 days of storage, the TPC of the unheated milks were 1.7×10^5, 1.2×10^7 and 6.2×10^8 cfu/ml, respectively. In term of TPC quality remained *acceptable* after 4 days of storage, the TPC was well within 10^6 cfu/ml, above which quality problems begin to occur (Punch *et al.*, 1965).

Thermisation appeared to be effective in reducing the initial bacterial counts. The TPC was reduced 1 log cycle when milk was thermised immediately on arrival and the reduction in TPC increased to 2, 4 and 5 log cycles when thermisation was done after 2, 4 and 7 days of storage. It was interesting to note that the TPC of the thermised milks tested 1 day after thermisation were always lower than 1.0×10^4 cfu/ml, irrespective of their initial count

before thermisation. Gilmour *et al.* 1981 made similar observations. They reported that thermisation at 65 ^{o}C for 10 seconds was equally effective with milk of varying microbiological quality. However Bjorgum *et al.* (1978), noted that higher temperatures were required for effective thermisation of milk with high initial count.

Similar results were obtained when these same milk samples were examined for psychrotrophic bacteria. The reduction in counts was also dependent on the initial counts before thermisation. However, greater reduction were observed. This was expected since most psychrotrophs were heat labile (Senyk *et al.*, 1982). Even though heat resistant psychrotrophs have been reported to survive pasteurisation heat treatments (van den Berg, 1984) these were not the common cause of a rapidly increasing bacterial count during storage of thermised milk at 6 ^{o}C.

In the present study, the increases in PBC of the thermised milk during the first 3 days of storage were negligible when the milk was thermised fresh and 2 days after raw storage but were significant when the milk was thermised 4 and 7 days after storage.

Gilmour *et al.* (1981) observed that the effectiveness of thermisation becomes more obvious when prolonged raw milk storage times were employed, since the difference between the mean counts during storage following thermisation increased with the raw storage time.

The titratable acidity of milk is expressed as percentage of lactic acid. This value may be used as an

indirect measure of the presence of lactic acid-producing bacteria in milk. Nevertheless, it is not as good an indicator as would be expected. The pH value would be a better indicator, because the acid available for titration with the standard alkali is dependent on the buffering capacity of the milk.

The TA of the thermised milk increased during storage. TA value of < 0.16 would be expected to indicate an *acceptable* level of acidity for raw milk. It was observed that TA of the milk remained unchanged after 3 days of thermisation.

Numerous methods have been developed for measuring FFA in milk to estimate the intensity of lipolysed flavour. The most frequently method is the titrimetric BDI method. However, in the present experiments FFA were measured by converting the FFA to copper soaps, extracted and the copper reacted with a colour reagent. The colour complex was measured colorimetrically (section 2.2.17.1).

The FFA values obtained in the present study were comparable to those obtained by Shipe *et al*. (1980) and in earlier studies by Koops and Klomp, (1977). The increase in the FFA during storage is an indicator of the development of lipid hydrolysis. In the present study the FFA remained static in the first 7 days of storage of milks that were thermised fresh and after 2 days storage. However a small but significant difference in FFA content was observed beyond the 7^{th} day.

Tyrosine value is an index of amino acids and peptides soluble in 10 per cent TCA. It is therefore an index of

proteolysis (Juffs, 1973). It has been used to monitor proteolysis in thermised milk during extended storage (Griffiths *et al.*, 1986).

In the present study no significant increase in TV in the first 7 days of storage were observed except for milk that was thermised on the 7^{th} day of raw milk storage. Thermisation retards the onset of proteolysis in stored milk (Griffiths *et al.* (1986).

Juffs, (1975) proposed TV of 0.55 mg/ml as the upper limit in the natural TV of bulk milk. Milk containing TV exceeding 0.55 mg/ml may be regarded as having undergone proteolysis. None of the thermised milks in the present study exceeded this value. However, Juffs, (1973) reported large variation in TV between the raw samples studied.

Hsu and Shipe (1986) studied the effects of some physical treatments on proteolysis in milk. They reported that proteolysis can be increased by physical treatments such as agitation, ultrasonic treatments, freezing and thawing. These physical treatments increase the concentration of available proteolytic enzymes.

In general, the period of storage would elapse before any increase in TV was detected. This might be a direct consequence of variation in initial counts and the types of the microflora present. Variation in TV especially in milk with counts of less than 10^4 cfu/ml, were due perhaps to different levels of native protease and NPN (Reimerdes *et al.*, 1979 and Humbert *et al.*, 1985).

Gebre-Egziabher *et al.* (1985) reported that a more

consistent relationship between TV and PBC was found when the count exceeded 10^6 cfu/ml. This was similar to the result obtained by Suhren *et al*. (1982) who found that count in excess of 10^6 cfu/ml were needed before lower raw milk quality was detected, by means of the pyruvate test.

Juffs, (1973) studied the relationship between bacterial population counts and TV, both in cold-stored raw and pasteurised milks. He observed that the bacterial counts (TPC, PBC ond PPBC) in raw milk generally exceeded 10^6 cfu/ml before a definite increased in TV could be detected. However, no conclusive results were obtained when pasteurised milk was used.

Conclusions

1. Thermisation heat treatment of milk at 65 ^{o}C for 16 seconds completely eliminated the coliform bacteria. In addition, it is effective in reducing the initial mesophilic and psychrotrophic bacteria in milk.

2. The effectiveness of thermisation in reducing the bacterial counts is directly related to the initial counts. The higher the bacterial counts the greater is the percentage reduction due to thermisation. In addition, based on counts immediately after thermisation, the bacterial quality of the thermised milk in the present study appeared not to be affected by the length of the raw milk cold storage.

3. The TPC of the thermised milk in the present study, remained less than 10^4 cfu/ml after 3 days of storage at 6^{o}C when the milk was thermised fresh, 2 and 4 days of cold storage. Therefore, in addition to length of raw milk cold storage, the milk used in the present study was 7 days old post- miking without any significant increase in TPC.

4. The effectiveness of thermisation become more obvious when prolonged storage times were employed. The differences between the mean bacterial counts following thermisation increased with the length of the raw milk storage time. This effect cannot be easily explained because milk, as used in these experiments, contained many different types of microorganisms of unknown

physiological characteristics.

5. Thermisation heat treatment greatly inhibited subsequent lipolysis and proteolysis of milk during the storage period. In the present study, the mean FFA and TV of raw unheated milk through storage at 5 ^{o}C were higher than that of the thermised milk.

SECTION 5.3 STUDIES ON THE EFFECTS OF DOUBLE HEAT TREATMENTS ON MILK QUALITY.

Materials and methods

The milk used in the present experiment was also used in the earlier trial (section 4.2). A 2 x 3 x 5 factorial experiment was performed following each trial of the main (for dates see chapter 3). After 2 and 4 days of raw milk storage at 5 ^{o}C, 150 litres were drawn and thermised at 65 ^{o}C for 16 seconds as previously described (section 2.1). The thermised milks were stored in steam sterilised 50 litres milk cans and pasteurised (section 2.1) following 2, 4 and 7 days of storage after thermisation at 6 ^{o}C. Pasteurised milk samples were taken in 200 ml sterile sample bottles and analysed 1, 3, 7, 15 and 21 days after storage at 5 ^{o}C for TPC, PBC, TBC, PLBC, TA, FFA and TV using the procedures as described in sections 2.3.1, 2.3.2, 2.3.3, 2.3.2, 2.2.12, 2.2.17.1 and 2.2.20, respectively.

All data were subjected to the analysis of variance as described in section 2.4, using trial, storage time after pasteurisation (Day), the raw storage time before pasteurisation (CS2TCS4T) and the length of storage after thermisation as the main effects.

Results

The response of all the storage treatments to all the parameters measured varied highly significantly between trials ($p < 0.001$) with the exception of TV which was not as significant ($p < 0.05$) and TBC which was completely non significant.

The TPC of milks pasteurised after the storage treatments are shown in table 5.3.1. The TPC of unpasteurised thermised milk and the pasteurised thermised milks increase significantly ($p < 0.001$) during storage. The increases in TPC were significantly different ($p < 0.001$) when the unpasteurised thermised milks (thermised on the day of delivery) were compared to the milks pasteurised after 2, 4 and 7 days of themisation and storage at 6 ^{o}C.

The differences in TPC between milks pasteurised after 2, 4 and 7 days of thermisation were also significant ($p < 0.05$) when they were compared with each other. The TPC of the pasteurised milks increased when prepared from milks with longer storage after thermisation. The differences between pasteurised milks prepared from milks thermised after 2 and 4 days of raw cold storage were not significant ($p > 0.05$).

The PBC of milks pasteurised after the storage treatments are shown in table 5.3.2. The PBC of unpasteurised thermised milk and the pasteurised thermised

milks increased significantly during storage. The increases in the PBC were very highly significant ($p < 0.001$) when the unpasteurised thermised milks were compared to the milks pasteurised after 2, 4 and 7 days of thermisation and storage at 6 ^{o}C.

The differences in PBC between milks pasteurised after 2, 4 and 7 days of thermisation and storage at 6 ^{o}C were not significant ($p > 0.05$) when they were compared with each other. The difference between pasteurised milks prepared from milks thermised after 2 and 4 days of raw cold storage were also not significant ($p > 0.05$).

Significant CS2TCS4T.Storage interaction ($p < 0.001$) was observed. The increase in PBC through storage after pasteurisation of the pasteurised milks prepared from milks thermised after the extended raw cold storage was very highly variable

The TBC of milks pasteurised after the storage treatments are shown in table 5.3.3. The TBC of unpasteurised thermised milk and the pasteurised thermised milks increased significantly ($p < 0.01$) during storage. However, the increases were not of practical importance because the levels of the TBC in all the pasteurised milks were approximately less than 2 log units.

The PLBC of milks pasteurised after the storage treatments are shown in table 5.3.4. The response of PLBC to all the storage treatments followed approximately the similar pattern as that of the PBC. However, mean differences in PLBC between milk pasteurised after 2, 4

Table 5.3.1

Mean[a] total plate counts (TPC) (log_{10} cfu/ml) of milks pasteurised (73 ^{o}C for 16 seconds) after 2, 4 and 7 days of thermisation (65 ^{o}C/16 seconds) and storage (6 ^{o}C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 ^{o}C).

Storage		Pasteurised days after thermisation							
		2		4		7		Means[b]	
(days)	CST[c]	CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	3.40	3.05	2.74	2.73	3.04	3.08	2.43	2.95	2.74
3	3.85	3.16	3.24	3.09	3.32	3.23	2.79	3.16	3.12
7	4.96	3.76	3.78	3.86	3.64	3.50	3.05	3.71	3.49
15	7.87	4.21	4.37	4.23	4.34	4.48	3.57	4.31	4.09
21	9.74	4.45	5.28	4.68	5.36	3.96	3.62	4.37	4.75
Means	5.97	3.73	3.88	3.72	3.94	3.65	3.09	3.70	3.64

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 ^{o}C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.17	0.22	0.20	0.24	0.15	0.19

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.34	0.26	0.59

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	27.44	52.56***
Day	4	20.89	40.01***
Therm[a]	1	67.80	129.85***
Day.Therm	4	10.69	20.47***
CS2TCS4T[b]	1	0.08	0.16
Storage[c]	2	2.00	3.83*
Day.CS2TCS4T	4	0.30	0.58
Day.Storage	8	0.35	0.67
CS2TCS4T.Storage	2	1.39	2.67*
Day.CS2TCS4T.Storage	8	0.11	0.21
Trial.Day	8	0.97	1.85
Trial.CS2TCS4T	4	0.93	1.78
Mean square error	56	0.52	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, * significant $p < 0.05$, *** significant $p < 0.001$

Table 5.3.2

Mean[a] psychrotrophic bacterial counts (PBC) (log_{10} cfu/ml) of milks pasteurised (73 °C for 16 seconds) after 2, 4 and 7 days of thermisation (65 °C/16 seconds) and storage (6 °C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 °C).

Storage (days)	CST[c]	Pasteurised days after thermisation						Means[b]	
		2		4		7			
		CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	1.80	0.00	0.00	0.73	0.00	1.03	0.00	0.59	0.00
3	2.58	2.09	1.16	1.51	2.12	3.83	1.59	2.47	1.62
7	3.04	1.14	2.08	1.51	1.80	2.56	0.33	1.74	1.41
15	7.35	0.67	1.87	0.73	1.33	2.02	0.00	1.14	1.07
21	10.17	0.83	3.07	2.15	5.51	5.12	1.88	2.70	3.49
Means	4.99	0.94	1.64	1.33	2.15	2.91	0.76	1.73	1.52

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 °C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.33	0.43	0.39	0.47	0.29	0.36

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.66	0.51	1.14

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	25.08	12.85***
Day	4	34.94	17.91***
Therm[a]	1	145.84	74.74***
Day.Therm	4	23.90	12.25***
CS2TCS4T[b]	1	1.00	0.51
Storage[c]	2	2.54	1.30
Day.CS2TCS4T	4	1.79	0.92
Day.Storage	8	1.54	0.79
CS2TCS4T.Storage	2	21.17	10.85***
Day.CS2TCS4T.Storage	8	2.40	1.23
Trial.Day	8	2.05	1.05
Trial.CS2TCS4T	4	4.70	2.41
Mean square error	56	1.95	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, *** significant $p < 0.001$

Table 5.3.3

Mean[a] thermoduric bacterial counts (TBC) (log_{10} cfu/ml) of milks pasteurised (73 ^{o}C for 16 seconds) after 2, 4 and 7 days of thermisation (65 ^{o}C/16 seconds) and storage (6 ^{o}C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 ^{o}C).

Storage (days)	CST[c]	Pasteurised days after thermisation						Means[b]	
		2		4		7			
		CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	1.60	1.43	1.60	1.72	1.67	1.77	1.20	1.64	1.49
3	2.24	1.96	1.47	1.63	1.60	1.23	0.67	1.61	1.24
7	2.16	1.57	1.77	1.60	2.03	2.28	1.07	1.81	1.62
15	3.23	1.90	1.95	1.73	2.23	2.43	2.35	2.02	2.18
21	4.43	1.27	1.93	1.70	1.68	1.95	1.52	1.64	1.71
Means	2.73	1.63	1.74	1.68	1.84	1.93	1.36	1.74	1.65

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 ^{o}C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.16	0.21	0.19	0.23	0.14	0.17

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.32	0.24	0.55

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	0.71	1.55
Day	4	2.08	4.55**
Therm[a]	1	13.76	30.00***
Day.Therm	4	2.80	6.11***
CS2TCS4T[b]	1	0.20	0.45
Storage[c]	2	0.10	0.21
Day.CS2TCS4T	4	0.20	0.43
Day.Storage	8	0.37	0.80
CS2TCS4T.Storage	2	1.28	2.80
Day.CS2TCS4T.Storage	8	0.21	0.46
Trial.Day	8	1.45	3.17**
Trial.CS2TCS4T	4	0.39	0.85
Mean square error	56	0.46	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, ** significant $p < 0.01$, *** significant $p < 0.001$

Table 5.3.4

Mean[a] psychrotrophic lipolytic bacterial counts (PLBC) (log_{10} cfu/ml) of milks pasteurised (73 ^{o}C for 16 seconds) after 2, 4 and 7 days of thermisation (65 ^{o}C/16 seconds) and storage (6 ^{o}C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 ^{o}C).

Storage (days)	CST[c]	Pasteurised days after thermisation						Means[b]	
		2		4		7			
		CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	1.07	0.00	0.59	0.00	0.33	0.00	0.00	0.00	0.31
3	1.93	0.93	0.77	1.50	1.06	1.27	0.87	1.23	0.90
7	2.40	0.73	1.43	1.40	1.82	0.82	0.00	0.98	1.08
15	5.36	0.73	1.73	1.13	1.67	0.83	0.67	0.90	1.36
21	7.03	1.50	2.80	2.97	1.84	0.64	1.20	1.28	1.95
Means	3.57	0.78	1.47	1.40	1.34	0.46	0.55	0.89	1.12

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 ^{o}C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.21	0.28	0.25	0.30	0.19	0.23

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.42	0.33	0.73

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	30.60	37.85***
Day	4	12.56	15.54***
Therm[a]	1	84.14	104.06***
Day.Therm	4	11.56	14.30***
CS2TCS4T[b]	1	1.30	1.61
Storage[c]	2	6.03	7.46***
Day.CS2TCS4T	4	0.66	0.81
Day.Storage	8	1.32	1.64
CS2TCS4T.Storage	2	1.16	1.44
Day.CS2TCS4T.Storage	8	1.06	1.31
Trial.Day	8	2.36	2.91**
Trial.CS2TCS4T	4	5.48	6.78**
Mean square error	56	3.71	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, ** significant $p < 0.01$, *** significant $p < 0.001$

and 7 days of thermised storage was highly significant ($p < 0.001$). The PLBC of milk pasteurised 7 days after thermisation was lower than milk pasteurised after 2 or 4 day of thermisation.

The percentage lactic acid content of milks pasteurised after the storage treatments (expressed as TA) are shown in table 5.3.5. The TA of unpasteurised thermised milk and the pasteurised thermised milks increase highly significantly during storage ($p < 0.001$). The increases in TA were significantly different ($p < 0.001$) when the unpasteurised thermised milks was compared to the milks pasteurised after 2, 4 and 7 days of thermisation and storage at 6 ^{o}C. The mean TA of the unpasteurised thermised milks were significantly higher ($p < 0.01$) than that of pasteurised thermised milk.

The differences in TA between milks pasteurised after 2, 4 and 7 days of thermisation were slightly significant ($p < 0.05$) when they were compared with each other. The differences between pasteurised milks prepared from milks thermised after 2 and 4 days of raw cold storage were not significant ($p > 0.05$).

Significant Day.Therm interaction ($p < 0.05$) was observed.

The FFA content of milks pasteurised after the storage treatments are shown in table 5.3.6. The FFA of the unpasteurised thermised milk and all the pasteurised thermised milks increase significantly during storage ($p < 0.001$). The increases in FFA were significantly different when the unpasteurised thermised milks were compared to

the milks pasteurised after 2, 4 and 7 days of thermisation and storage at 6 ^{o}C.

The differences in FFA content between milks pasteurised after 2, 4 and 7 days of thermisation were also significant ($p < 0.001$) when they were compared with each other. The differences incresed with prolonged storage after thermisation.

The differences in FFA content between pasteurised milks prepared from milks thermised after 2 and 4 days of raw cold storage were highly significant ($p < 0.001$). The FFA content of pasteurised milks prepared from milks thermised after 2 days of raw cold storage was lower than that prepared from milks thermised after 4 days of raw cold storage.

Significant CS2TCS4T.Storage and Day.Therm interactions were observed.

The TV of milks pasteurised after the storage treatments are shown in table 5.3.7. The TV of unpasteurised thermised and the pasteurised milks increase significantly during storage. The increases in TV were not significant ($p < 0.05$) when the unpasteurised thermised milks were compared to the milks pasteurised after 2, 4 and 7 days after of thermisation and storage at 6 ^{o}C. However, the differences in TV between milks pasteurised after thermisation were significant ($p < 0.001$). The TV of the pasteurised milks increase with prolonged thermised storage period.

Table 5.3.5

Mean[a] titratable acidity (TA) (as percentage lactic acid) of milks pasteurised (73 °C for 16 seconds) after 2, 4 and 7 days of thermisation (65 °C/16 seconds) and storage (6 °C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 °C).

