# CHANGES IN THE CHARACTERISTICS AND PROPERTIES OF MILK FROM PRODUCTION TO CONSUMPTION - CHEESE MANUFACTURE AND QUALITY

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## SUMMARY

The purpose of this study was to investigate the influence of cold storage of raw milk on its composition and on the yield, composition and characteristics of Cheddar cheese made from it.

A preliminary study was carried out on bulk herd raw milk which was stored at 5°C with or without preservative (merthiolate) for up to 7 days. The experiment was done on three occasions. There were clear differences in the composition of milk of different trials. The results indicated clear evidence of proteolysis of milk proteins during cold storage as detected by the increase in the number of free amino acids spots on thin layer chromatography plates, and by the reduction in casein and protein nitrogen. Changes in the state of calcium and phosphorus took place in stored milk. Milk stored with preservative remained in a better quality than milk stored without preservative for up to 7 days indicating the value of improving the storage condition of milk prior to manufacture.

The main experiment involved a study of the quality of raw milk from a bulk silo purchased from a dairy factory in the West of Scotland. Four trials were undertaken during the period 1979-1980. The raw milks were delivered by road tanker in quantities of around 2,500 litres for each trial. On delivery a portion of the consignment was immediately manufactured and the remainder was split into two equal amounts, one of which was held at 2°C for 2, 4 and 7 days and the other at 6°C for the same periods of time. The use of 2°C for the storage of raw milk was carried out in an attempt to increase the keeping quality of milk prior to manufacture. Cheddar cheese was manufactured on the day of delivery immediately after pasteurization and after 2, 4 and 7 days of cold storage at each temperature. Raw milk was analysed on every day of cheese manufacture. Cheddar cheese was analysed after 1 week and 1, 2, 4, 8 and 12 months of curing.

Milk analyses indicated that at both temperatures (2°C and 6°C) the total solids, fat, protein, total calcium, total phosphorus, total ash and non-protein nitrogen remained unchanged during the storage of up to 7 days. Non-casein nitrogen increased slightly after 7 days

at $2^{o}C$, but it had increased even after 2 days in the milk held at $6^{o}C$. Storage of milk at $2^{o}C$ for seven days and up to 4 days at $6^{o}C$ had not affected the soluble calcium, soluble phosphorus, titratable acidity and freezing point depression. The pH showed significant decrease in milks held for 4 and 7 days at $6^{o}C$. Free sulphydryl groups increased during the storage of milk at $2^{o}C$ while at $6^{o}C$ there was no such significant increase. The acid degree value increased in milks held at $6^{o}C$ more than in milks held at $2^{o}C$. Starter cultures appeared to act faster on cold-stored milk causing higher development of acidity. The rennet clotting time increased in milks held for 2 and 4 days at $2^{o}C$ but it was similar to the initial milks after 7 days. In the milks held for 4 and 7 days at $6^{o}C$ the rennet clotting time decreased due to the development of acidity and its effect on rennet activity. The rennet clotting time was correlated to the milk contents of protein, non-protein nitrogen, soluble calcium, ash and fat content. The major peaks of free amino acids in the separation chromatogram of milk stored at low temperature represents ammonia, glutamic acid and glycine. Ammonia and lysine increased as a result of storage. Other amino acids were either slightly decreased or remained unchanged.

The results of whey analyses showed that the storage of milk at $2^{o}C$ produced either no significant differences in the whey composition or a reduction in the amount of some milk components lost in the whey (i.e. protein, calcium and phosphorus). On the other hand, storage of milk at $6^{o}C$ showed an increase of more milk components to the whey.

The yield of cheese (calculated at 35 per cent moisture level) was not affected by storage of milk for up to 7 days at $2^{o}C$. Storage of milk at $6^{o}C$, caused reduction in cheese yield and this loss was evident with milk held for 2 days after receipt.

Cheeses produced from milks held at $2^{o}C$ contained more calcium, phosphorus, ash and slightly more protein, fat, the free fatty acids value was higher. These cheeses contained less moisture and soluble nitrogen than the control cheese (made from milk on the day of delivery) and had lower elasticity and were slightly softer. Cheese produced from milks held at $6^{o}C$ retained less calcium, phosphorus, ash, protein, soluble nitrogen and had a slightly lower fat content.

The free fatty acids content was higher than that of the control cheese as was the moisture level and the texture of these cheeses was softer and less elastic. The results indicated that the composition of cheese produced from milks held at 6°C for 7 days are bad for moisture. The cheese did not meet the U.K. legal standard for Cheddar cheese in moisture. The high values obtained for free fatty acids in these cheeses indicated inferior quality to the control and other cheeses made during the trials.

Increases in the moisture content and soluble nitrogen content of cheese were associated with softer cheese. If the cheese contained higher total protein and or soluble nitrogen, the elasticity of the cheese was higher.

Quality assessment of cheese showed that cheeses manufactured from milks held at 2°C were higher scored (for flavour, taste, body, texture, openness and colour) than the cheeses made from unstored milks, but this increase was not significant. Cheeses made from milks held at 6°C were given dramatically lower scores than the cheeses made from unstored milks for all the grade characteristics.

Off-flavours were not observed in the cheeses made from milks held at 2°C before 8 months of ripening. But cheeses made from milks held for even 2 days at 6°C, showed off-flavour at 2 months old. With the increasing of repening time, the off-flavour of the cheeses were more distinct.

The electrophoretic studies showed that the cold storage of raw milk resulted in increased concentration of minor bands in the area of κ-casein. This increase was higher in milks held at 6°C than milks held at 2°C. The major β- and $\alpha_s$-caseins bands decreased in concentration while the small band intensity was increased so indicating the occurrence of proteolysis during cold storage. β-casein was degraded faster in milks held at 6°C than in milks held at 2°C. The rate of breakdown of β-casein which is an important factor in the ripening of Cheddar cheese was faster in cheeses made from milks held at 6°C than milks held at 2°C. Milks held for 7 days at 2°C showed fast breakdown in the β-casein.

$\alpha_s$-casein showed the normal breakdown during repening of the cheese.

No variation resulted from the storage of milk.

The statistical correlation studies indicated close correlations between the different organoleptic characteristics. Cheeses with higher scores for organoleptic characteristics retained less moisture and free fatty acids and higher calcium. Close correlations were observed between non-protein nitrogen, acid degree value, soluble calcium, soluble phosphorus, soluble ash, titratable acidity, pH, freezing point depression, rennet clotting time and starter activity value (as measured by titratable acidity of milks inoculated with starter culture and incubated for $5\frac{1}{2}$ hours) and the bacteriological quality of the milk.

The cheese yield was positively correlated with the level of fat, total solids and fat content of the milk. The cheese yield decreased when the levels of non-protein nitrogen, soluble calcium and soluble ash increased in milks during storage.

Using milk stored for up to 4 days at $2^{o}C$ and the unstored milks for cheesemaking resulted in cheeses being awarded higher scores than cheeses produced from milks stored at $6^{o}C$. Lower scored cheeses were associated with higher free fatty acids, moisture and lower calcium and ash contents.

## 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:-

| | | |
|---|---|---|
| Invstor | = | The day of delivery (initial) compared with the mean value of the other days of storage |
| DF | = | Degree of freedom = n - 1 |
| MS | = | Mean of Sum Squares = $\frac{1}{N} \Sigma (x - \bar{x})^2$ |
| REP | = | Replicates |
| SED | = | Standard Error of Differences of Means = V Residual MS/N (individual variants) |
| VR | = | Variance Ratio = MS (individual variants)/MS (Residual) |
| ADV | = | Acid degree value |
| FFA | = | Free fatty acids |
| FPD | = | Freezing point depression |
| mEq | = | milliequivalent |
| RCT | = | Rennet clotting time |
| SAV | = | Starter activity value as measured by determining the titratable acidity and pH of milk inoculated with the starter culture and incubated for $5\frac{1}{2}$ h |
| NPN | = | Non-protein nitrogen |
| NCN | = | Non-casein nitrogen |
| t.l.c. | = | Thin layer chromatography |
| TA | = | Titratable acidity |
| B.C.T | = | Ball compressor total |

| | | |
|---|---|---|
| Lys | = | Lysine |
| His | = | Histidine |
| Arg | = | Arginine |
| Asp | = | Aspartic acid |
| Thr | = | Threonine |
| Ser | = | Serine |
| Glu | = | Glutamic acid |
| Ala | = | Alanine |
| Cys | = | Cystine |
| Val | = | Valine |
| Ile | = | Isoleucine |
| Leu | = | Leucine |
| Tyr | = | Tyrosine |
| Phe | = | Phenylalanine |

# EFFECT OF COLD STORAGE ON THE QUALITY OF MILK

## INTRODUCTION

Milk is a good medium for the growth of micro-organisms which find their way into it during the various stages from production to processing and consumption. Once the milk has been produced, the dairyman gives great attention to minimizing the possibility of contamination and to cooling the milk in the shortest possible time to preserve its freshness and limit bacterial growth.

With the advent of larger cheese factories, and especially in countries where the collection of milk held cold on farms in refrigerated tanks is practised, the storage of milk prior to use has become a most important stage in dairy manufacturing processes in recent years. The advent of a five or six-day working week for creamery workers will make the storage of milk even more important (Scott, 1981).

Reports in the literature indicate that the cold storage of milk prior to cheesemaking adversely affects the yield and organoleptic quality of cheese. In a recent study Banks, Griffiths, Muir and Phillips (1982) showed the necessity to study the effect of cold storage of milk at the creamery on the yield and quality of cheese.

In some countries milk is held for 3 to 5 days before it is made into cheese. The situation in Iraq regarding the weather and milk collection is rather different from Northern Europe. The temperature in Baghdad in 1974 in January, the coldest time of the year ranged from 3.5°C to 13.2°C and in August the hottest period from 24.5°C to 44.8°C (Al-Darwash, 1975). Milk is disposed of by the farmer in Iraq in two ways: (i) under the control of the State Enterprize for Dairy Products which have 18 milk collection centres of from 20 to 60 tonnes capacity of raw milk. These centres are located in areas of milk production. The overall capacity of these centres is 450 tonnes of milk per day. A further sixteen centres, each of from 10-20 tonnes capacity, are under construction at the present time. Most of the milk arriving at these centres is uncooled (Al-Shabibi et al., 1980). Milk is transferred in sterilized cans supplied by milk collection centres. The farmers are paid for their milk according to the percentage of fat in

milk and the quality of the milk (tested by Resazurin and methylene blue tests and organoleptically). The accepted milk is cooled down to 5-7$^{o}$C and held in the centres and thereafter transferred to the creameries by road tankers. (ii) some farmers owning only a few animals and producing small amounts of milk provide milk for their families and for local sale either unprocessed or in the form of milk products.

There is therefore the necessity for improving the keeping quality of milk. This can be achieved by:

1. Storage of milk at the lower temperature of 1-2$^{o}$C.
2. Thermisation at 63$^{o}$C for 10-15 seconds of the milk on arrival at the factory and prior to further cold storage or use. This method is widely used in the Netherlands (Emmons, 1978).
3. Addition of small amount of starter culture to the milk at the creamery to inhibit growth of psychrotrophs with or without thermisation (Hadland, 1978).
4. Addition of some preservatives like carbon dioxide as has been suggested by King and Mabbitt (1982).

In this study Cheddar cheese was made from milk pasteurized after various periods of storage at 2$^{o}$C and 6$^{o}$C. The effect of this storage was examined in relation to the properties of the milk and the resultant cheese.

## 1. Effect of cold storage on milk proteins

### 1.1 Proteolysis

Free amino acids (FAA) were studied (Prodanski, 1962) in milk samples immediately after milking and after 24 h of storage at 4-5$^{o}$C and 15-16$^{o}$C. Immediately after milking the FAA in cow's, ewe's, buffalo's and goat's milks were detected as 12-14 spots on paper chromatograms. After the period of low temperature storage 11-12 spots were evident - and after the high temperature storage 15-17 spots were present. However, different results were reported by Natarajan and Nambudripad (1978) who found that the FAA content of the initial raw milks (farm and market, the type of animal was not stated) gave only 3 and 5 spots, respectively, with analysis by paper chromatography.

On storage at 5°C, the number of spots increased to 6 and 10, respectively.

Scandinavian workers (Storgårds and Lindqvist, 1962, Lindqvist and Storgårds, 1966) have reported that the majority of amino acids were affected only slightly or not at all by ageing during cold storage. Lysine, however, and other compounds ethanolamine and ammonia increased rapidly. In the same studies, certain components like glutamic acid glycerophosphoethanolamine and phosphoethanolamine were found to be destroyed rapidly. Glycine showed a slight initial decrease followed by a rapid increase.

The steady increase in ammonia concentration which is suggested as a criterion of the "biological" age of milk, was noted in raw cold stored milk (Ludzinska et al., 1970; Kaczorek et al., 1973). However, proteose and peptone contents were found to increase during cold storage (Kaczorek et al., 1973). The casein nitrogen of milk decreased while non-protein nitrogen and non-casein nitrogen increased (Youssef et al., 1975).

Juffs (1975b) stated that the first evidence of proteolysis in raw milk stored at 5°C was the formation of para-κ-casein. In his study Vujicic (1973) collected samples of raw milk from individual farms directly after milking or at reception at the dairy and stored them at 5°C or 10°C for 14 d. Proteolysis amounting to 1-5 per cent of the protein content took place in the first 2 d and then tended to decrease on further storage. Cousin and Marth (1977b) found higher amounts of non-protein nitrogen and non-casein nitrogen in milk inoculated with psychrotrophic bacteria and held at 4.4°C for 7 d compared with control uninoculated milk.

Te Whaiti and Fryer (1978) reported that proteinase produced by psychrotrophic Pseudomonas spp. in refrigerated milk is low and that the proteinases produced were relatively inactive at refrigeration temperature. These enzymes could, however, markedly influence the keeping quality of milk products since they are not completely inactivated even at 130°C for 20 s.

### 1.2 Caseins

Kappa casein (as a percentage of total casein) was found to be an important factor influencing micelle size in bovine milk (Rose and

Colvin, 1966). Rose (1968) reported that some of the β-casein incorporated into the micelles in the cow's udder, dissolved when the milk was chilled and ceased to be a part of the micelles. However, when the milk was warmed that part of the β-casein did not re-enter the micelles but was deposited on the surface of the micelles.

Electrophoretic patterns showed that even on storage for 12 d at 6°C only minor changes occurred in the casein (Nakanishi et al., 1968). The total casein in the supernatant prepared by high-speed centrifugation of mid-lactation milks increased from approximately 6 to 15 per cent on cooling from 30° to 5°C, and β-casein was about 46 per cent of this increase. $\alpha_s$-casein was 30 per cent and κ-casein was 23 per cent.

Anderson et al. (1972) stated that when milk was aged there was a complete loss of the protein band of greatest mobility (molecular weight 16,000) and partial loss of material in the 250,000 molecular weight region from the deoxylate soluble fraction of the fat globule membrane. Ubrene and Ramanauskas (1971) found that the major changes observed during the storage of milk at 6, 8 or 10°C, were alterations of the quantitative relationship between the casein particle size groups, some decrease in the stability of the protein after various pasteurization treatments and increase in alkaline buffer capacity. Bloomfield and Mead (1975) reported that at least part of the β-casein is rather loosely connected to the micelles since it reversibly dissociates from the micelles at temperatures around 5°C. This may be connected with a conformational change in the protein in going from the physiological temperature to 5°C.

It has also been confirmed (Flükiger, 1976, Reimerdes and Klostermeyer, 1976) that transfer of micellar β-casein and proteinase to serum increased as the temperature decreased from 35°C to 5°C and during 20 h holding at a temperature of 5°C. However, only below 10°C did storage affect the distribution of $\alpha_s$-casein (Reimerdes and Klostermeyer, 1976). Shidlovskaya and Patratii (1976) however, stated that the average diameter of casein micelles and the molecular weight of casein decreased proportionally with the duration of storage (for over 3 days in the range of 3-15°C). Flükiger (1976) stated that the casein micelles consist of the different caseins, calcium, phosphates, citrates and other constituents of milk that are only found in small amounts. The main changes which occur during cooling concern the discharge of the

calcium, phosphate, β-casein and protease from the linkage to the micelles. The amount of β-casein in the serum of milk is increased by 100 per cent when the milk is cooled from 20°C to 5°C. Cooling to 0°C causes a considerable decrease in the amount of β-casein in milk serum. The dissolution of the micelles spreads over many hours, but a corresponding length of time is required for the resynthesis or the regeneration of the micelles. It is difficult to reverse these changes in the composition of micelles to get milk suitable for cheesemaking. The prolongation of the coagulation time and the deterioration of the curd firmness observed in the case of cold-stored milk can be correlated to the above mentioned changes of the micelles.

Sabarwal and Ganguli (1972) found that casein micelles from chilled milk showed different patterns on starch-gel electrophoresis compared with normal micelles. The intensity of the κ-casein band was deeper in chilled milk samples and the γ-casein band from cow's milk gradually diminished with chilling. Gel filtration studies, however, suggested that some molecular rearrangement occurs in the casein micelle when the milk is chilled.

Feeney and Whitaker (1977) reported that β-casein undergoes a temperature-dependent precipitation in the presence of $Ca^{++}$ being soluble at 4°C and insoluble at 35°C. Kappa casein was found to resist such precipitation and when mixed with appropriate proteins of $\alpha_s$ and β-casein, it forms a complex of micelles with casein and stabilizes them against precipitation by $Ca^{++}$.

Heikonen and Linko (1977) suggested further studies on the behaviour and subsequent effect on quality of casein component during cooling operation. Cheeseman (1977), however, suggested the need to study details of colloidal micelle structure and the behaviour of the milk colloidal system at various stages of normal processing i.e. cold storage of milk and its effect on cheese manufacture, freezing and thawing of milks and concentrates.

Niki et al. (1978) indicated that β-casein occurs in the micelle in 3 distinct forms of combination:-

(a) linked by hydrophobic binding to the micelle at or near its surface;

(b) similarly linked but in less accessible areas; and

(c) firmly linked to $\alpha_s$-casein by covalent forces.

It was stated by Ekstrand and Larsson-Raznikiewicz (1978) that in bulk skim milk the $\alpha_s$, β- and κ-caseins occurred in the following proportions of the total casein: 52, 33 and 15 per cent, respectively. The distribution varies with the size of the micelle. In large and medium size micelles the $\alpha_s$-casein content is almost constant, β-casein and κ-casein appear to be complementary so that the κ-casein content increases with the decrease in the size of the micelle. In small micelles the relative β-casein content is about 50 per cent, $\alpha_s$-casein is only about 33 per cent. The same authors suggested that β-casein stabilizes the structure of the larger micelles.

However, Dalgleish (1978) reported that all the major casein fractions are known to aggregate, even in the absence of $Ca^{++}$ but the mechanism of the three aggregations are not identical. $\alpha_s$-casein does not aggregate extensively, but β- and κ-caseins form micelle-like aggregates, and in the case of β-casein, the aggregation is highly temperature-dependent.

Recently, Ali et al. (1980a) found that storage of milk at 4 or 7°C resulted in a dissociation of micellar caseins, particularly β-casein, into the soluble phase during the first 48 h, but on further storage there was a partial reversal of this process. At higher temperatures (10-20°C) the contents of all the individual caseins in the soluble phase decreased throughout the storage. The same authors Ali et al. (1980b) studied a number of factors in addition to cold storage time and temperature of the milk on the equilibrium between the soluble and micellar phases. The effect of pH was not apparent in fresh milk, but after storage at 4°C there was a minimum in the soluble casein concentration at about pH 6.6, largely due to variations in β-casein concentration.

With milk of higher somatic cell counts (2-3 x $10^6$/ml), increases of up to 37 per cent in total casein in the soluble phase were observed, most of which was contributed by β-casein, while κ- and $\alpha_s$-casein increased only slightly. Where milk was held at 4°C, the concentrations

of all the caseins, calcium and phosphate in the soluble phase increased substantially during the first 48 h, but this was followed by a slight decline on further storage (Ali et al., 1980c).

### 1.3 Effect of cold storage of milk on micellar and serum salt equilibrium

Rose and Colvin (1966) found that the total soluble calcium is one of the major factors influencing micelle size in bovine milk. Citrate concentration was also related to the micelle diameter but this is probably an indirect effect. It was also indicated (Rose, 1968) that at a fixed level of calcium caseinate the calcium content of the micelles and the degree of polymerization of temperature sensitive casein components (mostly β-casein) are the major factors controlling the proportion of casein present in micellar form.

Sainclivier (1959) found that the ultrafiltrate of the cooled milk had a lower content of Ca and P salts than the fresh milk. However, Nakanishi et al. (1968) observed no changes in the content of P and sialic acid in casein. The results of Ubren and Ramanauskas (1971) indicated that the concentration of soluble Ca and P increased during cold storage at 6-8$^{o}$C or 10$^{o}$C.

In their article, Sabarwal and Ganguli (1972) indicated that when milk was chilled for 12 and 24 h at 0$^{o}$C, the Ca and P contents of the casein micelle of both buffalo's and cow's milk decreased; and this decrease was directly proportional to the cooling period. A proportionate increase with cooling in the sialic acid content of the casein micelle of chilled milk was also noticed (Sood, Sidha and Dewan, 1977). A significant negative correlation was found to exist between the calcium content of casein micelles and their solvation (derived from viscosity data) (Sood, Gaind and Dewan, 1979).

It has been mentioned (Schmidt, 1980) that the colloidal phosphate in a finely divided form acts as a cementing agent, binding casein sub-micelles together into casein micelles. The sub-micelles are composed of $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-caseins in an average ratio of 3:1:3:1. The hydrophobic residues are buried in the interior of the sub-micelles whereas the charged groups, notably the ester groups surround this core. The κ-casein is localised in one area of the surface. The sub-micelles

are cemented together into micelles by colloidal phosphate, which binds to the ester phosphate groups of $\alpha_{s1}$-, $\alpha_{s2}$- and β-caseins but not to the κ-casein. Sub-micelles with a low κ-casein content are buried in the interior of the micelle and those with much κ-casein have a surface position, thus preventing indefinite growth of the micelles.

### 1.4 Effect of cold storage and post heat treatment on renneting activity

Many workers agree that cold storage of raw milk increases the rennet coagulation time (RCT) (Fricker, 1958; Peltola and Vogt, 1959; Swartling, 1965; Swartling and Johansson, 1965; Vassal and Auclair, 1966; Aapola and Antila, 1970; Antila, 1971; Thomas, 1971; Losi et al., 1974; Szakaly, 1974; Youssef et al., 1975; Wiles, 1977; Amram and Lenoir, 1978 and Ali et al., 1980a).

The average increase in RCT of mixed evening and morning bulk milk held at 2.2°C (36°F) was 4 per cent after one day, 7.2 per cent after 2 days and 12.5 per cent after 3 days (Rapp and Calbert, 1954). However, it was indicated that cold storage lasting 24 h increased the RCT by about 23 per cent but the increase during further 24 h at the same temperature(2-4°C) was only about 4 per cent (Peltola and Vogt, 1959). Heating skim milk and dispersions of caseinate in centrifuged whey to 85°C for 30 min caused an immediate increase in the RCT at 35°C and a further prolongation (hysteresis) when the heated samples are held following heating (Kannan and Jenness, 1961). In his work, Aule (1961) found that cold storage before pasteurization had little effect on RCT and coagulum firmness while cold storage after pasteurization prolonged the RCT and caused a marked decrease in coagulum firmness. Furthermore, cold storage of both raw and pasteurized milk had the effect of reducing whey separation. Rennet coagulation time was found to be correlated negatively with α-casein, β-casein and total Ca contents and positively with total protein and total casein contents. There was no relation between RCT and milk fat, total and dialysable inorganic P, and dialysable and non-dialysable Ca content. Forewarming milk for 30 min at 80°C can introduce or accentuate a RCT minimum when the milk is subsequently heated at a higher temperature. Sweetsur and White (1974) reported that forewarming heating (30 min. at 80°C) was sufficient to cause almost complete denaturation of the albumin and globulin.

On the other hand, the curd prepared from a mixture of cold and uncooled milk was found to be softer than that prepared from uncooled milk (Losi et al., 1974). The rate of curd synersis increased as the pH decreased during cold storage of milk and was dependent on the kind of milk used and the length of storage. The rate of such an increase in RCT was inversely related to the increase in the diameter of casein micelles during cold storage (Youssef et al., 1975).

In his study, Hossain (1976) found that RCT is influenced by the κ-casein content of the milk, and the curd firmness by the $\alpha_s$ and β-casein contents. After keeping the samples for 48 h and 3 d at 2-3°C, the RCT increased by 10-15 per cent, the curd firmness was much reduced and shearing strength reduced by 25-30 per cent; the curd was more friable, and curd losses in whey increased by 20-23 per cent (Amram and Lenoir, 1978).

Cousin and Marth (1977a) found that the longer raw milks with or without added psychrotrophs were incubated at 7°C the more rapidly they were coagulated by rennet extract (100 per cent strength No. 002045, from Marschall Division of Miles Laboratories). Raw milk inoculated with Lactobacillus spp. (No. 29 and No. 34) and Pseudomonas spp. (No. 1, 13 and 36) was coagulated more slowly by rennet than was uninoculated milk. All pasteurized and inoculated milks clotted in substantially less time than did pasteurized control milk when rennet was added. The clotting times of milks varied when different species isolates of the same genus or of different genera served as the inoculum. The coagulation of raw milks by rennet took less time than pasteurized inoculated milks.

Cousin and Marth (1977b) reported that RCT and curd firmness are not related to concentration of casein in milk but that milk which coagulates quickly gives a firmer curd than that which coagulates slowly. The speed at which rennet coagulates milk is governed to some extent by acidity, RCT increases as the pH increase from 6.32 to 7.45.

Ritter (1970) and Knoop and Peters (1978a) on the other hand, are agreed that in rennet curds of milk stored at 4°C for 24-72 h only unimportant structural changes occur as compared with those in curd from fresh milk. These changes can be eliminated by adding small amounts of calcium to

the milk. It was found however, that prolonged storage of milk at 5°C delayed firmness properties of the rennet coagulum and increased slightly the fat lost into whey and the moisture held in the curd at milling (Chapman et al., 1978).

The results obtained by Ekstrand et al. (1980) showed that RCT varied with the micelle size. The effect, which is more pronounced with chymosin (EC 3.4.23.4) than with rennet (contains about 85 per cent chymosin and 15 per cent pepsin), appears to be related to the availability of κ-casein. Therefore, the largest micelles, with a lower κ-casein content showed longer RCT than medium size. In the case of the smallest micelles the RCT increases again, probably due to an increase in β-casein content.

## 2. Effect of cold storage of milk on lipolysis

### 2.1 Effect of agitation and aeration

Agitation and air admission was found to increase lipolysis (Herrington and Krukovsky, 1942; Hadland and Solberg, 1965; National Agricultural Advisory Service Milk Group, 1965; Danils, 1966; Morrissey and Hickey, 1974; Deeth and Fitz-Gerald, 1978). The chief factors involved in inducing rancidity in pipeline milkers were found to be the admission of air to the milk line, the low milk flow-rate, the inclusion of a filter and numerous fittings in the vacuum section of the milk line and the continuous operation of the centrifugal pump (Kelley and Dunkley, 1954; Thomas et al., 1955b).

Olson, et al. (1956) found that pipeline milkers installed properly so as to avoid foam formation, will not cause an increase in rancidity development of raw milk. Irvin (1959) found that shortening the length of pipeline in a milking installation caused a marked reduction in the incidence of hydrolytic activity. Rancidity trouble associated with changing from can to bulk tank can be reduced. In one case, the cleaning difficulties of the pipeline system were solved and, in the other, the milk inlet to the bulk tank was extended to near the bottom of the tank. Koops and Tarassuk (1959) found that any kind of agitation of milk will cause the phosphatide to move from the fat phase into the skim milk.

The people in the milk room should be instructed not to start the stirring device until the agitator is well covered by the milk. Drivers of the collection tankers should be required to see that all pipe fittings are well tightened so as to limit the amount of air drawn into the milk (Janzen, 1964; Danils, 1966). It is recommended (National Agricultural Advisory Service Milk Group, 1965) that the milk should be pasteurized within 24 h of arrival at the dairy and should be stored at 4°C to discourage growth of psychrotrophs.

It was found (Swartling, 1965; Storgårds, 1966; Jellema, 1973; O'Halloran et al., 1975) that bulk tank milk in general had a higher content of FFA than normal can milk. However, no flavour defects appeared in the milk due to the use of tank equipment and no rise in FFA was observed in a study carried out in Finland by Kylä-Siurola and Anna-Liisa (1966).

The amount of air entering the pipeline is controllable to some extent. Besides the air entering the system as part of the functioning of the milking machine, air leaks past the teat cup during milking and during machine stripping when the milkers are pulling on the teat cups, as may also happen in some cases during changing of the units from cow to cow. Through careful selection of the equipment and planning the milking installation the off-flavour may be affectively controlled (Crawford, 1967).

Hydrolysis of milk fat was found to occur during machine milking (Atramentova and Atramentov, 1970). Furthermore, Te Whaiti and Fryer (1973 and 1975) found that stirring the milk in the farm vat increased the susceptibility of cream to release free fat upon blending. However, the activity of lipolytic enzymes is increased when milk is subjected to mechanical agitation which causes disruption of the fat globule membrane (Downey, 1975). Agitation at low temperature resulted in a maximum degree of lipase activation in fresh milk after 2-4 h cold storage (Deeth and Fitz-Gerald, 1977).

Fleming (1980) concluded that the effect of mechanical agitation on lipolysis depends not only on the previous temperature history of the milk but also on the temperature during agitation, the nature of the mechanical treatment, and characteristics of the milk, some of which are not fully understood. However, agitation of raw milk at low

temperatures following cold storage ($4^{o}C$) tends to produce greater activation of lipase arising perhaps from a greater transfer of lipase activity to the cream due to agitation under these conditions.

## 2.2 Seasonal variations

Dunkley (1946) found that the lipolysis problem in milk is more common in winter than in other seasons. It can not be prevented or cured in all cases by feeding. However, Tarassuk and Henderson (1942) stated that a proportion of cows producing milk containing a naturally active lipase which, on cooling and ageing of the milk causes a rapid development of rancid flavour. The total concentration of individual FFA of homogenized raw mixed herd milk was found (Claypool and Jezeski, 1965) to be about 30 per cent higher for winter months, the increase almost occurring exclusively in short chain acids.

It was stated (Dunkley, 1946; Doody et al.,1975; Saito,1981) that milk was most susceptible to lipolysis in late lactation, least susceptible in mid lactation and was of intermediate susceptibility in early lactation. Even in late lactation, abnormal ADV were found only in milk from milking installation with risers (2 x 0.91 m used in milking line).

Salih and Anderson (1978) found that the milk lipoprotein lipase activity was increased from the first milking post partum to day 10 and then remained unchanged. The acid degree value was stable during the first four days of lactation, increased until day 10 and then decreased.

Weather conditions such as atmospheric temperature and relative humidity have not been found to have a significant effect. The reason for the greater incidence of spontaneous lipolysis during some seasons appeared to relate to the proportion of cows in late lactation and the quality of the feed (Deeth and Fitz-Gerald, 1976).

It may be concluded therefore, that season and climate have no direct influence but there is a coincidence with other factors especially nutrition (Jellema, 1980).

## 2.3 Effect of temperature and period of storage

It was found by Krukovsky and Herrington (1939) that warming of cold raw milk accelerate lipolysis. To secure a maximum of activation,

milk should be pre-cooled to 5°C or lower, re-warmed to 30°C, and then re-cooled below 10°C. The rate of lipolysis seems dependent upon the crystalline state of the fat, and consequently upon the previous temperature history of the milk. Herrington and Krukovsky (1939) stated however that lipolysis at low temperature was most rapid during the succeeding 6 days.

There is a complete absence of lipolysis while the milk is in the cow's udder. Milk lipase is activated by cooling the milk (Tarassuk and Richardson, 1941). The enzyme is particularly inactive in milk held at 20°C to 37°C but cooling to 15 to 20°C or lower causes activation. Holding the milk at 32 to 37°C for 1 to 3 h after milking retards the activity of lipase profoundly even if the milk is cooled afterwards. Development of rancidity is postponed for over 30 h by holding the milk for 2½ h at 33°C. The retarding effect is inversely related to the concentration of lipase and is progressively increased up to 3 to 3½ h of holding.

Rapid cooling markedly reduced lipolysis (Herrington and Krukovsky, 1942; Willart, 1963);a critical temperature range was found in which the rate of cooling is very important. The upper limit is 20-25°C and the lower one is 0°C for natural milk and 10°C for activated milk (warmed and then cooled).

Fresh milk just after milking (no count given) could be kept at 2°C for about 5 days, but the milk with a count of 0.1-1 million/ml could not be kept even at 2°C. At 5-6°C even fresh milk could be kept only for 2 days (Arima et al., 1965).

The effect of different lipase assay temperature on distribution of FFA at pH 8.6 and at temperatures of 4, 10, 25 and 37°C was studied by Robertson et al.(1966). The area percentages for the lower molecular weight fatty acids esters (C-6 to C-12) and for C18 were found to be greater for the lower temperature of 4°C than the higher temperature of 37°C. These results reveal, strikingly, the alteration in the specificity of lipase action due to temperature difference with the lower temperature favouring the release of the lower molecular weight and unsaturated fatty acid and the higher temperature having an opposite effect.

Willart and Sjostrom (1966) showed that cooling milk slowly to temperatures above 24°C gives lower amounts of FFA than slow cooling to temperatures below 24°C. However, it is striking that at temperatures where the fat phase is liquid (in the fat globules) the stability toward lipolysis seems to be greatest. When the fat solidified, the formation of big crystals is favoured by slow cooling, while small ones are formed by rapid coolings. The stability of the fat globule membrane which normally protects the fat from the lipases in the milk is probably disturbed by the formation of these crystals of fat. However, lipolytic changes were marked at 15°C with appearance of rancid flavour within 48 h (Zmarlicki, 1971).

Speakers at an IDF meeting on this topic (IDF-FIL, 1975) agreed that lipolysis is mainly caused by lipolytic enzymes. Fredman (1978) found no relation between the number of psychrotrophic bacteria and the content of FFA. This indicated that the principal factor in lipolysis development is the original lipases present in the milk (induced lipolysis). On the other hand, Muir et al. (1978) suggested that the lipolysis on storage was of bacterial origin and that exo-enzymes produced by the psychrotrophic bacteria were the principal factor responsible for milk spoilage.

However, original lipase activity of milk does not have any demonstrable effect on the extent of glyceride synthesis but there is a slight positive correlation between the cell count of milk and the extent of glyceride synthesis (Luhtala et al., 1970). Anderson and Cheeseman (1975) stated that cooling for 24 h at 4°C resulted in a loss of fat globule membrane, the principle component of which was protein; some complete breakdown of globule also occurs.

Law et al. (1976) indicated that the highest incidence of lipolytic activity among the psychrotrophic Gram-negative flora of commercial raw milks was found in strains of Pseudomonas fluorescens and Ps. fragi. The lipases of all of the lipolytic strains remained wholly or partly active after heat treatment at 63°C for 30 minutes.

Olivecrona (1980) stated that even after 24 h storage, good quality milk usually contains less than 1µ mol FFA per ml. In contrast, the lipase of milk would release this amount of fatty acid in about 1 minute, if conditions were optimal for its action. Thus some factor(s) must strongly inhibit the action of the lipase in milk. Studies in model

systems have demonstrated that when triglyceride emulsions are covered by a surface film of proteins and/or polar lipids, lipase action is strongly inhibited. Fleming (1980) reported that the practice now adopted by many dairy factories of heating the milk at reception to at least thermisation temperature of 63°C for 15 sec, in the interest of arresting lipolysis and controlling microbial activity, is expedient. Additionally, the improved stability of the raw material resulting from this treatment offers greater flexibility in the processing timetable, a factor of considerable advantage in the optimisation of processing resources.

## 3. Effect of cold storage of milk on dairy products

### 3.1 Processing, quality and yield of cheese

Mattsson (1958) stated that cold-stored milk (overnight at 4° to 11°C) required 15 per cent more $CaCl_2$, 19 per cent more rennet and a slightly higher heating temperature than fresh milk in order to achieve normal cheesemaking process. Prolonged storage of raw milk for about two days at 1-2°C or milk heated to 62°C for 5 min and stored at 7-8°C was generally associated with some loss of quality in the resultant cheese (Stadhouders et al., 1962). However, milk which had been pasteurized by the H.T.S.T. method could be stored at 3-9°C depending on the season, without adverse effect on cheese quality (Futschick, 1962).

It was indicated by Futschick (1962) that storage of raw milk from one day to the next before processing had adverse effects on the taste, eye formation and consistency of Edam and Austrian Stangen cheese. Balinskaite (1964) found that the best curd and cheese was obtained (type of cheese was not mentioned) when fresh whole milk was aged at 10°C for 12-18 h prior to the addition of rennet while storage at 4°C or premature addition of rennet gave poor results. It was concluded that the quality of the Swedish Tilsit and Swedish Steppe cheese was not adversely affected by the use of bulk milk from refrigerated tanks collected on an every other day basis compared with milk collected on a daily basis (Swartling and Johansson, 1965). Bolliger (1967) stated that high quality Emmental cheese may be produced from normal milk cooled to at least 5°C and stored for 48 h. Gorgonzola, Mozzarella and Taleggio cheeses are not affected by preparation from refrigerated

milk if it was initially of good quality (Bottazzi, 1970). Milk stored in farm tanks 3 to 4 d and 2 d at 0 to 1°C and 3 to 4°C respectively, was found to be suitable for the manufacture of Finnish Edam cheese (Aapola and Antila, 1970). Vitagliano et al. (1971) prepared cheese, from 7 samples of cow's milk and 4 of ewe's milk previously refrigerated at 4°C for 48 h,equal in quality to those from the unrefrigerated milks but somewhat higher in moisture and averaged respectively 2.4 and 3.0 per cent lower in total yields and 5.6 and 3.5 per cent in yield of dry matter. On the other hand, it was recommended (Ubrene and Ramanauskas, 1971) that for milk for cheese-making a storage temperature of 10°C is considered optimal. Antila (1971) stated that bulk stored milk (1, 2 and 3 days at 2-3°C) did not present any difficulties in Edam cheese-making. Boer (1973) in a later study found that the number of milkings (4 or 6) had no effect on the quality of Edam cheese even if the milk was stored untreated in the dairy for 24 h.

Juffs (1974) incorporated small amounts of proteinase produced by Pseudomonas flourescens and Ps. aeroginosa in the milk used to manufacture Cheddar cheese. The formation of soft bodied curd which did not drain freely during cheddaring was observed. Proteolytic activity of these proteinases relative to that of rennet or starter appeared to be diminished as acidity increased during the cheesemaking process. Acid soluble tyrosine and tryptophan were at higher levels in cheese with added proteinase than in control cheese after 3 weeks of storage. It was found (Cousin and Marth, 1977b) that the production time was decreased and a firmer curd resulted when Cheddar cheese was made from milk cultured with psychrotrophic bacteria rather than control milk. Differences were minor for milk fat, moisture, nitrogen content, and pH between cheeses made from control and pre-cultured milks. Sensory evaluation revealed little difference between cheeses from control and treated milks after 3 months of ripening at 4.4 or 10°C. Decrease in organoleptic desirability became evident at 6 months, especially for cheeses ripened at 10°C.

Camembert-type cheeses made from milk stored for 9 days at 5°C had a bitter and rancid flavour and these cheese samples contained also more FFA, 2-alkanones and 2-alkanols than cheeses made from milk stored for 2 days at 5°C (Dumont et al., 1977).

Chapman et al. (1978) stored raw milk from a bulk supply at $5^{o}$, 7.5 or $5^{o}C$ rising slowly to $10^{o}C$ and made it into Cheddar cheese 24, 48 or 72 h later. Reduction in casein content and weak rennet gel which indicated proteolytic changes in milk for cheesemaking was observed. Chemical analysis and grading at 8 weeks old showed that the Cheddar cheese was of good quality, although the moisture in fat-free substance increased slightly with prolonged milk storage. Barabanschikov et al. (1978) stated that milk with high protein content was characterized by larger casein micelles and found to be more suitable for cheese manufacture than the milk of low protein.

Knoop and Peters (1978b) found that the milk held at $4^{o}C$ for up to 78 h and then treated with 10-20 g $CaCl_2$/100 l renneted normally and produced good cheese. Milk held at $10\text{-}12^{o}C$ for > 24 h, however, ripened abnormally, coagulated slowly with rennet and yielded a soft poorly syneresing and loose bodied cheese which ripened over rapidly. These effects were associated with a slower and less complete aggregation of the casein micelles, a condition presumably resulting from proteolytic hydrolysis of certain aggregation-promoting micellar surface groups.

It was stated by Reimerdes (1978) that the following changes have been observed in the properties of refrigerated milk compared to fresh raw milk:-

1. the cold stored bulk milk differs significantly in renneting properties;
2. there are serious difficulties with curd formation and properties;
3. losses in cheese yield have been mentioned where refrigerated milk is used;
4. the normal, well established schedules for milk processing are not reliable for the processing of cooled milk;
5. variations in these changes are related to time and temperature of storage of milk.

There is some correlation between the above results with alterations in the physico-chemical equilibrium of milk components.

Mikolajcik (1979) reported that the principal off-flavours in Cheddar

cheese which may be associated with growth of psychrotrophs are referred to as 'bitter', 'rancid', and 'unclean'. The flavour score of Cheddar cheese made from milk containing proteases isolated from Pseudomonas spp. did not differ significantly from control cheeses even after 6 months storage. Some lots of the test cheese had a weak curd, and soft body.

Hicks et al. (1980) stated that in all varieties of cheese, yield on a dry matter basis decreased with the storage time of the milk used for its production. The rate of loss of yield of Cheddar cheese was dependent on the initial bacterial quality of the milk. Certainly as storage time increased to where bacterial population reached $10^6$ c.f.u./ml yield losses were apparent. Very high quality milk shows a lag period of up to 3 to 4 days from time of milking until big losses in yield occur. Low quality milk had a linear yield loss. Proteolytic degradation of casein by psychrotrophs caused additional water to be bound by the altered casein. Cheese manufactured from stored milk often incorporates moisture in excess of legal and plant standards. When graded at 6 months of age the cured Cheddar cheese produced from stored milk had an inferior quality related to gassy texture with fruity and unclean flavour by comparison with the cheese made from fresh milk.

