MICROBIOLOGICAL QUALITY OF HEAT PROCESSED MILKS

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SUMMARY

1. The aim of this work was to investigate the quality of pasteurized milk. The work involved a study of the quality of pasteurized milk from retail sources in Ayr and district. One hundred and thirty-two packs of pasteurized milk were purchased over a five month period from six retail sources - four shops and two supermarkets. The milks were processed by six different dairies. The quality of the milk was assessed using total colony count, psychrotrophic count, lipolytic count, proteolytic count, Bacillus cereus count, coliform count, pH, titratable acidity and phosphatase test. It was found that 6.06 per cent of the samples failed the phosphatese test. However, 13.6 per cent failed the standard for coliform bacteria in pasteurized milk in Scotland i.e. absent in 1/1000 ml of milk. 15.9 per cent of the samples showed a higher total colony count than the maximum count for the 'standard' grade of raw milk in Scotland (< 50,000 per ml).

2. A comparison was made of the microbiological quality of pasteurized milk packaged in glass bottles and single-service containers (SSC) using samples of pasteurized milk obtained from two commercial dairies. The bacteriological tests referred to above were used. The effect of the following factors on the microbiological quality was investigated. (i) storage at $5^{\circ}C$ for 2, 4 and 6 days, (ii) the package i.e. either glass bottles or single-service containers, and (iii) exposure treatment for 3 h to light and temperature conditions simulating the doorstep delivery system. It was concluded from the tests that exposure to simulated doorstep delivery conditions had no significant effect on the microbiological quality during the trials which took place in winter time. The mean microbial counts of pasteurized milk processed in dairy A and packaged in glass bottles were significantly higher than those obtained from pasteurized milk from the same tank packaged in SSC. These differences appeared in some tests made on the day of processing. However, the mean of similar microbial counts of milk processed in dairy B, gave the exact opposite results of those obtained at dairy A. The results showed that there was no significant difference in the microbiological quality of pasteurized milk packaged

in SSC in the two dairies. However, a highly significant difference was found in the bacteriological quality of the samples of pasteurized milk packaged in glass bottles between the two dairies. The empty glass bottles were found to be an important source of post-pasteurization contamination. The micro-organisms which were responsible for the contamination were Aeromonas species, Citrobacter freundii, Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter agglomerans, Pseudomonas species and Serratia liquefaciens. The total colony count of samples of pasteurized milk taken from the pasteurized milk tank in both dairies was not more than 16,000 per ml after 6 days of storage at 5°C.

3. The chemical quality of pasteurized milk packaged in glass bottles and SSC was determined using pH, titratable acidity, dissolved oxygen content, acid degree value, phosphatase test and vitamin C content (ascorbic and dehydroascorbic acid) on the day of processing and after 2, 4 and 6 days of storage at 5°C. From the practical point of view there was no significant difference in the pH and titratable acidity between the milks packaged in the two types of container. The dissolved oxygen content decreased significantly in pasteurized milk packaged in glass bottles during the period of storage. However, there was no significant changes in the dissolved oxygen content in the case of milk in SSC. A highly significant negative correlation was found between the dissolved oxygen content and the total colony count in the samples of milk packaged in glass bottles but not with the milk packaged in SSC. The acid degree value (ADV) of the milk packaged in glass bottles was significantly higher than the milk packaged in SSC during the storage. Pasteurized milk packaged in glass bottles lost 40 per cent of its ascorbic acid content on exposure for 3 h to the light and temperature conditions simulating the doorstep delivery system. Exposure to simulated doorstep delivery conditions had no significant effect on the ascorbic acid content of milk in single service containers.

4. Assessment of the quality of pasteurized milk packaged in the

above two different packaging systems was determined. The appearance scores showed no significant difference between the two packaging systems. There was a slight decrease in the appearance scores during the storage at $5^{o}C$. However, there was a significant decrease in the odour and flavour scores during the storage at $5^{o}C$. There was no significant difference between the two packaging systems from the practical point of view. Fruity, bitter, oxidized, malty and cooked flavours were identified in pasteurized milk in both types of container.

5. All of the pure strains of Bacillus species tested showed no growth after 24 h at the optimum temperature of each on plate count agar to which was added Benzalkon A-50 per cent (100 mg/l) or Janus green (1000 mg/l). However, strains of Pseudomonas, E. coli and Proteus grew on the above medium under the same conditions.

A highly significant correlation was found between the total colony count of pasteurized milk after pre-incubation for 24 h at $25^{o}C$ on the above two selective media and the total colony count of the same milk after 7 days of storage at $5^{o}C$ using a non-selective medium plate count agar.

ABBREVIATIONS

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

ADV	=	Acid degree value
DF	=	Degree of Freedom = n - 1
Glass-E	=	Samples of pasteurized milk packaged in glass bottles and exposed for 3 h to light and temperature conditions simulating doorstep delivery system prior to storage at $5^{o}C$.
Glass-N	=	Samples of pasteurized milk packaged in glass bottles and stored in darkness at $5^{o}C$.
MS	=	Mean of Sum Squares $= \frac{1}{N} \Sigma (x - \bar{x})^2$ = Standard Deviation
REP	=	Replicates
SE	=	Standard Error = V Residual MS
SED	=	Standard Error of Differences of Means = V Residual MS/N (individual variants)
SSC	=	Single-service containers
SSC-E	=	Samples of pasteurized milk packaged in single-service containers and exposed for 3 h to light and temperature conditions simulating doorstep delivery system prior to storage at $5^{o}C$.
SSC-N	=	Samples of pasteurized milk packaged in single-service containers and stored in darkness at $5^{o}C$.
Tank	=	Samples of pasteurized milk taken from pasteurized milk tank
VR	=	Variance Ratio = MS (individual variants/MS Residual.

FOOTNOTE

In referring to studies in which the terms psychrophilic and psychrotrophic are used, the author of this thesis has reproduced the term given in the original work.

MICROBIOLOGICAL QUALITY OF HEAT PROCESSED MILKS

INTRODUCTION

Types of heat processed milks in the United Kingdom and other countries including Iraq

There are three types of heat processed milks which have been produced in the U.K. and also in many other countries.

1. Pasteurized Milk

A pasteurized product is a product which has been subjected to pasteurization; which if retailed as such has been cooled without delay and has been then packaged with minimum delay under conditions which minimize contamination. The product must give a negative phosphatase test immediately after heat treatment (IDF/FIL, 1980).

Pasteurized milk is produced extensively in the U.K., European countries, the U.S.A., the U.S.S.R., Canada, Australia, New Zealand and some other countries with temperate climates.

Ridgway (1970) reported that, in commercial practice, refrigeration could prolong the keeping quality of pasteurized milk to 6-8 days but it was essential that the milk be held at temperatures below 7.2^{o}C (45^{o}F) at all times, to achieve this length of life.

The E.E.C. Regulation (1971) (as amended by E.E.C. Regulation, 1976) permitted the sale of heat treated milk (pasteurized, UHT and sterilized) in three grades according to fat content. These are:

a. * Whole milk with a minimum fat content of 3.5 per cent,

b. Semi-skimmed milk with a fat content of between 1.5 and 1.8 per cent, and

c. Skimmed milk with a fat content of not more than 0.3 per cent.

The E.E.C. also allowed members States to provide for an additional whole milk category with a fat content fixed by them at no less than

*The minimum legally permitted fat content of unstandardised whole milk in the United Kingdom is 3 per cent. (U.K. Drinking Regulations, 1976).

3.8 (e.g. Channel Islands and premium South Devon milks in the U.K.).

In some E.E.C. countries the semi-skimmed milk may be fortified. Further development has been made on this type of milk by the addition of vitamins and the production of flavoured milk (Ashton, 1974).

Ashton et. al. (1979) reported that pasteurized milk has a relatively short keeping quality. Under very well controlled conditions the maximum keeping quality (shelf life) of commercially produced pasteurized milk, stored under refrigeration from the time of production is considered to be of the order of 21-28 days. A shorter keeping quality of 3-7 or 9 days under refrigerated storage could, in certain countries, be considered sufficiently long and satisfactory, whilst in others, a 2-3 days shelf-life might be customary and generally regarded as acceptable.

They reported that pasteurized milk is facing many problems which are related to:

a) the bacterial flora of raw milk,

b) the lipases and proteinases (naturally or bacteriologically developed) which are present in the raw milk prior to the process of pasteurization and the effects of the residual amounts of the heat-stable enzymes present after heat treatment, and

c) the efficiency of handling and treatment of milk which includes preparation of the plant for use, the suitability of the process with plant used and the operational skills involved in processing treatment.

2. UHT Milk

UHT milk is now marketed in the U.K. and most European countries, Kenya, Mexico, Zambia, the U.S.A., Canada, the U.S.S.R. and Iraq.

The UHT product is defined by the IDF/FIL (1980) as a product which has been subjected to UHT treatment, which is packaged in sterile containers under aseptic conditions, and which satisfies the keeping quality tests given in IDF/FIL (1969).

Lang and Lang (1975) stated that UHT milk sold in several variants differing in fat content such as whole milk with 3-4 per cent fat; partially skimmed milk with 1.5 - 1.8 per cent fat, and skim milk with less than 0.3 per cent fat. They said that in some countries such as Switzerland and the German Federal Republic, UHT - market milk of reduced fat content, 1.8 per cent, and enriched with protein, is also available. Flavoured UHT milk also is produced in some European countries.

Burton (1977) reported that this type of heat processed milk was rapidly developed in many European countries, (Italy, West Germany, Switzerland and France), the U.S.A. and Canada, while it was very slow to develop in the U.K. compared to pasteurized milk. Slow development of sales of UHT milk in the U.K. was related to the availability of high-quality pasteurized milk and the maintenance of the household (door-step) delivery system.

The Scottish Milk Marketing Board (1980) reported that UHT milk has been commercially sold in the U.K. since 1965, but in 1979 sales were still under 1 per cent of the total liquid milk market. However, it was 48 per cent in Italy, 37 per cent in West Germany, 36 per cent in Switzerland, 27 per cent in France, 12 per cent in Belgium and 5 per cent in Holland.

3. Sterilized Milk

The sterilized product is defined by the IDF/FIL (1980) as a product which has been subjected to sterilization, either in sealed containers or in continuous flow, and subsequently packaged in sterile containers under aseptic conditions, which satisfies the keeping quality test described in IDF/FIL (1969), and which does not give turbidity when subjected to the modified turbidity test described in IDF/FIL (1972).

Ashton & Romney (1979) reported that sterilized milk has been marketed in Europe since the turn of the 20th century. Originally, it was thought that subjecting milk contained in a glass bottle to a temperature exceeding boiling point for about 30 minutes in a sealed vessel i.e. an autoclave, would be sufficient to kill all the micro-organisms which were present, thus making it sterile. Such treatment, it was considered, would permit milk to have an extended keeping quality of weeks or even months. They defined sterilized milk as milk which after filling into containers in which it is sealed, has been heated to a temperature within the limits 100-135^{o}C for 15 minutes and which gives no precipitate when a sample is subjected to a modified turbidity test.

The higher temperature, which is used to sterilize the milk, causes caramelization of the lactose producing a 'cooked' flavour and a more creamy appearance (National Dairy Council, 1974).

This type of milk is not increasing to the same extent as pasteurized and UHT milks.

The Federation of the U.K. Milk Marketing Boards (1979) reported that the percentage of sterilized milk sold in England and Wales in that year was 7.1, while in 1975 it was 7.6.

Methods of processing - Control requirement - Standards (commercial and legal).

1. Methods of processing used for pasteurized milk

Pasteurization is a process applied to a product with the object of minimizing possible health hazards, arising from pathogenic micro-organisms associated with milk, by heat treatment which is consistent with minimal chemical, physical and organoleptic changes in the product (IDF/FIL, 1980).

Methods of processing used for pasteurized milk are:

a) Holder Method:

In this method raw milk is heated to temperatures in the range of

60 to 62.8^{o}C (140 to 145^{o}F) for 30 min. Most regulations agree that the milk should be heated for 30 min. After pasteurization the milk is cooled to 10^{o}C (50^{o}F) (Herrington, 1948, Hall and Trout, 1968).

In Scotland, pasteurized milk may be produced by the above method, using temperatures in the range of 62.8^{o}C (145^{o}F) to 65.6^{o}C (150^{o}F). The milk should be cooled immediately to not more than 7.2^{o}C (45^{o}F). Indicating and recording thermometers should be installed in suitable places and each recording thermometer chart should be dated and kept for not less than three months. Immediately after cooling, the milk should be put into retail containers or unventilated bulk containers (The Milk(Special Designations)(Scotland)Order, 1965). Different equipment has been used with this method such as either a Vat pasteurizer or a Batch heating system with continuous cooling.

b) HTST Method

This method was developed in the period between 1920 to 1927, and literally means high temperature short time (Burton, 1940). In this method raw milk is heated to 71.1^{o}C (160^{o}F) or higher for 15.5 s or longer (Herrington, 1948). Many different types of equipment are used in this method such as plate heat exchangers, tubular, and scraped surface heaters etc.

Recently in the Annual Session of the IDF in Switzerland, Ashton et.al. (1979) defined pasteurization as a process which involves heating a product to a temperature of less than 100^{o}C for a time sufficient to render it bacteriologically safe for human consumption. They add that, in the case of liquid milk for human consumption, an appropriate heat treatment would be 72^{o}C for 15 s or an equivalent time/temperature combination. In practice the process should be such as to satisfy a test such as that described in the IDF/FIL (1974) for the absence of phosphatase. In certain countries, time/temperature combinations are permitted that will ensure the destruction of the peroxidase enzyme. For liquid milk for human consumption, pasteurization should be followed by immediate cooling and packaging in such a manner as to minimize post-pasteurization contamination.

Pasteurized milk which is produced in Scotland by this method should be heated to not less than 71.7°C (161.5°F) and not more than 78.3°C (173°F) for not less than 15 s. The milk should be cooled immediately to not more than 7.2°C (45°F). The apparatus should be provided with a device (flow diverion valve) which will automatically prevent any milk which has not reached a temperature of 71.7°C (161.5°F) from flowing into the cooler (The Milk(Special Designation) (Scotland)Order, 1965).

2. Methods of processing used for UHT milk

UHT treatment is a process applied to a product in continuous flow with the object of destroying all micro-organisms, or at least inhibiting the growth of any residual micro-organisms, by a high temperature (not less than 132°C)/short time (not less than 0.5 s) heat treatment which gives minimum chemical, physical and organoleptic changes in the product in relation to the severity of the heat treatment required (IDF/FIL, 1980).

The methods of processing used for UHT milk are:

a) Direct heating method

In this method raw milk is heated to temperatures in the range of 135 to 150°C with a holding time of a few seconds followed by aseptic filling of the cooled milk (Burton, 1977). However, Ball (1977) reported that the 'direct' method is to heat the raw milk to 80°C by either vapour or some form of regeneration, after which it passes through a booster pump before steam injection raises the temperature from 145 to 150°C. After a holding period of 2-5 s, the milk is flash cooled to 80°C, then homogenized aseptically and further cooled to a temperature of about 20°C when the milk is packaged. The above method utilises steam injection into the milk or vice versa. Steam is normally used as the heating medium in this method (Aggarwal, 1974; Burton, 1977).

b) Indirect heating method

Ball (1977) reported that the 'indirect' method for producing a

UHT-product was to heat the milk to 85^{o}C by regeneration, homogenize, further heat to 138^{o}C and hold for 2 s before cooling either by cold water, regeneration and final cold water only, to give a filling temperature of approximately 20^{o}C. UHT-temperature, homogenization temperature and final cooling temperatures are controlled.

The Milk (Special Designations)(Scotland) Amendment Order (1966) stipulates that milk should be retained at a temperature of not less than 132.2^{o}C (270^{o}F) for not less than 1 s. The apparatus should be equipped with a device which shall automatically divert the flow of any milk not raised to the authorised temperature. Indicating and recording thermometers should be fitted where necessary by the Local Authority and the recording thermometer charts should be dated and kept for not less than 3 months. Immediately after treatment, the milk shall be aseptically filled into sterile retail containers.

Evette (1976) reported that the equipment used for UHT-treatment of milk is as follows:

a) Direct heating system, this system includes:

i. Steam injection into the milk: The equipment used with this system is the APV Uperiser, Alfa Laval VTIS, and Cherry-Burrell steam injection.

ii. Milk injection into the steam: The equipment used with this system is the Laghuilharre UHT-Sterilizer, Paasch and Silkeborg polaristator).

iii. Friction sterilization e.g. (Seffac UF 400)

iv. IR sterilization (e.g. Actini UHT): In this apparatus homogenized milk at 50^{o}C is heated to 115^{o}C before entering the IR Irradiation section where the temperature is increased to 135^{o}C at a rate of 2.4^{o}C/s. After maintenance at this temperature for 15 s in a high turbulence tubular holder, the milk is cooled by regeneration.

b) Indirect heating system: with this system the equipment includes:

i. Tubular heat exchangers (e.g. the commercial equipment from Stork, Sterideal Cherry-Burrell, Spiratherm etc.).

ii. Plate heat exchangers (e.g. Sordi steriplak, Ahlborn IHS, APV Ultramatic and Alfa Laval Thermodule).

Burton (1977) summarized the sterilization methods which are used to sterilize UHT-milk containers. These methods are, super heated steam, hydrogen peroxide with heat, alchohols and sterile air. He indicated also that the filling methods used with UHT-milk are flow volume determined by time, flow volume determined by carton and volumetric methods.

3. Method of processing used for sterilized milk

Sterilization is a process applied to a product with the object of destroying all micro-organisms, or at least inhibiting the growth of any residual micro-organisms by heat treatment at a temperature exceeding 100^{o}C (IDF/FIL, 1980).

The methods of processing used for sterilized milk are:

a) Batch method

In this method the milk is preheated, homogenized, and filled into bottles prior to sterilization by the batch method using a temperature in the range of 104 to 110^{o}C for 30 to 40 min after which the bottles are removed and left to cool (National Dairy Council, 1974). Ashton and Romney (1979) reported that the process of batch sterilization can be carried out by three methods:

i. in stacks of crates in a static pressure vessel (autoclave),

ii. in a cage which can be rotated within a static pressure vessel (autoclave) or,

iii. in a rotary pressure vessel (autoclave).

b) Continuous method

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

Ridgway (1970) reported that sterilized milk was processed by preheating the milk in a plate heat exchanger to 41.7^{o}C (107^{o}F) after which the milk was homogenized and filled into bottles, sealed and heated to 107.2 to 112.8^{o}C (225 to 235^{o}F) for 30 to 40 min in a batch or continuous sterilizer.

The Milk (Special Designations)(Scotland) Order (1965) stipulates that sterilized milk should be heated to a temperature of not less than 100^{o}C (212^{o}F) for such a period as to ensure that it will comply with the turbidity test. The milk should be treated in capped bottles, so that on completion they are hermetically sealed. The apparatus in which the milk is sterilized should be approved by the Local Authority. Thermometers and pressure gauges should be installed in suitable places.

Standards and legal requirement

The milk (Special Designation)(Scotland) Order (1965 and 1976) stipulates that

a) pasteurized milk should be cooled to 7.2^{o}C or lower, have 10 micrograms or less of p-nitrophenol per ml. in the phosphatase test, and coliform organisms must be absent in 1/1000 ml.

b) Sterilized milk must give a negative reaction when tested for turbidity.

c) UHT milk: the colony count on yeastral milk agar(YMA) must not exceed 1,000/ml after storage for 24 h at 30^{o}C (1 ml of dilution plated in duplicate on YMA at 30^{o}C).

In England and Wales, the half-hour methylene blue test was the official test prescribed by the Milk (Special Designation) Regulation (1963) for pasteurized milk. The milk is aged by storing at atmospheric shade temperature from the time of arrival in the laboratory until testing at 09.30 h on the following day. During the winter months (1 November to 30 April) storage over night (1700 to 0930) is at 18.3 ±1.1°C (65 ±1°F). Samples are regarded as satisfactory provided the methylene blue is not decolorised after 30 min at 37-38°C.

The legal requirements for heat processed milk which were prescribed by The Milk and Dairies (Channel Islands and South Devon Milk) Regulations (1956), EEC Regulation (1971 and 1976), and UK Drinking Regulations (1976) are:

a. Whole heat-treated milk (home production): 3 per cent of fat is the legal minimum.

b. Whole heat-treated milk (imported) 3.84 per cent of fat is the legal minimum.

c. Semi-skimmed milk: 1.5 and 1.8 per cent of fat are the legal minimum and maximum respectively.

d. Skimmed milk: 0.3 per cent of fat is the legal maximum.

e. Channel Islands and South Devon milk: 4 per cent of fat is the minimum.

Industrial procedures including storage of milk before and after processing, distribution and retailing patterns

1. Storage of milk before processing

The temperature of the milk after milking is usually nearly the temperature of the cow. So, if there are contaminants present the milk becomes sour very quickly if it is not cooled. Therefore it must be cooled immediately after milking to a low temperature. Many investigators have studied the condition of the milk before processing and the factors which affect the quality of milk.

In 1959, it was found that storage of milk before processing at a low temperature is an important factor which has an effect on the keeping quality of the products. The milk should be cooled quickly to avoid the growth of psychrotrophic bacteria which are mainly caseolytic, lipolytic, Gram-negative rods and coli-aerogenes organisms and grow in raw milk stored at 3-5^{O}C for three days (Thomas et. al., (1959).

Thomas and Druce (1963) found that Pseudomonas species predominated amongst the 235 psychrophilic bacteria isolated from raw milk after storage at 3 to 5^{O}C for three days. Franklin (1969) indicated that the increase in growth of psychrotrophs in raw milk during collection and storage before processing is due (i) to the increasing use of refrigerated bulk tanks on the farms,(ii) to alternate-day collection of ex-farm milk and (iii) to the large quantity of milk stored at dairies. He found that many strains of psychrotrophs produce lipolytic and proteolytic enzymes during the long storage at low temperature and finally these enzymes produce undesirable off-flavours in the milk after processing because they are not all destroyed by pasteurization.

Crawford (1967) observed that alternate day collected tanker milk of good quality could be held at 5^{O}C at the creamery for one day with very little increase in the bacterial content, but on holding at this temperature for a second day, the bacterial numbers increased sharply.

In another study in 1969, it was found that the psychrotrophic count of raw milk, stored at 7.2^{O}C for 3 days, increased 10-fold while the coliform count doubled and thermoduric and enterococci counts remained unchanged (Hartley, et. al., 1969).

Von Bockelmann (1969) made daily examinations for up to three days of raw milk stored at 5^{O}C in 10,000 l tanks at the creamery. He found that the average total colony count per ml at 30^{O}C for one, two and three days respectively of cold storage were 1.6×10^6, 3.8×10^6 and 17.0×10^6; the average psychrotrophic count per ml was 0.4×10^6, 2.1×10^6 and 11.0×10^6; the coliform count per ml was 87×10^3, 230×10^3 and 780×10^3 and the thermoduric count per ml was 45×10^3, 45×10^3 and 55×10^3. He concluded that milk containing $< 10^6$ bacteria per ml with < 50 per cent of the organisms being psychrotrophs, could be satisfactorily cold-stored at 5^{O}C for 2-3 days before pasteurization.

Thomas (1974) reported that the bulk collected milk sampled from transport tankers on arrival at the creamery usually has a higher total bacterial content than that of milk sampled from tanks at the time of collection. This increase is mainly due to psychrotrophs derived from the road tankers and the ancillary filling and pumping equipment.

In 1975, it was found that refrigeration directly after milking has an effect on the hygienic quality of raw milk. It appeared in the same study that the bacterial growth was very slow during the first 48 h of storage at 5°C or 7°C. A rapid growth of psychrophilic bacteria occurred after 48 h at 7°C and 72 h at 5°C (Vukelic et. al., 1975).

2. Storage of milk after processing

a). Pasteurized milk

It is very important to hold pasteurized milk at low temperature after processing because, in this type of milk, not all the micro-organisms and spores have been destroyed. Therefore it must be kept at a cold temperature until it has reached the consumer.

Many studies have been done on the storage of heat processed milks and the effect of this processing on the quality of the milk. Hempler (1955) found that strains of Achromobacter, Pseudomonas and Alcaligenes predominated in commercially pasteurized milk stored at 7°C for 2 to 5 days. The psychrophilic count had increased slightly in two days, and rapidly after the third day. High counts of psychrophilic bacteria were found in pasteurized milk stored at 7.2°C (45°F) for 5 days (Elliker et. al., 1964). Ciblis (1965) found that streptococci predominatad in pasteurized milk prepared by the HTST method and stored at 10 to 15°C. But when the pasteurised milk was stored at 5°C there was a large number of psychrophilic short rods present. Maxcy (1966) demonstrated that the spoilage of pasteurized milk stored for 14 to 21 days at 5°C was due to Gram-negative organisms. In a study on storage stability of commercial milk, it was found that summer pasteurized milk had twice the shelf-life of winter milk. Summer pasteurized milk stored at 0°C (32°F) was acceptable for 4.4 weeks (Finley _et. al._, 1968).

Mourgues and Auclair (1969) studied the effect of storing commercially pasteurized milk at 4, 6 and 8°C. They found that the average total counts after 8 days at these temperatures were 3.02, 31.5 and 188 million per ml respectively. However, with other samples of pasteurized milk which had a high quality (initial total count 9,350 per ml) after the same conditions of storage were 31,200, 390,000 and 8,100,000 per ml. They found that the flavour defects occurred in the samples when the psychrophilic counts were 2 to 120 million per ml.

Tormo Iguacel _et. al._, (1971) found that the log total count per ml of pasteurized milk packaged in cartons and plastics bottles and stored at 5 and 10°C respectively increased from 3.77 to 5.79 and 6.89. They found coliform bacteria after four days in four samples stored at 5°C and six samples stored at 10°C. They identified two _Escherichia coli_, three _Aerobacter aerogenes_ and one intermediate species.

Credit _et. al._, (1972) indicated that the problem in pasteurized milk stored for a long time at 4.5°C was psychrotrophic bacilli, which can produce off-flavours in pasteurized milk.

In 1973, pasteurized milk was stored at 4 and 8°C. The total count gradually decreased and was followed by a rapid increase. Off-flavour defects appeared after thirty and ten days at the two temperatures respectively. The organisms which were responsible for flavour defects were _Bacillus cereus_, _Bacillus licheniformis_ and _Microbacterium_ species (Mourgues and Auclair, 1973). After two years these investigators found that the temperature of storage for pasteurized milk should be near to 0°C because pasteurized milk still containes sporulating bacteria mainly *B. cereus* ,which survive heat treatment and may grow under refrigerated conditions causing deterioration and off-flavours (Mourgues and Auclair, 1975).

Washam *et. al.*, (1977) isolated 700 cultures of psychrotrophic bacteria from 227 pasteurized milk samples which had a shelf life of > 20 days at 7.2^{o}C. They found that 135 cultures were resistant to heating at 72^{o}C for 16 s and were able to re-establish growth at 7.2^{o}C. The genus *Bacillus* occurred most frequently. The non-sporing types were assigned to the genera *Arthrobacter*, *Microbacterium*, *Streptococcus* and *Corynebacterium*.

Becker *et. al.* (1977) found that the storage life of pasteurized milk at 10^{o}C was much shorter than at 5^{o}C but it did not fall below 5 days. They concluded that the major factors affecting the keeping quality of pasteurized milk were post pasteurization contamination and storage temperature.

b) UHT Milk

UHT milk after processing could be deteriorated by the micro-organisms which either survive the UHT treatment or by post sterilization contamination. Franklin (1970) found that a strain of *B. cereus* produced spores which would survive 135^{o}C for 4 h.

The other limitation to the storage life of UHT milk after processing is the coagulation after prolonged storage which is caused by the slow action of proteolytic enzymes produced by some *Pseudomonas* species in cold storage before processing (Bengtsson, Gardhage and Isaksson, 1973).

Burton (1977) reported that post sterilization is a greater danger which affects the long-life of UHT milk. This may occur either through inadequate plant cleaning and sterilization, through direct atmospheric contamination, or through the aseptic homogenizer.

c) Sterilized milk

Franklin (1970) indicated that the failure level of well produced sterilized milk during storage was about 5-10 per cent. He stated that this failure rate was due to large numbers of thermo-tolerant spore contamination arising from faults in the containers.

Ashton and Romeny (1979) reported that sterilized milk should be stored at a moderate temperature, between 10-15^{O}C (50-60^{O}F) in a manner which would permit daily examination for faulty containers, which, if present, should be removed. They advised that the storage area should be washed at least once per week.

3. Distribution and retailing patterns

Milk is usually distributed direct to consumers or by sub-branches of the same firm or to independent retailers (National Dairy Council, 1974). Storage of pasteurized milk at low temperatures in shops and houses is a very important factor in the quality of pasteurized milk after it has been distributed. In 1969, the poorest quality of pasteurized milk from retail sources was obtained from shops without refrigeration (McLarty and Robb, 1969).

Labuschagne et. al., (1971) reported that bottled milk must be delivered at a temperature of not more than 15^{O}C. These investigators found that the increase in the rate of temperature between the milk and the surroundings was 0.003^{O}C/min for a partially insulated vehicle. This increase in temperature proved unsuitable for delivery of milk below 15^{O}C. They suggested that the increase in temperature must be not more than 0.001^{O}C/min.

Gray (1973) reported that all stages in distribution and handling should be in the temperature range 3.3^{O}C (38^{O}F) to 7.2^{O}C (45^{O}F).

Microbiological and other control tests applied to heat processed milks

There are many tests (microbiological and chemical) which are used to detect the correct treatment of heat processed milks. These tests, which are applicable to heat processed milks may be (i) statutory, (ii) advisory on National or Government level, (iii) advisory within industry, or (iv) laid down by Specification.

1. Microbiological and other control tests applied to pasteurized milk

Järvik (1951) found that the Bacterium coli test afforded a useful check on the extent of post pasteurization contamination of milk, the absence of Bacterium coli in 0.1 ml of milk indicating a satisfactory result, and the absence of this organism in 0.01 ml an excellent condition of cleanliness.

Feagan (1952) used plate and coliform counts, dye reduction tests (modified methylene blue and resazurin) the alcohol test and keeping quality tests to examine samples of pasteurized milk which were all stored at 18^{o}C (64.5^{o}F) for 18 h. He found that the dye reduction tests and alcohol test were the most reliable for the determination of the keeping quality of pasteurized milk.

Day and Doan (1956) used a tetrazolium reduction test to assess the keeping quality of pasteurized milk. They found that this test gave useful results when the milk was stored at 4.4^{o}C (40^{o}F).

Thomasow (1956) determined the keeping quality of pasteurized milk (HTST and flash methods) by the resazurin test (30 min at 37^{o}C), and by taste, smell and coliform titre. He found a general relationship between the keeping quality of the pasteurized milk and the total count but not between the keeping quality and the coliform titre. However, McCallum (1960) reported that the presumptive coliform test was the most reliable for detecting poor quality of pasteurized milk during 1956-1959 in Glasgow, Scotland. He found this result after a comparison between the presumptive coliform test, resazurin test and psychrophilic count.

The Milk (Special Designations)(Scotland) Orders (1965 and 1976) stipulates that any samples of milk taken after pasteurization should be

a) subjected to the phosphatase test and give a reading not exceeding 10 micrograms of p-nitrophenol per ml of milk, and

b) found to contain no coliform organisms in 1/1000 ml of milk.

Elliker et. al. (1964) reported that the psychrophilic count was a more sensitive method than the coliform test for assessing post pasteurization contamination.

In their study on the bacteriological quality of pasteurized milk from a retail source in Edinburgh (Scotland), McLarty and Robb (1969) used the bacterial count (YMA), the Violet Red Bile Agar (VRBA) test, the Most Probable Number (MPN) test, and the Aschaffenburg and Mullen phosphatase test.

Cox (1970) summarised advisory bacteriological tests for pasteurized milk:

a) presumptive coliform test:

(i) Direct sample - coliform must be absent in 1 ml.

(ii) Incubated sample - coliform must be absent in 1 ml.

b) plate count ($30^{0}C$):

No standard (< 30,000/ml noted).

c) keeping quality tests:

(i) High temperature keeping quality (24 h/$26^{0}C$)

(1) Direct sample - < 0.2 per cent acidity, and

(2) Laboratory pasteurized - < 0.2 per cent acidity.

(ii) Methylene Blue Reduction test:

(1) Statutory test - > ½ h reduction time

(2) After 48 h incubation - > ½ h reduction time.

He reported that the failure of any of these tests can be followed up to show whether (a) poor quality raw milk (b) plant contamination or (c) inefficient pasteurization are responsible for potentially poor keeping quality.

Watrous et. al. (1971) assessed the keeping quality of pasteurized milk by the standard plate count, coliform count, and psychrotrophic count. All these tests had been used with commercially and laboratory pasteurized samples held at 4.4 and 7.2°C for 0, 5 and 10 days. In another study in 1971, it was found that the total count was not significantly related to the keeping quality test (assessed by using 68 per cent (v/v) alcohol after pre-incubation at 18°C for 18, 21 and 24 h) on pasteurized milk. However, psychrotrophic and coliform counts showed a correlation coefficient of 0.7259 and 0.7266 respectively (Jooste and Groeneveld, 1971).

Langeveld <u>et. al</u>. (1976) developed a rapid method for the detection of post-pasteurization contamination in HTST - pasteurized milk. In this method the filled package or a line sample of equal volume, is pre-incubated for 24 h at 25°C. Then 0.01 ml of the pre-incubated milk is spread on a plate containing plate count agar to which 100 mg of Benzyldimethyl-alkyl ammonium chloride 50 per cent aqueous solution (Benzalkon A-50 per cent) has been added. It is concluded that re-infection has taken place if colonies have developed with a 24 h incubation period at 30°C. This method is identified as pre-incubation plate count Benzalkon A-50 per cent (P.I.PCBA).

Recently, Schilhabel <u>et. al</u>. (1978) suggested a quantitative test to determine the recontamination in pasteurized milk. The test consists of streaking different amounts of milk (after 24 h enrichment at room temperature) onto 2:1 plate count: violet red bile agar, and incubating at room temperature for 48 h. The re-contamination titre is determined by the MPN method. They reported that the test will detect recontamination rates of a few bacteria per 100 ml milk.

2. <u>Microbiological and other control tests applied to UHT milk</u>

The IDF/FIL (1969) suggested standard tests for UHT milk.

a) Tests to be done before incubation in one of the container/ samples.

(i) Stability test with aqueous ethanol 68 per cent (v/v)

(ii) Titratable acidity expressed as g of lactic acid per 100 ml of milk

(iii) Direct microscopic count.

(iv) Organoleptic examinations: presence or absence of precipitate, sediment, abnormal odour or taste.

If the milk passes the ethanol stability test, the following tests require to be done.

b) Tests to be done after incubation at 30 ±1°C for 14 days and 55 ±1°C for 7 days (under conditions of temperate climate, incubation at 55 ± 1°C can be omitted).

(i) Stability test with aqueous ethanol 68 per cent (v/v).
(ii) Titratable acidity expressed as g of lactic acid per 100 ml of milk.
(iii) Colony count according to the IDF/FIL (1970) using an inoculum equivalent to 0.1 ml of milk.
(iv) Organoleptic examinations: presence or absence of precipitate, sediment, abnormal odour or taste.

c) Specification of satisfactory sample.

(1) Before incubation: samples must pass the ethanol stability test.

(2) After incubation:

(i) Sample must pass the ethanol stability test.
(ii) Titratable acidity should not differ from what it was before incubation by more than 0.02 expressed as g of lactic acid per 100 ml of milk.

(iii) Colony count should not exceed 10 per 0.1 ml of milk.

(iv) The odour and taste of the samples should not be different from that normally obtained by prolonged incubation of UHT milk.

(v) The physical appearance should be normal and no trace of coagulation or proteolysis should be present.

The Milk (Special Designations)(Scotland) Order (1976) stipulates a colony count for UHT milk, the count of the milk is determined on an unopened package held for 24 h at 30°C. The count should not exceed 1000 per ml.

Nakanishi., et. al. (1976) used the standard plate count, psychrophilic count, pH, acidity, alcohol test, clot-on-boiling, protein and non-protein N or free tyrosine to assess the quality of UHT milk. However, Burton (1977) used oxygen content, ascorbic acid, and folic acid to assess the quality of UHT milk.

3. Microbiological and other control tests applied to sterilized milk

Many tests have been suggested for sterilized milk. The IDF/FIL (1969) suggested a control test for sterilized milk as described in the UHT milk referred to above.

The turbidity test is the best one used to date. However, Ridgway (1955) found that there was a relationship between the spore count of milk and the keeping quality after commercial sterilization, and he suggested that the spore count is a more suitable test for sterilized milk.

Packaging of heat processed milks and the relationship between the package and the microbiological quality

The microbiological quality of milk can be affected by the packaging material and the packaging machinery. In 1957, it was found that the paper used to prepare the package in the Tetra Pak system was bacteriologically satisfactory and no significant increase in bacterial content of the milk was caused by the packaging process (Pedersen et. al., 1957). Hamann (1960) compared the quality of milk in Tetra Pak containers with that packaged in glass bottles. He found that bacterial growth in milk packaged in Tetra Pak containers and stored for 16 h at 13 to 17°C proceeded more slowly than in milk packaged in glass containers under the same conditions, but at 5 to 7°C the difference was small. Smith and his co-workers (Smith et. al., 1967) reported on the quality of pasteurized milk packaged in single-service containers and stored at 1.6°C and 4.4°C

(35^{O}F and 40^{O}F). They found that the bacterial counts of milk packaged in a plastics and paper laminate reached the maximum bacterial standard of the U.S.A. (< 25,000 per ml) for pasteurized milk after 3 to 9 days storage, whereas milk packaged in pre-formed waxed paper containers did not reach the maximum count standard until 6 to 12 days at 1.6^{O}C and 4.4^{O}C respectively.

Cox and co-workers (1970) reported that most milk sold in Australia was packed in glass bottles and capped with aluminium foil. Wax coated cartons have been available for many years but have been used only in relatively small quantities. The Tetra Pak and Tetra Brik packages have also been used. Plastics sachets had been introduced but at that time it was too soon to assess the acceptability of them. Interest was also being shown in single-service blow-moulded plastics bottles.

Langeveld (1971) compared the quality of samples of pasteurized milk packaged in single-service packages and bottles and stored at 5^{O}C. The shelf-life of the samples was 8 to 23 days. In the same study when pasteurized milk was packaged in Tetra Brik packs with an intermediate aluminium layer in the laminate and stored at 5 and 7^{O}C the shelf-life at 5^{O}C was 16 to 28 days. It was found that storage at 7^{O}C reduced the shelf-life of pasteurized milk. However, there was no conclusion drawn from a comparison of the milk packaged by the two different systems.

Lubieniecki (1972) reported that the influence of packaging on the contamination of food may be directly due to the presence of microorganisms on the packaging material or indirectly due to the permeability or impermeability to air and water vapour.

Abdus Sattar and de Man (1973) studied the effect of packaging material on light-induced quality deterioration of milk. They exposed the milk to fluorescent light at intensities of 100 or 200 ft.C at a temperature of 5^{O}C for periods of 3, 6, 12 or 24 h. Four different types of packaging material were used, i.e. clear and opaque pouches, cartons, and plastics jugs. Off-flavours were detected in all the containers except in the opaque pouches. Significant losses of ascorbic acid and riboflavin occurred in milk packaged in all the containers except in the opaque pouches.

Schonbörn et. al., (1975) compared pasteurized milk packaged in three different types of container:

(i) with no air space,

(ii) upright package with roof-shaped air space, and

(iii) an oblong package with roof-shaped air space.

Samples were taken after storage for 1, 5 and 8 days and examined organoleptically as well as bacteriologically. The results showed that milk in container type (i) had a higher flavour score than that packaged in types (ii) and (iii) after one day's storage at 4 to 5^{o}C. Bacteriological results showed that the milk packed in system (iii) containers had a significantly higher colony count than that packaged in type (i).

Recently, Hankin et. al., (1977) showed that pasteurized milk packaged in glass bottles deteriorated in a shorter time than that packaged in paper cartons, Takeda et. al. (1979) found with UHT milk, that there was no difference in the keeping quality of milks packaged in bottles or Tetra Pak cartons and stored at 7^{o}C for 9-10 days.

Luquet et. al. (1977) packaged UHT milk in five different types of package. These investigators studied the effect of the method and the type of package on ascorbic acid, riboflavin, folic acid, and organoleptic properties. They found that UHT milk in plastics bottles rapidly became unacceptable in relation to smell and taste when the bottles were exposed to light.

The effect of the thickness of the package has been studied, Leali and Quaroni (1967) used three thicknesses (9/100, 12/100 and 15/100 mm) of polythene for packaging sterilized milk. Samples were stored at 20 and 32^{o}C for 20 days and at 55^{o}C for 8 days. On examination they found that a number of samples of milk in containers of the different thicknesses of material were not sterile; 32 of the 35 samples of milk packaged in 9/100 mm polythene were unsterile, 3 out of 92 samples packaged in 12/100 mm were unsterile and 2 out of 94 samples of milk packed in 15/100 mm polythene were unsterile.

Recently, Burton (1977) reported that oxygen-permeable containers such as blow-moulded polythene containers are not suitable for UHT milk. He summarised the type of containers available for UHT milk as follows: cans, cartons formed from roll material, cartons assembled from pre-formed blanks, plastic film, form-fill-seal, plastics film sachets and blow-moulded thermoplastic bottles. In another study in the same year, Tylkin et. al. (1977) packaged UHT milk (140^{0}C for 4 s) in (i) normal polyethylene-coated Al-foil paper packages, (ii) waxed paper packages with non-pigmented polyethylene and (iii) experimental packages without Al-foil but with black-pigmented polyethylene. After the packs of UHT milk had been stored at 19 to 22^{0}C for 30 days they were examined and it was concluded that UHT milk packaged in laminated polythene without foil cannot be guaranteed as satisfactory for more than five days.

The microbiology of raw milk and heat-processed milks and the inter-relationship of the same:

Franklin et. al. (1956) reported that the raw milk in the U.K. contained about one resistant spore per ml. They indicated that the spore content of raw milk is higher in winter than in summer.

Thomas and Druce (1963) found that *Pseudomonas* species predominated amongst the 235 psychrophilic bacteria isolated from raw milk, but about 36.6 per cent of the organisms present were fluorescent strains and 57.6 per cent non-fluorescent or *Achromobacter*.

Franklin (1969) reported that *B. cereus* was invariably found in raw milk even under the best production conditions.

Druce and Thomas (1972) indicated that the main direct source of psychrotrophic bacterial contamination of raw milk was poorly cleaned pipeline milking plants and bulk tanks.

In a review, Thomas and Thomas (1976) reported that the microflora of farm milk tanks showed a low incidence of streptococci and a relatively low incidence of micrococci and corynbacteria, but a remarkably high incidence of Gram-negative rods especially in poorly cleaned tanks.

Davis (1977) studied the bacteriological quality of the bulk and churn collected milk supplies in Wales. He found that the colony count in churn milk was more than in the bulk supplies.

In sterilized milk, it has been found that spores of Bacillus subtilis predominated. The maximum spore count (after 105°C for 15 min) of mesophilic bacteria was 60 spores per 100 ml. The maximum count of resistant thermophilic spores was about 80 per ml. In the same study 204 cultures were isolated from spoiled samples of sterilized milk and it was established that 90 per cent were B. subtilis. Strains of Bacillus pumilus, Bacillus coagulans, Bacillus cereus and Bacillus licheniformis were also present (Ridgway, 1955).

In another study, it was found that the spoilage of in-bottle sterilized milk was due to Bacillus stearothermophilus and B. subtilis (Grinsted and Clegg, 1955).

Franklin (1970) reported that 20 per cent of in-bottle sterilized milk in the U.K. contained mesophilic bacterial spores after processing.

B. subtilis, B. licheniformis, Bacillus megatherium, B. cereus, B. pumilus, B. stearothermophilus and Bacillus pulvifaciens were isolated from the Tetra Pak containers of uperised milk (Garibaldi et. al. 1965).

Cox (1975) reported that it was very rare for the spores from normal raw bulked milk to survive the UHT heat process. He indicated that in the absence of specific packaging problems such as inadequate steam or faulty H_2O_2 sterilization of packages, the spoilage rate was less than 0.1 per cent.

Several authors (Randolph et. al., 1965; Langlois et. al., 1966 and Overcast, 1967) have suggested that the most important bacteriological problems in pasteurized milk in the supermarket today are related to the psychrotrophic bacteria.