Storage		Pasteurised days after thermisation							
		2		4		7		Means[b]	
(days)	CST[c]	CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	0.140	0.137	0.137	0.127	0.143	0.140	0.150	0.134	0.143
3	0.140	0.133	0.127	0.137	0.143	0.143	0.147	0.137	0.139
7	0.167	0.127	0.140	0.134	0.143	0.143	0.147	0.139	0.143
15	0.183	0.153	0.150	0.150	0.147	0.167	0.140	0.157	0.146
21	0.183	0.170	0.157	0.167	0.162	0.161	0.154	0.166	0.158
Means	0.163	0.144	0.142	0.143	0.145	0.152	0.150	0.147	0.146

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 °C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.003	0.004	0.004	0.004	0.003	0.003

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.006	0.005	0.011

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	0.00114	6.54***
Day	4	0.00272	15.63***
Therm[a]	1	0.00347	19.98***
Day.Therm	4	0.00052	3.00*
CS2TCS4T[b]	1	0.00002	0.13
Storage[c]	2	0.00063	3.63*
Day.CS2TCS4T	4	0.00032	1.87
Day.Storage	8	0.00028	1.58
CS2TCS4T.Storage	2	0.00004	0.25
Day.CS2TCS4T.Storage	8	0.00015	0.85
Trial.Day	8	0.00048	2.75*
Trial.CS2TCS4T	4	0.00045	2.56*
Mean square error	56	0.00017	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, * significant $p < 0.05$, *** significant $p < 0.001$

Table 5.3.6

Mean[a] free fatty acids (FFA) (mEq/100 ml) of milks pasteurised (73 °C for 16 seconds) after 2, 4 and 7 days of thermisation (65 °C/16 seconds) and storage (6 °C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 °C).

Storage (days)	CST[c]	Pasteurised days after thermisation						Means[b]	
		2		4		7			
		CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	0.56	0.46	0.38	0.34	0.51	0.52	0.58	0.44	0.49
3	0.58	0.40	0.41	0.43	0.49	0.54	0.59	0.45	0.49
7	0.68	0.42	0.44	0.56	0.64	0.53	0.74	0.59	0.61
15	0.76	0.60	0.54	0.51	0.58	0.59	0.63	0.56	0.58
21	0.87	0.66	0.67	0.54	0.81	0.68	0.86	0.63	0.78
Means	0.69	0.51	0.49	0.48	0.61	0.57	0.68	0.52	0.59

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 °C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.03	0.03	0.03	0.04	0.02	0.03

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.05	0.04	0.09

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	29.86	25.48***
Day	4	22.14	18.89***
Therm[a]	1	48.87	41.69***
Day.Therm	4	4.60	3.92**
CS2TCS4T[b]	1	11.83	10.10***
Storage[c]	2	12.58	10.73***
Day.CS2TCS4T	4	1.34	1.14
Day.Storage	8	1.14	0.97
CS2TCS4T.Storage	2	4.83	4.12*
Day.CS2TCS4T.Storage	8	0.58	0.50
Trial.Day	8	6.57	5.61***
Trial.CS2TCS4T	4	4.36	3.72**
Mean square error	56	1.17	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$

Table 5.3.7

Mean[a] tyrosine value (TV) (mg/ml) of milks pasteurised (73 °C for 16 seconds) after 2, 4 and 7 days of thermisation (65 °C/16 seconds) and storage (6 °C). The thermised milks were prepared fresh[c] and after 2 and 4 days of cold storage (5 °C).

Storage		Pasteurised days after thermisation							
		2		4		7		Means[b]	
days	CST[c]	CS2T[d]	CS4T[e]	CS2T	CS4T	CS2T	CS4T	CS2T	CS4T
1	0.077	0.053	0.081	0.053	0.095	0.076	0.084	0.061	0.087
3	0.077	0.055	0.083	0.064	0.090	0.081	0.081	0.066	0.084
7	0.088	0.064	0.085	0.075	0.101	0.089	0.091	0.076	0.092
15	0.100	0.083	0.098	0.072	0.112	0.109	0.102	0.088	0.104
21	0.124	0.090	0.109	0.101	0.098	0.098	0.101	0.096	0.103
Means	0.093	0.069	0.091	0.073	0.099	0.090	0.092	0.077	0.094

Note: (a) Mean of 3 trials, (b) mean after pasteurised storage by storage treatments, (c) initial thermisation with no storage, (d) thermised 2 days and (e) 4 days after storage at 5 °C

SED of means:

Table	Trial	Day	Therm	Day Thermised	CS2TCS4T	Storage
SED	0.003	0.004	0.004	0.005	0.003	0.004

Table	Day CS2TCS4T	Storage CS2TCS4T	Storage.Day CS2TCS4T
SED	0.007	0.005	0.012

(...contd)

(Contd...)

Analysis of variance

Source of variation	df	MS	F
Trial	2	0.00096	4.76*
Day	4	0.00340	16.93***
Therm[a]	1	0.00071	3.54
Day.Therm	4	0.00024	1.18
CS2TCS4T[b]	1	0.00620	30.84***
Storage[c]	2	0.00095	4.71*
Day.CS2TCS4T	4	0.00022	1.09
Day.Storage	8	0.00009	0.43
CS2TCS4T.Storage	2	0.00130	6.48**
Day.CS2TCS4T.Storage	8	0.00018	0.89
Trial.Day	8	0.00020	1.02
Trial.CS2TCS4T	4	0.00273	13.56***
Mean square error	56	0.00020	

Note: (a) = CST vs CS2TCS4T, (b) = CS2T vs CS4T, (c) = thermised storage, * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$

The TV of pasteurised milks prepared from milk thermised after 2 days of raw cold storage was significantly lower ($p < 0.001$) than that prepared from milks thermised after 4 days of raw cold storage.

Discussion

The results of bacterilogical analysis of pasteurised milks in the present study indicated that the extended raw milk cold storage prior to thermisation and pasteurisation, has no effects on the bacterial counts of the corresponding pasteurised milk.

But, the extended storage following thermisation did have some influence on mesophilic bacteria but not on psychrotrophic bacteria. The psychrotrophic bacteria was eradicated by pasteurisation regardless of the storage treatments before pasteurisation.

The mesophilic bacteria increased significantly after 3 days of unpasteurised thermised milk storage. The reason for the increase in the microbial numbers due to the microorganisms entering the logarithmic phase of growth was not obvious. But it was possible that differences in the species of the microflora present might have been responsible (Cousin, 1982). The unpasteurised thermised milk was thermised at the start of the experiments and was not be more than 12 hours old. Thus, the duration of storage and the storage conditions should not have been responsible for the increase in counts.

Karlikanova, (1977) heat treated milk at 65 ^{o}C for 10-

20 seconds, held the resultant milk at 5 ^{o}C for 24 hours and heat treated the milk again at 72 ^{o}C for 15-20 seconds. It was observed that the double heat treatment was best for maintaining the initial physicochemical and processing quality of milk while improving quality and enabling storage at 5 ^{o}C for up to 72 hours.

Meislahn, (1985) thermised milk at 58 ^{o}C for 20 seconds followed by pasteurisation. He reported that reported thermisation has little effect on the quality of pasteurised milk during the subsequent storage at 10 ^{o}C.

Gilmour *et al.* (1981), carried out a trial to study the effects of double heat treatments on milk quality, using a laboratory scale pasteuriser. Milks were thermised by various time temperature combinations including 65 ^{o}C for 15 seconds before pasteurisation. They reported that thermisation alone was effective in reducing all types of microorganisms compared to an unthermised control. When pasteurisation followed thermisation, there were no significant differences in bacterial count when compared to those of milks which were obtained pasteurisation alone. They concluded that there are no benefits to be gained in terms of bacterial numbers obtained since the pasteurised unthermised and pasteurised thermised milks gave broadly similar counts.

The observation was supported by an earlier studies (Bjorgum *et al.*, 1978) that pasteurisation alone was capable of reducing microbial numbers to a common level regardless of the initial count (as gained by

thermisation).

Mikolajcik (1979) contrasted these suggestions and reported that there are benefits to be gained when milk was thermised before pasteurisation. They reported that in terms of microbial numbers higher counts were obtained in unthermised pasteurised milk than in thermised pasteurised milk especially after longer storage times after pasteurisation. In addition, he reported that it was unlikely that the heat applied during thermisation would be sufficient to activate the spores of mesophilic organisms and allowing the resulting vegetative cells to be killed by the subsequent pasteurisation procedure.

Collins (1981) indicated that the period of heating required to activate spores depends on the temperature and strain of organism present. But in general, a considerable number of minutes rather than seconds would be required when heating at thermisation or pasteurisation temperatures. However, it would have been expected that thermisation would be lethal to cells which, although not bearing spores at the time of treatment, had the potential to do so. The effects would have been to reduce the number of spores occurring in the thermised milks both before and after pasteurisation.

Conclusions

1. The interval of raw milk cold storage and the length of the storage period after thermisation and before pasteurisation have no significant impact on the the

bacterial counts of the milks during subsequent storage after pasteurisation.

2. In terms of bacterial counts, thermised milks in the present study could be stored up to 7 days in addition to the 4 days raw milk cold storage prior to thermisation without any significant deterioration in quality, However, in terms of FFA concentration unpasteurised thermised milk will begin to deteriorate from the 15^{th} day of storage onwards.

3. In terms of bacterial counts, the pasteurised thermised milks in the present study remained *acceptable* (count of less than 10^6 cfu/ml) up to the 21^{st} day of storage when the pasteurised milks were prepared from milks stored at 6 ^{o}C, after 2 and 4 days of thermisation.

4. Pasteurisation is the basic heat treatment required in the manufacture of various dairy products for safety and quality reasons. Thermisation prior to pasteurisation will not only increase the safety and quality of the products, but in addition, will enhance the manufacturing schedules since the thermised milks remained in *acceptable* quality for longer periods.

CHAPTER 6

THE EFFECTS OF RAW MILK STORAGE AT 2 °C AND 5 °C, AND STORAGE AT 6°C AFTER THERMISATION ON SELECTED MILK PROPERTIES

Introduction

It has been found that moderate heating, such as pasteurisation could cause a small change in pH (Webb and Johnson (1965). When milk is heated other changes also occur, such as the ratio of colloidal calcium to ionic calcium. The movement of ions is mainly from calcium citrate and calcium phosphate to colloidal calcium located in the casein micelle. This can affect the sensitivity of the stability of casein and other protein to heat. Milk with altered protein stability may have an effect on certain manufacturing processes such as in cheese manufacture when calcium chloride often has to be added to pasteurised milk to make up for loss of calcium due to the heat treatment (Webb and Johnson, 1965).

Heat treatment of milk can cause combination of casein and whey proteins and decrease the amount of ionic calcium in milk serum (Morr, 1975). Cold storage causes Calcium and casein components to migrate from the micelles to the serum (Ali *et al.* 1980). These changes lead in turns to alteration in the coagulation and the syneresis of gels prepared from such milk.

The purpose of this study was to investigate the

effects of raw milk storage at 2 ^{o}C and 5 ^{o}C, and storage at 6 ^{o}C after thermisation (i) on the changes of calcium and phosphorous bound to the casein micelle, (ii) the changes on free sulphydryl and disulphide groups and (iii) on the changes in distribution of N partitions in milk.

Material and method

Milks analysed for the studies were milk samples obtained from the earlier trials (section 4.1 and section 5.1). The samples from fresh blended milk were treated as the initial samples. Milk samples were drawn from raw milks stored at 2 (Deep Cooling, DC) and 5 ^{o}C (Cold Storage, CS) after 1, 3, 7 and 9 days of storage (section 4.1), fresh thermised (Thermised on arrival, TO) and fresh pasteurised (Pasteurised on arrival, PO) milks (section 5.2) after 1, 3, 7 and 9 days of storage at 6 ^{o}C and 5 ^{o}C, respectively. The samples were deep frozen to - 20 ^{o}C and thawed to 20 ^{o}C immediately before analysis.

Samples were analysed for total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) as described in sections 2.2.3, 2.2.4 and 2.2.5, respectively. Total free sulphydryl (SH) and disulphide (SS) groups were analysed using the method as described in section 2.2.16; and total calcium, phosphorous and their soluble forms were analysed using the method as described in sections 2.2.10 and 2.2.11, respectively.

Milk ultrafiltrates for the analysis of soluble calcium and phosphorous were prepared *fresh*, that is, the samples

were prepared on the day of sampling, as described section 2.1.1.

Data were subjected to the analysis of variance as described in section 2.4, using trial, treatments and storage times as the main effect.

Results

The total protein nitrogen (PN), non-casein nitrogen (NCN) and the non-protein nitrogen (NPN) contents of the milk studied are given in tables 6.1, 6.2 and 6.3, respectively. The results of the present study for all the above constituents of the milk tested were comparable to an earlier studies (Al-Darwash, 1982).

The differences in PN, NCN and NPN between trials in all the experimental treatments were very highly significant ($p < 0.001$).

The PN of milks for all the treatments are given in table 6.1. The mean PN of the milks decreased very highly significantly ($p < 0.001$) during storage. The decreased was significant ($p < 0.05$) in all treatments after 3 days of storage. The decrease in PN was greater in pasteurised milk than in thermised or raw milks stored at 5 ^{o}C and 2 ^{o}C, respectively.

The interaction Trial.Treatment was significant ($p < 0.05$)

The NCN content of milk increased significantly for all treatments during storage ($p < 0.01$) (Table 6.2). The

increase was significant 3 days following storage for all milks except the NCN of the raw milk stored at 2 ^{o}C which was detected 7 days after storage. The mean NCN content of raw milk stored at 5 ^{o}C was higher than that of milks which had been stored at 2 ^{o}C, thermised or pasteurised.

No significant interactions ($p > 0.05$) were observed

The means of NPN content of milk in all the treatments are given in table 6.3. The NPN content of all the milks increase significantly ($p < 0.01$) during storage. The increase in the NPN content of the raw milk stored at 5 ^{o}C was significantly higher ($p < 0.01$) than the raw milk stored at 2 ^{o}C or thermised and pasteurised milks, respectively. Similar results were obtained when the means of raw milk stored at 2 ^{o}C were compared to both thermised and pasteurised milks. However, no significant difference ($p > 0.05$) was observed when the thermised milk and the pasteurised milk was compared to each other.

The increase in NPN was significant ($p < 0.01$) after 1, 3, 7, and 9 days following storage of raw milk at 5 ^{o}C and at 2 ^{o}C and after thermisation and pasteurisation, respectively.

The interaction Trial.Storage was significant ($p < 0.01$) and the interaction Storage.Treatment was highly significant ($p < 0.001$).

Tables 6.4 and 6.5 illustrate the changes that occurred during storage in free sulfhydryl (SH) and disulfide (SS) groups of the milk, respectively and table 6.6 described the total SH (SH + reduced SS) during storage.

Very highly significant ($p < 0.001$) increases were

found in the SS and SH content of milk during storage for all the treatments. The increase in SS and SH groups was significantly higher ($p < 0.01$) in raw milk stored at 5 ^{o}C than the raw milk stored at 2 ^{o}C, or in the thermised and pasteurised milks, respectively. A similar observation was made when the SH value for the raw milk stored at 2 ^{o}C was compared to the thermised and the pasteurised milks. However, no significant difference ($p > 0.05$) was observed when SH value of thermised and pasteurised milk was compared with each other. In addition, no significant difference ($p > 0.05$) was observed when the SS value of the raw milk stored at 2 ^{o}C was compared to the thermised and the pasteurised milks, and when the thermised and the pasteurised milks were compared with each other.

The changes in the SH group were significant ($p < 0.01$) from the 1st day of storage in all treatments. The changes in SS group were significant ($p < 0.01$) 3 days after storage for the raw milks stored at 5 ^{o}C and 2 ^{o}C, and after 1 day of storage for thermised and the pasteurised milks. The difference between the effects of storage times for both SS and SH groups increased significantly ($p < 0.01$) with time in all the treatments.

The interactions Trial.Storage and Storage.Treatment for both SH and the SS groups were significant ($p < 0.05$ and $p < 0.001$, respectively). In addition, the interaction Trial.Treatment for SH group was also significant ($p < 0.05$).

Table 6.1

Total protein nitrogen content (PN) (per cent) of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	3.00	3.00	3.00	3.00	3.10	3.10	3.10	3.10	2.95	2.95	2.95	2.95	3.02	3.02	3.02	3.02
1	2.96	2.99	3.10	2.99	3.29	2.99	2.95	2.96	2.94	2.96	2.92	2.95	3.06	2.98	2.99	2.97
3	2.95	2.92	2.96	2.99	3.10	2.86	2.93	2.94	2.79	2.85	2.91	2.93	2.95	2.88	2.93	2.93
7	2.84	2.85	2.96	2.96	2.86	2.80	2.82	2.90	2.72	2.82	2.85	2.94	2.81	2.82	2.88	2.93
9	2.82	2.86	2.97	2.96	2.86	2.82	2.82	2.86	2.69	2.79	2.82	2.92	2.79	2.82	2.86	2.91
$\bar{x}$	2.91	2.92	3.00	2.98	3.04	2.91	2.92	2.95	2.82	2.87	2.89	2.94	2.92[b]	2.90	2.94	2.96

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.02	0.02	0.02	0.03	0.04	0.04

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	0.076	0.038	17.8***
Storage	4	0.289	0.072	33.9***
Treatment	3	0.022	0.007	3.4*
Trial.Storage	8	0.044	0.006	2.6
Trial.Treatment	6	0.092	0.015	7.3**
Storage.Treatment	12	0.061	0.005	2.4
Mean square error	24	0.051		
Total	59	0.037		

Statistical significance:
* = p < 0.05
** = p < 0.01
*** = p < 0.001

Table 6.2

Total non-casein nitrogen content (NCN) (per cent) of blended milk tested fresh[a] and after raw storage at 2 °C (DC), 5 °C (CS), thermisation (65 °C/16 seconds) and storage at 6 °C (TO) and pasteurisation (73 °C/16 seconds) and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	0.40	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
1	0.41	0.40	0.41	0.40	0.40	0.42	0.41	0.40	0.40	0.41	0.40	0.41	0.40	0.41	0.41	0.40
3	0.43	0.41	0.41	0.42	0.41	0.40	0.42	0.41	0.41	0.40	0.41	0.41	0.42	0.40	0.41	0.41
7	0.45	0.42	0.40	0.43	0.46	0.44	0.44	0.41	0.42	0.40	0.41	0.40	0.44	0.42	0.42	0.41
9	0.47	0.43	0.41	0.40	0.48	0.45	0.44	0.42	0.42	0.41	0.40	0.42	0.46	0.43	0.42	0.42
$\bar{x}$	0.42	0.42	0.41	0.41	0.43	0.42	0.42	0.41	0.41	0.40	0.40	0.41	0.42[b]	0.41	0.41	0.41

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.003	0.004	0.004	0.01	0.01	0.01

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	0.0024	0.0012	11.9***
Storage	4	0.0063	0.0016	15.6***
Treatment	3	0.0020	0.0007	6.6**
Trial.Storage	8	0.0020	0.0003	2.5
Trial.Treatment	6	0.0014	0.0002	2.4
Storage.Treatment	12	0.0032	0.0003	2.7
Mean square error	24	0.0024	0.0001	
Total	59	0.0199	0.0003	