Ali et al. (1980a) indicated that cheese-making losses of fat and curd fines in whey were greater with increased soluble phase casein on storage of milk at 10-15$^{o}$C. The curd structure was weaker, curd was more moist and slightly lower cheese yield was obtained where stored milk with an increase content of soluble phase casein was used. When milks were stored for up to 3 d at 4$^{o}$C prior to cheese production the cheese gradings were virtually unaffected by storage, but higher temperatures of storage (10-15$^{o}$C) led to cheese being down graded, largely for body and texture defects and also for flavour when the storage period was more than 3 days.

Downey (1980 a) reported that the quality deterioration in various dairy products, such as flavour impairment in cheese, is attributed to heat-resistant enzymes. He pointed out that microbial proteinases seemed to be more heat-resistant than the microbial lipases and hence posed a greater risk to product quality. However, Ali et al. (1980c) found that the rennet clotting time, losses of fat in whey, curd

moisture, and losses in curd yield and rigidity were all greater as the higher the somatic cell count of milk increased. The differences were detectable in these parameters between milks of very low cell count (e.g. $5 \times 10^4$ cells/ml) and milks with counts more typical of those found in bulk supplies (e.g. $5 \times 10^5$ cells/ml).

Cogan (1980) reported that the flavour of both Cheddar and Cottage cheese made from milk containing added proteinase was significantly lower than the control cheese. However, he reported that there is increase in the FFA of cheese (after 6 weeks ripening) made from milk to which a broth culture of Alcaligenes viscolactis was added before pasteurization. The initial count was $36 \times 10^5$/ml while the pasteurization treatment ($74^{\circ}C \times 10$ s) would have totally inactivated the organism, it would have inactivated only the enzyme level by about 60 per cent. Incubation of Pseudomonas fragi and Ps. mucidolens in milk at $5^{\circ}C$ for 20 h before heat treatment at $63^{\circ}C$ for 30 min led to increase FFA levels in Swiss cheese when examined after 60 days at $13^{\circ}C$.

### 3.2 Quality of butter

It was stated (Scheib et al., 1942) that pasteurization at $73.8^{\circ}C$ ($165^{\circ}F$) for 30 min was required to destroy the harmful natural enzymes in cream used for butter production. The liberation of FFA during churning had a marked effect upon churning time, e.g. in the samples held at $125^{\circ}F$, the churning times were 80, 65, 50, 43 and 45 mins, while the initial acid degree values of the butter were 1.56, 0.94, 0.58, 0.42 and 0.40 respectively (Krukovsky and Herrington, 1942). McDowell (1964) used good and poor quality milk for butter production. Butter made from cream separated from milk accumulated in a refrigerated tank from morning and evening milkings for three days was compared with butter from similar milk unchilled and separated and pasteurized for churning daily. It was concluded that when milk was of good quality there was no difference in the butters either when fresh or after storage of butter for 4 or 8 months.

However, the chemical analysis (acid and peroxide numbers, Cu, Fe, and aldehyde) of butter which was produced from milk with and without pronounced oxidized flavour did not give such differences as permit the drawing of any definite conclusion (Bergman, Berglöf and Kjell, 1962). The

quality of butter manufactured from milk from a refrigerated farm tank was found to be slightly impaired by the lipolytic activity or by some other factor (Swartling, 1965). It was indicated by Antila (1971) that cold storage impairs creaming and is probably connected with the fat globules and their membrane. Though, butter graded equally well when churned from milk which had been separated after 0 or 1 d storage at either of 10°C or 4.5°C, the butter was found to be poorer, when the milk was stored for a longer period (Fryer, 1972).

Barbanshchikov and Tolstyakova (1977) found that passing milk through a 160 m long pipeline milker caused the diameter of fat globules to decrease and their numbers to increase due to partial breakup of fat globules, mainly those with diameter > 2 μ m. The churning time of the cream obtained from the milk was increased, as were the fat losses in the buttermilk; the flavour and odour of the butter were adversely affected.

Downey (1980b) found that butters with low pH (4.75) and high salt content (2 per cent) are most susceptible to lipid auto-oxidation but are inhibitory to lipolysis and associated microbial growth; conversely butter with a pH of 6.6 and no added salt is susceptible to lipolysis and microbial growth but not lipid auto-oxidation.

Connolly et al. (1980) found that little lipolysed flavour was detected in fresh sweet cream butter even though the level of FFA showed wide variability (20-60 mg NaOH/100 g fat). Butter stored at 15°C showed increased FFA with time which correlated with an increased development of lipolysed flavour. By contrast, butter stored at -18°C showed little if any change in FFA levels although lipolysed flavour was reported.

Downey (1980a) concluded that an increase in FFA levels in milk and dairy products are not deemed a serious commercial problem unless they impair the flavour quality and impart a distinct off-flavour which can be detected by the consumer. Depending on the preferred approach, significantly different tolerance limits may be set for FFA levels in milk and dairy products. Indeed, with the second approach, lipolysis of milk (pre-manufacture lipolysis) may not be as serious a threat to the flavour quality of butter and other high fat products as is generally

assumed. Because of the interfacial blocking phenomenon (fatty acids accumulate at the oil/water interface and non-covalent interaction with the hydrophobic moieties of the substrate may lead to unfolding of the enzyme, thus blocking further the oil/water interface and resulting in cessation of lipolysis) preventing FFA accumulation in normal milk in excess of a predetermined notional threshold (usually less than 1.5-2.0 m-equiv/l milk), which may not be sufficient to confer a distinct off-flavour on products made from the milk, especially if there is a preferential loss of the more highly flavoured FFA during cream separation and churning. It might be concluded that the potential risk of flavour impairment of dairy products from lipolysis of milk has been overstated.

### 3.3 Pasteurized milk

Jensen (1960) found that the most frequent defect in flavour quality after pasteurization was of feed origin, however, rancidity was more prevalent in milk from pipeline milking installation than from non-pipeline milkers. Bergman, Berglöf and Kjell (1962) stated that milk with a pronounced oxidized flavour before H.T.S.T. pasteurization produced milk which within 1-2 d of heat treatment developed an oxidized flavour on cold storage at the dairy. Pasteurization (63$^{o}$C for 30 min) of milk prior to cold storage leads to a higher frequency of oxidation flavour. However, the cold storage of unpasteurized milk at the dairy involved an increased risk of oxidation flavour (Bergman et al., 1962). In their study, Dunkley and Franke (1967) reported that pasteurization increases the susceptibility of milk to development of oxidized flavour.

Thomas (1969) indicated that raw milk produced under satisfactory hygienic conditions led to satisfactory keeping quality of the milk after pasteurization, but the addition of an unsatisfactory raw milk supply to the bulk before commercial pasteurization caused a marked deterioration in the keeping quality of the pasteurized milk. Mergl and Cerna (1969) however, stated that milk pasteurized after low temperature storage of the raw supply, kept for > 48 h without organoleptic change. Hadland and Hoy (1974) indicated that milk stored in bulk for 1 day at 2$^{o}$C before pasteurization had a satisfactory keeping quality of 14 d after pasteurization. Storage for 4 d at 2$^{o}$C however, reduced the keeping quality of the pasteurized milk to 6-8 d.

Hankin et al. (1977), collected samples of commercial pasteurized milks in their original container direct from the filler. Samples stored at 1.7, 5.6 and 10°C, remained organoleptically acceptable for an average of 17.5, 12.1 and 6.9 d respectively. The most common defect in samples stored at 1.7 or 5.6°C was lack of freshness, whilst at 10°C putrid and/or curdled off-flavour frequently occurred.

### 3.4 Effect of storage on UHT milk

Lindqvist (1970) stated that when UHT milk was stored at a temperature of 20-30°C for more than one month it frequently showed some changes varied from a slight flocculation to complete coagulation with serum separation. Taste and colour often remained unchanged. A marked increase in the amino acid content of the milk occurred in certain instances at the same time as the electrophoresis and gel filtration curves indicated the break-down of the protein. It was concluded therefore, that in order to have UHT products of maximum durability one must use a bacteriologically satisfactory raw milk and store the final UHT product all the way to the consumers at as low a temperature as possible with a maximum of 18°C (Lindqvist, 1970). Langsrud (1970) stated that sedimentation and gelation in UHT processed cow's milk during storage, could generally be inhibited by homogenization. Storing of UHT processed goat's milk at 50°C, caused significant increase in non-protein nitrogen after 71 d storage. The flavour was sharp and bitter after 7 d, and the content of available lysine was reduced. After 14 d the milk was distinctly brown in colour due to the browning reaction. The NPN increased at 37°C and to lesser extents at 30°C and room temperature. The increase in NPN on storage was significant in milk held at 4°C for 2 years. However, as the proteose-peptone content increased the casein content decreased. This suggests that the increase in proteose-peptone was caused by enzymatic degradation of the casein fraction. There is a natural proteolytic enzyme associated with the κ-casein component of the casein complex and this may have been reactivated during storage. The bitter, soapy flavour appeared when the proteose-peptone fraction had increased to about twice its normal value. O'Sullivan (1970) concluded that conventional in-can sterilization in a pilot rotary sterilizer at 120°C for 20 min did not complete denaturation of the whey proteins, though it was more severe than UHT sterilization (at 137.7°C for 4 s). The results for

the latter process confirm that denaturation of the whey proteins reaches a maximum value of approximately 80 per cent.

Andrews and Cheeseman (1971) found that storage of aseptically packed UHT milk at ambient or higher temperature produced changes in the electrophoretic pattern of the milk caseins. Storage at 4°C did not give rise to these changes. Changes in the sensitivity to calcium ions of individual caseins, whole casein and milk that had been subjected to various heat treatments or to treatment with acetaldehyde showed that all these different treatments gave rise to modified casein which in general, became less sensitive to calcium.

Studies at the National Institute for Research in Dairying (1973) compared the flavour assessment with aseptically-bottled milk given a UHT process with 5 min preholding at 70, 80 or 90°C and milk treated by the same process without preholding. Milk preheld at 70°C remained acceptable but at 90°C acceptability was greatly diminished from 7 to 56 d after sterilization.

Cheeseman and Knight (1974) found that heat treatment at 100°C for 40 min gave high molecular weight aggregates of whey proteins. It was concluded that increase in the length of storage of UHT milk gave rise to an increase in the proportion of casein aggregates excluded from sepharose 6-B gel. The size distribution of sub-micellar aggregates of casein as determined from the gel-column elution profiles suggests that there was no gradual increase in sub-micellar aggregate size with length of storage as might have occurred if association took place between aggregates.

Andrews (1975) stated that after some months of storage at the higher temperatures of 30 and 37°C, the extent of polymerization of the caseins and whey proteins due to reactions of the Maillard type was several times greater than the heat-induced changes resulting from the UHT processing itself. In addition, further amounts of polymer were formed by disulphide bonding, the contribution of such polymers diminishing gradually in a temperature-dependent manner during storage due to continuing polymerization reactions. It appeared that $\alpha_{s1}$-casein may be preferentially involved in these polymerizations with β-casein reacting at a somewhat slower rate. Polymerization and associated

reactions modifying molecular charge led to the expected alterations in electrophoretic mobility and a loss of definition in the bands due to the various protein components. However, proteolysis was of minor significance (Andrews, 1975; Andrews et al., 1977; Cheeseman, 1977; Bottazi and Pecis, 1978).

Zadow and Chituta (1975) indicated that neither the pH of raw milk in the range 6.6 to 7.7 nor incubation for 4 h at 30°C affected gel time of UHT milk. Preheating raw milk extended gel time, greater effects being obtained with increasing severity of the treatment.

Roberts (1977) stated that milk of 24 to 48 h since milking is the best for UHT milk. This usually ensures that the normal slight odour and taints associated with very fresh milk will have dispersed.

Renner (1977) concluded that with UHT milk correctly produced by the 'direct' method and irrespective of fat content (1.5 or 3.5 per cent), no perceptible taste change took place on storage at room temperature for 6 weeks, whereas UHT milk with 3.5 per cent fat produced by the 'indirect' method showed appreciable changes under these conditions, reduction of fat content of 1.5 per cent leading to marked improvement. In their research, Tylkin and Tsaberyabaya (1976) found that immediately after processing the UHT milk was of a cream-like colour, thin and contained sediment. During storage, separation of fat was observed as well as precipitation of protein and development of oxidized flavour. The stability of the fat phase in stored sterilized milk depended on the homogenization and the stability of the protein colloidal phase was affected by the thermostability of the milk.

Burton (1977) observed that coagulation after prolonged storage is caused by the slow action of proteolytic enzymes, which ultimately destabilize the casein.

Wiles (1977) stated that there are two concepts for the production of long-life products by UHT treatment. Either batches of product are prepared, mixed, partially or wholly cooked all using conventional techniques and equipment. Finally the product is continuously heat treated to give it long-life properties before packaging aseptically. The alternative is a wholly continuous operation in which the UHT

or heat treatment plant is part of a more fully automatic system, with in-line metering of ingredients and in-line mixing. The product will be cooked and sterilized in a continuous operation by adjusting the time and temperature profile of the heat treatment plant. The product may then be further processed, textured, shipped, homogenized etc. under aseptic conditions and then packed aseptically.

Moller et al. (1977a)indicated that casein from stored UHT milk was found to be more resistant to proteolysis than casein from unheated milk. Resistance to proteolysis was attributed to the Maillard reaction between milk proteins and lactose during storage of UHT milk. However, it was stated that after 34 months the milk had all gelled and casein micelles in the gel phase (at 4°C storage) had become very spiky and long tendrils often bridge micelles forming large network (Andrews et al., 1977).

Moller (1977b) found that lactuloselysine (e-N-deoxylactosyl-L-lysine) formation was comprehensive and involved 10-30 per cent of lysine residues in UHT milk stored at 30-37°C for 6 months to 3 years. Fructoselysine (e-N-deoxyfructosyl-L-lysine) concentration was generally about 10 per cent of the lactose lysine concentration.

Andrews et al. (1977) stated that within a 14 month period milk remained liquid and no evidence of any proteolytic breakdown was seen on electrophoretic examination. Electron microscopic changes were striking and depended on storage temperature. However, clear evidence of the coalescence of micelles was observed. After storage at 37°C the micelles were much larger than in the other samples.

Mehta and Bassette (1978) observed significant differences in browning between UHT milk and freshly pasteurized milks and between 2 and 12 d old UHT milk but none beyond 12 d. Bottazi and Pecis (1978) stated that the organoleptic quality of UHT milk stored for more than 6 months at ambient temperature remained satisfactory. The UHT treatment did not increase the rennetability of the milk and it gave rise to stable micellar complexes and slowed down the gelling process. It was indicated by Soeren and Evers (1978) that casein was rapidly broken down to small peptides when milk was sterilized by the direct UHT treatment of 4s at 140°C and then stored at 28°C in one litre single service packs. The rate of proteolysis decreased with increasing fat content of the milk.

*The purpose of this study was to investigate the influence of storing the raw milk at 2°C and 6°C on its composition and characteristics and the resultant effect on the quality of Cheddar cheese.*

# CHAPTER ONE

## MATERIALS AND METHODS

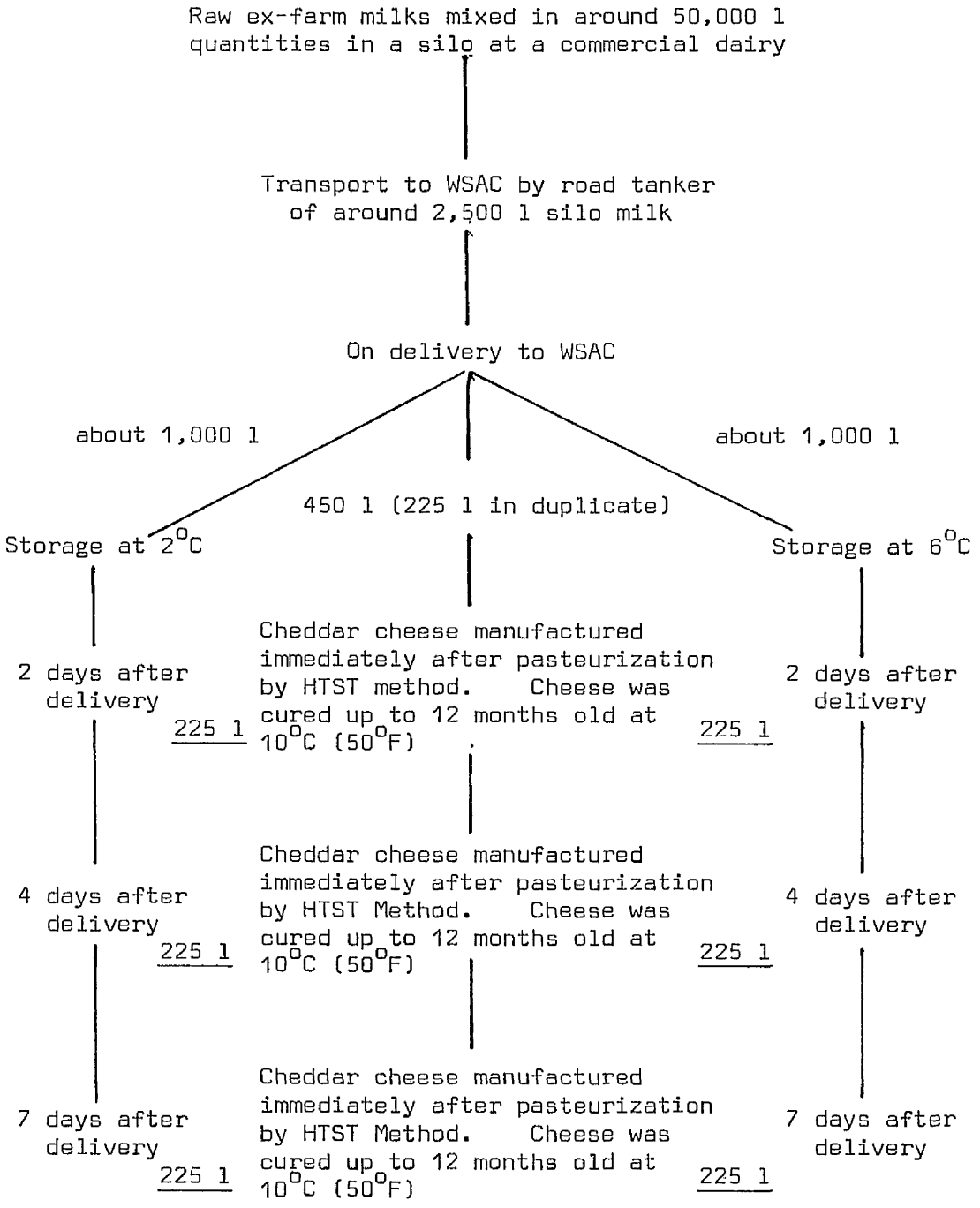
### SECTION 1 - SAMPLES COLLECTION

#### a. Preliminary trials

Two 5 l lots of bulk herd milk from the College were stored at 5°C, one without preservative and the other with the addition of 0.02 per cent of preservative (thiomersal $C_2H_5HgS.C_6H_4.COONa$ supplied by BDH Chemicals Ltd., Poole, England). Each lot was stored in a stainless steel jug covered with stretch-and-seal film, and stirred by a magnetic stirrer for ½ h every 6 h. The stirring was controlled by a timer. Samples were collected from each lot on the day of analysis. The samples were stored in a cold bath using ice bags to keep the temperature of the samples at not more than 4°C until the time of analysis. The milk samples were analysed for free and total sulphydryl groups, disulfide groups, total solids, protein, casein, non-casein nitrogen (NCN), non-protein nitrogen (NPN), fat, pH, total acidity, density and renneting clotting time (RCT). In another trial the following characteristics were studied:- ash, calcium, phosphorus, soluble ash, soluble-calcium, soluble phosphorus and free amino acids. Each experiment was carried out on three occasions.

#### b. The main experiment

This study was carried out using around 2,500 l quantities of milk from a bulk silo of around 50,000 l commercially produced milk at a dairy factory in the West of Scotland. The milk was delivered by road tanker and the experiment was carried out on four occasions. Two lots (1,000 l each) were stored, one at 2°C and the other at 6°C with intermittent mechanical agitation for ½ h every 6 h. Samples of raw milk were taken immediately on delivery and after 2, 4 and 7 days of storage. Cheddar cheese was manufactured from pasteurized (71.7°C for a minimum of 15 s) portions of each of the two lots of milk after the same period of storage and analysed after curing at 10°C (50°F) for 1 week, and 1, 2, 4, 8 and 12 months as described in the following diagram.




Tests made on raw milks on delivery and after 2, 4 and 7 days of storage at 2°C and 6°C. Tests made on Cheddar cheese after 1 week, 1, 2, 4, 8 and 12 months of curing.

## SECTION 2 - MILK ANALYSIS

### 2.1 Total solids

Throughout the study the total solids content was determined by drying at 102°C according to the IDF standard method 21 (IDF/FIL, 1962). Hot air oven supplied by Townson and Mercer Ltd., Croydon, England was used.

### 2.2 Fat

Throughout the primary work fat was determined according to the IDF standard method 4 (IDF/FIL, 1969) which is based on the Rose-Gottlieb procedure. During the main experiment work the fat content was determined according to B.S. 696, Part 2 (British Standards Institution, 1969).

### 2.3 Total nitrogen

The improved micro-Kjeldhal method of the Association of Official Agricultural Chemists (1965) was used to determine the total nitrogen content of milk (expressed as protein), with the use of Kjeldhal copper catalyst tablets supplied by BDH Chemicals Ltd., England, instead of mercuric oxide and standard hydrochloric acid as the receiver in distillation. The excess of the acid was titrated with standard sodium hydroxide solution.

### 2.4 Non-casein nitrogen

The IDF standard method 29 (IDF/FIL, 1964) was used to precipitate the casein of the milk. The nitrogen content of the filtrate was determined according to the improved micro-Kjeldhal method previously described (Section 2.3). Non-casein nitrogen is expressed as protein by multiplying the nitrogen content by the factor 6.38. Casein was calculated using the following formula:-

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

### 2.5 Non-protein nitrogen

Twelve per cent trichloroacetic acid soluble nitrogen was prepared by weighing 10 ml of milk into a 50 ml volumetric flask which was filled

to the mark with 15 per cent (w/v) trichloroacetic acid. The mixture was filtered through filter paper (Whatman number 42). The nitrogen content of the filtrate was determined according to the improved Kjeldahl method previously described (Section 2.3). The protein nitrogen was then calculated using the following formula:-

Protein nitrogen (per cent) = 6.38 (Total nitrogen - NPN)

## 2.6 Ash content

A weighed milk sample was dried, charred and ashed at 540$^{o}$C according to the method of Pearson (1976) using a muffle furnace supplied by Baird and Tatlock (London) Ltd., England.

## 2.7 Calcium content

Throughout the primary experiments the total calcium content in milk was determined according to the IDF standard method 36 (IDF/FIL, 1966). The principle of this method is based on precipitating the protein substances with trichloroacetic acid. The calcium contained in the filtrate is precipitated as calcium oxalate which is separated by centrifuging and titrated with potassium permanganate. This method takes a long time to perform and is a reference method for determining the calcium content of milk but may not be suitable for cheese, whey and ultrafiltrate.

Pearce (1977) modified the complexometric method for determination of calcium in dairy products. This method is based on titration with ethylenediaminetetra-acetic acid (EDTA). He proved that Patton and Reeder's indicator (P and R) (2-Hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid) is specific for calcium. He recommended the use of high pH (> 13), P and R indicator and direct titration for determination of only calcium in dairy products. The addition of a little less than the expected titre of EDTA (found by a preliminary titration) before addition of alkali minimizes the problems caused by precipitation of calcium phosphate and the co-precipitation of calcium and magnesium hydroxides.

This method was used for milk, whey and cheese, throughout the main experiments. It is also used for milk ultrafiltrate using 10 ml

amounts of sample. The P and R indicator was supplied by Fisons Scientific Apparatus, Loughborough, Leicestershire, England; a magnetic stirrer was used for continuous stirring during titration.

Duplicate samples were analysed at the same time. Experience is required to detect the end point.

## 2.8 Phosphorus content

The method of the IDF standard method 42 (IDF/FIL, 1967) was used. The ash obtained above (Section 2.6) was used to determine phosphorus colorimetrically by reduction of the ammonium phosphomolybdate by diaminophenol (amidol) and the optical density of the solution obtained was measured at 750 nm using a Spectronic 20 supplied by Bausch and Lomb Inc., Rochester, N.Y., U.S.A.

## 2.9 Titratable acidity

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

## 2.10 Hydrogen ion concentration (pH)

The hydrogen ion concentration (pH) of milk was measured by a Pye 290 pH meter fitted with a combined glass electrode (Activion Glass Ltd., Scotland). Buffer solutions of pH 4 and pH 7 were used to standardize the equipment before use, taking into consideration the temperature of the sample and the buffer solutions.

## 2.11 Extraneous water

The FPD of milk was determined using the Advanced Milk Cryoscope (Model 41) provided by Advanced Instruments Inc., Needham Heights, Massachusetts. This instrument meets the requirements specified by the Association of Official Agricultural Chemists.

## 2.12 Antibiotic residues in milk

The disc assay method, based on the procedure of Galesloot and Hassing (1962) and as used by the Scottish Milk Marketing Board for the control of antibiotic residues in ex-farm milks was used throughout the study.

## 2.13 Total and free sulphydryl (-SH) and disulfide groups

Haschemeyer and Haschemeyer (1973) stated that 5, 5' -dithiobis-2-nitrobenzoic acid, commonly referred to as DTNB or Ellman's reagent is an excellent and very specific reagent for the quantitative determination of free sulphydryl groups in proteins. The method of Beveridge et al. (1974) was used in this study. For determination of SH groups, 0.5 ml of skim milk which was prepared as described in Section 2.16 was added to 2.5 ml of 8 M urea in Tris-glycine buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per litre, pH 8.0, denoted as Tris-Gly) and 0.02 ml of Ellman's reagent in Tris-Gly (4 mg/ml) supplied by BDH Chemicals Ltd., Poole, England).

For SS, 0.2 ml of skim milk, 1 ml of 10 M urea in Tris-Gly and 0.02 ml of 2-mercaptoethanol were incubated at 25°C for 1 h. After an additional 1 h incubation with 10 ml of 12 per cent TCA, the tubes were centrifuged at 4,000 g for 10 min using a BTL Bench centrifuge. The precipitate was twice resuspended in 5 ml of 12 per cent TCA and centrifuged to remove 2-mercaptoethanol. The precipitate was dissolved in 3 ml of 8 M urea in Tris-Gly and 0.03 ml of Ellman's reagent was added for colour development. Absorbance was measured at 412 nm on a Pye Unicam SP 1800 ultraviolet spectrophotometer.

The total solids of skim milk were determined by drying at 102°C for 24 h. The following formula was then used:-

$$\mu MSH/g = \frac{73.53\ A_{412}.D}{C}$$

where, $A_{412}$ = the absorbance at 412; C = the sample concentration in mg solids/ml; D = the dilution factor, 6.04 and 15 for SH and total SH (SH + reduced SS) in milk, respectively.

## 2.14 Free amino acids

### a. Qualitative detection

The technique of Stahl (1969) was used for preparing and spreading

plates for thin-layer chromotography. Plates of 20 x 20 cm were covered with a layer of silica gel G type 60, art 7731 (supplied by E. Merck, Darmstadt, Germany) of a 250μ thickness using a suspension of 30 g of silica gel in 60 ml of distilled water. Plates were eluted with chloroform; methanol; 17 per cent ammonia (2:2:1) Ninhydrin solution (0.3 g Ninhydrin + 100 ml butanol + 3 ml glacial acetic acid) was used to spray the dried plates (Pataki, 1968). The plates were then put in an oven at 102°C for 10 min. The spots of amino acids observed were immediately marked with a pin. Ten ml of the ultrafiltrate which was prepared according to Section 2.16 were dried in the freeze drier EF03 supplied by Edwards High Vacuum, Manor Royal, Crawley, Sussex. The freeze-dried ultrafiltrates were dissolved in 2 ml of distilled water containing 10 per cent (v/w) n-propanol. Five microlitres of each sample were applied on the prepared plate, using hot air for drying during application. The Rf value of each spot was compared with the Rf values of the amino acids which were applied on the same plate and at the same conditions. The standard amino acids solutions (set NO. 1, Cat. No. 2058) were supplied by Shandon Scientific Company Ltd., Willesden, London, England. These amino acids were DL-alpha-Alanine, aspartic acid, glutamic acid, glycine, DL-leucine, DL-iso-leucine, proline, DL-valine and a mixture of standard amino acids. These amino acids were supplied in a form of solution in 10 per cent (v/v) iso-propanol in water.

b. Quantitative analysis

The method of Spackman et al. (1958) was used in analysing the free amino acids in milk. Eight ml of the milk ultrafiltrate which was prepared according to Section 2.16 were freeze-dried and kept in the freezer until the day of analysis. A Jeol JLC-5AH automatic amino acid analyser supplied by Jeol (UK) Ltd., Grove Park, London, England was used.

## 2.15 Polyacrylamide slab gel electrophoresis

Skim milk was prepared by centrifugation at 1,500 g for 20 min. Caseins in 30 ml of the skim milk were precipitated at pH 4.6 by adding drops of 1 N hydrochloric acid. The precipitation was

repeated twice according to the method of McKenzie (1970). The precipitates were separated by centrifugation at 1,500 g for 10 min, discarding the supernatant and then the precipitates were washed twice with distilled water. The precipitated caseins were freeze-dried and stored until the time of analysis. Samples for electrophoresis were prepared by dissolving 20 mg of the freeze-dried casein in 5 ml of gel buffer (GB) using a Whirlimixer supplied by Fisons Scientific Apparatus, Bishop Meadow Road, Loughborough, Leicestershire, England. Standard casein was prepared by dissolving 20 mg casein (light white soluble, supplied by BDH Laboratory Chemicals, Poole, England), in 5 ml GB. Mercaptoethanol was added to the samples (1 µ l per mg of casein). The GB used was composed of 4.5 g tris (hydroxymethyl) methylamine + 0.53 g citric acid + 270.27 g urea and the volume was completed to 1 litre in a volumetric flask.

A model 220 vertical slab electrophoretic cell supplied by Bio Rad Laboratories Ltd., Caxton Way, Watford, Hertfordshire, England was used. This cell consisted of a lower chamber into which the assembled cell core was inserted just before sample application. The assembled cell core contains a center filled with cold water and connected in a water circle to keep the two slabs on both sides cold during running.

The separating gel was made from a solution containing 8 per cent (w/v) cyanogum in GB. It was poured into the sandwich very gently from the top, avoiding the formation of air bubbles. The gel forming plate was inserted very gently pressing onto the outside glass plate. The separating gel was left to polymerize overnight (as recommended by Bio Rad). The gel forming plate was pulled out very gently, and the top surface of the separating gel was washed twice with distilled water and then with the electrophoresis buffer.

The stacking gel was made from GB containing 5 per cent (w/v) cyanogum. This solution was poured on the top of the separating gel. The sample well forming comb (20 slots) was inserted very gently to avoid air bubble formation and left for 1 h to polymerize. The comb was taken out and the slots were washed twice with the electrode buffer.

The lower buffer chamber was put on the levelling table and then the

core assembly was put inside it. The lower buffer chamber was filled with the buffer up to within 2-3 cm of the top of the glass plates. However, the upper buffer chamber was filled with the same buffer. Air bubbles were removed by L-shaped glass rods. Gels were pre-run for 2 h to remove the impurities.

Ten microlitres of each of the samples were applied after addition of a drop of glycerine to each sample to increase the density of the samples. The cell lid was dried and the cell was connected to the water circle and then into a DC power unit supplied by Associated Electrical Industries Ltd., Woolwich, England, using a constant current of 150 V for 2 h.

After completion of the run, the slabs were separated from the glass plates very gently and transferred on one of the glass plates into the staining tank where the slab was stained overnight with 0.1 per cent (w/v) Coomassie blue R (Kenacid blue R) supplied by BDH Laboratories, in 10 per cent (w/v) acetic acid. Destaining the gel was carried out by immersing the gel in a tank containing 5 per cent acetic acid solution (v/v) and applying an electrical current of 12 volts (0.6-1.0 AMP) through the acid for 4 h using a battery charger (popular model) supplied by F.C. Heayberd & Co. Ltd., London. Acid was changed once during destaining.

When the gel became clear, the slabs were then transferred into a cellophane/polythene pouch and vacuum sealed. The slabs were photographed and scanned using a scanner described in Chapter Six.

All the reagents were obtained from BDH Chemicals Ltd., Poole, England, except the 1 N hydrochloric acid which was obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex, England

## 2.16 Preparation of milk ultrafiltrate

The skim milk was prepared by centrifugation of whole milk at 1,500 g for 20 min, pipetting off the serum from below the fat layer. Amicon ultrafiltration cell model 202 supplied by Amicon Corp., Lexington, Mass. 02173, U.S.A. was used. A UM2 membrane which retains the molecules with molecular weight > 1000 was used. The ultrafiltration

was done at 4°C for 24 h to collect 50 ml using 3.5 kg/cm$^2$ (50 p.s.i.) pressure supplied from a nitrogen cylinder.

The ultrafiltrate was used to study the following characteristics:

a. Soluble calcium and phosphorus

Ten ml of the ultrafiltrate were used to determine either the soluble calcium according to one of the two methods described in Section 2.7 or the soluble phosphorus according to the method described in Section 2.8.

b. Free amino acids

Free amino acids were detected either qualitatively or quantitatively according to the methods described in Section 2.14 a or b, respectively.

## SECTION 3 - CLOTTING AND STARTER ACTIVITY

### 3.1 Clotting activity

The clotting activity of milk samples was measured using Hansen's standard cheese rennet (reference No. 351) supplied by Chr. Hansen's Laboratory Ltd., Reading. One of the following two methods was used:-

a. The method of B.S. 3624 (British Standards Institution, 1963) which was developed originally to measure the coagulation power of rennet. The author modified this method to study the changes in the coagulation time of raw and cold stored milks. The rennet solution was diluted by the buffer solution used by Eisses (1977), one ml of the rennet solution was diluted into 100 ml buffer. This buffer composed of 1 N acetic acid 3.5 ml, sodium acetate 10 g, diluted to 1 litre with distilled water with drops of chloroform as preservative. One ml of the diluted rennet solution was used for 10 ml of milk sample. The time required from the addition of rennet to the first evidence of clotting was expressed as the RCT. A lamp and magnifying glass were used to help to see the first evidence of coagulation in the milk under test. Triplicate readings were obtained.

b. The Dutch method (Eisses, 1977) differs from the

previously described method (B.S. 3624), by using 50 ml of milk. Bottles are used instead of test tubes and are rotated at 16-18 rpm instead of 2-4 rpm. The substrates used in this experiment were raw or cold stored milk without addition of either calcium chloride or lactic acid.

3.2 a. Starter activity

The starter cultures used were supplied by Chr. Hansen's Laboratorium A/s, Sankt Annae Plads 3, 1250, Copenhagen. The type of cultures were Redi-Set freeze-dried blue label type O. These cultures are a mixture containing a few strains of Streptococcus cremoris (95-98 per cent) and Streptococcus lactis (2-5 per cent). These cultures contain approximately $2 \times 10^9$ viable cells per g, and do not produce either aroma components or $CO_2$. To avoid the possibility of phage action it is recommended that O-cultures are used in a rotation scheme. Three different CHL cultures were used; for trials 1 and 2 the CHL number 173 (production batch 19) was used, while for trials 3 and 4 the CHL numbers 172 (production batch 219) and 96 (production batch 239) were used, respectively.

To study the activity of each culture used in each trial and on each day of cheese manufacturing the raw or cold stored milks were dispensed by 100 ml pipette into each of five 100 ml sterilized bottles. Milks were pasteurized at 63°C for 35 min in a water bath, then cooled to 30°C. The milk samples were incubated with 1 g of the same starter to be used for cheese-making, and incubated at 30°C for 5½ h. The titratable acidity and pH were measured for each bottle according to the procedure described in Sections 1.9 and 1.10 respectively.

b. Preparation of starter for cheesemaking

The freeze-dried Redi-Set lactic culture described in Section 3.2 a. which is enough to make 1,000 l of starter was used. All of the contents of the pack of the culture were transferred into a weighed sterilized bottle which was reweighed again to find the total weight of the starter.

Antibiotic-free skimmed milk powder was added to de-ionized water to give a 10 per cent (w/w) solution. A Silverson mixer (Silverson Machines Ltd., London) was used to reconstitute the skim milk powder. The skimmed milk required for each trial (32 l) was prepared 2 days before the receipt of the raw milk for cheesemaking. The prepared skimmed milk was dispensed in 3.5 l quantities in 5 l conical flasks and sterilized at 121°C (1.06 kg/cm$^2$ or 15 p.s.i.) for 5 min using an autoclave (Astel model 2,000 supplied by Astel Hearson Ltd., 172 Brownhill Road, Catford, London, England). The milks were cooled in a water bath and then kept in a refrigerated room at 4°C until the day of use.

Slightly more than the quantity of the freeze-dried starter culture required for 7 l of milk medium was weighed into a sterilized 25 ml bottle, dissolved in the required volume of sterilized distilled water so that the quantity of cells required to inoculate each of the two 3.5 l lots of sterilized skimmed milk could be transferred by adding an inoculum of 5 ml. The sterilized skimmed milks were warmed up to 22°C in a 30°C water bath before inoculation. The temperature of milks was controlled by a sterilized thermometer which had been inserted aseptically into each flask. The inoculated flasks were mixed very well and incubated at 22°C for 20-22 h until coagulation. Starter inoculations and transfers were made in a room fitted with an ultraviolet bactericidal light with extreme attention to aseptic technique.

## SECTION 4 - CHEESEMAKING SYSTEM

Throughout the study, Cheddar cheese was made using 180 l (40 gal) quantities of milk.

### 4.1 Milk reception and treatment

The milk was received as described in Section 1 b. and was divided into three portions:-

a. Initial day: Four hundred and fifty litres (100 gal) of milk were pasteurized at 71.7°C for 15 s immediately before

cheesemaking using a pasteurizer supplied by the APV Company Ltd., PO Box No. 4, Manor Royal, Crawley, West Sussex, England. The pasteurized milk was added to a number of sterilized 45 l (10 gal) capacity milk cans made of aluminium alloy. The first 5-15 gal of milk from the pasteurizer were discarded. Checks were made on the milk in the cans for FPD as an indication of extraneous water. The milk in the cans was then weighed to the nearest 5 g using an Avery single pan balance (accuracy 1 in 2000) and added to each of two 225 l (50 gal) stainless steel cheese vats. Vats were sterilized before use by the addition of a solution of 100 ppm sodium hypochlorite for 30 min and thereafter rinsing thoroughly. The surfaces of the vat were dried by tissues just before the pasteurized milk was added to the vat.

The contents of the vats were maintained at the required temperature or warmed by injecting steam into water in a jacket of the vat. The contents of the vat were mixed with an electrically operated paddle stirrer.

b. Milk stored at $2^{o}C$: The second portion of milk of 900 l (200 gal) was pumped from the receiving tank into a stainless steel refrigerated tank supplied by the Dairy Supply Co. Ltd., London, England. The temperature of the milk was maintained at $2^{o}C$. This tank is supplied with a mixer which was timed to mix the milk for ½ h every 6 h. The tank and agitation meets the requirements of B.S. 3976 (British Standards Institution, 1966).

c. Milk stored at $6^{o}C$: The same quantity of milk i.e. 900 l (200 gal) was stored in a refrigerated room at $6^{o}C$. The storage tank used is supplied with a Rotamilk mixer supplied by the Motor Gear and Eng. Co. Ltd., Essex. The same mixing interval was used as in the other tank.

Two hundred and twenty five litres (50 gal) of each of the two stored portions of milk were pumped into the cheese room after pasteurization. The same procedure was used as in the initial day to ensure that the pasteurized milk did not contain water.

### 4.2 Starter and rennet addition

The quantity of starter required for each vat was weighed from a mixture of the two flasks which had been prepared as in Section 3.2b. It was added to give 1.75 per cent (w/w). The required quantity of rennet (as recommended by the supplier) was diluted with water, weighed and added after 20 minutes from the addition of the starter. Three minutes were given to mix the rennet with the milk in the vat before being left undisturbed during the coagulation period.

### 4.3 Curd treatment and cheesemaking operations

Scalding of the curd, draining the whey, cheddaring, milling, moulding and pressing were done as described by Al-Obaidi (1980) with the separate collection of whey at draining, at cheddaring and at pressing. Each of the wheys were weighed and then samples were collected for chemical analysis.

### 4.4 Packaging and curing

The cheese blocks were weighed and cut into four smaller blocks each weighing around 4.54 kg (10 lb) which were packed in cellulose/ polythene pouches and vacuum sealed by the Autovac vacuum packing and sealing machine (supplied by Interfood Tech. Ltd., Industrial Estate, Harefield Road, Rickmansworth, Herts., England). The packed blocks of cheese were placed in a fibreboard case and stored at $10^{o}C$ ($50^{o}F$).

## SECTION 5 - CHEESE ANALYSIS

### 5.1 Moisture

The moisture content of cheese samples was determined according to B.S. 770 (British Standards Institution, 1963). This method is based on drying 3 g of grated cheese sample until a constant weight. An aluminium foil container (Foilpak supplied by Brodie Ltd., Glasgow) of a diameter of 8.26 cm (3.25 in) and a depth of 1.91 cm (0.75 in) was used. The temperature of drying was $102^{o}C$ using a fan ventilated hot air oven.

### 5.2 Fat content

Fat was determined according to B.S. 696, Part 2 (British Standards

Institution, 1969) which is developed from the 'Gerber' method.