It has been found that strains of Achromobacter, Pseudomonas and Alcaligenes predominated in pasteurized milk stored at 7°C for 2 to 5 days. Small numbers of coli-aerogenes bacteria have sometimes been found (Hempler, 1955). Papavassiliou (1956) found that Clostridium perfringens, Clostridium sporogenes and Clostridium butyricum were the most common anaerobic spore formers in pasteurized milk.

Many studies have been made of the effect of the quality of raw milk on the keeping quality of heat processed milk. Ashton (1950) found that the ageing of raw milk at 4.4 to 10°C for 24 h before pasteurization increased the peptonising microflora and reduced the keeping quality of the corresponding pasteurized milk. In a study designed to determine the relationship of the numbers and types of bacteria in raw milk to the initial flavour score of the processed milk, Glenn (1958) obtained results which indicated that the numbers and kinds of bacteria in raw milk had an effect on the initial flavour score of processed milk.

In India, Varma et. al. (1959) found that the bacterial count after pasteurization was related to the count of raw milk. However, Storgårds (1961) showed that the bacterial content of raw milk had little effect on the bacterial content, flavour, and keeping quality of HTST pasteurized milk. He also reported that there was a good correlation between the thermoduric bacteria in raw milk and the keeping quality of HTST pasteurized milk.

Franklin (1965) found that there was no correlation between the bacterial counts of raw or pasteurized milk and the keeping quality of pasteurized milk. The most important factor was the type of organisms which were present in pasteurized milk. He indicated that B. cereus is the most important organism which affects the keeping quality of pasteurized milk.

Thomas and Druce (1969) reported that raw milk produced under satisfactory hygienic conditions resulted in pasteurized milk with a satisfactory keeping quality, but the addition of 1 per cent of an unsatisfactory raw milk supply to the bulk tank before commercial pasteurization caused a marked deterioration in the keeping quality of the pasteurized milk. Lerpido et. al. (1970) found that raw milk with high bacterial counts gave a correspondingly high count after pasteurization. In another study, it was found that pasteurized milk produced from raw milk containing more than one million bacteria per ml frequently developed objectionable flavour on storage (Patel and Blankenagel, 1972). Recently, Davis (1975) indicated that the troubles with pasteurized milk are nearly always caused by the poor quality of raw milk.

The effect of various stages of handling, storage etc. on the keeping quality of heat processed milks

In 1954, it was reported that the open cooler, vacuum storage tanks, pipelines leading to the bottle-filling machine and the bottles were important sources of re-contamination of pasteurized milk (Pothmann and Hillebrand, 1954). In two other studies in 1959 and 1973, it was shown that the bottle filler was the most common source of coliform contamination in commercially pasteurized milk (Glenn and Olson, 1959; Kalina et. al., 1973). Ogawa et. al. (1966) reported that the contamination with psychrophilic bacteria during the bottling process was an airborne contamination. Cannon and Reddy (1970) found that the air pulled through the filler bowl at a rate of 3000-6000 l per min by the defoamer is an important source of the contamination of pasteurized milk with psychrotropic bacteria.

Franklin (1969) found that not all spoilage enzymes were destroyed by the pasteurization temperature and he indicated that extended cold storage of raw milk before processing affected the keeping quality of pasteurized milk because of the action of enzymes produced by the proteolytic and lipolytic bacteria. Labuschagne et al. (1971) indicated that high temperature of delivery and distribution affect the keeping quality of pasteurized milk.

It has been found that the homogenising process may split up clumps of bacteria and disrupt the fat globule membrane so possibly activating bacterial growth. The inclusion of a homogeniser in the processing line may introduce another source of contamination (Davis, 1975).

Hermier and Bergere (1959) found that the source of contamination of the sterilized milk was the bottle-filler, which is difficult to clean.

In the case of UHT milk, it has been found that there are many sources of contamination with mesophilic spores. These sources are the equipment, pipelines and the air of the dairy (Labots et. al., 1960). It has also been shown that the cooling section of the processing equipment is an important source of contamination (Wasserfall, 1972).

Burton (1977) reported that the sources of post-sterilization contamination in UHT milk were (i) inadequate plant cleaning and sterilization, (ii) direct atmospheric contamination, (iii) the aseptic homogeniser, (iv) aseptic balance tank and (v) aseptic filler.

Comparison of different methods of assessing the quality of heat processed milks

Several investigators have given particular attention to the development of suitable methods for assessing the quality of heat-processed milk. Their attempts were based on comparing different methods to find an accurate as well as quicker test for estimating the quality of heat-processed milks.

Kheshgi (1948) compared brilliant-green lactose bile broth, formate ricinoleate broth, violet-red agar and desoxycholate agar for the detection of coliform organisms in milk. He found that the latter medium was the best presumptive medium for pasteurized milk. He observed a general relationship between the total colony count and the coliform count in raw milk but not in pasteurized milk.

Pirauz et. al. (1953) indicated that, with pre-incubated samples, methylene blue test is as accurate as and quicker than coliform test for estimating the keeping quality of pasteurized milk. They also found that the keeping quality of pasteurized milk is related to the coliform count rather than the thermoduric count. However, Thomasow (1956) found that there is a general relationship between the keeping quality and the total colony count, but not between the keeping quality and the coliform titre when these methods were used to assess the quality of pasteurized milk.

In studies concerned with methods for assessing the keeping quality of pasteurized milk Ciblis and Schwarz (1960) reported that the number of coliform bacteria determined on metachrome - yellow agar and on fuchsin - lactose agar was higher than on the triphenyl tetrazolium chloride agar (TTC Agar). However, in another study in the same year Havelka and Hudec (1960) found that the TTC medium was the most suitable of seven different media to estimate coliform bacteria in the milk.

In 1964, it was found that the psychrotrophic count was more sensitive than the coliform test in detecting post-pasteurization contamination (Elliker et. al., 1964). Labots and Hup (1964) enumerated Bacillus cereus in pasteurized milk using the following methods:

(i) flocs count: the number of flocs formed in the cream layer of pasteurized milk,

(ii) estimation of the most probable number (MPN) of B. cereus spores using a dilution method,

(iii) centrifugation method: spore count in the sediment of centrifuged milk using incubation in egg-yolk medium, and

(iv) filtration method: enumeration using a membrane filter technique.

They found that the MPN method gave the highest numbers, followed by the centrifugation method, then the flocs count. The filtration method gave the lowest results.

Recently, the spiral plate count method (SPLPC) and standard plate count (SPC) methods were used to detect raw and pasteurized milk. The results showed that the variance of SPLPC was slightly less than that of the SPC method. It was suggested that the SPLPC method could be substituted for the SPC method (Donnelly et. al., 1976).

In the Netherlands, Langeveld et. al.,(1976) used different selective growth inhibitors with plate count agar (Difco) in attempts to find a rapid and sensitive method to detect post-pasteurization contamination in pasteurized milk. They found that 100 mg of Benzalkon A-50 per kg. is better than other selective agents. They compared this method with the pre-incubation coliform test, and found that re-infection was detected more effectively by the plate count method with Benzalkon A-50 added than by the coliform method.

Possibilities for establishing a new procedure for the quality control of heat processed milks

There have been many attempts to find a more effective method for use as a quality control test for heat processed milk. One of these attempts is based on the possibility of using the pyruvate content in the milk as an indication of keeping quality. The second is based on preliminary incubation of the milk sample before dye reduction tests. The third is to use a selective medium to detect post-pasteurization contamination.

In the Netherlands Institute for Dairy Research (NIZO) (1976), a rapid method was developed for the detection of post-pasteurization contamination in milk using plate count agar (Difco) with 100 mg per kg of benzyl-dimethyl-alkyl-ammonium chloride (50 per cent aqueous solution). This medium prevents any Gram-positive bacteria from growing. This method was designed especially to detect post-pasteurization contamination with Gram-negative psychrotrophic bacteria.

Many studies have been made of the use of the pyruvate content as an indicator of the quality of milk. Heeschen _et. al._ (1975) reported that pyruvate is not affected by pasteurization, and so it could be used to indicate the quality of milk from the farm to the consumer.

Springmeyer _et. al._ (1976) estimated the quality of raw milk by measuring the pyruvate content using a Technicon Auto-Analyser. They found that this test is suitable for cold milk ($< 4^{o}C$) delivered once daily. They also indicated that the pyruvate content reflected the presence of non-acid forming and acid forming organisms. Suhren _et. al._ (1976) used measurements of the pyruvate content to assess the spoilage of pasteurized milk. They found that the pyruvate content increased at the same rate as the bacterial content with the storage time. They suggested that further studies on a large number of milk samples of different origin were needed to evaluate the method.

Recently, Marshall and Harman (1978) indicated that somatic cells contribute to the pyruvate content of raw and pasteurized milk. They suggested that it is possible to use the pyruvate test in controlling the quality of bulked raw milk and the keeping quality of pasteurized milk.

Scope of work

Considerable attention has been directed recently to the keeping quality of heat-processed milk due to the extensive development of relatively prolonged refrigeration of the milk at the farm, dairy, retail sale and the consumer's house.

Quality of pasteurized milk could be affected by many factors such as quality of raw milk, handling, processing, distribution and the storage in the market and consumer's refrigerator.

This study was concerned with the microbiological, chemical and organoleptic quality of pasteurized milk because pasteurized milk is the main heat-processed milk produced in Scotland.

This study started .. a short survey on the quality of pasteurized milk from retail sources in Ayr and district in south-west Scotland. Then a comparison of the microbiological, chemical and organoleptic quality of pasteurized milk packaged in glass bottles and single service containers was made.

CHAPTER ONE

MATERIALS AND METHODS

SECTION 1. MICROBIOLOGICAL METHODS

1. Total plate count

The total plate count was determined according to the IDF standard method 3; 1958 (IDF/FIL, 1958). Serial 10-fold dilutions of the milk were made in quarter strength Ringer's solution. The dilutions were then plated in duplicate by adding 1 ml to each of two petri dishes to which was then added 10 ml of plate count agar (yeast extract 2.5 g, tryptone 5 g, dextrose 1 g, agar 15 g, distilled water 1000 ml, separated milk 10 ml, pH 7). When used the medium was tempered to 45^{o}C. The colonies were counted after 72 h incubation at 30^{o}C. Only plates with colony counts between 30 and 500 were used for recording results. If more than one dilution gave a count within this range, the higher result was selected.

2. Psychrotrophic count

Psychrotrophic bacteria were determined as described by the American Public Health Association (1978). Serial ten-fold dilutions of the milk were made using a phosphate - buffered sterile water medium. The dilutions were selected so that the total number of colonies on a plate were likely to be between 30 to 300. The selected dilutions were plated in duplicate and the dishes were incubated at 7^{o}C for 10 days. The plating medium had the following composition:

> pancreatic digest of casein 5 g, yeast extract 2.5 g, glucose 1 g, agar 15 g and distilled water to 1 litre. The final pH was 7 ±0.2 after sterilization at 121^{o}C for 15 min.

3. Lipolytic count

These bacteria were determined according to the IDF standard method 41; 1966 (IDF/FIL, 1966). The outline of the method is as follows:

a. preparation of the dilutions. The samples of milk were diluted ten-fold using sterile quarter strength Ringer's Solution.

b. preparation of the butter fat. Fresh butter was melted, and the serum separated and filtered.

c. preparation of victoria blue dye base. 2 - 3 g of victoria blue were boiled with 200 ml tap water until dispersed. 10 per cent (W/V) NaOH was added until the blue colour changed to brown. The insoluble precipitate was allowed to settle before filtration. The dye base was washed with water which was made slightly alkaline with NH_4 OH and dried at 30^{o}C.

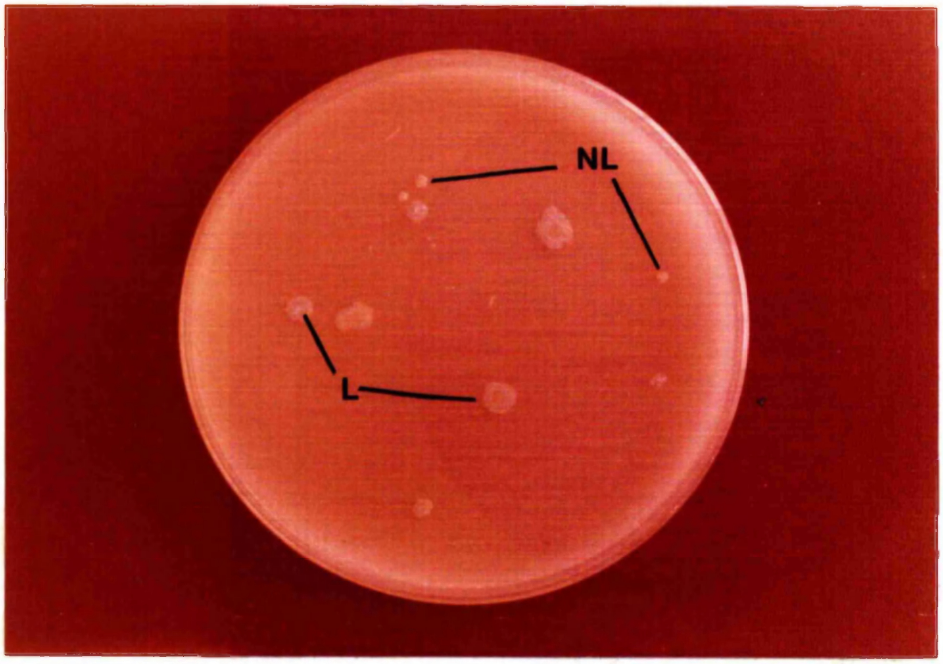
d. dissolving of victoria blue dye base in the fat. 75 mg of victoria blue dye base were dissolved in 100 g of butter fat. Then the mixture was stirred while using a water bath to maintain the temperature at 90^{o}C until no undissolved dye particles were seen. Then the coloured butter fat was filtered, dispensed in 5 ml quantities and sterilized for 10 min at 115^{o}C. The dyed fat was stored at 5^{o}C until required but the storage time was not more than two months.

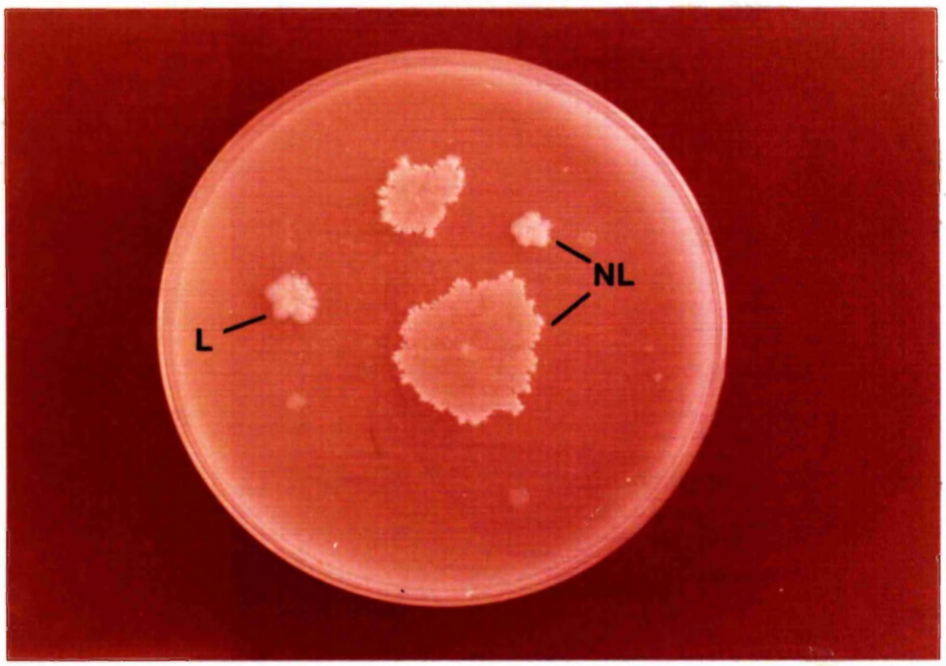
e. preparation of the nutrient agar. The medium was prepared to the following specification: peptone 5 g, meat extract 5 g, agar 20 g, distilled water to make the volume up to 1 litre. The medium was boiled until the ingredients were dissolved and the pH adjusted to 7.5. The nutrient agar was dispensed in 100 ml quantities in screw cap bottles and sterilized by heating for 15 min at 121^{o}C. The medium was stored at 5^{o}C until used.

f. method of use. The nutrient agar was melted and cooled to 45^{o}C. Five ml of the melted dye was added to 100 ml quantities of nutrient agar. The bottles were closed and the dye fat emulsified by vigorous shaking and poured immediately into petri dishes. These were dried for 20-24 h in the dark. o.1 ml of

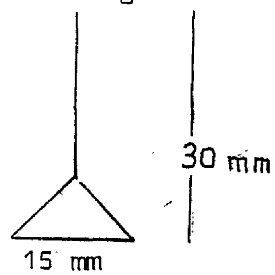
PLATE 1:1

Lipolytic (L) and non Lipolytic (NL) colonies of bacteria from pasteurized milks on nutrient agar containing Victoria blue dye after 3 d at 30°C





the sample dilution was spread on the surface of the agar using a platinum wire spreader. The dimensions of the spreader are shown in the drawing below.



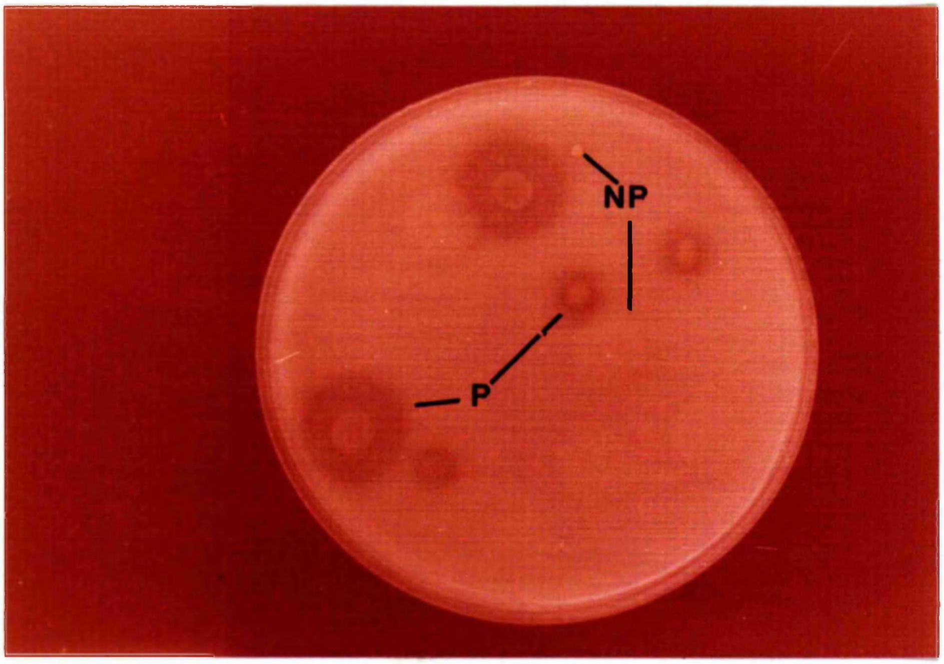
The inoculated plates were incubated for three days at 30^{o}C and then examined. Clear blue colonies, indicative of the growth of lipolytic organisms were counted with the naked eye. Only plates with between 6 and 60 blue colonies were counted.

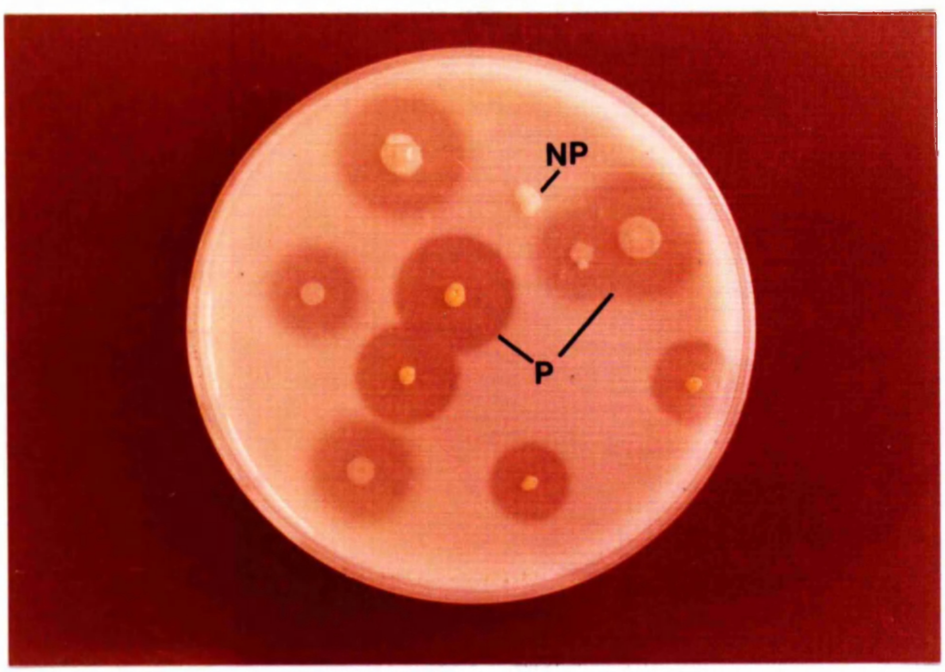
4. Proteolytic count

These bacteria were detected by an improved agar medium by Martley et. al. (1970). The composition of the medium was: standard method agar (SMA) 23.5 g (pancreatic digest of casein 5 g, yeast extract 2.5 g, glucose 1 g and agar 15 g), sodium caseinate 10 g, hydrated trisodium citrate 4.41 g and $CaCl_2.6H_2O$ 4.38 g. The sodium caseinate was dispersed in half of the 0.015 M sodium citrate solution using a Silverson blender and was then added to the SMA which had been rehydrated in the other half of the sodium citrate before autoclaving at 121^{o}C for 15 min. Finally, 20 ml of sterile 1M $CaCl_2$ solution were added to the molten medium. The pH is 6.6. The complete medium, referred to as standard methods caseinate agar (SMCA), was mixed thoroughly by gentle agitation before being dispensed in 12 ml amounts in flat-bottomed petri dishes to form a layer 2 mm thick. The plates were held at 30^{o}C for 48 h and then stored at 5^{o}C until used. Duplicate 0.1 ml solutions of 10-fold dilutions in quarter strength Ringer's solution of the pasteurized milk were spread on the surface of SMCA plates with sterile bent glass rods. The plates were allowed to dry for 15 min and were incubated for 72 h at 30^{o}C. Organisms which formed a white or off-white precipitate

PLATE 1:2

Proteolytic (P) and non-proteolytic (NP) colonies of bacteria from pasteurized milks on a standard method caseinate Agar after 3 d at 30^{o}C





around the colony on SMCA were considered to be proteolytic bacteria.

5. *B. cereus* count

The number of these bacteria was determined according to the method described by Harrigan and McCance (1976).

The medium used in this method was prepared as follows:-

a. Basal medium:- peptone 10 g, meat extract 1 g, D-mannitol 10 g, sodium chloride 10 g, phenol red 0.2 per cent (w/v) solution 12.5 ml, agar 15 g and distilled water 887.5 ml with a final pH 7.1. The ingredients were dissolved in the distilled water and the medium was distributed in 90 ml amounts in screw-capped bottles and sterilized by heating at 121^{o}C for 15 min.

b. preparation of the antibiotic:- 50 mg of polymyxin B sulphate were dissolved in 50 ml of distilled water and sterilized by filtration.

c. preparation of the complete medium:- 10 ml of egg-yolk emulsion was added to 90 ml of molten medium at 45 to 50^{o}C. Then 1 ml of sterile polymyxin B sulphate solution was added. The final concentration of the antibiotic in the medium was thus 10 mg per ml of the medium. The medium was mixed well and 15 ml quantities were added to petri dishes. The poured plates were dried for 1 h at 45^{o}C before being used. The complete medium was called mannitol egg-yolk phenol red polymyxin agar (MEPP agar).

d. procedure:- 0.1 ml amounts were transferred from serial dilutions of pasteurized milk to duplicates of poured, dried MEPP agar. The inoculum was spread evenly over the surface of each plate with a sterile right angled glass rod. The plates were inverted and incubated for 48 h at 30^{o}C. The colonies with a rough and dry form and with a violet-red background and a halo of dense white precipitate were presumed to be *B. cereus*

The colonies were confirmed to be B. cereus by streaking them on egg-yolk agar followed by culture on nutrient agar slopes and microscopic examination for the presence of gram-positive rods and capability of sporulation.

6. Coliform bacteria

These bacteria were determined according to the IDF standard method 40; 1966 (IDF/FIL, 1966) using 2 per cent Oxoid brand brilliant green lactose bile broth which contained peptone 10 g, lactose 10 g, oxgall (dry) 20 g and brilliant green 0.0133 g.

a. preparation of the medium: 40 g of the dehydrated prepared mediums were dissolved in distilled water. The final pH was 7.2. The liquid medium was dispensed in 10 ml amounts in test tubes each containing a Durham gas tube and sterilized in the autoclave at 121°C for 15 min.

b. procedure: serial dilutions of pasteurized milk were prepared in quarter strength Ringer's solution. Each dilution was inoculated in parallel into three test tubes using 1 ml to each tube. The inoculated tubes were incubated for three days at 30°C. The visible formation of gas was evaluated as a positive result. The number of positive tubes was used in reading the most probable number (MPN) of coliform bacteria from the McCrady table for three parallel series.

7. Rapid test of post-pasteurization contamination for HTST Pasteurized milk

To detect post-pasteurization contamination, a rapid test was done according to the method described by Langeveld et. al. (1976). Duplicate packs or bottles from each source were incubated at 25°C for 24 h. Then 0.01 ml from each container was spread by a special platinum loop on each of two petri dishes which contained the well-dried selective medium. Plate count agar (Difco) was used in this method with one of the following selective agents:

a. 50 mg/kg of Benzalkon A-50 per cent (Merck-Schuchardt).

b. 1000 mg/kg of Janus green (Hopkins and Williams).

c. 1000 mg/kg of Bromothymol blue (Schuchardt).

The plates were inverted and a drop of glycerol was placed on the lid to prevent the spreading of the colonies on the surface of the plates. The plates were incubated at 30^{o}C for 24 h. The results were recorded as positive if more than one colony developed during the above incubation period.

SECTION 2. CHEMICAL METHODS

1. pH

pH readings were obtained using a Model 290 Mk2 pH meter made by Pye Unicam. Before measurement, the equipment was adjusted using buffers of known pH taking into consideration the temperature of the sample. Twenty ml of the pasteurized milk in a small plastic container was used for the determination of pH. The average of two readings was recorded.

2. Titratable acidity

The titratable acidity of milk was measured according to B.S. 1741 (British Standards Institution, 1963), using 1 ml of a 0.5 per cent (w/v) solution of phenolphthalein as indicator. Ten ml of the sample were titrated against N/9 sodium hydroxide solution. The titratable acidity was expressed as percentage lactic acid.

3. Dissolved oxygen content

The dissolved oxygen content of samples of pasteurized milk was measured using the Dissolved Oxygen Probe Model No. 8012 made by Electronic Instruments Limited (EIL). About 120 ml of the sample were measured into a conical flask with glass stopper and warmed in a water bath to 17°C immediately before the oxygen content was measured. The instrument was fixed at zero oxygen level with 5 per cent (w/v) of sodium sulphite solution before it was used.

4. Phosphatase test

The phosphatase test was determined according to the IDF standard method 82; 1978 (IDF/FIL, 1978). One ml of sample was added to 5 ml disodium p-mitrophenyl phosphate buffer solution (pH 10.2) in a test tube. The tubes were incubated at a temperature of 37°C for 2 h. The p-nitrophenol liberated was measured in direct comparison with standard colour glasses in a simple comparator using reflected light. The result was positive if the reading was more than 10 mg p-nitrophenol per ml.

5. Vitamin C (ascorbic acid and dehydroascorbic acid)

Vitamin C was determined using the 2,6-dichlorophenolindophenol (DCP) visual titration method described by Toothill *et. al.* (1970). Cucumber juice (variety, Farbiola) was used for oxidation and a suspension of *Escherichia coli* for reduction. DCP (BDH Ltd.) was used as an oxidizing agent during the titration of the milk samples.

The following procedure was used for the determination of vitamin C:

a. determination of ascorbic acid plus interfering substances.

b. determination of ascorbic acid, dehydroascorbic acid, and interfering substances.

c. determination of ascorbic acid, dehydroascorbic acid, and a blank control.

Preparation of the bacterial suspension:

The suspension was prepared as recommended by Stewart and Sharp (1945) using *E. coli* 904 (National Collection of Dairy Organisms). The reduction activity of the bacterial suspension was checked with a pure ascorbic acid solution before each test. The results were multiplied by a factor which was calculated from the percentage activity of the bacterial suspension.

6. Acid degree value

The acid degree value (ADV) was determined according to the method described by Thomas, Neilson and Olsen (1955) as modified by Hunter, Wilson and Barclay (1966, 1968).

The principle of this method is the extraction of the fat from the milk using B.D.I. reagent which consists of 30 g Triton x-100 and 70 g of sodium hexametaphosphate made up to one litre. About 1 g of the extracted fat is weighed into a conical flask fitted with a glass stopper. The fat is dissolved in 5 ml of fat solvent, which consisted of 4 parts of petroleum ether (40/60) and 1 part of absolute methanol (w/v) and titrated against a previously standardised alcoholic solution of KOH after the addition of 5 drops of phenolphthalein 1 per cent (w/v) solution. ADV is defined as ml of N KOH required to neutralise the free fatty acids present in 100 g of milk fat.

CHAPTER TWO

THE QUALITY OF PASTEURIZED MILK FROM RETAIL SOURCES

INTRODUCTION

Many changes have occurred in recent years in the dairy industry. These include methods of milk production, handling, processing and distribution of milk and dairy products. Recently heat-processed milks have been marketed in areas which are considerable distances from the processing plants.

Processing dairies prepare their milks for distribution to the public either directly by the door-step delivery system or through sub-purchasers. The processing may either be pasteurization (low temperature method or HTST), sterilization, or since 1st October 1965, ultra heat treated.

Recently the sales of heat-processed milks were increased in the U.K. It was 7,136 million litres during 1978-1979. Of this amount, 6,318 million litres were sold in England and Wales, while 605 million litres were sold in Scotland and 213 million litres in Northern Ireland (The Federation of U.K. Milk Marketing Boards, 1979).

It has been previously stated by many investigators that the quality of pasteurized milk after processing can be affected in many ways. The temperature of milk during distribution has been shown to vary considerably (Ratzlaff, 1955; Labuschagne et. al.,1971, and Gray, 1973). The other factor is the condition of the milk in the shops. McLarty and Robb (1969), found that the poorest quality of pasteurized milk was that stored in shops where there was no means of refrigeration.

There are many different dairy plants producing pasteurized milk in Scotland, and these make deliveries to a wide area. Pasteurized milk may be sold either by the supermarkets or in smaller shops. It would seem that with increasing sales of processed milks through shops that now is a suitable time to study the quality of pasteurized milk on the market and compare the results with standards which are laid down by The Milk (Special Designations) (Scotland) Order (1976).

The procedures used in the short survey are described below.

EXPERIMENTAL

1. Collection of Milk Samples

During the period between 10th April and 28th August 1979, 12 samples of pasteurized milk were collected every two weeks from four different shops and two supermarkets in Ayr and districts in south-west Scotland. Two samples from each source were chosen at random. The samples were produced by six different dairy plants coded A, B, C, D, E and F. Samples were all collected within one hour in the morning and packed in ice packs while being transported to the laboratory. All 132 samples taken in the survey were analyzed within four hours of purchase.

2. Microbiological analysis

Microbiological tests were made on the samples of pasteurized milk by the methods referred to in Chapter One (Section 1:1, 2, 3, 4, 5, 6 and 7). These included total colony count, psychrotrophic count, lipolytic count, proteolytic count, B. cereus count and coliform count. The rapid test for post-pasteurization contamination was made on a number of samples from each retail source.

3. Chemical analysis

Tests for pH, titratable acidity (per cent of lactic acid) and phosphatase were made on the samples by the methods referred to in Chapter One (Section 2:1, 2, 4 and 6). The acid degree value was determined for six samples from each source, i.e. 36 samples in total.

RESULTS

The $\log_{10}$ of mean, minimum and maximum counts of the bacteriological tests are given in Table 2:1. The range and means of pH and titratable acidity are also given.

Distribution of the total colony count and coliform count are presented in Tables 2:2 and 2:3 respectively. Correlation coefficients between the bacteriological and chemical tests which were used in the study are given in Table 2:4.

Tables 2:5 to 2:11 contain the results of the statistical analysis of variance for the bacteriological and chemical tests related to the source of milk and the date of testing.

Variations of the different bacteriological and chemical tests with the date of testing are illustrated in Graphs 2:1 and 2:2 respectively.

None of the 72 samples of pasteurized milk tested by the rapid post-pasteurization contamination test passed the test (Table 2:14).

Of 132 samples subjected to the phosphatase test, 8 samples (6.06 per cent) failed the test (10 mg or less of p-nitrophenol/ml milk). The acid degree values (ADV) are shown in Table 2:13.

TABLE 2:1

The bacteriological quality of milk sold in retail containers in Ayr and district

Test	Code of retail source of pasteurized milk samples A	B	C	D	E	F
	The $\log_{10}$ of the mean (uppermost figure for each test) and minimum and maximum (lower figures for each test) obtained by plating milks obtained from retail sources on various media					
Total colony count	4.46 3.53 -5.16	4.49 3.05 -5.33	4.37 3.20 -4.97	5.41 3.82 -6.22	4.37 3.61 -5.00	4.00 3.45 -4.96
Psychrotrophic "	4.34 1.00 -5.15	4.35 1.00 -5.31	4.72 1.00 -4.94	5.25 2.59 -6.16	4.06 1.00 -4.87	3.65 1.00 -4.65
Lipolytic "	3.89 1.77 -4.51	3.63 1.77 -4.62	3.74 1.70 -4.72	4.84 2.19 -5.72	4.03 1.70 -4.81	3.54 1.70 -4.29
B. cereus "	2.96 0.30 -3.83	2.78 0.30 -3.38	3.16 0.30 -4.43	3.94 2.16 -4.92	3.27 0.30 -4.47	2.45 0.30 -2.93
Proteolytic "	3.20 2.39 -3.81	3.56 0.30 -4.14	4.06 1.69 -4.64	4.43 1.47 -5.24	3.59 0.30 -4.54	3.22 2.65 -4.04
Coliform "	1.41 0.00 -2.14	1.13 0.00 -2.14	0.06 0.00 -1.17	1.82 0.00 -2.14	0.90 0.00 -2.04	1.25 0.00 -2.14
	The mean and range of values for pH and titratable acidity of the samples					
Titratable acidity (% of lactic acid)	0.151 0.135-0.161	0.152 0.140-0.162	0.151 0.130-0.170	0.151 0.136-0.174	0.149 0.136-0.169	0.155 0.143-0.170
pH	6.72 6.68 -6.78	6.70 6.66 -6.75	6.72 6.65 -6.78	6.70 6.62 -6.76	6.70 6.61 -6.76	6.70 6.60 -6.76

TABLE 2:2

Distribution of the total colony count/ml (30^{o}C for 3 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The result is expressed as a percentage within the range shown.

Total Colony Count/ml	Code of retail source of milk					
	A	B	C	D	E	F
$< 3 \times 10^4$	81.8	72.7	68.2	31.8	77.3	90.9
3×10^4 to 5×10^4	4.5	13.7	22.7	22.7	9.1	9.1
5×10^4 to 10^5	0.0	4.5	9.1	0.0	9.1	0.0
$> 10^5$	13.7	9.1	0.0	45.5	4.5	0.0

TABLE 2:3

Distribution of the count of coliform organisms/ml (30°C for 3 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The result is expressed as a percentage within the range shown*.

	Code of source of milk					
	A	B	C	D	E	F
Absent in 1 ml	72.7	72.7	68.2	9.1	77.3	27.3
Present in 1 ml	18.2	18.2	31.8	45.5	13.6	63.6
Present in 1/1000 ml	9.1	9.1	0.0	45.4	9.1	9.1

*Standard in Scotland - Coliform organisms should be absent in 1/1000 ml (The Milk (Special Designations)(Scotland) Order, 1976)

TABLE 2:4

The correlation coefficient between the microbiological and chemical tests which were used to assess the quality of pasteurized milks at the point of sale to consumer, in Ayr and district.

(1)	Total colony count	1.00							
(2)	Psychrotrophic count	0.70***	1.00						
(3)	Lipolytic count	0.77***	0.79***	1.00					
(4)	*B. cereus* count	0.68***	0.67***	0.73***	1.00				
(5)	Proteolytic count	0.65***	0.51***	0.56***	0.58***	1.00			
(6)	Coliform count	0.60***	0.58***	0.55***	0.38***	0.31**	1.00		
(7)	Titratable acidity (% lactic acid)	0.02	0.20	0.02	0.04	0.08	0.02	1.00	
(8)	pH	-0.03	-0.03	0.02	-0.02	0.02	-0.11	0.01	1.00
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)

DF = 132

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

TABLE 2:5

The total colony count/ml (30°C for 3 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source

	Code of source of milk					
	A	B	C	D	E	F
Total colony count	9.55	9.59	9.68	11.22	9.16	8.77

Date of testing (1979)	Total colony count	Date of testing (1979)	Total colony count
10 April	9.84	3 July	10.60
24 April	9.17	17 July	9.28
8 May	9.83	31 July	9.55
22 May	8.94	14 August	9.73
5 June	9.99	28 August	9.22
19 June	10.45		
	Grand mean 9.742		

	DF	MS	VR
Source of milk	5	14.182	11.31***
Date of testing	10	2.979	2.37*
Residual	116	1.253	
Total	131		

Table	Source	Date
REP	22	12
SED	0.3375	0.4570

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

TABLE 2:6

The count of psychrotrophic bacteria/ml (7^{o}C for 10 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source.

	Code of source of milk					
	A	B	C	D	E	E
Psychrotrophic count	7.07	6.54	6.06	8.15	6.28	7.48

Date of testing (1979)	Psychrotrophic count	Date of testing (1979)	Psychrotrophic count
10 April	8.57	3 July	7.85
24 April	8.12	17 July	5.52
8 May	8.15	31 July	6.16
22 May	4.67	14 August	5.14
5 June	8.20	28 August	5.71
19 June	8.15		

	DF	MS	VR
Source of milk	5	13.863	1.75
Date of testing	10	26.321	3.33***
Residual	116	7.903	
Total	131	9.537	

Table	Source	Date
REP	22	12
SED	0.8480	1.1480

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

TABLE 2:7

The count of lipolytic organisms/ml (30^{o}C for 3 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source.

	Code of source of milk					
	A	B	C	D	E	F
Lipolytic count	7.28	6.89	7.00	8.83	7.55	7.07

Date of testing (1979)	Lipolytic count	Date of testing (1979)	Lipolytic count
10 April	8.26	3 July	8.92
24 April	7.74	17 July	6.97
8 May	7.88	31 July	7.95
22 May	6.47	14 August	7.04
5 June	7.34	28 August	5.87
19 June	7.37		

Grand mean 7.44

	DF	MS	VR
Source of milk	5	11.516	3.01*
Date of testing	10	8.651	2.26*
Residual	116	3.822	
Total	131	4.484	

Table	Source	Date
REP	22	12
SED	0.589	0.798

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

TABLE 2:8

The count of *B. cereus*/ml ($30^{o}C$ for 2 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source.

	Code of source of milk					
	A	B	C	D	E	F
B. cereus count	4.93	4.64	4.47	7.29	5.16	5.09

Date of testing (1979)	*B.cereus* count	Date of testing (1979)	*B.cereus* count
10 April	5.56	3 July	2.05
24 April	6.39	17 July	5.19
8 May	6.09	31 July	5.36
22 May	4.15	14 August	5.44
5 June	4.22	28 August	4.03
19 June	5.42		

Grand mean 5.26

	DF	MS	VR
Source of milk	5	23.161	4.87***
Date of testing	10	7.890	1.66
Residual	116	4.754	
Total	131	5.696	

Table	Source	Date
REP	22	12
SED	0.657	0.890

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

TABLE 2:9

The count of proteolytic organisms/ml (30^{o}C for 3 d) of pasteurised milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source.