Statistical significance: ** = $p < 0.01$
*** = $p < 0.001$

Table 6.3

Total non-protein nitrogen content (NPN) (per cent) of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
1	0.22	0.21	0.22	0.22	0.22	0.21	0.20	0.20	0.22	0.20	0.21	0.21	0.22	0.21	0.21	0.21
3	0.23	0.23	0.21	0.23	0.26	0.23	0.21	0.22	0.23	0.22	0.22	0.22	0.24	0.23	0.21	0.22
7	0.26	0.26	0.23	0.22	0.28	0.24	0.22	0.21	0.26	0.24	0.21	0.21	0.27	0.25	0.22	0.21
9	0.28	0.25	0.24	0.22	0.31	0.26	0.23	0.21	0.28	0.26	0.22	0.23	0.29	0.26	0.23	0.22
$\bar{x}$	0.24	0.24	0.22	0.22	0.26	0.23	0.21	0.21	0.24	0.23	0.21	0.22	0.25[b]	0.23	0.22	0.22

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.002	0.003	0.003	0.01	0.01	0.01

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	0.0005	0.0002	5.1*
Storage	4	0.0125	0.0031	65.4***
Treatment	3	0.0088	0.0029	61.3***
Trial.Storage	8	0.0005	0.0001	1.2
Trial.Treatment	6	0.0012	0.0002	4.2*
Storage.Treatment	12	0.0070	0.0006	12.2***
Mean square error	24	0.0012	0.0001	
Total	59	0.0317	0.0005	

Statistical significance: * = $p < 0.05$
*** = $p < 0.001$

Table 6.4

Total free sulphydryl group (umSH/g) of blended milk tested fresh[a] and after storage at 2 ^{o}C (DC), 5 ^{o}C (CS), thermisation and storage at 6 ^{o}C (TO), and pasteurisation and storage at 4 ^{o}C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	3.36	3.36	3.36	3.36	4.20	4.20	4.20	4.20	3.80	3.80	3.80	3.80	3.79	3.79	3.79	3.79
1	3.42	3.41	3.51	3.44	4.26	4.28	4.21	4.20	3.86	3.82	3.80	3.84	3.85	3.84	3.84	3.83
3	4.42	3.90	3.65	3.60	4.29	4.28	4.23	4.31	3.92	3.88	3.92	3.86	4.21	4.02	3.93	3.92
7	5.20	4.45	4.20	4.50	5.10	5.36	4.43	4.40	3.96	3.92	3.96	3.91	4.75	4.58	4.20	4.27
9	6.94	5.90	5.20	5.10	5.20	5.20	4.48	4.42	4.10	3.96	3.95	3.94	5.41	5.02	4.54	4.49
$\bar{x}$	4.67	4.20	3.98	4.00	4.61	4.66	4.31	4.31	3.93	3.88	3.89	3.87	4.40[b]	4.25	4.06	4.06

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.001	0.01	0.01	0.15	0.14	0.17

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	3.408	1.704	37.2***
Storage	4	10.070	2.517	54.9***
Treatment	3	1.237	0.412	9.0***
Trial.Storage	8	7.807	0.976	21.3***
Trial.Treatment	6	0.847	0.141	3.1*
Storage.Treatment	12	1.251	0.104	2.3*
Mean square error	24	1.101	0.046	
Total	59	25.719	0.436	

Statistical significance: * = $p < 0.05$
*** = $p < 0.001$

Table 6.5

The disulphide groups (uM SS/g) content of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	28.25	28.25	28.25	28.25	26.50	26.50	26.50	26.50	27.65	27.65	27.65	27.65	27.47	27.47	27.47	27.47
1	28.54	29.62	30.30	32.20	26.60	26.70	27.00	27.10	26.97	27.67	28.10	27.65	27.37	28.00	28.47	28.98
3	32.62	30.20	32.45	32.00	27.85	27.10	27.10	28.15	28.96	28.15	29.20	28.10	29.81	28.48	29.55	29.42
7	38.75	34.25	34.40	36.45	29.10	28.20	27.20	27.16	29.96	29.20	28.10	29.10	32.60	30.75	29.90	30.90
9	40.26	36.60	35.00	35.46	29.55	29.10	28.28	28.15	32.45	30.36	28.28	29.25	34.09	32.02	30.52	30.95
$\bar{x}$	33.68	31.78	32.08	32.87	27.92	27.64	27.22	27.41	29.20	28.61	28.25	28.35	30.27[b]	29.34	29.18	29.55

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.24	0.30	0.27	0.53	0.47	0.61

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	284.88	142.44	256.2***
Storage	4	167.01	41.75	75.1***
Treatment	3	10.34	3.45	6.2**
Trial.Storage	8	82.44	10.30	18.5***
Trial.Treatment	6	4.69	0.78	1.4
Storage.Treatment	12	31.24	2.60	4.7**
Mean square error	24	13.34	0.56	
Total	59	593.95	10.07	

Statistical significance: ** = $p < 0.01$
*** = $p < 0.001$

Table 6.6

Total sulphydryl and disulphite (uMSSH/g) content of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	31.61	31.61	31.61	31.61	30.70	30.70	30.70	30.70	31.45	31.45	31.45	31.45	31.25	31.25	31.25	31.25
1	31.96	33.03	33.81	35.65	30.86	30.98	31.21	31.30	30.83	31.49	31.90	31.49	31.22	31.83	32.31	32.81
3	37.04	34.10	36.10	35.60	32.14	31.38	31.33	32.46	32.88	32.03	33.12	31.96	34.02	32.50	33.48	33.34
7	43.95	38.70	38.60	40.95	34.20	33.56	31.63	31.56	33.92	31.12	32.06	33.10	37.36	35.33	34.20	35.17
9	47.20	42.50	40.20	40.56	34.75	34.30	32.76	32.57	36.55	34.32	32.23	33.19	39.50	37.04	35.06	35.44
$\bar{x}$	38.35	35.99	36.06	36.87	32.53	32.30	31.53	31.72	33.13	32.48	32.13	32.22	34.67[b]	33.59	33.24	33.60

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.28	0.36	0.32	0.62	0.56	0.72

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	279.98	139.99	181.5***
Storage	4	256.87	64.22	83.3***
Treatment	3	17.23	5.74	7.4***
Trial.Storage	8	131.37	16.42	21.3***
Trial.Treatment	6	7.25	1.21	1.6
Storage.Treatment	12	43.75	3.65	4.7***
Mean square error	24	18.51	0.77	
Total	59	754.97	12.80	

Statistical significance: *** = $p < 0.001$

The differences in the levels of total calcium content of milks (Table 6.7) between the treatments were not significant ($p > 0.05$). However, lower level of calcium was observed in the pasteurised milk. The changes in calcium content of milk during storage was very highly significant ($p < 0.01$).

The increase in soluble calcium content of milks during storage were very highly significant ($p < 0.001$) (Table 6.8). However, no significant increase was observed in the values for pasteurised milk. The increase in soluble calcium was significant ($p < 0.01$) after 3 of storage for the raw milks stored at 5 ^{o}C and at 2 ^{o}C and after 9 days of storage for the thermised milk.

The differences in soluble calcium content between the raw milks stored at 5 ^{o}C and 2 ^{o}C were not significant ($p > 0.05$). A similar observation was made when the thermised and the pasteurised milks were compared with each other. However, highly significant differences ($p < 0.001$) were observed when the soluble calcium content of raw milks stored at 5 ^{o}C and 2 ^{o}C were compared to those of thermised and the pasteurised milks.

The interactions Trial.Storage and Storage.Treatments were very highly significant ($p < 0.001$). These interactions explained the variations in responses between the treatments in each trial.

The phosphorous content of milks for all the treatments decreased very highly significant ($p < 0.001$)

Table 6.7

Total calcium (mg/100 ml milk) content of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	110.0	110.0	110.0	110.0	126.0	126.0	126.0	126.0	106.2	106.2	106.2	106.2	114.0	114.0	114.0	114.0
1	110.0	115.4	114.3	110.2	126.0	126.2	126.8	126.4	117.2	117.1	116.8	117.3	117.7	119.6	119.3	118.0
3	116.4	118.2	118.8	116.7	128.5	127.7	127.2	126.8	118.9	118.2	117.2	117.4	121.3	121.4	121.1	123.6
7	120.0	116.3	119.4	121.9	136.8	134.2	129.5	127.6	120.2	119.9	118.2	118.4	125.6	123.5	122.4	122.6
9	118.2	119.6	125.7	120.3	137.3	136.5	130.6	128.4	122.5	121.3	119.6	118.8	126.0	125.8	125.1	122.5
$\bar{x}$	114.9	115.9	117.6	115.8	130.7	129.9	128.2	127.0	118.9	118.5	117.6	117.6	120.9[b]	121.9	120.4	121.1

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.6	0.8	0.7	1.4	1.3	1.7

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	1834.87	917.44	221.0***
Storage	4	502.64	125.66	30.3***
Treatment	3	22.86	7.62	1.8
Trial.Storage	8	84.40	10.55	2.5*
Trial.Treatment	6	59.97	10.00	2.4
Storage.Treatment	12	35.69	2.97	0.7
Mean square error	24	99.65	4.15	
Total	59	2640.09	44.74	

Statistical significance: * = $p < 0.05$
*** = $p < 0.001$

Table 6.8

The milk ultrafiltrate soluble calcium content of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	14.24	14.24	14.24	14.24	15.50	15.50	15.50	15.50	14.45	14.45	14.45	14.45	14.73	14.73	14.73	14.73
1	13.32	13.32	12.20	13.00	16.20	16.15	16.00	15.58	15.10	14.98	14.50	14.45	14.87	14.82	14.23	14.34
3	14.20	14.65	14.20	14.10	16.39	16.40	16.10	15.92	15.20	15.10	14.67	14.60	15.26	15.38	14.99	14.87
7	14.48	14.70	13.30	13.40	17.10	16.89	16.15	16.10	15.80	15.30	14.92	14.82	15.79	15.63	14.79	14.77
9	15.20	15.85	14.15	13.25	17.15	17.10	16.79	16.10	16.00	15.60	14.95	14.91	16.12	16.18	15.30	14.75
$\bar{x}$	14.29	14.55	13.62	13.60	16.47	16.41	16.11	15.84	15.31	15.09	14.70	14.65	15.36[b]	15.35	14.81	14.70

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.08	0.10	0.09	0.17	0.15	0.20

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	48.457	24.225	425.0***
Storage	4	8.035	2.009	35.2***
Treatment	3	5.509	1.836	32.2***
Trial.Storage	8	4.505	0.563	9.9***
Trial.Treatment	6	0.744	0.124	2.2
Storage.Treatment	12	2.828	0.236	4.1***
Mean square error	24	1.368	0.057	
Total	59	71.445	1.211	

Statistical significance: *** = $p < 0.001$

Table 6.9

Total phosphorous (mg/100 g) content of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	66.54	66.54	66.54	66.54	75.62	75.62	75.62	75.62	68.75	67.75	68.75	68.75	70.30	70.30	70.30	70.30
1	64.78	65.56	65.66	66.60	74.65	75.77	74.20	74.15	67.25	66.5	67.10	66.20	68.89	69.28	68.99	68.98
3	64.20	62.25	62.32	60.25	72.25	73.16	73.36	73.46	65.66	65.96	66.94	65.10	67.37	67.12	67.54	66.27
7	62.36	63.36	63.31	61.10	71.96	72.10	73.96	73.32	64.21	65.84	65.10	65.16	66.18	67.10	67.46	66.69
9	60.25	64.10	62.56	62.20	70.27	71.19	72.15	72.76	62.96	65.25	65.10	64.75	64.49	66.85	66.60	66.57
$\bar{x}$	63.63	64.36	64.08	63.34	72.95	73.57	73.86	73.96	65.77	66.46	66.60	65.99	67.45[b]	68.13	68.18	67.76

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.27	0.35	0.31	0.60	0.54	0.69

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	1031.66	515.83	713.5***
Storage	4	143.63	35.91	49.7***
Treatment	3	5.28	1.76	2.4
Trial.Storage	8	9.31	1.16	1.6
Trial.Treatment	6	3.24	0.54	0.7
Storage.Treatment	12	11.39	0.95	1.3
Mean square error	24	17.35	0.72	
Total	59	1221.86	20.71	

Statistical significance: *** = $p < 0.001$

Table 6.10

The milk ultrafiltrate soluble phosphorous (mg/100 gm) of blended milk tested fresh[a] and after storage at 2 °C (DC), 5 °C (CS), thermisation and storage at 6 °C (TO), and pasteurisation and storage at 4 °C (PO).

Trial	1				2				3				Means[a]			
Storage (days)	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO	CS	DC	TO	PO
Fresh[a]	36.25	36.25	36.25	36.25	32.20	32.20	32.20	32.20	29.58	29.58	29.58	29.58	32.67	32.67	32.67	32.67
1	37.61	36.25	36.27	36.82	32.40	32.30	32.42	32.37	29.72	29.80	29.60	29.60	33.24	32.78	32.76	32.93
3	38.26	37.20	37.10	37.10	33.25	32.67	32.67	32.52	29.89	30.00	29.76	30.00	33.80	33.29	33.18	33.21
7	38.91	38.20	37.25	38.15	33.75	33.75	33.15	33.06	30.75	30.75	30.10	31.00	34.30	34.07	33.50	34.07
9	40.15	38.65	38.10	38.00	34.20	33.60	33.42	33.15	31.60	30.90	30.15	31.00	35.32	34.38	33.89	34.05
$\bar{x}$	38.24	37.31	36.99	37.76	33.16	32.80	32.77	32.60	30.20	30.20	29.83	30.23	33.87[b]	33.44	33.20	33.30

Note: (a) tested immediately after storage (b) treatment means by storage time (c) overall treatment means

SED of means:

Factor	Trial	Storage	Treatment	Trial Storage	Trial Treatment	Storage Treatment
SED	0.08	0.10	0.09	0.17	0.15	0.20

(...contd)

(...contd)

Analysis of variance

Source of variation	df	SS	MS	F
Trial	2	549.49	274.70	4736.8***
Storage	4	25.09	6.27	108.2***
Treatment	3	3.58	1.19	20.6***
Trial.Storage	8	2.06	0.26	4.4***
Trial.Treatment	6	2.07	0.34	5.9***
Storage.Treatment	12	2.35	0.20	3.4**
Mean square error	24	1.39	0.06	
Total	59	585.93	9.93	

Statistical significance: ** = $p < 0.01$
*** = $p < 0.001$

during storage. The decrease was significant ($p < 0.01$) after 1 day of storage. The differences in phosphorous content between between the treatment means were not significant ($p > 0.05$). No significant interactions ($p > 0.05$) were observed.

The soluble phosphorous content of milks increased very significantly ($p < 0.001$) during storage (Table 6.10). The increase was significantly higher ($p < 0.01$) in raw milk stored at 5 ^{o}C than that stored at 2 ^{o}C or the milks stored after thermisation or pasteurisation.

No significant differences ($p > 0.05$) were observed when the soluble phosphorous values for raw milk stored at 2 ^{o}C, thermised milk and those of pasteurised milk were compared with each other.

The interactions Trial.Storage, Trial.Treatment and Treatment.Storage were very significant ($p < 0.01$). These interactions explained the differences in the response of treatments veried with trials when the responses were measured over the storage time.

Discussion

Besides heat treatment, storage of milk at 2-6 ^{o}C is the most suitable procedure to avoid undesirable quality changes caused by contaminating microrganisms. However, cold storage has brought about changes in the processing parameters of raw milk. These changes were related to the proteolytic activities and the changes in the micellar

characters of the casein fraction of the milk proteins.

Proteolysis in milk caused the modification of nitrogen distribution (Reiter *et al.*, 1979). The degree of proteolysis can be described by the levels of the nitrogen components released into the growth medium (Humbert and Alais, 1979).

In dairy products and in commercial proteins or proteins prepared in the laboratory, proteolysis led to the appearance of NCN, soluble at pH 4.6; and NPN soluble in TCA. The increase in NCN has been observed in milk (Nakai *et al.*, 1964; and Corradini and Pecis, 1979).

Adams *et al.* (1975)studied the effects of heat resistant proteases produced in milk by psychrotrophic bacteria. They reported the increase in both the NCN and NPN values in cold-stored raw milk that has been treated with heat resistant protease isolated from psychrotrophic bacteria.

Richardson and Newstead (1979) investigate the effects of heat stable protease on the storage life of UHT milk. They reported that the level of NPN in the UHT milk following storage increased with increasing protease concentration.

Nakanishi and Tanabe (1970) studied changes in milk protein caused by psychrotrophic bacteria during cold storage of milk and observed that casein nitrogen in milk decreased, whereas NPN increased during storage.

It appeared from the present study that storage of raw milk at 2 ^{o}C and thermisation can effectively reduce the level of proteolysis in milk during storage. And based on the level of NPN and NCN measured, thermisation of milk

was more effective than cold storage at 2 ^{o}C

Cousin and Marth (1977a) reported increase in NPN and NCN in skim milk inoculated with psychrotrophic bacteria before processing and a decrease in manufacturing time of cottage cheese made from it. They found that yield and moisture content of cottage cheese depended on milk storage time at low temperature.

Snoren and Both (1981) monitored the level of proteolysis in UHT-sterilised whole milk during storage. They reported an increase in NPN of the UHT milk during storage at 15 ^{o}C. They attributed the increase to the presence of a thermoresistant bacterial protease which survived the heat treatment.

In addition to changes in the nitrogen distribution of milk proteins due to heating and cooling, heating of milk above 74 ^{o}C have been known to produce cooked flavour. This flavour arise from the SH groups activated by heat denaturation of *B*-lactoglobulin and proteins of the fat globule membrane. Flavour was specifically due to volatile sulfides, and hydrogen sulfides (H_2S) in particular.

According to Kitchen (1976), Kiermeier and Petz (1967) first reported the presence of an enzyme called sulphydryl oxidase in milk which oxidises SH groups to form SS. In a model system it was shown that the addition of partially purified sulphydryl oxidase enzyme solution to heated skim milk considerably reduced its SH content. They suggested its use in the elimination of cooked flavour in UHT milk. The enzyme survived pasteurisation.

Therefore, heat-induced cooked flavours produced as a result of SH group formation, were removed upon storage of milk. In addition, Jenness and Patton (1959) reported that the SH group in milk can be activated by heat treatment.

Sulphydryl oxidase in milk and dairy products may have important consequences with regard to flavour and oxidative stability. It is inactivated only partially by pasteurisation. Consequently, cooked flavours do not persist in pasteurised milk. Enzymatic oxidation of sulfhydryl groups may result in a retardation of subsequent lipid oxidation in milk since strong oxidants such as hydroxyl radical and superoxide were not intermidiates in the enzyme-catalysed reaction. In contrast, these oxidants were formed during nonenzymatic auto-oxidation process.

Allen and Joseph, (1985) reported that the O_2 consumed by SH group oxidation in heated milk was low as few oxidisable free sulphydryls were formed as the result of the heat treatment.

Jenness and Patton (1959) reported the presence of SH substances originated in the *B*-lactoglobulin and proteins associated with the milk fat globulin membrane. They were found to be strongly antioxygenic and were thought to be effective in preventing oxidised flavour in heated milk.

In addition to causing changes in the SH and the SS groups, cooling and heat treatments of milk, brought about ionic migration from the casein micelle complex that could alter the processing properties of the raw milk.