### 5.3 Total nitrogen

Sodium citrate cheese extract was prepared according to the method used by Al-Obaidi (1980) which was obtained from Vakaleris and Price (1959). Ten grams of cheese, 40 ml of 0.5 M sodium citrate solution, and approximately 80 ml distilled water were mixed by a Silverson mixer at high speed until a good homogeneous consistency was obtained. The homogeneous mixture was then transferred quantitatively into a 200 ml volumetric flask and filled to the mark with distilled water. The total nitrogen content of this extract was determined by the improved micro-Kjeldahl method as described in Section 2.3

### 5.4 Soluble nitrogen

The method used by Al-Obaidi (1980) which is based on the method of Vakaleris and Price (1959) was used to prepare a hydrochloric acid filtrate from the sodium citrate extract. Ten ml of 1.41 N hydrochloric acid was mixed with 100 ml of sodium citrate cheese extract which is described in the previous paragraph and the volume was made up to 125 ml. The mixture was then filtered through Whatman number 42 filter paper, and the nitrogen content was determined in the filtrate by the improved micro-Kjedahl method (Section 2.3).

### 5.5 Ash content

Ash was determined according to IDF Standard method 27 (IDF/FIL, 1964).

### 5.6 Salt

The method described in B.S. 770 (British Standards Institution, 1963) was used for determination of salt in cheese.

### 5.7 Calcium

The same method as described in Section 2.7 was used. Grated cheese sample (0.5 g) was soaked for 1 h in distilled water. Addition of acid to the cheese was not required.

### 5.8 Phosphorus

Phosphorus was determined according to the method of IDF 33 (IDF/FIL) 1971). The principle of this method is based on digesting a weighed

sample of grated cheese in a 25 ml Kjeldahl flask with sulphuric acid in the presence of hydrogen hydroxide. The phosphate is treated with sodium molybdate and hydrazine sulphate as a reducing agent. The molybdenum blue so formed was measured photometrically and the phosphorus content was then calculated. A cigarette paper was used for weighing the cheese sample, one was also used with the blank.

5.9 Free fatty acids

The method of Al-Obaidi (1980) was used with a slight modification. A mixture of 1 g grated cheese sample, 10 ml distilled water, and 10 ml of a 95 per cent (v/v) neutralized ethanol was prepared in a 50 ml centrifuge tube, shaken vigorously for 1 minute. Fifteen ml of the mixture consisting of 4 parts ethyl ether and 6 parts petroleum spirit were then added and the tube was shaken vigorously for another 1 min. The tube was then centrifuged for 3 min at 400 g and 5 ml of the supernatant was added to 15 ml of 95 per cent (v/v) ethanol containing five drops of 1 per cent /w/v) of phenolphthalein in alcohol (the 15 ml of ethanol was neutralized to a pink colour before the addition of the 5 ml supernatant). The mixture was titrated with 0.025 N alcoholic solution of potassium hydroxide to the same pink colour using a 10 ml burette graduated to 0.01 ml. The volume required to neutralize the total ether layer was obtained by multiplying this volume by 3. The results are expressed as free fatty acids and as a percentage of the fat content in cheese.(mEq/100 g).

5.10 Hydrogen ion concentration (pH)

Ten grams of the grated cheese samples were weighed and placed in a 25 ml plastic beaker with 10 ml distilled water. The cheese was thoroughly mixed in water using a Silverson mixer supplied by Silverson Machines, England. The pH was then measured as in Section 2.10.

5.11 Polyacrylamide slab gel electrophoresis

The method described in Section 2.15 was used.

One hundred and fifty mg of grated cheese sample were dissolved in 20 ml of GB. Fat was separated by centrifugation at 1,500 g for 10 min, and the protein suspension was taken from below the fat layer by Pasteur pipette.

5.12 Measurement of cheese firmness and elasticity

The method described by Al-Dahhan (1977) was used to measure the firmness of cheese. The test was made using a block of 4.54 kg (10 lb) rindless cheese on a table in the curing room. All the readings obtained were corrected to a temperature of 15.6°C (69°F) adding one B.C.T. unit per degree Fahrenheit (1.8 B.C.T. per degree Centigrade) when the temperature of the tested cheese was below 15.6°C (60°F), subtracting the same when the temperature of the cheese was more than 15.6°C (60°F).

## SECTION 6 - WHEY ANALYSIS

Whey samples at running, cheddaring and pressing were analysed for fat, total nitrogen, non-protein nitrogen, ash, calcium and phosphorus according to corresponding analysis for milks which are described in Sections 2.2, 2.3, 2.5, 2.6, 2.7 and 2.8 respectively. Three ml of the whey sample were used for total nitrogen determination while for the other analysis ten ml quantities of sample were used.

## SECTION 7 - STATISTICAL ANALYSIS

The methods of Snedecor and Cohran (1976) were used in the analysis of variance of results. The term 'Invstor ' was used to minimize the possible source of variation between the values obtained for the analysis of milk on delivery and stored milks. This factor was also used for the statistical analysis of the values obtained for cheeses made from these milks. Cheese was made in duplicate on the day of delivery. Thereafter cheese was made from milk stored at 2°C and 6°C after 2, 4 and 7 days using a single lot for each treatment.

Mean figures given in the Tables (except 4:28 and 4:35) in Chapters 4 and 5 were arrived at by adding the duplicate initial values (shown in the tables as means) and the values obtained after storage and dividing by 8.

CHAPTER TWO

# PRELIMINARY STUDY OF THE EFFECT OF COLD STORAGE OF MILK ON ITS PROPERTIES

## INTRODUCTION

The storage of bulk milk at 2-6$^{o}$C is the most suitable procedure beside heat treatment to avoid deterioration of quality by contaminating micro-organisms. A great amount of study has taken place and many research reports have appeared during the past twenty to thirty years as the bulk milk collection system has progressed. This system has resulted in changes in the techniques of milking, agitation and refrigeration of milk and collection and transport to the dairy, which gave rise to changes in the chemico-physical and bacteriological quality of milk.

In Scotland bulk milk collection from refrigerated farm tanks has been the sole system since 1976/77. The Milk Marketing Board for England and Wales completed its bulk collection programme in July 1979. It is only in Northern Ireland that churns are still used as part of the collection and delivery arrangements. (The Federation of U.K. Milk Marketing Boards, 1980).

The aim of this experiment is to study compositional and properties changes in milk during cold storage at 5$^{o}$C and associated changes in the colloidal and soluble salts.

## EXPERIMENTAL

Three trials were carried out on milk samples collected on 10.4.79, 24.4.79 and 23.7.79 as described in Chapter One, Section 1a. Milk samples, with and without added preservative, were analysed for total solids, fat, total nitrogen, non-casein nitrogen (NCN), non-protein nitrogen (NPN), titratable acidity, pH, total and free sulphydryl groups and free amino acids (FAA) according to the methods referred to in Chapter One, Sections 2.1, 2.2, 2.3, 2.4, 2.5, 2.9, 2.10, 2.13 and 2.14a, respectively. The density of milk samples was determined according to the method of Pearson (1976) using the density lactometer. Solids-not-fat (SNF) were calculated as the difference between total

solids and fat contents. Rennet clotting time (RCT) was measured according to the method described in Chapter One, Section 3.1b.

Three further trials (29.5.79, 11.6.79 and 9.7.79) were done to study the changes in total and soluble salts. Milk ultrafiltrates were prepared at $4^{o}C$ and analysed for soluble calcium and phosphorus and FAA. The ultrafiltrates were prepared according to the procedure described in Chapter One, Section 2.16 from fresh mixed evening and morning milk, held cold in a refrigerated farm tank and from the same milk after storage at $5^{o}C$ for 1, 2 and 7 days. The milk samples and the corresponding ultrafiltrates were analysed for ash, calcium and phosphorus according to the methods referred to in Chapter One, Sections 2.6, 2.7 and 2.8, respectively.

RESULTS

No significant differences were observed in the total solids, fat, SNF, density and protein values obtained from fresh and stored samples in any one trial (Tables 2:1, 2:2, 2:3, 2:4 and 2:7 respectively). Significant differences were found in titratable acidity (Table 2:5) with the storage time. Milk samples stored without preservative were significantly higher in titratable acidity than milk to which preservative had been added.

The analysis of variance of pH results (Table 2:6) showed significant differences between the milk in different trials which may be related to variations in the milks used. The pH value of samples in trial 3 showed the lowest value. The pH of samples showed significant decreases in pH with storage.

The casein content of samples (Table 2:8) showed very highly significant (at 0.1 per cent level) differences between the milk in different trials. The addition of preservative had a highly significant effect on the casein values in milks after storage.

The casein content of milk samples without added preservative was found to be lower after storage, so indicating the breakdown of casein due to proteolytic organisms. However, a slight reduction was found in the casein content of milk samples stored with preservative. This

TABLE 2:1

The total solids (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 12.44 | 11.57 | 12.26 | 12.09 | 12.09 |
| 1 | | 12.43 | 11.57 | 12.23 | 12.08 | |
| 2 | | 12.45 | 11.58 | 12.17 | 12.07 | 12.09 |
| 7 | | 12.44 | 11.81 | 12.11 | 12.13 | |
| 1 | With pre-servative | 12.47 | 11.53 | 12.19 | 12.06 | |
| 2 | | 12.42 | 11.52 | 12.14 | 12.03 | 12.09 |
| 7 | | 12.46 | 11.87 | 12.18 | 12.17 | |
| Means | | 12.44 | 11.64 | 12.18 | 12.09 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 1.5262318 | 2706.614*** |
| Invstor | 1 | 0.0043460 | 7.707* |
| Trial Invstor | 2 | 0.0019389 | 3.438 |
| Storage | 2 | 0.002422 | 4.296 |
| Treatment | 1 | 0.0003556 | 0.631 |
| Trial Storage | 4 | 0.0010972 | 1.946 |
| Trial Treatment | 2 | 0.0009056 | 1.606 |
| Storage Treatment | 2 | 0.0016889 | 2.995 |
| Residual | 4 | 0.0005639 | |
| Total | 20 | 0.1538858 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.01269 | 0.01679 | 0.01583 | 0.02908 | 0.02742 | 0.01939 |

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

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:2

The fat (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 3.51 | 2.48 | 3.44 | 3.14 | 3.14 |
| 1 | | 3.50 | 2.48 | 3.36 | 3.11 | |
| 2 | | 3.48 | 2.49 | 3.31 | 3.09 | 3.16 |
| 7 | | 3.48 | 2.92 | 3.40 | 3.27 | |
| 1 | With preservative | 3.50 | 2.42 | 3.41 | 3.11 | |
| 2 | | 3.48 | 2.51 | 3.42 | 3.14 | 3.17 |
| 7 | | 3.46 | 2.95 | 3.41 | 3.27 | |
| Means | | 3.49 | 2.61 | 3.39 | 3.16 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 2.191633 | 1843.418*** |
| Invstor | 1 | 0.002229 | 1.875 |
| Trial Invstor | 2 | 0.000506 | 0.425 |
| Storage | 2 | 0.000022 | 0.019 |
| Treatment | 1 | 0.001606 | 1.350 |
| Trial Storage | 4 | 0.001256 | 1.056 |
| Trial Treatment | 2 | 0.001672 | 1.407 |
| Storage Treatment | 2 | 0.000822 | 0.692 |
| Residual | 4 | 0.001189 | |
| Total | 20 | 0.220146 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.01843 | 0.02438 | 0.02299 | 0.04223 | 0.03981 | 0.02815 |

***significant at 0.1 per cent level

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:3

The solids-not-fat (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 8.92 | 9.09 | 8.82 | 8.94 | 8.94 |
| 1 | | 8.93 | 9.09 | 8.87 | 8.96 | |
| 2 | | 8.97 | 9.09 | 8.86 | 8.97 | 8.94 |
| 7 | | 8.96 | 8.91 | 8.71 | 8.89 | |
| 1 | With preservative | 8.97 | 9.11 | 8.78 | 8.95 | |
| 2 | | 8.94 | 9.01 | 8.72 | 8.89 | 8.91 |
| 7 | | 9.00 | 8.92 | 8.77 | 8.89 | |
| Means | | 8.96 | 9.03 | 8.80 | 8.92 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.132290 | 64.097*** |
| Invstor | 1 | 0.000179 | 0.087 |
| Trial Invstor | 2 | 0.001610 | 0.780 |
| Storage | 2 | 0.002867 | 1.389 |
| Treatment | 1 | 0.003472 | 1.682 |
| Trial Storage | 4 | 0.002108 | 1.022 |
| Trial Treatment | 2 | 0.002289 | 1.109 |
| Storage Treatment | 2 | 0.003622 | 1.755 |
| Residual | 4 | 0.002064 | |
| Total | 20 | 0.015285 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.02428 | 0.03212 | 0.03029 | 0.05564 | 0.05246 | 0.03709 |

***significant at 0.1 per cent level

+sample of mixed evening and morning milk taken at around 7.00 a.m, held in iced water and analysed on the same day.

TABLE 2:4

The density of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 29.70 | 30.80 | 29.50 | 30.00 | 30.00 |
| 1 | | 29.70 | 30.70 | 29.20 | 29.87 | |
| 2 | | 29.70 | 30.60 | 29.50 | 29.93 | |
| 7 | | 29.40 | 29.95 | 29.60 | 29.65 | 29.82 |
| 1 | With preservative | 29.70 | 30.80 | 29.40 | 29.97 | |
| 2 | | 29.70 | 30.70 | 29.50 | 29.97 | |
| 7 | | 29.60 | 30.05 | 29.50 | 29.72 | 29.89 |
| Mean | | 29.64 | 30.51 | 29.46 | 29.87 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 3.380455 | 380.321*** |
| Invstor | 1 | 0.009603 | 1.080 |
| Trial Invstor | 2 | 0.000079 | 0.009 |
| Storage | 2 | 0.00222 | 0.250 |
| Treatment | 1 | 0.02722 | 3.063 |
| Trial Storage | 4 | 0.03555 | 4.000 |
| Trial Treatment | 2 | 0.003889 | 0.437 |
| Storage | 2 | 0.00222 | 0.250 |
| Residual | 4 | 0.00888 | |
| Total | 20 | 0.349617 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.0504 | 0.0667 | 0.0629 | 0.1155 | 0.1089 | 0.0770 |

***significant at 0.1 per cent level

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:5

Acidity (expressed as percentage lactic acid) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 0.14 | 0.15 | 0.14 | 0.14 | 0.14 |
| 1 | | 0.15 | 0.15 | 0.15 | 0.15 | |
| 2 | | 0.15 | 0.15 | 0.17 | 0.16 | 0.16 |
| 7 | | 0.16 | 0.15 | 0.20 | 0.17 | |
| 1 | With preservative | 0.14 | 0.15 | 0.14 | 0.14 | |
| 2 | | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| 7 | | 0.15 | 0.14 | 0.16 | 0.15 | |
| Mean | | 0.15 | 0.15 | 0.16 | 0.15 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.00018571 | 6.078 |
| Invstor | 1 | 0.00031746 | 10.390* |
| Trial Invstor | 2 | 0.00008651 | 2.831 |
| Storage | 2 | 0.0004222 | 13.818* |
| Treatment | 1 | 0.000555 | 18.182* |
| Trial Storage | 4 | 0.00013056 | 4.273 |
| Trial Treatment | 2 | 0.00017222 | 5.636 |
| Storage Treatment | 2 | 0.00008889 | 2.909 |
| Residual | 4 | 0.00003056 | |
| Total | 20 | 0.00017143 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.00295 | 0.00391 | 0.00369 | 0.10067 | 0.00638 | 0.00451 |

*significant at 5 per cent level

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:6

The pH value of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 6.75 | 6.74 | 6.73 | 6.74 | 6.74 |
| 1 | | 6.75 | 6.74 | 6.71 | 6.73 | |
| 2 | | 6.75 | 6.74 | 6.51 | 6.67 | |
| 7 | | 6.71 | 6.72 | 6.38 | 6.60 | 6.67 |
| 1 | With preservative | 6.75 | 6.74 | 6.74 | 6.74 | |
| 2 | | 6.76 | 6.74 | 6.70 | 6.73 | |
| 7 | | 6.75 | 6.73 | 6.65 | 6.71 | 6.73 |
| Mean | | 6.75 | 6.74 | 6.63 | 6.70 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.028043 | 14.695* |
| Invstor | 1 | 0.004464 | 2.339 |
| Trial Invstor | 2 | 0.003457 | 1.812 |
| Storage | 2 | 0.010017 | 5.249 |
| Treatment | 1 | 0.018050 | 9.459* |
| Trial Storage | 4 | 0.006342 | 3.323 |
| Trial Treatment | 2 | 0.011267 | 5.904 |
| Storage Treatment | 2 | 0.004017 | 2.105 |
| Residual | 4 | 0.001908 | |
| Total | 20 | 0.008456 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.02335 | 0.03089 | 0.02912 | 0.05350 | 0.05044 | 0.03567 |

*significant at 5 per cent level

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:7

The total protein (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without Preservative | 3.22 | 3.20 | 3.10 | 3.17 | 3.17 |
| 1 | | 3.20 | 3.18 | 3.08 | 3.15 | |
| 2 | | 3.23 | 3.18 | 2.97 | 3.18 | 3.15 |
| 7 | | 3.22 | 3.07 | 3.03 | 3.11 | |
| 1 | With pre-servative | 3.19 | 3.19 | 3.10 | 3.16 | |
| 2 | | 3.22 | 3.20 | 3.11 | 3.18 | |
| 7 | | 3.19 | 3.07 | 3.08 | 3.13 | 3.16 |
| Mean | | 3.21 | 3.17 | 3.07 | 3.15 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.0386904 | 69.296*** |
| Invstor | 1 | 0.0018286 | 3.275 |
| Trial Investor | 2 | 0.0001595 | 0.286 |
| Storage | 2 | 0.001050 | 1.881 |
| Treatment | 1 | 0.001800 | 3.224 |
| Trial Storage | 4 | 0.0012917 | 2.313 |
| Trial Treatment | 2 | 0.0030167 | 5.403 |
| Storage Treatment | 2 | 0.0010167 | 1.821 |
| Residual | 4 | 0.0005583 | |
| Total | 20 | 0.0049447 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.01263 | 114.2 | 107.6 | 197.8 | 186.5 | 131.8 |

***significant at 0.1 per cent level.

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:8

The casein (per cent) content of mixed evening and morning farmsupply of raw milk tested fresh+ (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 2.81 | 2.78 | 2.69 | 2.76 | 2.76 |
| 1 | | 2.79 | 2.78 | 2.58 | 2.72 | |
| 2 | | 2.80 | 2.77 | 2.48 | 2.68 | 2.69 |
| 7 | | 2.76 | 2.67 | 2.48 | 2.68 | |
| 1 | With preservative | 2.75 | 2.78 | 2.69 | 2.74 | |
| 2 | | 2.79 | 2.79 | 2.69 | 2.76 | |
| 7 | | 2.76 | 2.66 | 2.64 | 2.70 | 2.73 |
| Mean | | 2.78 | 2.76 | 2.61 | 2.72 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.0634905 | 174.478*** |
| Invstor | 1 | 0.0065722 | 18.061* |
| Trial Invstor | 2 | 0.0014151 | 3.889 |
| Storage | 2 | 0.0040056 | 11.008* |
| Treatment | 1 | 0.0102722 | 28.229** |
| Trial Storage | 4 | 0.0009805 | 2.695 |
| Trial Treatment | 2 | 0.0142721 | 39.221* |
| Storage Treatment | 2 | 0.0009389 | 2.580 |
| Residual | 4 | 0.0003639 | |
| Total | 20 | 0.0095233 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.01020 | 0.01349 | 0.01272 | 0.02336 | 0.02203 | 0.01558 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

reduction in casein content might be due to proteolytic enzymes either naturally occurring or formed by bacteria prior to preservative addition.

Very highly significant differences were observed in the protein nitrogen contents (Table 2:9) of the milk in the different trials. Significant differences were also found due to the storage, preservative, interaction between trial X storage and storage X preservative.

No significant differences were observed in the NCN levels of the samples in all trials (Table 2:10). On the other hand NPN (Table 2:11) showed highly significant differences (at 1 per cent level) due to the trial, the addition of preservative and the interaction between trial X storage. The storage time and the interaction between trial X preservative and storage X preservative had a significant effect on NPN levels.

The rennet clotting time (RCT, Table 2:12) of milks differed significantly between trials. The addition of preservative had a significant effect on the RCT. The milk stored without preservative gave a slightly longer RCT after 1 and 2 days of storage but further storage resulted in a lower RCT when the milks had been stored for 7 days, this effect of storage being probably due to the development of acidity (Table 2:5). The milk stored with added preservative showed a very slight drop in RCT after 7 days of storage but the reduction in RCT was much less than was obtained with the sample containing no preservative.

There was a significant difference in free sulphydryl groups (Table 2:13) in different trials and an interaction between storage X preservative was observed. The amount of total sulphydryl groups showed significant difference between trials and were affected by storage time (Table 2:14). On the other hand no significant differences were found in the level of disulphide groups of all the milks before and after storage (Table 2:15).

The total ash content of the milk (Table 2:15) showed a highly significant difference due to the trial. The ultrafiltrate soluble ash (Table 2:17) showed a significant increase on storage of the milk prior to ultrafiltration, which indicates the transfer of ash from the colloidal phase to the soluble phase during cold storage of the milk.

TABLE 2:9

The protein nitrogen (as protein per cent) content of a mixed evening and morning farm supply of raw milk tested fresh+ and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 3.00 | 2.99 | 2.79 | 2.93 | 2.93 |
| 1 | | 2.98 | 2.97 | 2.89 | 2.95 | |
| 2 | | 2.99 | 2.92 | 2.74 | 2.88 | 2.91 |
| 7 | | 2.97 | 2.82 | 2.84 | 2.89 | |
| 1 | With preservative | 2.97 | 2.98 | 2.89 | 2.95 | |
| 2 | | 3.02 | 2.99 | 2.87 | 2.96 | 2.94 |
| 7 | | 2.96 | 2.80 | 2.89 | 2.91 | |
| Mean | | 2.98 | 2.94 | 2.84 | 2.92 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.0360190 | 121.186*** |
| Invstor | 1 | 0.0000389 | 0.131 |
| Trial Invstor | 2 | 0.0032198 | 10.833* |
| Storage | 2 | 0.0032722 | 11.009* |
| Treatment | 1 | 0.0053389 | 17.963* |
| Trial Storage | 4 | 0.0038139 | 12.832* |
| Trial Treatment | 2 | 0.0012389 | 4.168 |
| Storage Treatment | 2 | 0.0022722 | 7.645* |
| Residual | 4 | 0.0002972 | |
| Total | 20 | 0.0056933 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.00922 | 0.01219 | 0.01149 | 0.0211 | 0.01991 | 0.01408 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:10

The non-casein nitrogen (expressed as protein per cent) content of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 0.41 | 0.40 | 0.42 | 0.41 | 0.41 |
| 1 | | 0.41 | 0.41 | 0.40 | 0.40 | |
| 2 | | 0.43 | 0.40 | 0.49 | 0.44 | 0.44 |
| 7 | | 0.45 | 0.40 | 0.56 | 0.47 | |
| 1 | With preservative | 0.44 | 0.40 | 0.41 | 0.42 | |
| 2 | | 0.42 | 0.40 | 0.42 | 0.41 | 0.42 |
| 7 | | 0.43 | 0.42 | 0.43 | 0.43 | |
| Mean | | 0.43 | 0.41 | 0.45 | 0.43 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.0025210 | 3.933 |
| Invstor | 1 | 0.0009013 | 1.406 |
| Trial Invstor | 2 | 0.002264 | 0.353 |
| Storage | 2 | 0.0025144 | 3.923 |
| Treatment | 1 | 0.0019845 | 3.096 |
| Trial Storage | 4 | 0.0010652 | 1.662 |
| Trial Treatment | 2 | 0.0019235 | 3.001 |
| Storage Treatment | 2 | 0.0013715 | 2.140 |
| Residual | 4 | 0.0006410 | |
| Total | 20 | 0.0013412 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.01353 | 0.01790 | 0.01688 | 0.03101 | 0.02923 | 0.02067 |

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:11

The non-protein nitrogen (as proten per cent) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 |
| 1 | | 0.22 | 0.21 | 0.19 | 0.21 | |
| 2 | | 0.25 | 0.26 | 0.23 | 0.25 | 0.23 |
| 7 | | 0.25 | 0.25 | 0.19 | 0.23 | |
| 1 | With preservative | 0.23 | 0.20 | 0.21 | 0.22 | |
| 2 | | 0.21 | 0.21 | 0.24 | 0.22 | |
| 7 | | 0.23 | 0.22 | 0.19 | 0.21 | 0.22 |
| Mean | | 0.23 | 0.22 | 0.21 | 0.22 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.00102033 | 19.414** |
| Invstor | 1 | 0.00038064 | 7.243 |
| Trial Invstor | 2 | 0.00020017 | 3.809 |
| Storage | 2 | 0.00086317 | 16.424* |
| Treatment | 1 | 0.00116805 | 22.225** |
| Trial Storage | 4 | 0.00104933 | 19.966** |
| Trial Treatment | 2 | 0.00070072 | 13.333* |
| Storage Treatment | 2 | 0.00062039 | 11.804* |
| Residual | 4 | 0.00005256 | |
| Total | 20 | 0.00063829 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 0.00388 | 0.00513 | 0.0483 | 0.00888 | 0.00837 | 0.00592 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:12

The rennet clotting time (RCT in s) of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage at 5°C for 1, 2 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means | Overall mean |
|---|---|---|---|---|---|---|
| Initial | Without preservative | 1205 | 1383 | 1387 | 1325 | 1325 |
| 1 | | 1426 | 1461 | 1453 | 1447 | |
| 2 | | 1450 | 1517 | 472 | 1146 | 1169 |
| 7 | | 998 | 1399 | 344 | 914 | |
| 1 | With preservative | 1467 | 1420 | 1553 | 1480 | |
| 2 | | 1553 | 1528 | 1396 | 1492 | 1455 |
| 7 | | 1500 | 1558 | 1118 | 1392 | |
| Mean | | 1371 | 1467 | 1103 | 1314 | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 285163 | 10.937* |
| Invstor | 1 | 1 | 0.000 |
| Trial Invstor | 2 | 70894 | 2.719 |
| Storage | 2 | 111906 | 4.292 |
| Treatment | 1 | 393680 | 15.099* |
| Trial Storage | 4 | 127515 | 4.891 |
| Trial Treatment | 2 | 111297 | 4.269 |
| Storage Treatment | 2 | 87328 | 3.349 |
| Residual | 4 | 26074 | |
| Total | 20 | 117061 | |

| Table | Trial | Storage | Treatment | Trial Storage | Trial Treatment | Storage Treatment |
|---|---|---|---|---|---|---|
| SED | 86.3 | 114.2 | 107.6 | 197.8 | 186.5 | 131.8 |

*significant at 5 per cent level

[+]sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:13

The free sulphydryl groups (in μMSH/g) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means |
|---|---|---|---|---|---|
| Initial | Without preservative | 2.29 | 5.75 | 3.81 | 3.95 |
| 2 | | 3.27 | 6.21 | 4.59 | 4.69 |
| 4 | | 3.60 | 5.82 | 4.62 | 4.68 |
| 7 | | 5.40 | 6.34 | 7.05 | 6.26 |
| Initial | With preservative | 2.10 | 6.83 | 2.83 | 3.92 |
| 2 | | 2.96 | 4.37 | 4.03 | 3.91 |
| 4 | | 3.07 | 3.40 | 3.78 | 3.42 |
| 7 | | 5.11 | 5.16 | 4.72 | 5.00 |
| Means | | 3.48 | 5.53 | 4.43 | 4.48 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 8.4605 | 9.711** |
| Storage | 3 | 3.6777 | 4.221 |
| Preservative | 1 | 4.1917 | 4.811 |
| Storage X Preservative | 3 | 0.5102 | 0.586 |
| Residual | 14 | 0.8712 | |
| Total | 23 | 1.9945 | |

| Table | Trial | Storage | Preservative | Storage Preservative |
|---|---|---|---|---|
| SED | 0.467 | 0.539 | 0.381 | 0.762 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysied on the same day.

TABLE 2:14

The total sulphydryl groups (μMSH/g) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means |
|---|---|---|---|---|---|
| Initial | Without preservative | 25.57 | 27.46 | 29.43 | 27.49 |
| 2 | | 25.53 | 25.78 | 29.69 | 27.00 |
| 4 | | 27.37 | 32.39 | 30.34 | 30.03 |
| 7 | | 27.74 | 33.90 | 30.66 | 30.77 |
| Initial | With preservative | 24.88 | 26.92 | 29.48 | 27.09 |
| 2 | | 28.24 | 27.40 | 29.06 | 28.23 |
| 4 | | 27.26 | 26.17 | 28.30 | 27.24 |
| 7 | | 27.81 | 33.86 | 30.80 | 30.82 |
| Means | | 26.80 | 29.23 | 29.72 | 28.58 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 19.588 | 6.309* |
| Storage | 3 | 15.003 | 4.832* |
| Preservative | 1 | 1.344 | 0.433 |
| Storage X Preservative | 3 | 4.283 | 1.380 |
| Residual | 14 | 3.105 | |
| Total | 23 | 6.167 | |

| Table | Trial | Storage | Preservative | Storage . Preservative |
|---|---|---|---|---|
| SED | 0.881 | 1.017 | 0.0719 | 1.439 |

*significant at 5 per cent level

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:15

The disulphide groups (μMSS/g) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days with and without preservative

| Storage d | Trial | 1 | 2 | 3 | Means |
|---|---|---|---|---|---|
| Initial | Without preservative | 23.27 | 21.71 | 25.62 | 23.53 |
| 2 | | 22.25 | 19.57 | 25.10 | 22.31 |
| 4 | | 23.77 | 26.57 | 25.72 | 25.35 |
| 7 | | 22.63 | 27.55 | 23.61 | 24.60 |
| Initial | With preservative | 22.78 | 20.09 | 26.65 | 23.17 |
| 2 | | 25.28 | 22.63 | 25.03 | 24.31 |
| 4 | | 24.19 | 22.77 | 24.52 | 23.83 |
| 7 | | 22.41 | 28.71 | 26.08 | 25.73 |
| Means | | 23.32 | 23.70 | 25.29 | 24.10 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 8.734 | 1.721 |
| Storage | 3 | 5.112 | 1.007 |
| Preservative | 1 | 0.592 | 0.117 |
| Storage X Preservative | 3 | 3.692 | 0.728 |
| Residual | 14 | 5.075 | |
| Total | 23 | 5.022 | |

| Table | Trial | Storage | Preservative | Storage Preservative |
|---|---|---|---|---|
| SED | 1.126 | 1.301 | 0.920 | 1.839 |

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:16

The total ash (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 0.61 | 0.81 | 0.80 | 0.74 |
| 2 | 0.69 | 0.82 | 0.75 | 0.75 |
| 4 | 0.68 | 0.82 | 0.73 | 0.74 |
| 7 | 0.68 | 0.77 | 0.74 | 0.73 |
| Means | 0.66 | 0.81 | 0.75 | 0.74 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.022626 | 14.062** |
| Storage | 3 | 0.000246 | 0.153 |
| Residual | 5 | 0.001609 | |
| Total | 10 | 0.005403 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 0.0284 | 0.0328 |

**significant at 1 per cent level

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced ater and analysed on the same day.

TABLE 2:17

The ultrafiltrate soluble ash (per cent) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 0.28 | 0.39 | 0.35 | 0.34 |
| 2 | 0.38 | 0.42 | 0.39 | 0.40 |
| 4 | - | 0.44 | 0.42 | 0.41 |
| 7 | 0.34 | 0.42 | 0.41 | 0.39 |
| Means | 0.34 | 0.42 | 0.39 | 0.38 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 0.0062601 | 19.018** |
| Storage | 3 | 0.0027131 | 8.243* |
| Residual | 5 | 0.0003292 | |
| Total | 10 | 0.0022305 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 0.01283 | 0.01481 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:18

The total calcium (mg/100 ml)content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 106.00 | 128.73 | 116.54 | 117.09 |
| 2 | 106.00 | 129.05 | 118.37 | 117.81 |
| 4 | 115.30 | 129.11 | 117.50 | 120.64 |
| 7 | 115.30 | 129.95 | 118.58 | 121.28 |
| Means | 110.65 | 129.21 | 117.75 | 119.20 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 400.265 | 66.203** |
| Storage | 3 | 10.068 | 1.629 |
| Residual | 5 | 6.181 | |
| Total | 10 | 87.964 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 1.758 | 2.030 |

***significant at 0.1 per cent level

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and anslysed on the same day.

TABLE 2:19

The ultrafiltrate soluble calcium (mg/100 ml) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 12.24 | 12.84 | 13.19 | 12.76 |
| 2 | 12.09 | 12.99 | 14.54 | 13.21 |
| 4 | 13.98 | 14.86 | 14.50 | 14.45 |
| 7 | 15.02 | 16.18 | 17.63 | 16.28 |
| Means | 13.33 | 14.22 | 14.96 | 14.17 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 3.3427 | 9.691* |
| Storage | 3 | 7.3497 | 21.308*** |
| Residual | 5 | 0.3449 | |
| Total | 10 | 3.0459 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 0.415 | 0.480 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:20

The total phosphorus (mg/100 g ) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 65.55 | 89.59 | 94.23 | 83.12 |
| 2 | 67.12 | 88.40 | 94.50 | 83.34 |
| 4 | 67.62 | 86.52 | 87.73 | 80.62 |
| 7 | 59.22 | 83.59 | 89.98 | 77.60 |
| Means | 64.88 | 87.02 | 91.61 | 81.17 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 928.254 | 385.570** |
| Storage | 3 | 27.602 | 11.465* |
| Residual | 5 | 2.407 | |
| Total | 10 | 195.135 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 1.097 | 1.267 |

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

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:21

The ultrafiltrate soluble phosphorus (mg/100 g) content of a mixed evening and morning farm supply of raw milk tested fresh+ (initial) and after storage at 5°C for 2, 4 and 7 days

| Storage d | Trial | | | Means |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Initial | 22.51 | 17.20 | 20.50 | 20.07 |
| 2 | 23.95 | 18.59 | 23.20 | 21.91 |
| 4 | 24.98 | 20.57 | 26.94 | 24.16 |
| 7 | 24.07 | 22.87 | 33.06 | 26.67 |
| Means | 23.88 | 19.81 | 25.92 | 23.20 |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 2 | 38.599 | 5.226 |
| Storage | 3 | 24.220 | 3.279 |
| Residual | 5 | 7.386 | |
| Total | 10 | 18.697 | |

| Table | Trial | Storage |
|---|---|---|
| SED | 1.922 | 2.219 |

+sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

TABLE 2:22

The free amino acids of a mixed evening and morning farm supply of raw milk tested fresh[+] (initial) and after storage for 2, 4 and 7 days

| Storage d | 1st trial | 2nd trial | 3rd trial |
|---|---|---|---|
| Initial | Lysine, unidentified spot | Lysine, Alanine, Leucine | Lysine, Alanine |
| 2 | Lysine, unidentified spot | Lysine, Alanine, Aspartic, Leucine | Lysine, Alanine |
| 4 | Lysine, Glutamic Alanine | Lysine, Alanine, Leucine, Aspartic | Lysine, Alanine, Aspartic, Glysine, Leucine |
| 7 | Glycine, Lysine, Alanine, unidentified spot | Lysine, Alanine, Leucine, Valine Aspartic, unidentified spot | Cysine, Alanine, Aspartic, Glycine, Leucine |

[+] sample of mixed evening and morning milk taken at around 7.00 a.m., held in iced water and analysed on the same day.

Total calcium levels (Table 2:18) varied with trials (at 0.1 per cent level) but no significant difference was caused in calcium level by storage. The ultrafiltrate soluble calcium (Table 2:19) was significantly affected by storage. The total phosphorus (Table 2:20) level varied with the trial (at 1 per cent level) and storage had a significant effect. The ultrafiltrate soluble phosphorus did not vary significantly either due to trials or as the result of storage.

The results of free amino acids analysis by t.l.c. showed that there are differences in the number and type of the free amino acids occupying the positions of the standard amino acids referred to in Table 2:22. Storage of the milk samples resulted in more spots appearing on the t.l.c. plates.

## DISCUSSION

The purpose of the cold storage of raw milk is to minimize the deterioration in its quality. The results of this experiment indicate that the change in the composition of milk during cold storage can not be determined by the routine analysis such as total solids, fat, total protein or density. However, the results of these analyses showed wide differences from one trial to another. The changes in the titratable acidity and pH may be due to the enzymic activity of the bacterial cells which might increase in number during the storage of unpreserved samples.

Proteolysis during storage is easily detected by determining the number of spots of free amino acids using t.l.c. and by a reduction in the casein and protein contents of milk. The occurence of proteolysis due to cold storage of milk has been reported by many workers who detect proteolysis by increase in ammonia concentration (Ludzinska et al., 1970; Kaczorek et al., 1973) or by increases in the protease peptone content (Kaczorek et al., 1973) or by detecting the decrease in casein nitrogen, the increase in NCN (Youssef et al., 1975) or NPN (Cousin and Marth, 1977b).

It is well known that the reason of proteolysis in milk is either native enzymes or enzymes produced by microbial contaminants or both. The degree of significance of difference due to preservative is more

than the effect of storage time which indicates that proteolytic micro-organisms are more important in the proteolysis of milk than the native milk enzymes. These results are in agreement with the findings of Shipe et al. (1981) who reported that inhibition of microbial growth with merthiolate suppressed the acceleration of rates of changes (ADV, tyrosine value, and pyruvate contents) indicating that microbial activity was responsible. The increase in the number of free amino acids spots with increase storage time is in agreement with the results of Prodanski, (1962) and Natarjan and Nambudripad (1978).

The results of RCT are in agreement with the results of most other workers who have reported that the cold storage of milk prolonged the RCT (see Chapter One Section 1.4 for references). However, the development of acidity in the milk samples stored without preservative caused a reduction in RCT after 7 days of storage.

There is slight increase in free sulphydryl groups of milk with the increase of time of storage whereas there was no clear difference in the disulphide groups. The results of this study showed that the quantity of free and total sulphydryl groups in raw milks varies. These findings are in agreement with the results of Zweig and Block (1953). The sulphydryl groups may participate in a variety of reactions, e.g.:-

a. they may be lost by volatilization;

b. they may oxidize to disulphide; or

c. they may be reburied in the protein structures,

(Patrick and Swaisgood, 1976). Further studies were carried out by Al-Saltan (1981) on the effect of cold storage of raw milk on free sulphydryl groups.

The results of determinations of soluble ash and calcium indicated that as the storage time increases the quantity of soluble ash and calcium found were greater. Total phosphorus values are in agreement with the results of Kervina and Slanovec (1970).

The results of determination of total calcium in milk are in agreement with the results of Davies and White (1962) and Kervina and Slanovec (1970). On the other hand, the mean value for soluble calcium was

14.11 mg/100 ml which is very much less than the value of 43.4 mg/100 ml reported by Davies and White. The difference in results may be due to the fact that Davies and White made the ultrafiltration at 20°C whereas in this study a temperature of 4°C was used.

Kapsimalis and Zall (1981) found that permeates obtained at 45°C were cloudy in appearance compared to the virtually clear permeates obtained at 15°C from large pore membranes. The cloudy appearance may be from colloidal particles of sufficient particle size to scatter visible light, probably protein molecules or colloidal calcium phosphate. The higher conductivities and total solids of the permeates indicate that more solutes of higher molecular weight passed through the small and large pore membrane during ultrafiltration of skim milk at 45° compared to 15°C. This suggests that temperature of the feed may: (1) alter the membrane's configuration in such a way that the effective molecular size cut-off is changed; (2) alter the grouping of the molecules or (3) affect material deposit formations on the membrane accumulated during ultrafiltration.

These results indicate the remarkable effect of temperature on the distribution of calcium between the colloidal and the soluble phases. Parker and Dalgleish (1981) reported on the impossibility of precipitating a β-casein-$Ca^{+2}$ complex at 4°C.

## CONCLUSION

1. There are clear differences in the composition of milk in different trials.

2. Clear evidence of proteolysis of milk proteins during cold storage was detected by the increase in the number of free amino acid spots on t.l.c. plates, and by the reduction in casein and protein nitrogen.

3. There was observable change in the state of equilibrium of mineral salts due to the cold storage of raw milk. This may be due to differences in the capability of different types of caseins in binding salts particularly $Ca^{+2}$. However, the size and changes of the casein micelle plays a part in the possibility of binding.

4. Treatment of milk with preservative prevented the change during storage of milk in acidity, pH, protein nitrogen and RCT. These findings indicate that a temperature of 5°C is not sufficient in itself to inhibit the microbiological deterioration of milk stored for an extended time prior to processing.

# CHAPTER THREE

## THE EFFECT OF COLD STORAGE ON THE COMPOSITION AND PROPERTIES OF RAW MILK PRIOR TO MANUFACTURING OF CHEESE

### INTRODUCTION

From the start of bulk milk collection in the United States in 1940, the system was later introduced in Europe where recent progress in some regions has brought the amount of bulk collected milk up to 80-100 per cent of the total milk produced (Hadland, 1978). The bulk milk collection system has raised a series of new technical problems and has resulted in a higher demand for technical and quality control and responsibility, at all stages of milk treatment.

Reimerdes (1978) considered that the chemical status of the milk system resulting in variation of processing parameters especially during cheese-making and cream production was of significance in relation to the bacterial properties of bulk milk stored at a low temperature value of 2-6$^{o}$C.

Hadland (1978) reviewed the physico-chemical reactions in milk subjected to the farm bulk collection system. He suggested that variations in temperatures caused by addition of warmer milk to the refrigerated milk already in the tank might result in a series of physico-chemical reactions in the milk which may be of importance in respect of milk quality. Control of these reactions is desirable for better regulation of properties and final quality of milk and resulting products.

The purpose of this study was to find out the effect of cold storage of raw milk prior to its use for cheesemaking, at 2$^{o}$C and 6$^{o}$C on its composition and characteristics.

### EXPERIMENTAL

Bulk silo milk was collected as described in Chapter One, Section 1b. Milk was analysed for total solids, fat, total nitrogen (protein), NCN, NPN, ash, calcium, phosphorus, titratable acidity, pH, FPD and anti-biotic residues according to methods stated in Chapter One, Section 2.1

to 2.12, respectively. The acid degree value (ADV) was determined according to the method of Thomas et al. (1955a) as modified by Hunter et al. (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 with distilled water. 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 by volume of petroleum ether (40/60) and 1 part of absolute methanol and titrated against a standardized alcoholic solution of potassium hydroxide immediately before titration, with the use of 5 drops of 1 per cent (w/v) phenolphthalein.