	Code of source of milk					
	A	B	C	D	E	F
Proteolytic count	6.92	7.03	8.46	8.48	6.91	7.15

Date of testing (1979)	Proteolytic count	Date of testing (1979)	Proteolytic count
10 April	7.97	3 July	8.70
24 April	7.82	17 July	7.26
8 May	7.50	31 July	7.22
22 May	6.32	14 August	8.01
5 June	8.36	28 August	4.66
19 June	8.59		
	Grand mean 7.49		

	DF	MS	VR
Source of milk	5	12.828	4.95***
Date of testing	10	16.272	6.28***
Residual	116	2.590	
Total	131	4.025	

Table	Source	Date
REP	22	12
SED	0.485	0.657

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

TABLE 2:10

The count of coliform organisms (30^{o}C for 3 d) of pasteurized milks at the point of sale to the consumer in Ayr and district. The results are expressed as the means of the $\log_e$. The data represents 22 samples from each source

	Code of source of milk					
	A	B	C	D	E	F
Coliform count	0.99	0.72	0.36	2.84	0.63	1.71

Date of testing (1979)	Coliform count	Date of testing (1979)	Coliform count
10 April	0.83	3 July	2.05
24 April	1.01	17 July	1.01
8 May	0.61	31 July	1.75
22 May	0.24	14 August	1.09
5 June	1.40	28 August	1.35
19 June	1.96		

Grand mean 1.21

	DF	MS	VR
Source of milk	5	18.768	7.54***
Date of testing	10	3.802	1.52
Residual	116	2.486	
Total	131	3.208	

Table	Source	Date
REP	22	12
SED	0.475	0.644

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

TABLE 2:11

The titratable acidity of pasteurized milks at the point of sale to the consumer in Ayr and district. The date represents 22 samples from each source

	Code of source of milk					
	A	B	C	D	E	F
Titratable acidity	0.151	0.152	0.151	0.151	0.150	0.155

Date of testing (1979)	Titratable count	Date of testing (1979)	Titratable count
10 April	0.153	3 July	0.146
24 April	0.155	17 July	0.157
8 May	0.160	31 July	0.145
22 May	0.153	14 August	0.141
5 June	0.157	28 August	0.149
19 June	0.152		

Grand mean 0.152

	DF	MS	VR
Source of milk	5	0.00006810	1.82
Date of testing	10	0.00040979	10.98***
Residual	116	0.00003736	
Total	131	0.00006691	

Table	Source	Date
REP	22	12
SED	0.001841	0.002493

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

TABLE 2:12

The pH value of pasteurized milks at the point of sale to the consumer in Ayr and district. The data represents 22 samples from each source

	Code of source of milk					
	A	B	C	D	E	F
pH	6.72	6.70	6.72	6.70	6.70	6.70

Date of testing (1979)	pH	Date of testing ((1970)	pH
10 April	6.69	3 July	6.71
24 April	6.71	17 July	6.74
8 May	6.70	31 July	6.71
22 May	6.70	14 August	6.68
5 June	6.73	28 August 6.70	6.70
19 June	6.74		
	Grand mean	6.71	

	DF	MS	VR
Source of milk	5	0.002265	2.17
Date of testing	10	0.00445	4.27***
Residual	116	0.001043	
Total	131	0.001350	

Table	Source	Date
REP	22	12
SED	0.0097	0.0131

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

TABLE 2:13

The acid degree value of pasteurized milks at the point of sale to the consumer in Ayr and district

Date of testing (1979)	No. of sample	Code of source of milk A	B	C	D	E	F
31 July	1	0.68	0.57	0.88	0.66	0.75	0.65
	2	0.77	0.60	0.78	0.66	0.69	0.76
14 August	3	0.90	0.75	0.73	0.83	0.76	0.94
	4	0.85	0.78	0.84	0.88	0.77	0.81
28 August	5	0.95	0.75	0.65	0.85	0.85	0.78
	6	0.92	0.68	0.71	0.92	0.81	0.81
The mean		0.84	0.72	0.76	0.80	0.77	0.79

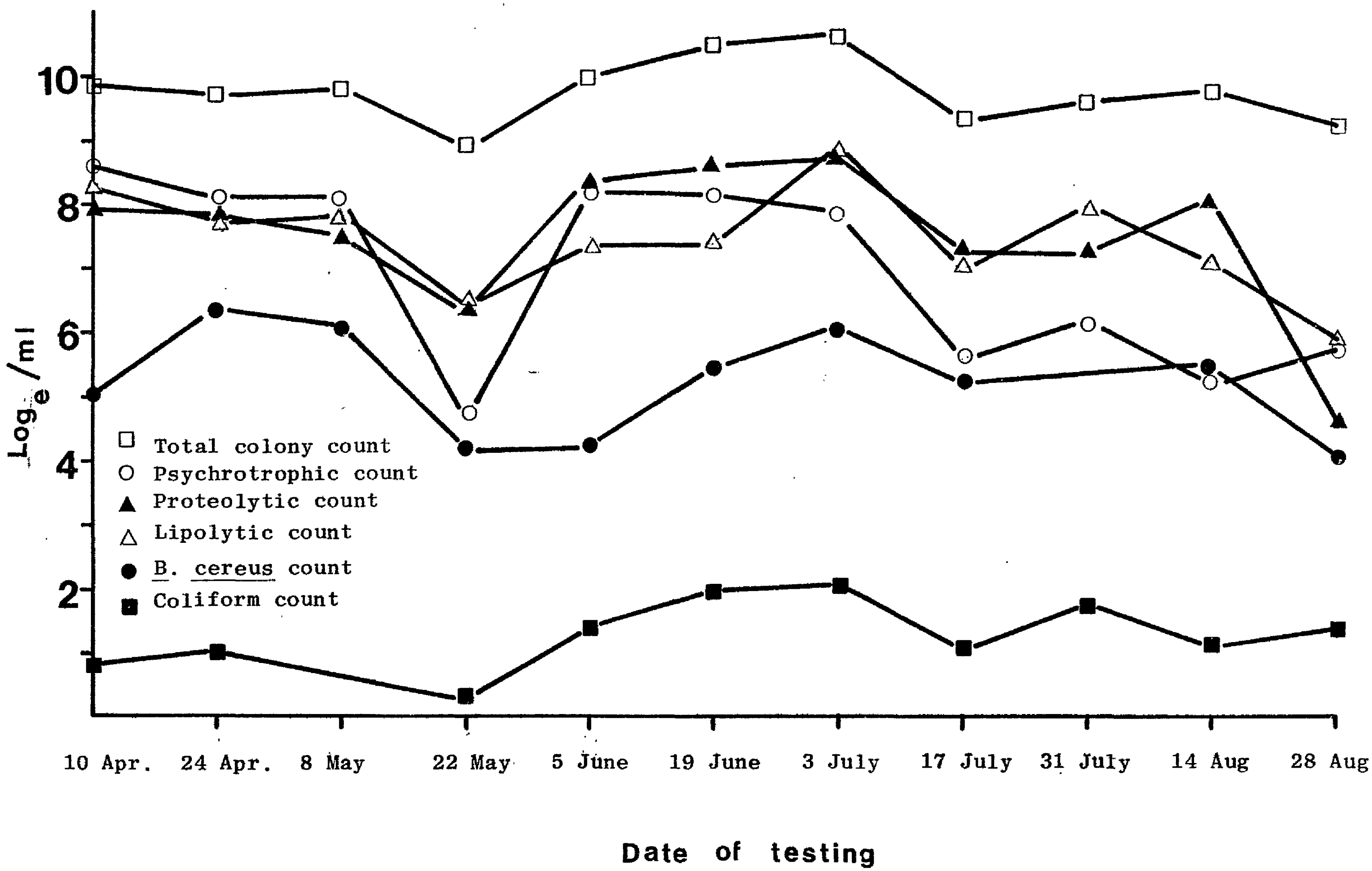
TABLE 2:14

The results of the rapid test for post-pasteurization using three different selective media. The results are expressed as positive (+) if more than one colony grow on the selective media after 24 h at 30^{o}C

Date of testing (1979)	No. of sample	Benzalkon A-50% 100 mg/kg						Janus green 1000 mg/kg						Bromothymol blue 1000 mg/kg					
		Code of source of milk																	
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
19 June	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3 July	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17 July	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31 July	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14 August	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28 August	11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

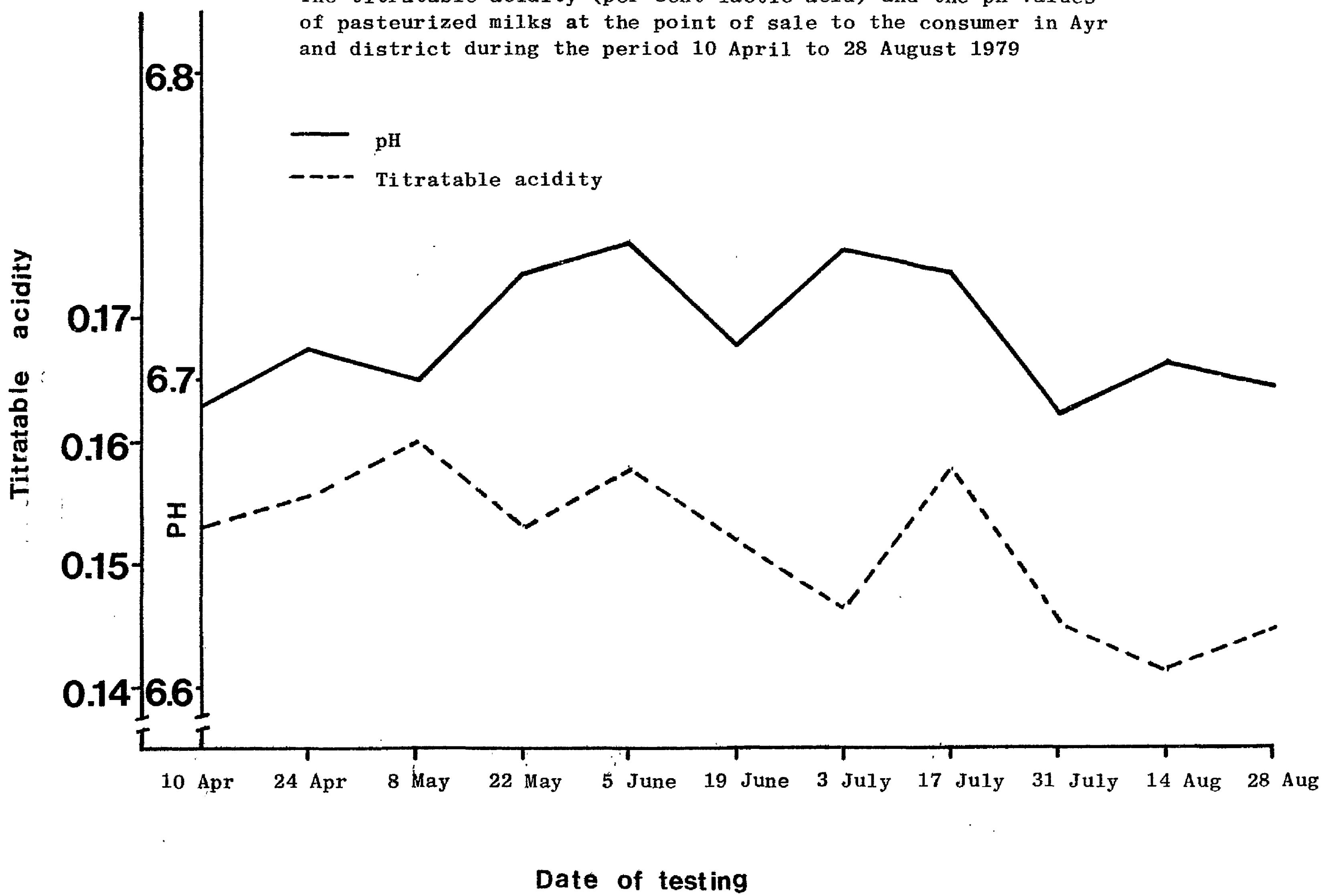
GRAPH 2:1

Microbial counts of pasteurized milks at the point of sale to the consumer in Ayr and district during the period 10 April and 28 August 1979



GRAPH 2:2

The titratable acidity (per cent lactic acid) and the pH values of pasteurized milks at the point of sale to the consumer in Ayr and district during the period 10 April to 28 August 1979



DISCUSSION

1. Total colony count

In Scotland the total colony count for the 'Standard'grade of raw milk must be less than 50,000 per ml (The Milk (Special Designations) (Scotland) Order, 1976). The result of this study showed that 15.9 per cent of the samples of pasteurized milk tested were above this level. But when the results were compared with the standard in the U.S.A. for grade A of pasteurized milk of not more than 30,000 bacteria per ml (Milk Ordance and Code, 1979), 28.7 per cent of the samples of pasteurized milk were in excess of this level.

The poorest quality of pasteurized milk was found in the samples from the shops without refrigeration. These findings confirm those of McLarty and Robb (1969).

The statistical analysis results (Table 2:5) show a significant difference at 0.1 per cent level in the total colony count between the sources of milk, while the dates of testing (indicative of ambient conditions) gave a significant difference at 5 per cent level when the variance ratio (VR) is compared with the F-value in the statistical tables. The highest level of total colony count appeared in June and July and this is shown in Graph 2:1. These results are in agreement with those obtained by Burgwald and Josephson (1947), Rogick and Burgwald (1962), Finley et. al. (1968), McLarty and Robb, (1969) and Jones and Langlois (1977). There is a highly significant difference ($p < 0.001$) between the colony count from samples from source D and the others. This may be related to the unsuitable temperatures of the storage cabinets in the supermarket. The processing source of this milk was from an area which was at some distance from Ayr. In this case, the milk took 1 or 2 days after processing to reach the supermarket. However, when compared with source E where the processed milk was delivered from the same distance, there is a highly significant difference at 0.1 per cent level in total colony count. This may be due to different quality standards at the dairies and also to the packaging systems, milk from source E being packaged in Tetra Pak containers and D in

Pure Pak, the latter pack having a head space which could give the aerobic bacteria a chance to develop more rapidly than in the milk from source E (Schönbornm Kinkel and Müller, 1965)

2. Psychrotrophic count

Increased attention has been given recently to the psychrotrophic bacteria. This is because milk is held for longer periods at low temperature before processing and after processing and in handling and distribution. Most of the psychrotrophic bacteria in pasteurized milk are caused by post-pasteurization contamination which has been estimated as accounting for about 80 per cent of the problems in the shelf-life of pasteurized milk, the remaining 20 per cent being caused by heat-resistant psychrotrophs (Bedfelt, 1980).

In this study, none of the samples of pasteurized milk tested by the post-pasteurization contamination test passed (Table 2:14). The results of the statistical analysis (Table 2:6) showed that there was a significant difference at 0.1 per cent level in the psychrotrophic count obtained on different days of sampling. This is because some of the milk could be tested 1, 2 or 3 days after processing. Thomas, Griffiths and Foulkes (1960) stated that the rate of multiplication of psychrotrophic bacteria in refrigerated milk varies from sample to sample, depending on the types of bacteria dominant in the milk. Others found that psychrotrophic bacteria increased slightly in two days, and rapidly after the third day (Watrous et. al., 1952; Hempler, 1955).

Vukelic et. al. (1975) found that a rapid growth of psychrotrophic bacteria occurred after 48 h at 7^{o}C and after 72 h at 5^{o}C.

The correlation coefficient between the psychrotrophic count and total colony count was 0.70 ($p < 0.001$) (Table 2:4). Jooste and Groenveld(1971) found it to be 0.72 . Thomas (1958) stated that pasteurized milk should be sold to the consumer with less than 10,000/ml of psychrotrophs. In this study 22.7 per cent of the samples failed this suggested standard. In a study made by McLarty and Robb (1969), 20 per cent failed this level when the pasteurized milk from a retail source was held at 5°C for 24 h.

3. Lipolytic count

Lipolytic organisms which are predominant in pasteurized milk include species of Pseudomonas, Achromobacter, Staphylococcus, Serratia, Flavobacterium, Alcaligenes and Micrococcus. These lipolytic organisms are also found in dairy products. The effect of these species is to produce lipase enzymes which together with the natural milk lipase enzyme cause hydrolysis of the fat giving rise to rancidity in the milk. Thomas (1970) reported that organisms of the genus Pseudomonas accounted for 62.1 to 70.1 per cent in the total colony count in pasteurized milk.

From the results shown in Table 2:7, there is a significant difference at 5 per cent level in lipolytic count between the sources of milk. The same level of difference was found between the days of sampling. The total colony and psychrotrophic counts showed a correlation coefficient of 0.77 ($p < 0.001$) and 0.79 ($p < 0.001$) respectively with the lipolytic count (Table 2:4). The higher lipolytic count was found in pasteurized milk from source D. This is related to the high total colony count in this milk. There is a difference between the other dairies, but it is not significant. This difference might be due to the variation in the source of milk, processing conditions and the degree of post-pasteurization contamination.

4. B. cereus count

This is a spore-forming organism which was first isolated from air by Frankland and Frankland (1887). This bacterium is a very important factor affecting the keeping quality of pasteurized milk by causing a fault similar to that found in bitty cream. Franklin (1969) reported that B. cereus spores can get into the milk from the skin of cows, dust, dirt, faeces, soil, fodder, flies and the surfaces of utensils and dairy plant. Billing and Cuthbert (1958) found a very high number of *B. cereus* spores in washed milk cans.

From the results presented in Table 2:8, the lowest count was found in July. These findings confirmed the results of Ridgway (1954) and Franklin, Williams & Clegg (1956). There was no significant difference in the count of *B. cereus* during the period of this study. This might be due to the slow germination of the spores of *B. cereus* in the milk which were found to have a lower germination rate than those which were isolated from soil and dung (Labots and Hup, 1964; Labots, Hup and Galesloot, 1965).

5. Proteolytic count

The common proteolytic bacteria are the species of *Bacillus*, *Clostridium*, *Pseudomonas* and *Proteus*. These bacteria are very important in causing deterioration in pasteurized milk. The common psychrotrophic bacteria are strongly proteolytic and cause an undesirable flavour in the milk due to hydrolysis of the milk protein. There is a highly significant difference at 0.1 per cent level in the proteolytic count between the sources of milk and the same level of difference found between the days of sampling (Table 2:9).

These differences are due to the difference in the source of milk and the seasonal variation. The proteolytic count depends on the number of spore-forming proteolytic bacteria which survive heat treatment together with the post-pasteurization contamination by non-spore-forming proteolytic bacteria (e.g. *Pseudomonas* and *Proteus*). The highest count of proteolytic organisms was found in milk from sources C and D (Table 2:9). In the case of source C the majority of the proteolytic bacteria was from the raw milk as spore-forming bacteria because there was not the same increase in the total colony count compared with milk from source D. In the case of source D most of the contaminating proteolytic organisms entered the milk after processing. There is no significant difference in the proteolytic count between the other sources (A, B, E and F) (Table 2:9).

6. Coliform count

The Milk (Special Designations) (Scotland) Order (1976) stipulates that coliform bacteria should be absent in 1/1000 ml of pasteurized milk. In this study 13.6 per cent of the samples failed this legal standard. About half of the samples failing the standard were from source D.

Many investigators have demonstrated that coliform bacteria develop rapidly in the summer time when ambient conditions are at their highest in commercially pasteurized milk (Dahlberg, 1945; McCallum, 1960 and McLarty and Robb, 1969). In this study the highest count of coliform bacteria was obtained in the summer months.

The number of coliform bacteria found in pasteurized milk from source D was significantly higher ($p < 0.001$) than that obtained from the other sources (Table 2:10). The correlation coefficient between the total colony count and the coliform count was 0.60 ($p < 0.001$). However, Jooste and Groeneveld (1971) found it to be 0.72.

7. Titratable acidity and pH

The titratable acidity of milk is expressed as the per cent lactic acid of the sample. This value may be used as an indirect measure of lactic-acid-producing bacteria in the milk. The pH is a more accurate indicator than the titratable acidity because the acid available to be neutralized with standard alkaline depends on the buffering capacity of the milk.

Cox (1975) reported that the titratable acidity in pasteurized milk should be lower than 0.2 per cent. None of the samples which were tested reached this level. It means that there was not a significant contamination after processing by acid-forming bacteria. The acid-forming streptococci which survive pasteurization are types commonly requiring relatively high temperatures for their active development. Pasteurization usually reduces the content of acid-forming bacteria and the other types of organisms have a greater opportunity for growth in pasteurized milk. From the result in

Table 2:11 the variation in the range of acidity was from 0.141 to 0.160 per cent and in the pH value in Table 2:12 was from 6.688 to 6.745.

From the result there is no indication of the quality of pasteurized milk related to the titratable acidity and the pH. Schönborn, Kinkel and Müller (1975) found that there was no change in the pH during the storage of pasteurized milk except for a slight decrease up to 0.5 unit in a few samples stored for 8 days at 4 to 5^{0}C.

8. Phosphatase test

6.06 per cent of all the samples failed the legal standard for pasteurized milk (The Milk) (Special Designations) (Scotland) Order (1976). 75 per cent of these samples were from source F. Failure in the phosphatase test indicates defective pasteurizing techniques including faulty equipment. McLarty and Robb (1969) found that the percentage of the samples of pasteurized milk which failed this test was 1.2 per cent.

9. Acid degree value (ADV)

The American Public Health Association (1978) interpret the levels of ADV as follows:

(i) < 0.4 Normal

(ii) 0.7-1.1 Borderline (indefinite)

(iii) 1.2 Slight rancidity

(iv) 1.5 and above Unsatisfactory (extreme hydrolytic rancidity)

From the data which is presented in Table 2:13 the number of samples with an ADV of < 0.7 represents 22.3 per cent of the total. Dillman and Anderson (1970) in their study in the U.S.A. on commercial pasteurized milk from retail sources recorded a figure of 25 per cent below 0.7. In the present study the percentage of samples with an ADV of > 0.7 was 77.7 which is higher than was found by the above investigators, who recorded 68.6 per cent of supplies above the 0.7 level.

Krukovsky and Herrigton (1941) found that the threshold at which rancidity can be recognized is near an ADV of 0.80. In the present study 41.6 per cent of the samples had ADVs higher than this level (Table 2:13).

The percentage of the ADV could be affected by many factors. The mechanical agitation of raw milk is the major cause of induced lipolysis. The breakdown could happen to the fat globule membrane releasing the free fat and this will be hydrolyzed by either the native lipase enzyme or by microbial lipase enzymes produced by some psychrotrophic bacteria during the storage of raw milk on the farm. Some of these enzymes are heat-resistant and will cause lipolysis in the milk after processing.

CONCLUSION

The supplies of pasteurized milk analysed in the survey were not of the same quality. All the samples were contaminated by post-pasteurization contamination, but the degree of contamination differed from one source to another. The poorest quality was found in milk from a supermarket without refrigeration. A small number of samples failed the phosphatase test, and over thirteen per cent failed the legal standard for coliform organisms. Some shops and supermarkets keep pasteurized milk at room temperature before sale. This temperature is suitable for the growth of most micro-organisms and leads to poorer quality milk on retail sale to the consumer. Maintainance of milk at temperatures below $5^{o}C$ would lead to improved quality.

CHAPTER THREE

A COMPARISON OF THE MICROBIOLOGICAL QUALITY OF PASTEURIZED MILK PACKAGED IN GLASS BOTTLES AND IN SINGLE-SERVICE CONTAINERS.

INTRODUCTION

The retail sale of packaged milk began in the U.K. dairy industry about a century ago with the production of sterilized milk in glass bottles. The glass bottle continued to be the only form of packaging until 1933 when the first single service packages in the form of cartons were introduced.

During the forties there was a rapid development in the manufacture of various types of packaging materials which resulted in a wide range of containers suitable for the packaging of milk.

Hall (1973) summarised the various retail packages for liquid milks. These packages are presented in Table 3:1.

Many investigators have studied the quality of milk packaged in two or three different types of cartons. Schönborn et. al. (1975) investigated the quality of pasteurized milk packaged in (i) square package without air space and (ii) oblong package with roof-shaped air space.

During the investigation samples of pasteurized milk were taken on storage days 1, 5 and 8 and examined organoleptically as well as bacteriologically. The results showed that milk packaged in package (ii) contained a significantly higher colony count than in package (i).

Pasteurized milk packaged in glass bottles or single-service containers may be contaminated by the pipeline between the pasteurized milk tank

TABLE 3:1

Retail packages for milk

Glass bottles	Plastics bottles	Cartons		Sachets		Plastics bottles	Bag-in-box
		pre-formed or supplied as blanks	form-fill-seal	pillow	bag		
Rockware	Kempro	Perga	Jiropak	Bertopack**		Bekum	Bertoblock
United		Pergaplas	Tetra Pak*	Finnpack		GF(Uniloy)	
Glass etc.		————		Hassia		Hamba	
						Metal Box	
		Block Pack	Selfpack*	prepac***		Sanc	
		Pure-Pack*	Tetra Brik*	Rotapack		Sidel	
		Tetra Rex		Thimpak	Doypack*	Stork	
		Sealking	Zupack**		Franspack	Bottlepack*	
						Mecaplast	
						Hessel	

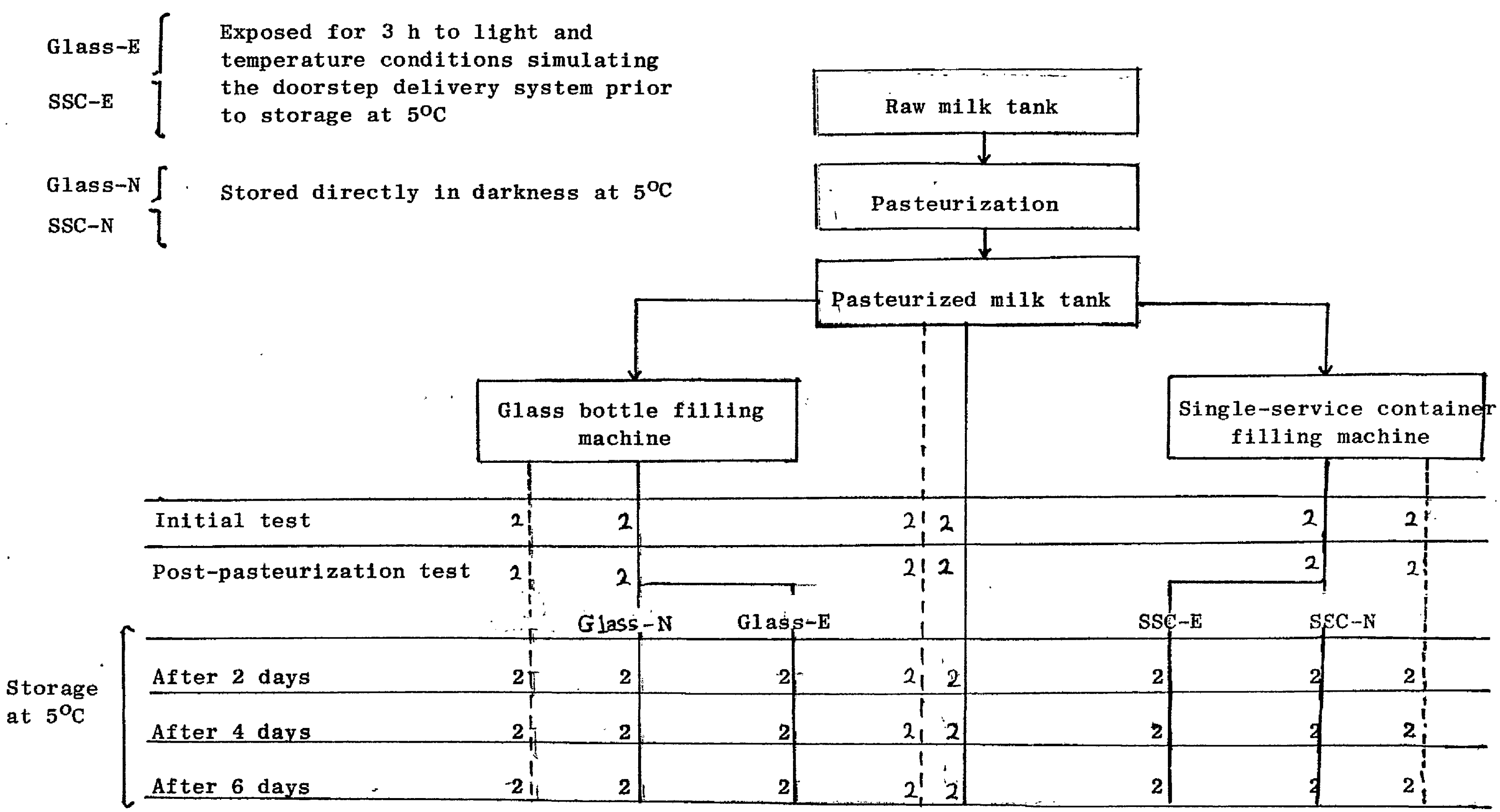
* Aseptic version in commercial use.
** Aseptic version under commercial test.
*** Aseptic version in development.

and the filling machine or by the filling machines (Thomas, Thomas and Ellison, 1949; Ford and Babel, 1951).

The washed milk bottles were found to be an important source of contamination of pasteurized milk by psychrotrophic bacteria. The count of these bacteria was found to be about 10 to 16 per bottle, with maximum counts of > 10^3 per bottle (Rogick and Burgwald, 1952; Thomas, Griffiths and Foulkes, 1960).

The aim of the work described in this chapter was to investigate the microbiological quality of pasteurized milk packaged in two different packaging systems and comparing the results with the quality of the same milk from the pasteurized tank. The effect on the milks of exposure to 3 h of light and temperature conditions simulating doorstep delivery was studied.

Diagram of the collection, treatment and testing of samples of pasteurized milks from dairy A (——) and dairy B (----)



EXPERIMENTAL

The study recorded in this chapter was made on two commercial dairies coded A and B.

Dairy A

1. Collection of the samples

Samples of pasteurized milk were collected and transported to the laboratory in a polystyrene box packed with ice blocks. The samples were taken at the same time from three sources:-

(i) *the pasteurized milk tank using a sterile container;

(ii) the bottle filler - filled and capped bottles from the production line were taken at random.

(iii) the filling machine for the single-service containers - filled and sealed SSC from the production line were taken at random.

Arrangements were made with the management to ensure that all pasteurized milk came from the same source i.e. the same raw storage tank and the same pasteurized milk storage tank. This procedure was followed on six occasions.

2. Treatment of the samples

The samples were grouped as follows:-

Group A and B contained two samples from each of the above sources marked (i), (ii) and (iii).

Group C and D contained six samples from source (ii).

Group E and F contained six samples from source (iii).

Group G contained six samples from source (i).

Samples in group A were used for the initial test on the day of the processing. Samples in group B were used for the test for post-pasteurization contamination. Samples in group C and E were exposed

*Samples from this source were tested in trials 2, 3, 4, 5 and 6 only. The results of the first trial were estimated by the computer.

to simulated daylight for 3 h, equivalent to the doorstep delivery system. The intensity of the light was 1000 to 2000 lumen. Lighting consisted of 40 watt cool white fluorescent tubes and samples were placed at the proper distance from the light source to obtain the desired intensity, which was measured by means of a type C, E22 - light meter produced by Megatron Ltd.

The temperatures during the period of exposure to light were based on ambient temperatures for the College area as recorded by Channon and Kirkwood (1979). After the exposure treatment the samples in the two groups were stored at $5^{o}C$ in the dark. Samples in groups D, F and G were stored directly at $5^{o}C$ in the dark without exposure treatment.

3. Preparation of the samples for testing

Samples from groups A, C, D, E and F on each day of testing were divided into three portions after they had been taken from the cold store and shaken thoroughly.

a. 10 ml from each container was poured aseptically into a sterile jar for the bacteriological analysis.

b. About 200 ml were used for the chemical analysis.

c. The remainder of the milk in each container was used for the assessment of the organoleptic quality.

4. Microbiological analysis

Samples in group A were tested on the day of processing i.e. the initial test. Two samples from groups C, D, E, F and G were tested during the period of 2, 4 and 6 days, for total colony count, psychrotrophic count, lipolytic count, proteolytic count, B. cereus count and coliform count using the methods described in Chapter One (Section 1:1, 2, 3, 4, 5 and 6).

Samples in group B were tested for the detection of post-pasteurization contamination using the rapid method described in Chapter One (section 1:7).

5. Identification methods

Random colonies, which appeared in the test for post-pasteurization contamination as referred to above, were identified using the 'Oxi/Ferm' and 'Enterotube' systems which were supplied by F. Haffmann - La Roche and Co. AG., Basle, Switzerland.

6. Quality of the packaging material

a. Glass bottles:-

The sterility of the glass bottles was tested according to B.S. 4285, part 2, 1968 (British Standards Institution, 1968). Three samples in each trial were used for this test.

b. Cartoning material:-

As described in B.S. 4285, part 2, 1968 (British Standards Institution, 1968), a swabbing technique was used for testing the carton material just before it entered the filling machine. Three areas which formed three single-service cartons were swabbed in each trial.

Dairy B

1. Collection of the samples

Samples of pasteurized milk were collected from the dairy as described in Dairy A. This procedure was followed on 3 occasions.

2. Treatment of the samples

Two samples from each source were used for the initial test (on the same day as the milk was processed). The remaining samples, which were kept at $5^{o}C$ for 6 days were tested after 2, 4 and 6 days of storage. Two samples from each source were used.

3. Microbiological analysis

The samples were tested for total colony count, psychrotrophic count, lipolytic count, proteolytic count, *B. cereus* count and coliform count using the methods described in chapter one (section 1:1, 2, 3, 4, 5 and 6).

RESULTS

Dairy A

1. Total colony count

The results of the total Colony Count are presented in Tables 3:2, 3:3, 3:4 and 3:5. Table 3:2 shows the results of samples tested on the day of processing. In these results, the total colony count found in pasteurized milk packaged in glass bottles was significantly higher ($p < 0.001$) than that obtained from pasteurized milk packaged in the SSC system. However, pasteurized milk in both packaging systems gave significantly higher ($p < 0.001$) total colony counts than pasteurized milk which was obtained from the pasteurized milk tank. These differences were the same after 2, 4 and 6 days of cold storage at $5^{o}C$. The results show that exposure to light and temperature conditions simulating the doorstep delivery system had no significant effect on the total colony count. There was a significant difference between the trials through the experiment. From the mean of the results presented in Table 3:4, the total colony count in the samples of glass bottles was nearly 2.153×10^5 per ml after 4 days of storage at $5^{o}C$. In trials 3 and 5 the count was $>10^6$ per ml. When this was compared with the total colony count obtained from the SSC the highest total colony count in all the trials was 6.35×10^4 per ml. The mean total colony count found in the samples from the pasteurized milk tank was 1826 per ml after 4 days of cold storage at $5^{o}C$. After 6 days of cold storage the mean total colony count obtained from pasteurized milk packaged in glass bottles was 1.830×10^6 per ml compared with the mean of pasteurized milk packaged in SSC which was 2.821×10^5 per ml.

The mean of the total colony count of pasteurized milk obtained from the pasteurized tank was 4628 per ml. The variation in the total colony count obtained from different treatments with the storage time are illustrated in graph 3:1.

TABLE 3:2

The total colony count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers on the day of processing

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	9.04	8.91	7.94	8.63
2	9.06	8.83	8.05	8.64
3	9.83	8.94	8.35	9.04
4	8.39	8.80	7.36	8.19
5	8.67	8.06	6.86	7.86
6	8.31	8.35	7.85	8.17
Mean	8.88	8.65	7.74	8.42

	DF	MS	VR
Trials	5	1.085739	149.99***
Sample	2	4.404881	608.54***
Trials sample	9	0.205147	28.34***
Residual	17	0.007238	
Total	33	0.491146	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.0491	0.0347	0.0851

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

TABLE 3:3

The total colony count/ml (30^{o}C for 3 d).expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5^{o}C in darkness and in light and temperature conditions simulating the door step delivery system. The total colony count of pasteurized milk from pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E+	Glass-N+	SSC-E+	SSC-N+	Tank	Mean
1	9.18	9.15	8.99	9.03	7.80	8.85
2	8.96	8.80	8.88	8.90	8.09	8.73
3	11.19	11.18	8.44	8.48	8.42	9.54
4	8.27	8.24	8.67	8.73	6.23	8.03
5	8.68	8.38	8.22	8.13	6.63	8.01
6	8.13	8.52	7.90	8.15	7.77	8.09
Mean	9.07	9.06	8.52	8.57	7.49	8.54

	DF	MS	VR
Trial	5	3.74965	152.08***
Treatment	4	4.97174	201.65***
Trial treatment	19	0.85358	34.62***
Residual	29	0.02465	
Total	57	0.97488	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.0702	0.0641	0.1570

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

+ E - exposed to light and temperature conditions simulating doorstep delivery system for 3 h.

N - not exposed to light and temperature conditions simulating doorstep delivery system.

TABLE 3:4

The total colony count/ml (30°C for 3 d) expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at 5°C in darkness and in light and temperature conditions simulating the door step delivery system. The total colony count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	10.29	10.14	9.24	9.31	5.92	8.98
2	11.33	10.78	11.06	10.74	8.12	10.41
3	15.11	14.61	10.89	10.94	8.65	12.04
4	12.80	12.79	10.41	10.28	7.20	10.70
5	14.14	13.77	10.04	9.72	7.13	10.96
6	10.58	11.02	11.05	10.87	8.02	10.31
Mean	12.37	12.19	10.45	10.31	7.51	10.57

	DF	MS	VR
Trial	5	9.8939	61.69***
Treatment	4	45.9344	286.44***
Trial treatment	19	2.1518	13.41***
Residual	29	0.1604	
Total	57	4.8902	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1791	0.1635	0.4005

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 3:5

The total colony count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The total colony count of pasteurized milk from pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	13.92	14.04	10.53	10.48	7.20	11.24
2	14.09	13.52	15.21	15.27	8.96	13.41
3	15.83	14.96	11.83	11.69	8.83	12.68
4	14.35	14.14	14.08	14.01	8.28	12.97
5	14.51	15.12	11.44	11.25	8.09	12.08
6	13.59	14.98	12.01	12.52	9.30	12.48
Mean	14.38	14.46	12.52	12.58	8.44	12.48

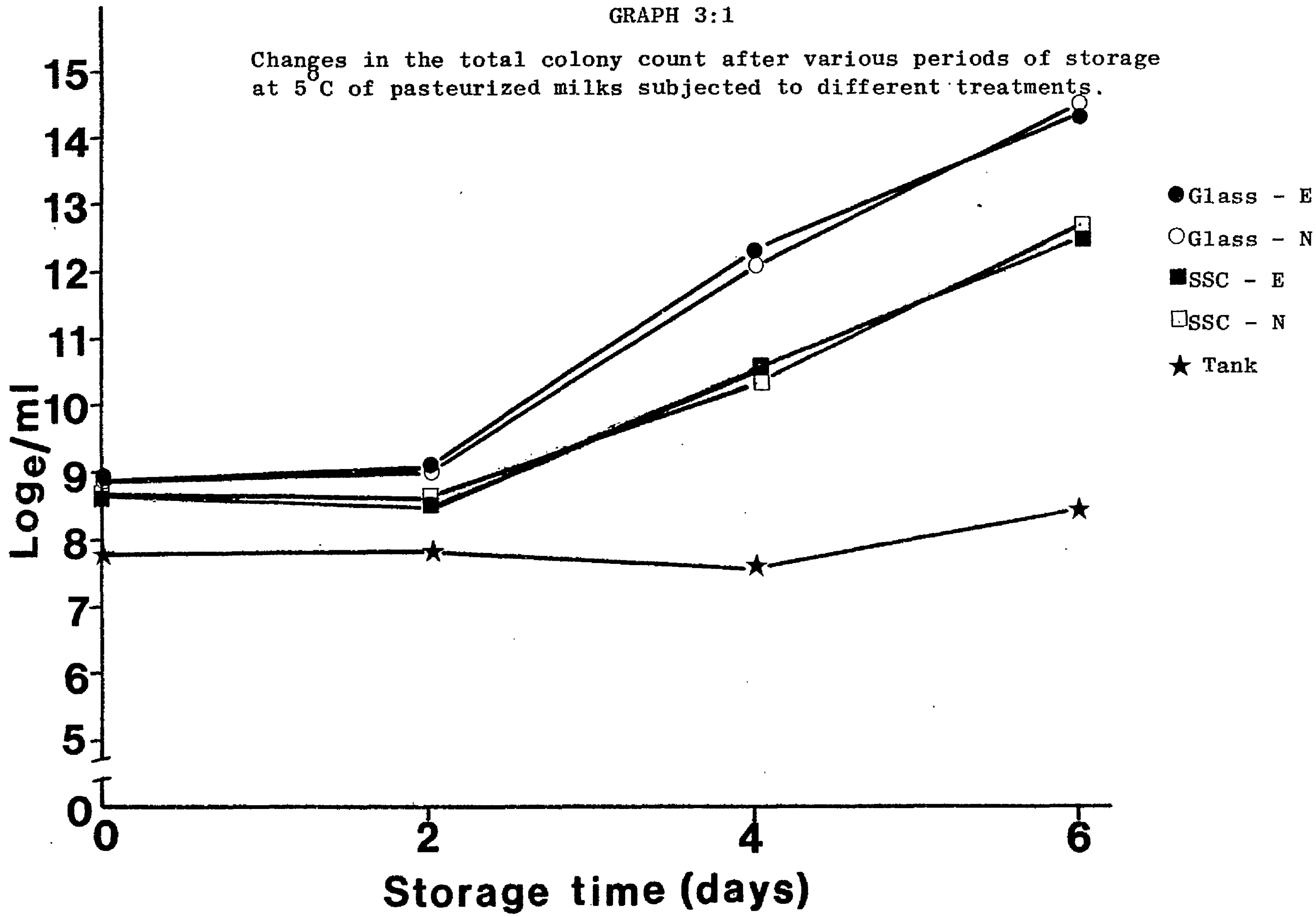
	DF	MS	VR
Trial	5	5.7027	20.84***
Treatment	4	71.5506	261.55***
Trial treatment	19	2.6431	9.66***
Residual	29	0.2736	
Total	57	6.5415	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2339	0.2135	0.5230

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

GRAPH 3:1

Changes in the total colony count after various periods of storage at 5°C of pasteurized milks subjected to different treatments.



2. Psychrotrophic count

The mean psychrotrophic counts (Tables 3:6, 3:7, 3:8 and 3:9) of pasteurized milk packaged in glass bottles were higher than the counts of the same milk from the pasteurized milk tank and single service containers (SSC). However, pasteurized milk packaged in SSC gave higher psychrotrophic counts (significant at 5 per cent level) than that from the pasteurized milk tank on the day of processing. The mean of the six trials showed that the psychrotrophic count of milk in glass bottles was significantly higher ($p < 0.001$) than that obtained from SSC after 2, 4 and 6 days of storage at $5^{o}C$. However, the mean of the psychrotrophic count from the pasteurized milk tank was 757 per ml after 6 days of cold storage at $5^{o}C$.

Exposure of pasteurized milk in glass bottles and in SSC to light and temperature conditions simulating doorstep delivery had no significant effect on the count of psychrotrophic organisms in each type of container. The variations in the psychrotrophic count with storage time is illustrated in graph 3:2. There was a highly significant difference ($p < 0.001$) between the psychrotrophic counts of the milks taken in each of the trials. Psychrotrophic counts showed correlation coefficients with total colony counts, 0.4734, 0.5859, 0.8244 and 0.9252 on 0, 2, 4 and 6 days of storage at $5^{o}C$ respectively. After six days of storage at $5^{o}C$ the mean of the psychrotrophic count of pasteurized milk packaged in glass bottles was 1.506×10^{6} per ml, while in the SSC samples it was 2.298×10^{5} per ml.