An ultrafiltrate with a composition closely similar to

an equilibrium diffusate was prepared by pressure filtration of skim milk through a semipermeable membrane with 10,000 MW cut off and 15 psi pressure which was low enough to avoid seiving effects. Estimation of the diffusable calcium and phosphorous concentration was achieved by analysis from the composition of the diffusate, and a colloidal concentration calculated as the total skim milk concentration less the diffusable concentration.

It is generally accepted that the casein micelle is composed of spherical subunits which are bound together by colloidal micellar calcium phosphate (MCP). Removal of the MCP resulted in the disintegration of native casein micelles (Visser *et al.*, 1979).

According to Holt and Muir (1979) about two-third of the Ca, one-third of the Mg and one-half of the phosphorous are in colloidal state in a typical milk. It was presumed that all colloidal phosphorous was present in the MCP and the colloidal calcium were partly incorporated in the MCP and partly bound in a more direct manner to casein. A small proportion of colloidal calcium was also bound to the β-lactoglobulin and the β-lactalbumin.

The mean calcium content of milk in the present study were 121.5, 121.4, 120.9 and 119.9 mg/100 ml for milks stored raw at 5 ^{o}C and 2 ^{o}C and thermised milk (stored at 6 ^{o}C) and pasteurised milk (stored at 5 ^{o}C), respectively. The mean phosphorous contents were 67.45, 68.13, 68.18 and 67.76 mg/100 for raw milks stored at 5 ^{o}C and 2 ^{o}C,

thermised and pasteurised milks respectively. These values were comparable to those reported earlier (Jenness and Patton, 1959 and Al-Darwash, 1982).

The means of calcium and phosphorous content of milk stored raw at 2 and 5 ^{o}C and those of thermised and the pasteurised milks were not significantly different between each storage treatments. However, the reduction of phosphorous content of the milks through storage were significant.

Soluble calcium and soluble phosphorous of milks in the present study were observed to increase through storage time. The increases were greater in the raw milks stored at 2 ^{o}C and 5 ^{o}C than that of the thermised and the pasteurised milks.

Storage temperature and heat treatment of milk are known to induce changes in the nature of the MCP and alter their distribution between the soluble and the colloidal phases. Heating at 63 ^{o}C for 30 minutes had been shown to reduce the diffusable calcium and phosphorous of milk (Jenness and Patton, 1959).

Payne, (1962) made similar observations. Heating of milk leads to many changes including a decrease in diffusable calcium and phosphorous. However, to some extend these changes were reversibled on cooling.

Chilling of milk causes a slight increase in soluble calcium and phosphorous (Holt and Muir 1979). Davies and white (1966) cooled skim milk to 3 ^{o}C and observed that almost 0.5 mM more phosphorous and calcium became diffusible within 24 hours. The increase in the diffusible

calcium and phosphorous was due to dissolution of colloidal phosphate (Davies and White, 1966).

Visser *et al.* (1979) made similar observations. They reported that the distribution of calcium in milk serum increased by 8.8 per cent during cold storage. It was suspected that these changes were at the expense of MCP.

The changes in the calcium phosphate/citrate equilibrium were directly related to the solubility of the casein micelle and the protein equilibrium. Ali *et al.* (1980) reported that up to 42 per cent of the total calcium was dissolved in the milk serum after storage at 4 ^{o}C for 48 hours.

Payne (1962) summarised the effect of temperature changes on the milk salts equilibrium as follows:-

On cooling some of the colloidal salts were dissolved, and the previous ionic partitions due to cooling during storage may be re-established by rewarming the milk. Likewise, when milk is gently warmed and then cooled, the original state of the equilibrium can be re-establish. However, prolonged storage of frozen milk or severe heating, may cause changes in the milk salt partition that were irreversible.

Conclusions

1. The response of all parameters to the all treatments were highly variable between trials.

2. In terms of the NCN and NPN values, cooling of raw milk

to 2 ^{o}C reduced the level of proteolysis in milk during raw storage. The NCN and NPN content of the raw milk stored at 2 ^{o}C increased significantly 4 days later than the milk stored at 5 ^{o}C. Thermisation reduced the NCN content further. The effects of thermisation on the NCN and NPN content of milk were comparable to that of pasteurisation.

3. Low temperature storage of raw milk at 2 ^{o}C and 5 ^{o}C results in the increase appearance and increase of soluble calcium. Thermisation effectively controlled the increase in soluble calcium by inducing changes in the nature of the micellar calcium phosphate, hence altering their distribution between the soluble and the colloidal phases. The effectiveness was comparable to that of pasteurisation.

4. The migration of phosphorous from the colloidal phase to the soluble phase was significant during storage of raw milk at 5 ^{o}C. The migration, was significantly reduced when the raw milk was stored at a lower temperature of 2 ^{o}C. And further, when the milk was thermised. The corresponding effects of thermisation equalled to that of pasteurisation.

5. Further investigation is recommended on the role of free and reduced SH groups to flavour and its role as natural antioxidants in heat treated milk.

CHAPTER SEVEN

FREE FATTY ACIDS PROFILE STUDIES

Introduction

Triglyceride is the major component of cow's milk lipid since it accounts for 97-98 per cent of the total lipids. But additionally, there are small amounts of diglyceride (0.25-0.48 per cent), monoglyceride (0.016-0.038 per cent), cholesterol esters (trace), cholesterol (0.22-0.41 per cent) FFA (0.10-0.44 per cent) and phospholipid (0.2-1.0 per cent) (Jennes and Patton, 1959). Higher values for the proportion of the diglyceride and FFA may be observed in samples that have been subjected to lipolysis (Banks *et al.*, 1986).

The major fatty acids of the lipid contain 16 and 18 carbon (C) atoms, and a high proportion of the C:18 fatty acids are mono-unsaturated (Walstra and Jenness, 1984).

Cow's milk contains a high proportion of short chain (C:4 to C:14) fatty acids and very small amount of di-unsaturated fatty acids, as other herbivores species. There are, in addition, trace amounts of a large number of branched-chain saturated acids and positional and configurational isomers of unsaturated acids (Walstra and Jenness, 1984).

The fatty acids present in cow's milk fat include all the saturated fatty acids from C:2 to C:28, mono-methyl branched-chain fatty acids from C:16 to C:28, multi-methyl

branched-chain fatty acids from C:16 to C:28, cis- and trans-mono-enoic fatty acids from C:10 to C:26, numerous di- and polyenoic fatty acids, keto and hydroxy fatty acids and cyclohexyl fatty acids (Walstra and Jenness, 1984)

The lipolytic off-flavour has been attributed to the presence of free butyric (C:4) and other water-soluble volatile acids, such as caproic (C:6), caprylic (C:8), capric (C:10) and lauric (C:12) acids (Al-Shabibi *et al.*, 1964). Therefore, the objective of this study was to illustrate further the FFA profile of these fatty acids in relation to bacterial counts and the storage treatments of milks.

Material and method

The milks used in the following studies were milks sampled from the previous trials (Sections 4.1, 4.2, 5.2 and 5.3). Milk sampled from section 4.1, namely deep-cooled (DC, 2 ^{o}C) and cold-stored milk (CS, 5 ^{o}C) were drawn fresh and after 3, 4, 7 and 15 days of storage.

Milk sampled from section 4.2, namely, milks that were pasteurised after 2 , 4 and 7 days of deep-cooled storage at 2 ^{o}C (DC2P, DC4P and DC7P, respectively) and cold storage at 5 ^{o}C (CS2P, CS4P and CS7P, respectively), were drawn after 1, 3, 7, 15 and 21 days of storage.

The milk samples from sections 5.2 and 5.3, namely, thermised milks (TO); and thermised milks, pasteurised 2,

4 and 7 days after storage (TP2, TP4 and TP7, respectively), were drawn after 1, 3, 7, 15 and 21 days of storage.

Milk samples for FFA analysis were deep frozen to -20 ^{o}C on the day of sampling and thawed to 20 ^{o}C immediately before analysis. Microbiological samples of milk sampled from sections 4.1, 4.2 and 5.2 were tested fresh on the day of sampling.

Milk samples from sections 4.1, 4.2 and 5.2 were analysed for total plate count (TPC) and psychrotrophic bacterial count (PBC) using the procedures as described in sections 2.3.1 and 2.3.2, respectively. In addition, all samples were analysed for FFA using the method described in section 2.2.17.2. The study was conducted during the third trial only.

All data were subjected to the analysis of variance as described in section 2.4, using treatments and storage time as the main effects.

Results

The results of the study will be presented in graphic form and accompanied with the statistical analysis and according to the sectional grouping. To simplify description of the FFA studied, the FFA were grouped into (i) short chain, namely C:4 to C:12 and (ii) long chain FFA, namely, C:14 to C18:2 fatty acids.

The FFA standards used in the analysis and their concentration are shown in table 7.1. A clear and distinct

seperation was obtained for all the FFA standards. A sample of chromatogram of the standards is shown in figure 7.1.

The correlation coefficients of the various variables are shown in table 7.2. The TPC and the PBC of the DC milk correlate very highly significantly ($p < 0.001$) with each other. Similar observations were noted with the TO and the CS milks, but with lower significant level ($p < 0.01$ and $p < 0.05$, respectively). No significant ($r = 0.23$, $p > 0.05$) correlation was observed between the TPC and PBC of the pasteurised milk.

For the DC milk the TPC showed significantly direct correlation with fatty acids C:12, C18:1 ($r = > 0.75$, $p < 0.05$); C:14, C18:2, long chain and the total FFA ($r = > 0.98$, $p < 0.001$). The PBC were very highly significantly ($r = > 0.95$, $p < 0.001$) correlated with C:14, C18:2, long chain and the total FFA.

Table 7.1

The fatty acids standard used in the gas liguid chromatography (GLC) studies

Chain length	Nomenclature and chemical formula	Molecular weight	mg/100 ml[a] in standard
C 4:0	*n*-Butyric acid $CH_3\ (CH_2)_2.COOH$	88.11	12.4
C 6:0	*n*-Hexanoic acid (n-caproic acid) $CH_3\ (CH_2)_4.COOH$	116.16	10.4
C 8:0	*n*-Octanoic acid (n-caprylic acid) $CH_3\ (CH_2)_6.COOH$	144.24	8.7
C 9:0	*n*-Nonanoic acid $CH_3\ (CH_2)_7.COOH$	158.24	10.8
C 10:0	*n*-Decanoic acid (*n*-capric acid) $CH_3\ (CH_2)_8.COOH$	172.27	13.7
C 12:0	Lauric acid $CH_3\ (CH_2)_{10}.COOH$	200.32	20.8
C 14:0	Myristic acid $CH_3\ (CH_2)_{12}.COOH$	228.38	13.1
C 16:0	Palmitic acid $CH_3\ (CH_2)_{14}.COOH$	256.43	31.3
C 18:0	Stearic acid $CH_3\ (CH_2)_{16}.COOH$	284.49	32.6
C 18:1	Oleic acid $CH_{17}\ H_{33}\ COOH$	282.47	60.0
C 18:2	Linoleic acid $C_{17}\ H_{31}\ COOH$	280.45	59.3
	Total fatty acids		273.4

Note: (a) = The fatty acids standard were dissolved in di-isopropyl ether containing 4 per cent formic acid

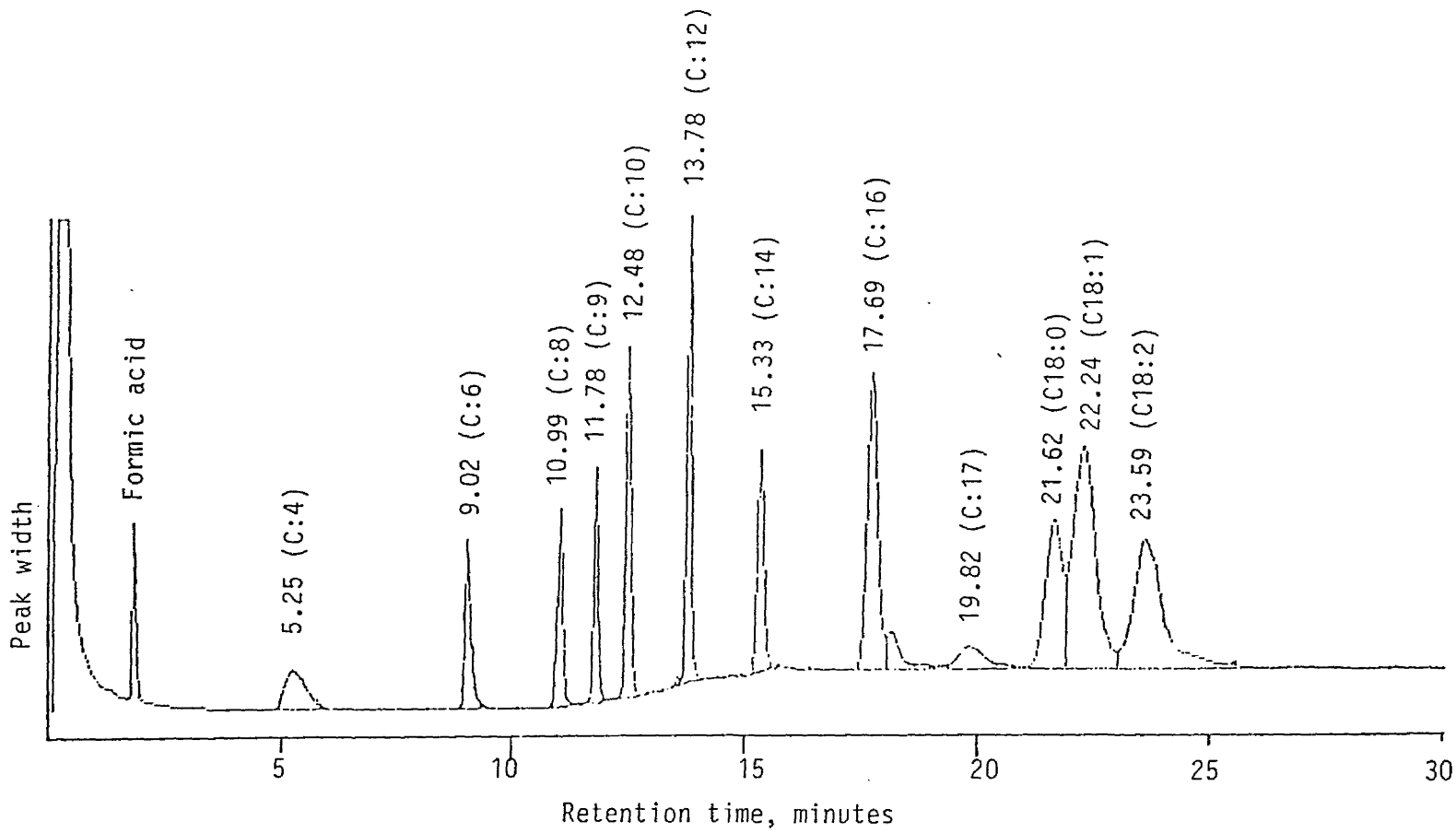


Figure 7.1: Sample chromatogram showing the retention times (minutes) of the FFA standards.

Table 7.2

The correlation coefficients of total plate count (TPC) and psychrotrophic bacterial count (PBC) of milk stored at 2 ^{o}C (DC) and 5 ^{o}C, thermised (TO) and pasteurised (PO) milk stored at 6 and 4 ^{o}C, respectively, against the measured FFA.

	DC		CS		TO		PO	
	TPC	PBC	TPC	PBC	TPC	PBC	TPC	PBC
PBC	0.979***	--	0.809*	--	0.941**	--	-0.231	--
FFA[a]	0.843*	0.733	0.816*	0.945**	1.000***	0.935**	0.931**	-0.034
C:4	0.691	0.678	0.984***	0.806*	0.298	0.594	0.563	-0.632
C:6	0.688	0.676	0.979***	0.885**	0.153	0.474	0.147	-0.553
C:8	0.598	0.588	0.967***	0.640	0.698	0.717	0.323	-0.431
C:10	0.650	0.639	0.906**	0.505	0.761*	0.590	0.067	-0.173
C:12	0.759*	0.744	0.917**	0.579	0.736	0.498	0.145	-0.088
C:14	0.990***	0.995***	0.860*	0.510	0.553	0.352	0.722	-0.221
C:16	0.579	0.609	0.629	0.322	-0.555	-0.450	0.821*	-0.335
C18:0	0.661	0.653	0.745	0.367	-0.662	-0.486	0.836*	-0.484
C18:1	0.859*	0.845	0.959***	0.616	0.569	0.279	0.185	0.358
C18:2	0.989***	0.958***	0.922**	0.549	-0.623	-0.420	0.699	-0.260
Low[b]	0.684	0.672	0.987***	0.706	0.798*	0.854*	0.263	-0.386
High[c]	0.992***	0.988***	0.899**	0.540	0.014	-0.112	0.761*	-0.278
Total[d]	0.973***	0.967***	0.917**	0.566	0.207	0.109	0.743	-0.290

Note: (a) = Total FFA measured qualitatively, (b) = short (C:4-C:12) and (c) = long chain FFA (C:14-C18:2), (d) total FFA measured by GLC. * = Significant $p < 0.05$, ** = highly significant $p < 0.01$ and *** = very highly significant $p <$

The TPC of milk stored at 5 ^{o}C correlate significantly with the FFA ($r = 0.82$, $p < 0.05$), C:4, C:6 and C:8 ($r = > 0.96$, $p < 0.001$); C:10 and C:12 ($r = > 0.91$, $p < 0.01$); C:14 ($r = 0.86$, $p < 0.05$); C:18:1 ($r = 0.95$, $p < 0.01$); C18:2 ($r = 0.92$, $p < 0.01$); short chain FFA ($r = 0.99$, $p < 0.001$); long chain FFA and the total FFA ($r = 0.92$, $p < 0.01$). Fewer variables were observed to correlate significantly with the PBC, namely; the FFA ($r = 0.95$, $p < 0.01$) and the short chain FFA, namely the C:4 ($r = 0.81$, $p < 0.05$) and C:6 ($r = 0.89$, $p < 0.01$).

The TPC of the thermised milk correlate significantly with the FFA ($r = 1.00$, $p < 0.001$), C:10 and the short chain FFA ($r = 0.80$, $p < 0.05$). The PBC correlate significantly with the FFA ($r = 0.94$, $p < 0.01$) and the short chain FFA. No significant ($p > 0.05$) correlations were observed with all the FFA when measured singularly.

The TPC of the pasteurised milk correlate significantly with the FFA ($r = 0.93$, $p < 0.01$), C:16 and C18:0 ($r = > 0.0.82$, $p < 0.05$) and with the long chain FFA ($r = 0.76$, $p < 0.05$). The correlation between the PBC and all the fatty acids measured were not significant ($p > 0.05$).

Section 7.1 The FFA profile of deep-cooled (DC, 2 °C), cold-stored (CS, 5 °C) milk and thermised (65 °C/16 seconds) milk during storage.

The TPC, PBC and FFA content of milk stored at 2 and 4 °C are shown in table 7.1.1 and the FFA content of thermised milk are shown in table 7.1.2. The changes in the FFA through storage are illustrated in figures 7.1.1, 7.1.2 and 7.1.3.

A general trend was observed that the FFA of all the treatments studied increased during storage. The FFA content of the CS milk, both short and long chain FFA changed radically and immediately after storage. For the DC and TO milks the short and long chain FFA remained almost unaltered for the first 3 days of storage and then increased gradually through storage.

The means of the short and long chain FFA for the CS milk were significantly higher ($p < 0.05$) than the means of DC and the TO milks. No significant difference ($p > 0.05$) was observed when the means of DC and TO milk were compared with each other.