Milk ultrafiltrates were prepared according to the methods given in Chapter One, Section 2.16. Soluble ash, soluble calcium and soluble phosphorus were determined according to the procedures described in Chapter One, Section 2.6, 2.7 and 2.8 respectively. The FAAs were determined according to the procedure described in Chapter One, Section 2.14b. Rennet clotting time (RCT) and starter activity were determined according to the methods given in Chapter One, Sections 3.1a and 3.2a, respectively.

RESULTS

Tables 3:1 to 3:16 show the effect of cold storage of raw milk on its composition. Neither storage time nor temperature of storage produced significant changes in total solids, fat and protein (Tables 3:1, 3:2 and 3:3). However, the milks of different trials showed significantly different values for these three properties.

Highly significant differences occurred in the non-casein nitrogen (NCN) and non-protein nitrogen (NPN) of the milks used in the different trials (Tables 3:4 and 3:5). Storage of the milks brought about a significant increase in NCN levels ($p < 0.05$) in the milks in all four trials but storage had no influence on the NPN levels of the milks in any trial. Storage of milks at $6^{o}C$ resulted in a significant increase in the NCN level compared to that of milks held at $2^{o}C$.

Total ash, calcium, phosphorus and soluble ash levels were unaffected by either the duration or the temperature of storage (Tables 3:6, 3:7, 3:8 and 3:9, respectively). The phosphorus level of milks used in the

TABLE 3:1

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the total solids content (per cent)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | Means |
| 1 | 12.27 | 12.12 | 12.27 | 12.24 | 12.32 | 12.31 | 12.27 | 12.26 |
| 2 | 12.22 | 12.25 | 12.27 | 12.23 | 12.25 | 12.20 | 12.12 | 12.22 |
| 3 | 12.11 | 12.20 | 12.11 | 12.08 | 12.11 | 12.15 | 12.08 | 12.12 |
| 4 | 12.27 | 12.24 | 12.27 | 12.18 | 12.20 | 12.26 | 12.18 | 12.23 |
| Mean Values | 12.22 | 12.20 | 12.23 | 12.18 | 12.22 | 12.23 | 12.16 | 12.21 |
| | | 12.21 | | | 12.20 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.025319 | 9.972* |
| Invstor | 1 | 0.000882 | 0.347 |
| Trial Invstor | 3 | 0.000554 | 0.218 |
| Storage | 2 | 0.007392 | 2.911 |
| Temperature | 1 | 0.000009 | 0.004 |
| Trial Storage | 6 | 0.001923 | 0.757 |
| Trial Temperature | 3 | 0.006171 | 2.430 |
| Storage Temperature | 2 | 0.00632 | 0.249 |
| Residual | 6 | 0.002539 | |
| Total | 27 | 0.005179 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.02693 | 0.03086 | 0.02909 | 0.06171 | 0.05818 | 0.03563 |

*significant at 5 per cent level

TABLE 3:2

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the fat content (per cent)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | Means |
| 1 | 3.85 | 3.85 | 3.80 | 3.85 | 3.85 | 3.85 | 3.83 | 3.84 |
| 2 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.85 | 3.81 |
| 3 | 3.55 | 3.60 | 3.60 | 3.60 | 3.55 | 3.60 | 3.60 | 3.59 |
| 4 | 3.60 | 3.60 | 3.60 | 3.60 | 3.60 | 3.65 | 3.60 | 3.61 |
| Mean Values | 3.70 | 3.71 | 3.70 | 3.71 | 3.70 | 3.72 | 3.72 | 3.71 |
| | | 3.71 | | | 3.71 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.1221809 | 306.517*** |
| Invstor | 1 | 0.0004667 | 1.171 |
| Trial Invstor | 3 | 0.0004190 | 1.051 |
| Storage | 2 | 0.0002042 | 0.512 |
| Temperature | 1 | 0.0002667 | 0.669 |
| Trial Storage | 6 | 0.0004542 | 1.139 |
| Trial Temperature | 3 | 0.0003778 | 0.948 |
| Storage Temperature | 2 | 0.0007042 | 1.767 |
| Residual | 6 | 0.0003986 | |
| Total | 27 | 0.0139481 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01067 | 0.01223 | 0.01153 | 0.02445 | 0.02305 | 0.01412 |

***significant at 0.1 per cent level

TABLE 3:3

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the total protein (per cent)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | Means |
| 1 | 2.84 | 2.91 | 3.00 | 2.88 | 3.05 | 2.94 | 3.01 | 2.95 |
| 2 | 2.91 | 2.95 | 2.87 | 2.85 | 3.04 | 2.94 | 2.98 | 2.94 |
| 3 | 3.06 | 3.04 | 2.99 | 3.03 | 2.99 | 2.99 | 2.99 | 3.01 |
| 4 | 3.14 | 3.14 | 3.17 | 2.97 | 3.16 | 3.07 | 3.07 | 3.10 |
| Mean Values | 2.99 | 3.01 | 3.01 | 2.93 | 3.06 | 2.99 | 3.01 | 3.00 |
| | | 2.98 | | | 3.02 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.083947 | 20.602** |
| Invstor | 1 | 0.001300 | 0.319 |
| Trial Invstor | 3 | 0.011407 | 2.799 |
| Storage | 2 | 0.016751 | 4.111 |
| Temperature | 1 | 0.015158 | 3.720 |
| Trial Storage | 6 | 0.004703 | 1.154 |
| Trial Temperature | 3 | 0.009847 | 2.417 |
| Storage Temperature | 2 | 0.010875 | 2.669 |
| Residual | 6 | 0.004075 | |
| Total | 27 | 0.016296 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.02413 | 0.02764 | 0.02606 | 0.05528 | 0.05212 | 0.03192 |

**significant at 2 per cent level

TABLE 3:4

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the non-casein nitrogen (calculated as protein and expressed as a percentage)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | Means |
| 1 | 0.62 | 0.66 | 0.65 | 0.65 | 0.68 | 0.68 | 0.65 | 0.65 |
| 2 | 0.56 | 0.54 | 0.61 | 0.68 | 0.56 | 0.65 | 0.75 | 0.62 |
| 3 | 0.63 | 0.65 | 0.67 | 0.67 | 0.63 | 0.67 | 0.66 | 0.66 |
| 4 | 0.68 | 0.66 | 0.67 | 0.62 | 0.73 | 0.71 | 0.65 | 0.67 |
| Mean Values | 0.63 | 0.63 | 0.65 | 0.65 | 0.65 | 0.67 | 0.68 | 0.65 |
| | | 0.64 | | | 0.67 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0062086 | 13.067** |
| Invstor | 1 | 0.0062488 | 13.161* |
| Trial Invstor | 3 | 0.0016272 | 3.427 |
| Storage | 2 | 0.0037032 | 7.799* |
| Temperature | 1 | 0.0056985 | 12.002* |
| Trial Storage | 6 | 0.0101678 | 21.414*** |
| Trial Temperature | 3 | 0.0018212 | 3.836 |
| Storage Temperature | 2 | 0.0000446 | 0.094 |
| Residual | 6 | 0.0004748 | |
| Total | 27 | 0.0041581 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.00824 | 0.00944 | 0.00890 | 0.01887 | 0.01779 | 0.01090 |

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

TABLE 3:5

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the non-protein nitrogen (calculated as protein and expressed as a percentage)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | Means |
| 1 | 0.12 | 0.13 | 0.12 | 0.15 | 0.13 | 0.15 | 0.15 | 0.14 |
| 2 | 0.18 | 0.18 | 0.19 | 0.19 | 0.18 | 0.18 | 0.18 | 0.18 |
| 3 | 0.16 | 0.16 | 0.17 | 0.15 | 0.15 | 0.19 | 0.23 | 0.17 |
| 4 | 0.19 | 0.19 | 0.20 | 0.18 | 0.19 | 0.19 | 0.19 | 0.19 |
| Mean Values | 0.16 | 0.16 | 0.17 | 0.17 | 0.16 | 0.18 | 0.19 | 0.17 |
| | | 0.17 | | | 0.18 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.00804249 | 11.916** |
| Invstor | 1 | 0.00065744 | 0.974 |
| Trial Invstor | 3 | 0.00018369 | 0.272 |
| Storage | 2 | 0.00115152 | 1.706 |
| Temperature | 1 | 0.00108300 | 1.605 |
| Trial Storage | 6 | 0.00037608 | 0.557 |
| Trial Temperature | 3 | 0.00086594 | 1.283 |
| Storage Temperature | 2 | 0.00027381 | 0.406 |
| Residual | 6 | 0.00067492 | |
| Total | 27 | 0.00141383 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.009819 | 0.011249 | 0.010606 | 0.022499 | 0.021212 | 0.012990 |

**significant at 1 per cent level

TABLE 3:6

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the total ash (per cent)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.76 | 0.75 | 0.76 | 0.77 | 0.74 | 0.76 | 0.67 | 0.74 |
| 2 | 0.72 | 0.73 | 0.74 | 0.73 | 0.74 | 0.72 | 0.74 | 0.73 |
| 3 | 0.75 | 0.74 | 0.71 | 0.71 | 0.76 | 0.74 | 0.71 | 0.73 |
| 4 | 0.72 | 0.75 | 0.74 | 0.72 | 0.72 | 0.74 | 0.71 | 0.73 |
| Mean Values | 0.74 | 0.74 | 0.74 | 0.73 | 0.74 | 0.74 | 0.71 | 0.73 |
| | | 0.74 | | | 0.73 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0006731 | 0.688 |
| Invstor | 1 | 0.0003344 | 0.342 |
| Trial Invstor | 3 | 0.0005119 | 0.524 |
| Storage | 2 | 0.0024025 | 2.457 |
| Temperature | 1 | 0.0007632 | 0.781 |
| Trial Storage | 6 | 0.0004917 | 0.503 |
| Trial Temperature | 3 | 0.0014212 | 1.454 |
| Storage Temperature | 2 | 0.0007957 | 0.814 |
| Residual | 6 | 0.0009777 | |
| Total | 27 | 0.0008937 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01182 | 0.01354 | 0.01276 | 0.02708 | 0.02553 | 0.01563 |

TABLE 3:7

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the calcium content (mM/kg)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 28.38 | 28.37 | 28.38 | 28.36 | 28.34 | 28.37 | 28.38 | 28.37 |
| 2 | 28.38 | 28.28 | 28.38 | 28.38 | 28.38 | 28.38 | 28.38 | 28.38 |
| 3 | 28.77 | 28.95 | 28.71 | 28.61 | 29.01 | 28.84 | 28.68 | 28.80 |
| 4 | 28.44 | 28.20 | 28.43 | 28.40 | 28.29 | 28.27 | 28.36 | 28.34 |
| Mean Values | 28.49 | 28.47 | 28.47 | 28.44 | 28.50 | 28.46 | 28.45 | 28.47 |
| | | 28.46 | | | 28.47 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.329002 | 122.670*** |
| Invstor | 1 | 0.002072 | 0.772 |
| Trial Invstor | 3 | 0.003396 | 1.266 |
| Storage | 2 | 0.004305 | 1.605 |
| Temperature | 1 | 0.000704 | 0.263 |
| Trial Storage | 6 | 0.021004 | 7.832* |
| Trial Temperature | 3 | 0.004216 | 1.572 |
| Storage Temperature | 2 | 0.000804 | 0.300 |
| Residual | 6 | 0.002682 | |
| Total | 27 | 0.043146 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.02768 | 0.03171 | 0.02990 | 0.06343 | 0.05980 | 0.03662 |

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

TABLE 3:8

The effect of storage of bulked raw milk, at 2°C and 6°C for various periods of time prior to cheese manufacture, on the total phosphorus content (mg/100 g)

| Trial | Storage at 2°C | | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 88.45 | 90.35 | 92.90 | 91.35 | 81.30 | 87.70 | 72.40 | 86.35 |
| 2 | 82.65 | 88.15 | 90.85 | 90.80 | 89.25 | 86.50 | 93.90 | 88.87 |
| 3 | 96.40 | 91.25 | 90.35 | 90.80 | 96.80 | 89.20 | 93.85 | 92.66 |
| 4 | 97.25 | 98.05 | 102.75 | 94.45 | 95.45 | 97.60 | 93.00 | 96.94 |
| Mean Values | 91.19 | 91.95 | 94.21 | 91.85 | 90.70 | 90.25 | 88.29 | 91.20 |
| | | 92.67 | | | 89.75 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 299.396 | 12.712** |
| Invstor | 1 | 0.010 | 0.000 |
| Trial Invstor | 3 | 44.133 | 1.874 |
| Storage | 2 | 19.701 | 0.836 |
| Temperature | 1 | 100.341 | 4.260 |
| Trial Storage | 6 | 42.486 | 1.804 |
| Trial Temperature | 3 | 105.278 | 4.470 |
| Storage Temperature | 2 | 8.175 | 0.347 |
| Residual | 6 | 23.552 | |
| Total | 27 | 70.324 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 1.834 | 2.101 | 1.981 | 4.203 | 3.963 | 2.427 |

**significant at 1 per cent level

TABLE 3:9

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the soluble ash content (per cent)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.37 | 0.39 | 0.39 | 0.34 | 0.42 | 0.36 | 0.50 | 0.40 |
| 2 | 0.42 | 0.42 | 0.39 | 0.49 | 0.42 | 0.47 | 0.52 | 0.45 |
| 3 | 0.38 | 0.39 | 0.48 | 0.43 | 0.36 | 0.39 | 0.48 | 0.42 |
| 4 | 0.43 | 0.48 | 0.46 | 0.38 | 0.39 | 0.42 | 0.60 | 0.45 |
| Mean Values | 0.40 | 0.42 | 0.43 | 0.41 | 0.40 | 0.41 | 0.53 | 0.43 |
| | | 0.42 | | | 0.44 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0092516 | 1.294 |
| Invstor | 1 | 0.0061783 | 0.864 |
| Trial Invstor | 3 | 0.0000883 | 0.012 |
| Storage | 2 | 0.0159600 | 2.233 |
| Temperature | 1 | 0.0063941 | 0.895 |
| Trial Storage | 6 | 0.0021559 | 0.302 |
| Trial Temperature | 3 | 0.0026417 | 0.370 |
| Storage Temperature | 2 | 0.0250280 | 3.502 |
| Residual | 6 | 0.0071475 | |
| Total | 27 | 0.0069005 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.03195 | 0.03661 | 0.03451 | 0.07322 | 0.06903 | 0.04227 |

trials varied significantly ($p < 0.01$). The soluble calcium values (Table 3:10) increased significantly ($p < 0.05$) during the storage period of seven days. Increase in soluble calcium levels was greater at a storage temperature of $6^{o}C$ than at $2^{o}C$. The soluble phosphorus values increased during the period of storage (Table 3:11). The significant interaction between storage and temperature effects, indicate that an increase in the storage period at $6^{o}C$ resulted in a significant increase in the soluble phosphorus level of the milks. No such increase occurred on storage at $2^{o}C$.

The titratable acidity and pH values of the milks (Tables 3:12 and 3:13) changed ($p < 0.001$) during the period of storage. The interaction between storage and temperature were very highly significant.

Highly significant differences were observed in the initial FPD of milks (Table 3:14) in different trials. The FPD of milks held at $2^{o}C$ did not alter over a period of storage of 7 days. The uncorrected FPD values of milks held at $6^{o}C$ increased. When the correction was made for acidity development in the sample held at $6^{o}C$ for 4 days it is still higher than the previous FPD of the same milk. The titratable acidity of milks stored for 7 days was higher than 0.3 per cent lactic acid which is the maximum limit for correction.

The free sulphydryl groups values (Table 3:15) showed significant differences in different trials and with different storage temperature. The sulphydryl groups values increased during storage of the milks at $2^{o}C$ and $6^{o}C$ and the increase was greater at the lower temperature.

The acid degree value (ADV) (Table 3:16) of milks in different trials showed significant difference ($p < 0.001$). Significant increases in the ADV took place on storage of raw milk at both $2^{o}C$ and $6^{o}C$.

Tables 3:17 and 3:18 represent starter activities in milk held at $2^{o}C$ and $6^{o}C$.* The activity (SAV) was determined by measuring the titratable acidity and pH of inoculated heat treated milk which had previously been cold stored at the two temperatures for various periods of time. Very highly significant differences were found in both SAV (by titration) and SAV (by pH) due to the temperature and period of storage. These changes are higher at $6^{o}C$ than at $2^{o}C$.

*Result of antibiotic tests: 1st and 4th trials -ve at 0.02 i.u.penicillin/ml
2nd and 3rd trials +ve at 0.02 i.u./ml.

TABLE 3:10

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the soluble calcium content (mM/kg)

| Trial | Storage at $2^{o}C$ | | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | Initial | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 3.70 | 4.18 | 4.13 | 3.29 | 4.53 | 4.48 | 8.87 | 4.74 |
| 2 | 4.79 | 5.66 | 5.81 | 7.48 | 5.82 | 6.61 | 11.37 | 6.79 |
| 3 | 4.30 | 7.18 | 7.20 | 4.46 | 4.53 | 4.35 | 14.29 | 6.62 |
| 4 | 5.39 | 5.96 | 7.65 | 5.68 | 4.96 | 5.47 | 18.01 | 7.59 |
| Mean Values | 4.54 | 5.75 | 6.20 | 5.23 | 4.96 | 5.23 | 13.13 | 6.43 |
| | | 5.72 | | | 7.78 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 20.3575 | 2.193 |
| Invstor | 1 | 33.3459 | 3.592 |
| Trial Invstor | 3 | 0.7917 | 0.085 |
| Storage | 2 | 71.4459 | 7.695* |
| Temperature | 1 | 50.4914 | 5.438* |
| Trial Storage | 6 | 4.0412 | 0.435 |
| Trial Temperature | 3 | 1.5549 | 0.167 |
| Storage Temperature | 2 | 102.9365 | 11.087** |
| Residual | 6 | 9.2841 | |
| Total | 27 | 21.5061 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 1.1517 | 1.3194 | 1.2439 | 2.6388 | 2.4879 | 1.5235 |

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

TABLE 3:11

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the soluble phosphorus content (mg/100 g)

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 31.95 | 30.05 | 33.35 | 34.20 | 34.80 | 37.70 | 50.80 | 36.12 |
| 2 | 31.45 | 34.25 | 32.55 | 47.85 | 38.75 | 39.10 | 54.45 | 39.77 |
| 3 | 38.15 | 33.70 | 38.65 | 36.10 | 28.80 | 30.45 | 49.65 | 36.50 |
| 4 | 37.50 | 37.25 | 36.50 | 30.25 | 32.35 | 33.15 | 58.70 | 37.96 |
| Mean Values | 34.76 | 33.81 | 35.26 | 37.10 | 33.67 | 35.10 | 53.40 | 37.59 |
| | | 35.39 | | | 40.72 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 38.445 | 0.638 |
| Invstor | 1 | 74.486 | 1.236 |
| Trial Invstor | 3 | 44.844 | 0.744 |
| Storage | 2 | 628.906 | 10.437* |
| Temperature | 1 | 341.333 | 5.664 |
| Trial Storage | 6 | 15.201 | 0.252 |
| Trial Temperature | 3 | 39.584 | 0.657 |
| Storage Temperature | 2 | 360.804 | 5.988* |
| Residual | 6 | 60.259 | |
| Total | 27 | 119.134 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Trial Temperature |
|---|---|---|---|---|---|---|
| SED | 2.934 | 2.964 | 5.929 | 3.361 | 3.169 | 6.338 |

*significant at 5 per cent level

TABLE 3:12

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the titratable acidity (per cent lactic acid)

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.150 | 0.155 | 0.155 | 0.165 | 0.155 | 0.165 | 0.282 | 0.175 |
| 2 | 0.155 | 0.165 | 0.168 | 0.175 | 0.165 | 0.175 | 0.370 | 0.196 |
| 3 | 0.155 | 0.160 | 0.165 | 0.170 | 0.165 | 0.170 | 0.375 | 0.194 |
| 4 | 0.155 | 0.160 | 0.170 | 0.175 | 0.165 | 0.175 | 0.380 | 0.197 |
| Mean Values | 0.154 | 0.160 | 0.164 | 0.171 | 0.162 | 0.171 | 0.352 | 0.191 |
| | | 0.165 | | | 0.228 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0007504 | 2.436 |
| Invstor | 1 | 0.0063763 | 20.696** |
| Trial Invstor | 3 | 0.0000724 | 0.235 |
| Storage | 2 | 0.0251461 | 81.618*** |
| Temperature | 1 | 0.024033 | 77.908*** |
| Trial Storage | 6 | 0.0002901 | 0.941 |
| Trial Temperature | 3 | 0.0002979 | 0.967 |
| Storage Temperature | 2 | 0.0206303 | 66.961*** |
| Residual | 6 | 0.0003081 | |
| Total | 27 | 0.0047735 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.00938 | 0.01075 | 0.01013 | 0.02150 | 0.02027 | 0.01241 |

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

TABLE 3:13

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture on the pH

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 6.75 | 6.75 | 6.73 | 6.66 | 6.75 | 6.67 | 5.85 | 6.59 |
| 2 | 6.71 | 6.67 | 6.61 | 6.51 | 6.67 | 6.53 | 5.41 | 6.44 |
| 3 | 6.74 | 6.72 | 6.62 | 6.55 | 6.70 | 6.48 | 5.53 | 6.48 |
| 4 | 6.64 | 6.65 | 6.49 | 6.48 | 6.64 | 6.21 | 5.50 | 6.37 |
| Mean Values | 6.71 | 6.70 | 6.61 | 6.55 | 6.69 | 6.47 | 5.57 | 6.47 |
| | | 6.62 | | | 6.24 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.058870 | 16.642** |
| Invstor | 1 | 0.264815 | 74.860*** |
| Trial Invstor | 3 | 0.003412 | 0.964 |
| Storage | 2 | 0.876429 | 247.755*** |
| Temperature | 1 | 0.847504 | 239.578*** |
| Trial Storage | 6 | 0.010240 | 2.895 |
| Trial Temperature | 3 | 0.004837 | 1.367 |
| Storage Temperature | 2 | 0.556303 | 157.260*** |
| Residual | 6 | 0.003537 | |
| Total | 27 | 0.157845 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.0318 | 0.0364 | 0.0343 | 0.0728 | 0.0687 | 0.0421 |

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

TABLE 3:14

The effect of storage of bulked raw milk, at 2°C and 6°C for various periods of time prior to cheese manufacture, on the freezing point depression (°C)

| Trial | Initial | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.529 | 0.532 | 0.528 | 0.529 | 0.531 | 0.533 | (0.547') 0.582 | 0.536 |
| 2 | 0.531 | 0.533 | 0.530 | 0.532 | 0.531 | 0.532 | 0.592 | 0.540 |
| 3 | 0.537 | 0.538 | 0.537 | 0.536 | 0.538 | 0.541 | 0.589 | 0.545 |
| 4 | 0.536 | 0.536 | 0.539 | 0.537 | 0.538 | 0.543 | 0.607 | 0.548 |
| Mean Values | 0.533 | 0.532 | 0.533 | 0.533 | 0.534 | 0.537 | 0.592 | 0.542 |
| | | 0.533 | | | 0.555 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.00018985 | 11.613** |
| Invstor | 1 | 0.00039010 | 23.863** |
| Trial Invstor | 3 | 0.00000490 | 0.300 |
| Storage | 2 | 0.00219304 | 134.153*** |
| Temperature | 1 | 0.00281667 | 172.302*** |
| Trial Storage | 6 | 0.00001338 | 0.818 |
| Trial Temperature | 3 | 0.00001189 | 0.727 |
| Storage Temperature | 2 | 0.00209179 | 127.960*** |
| Residual | 6 | 0.00001635 | |
| Total | 27 | | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.002161 | 0.002476 | 0.002334 | 0.004952 | 0.004669 | 0.002859 |

'corrected to the milk titratable acidity.

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

TABLE 3:15

The effect of storage of bulked milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture, on the free sulphydryl groups (μM/kg).

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 5.15 | 4.83 | 5.02 | 8.85 | 4.39 | 5.02 | 6.41 | 5.67 |
| 2 | 3.59 | 4.15 | 4.94 | 4.02 | 3.15 | 2.32 | 3.88 | 3.72 |
| 3 | 3.33 | 6.44 | 6.28 | 3.96 | 4.15 | 3.85 | 4.30 | 4.62 |
| 4 | 3.67 | 4.10 | 6.20 | 6.86 | 4.14 | 4.85 | 6.34 | 5.21 |
| Mean Values | 3.93 | 4.95 | 5.61 | 5.92 | 3.96 | 4.01 | 4.23 | 4.80 |
| | | 5.50 | | | 4.40 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 4.9376 | 5.721* |
| Invstor | 1 | 3.5177 | 4.076 |
| Trial Invstor | 3 | 0.5016 | 0.581 |
| Storage | 2 | 2.6285 | 3.045 |
| Temperature | 1 | 7.2051 | 8.348* |
| Trial Storage | 6 | 2.0946 | 2.427 |
| Trial Temperature | 3 | 0.1624 | 0.188 |
| Storage Temperature | 2 | 0.4286 | 0.497 |
| Residual | 6 | 0.8631 | |
| Total | 27 | 1.9033 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.497 | 0.569 | 0.536 | 1.138 | 1.073 | 0.657 |

*significant at 5 per cent level

TABLE 3:16

The effect of storage of bulked raw milk, at 2°C and 6°C for various periods of time prior to use for cheese manufacture, on the acid degree value

| Trial | Initial | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d. | 4 d | 7 d | 2 .d | 4 .d | 7 d | |
| 1 | 1.145 | 1.756 | 1.793 | 2.096 | 1.689 | 1.970 | 2.131 | 1.797 |
| 2 | 1.423 | 1.782 | 1.762 | 2.745 | 1.753 | 1.884 | 2.570 | 1.988 |
| 3 | 1.051 | 1.487 | 1.734 | 1.587 | 1.381 | 1.814 | 1.615 | 1.524 |
| 4 | 1.260 | 1.345 | 1.600 | 1.772 | 1.338 | 1.924 | 1.957 | 1.599 |
| Mean Values | 1.220 | 1.592 | 1.722 | 2.050 | 1.540 | 1.898 | 2.068 | 1.727 |
| | | 1.788 | | | 1.835 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.304944 | 94.320*** |
| Invstor | 1 | 1.202098 | 371.814*** |
| Trial Invstor | 3 | 0.020886 | 6.460* |
| Storage | 2 | 0.485624 | 150.206*** |
| Temperature | 1 | 0.013395 | 4.143 |
| Trial Storage | 6 | 0.100107 | 30.963*** |
| Trial Temperature | 3 | 0.011077 | 3.426 |
| Storage Temperature | 2 | 0.027254 | 8.430* |
| Residual | 6 | 0.003233 | |
| Total | 27 | 0.143407 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.0304 | 0.0348 | 0.0328 | 0.06696 | 0.0657 | 0.0402 |

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

TABLE 3:17

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture, on the titratable acidity of the incubated inoculated milks (SAV, per cent lactic acid)

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.39 | 0.39 | 0.41 | 0.37 | 0.44 | 0.51 | 0.63 | 0.45 |
| 2 | 0.34 | 0.33 | 0.43 | 0.47 | 0.36 | 0.44 | 0.72 | 0.44 |
| 3 | 0.33 | 0.35 | 0.37 | 0.36 | 0.43 | 0.43 | 0.58 | 0.41 |
| 4 | 0.27 | 0.40 | 0.41 | 0.54 | 0.46 | 0.45 | 0.75 | 0.47 |
| Mean Values | 0.33 | 0.36 | 0.40 | 0.44 | 0.42 | 0.46 | 0.67 | 0.44 |
| | | 0.40 | | | 0.52 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0046364 | 10.635** |
| Invstor | 1 | 0.0543025 | 124.564*** |
| Trial Invstor | 3 | 0.0045409 | 10.416** |
| Storage | 2 | 0.0551914 | 126.603*** |
| Temperature | 1 | 0.0798104 | 183.076*** |
| Trial Storage | 6 | 0.0052369 | 12.013 |
| Trial Temperature | 3 | 0.0003974 | 0.912 |
| Storage Temperature | 2 | 0.0213161 | 48.897*** |
| Residual | 6 | 0.0004359 | |
| Total | 27 | 0.0129589 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01116 | 0.01279 | 0.01205 | 0.02557 | 0.02411 | 0.01476 |

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

TABLE 3:18

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture, on the pH values of incubated inoculated milk (SAV by pH)

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 5.25 | 5.10 | 5.33 | 5.38 | 5.00 | 5.06 | 4.84 | 5.14 |
| 2 | 5.69 | 5.74 | 5.26 | 5.19 | 5.71 | 5.20 | 4.52 | 5.33 |
| 3 | 5.25 | 5.35 | 5.42 | 5.46 | 5.38 | 5.45 | 5.09 | 5.34 |
| 4 | 5.62 | 5.05 | 5.22 | 4.52 | 5.05 | 5.22 | 4.45 | 5.02 |
| Mean Values | 5.45 | 5.31 | 5.31 | 5.14 | 5.28 | 5.23 | 4.72 | 5.21 |
| | | 5.25 | | | 5.08 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.173941 | 17.575** |
| Invstor | 1 | 0.282735 | 28.568** |
| Trial Invstor | 3 | 0.105239 | 10.634** |
| Storage | 2 | 0.333017 | 33.649*** |
| Temperature | 1 | 0.177505 | 17.935** |
| Trial Storage | 6 | 0.121898 | 12.317** |
| Trial Temperature | 3 | 0.025204 | 2.547 |
| Storage Temperature | 2 | 0.090264 | 9.120* |
| Residual | 6 | 0.009897 | |
| Total | 27 | 0.111508 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.0532 | 0.0609 | 0.0574 | 0.1218 | 0.1149 | 0.0703 |

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

Calculation of the developed acidity in the inoculated milk was made by subtracting the original titratable acidity of inoculated milks from the titratable acidities after 5½ h of incubation (Table 3:17). The results are presented in Table 3:19. In these calculations it was assumed that the increase in acidity due to incubation is zero. The same calculation were used to measure the change in pH using the data presented in Tables 3:13 and 3:18. The results are presented in Table 3:20. Analyses of variance showed that trials had a significant ($p < 0.01$) effect on the level of development of acidity in the inoculated milk. Storage of milk at 2°C produced an increase in the development of acidity in the inoculated milks ($p < 0.01$). The increase was higher at 6°C ($p < 0.001$). Temperature of storage had a highly significant effect ($p < 0.01$). The pH values showed a significant effect ($p < 0.001$) due to trial. Storage, temperature and the interaction between them showed highly significant differences. The mean values of pH were not changed significantly up to 4 days of storage at 2°C. Further storage of 7 days was associated with a reduction in the pH value of milk ($p < 0.05$). Storage at 6°C for 2 days did not change the pH value of the milk but increasing the period of storage to 4 and 7 d caused reduction in the pH values of the milk.

The rennet clotting times (RCT) of milks in different trials varied significantly ($p < 0.001$, Table 3:21). Temperature and period of storage showed very highly significant differences at a storage temperature of 6°C, the RCT values after 2 d were higher than the initial values in two of the four trials. In the remaining two trials the values after 2 d were similar to the initial values. In all four trials the RCT values after 4 d of storage at 6°C were significantly less than the initial value. When the RCT test was made with milk held at 6°C for 7 d the values were dramatically lower ($p < 0.001$). Calculating the changes in RCT as a percentage of the initial RCT of milk, the storage at 2°C increased the RCT by 6.45, 6.64 and 3.04 per cent after 2, 4 and 7 d of storage. Storage at 6°C increased the RCT by 3.41 per cent and decreased it by 0.14 and 0.82 per cent after 2, 4 and 7 d of storage respectively.

Comparing the means for the different periods of storage, the mean values of RCT at 2°C after 2 and 4 d showed significant increase ($p < 0.05$) while after 7 d the RCT did not vary significantly from the initial.

TABLE 3:19

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture, on developed titratable acidity in inoculated milk after $5\frac{1}{2}$ h incubation

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.240 | 0.235 | 0.255 | 0.205 | 0.285 | 0.345 | 0.348 | 0.275 |
| 2 | 0.185 | 0.165 | 0.262 | 0.295 | 0.195 | 0.265 | 0.350 | 0.245 |
| 3 | 0.175 | 0.190 | 0.205 | 0.190 | 0.265 | 0.260 | 0.205 | 0.213 |
| 4 | 0.115 | 0.240 | 0.240 | 0.365 | 0.295 | 0.275 | 0.370 | 0.271 |
| Mean Values | 0.179 | 0.207 | 0.240 | 0.264 | 0.260 | 0.286 | 0.318 | 0.251 |
| | | 0.237 | | | 0.288 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0056028 | 7.754** |
| Invstor | 1 | 0.0241680 | 33.449** |
| Trial Invstor | 3 | 0.0038616 | 5.345* |
| Storage | 2 | 0.0065578 | 9.076* |
| Temperature | 1 | 0.0155550 | 21.528** |
| Trial Storage | 6 | 0.0042067 | 5.822* |
| Trial Temperature | 3 | 0.0013640 | 1.888 |
| Storage Temperature | 2 | 0.0000420 | 0.058 |
| Residual | 6 | 0.0007225 | |
| Total | 27 | 0.0042586 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01437 | 0.01646 | 0.01552 | 0.03292 | 0.03104 | 0.01901 |

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

TABLE 3:20

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on changes in pH of inoculated milks after $5\frac{1}{2}$ h incubation

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 1.50 | 1.65 | 1.40 | 1.28 | 1.75 | 1.61 | 1.05 | 1.46 |
| 2 | 1.02 | 0.93 | 1.35 | 1.32 | 0.96 | 1.33 | 0.89 | 1.11 |
| 3 | 1.49 | 1.37 | 1.20 | 1.09 | 1.32 | 1.03 | 0.44 | 1.13 |
| 4 | 1.02 | 1.60 | 1.27 | 1.96 | 1.59 | 0.99 | 1.05 | 1.35 |
| Mean Values | 1.26 | 1.39 | 1.30 | 1.41 | 1.41 | 1.24 | 0.86 | 1.27 |
| | | 1.37 | | | 1.17 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.202795 | 25.820*** |
| Invstor | 1 | 0.000372 | 0.047 |
| Trial Invstor | 3 | 0.096534 | 12.291** |
| Storage | 2 | 0.136629 | 17.396** |
| Temperature | 1 | 0.242004 | 30.812** |
| Trial Storage | 6 | 0.127062 | 16.178** |
| Trial Temperature | 3 | 0.051537 | 6.562* |
| Storage Temperature | 2 | 0.191554 | 24.389** |
| Residual | 6 | 0.007854 | |
| Total | 27 | 0.102253 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.0474 | 0.0543 | 0.0512 | 0.1085 | 0.1023 | 0.0627 |

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

TABLE 3:21

The effect of storage of bulked raw milk, at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture, on the rennet clotting time (s)

| Trial | Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d. | |
| 1 | 444 | 465 | 508*** | 517*** | 484** | 417* | 95** | 419 |
| 2 | 509 | 572*** | 543* | 578*** | 553** | 460** | 88** | 472 |
| 3 | 548 | 558 | 559 | 536 | 545 | 441** | 99*** | 469 |
| 4 | 608 | 650** | 638* | 543*** | 599 | 492*** | 90*** | 517 |
| Percentage of change+ | | 6.45 | 6.64 | 3.04 | 3.41 | -14.23 | -82.35 | |
| Mean Values | 527 | 561* | 562* | 543 | 545 | 452*** | 93*** | 469 |
| | | 556 | | | 364 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 11360.6 | 42.267*** |
| Invstor | 1 | 15653.6 | 58.239*** |
| Trial Invstor | 3 | 1166.9 | 4.342* |
| Storage | 2 | 123815.8 | 460.659*** |
| Temperature | 1 | 221247.6 | 823.155*** |
| Trial Storage | 6 | 1835.8 | 6.830* |
| Trial Temperature | 3 | 689.8 | 2.566 |
| Storage Temperature | 2 | 104590.4 | 389.130*** |
| Residual | 6 | 268.8 | |
| Total | 27 | 27629.4 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 8.76 | 10.02 | 9.47 | 20.08 | 18.93 | 11.59 |

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

+calculated as the percentage of increase in RCT of the initial samples

At 6°C the storage for 2 d did not show significant difference in RCT. Whereas a very highly significant difference was observed after 4 and 7 d.

Table 3:22 shows the correlation coefficient between RCT, SAV by titration, SAV by pH and milk composition. Highly significant correlations were observed between RCT and protein, NPN and soluble calcium while a significant correlation was observed between RCT and the soluble ash. Starter titratable acidity showed highly significant correlation with milk titratable acidity. Starter pH showed no significant correlation with the milk components which had been studied. The regression analysis for the correlation between RCT and protein and NPN; soluble, calcium, phosphorus and ash are presented in Tables 3:23 and 3:24 respectively.

The correlation coefficient and regression analysis were done excluding values for the milk held at 6°C for 7 d. Including these milk data would give a very highly significant variation than the mean values for all other treatments. This describes the absence of significance in the correlation between the FPD and the titratable acidity.

Fig. 3:1 showed that the increase of total milk protein and NPN contents increased the RCT. Fig. 3:2 showed that both the level of soluble calcium and soluble phosphorus in the milks increased the RCT to a similar degree whereas increased soluble ash in the milk was associated with greater increase in RCT values.

Slight relation was observed between the titratable acidity and pH of milk with the STA and SpH.

The results of determinations of FAAs in trials 3 and 4 are presented as reduced-size graphs of the amino acid analyser output and show the variations during the storage of raw milk at 2°C and 6°C.