3. Lipolytic count

The results of the lipolytic counts are presented in Tables 3:10, 3:11, 3:12 and 3:13. The results obtained from tests made on the day of processing (Table 3:10) show a highly significant difference between the lipolytic count of the samples from the pasteurized milk tank, the glass bottles and single-service containers. However, the mean lipolytic count of the samples of pasteurized milk packaged in glass bottles was highly significant ($p < 0.001$) than that of samples of pasteurized milk packaged in SSC. But the latter showed a higher

TABLE 3:6

The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.32	1.95	1.13	2.80
2	5.94	6.26	2.30	4.83
3	8.96	1.93	0.00	3.63
4	4.91	0.69	3.06	2.89
5	6.85	1.49	2.39	3.58
6	6.21	2.13	2.39	3.58
Mean	6.35	2.41	1.88	3.55

	DF	MS	VR
Trial	5	3.1766	12.598***
Sample	2	72.0925	285.915***
Trial sample	9	6.1659	24.454***
Residual	17	0.2521	
Total	33	6.6620	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.2899	0.2050	0.5021

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

TABLE 3:7

The psychrotrophic count/ml (7^0C for 10 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The psychrotrophic count of pasteurized milk from pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	7.49	7.56	2.90	2.77	2.52	4.56
2	5.99	4.61	6.67	6.09	2.30	5.13
3	10.85	10.93	5.53	4.47	0.00	6.35
4	6.57	6.73	4.09	3.96	5.86	5.44
5	8.52	6.88	2.19	2.35	2.39	4.47
6	5.84	5.72	3.15	3.01	4.70	4.48
Mean	7.54	7.07	4.09	3.77	2.96	5.09

	DF	MS	VR
Trial	5	5.3476	10.37***
Treatment	4	51.5902	100.06***
Trial treatment	19	7.7136	14.96***
Residual	29	0.5156	
Total	57	6.9229	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.3211	0.2931	0.7180

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

TABLE 3:8

The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 4 days of storage at $5^{o}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The psychrotrophic count of pasteurized milk from pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	9.99	10.19	7.14	6.64	3.38	7.47
2	11.04	10.58	10.98	10.45	6.66	9.94
3	14.34	14.10	7.66	7.46	0.00	8.71
4	12.71	10.35	10.26	10.17	6.85	10.07
5	14.06	13.65	8.56	7.54	6.01	9.97
6	8.32	8.73	8.86	8.38	6.98	8.25
Mean	11.74	11.27	8.91	8.44	4.98	9.07

	DF	MS	VR
Trial	5	11.8428	61.39***
Treatment	4	87.4056	453.12***
Trial treatment	19	8.7499	45.36***
Residual	29	0.1929	
Total	57	10.1873	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1964	0.1793	0.4392

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

TABLE 3:9

The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 6 days of storage at $5^{o}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The psychrotrophic count of pasteurized milk from pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1.	13.75	13.76	10.07	10.10	5.27	
2	14.00	13.48	15.02	15.10	7.76	
3	15.46	14.40	11.54	11.86	2.33	
4	14.04	13.87	13.99	13.74	7.44	
5	14.46	15.11	11.14	11.10	8.05	
6	13.46	14.96	11.98	12.48	8.95	
Mean	14.19	14.26	12.29	12.40	6.63	

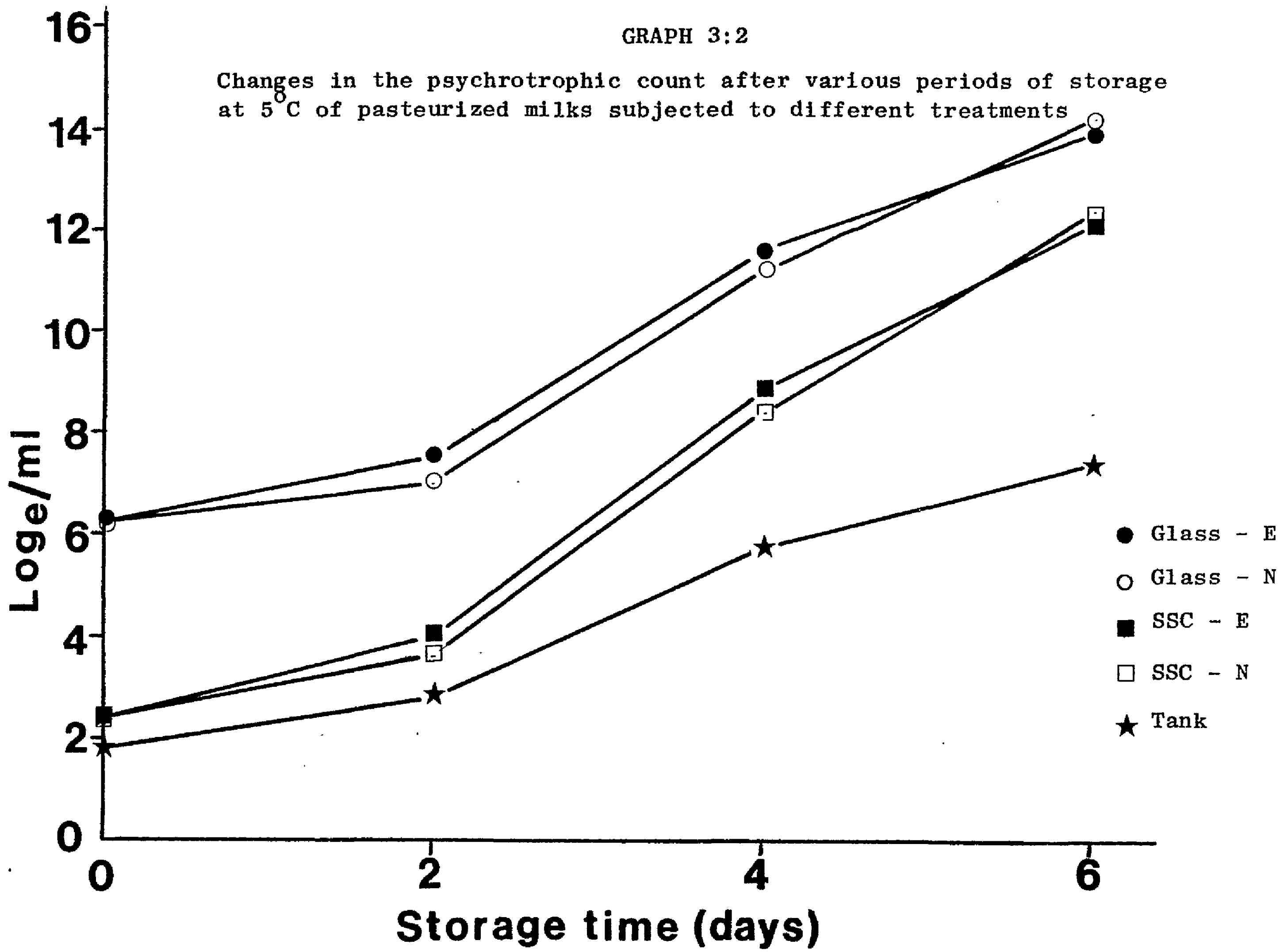
	DF	MS	VR
Trial	5	8.8303	13.17***
Treatment	4	116.9224	174.47***
Trial treatment	19	4.8275	7.20***
Residual	29	0.6701	
Total	57	10.9298	

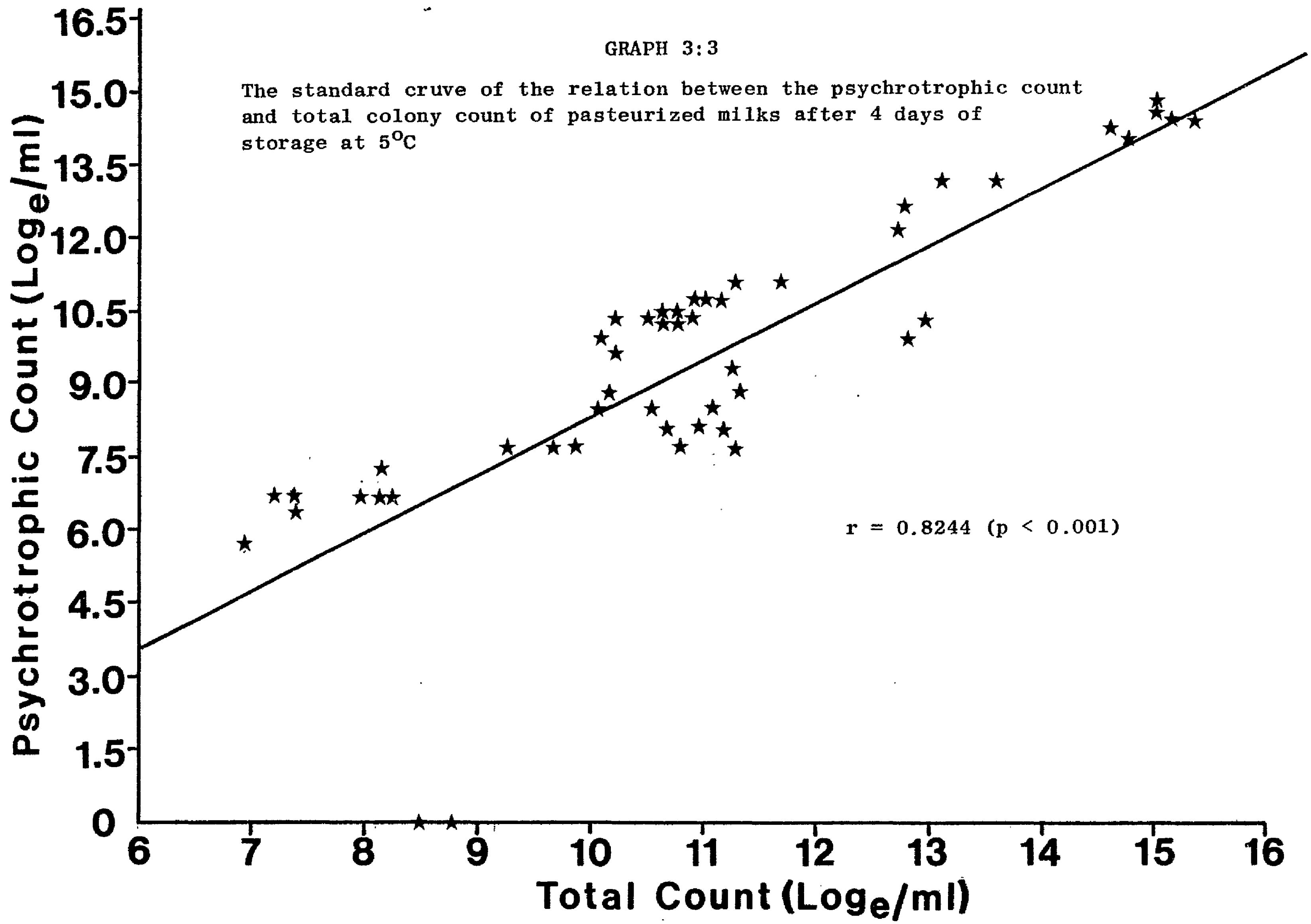
Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.3660	0.3340	0.8190

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

GRAPH 3:2

Changes in the psychrotrophic count after various periods of storage at 5°C of pasteurized milks subjected to different treatments




GRAPH 3:3
The standard cruve of the relation between the psychrotrophic count and total colony count of pasteurized milks after 4 days of storage at 5°C
r = 0.8244 (p < 0.001)
16.5
15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
6
7
8
9
10
11
12
13
14
15
16
Psychrotrophic Count (Loge/ml)
Total Count (Loge/ml)

GRAPH 3:4

The standard curve of the relation between the psychrotrophic count and total colony count of pasteurized milks after 6 days of storage at 5°C

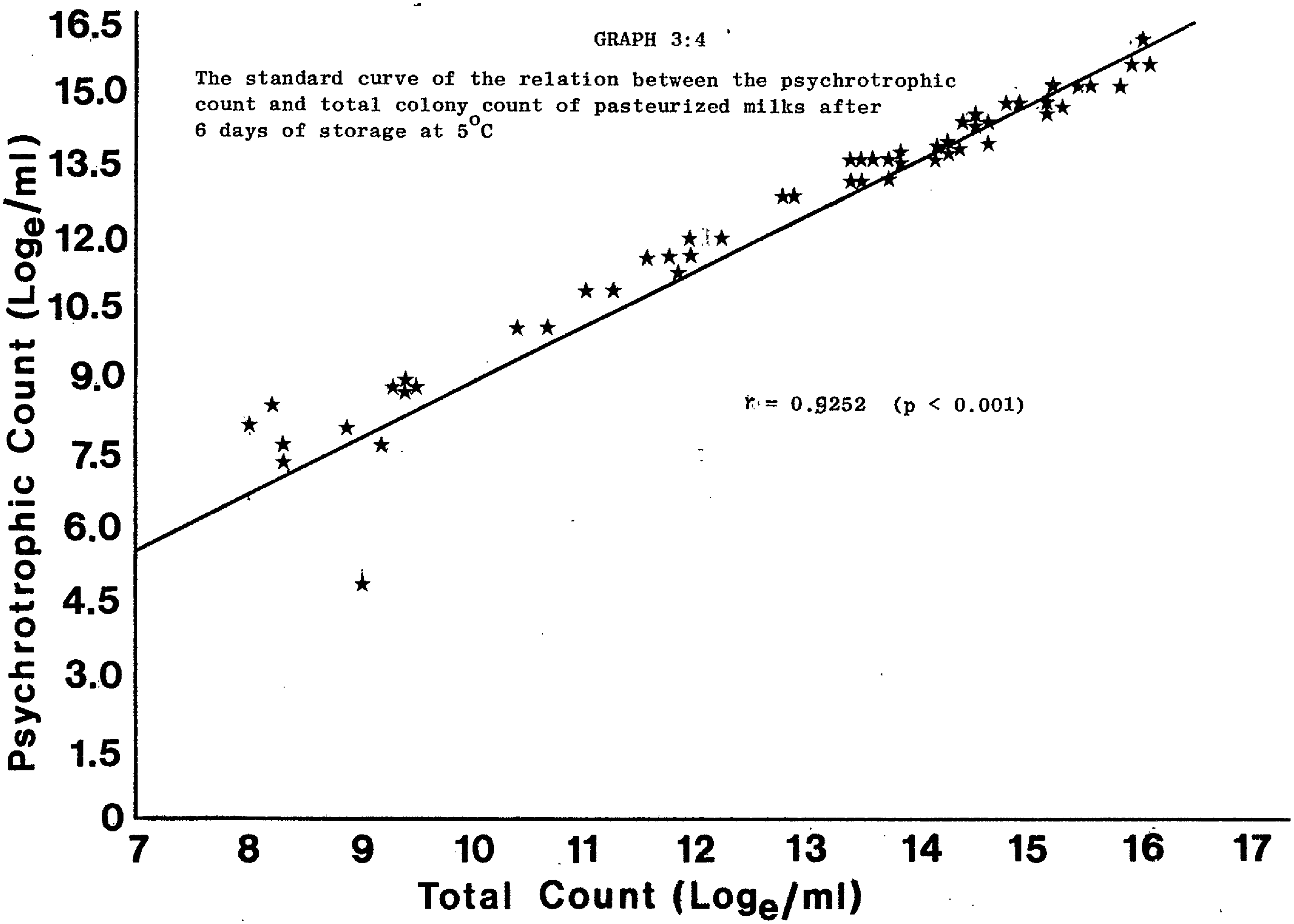


TABLE 3:10

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single service containers on the same day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.83	4.45	1.50	3.93
2	5.69	6.05	0.00	3.91
3	8.55	4.96	0.00	4.50
4	4.09	4.09	4.09	4.09
5	4.58	4.09	0.00	2.89
6	4.45	4.61	3.33	4.13
Mean	5.53	4.71	1.48	3.93

	DF	MS	VR
Trial	5	1.7632	12.42***
Sample	2	54.8992	386.99***
Trial sample	9	6.3345	44.65***
Residual	17	0.1419	
Total	33	5.3951	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.2175	0.1538	0.3766

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

TABLE 3:11

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The lipolytic count of pasteurized milk from the pasteurized milk tank is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	6.22	5.97	5.46	4.99	2.62	5.05
2	7.16	7.11	7.33	7.25	4.42	6.66
3	10.41	10.73	5.27	5.43	0.00	6.37
4	4.29	4.25	4.53	4.55	4.00	4.34
5	7.89	6.00	4.09	4.35	3.57	5.18
6.	4.42	4.84	4.39	4.29	2.39	4.07
Mean	6.73	6.48	5.18	5.15	2.85	5.28

	DF	MS	VR
Trial	5	10.96879	125.01***
Treatment	4	28.49561	324.78***
Trial treatment	19	5.46563	62.29***
Residual	29	0.08774	
Total	57	4.82838	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1325	0.1209	0.2962

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 3:12

The lipolytic count/ml ($30^{\circ}C$ for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 4 days of storage at $5^{\circ}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The lipolytic count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	7.98	8.32	7.45	7.22	3.84	6.96
2	9.80	9.74	10.09	9.41	6.38	9.08
3	13.39	13.52	7.60	6.85	4.37	9.15
4	12.12	12.15	9.88	9.98	6.71	10.17
5	12.86	12.66	7.34	6.88	6.51	9.25
6	7.06	6.99	7.49	7.29	4.79	6.72
Mean	10.53	10.56	8.31	7.94	5.43	8.55

	DF	MS	VR
Trial	5	19.21619	212.17***
Treatment	4	54.48410	601.58***
Trial treatment	19	4.96624	54.83***
Residual	29	0.09057	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1346	0.1229	0.3009

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

TABLE 3:13

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The lipolytic count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	11.97	12.31	9.37	9.38	4.62	9.53
2	13.53	12.45	14.18	14.20	7.20	12.31
3	13.37	14.12	11.76	11.70	4.59	11.11
4	13.44	13.31	13.27	13.13	7.21	12.07
5	14.20	13.93	11.08	10.73	6.85	11.36
6	13.04	12.86	11.73	12.06	7.97	11.53
Mean	13.26	13.16	11.90	11.87	6.41	11.32

	DF	MS	VR
Trial	5	9.7048	22.15***
Treatment	4	95.7558	218.64***
Trial treatment	19	2.1613	4.93***
Residual	29	0.4380	
Total	57	8.5143	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2960	0.2702	0.6618

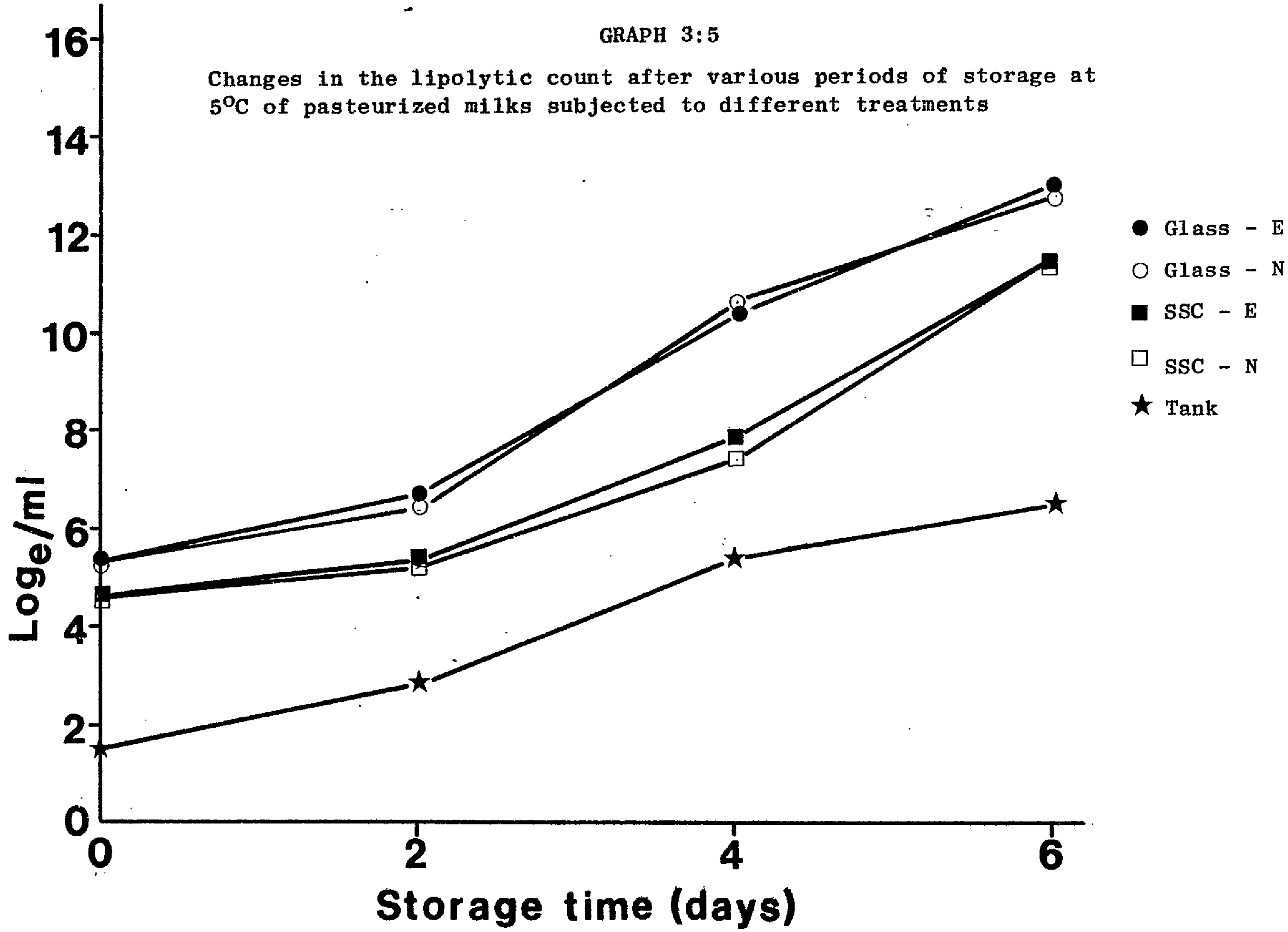
* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

GRAPH 3:5

Changes in the lipolytic count after various periods of storage at $5^{o}C$ of pasteurized milks subjected to different treatments



lipolytic count ($p < 0.001$) than was obtained from the samples from the pasteurized milk tank. The same differences were found in the respective samples after 2, 4 and 6 days of storage at 5°C. It is clear from the results that there was no significant effect of exposure for 3 h to light and temperature conditions simulating the doorstep delivery system on the lipolytic count. The variations on the lipolytic count due to the storage time at different treatments are illustrated in graph 3:3. The lipolytic count showed a correlation coefficient of ($r = 0.5724$, $p < 0.001$) with the acid degree value. After 6 days of storage at 5°C the mean of the lipolytic count of pasteurized milk in glass bottles was 5.458×10^5 per ml. However in SSC samples it was 1.45×10^5 per ml. It is interesting to note that the mean lipolytic count obtained from the milk from the pasteurized milk tank using the same conditions, was 608 per ml.

4. Bacillus cereus count

The results of the B. cereus counts are presented in Tables 3:14, 3:15, 3:16 and 3:17. The results show that differences occurred in the count of B. cereus in the different samples taken on the day of processing. The B. cereus count obtained from pasteurized milk packaged in glass bottles was significantly higher ($p < 0.001$) than that obtained from pasteurized milk packaged in SSC. The latter gave a significantly higher count ($p < 0.001$) than that from the pasteurized milk tank. These differences are shown for all of the days of storage at 5°C. Exposure to light and temperature conditions simulating the doorstep delivery system had no significant effect on the count of B. cereus. There was a significant difference ($p < 0.001$) between the B. cereus counts obtained in the six different trials. The variations in the B. cereus count with storage time are illustrated in graph 3:4. The count of B. cereus increased in all the samples during the storage time. The mean count of B. cereus in milks purchased in glass bottles in the six trials was 67,846 per ml after 6 days of storage at 5°C. However, in the SSC samples the corresponding count was 11,614 per ml, while in the samples from the pasteurized milk tank it was 200 per ml.

TABLE 3:14

The *B. cereus* count/ml (30°C for 2 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, glass bottles and single service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	3.18	2.09	0.71	2.00
2	2.72	2.59	0.00	1.77
3	5.12	2.91	0.00	2.68
4	1.10	1.10	1.10	1.10
5	2.46	1.10	1.10	1.55
6	4.43	2.30	1.10	2.61
Mean	3.17	2.01	0.67	1.95

	DF	MS	VR
Trial	5	2.2521	5.01**
Sample	2	18.7835	41.85***
Trial sample	9	2.0185	4.49**
Residual	17	0.4487	
Total	33	2.2613	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.387	0.273	0.670

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

TABLE 3:15

The B. cereus count/ml (30°C for 2 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The B. cereus count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	4.34	4.62	4.29	3.70	1.08	3.61
2	3.73	3.17	4.51	3.63	0.00	3.01
3	5.02	4.96	3.70	3.86	0.00	3.51
4	3.80	3.82	4.10	4.41	1.09	3.44
5	4.57	4.70	1.09	1.09	1.09	2.51
6	4.68	5.10	3.75	2.46	1.09	3.42
Mean	4.36	4.40	3.58	3.19	0.73	3.25

	DF	MS	VR
Trial	5	1.7330	7.55**
Treatment	4	27.0501	117.94***
Trial treatment	19	1.7419	7.59***
Residual	29	0.2294	
Total	57	2.7476	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2142	0.1955	0.4789

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

TABLE 3:16

The B. cereus count/ml (30^oC for 2 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at 5^oC in darkness and in light and temperature conditions simulating the doorstep delivery system. The B. cereus count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	6.61	6.51	5.41	5.76	3.31	5.48
2	7.37	7.36	7.36	7.26	2.58	6.38
3	8.88	8.80	4.56	4.56	0.00	5.36
4	8.20	6.49	6.78	6.44	4.61	6.50
5	7.27	5.71	4.75	4.08	4.42	5.24
6	5.85	6.46	5.69	5.62	6.03	5.93
Mean	7.36	6.89	5.76	5.62	3.46	5.82

	DF	MS	VR
Trial	5	2.9181	6.92***
Treatment	4	27.3766	64.95***
Trial treatment	19	4.1197	9.77***
Residual	29	0.4214	
Total	57	3.7648	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2903	0.2650	0.6492

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

TABLE 3:17

The B. cereus count/ml ($30^{o}C$ for 2 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The B. cereus count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	9.45	9.05	7.26	7.15	3.28	7.24
2	12.27	11.43	11.97	12.07	6.65	10.88
3	12.61	12.67	8.97	8.91	3.57	9.35
4	11.58	11.39	11.67	11.36	5.61	10.32
5	11.48	10.56	7.12	6.82	5.52	8.30
6	10.49	10.52	9.15	9.87	7.16	9.44
Mean	11.31	10.94	9.36	9.36	5.30	9.25

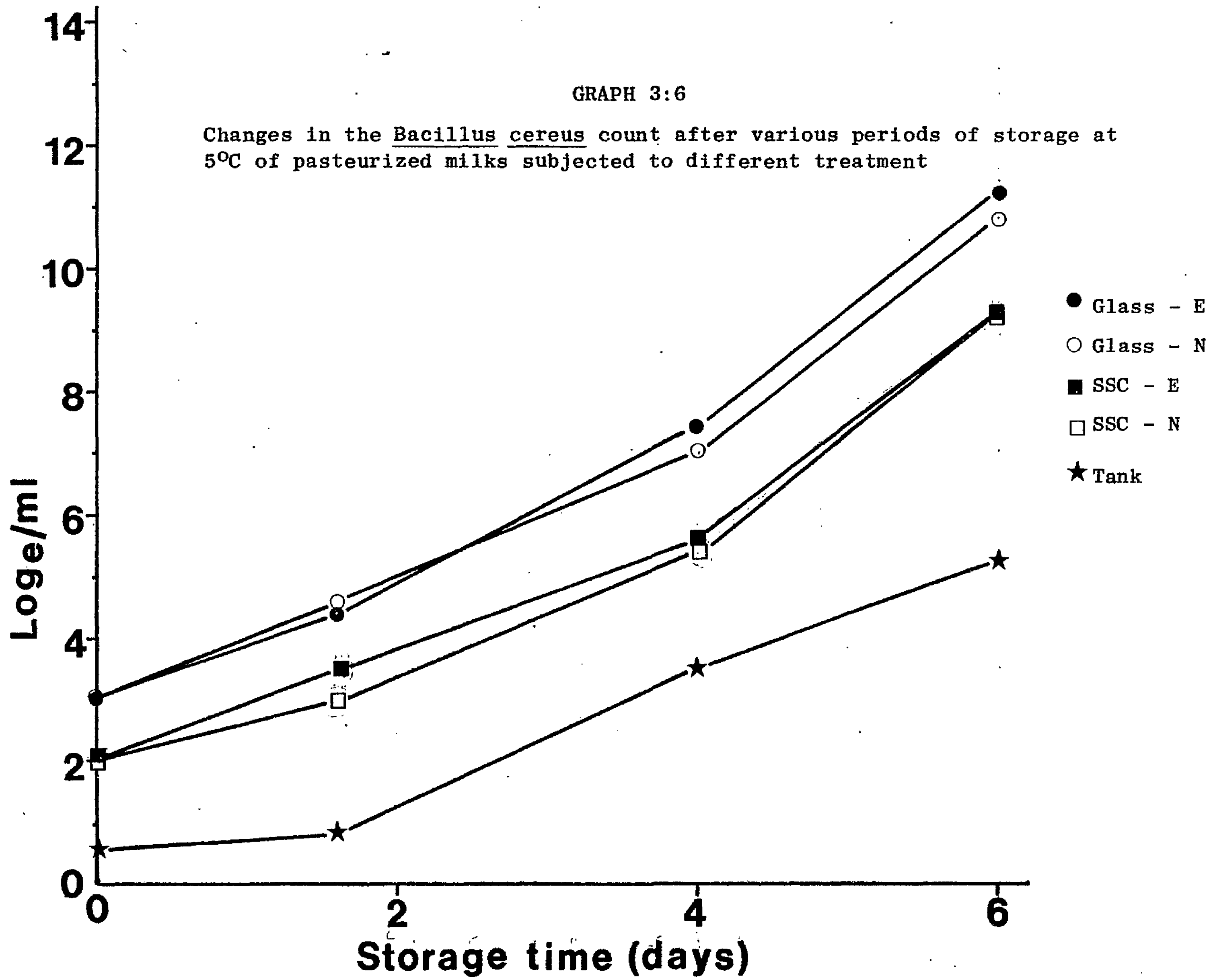
	DF	MS	VR
Trial	5	17.5975	106.16***
Treatment	4	68.1796	411.29***
Trial treatment	19	2.9131	17.57***
Residual	29	0.1658	
Total	57	7.3833	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1821	0.1662	0.4071

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

GRAPH 3:6

Changes in the *Bacillus cereus* count after various periods of storage at 5°C of pasteurized milks subjected to different treatment



The correlation coefficient between the B. cereus count and the total colony count was 0.8974 which is highly significant ($p < 0.001$). This correlation coefficient was calculated from all the samples from different sources.

5. Proteolytic count

The results of the proteolytic count are presented in Table 3:18, 3:19, 3:20 and 3:21. Differences existed in the proteolytic count of samples on the day of processing. The mean of the proteolytic count obtained from the pasteurized milk packaged in glass bottles was significantly higher ($p < 0.001$) than that of pasteurized milk packaged in SSC. The latter showed a significantly higher ($p < 0.001$) count than that found in the samples taken from the pasteurized milk tank. Differences were found to be the same as at 2, 4 and 6 days of storage at 5^oC. Exposure to light and temperature conditions simulating the doorstep delivery system had no significant effect on the proteolytic count.

A highly significant difference ($p < 0.001$) was found between the proteolytic counts obtained in different trials after various periods of storage.

After 6 days of storage at 5^oC the means of the proteolytic counts of the pasteurized milk packaged in glass bottles, SSC and from the pasteurized milk tank were 4.146×10^5 per ml, 8.082×10^4 per ml, and 1.686×10^3 per ml respectively.

The variations in the proteolytic count with storage time are illustrated in graph 3:5. The increase in the proteolytic count was greater in milk packaged in glass bottles than in the other samples.

The proteolytic count showed a highly significant correlation coefficient ($r = 0.9549$, $p < 0.001$) with the total colony count. The correlation coefficient with the psychrotrophic count was highly significant ($r = 0.9104$, $p < 0.001$).

TABLE 3:18

The proteolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	6.08	5.77	3.73	5.19
2	7.04	6.69	0.00	4.57
3	9.08	6.64	6.44	7.39
4	5.55	5.97	6.06	5.86
5	6.66	5.41	4.32	5.46
6	6.62	5.71	4.93	5.75
Mean	6.84	6.03	4.25	5.71

	DF	MS	VR
Trial	5	5.3468	48.29***
Sample	2	21.0944	190.54***
Trial sample	9	4.9504	44.71***
Residual	17	0.1107	
Total	33	3.4957	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.1921	0.1358	0.3327

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

TABLE 3:19

The proteolytic count/ml ($30^{o}C$ for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at $5^{o}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The proteolytic count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	7.13	6.90	6.57	6.71	5.69	6.60
2	6.65	6.85	6.58	6.55	6.09	6.54
3	10.86	10.87	6.63	6.64	6.74	8.35
4	7.01	6.78	6.99	7.07	5.01	6.57
5	8.03	6.61	6.26	6.43	5.58	6.58
6	6.20	6.16	6.19	6.18	6.28	6.20
Mean	7.64	7.36	6.54	6.80	5.90	6.81

	DF	MS	VR
Trial	5	5.92891	88.15***
Treatment	4	5.86081	87.13***
Trial treatment	19	1.81483	26.98***
Residual	29	0.06726	
Total	57	1.57053	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.1160	0.1059	0.2593

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

TABLE 3:20

The proteolytic count/ml ($30^{\circ}C$ for 3 d), expressed as $\log_e$, of pasteurised milk packaged in glass bottles and single-service containers after 4 days of storage at $5^{\circ}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The proteolytic count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	9.61	10.25	7.75	7.62	5.50	8.15
2	8.05	8.22	8.88	8.83	6.66	8.13
3	13.62	13.96	9.81	9.96	6.45	10.76
4	11.33	8.60	8.81	8.61	6.59	8.79
5	11.99	12.20	8.08	8.81	6.00	9.42
6	9.91	10.30	9.43	9.23	7.39	9.25
Mean	10.75	10.59	8.79	8.84	6.43	9.08

	DF	MS	VR
Trial	5	9.6652	28.90***
Treatment	4	36.6489	109.59***
Trial treatment	19	2.8450	8.507***
Residual	29	0.3344	
Total	57	4.5381	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2586	0.2361	0.5783

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

TABLE 3:21

The proteolytic count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The proteolytic count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	12.52	12.41	9.59	9.70	6.37	10.12
2	11.49	12.23	14.34	14.41	7.22	11.94
3	15.50	14.50	10.73	10.95	7.55	11.84
4	12.91	12.63	13.55	13.70	7.41	12.04
5	12.49	13.23	8.71	8.60	7.39	10.08
6	12.49	12.81	10.21	11.11	8.65	11.05
Mean	12.90	12.97	11.19	11.41	7.43	11.18

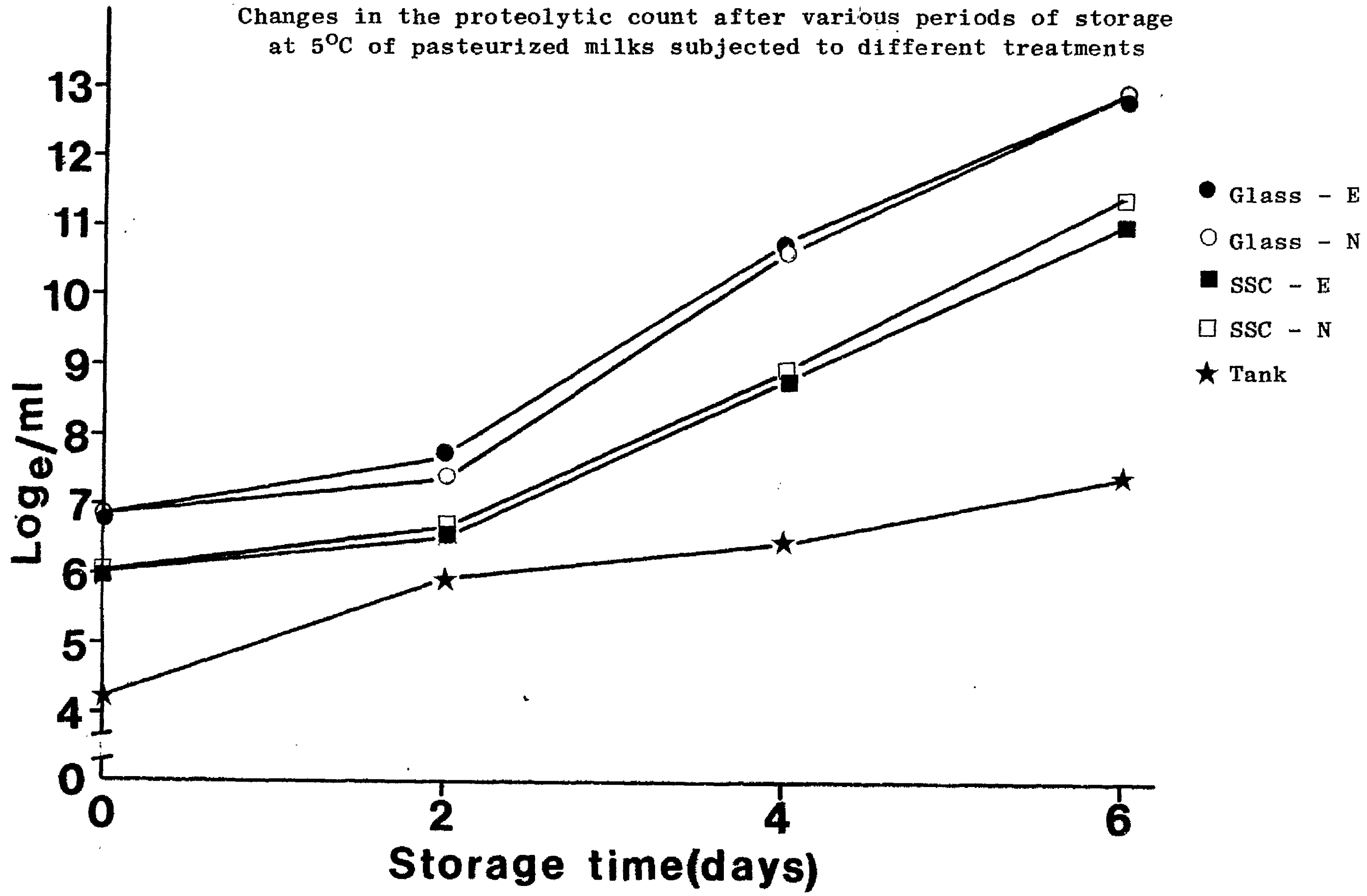
	DF	MS	VR
Trial	5	8.1899	31.56***
Treatment	4	60.8102	234.40***
Trial treatment	19	4.7904	18.46***
Residual	29	0.2594	
Total	57	6.7146	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.2278	0.2079	0.5093

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

GRAPH 3:7

Changes in the proteolytic count after various periods of storage at 5°C of pasteurized milks subjected to different treatments



6. Coliform count

The results of the coliform counts on samples of pasteurized milk are presented in tables 3:22, 3:23, 3:24 and 3:25. Differen levels of coliform infection were found in the various milks on the day of processing (Table 3:22). The coliform count of pasteurized milk packaged in glass bottles was significantly higher ($p < 0.001$) than that obtained from pasteurised milk packaged in SSC. Similar differences in the coliform count of milk packaged in glass bottles and SSC were found after 2, 4 and 6 days of storage at 5°C. Coliform organisms were absent in 1 ml in the samples of milk in SSC on the day of processing and after 2 and 4 days of storage except in the first trial. Coliform bacteria were found in the samples of SSC after 6 days of storage at 5°C. None of the samples taken from the pasteurized milk tank gave a positive result for coliform in 1 ml in all the experiments. The results in the first trial were estimated by the computer.

Positive coliform tests at 1 ml level were found on the day of processing in pasteurized milk packaged in glass bottles. The count of coliforms increased in this form of packaged milk during the storage time at 5°C. (graph 3:8).

Exposure to light and temperature conditions simulating the doorstep delivery system had no significant effect on coliform numbers.

Highly significant differences ($p < 0.001$) were found between coliform numbers obtained in different trials on the day of processing and after 2 days storage at 5°C. After 4 and 6 days storage the differences in coliform levels between trials was significant at 1 per cent level.

7. Results of the rapid post pasteurization test

All of the samples which were tested failed this test. The identification of the colonies which grew on the selective media used in this test is summarised in Table 3:26.

TABLE 3:22

The coliform count/ml (30^{o}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.34	1.96	2.14	3.14
2	1.50	0.00	0.00	0.50
3	4.82	0.00	0.00	1.61
4	2.78	0.00	0.00	0.93
5	3.57	0.00	0.00	1.19
6	2.43	0.00	0.00	0.81
Mean	3.41	0.33	0.36	1.36

	DF	MS	VR
Trial	5	5.4010	8.04***
Sample	2	37.5701	55.93***
Trial sample	9	0.9377	1.39
Residual	17	0.6716	
Total	33	3.6970	

Table	Trial	Sample	Trial sample
REP	6	12	2
SED	0.473	0.335	0.820

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

TABLE 3:23

The coliform count/ml ($30^{\circ}C$ for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at $5^{\circ}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The coliform count of pasteurized milk from the pasteurized milk tank is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	4.39	6.55	0.80	1.15	1.87	2.58
2	1.95	0.80	0.00	0.00	0.00	0.55
3	5.04	5.81	0.00	0.00	0.00	2.17
4	3.54	3.66	0.00	0.00	0.00	1.44
5	1.15	0.00	0.00	0.00	0.00	0.23
6	3.06	1.95	0.00	0.00	0.00	1.00
Mean	3.19	3.13	0.13	0.19	0.31	1.39

	DF	MS	VR
Trial	5	10.5285	20.48***
Treatment	4	31.3903	61.06***
Trial treatment	19	2.6114	5.08***
Residual	29	0.5140	
Total	57	4.2584	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.3206	0.2927	0.7170

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

TABLE 3:24

The coliform count/ml ($30^{o}C$ for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at $5^{o}C$ in darkness and in light and temperature conditions simulating the doorstep delivery system. The coliform count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	5.04	6.56	0.80	1.61	0.75	2.95
2	4.65	1.39	0.00	0.00	0.00	1.21
3	5.53	6.11	0.00	0.00	0.00	2.49
4	7.24	5.13	0.00	0.00	0.00	2.64
5	7.12	7.12	0.00	0.00	0.00	2.85
6	4.04	5.04	0.00	0.00	0.00	1.82
Mean	5.61	5.23	0.13	0.54	0.13	2.33

	DF	MS	VR
Trial	5	4.602	4.39**
Treatment	4	96.032	91.67***
Trial treatment	19	2.241	2.14*
Residual	29	1.048	
Total	57	8.423	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.4580	0.4180	1.0230

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

TABLE 3:25

The coliform count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system. The coliform count of pasteurized milk from the pasteurized milk tanks is shown.

No. of trial	Sample and treatment					
	Glass-E	Glass-N	SSC-E	SSC-N	Tank	Mean
1	6.01	6.55	1.60	0.80	0.38	2.91
2	7.00	4.19	2.95	2.30	0.00	3.29
3	6.38	7.24	0.00	0.00	0.00	2.72
4	7.24	6.67	1.60	2.19	0.00	3.54
5	6.13	6.13	3.91	3.91	0.00	4.01
6	6.67	7.24	0.80	0.00	0.00	2.94
Mean	6.57	6.34	1.81	1.53	0.06	3.24

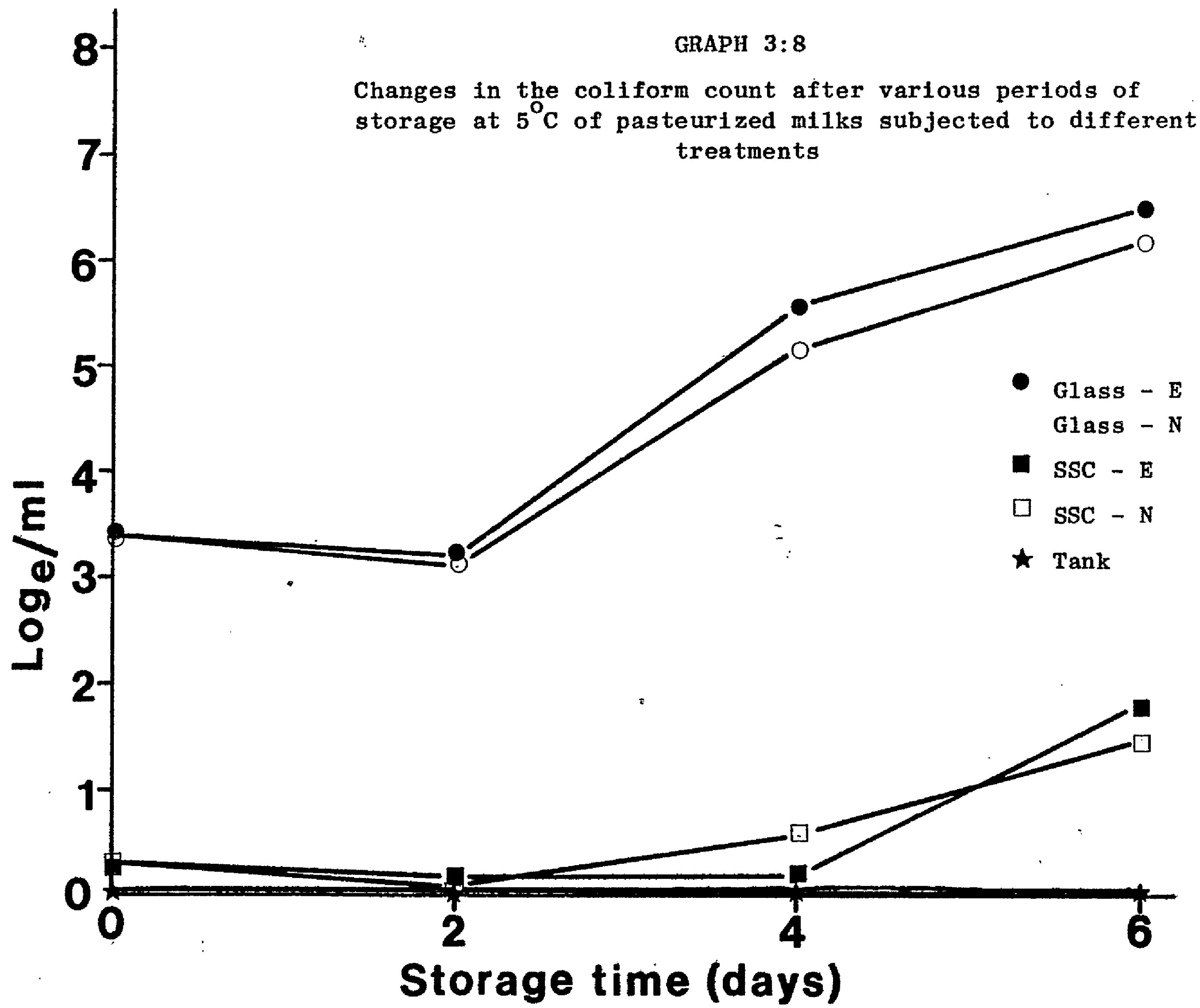
Trial	5	2.3055	4.12**
Treatment	4	109.8213	196.36***
Trial treatment	19	2.5259	4.51***
Residual	29	0.5593	
Total	57	9.0355	

Table	Trial	Treatment	Trial treatment
REP	10	12	2
SED	0.3345	0.3053	0.7479

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

GRAPH 3:8

Changes in the coliform count after various periods of storage at 5°C of pasteurized milks subjected to different treatments



8. Quality of the packaging material

The results of the sterility tests on glass bottles and the packaging material are presented in Table 3:28. The results show that the empty glass bottles were an important source of post-pasteurization contamination. The cartoning material was found to be less contaminated than the glass bottles.

TABLE 3:26

Types and numbers of the micro-organisms which were isolated from two different selective media* used in the rapid test for post pasteurization contamination and identified using an 'Oxi/Ferm' tube and 'Enterotube' systems.

Micro-organisms	Number
Aeromonas spp	1
Citrobacter freundii	1
Escherichia coli	5
Enterobacter aerogenes	6
Enterobacter cloacae	3
Enterobacter agglomerans	2
Pseudomonas Spp.	14
Serratia liquefaciens	4
Total number of isolates	36

*Plate count agar (Difco) was prepared with one of the following additions:-

1. Benzalkon A-50%(Alkyl benzyl diamethyl ammonium chloride) (Merck-Schuchardt) 100 mg/kg medium.

2. Janus green (Hopkins and Williams) 1000 mg/kg medium.

TABLE 3:27

The correlation coefficient matrix between the different microbiological tests used to assess the microbiological quality of pasteurized milk packaged in glass bottles and single-service containers

1	Total colony count	1.0000					
2	Psychrotrophic "	*** 0.9173	1.000				
3	Lipolytic "	*** 0.9450	*** 0.9462	1.0000			
4	Proteolytic "	*** 0.9549	*** 0.9104	*** 0.9121	1.000		
5	*B. cereus* "	*** 0.8974	*** 0.9039	*** 0.9069	*** 0.8950	1.000	
6	Coliform "	*** 0.5993	*** 0.6036	*** 0.5435	*** 0.5716	*** 0.5068	1.000
		1	2	3	4	5	6

DF = 166

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

TABLE 3:28

Total colony count of washed bottles and cartoning material of single-service containers sampled at Dairy A during packaging of pasteurized milk in the six different trials.