The FFA content of CS milk increased immediately during storage. For the DC and TO milk, the short chain FFA, namely C:4-C:12, remained relatively unaltered up to 7 days of storage and then increased substantially thereafter. Similar trend was observed with the long chain FFA but the changes occurred after 3 days of storage.

Table 7.1.1

Changes in FFA profile of a raw milk during storage at 2 and 4 °C in relation to total plate counts (TPC), psychrotrophic bacterial count (PBC) and FFA measured quantitatively.

Storage		cfu/ml		mg/100 ml									
°C	Days	TPC	PBC	C4	C6	C8	C10	C12	C14	C16	C18:0	18:1	C18:2
2 °C	Initial	4.42	4.30	0.255	0.219	0.226	0.316	0.311	0.529	1.581	1.165	4.571	0.629
	3	4.71	4.60	0.250	0.215	0.222	0.318	0.300	0.543	2.091	1.267	5.085	0.711
	4	6.11	6.21	0.205	0.197	0.200	0.289	0.304	0.716	3.298	1.627	5.238	0.794
	7	8.78	7.25	0.313	0.242	0.212	0.322	0.318	0.875	4.018	2.073	6.707	1.135
	15	9.25	7.89	1.031	0.564	0.428	0.658	0.597	0.969	2.303	1.420	10.302	1.268
Mean		6.66	6.05	0.410	0.287	0.257	0.381	0.374	0.726	2.658	1.510	6.38	0.907
4 °C	Initial	4.45	4.20	0.159	0.149	0.129	0.249	0.188	0.319	0.878	0.567	2.480	0.235
	3	5.38	4.85	0.504	0.300	0.280	0.396	0.415	0.690	1.798	1.251	5.997	0.983
	4	6.23	5.92	0.438	0.314	0.286	0.435	0.518	0.985	4.213	1.844	6.394	0.855
	7	8.31	6.29	0.921	0.467	0.525	0.968	0.828	1.507	5.223	2.642	13.453	1.734
	15	8.39	8.37	1.031	0.564	0.428	0.658	0.597	0.969	2.303	1.420	10.302	1.268
Mean		6.64	5.93	0.610	0.359	0.330	0.541	0.509	0.894	2.883	1.545	7.725	1.015

Table 7.1.2

Changes in FFA profile of thermised (65 °C/16 sec) during storage at 6 °C; in relation to TPC and PBC

Treatment	Days storage	cfu/ml		mg/100 ml									
		TPC	PBC	C4	C6	C8	C10	C12	C14	C16	C18:0	18:1	C18:2
A	1	2.48	1.30	0.297	0.203	0.165	0.276	0.310	0.618	1.906	1.069	3.980	0.476
	3	2.78	2.08	0.393	0.203	0.213	0.316	0.365	0.747	2.487	1.212	4.221	0.673
	7	7.01	4.40	0.544	0.209	0.215	0.320	0.327	0.654	2.021	1.110	3.813	0.557
	15	9.62	5.14	0.347	0.202	0.224	0.355	0.481	0.808	1.834	1.001	4.833	0.381
	21	*	*	0.210	0.181	0.190	0.266	0.265	0.617	2.801	1.549	4.425	0.692
	Mean	5.47	3.23	0.358	0.200	0.201	0.303	0.349	0.689	2.206	1.188	4.254	0.556

Note: (A) = Thermisation

Table 7.1.3

Means of FFA concentration (mg/100 ml) of milk measured 1, 3, 4, 7 and 15 days after storage at 2 ^{o}C, 4 ^{o}C and after thermisation and storage at 6 ^{o}C

FFA	Treatments			Means	SED[h]
	CS[a]	DC[b]	TO[c]		
C:4	0.611	0.358	0.411	0.460	0.276
C:6	0.359	0.200	0.287	0.282	0.053
C:8	0.330	0.201	0.258	0.263	0.047
C:10	0.541	0.303	0.381	0.408	0.086
C:12	0.509	0.350	0.374	0.411	0.071
C:14	0.894	0.689	0.726	0.770	0.111
C:16	2.883	2.206	2.660	2.582	0.502
C18:0	1.545	1.188	1.510	1.414	0.217
C18:1	7.725	4.254	6.381	6.120	1.129
C18:2	1.016	0.556	0.907	0.826	0.153
Short chain FFA[d]	2.349	1.412	1.710	1.824	0.380
Long chain FFA[e]	14.062	8.893	12.183	11.713	1.871
Total[f]	16.413	10.305	13.893	13.537	2.179

Note: a = Cold storage at 5 ^{o}C, (b) = deep cooling at 2 ^{o}C, (c) = thermisation and storage at 6 ^{o}C, (d) = C:4-C14, (e) C:16-C18:2, (f) = C:4-C18:2, (g) = SED of treatments mean.

(...contd)

(...contd)

F values for treatment and storage effects and mean square error

Source	df	C:4	C:6	C:8	C:10	C:12	C:14
Treatment	2	1.17	2.91	2.33	2.47	1.85	1.22
Storage	4	1.51	2.45	1.67	1.47	1.90	2.82
Mean square error	8	0.08	0.01	0.01	0.03	0.02	0.05

Source	df	C:16	C18:0	C18:1	C18:2	Short chain FFA	Long chain FFA	Total FFA
Treatment	2	0.58	1.03	3.01	3.06	1.98	2.44	2.48
Storage	4	2.29	2.06	2.62	2.26	1.68	2.72	2.64
Mean square error	8	1.01	0.19	5.09	0.09	0.58	14.00	19.00

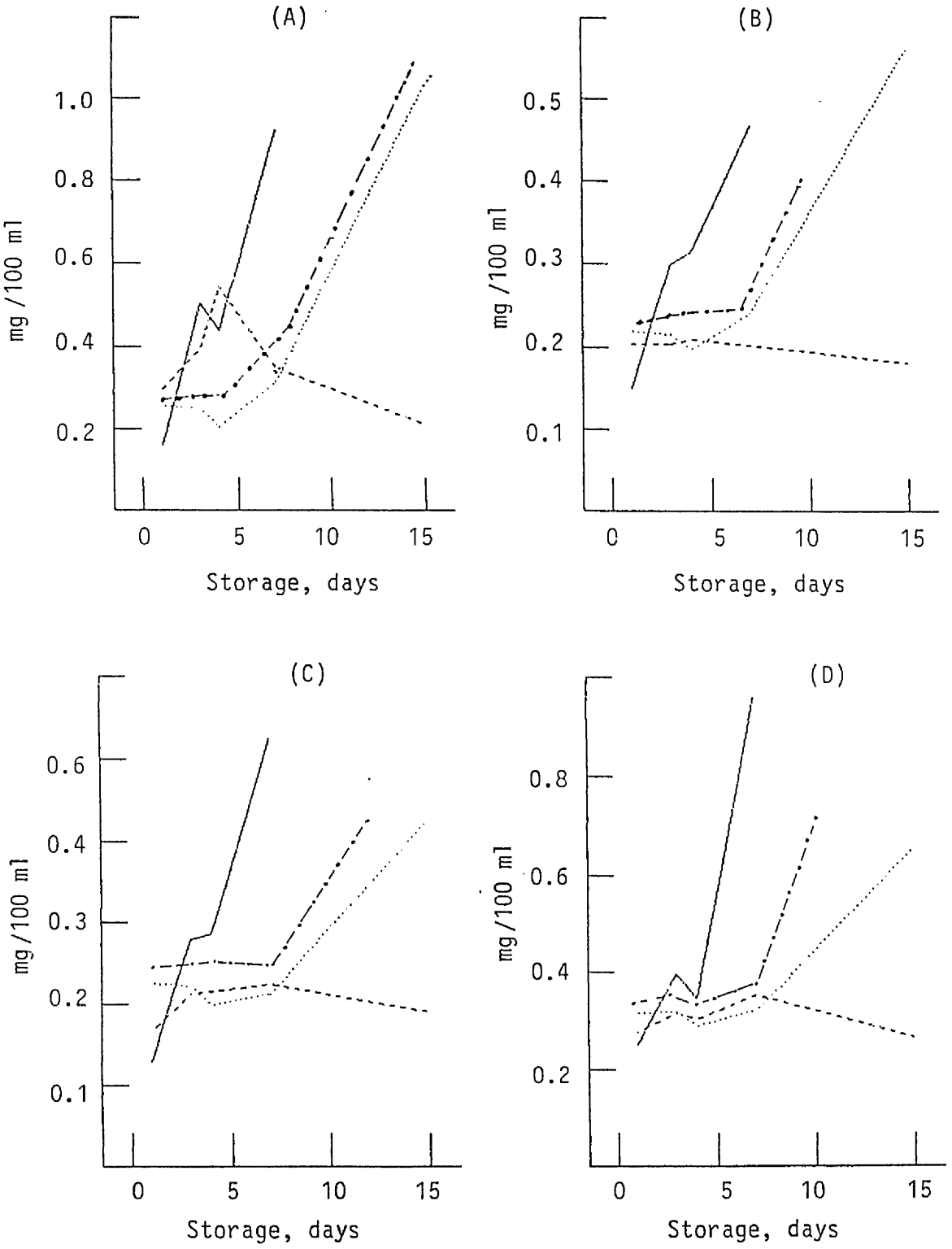


Figure 7.1.1: The response of (A) C:4, (B) C:6, (C) C:8 and (D) C:10 FFA to storage at 5°C (-) and 2°C (-·-); thermisation and storage at 6°C (---) and pasteurisation and storage at 5°C (···).

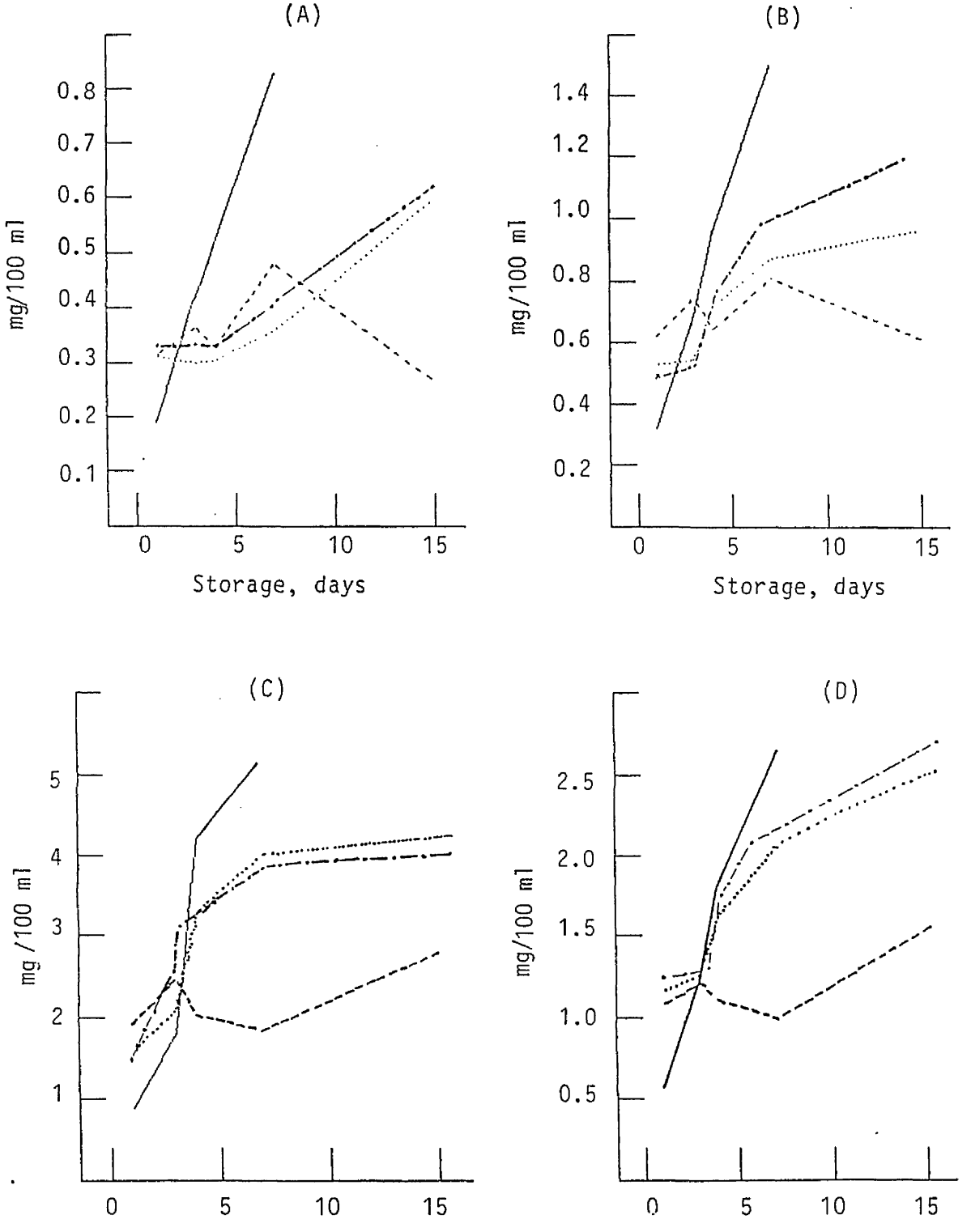


Figure 7.1.2: The response of (A) C:12, (B) C:14, (C) C:16 and (D) C18:0 FFA to stroage at 5°C (-) and 2°C (-··-); thermisation and storage at 6°C (---) and pasteurisation and storage at 5°C (···).

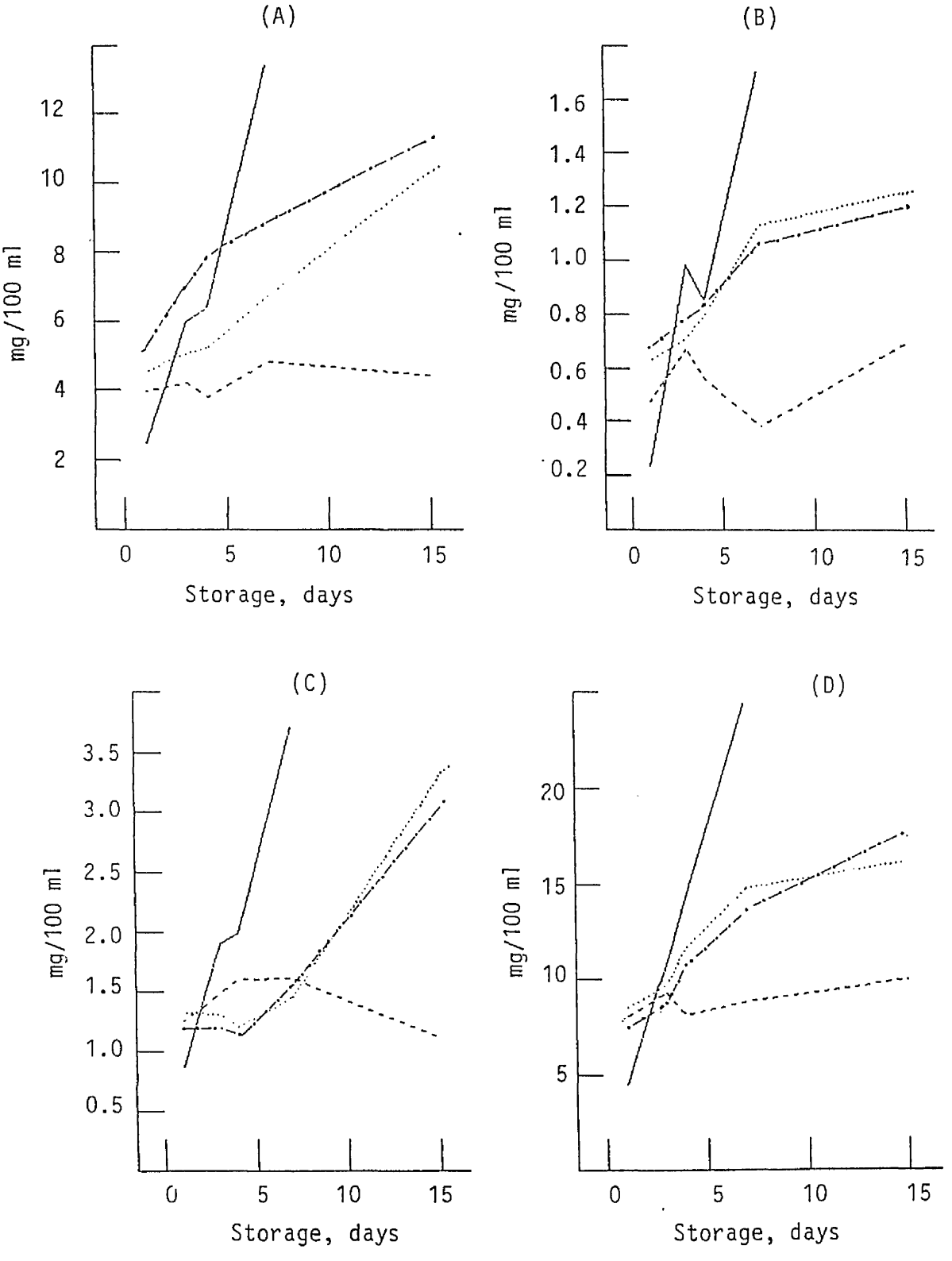


Figure 7.1.3: The response of (A) C18:1, (B) C18:2, (C) short chain (C:4 - C:12), and (D) long-chain (C:14 - C18:2) FF to storage at 5°C (-) and 2°C (-·-); thermisation and storage at 6°C (---) and pasteurisation and storage at 5°C (···).

The storage treatment affects the mean FFA concentration (Table 7.1.1). After 4 days of storage and when the TPC and PBC were > 1.0×10^6 cfu/ml, the short chain FFA of the DC milk ranged between 0.205 to 0.304 mg/100 ml and the long chain FFA ranges between 0.969 to 10.302 mg/100 ml. For the CS milk the ranges were higher for the short chain FFA (0.286 to 0.518 mg/100 ml) and lower for the long chain FFA (0.985 to 6.394 mg/100 ml).

For the TO milk and under the same conditions except with lower PBC (4.40 cfu/ml) the range for the short chain FFA were from 0.209 to 0.544 mg/100 ml and 0.557 to 3.813 mg/100 ml for the long chain FFA.

Section 7.2 The FFA profile of milk pasteurised after 2, 4 and 7 days of storage at 5 ^{o}C.

The mean FFA content of the pasteurised milks are given in table 7.2.1 and the changes through storage are illustrated in figures 7.2.1, 7.2.2 and 7.2.3. Analysis of the data indicated that no significant ($p > 0.05$) changes occurred in all the FFA studied after 7 days of pasteurised milk storage and after more than 7 days, for the CS2P and CS4P milks. The FFA remained level and increased only slightly over the storage period. But large increases were observed in the CS7P milk for all the FFA after 7 days of storage.

The treatment means increased with the extended raw milk storage time. The means of the CS2P milk was observed to be consistently lower than those of CS4P and CS7P milks. No significant differences ($p > 0.05$) were observed when the means of treatments CS2P and CS4P were compared with each other for all the FFA studied. However, highly significant differences ($p < 0.01$) were observed when treatments CS2P and CS4P were compared to CS7P for all the FFA, except C18:1.

Table 7.2.1

The mean FFA concentration (mg/100 ml) of milk pasteurised after 2 (CS2P), 4 (CS4P) and 7 (CS7P) days at 5 °C and analysed after 1, 3, 7, 15 and 21 days of storage at 5 °C.