Some of the peaks of ammonia, Gly and Glu acid were recorded in high concentration and in particular cases it is not possible to judge the changes in the concentration of these compounds unless the area of each is calculated. In such instances the area of the peaks were calculated and the numerical values are shown in Figs 3:3 to 3:6 These areas were calculated from the reduced chromatograms for each component. Each amino acid is usually represented in two peaks in the original

TABLE 3:22

The correlation coefficient between rennet clotting time (RCT) starter activity (SAV) by titratable acidity and by pH (SpH) with the milk composition initially and after storage for 2, 4 and 7 days at 2°C and for 2 and 4 days at 6°C

| | RCT | SAV by titratable acidity | SAV by pH |
|---|---|---|---|
| Protein | 0.5797** | -0.1808 | -0.0362 |
| NPN | 0.5231** | 0.0685 | 0.0863 |
| Soluble calcium | 0.5344** | 0.0515 | -0.0242 |
| Soluble phosphorus | 0.2862 | -0.0614 | 0.0969 |
| Soluble ash | 0.4458* | -0.1336 | 0.1155 |
| Fat | -0.5237** | 0.1158 | 0.0724 |
| Titratable acidity | 0.0829 | 0.5399** | -0.2159 |
| pH | -0.0444 | -0.3875 | 0.1847 |
| FPD | 0.3281 | 0.0913 | 0.0913 |

DF = 22

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

TABLE 3:23

The regression analysis for the effect of protein, NPN and fat contents of milk on the rennet clotting time (RCT)

| | Estimate | S.E. | T |
|---|---|---|---|
| Y Intercept (Protein) | 2.50991344 | 0.14707887 | 17.07 |
| " (NPN) | 0.05561129 | 0.03941159 | 1.41 |
| " (Fat) | 4.2446566 | 0.1871848 | 22.68 |
| Slope (Protein) | 0.00091691 | 0.00027477 | 3.34 |
| (NPN) | 0.00021199 | 0.00007373 | 2.88 |
| (Fat) | 0.0010083 | 0.0003497 | -2.88 |

Analysis of Variance

| | DF | Protein | | NPN | | Fat | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 0.0722 | 0.072198 | 0.00386 | 0.0038593 | 0.0873 | 0.08730 |
| Residual | 22 | 0.1426 | 0.006483 | 0.01024 | 0.0004655 | 0.2310 | 0.01050 |
| Total | 23 | 0.2148 | 0.009341 | 0.01410 | 0.0006131 | 0.3183 | 0.01384 |

Percentage variance accounted for 30.6 (Protein)
" " " " 24.1 (NPN)
" " " " 24.1 (Fat)

TABLE 3:24

The regression analysis for the effect of soluble calcium, phosphorus and ash on the rennet clotting time (RCT)

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (soluble calcium) | -0.375184 | 1.931963 | -0.19 |
| " (soluble phos.) | 24.671890 | 7.382710 | 3.34 |
| " (soluble ash) | 0.2483856 | 0.06998080 | 3.55 |
| Slope (soluble calcium | 0.010703 | 0.003609 | 2.97 |
| " (soluble phos.) | 0.019326 | 0.013792 | 1.40 |
| " (soluble ash) | 0.00030539 | 0.00013074 | 2.34 |

Analysis of Variance

| | DF | Soluble calcium | | Soluble phosphorus | | Soluble ash | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 9.84 | 9.838 | 32.1 | 32.07 | 0.00801 | 0.008009 |
| Residual | 22 | 24.61 | 1.119 | 359.4 | 16.34 | 0.03229 | 0.001468 |
| Total | 23 | 34.45 | 1.498 | 391.5 | 17.02 | 0.04030 | 0.001752 |

Percentage variance accounted for 25.3 (Soluble calcium)
" " " " 4.0 (Soluble phosphorus)
" " " " 16.2 (soluble ash)

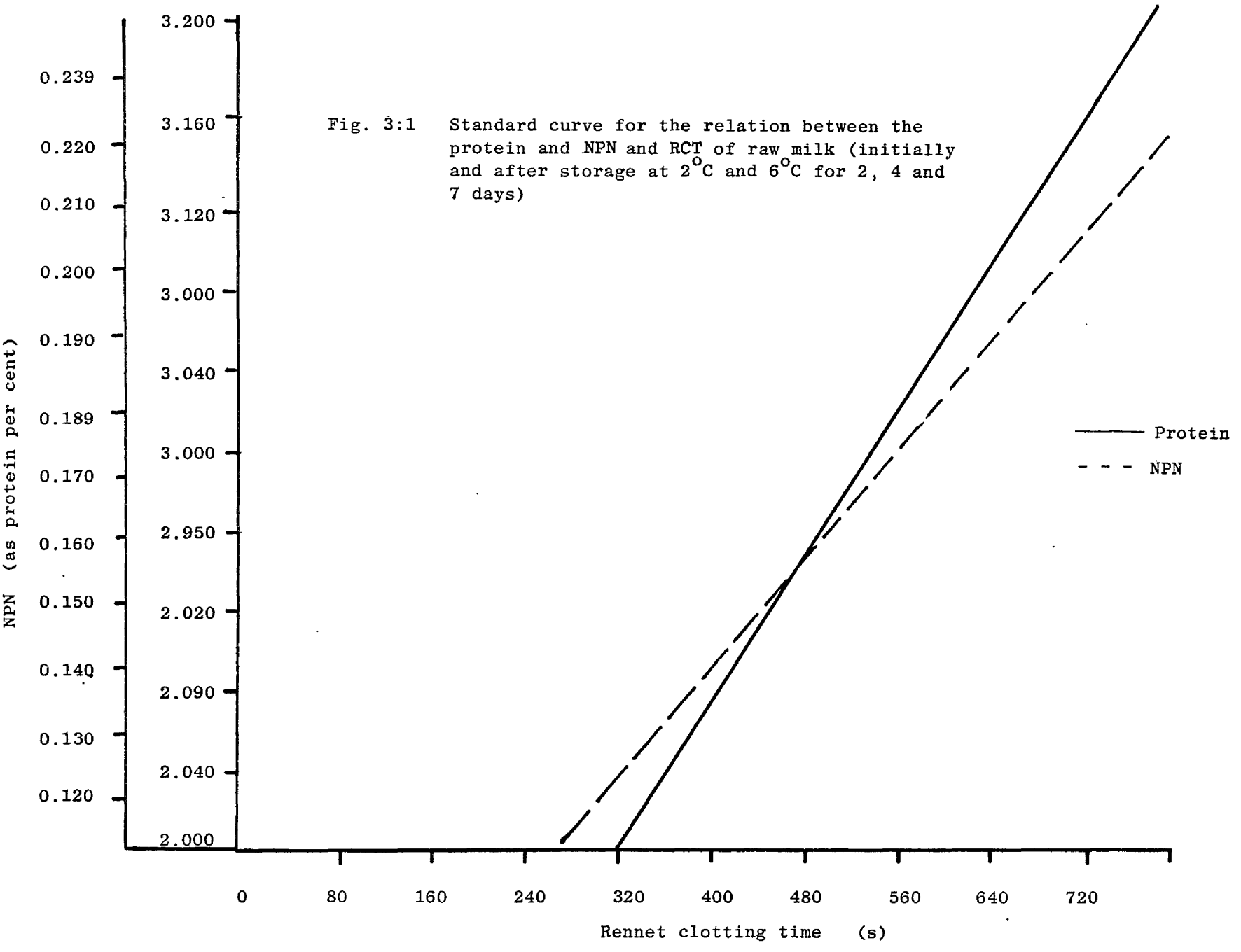
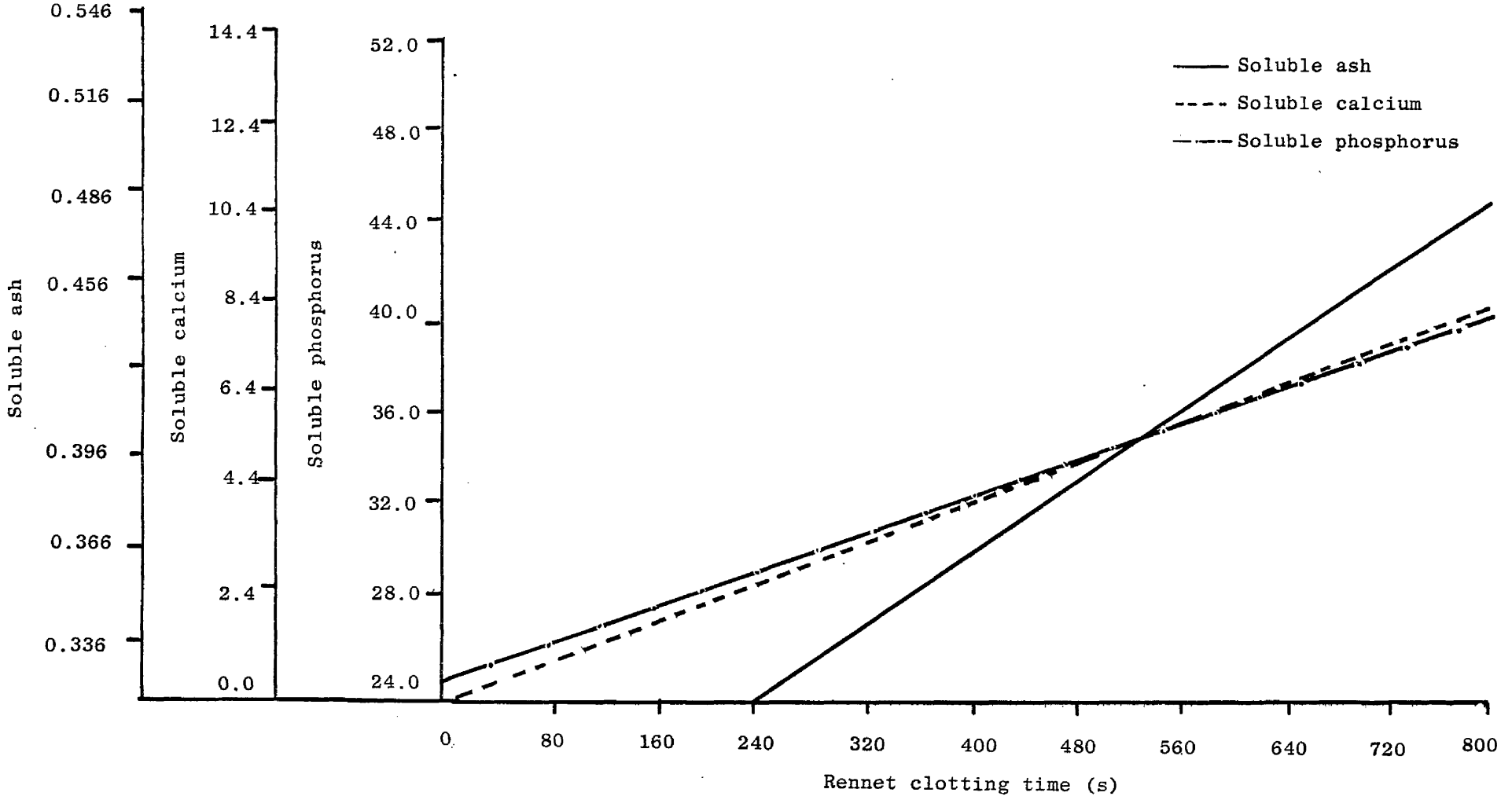



Fig. 3:1 Standard curve for the relation between the protein and NPN and RCT of raw milk (initially and after storage at 2°C and 6°C for 2, 4 and 7 days)

Fig. 3:2 Standard curve for the relation between the soluble calcium, phosphorus and ash and RCT of raw milk (initially and after storage at 2°C for 2, 4 and 7 days and at 6°C for 2 and 4 days)



chromatogram, the small peak represents the concentration of amino acid and the large peak represents a x3 concentration. Where the values are shown, the areas of peaks which were too big to be drawn in the chromatogram, were calculated from corresponding small peaks and then multiplied by a factor of three.

a. Trial Three

Fig. 3: 3 shows the effect of storage of raw milk at $2^{o}C$ on the level of FAAs. The highest determined peaks were ammonia, Glu acid and Gly in milks. The other amino acids observed were Lys, His, Arg, Asp, Thr, Ser, Pro, Ala, Cys, Val, Ile, Leu, Tyr and phe. A group of five peaks were also observed and represent acidic phosphoseryl peptides.

During the storage of milk at $2^{o}C$, Lys increased after 2 and 4 d but further storage to 7 d caused slight reduction in its concentration in the milk. The ammonia peak decreased after 2 and 4 d of storage, but increased from the 4th to the 7th d of storage. Asp, Glu, Ala and Val amino acids decreased in the milk over the period of storage of 7 d at $2^{o}C$ but the values after 4 d of storage were slightly higher than the values at 2 d or 7 d. Gly and Thr had decreased after 2 d of storage but thereafter increased during the remainder of the 7 d period at $2^{o}C$. His, Ser, Pro, Cys, Ile, Leu, Thr and Phe decreased as the period of storage increased. The phosphoryl peptides had decreased after 2 d of storage at $2^{o}C$, but further storage for 4 and 7 d resulted in an increase in these compounds.

Storage of milk at $6^{o}C$ for up to 4 d resulted in increases in the concentration of Lys and ammonia. Further storage to 7 d caused a slight decrease in the concentration in the milk. Levels of Arg, His, Ile, Leu, Try and Phe showed a slight decrease after 2 d of storage, and after 4 and 7 d these compounds had almost disappeared. Asp acid increased markedly after 2 d of storage, but then decreased after 4 and 7 d of storage. Thr showed a slight increase after 2 d of storage and then decreased dramatically after 4 and 7 d of milk storage. Levels of Ser decreased rapidly during the storage of milk while Pro and Cys were decreased slightly (Fig. 3:4).

Fig. 3:3 Chromatographic analysis of the free amino acids of bulked milk on delivery by road tanker and after storage at 2°C for various periods of time prior to cheese manufacture, Trial 3.

Fig. 3:4 Chromatographic analysis of the free amino acids of bulked milk on delivery by road tanker and after storage at 6°C for various periods of time prior to cheese manufacture, Trial 3.

Glu acid decreased in amount during the period of storage. Gly increased over the first 4 d of storage then decreased to about half its original concentration by the 7th d of storage. The concentration of Ala was unchanged after 2 d of storage and thereafter was slightly reduced when determined after a storage of 7 d. The value for Ala was higher than the original. The level of Val showed an increase after 2 days of storage but thereafter decreased and was absent after 7 d of storage.

The peaks for phosphoseryl peptides, 1, 3 and 4 increased after storage for 2 d but decreased after further storage of 4 and 7 d. The peak for phosphoseryl peptide No. 2 decreased after 2 and 4 d of storage but thereafter increased during the remainder of the 7 d of storage.

b. Trial Four

The same amino acids observed in trial 3 were observed in trial 4 but in the case of the initial sample (raw milk on delivery by road tanker) the duplicate did not show the presence of Ile, Leu, Try, Phe (Cys could be seen on the original chromatogram). As a result of storage of the milk at 2°C (Fig. 3:5) for 2 to 7 d ammonia increased. Lys increased after 2 d of storage and thereafter decreased on further storage. All other amino acids decreased as the period of storage was increased.

The storage of milk at 6°C for 7 d resulted in an increase in the concentration of ammonia but the quantitative value for this increase could not be calculated. Lys decreased after 2 d of storage of the milk at 6°C and remained at this level during further storage. His appeared more clearly after 2 and 4 d of storage and showed a slight increase when the milk was stored a further 3 d. The small amount of Arg present in the milk on delivery was absent in the stored milks. The level of Asp acid decreased after storage of the milk for 2 d at 6°C. Thr and Ser peaks were absent on examination of the milk after 2 and 4 d of storage but after 7 d they were present. Glu acid was reduced by storage for 2 and 4 d. However, after the milk had been held for 7 d this amino acid was present at about one-third of its initial level in the milk. Almost all of the Pro disappeared on storage

Fig. 3:5 Chromatographic analysis of the free amino acids of bulked milks on delivery by road tanker and after storage at 2°C for various periods of time prior to cheese manufacture, Trial 4.

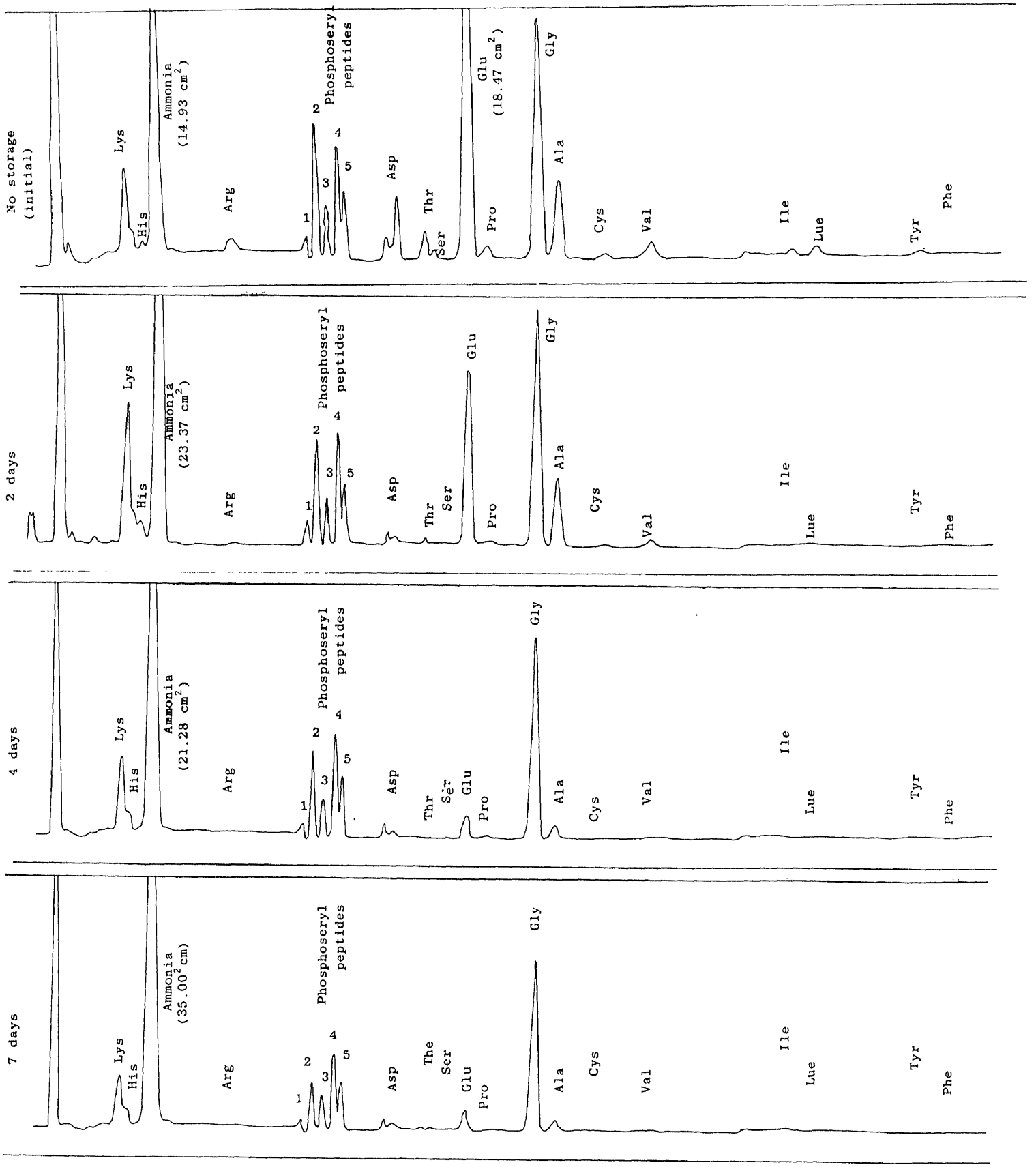
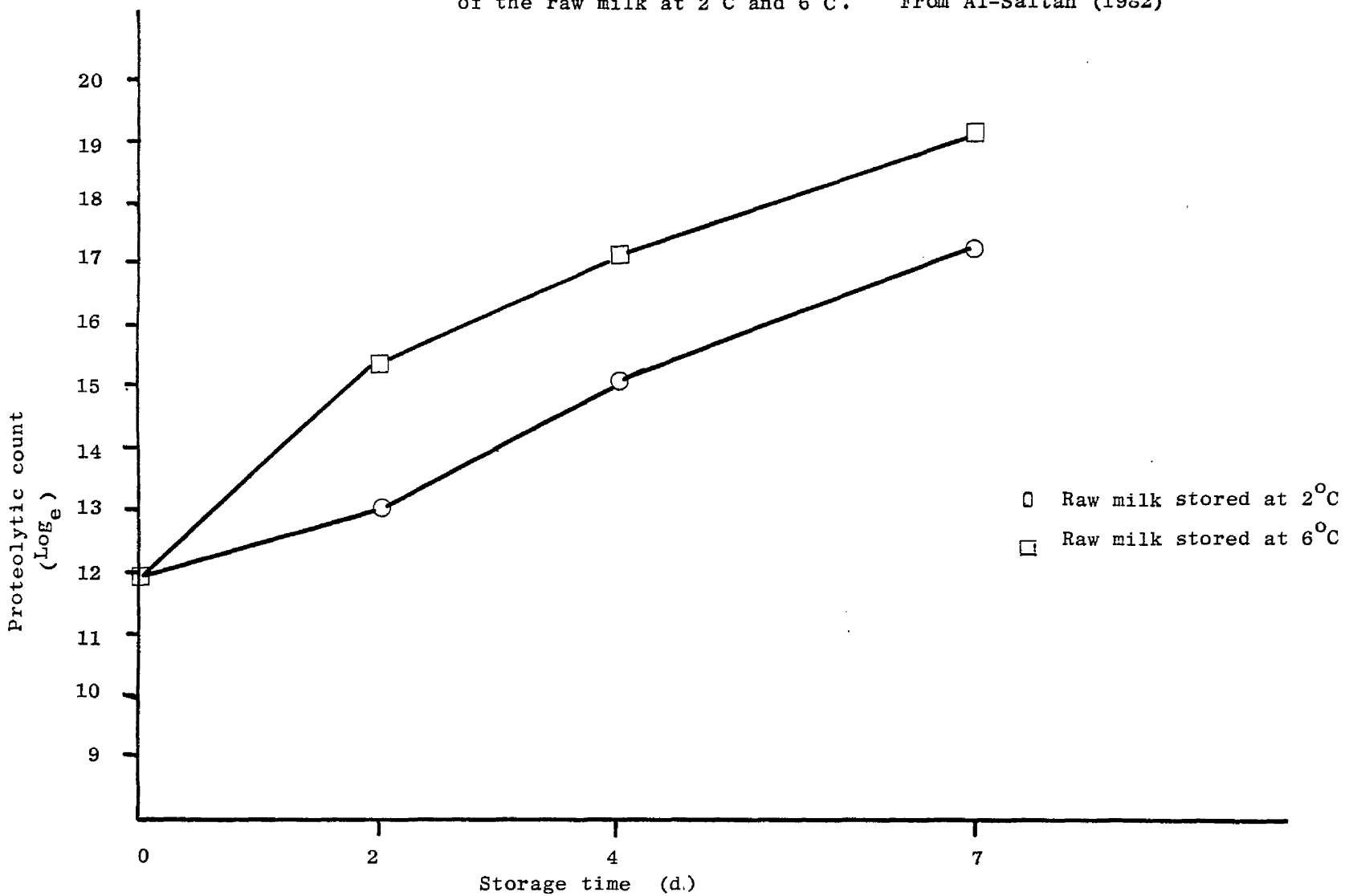


Fig. 3:6 Chromatographic analysis of the free amino acids of bulked milks on delivery by road tanker and after storage at 6°C for various periods of time prior to cheese manufacture, Trial 4.

Fig. 3:7 Changes in the proteolytic count after various periods of storage of the raw milk at 2°C and 6°C. From Al-Saltan (1982)



on the distribution of micellar and soluble proteins (Nakanishi et al., 1968).

The increase in the amount of soluble calcium and phosphorus which were observed after the storage of milk may be due to transfer of micellar calcium and phosphorus into the soluble phase. These results are in agreement with the results of Ubren and Ramananskas (1971), Sabarawal and Ganguli (1972; Youssef et al. (1975), Flükiger (1976) and Ali et al. (1980a). Storage at 2°C resulted in smaller increases in soluble calcium and phosphorus than occurred at 6°C. This result might be associated with microbial action which is greater at the higher temperature.

Recently Ali et al. (1980b) reported that both decreasing and increasing level of pH caused increases in soluble caseins. Both soluble Ca and soluble phosphate showed a steady pH dependence, being lowest at the highest pH used (6.2 to 7.0). Rajput and Ganguli (1981) stated that more than 90 per cent of the calcium from casein micelles of cow's, buffalo's and goat's milks was released with lowering of the pH of milk to 5.0. Decalcification from large micelles was relatively faster than from medium or small sized micelles as the pH was lowered. They suggested involvement of phosphate, probably linked to threonine and serine and imidazole groups, in calcium-casein interaction.

The titratable acidity of milk held at 2°C up to 7 d and at 6°C for 4 d did not vary significantly in all trials. Storage of milk at 6°C up to 7 d resulted in significant increase ($p < 0.001$). The pH values of milk held at 2°C was decreased ($p < 0.05$) after 4 d of storage and after 7 d further decrease in pH occurred ($p < 0.01$). Storage at 6°C showed more decrease ($p < 0.001$) after 4 and 7 d.

Complementary to the studies in which Cheddar cheese was produced from milks held at 2°C and 6°C for periods up to 7 d (Chapter Four) were the laboratory tests to determine the effect of the milk storage conditions on acid production by the starter bacteria used in the cheese vats. The difference between the titratable acidities and pH values on inoculation of milk and after incubation at a suitable growth temperature for mesophilic starters such as 30°C for 5½ h is a 'measure of the acidity' of the starter bacteria under the defined conditions.

of the milk. Gly decreased to about half of its original level when the milk was stored for 2 and 4 d and by the end of the 7 d storage period at 6°C was almost absent. The level of Ala had decreased after 2 d of storage but thereafter increased in milk held at 6°C for 4 and 7 d. Cys, Val, Ile and Leu were absent in milks tested after 2 and 4 d of storage but appeared in the milks held for 7 d. The phosphoseryl peptide peaks decreased after 2 d of storage and remained unchanged during the further storage of up to 7 d. (Fig. 3:8)

There were variations between the two trials in the influence of cold storage on the quantity of some amino acids.

## DISCUSSION

Milk consists of more than 85 per cent water. The lactose and the salts chiefly form genuine solutions. The protein substances form colloidal solutions. Fat exists in the form of small globules. Milk is simultaneously a genuine and a colloidal solution and at the same time is also an emulsion. Cooling, heating and physical treatments may result in distinct shifting of the equilibrium which exist when the milk leaves the udder of the cow.

The casein exists in micelle form. These consist of the different caseins and calcium, phosphate, citrate and other ingredients of milk that are only found in small amounts. The composition of the casein micelles and the serum of the milk maintain a very unstable equilibrium. During cooling of milk, calcium, phosphate, beta casein and proteose are discharged from the linkage to the micelles (Flückiger, 1976).

In this study the major components of milk (total solids, fat, protein, ash, calcium, phosphorus) did not vary in quantity from their original level during the storage period of seven days but changes took place in the type and the state of these components.

During storage there were increases in NCN which indicates the occurrence of proteolysis of caseins. The level of NPN did not show any statistical variation during storage but this may be due to the low NPN content of milk and the inability to detect minor changes by micro-Kjeldahl method. The temperature of milk plays a significant factor

In the trials it was found that at 2°C the titratable acidity of the inoculated stored milks was increased significantly after 4 and 7 d. There was an increase in the titratable acidity in the fourth trial even after 2 d. But, at 6°C this increase was higher.

Subtracting the original titratable acidity of the milk before inoculation from the final titratable acidity after inoculation, the resultant figures showed that storage at 2°C for 4 d did result in change, except the fourth trial. Storage at 6°C increased significantly the titratable acidity of the inoculated milk even after subtracting the original development in the stored milks.

The pH values for the inoculated milks showed the same variation as for the titratable acidity of milk. The changes in pH due to incubation with the added starter culture (Table 3:20), showed variation between trials. The change in pH was greater in milks after 2 d than at 4 and 7 d in those milks held at 2°C. At 6°C the change in pH was lower after 4 and 7 d than after 2 d. This decrease was greater at 6°C than at 2°C.

The reduction in change in pH after 4 and 7 days at both temperatures may be due to the accumulation of lactic acid due to the action of starter micro-organisms on longer stored milk or might be due to the presence of heat resistant inhibitors. Duthie et al. (1976) found that when normal micro-organisms in raw milk increased in sufficient numbers to decrease the pH of milk to 6.1, heat sensitive inhibitors were produced. They reported that lactic acid was not responsible for inhibition at pH values between 6.5 and 6.1. But, as the bacterial numbers from a starter culture added to raw milk increased to millions and pH decreased to 6.3 and 6.1, compounds were synthesised that inhibited growth of Bacillus subtilis and Sarcina lutea.

The difference observed between the acidity as measured by titratable acidity and pH is mainly due to buffer capacity of milk. The buffer capacity of milk, which provides such a stable system involves protein, phosphates, citrates and carbonates mainly. The calcium and magnesium in the system are present both as free ions and as complexes with casein, phosphates and citrates. Furthermore, both the calcium and magnesium influence the titration of milk, since they can precipitate

as colloidal phosphates. The buffering capacity is low at the end point of phenolphthalein (pH 8.3), which makes this indicator useful for titration.

On the other hand, the pH value is a measure of the hydrogen ions dissociated in a solution. It does not measure the acidity as, for instance, by a titration. The pH value is a measure of the ionic dissociation in a solution and therefore measures the reactivity of the elements in that solution. It is therefore, a value which affects biological or biochemical reactions, as opposed to the titration acidity in the solution which involves buffer capacity (pH of milk up to pH 8.3). There is no correlation between pH values and titratable acidity measurements, so the cheesemaker cannot automatically switch from one form of measurement to the other (Scott, 1981).

The FPD did not vary even in milk held for 7 d at $2^{o}C$. At $6^{o}C$ up to 4 d,only slight increase in the FPD took place and this was accompanied by a slight increase in titratable acidity. After 7 d the maximum depression in freezing point was observed ($p < 0.001$) which is associated with the maximum increase in titratable acidity of that milk.

Souring, which involves a net increase in the number of molecules in solution as lactose (and sometimes citrate) is degraded, results in a lowering of the freezing point. On the other hand, the freezing point is determined principally by the concentration of small molecules and ions in solution (Jenness and Patton, 1959).

Van der Have et al. (1980) stated that notwithstanding considerable differences in the composition of the separate milkings, the sum of the contribution of lactose, chloride and phosphates to the FPD does not show much variation. The role of phosphate is of less importance than of the other constituents in this function.

The main origin of -SH groups in milk is serum protein. Of the milk serum protein, β-lactoglobulin is the main origin of -SH groups. The proteins associated with the fat globule membrane, which may or may not be similar to or identical with the milk serum proteins, also serve as an origin. Sulphydryl groups are considered to be occluded or bonded in such a way in the native protein as to be relatively unreactive (Jenness and Patton, 1959). The same authors considered

that when the protein particle is subjected to sufficient heat it uncoils and the groups become more accessible and reactive. Thus, SH groups may not be essential for complex formation, although they may be involved in preliminary self-aggregation of the β-lactoglobulin (McKenzie, 1970).

Watanabe and Klostermeyer (1976) found that the -SH groups of β-lactoglobulin A decreased with increasing time and temperature (75 and 95$^{o}$ for up to 40 min at pH 6.9). This effect was only small when $O_2$ was excluded. The decreases of -SH groups in the $O_2$- containing system were reflected in increases of -SS groups. They observed losses in the total -SH and -SS groups which may be due to the formation of volatile compounds such as $H_2S$, mercaptans, sulphides and disulphides and of non-volatile compounds such an lanthionine, sulfenic and sulfinic groups.

In this study, storage up to 7 d at both temperatures did not significantly affect the level of free -SH groups. However, there was a slight increase with the increase of period of storage at both temperatures. Milks held at 6$^{o}$C gave lower -SH values than the same milks held at 2$^{o}$C. It would appear possible that more rapid oxidation or increased bacterial enzyme activity at the higher temperature brought about this result.

The results which relate to the sulphydryl groups would need further discussion in details on the possible aggregation within κ-casein itself and between α- and κ-caseins referring to McKenzie (1970). Most of the κ-caseins are highly associated in solution at neutral pH. The chain of κ-casein is linked by an intermolecular disulphide bridge, and the polymers so formed are themselves able to undergo considerable polymerization through non-covalent forces. Noble and Waugh, 1965 (as cited by McKenzie, 1970), found, on sedimentation of synthetic mixture of $\alpha_s$- and κ-casein that α-κ interaction occurs readily at 37$^{o}$C but require pretreatment with urea or alkali at 20$^{o}$C, and this does not occur at 5$^{o}$C. Thus it was concluded that there is no appreciable α -κ-casein interaction at 2$^{o}$C. Swaisgood et al., 1964; Mackinlay and Wake, 1964 (as cited by McKenzie, 1970) stated that the disulphide bonds linked together individual κ-casein molecules to form aggregates. Swaisgood et al., 1964 (McKenzie, 1970) however stated that it seems likely that the cross-linking of κ-caseins

by disulphide bonds occurs randomly and that the degree of cross-linking varies widely. In the view of the author, cooling of milk may make the peptides within the molecules close to each other through different bonds such as SS-groups which would reduce the free -SH in the milk.

Storing the milk significantly increased the release of free fatty acids due to native and microbial derived lipases. The correlation coefficient between lipolytic count and ADA is discussed in Chapter Seven.

There was an increase in the RCT of milk held at $2^{o}C$ for up to 7 d. However, milks stored at $6^{o}C$ gave an increase in RCT values when stored for up to 2 d but thereafter the RCT values declined. This decline in RCT values is associated with the development of acidity in the milk. Al-Obaidi (1980) found that calf rennet activity was decreased gradually with increase in pH, and at pH 6.9 the coagulant was inactive. Decrease in pH increases the rate of coagulation (Cheeseman 1981). However, he stated that the nature of the gel formed also changes: lower pHs tend to give rise to a coarse type coagulum, while addition of calcium yields a harder gel.

In the milk stored at $2^{o}C$ the increase in RCT may be due to size and composition of casein micelles.

These results are in agreement with the results of other workers who noticed increases in RCT of the cold stored milk (Fricker,1958; Peltola and Vogt, 1959; Swartling, 1965; Swartling and Johansson, 1965; Vassal and Auclair, 1966; Aapola and Antila, 1970; Antila, 1971; Thomas, 1971; Losi et al., 1974; Szakaly, 1974; Youssef et al., 1975; Wiles, 1977; Amram and Lenoir, 1978; Ali et al., 1980a; Garnot, et al., 1981).

It was reported by Rose (1968) that some of the β-casein incorporated into the micelles in the cow's udder dissolves after milking when the milk is chilled and ceases to be a part of the micelle. Nakanishi et al. (1968) found that from 6 to 15 per cent of the total casein moved out of the micelles on cooling from $30^{o}$ to $5^{o}C$, and β- and the $\alpha_{s}$- and κ-caseins represent 46, 30 and 23 per cent of this decrease, respectively.

Flükiger (1976) found that the amount of β-casein in the serum of milk is increased by 100 per cent when the milk is cooled from 20° to 5°C. Shidlovskaya and Patratii (1976) reported that the average diameter of casein micelles decreased proportionally to the duration of storage (for over 3 d in the range of 3-15°C).

Two stages are involved in the formation of a gel or coagulum when rennet is added to milk (Cheeseman, 1981). The first is an enzymic stage characterized by increase in nitrogenous components soluble in 12 per cent TCA - soluble peptides are derived from κ-casein molecules in which the enzyme hydrolyses a single phenylalanine - methionine bond to yield para-κ-casein and a glycomactopeptide soluble in TCA. The second stages involve the aggregation of the enzymically altered casein micelles to form gel structure.

Cheeseman (1981) stated that increasing temperature and calcium ion concentration increases the rate of gel formation. Ekstrand (1980) found that the coagulation time for milk with chymosin and commercial rennet extract varies with the micelle size. The effect is more pronounced with chymosin than with the rennet extract and appears to be related to the availability of κ-casein. Therefore, the largest micelles, with a lower κ-casein content showed longer coagulation times than medium size micelles. In the region of the smallest micelles this time increases again, probably due to an increased β-casein content. Ekstrand et al. (1981) found also that the largest and the smallest micelles have lower rennetability than the medium size micelles.

In general, the storage of bulked milk at 2°C and 6°C for up to 7 d resulted in decreased levels of amino acids. On the other hand there were cases where storage had a different effect on individual amino acids.

The major peaks detected of chromatograms of raw milk were ammonia, glutamic acid and glycine. These findings are in agreement with the findings of Storgårds and Lindqvist (1962).

Milk is a complex biological system and changes in the FAA content should be seen in this context and also in relation to the possibility of various enzymatic and microbiological activities.

The increase in FAAs was higher where milks were held at 6°C than 2°C and these changes corresponded with the increases in the numbers of

proteolytic bacteria of these milks (Fig. 3:7). The growth of these organisms requires some essential amino acids such as Phe, Val, Leu, Ile, Ayg and Lys.(Berkeley and Campbell,1979). These FAAs resulting from the breakdown of milk proteins either decreased as a result of bacterial action or remained steady in some cases where the rate of milk protein breakdown was equal to the rate of their use in bacterial metabolism.

The effect of different temperatures and period of storage on the FAAs is in agreement with the variations observed in the NCN content of milk during cold storage (see Table 3:4).

The acidic peptides originate from the partial breakdown of caseins (proteolysis). These peptides are acidic due to the presence of phosphate in their composition. As a result of storage, some of these peptides decreased while others increased. Storgårds and Lindqvist (1962) found that the p-ethanolamine and glycero-p-ethanolamine showed the fastest decrease of all ninhydrin-positive substances and it may be assumed that they are attacked by the bacteria chiefly to liberate energy. They also reported that the 'simultaneous' rapid increase in the level of ethanolamine confirms that the splitting away of phosphate is the primary factor in degradation.

These four peptides observed in this study occupy the same positions on the chromatogram of ninhydrin positive peptides which were named by Storgårds and Lindqvist (1962) as glycero-phospho-ethanolamine, phosphoethanolamine, taurine and urea. However, no peak was observed in this study in the positions detected by the same authors and identified as phosphoserine. In the opinion of Manson (1982) it is rather difficult to assume that phosphoserine will be free in the milk.

Although Deutch and Samuelsson (1959) detected phosphoserine free in the milk besides ethanolamine, glyceroethanolamine, citrullin, urea, hexosamines and many other substances. The author believes that with the background of development, since the work of these authors, in amino acid determination techniques, it would be of interest to isolate these peptides and confirm their composition and also determine the factors which encourage the presence of these peptides at this stage of proteolysis.

## CONCLUSION

Differences were observed between different trials in relation to the composition and properties of the milk subjected to storage at low temperature. Less deterioration took place in the properties and characteristics of the milk held at $2^{o}C$ than at $6^{o}C$. Storage at both temperatures had no significant effect on the total solids, fat, protein, total calcium, total phosphorus, total ash and NPN. Non-casein nitrogen increased slightly after 7 d at $2^{o}C$ but it increased even after 2 d in the milk held at $6^{o}C$. Storage of milk at $2^{o}C$ for 7 d and up to 4 d at $6^{o}C$ had no significant effect on soluble calcium, soluble phosphorus, titratable acidity and FPD. The pH values showed significant decrease only after 4 and 7 d of storage at $6^{o}C$. Free sulphydryl groups increased during the storage of milks at $2^{o}C$ even after 2 d. On the other hand, at $6^{o}C$ there was no significant increase in free -SH groups. The ADV increased at both temperatures after storage of 2 d and it showed further increase with further storage. The highest values noticed in milk held at $6^{o}C$ for 7 d.

The development of acidity as a result of the addition of starter culture was higher in the milk stored for 7 d at $2^{o}C$ compared with the initial milk. In the case of storage at $6^{o}C$, this development in acidity appeared even after 2d. Starter culture seems to grow more actively in cold-stored milk compared with initial milks.

Rennet clotting time increased in the milks held for 2 and 4 d at $2^{o}C$ but it was similar to the initial after 7 d. The increase in RCT is believed to be due to the changes in size of micelles and the decrease of calcium ions. In the milks held for 7 d at $2^{o}C$ and 2 d at $6^{o}C$, the slight development in the acidity resulting from bacterial activity diminished the possible increase in the RCT caused by the storage of milk. In the milks held at $6^{o}C$ for 4 and 7 d the RCT was decreased due to the development of acidity. There was a significant correlation between RCT and protein, NPN, soluble calcium, ash and fat content of milks.

The major peaks of FAAs observed in the chromatograms of milk stored at low temperature represents ammonia, Glu and Gly. Ammonia and Lys increased as a result of storage, so indicating the proteolytic activity in the milk. Other essential amino acids were either slightly decreased or remained unchanged during storage.

Unidentified acidic peptides resulting from the partial breakdown of casein were also detected.

# CHAPTER FOUR

## THE EFFECT OF COLD STORAGE OF RAW MILK PRIOR TO CHEDDAR CHEESE MANUFACTURE ON ITS YIELD, COMPOSITION AND RIPENING

### INTRODUCTION

In the last nineteen years the quantity of milk produced in the United Kingdom and other EEC countries (Germany, France, Italy, Netherlands, Belgium, Luxemburg, Denmark and Republic of Ireland) has increased. The annual milk yield in kg per cow range from 3,577 to 4,790 kg in the U.K. and 2,943 to 4,055 in the EEC countries at 1960 (the nine member states*) and 1979, respectively. The milk delivered to dairies in these countries during the same period of time has increased from 10,222 to 15,093 thousand tonnes and from 57,315 to 93,157 thousand tonnes, respectively (Milk Marketing Board, 1980).

With the advent of refrigeration of milk on the farm and the development of current milk transportation procedures, a new factor has crept into the cheese yield picture. This factor is the growth of psychrotrophic bacteria in cold stored milk. Although much research has been done to characterize the effect of these micro-organisms on milk, little has been done to determine how they effect cheese yield. Under U.K. conditions most milk is collected from farms on a daily basis. In some seasons of low production however, the milk may be transported from farms to factories, on alternate day collection. In some countries alternate day collection is more common and some portion of that milk is at least 36 hours old. When received at the dairy factory many cheese factories operate under optimum plant utilisation where milk is stored for the following days operation. Therefore, under these conditions some of the milk would be 60 to 72 hours old (Hicks et. al.,1980).

These authors have also stated that some cheese plants purchase surplus grade A milk (the quality specified for the liquid milk market in U.S.A.) that has been pumped and chilled through a transfer station before it is shipped to the cheese factory. The practice often adds more than

*Figures for countries who were not members of the EEC at 1960 were also included.

48 h to the storage time before the milk can be manufactured. Therefore, surplus grade A milk used for cheese production in U.S.A. is often more than 5 d old before it reaches the vat. Obviously, these storage practices can greatly increase the final bacterial count.

A special temperature treatment or thermization of bulk collected milk may be desirable for the reduction of the bacterial count and also for the chemico-physical recovery of the casein structures (Hadland, 1978). Thermization is a means of controlling the microbial flora of collected milk for better keeping quality of the dairy, by destroying most of the psychrotrophic flora during warming to 63-65$^{o}$C for about 15 s. If thermization could be adopted to include counteracting the effect of Clostridia in cheese production, this would mean an important additional effect of this type of treatment.

The purpose of this study is to find out the effect of cold storage of milk at 2$^{o}$C and 6$^{o}$C for 2, 4 and 7 d on the yield, composition and the ripening of Cheddar cheese.

## EXPERIMENTAL

Bulk silo milk for cheesemaking was supplied by the Scottish Milk Marketing Board. The milk delivered by road tanker was divided into three portions, the first to be pasteurized and pumped to the cheese vat for immediate cheesemaking, the second portion to be stored at 2$^{o}$C and the third to be stored at 6$^{o}$C according to the procedure described in Chapter One, Section 1b.

Cheese was manufactured from pasteurized milk after storage of raw milk of the two temperatures for 2, 4 and 7 d according to the procedure described in Chapter One, Section 4.

A composite sample was made of four cores from the cheese block (4.5 kg or 10 lb) after 1 week and 1 and 2 months of curing. The next samples were taken after 4, 8 and 12 months of curing. After removing the nylon polythene laminate pouch from the cheese block, the top 1 cm of cheese was discarded and another 1 cm thick slice was cut and used as sample for chemical analysis. The cheese plugs or slices were grated using a domestic cheese grater. The moisture and pH were determined immediately after sampling and the remainder of each sample was kept in

the freezer (-20°C) until the time of analysis.

Cheese samples were analysed for fat, ash, salt, calcium and phosphorus at 1 month old. Moisture, total nitrogen (protein), soluble nitrogen, hydrogen ion concentration (pH), firmness and elasticity were determined when the cheese was 1 week and 1, 2, 4, 8 and 12 months old. Free fatty acids were determined after 9 and 12 months. The analyses of cheese were carried out according to the methods described in Chapter One, Section 5.

The wheys were collected at running during cheddaring and after pressing. The amounts of whey were weighed, mixed thoroughly and then sampled. The samples were analysed for fat, total nitrogen (protein), NPN, ash, calcium and phosphorus according to the methods given in Chapter One, Section 6.

The mean values of the results were taken and tabulated as rounded figures to the first or second decimal point.

## RESULTS

### (a) Analysis of whey samples

Tables 4:1 to 4:18 show the effect of cold storage of raw milk at 2°C and 6°C for 2, 4 and 7 d prior to Cheddar cheese making on the composition of whey at running, during cheddaring and pressing.

Different trials showed a significant difference ($p < 0.01$) in the calcium content of wheys at running (Table 4:1). Both the period and temperature of storage resulted in very highly significant differences in the calcium content of whey at running. The length in the period of storage at 2°C had no significant effect on calcium content whereas the duration of storage at 6°C had a significant effect ($p < 0.001$).

In the case of wheys collected during cheddaring (Table 4:2) both the trial and storage time resulted in differences in calcium content ($p < 0.001$). However, there was a decrease in calcium content in the whey collected during cheddaring where cheese was made from milk stored at both temperatures for 7 d ($p < 0.001$). There was no significant change due to temperature of storage.