No. of trial	No. of bottle	Total colony count/bottle	No. of carton	Total colony count/carton
First	1	5	1	0
	2	15	2	0
	3	10	3	2
Second	4	8	4	18
	5	2	5	35
	6	6	6	23
Third	7	10	7	0
	8	15	8	1
	9	10	9	0
Fourth	10	9	10	0
	11	4	11	0
	12	18	12	0
Fifth	13	10	13	2
	14	15	14	5
	15	30	15	0
Sixth	16	5	16	2
	17	27	17	1
	18	13	18	0

Dairy B

1. Total colony count

The results of the total colony count are presented in Table 3:29, 3:30, 3:31 and 3:32. The results show a highly significant difference ($p < 0.001$) in the total colony count of the different samples of pasteurized milk on the day of processing (Table 3:29). The total colony counts of pasteurized milk packaged in SSC and stored at $5^{o}C$ for up to 6 days showed greater increases than those of milk stored under the same conditions in glass bottles. The total colony count in glass bottles was higher ($p < 0.001$) than that of samples taken from the pasteurized milk tank. The differences referred to above were found after 2, 4 and 6 days of storage at $5^{o}C$. The mean of the total colony count obtained from pasteurized milk packaged in glass bottles, SSC and the pasteurized milk tank were about 4.62×10^4, 1.989×10^5 and 10,940 per ml respectively after 6 days of storage at $5^{o}C$.

The variations of the total colony count with the time of storage are shown in graph 3:9.

From the results presented in Table 3:31, it is clear that the mean of the total colony count in samples in each types of package both of the packaging systems was $< 3 \times 10^4$ per ml after 4 days of storage at $5^{o}C$.

Significant differences (at 0.1 per cent level) were found in the total colony count of samples taken in each of the three trials when tested on the day of processing and after 2, 4 and 6 days of storage at $5^{o}C$.

The variation in the total colony count with the storage time of the different samples is illustrated in graph 3:9.

TABLE 3:29

The total colony count/ml (30°C for 3d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank and from glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	8.80	9.00	8.50	8.77
2	8.57	8.74	7.57	8.29
3	9.61	9.68	9.44	9.58
Mean	8.99	9.14	8.50	8.88

	DF	MS	VR
Trial	2	2.52643	229.73***
Sample	2	0.66747	60.69***
Trial sample	4	0.14488	13.17***
Residual	9	0.01100	
Total	17	0.41566	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.0605	0.0605	0.1049

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

TABLE 3:30

The total colony count/ml (30^oC for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days of storage at 5^oC.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	8.55	9.07	8.63	8.75
2	8.56	8.64	7.83	9.16
3	9.67	9.76	9.58	8.68
Mean	8.93	9.16	8.68	8.92

	DF	MS	VR
Trial	2	2.788398	639.46***
Sample	2	0.341366	78.28***
Trial sample	4	0.116434	26.70***
Residual	9	0.004361	
Total	17	0.397912	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.0381	0.0381	0.0660

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

TABLE 3:31

The total colony count/ml (30^{o}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 4 days of storage at 5^{o}C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	9.17	11.99	0.72	9.96
2	8.52	8.60	8.68	8.60
3	9.55	9.66	9.57	9.59
Mean	9.08	10.08	8.99	9.38

	DF	MS	VR
Trial	2	2.98797	37.76***
Sample	2	2.20116	54.34***
Trial sample	4	2.04990	50.60***
Residual	9	0.04051	
Total	17	1.11426	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1162	0.1162	0.2013

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 3:32

The total colony count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized tank, and from glass bottles and single-service containers after 6 days of storage at 5°C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	10.81	14.52	8.83	11.39
2	11.40	12.29	9.40	11.03
3	10.01	9.81	9.67	9.83
Mean	10.74	12.20	9.30	10.75

	DF	MS	VR
Trial	2	3.99126	52.36***
Sample	2	12.63221	165.73***
Trial sample	4	4.22520	55.43***
Residual	9	0.07622	
Total	17	2.99022	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1594	0.1594	0.2761

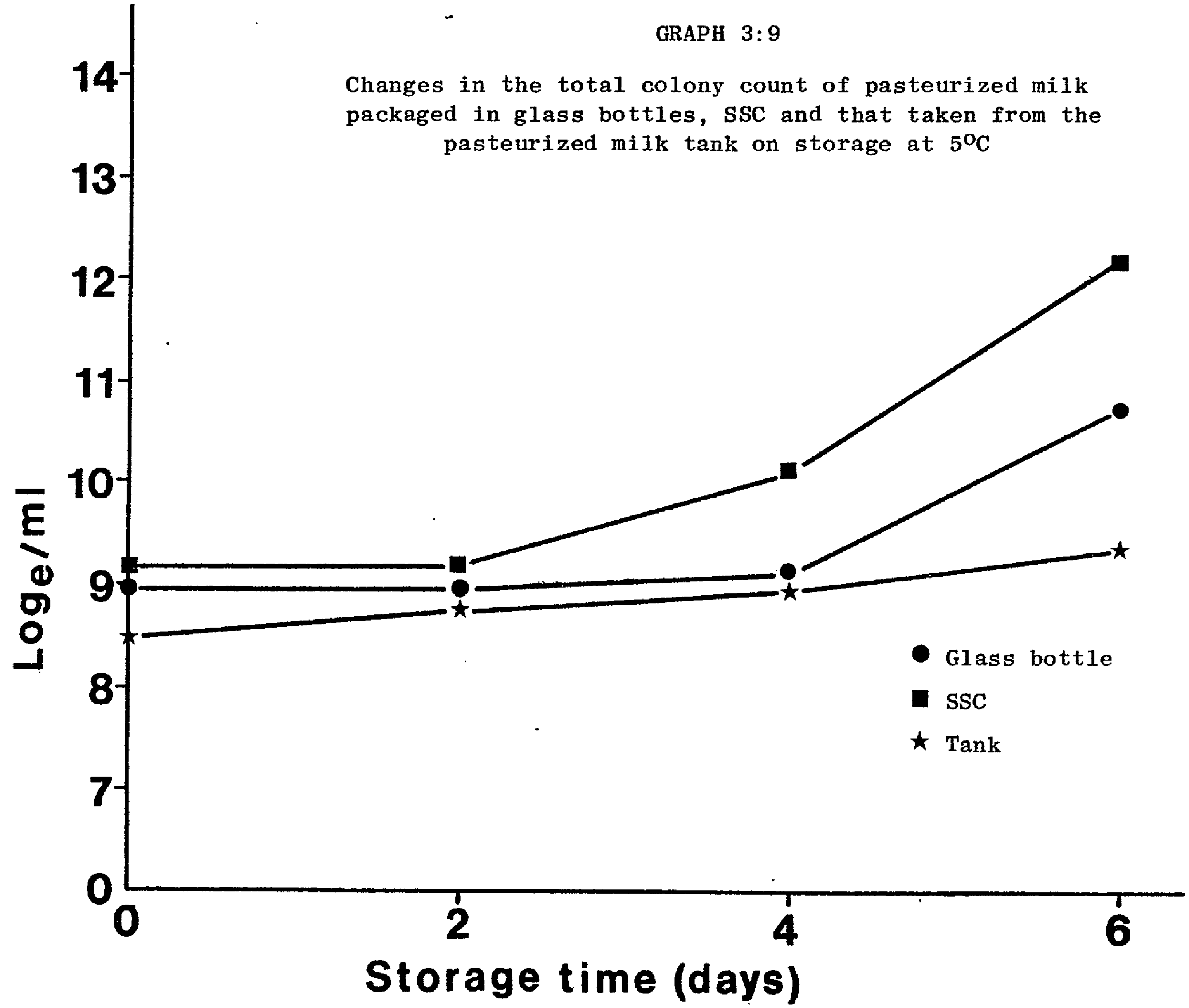
* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

GRAPH 3:9

Changes in the total colony count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5^{o}C



2. Psychrotrophic count

The results of the psychrotrophic count are presented in Tables 3:33, 3:34, 3:35 and 3:36. When tested on the day of processing the mean of the psychrotrophic count of pasteurized milk packaged in SSC was significantly higher ($p < 0.01$) than that of pasteurized milk packaged in glass bottles and also of the samples taken from the pasteurized milk tank. There was no significant difference between the counts from the milk in glass bottles and from the pasteurized milk tank on the day of processing. After 2 days of storage, the psychrotrophic count obtained from SSC samples was significantly higher ($p < 0.001$) than of the milk in glass bottles and the samples from the pasteurized milk tank. These differences were also found after 4 and 6 days of storage at $5^{O}C$. After the milks has been stored at $5^{O}C$ for 4 and 6 days, significantly higher ($p < 0.001$) psychrotrophic counts were found in milks packaged in glass bottles compared to the samples of milk from the pasteurized milk tank. The mean of the psychrotrophic count of the samples of milk in glass bottles, SSC and that from the pasteurized milk tank were 6,905, 7.921×10^4 and 1,845 per ml respectively after 6 days of storage at $5^{O}C$.

The differences between the psychrotrophic counts in the three trials on the day of processing and after 2, 4 and 6 days of storage at $5^{O}C$ were significant at 1, 0.1, 0.1 and 0.1 per cent level respectively.

The variation in the psychrotrophic count in respect of storage time of the different samples is illustrated in graph 3:10.

3. Lipolytic count

The results of the lipolytic count are presented in Tables 3:37, 3:38, 3:39 and 3:40. The results of tests made on the day of processing showed that there was no significant difference in the lipolytic count in all the samples of pasteurized milk.

After 2 days of storage at $5^{O}C$, the mean lipolytic counts of pasteurized milk packaged in SSC was significantly higher ($p < 0.001$) than that of the milk packaged in glass bottles or of the samples

TABLE 3:33

The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	0.34	3.07	0.89	1.43
2	1.09	1.58	0.89	1.19
3	0.34	0.89	0.34	0.53
Mean	0.59	1.85	0.71	1.05

	DF	MS	VR
Trial	2	1.3284	10.70**
Sample	2	2.8906	23.28**
Trial sample	4	0.8635	6.95**
Residual	9	0.1241	
Total	17	0.7652	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2034	0.2034	0.3523

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

TABLE 3:34

The psychrotrophic count/ml (7^{o}C for 10 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days of storage at 5^{o}C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	1.24	6.70	1.10	3.01
2	1.50	2.17	1.24	1.64
3	2.51	1.39	1.24	1.71
Mean	1.75	3.42	1.19	2.12

	DF	MS	VR
Trial	2	3.5900	16.90***
Sample	2	8.0406	37.85***
Trial sample	4	6.8733	32.36***
Residual	9	0.2124	
Total	17	3.0980	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2660	0.2660	0.4610

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

TABLE 3:35

The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 4 days of storage at $5^{o}C$

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.43	10.87	6.77	7.69
2	7.04	7.77	5.10	6.64
3	5.80	5.97	4.74	5.50
Mean	6.09	8.20	5.54	6.61

	DF	MS	VR
Trial	2	7.19747	541.42***
Sample	2	11.86854	892.80***
Trial sample	4	4.43827	333.86***
Residual	9	0.01329	
Total	17	3.29439	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.0666	0.0666	0.1153

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

TABLE 3:36

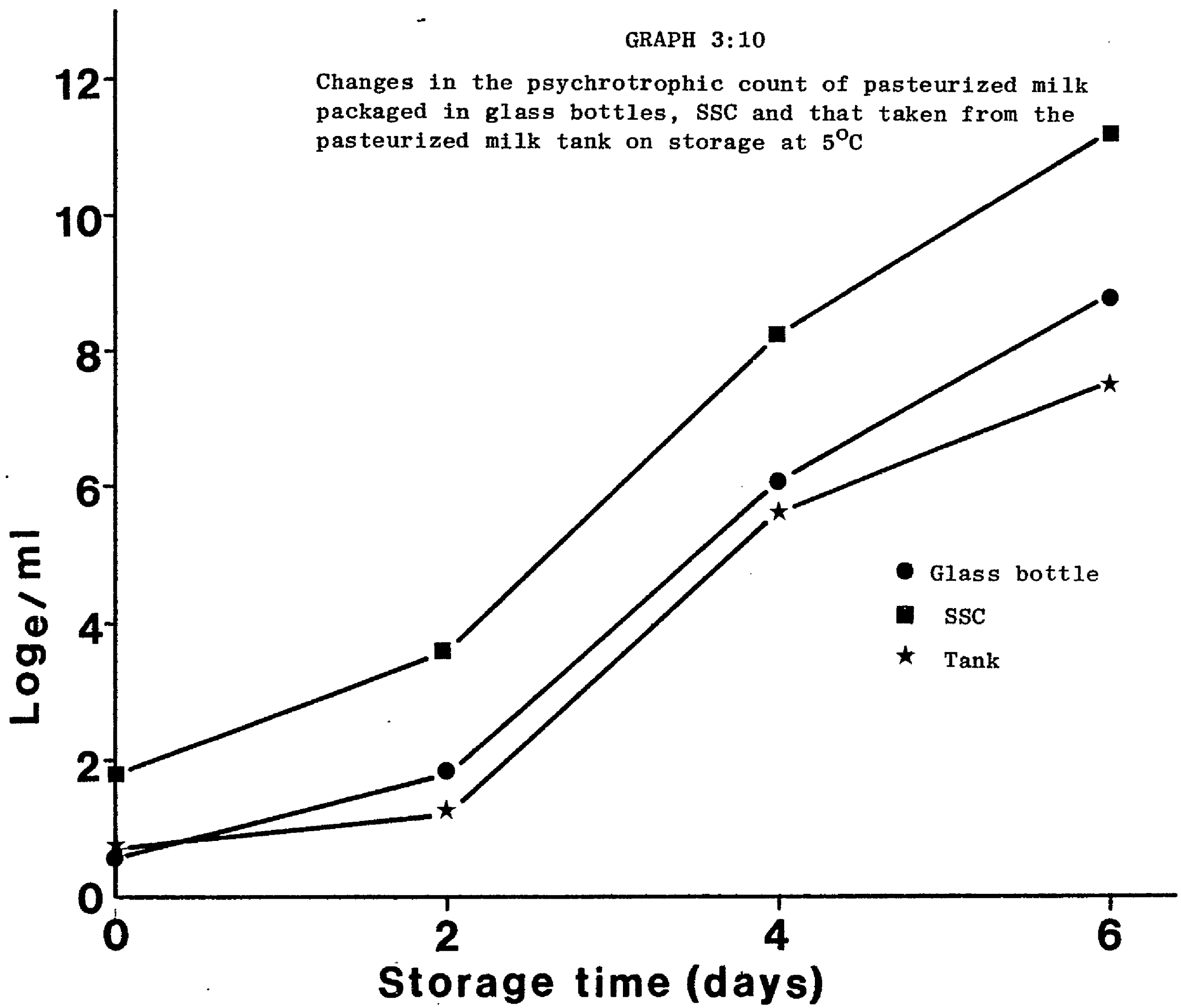
The psychrotrophic count/ml ($7^{o}C$ for 10 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 6 days of storage at $5^{o}C$.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	8.03	14.32	6.37	9.57
2	11.33	12.25	9.12	10.90
3	7.16	7.26	7.08	7.17
Mean	8.84	11.28	7.52	9.21

	DF	MS	VR
Trial	2	21.4665	80.11***
Sample	2	21.7655	81.22***
Trial sample	4	9.3257	34.80***
Residual	9	0.2680	
Total	17	7.4223	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2990	0.2990	0.5180

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



GRAPH 3:10

Changes in the psychrotrophic count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5°C

TABLE 3:37

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles, and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	4.88	4.88	4.73	4.83
2	2.58	3.23	2.77	2.86
3	4.18	4.26	3.97	4.14
Mean	3.88	4.12	3.82	3.94

	DF	MS	VR
Trial	2	5.99072	144.14***
Sample	2	0.15316	3.68
Trial sample	4	0.06632	1.59
Residual	9	0.04156	
Total	17	0.76042	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1177	0.1177	0.2039

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

TABLE 3:38

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days of storage at 5°C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	3.64	5.83	4.04	4.50
2	3.54	3.70	3.50	3.58
3	6.21	6.08	5.69	5.99
Mean	4.46	5.20	4.41	4.69

	DF	MS	VR
Trial	2	8.87910	146.36***
Sample	2	1.17756	19.41***
Trial sample	4	0.84711	13.96***
Residual	9	0.66066	
Total	17	1.41457	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1422	0.1422	0.2463

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 3:39

The lipolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 4 days of storage at 5°C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.56	12.16	6.48	8.07
2	6.48	6.11	4.71	5.77
3	7.06	7.55	5.62	6.74
Mean	6.37	8.61	5.60	6.86

	DF	MS	VR
Trial	2	7.9842	23.92***
Sample	2	14.6461	43.89***
Trial sample	4	7.3475	22.02***
Residual	9	0.3336	
Total	17	4.5678	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.3330	0.3330	0.5780

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

TABLE 3:40

The lipolytic count/ml (30^{o}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 6 days of storage at 5^{o}C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	9.76	14.33	7.11	10.40
2	11.15	11.98	8.98	10.70
3	9.06	9.21	7.56	8.81
Mean	9.99	11.84	7.88	9.90

	DF	MS	VR
Trial	2	7.66172	86.57***
Sample	2	23.48816	265.37***
Trial sample	4	4.82399	54.50***
Residual	9	0.08850	
Total	17	4.84637	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1718	0.1718	0.2975

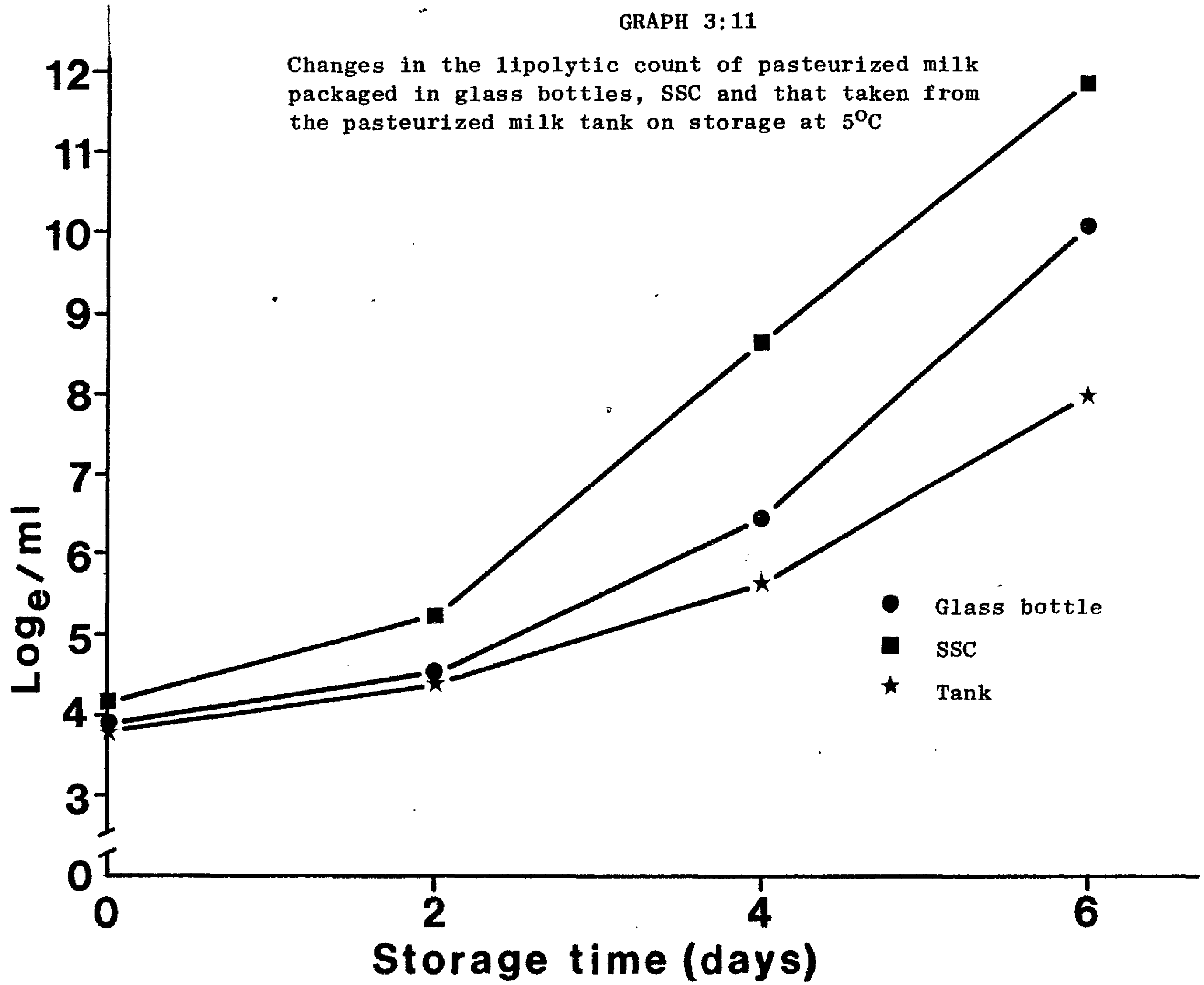
* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

GRAPH 3:11

Changes in the lipolytic count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5°C



taken from the pasteurized milk tank. These differences were also found at the same level of significance after 4 and 6 days of storage at $5^{o}C$.

After the samples had been stored at $5^{o}C$ for 4 and 6 days the lipolytic count of the milk packaged in glass bottles was significantly higher ($p < 0.5$ and $p < 0.1$ respectively) than the corresponding samples from the pasteurized milk tank.

After samples had been stored at $5^{o}C$ for 6 days the mean lipolytic counts for milk in glass bottles, SSC and from the pasteurized milk tank were 2.181×10^{4}, 1.387×10^{5} and 2.644×10^{3} per ml respectively.

The difference between the lipolytic counts in the three trials on the day of processing and after 2, 4 and 6 days of storage at $5^{o}C$ were highly significant ($p < 0.001$).

The variations in the lipolytic count with the storage time obtained from the three different samples are shown in graph 3:11.

4. Bacillus cereus count

The results of the Bacillus cereus count are presented in Tables 4:41, 3:42, 4:43 and 3:44. The results of tests made on the day of processing show that B. cereus was absent in 1 ml in all of the samples of pasteurized milk (Table 3:41).

After milks had been stored for 2 days at $5^{o}C$ there was no significant difference in the B. cereus counts of each of the samples of pasteurized milk. There were significant differences (0.1 per cent level) between the B. cereus counts of all the samples after 4 and 6 days of storage at $5^{o}C$. After 4 days of storage at $5^{o}C$ the mean B. cereus count of SSC samples was significantly higher ($p < 0.5$) than that of milk in glass bottles. However, the B. cereus counts of milk in SSC and glass bottles were greater than ($p < 0.001$) those of samples taken from the pasteurized milk tank.

After 6 days of storage, there was highly significant difference

TABLE 3:41

The *B. cereus* count/ml* ($30^{o}C$ for 2 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank and from glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00

* 0 ≡ no count when 1ml of undiluted milk was plated.

TABLE 3:42

The *B. cereus* count/ml ($30^{o}C$ for 2 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days of storage at $5^{o}C$.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	4.81	4.91	4.72	4.81
2	3.49	3.43	2.77	3.23
3	2.28	2.28	1.20	1.92
Mean	3.53	3.54	2.90	3.32

	DF	MS	VR
Trial	2	12.5740	25.96***
Sample	2	0.8102	1.67
Trial sample	4	0.1529	0.31
Residual	9	0.4843	
Total	17	1.8670	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.402	0.402	0.696

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

TABLE 3:43

The B. cereus count/ml ($30^{\circ}C$ for 2 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 4 days of storage at $5^{\circ}C$.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	4.95	6.48	2.39	4.61
2	3.50	3.01	2.99	3.17
3	2.39	3.15	1.79	2.44
Mean	3.62	4.21	2.39	3.41

	DF	MS	VR
Trial	2	7.2934	53.28***
Sample	2	5.1840	37.87***
Trial sample	4	2.2162	16.19***
Residual	9	0.1369	
Total	17	2.0619	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2136	0.2136	0.3700

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

TABLE 3:44

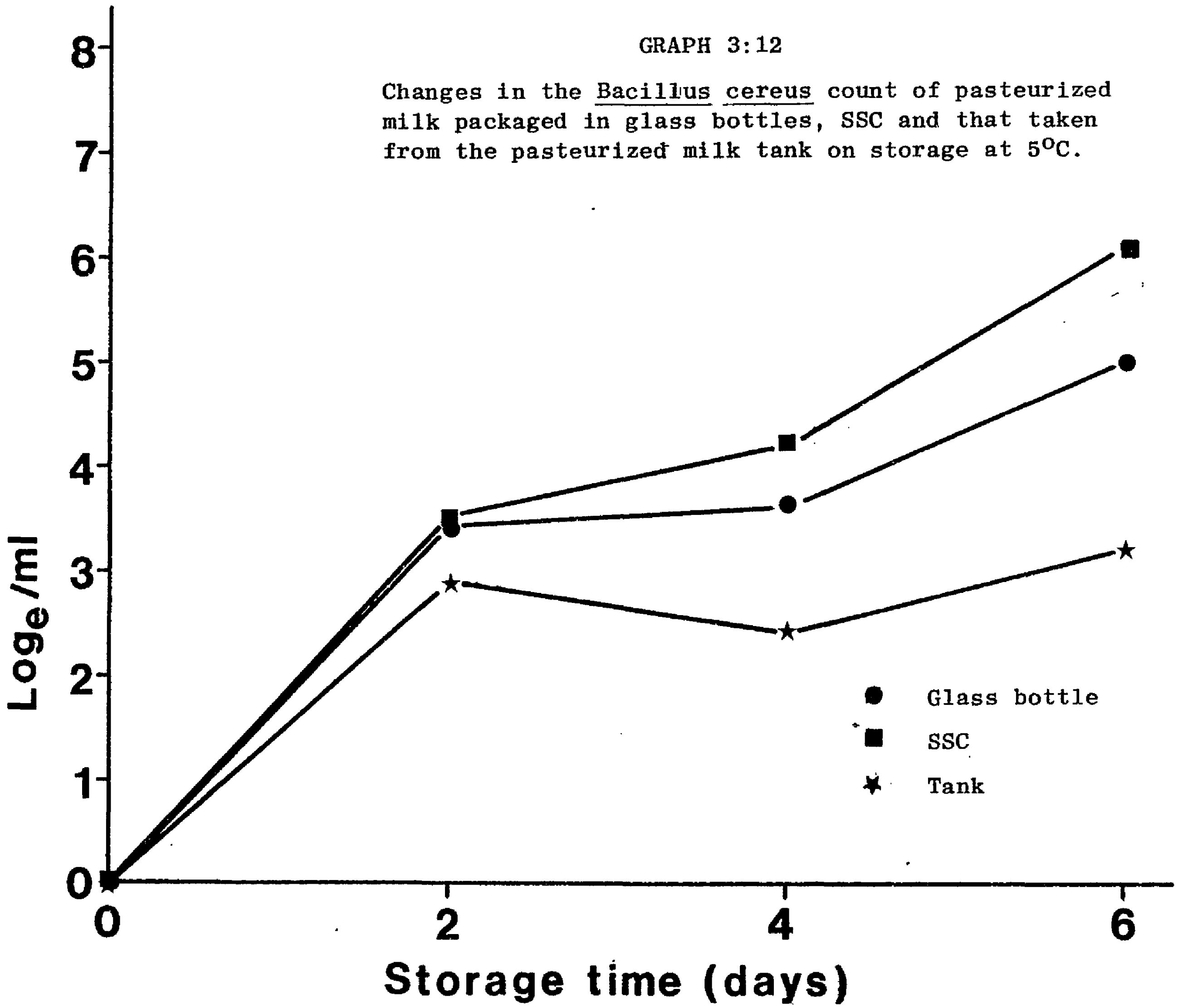
The *B. cereus* count/ml (30°C for 2 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 6 days of storage at 5°C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.97	9.62	3.35	6.31
2	4.63	4.82	3.35	4.26
3	4.60	3.96	2.91	3.82
Mean	5.07	6.13	3.20	4.80

	DF	MS	VR
Trial	2	10.5881	50.15***
Sample	2	13.1963	62.50***
Trial sample	4	4.6842	22.18***
Residual	9	0.2111	
Total	17	4.0121	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2650	0.2650	0.4590

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



GRAPH 3:12

Changes in the <u>Bacillus cereus</u> count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5°C.

(0.1 per cent level) in the B. cereus count between the samples from the different types of containers. The B. cereus count in the pasteurized milk packaged in the SSC was higher ($p < 0.01$) than that of the milk in glass bottles. The pasteurized milk packaged in SSC and glass bottles gave higher ($p < 0.001$) counts of B. cereus than milk taken from the pasteurized milk tank and treated in the same way.

A highly significant difference (0.1 per cent level) in B. cereus counts was found between the trials after samples had been stored for 2, 4 and 6 days at 5^oC.

After 6 days of storage at 5^oC, the mean of B. cereus counts of samples of milk in glass bottles, SSC and that from the pasteurized milk tank were about 160, 460 and 25 per ml respectively.

The variations on storage of the B. cereus count of samples in glass bottles, SSC and from the pasteurized milk tank are illustrated in graph 3:12.

5. Proteolytic count

The results of the proteolytic count are presented in Tables 3:45, 3:46, 3:47 and 3:48. The results of tests made on the day of processing and after 2 days of storage at 5^oC (Tables 3:45 and 3:46) showed that there was no significant difference in the proteolytic count of the samples of pasteurized milk.

After 4 days of storage at 5^oC the results showed (Table 3:47) that there was no significant difference between the proteolytic counts of pasteurized milk packaged in glass bottles and SSC. However, a signficant difference ($p < 0.01$) was found in the proteolytic count of milk in glass bottles and SSC compared with the count of the samples taken from the pasteurized milk tank.

When the samples had been stored for 6 days at 5^oC, the proteolytic count of pasteurized milk packaged in SSC was significantly higher ($p < 0.001$) than that of pasteurized milk packaged in glass bottles.

TABLE 3:45

The proteolytic count/ml ($30^{o}C$ for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.57	5.53	5.59	5.56
2	4.19	4.49	4.36	4.35
3	3.64	4.10	3.77	3.84
Mean	4.46	4.71	4.57	4.58

	DF	MS	VR
Trial	2	4.73080	74.46***
Sample	2	0.09042	1.42
Trial sample	4	0.03682	0.58
Residual	9	0.06353	
Total	17	0.60950	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1455	0.1455	0.2521

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

TABLE 3:46

The proteolytic count/ml ($30^{o}C$ for 3 d), expressed as log_e, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days storage at $5^{o}C$

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	5.68	5.60	5.51	5.60
2	4.06	3.58	3.54	3.73
3	3.82	3.92	4.22	3.99
Mean	4.52	4.37	4.42	4.44

	DF	MS	VR
Trial	2	6.15874	115.43***
Sample	2	0.03754	0.70
Trial sample	4	0.11686	2.19
Residual	9	0.05335	
Total	17	0.78472	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1334	0.1334	0.2310

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

TABLE 3:47

The proteolytic count/ml (30°C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 4 days storage at 5°C

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	7.60	8.63	6.54	7.59
2	4.85	4.65	3.86	4.46
3	7.37	6.90	6.78	7.02
Mean	6.61	6.73	5.73	6.36

	DF	MS	VR
Trial	2	16.7060	131.80***
Sample	2	1.7850	14.08**
Trial sample	4	0.5690	4.48*
Residual	9	0.1267	
Total	17	0.3764	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2055	0.2055	0.3560

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

TABLE 3:48

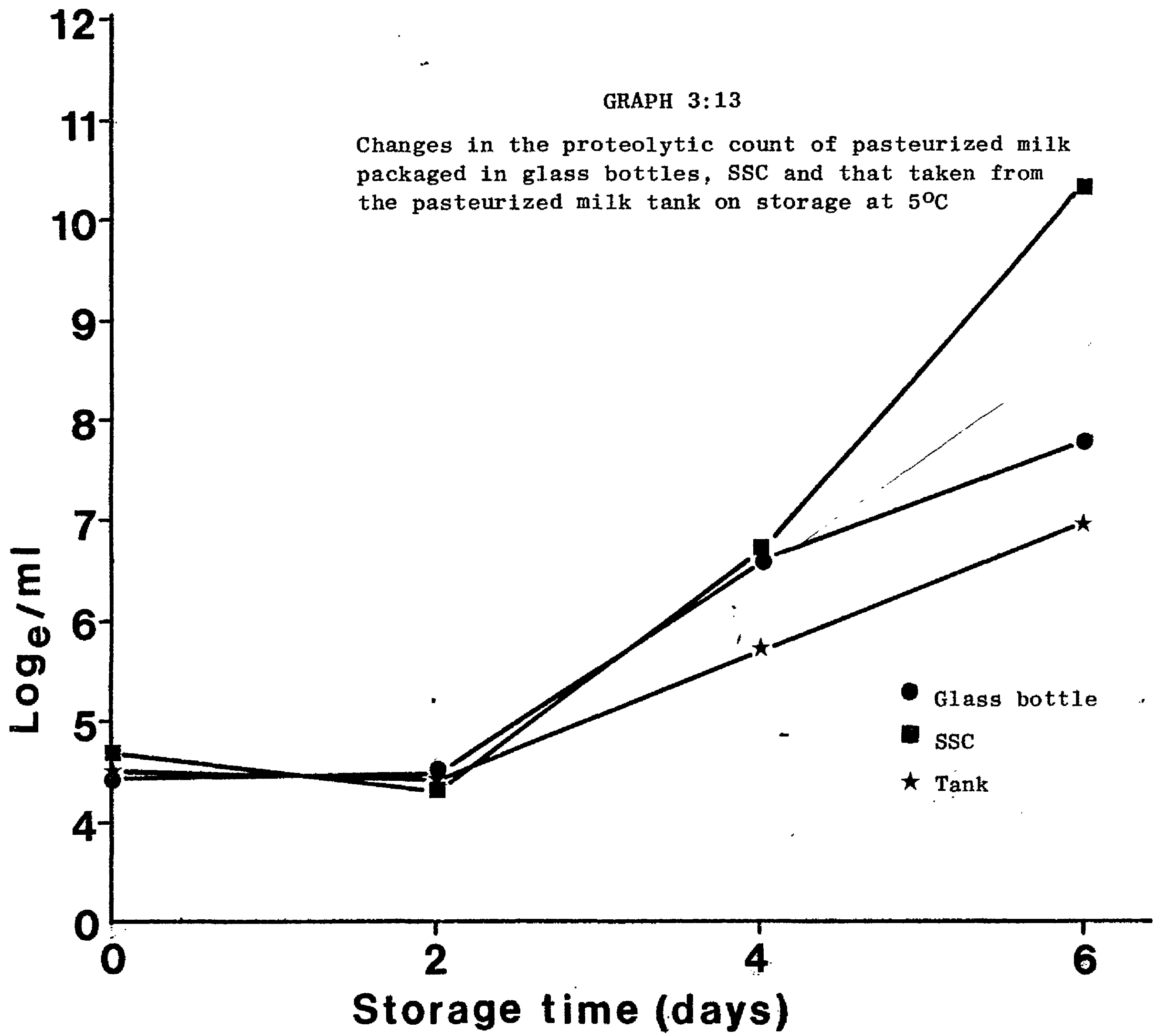
The proteolytic count/ml (30^{o}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 6 days of storage at 5^{o}C.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	6.77	13.90	7.71	9.46
2	7.53	8.25	7.72	7.84
3	9.20	9.24	5.61	8.02
Mean	7.83	10.46	7.02	8.44

	DF	MS	VR
Trial	2	4.73042	141.59***
Sample	2	19.47552	582.95***
Trial sample	4	9.75383	291.95***
Residual	9	0.03341	
Total	17	5.16046	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.1055	0.1055	0.1828

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



GRAPH 3:13

Changes in the proteolytic count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5°C

The latter had a higher count ($p < 0.001$) than that of the pasteurized milk taken from the pasteurized milk tank.

The mean proteolytic counts after storage for 6 days at 5^oC of the samples of milk in glass bottles, SSC and that taken from the pasteurized milk tank were 2.515, 3.49×10^4 and 1.119 per ml respectively.

There was a highly significant difference (0.1 per cent level) between trials in the proteolytic count of samples on the day of processing and after 2, 4 and 6 days of storage at 5^oC.

The variations of the proteolytic counts of pasteurized milk samples in glass bottles, SSC and that taken from the pasteurized milk tank with the storage time are illustrated in graph 3:13.

6. Coliform count

The results of the coliform count are presented in Tables 4:49, 3:50, 3:51 and 3:52.

The results of tests made on the day of processing show that coliform bacteria were absent in 1 ml in all the samples.

In the first trial coliform bacteria were found in the samples of milk in the SSC after 2 days of storage at 5^oC. None of the remaining samples of milk in glass bottles or from the pasteurized milk tank gave any positive results.

After 4 days of storage at 5^oC, coliform bacteria were found only in the samples of milk from SSC in the first trial. After 6 days of storage positive coliform tests (1 ml) were found in all of the samples of pasteurized milk in glass bottles except in the samples of the second trial. After a similar storage time all samples of milk in SSC were positive at 1 ml with the exception of the samples of the third trial. Coliform bacteria were found to be absent at 1 ml level in all of the samples taken from the pasteurized milk tank.

The count of coliform bacteria of the samples of pasteurized milk packaged in SSC was significantly higher ($p < 0.001$) than that of the samples of pasteurized milk packaged in glass bottles.

The variations of the coliform count of samples of pasteurized milk in glass bottles, SSC and that taken from the pasteurized milk tank after storage at $5^{O}C$ are illustrated in graph 3:14.

TABLE 3:49

The coliform count/ml* (30^{o}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank and from glass bottles and single-service containers on the day of processing.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3.	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00

* No coliform present when 1 ml of undiluted milk was tested.

TABLE 3:50

The coliform count/ml ($30^{o}C$ for 3 d) expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 2 days storage at $5^{o}C$.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	0.00	1.15	0.00	0.38
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
Mean	0.00	0.38	0.00	0.13

	DF	MS	VR
Trial	2	0.2945	1.00
Sample	2	0.2945	1.00
Trial sample	4	0.2945	1.00
Residual	9	0.2945	1.00
Total	17	0.2945	1.00

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.313	0.313	0.543

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

TABLE 3:51

The coliform count/ml (30^{0}C for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank and from glass bottles and single-service containers after 4 days of storage at 5^{0}C.

No. of trial	Sample: Glass bottle	SSC	Tank	Mean
1	0.00	6.15	0.00	2.05
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
Mean	0.00	2.05	0.00	0.68

	DF	MS	VR
Trial	2	8.4149	52.37***
Sample	2	8.4149	52.37***
Trial sample	4	8.4149	52.37***
Residual	9	0.1607	
Total	17	4.0450	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.2314	0.2314	0.4009

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

TABLE 3:52

The coliform count/ml (30^oC for 3 d), expressed as $\log_e$, of samples of pasteurized milk taken from the pasteurized milk tank, and from glass bottles and single-service containers after 6 days of storage at 5^oC.

No. of trial	Sample			
	Glass bottle	SSC	Tank	Mean
1	2.72	7.24	0.00	3.32
2	0.00	1.45	0.00	0.48
3	0.06	0.00	0.00	0.00
Mean	0.91	2.90	0.00	1.27

	DF	MS	VR
Trial	2	19.3266	64.35***
Sample	2	13.1739	43.87***
Trial sample	4	7.5036	24.98***
Residual	9	0.3003	
Total	17	5.748	

Table	Trial	Sample	Trial sample
REP	6	6	2
SED	0.3160	0.3160	0.548

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

GRAPH 3:14

Changes in the coliform count of pasteurized milk packaged in glass bottles, SSC and that taken from the pasteurized milk tank on storage at 5°C

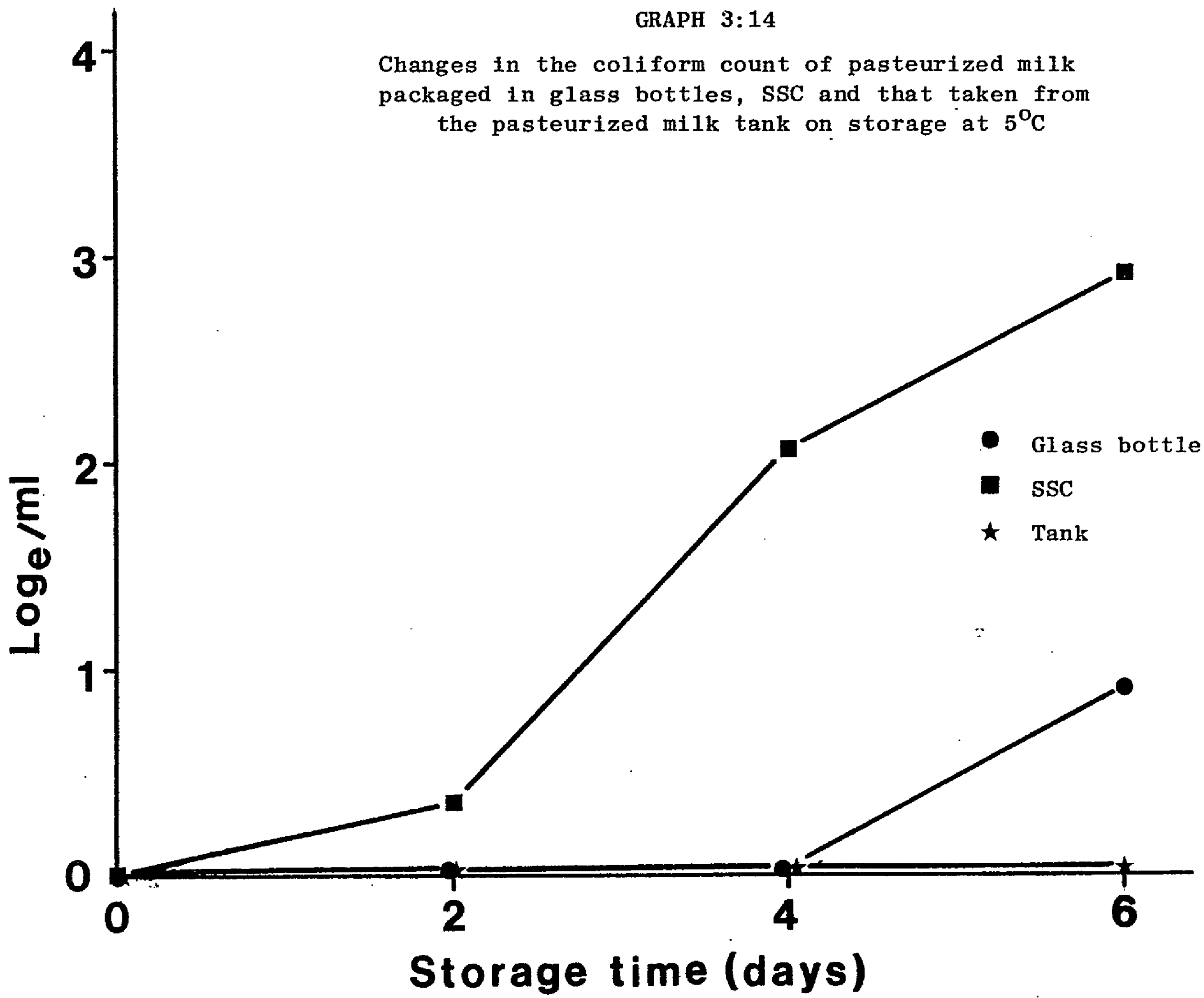
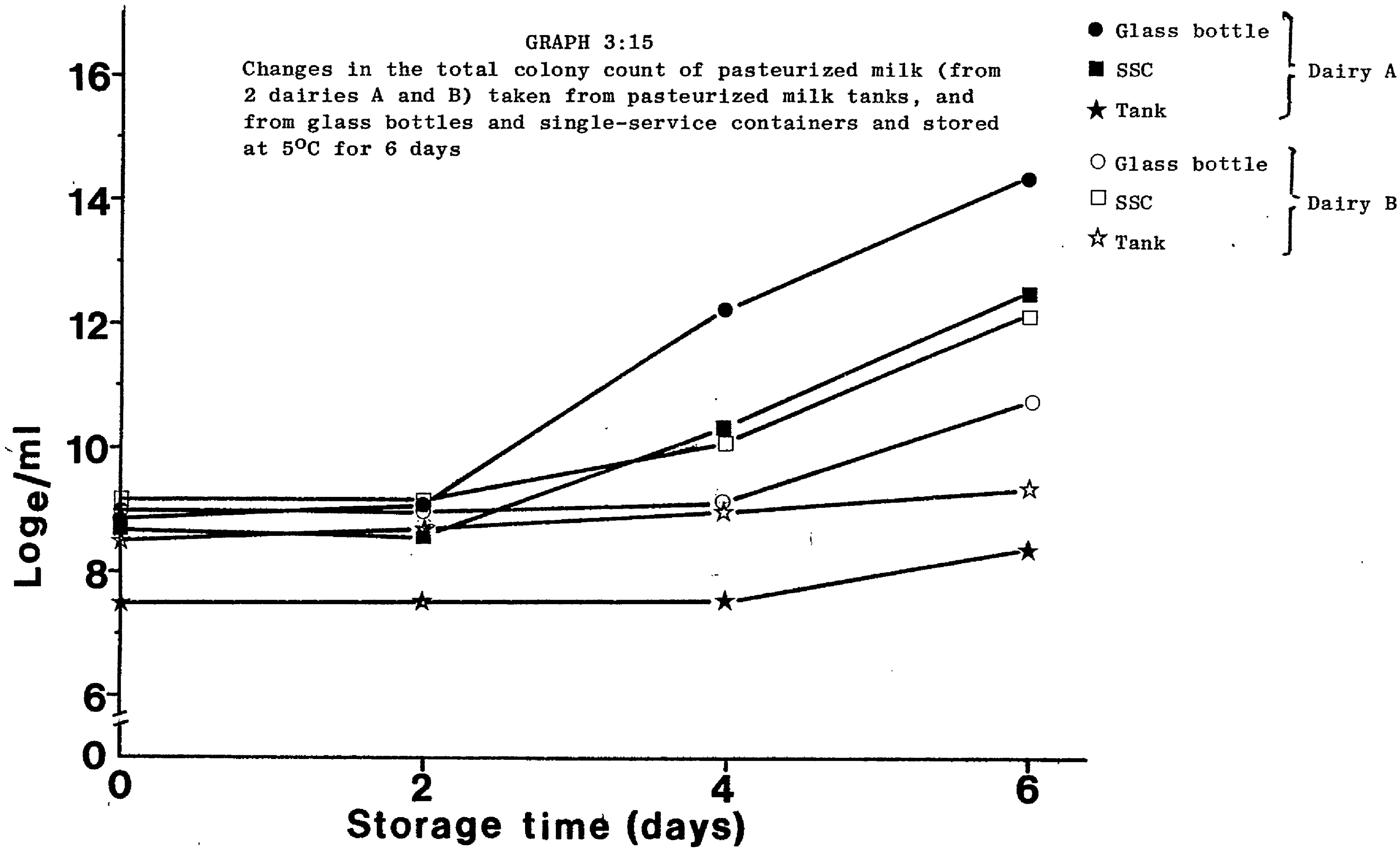


TABLE 3:53

A comparison of results from dairies A and B of the total colony count/ml (30°C for 3 d), expressed as $\log_e$, of pasteurized milk packaged in glass bottles and single service containers and from pasteurized milk tanks after different periods of storage at 5°C

Period of testing	Dairy A			Dairy B		
	Glass bottle	SSC	Tank	Glass bottle	SSC	Tank
On the day of processing	8.88	8.65	7.74	8.89	9.14	8.50
After 2 days of storage	9.06	8.57	7.49	8.93	9.16	8.68
After 4 days of storage	12.19	10.31	7.51	9.08	10.08	8.99
After 6 days of storage	14.46	12.58	8.44	10.74	12.20	9.30



GRAPH 3:15

Changes in the total colony count of pasteurized milk (from 2 dairies A and B) taken from pasteurized milk tanks, and from glass bottles and single-service containers and stored at 5°C for 6 days

DISCUSSION

Dairy A

Counts obtained by various microbial tests on samples of pasteurized milk packaged in glass bottles were higher than those obtained from pasteurized milk packaged in single-service containers. The differences were present in samples tested on the day of processing.