Fatty Acid	Treatments				SED[d]
	CS2P	CS4P	CS7P	Means	
C:4	0.407	0.497	3.400	1.435	0.613
C:6	0.270	0.330	1.435	0.678	0.227
C:8	0.233	0.277	1.382	0.631	0.234
C:10	0.378	0.453	2.581	1.137	0.454
C:12	0.475	0.538	1.864	0.959	0.290
C:14	0.926	1.083	3.004	1.671	0.447
C:16	3.504	3.887	9.164	5.518	1.337
C18:0	1.817	1.879	10.658	4.785	2.808
C18:1	7.165	7.246	3.794	13.402	3.708
C18:2	1.166	1.311	3.481	1.986	0.465
Short chain FFA[a]	1.764	2.094	10.663	4.840	1.817
Long chain FFA[b]	14.579	15.406	52.101	27.360	7.778
Total[c]	16.342	17.500	62.764	27.250	9.585

Note: (a) = C:4-C:12 FFA, (b) = C:14-C18:2, (c) = total of all FFA measured, (d) = SED of treatments mean.

(contd...)

(...contd)

F values for treatment and storage effects and mean square error

Source	df	C:4	C:6	C:8	C:10	C:12	C:14
Treatment	2	2.87	3.27	2.93	2.90	2.94	2.84
Storage	4	1.27	1.32	1.10	1.16	1.19	1.69
Mean square error	16	3.01	0.412	0.441	1.650	0.675	1.60

Source	df	C16	C18:0	C18:1	C18:2	Short FFA	Long FFA	Total
Treatment	2	2.58	1.24	3.37	3.31	2.95	3.07	3.05
Storage	4	2.38	1.42	1.64	2.05	1.21	1.87	1.73
Mean square error	16	14.30	63.10	110.00	1.73	26.40	4.84	7.35

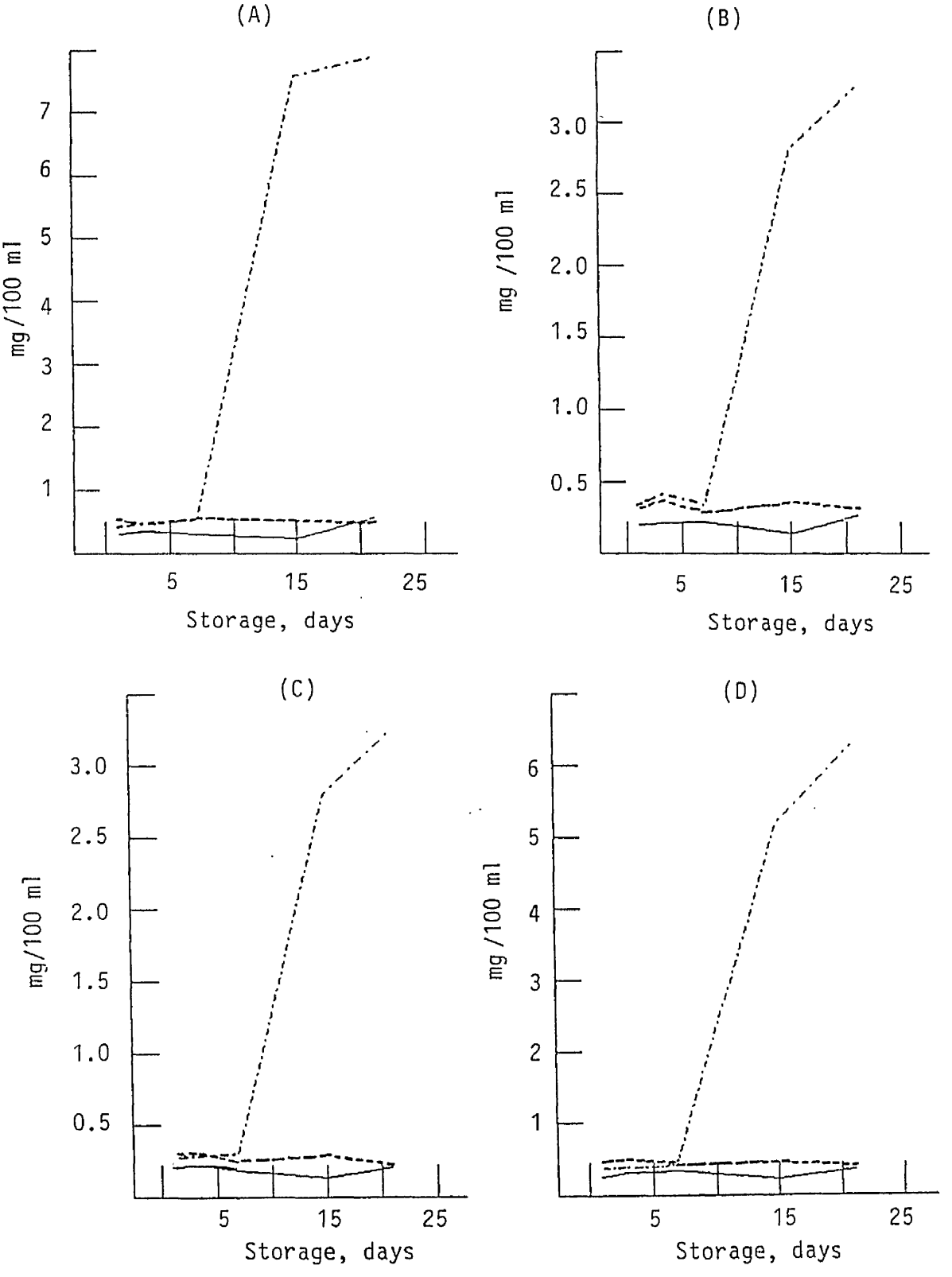


Figure 7.2.1: The response of (A) C:4, (B) C:6, (C) C:8 and (D) C:10 FFA to pasteurisation following 2(-), 4 (----) and 7 (-·-) days of cold storage (CS) at 5°C.

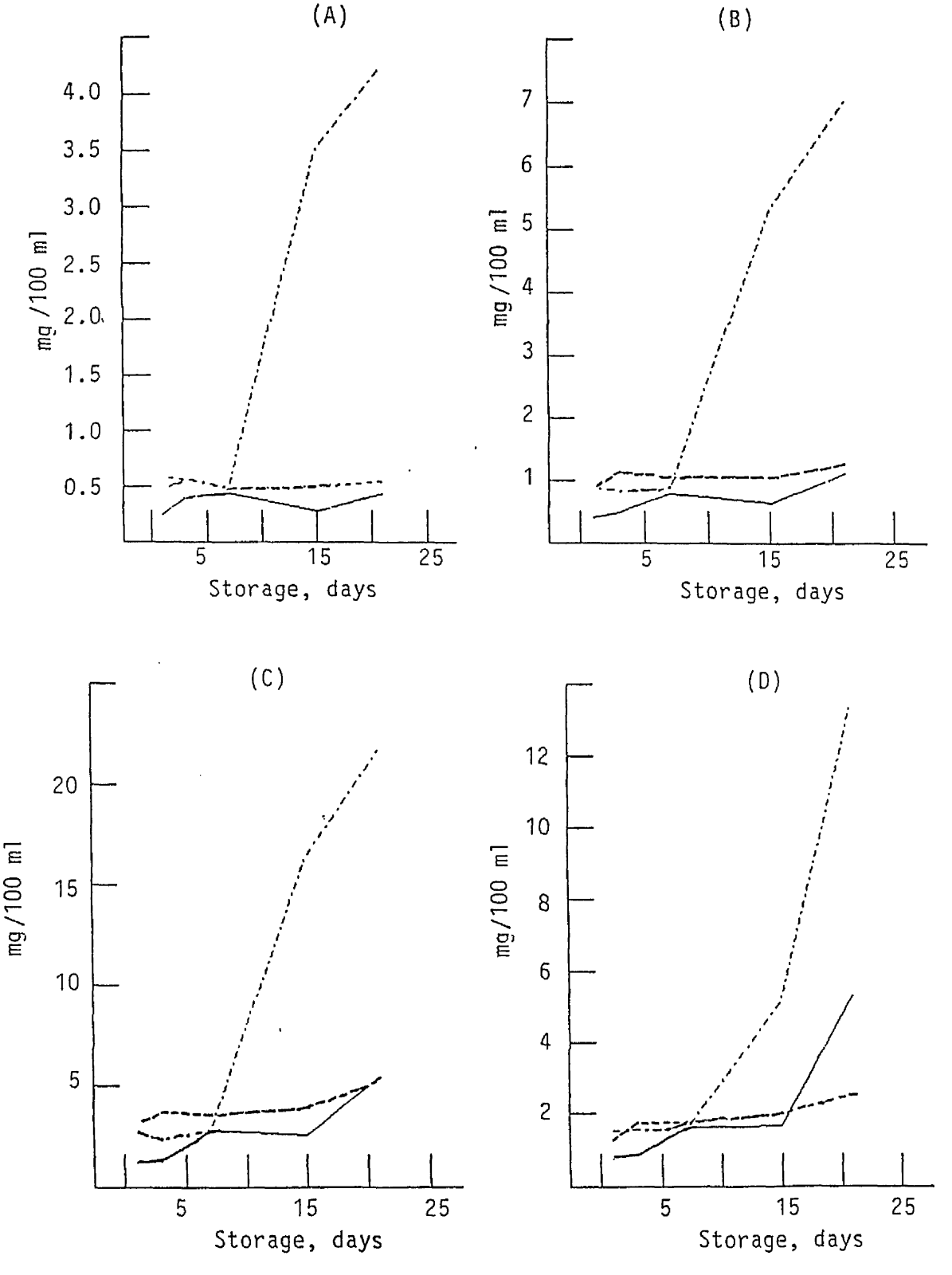


Figure 7.2.2: The response of (A) C:12, (B) C:14, (C) C:16 and (D) C18:0 FFA to pasteurisation following 2 (-), 4 (---) and 7 (-·-) days of cold storage (CS) at 5°C.

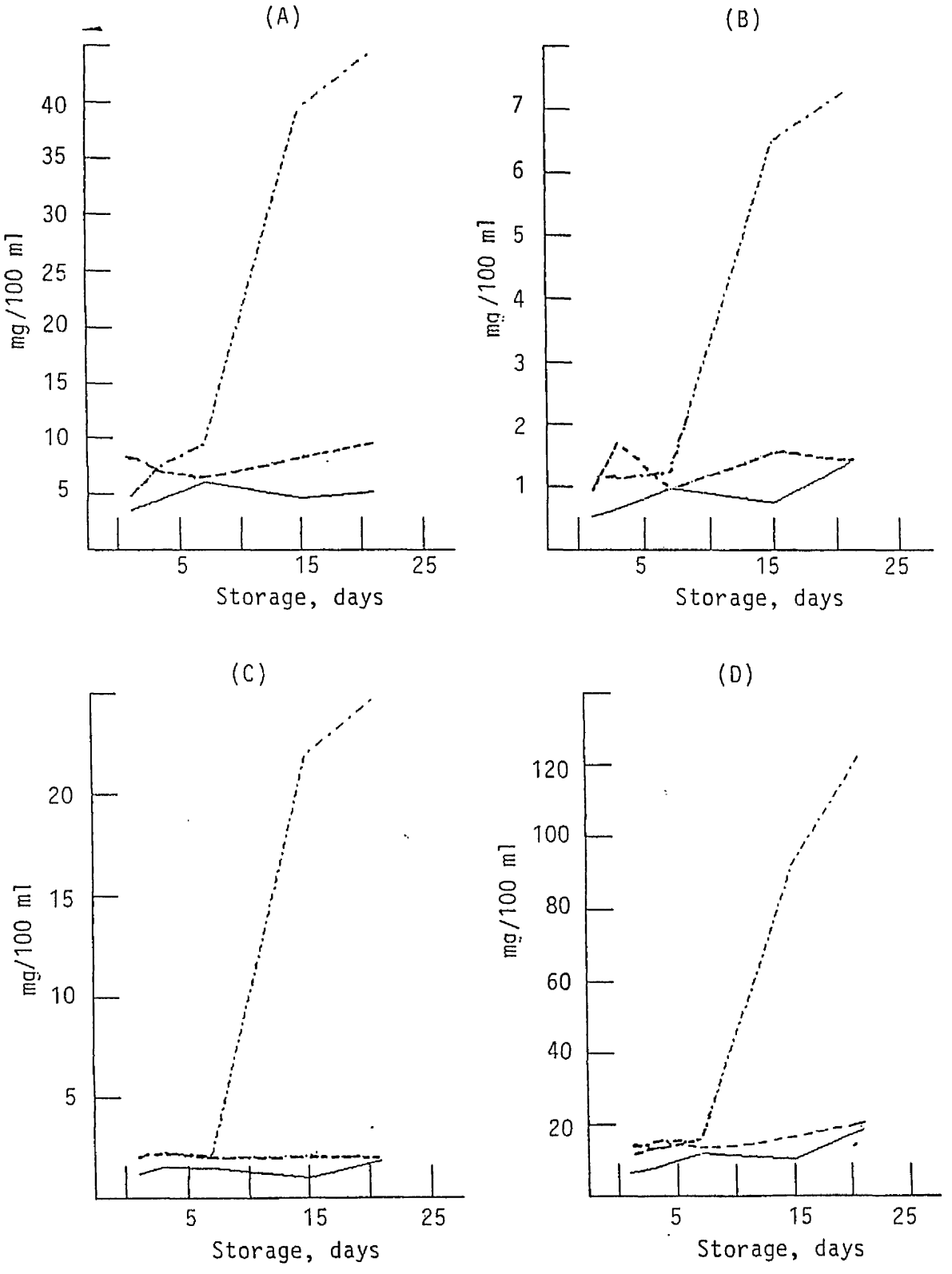


Figure 7.2.3: The response of (A) C18:1, (B) C18:2, (C) short chain (C:4 - C:12) and (D) long chain (C:14 - C18: 2) FFA to pasteurisation following 2 (-), 4 (---) and 7 (...) days of cold storage (C) at 5°C.

Section 7.3 The FFA profile of milk pasteurised after 2, 4 and 7 days of storage at 2 °C.

The mean FFA content of the pasteurised milks are given in table 7.3.1 and the changes in the FFA profile of the milk through storage at 4 °C after pasteurisation are shown in figures 7.3.1, 7.3.2 and 7.3.3.

Pasteurisation affects the level of fatty acids studied. It appeared that the level of all the FFA in all the treatments originated from the same point and diverged as the storage time increases. The mean concentration of the short chain FFA of milk pasteurised fresh were lower than DC2P, DC4P or DC7P. A similar pattern was observed with the long chain FFA except for C18:0 when the fresh pasteurised milk was higher than the milk pasteurised after 2 and 4 days of storage. The FFA concentration of DC7P was consistently higher than the other treatments.

Significant difference ($p < 0.05$) was observed when the fresh pasteurised and DC7P milk were compared for C:10, C18:1 and C18:2 fatty acids. The DC7P was consistently higher. No significant differences were observed when the fresh pasteurised milk were compared either to DC2P or DC4P milks. Similar observation was made when the DC2P and DC4P milks were compared with each other.

In the present study it was observed that the level of fatty acids studied remained at the level of immediately after pasateurisation up to 15 days and increased only

Table 7.3.1

The mean FFA concentration (mg/100 ml) of milk pasteurised fresh[a] and after 2 (DC2P), 4 (DC4P) and 7 (DC7P) days of storage at 2 ^{o}C.

Fatty acids	Treatment					SED[e]
	Fresh[a]	DC2P	DC4P	DC7P	Means	
C4	0.352	0.510	0.392	0.785	0.510	0.144
C6	0.207	0.319	0.334	0.463	0.331	0.059
C8	0.193	0.260	0.273	0.357	0.271	0.034
C10	0.293	0.416	0.419	0.594	0.430	0.060
C12	0.356	0.455	0.450	0.619	0.470	0.066
C14	0.687	1.024	0.801	1.211	0.931	0.154
C16	2.642	3.577	2.724	3.569	3.128	0.406
C18:0	2.062	1.969	1.267	2.208	1.877	0.301
C18:1	4.754	6.799	5.220	10.194	6.742	0.984
C18:2	0.862	1.029	0.868	1.445	1.051	0.108
Short chain[b]	1.401	1.959	1.868	2.818	2.012	0.324
Long chain[c]	11.007	14.398	10.881	18.627	13.728	1.602
Total[d]	12.408	16.357	12.749	21.445	15.740	1.893

Note: (a) = Pasteurised on arrival, (b) = C:4-C12, (c) = C:14-C18:2, (d) = C:4-C18:2, (e) = SED of treatments mean.

(contd...)

(...contd)

F values for treatment and storage effects and mean square error

Factor	df	C:4	C:6	C:8	C:10	C:12	C:14
Treatment	3	2.43	2.74	3.35	3.54*	3.00	1.92
Storage	4	5.22*	4.06*	3.65*	5.52**	6.27**	8.15***
Mean square error	12	0.079	0.021	0.007	0.022	0.02	60.142

Factor	df	C:16	C18:0	C18:1	C18:2	Short FFA	Long FFA	Total
Treatment	3	1.35	1.61	5.22*	5.38*	2.77	4.33*	4.10*
Storage	4	14.97***	10.19***	5.73**	13.21***	5.32*	12.70***	11.61***
Mean square error	12	0.987	0.54	5.81	0.07	0.63	15.40	21.50

Note: * = Significant ($p < 0.05$), ** = significant ($p < 0.01$), *** = significant ($p < 0.001$).