TABLE 4:1

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the calcium content in the whey at running (mM/kg)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 13.09 | 12.93 | 14.09 | 14.10 | 12.79 | 15.35 | 16.80 | 14.03 |
| 2 | 12.45 | 12.05 | 12.80 | 13.30 | 11.25 | 14.35 | 15.20 | 12.98 |
| 3 | 12.13 | 12.81 | 12.06 | 13.17 | 12.70 | 12.97 | 16.08 | 13.01 |
| 4 | 12.48 | 11.24 | 11.14 | 11.17 | 12.27 | 12.99 | 17.32 | 12.64 |
| Mean Values | 12.54 | 12.26 | 12.52 | 12.94 | 12.25 | 13.92 | 16.35 | 13.16 |
| | | 12.57 | | | 14.17 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 5.7992 | 11.439** |
| Invstor | 1 | 8.3759 | 16.521** |
| Trial Invstor | 3 | 0.6944 | 1.370 |
| Storage | 2 | 23.0668 | 45.499*** |
| Temperature | 1 | 30.7791 | 60.712*** |
| Trial Storage | 6 | 1.3492 | 2.661 |
| Trial Temperature | 3 | 2.7389 | 5.402* |
| Storage Temperature | 2 | 11.8426 | 23.359*** |
| Residual | 10 | 0.5070 | |
| Total | 31 | 4.8334 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.2517 | 0.2517 | 0.2298 | 0.5035 | 0.4596 | 0.3083 |

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

TABLE 4:2

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the calcium in the whey collected during cheddaring (mM/kg)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 25.32 | 22.85 | 24.80 | 21.91 | 24.56 | 25.55 | 20.50 | 23.85 |
| 2 | 23.92 | 19.80 | 23.20 | 21.15 | 20.70 | 25.35 | 21.45 | 22.44 |
| 3 | 21.23 | 20.32 | 19.60 | 18.08 | 20.48 | 21.71 | 18.25 | 20.11 |
| 4 | 21.48 | 20.19 | 21.14 | 18.20 | 20.67 | 20.45 | 18.42 | 20.26 |
| Mean Values | 22.99 | 20.79 | 22.19 | 19.84 | 21.60 | 23.27 | 19.66 | 21.66 |
| | | 20.94 | | | 21.51 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 52.1242 | 24.657*** |
| Invstor | 1 | 37.3651 | 17.676** |
| Trial Invstor | 3 | 0.1799 | 0.085 |
| Storage | 2 | 35.5061 | 16.796*** |
| Temperature | 1 | 3.9199 | 1.854 |
| Trial Storage | 6 | 4.2418 | 2.007 |
| Trial Temperature | 3 | 0.7283 | 0.345 |
| Storage Temperature | 2 | 1.7613 | 0.833 |
| Residual | 10 | 2.1139 | |
| Total | 31 | 10.3712 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.5140 | 0.5140 | 0.4693 | 1.0281 | 0.9385 | 0.6296 |

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

In the samples of whey obtained after pressing (Table 4:3), calcium content varied in different trials ($p < 0.001$). Neither the period nor the temperature of storage gave significant differences.

Trials showed differences ($p < 0.001$, Table 4:4) in the phosphorus content of whey at running. While storage has not affected the phosphorus content the temperature at which the milk was stored did affect the results. There was a significant increase in the case of whey from milk stored at $6^{o}C$ compared to $2^{o}C$ ($p < 0.001$). Storage at $2^{o}C$ resulted in a decrease in the amount of phosphorus lost in the whey at running while storage at $6^{o}C$ resulted in an increase in phosphorus level compared to the control.

Neither trial nor storage period affected the amount of phosphorus lost in whey during cheddaring (Table 4:5), but the period of storage caused significant differences in phosphorus levels.

Trials showed variations ($p < 0.001$) in the phosphorus content of whey collected during pressing whereas neither the period nor the temperature of storage resulted in significant differences (Table 4:6).

The ash content of the whey at both running and during cheddaring (Tables 4:7 and 4:8) showed no significant variations with time or temperature of storage of the milk. The ash content of whey obtained during pressing showed highly significant differences due to the trials (Table 4:9). The amount of fat lost to the whey at running and during cheddaring did not show any significant difference due to trial ($p < 0.05$), and storage ($p < 0.01$). The interaction between temperature and period of storage was significant. Storage at $2^{o}C$ for up to 7 d did not increase significantly the fat content of wheys but storage at $6^{o}C$ resulted in a significant increase in fat level in the resultant whey.

The amount of protein lost in the whey at running (Table 4:13) showed significant differences ($p < 0.001$) between trials. Both the period and temperature of storage significantly increased the protein content of the whey at running. This increase was greater at the higher temperature. In the case of wheys obtained during cheddaring (Table 4:14) the protein lost in the whey only varied due to different trials

TABLE 4:3

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the calcium content in the whey from pressed cured (mM/kg)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 72.05 | 63.14 | 68.21 | 56.27 | 62.04 | 61.17 | 59.56 | 64.31 |
| 2 | 55.75 | 55.65 | 56.75 | 55.55 | 51.05 | 52.75 | 54.40 | 54.71 |
| 3 | 52.13 | 56.20 | 53.60 | 44.66 | 56.42 | 55.24 | 60.90 | 53.91 |
| 4 | 64.03 | 52.36 | 53.44 | 54.90 | 58.45 | 54.47 | 49.40 | 56.39 |
| Mean values | 60.99 | 56.84 | 58.00 | 52.85 | 56.99 | 55.91 | 56.06 | 57.33 |
| | | 55.89 | | | 56.32 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 363.6499 | 11.967** |
| Invstor | 1 | 286.0144 | 9.412* |
| Trial Invstor | 3 | 122.6092 | 4.035* |
| Storage | 2 | 32.8098 | 1.080 |
| Temperature | 1 | 2.1879 | 0.072 |
| Trial Storage | 6 | 13.8511 | 0.456 |
| Trial Temperature | 3 | 49.1352 | 1.617 |
| Storage Temperature | 2 | 28.4090 | 0.935 |
| Residual | 10 | 30.3887 | |
| Total | 31 | 77.5424 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 1.949 | 1.949 | 1.779 | 3.898 | 3.558 | 2.387 |

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

TABLE 4:4

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the phosphorus in the whey at running (mg/100 g of milk)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 204.8 | 202.5 | 203.9 | 198.7 | 218.9 | 225.9 | 229.4 | 211.1 |
| 2 | 176.0 | 176.5 | 172.0 | 163.5 | 175.4 | 181.4 | 206.8 | 178.5 |
| 3 | 204.4 | 261.4 | 194.8 | 190.5 | 231.1 | 203.7 | 215.8 | 213.3 |
| 4 | 195.5 | 196.0 | 203.6 | 190.4 | 210.2 | 242.0 | 302.6 | 217.0 |
| Mean values | 195.2 | 209.1 | 193.6 | 185.8 | 208.9 | 213.3 | 238.7 | 205.0 |
| | | 196.2 | | | 220.3 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 5085.02 | 19.867*** |
| Invstor | 1 | 2043.63 | 7.984* |
| Trial Invstor | 3 | 362.98 | 1.418 |
| Storage | 2 | 318.96 | 1.246 |
| Temperature | 1 | 6984.17 | 27.287*** |
| Trial Storage | 6 | 1471.58 | 5.749** |
| Trial Temperature | 3 | 1521.33 | 5.944** |
| Storage Temperature | 2 | 2872.04 | 11.221*** |
| Residual | 10 | 255.95 | |
| Total | 31 | 1538.93 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 5.656 | 5.656 | 5.164 | 11.313 | 10.327 | 6.928 |

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

TABLE 4:5

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the phosphorus content in the whey collected during cheddaring (mg/100 g milk)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 267.5 | 269.8 | 265.4 | 252.3 | 255.8 | 256.3 | 256.4 | 261.4 |
| 2 | 232.3 | 225.0 | 230.7 | 211.2 | 224.8 | 236.3 | 224.1 | 227.1 |
| 3 | 258.4 | 268.7 | 245.4 | 229.4 | 228.8 | 244.8 | 226.7 | 245.1 |
| 4 | 255.0 | 248.2 | 247.0 | 243.8 | 243.3 | 239.2 | 229.2 | 245.1 |
| Mean values | 253.3 | 252.9 | 247.1 | 234.2 | 238.2 | 244.1 | 234.1 | 244.7 |
| | | 244.7 | | | 238.8 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 3137.63 | 30.097*** |
| Invstor | 1 | 1592.17 | 15.273** |
| Trial Invstor | 3 | 73.21 | 0.702 |
| Storage | 2 | 698.85 | 6.704* |
| Temperature | 1 | 422.45 | 4.052 |
| Trial Storage | 6 | 69.74 | 0.669 |
| Trial Temperature | 3 | 227.00 | 2.177 |
| Storage Temperature | 2 | 240.88 | 2.311 |
| Residual | 10 | 104.25 | |
| Total | 31 | 505.44 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 3.610 | 3.610 | 3.295 | 7.220 | 6.691 | 4.421 |

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

TABLE 4:6

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the phosphorus content in the whey obtained after pressing (mg/100 g milk)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 630.6 | 581.8 | 563.8 | 538.7 | 584.2 | 580.6 | 560.1 | 583.8 |
| 2 | 503.6 | 478.0 | 470.3 | 479.4 | 491.2 | 476.2 | 488.5 | 486.3 |
| 3 | 503.6 | 492.3 | 473.8 | 419.6 | 506.1 | 500.3 | 543.0 | 492.8 |
| 4 | 564.0 | 493.4 | 473.2 | 472.9 | 534.1 | 485.6 | 443.4 | 503.8 |
| Mean values | 550.4 | 511.4 | 495.3 | 477.7 | 528.9 | 510.7 | 508.8 | 516.7 |
| | | 494.8 | | | 516.1 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 32860.81 | 18.429*** |
| Invstor | 1 | 24286.47 | 13.620** |
| Trial Invstor | 3 | 2960.95 | 1.661 |
| Storage | 2 | 2972.59 | 1.667 |
| Temperature | 1 | 5467.72 | 3.066 |
| Trial Storage | 6 | 615.73 | 0.345 |
| Trial Temperature | 3 | 1488.84 | 0.835 |
| Storage Temperature | 2 | 288.54 | 0.162 |
| Residual | 10 | 1783.15 | |
| Total | 31 | 5475.28 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 14.930 | 14.930 | 13.629 | 29.859 | 27.258 | 18.285 |

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

TABLE 4:7

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the ash content (per cent) in the whey at running

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.57 | 0.58 | 0.54 | 0.57 | 0.54 | 0.61 | 0.62 | 0.57 |
| 2 | 0.54 | 0.55 | 0.54 | 0.58 | 0.55 | 0.57 | 0.60 | 0.56 |
| 3 | 0.54 | 0.58 | 0.50 | 0.56 | 0.57 | 0.56 | 0.56 | 0.55 |
| 4 | 0.56 | 0.55 | 0.60 | 0.56 | 0.56 | 0.57 | 0.59 | 0.57 |
| Mean values | 0.55 | 0.56 | 0.54 | 0.57 | 0.55 | 0.58 | 0.59 | 0.56 |
| | | 0.56 | | | 0.57 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0016730 | 1.694 |
| Invstor | 1 | 0.0023436 | 2.373 |
| Trial Invstor | 3 | 0.0000634 | 0.064 |
| Storage | 2 | 0.0022287 | 2.256 |
| Temperature | 1 | 0.0031153 | 3.154 |
| Trial Storage | 6 | 0.0014280 | 1.446 |
| Trial Temperature | 3 | 0.0003690 | 0.374 |
| Storage Temperature | 2 | 0.0022097 | 2.237 |
| Residual | 10 | 0.0009878 | |
| Total | 31 | 0.0012612 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01111 | 0.01111 | 0.01014 | 0.02222 | 0.02029 | 0.01361 |

TABLE 4:8

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the ash content (per cent) in the whey collected during cheddaring

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.72 | 0.72 | 0.73 | 0.69 | 0.73 | 0.73 | 0.57 | 0.70 |
| 2 | 0.70 | 0.65 | 0.70 | 0.68 | 0.67 | 0.63 | 0.71 | 0.68 |
| 3 | 0.71 | 0.68 | 0.71 | 0.67 | 0.67 | 0.68 | 0.68 | 0.69 |
| 4 | 0.72 | 0.68 | 0.67 | 0.66 | 0.64 | 0.67 | 0.63 | 0.67 |
| Mean values | 0.71 | 0.68 | 0.70 | 0.68 | 0.68 | 0.68 | 0.65 | 0.69 |
| | | 0.69 | | | 0,67 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0024019 | 0.972 |
| Invstor | 1 | 0.0139367 | 5.642* |
| Trial Invstor | 3 | 0.0008432 | 0.341 |
| Storage | 2 | 0.0027454 | 1.111 |
| Temperature | 1 | 0.0041033 | 1.661 |
| Trial Storage | 6 | 0.0039323 | 1.592 |
| Trial Temperature | 3 | 0.0004414 | 0.179 |
| Storage Temperature | 2 | 0.0006102 | 0.247 |
| Residual | 10 | 0.0024700 | |
| Total | 31 | 0.0027130 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01757 | 0.01757 | 0.01604 | 0.03514 | 0.03208 | 0.02152 |

*significant at 5 per cent level

TABLE 4:9

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the ash content (per cent) in the whey obtained after pressing

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 2°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 10.03 | 9.44 | 9.63 | 11.57 | 10.37 | 10.23 | 11.24 | 10.32 |
| 2 | 11.44 | 10.53 | 10.28 | 12.14 | 11.97 | 11.92 | 10.06 | 11.22 |
| 3 | 12.03 | 11.92 | 11.43 | 13.65 | 11.04 | 13.18 | 12.14 | 12.18 |
| 4 | 10.09 | 10.09 | 11.30 | 10.82 | 11.69 | 11.56 | 10.44 | 10.76 |
| Mean values | 10.90 | 10.49 | 10.66 | 12.05 | 11.27 | 11.72 | 10.97 | 11.12 |
| | | 11.07 | | | 11.32 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 10.13151 | 7.668** |
| Invstor | 1 | 1.05882 | 0.801 |
| Trial Invstor | 3 | 0.72398 | 0.548 |
| Storage | 2 | 1.57028 | 1.188 |
| Temperature | 1 | 0.77181 | 0.584 |
| Trial Storage | 6 | 1.36370 | 1.032 |
| Trial Temperature | 3 | 0.30057 | 0.227 |
| Storage Temperature | 2 | 5.37335 | 4.067 |
| Residual | 10 | 1.32123 | |
| Total | 31 | 2.27679 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.4064 | 0.4064 | 0.3710 | 0.8128 | 0.7420 | 0.4977 |

**significant at 1 per cent level

TABLE 4:10

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture on the fat (per cent) in the whey at running

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.30 | 0.35 | 0.35 | 0.30 | 0.30 | 0.22 | 0.30 | 0.30 |
| 2 | 0.27 | 0.40 | 0.35 | 0.30 | 0.32 | 0.30 | 0.22 | 0.31 |
| 3 | 0.30 | 0.32 | 0.35 | 0.35 | 0.30 | 0.25 | 0.25 | 0.30 |
| 4 | 0.20 | 0.30 | 0.20 | 0.20 | 0.30 | 0.25 | 0.72 | 0.30 |
| Mean values | 0.27 | 0.34 | 0.31 | 0.29 | 0.31 | 0.26 | 0.37 | 0.30 |
| | | 0.31 | | | 0.31 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0002229 | 0.017 |
| Invstor | 1 | 0.0247521 | 1.884 |
| Trial Invstor | 3 | 0.0104187 | 0.793 |
| Storage | 2 | 0.0103646 | 0.789 |
| Temperature | 1 | 0.0000521 | 0.004 |
| Trial Storage | 6 | 0.0201562 | 1.534 |
| Trial Temperature | 3 | 0.0501909 | 3.821* |
| Storage Temperature | 2 | 0.0244271 | 1.859 |
| Residual | 10 | 0.0131371 | |
| Total | 31 | 0.0170707 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.04052 | 0.04052 | 0.03698 | 0.08105 | 0.07398 | 0.04963 |

*significant at 5 per cent level

TABLE 4:11

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to cheese manufacture on the fat (per cent) in the whey collected during cheddaring

| Trial | No storage (Initial | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.15 | 0.20 | 0.10 | 0.30 | 0.10 | 0.10 | 0.20 | 0.16 |
| 2 | 0.17 | 0.15 | 0.10 | 0.10 | 0.10 | 0.10 | 0.12 | 0.13 |
| 3 | 0.27 | 0.20 | 0.20 | 0.15 | 0.20 | 0.15 | 0.25 | 0.21 |
| 4 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.60 | 0.16 |
| Mean values | 0.17 | 0.16 | 0.12 | 0.16 | 0.12 | 0.11 | 0.29 | 0.17 |
| | | 0.15 | | | 0.17 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.01930990 | 1.414 |
| Invstor | 1 | 0.00157552 | 0.115 |
| Trial Invstor | 3 | 0.01754773 | 1.285 |
| Storage | 2 | 0.05255201 | 3.848 |
| Temperature | 1 | 0.00880208 | 0.645 |
| Trial Storage | 6 | 0.01852427 | 1.356 |
| Trial Temperature | 3 | 0.02963535 | 2.170 |
| Storage Temperature | 2 | 0.03317705 | 2.429 |
| Residual | 10 | 0.01365623 | |
| Total | 31 | 0.03039105 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.041316 | 0.041316 | 0.037716 | 0.082632 | 0.075433 | 0.050602 |

TABLE 4:12

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the fat (per cent) in the whey obtained after pressing

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.86 | 1.50 | 1.65 | 1.90 | 1.55 | 1.65 | 1.50 | 1.43 |
| 2 | 2.01 | 1.80 | 1.95 | 2.45 | 1.55 | 2.20 | 6.20 | 2.52 |
| 3 | 1.12 | 1.20 | 1.65 | 2.05 | 1.00 | 1.35 | 2.15 | 1.46 |
| 4 | 1.22 | 1.40 | 1.10 | 1.65 | 1.20 | 1.22 | 4.85 | 1.73 |
| Mean values | 1.31 | 1.47 | 1.59 | 2.01 | 1.32 | 1.61 | 3.67 | 1.79 |
| | | 1.69 | | | 2.20 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 4.141599 | 4.583* |
| Invstor | 1 | 4.924082 | 5.568* |
| Trial Invstor | 3 | 0.057409 | 0.065 |
| Storage | 2 | 9.807640 | 11.089** |
| Temperature | 1 | 3.126297 | 3.535 |
| Trial Storage | 6 | 1.618070 | 1.830 |
| Trial Temperature | 3 | 1.636852 | 1.851 |
| Storage Temperature | 2 | 4.010350 | 4.534* |
| Residual | 10 | 0.884423 | |
| Total | 31 | 2.314427 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.33249 | 0.033249 | 0.30352 | 0.66499 | 0.60705 | 0.40722 |

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

TABLE 4:13

The effect of storage of bulked milk at 2°C and 6°C for various period of time prior to use for cheese manufacture on the protein content (per cent) in the whey at running

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.77 | 0.77 | 0.84 | 0.81 | 0.80 | 0.83 | 0.84 | 0.80 |
| 2 | 0.80 | 0.79 | 0.81 | 0.82 | 0.83 | 0.83 | 0.84 | 0.81 |
| 3 | 0.80 | 0.83 | 0.78 | 0.81 | 0.83 | 0.81 | 0.82 | 0.81 |
| 4 | 0.86 | 0.87 | 0.91 | 0.94 | 0.89 | 0.91 | 0.98 | 0.90 |
| Mean values | 0.80 | 0.82 | 0.83 | 0.84 | 0.84 | 0.85 | 0.87 | 0.83 |
| | | 0.83 | | | 0.85 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0346712 | 56.873*** |
| Invstor | 1 | 0.0162987 | 26.736*** |
| Trial Invstor | 3 | 0.0012754 | 2.092 |
| Storage | 2 | 0.0035456 | 5.816* |
| Temperature | 1 | 0.0044853 | 7.358* |
| Trial Storage | 6 | 0.0024200 | 3.970* |
| Trial Temperature | 3 | 0.0000867 | 0.142 |
| Storage Temperature | 2 | 0.0001376 | 0.226 |
| Residual | 10 | 0.0006096 | |
| Total | 31 | 0.0050602 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.00873 | 0.00873 | 0.00797 | 0.01746 | 0.01594 | 0.01069 |

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

TABLE 4:14

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the protein content (per cent) in the whey collected during cheddaring

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.85 | 0.82 | 0.87 | 0.86 | 0.82 | 0.83 | 0.76 | 0.83 |
| 2 | 0.84 | 0.88 | 0.83 | 0.82 | 0.84 | 0.82 | 0.92 | 0.85 |
| 3 | 0.99 | 0.93 | 0.89 | 0.89 | 0.91 | 0.91 | 0.92 | 0.93 |
| 4 | 0.92 | 0.94 | 0.94 | 0.91 | 0.91 | 0.94 | 0.96 | 0.93 |
| Mean values | 0.90 | 0.89 | 0.88 | 0.87 | 0.87 | 0.88 | 0.89 | 0.89 |
| | | 0.88 | | | 0.88 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0443359 | 17.756*** |
| Invstor | 1 | 0.0044564 | 1.785 |
| Trial Invstor | 3 | 0.0061626 | 2.468 |
| Storage | 2 | 0.0000171 | 0.007 |
| Temperature | 1 | 0.0000141 | 0.006 |
| Trial Storage | 6 | 0.0015361 | 0.615 |
| Trial Temperature | 3 | 0.0026001 | 1.041 |
| Storage Temperature | 2 | 0.0018788 | 0.752 |
| Residual | 10 | 0.0024970 | |
| Total | 31 | 0.0065079 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.01767 | 0.01767 | 0.01613 | 0.03533 | 0.03226 | 0.02164 |

*significant at 5 per cent level

($p < 0.001$). The protein content of whey at pressing (Table 4:15) showed variations between trials ($p < 0.001$). No significant differences were found due to the period or temperature of storage. However, the interaction between the storage time and temperature was significant. Storage of milk up to 7 d at 6°C caused a slight increase in the amount of protein lost into the whey at pressing. The non-protein nitrogen contents of whey at running and during cheddaring (Tables 4:16 and 4:17) showed significant variations due to different trials. No significant differences were found due to temperature or storage of the milk. The NPN of wheys taken during pressing (Table 4:18) increased significantly with the storage of milk at 6°C for 7 d but not with the storage at 2°C.

(b) Cheese yield

Table 4:19 shows the effect of cold storage of milk prior to processing on the yield of cheese. Significant variations ($p < 0.001$) were observed between different trials in the yield of cheese. Irrespective of the temperature of storage, no significant difference was observed due to the storage in the yield of cheese from milk held for up to 7 d before manufacture. There was an increase ($p < 0.001$) in the yield of cheese produced from milk held at 6°C compared to that obtained from milk stored at 2°C for 2, 4 and 7 d. This increase in the yield at 6°C is due to the retention of more moisture in these cheeses. When the yield was then calculated on a moisture level of 35 per cent (Table 4:20), the overall mean of yield of cheese produced from milk stored at 6°C was significantly lower than that produced from milk held at 2°C. The length of storage of milk prior to manufacture had a significant effect on the yield. In each of the four trials, storage of milk at 2°C for up to 7 d did not result in any significant difference in the yield of cheese. However, there was a slight increase in the yield of cheese made from milk held for 2 and 4 d at 2°C. While this difference is statistically not significant it could be of importance from the commercial point of view. On the other hand, storage of milk at 6°C for 7 d brought about a significant reduction in the cheese yield. In the case of cheeses made from milk after 4 d of storage at 6°C similar yield was obtained to the yield of cheese made from unstored milk.

TABLE 4:15

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the protein (per cent) in the whey obtained after pressing

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 1.06 | 1.03 | 1.04 | 0.94 | 0.98 | 1.06 | 1.15 | 1.04 |
| 2 | 0.98 | 0.97 | 1.08 | 1.00 | 0.94 | 1.05 | 1.04 | 1.00 |
| 3 | 1.14 | 1.09 | 1.08 | 1.07 | 1.11 | 1.07 | 1.36 | 1.13 |
| 4 | 1.21 | 1.10 | 1.05 | 1.15 | 1.07 | 1.13 | 1.17 | 1.14 |
| Mean values | 1.10 | 1.05 | 1.06 | 1.04 | 1.02 | 1.08 | 1.18 | 1.08 |
| | | 1.05 | | | 1.09 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0721004 | 12.639*** |
| Invstor | 1 | 0.0065567 | 1.149 |
| Trial Invstor | 3 | 0.0090351 | 1.584 |
| Storage | 2 | 0.0213527 | 3.743 |
| Temperature | 1 | 0.0242550 | 4.251 |
| Trial Storage | 6 | 0.0075534 | 1.324 |
| Trial Temperature | 3 | 0.0069362 | 1.216 |
| Storage Temperature | 2 | 0.0302129 | 5.296* |
| Residual | 10 | 0.0057051 | |
| Total | 31 | 0.0161465 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.02670 | 0.02670 | 0.02438 | 0.05341 | 0.04876 | 0.03271 |

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

TABLE 4:16

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the non-protein nitrogen (per cent) in the whey at running

| Trial | No storage (initial) | Storage at 2°C | | | Storage at 6°C | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| 2 | 0.23 | 0.23 | 0.26 | 0.26 | 0.23 | 0.23 | 0.24 | 0.24 |
| 3 | 0.21 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |
| 4 | 0.28 | 0.26 | 0.26 | 0.26 | 0.27 | 0.26 | 0.27 | 0.27 |
| Mean values | 0.24 | 0.24 | 0.24 | 0.25 | 0.24 | 0.24 | 0.24 | 0.24 |
| | | 0.24 | | | 0.24 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.00709187 | 94.922*** |
| Invstor | 1 | 0.00012675 | 1.697 |
| Trial Invstor | 3 | 0.00040079 | 5.364* |
| Storage | 2 | 0.00013431 | 1.798 |
| Temperature | 1 | 0.00002133 | 0.286 |
| Trial Storage | 6 | 0.00016440 | 2.200 |
| Trial Temperature | 3 | 0.00019483 | 2.608 |
| Storage Temperature | 2 | 0.00002952 | 0.395 |
| Residual | 10 | 0.00007471 | |
| Total | 31 | 0.00081522 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.003056 | 0.003056 | 0.002790 | 0.00612 | 0.005579 | 0.003743 |

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

TABLE 4:17

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the non-protein nitrogen (per cent) in the whey collected during cheddaring

| Trial | No Storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.29 | 0.28 | 0.29 | 0.27 | 0.28 | 0.29 | 0.27 | 0.28 |
| 2 | 0.26 | 0.26 | 0.28 | 0.26 | 0.23 | 0.28 | 0.26 | 0.26 |
| 3 | 0.27 | 0.28 | 0.26 | 0.27 | 0.25 | 0.26 | 0.26 | 0.27 |
| 4 | 0.33 | 0.31 | 0.30 | 0.32 | 0.31 | 0.31 | 0.27 | 0.31 |
| Mean values | 0.29 | 0.28 | 0.28 | 0.28 | 0.27 | 0.28 | 0.27 | 0.28 |
| | | 0.28 | | | 0.27 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.00790540 | 17.837*** |
| Invstor | 1 | 0.00123018 | 2.776 |
| Trial Invstor | 3 | 0.00035330 | 0.797 |
| Storage | 2 | 0.00029818 | 0.673 |
| Temperature | 1 | 0.00064533 | 1.456 |
| Trial Storage | 6 | 0.00036030 | 0.813 |
| Trial Temperature | 3 | 0.00005422 | 0.122 |
| Storage Temperature | 2 | 0.00037727 | 0.851 |
| Residual | 10 | 0.0004432 | |
| Total | 31 | 0.00122 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.007443 | 0.007443 | 0.006795 | 0.014886 | 0.013589 | 0.009116 |

***significant at 0.1 per cent level

TABLE 4:18

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the non-protein nitrogen (per cent) in the whey obtained after pressing

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 0.33 | 0.34 | 0.34 | 0.31 | 0.32 | 0.34 | 0.38 | 0.34 |
| 2 | 0.29 | 0.33 | 0.33 | 0.31 | 0.28 | 0.27 | 0.37 | 0.31 |
| 3 | 0.38 | 0.37 | 0.38 | 0.36 | 0.37 | 0.36 | 0.51 | 0.39 |
| 4 | 0.42 | 0.38 | 0.41 | 0.42 | 0.39 | 0.40 | 0.46 | 0.41 |
| Mean values | 0.35 | 0.35 | 0.37 | 0.35 | 0.34 | 0.34 | 0.43 | 0.36 |
| | | 0.36 | | | 0.37 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.03609800 | 29.674*** |
| Invstor | 1 | 0.00139213 | 1.144 |
| Trial Invstor | 3 | 0.00070392 | 0.579 |
| Storage | 2 | 0.00864950 | 7.110* |
| Temperature | 1 | 0.00256669 | 2.110 |
| Trial Storage | 6 | 0.00087027 | 0.715 |
| Trial Temperature | 3 | 0.00124513 | 1.024 |
| Storage Temperature | 2 | 0.01352703 | 11.120** |
| Residual | 10 | 0.00121650 | |
| Total | 31 | 0.00580127 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.012331 | 0.012331 | 0.011257 | 0.024663 | 0.022514 | 0.0015103 |

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

TABLE 4:19

The effect of storage of bulk milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the yield of cheese (expressed as percentage)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 10.47 | 10.30 | 10.21 | 10.16 | 10.43 | 10.55 | 11.31 | 10.49 |
| 2 | 10.15 | 9.88 | 9.89 | 10.11 | 10.05 | 9.95 | 10.46 | 10.08 |
| 3 | 9.74 | 9.75 | 9.75 | 9.77 | 9.84 | 9.89 | 10.28 | 9.85 |
| 4 | 10.18 | 10.23 | 10.03 | 10.18 | 10.58 | 10.43 | 10.07 | 10.25 |
| Mean values | 10.15 | 10.04 | 9.97 | 10.06 | 10.22 | 10.20 | 10.53 | 10.17 |
| | | 10.02 | | | 10.32 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.58136 | 12.767*** |
| Invstor | 1 | 0.00259 | 0.057 |
| Trial Invstor | 3 | 0.01227 | 0.270 |
| Storage | 2 | 0.09364 | 2.057 |
| Temperature | 1 | 0.53104 | 11.664** |
| Trial Storage | 6 | 0.04889 | 1.074 |
| Trial Temperature | 3 | 0.0383 | 0.875 |
| Storage Temperature | 2 | 0.04831 | 1.061 |
| Residual | 10 | 0.04553 | |
| Total | 31 | 0.11181 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.1067 | 0.1067 | 0.0974 | 0.2134 | 0.1948 | 0.1307 |

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

TABLE 4:20

The effect of storage of bulk milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the yield of cheese (adjusted to a moisture level of 35 per cent and expressed as a percentage of the milk used)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 10.06 | 10.02 | 9.91 | 9.93 | 10.00 | 10.14 | 10.27 | 10.05 |
| 2 | 9.77 | 9.70 | 9.58 | 9.56 | 9.71 | 9.71 | 9.75 | 9.69 |
| 3 | 9.42 | 9.63 | 9.60 | 9.45 | 9.59 | 9.61 | 9.42 | 9.52 |
| 4 | 9.95 | 9.95 | 9.86 | 10.02 | 9.86 | 9.82 | 9.10 | 9.81 |
| Mean values | 9.80 | 9.83 | 9.74 | 9.74 | 9.79 | 9.82 | 9.64 | 9.77 |
| | | 9.77 | | | 9.75 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.40088 | 14.355*** |
| Invstor | 1 | 0.00988 | 0.354 |
| Trial Invstor | 3 | 0.02674 | 0.958 |
| Storage | 2 | 0.03237 | 1.159 |
| Temperature | 1 | 0.024 | 0.089 |
| Trial storage | 6 | 0.2083 | 0.746 |
| Trial temperature | 3 | 0.08346 | 2.989 |
| Storage temperature | 2 | 0.01701 | 0.609 |
| Residual | 10 | 0.02793 | |
| Total | 31 | 0.06608 | |

| Table | Trial | Storage | Temperature | Trial storage | Trial temperature | Storage temperature |
|---|---|---|---|---|---|---|
| SED | 0.0836 | 0.0836 | 0.0763 | 0.1671 | 0.1526 | 0.1023 |

***significant at 0.1 per cent level

Cheese moisture content at 1 week old

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | |
|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d |
| 1 | 37.52 | 36.74 | 36.87 | 36.47 | 37.66 | 37.52 | 40.96 |
| 2 | 37.46 | 36.18 | 37.05 | 38.57 | 37.21 | 36.58 | 39.43 |
| 3 | 37.19 | 35.77 | 36.01 | 37.13 | 36.61 | 36.84 | 40.45 |
| 4 | 36.78 | 36.77 | 36.09 | 36.04 | 39.44 | 38.81 | 41.25 |

When the mass recovery was determined there was no significant difference between the total output material (when calculated as percentage) and the total input materials resulting from different periods or temperature of storage or due to the different trials (Table 4:21). This indicates the consistency in the weighing procedure of milk, cheese and whey throughout the course of this study.

(c) Composition of cheese

Table 4:22 indicates that the calcium content of cheese produced from milk held at $2^{o}C$ for up to 7 d and for up to 4 d at $6^{o}C$ retained more calcium ($p < 0.001$) than cheese produced from freshly manufactured milk. Significant variations ($p < 0.001$) were observed in the calcium content of cheese due to the period and temperature of milk storage and the interaction between them. Milk held at $6^{o}C$ for 7 d gave cheese with dramatically lower calcium content ($p < 0.001$).

The analysis of variance of phosphorus content of the cheese (Table 4:23) showed significant differences ($p < 0.05$) between trials. Cheeses from milk held at $2^{o}C$ retained a greater quantity of phosphorus than cheese made from the same milk held at $6^{o}C$.

The ash content was lower ($p < 0.05$) in the cheese made from milk held at $6^{o}C$ than at $2^{o}C$. However, the duration of the storage did not cause significant differences. On the other hand the salt content of cheeses (Table 4:25) and the salt in aqueous phase (Table 4:26) did not show any significant variation because of any factor.

The fat content of cheese (Table 4:27) showed significant variations due to trials, and temperature of milk storage. Cheeses made from the same milk stored at $6^{o}C$ contained less fat than cheeses made from milk held at $2^{o}C$. This variation may be due to the higher moisture content of cheeses manufactured from milk stored at $6^{o}C$. When the fat content of cheese is calculated on a dry matter basis (Table 4:28) the variation is still present but is no longer statistically significant.

The FFA content of cheeses at nine and twelve months old are presented in Tables 4:29 and 4:30 respectively. A significant increase due to the period ($p < 0.05$) and temperature ($p < 0.01$) of storage of milk used for cheese production is indicated. Storage at $2^{o}C$ did not result in any

TABLE 4:21

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the mass recovery (expressed as a percentage of the total out put from the masses used)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 99.00 | 98.95 | 99.08 | 99.17 | 99.14 | 98.97 | 99.14 | 99.06 |
| 2 | 99.06 | 99.10 | 99.07 | 99.43 | 99.13 | 98.92 | 99.19 | 99.12 |
| 3 | 98.88 | 98.95 | 98.91 | 98.89 | 99.17 | 99.22 | 98.89 | 98.97 |
| 4 | 99.02 | 99.04 | 99.03 | 98.86 | 98.93 | 99.30 | 99.13 | 99.04 |
| Mean values | 98.99 | 99.01 | 99.02 | 99.09 | 99.09 | 99.10 | 99.09 | 99.05 |
| | | 99.04 | | | 99.09 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.02829 | 1.364 |
| Invstor | 1 | 0.0337 | 1.609 |
| Trial Invstor | 3 | 0.002 | 0.114 |
| Storage | 2 | 0.00275 | 0.133 |
| Temperature | 1 | 0.01760 | 0.848 |
| Trial Storage | 6 | 0.03264 | 1.573 |
| Trial Temperature | 3 | 0.02735 | 1.318 |
| Storage Temperature | 2 | 0.00440 | 0.212 |
| Residual | 10 | 0.02075 | |
| Total | 31 | 0.02073 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.0720 | 0.0720 | 0.0675 | 0.1440 | 0.1315 | 0.0882 |

TABLE 4:22

The effect of storage of bulked milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on calcium in cheese ($_{m}M$/kg)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 176.78 | 189.34 | 189.68 | 194.23 | 182.71 | 191.11 | 149.95 | 181.32 |
| 2 | 187.82 | 185.52 | 188.34 | 201.81 | 197.00 | 200.78 | 158.45 | 188.45 |
| 3 | 191.09 | 192.10 | 186.58 | 199.59 | 189.09 | 188.18 | 153.92 | 186.46 |
| 4 | 186.41 | 193.27 | 194.68 | 196.83 | 176.53 | 184.13 | 152.93 | 183.90 |
| Mean values | 185.52 | 190.06 | 189.82 | 198.12 | 186.33 | 191.51 | 153.81 | 185.09 |
| | | 192.67 | | | 177.22 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 150.62 | 4.198 |
| Invstor | 2 | 7.36 | 0.205 |
| Trial Invstor | 6 | 70.60 | 1.967 |
| Storage | 2 | 991.37 | 27.628*** |
| Temperature | 1 | 2863.34 | 79.798*** |
| Trial Storage | 6 | 19.36 | 0.539 |
| Trial Temperature | 3 | 140.47 | 3.915 |
| Storage Temperature | 2 | 2527.44 | 70.437*** |
| Residual | 6 | 35.88 | |
| Total | 31 | 372.39 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 2.118 | 2.594 | 2.445 | 5.188 | 4.891 | 2.995 |

***significant at 0.1 per cent level

TABLE 4:23

The effect of storage of bulk milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the phosphorus content in cheese (expressed in mg/100 g)

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 511.0 | 542.0 | 509.5 | 564.5 | 498.0 | 500.0 | 418.0 | 506.8 |
| 2 | 437.7 | 515.5 | 429.0 | 443.0 | 458.5 | 437.0 | 373.0 | 441.4 |
| 3 | 434.0 | 521.0 | 435.5 | 447.5 | 392.0 | 417.0 | 449.0 | 441.3 |
| 4 | 469.2 | 451.0 | 419.0 | 439.0 | 384.5 | 412.0 | 341.5 | 423.3 |
| Mean values | 463.0 | 507.4 | 448.4 | 473.5 | 433.2 | 441.5 | 394.4 | 453.2 |
| | | 476.4 | | | 423.4 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 21577.4 | 8.696* |
| Invstor | 2 | 1058.4 | 0.427 |
| Trial Invstor | 6 | 1783.5 | 0.719 |
| Storage | 2 | 5443.1 | 2.194 |
| Temperature | 1 | 33761.0 | 13.606* |
| Trial Storage | 6 | 1274.7 | 0.514 |
| Trial Temperature | 3 | 400.7 | 0.161 |
| Storage Temperature | 2 | 6410.7 | 2.583 |
| Residual | 6 | 2481.4 | |
| Total | 31 | 5121.2 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 17.61 | 21.57 | 20.34 | 43.14 | 40.67 | 26.91 |

*significant at 5 per cent level

TABLE 4:24

The effect of storage of bulk milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on the ash content in cheese (include the added NaCl, per cent)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 3.58 | 3.56 | 4.14 | 3.90 | 3.57 | 3.69 | 3.15 | 3.65 |
| 2 | 3.65 | 3.97 | 3.84 | 3.69 | 3.79 | 3.84 | 3.75 | 3.77 |
| 3 | 3.68 | 3.65 | 3.87 | 3.84 | 3.77 | 3.50 | 3.36 | 3.67 |
| 4 | 3.58 | 3.75 | 3.80 | 3.87 | 3.44 | 3.76 | 3.55 | 3.67 |
| Mean values | 3.62 | 3.37 | 3.91 | 3.83 | 3.64 | 3.70 | 3.46 | 3.69 |
| | | 3.82 | | | 3.60 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.049387 | 0.720 |
| Invstor | 2 | 0.113243 | 1.653 |
| Trial Invstor | 6 | 0.011874 | 0.173 |
| Storage | 2 | 0.111555 | 1.629 |
| Temperature | 1 | 0.606825 | 8.860* |
| Trial Storage | 6 | 0.047898 | 0.699 |
| Trial Temperature | 3 | 0.063880 | 0.933 |
| Storage Temperature | 2 | 0.090275 | 1.172 |
| Residual | 6 | 0.068491 | |
| Total | 31 | 0.075034 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.09253 | 0.11332 | 0.10684 | 0.022665 | 0.21368 | 0.13085 |

*significant at 5 per cent level

TABLE 4:25

The effect of storage of bulk milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on salt (NaCl) content (per cent) in cheese

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 1.66 | 1.71 | 1.67 | 1.61 | 1.55 | 1.70 | 1.24 | 1.60 |
| 2 | 1.53 | 1.53 | 1.75 | 1.45 | 1.62 | 1.54 | 1.71 | 1.58 |
| 3 | 1.56 | 1.60 | 1.69 | 1.71 | 1.63 | 1.45 | 1.66 | 1.61 |
| 4 | 1.61 | 1.69 | 1.60 | 1.76 | 1.46 | 1.74 | 1.94 | 1.68 |
| Mean values | 1.58 | 1.63 | 1.68 | 1.63 | 1.56 | 1.61 | 1.64 | 1.62 |
| | | 1.65 | | | 1.60 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.0280281 | 0.536 |
| Invstor | 2 | 0.0368343 | 0.704 |
| Trial Invstor | 6 | 0.0229886 | 0.439 |
| Storage | 2 | 0.0092335 | 0.176 |
| Temperature | 1 | 0.0234967 | 0.449 |
| Trial Storage | 6 | 0.0545498 | 1.042 |
| Trial Temperature | 3 | 0.03049 | 0.574 |
| Storage Temperature | 2 | 0.0075842 | 0.145 |
| Residual | 6 | 0.0523384 | |
| Total | 31 | 0.0349770 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.00088 | 0.09906 | 0.09340 | 0.19813 | 0.18679 | 0.11439 |

TABLE 4:26

The effect of storage of bulk milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on salt (NaCl) in aqueous phase (per cent) in cheese

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 4.41 | 4.66 | 4.53 | 4.40 | 4.12 | 4.51 | 3.04 | 4.26 |
| 2 | 4.12 | 4.18 | 4.73 | 3.96 | 4.38 | 4.25 | 4.35 | 4.26 |
| 3 | 4.27 | 4.35 | 4.76 | 4.84 | 4.52 | 3.92 | 4.28 | 4.40 |
| 4 | 4.27 | 4.52 | 4.47 | 4.69 | 3.62 | 4.52 | 4.66 | 4.38 |
| Mean values | 4.17 | 4.43 | 4.62 | 4.47 | 4.16 | 4.30 | 4.08 | 4.33 |
| | | 4.51 | | | 4.18 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.091786 | 0.230 |
| Invstor | 2 | 0.289476 | 0.726 |
| Trial Invstor | 6 | 0.142216 | 0.357 |
| Storage | 2 | 0.168334 | 0.422 |
| Temperature | 1 | 1.269774 | 3.186 |
| Trial Storage | 6 | 0.375480 | 0.942 |
| Trial Temperature | 3 | 0.242077 | 0.607 |
| Storage Temperature | 2 | 0.016372 | 0.041 |
| Residual | 6 | 0.398558 | |
| Total | 31 | 0.281201 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.22320 | 0.27337 | 0.25773 | 0.54673 | 0.51547 | 0.31566 |

TABLE 4:27

The effect of storage of bulk milk at $2^{o}C$ and $6^{o}C$ for various periods of time prior to use for cheese manufacture on the fat content (per cent) in cheese

| Trial | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 32.63 | 33.07 | 33.10 | 32.85 | 32.82 | 33.11 | 29.86 | 32.51 |
| 2 | 32.70 | 33.52 | 32.67 | 32.20 | 32.27 | 33.26 | 30.61 | 32.49 |
| 3 | 32.80 | 32.45 | 34.90 | 34.82 | 31.90 | 31.97 | 31.07 | 32.84 |
| 4 | 32.00 | 32.20 | 32.70 | 31.00 | 29.90 | 31.00 | 27.60 | 31.05 |
| Mean values | 32.53 | 32.81 | 33.34 | 32.72 | 31.72 | 32.34 | 29.78 | 32.22 |
| | | 32.96 | | | 31.28 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 11.1207 | 11.278** |
| Invstor | 2 | 0.9494 | 0.963 |
| Trial Invstor | 6 | 0.8876 | 0.900 |
| Storage | 2 | 8.7143 | 9.939* |
| Temperature | 1 | 30.1150 | 30.541** |
| Trial Storage | 6 | 2.4028 | 2.437 |
| Trial Temperature | 3 | 2.0269 | 2.056 |
| Storage Temperature | 2 | 3.6220 | 3.673 |
| Residual | 6 | 0.9860 | |
| Total | 31 | 3.9286 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.3511 | 0.4300 | 0.4054 | 0.8600 | 0.8108 | 0.4965 |

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

TABLE 4:28

The effect of storage of bulk milk at 2°C and 6°C for various periods of time prior to use for cheese manufacture on fat in cheese (on dry matter base)

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 52.69 | 52.95 | 52.04 | 51.96 | 52.82 | 52.80 | 51.96 | 52.46 |
| 2 | 52.06 | 52.87 | 51.76 | 50.90 | 51.21 | 52.10 | 50.52 | 51.63 |
| 3 | 51.75 | 51.23 | 54.17 | 53.89 | 54.50 | 50.75 | 50.69 | 52.43 |
| 4 | 51.31 | 51.50 | 51.03 | 49.65 | 50.06 | 50.41 | 47.32 | 50.18 |
| Mean values | 51.95 | 52.14 | 52.25 | 51.60 | 52.15 | 51.51 | 50.12 | 51.67 |
| | | 52.00 | | | 51.26 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 2.874 | 1.785 |
| Invstor | 1 | 0.575 | 0.357 |
| Trial Invstor | 3 | 0.248 | 0.154 |
| Storage | 2 | 0.350 | 0.218 |
| Temperature | 1 | 6.647 | 4.129 |
| Trial Storage | 6 | 1.798 | 1.117 |
| Trial Temperature | 3 | 4.869 | 3.052 |
| Storage Temperature | 2 | 0.797 | 0.495 |
| Residual | 6 | 1.610 | |
| Total | 27 | 1.998 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 0.678 | 0.777 | 0.732 | 1.554 | 1.465 | 0.897 |

Cheese moist content at 1 month old

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | |
|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d |
| 1 | 38.07 | 37.54 | 36.39 | 36.78 | 37.86 | 37.29 | 42.53 |
| 2 | 37.19 | 36.60 | 36.88 | 36.74 | 36.98 | 36.16 | 39.41 |
| 3 | 36.62 | 36.67 | 35.57 | 35.39 | 35.96 | 37.00 | 38.71 |
| 4 | 37.63 | 37.48 | 35.92 | 37.56 | 40.27 | 38.51 | 41.67 |

TABLE 4:29

The effect of storage of bulk milk at 2°C and 6°C for various periods of time prior to cheese manufacture on free fatty acid content (FFA in mEq/100 g) in the fat of the cheese at nine months old.