Since the milk filled into each type of container was from the same source,i.e. from the pasteurized milk tank,the differences in microbial counts point to different levels of post-pasteurization infection. In this case the post-pasteurization contamination appears to have been greater with glass bottles and associated filling equipment than with SSC and corresponding filling ancillaries.

The contamination after processing could have occurred in glass bottle packaging system due to:-

(i) contamination from the pipeline leading to the bottle-filling machine and the filling machine itself(Pothmann and Hillebrand, 1954; Glenn and Olson, 1959; and Kalina et. al., 1973),

(ii) the airborne contamination during the bottling process (Ogawa et. al., 1968), and

(iii) contaminants in the empty bottles, particularly if washing has not been adequate.

The results in Table 3:28 show that the empty glass bottles were a source of contamination. These results agree with those of Rogick and Burgwald (1952) and Pothmann and Hillebrand.(1954).

The results given in Table 3:28 also indicate that post-pasteurization contamination occurred in the SSC packaging system, but to a lesser extent than in the glass bottle packaging system. The contamination in the SSC packaging system could be due to:-

(i) cartoning material which was found to harbour some micro-organisms (Table 3:28). These results agree with those of Overcast (1967) who found that paper cartons were contaminated with small numbers of psychrotrophs,

(ii) airborne contamination (Overcast, 1967) and

(iii) contaminants on the contact surfaces of the filling machine.

The results of tests made on samples from the pasteurized milk tank showed a very low bacterial count compared with milk packaged in glass bottles and SSC. The increase in the bacterial count of samples from the pasteurized milk tank was very slow during the period of storage at 5°C. This result indicates that the contaminants in the bulk pasteurized milk were thermoduric bacteria which survived pasteurization temperature. Most of these bacteria are likely to be sporeforming organisms which have a long multiplication time at 5°C (Langveld et. al., 1973). In these studies none of the samples of pasteurized milk from the pasteurized milk tank held at 5°C for 6 days exceeded 10,000 per ml.

The results in Table 3:4 showed that after 4 days of storage at 5°C, the mean of the total colony count of pasteurized milk packaged in glass bottles was 2.15×10^5 per ml. The time which elapses between processing and consumption of milk varies, depending on distribution and retailing patterns. Under certain conditions milk will have been subjected to storage conditions similar to those used in the trials and so levels of bacterial contamination will be similar in practice. When these results are compared with those obtained from SSC samples subjected to the same conditions, it is evident that the bacteriological quality of milk in SSC is better, since the mean of the total colony count was 3.2×10^4 per ml.

It is clear from the results that psychrotrophic bacteria were predominant organisms after 4 and 6 days of storage at 5°C in pasteurized milk. The correlation coefficients with total colony counts being 0.8244 and 0.9252 respectively.

Most of the psychrotrophic bacteria were found to be either lipolytic or proteolytic or both. The counts of these organisms had significant correlation coefficients, 0.9462 and 0.9104 respectively, with the count of psychrotrophic bacteria (Table 3:27). Schultze (1960) studied 586 cultures of psychrotrophs isolated from pasteurized dairy products. He found about 90 per cent of the cultures were either lipolytic or proteolytic and 66 per cent had both characteristics.

A comparison of the results of tests for B. cereus on pasteurized milk, packaged in glass bottles and SSC with those of samples from the pasteurized milk tank, showed that post-pasteurization contamination with B. cereus had occurred. Stewart (1975) found that contamination by B. cereus in the milk plant was due to the air, water, milk handling equipment and personnel. Cannon (1972) found that the pipeline gaskets were a major source of psychrotrophic sporeformers.

Coliform bacteria were found in 1 ml of pasteurized milk packaged in glass bottles on the day of processing but were absent in the same quantity of pasteurized milk packaged in SSC. Glenn and Olson (1959) and Kalina et. al. (1973) found that the bottle-filler was the most common source of coliform contamination in commercially pasteurized milk. Contamination due to these bacteria was found in SSC milks (+ve 1 ml) after 6 days of storage at 5°C. The absence of coliform bacteria (in 1 ml) in the samples obtained from the pasteurized milk tank, when tested on the day of processing and after 2, 4 and 6 days of storage at 5°C means that the pasteurized milk was contaminated by these organisms after processing.

The effect of light and temperature conditions simulating the doorstep delivery system on the microbiological quality of pasteurized milk is related to a combination of three factors:-

(i) the source of light similar to day-light,

(ii) temperature during exposure treatment,

(iii) the time of exposure to the light and temperature.

Factors (ii) and (iii) could affect the microbiological quality of pasteurized milk. However, it is clear from the results of the different bacteriological tests used in this study that exposure for 3 h to light and temperature conditions simulating the doorstep delivery system had no effect on the microbiological quality of pasteurized milk.

Since the studies were made in the period October 1979 - March 1980 - a period of cold weather - the temperatures used during exposure treatments were not high enough to affect the microbiological quality of the exposed samples. The temperatures of exposure were chosen according to the mean temperatures at Auchincruive during 1976, 1977 and 1978 (Channon and Kirkwood, 1979). Mean ambient temperatures on which the procedure was based were 11.1, 6.6, 6.6, 3.6, 3.7 and 5.8°C for the months October-March respectively. It will be seen that the exposure temperatures were similar to the chosen temperature of 5°C at which storage took place. However, if this work had been done in summer-time the temperature could have had an affect on the microbiological quality due to exposure because the temperatures in the morning in summer-time could have been more than 10°C. Further work requires to be done in summer time to find the effect of the exposure treatment on the microbiological quality of pasteurized milk.

Table 3:26 shows the Gram-negative bacteria which were isolated and identified from the selective media. These types of bacteria were responsible for contamination of pasteurized milk after processing. Pseudomonas were the predominant organisms. This is in agreement with Overcast and Skeen (1959), Schultze and Olson (1960), Dempster (1968) and Thomas (1970).

Dairy B

The results of the various microbial tests which were used to assess the microbiological quality of pasteurized milk produced at dairy B showed that pasteurized milk packaged in glass bottles was superior in quality to that which was packaged in SSC.

The results indicated that very low post-pasteurization contamination occurred in the glass bottle packaging system in this dairy. The mean of the total colony count found in the glass bottle samples after 6 days of storage at $5^{o}C$ was 4.6×10^{4} bacteria per ml (Table 3:32). However, it is clear from the same table that the mean of the total colony count obtained from SSC samples was 1.98×10^{5} bacteria per ml, while the samples from the pasteurized milk tank have a mean total colony count of about 1.09×10^{4} per ml given the same conditions referred to above.

After various periods of storage at $5^{o}C$, pasteurized milk packaged in the SSC showed higher psychrotrophic, lipolytic, proteolytic and B. cereus counts than those of pasteurized milk packaged in glass bottles.

Dairies A and B

The post-pasteurization contamination in the SSC packaging system was similar in both dairies. However, the mean of the total colony count of SSC samples from dairy B was lower than that from dairy A. The results of the total colony count of pasteurized milks in glass bottles obtained from dairy A were very much higher compared with those obtained from dairy B (Table 3:53). This fact indicates the importance of cleaning and sanitation in the dairies and the effect of these treatments in producing milk of good or poor microbiological quality.

Graph 3:15 shows the variations of the total colony count, of samples of pasteurized milks obtained from dairies A and B, with storage time. The graphs indicate that there was little difference in the bacteriological quality of SSC samples obtained from the two dairies. The counts of B. cereus were lower in samples taken from dairy B than those from dairy A. Coliform bacteria were absent at 1 ml in all the samples from the pasteurized milk tanks at both dairies.

The results indicated good hygienic conditions in dairy B compared with those in dairy A. The mean of the total colony count obtained from the pasteurized milk tank in dairy B showed higher level than that obtained in dairy A (Table 3:53). This was due to a higher level of sporeforming bacteria in the raw milk which was used in dairy B.

It is evident from the six trials at dairy A and three trials at dairy B that the quality of the pasteurized milk is dependent on several factors of which the post pasteurization contamination is the most important where milk is under storage at $5^{o}C$.

The highest count in milk taken from the pasteurized milk tank at both dairies and stored at $5^{o}C$ for 6 days was less than 16,000 per ml. The serious effect of contamination after this stage in the process is demonstrated.

CONCLUSION

1. It is concluded from the study reported in this chapter that the post-pasteurization contamination by psychrotrophic bacteria is the most important bacteriological problem in determining the quality of pasteurized milk. This conclusion is reached by comparing the results of various microbial counts obtained from the pasteurized milk tank with those obtained from milk packaged in glass bottles and single-service containers.

2. Microbiological quality of pasteurized milk could be improved by the adoption of vigorous hygienic and sanitary conditions in the dairy and by holding the packaged milk at or below 5°C during transport and in the supermarket until it reaches the consumer.

3. Exposure of the packaged milk on the day of processing, to 3 h of conditions of light and temperature simulating the doorstep delivery system during winter time had no effect on the microbiological quality of the pasteurized milk, but there could be an effect in summer time when temperatures are higher. Further work requires to be done in this field.

4. The changes on storage at 5°C on the microbiological quality of pasteurized milk packaged in SSC systems in the two dairies was similar. However there was a significant difference in the microbiological quality of milk packaged in glass bottles at the two dairies.

5. Pseudomonas species were found to be the predominating organisms contaminating the pasteurized milk after processing when random samples of micro-organisms were selected and identified.

6. There was a highly significant correlation between the various microbial tests used to assess the microbiological quality of pasteurized milk.

7. The microbiological quality of pasteurized milk prior to packaging at each of the dairies was of a high standard and coliform bacteria were absent in 1 ml quantities after 6 days of storage at $5^{0}C$.

CHAPTER FOUR

A COMPARISON OF THE CHEMICAL QUALITY OF PASTEURIZED MILK PACKAGED IN GLASS BOTTLES AND SINGLE-SERVICE CONTAINERS

INTRODUCTION

Milk is an important item in our food. It contains proteins, fat, carbohydrates, minerals and vitamins. The conditions of these constituents could be affected by many factors, e.g. processing, handling, storage, packaging materials, micro-organisms and enzymes. The changes in the fat e.g. are due to the action of the natural and microbial enzymes in milk. This action affects the quality of the milk due to the production of rancidity.

Vitamins in the milk particularly vitamin C are sensitive to heat, light etc. The light and oxygen are important factors affecting this vitamin.

The aim of this work is to study:-

(i) the effect of exposure of vitamin C, in pasteurized milk, packaged in glass bottles and single-service containers, to 3 h of light and temperature conditions simulating doorstep delivery,

(ii) the change in the acid degree value (ADV) in pasteurized milk packaged in both types of containers referred to above,

(iii) the changes in the dissolved oxygen content of pasteurized milk packaged in both types of container.

Titratable acidity, pH and phosphatase were measured as control tests in the experiments.

EXPERIMENTAL

1. Treatment of the samples

Two hundred ml of each sample were used for the chemical analysis described in chapter three (Experimental, Dairy A, 3b).

Seventy ml from each sample were poured into a conical flask with a glass stopper and used to measure the dissolved oxygen content. Twenty and forty ml were used for the determination of titratable acidity and the pH respectively. Sixty ml from each sample were used for the determination of acid degree value (ADV). The remaining milk from each duplicate sample were mixed and kept in a flask covered with black paper at 5^oC until the following day when the vitamin C content was measured.

2. Chemical analysis

Two samples of pasteurized milk packaged in glass bottles and single-service containers, as described in chapter three (Experimental, Dairy A), were tested on the day of processing and after 2, 4 and 6 days of storage at 5^oC for acid degree value (ADV), dissolved oxygen content, pH and titratable acidity using the methods described in chapter one (section 2:1, 2:2, 2:3 and 2:6). The phosphatase test was made on the samples on the day of processing using the method described in chapter one (section 2:4). Vitamin C was determined after 1, 3 and 7 days of storage at 5^oC using the method described in chapter one (section 2:5).

The test for vitamin C was made on 4 trials only (numbers 3, 4, 5 and 6) because of difficulties with the method.

RESULTS

1. Titratable acidity

The results of measurements of the titratable acidity are presented in Tables 4:1, 4:2, 4:3 and 4:4 after milk was held under storage at $5^{o}C$ There was a significant difference (at 0.1, 1 and 0.1 per cent level respectively) between the trials in the titratable acidity of samples tested on the day of processing and after 2 and 4 days of storage at $5^{o}C$. However, there was no significant difference between the trials in titratable acidity of milk after 6 days of storage at $5^{o}C$.

In relation to the titratable acidities of milks packaged in different containers the difference appeared on the fourth day of storage. The milk samples from the glass bottles showed higher acidities ($p < 0.1$) than the milk samples packaged in SSC.

There was no significant difference in titratable acidity of the samples exposed for 3 h to light and temperature conditions simulating the doorstep delivery system and the samples which were not exposed to the above mentioned conditions.

The variation in the titratable acidity with the storage time at $5^{o}C$ are shown in Graph 4:1. It is clear that there was a small but definite decrease in titratable acidity in all samples after the fourth day of storage.

The maximum titratable acidity found through the experiment was 0.157 per cent lactic acid and the minimum was 0.140 per cent.

2. pH

The results of test for the pH value are presented in Tables 4:5, 4:6, 4:7 and 4:8 after 0, 2, 4 and 6 days of storage at $5^{o}C$ respectively. There was a significant difference (0.1 per cent level) in the pH levels of samples taken in the six trials on each day of the storage when the VR was compared with F-value in the statistical tables.

TABLE 4:1

The titratable acidity (per cent lactic acid) of samples of pasteurized milk packaged in glass bottles and single-service containers on the day of processing.

No. of trial	Sample		
	Glass bottle	SSC	Mean
1	0.1500	0.1450	0.1475
2	0.1450	0.1450	0.1450
3	0.1425	0.1400	0.1412
4	0.1550	0.1575	0.1562
5	0.1525	0.1500	0.1512
6	0.1550	0.1550	0.1550
Mean	0.1500	0.1487	0.1493

	DF	MS	VR
Trial	5	0.000136875	43.80***
Sample	1	0.000009375	3.00
Trial sample	5	0.000006875	2.20
Residual	12	0.000003125	
Total	23	0.000033288	

Table	Trial	Sample	Trial sample
REP	4	12	2
SED	0.0012	0.0007	0.0017

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

TABLE 4:2

The titratable acidity (per cent lactic acid) of samples of pasteurized milk packaged in glass bottles and single service containers after 2 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E+	Glass-N+	SSC-E+	SSC-N+	Mean
1	0.1500	0.1500	0.1500	0.1500	0.1500
2	0.1500	0.1475	0.1475	0.1500	0.1487
3	0.1475	0.1475	0.1500	0.1500	0.1487
4	0.1500	0.1500	0.1500	0.1500	0.1500
5	0.1500	0.1500	0.1450	0.1450	0.1475
6	0.1500	0.1500	0.1525	0.1500	0.1506
Mean	0.1495	0.1491	0.1491	0.1491	
	0.1493		0.1491		
Overall mean		0.1492			

	DF	MS	VR
Trial	5	0.000010521	4.04**
Sample	1	0.000000521	0.20
Exposed	1	0.000000521	0.20
Trial sample	5	0.000013021	5.00**
Trial exposed	5	0.000000521	0.20
Sample exposed	1	0.000000521	0.20
Trial sample exposed	5	0.000003021	1.16
Residual	24	0.000002604	
Total	47	0.000004244	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0008	0.0004	0.0006	0.0016

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

+E - exposed to light and temperature conditions simulating doorstep delivery system for 3 h

N - not exposed to light and temperature conditions simulating doorstep delivery system.

TABLE 4:3

The titratable acidity (per cent lactic acid) of samples of pasteurized milk packaged in glass bottles and single service containers after 4 days of storage at 5°C in darkness and in light conditions simulating the doorstep delivery system.

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	0.1500	0.1525	0.1450	0.1450	0.1481
2	0.1475	0.1450	0.1500	0.1450	0.1468
3	0.1500	0.1500	0.1500	0.1500	0.1500
4	0.1500	0.1500	0.1500	0.1500	0.1500
5	0.1550	0.1525	0.1500	0.1500	0.1518
6	0.1475	0.1475	0.1450	0.1475	0.1468
Mean	0.1500	0.1483	0.1499	0.1475	
	0.1497		0.1481		
Overall mean		0.1489			

	DF	MS	VR
Trial	5	0.000032083	10.26**
Sample	1	0.000033333	10.66**
Exposed	1	0.000002083	0.66
Trial sample	5	0.000015833	5.06**
Trial exposed	5	0.000007083	2.26
Sample exposed	1	0.000000000	0.00
Trial sample exposed	5	0.000002500	0.80
Residual	24	0.000003125	
Total	47	0.000008466	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0008	0.0005	0.0007	0.0017

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

TABLE 4:4

The titratable acidity (per cent of lactic acid) of samples of pasteurized milk packaged in glass bottles and single service containers after 6 days of storage at 5°C and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	0.1450	0.1450	0.1450	0.1500	0.1462
2	0.1450	0.1475	0.1475	0.1425	0.1456
3	0.1450	0.1500	0.1475	0.1450	0.1468
4	0.1450	0.1475	0.1450	0.1450	0.1456
5	0.1450	0.1475	0.1475	0.1450	0.1462
6	0.1450	0.1400	0.1475	0.1450	0.1443
Mean	0.1450	0.1462	0.1466	0.1454	
	0.1456		0.1460		
Overall mean		0.1458			

	DF	MS	VR
Trial	5	0.000005833	1.40
Sample	1	0.000002083	0.50
Exposed	1	0.000000000	0.00
Trial sample	5	0.000009583	2.30
Trial exposed	5	0.000001000	2.40
Sample exposed	1	0.000018750	4.50*
Trial sample exposed	5	0.000013750	3.30*
Residual	24	0.000004167	
Total	47	0.000006738	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0010	0.0005	0.0008	0.0020

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

GRAPH 4:1

The variations in the titratable acidity (per cent lactic acid) after storage at 5°C of pasteurized milks subjected to different treatments

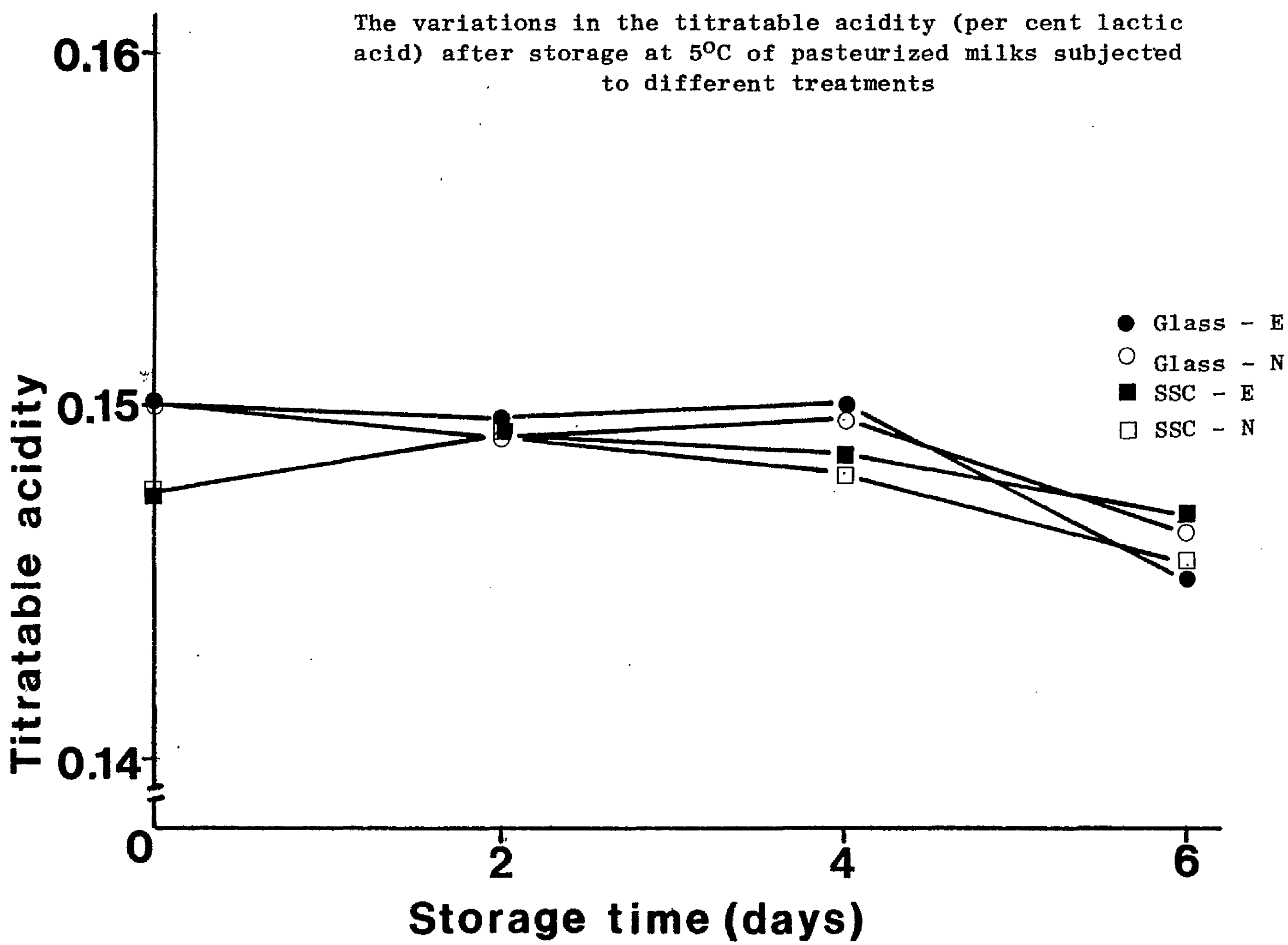


TABLE 4:5

The pH value of samples of pasteurized milk packaged in glass bottles and single service containers on the day of processing

No. of trial	Sample		
	Glass bottle	SSC	Mean
1	6.550	6.615	6.582
2	6.645	6.650	6.647
3	6.575	6.585	6.580
4	6.555	6.540	6.547
5	6.675	6.685	6.680
6	6.690	6.690	6.690
Mean	6.615	6.627	6.621

	DF	MS	VR
Trial	5	0.01400746	305.60***
Sample	1	0.00093753	20.45***
Trial sample	5	0.00074750	16.30***
Residual	12	0.00004583	
Total	23	0.00327227	

Table	Trial	Sample	Trial sample
REP	4	12	2
SED	0.0047	0.0027	0.0067

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

TABLE 4:6

The pH value of samples of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	6.670	6.680	6.660	6.670	6.670
2	6.680	6.680	6.660	6.645	6.666
3	6.570	6.570	6.570	6.580	6.572
4	6.615	6.595	6.640	0.650	0.625
5	6.715	6.735	6.720	6.715	6.721
6	6.760	6.760	6.765	6.765	6.762
Mean	6.668	6.670	6.669	6.870	
	6.669		6.670		
Overall mean		6.669			

	DF	MS	VR
Trial	5	0.03636318	484.83***
Sample	1	0.00000833	0.11
Exposed	1	0.00003333	0.44
Trial sample	5	0.00102332	13.64***
Trial exposed	5	0.00009833	1.31
Sample exposed	1	0.00000000	0.00
Trial sample exposed	5	0.00018500	2.467
Residual	24	0.00007500	
Total	47	0.00404661	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0043	0.0025	0.0035	0.0086

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

TABLE 4:7

The pH value of samples of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at 5^{o}C in darkness, and in light and temperature conditions simulating the doorstep delivery

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	6.550	6.565	6.535	6.565	6.555
2	6.575	6.580	6.555	6.520	6.557
3	6.530	6.540	6.570	6.585	6.556
4	6.556	6.585	6.610	6.645	6.597
5	6.680	6.705	6.715	6.715	6.703
6	6.760	6.735	6.735	6.740	6.742
Mean	6.608	6.618	6.620	6.628	
	6.613		6.624		
Overall mean		6.618			

	DF	MS	VR
Trial	5	0.0555398	429.98***
Sample	1	0.0014083	10.90**
Exposed	1	0.0010083	7.80*
Trial sample	5	0.0028033	21.70***
Trial exposed	5	0.0007033	5.44**
Sample exposed	1	0.0000083	0.06
Trial sample exposed	5	0.0003533	2.73*
Residual	24	0.0001292	
Total	47	0.0064367	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0056	0.0032	0.0046	0.0113

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

TABLE 4:8

The pH value of samples of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	6.630	6.625	6.635	6.625	6.628
2	6.600	6.590	6.620	6.600	6.602
3	6.650	6.600	6.615	6.615	6.620
4	6.570	6.555	6.615	6.585	6.581
5	6.685	6.690	6.730	6.715	6.705
6	6.675	6.685	6.720	6.725	6.701
Mean	6.635	6.624	6.655	6.644	
	6.629		6.650		
Overall mean	6.639				

	DF	MS	VR
Trial	5	0.0213770	133.25***
Sample	1	0.0050021	31.18***
Exposed	1	0.0015188	9.46**
Trial sample	5	0.0009071	5.65**
Trial exposed	5	0.0002938	1.83
Sample exposed	1	0.0000021	0.01
Trial sample exposed	5	0.0003271	2.03
Residual	24	0.0001604	
Total	47	0.0026574	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0063	0.0036	0.0051	0.0126

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

The difference in the pH of the samples on the day of processing and after 4 and 6 days of storage at $5^{o}C$ was significant (0.1, 1 and 0.1 per cent level respectively). However, there was no significant difference after 2 days of storage.

The milk in glass bottles showed a lower pH than the samples in SSC. Exposure to light and temperature conditions simulating the doorstep delivery system for 3 h affected the pH value after 4 and 6 days from the processing time. The pH value of milk in glass bottles stored in darkness was higher ($p < 0.5$) than that of samples exposed to simulated doorstep delivery conditions.

The highest reading in the pH value of all the samples was 6.760 and the lowest was 6.520. This means that there was no significant change in the pH value which might affect the chemical quality of pasteurized milk in the two different packaging systems.

3. Dissolved oxygen content

The results of determinations of the dissolved oxygen content are presented in Tables 4:9, 4:10, 4:11 and 4:12. There was significant difference (at 0.1 per cent level) between the dissolved oxygen content of samples taken in the six trials on the day of processing and after 2 and 4 days of storage at $5^{o}C$ and at 5 per cent level in the samples stored for 6 days.

The dissolved oxygen content of milk packaged in single-service containers was significantly higher ($p < 0.001$) than that of milk packaged in glass bottles on the day of processing and after 2, 4 and 6 days of storage at $5^{o}C$.

A negative correlation was found between the $\log_e$ of the total colony count and the dissolved oxygen content in the samples of milk in glass bottles. This correlation coefficient was significant at 0.1 per cent level ($r = 0.8092$). The regression equation was found to be:-

$$\text{Oxygen (ppm)} = 13.86373 - 0.51118 \times \log_e \text{ of total colony count.}$$

TABLE 4:9

The dissolved oxygen content (ppm) of samples of pasteurized milk packaged in glass bottles and single-service containers on the day of processing

No. of trial	Sample		
	Glass bottle	SSC	Mean
1	9.125	9.580	9.352
2	9.715	9.590	9.652
3	9.780	10.015	9.897
4	8.715	10.195	9.455
5	10.050	10.240	10.145
6	9.515	10.315	9.915
Mean	9.483	9.989	9.736

	DF	MS	VR
Trial	5	0.36673	11.34***
Sample	1	1.53520	47.51***
Trial sample	5	0.32183	9.96***
Residual	12	0.03231	
Total	23	0.23329	

Table	Trial	Sample	Trial sample
REP	4	12	2
SED	0.1271	0.0734	0.1798

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

TABLE 4:10

The dissolved oxygen content (ppm) of samples of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	8.950	8.915	9.255	9.220	9.085
2	9.040	9.610	10.550	10.240	9.860
3	8.715	8.595	10.325	10.335	9.492
4	8.655	9.910	11.620	11.415	10.400
5	9.415	9.700	10.730	10.855	10.175
6	9.380	9.676	10.460	10.405	9.980
Mean	9.026	9.401	10.490	10.412	
	9.213		10.451		
Overall mean		9.832			

	DF	MS	VR
Trial	5	1.81797	22.90***
Sample	1	18.38686	231.52***
Exposed	1	0.26403	3.32
Trial sample	5	0.87783	11.05***
Trial exposed	5	0.08847	1.11
Sample exposed	1	0.61653	7.76*
Trial sample exposed	5	0.18379	2.31
Residual	24	0.07937	
Total	47	0.76601	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.1409	0.1409	0.0813	0.2817

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

TABLE 4:11

The dissolved oxygen content (ppm) of samples of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	7.700	7.730	9.170	9.160	8.440
2	8.710	9.345	10.645	10.665	9.841
3	6.810	7.025	10.780	10.560	8.794
4	7.180	8.410	9.225	9.675	8.622
5	7.055	9.430	10.620	10.615	9.430
6	7.600	7.575	10.510	10.560	9.061
Mean	7.509	8.252	10.158	10.206	
	7.881		10.182		
Overall mean		9.031			

	DF	MS	VR
Trial	5	2.22250	30.96***
Sample	1	63.54893	885.26***
Exposed	1	1.87625	26.13***
Trial sample	5	1.65016	22.98***
Trial exposed	5	0.51169	7.12***
Sample exposed	1	1.45255	20.23***
Trial sample exposed	5	0.39424	5.49
Residual	24	0.67179	
Total	47	1.96795	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.1340	0.07 73	0.1094	0.2679

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

TABLE 4:12

The dissolved oxygen content (ppm) of samples of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	6.580	6.220	9.710	10.100	8.150
2	6.390	7.410	11.540	11.240	9.140
3	6.650	6.360	9.390	10.190	8.150
4	7.120	5.980	10.250	10.110	8.370
5	5.100	4.850	10.000	9.990	7.490
6	6.370	4.470	9.640	8.230	7.180
Mean	6.370	5.880	10.09	9.98	
	6.130		10.030		
Overall mean			8.080		

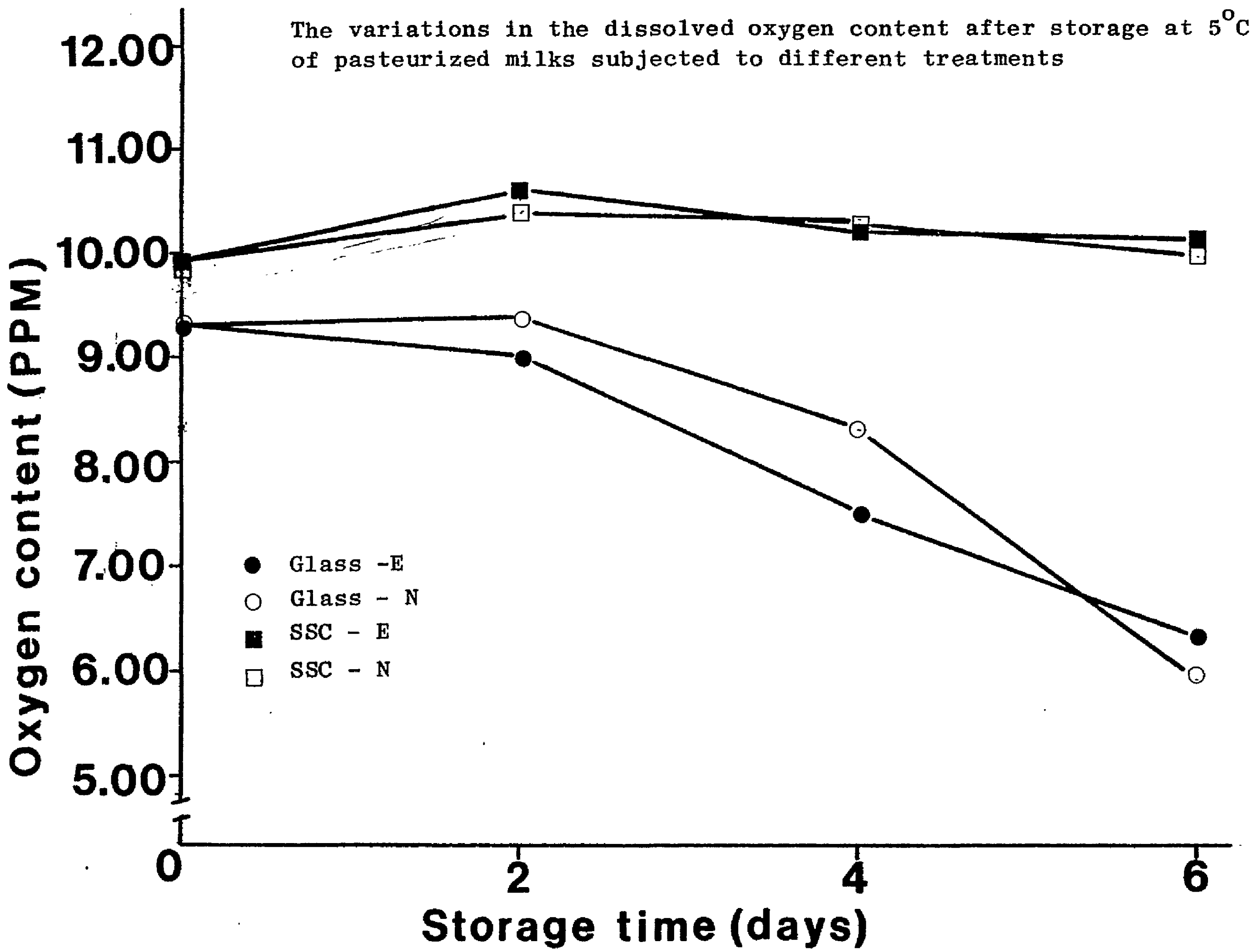
	DF	MS	VR
Trial	5	3.819	2.68*
Sample	1	183.262	128.74***
Exposed	1	1.083	0.76
Trial sample	5	0.939	0.66
Trial exposed	5	1.131	0.79
Sample exposed	1	0.428	0.30
Trial sample exposed	5	0.396	0.27
Residual	24	1.423	
Total	47	5.327	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.5970	0.3440	0.4870	1.1930

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

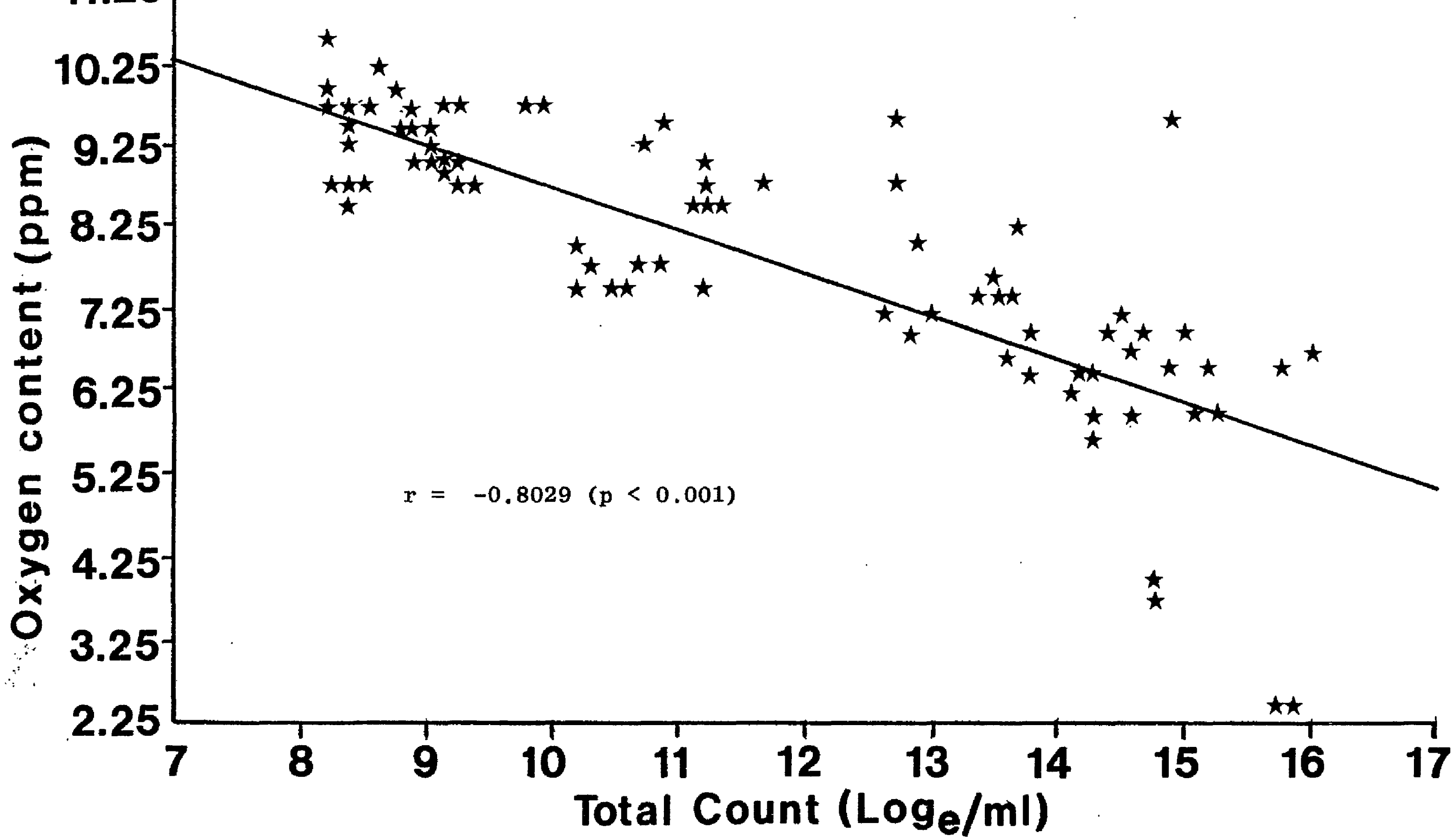
GRAPH 4:2

The variations in the dissolved oxygen content after storage at 5°C of pasteurized milks subjected to different treatments



GRAPHS 4:3

The standard curve for the relation between the dissolved oxygen content and the total colony count of pasteurized milk packaged in glass bottles after various periods of storage at $5^{o}C$



This negative correlation is presented in Graph 4:3. There was no correlation between the oxygen content and the total colony count in the samples of SSC.

The variations in the oxygen content during the different periods of storage are illustrated in Graph 4:2. There was a decrease in the oxygen content of the samples of milk in glass bottles during the storage time. However, in the case of samples of milk in SSC there was a slight increase in dissolved oxygen level after 2 days of storage.

The range of the dissolved oxygen content was 4.47 to 10.05 ppm in the samples of milk in glass bottles, during the period of storage, while it ranged from 8.23 to 11.62 ppm in the samples of milk in single service containers.

4. Acid degree value (ADV)

The results of the tests for ADV are presented in Tables 4:13, 4:14, 4:15 and 4:16. There was a singnificant difference between the trials (5 per cent level) in the acid degree values of milks tested on the day of processing. After the milks had been held for 4 and 6 days at $5^{o}C$ the ADV of milks packaged in glass bottles were significantly higher ($p < 0.001$) than those of milk packaged in SSC. Exposure to simulated doorstep delivery conditions for 3 h on the 2nd, 4th and 6th day after processing had no significant effect on ADV levels.

The ADVs showed a correlation coefficient 0.57, 0.56 and 0.53 with the $\log_e$ of the total colony count, psychrotrophic count and lipolytic count respectively.

The variations in the ADV during the periods of storage are illustrated in Graph 4:3. There is an increase in the ADV during the storage time in milk packaged in both types of container.

The range of the ADV in the samples of milk in glass bottles was 0.795 to 1.630 per cent, while in the samples of SCC it was 0.775 to 1.520 per cent.