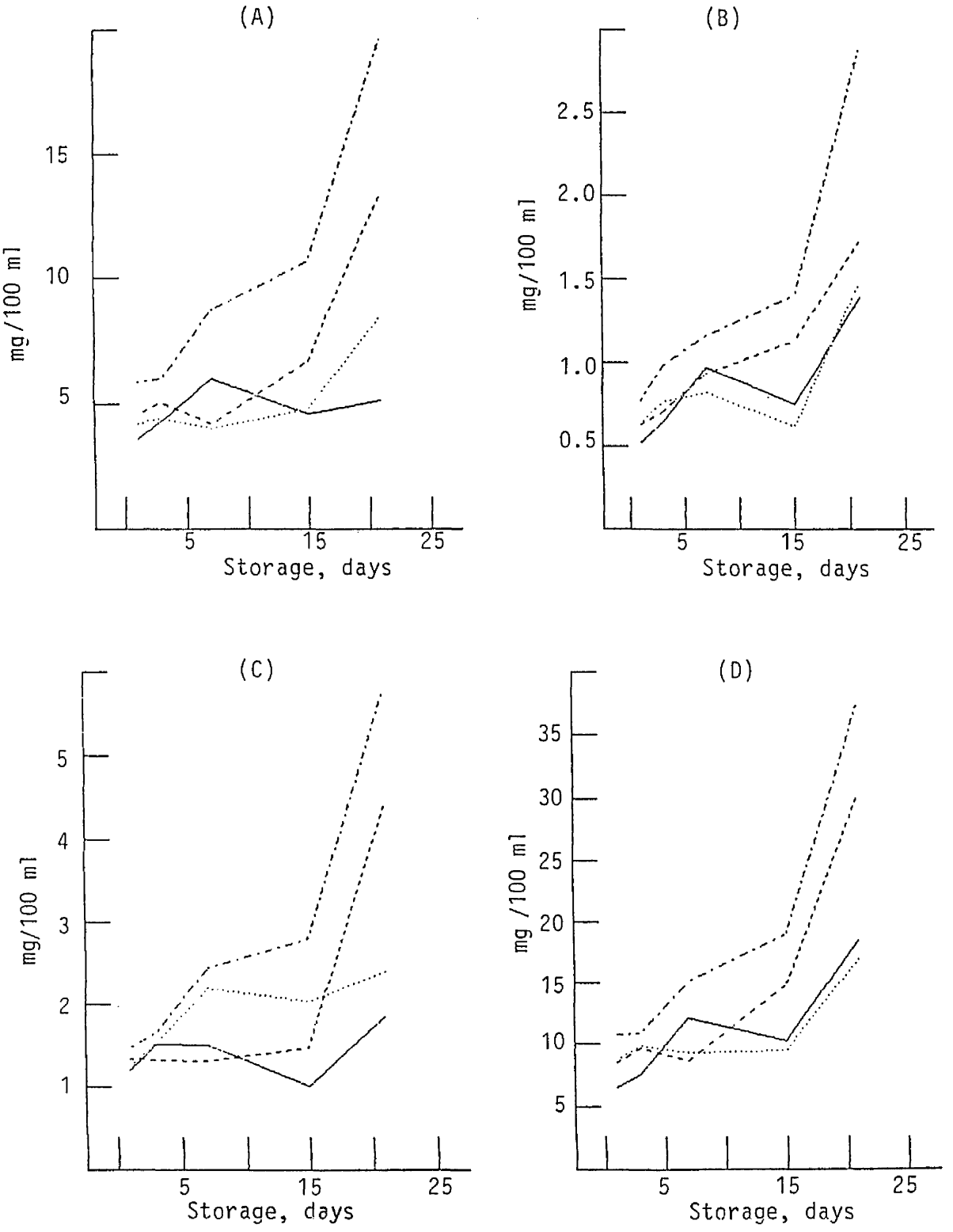


Figure 7.3.1: The response of (A) C18:1, (B) C18:2, (C) short chain (C:4 - C:12) and (D) long chain (C:14 - C18:2) FFA to pasteurisation on arrival (-), after 2 (---), 4 (....) and 7 (-·-) days of storage at 2°C (DC)

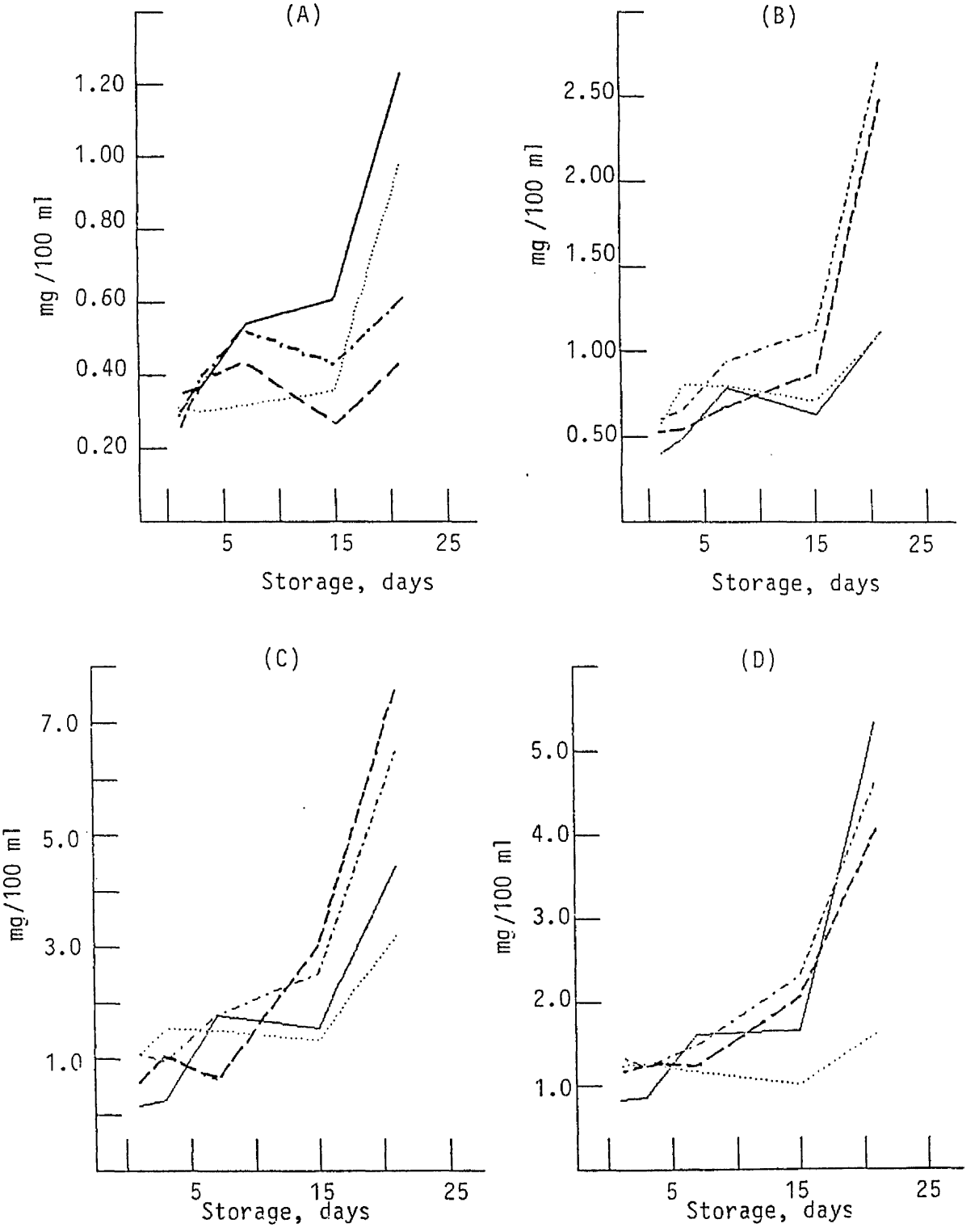


Figure 7.3.2: The response of (A) C:12, (B) C:14, (C) C:16 and (D) C18:0 FFA to pasteurisation on arrival (-), after 2 (---), 4 (...) and 7 (-·-) days of storage at 2°C (DC).

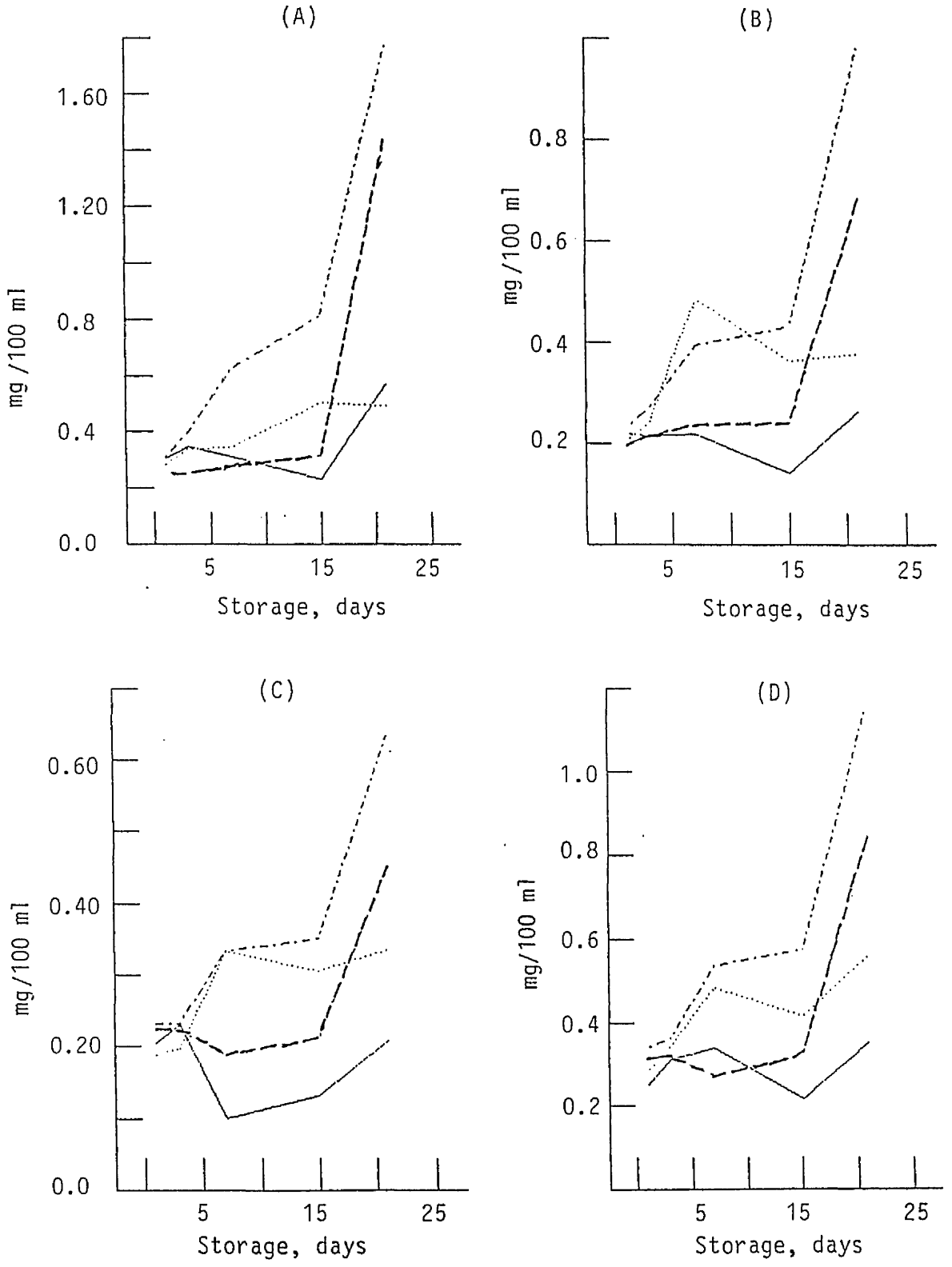


Figure 7.3.3: The response of C:4, C:6, C:8 and C:10 FFA to pasteurisation on arrival (-) , after 2 (---), 4 (...) and 7 (-·-) days after storage at 2°C (DC).

slightly after 21 days of storage. Extending the raw storage period before pasteurisation changed the FFA profile of the milk during storage after pasteurisation. Significant changes in the FFA occured earlier as the extended storage time lengthened. The FFA level of the DC2P and DC4P milks increased significantly ($p < 0.01$) after 7 days of storage. The value of the DC7P milk increased more significantly ($p < 0.001$) and at much earlier time, i.e 3 days.

Section 7.4 The FFA profile of milk pasteurised after 2, 4 and 7 days of storage at 6 ^{o}C following thermisation.

The mean FFA content of the pasteurised thermised milks are given in table 7.4.1 and the changes through storage are illustrated in figures 7.4.1, 7.4.2 and 7.4.3. Analysis of the data indicated that except for C:4 no significant ($p > 0.05$) changes occurred in the short chain FFA over the pasteurised storage time. However, the changes in the long chain FFA were significant, namely C:16 ($p < 0.001$); C18:0, C18:1 ($p < 0.05$) and C18:2 ($p < 0.001$).

Analysis of the treatment means indicated that no significant differences were observed when the short chain FFA were compared with each other. However it was noted that except for C:10 the means level of TP4 milk were higher than the TP2 milk. Nevertheless, the short chain FFA of the TP7 milk were always higher than the TP4 milk.

The longer chain FFA indicated no significant difference ($p > 0.05$) between treatments and the the levels of FFA increased with longer storage time after thermisation.

Table 7.4.1

The mean FFA concentration (mg/100 ml) of milk pasteurised 2 (TP2), 4 (TP4) and 7 (TP7) days after thermisation and storage at 6 ^{o}C. The milk was analysed after 1, 3, 7, 15 and 21 days of pasteurised storage at 5 ^{o}C.

FFA	Treatments				SED[d]
	TP2	TP4	TP7	Means	
C:4	0.352	0.250	0.337	0.313	0.042
C:6	0.207	0.204	0.249	0.220	0.017
C:8	0.193	0.176	0.221	0.196	0.026
C:10	0.293	0.338	0.351	0.327	0.024
C:12	0.356	0.314	0.339	0.336	0.042
C:14	0.687	0.596	0.568	0.617	0.067
C:16	2.642	2.124	1.798	2.188	0.341
C18:0	2.062	1.272	1.314	1.550	0.391
C18:1	4.754	4.599	5.543	4.965	0.702
C18:2	0.862	0.755	0.921	0.846	0.096
Short chain FFA[a]	1.401	1.282	1.497	1.394	0.128
Long chain FFA[b]	11.007	9.346	10.144	10.166	0.765
Total[c]	12.408	10.629	11.641	11.559	0.771

Note: (a) = C:4-C12, (b) = C:14-C18:2, (c) = C:4-C18:2
(d) = SED of treatments mean.

(contd...)

F values for treatment and storage effects and mean square error

Source	df	C:4	C:6	C:8	C:10	C:12	C:14	C:16	C18:0
Treatment	2	2.32	2.59	1.62	2.05	0.33	1.07	1.95	1.62
Storage	4	4.88*	3.97	1.00	1.84	2.71	11.20**	13.22**	5.11*
Mean square error	8	0.007	0.001	0.003	0.002	0.007	0.018	0.46	0.610

Source	df	C18:1	C18:2	Short chain FFA	Long chain FFA	Total
Treatment	2	0.65	0.96	0.89	1.47	1.67
Storage	4	4.45*	11.89**	3.78	29.38**	32.37**
Mean square error	8	1.970	0.367	0.065	2.34	2.38

Note: * = Significant ($p < 0.05$), ** = significant ($p < 0.001$)

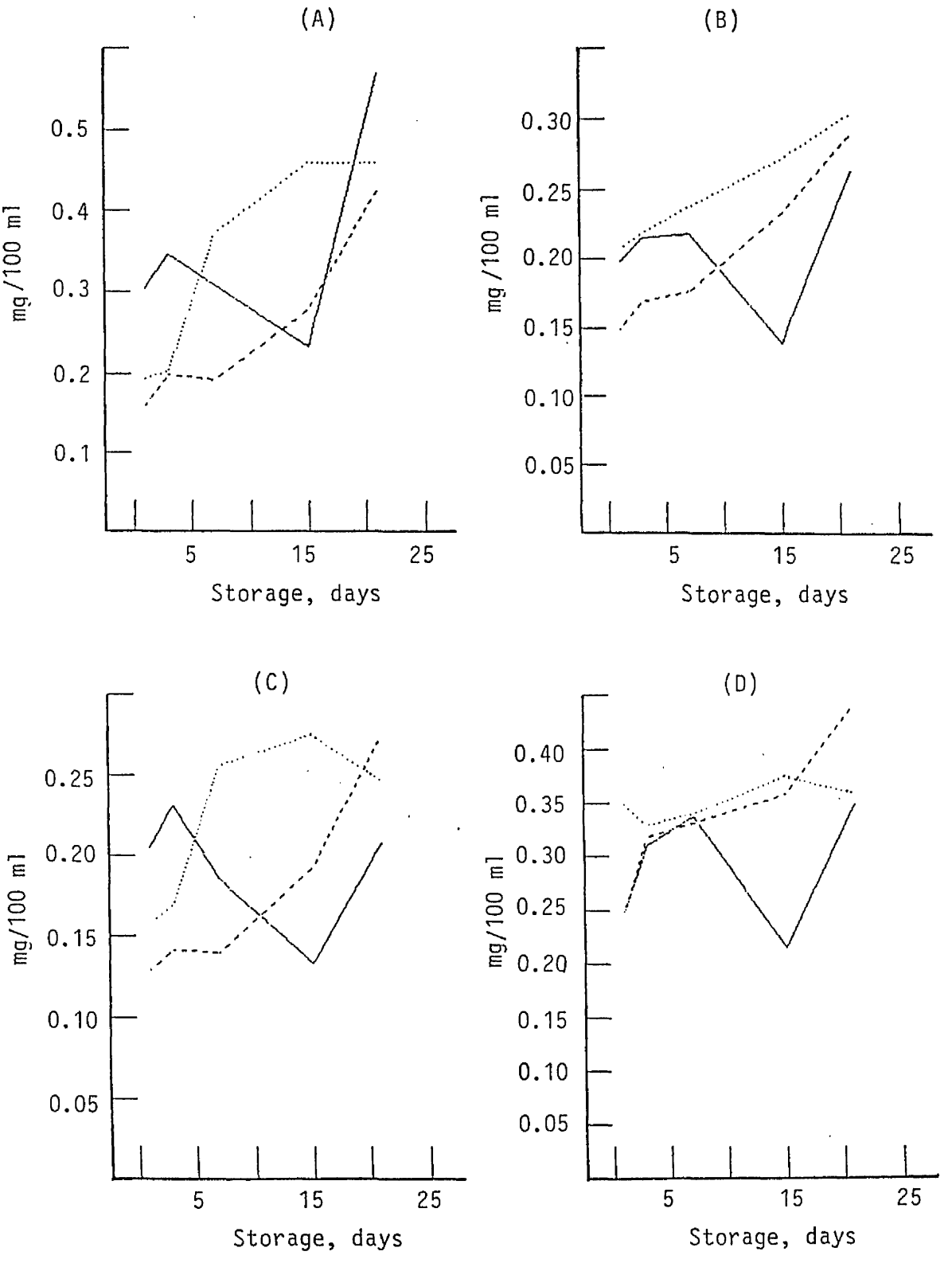


Figure 7.4.1: The response of (A) C:4, (B) C:6, (C) C:8 and (D) C:10 FFA to pasteurisation after 2 (-), 4 (---) and 7 (...) days of thermisation and storage at 6°C.