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 1.72 | 2.22 | 1.43 | 1.78 | 1.86 | 3.02 | 13.66 | 3.43 |
| 2 | 0.78 | 1.09 | 1.40 | 2.89 | 1.35 | 15.82 | 21.68 | 5.72 |
| 3 | 0.61 | 0.97 | 1.32 | 2.30 | 1.08 | 4.78 | 7.72 | 2.42 |
| 4 | 0.92 | 0.90 | 0.41 | 1.91 | 2.59 | 3.56 | 5.04 | 2.03 |
| Mean values | 1.01 | 1.30 | 1.14 | 2.22 | 1.72 | 6.79 | 12.02 | 3.40 |
| | | 1.55 | | | 6.84 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 43.75296 | 2.319 |
| Invstor | 2 | 61.08366 | 3.237 |
| Trial Invstor | 6 | 7.96715 | 0.422 |
| Storage | 2 | 126.59221 | 6.709* |
| Temperature | 1 | 335.94092 | 17.804** |
| Trial Storage | 6 | 20.39931 | 1.081 |
| Trial Temperature | 3 | 47.30620 | 2.507 |
| Storage Temperature | 2 | 88.28548 | 4.679 |
| Residual | 6 | 18.86868 | |
| Total | 31 | 46.591518 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 1.5358 | 1.8809 | 1.7734 | 3.7618 | 3.5467 | 2.1719 |

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

TABLE 4:30

The effect of storage of bulked milk at 2°C and 6°C for various periods of time prior to cheese manufacture on free fatty acid content (FFA in mEq/100 g) in the fat of the cheese at twelve months old

| Trial | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 | 1.19 | 1.01 | 0.94 | 1.52 | 1.28 | 2.45 | 13.09 | 2.83 |
| 2 | 0.90 | 1.39 | 1.04 | 2.40 | 1.44 | 13.21 | 23.12 | 5.55 |
| 3 | 0.69 | 0.97 | 0.76 | 4.08 | 1.35 | 7.46 | 11.24 | 3.40 |
| 4 | 0.57 | 0.73 | 0.89 | 1.35 | 1.65 | 2.19 | 4.19 | 1.52 |
| Mean values | 0.83 | 1.03 | 0.91 | 2.34 | 1.43 | 6.33 | 12.91 | 3.33 |
| | | 1.42 | | | 6.89 | | | |

| | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 45.1640 | 1.829 |
| Invstor | 2 | 66.2019 | 2.681 |
| Trial Invstor | 6 | 7.0089 | 0.284 |
| Storage | 2 | 145.5570 | 5.895* |
| Temperature | 1 | 358.2083 | 14.507** |
| Trial Storage | 6 | 24.5009 | 0.992 |
| Trial Temperature | 3 | 46.1671 | 1.870 |
| Storage Temperature | 2 | 88.9223 | 3.601 |
| Residual | 6 | 24.6924 | |
| Total | 31 | 50.6702 | |

| Table | Trial | Storage | Temperature | Trial Storage | Trial Temperature | Storage Temperature |
|---|---|---|---|---|---|---|
| SED | 1.7569 | 2.1517 | 2.0286 | 4.3034 | 4.0573 | 2.4846 |

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

significant increase in FFA even where the duration of storage was 7 d but with storage at 6°C the increase in FFA was significant in cheese made from milk held for 4 and 7 d.

(d) Effect of cold storage of milk on the curing of cheese

1. Moisture

Very highly significant variations were observed in moisture content due to trial, curing time, period and temperature of milk storage, and the interaction between milk storage time and temperature (Table 4:31). The effect of temperature on the moisture content of cheese was greater than the effect of the period of storage of milk up to 7 d. Cheese produced from milk held at 6°C retained a higher moisture level than those made from milks held at 2°C for equivalent amounts of time. Cheeses produced from milks held at 2°C showed a lower moisture content compared with the cheese made from unstored (initial) milks. In the case of cheese made from milk stored for 7 d at 6°C the moisture content was 39.26 per cent which is above the legal requirements for Cheddar cheese. The curing of cheese was associated with an apparent reduction in its moisture content. This reduction in moisture content was at a maximum in the cheeses manufactured from milks held at 6°C for 7 d. The significant interaction ($p < 0.001$) between trial X temperature and trial X storage indicate that the effect of temperature and period of storage are affected by the quality of the original milks. However, the interaction between curing time and temperature of storage of milks indicates that the storage of milk at 2°C was associated with less moisture being lost during curing as compared to cheeses made from the same milk held at 6°C. The period of storage at different temperature showed a significant interaction with the curing time.

The analysis of variance of calculated moisture in fat free cheese (MFFC) showed the same pattern of variation that was found for the moisture content of cheese (Table 4:32).

2. Total and soluble nitrogen levels in cheese

Very highly significant differences were observed in total nitrogen due to trial, curing, temperature and period of storage and the

TABLE 4:31

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the moisture content (per cent w/w) of cheese during curing

| Milk storage condition / Curing period | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 37.24 | 36.37 | 36.51 | 37.05 | 37.73 | 37.44 | 40.52 | 37.51 |
| 1 Month | 37.38 | 37.08 | 36.19 | 36.62 | 37.64 | 37.25 | 40.58 | 37.52 |
| 2 Months | 37.01 | 36.31 | 35.89 | 35.88 | 37.38 | 36.81 | 40.15 | 37.06 |
| 4 Months | 36.57 | 35.56 | 35.74 | 36.24 | 36.29 | 36.72 | 39.12 | 36.73 |
| 8 Months | 36.43 | 35.90 | 35.24 | 35.99 | 36.91 | 36.65 | 38.58 | 36.52 |
| 12 Months | 35.34 | 35.22 | 34.76 | 35.60 | 35.93 | 36.14 | 36.62 | 35.62 |
| Mean values | 36.66 | 36.07 | 35.72 | 36.23 | 37.15 | 36.84 | 39.26 | 36.82 |
| | | 36.01 | | | 37.75 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 37.35 | 36.68 | 36.05 | 37.21 |

| Source of variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 33.37233 | 58.135*** |
| Curing | 5 | 32.94315 | 57.388*** |
| Invstor | 1 | 3.35406 | 5.843* |
| Trial Curing | 15 | 1.31448 | 2.290* |
| Trial Invstor | 3 | 4.30213 | 7.494 |
| Curing Invstor | 5 | 0.19524 | 0.340 |
| Temperature | 1 | 218.12601 | 379.981*** |
| Storage | 2 | 56.88414 | 99.094*** |
| Trial Curing Invstor | 15 | 0.67655 | 1.179 |
| Trial Temperature | 3 | 20.06439 | 34.953*** |
| Curing Invstor Temperature | 5 | 1.64520 | 2.866** |
| Trial Storage | 6 | 3.87491 | 6.750*** |
| Curing Storage | 10 | 1.10713 | 1.929 |
| Temperature Storage | 2 | 30.08444 | 52.408*** |
| Trial Curing Temperature | 15 | 0.45610 | 0.795 |
| Trial Curing Storage | 30 | 0.78282 | 1.364 |
| Trial Temperature Storage | 6 | 4.73629 | 8.251*** |
| Curing Temperature Storage | 10 | 2.38897 | 4.162*** |
| Residual | 54 | 0.57404 | |
| Total | 191 | 4.81868 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.1094 | 0.1339 | 0.2679 | 0.0998 | 0.1094 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temp. Storage |
|---|---|---|---|---|---|---|
| SED | 0.4374 | 0.1997 | 0.2445 | 0.2187 | 0.2679 | 0.1339 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temperature Storage | Curing Temperature Storage |
|---|---|---|---|---|
| SED | 0.4891 | 0.5357 | 0.2679 | 0.3281 |

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

TABLE 4:32

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the moisture-in-fat-free cheese (expressed as percentage)

| Milk storage condition / Curing period | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 55.50 | 54.09 | 54.74 | 54.99 | 55.25 | 55.31 | 57.98 | 55.42 |
| 1 Month | 55.49 | 55.14 | 54.27 | 54.38 | 55.13 | 55.03 | 58.09 | 55.38 |
| 2 Months | 54.88 | 54.01 | 53.82 | 53.31 | 54.76 | 54.36 | 57.21 | 54.65 |
| 4 Months | 54.35 | 52.89 | 53.59 | 53.82 | 54.63 | 54.25 | 55.76 | 54.20 |
| 8 Months | 54.14 | 53.41 | 52.84 | 53.41 | 54.05 | 54.15 | 55.16 | 53.91 |
| 12 Months | 52.50 | 52.57 | 52.52 | 52.01 | 52.62 | 53.39 | 52.40 | 52.57 |
| Mean values | 54.48 | 53.69 | 53.63 | 53.65 | 54.41 | 54.42 | 56.10 | 54.36 |
| | | 53.66 | | | 54.98 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 55.32 | 54.38 | 53.73 | 53.99 |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 46.9472 | 27.352*** |
| Curing | 5 | 72.8555 | 42.422*** |
| Invstor | 1 | 1.8544 | 1.080 |
| Trial Curing | 15 | 3.1491 | 1.834 |
| Trial Invstor | 3 | 10.3534 | 6.029** |
| Curing Invstor | 5 | 0.2478 | 0.144 |
| Temperature | 1 | 125.3865 | 73.010*** |
| Storage | 2 | 22.7832 | 13.266*** |
| Trial Curing Invstor | 15 | 1.6621 | 0.968 |
| Trial Temperature | 3 | 20.6543 | 12.027*** |
| Curing Invstor Temperature | 5 | 2.5577 | 1.489 |
| Trial Storage | 6 | 15.7872 | 9.193*** |
| Curing Storage | 10 | 3.6661 | 2.135* |
| Temperature Storage | 2 | 22.9627 | 13.371*** |
| Trial Curing Temperature | 15 | 0.9965 | 0.580 |
| Trial Curing Storage | 30 | 1.6079 | 0.936 |
| Trial Temperature Storage | 6 | 5.5239 | 3.216** |
| Curing Temperature Storage | 10 | 3.3508 | 1.951 |
| Residual | 54 | 1.7174 | |
| Total | 191 | 6.5817 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.1892 | 0.2317 | 0.4633 | 0.1727 | 0.1892 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temp. Storage |
|---|---|---|---|---|---|---|
| SED | 0.7566 | 0.3453 | 0.4230 | 0.3783 | 0.4633 | 0.2317 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temperature Storage | Curing Temp. Storage |
|---|---|---|---|---|
| SED | 0.8459 | 0.9269 | 0.4633 | 0.5675 |

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

interaction between temperature and the period of storage (Table 4:33). Cheese manufactured from milk held at $2^{o}C$ contained slightly higher protein contents than the cheese made from unstored (initial) milk. On the other hand, cheese produced from milk held at $6^{o}C$ was significantly lower in protein content compared to cheese made from the unstored milk and compared with the milk stored at $2^{o}C$. The reduction in the protein content of cheese was not significant in the case of cheese made from milk stored for 4 d at $6^{o}C$ while it was significant ($p < 0.001$) in the case of cheese made from milk held at the same temperature for 7 d.

A strong interaction ($p < 0.001$) between trial and storage period indicates the effect of the quality of milk on the protein content of the resulting cheese. Significant interactions ($p < 0.05$) were found between curing time X storage time, trial X curing X temperature and trial X temperature X storage. These interations indicated that the changes which took place in the protein during storage of milk at the different period and temperature of storage had affected the protein content of the resultant cheese. Also, these effects varied depending on the quality of the milk in different trials used.

When the mean values for the protein content are calculated on a dry matter basis for the cheese produced from unstored milk, and milk held at $2^{o}C$ and $6^{o}C$, the protein contents obtained are 14.14, 14.44 and 13.74 per cent, respectively. This indicates that even after the effect of moisture is removed, similar variations in the protein content of cheese were observed. This variation may be of importance in terms of yield and the quality of cheese.

Significant variations ($p < 0.001$) occurred in the soluble nitrogen content of the cheese made in different trials (Table 4:34). Neither the temperature nor the period of storage affected the soluble nitrogen content significantly. The soluble nitrogen content in the cheese increased during the curing period of 12 months ($p < 0.001$). There were significant effects for the interactions between curing time and the temperature of storage of milks and between the temperature and the period of storage of milk ($p < 0.001$). This indicates that the temperature of storage of milks affects the development of soluble nitrogen in the cheese.

Analysis of variance for the soluble nitrogen expressed as a percentage of the total nitrogen in the cheese during curing is represented in Table 4:35. The SN/TN values for cheese made from milks held at $6^{o}C$ were greater than for cheese made from the milks stored for 2-7 d at

TABLE 4:33

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the total nitrogen of cheese (expressed as protein in per cent) during curing.

| Milk storage condition | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| Curing period | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 21.20 | 21.66 | 22.03 | 21.72 | 21.81 | 21.53 | 20.62 | 21.47 |
| 1 Month | 21.86 | 21.80 | 22.31 | 22.21 | 21.47 | 21.33 | 20.90 | 21.72 |
| 2 Months | 22.32 | 22.56 | 23.11 | 22.60 | 22.36 | 22.32 | 21.38 | 22.37 |
| 4 Months | 22.26 | 22.55 | 22.44 | 22.23 | 22.88 | 22.06 | 20.79 | 22.18 |
| 8 Months | 22.95 | 22.85 | 23.31 | 22.97 | 22.73 | 22.80 | 21.93 | 22.81 |
| 12 Months | 23.27 | 23.03 | 23.33 | 23.47 | 23.07 | 22.90 | 22.53 | 23.11 |
| Mean values | 22.31 | 22.41 | 22.75 | 22.53 | 22.39 | 22.16 | 21.40 | 22.28 |
| | | 22.56 | | | 21.97 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 21.29 | 21.92 | 22.40 | 23.50 |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 83.4362 | 212.537*** |
| Curing | 5 | 25.0296 | 63.758*** |
| Invstor | 1 | 0.1433 | 0.365 |
| Trial Curing | 15 | 4.3291 | 10.798*** |
| Trial Invstor | 3 | 1.0491 | 2.672 |
| Curing Invstor | 5 | 0.6064 | 1.545 |
| Temperature | 1 | 25.7044 | 65.477*** |
| Storage | 2 | 7.4581 | 18.998*** |
| Trial Curing Invstor | 15 | 0.5682 | 1.447 |
| Trial Temperature | 3 | 0.6234 | 1.588 |
| Curing Invstor Temperature | 5 | 0.3474 | 0.885 |
| Trial Storage | 6 | 2.3034 | 5.868*** |
| Curing Storage | 10 | 0.8507 | 2.167* |
| Temperature Storage | 2 | 7.9119 | 20.154*** |
| Trial Curing Temperature | 15 | 0.8987 | 2.289* |
| Trial Curing Storage | 30 | 0.5316 | 1.354 |
| Trial Temperature Storage | 6 | 1.0464 | 2.666* |
| Curing Temperature Storage | 10 | 0.1247 | 0.318 |
| Residual | 54 | 0.3926 | |
| Total | 191 | 3.1131 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.0904 | 0.1108 | 0.2215 | 0.0826 | 0.0904 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temperature Storage |
|---|---|---|---|---|---|---|
| SED | 0.3617 | 0.1651 | 0.2022 | 0.1809 | 0.2215 | 0.1108 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temperature Storage | Curing Temperature Storage |
|---|---|---|---|---|
| SED | 0.4044 | 0.4430 | 0.2215 | 0.2713 |

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

TABLE 4:34

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the soluble nitrogen content (per cent) of cheese during curing

| Milk storage condition | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| Curing period | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 1.53 | 1.40 | 1.34 | 1.27 | 1.39 | 1.44 | 1.57 | 1.43 |
| 1 Month | 2.52 | 2.37 | 2.39 | 2.34 | 2.34 | 2.45 | 2.69 | 2.45 |
| 2 Months | 3.43 | 3.30 | 3.16 | 3.01 | 3.14 | 3.15 | 3.45 | 3.26 |
| 4 Months | 4.51 | 4.43 | 4.35 | 4.24 | 4.31 | 4.42 | 4.51 | 4.41 |
| 8 Months | 5.73 | 5.70 | 5.65 | 5.54 | 5.59 | 5.67 | 5.68 | 5.66 |
| 12 Months | 6.61 | 6.65 | 6.57 | 6.40 | 6.39 | 6.28 | 6.22 | 6.47 |
| Mean values | 4.05 | 3.97 | 3.91 | 3.80 | 3.86 | 3.90 | 4.02 | 3.95 |
| | | 3.89 | | | 3.93 | | | |

| Trial means | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | 3.57 | 3.72 | 4.12 | 4.37 |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 12.95633 | 203.539*** |
| Curing | 5 | 236.98285 | 3722.904*** |
| Invstor | 1 | 1.46761 | 23.056*** |
| Trial Curing | 15 | 0.73055 | 11.477*** |
| Trial Invstor | 3 | 0.01172 | 0.184 |
| Curing Invstor | 5 | 0.03670 | 0.577 |
| Temperature | 1 | 0.07379 | 1.159 |
| Storage | 2 | 0.00294 | 0.046 |
| Trial Curing Invstor | 15 | 0.05461 | 0.858 |
| Trial Temperature | 3 | 0.07259 | 1.140 |
| Curing Invstor Temperature | 5 | 0.23399 | 3.676** |
| Trial Storage | 6 | 0.04697 | 0.738 |
| Curing Storage | 10 | 0.06323 | 0.993 |
| Temperature Storage | 2 | 0.69588 | 10.932*** |
| Trial Curing Temperature | 15 | 0.02969 | 0.466 |
| Trial Curing Storage | 30 | 0.08856 | 1.391 |
| Trial Temperature Storage | 6 | 0.06811 | 1.070 |
| Curing Temperature Storage | 10 | 0.03526 | 0.554 |
| Residual | 54 | 0.06366 | |
| Total | 191 | 6.53570 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.03642 | 0.0446 | 0.08920 | 0.03324 | 0.03642 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temp. Storage |
|---|---|---|---|---|---|---|
| SED | 0.14567 | 0.06649 | 0.08143 | 0.07283 | 0.08920 | 0.04460 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temperature Storage | Curing Temp. Storage |
|---|---|---|---|---|
| SED | 0.16286 | 0.17849 | 0.08920 | 0.10925 |

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

TABLE 4:35

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the ratio of SN/TN (expressed as a percentage) of cheese during curing

| Milk storage condition | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| Curing period | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 7.22 | 6.46 | 6.00 | 5.85 | 6.37 | 6.69 | 7.61 | 6.60 |
| 1 Month | 11.53 | 10.87 | 10.71 | 10.54 | 10.90 | 11.49 | 12.87 | 11.27 |
| 2 Months | 15.37 | 14.63 | 13.67 | 13.32 | 14.04 | 14.11 | 16.14 | 14.47 |
| 4 Months | 20.26 | 19.64 | 19.38 | 19.07 | 18.84 | 20.04 | 21.69 | 19.85 |
| 8 Months | 24.97 | 24.94 | 24.24 | 24.12 | 24.49 | 24.87 | 25.90 | 24.79 |
| 12 Months | 28.41 | 28.87 | 28.16 | 27.27 | 27.70 | 27.42 | 27.61 | 27.92 |
| Mean values | 17.96 | 17.57 | 17.03 | 16.69 | 17.06 | 17.44 | 18.64 | 17.48 |
| | | 17.10 | | | 17.71 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 16.67 | 16.84 | 18.28 | 18.50 |

| Source of variation | DF | MS | VR |
|---|---|---|---|
| Curing | 5 | 467.59668 | 5332.684*** |
| Invstor | 1 | 1.59365 | 18.175** |
| Curing Invstor | 5 | 0.07962 | 0.908 |
| Invstor Temperature | 1 | 3.38560 | 38.611*** |
| Invstor Storage | 2 | 0.63975 | 7.296* |
| Curing Invstor Temperature | 5 | 0.49004 | 5.589* |
| Curing Invstor Storage | 10 | 0.25653 | 2.926 |
| Temperature Storage | 2 | 4.60714 | 52.542*** |
| Residual | 10 | 0.08769 | |
| Total | 41 | 57.55476 | |

| Table | Curing | Temperature | Storage | Curing Temperature | Curing Storage | Temperature Storage |
|---|---|---|---|---|---|---|
| SED | 0.1583 | 0.1396 | 0.1481 | 0.3419 | 0.3627 | 0.1710 |

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

$2^{o}C$ ($p < 0.001$). The storage of milk at $2^{o}C$ and $6^{o}C$ caused a decrease in the ratio of SN/TN in the cheese compared to cheese made from unstored milks.

3. Firmness and elasticity

The firmness of cheese was measured using the ball compressor by which means the penetration of a weighed semi-spherical ball into the surface of cheese under standard conditions of time and temperature is measured. An increase in ball compressor values indicates decreasing firmness. A measure of the elasticity or springiness of the cheese is obtained by observing the recovery values for the weighted ball in a standard time.

The firmness of cheese varied ($p < 0.001$) and was affected by trial, curing time, temperature and period of storage of milk before use (Table 4:36). The interaction between curing time and temperature of milk storage and also between temperature and storage period for milk were significant ($p < 0.001$). The mean values for the ball compressor readings dropped from 72.67 to 54.53 with increase of the age of cheese from one week to 8 months and thereafter increased to 59.49 for cheese aged 12 months. (Fig. 4:1)

The ball compressor values of cheese (at 1 week old) made from milk stored at $2^{o}C$ for 2, 4 and 7 d were lower than those for cheese made from unstored milk. On the other hand, where cheeses were produced from milk held at $6^{o}C$, the mean ball compressor penetration readings increased in the cheese made from milk stored for 2 d and then decreased where the milk was held for 4 and 7 d before use but was still higher than those obtained with cheese made from the same milk on the day of delivery. In the case of milk held for 7 d at $6^{o}C$ the mean reading for the curing period was 69.88 compared with 60.97 for the unstored milks. Significant interactions ($p < 0.001$) were observed between trial X temperature, curing X temperature, trial X storage time and trial X temperature X storage time. The interactions between curing X temperature X storage was significant ($p < 0.01$) and it was slightly significant ($p < 0.05$) for trial X curing X temperature and time X curing X storage time. Elasticity of cheeses (Table 4:37)

TABLE 4:36

The effect of cold storage of bulk milk at 2°C and 6°C on the firmness of cheese during the curing (the results are expressed as total ball compressor readings at 15.6°C (60°F)

| Milk storage condition / Curing period | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 74.02 | 71.04 | 67.68 | 68.89 | 78.63 | 74.83 | 91.30 | 75.18 |
| 1 Month | 64.95 | 63.81 | 64.04 | 66.94 | 66.44 | 65.97 | 75.68 | 66.60 |
| 2 Months | 62.82 | 62.03 | 65.22 | 72.32 | 64.04 | 62.89 | 72.02 | 65.52 |
| 4 Months | 57.74 | 58.82 | 61.75 | 64.10 | 60.27 | 57.77 | 67.27 | 60.68 |
| 8 Months | 53.18 | 49.78 | 54.40 | 55.76 | 57.09 | 54.77 | 58.06 | 54.53 |
| 12 Months | 59.74 | 57.04 | 58.84 | 59.05 | 63.45 | 55.50 | 62.54 | 59.49 |
| Mean values | 62.07 | 60.42 | 61.99 | 64.51 | 64.99 | 61.96 | 71.31 | 63.67 |
| | | 62.31 | | | 66.09 | | | |
| | | 1 | | 2 | 3 | | 4 | |
| Trial means | | 66.02 | | 60.45 | 61.40 | | 66.79 | |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 492.60 | 47.764*** |
| Curing | 5 | 1628.37 | 157.890*** |
| Invstor | 1 | 162.51 | 15.757** |
| Trial Curing | 15 | 47.10 | 4.567*** |
| Trial Invstor | 3 | 25.76 | 2.498 |
| Curing Invstor | 5 | 14.31 | 1.387 |
| Temperature | 1 | 514.42 | 49.879*** |
| Storage | 2 | 503.44 | 48.814*** |
| Trial Curing Invstor | 15 | 12.97 | 1.258 |
| Trial Temperature | 3 | 88.98 | 8.627*** |
| Curing Invstor Temperature | 5 | 134.04 | 12.997*** |
| Trial Storage | 6 | 77.88 | 7.551*** |
| Curing Storage | 10 | 26.30 | 2.550 |
| Temperature Storage | 2 | 145.60 | 14.118*** |
| Trial Curing Temperature | 15 | 20.04 | 1.943* |
| Trial Curing Storage | 30 | 18.26 | 1.771* |
| Trial Temperature Storage | 6 | 131.02 | 12.704*** |
| Curing Temperature Storage | 10 | 33.34 | 3.233** |
| Residual | 54 | 10.31 | |
| Total | 191 | 88.15 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.656 | 0.803 | 1.606 | 0.598 | 0.656 |

| | Trial Curing Invstor | Trial Invstor Temperature | Curing Temp. | Trial Storage | Curing Storage | Temperature Storage |
|---|---|---|---|---|---|---|
| SED | 2.622 | 1.197 | 1.466 | 1.311 | 1.606 | 0.803 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temp. Storage | Curing Temp. Storage |
|---|---|---|---|---|
| SED | 2.932 | 3.211 | 1.606 | 1.967 |

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

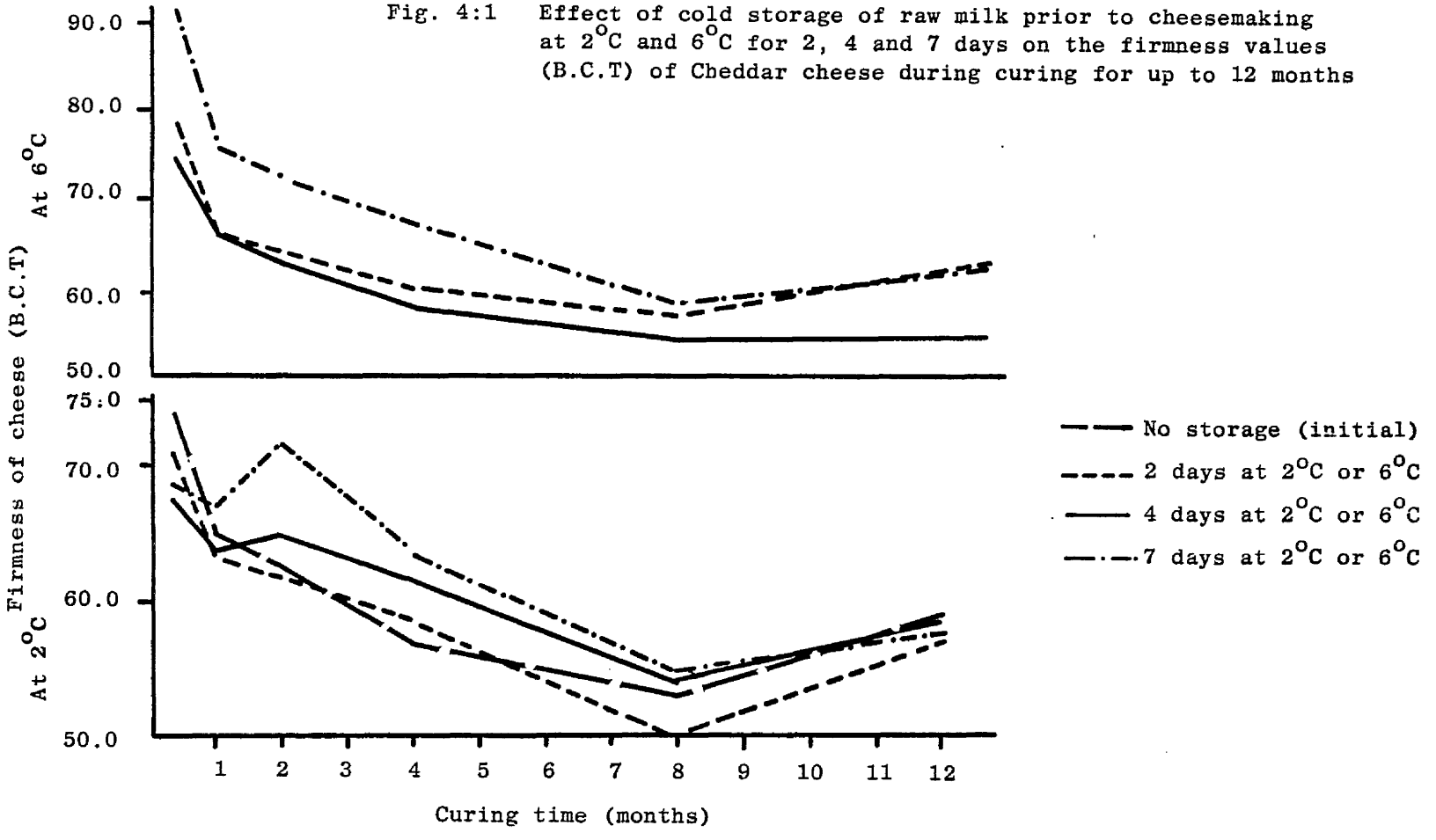



Fig. 4:1 Effect of cold storage of raw milk prior to cheesemaking at 2°C and 6°C for 2, 4 and 7 days on the firmness values (B.C.T) of Cheddar cheese during curing for up to 12 months

TABLE 4:37

The effect of cold storage of bulk milk at 2°C and 6°C on the elasticity of cheese during curing (expressed as a percentage of the total ball compressor readings at 15.6°C (60°F))

| Milk storage condition / Curing period | No storage (Initial) | Storage at 2°C | | | Storage at 6°C | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 83.63 | 79.46 | 70.79 | 69.60 | 79.20 | 73.80 | 72.35 | 74.63 |
| 1 Month | 82.95 | 84.74 | 81.66 | 81.83 | 76.75 | 81.31 | 71.61 | 78.63 |
| 2 Months | 73.94 | 77.16 | 74.25 | 72.01 | 74.20 | 75.21 | 68.69 | 72.24 |
| 4 Months | 72.41 | 70.58 | 71.69 | 70.06 | 69.02 | 60.19 | 57.99 | 69.17 |
| 8 Months | 73.50 | 77.32 | 76.66 | 73.69 | 71.50 | 70.71 | 61.25 | 72.27 |
| 12 Months | 67.69 | 71.56 | 69.93 | 70.13 | 66.45 | 70.99 | 62.33 | 68.35 |
| Mean values | 75.28 | 76.37 | 73.47 | 72.06 | 72.45 | 72.84 | 63.12 | 72.55 |
| | | 73.96 | | | 69.31 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 68.24 | 73.18 | 73.80 | 74.97 |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 421.94 | 53.530*** |
| Curing | 5 | 451.89 | 57.169*** |
| Invstor | 1 | 478.081 | 60.561*** |
| Trial Curing | 15 | 161.99 | 20.520*** |
| Trial Invstor | 3 | 47.89 | 6.039** |
| Curing Invstor | 5 | 103.49 | 13.067*** |
| Temperature | 1 | 781.370 | 98.980*** |
| Storage | 2 | 616.04 | 78.046*** |
| Trial Curing Invstor | 15 | 24.32 | 3.081** |
| Trial Temperature | 3 | 76.85 | 9.743*** |
| Curing Invstor Temperature | 5 | 56.419 | 7.147*** |
| Trial Storage | 6 | 94.669 | 11.992*** |
| Curing Storage | 10 | 31.17 | 3.948*** |
| Temperature Storage | 2 | 188.307 | 23.854*** |
| Trial Curing Temperature | 15 | 9.789 | 1.240 |
| Trial Curing Storage | 30 | 22.130 | 2.803** |
| Trial Temperature Storage | 6 | 62.735 | 7.947*** |
| Curing Temperature Storage | 10 | 11.532 | 1.461 |
| Residual | 54 | 7.894 | |
| Total | 191 | 77.133 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.574 | 0.702 | 1.405 | 0.524 | 0.574 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temperature Storage |
|---|---|---|---|---|---|---|
| SED | 2.294 | 1.047 | 1.282 | 1.147 | 1.405 | 0.702 |

| | Trial Curing Temp. | Trial Curing Storage | Trial Temperature Storage | Curing Temp. Storage |
|---|---|---|---|---|
| SED | 2.565 | 2.810 | 1.405 | 1.721 |

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

showed significant differences ($p < 0.001$) due to trial, curing and temperature and period of milk storage prior to use. Significant interactions ($p < 0.001$) were observed between curing X temperature, curing X storage, temperature X storage time, trial X curing, trial X temperature, curing X temperature, trial X storage time, curing X storage time and trial X temperature X storage time. This indicates that the period, temperature and trial have affected the curing of cheese. During curing of the cheese the elasticity figure for one month old cheese had increased compared with the one week old cheese. Further curing resulted in reduced elasticity values. The storage at both $2^{o}C$ and $6^{o}C$ of milk prior to use resulted in significantly lower elasticity values during the curing of cheese. The elasticity values were lower for cheeses produced from milks held at $6^{o}C$. Storage of milk at $2^{o}C$ for 2 d was associated with an increase in the elasticity of cheese. Further storage of milk at $2^{o}C$ caused decreasing elasticity in the resulting cheese. (Fig. 4:2)

4. pH

The pH of cheese (Table 4:38) fell during the first two months of curing and thereafter rose over the remainder of the period of one year. The individual trial, the curing period and the temperature of storage of milk prior to cheesemaking, had significant effects ($p < 0.001$) on pH values during curing. Storage of milk up to 7 d did not affect the pH values of the resulting cheese during curing. Significant interactions ($p < 0.001$) were observed between trial X curing period and temperature X storage time of milk prior to use. The interactions between trial X temperature of milk before use, curing period X milk storage time, trial X temperature X storage time were significant ($p < 0.05$).

Significant correlations (Table 3:39) were observed between firmness values and the soluble N ($p < 0.05$) and moisture contents of cheese ($p < 0.05$). Negative correlation was observed between the firmness of cheese and its fat content ($p < 0.01$). The elasticity was positively correlated with protein ($p < 0.001$) and soluble N levels ($p < 0.01$), but, negatively correlated with moisture content ($p < 0.01$).