TABLE 4:13

The acid degree value (ADV) of samples of pasteurized milk packaged in glass bottles and single service containers on the day of processing

No. of trial	Sample		
	Glass bottle	SSC	Mean
1	0.805	0.775	0.790
2	0.815	0.825	0.820
3	0.795	0.785	0.790
4	0.800	0.865	0.832
5	0.965	0.830	0.897
6	0.995	0.950	0.972
Mean	0.862	0.838	0.850

	DF	MS	VR
Trial	5	0.020534	4.94*
Sample	1	0.003504	0.84
Trial sample	5	0.004414	1.06
Residual	12	0.004154	
Total	23	0.007743	

Table	Trial	Sample	Trial sample
REP	4	12	2
SED	0.0456	0.0263	0.0645

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

TABLE 4:14

The acid degree value (ADV) of samples of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	0.895	0.960	0.815	0.835	0.876
2	0.875	0.820	0.830	0.855	0.845
3	0.930	0.905	1.035	1.040	0.977
4	0.960	0.940	0.960	0.975	0.959
5	1.230	1.305	1.345	1.120	1.250
6	1.180	1.240	1.165	1.135	1.180
Mean	1.012	1.028	1.025	0.993	
	1.020		1.009		
Overall mean		1.015			

	DF	MS	VR
Trial	5	0.216273	33.66***
Sample	1	0.001408	0.21
Exposed	1	0.000675	0.10
Trial sample	5	0.011743	1.82
Trial exposed	5	0.003060	0.47
Sample exposed	1	0.007008	1.09
Trial sample exposed	5	0.009463	1.47
Residual	24	0.006425	
Total	47	0.029064	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0401	0.0231	0.0327	0.0802

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

TABLE 4:15

The acid degree value (ADV) of samples of pasteurized milk packaged in glass bottles and single-service containers after 4 days of storage at 5^{o}C in darkness, and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	1.025	1.035	0.970	0.945	0.994
2	1.000	0.935	0.950	0.935	0.955
3	1.245	1.195	1.100	0.110	1.162
4	1.160	1.170	0.980	1.050	1.090
5	1.460	1.425	1.320	1.205	1.352
6	1.265	1.565	1.295	1.270	1.324
Mean	1.192	1.221	1.102	1.069	
	1.207		1.086		
Overall mean		1.146			

	DF	MS	VR
Trial	5	0.21969	21.68***
Sample	1	0.17521	17.29***
Exposed	1	0.00007	0.00
Trial sample	5	0.00788	0.77
Trial exposed	5	0.00676	0.66
Sample exposed	1	0.01141	1.12
Trial sample exposed	5	0.01751	1.72
Residual	24	0.01013	
Total	47	0.03594	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0503	0.0291	0.0411	0.1007

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

TABLE 4:16

The acid degree value (ADV) of samples of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness, and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample				
	Glass-E	Glass-N	SSC-E	SSC-N	Mean
1	1.210	1.150	1.125	1.080	1.141
2	1.125	1.200	1.265	1.215	1.201
3	1.330	1.305	1.150	1.205	1.247
4	1.125	1.245	0.990	1.110	1.117
5	1.630	1.620	1.295	1.520	1.516
6	1.630	1.620	1.295	1.520	1.516
Mean	1.330	1.346	1.218	1.250	
	1.338		1.234		
Overall mean		1.286			

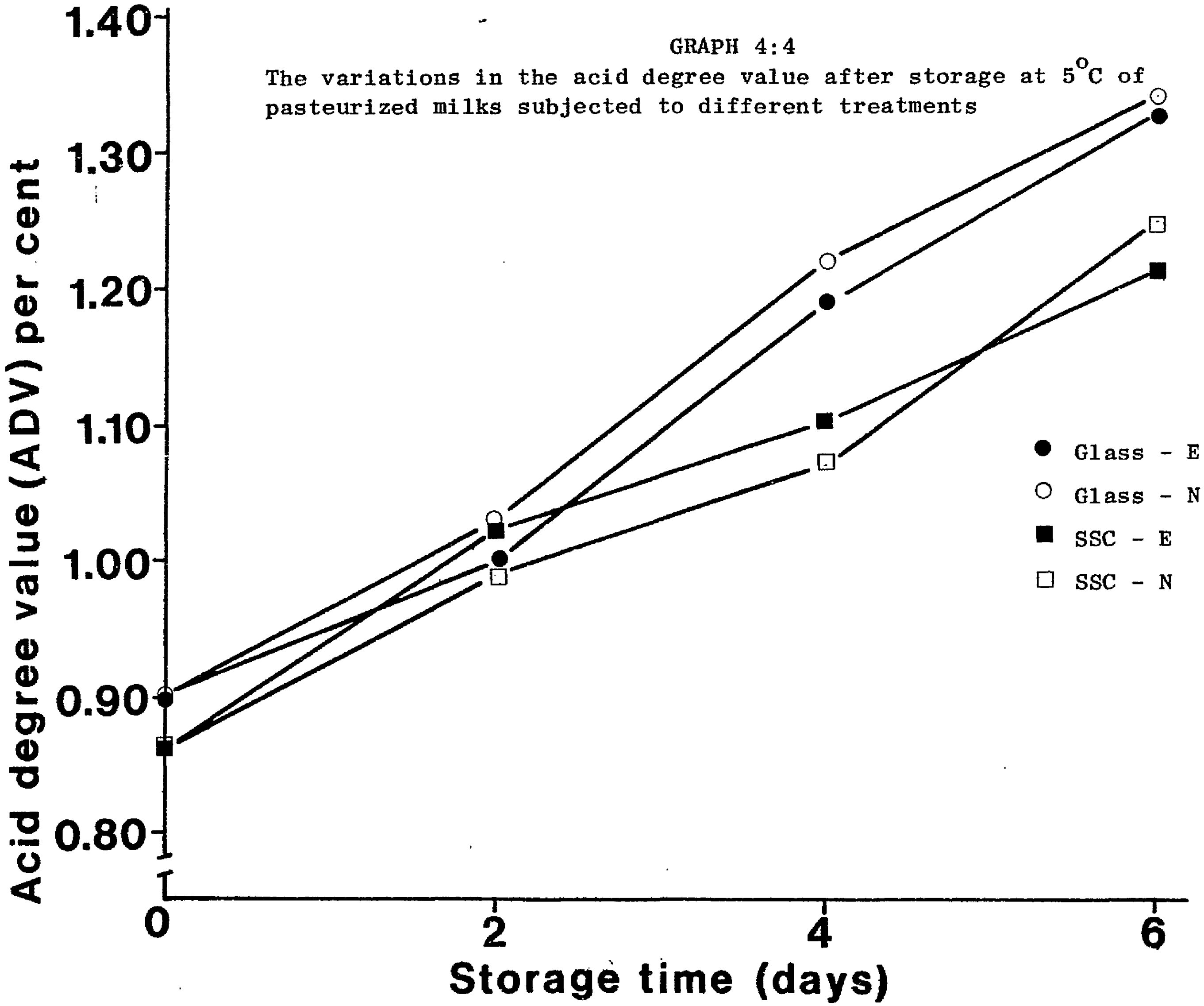
	DF	MS	VR
Trial	5	0.247527	113.91***
Sample	1	0.129169	59.44***
Exposed	1	0.007252	3.33
Trial sample	5	0.019784	9.10***
Trial exposed	5	0.011397	5.24**
Sample exposed	1	0.000752	0.34
Trial sample exposed	5	0.008807	4.05**
Residual	24	0.002173	
Total	47	0.034615	

Table	Trial	Sample	Sample exposed	Trial sample exposed
REP	8	24	12	2
SED	0.0233	0.0134	0.0190	0.0466

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

GRAPH 4:4

The variations in the acid degree value after storage at 5°C of pasteurized milks subjected to different treatments



5. Phosphatase test

None of the samples which were tested for phosphatase failed. This means that the temperature of pasteurization was sufficient to destroy the phosphatase enzymes in the milk and no recontamination with raw milk had occurred.

6. Vitamin C content (Ascorbic and Dehydroascorbic Acid)

The results of ascorbic and dehydroascorbic acid are presented in Tables 4:17, 4:18, 4:19 and 4:20. The results of determinations of ascorbic acid in pasteurized milk after 1 day of cold storage showed that there was no significant difference in ascorbic acid level between the trials or between the milk which was packaged in glass bottles and SSC (Table 4:17).

But, after 3 and 7 days of cold storage there were highly significant changes in the ascorbic acid content of milks. Table 4:18 shows a significant (1 per cent) difference in the ascorbic acid level of samples in different trials. The milk in glass bottles kept in darkness during storage contained a higher ascorbic acid content ($p < 0.01$)than milks exposed to the light and temperature conditions simulating the doorstep delivery system after 3 days of storage. However, there was no significant difference in ascorbic acid level of samples of milk in single-service containers kept in darkness and subjected to simulated doorstep delivery.

After 7 days of storage there was no signifificant difference in the ascorbic acid levels of all samples.

The effect of exposure to day-light conditions was detected in the samples of milk in glass bottles after 3 days of storage when a loss of40.8 per cent of the ascorbic acid content was recorded.

After 7 days of storage there was no ascorbic acid present in any of the samples.

The effect of the storage and exposure interaction showed a significant difference at 5 per cent level.

The degradation in the ascorbic acid during the period of storage of the milk in the two types of container is illustrated in Graph 4:5 where the differences referred to above are seen.

The results presented in Table 4:19 show a difference (0.119 mg/100 ml of milk) between the dehydroascorbic acid content of milk in glass bottles and SSC. This may be due to an oxidation reaction affecting the ascorbic acid in the glass bottle more than that in the SSC samples.

There was a difference between the four trials in the dehydroascorbic acid content of all milks. It was not significant however after 1 day of storage, but was significant at 1 per cent level after storage for 3 days.

The dehydroascorbic acid content decreased to zero in milk packaged in glass bottles irrespective of exposure or non-exposure to simulated doorstep delivery conditions, but in the case of milk in SSC some dehydroascorbic acid was detectable after 7 days.

TABLE 4:17

The ascorbic acid content (mg/100 ml milk) of pasteurized milk packaged in glass bottles and after 1 day of storage at 5°C.

No. of trial	Ascorbic acid (mg/100 ml milk)
1	1.41
2	1.05
3	1.49
4	1.96
Mean	1.48

Sample	Ascorbic acid (mg/100 ml milk)
Glass bottle	1.23
SSC	1.72

	DF	MS	VR
Trial	3	0.2851	0.82
Sample	1	0.4901	1.42
Residual	3	0.3443	
Total	7	0.3398	

Table	Trial	Sample
REP	2	4
SED	0.5870	0.4150

TABLE 4:18

The ascorbic acid content (mg/100 ml milk) of samples of pasteurized milk packaged in glass bottles and single-service containers after 3 and 7 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system.

	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
After 3 days	0.474	0.802	1.084	1.131
After 7 days	0.044	0.113	0.141	0.047

SED = 0.0405

	DF	MS	VR
Trial	3	0.602316	122.92**
Storage	1	4.949440	1010.10***
Sample	1	0.470207	95.96***
Exposed	1	0.061338	12.51*
Trial storage	3	0.755671	154.22***
Trial sample	3	0.052794	10.77*
Storage sample	1	0.412458	84.17**
Trial exposed	3	0.006645	1.35
Storage exposed	1	0.079900	16.30
Sample exposed	1	0.098901	20.18*
Trial storage sample	3	0.091274	18.62*
Trial storage exposed	3	0.000905	0.18
Trial sample exposed	3	0.009289	1.89
Storage sample exposed	1	0.006992	1.42
Residual	3	0.004900	
Total	31	0.343568	

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

GRAPH 4:5

The variations in the ascorbic acid content after storage at 5°C of pasteurized milks subjected to different treatments

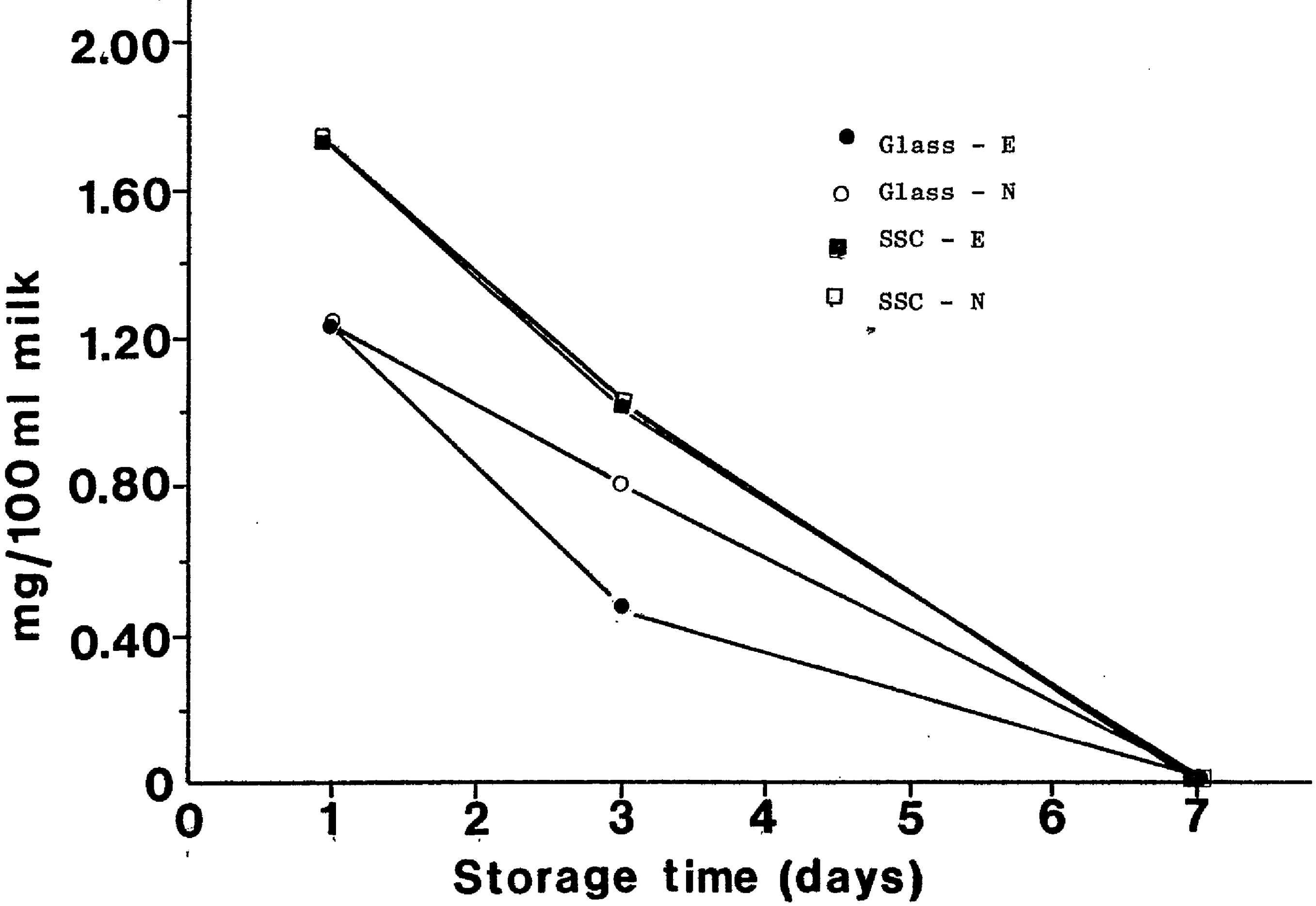


TABLE 4:19

The dehydroascorbic acid content (mg/100 ml milk) of samples of pasteurized milk packaged in glass bottles and single-service containers after 1 day of storage at 5^{o}C

No. of trial	Dehydroascorbic acid (mg/100 ml milk)
1	0.270
2	0.186
3	0.202
4	0.748
Mean	0.351

Sample	Dehydroascorbic acid (mg/100 ml milk)
Glass bottle	0.411
SSC	0.292

	DF	MS	VR
Trial	3	0.14240	6.55
Sample	1	0.02856	1.31
Residual	3	0.02174	
Total	7	0.07442	

Table	Trial	Sample
REP	2	4
SED	0.1474	0.1043

TABLE 4:20

The dehydroascorbic acid content (mg/100 ml) of samples of pasteurized milk packaged in glass bottles and single service containers after 3 and 7 days of storage at 5°C in darkness and in light and temperature conditions simulating the door-step delivery system

	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
After 3 days	0.281	0.357	0.315	0.257
After 7 days	0.075	0.081	0.126	0.220

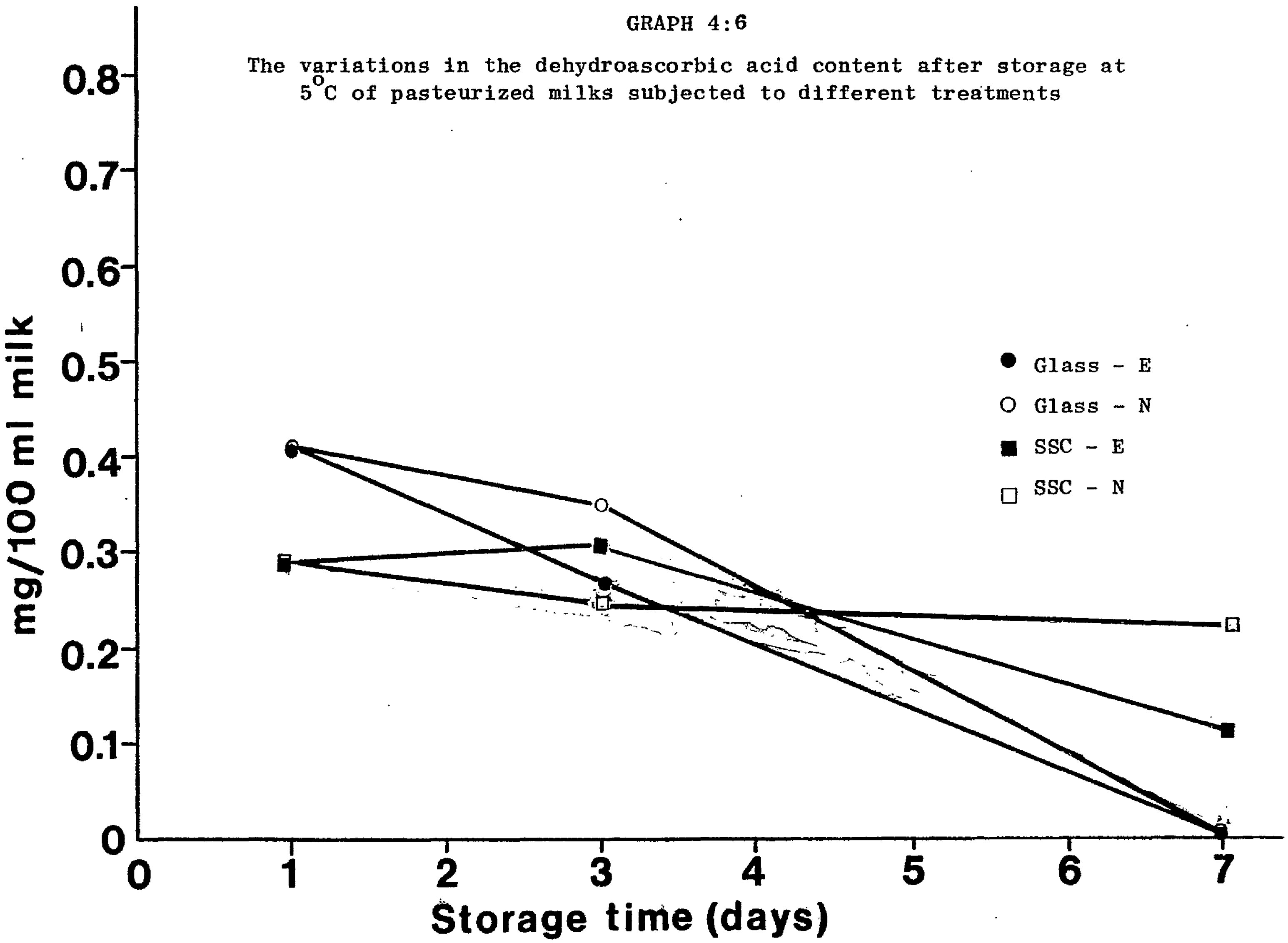
SED = 0.0652

	DF	MS	VR
Trial	3	0.269984	34.60**
Storage	1	0.250455	32.10*
Sample	1	0.007719	0.98
Exposed	1	0.006992	0.89
Trial storage	3	0.007074	0.90
Trial sample	3	0.006397	0.82
Storage sample	1	0.032832	4.20
Trial exposed	3	0.010220	1.31
Storage exposed	1	0.003383	0.43
Sample exposed	1	0.001093	0.14
Trial storage sample	3	0.005675	0.72
Trial storage exposed	3	0.004765	0.61
Trial sample exposed	3	0.013957	0.78
Storage sample exposed	1	0.024476	3.13
Residual	3	0.007802	
Total	31	0.042083	

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

GRAPH 4:6

The variations in the dehydroascorbic acid content after storage at $5^{o}C$ of pasteurized milks subjected to different treatments



DISCUSSION

1. Titratable acidity

From the results shown in Tables 4:1, 4:2, 4:3 and 4:4, it is clear that there was no obvious change in the titratable acidity of pasteurized milk packaged in glass bottles and SSC. This is due to the fact that the temperature of pasteurization kills most of the lactic acid organisms except a few species of thermoduric streptococci which are thermophilic and grow very slowly in milk stored at $5^{O}C$.

The difference between the trials was significant on the day of processing ($p < 0.001$) and after 2 days ($p < 0.01$) and 4 days ($p < 0.001$) of storage at $5^{O}C$. But there was no significant difference after 6 days of storage. These differences between the trials were due to the differences in the natural composition of the milk during the period of study.

There was no significant difference in the titratable acidity of milk packaged in different containers exposed to light and temperature conditions simulating doorstep delivery system and those which were stored in darkness.

The difference in the titratable acidity of milk packaged in different types of containers was not significant during the different periods of storage except after 4 days when there was a significant difference at 1 per cent level. On the 4th day of storage at $5^{O}C$ the milk in glass bottles showed the very slight increase of 0.0016 per cent over the milk packaged in SSC.

The results of all the tests for titratable acidity show that this test is not an effective measure of the quality of pasteurized milk when the milk is stored below $5^{O}C$ because while the range of titratable acidity in all of the samples was only 0.1400 to 0.1575 (per cent lactic acid) there were high levels of bacterial growth, and off-flavour had developed.

The slight decrease in the titratable acidity in all the samples after

6 days of storage at 5°C may be due to the diffusion of the carbon dioxide in pasteurized milk.

2. pH

The results of the pH value which are presented in Tables 4:5, 4:6, 4:7 and 4:8 show a highly significant difference ($p < 0.001$) in the pH values between the samples of the 6 trials on the day of processing and after 2, 4 and 6 days of storage at 5°C. This difference was related to the different composition of the milk during the period of testing. It has been found that Na, Cl, Ca, Mg, lactose, total N, non-casein nitrogen and casein levels affect the pH value of milk (Lück and Smith, 1975).

The pH of the pasteurized milk packaged in glass bottles was significantly lower than that of the same milk packaged in SSC except after 2 days of storage at 5°C when there was no significant difference. From an examination of the mean of the results, it is clear that the change in the pH value during the period of storage was very small. This level of change in the pH does not effect the quality of pasteurized milk packaged by the two systems.

There was a significant difference after 4 and 6 days of storage at 5°C between the pH value of samples which were exposed to light and temperature conditions simulating doorstep delivery and those which were stored in darkness. This difference was not due to the exposure treatment, but was probably due to the variation in the pH level of the milk in different trials.

The difference between the highest and the lowest pH value of all samples tested was 0.24. This very small change indicates that there was no alteration in the acidity level due to the microbiological content of the milk.

3. Dissolved oxygen content

Oxygen is necessary for the growth of aerobic bacteria. The dissolved oxygen in the milk and that present in the head space of containers may increase the growth of the micro-organisms present. Schönborn <u>et. al.</u> (1975) found that the bacteriological quality of pasteurized milk packaged in containers with a head space was significantly poorer than that of milk which was packaged in containers without a head space.

From the results of the dissolved oxygen content estimations which are presented in Tables 4:9, 4:10, 4:11 and 4:12, it is clear that there was a highly significant difference in the dissolved oxygen content of the samples from the six trials on the day of processing ($p < 0.001$), after 2 days ($p < 0.001$), after 4 days ($p < 0.001$) and after 6 days ($p < 0.5$) of storage at $5^{o}C$. The differences between the trials may be due to different amounts of oxygen incorporated in the milk at the time of processing.

The dissolved oxygen content of pasteurized milk packaged in SSC was significantly higher ($p < 0.001$) than that of pasteurized milk packaged in glass bottles when tested on the day of processing and after 2, 4 and 6 days of storage at $5^{o}C$.

It is clear from graph 4:2 that there was a decrease in the dissolved oxygen content of the samples in glass bottles during the storage. This decrease was probably due to the growth of the aerobic bacteria which utilize oxygen. No similar reduction in dissolved oxygen content was found with the milk packaged in SSC.

4. <u>Acid degree value (ADV)</u>

ADV is defined as the number of ml of 1 N KOH required to neutralize the free fatty acid present in 100 g of fat. It is considered to be an indication of rancidity in a dairy product.

The results of this study showed that pasteurized milk which was packaged in glass bottles contained a higher level of the ADV ($p < 0.001$) after 4 and 6 days of storage at $5^{o}C$ compared with the samples of the same milk packaged in SSC. This might have been due to the action of the larger numbers of bacteria of which the colony count showed a significant correlation with the ADV ($r = 57$, $p \leq 0.001$).

There was no significant difference in the ADV levels of samples due to exposure treatment to the light and temperature conditions simulating the doorstep delivery system.

There was a significant difference ($p < 0.5$) in ADV level between the milks in the different trials on the day of processing. However, the differences were highly significant ($p < 0.001$) after 2, 4 and 6 days of storage at $5^{o}C$.

The correlation coefficient between the ADV and the $\log_e$ of the lipolytic count was $r = 0.56$ ($p < 0.001$). This result indicates that the other reason for the increase in the ADV of milk on storage at $5^{o}C$ is the natural lipase enzymes which are present in milk. Mechanical operation in milk handling on the farm and in the dairy may increase the ADV of milk by disrupting the fat-globule membrane and so result in the enzymes gaining access to the triglycerides which are thereafter hydrolyzed. The standard method for the Examination of Dairy Product, APHA (1978) suggest that extreme rancidity is evident when milk contains > 1.5 of ADV. In this study, 14.2 per cent of pasteurized milk packaged in glass bottles exceeded this level, while only 4.7 per cent of milks in SSC exceeded this level.

5. Vitamin C (ascorbic and dehydroascorbic acid)

Vitamin C is one of the many vitamins present in milk. It is present in the milk as ascorbic acid and dehydroascorbic acid both of which are biologically active. The lose in this vitamin could occur during processing and storage. Light is one of the most importance factors affecting this vitamin. It is clear from the results shown in Table 4:18 that exposure to light and temperature conditions simulating doorstep delivery for 3 h before storage caused a 40.8 per cent loss in the ascorbic acid in pasteurized milk which was packaged in glass bottles, while the loss in pasteurized milk packaged by SSC, was 4.1 per cent. The other factor found to affect the vitamin C content in pasteurized milk was the storage time. It is known that reductons are formed during storage in the milk and these catalize the oxidation reaction of vitamin C in the milk. It is appeard from the results that there was a difference in vitamin C content in pasteurized milk during the period of study, which was expected, because of the change in the composition of the milk from time to time. The results of the dehydroascorbic acid (Table 4:19) showed that pasteurized milk in glass bottles gave a higher reading than that which was packaged in SSC. But, the difference was not significant. This means that ascorbic acid in the samples of milk in glass bottles changed more quickly to the dehydroascorbic acid form than in the samples of milk in the SSC. The other factor which affected the content of the dehydroascorbic acid was the storage time. There was a decrease in dehydroascorbic acid in all the samples, but the unexposed milks packaged in SSC contained a higher amount of dehydroascorbic acid than the other milks after 7 days of storage.

CONCLUSION

1. In this study, the titratable acidity and the pH were not affected after 6 days when the milk was stored at $5^{\circ}C$. There was no significant difference between the milk which was packaged in glass bottles and the SSC system. Exposure to light and temperature conditions simulating the doorstep delivery system had no effect on the titratable acidity and the pH.

2. The study showed that the dissolved oxygen content of pasteurized milk packaged in glass bottles decreased during the period of storage and had a negative correlation with the $\log_e$ of total colony count. It is interesting to note that in the case of the pasteurized milk packaged in single service containers the dissolved oxygen content after 6 days of storage at $5^{\circ}C$ was at a similar level to what it was on the day of processing. One may speculate that if the aerobic bacteria present in the milk used up the oxygen during this period then the oxygen level was maintained by the diffusion of oxygen from the atmosphere through the package. The package did not contain a layer of aluminium foil.

Further work is required to find the effect of different levels of dissolved oxygen in the milk on the growth of the organisms and shelf life of processed milks.

3. It is concluded that pasteurized milk packaged in glass bottles gave higher acid degree values after 4 and 6 days of storage at $5^{\circ}C$ than the same pasteurized milk packaged in the SSC system. Exposure to light and temperature conditions simulating the doorstep delivery system had no significant effect on acid degree values.

4. It is clear that the effect of exposure of pasteurized milk packaged in glass bottles to light and temperature conditions simulating the doorstep delivery system brought about a decrease of 40.8 per cent in the ascorbic acid content after 3 days of storage at $5^{\circ}C$ compared to the level of ascorbic acid in milk packaged in glass bottles which was

not exposed. However, there was no significant difference in the ascorbic acid levels of the same milk in either exposed or unexposed in single service containers. Pasteurized milk packaged in the SSC system contain more ascorbic acid after 3 days of storage at 5^{o}C than that which was packaged in glass bottles. The loss in the ascorbic acid during the storage was significant in both types of packaging systems.

CHAPTER FIVE

QUALITY ASSESSMENT OF PASTEURIZED MILK PACKAGED IN GLASS BOTTLES AND SINGLE-SERVICE CONTAINERS.

INTRODUCTION

The quality of pasteurized milk is the most important factor affecting the acceptability of pasteurized milk to the consumers. Consumers do not pay too much attention to the bacterial count as long as there is no effect on public health. The quality of pasteurized milk can be assessed for appearance, odour and flavour.

Appearance of the milk is usually chalk-white. But this could be affected by the physical, chemical and microbial changes in the milk during processing and storage. Appearance of the milk is an important factor from the consumer point of view so that any defect in the appearance of the milk will affect its quality.

The odour test is an excellent guide for judging the acceptability of pasteurized milk. Odours could be present in the pasteurized milk due to feeding stuffs, rancidity, products of bacterial growth and absorption after the milk is produced.

Flavour is the most important factor in assessing the quality of pasteurized milk. Johnson (1979) reported that there are many types of flavours which can be found in processed milks and each is caused by different sets of circumstances in the production or storage (Table 5:1).

The effect of light on flavour has been investigated by many workers. They found that the intensity of the light and the wave length are related to the development of off-flavours (Stull, 1954; Smith and MacLeod, 1955).

Milk is considered to be important in our food supply. The loss in the organoleptic quality is a serious problem from the consumer point

TABLE 5:1

Categories of off-flavours in milk summarized by Johnson (1979)

Causes	Descriptive or associative terms
Heat	Cooked, caramelized, heated and scorched.
Light induced	Light, sunlight and activated.
Lipolyzed	Rancid, butyric, bitter*, and goat flavour.
Microbial	Acid, bitter*, fruity, malty, putrid and unclean.
Oxidized	Papery, cardboard, metallic, oily and fishy.
Transmitted	Feed, weedy, cowy and byre.
Miscellaneous	Absorbed, astringent, bitter*, chalky, chemical, fat, foreign, lacks freshness and salty.

*Bitter flavour may arise from a number of different causes.

of view. The development in the packaging industry and the use of many different packaging systems for pasteurized milk could affect milk quality. This study was undertaken to assess the effect of the following factors on the quality of pasteurized milk:-

(i) the effect of two different containers and related packaging systems on the quality of pasteurized milk,

(ii) the effect of the exposure for 3 h to light and temperature conditions simulating doorstep delivery system,

(iii) the effect of the storage for 0, 2 and 6 days at $5^{o}C$.

EXPERIMENTAL

1. Quality assessment

The differences in the appearance, odour and flavour were assessed for each sample of milk using a hedonic scale. This scale contained eight categories of response defined as follows:-

8. like extremely;
7. like very much;
6. like moderately;
5. like slightly;
4. dislike slightly;
3. dislike moderately;
2. dislike very much and
1. dislike extremely.

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

1. very slight;
2. slight;
3. distinct and
4. pronounced.

2. Selecting the samples for quality assessment

The samples for the quality assessment were used only from sources (ii) and (iii) as described in chapter three (Experimental, Dairy A, 3c).

The quality assessment was made on the day of processing and after 2 and 6 days of storage at 5^oC.

3. The taste panel

The taste panel was chosen from the members of the staff of the Department of Dairy Technology, The West of Scotland Agricultural College, Ayr, who have good experience in grading milk and milk products.

4. Presentation of the samples for testing

The samples of milk were heated to 17°C and kept at this temperature for 3 h and filled into 50 ml beakers using the same procedure described by Schonborn *et. al.* (1975). Each sample was offered to four judges for the quality assessment.

RESULTS

1. Appearance

The mean of the scores given by the taste panel for the appearance of pasteurized milk packaged in glass bottles and single service containers and examined on the day of processing and after 2 and 6 days of storage at 5°C are presented in Tables 5: 2, 5:3 and 5:4.

The mean of the scores show that on the day of processing there was no difference in the appearance between the pasteurized milk packaged in glass bottles and that which was packaged in SSC. However, a highly significant difference (o.1 per cent level) was found in the appearance scores awarded in different trials. The difference between the judges in their grade scores for appearance was significant at 5 per cent level (Table 5:1).

After 2 days of storage at 5°C the mean of the scores showed that there was no significant difference in the appearance between the milks packaged in the two packaging systems. The scores, however, showed a highly significant difference (0.1 per cent level) between trials. Scores awarded by individual members of the panel differed at the same significant level. Samples of pasteurized milk in both types of packages which were exposed for 3 h on the day of processing to light and temperature conditions simulating the doorstep delivery system had a higher significance than the smples which were not exposed to the same conditions.

After 6 days of storage at 5°C the results showed that there was no significant difference in scores given to the different samples of pasteurized milk. A highly significant difference in scores was found between the trials and between the judges. Samples in both types of packages and which were not exposed to the simulated doorstep delivery conditions had scores significantly higher (5 per cent level) than the samples which were exposed.

The variations of the scores given for the appearance of the milks on

TABLE 5:2

Means of the scores given by a panel (4 judges) for the appearance of pasteurized milk packaged in glass bottles and single-service containers on the day of processing.

No. of trial	Sample	
	Glass bottle	SSC
1	6.25	6.62
2	6.83	6.87
3	6.62	7.25
4	6.75	7.00
5	6.89	6.98
6	5.99	4.60
Mean	6.55	6.55

	DF	MS	VR
Trial	5	6.6611	25.37***
Judges	3	0.8618	3.28*
Sample	1	0.0000	0.00
Trial judges	11	0.8955	3.41**
Trial sample	5	2.0205	7.69***
Judges sample	3	0.2686	1.02
Trial judges sample	11	0.6800	2.59**
Residual	40		
Total	79		

Table	Sample	Trial sample
RED	48	8
SED	0.1046	0.2562

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

TABLE 5:3

Means of the scores given by a panel (4 judges) for the appearance of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness (N) and in light and temperature conditions (E) simulating the doorstep delivery system.

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	6.75	6.62	6.50	6.37
2	6.44	6.44	6.76	6.52
3	6.87	6.75	7.25	6.87
4	6.93	6.72	7.19	6.92
5	6.84	6.71	7.36	7.09
6	5.27	4.60	4.59	4.02
Mean	6.52	6.30	6.61	6.30
Overall mean	6.41		6.45	

	DF	MS	VR
Trial	5	26.4425	164.98***
Judges	3	8.1009	50.54***
Sample	1	0.0813	0.50
Exposed	1	3.2738	20.42***
Trial judges	10	3.1880	19.89***
Trial sample	5	1.2838	8.01***
Judges sample	3	1.9792	12.34***
Trial exposed	5	0.2728	1.70
Judges exposed	3	1.8775	11.71***
Sample exposed	1	0.1112	0.69
Trial judges sample	10	2.3959	14.94***
Trial judges exposed	10	0.1773	1.10
Trial sample exposed	5	0.0372	0.23
Judges sample exposed	3	0.2555	1.59
Residual	86	0.1603	
Total	151	1.6668	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.0578	0.0817	0.2002

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

TABLE 5:4

Means of the scores given by a panel (4 judges) for the appearance of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	5.36	5.83	5.24	5.41
2	5.87	6.00	6.25	6.12
3	6.02	6.10	6.29	6.34
4	6.62	6.62	6.25	6.75
5	6.75	6.75	6.75	7.00
6	2.75	3.50	3.75	4.00
Mean	5.56	5.80	5.75	5.93
Overall mean	5.68		5.84	

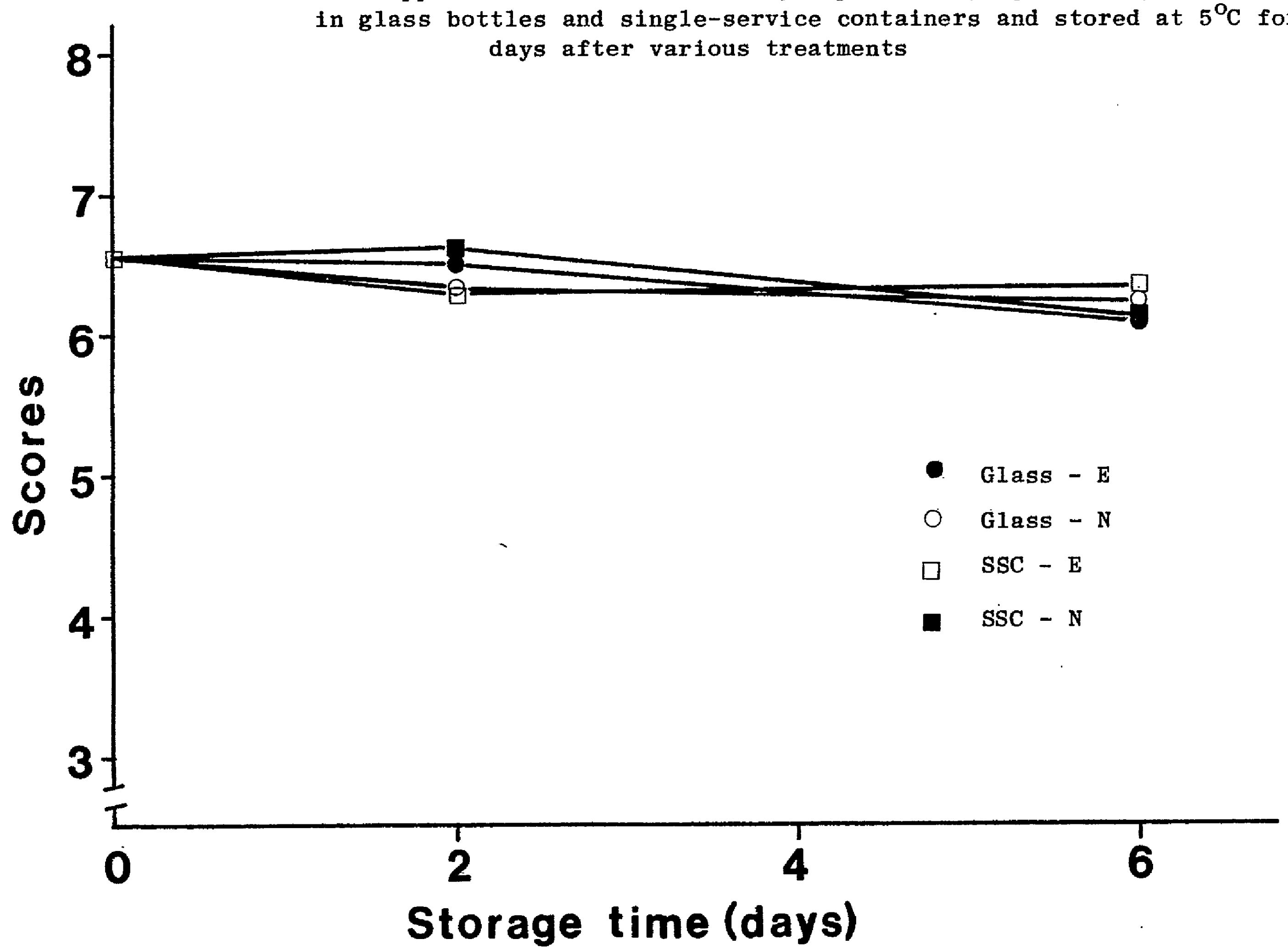
	DF	MS	VR
Trial	5	46.2376	131.53***
Judges	3	6.5848	18.73***
Sample	1	1.2963	3.68
Exposed	1	2.1048	5.98*
Trial judges	12	3.9640	11.27***
Trial sample	5	1.0064	2.86*
Judges sample	3	1.5243	4.33**
Trial exposed	5	0.2746	0.78
Judges exposed	3	0.2367	0.67
Sample exposed	1	0.0380	0.10
Trial judges sample	12	0.8296	2.36*
Trial judges exposed	12	0.2168	0.61
Trial sample exposed	5	0.2784	0.79
Judges sample exposed	3	0.2317	0.94
Residual	96	0.3515	
Total	167	2.1696	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	12
SED	0.0856	0.1210	0.2964

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

GRAPH 5:1

Mean appearance scores awarded by a panel (4 judges) for pasteurized milk in glass bottles and single-service containers and stored at 5°C for 6 days after various treatments



storage at 5^{o}C are shown in graph 5:1. There was only a very slight decrease in the scores given for the appearance of pasteurized milk during storage compared to the scores given on the day of processing.

2. Odour

The mean of the scores given by the taste panel for the odour of pasteurized milk packaged in glass bottles and single-service containers are presented in Tables 5:5, 5:6 and 5:7 on the day of processing and after 2 and 6 days of storage at 5^{o}C.

The scores given by the taste panel on the day of processing showed that there was no significant difference in the odour of the pasteurized milk packaged in the two different containers. A highly significant difference (0.1 per cent level) was found in the odour scores awarded in different trials and by different members of the panel.

After 2 days of storage at 5^{o}C, the results show that pasteurized milk packaged in the single-service containers had higher scores ($p < 0.001$) than pasteurized milk in glass bottles. Exposure or non-exposure to simulated doorstep delivery conditions had no significant effect on the odour scores of pasteurized milk in two types of containers after 2 days of storage at 5^{o}C.

After 6 days of storage at 5^{o}C, the odour scores showed that there was no significant difference between samples in the two different containers. However, pasteurized milk in glass bottles which were not exposed to simulated doorstep delivery conditions had higher scores ($p < 0.5$) than milk from the exposed samples.

It is clear that there was no significant difference between the members in their judgement of milk odour. However, the differences in odour scores of milk in the different trials was highly significant (0.1 per cent level.

The variation in the scores given for the odour of pasteurized milk in the two types of package during the storage time at 5^{o}C is illustrated

TABLE 5:5

Means of the scores given by a panel (4 judges) for the odour of pasteurized milk packaged in glass bottles and single-service containers on the day of processing.