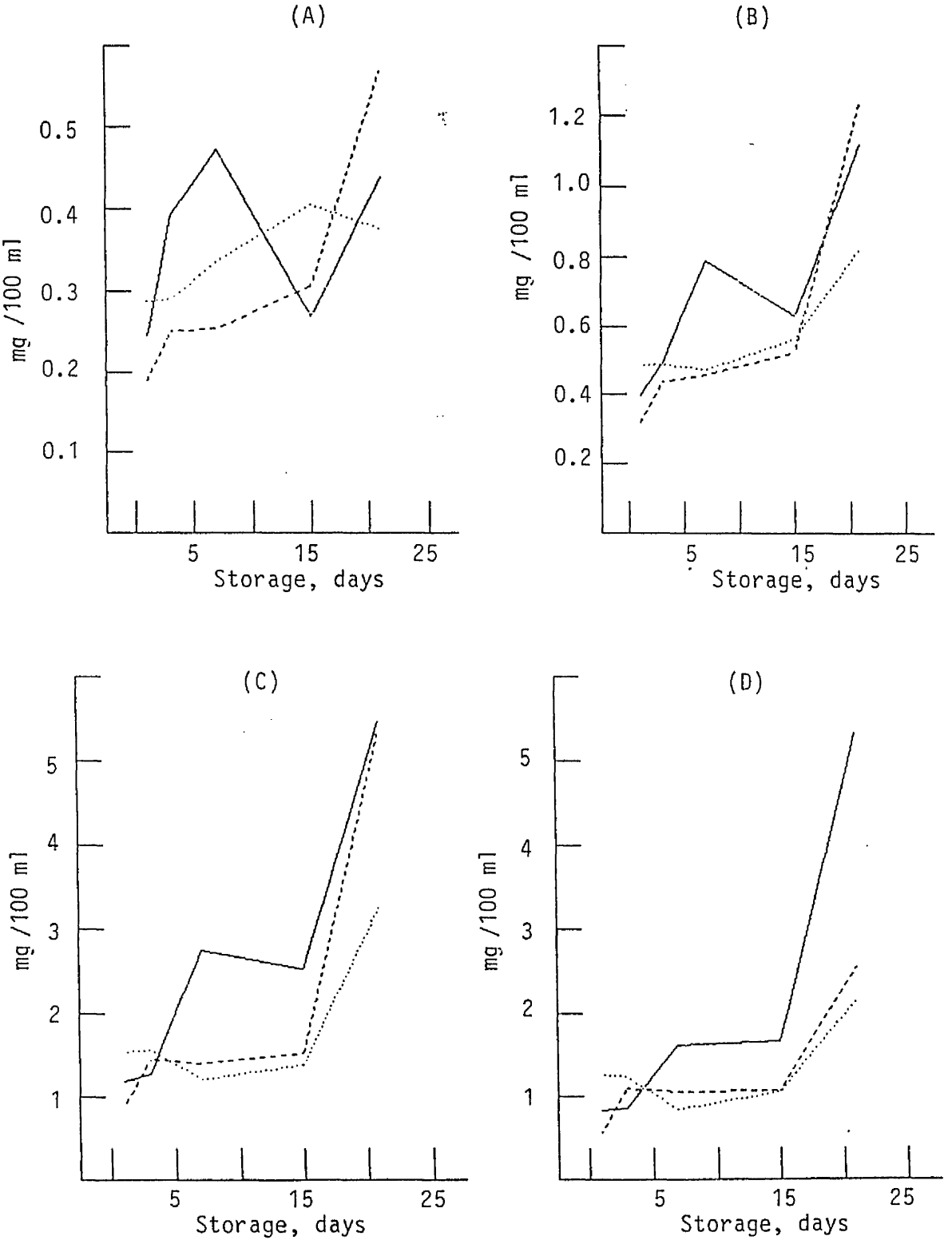


Figure 7.4.2: The response of (A) C:12, (B) C:14, (C) C:16 and (D) C18:0 FFA to pasteurisation after 2 (-), 4 (---) and 7 (...) days thermisation and storage at 6°C.

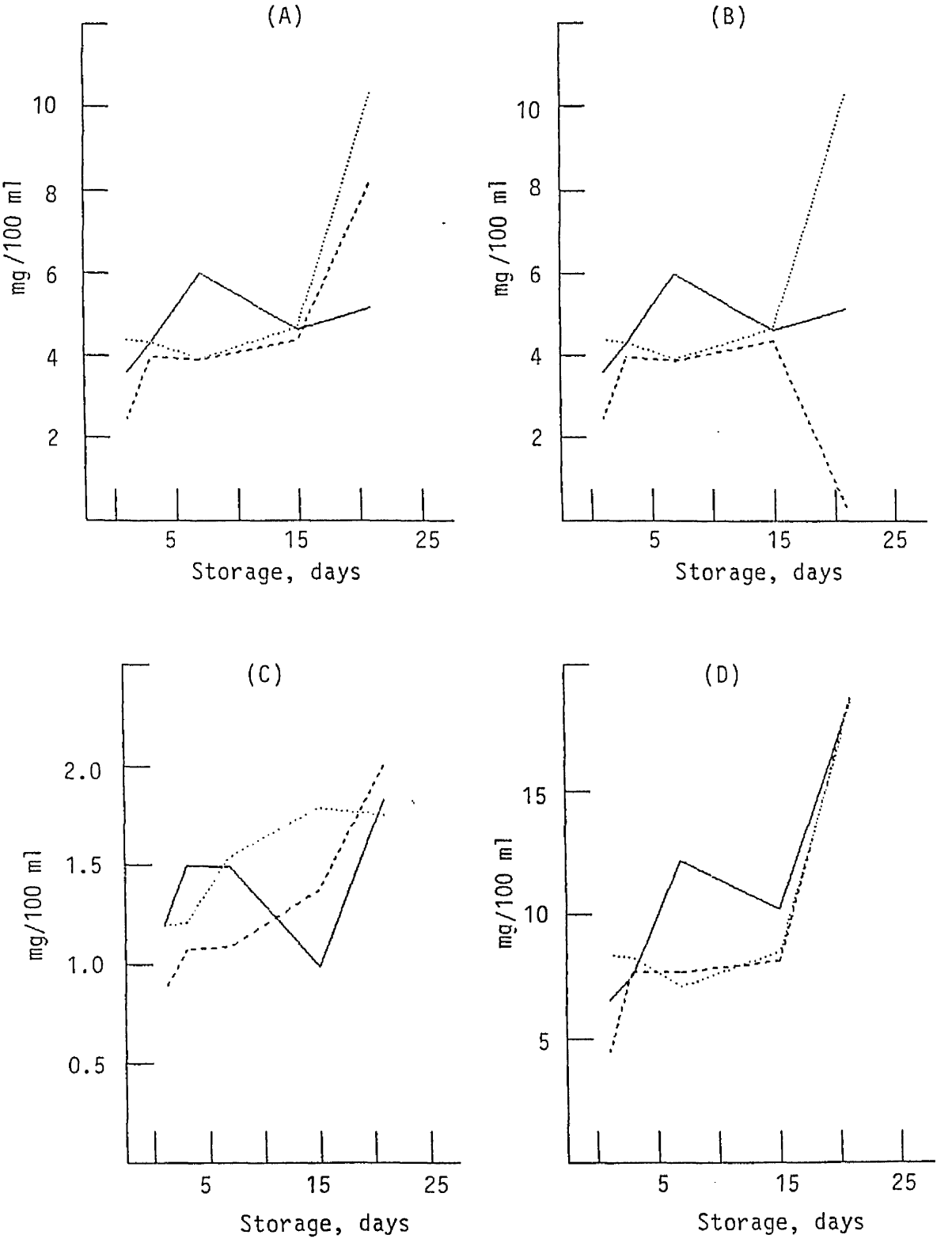


Figure 7.4.3: The response of (A) C18:1, (B) C18:2, (C) short chain (C:4 - C:12), and (D) long chain (C:14 - C18:2) FFA to pasteurisation after storage 2 (-), 4 (---) and 7 (...) days of thermisation and storage at 6°C.

Discussion

Free fatty acids (FFA) content of fresh raw milk is usually less than 1.0 per cent of the total milk lipids, however, they are important, because of their effects on flavours. The lipase catalysed hydrolysis of milk fat triglycerides causes a common defect in fluid milk described as rancid (Dunkley *et al.*, 1968) or preferably called lipolysed flavour (Shipe *et al.*, 1978 and Cousin, 1982). Other descriptive terms such as goaty, soapy and butyric have also been used to describe this off-flavour.

The growth of psychrotrophic bacteria in milk has been related to the flavour changes in the milk and the product made from it. The psychrotrophic bacteria secrete lipases that can break down milk fat triglycerides.

Kuzdzal-Savoie (1980) observed a significantly positive correlation between the lipolysed flavour, fat acidity and short chain fatty acids content of milk. The flavour producing properties of butyric acid is unquestionable as it possesses a foul odour when allowed to vaporise to the atomsphere. However, it is difficult to clearly identify the flavour of butyric acid when tasting a rancid dairy product (Al-Shabibi *et al.*, 1964). They suggested that other fatty acids might have substantially contributed to the rancid flavour of dairy product in combination with C:4.

Scanlan *et al.* (1965) evaluated the effects of added even numbered fatty acids from C4:0 to C18:1 to fresh milk flavour. They observed that no single FFA, added singly,

approximates the flavour of rancid milk.

In a subsequent trial, mixtures of fatty acids from C:4 to C:18 in different combinations were formulated in a different ratios. They observed that fatty acids from C:14 to C:18 contributed very little to the rancid flavour. They concluded that rancid flavour was derived from the lower even numbered fatty acids, C:4 to C:12, not singly, but in combination with one another. No single acid exerted a predominating influence in its flavour contribution.

Al-Shabibi *et al.* (1964) made a similar observation. They concluded that rancid flavour in milk was caused by several short chain fatty acids and most prominently C:10 and C:12.

Al-Shabibi *et al.* (1964) observed that the very short chain fatty acids, namely the formic, acetic and propionic acid do not contribute significantly to the lipolysed flavour defect in milk.

According to Al-Shabibi *et al.* (1964), the off flavors that accompanied the breakdown of milk fat were due to free fatty acids (FFA). Those of chain length C:4-C:8 caused part of the rancid flavor. C:10-C:12 were responsible for most of the unclean, soapy flavors, whereas C:14-C:18 fatty acids make little contribution to flavor.

Released unsaturated fatty acids were succeptible to oxidation, to aldehydes and ketones which give rise to off-flavors described as *cardboardy*, oxidised or metallic (Shipe *et al.*, 1978).

Walstra and Jenness (1984) postulated that typical off-flavor tresholds for total FFA are 1.2-1.5 mEq/100 gm fat for trained experts and 2.0-2.2 mEq/100 gm fat for the average consumer.

However, the sensitivity of the organoleptic detection of lipolysis in milk can be enhanced greatly by acidification (Kuzdzal-Savoie, 1980), but reduced by association of the FFA with proteins in milk (Park and Allen, 1979) and by heating of milk (Kintner and Day, 1965).

Proper pasteurisation destroys all pathogens and a very high percentage of non-pathogenic bacteria in milk. Properly pasteurised milk may be recontaminated with unsanitary equipment. Such contamination often includes psychrotrophic bacteria (Punch *et al.*, 1965; Shehata *et al.*, 1971 and Cousin, 1982) which are commonly responsible for flavour defects in pasteurised 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 (Shipe *et al.*, 1978). However, in milk which has not been cooled to below 4.4 ^{o}C immediately after pasteurisation 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).

In the present study, a strong positive correlation was observed between TPC, PBC and the FFA concentration of milk stored raw at 2 and 5 ^{o}C. Increased FFA concentration

has been reported in refrigerated raw milk in which psychrotrophs were growing (Suhren *et al.*, 1976; Muir *et al.*, 1978 and Sasano *et al.*, 1983). However, counts of about 1.0×10^6 cfu/ml (Punch *et al.*, 1965) were required before any lipolytic spoilage became apparent.

Using this value as a guide the raw milk stored at 2 ^{o}C and 5 ^{o}C would have spoiled 4 days after storage.

Muir *et al.* (1978) stored farm and creamery milk at 4, 6, and 8 ^{o}C and found poor correlation of FFA levels with psychrotrophic bacterial count. Lipolysis was observed only in milk with $> 5 \times 10^6$ cfu/ml, but some milks had about 1.0×10^8 cfu/ml without showing any flavor defects.

Other workers have reported wide variation in lipolytic activity associated with different strains of psychrotrophs (Thomas and Thomas, 1973b and Law and Mabbit, 1983).

Driessen (1983) studied the effects of PBC in raw milk destined for HTST pasteurisation processing in relation to flavour. They observed that 40 per cent of the UHT milk had flavor defects at the end of an average 10.7 day shelf life at 6.7 C, by which time viable counts (mostly psychrotrophic) averaged 1.9×10^6 cfu/ml.

The addition of lipase from psychrotrophic *Pseudomonas* species to milk before UHT treatment can cause flavor defects during subsequent storage. Andersson *et al.* (1981) found that rancidity developed within 1-7 months following storage. The lipase from *Pseudomonas* species was observed 52 per cent as active at room temperature as at 40 ^{o}C, hence, they concluded that storage temperature of UHT was

considered not to be a significant factor.

Mottar *et al.* (1987), observed a significant positive correlation between counts of psychrotrophic bacteria in raw milk and residual lipase in UHT-treated milk. They reported lipolysis after storage for 4 months at 20 ^{o}C. They conclude that only milk with a low count of psychrotrophs should be used for the manufacture of UHT-treated milk.

Most of the common organisms of milk spoilage are believed to have come originally from the soil and the associated plant life and had become indigenous to milk because of the excellence of milk for their growth and persistence (Shipe *et al.*, 1978). Rigorous sanitary procedures were required to limit the initial contamination of milk during its production. Rapid cooling to and holding at 4.4 ^{o}C or below to inhibit the multification of possible contaminants was imperative if the flavour quality of milk was to be maintained until pasteurisation.

Although bacteria may be responsible for a number of different flavour defects in both raw and pateurised milk, only those defects described as acids, malty and fruity (Shipe *et al.*, 1978) can be recognised as being of microbial origin by sensory perception alone.

The microbial genesis of these defects and the specific flavour compounds responsible have been demonstrated unequivocally (Mottar, 1981). The flavour described as stale, barny, unclean, bitter, foreign, rancid and feed

can be caused by bacteria, but determination of the actual cause is often difficult without bacteriological analysis because of the similarities of these flavours due to other causes.

Conclusions

1. The TPC, PBC and the FFA content of raw milk stored at 2 ^{o}C and 5 ^{o}C and that of thermised milk stored at 6 ^{o}C were strongly correlated. However, the relationship were dependent on the storage treatment of the milks.

   i. **Raw storage at 2 ^{o}C**

   The TPC and PBC correlate strongly with the long chain FFA (C:14 - C18:2) but not with the short chain FFA (C:4 - C:12)

   ii. **Raw storage at 5 ^{o}C**

   The TPC correlate with all the FFA (C:4 - C18:2) and more strongly with the short chain FFA (C:4 - C:12), but the PBC correlate only with the short chain FFA (C:4 - C:10)

   iii. **Storage after thermisation at 6 ^{o}C**

   The TPC correlate with the short chain FFA (C:4 - C:12) but not with the long chain FFA (C:14 - C18:2). A similar result were observed with the PBC. However, the correlation of the PBC with the short chain FFA (C:4 - C:12), was not as strongly, as that of the TPC.

2. The FFA (C:4 - C18:2) of the raw milk stored at 5 ^{o}C

increase immediately. In contrast, the FFA of the raw milk stored at 2 ^{o}C and that of thermised milk stored at 6 ^{o}C remain unaltered for 3 days following storage, then increases with storage time, but only slightly.

3. The pasteurisation treatment reduces slightly the FFA content of milks and it influences the FFA profile of milk during the subsequent storage. The FFA remain unchanged for a few days after pasteurisation. The length of time the FFA remain unaltered during subsequent storage were, in turn, influenced by the pre-processing milk storage treatments. The FFA profile of milk pasteurised after 2 and 4 days of raw storage at 2 ^{o}C and 5 ^{o}C were similar but the milk pasteurised after 7 days af raw storage both at 2 and 5 ^{o}C were significantly higher. In contrast, the FFA profile of the pasteurised thermised milk remained unchanged 21 days after storage.

Appendix 1

WSC MILK QUALITY SURVEY

Sample no..... Date.....

Farm's name.....

Address (optional).....

.....

ON-FARM MILK STORAGE

Storage tank size (insulated/refrigerated).....litres

Churns.....litres

MILK PICK-UP

Tanker registration no.....

Total volume of milk pick-up.....litres

Temperature of milk.....^{o}C

Pick-up time.....AM

Arrival time at WSC.....AM

Temperature on arrival at WSC.....^{o}C

NOTE

Please take 2 (two) 200 ml milk samples prior to pick-up. Sterile sample bottles and milk dippers are provided. Fill in the sample bottle then screw on tightly. Place all sample bottles in the insulated box provided.

Before sampling, turn on the milk tank agitator for at least 2 (two) minutes.

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Supplement...

EFFECTS OF LOW TEMPERATURE STORAGE AND THERMISATION ON THE QUALITY OF RAW AND HEAT TREATED MILK

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Submitted for the degree of Ph.D. in the Faculty of Science in the University of Glasgow.
December 1988

SUMMARY

The aim of this study was to investigate the effects of low temperature storage and thermisation on the quality of raw and pasteurised milk.

The study was conducted in two parts. In the first part, the quality of raw milk supplies in the West of Scotland, were investigated. In the second part, the effects of storage of raw milk at 2 ^{o}C and 5 ^{o}C and the effects of thermisation on the quality of pasteurised milk were studied.

The results of the on-farm milk quality study showed little cause for concern. The mean bacterial counts and the mean gross chemical compositions of raw milk supply in the West of Scotland were within the limits stipulated by the Scottish Milk Marketing Board for ex-farm milk quality.

The mean bacterial counts of raw milk increased during handling and transport. Blending of raw milk from various tanker loads, has no immediate effect on the bacterial counts. Storage of raw milk at 2 ^{o}C was found to increase its *keeping quality* by one day as compared to raw storage at 5 ^{o}C.

The development of lactic acid and free fatty acids contents was higher in raw milk stored at 2 ^{o}C than that stored at 5 ^{o}C. No correlation was observed between the development of lactic acid and bacterial counts during storage. Strong correlation between the changes in free fatty acids and the total plate count and between the free fatty acids and the psychrotrophic bacterial count was observed.

The length of raw storage before pasteurisation had no influence on the bacterial counts of the pasteurised milk. The total plate count was reduced to a common level in all

the pasteurised milks regardless of the length of storage and bacterial load before pasteurisation. The subsequent increase in the bacterial counts in pasteurised milk was influenced by the initial raw milk storage temperature.

The effects of thermisation heat treatments (55, 60, 63, 65 and 68 ^{o}C with 16 seconds holding time) on milk quality were studied. Heat treatment at 65 ^{o}C for 16 seconds was found to be the most effective and was recommended as the heat treatment of choice for thermisation, because it was the lowest of the heat treatments investigated which resulted in effective eradication of coliform bacteria and keeping quality extension of 4 days when stored at 6 ^{o}C, without the total plate count and the psychrotrophic bacterial count exceeding 10^{6} cfu/ml. In addition, it was the most severe of the heat treatments investigated that retained a detectable level of alkaline phosphatase activity, as such, if thermisation was followed by pasteurisation, the second and more severe heat treatment does not constitute a double pasteurisation.

The effects of extended storage of raw milk (5 ^{o}C) on effectiveness of thermisation and the quality of pasteurised milk were studied. In terms of bacterial counts, there are no benefits to be gained when the thermised milks were pasteurised. Pasteurisation alone was capable of reducing the total plate count and the psychrotrophic bacterial count to a common level regardless of the initial counts before the heat treatments. However, thermisation was found to be effective in reducing the lipolytic and proteolytic activities in the corresponding pasteurised milk during the subsequent storage.

The relationships between total plate count and

psychrotrophic bacterial count and free fatty acids studied (C:4 - C18:2) were observed to be dependent on storage temperature and the length of storage. At 2 ^{o}C of raw milk storage, the total plate count and psychrotrophic bacterial counts were correlated with the short chain fatty acids (C:4 - C:12). Storage of raw milk at 5 ^{o}C strengthened the correlations between the total plate count and the psychrotrophic bacterial count with all the fatty acids studied. The total plate count of the thermised milk were correlated only with the short chain fatty acids (C:4 - C:10).

The free fatty acids content of pasteurised milk prepared from raw milk stored at 5 ^{o}C were found to be higher than that prepared from milk stored at 2 ^{o}C, irrespective of the length of raw storage period before pasteurisation. The fatty acids content of the pasteurised milk prepared from thermised milk were lower than those prepared from raw milks stored at 2 ^{o}C and 5 ^{o}C and remained unaltered throughout storage.