No. of trial	Sample	
	Glass bottle	SSC
1	6.00	6.00
2	6.69	6.87
3	6.75	6.75
4	6.50	6.12
5	6.61	6.75
6	7.09	7.14
Mean	6.61	6.60

	DF	MS	VR
Trial	5	2.49059	28.46***
Judges	3	5.81539	66.46***
Sample	1	0.00003	0.00
Trial judges	11	0.70454	8.05***
Trial sample	5	0.15594	1.78
Judges sample	3	0.58911	6.73***
Trial judges sample	11	0.27136	3.10**
Residual	40	0.08750	
Total	79	0.59090	

Table	Sample	Trial sample
REP	48	8
SED	0.0604	0.1479

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

TABLE 5:6

Means of the scores given by a panel (4 judges) for the odour of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	6.12	5.87	5.87	5.99
2	6.70	7.18	7.16	7.31
3	5.12	5.12	5.87	5.74
4	5.29	5.71	6.14	5.96
5	5.81	5.29	5.27	5.96
6	6.70	6.85	7.33	5.82
Mean	5.96	6.00	6.27	6.30
Overall mean	5.98		6.29	

	DF	MS	VR
Trial	5	16.3244	54.97***
Judges	3	24.4984	82.50***
Sample	1	4.5430	15.29***
Exposed	1	0.0687	0.23
Trial judges	10	6.7019	22.56***
Trial sample	5	0.7153	2.40*
Judges sample	3	1.4510	4.88**
Trial exposed	5	0.1999	0.69
Judges exposed	3	0.3466	1.16
Sample exposed	1	0.0006	0.02
Trial judges sample	10	1.2842	4.32***
Trial judges exposed	10	0.2896	0.97
Trial sample exposed	5	0.8078	2.72*
Judges sample exposed	3	0.4979	1.67
Residual	86	0.2969	
Total	151	1.8776	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.0787	0.1112	0.2725

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 5:7

Means of the scores given by a panel (4 judges) for the odour of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	4.62	4.99	4.67	5.16
2	5.37	5.50	5.25	4.87
3	4.94	5.50	5.10	5.46
4	6.00	5.75	5.75	6.12
5	5.62	5.75	5.62	5.62
6	5.75	6.00	6.25	6.37
Mean	5.38	5.58	5.44	5.60
Overall mean	5.48		5.52	

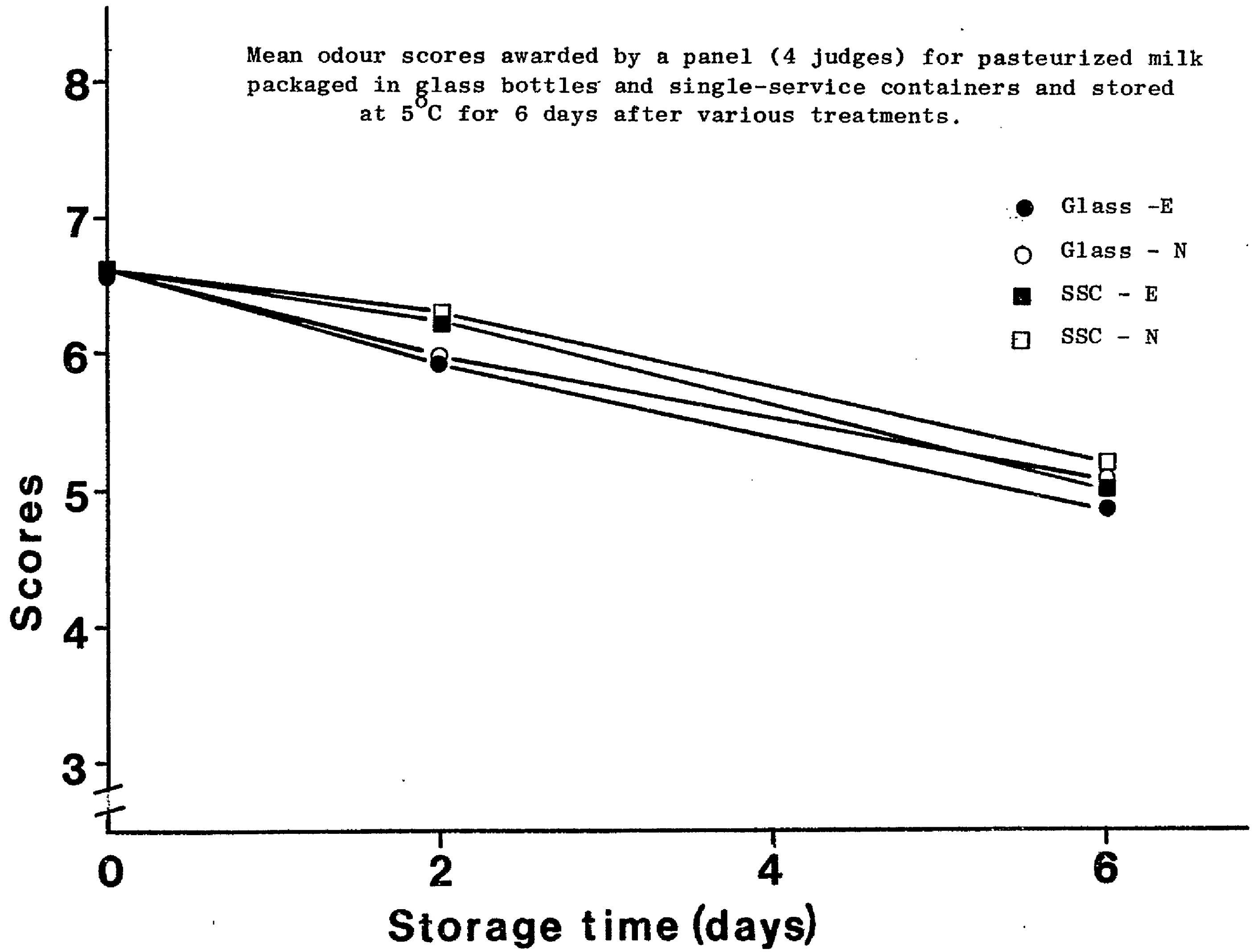
	DF	MS	VR
Trial	5	6.8507	30.79***
Judges	3	0.4878	2.19
Sample	1	0.0690	0.31
Exposed	1	1.5556	6.99*
Trial judges	12	6.7396	30.29***
Trial sample	5	0.5535	2.29*
Judges sample	3	1.2489	5.61**
Trial exposed	5	0.4206	1.89
Judges exposed	3	0.4124	1.85
Sample exposed	1	0.0130	0.05
Trial judges sample	12	1.1763	5.28***
Trial judges exposed	12	1.0948	4.92***
Trial sample exposed	5	0.2869	1.28
Judges sample exposed	3	0.0298	0.13
Residual	96	0.2225	
Total	167	1.0672	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.0681	0.0963	0.2358

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

GRAPH 5:2

Mean odour scores awarded by a panel (4 judges) for pasteurized milk packaged in glass bottles and single-service containers and stored at 5°C for 6 days after various treatments.



in Graph 5:2. The correlation coefficients between the scores given for odour and the $\log_e$ of the total colony count, the psychrotrophic count and the acid degree value are presented in Table 5:13.

3. Flavour (taste)

The scores given by the panel for the flavour (taste) of pasteurized milk packaged in glass bottles and single service containers on the day of processing and after 2 and 6 days of storage at $5^{o}C$ are presented in Tables 5:8, 5:9 and 5:10.

On the day of processing there was no significant difference between the flavour scores of pasteurized milk in the two different containers. However, there was a significant difference (1 per cent level) between the flavour scores given by the judges in the different trials. A highly significant difference was found between the judges in their scoring of the flavour of pasteurized milks on the day of processing.

After 2 days of storage at $5^{o}C$, the flavour of pasteurized milk packaged in SSC had higher ($p < 0.01$) scores than that packaged in glass bottles. There was a highly significant difference ($p < 0.001$) between the flavour scores awarded by the judges in the different trials. A highly significant difference ($p < 0.001$) was found between the judges in their assessment of the flavour of pasteurized milk after 2 days of storage at $5^{o}C$.

After 6 days of storage at $5^{o}C$, a highly significant ($p < 0.001$) difference was found between the judges in their assessment of the flavour of pasteurized milks. However, a significant difference ($p < 0.05$) was found between the flavour scores given for pasteurized milks from different trials.

Pasteurized milk in glass bottles had higher scores ($p < 0.05$) than that in single-service containers. There was no significant difference in flavour scores of pasteurized milk which was exposed to light and temperature conditions simulating the doorstep delivery system and that which was not exposed to these conditions. This was found for the

TABLE 5:8

Means of the scores given by a panel (4 judges) for the flavour of pasteurized milk packaged in glass bottles and single-service containers on the day of processing

No. of trial	Sample	
	Glass bottle	SSC
1	6.00	5.25
2	6.56	6.53
3	6.00	6.00
4	6.50	6.50
5	5.90	6.06
6	6.34	6.21
Mean	6.21	6.09

	DF	MS	VR
Trial	5	1.9931	4.19**
Judges	3	4.9817	10.48***
Sample	1	0.3750	0.78
Trial judges	11	0.7578	1.59
Trial sample	5	0.4084	0.86
Judges sample	3	0.8719	1.83
Trial judges sample	11	0.5983	1.26
Residual	40	0.4750	
Total	79	0.8084	

Table	Sample	Trial sample
REP	48	8
SED	0.1407	0.3446

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

TABLE 5:9

Means of the scores given by a panel (4 judges) for the flavour of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	5.50	4.87	6.00	5.00
2	4.52	5.16	4.84	5.14
3	4.50	3.87	5.25	4.87
4	4.12	4.34	4.42	4.17
5	5.72	5.23	5.60	5.80
6	6.85	6.33	7.17	6.81
Mean	5.20	4.97	5.54	5.30
Overall mean	5.08		5.42	

	DF	MS	VR
Trial	5	25.4601	21.28***
Judges	3	10.9308	22.01***
Sample	1	5.4646	11.00**
Exposed	1	2.7966	5.63**
Trial judges	10	9.8043	19.75***
Trial sample	5	0.6657	1.34
Judges sample	3	5.8901	11.86***
Trial exposed	5	1.5924	3.20*
Judges exposed	3	0.9888	1.99
Sample exposed	1	0.0016	0.03
Trial judges sample	10	1.7591	3.54***
Trial judges exposed	10	0.6833	1.37
Trial sample exposed	5	0.4130	0.83
Judges sample exposed	3	0.3712	0.74
Residual	86	0.4964	
Total	151	2.4412	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.1017	0.1438	0.3523

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

TABLE 5:10

Means of the scores given by a panel (4 judges) for the flavour of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5^{o}C in darkness and in light and temperature conditions simulating the doorstep delivery system

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	4.13	4.61	3.70	4.57
2	4.62	4.37	3.62	3.87
3	3.97	3.58	4.58	3.83
4	5.25	4.75	2.75	4.00
5	3.87	3.87	4.00	4.25
6	4.25	4.75	4.50	5.37
Mean	4.35	4.32	3.86	4.31
Overall mean	4.33		4.09	

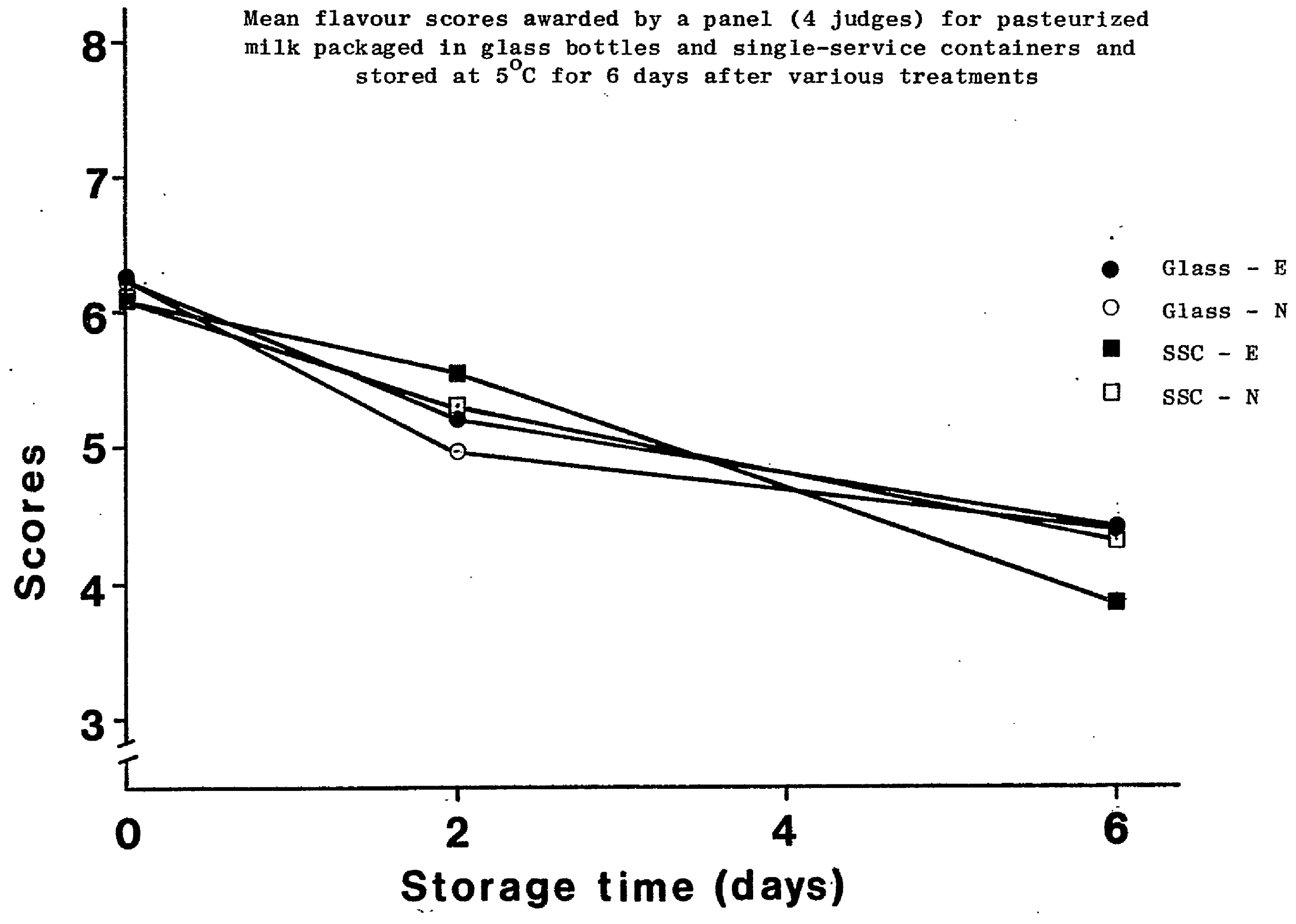
	DF	MS	VR
Trial	5	2.2993	3.57*
Judges	3	12.7768	19.86***
Sample	1	2.9482	4.58*
Exposed	1	2.2217	3.45
Trial judges	12	4.7249	7.34***
Trial sample	5	5.3297	8.28***
Judges sample	3	2.7005	4.19**
Trial exposed	5	1.8141	2.82**
Judges exposed	3	0.9545	1.48
Sample exposed	1	2.8399	4.41*
Trial judges sample	12	3.0460	4.73***
Trial judges exposed	12	1.3432	2.08*
Trial sample exposed	5	0.9522	1.48
Judges sample exposed	3	1.4759	2.29
Residual	96	0.6431	
Total	167	1.7055	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.1158	0.1637	0.4010

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

GRAPH 5:3

Mean flavour scores awarded by a panel (4 judges) for pasteurized milk packaged in glass bottles and single-service containers and stored at 5°C for 6 days after various treatments



milks in glass bottles and in single-service containers.

Changes in the flavour scores of pasteurized milk packaged in the two different containers with storage at $5^{o}C$ are illustrated in graph 5:3.

The flavour scores for pasteurized milk in both types of container showed correlation coefficients which were significant at the 0.1 per cent level with the $\log_e$ of the total colony count and psychrotrophic count (Table 5:13).

4. Total scores of the organoleptic tests

The total scores given for pasteurized milk packaged in glass bottles and single-service containers on the day of processing and after 2 and 6 days of storage at $5^{o}C$ are given in Tables 5:11, 5:12 and 5:13 respectively.

The total scores on the day of processing show that there was no significant difference between the samples in the two different containers. The differences between the trials were significant at 0.1 per cent level. The same significant difference was found between the members of the panel in the scores awarded.

After 2 days of storage of the milks, the results show that pasteurized milk packaged in SSC had a higher total score (significant at 0.1 per cent) than pasteurized milk packaged in glass bottles. The scores given to the milks in glass bottles which were exposed for 3 h to light and temperature conditions simulating the doorstep delivery system, were not significantly different from the scores given to the samples which were not exposed to these conditions. However, milk packaged in SSC which were exposed to simulated doorstep conditions had a higher score ($p < 0.05$) than the milk in SSC which were not exposed. The difference between the means of these was only 0.52, which is not a very great difference from the practical point of view. The differences were significantly higher ($p < 0.001$) between the trials and also between the samples.

TABLE 5:11

Means of the total scores given by a panel (4 judges) for appearance, odour and flavour of pasteurized milk packaged in glass bottles and single-service containers on the day of processing.

No. of trial	Sample	
	Glass bottle	SSC
1	18.25	17.87
2	20.09	20.28
3	19.37	20.00
4	19.75	19.62
5	19.41	19.81
6	19.43	19.97
Mean	19.39	19.26

	DF	MS	VR
Trial	5	9.812	8.92***
Judges	3	23.841	21.67***
Sample	1	0.382	0.34
Trial judges	11	1.845	1.67
Trial sample	5	2.222	2.02
Judges sample	3	4.816	4.37**
Trial judges sample	11	2.703	2.45*
Residual	40	1.100	
Total	79	3.045	

Table	Sample	Trial sample
REP	48	8
SED	0.214	0.524

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 5:12

Means of the total scores given by a panel (4 judges) for the appearance, odour and flavour of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5^{o}C in darkness and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	18.37	17.37	18.37	17.37
2	17.68	18.78	18.77	18.97
3	16.50	15.75	18.37	17.50
4	16.35	16.77	17.76	17.07
5	18.37	17.23	18.23	18.74
6	18.86	17.78	19.10	17.80
Mean	17.69	17.28	18.43	17.91
Overall mean	17.48		18.06	

	DF	MS	VR
Trial	5	14.616	10.30***
Judges	3	115.784	81.63***
Sample	1	22.624	15.95***
Exposed	1	10.464	7.37**
Trial judges	10	25.142	17.72***
Trial sample	5	3.316	2.33
Judges sample	3	20.837	14.69***
Trial exposed	5	3.693	2.60*
Judges exposed	3	8.213	5.79***
Sample exposed	1	0.164	0.11
Trial judges sample	10	7.907	5.57***
Trial judges exposed	10	0.714	0.50
Trial sample exposed	5	1.891	1.33
Judges sample exposed	3	1.527	1.07
Residual	86	1.418	
Total	151	6.958	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	12
SED	0.1719	0.2431	0.5955

* significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " " "

TABLE 5:13

Means of the total scores given by a panel (4 judges) for the appearance, odour and flavour of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5^{o}C in darkness and in light and temperature conditions simulating the doorstep delivery system.

No. of trial	Sample			
	Glass-E	Glass-N	SSC-E	SSC-N
1	14.12	15.43	13.62	15.15
2	15.87	15.87	15.12	14.87
3	14.95	15.21	15.97	15.64
4	17.87	17.12	14.75	16.87
5	16.25	16.37	16.37	16.87
6	12.75	14.25	14.50	15.75
Mean	15.30	15.71	15.05	15.86
Overall mean	15.50		15.46	

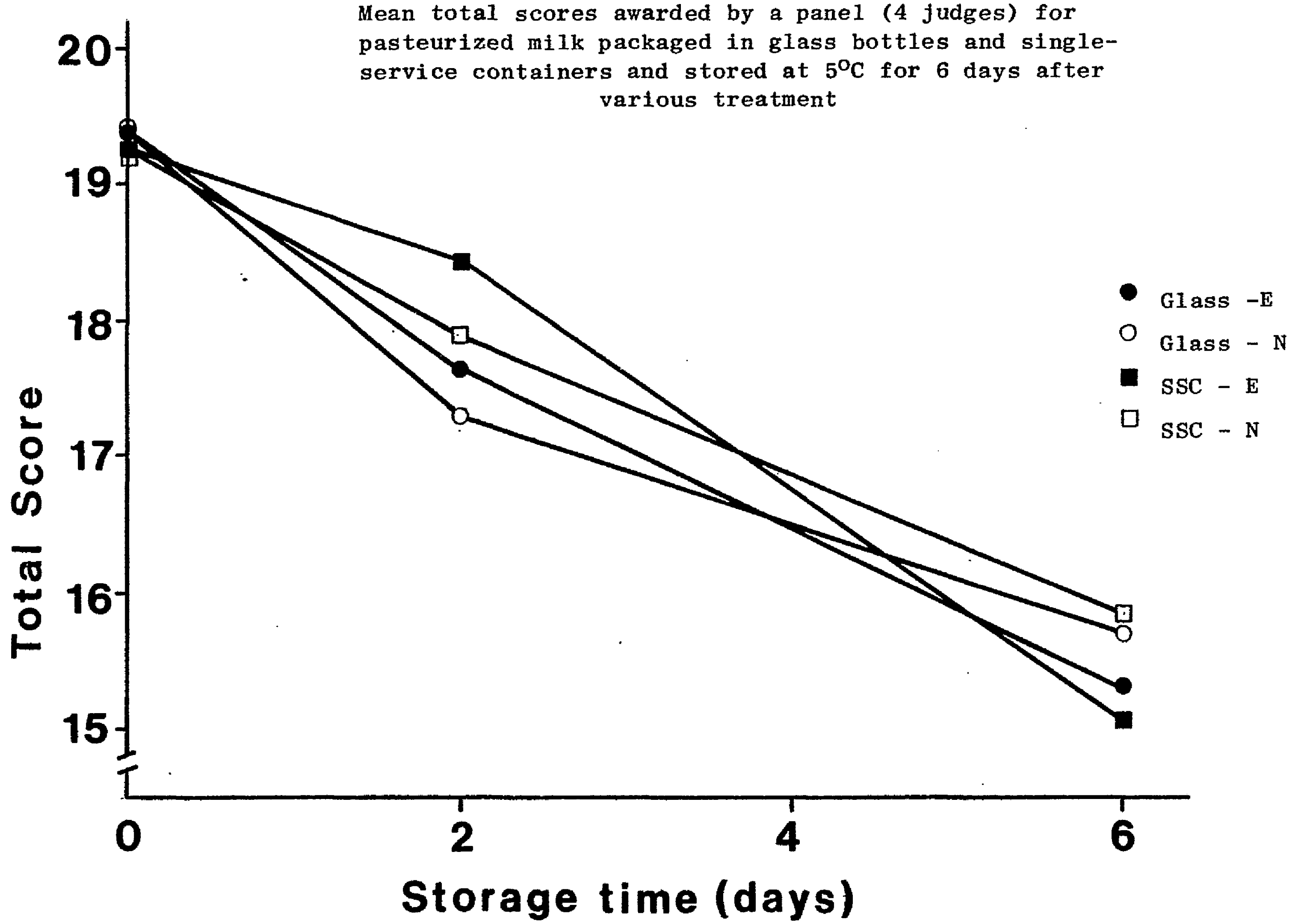
	DF	MS	VR
Trial	5	28.977	15.67***
Judges	3	18.270	9.88***
Sample	1	0.112	0.06
Exposed	1	17.516	9.47**
Trial judges	12	23.279	12.59***
Trial sample	5	11.225	6.07***
Judges sample	3	5.442	2.94*
Trial exposed	5	3.666	1.98
Judges exposed	3	3.494	1.89
Sample exposed	1	1.896	1.02
Trial judges sample	12	9.187	4.97***
Trial judges exposed	12	3.767	2.03*
Trial sample exposed	5	3.191	1.72
Judges sample exposed	3	0.841	0.45
Residual	96	1.849	
Total	167	5.696	

Table	Sample	Sample exposed	Trial sample exposed
REP	96	48	8
SED	0.1962	0.2775	0.6798

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

GRAPH 5:4

Mean total scores awarded by a panel (4 judges) for pasteurized milk packaged in glass bottles and single-service containers and stored at 5°C for 6 days after various treatment



The scores given by the taste panel to milks stored for 6 days at $5^{o}C$ showed that there was no significant difference between the quality of milk in the two packaging systems. Pasteurized milk in the single-service containers which were not exposed to simulated doorstep delivery had higher scores ($p < 0.01$) than that which was exposed. However, there was no significant difference in the scores of pasteurized milk in glass bottles which had been or had not been exposed to simulated doorstep delivery conditions.

The total scores for pasteurized milk packaged in single-service containers showed correlation coefficients of -0.7310 ($p < 0.001$), -0.7846 ($p < 0.001$) and -0.5862 ($p < 0.001$) with the $\log_e$ of the total colony count, $\log_e$ of the psychrotrophic count and the acid degree values respectively. However, in the case of pasteurized milk in glass bottles, the correlation coefficients with the above parameters were -0.7131 ($p < 0.001$), -0.7365 ($p < 0.001$) and -0.6614 ($p < 0.001$) respectively (Table 5:13).

The reduction in the total scores for pasteurized milk in the two types of containers with storage time is illustrated in graph 5:4.

5. Flavour defect

The flavour defects referred to by the members of the panel when the milks were examined on the day of processing and after 2 and 6 days of storage at $5^{o}C$ are given in Tables 5:14, 5:15 and 5:16.

The results indicate that on the day of processing (Table 5:14) the panel did not detect any flavour defect in the samples of milk packaged in either glass bottles or single service containers.

After 2 days of storage at $5^{o}C$ (Table 5:15) some of the milks in each type of container were judged to have very slight (level 1) and slight off-flavours (level 2). However, none of the samples showed distinct or pronounced off-flavours.

After 6 days of storage (Table 5:16) at $5^{o}C$ a few samples gave a distinct fruity flavour but none of these samples gave pronounced off-flavours. One sample from each type of package gave a pronounced oxidized flavour after 6 days of storage at $5^{o}C$.

TABLE 5:14

Categorization of comments by a panel (4 judges) of the flavours of pasteurized milk packaged in glass bottles and single-service containers when examined on the day of processing.

Flavour type	Flavour scale	No. of samples	
		Glass bottle	SSC
Malty	No flavour	12	12
	very slight	0	0
	slight	0	0
	distinct	0	0
	pronounced	0	0
Fruity	No flavour	12	12
	very slight	0	0
	slight	0	0
	distinct	0	0
	pronounced	0	0
Oxidised	No flavour	12	12
	very slight	0	0
	slight	0	0
	distinct	0	0
	pronounced	0	0
Bitter	No flavour	12	12
	very slight	0	0
	slight	0	0
	distinct	0	0
	pronounced	0	0
Cooked	No flavour	12	12
	very slight	0	0
	slight	0	0
	distinct	0	0
	pronounced	0	0

TABLE 5:15

Categorization of comments by a panel (4 judges) of the flavours of pasteurized milk packaged in glass bottles and single-service containers after 2 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system.

Flavour type	Flavour scale	No. of samples			
		Glass-E	Glass-N	SSC-E	SSC-N
Malty	No flavour	7	10	9	10
	very slight	4	2	3	2
	slight	1	0	0	0
	distinct	0	0	0	0
	pronounced	0	0	0	0
Fruity	No flavour	6	10	11	12
	very slight	6	2	1	0
	slight	0	0	0	0
	distinct	0	0	0	0
	pronounced	0	0	0	0
Oxidized	No flavour	10	11	8	9
	very slight	2	1	2	1
	slight	0	0	2	2
	distinct	0	0	0	0
	pronounced	0	0	0	0
Bitter	No flavour	11	12	12	12
	very slight	1	0	0	0
	slight	0	0	0	0
	distinct	0	0	0	0
	pronounced	0	0	0	0
Cooked	No flavour	10	12	12	12
	very slight	0	0	0	0
	slight	2	0	0	0
	distinct	0	0	0	0
	pronounced	0	0	0	0

TABLE 5:16

Categorization of comments by a panel (4 judges) of the flavours of pasteurized milk packaged in glass bottles and single-service containers after 6 days of storage at 5°C in darkness and in light and temperature conditions simulating the doorstep delivery system.

Flavour type	Flavour scale	No. of sample			
		Glass-E	Glass-N	SSC-E	SSC-N
Malty	No flavour	8	6	7	8
	very slight	4	4	5	3
	slight	0	2	0	1
	distinct	0	0	0	0
	pronounced	0	0	0	0
Fruity	No flavour	8	11	9	9
	very slight	2	0	1	1
	slight	1	0	0	2
	distinct	1	1	2	0
	pronounced	0	0	0	0
Oxidized	No flavour	4	8	9	7
	very slight	2	2	2	2
	slight	2	2	0	2
	distinct	1	0	0	1
	pronounced	3	0	1	0
Bitter	No flavour	10	11	10	12
	very slight	2	0	2	0
	slight	0	1	0	0
	distinct	0	0	0	0
	pronounced	0	0	0	0
Cooked	No flavour	10	9	12	11
	very slight	1	2	0	0
	slight	1	1	0	1
	distinct	0	0	0	0
	pronounced	0	0	0	0

TABLE 5:17

Correlation coefficients found between the organoleptic tests and the total colony count, psychrotrophic count and acid degree value obtained from pasteurized milk packaged in glass bottles and SSC which were stored for 6 days at 5^{o}C

Organoleptic test	Log_e of total colony count		Log_e of psychrotrophic count		Percentage of ADV	
	Glass bottle	SSC	Glass bottle	SSC	Glass bottle	SSC
Appearance	-0.3189*	-0.1701	-0.2912*	-0.2364	-0.4765***	-0.3936
Odour	-0.5472***	-0.4887***	-0.6184***	-0.4756***	-0.3052*	-0.3830**
Flavour	-0.6439***	-0.7166***	-0.6671***	-0.7539***	-0.4017**	-0.3327*
Total scores	-0.7131***	-0.7310***	-0.7365***	-0.7846***	-0.6614***	-0.5862***

DF = 58

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

DISCUSSION

1. Appearance

The scores which were given to the appearance of pasteurized milk on the day of processing showed that there was no difference between the glass bottle and SSC samples in this respect. There was a significant difference (at 0.1 per cent level) between the trials.

After 2 days of storage at 5°C, the scores for the appearance of the milks showed a slight decrease. There was no significant difference between the appearance of pasteurized milk packaged in glass bottles and that in single service containers under the above conditions. Samples of milk in bottles which were exposed to simulated doorstep delivery conditions had higher ($p < 0.01$) scores for appearance than the same milk in non-exposed containers. Similarly, milks in single-service containers exposed to light had a higher ($p < 0.001$) score for appearance than the unexposed milk in SSC. While the differences were significantly higher when analysed statistically.The result is of little importance from the practical point of view because the differences between the scores lay in the first decimal point.

After the milk had 6 days of storage at 5°C, the mean of the scores showed that the taste panel liked the appearance of the milk only moderately. There was no significant difference between the scores given to the appearance of the milk in glass bottles and single-service containers at this time.

The difference between the scores given for appearance of the milk on the day of processing and after 6 days of storage at 5°C was less than 1 point on the grading scale.

The results of the gradings for appearance are in agreement with those of Schönborn et. al. (1975), who reported that there was no difference in the appearance of pasteurized milk one day after delivery and no statistically significant difference five and eight days after delivery.

2. Odour

The mean of the scores for pasteurized milk on the day of processing showed that there was no difference in the odour between the milk in the glass bottles and that in single-service containers.

There was a highly significant difference (0.1 per cent level) between the scores awarded in different trials. There was also a significant difference (0.1 per cent level) between the judges.

After two days of storage at 5^{o}C the odour scores given for the SSC milks were significantly higher ($p < 0.001$) than those which were given for the milk in glass bottles. There was no significant difference between the odour scores of milks exposed to light and temperature conditions simulating the doorstep delivery system and those which were not exposed to these conditions. There was a highly significant difference (0.1 per cent level) between the scores awarded in different trials. The other highly significant difference at the 0.1 per cent occurred in the score awards made by individual graders.

After six days of storage at 5^{o}C, there was no significant difference between the odour scores given to the pasteurized milk packaged in glass bottles and SSC. The results showed that the samples of milk in glass bottles which were not exposed to the light and temperature conditions simulating the doorstep delivery system had higher ($p < 0.5$) odour scores (better quality) than those which were exposed. This may have been due to the affect of induced light in the case of the exposed samples. However, exposure to simulated doorstep delivery conditions had no significant effect in the case of SSC samples. A significant difference (0.1 per cent level) was found between the trials. The decrease in the ddour scores during the storage time was higher in the case of milk in glass bottles exposed to simulated doorstep delivery.

The results of the odour scores were in agreement with those of Schönborn et. al. (1975).

The odour scores showed highly significant negative correlations

($p < 0.001$) with the $\log_e$ of the total colony count and the psychrotrophic count. This suggests that the increase in these counts and the resulting enzymic activity was responsible to a certain degree for odour defects in the pasteurized milk.

3. Flavour

The flavour scores of pasteurized milks on the day of processing showed that there was no significant difference between the milks packaged in glass bottles and SSC. There was a significant difference (0.1 per cent level) between the flavour scores in the two types of package. The differences between the trials were significant at 1 per cent level.

After two days of storage at $5^{\circ}C$, the flavour scores given to pasteurized milk packaged in SSC were significantly higher than those given to pasteurized milk packaged in glass bottles. The differences were statistically significant. Some flavours specified by the taste panel in some milks in both types of container were classified as 'Very slight'. However, most of the samples showed no flavour defect after two days of storage at $5^{\circ}C$.

On the sixth day of storage, the flavour scores given to the pasteurized milk in the glass bottles were significantly higher ($p < 0.5$) than those which were given to the SSC samples. But, the differences were not of practical importance. On the other hand the difference between the two means was 0.24. There was no significant difference in the flavour score due to exposure to the simulated doorstep delivery conditions. There was clear evidence of the light-produced oxidized flavour in the samples of milk in glass bottles which were exposed to the day light for 3 h compared with those which were not exposed. Oxidized flavour appeared in some milks in SSC. This may have been due to the absorption of oxygen during storage. Highly significant negative correlation coefficients ($p < 0.001$) were found between the flavour scores and $\log_e$ of the total colony count, psychrotrophic count and the acid degree value. Randolph et. al. (1964) stated that the most common defects such as unclean, bitter, fruity, and stale flavours in pasteurized milk at the time of spoilage were those resulting from the growth of psychrotrophic bacteria.

It appeared from the results that the flavours in pasteurized milk were caused by microbial growth, and chemical and phsycial changes in the milk. There was a decrease in the flavour scores during the storage time at $5^{o}C$. The mean of the flavour scores after six days of storage at $5^{o}C$ showd on the scale between 'like slightly' and 'dislike slightly'.

4. Total scores

The combined scores for appearance, odour and flavour (taste) showed that there were no significant differences between the milks in glass bottles or single-service containers on the first day of processing and after six days of storage at $5^{o}C$. However, on the second day of storage, the overall total score given to the SSC samples was significantly higher ($p < 0.001$) than that given to the milks in glass bottles. There was a highly significant difference ($p < 0.001$) between the trials and between the judges in the scores awarded on the day of processing and after 2 and 6 days of storage at $5^{o}C$. From the practical point the effect of the exposure was not significant. Samples which were not exposed to simulated doorstep delivery conditions had a higher total score for quality than the samples which were exposed in both types of package, i.e. glass bottles and single service containers.

CONCLUSION

1. It is concluded that there was no significant difference in the appearance scores of pasteurized milk packaged in glass bottles or single service containers after 6 days of storage at $5^{O}C$. There was a wide difference between the judges in their scoring for appearance of pasteurized milk.

2. It is concluded that there was no difference in the odour scores of pasteurized milk packaged in the two types of container on the day of processing, but the milk packaged in SSC showed better odour after 2 days of storage at $5^{O}C$ than that packaged in glass bottles. However, there was no significant difference between the odour scores of milk in the two types of containers after 6 days of storage.

3. It is concluded that there was no significant difference in the flavour scores of milk packaged in glass bottles and single-service containers on the day of processing. After 2 days of storage the flavour scores for pasteurized milk packaged in SSC was better ($p < 0.01$) than those of pasteurized milk packaged in glass bottles.

4. The total scores for the assessment of quality of pasteurized milk packaged in SSC were higher ($p < 0.001$) than those of pasteurized milk packaged in glass bottles. However, there was no significant difference between the total scores of the milk in both types of container on the day of processing and after 6 days of storage at $5^{O}C$.

5. Oxidized flavour was the predominant off-flavour in the samples of milk packaged in glass bottles which were exposed to the light and temperature condition simulating the doorstep delivery system.

6. The results obtained in this study showed a highly significant difference between the scores given for the quality of the milk in the six trials. These differences may be related to differences in the milk because of composition, age, handling procedures etc. and also to differences in judging ability of the panel members.

CHAPTER SIX

EVALUATION OF INHIBITORS TO DETERMINE THE MICROBIOLOGICAL QUALITY OF PASTEURIZED MILK

INTRODUCTION

The incidence of psychrotrophic Gram-negative bacteria is the most important bacteriological problem in the market milk industry today. Post-pasteurization contamination in pasteurized milk is usually caused by psychrotrophic Gram-negative bacteria.

Many attempts have been made to find a rapid method for the enumeration of psychrotrophic bacteria, a test which could be used to determine the efficiency of cleaning and sanitizing in dairies. These attempts were based on the fact that the majority of psychrotrophic organisms in refrigerated milk are Gram-negative bacteria. The count of these organisms can be determined by using a selective agar containing inhibitors for the growth of Gram-positive bacteria. Many inhibitors were used for this purpose by Gyllenberg et. al. (1960) and Olson (1961), e.g. sodium deoxycholate and alkyldimethylbenzyl ammonium chloride.

Lightbody (1964, 1965 and 1966) found that penicillin agar was a useful medium for detecting post pasteurization contamination of milk, cream and butter. He found that only a few Gram-positive micrococci, corynebacteria and aerobic sporeformers produce colonies on it, and more than 80 per cent of the organisms which grew on it were Gram-negative rods.

Smith and Witter (1979) found that the presence of 2 mg of crystal violet per litre medium or 2 mg of neotetrazolium chloride per 1 of medium inhibited the growth of non-psychrotrophic bacteria while it did not reduce the counts of psychrotrophs.

Langeveld et. al. (1976) found that plate count agar (Difco) with the addition of 100 mg benzyl-dimethyl-alkyl-ammonium chloride (Benzalkon A-50 per cent) per kg of medium or 1000 mg Janus green per kg medium or 1000 mg Bromothymol blue per kg medium showed no growth when stroked with 0.01 ml of non-reinfected pre-incubated pasteurized milk. They pointed out that micro-organisms surviving HTST-pasteurization are Gram-positive except for *Alcaligenes tolerans*.

The aim of this study was:-

1. To evaluate some of the previously tested inhibitors with pure strains of spore-forming Gram-positive bacteria and non-spore forming Gram-negative bacteria.

2. To find the relationship between the total colony count of pasteurized milk on a selective medium after pre-incubation for 24 h at 25^{o}C and after 7 days of storage at 5^{o}C on plate count agar without any selective inhibitors.

EXPERIMENTAL

1. Pure strains of Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus firmus, Bacillus coagulans, Bacillus circulans, Bacillus brevis, Bacillus sphaericus, Bacillus polymyxa, Pseudomonas species, proteus and Escherichia coli 904 were grown for 24 h in nutrient broth at the optimum temperature for each organisms as described by Gibbs and Shapton (1968). Each organism was then stroked on Difco plate count agar medium to which addition had been made:-

(i) 100 mg of Benzalkon A-50 per cent per 1 and,

(ii) 1000 mg of Janus green per 1.

This experiment was repeated three times.

2. Eight samples of pasteurized milk were collected twice from the filling machines in a dairy - four samples in each trial from the bottling machine and the other four from the single-service filling machine. Each sample was mixed and divided aseptically into two sterile jars. The portions were treated as follows:-

Portion A This lot was incubated at 25^{o}C for 24 h and then 0.1 ml amounts of serial ten fold dilutions in quarter strength Ringer's solutions were plated in duplicate on plate count agar to which one of the following selective agents had been added:-

(i) 100 mg Benzalkon A-50 per cent per 1 medium,

(ii) 1000 mg Janus Green per 1 medium.

The plates were incubated for 24 h at 30^{o}C and examined for growth.

Portion B This lot was incubated at 5^{o}C for 7 days to allow psychrotrophs to develop and then the total colony count was determined according to the method described in chapter one/section 1:1).

TABLE 6:1

Regression analysis of Log_e TTC/JG[1] on Log_e of TCC[2]

	Log_e TTC/JG			
	Regression			Correlation coefficient
	Estimate	SE	T	
Y-intercept of log_e TTC	16.3826	2.9166	-5.62	0.9144***
Slope of log_e TTC	1.9999	0.2367	8.45	

The regression equation is:-

$$Log_e\ TTC/JG = -16.3826 + 1.9999 \times log_e\ TTC$$

Analysis of variance:-

	DF	SS	MS
Regression	1	194.64	194.64
Residual	14	22.49	1.60
Total	15	217.13	14.47
Change	-1	-194½64	194.642

% Variance accounted for 88.9.

1. Log_e of total colony count of pasteurized milk after pre-incubation for 24 h at 25°C using Janus green as an inhibitor for gram positive bacteria.

2. Log_e of total colony count of the same pasteurized milk referred to above, after 7 days of storage at 5°C using plate count agar.

*** significant at 0.1 per cent level

TABLE 6:2

Regression analysis of $\log_e$ TTC/B[1] on $\log_e$ TCC[2]

	$\log_e$ TTC/B			
	Regression			Correlation coefficient
	Estimate	SE	T	
Y-intercept of $\log_e$ TTC	-7.1864	2.1874	-3.29	0.8857***
Slope of $\log_e$ TCC	1.2670	0.1775	7.14	

The regression equation is:-

$$\log_e \text{TTC/B} = -7.1864 + 1.267 \times \log_e \text{TTC}$$

Analysis of variance:-

	DF	SS	MS
Regression	1	72.86	72.860
Residual	14	20.02	1.430
Total	15	92.88	6.192
Change	-1	-72.86	72.860

% variance accounted for 76.9

1. $\log_e$ of total colony count of pasteurized milk after pre-incubation for 24 h at 25°C using Banzalkon A-50 per cent as an inhibitor for gram-positive bacteria.

2. $\log_e$ of total colony count of the same pasteurized milk referred to above, after 7 days of storage at 5°C using plate count agar.

*** significant at 0.1 per cent level

TABLE 6:3

The effect of Benzalkon A-50 and Janus green as inhibitors on the growth of some Gram-negative and Gram-positive bacteria after 24 h at the optimum temperature growth for each

Type of organism	Benzalkon A-50 100 mg/l media	Janus green 1000 mg/l media
Bacillus subtilis	-	-
Bacillus cereus	-	-
Bacillus licheniformis	-	-
Bacillus pumilus	-	-
Bacillus firmus	-	-
Bacillus coagulans	-	-
Bacillus circulans	-	-
Bacillus brevis	-	-
Bacillus sphaericus	-	-
Pseudomonas spp.	+	+
Proteus	+	+
Escherichia coli 904	+	+

RESULTS AND DISCUSSION

Experiment 1

The results of this experiment are presented in Table 6:3. It is clear that the addition of 1000 mg Janus green per l medium completely inhibited the growth of the pure cultures of spore-forming bacteria. However, the non-sporeforming bacteria (*Pseudomonas*, *proteus* and *E. coli*) had grown after 24 h at 30°C. The same result was found where Benzalkon A-50 per cent (100 mg per l) was added to the agar. It could be possible, therefore, to use these agents in media for enumeration of the Gram-negative psychrotrophic bacteria in pasteurized milk.

HTST-pasteurization destroys most Gram-negative bacteria, but there are some Gram negative which survive this heat treatment.

Abd-El-Malek and Gibson (1952) found that *Alcaligenes tolerans* was the only Gram-negative rod occurring in milk after laboratory pasteurization at 63°C for 30 min. They reported that it grew at 10°C and at 37°C, the optimum being about 30°C. Stadhouders (1975) found some of the *Alcaligenes* and *Microbacterium* species as the thermoresistant bacteria commonly present in pasteurized milk. Therefore *Alcaligenes tolerans* is the only problem facing the attempts to find an efficient test for the quality of pasteurized milk using a selective medium which inhibits Gram-negative bacteria.

Martin (1974) reported that Bacillus spp. accounted for about 95 per cent of the sporeforming bacteria in market milk in the USA. Of these 43 per cent belonged to the species *Bacillus licheniformis*, 37 per cent were *Bacillus cereus*, and the remaining 20 per cent comprised other *Bacillus spp*.

The *Bacillus* species were chosen in this experiment because of this finding.

Experiment 2

The results of the second experiment presented in Tables 6:2 and 6:3 show that there was a highly significant correlation between the total colony count of pasteurized milk after pre-incubation for 24 h at 25°C using a selective media and the total colony count of milk after 7 days of storage at 5°C.

Janus green was a more effective inhibitor than Benzalkon A-50 per cent. The correlation coefficients were 0.9144 ($p < 0.001$) and 0.8857 ($p < 0.001$). The regression analysis also showed a highly significant correlation when the t-value was compared with the statistical tables.

In view of this one cannot conclude that the growth of Gram-negative bacteria on a selective medium is guaranteed identification of post-pasteurization contamination.

The results showed that the relationship between the counts on both selective media and the total colony count of pasteurized milk at 5°C for 7 days was not sufficiently accurate from the practial point of view. However, more work needs to be done on this project using other strains of micro-organisms.

CONCLUSION

1. The use of Janus green at 1000 mg per 1 of medium and Benzalkon A-50 (100 mg per litre medium) totally inhibited the strain of *Bacillus spp.* used in this experiment but not the growth of *Pseudomonas spp.*, *proteus*, and *Escherichia coli* 904

2. There was a highly significant correlation ($p < 0.001$) between the counts of pasteurized milk after pre-incubation at 25°C for 24 h and the total colony count of the milk after 7 days at 5°C.

3. The relationship between the counts on the two selective media and the total colony count after 7 days of storage was not enough to justify their use in practice.

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