As expected the yield was correlated significantly with moisture content of the cheese ($p < 0.001$). The yield is correlated with the firmness

Fig. 4:2 Effect of cold storage of raw milk prior to cheesemaking at 2°C and 6°C for 2, 4 and 7 days on the elasticity values of Cheddar cheese during curing for up to 12 months

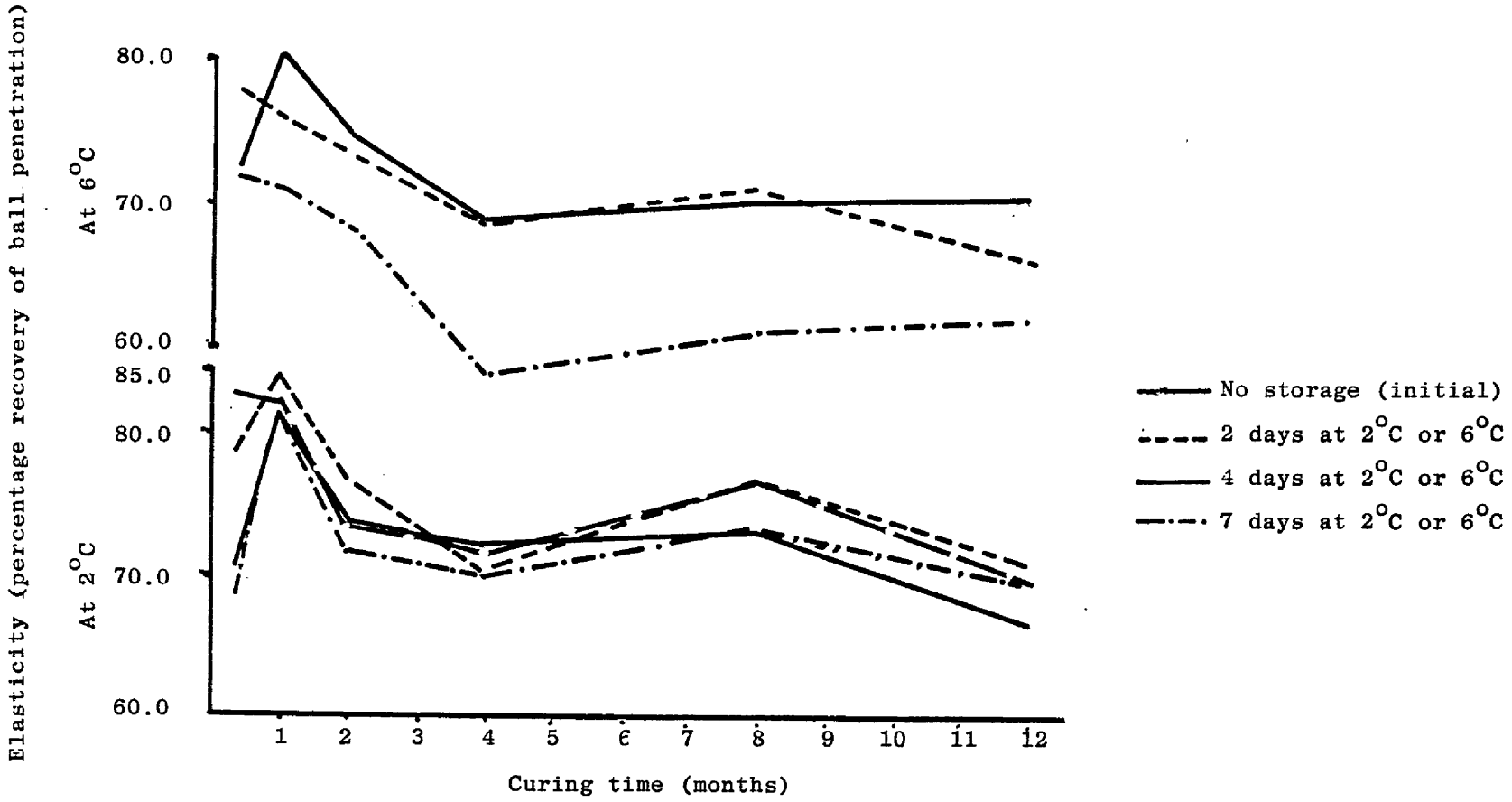


TABLE 4:38

The effect of cold storage of bulk milk at $2^{o}C$ and $6^{o}C$ on the pH of cheese during curing

| Milk storage conditions / Curing period | No storage (Initial) | Storage at $2^{o}C$ | | | Storage at $6^{o}C$ | | | Means |
|---|---|---|---|---|---|---|---|---|
| | | 2 d | 4 d | 7 d | 2 d | 4 d | 7 d | |
| 1 Week | 4.97 | 5.13 | 5.13 | 5.24 | 5.16 | 5.14 | 5.16 | 5.11 |
| 1 Month | 4.93 | 5.05 | 5.14 | 5.21 | 5.10 | 5.14 | 5.03 | 5.07 |
| 2 Months | 4.83 | 4.94 | 4.99 | 5.03 | 4.96 | 4.98 | 4.87 | 4.93 |
| 4 Months | 4.93 | 5.00 | 5.02 | 5.10 | 5.06 | 5.09 | 4.94 | 5.01 |
| 8 Months | 5.16 | 5.17 | 5.17 | 5.22 | 5.14 | 5.21 | 5.00 | 5.15 |
| 12 Months | 5.48 | 5.42 | 5.48 | 5.45 | 5.57 | 5.34 | 5.15 | 5.42 |
| Mean values | 5.05 | 5.12 | 5.15 | 5.21 | 5.16 | 5.15 | 5.02 | 5.12 |
| Temp. Means | | 5.16 | | | 5.11 | | | |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 5.10 | 5.04 | 5.19 | 5.12 |

| Source of Variation | DF | MS | VR |
|---|---|---|---|
| Trial | 3 | 0.3730071 | 28.977*** |
| Curing | 5 | 1.8232660 | 141.642*** |
| Invstor | 1 | 0.5295925 | 41.142*** |
| Trial Curing | 15 | 0.1730100 | 13.441*** |
| Trial Invstor | 3 | 0.0239478 | 1.860 |
| Curing Invstor | 5 | 0.1383915 | 10.751*** |
| Temperature | 1 | 0.1653131 | 12.842*** |
| Storage | 2 | 0.0370016 | 2.875 |
| Trial Curing Invstor | 15 | 0.0984147 | 7.645*** |
| Trial Temperature | 3 | 0.0412300 | 3.203* |
| Curing Invstor Temperature | 5 | 0.0132325 | 1.028 |
| Trial Storage | 6 | 0.0223606 | 1.737 |
| Curing Storage | 10 | 0.0394283 | 3.063* |
| Temperature Storage | 2 | 0.3456363 | 26.851*** |
| Trial Curing Temperature | 15 | 0.0233022 | 1.810 |
| Trial Curing Storage | 30 | 0.0157960 | 1.227 |
| Trial Temperature Storage | 6 | 0.0340716 | 2.647* |
| Curing Temperature Storage | 10 | 0.0213737 | 1.660 |
| Residual | 54 | 0.0128723 | |
| Total | 191 | 0.1004483 | |

| Table | Trial | Curing | Trial Curing | Temperature | Storage |
|---|---|---|---|---|---|
| SED | 0.01638 | 0.02006 | 0.04011 | 0.01495 | 0.01638 |

| | Trial Curing | Trial Temp. | Curing Temp. | Trial Storage | Curing Storage | Temp. Storage |
|---|---|---|---|---|---|---|
| SED | 0.06550 | 0.02990 | 0.03662 | 0.03275 | 0.04011 | 0.02006 |

| | Trial Curing Temperature | Trial Curing Storage | Trial Temperature Storage | Curing Temp. Storage |
|---|---|---|---|---|
| SED | 0.07324 | 0.08023 | 0.04011 | 0.04913 |

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

TABLE 4:39

The correlation coefficient between the mean figures for the compositional characteristics of cheese over a curing period of 12 months and the corresponding mean values for firmness, elasticity, yield and free fatty acids (FFA)

| | Firmness (B.C.T ) | Elasticity | Yield | Yield at 35 per cent moisture (Y35) | FFA at 9 months |
|---|---|---|---|---|---|
| Fat | -0.5291** | 0.0114 | -0.4700* | 0.0768 | 0.0446 |
| Protein | 0.3632 | 0.6570*** | -0.2988 | -0.3041 | -0.1572 |
| Soluble N | 0.4529* | 0.4694* | 0.0660 | -0.5870** | -0.2352 |
| Moisture | 0.4515* | -0.5873** | 0.8275*** | 0.1271 | 0.0616 |
| Firmness | - | -0.1228 | 0.5658** | 0.3183 | -0.1835 |
| Elasticity | -0.1228 | - | -0.6198*** | - | -0.0730 |
| FFA at 12 months | -0.2061 | 0.0231 | -0.1054 | -0.0018 | 0.9485*** |
| pH | 0.2988 | 0.2140 | -0.2323 | 0.0413 | -0.0520 |

TABLE 4:40

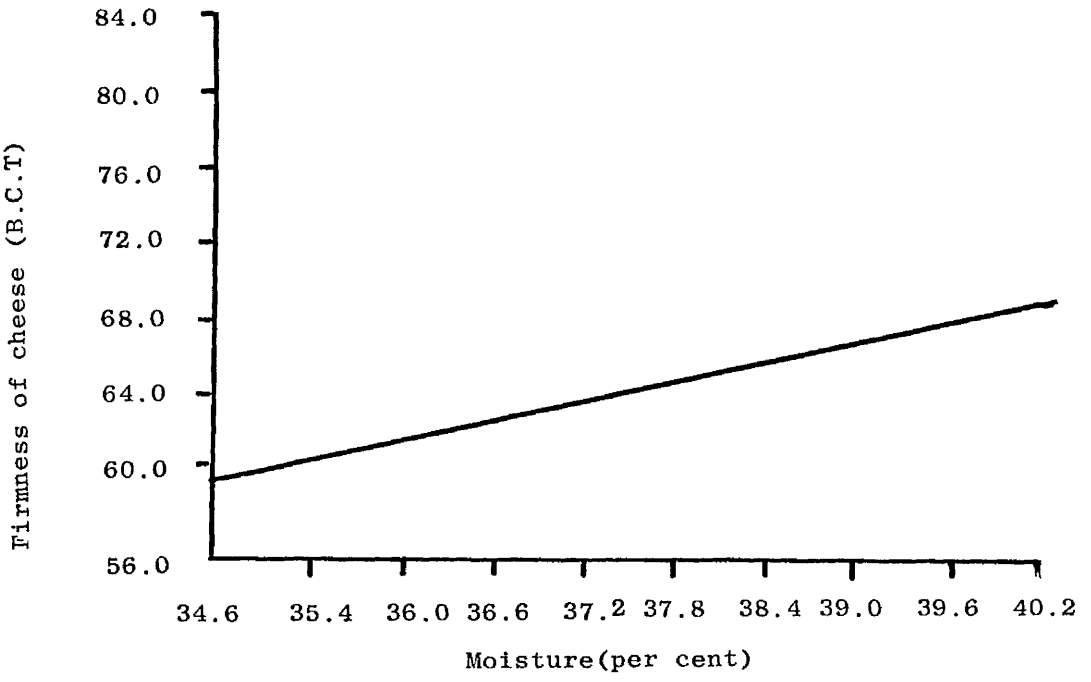
The regression analysis for the effect of moisture and soluble nitrogen content on the firmness of cheese

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (Moisture) | -0.51032 | 24.40717 | -0.02 |
| (Soluble N) | 47.4108 | 5.8317 | 8.13 |
| Slope (Moisture) | 1.72588 | 0.66894 | 2.58 |
| (Soluble N) | 3.8910 | 1.5020 | 2.59 |

Analysis of variance

| | DF | Moisture | | Soluble N | |
|---|---|---|---|---|---|
| | | SS | MS | SS | MS |
| Regression | 1 | 62.9 | 62.907 | 63.3 | 63.315 |
| Residual | 26 | 245.7 | 9.450 | 245.3 | 9.435 |
| Total | 27 | 308.6 | 11.430 | 308.6 | 11.430 |

Fig. 4:3 Standard curves for the correlation between the firmness (B.C.T) of Cheddar cheese and its content of moisture and soluble nitrogen



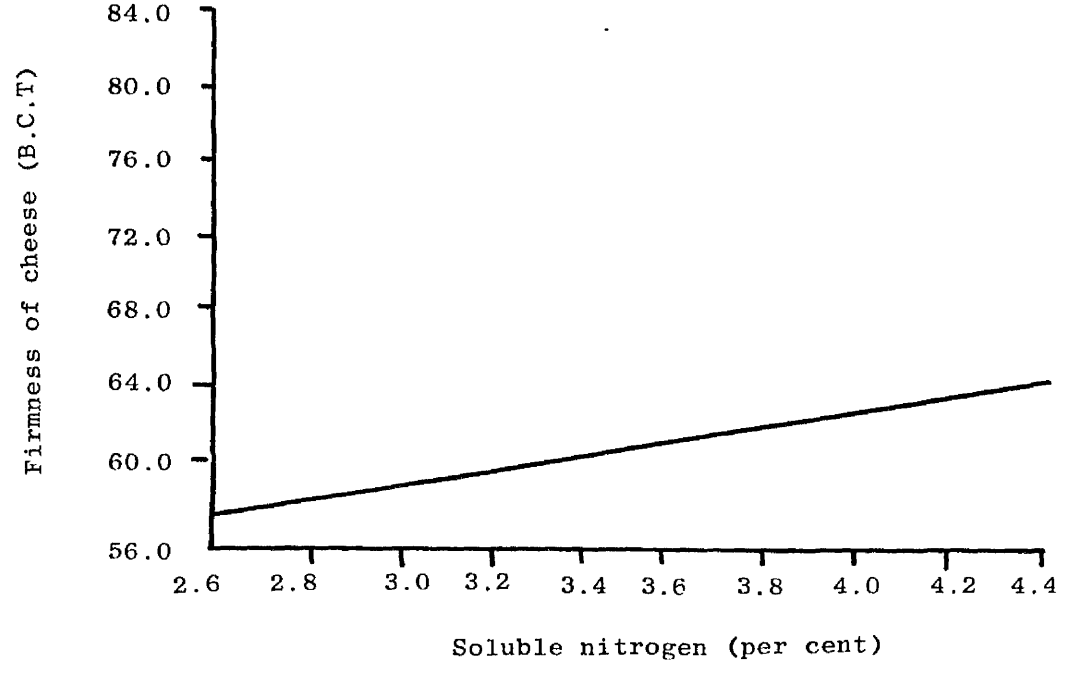



TABLE 4:41

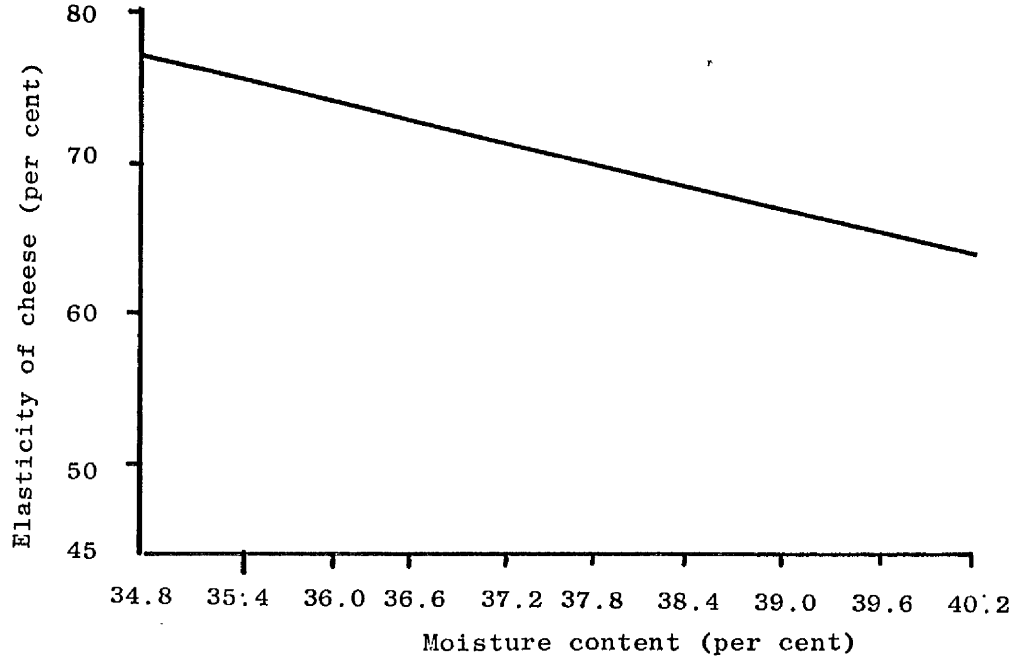
The regression analysis for the effect of moisture, total N and soluble N on elasticity

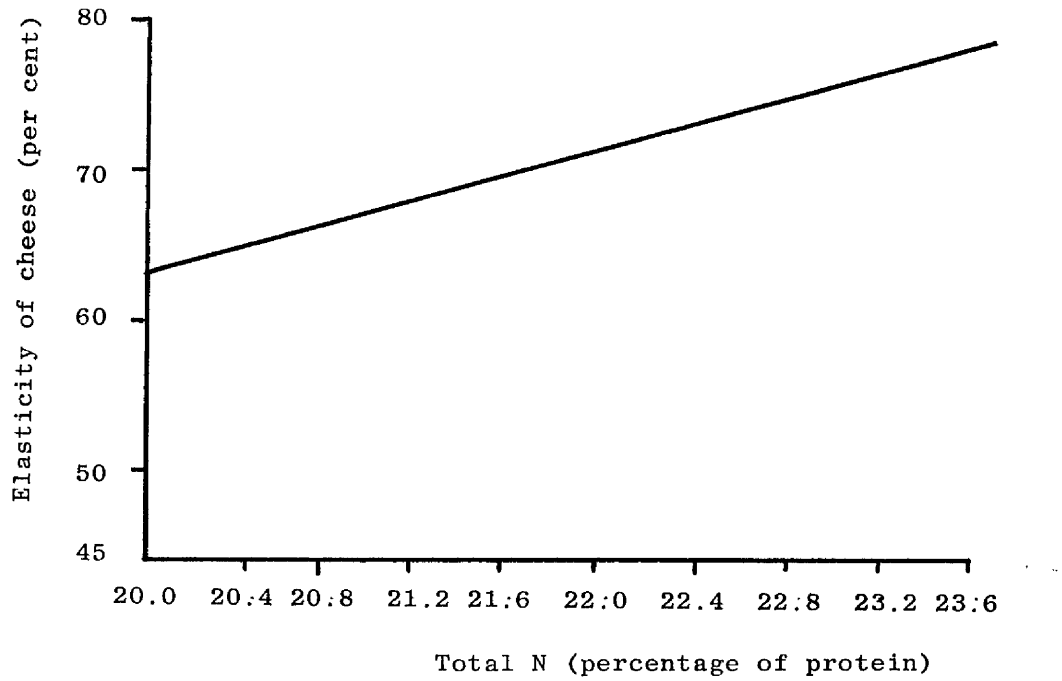
| | Estimate | S.E. | T |
|---|---|---|---|
| Y Intercept (Moisture) | 161.3856 | 28.1496 | 5.73 |
| (Total N) | -20.2523 | 16.3327 | -1.24 |
| (Soluble N) | 51.4172 | 6.6401 | 7.74 |
| Slope (Moisture) | -2.4189 | 0.7715 | -3.14 |
| (Total N) | 4.1791 | 0.7304 | 5.72 |
| (Soluble N) | 5.6264 | 1.7102 | 3.29 |

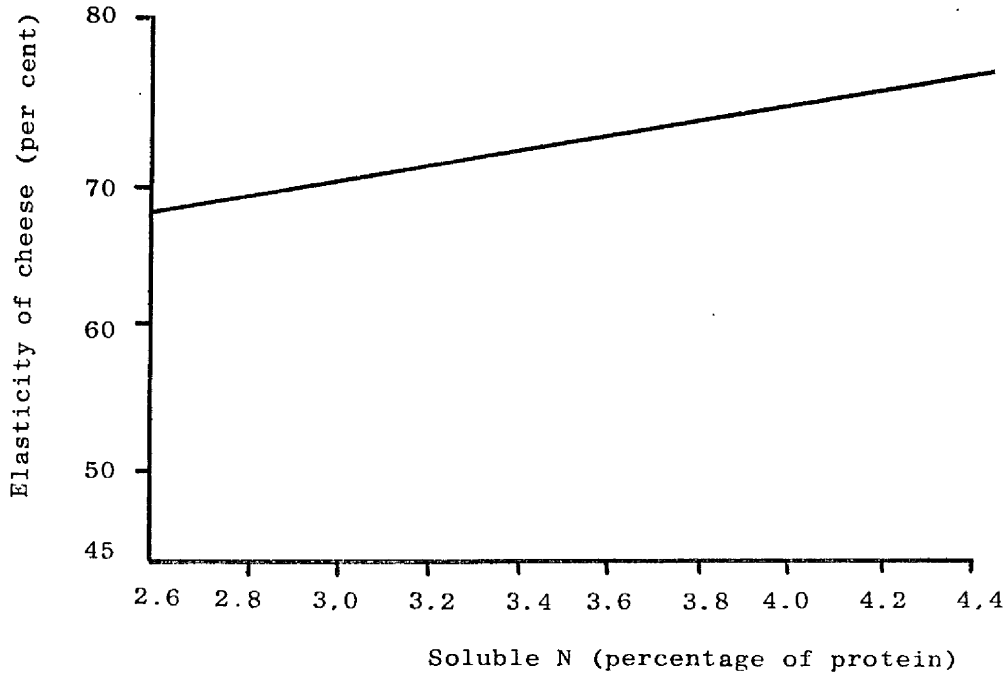
Analysis of variance

| | DF | Moisture | | Total N | | Soluble N | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 123.6 | 123.57 | 251.0 | 251.043 | 132.4 | 132.39 |
| Residual | 26 | 326.8 | 12.57 | 199.4 | 7.668 | 318.0 | 12.23 |
| Total | 27 | 450.4 | 16.68 | 450.4 | 16.682 | 450.4 | 16.68 |

of Cheddar cheese and its content of moisture and total nitrogen and soluble nitrogen.



of the cheese. The pH of the cheese did not show significant correlations with yield. There was a significant correlation between the FFA of cheese at 9 and 12 months of age.

The regression analysis (Table 4:40 and Table 4:41) and the standard curves (Figs 4:3 and 4:4) show the effect of each of these characteristics of cheese on its firmness and elasticity.

## DISCUSSION

Little information is available in the literature on the effect of cold storage of milk on the composition of cheese whey. Whey removed from curd at the running stage comprises the major part of the whey produced in cheesemaking.

### 1. Effect of cold storage on the calcium, phosphorus and ash contents of whey

#### (a) Calcium

Small but non-significant decreases were observed in the calcium level of wheys obtained in cheese production with milks stored at $2^{o}C$ for 2 and 4 d. No further decrease in calcium level occurred where storage of the milk was extended for a further 3 d prior to cheesemaking. Storage of milk at $6^{o}C$ for 2 d before use did not alter the calcium level of the whey. Storage of the milks for 4 and 7 d at this temperature however, resulted in significantly ($p < 0.001$) higher levels of calcium in the wheys. This increased loss of calcium into whey at the running stage might be due to the development of acidity in the milks prior to cheesemaking since it occurred in those wheys derived from milks with high titratable acidity (low pH) and which had increased levels of soluble calcium (Table 3:10). The findings reported here support the view of the effect of pH level expressed by Rajput and Ganguli (1981) who stated that more than 90 per cent of the calcium from the casein micelle of cow, buffalo and goat milks was released with lowering of the pH of milk to 5.0.

The calcium content of whey at cheddaring decreased significantly where the milk used for cheese production had been stored for 7 d at either temperature. In the case of wheys collected during

pressing, no significant differences in calcium content were found. The variations in the calcium concentration in the wheys collected during cheddaring and pressing are not important if we consider that the total loss of calcium into whey collected during cheddaring and pressing represent around 10 per cent of that lost into whey collected during running.

(b) Phosphorus

Storage of milk at 6°C prior to cheese production resulted in higher losses of phosphorus into the whey at running compared with unstored milks. These losses are slightly lower in the whey derived from milks stored at 2°C for 7 d than in whey derived from unstored milks. The storage of milk before use for 7 d at either temperature gave significant decreases in the phosphorus content of wheys collected during cheddaring. However, the wheys collected during pressing did not vary significantly in phosphorus content. Looking to the soluble phosphorus content in milk (Table 3:11) no significant change was found over a storage period at 2°C for up to 7 d but at 6°C the increase in soluble phosphorus was highly significant ($p < 0.001$).

(c) Ash

The ash content of whey did not vary significantly because of storage of milks prior to use for up to 7 d at either 2°C or 6°C. This finding is in agreement with results which showed that the storage of milk at 2°C and 6°C for up to 7 d did not affect the levels of soluble ash (Table 3:9).

2. Effect of cold storage on fat, protein and NPN lost into whey

(a) Fat

Cousin and Marth (1977b) found that the differences between the amounts of milk fat lost in wheys derived from unstored milk and milk held at 4.4°C for 1 week prior to use were negligible. Chapman et al. (1976) found no distinct pattern related to milk storage but reported that slightly higher fat losses occurred in wheys from refrigerated milks compared to whey from unstored milks. Chapman et al. (1978) reported that prolonged storage

of milk at 5°C for up to 72 h increased slightly the fat lost into the whey. Al-Obaidi (1980) did not find any significant effect of the storage of milk for up to 72 h at 4°C on the composition of the whey obtained in cheesemaking.

In this study the mean fat content of wheys at running did not vary significantly due to the period or temperature of storage of the milk prior to cheesemaking. This finding takes into account results obtained in one of the four trials where in the case of cheese production from milk held at 6°C for 7 d the fat content of the whey at running was three times that obtained with unstored milk. No significant variations occurred in fat losses during cheddaring except in the case of trial 4 where using milk held for 7 d at 6°C an increase in the fat lost into whey was detected.

In the case of whey obtained during pressing of curd made from milk held at 6°C for 7 d the mean fat content was significantly greater than the control whey from unstored milk. The tendency, however, was for the fat content of whey derived from stored milks to be higher than that of the control wheys (from unstored milks).

Hicks et al. (1980) reported that lipolytic enzymes cause the hydrolysis of triglycerides with the resulting increase in free fatty acids which are lost in the whey; the fat losses being greater as storage time increases.

(b) Protein

McCaskey and Babel (1966) found that the protein lost into the whey from cheese made with fresh raw milk was about 28 to 29 per cent of the total protein, losses were higher with pasteurized milk stored for several days at various low temperatures. Samples which were held at 2.2°C showed only a slight increase in protein loss during 3 d, even though the proteolytic and psychrophilic counts increased considerably. Milks stored for more than 3 d under these conditions resulted in an appreciable increase in protein in the whey from cheese-making and the increase accelerated with milk storage. Cousin and Marth (1977b) found that there was

a slight increase in per cent of nitrogen in wheys from refrigerated milks as compared to those from control milks. Al-Obaidi (1980) found no significant effect for milk stored on the protein content of the whey.

In this study, the protein contents in whey at running increased significantly with the increase of the period of storage of the milk prior to cheesemaking. This increase was higher at 6°C than it was when the milk was stored at 2°C. During cheddaring and pressing, the protein content of the two wheys did not vary significantly. While statistically not significant there appeared to be slight decreases in whey protein content where the milk used had been stored at 2°C up to 7 d and at 6°C for up to 4 d. In the case of milk held for 7 d at 6°C before use there was an increase in the protein lost into whey.

(c) Non-protein nitrogen (NPN)

Non-protein nitrogen content of the whey at running and cheddaring showed no significant effect due to the period or temperature of milk storage before use. These results are in agreement with the findings of Al-Obaidi (1980). The significant effect of storage of milk for 7 d at 6°C before cheesemaking on the whey composition at pressing might be due to proteolytic enzymes in the milk which accelerate the breakdown of protein during pressing.

It would be of interest to note that further studies are required to explain these observations. One possibility concerns the activity of proteolytic enzymes formed during the storage period by bacteria.

3. Effect of cold storage of milks on cheese yield and composition

Cheesemaking experts frequently complain about difficulties in manufacturing of cheese made from refrigerated milk (Knoop and Peters, 1978a). The defects mentioned include: delayed acidification, too soft a curd, poor syneresis, deformation of cheeses, increased release of water during ripening and, occasionally, too dry a curd. Reimerdes (1978) has related losses in cheese yield to cold storage of milk prior to

cheesemaking, but little information is available.

The significant increase in the cheese yield of milk stored at 6°C was due to the retention of higher moisture in these cheeses. This explanation is in agreement with the finding of Hicks et al. (1977) who stated that milk stored at 5, 7.5 and 10°C up to 12 d affected the moisture content of the cheese. Chapman et al. (1978) reported that prolonged storage of milk at 5°C resulted in an increase in the moisture held in the curd at milling. The effects of period and temperature of storage are more clear after adjusting the moisture content of the cheeses into 35 per cent level. There is a slight increase in the mean yield of cheese made from milk stored at 2°C whereas storage of milk prior to use at 6°C had an opposite effect.

This may be due to the difference in the microbial development at the two temperatures and the resulting difference in the extent of the breakdown of caseins. Aylward et al. (1980) reported that proteolysis of casein can reduce yield of cheese made from the milk affected by increased numbers of bacteria in stored milks. The psychrotrophic microflora of cold-stored raw milk has an important impact on its casein content, and then, on the yield of cheese from the milk.

Hicks et al. (1980) reported that milk considered by them to be of high bacteriological quality suffered no deterioration over a long period of 3 to 4 d from the time of milking. Thereafter heavy yield losses occurred. Low quality milk had a linear effect on yield loss.

Aylward et al. (1980) reported that after 10 d of storage at 5°C, the average decrease in cottage cheese yield from skim milk (grade A) was approximately 2 per cent per day. However, this average figure indicates the fact that there was little change in cheese yield from skim milk held at 5°C for 4 d before use - thereafter lower yield occurred. Al-Obaidi (1980) found no effect of storage of milk for 72 h at 4°C on the yield of Cheddar cheese. Onuorah et al. (1980) found that cheese yield measured as dry matter decreased as storage time increased at 5, 7.5 and 10°C for up to 12 d.

Recently Olson (1981) reported that the yield of cheese from cold-stored milk was about one to two per cent lower than unstored milk.

This is equivalent to about 0.1 to 1.8 pounds of cheese per hundredweight of milk. This effect on yield was not caused by the action of psychrotrophic bacteria since numbers were not high enough to cause problems.

4. Effect of cold storage of milk on calcium, phosphorus, ash, fat and FFA of Cheddar cheese

The calcium content of cheeses made from milk held at 2°C was significantly higher than that of cheese derived from the same milk held at 6°C before use. The maximum reduction occurred in calcium content in those cheeses made from milk held for 7 d at 6°C. This reduction in calcium content of cheese was reflected in an increase in the calcium lost into whey. With increase storage of milk at 2°C prior to use the calcium retained in the cheese was increased significantly. The mean phosphorus content of cheese produced from milk held at 2°C was higher than that of cheese made from milk held at 6°C. The storage period at 2°C increased the phosphorus content while an equal period of storage at 6°C had an opposite effect (these variations were not statistically significant). Changes in the ash content of the cheese made from milks after varying storage conditions are similar to those found for calcium and phosphorus.

This effect of cold storage conditions on the level of calcium, phosphorus and ash in cheese may be related to the changes in the state of the casein micelle and the composition of curds as an effect of microbial and enzymatic action. However, the equilibrium between the micellar and soluble state of these components changed in the milk as a result of storage of milk.

The fat content (calculated on dry matter basis) of the cheeses produced from milks held at 6°C was 1 per cent lower than the cheeses produced from milks held at 2°C but this difference was not significant statistically. This variation is probably due to the microbial lipolysis which took place in these milks (Chapter Three). These variations correspond with the higher fat losses in the whey. Al-Obaidi (1980) did not observe any significant effect of the storage of milk for 72 h at 4°C on the fat content of cheese.

The FFA of the cheese rose with increasing the period and temperature

of storage of milk prior to use. This increase in FFA corresponds with an increase in the ADV of the milk as a result of lypolysis in the original milk fat. It also corresponds with fat losses into whey. These variations in FFA occurred especially in the cheese produced from milk held at 6°C for 4 and 7 d. These cheeses were of a bad quality since the ADV were more than 3.0 (Deeth and Fitz-Gerald, 1976) while all other cheeses were of good quality by their criterion.

Milk lipase is destroyed by pasteurization prior to cheesemaking and in any case, being inactive at the pH and salt content of cheese, cannot contribute to the development of rancidity after manufacture. Rancidity in Cheddar cheese arises in most cases from lipolysis during storage, by lipase originating from microbial contamination of cheese or cheese milk. This is clearly shown in the development of FFA in cheeses made from milk held at 6°C for 4 and 7 d where the FFA values increased, from 9 to 12 months of curing. Cheeses made from milks given other treatments (i.e. no storage and storage at 2°C for up to 7 d and storage at 6°C for up to 2 d) did not show any increase in FFA during the curing period from the 9th to the 12th month.

## 5. Effect of cold storage of milk and the curing of Cheddar cheese on its composition

### 1. Moisture content

Scott (1981) divided the state in which the moisture is present in cheese into the following:

(a) that bound in the structure of a component of curd, i.e. protein;

(b) that loosely held by attractive forces in context with the curd particles, including fat, e.g. hygroscopic moisture, and

(c) that moisture which is free to move about and which carries the solubles about inside the curd.

As a result of the storage of milk at 6°C the cheese produced retained higher moisture content. On the other hand cheese produced from milks held at 2°C retained lower moisture content

as compared to cheese made from unstored milk. The retention of higher moisture content is due to the quality of milk. Hicks et al. (1980) found that milk of low microbiological quality resulted in cheese which retained more moisture than cheese made from milk of high microbiological quality. Their experiment was undertaken using the same manufacturing conditions and the results were indicative of the increased protein degradation in milks of poor microbiological quality. Onuorah et al. (1980) stated that the moisture content in cheese increased as storage time increased at 5, 7.5 and 10°C.

In these studies storage of the milk for 7 d at 6°C resulted in a moisture content above the legal standard for Cheddar cheese when a standard manufacturing procedure was used. Such a result was mentioned by Hicks et al. (1980) who found that Cheddar cheese manufactured from stored milk had a moisture content in excess of legal and plant standards. In other recent work Al-Obaidi (1980) reported that storing the milk at 4°C for up to 72 h before use resulted in Cheddar cheese having slightly higher moisture content but these increases were not significant statistically.

On the current experiments cheese produced from milk stored up to 7 d at 2°C or 4 d at 6°C contain a range of MFFC of 53.63 to 54.42 which is within the limits for good Cheddar cheese. Cheeses produced from milks held for 7 d at 6°C had a MFFC of 56.10 per cent which is higher than is consistent with good quality.

## 2. Protein degradation during curing

Cheeses produced from milks held at 6°C retained lower total protein than the cheese of the unstored milks or the milks held at 2°C. This is probably due to the higher extent of proteolysis at higher temperature which took place as an effect of microbial and enzymatic breakdown of milk proteins. This view was substantiated by determining the soluble nitrogen as a percentage of total nitrogen content of cheese (Table 4:35). These figures showed that in the case of cheese produced from milk held at 2°C and 6°C for 2 d the soluble nitrogen expressed as a percentage of total nitrogen of cheese was significantly lower than with cheese made from the

same milk on delivery. But after the milk had been stored for 7 d at either 2°C or 6°C the resulting cheeses contained higher SN/TN percentages. Several enzyme systems (rennet, microbial peptide hydrolases) work together and it is difficult to know the action of each system separately in cheese.

During the first 24 h after cheesemaking the temperature of the curd is high enough to increase the rate of enzyme acitivity compared to the curing temperature of 10°C (50°F). The temperature of the curd before pressing should be below the liquid fat temperature, i.e. 23.9°C (75°F) in summer-time, 26°C (79°F) in winter-time (Scott, 1981). This temperature is still high enough to accelerate the enzyme activity.

Gripon et al. (1977) differentiated soluble from insoluble nitrogen on the basis of solubility at pH 4.6. They reported that the purified acid protease from Penicillium roqueforti and neutral protease from P. caseicolum induced large increases in pH 4.6 soluble nitrogen and non-protein nitrogen but had little effect on production of free amino acids in the aseptic curds (containing 48 per cent dry matter) during ripening. Curd homogenates of Str.lactis led to little increase in pH 4.6 soluble nitrogen and NPN but to a high increase of free amino acids. The acid protease form P. roqueforti and the neutral protease from P. caseicolum play a fundamental role in the proteolysis induced by their micro-organisms during cheesemaking. Str.lactis strains exerts a slight endopeptidase activity on the caseins of curd. The extent of the activity would depend on the amount of enzymes contributed by the original milk and on the extent of growth of proteolytic micro-organisms and the related quantity of proteolytic enzymes produced by them. The correlation between the proteolytic count and the extent of protein degradation is discussed in Chapter Seven.

During ripening, the protein is degraded as a result of enzymatic and microbial action. The bacterial flora of cheese curd is continually changing both in numbers and in species (Scott, 1981). This may explain the slight difference in the pattern of proteolysis

observed between initial and stored milks at the two temperatures. A means of measuring the amount of enzyme present in milk at the time of cheesemaking, either from natural source or as a result of the growth of proteolytic micro-organisms before use of the milks would be advantageous.

Thus the growth of micro-organisms is more prolific in high moisture curds than in low moisture curds, and the ripening rate of high moisture cheese is faster than where the moisture content of cheese is low (Scott, 1981).

However the variation in the pH of cheese is another important factor to control the enzymatic action. Scott (1981) reported that some enzymes are active in two regions of pH (alkaline and acid values), but the end products may differ in each region of activity. Low pH values stimulate hydrolysis of fats and the production of soluble nitrogenous substances, amino nitrogen and ammonia. In most hard cheese, enzymes are active in the region pH 4.9-5.5 and less active at higher pH values.

The correlation between the pH of cheese during ripening and cheese characteristics was not significant but the pH values did not vary significantly except in the case where 7-day old milk ($6^{o}C$) was used. However, slight correlations between pH and firmness and elasticity of cheese were found and slight negative correlations were evident between pH and the cheese yield which is due to the higher moisture content of cheeses (made from 7-d old milk at $6^{o}C$) of lower pH.

The significant variations observed in the pH values of cheeses as affected by the temperatues and period of storage of milk is due to the different microbiological quality of milk. However, the type and rate of enzymatic action on milk components varied from one temperature to another and after different periods of storage.

3. Firmness and elasticity of cheese

Cheese produced from milk stored at $2^{o}C$ were firmer (at 1 week)

than the cheese made from unstored milk. On the other hand, storage of milk at 6°C for 2 d of storage resulted in cheese being softer in consistency.

Chapman et al. (1976) measured the firmness of rennet gels during the curd forming stage of cheesemaking. Storage of milk for 72 h at 5°C resulted in the formation of rennet gels which were firmer than those produced from the same milk before cold storage. These workers associated this finding with the fall in pH and the liberation of calcium ions. The combined effects of low temperature and prolonged storage of milk on firmness of rennet gels were to retard the rate of which the curds developed rigidity.

During curing of cheese in the author's work there were increases in the firmness value (i.e. decrease in B.C.T.). The interactions between temperature and period of storage of milk and the curing time indicated that the use of different temperatures (2 or 6°C) for different times of storage of milk affected the firmness of cheese to varying degrees depending on the various combinations of temperature, duration of storage of milk and length of curing time.

These variations may be due to enzymatic residues which remain active after pasteurization and to the cheesemaking procedure.

The firmness values (B.C.T.) were negatively correlated with fat content of cheese and positively correlated with soluble N and moisture. This indicates that cheeses with higher moisture and/or higher soluble N would be increasingly softer. On the other hand cheese with high levels of fat were found to be firmer. There was positive correlation between the firmness of cheese and total nitrogen content but this correlation was not significant.

Cousin and Marth (1977b) found that firmer curd resulted when Cheddar cheese was made from milk inoculated with psychrotrophic bacteria. Chen et al. (1979) found a close correlation between the textural measurement (for 11 types of cheeses including Cheddar cheese) and with composition and pH of the cheese. Increases in protein content and lower acidities (higher pH) resulted in increased cheese hardness. Similar interpretation can be drawn

for elasticity. Fat content does not contribute significantly to variations in hardness and elasticity. The elasticity independent variable follows this sequence:

protein > NaCl > water > pH > fat

Garnot et al. (1981) found that the maximum firmness was increased substantially with protein content of the cheese. The rate of firming of rennet gels after clotting increased markedly with increase in protein content of the milk.

In the present experiments significant correlations were observed between firmness and elasticity and the yield of cheese. Bynum and Olson (1981) found that the significant effects of using different curd firmness levels at the time of cutting on the yield and retention of milk constituents was observable only after the ninth month of curing of cheese in commercial trials. This suggests that monitoring curd firmness prior to cutting offers the potential for reducing losses in cheese production. The same authors found that the recovery of milk fat in cheese and the yield of cheese expressed per unit of milk fat were greater in lots of cheese made with a slightly increased curd strength at cutting compared to more typical curd firmness values. Pilot-scale trial showed no difference in yield parameter and recovery of milk components in cheese between treatments (two different curd firmness levels were used at cutting time).

The elasticity measurements which indicate the springiness of cheese showed lower values (less springiness) for the cheese made from stored milk at either temperature of storage than cheeses made from unstored milks. The springiness decreased as the curing time was extended.

The elasticity correlated significantly with the protein content of the cheese and to a lesser extent with soluble N. Elasticity showed a slight correlation with the pH of the cheese. But, it had a significant negative correlation with the moisture content of the cheese.

The correlation between the yield of the cheese and its characteristics

showed that the yield increased with the increase of moisture, and decreased firmness, in a decreasing order. Yield also decreased with increasing values for elasticity and ADV and for increasing content of fat, calcium, protein, ash and salt in water in this order of decreasing importance. But, the correlation between yield at 35 per cent moisture content (Y35) showed a different story (Table 4:39). The Y35 correlate negatively significant only with the soluble N content of cheese. Slight positive correlation with the fat content of cheese was also observed. This indicated that any correct conclusion to be drawn from yield result can not be achieved unless the moisture content of the cheese is adjusted to a constant figure.

Kairyukshtene (1971) stated that storage of milk at $4^{o}C$ for 24 h caused a reduction in the elasticity of the rennet coagulum. Olson (1977) found that the rennet curd tension of the milk increased with more extensive bacterial growth and proteolysis of milk protein prior to cheesemaking.

The FFA at nine and twelve months of age of cheese were correlated to each other ($p < 0.001$). The FFA of 12 months old cheese had not increased significantly from the levels existing at 9 months. This might be due to the presence of lipase inhibitors which might be produced by micro-organisms. Also, the salt content of cheese and its pH might inhibit lipase activity.

Measurements of FFA were made after sensory evaluation had indicated the presence of strongly rancid cheese and so no early curing time values were made.

## CONCLUSIONS

1. The results of whey analysis showed that storage of milk at $2^{o}C$ produced either no significant differences in the whey composition or a reduction in the amount of some milk components lost in the wheys (i.e. protein, calcium and phosphorus). On the other hand, storage of milk at $6^{o}C$ showed an increased loss of more milk components to the whey.

2. Cheese yield (calculated at a 35 per cent moisture level) was not significantly affected by storage of milk for up to 7 d at $2^{o}C$. There was a slight increase in the yield of cheese from milk stored for 2 d at $2^{o}C$. Although this increase was not significant statistically, it might be important commercially. It occurred in three of the four trials and in the overall analysis. Storage of milk at $6^{o}C$ on the other hand caused slight reduction in cheese yield.

3. Cheeses produced from milks held at $2^{o}C$ contained more calcium, phosphorus, ash and slightly more protein, fat and the free fatty acid level (FFA) was higher. These cheeses contained less moisture and soluble N than the control cheese (made from milk on the day of delivery) and had lower elasticity and were slightly softer. The mean pH (all 4 trials and values taken over entire curing period) of cheeses from milk held at $2^{o}C$ was 5.16 compared to 5.05 for the control cheese and 5.11 for cheese made from milks held at $6^{o}C$. Cheese produced from milks held at $6^{o}C$ retained less calcium, phosphorus, ash, protein, soluble N and had a slightly lower fat content. The free fatty acid content (FFA) was higher than that of the control cheese as was the moisture level and the texture of these cheeses was softer and less elastic.

   Milks held at $6^{o}C$ for 7 d gave a lower yield of cheese and the resultant cheese had lower contents of calcium, phosphorus, ash, fat and protein. The cheese was less elastic than the control. The pH was lower than the control. These cheeses had higher values than the control for free fatty acids (FFA), moisture, soluble N, SN/TN and ball compressor readings. These results indicated that the composition of cheese produced from milks held at $6^{o}C$ for 7d was unsatisfactory for moisture and fat in dry matter. The cheese did not meet legal standards for Cheddar cheese in the United Kingdom. While no standards exist for free fatty acid content of Cheddar cheese the high values obtained indicated inferior quality of the control and other cheese made during the trials.

4. The period and temperature of storage of milk prior to cheesemaking

affected the values obtained during the ripening of the resultant cheese. Lower moisture values associated with the increase in curing time may have been caused by exudation of 'whey' from the cheese especially in the case of cheese made from milks stored at $6^{o}C$. The total N showed slight increase which was due to the lower moisture contnet of the cheese. The soluble N, the ratio of SN/TN and the firmness of the cheese increased during the ripening period. The pH decreased during the early stages of ripening and thereafter increased in the normal way.

5. Yield of cheese (calculated on 35 per cent moisture basis) was correlated negatively with the soluble N content of the cheese. The correlation between yield and fat content of the cheese was positive but not statistically significant.

   Increases in the moisture content and soluble N content of cheese were associated with softer cheese. If the cheese contained higher total protein and or soluble N the elasticity of the cheese was higher.

# CHAPTER FIVE

## QUALITY ASSESSMENT OF RIPENED CHEDDAR CHEESE MADE FROM COLD STORED MILK

### INTRODUCTION

The sense of taste and smell may be considered as the human chemical senses because they provide data which in most cases could not be obtained by any other chemical test. By the taste and odour along with the closely associated tactile properties detected by touch, the consumer acceptability of the food could be determined.

The chemical and physical changes which take place in Cheddar cheese during ripening cause the cheese to change from a tough and curdy state to one which is soft and mellow. During this process, the insoluble nitrogenous components undergo change to soluble forms. In the course of this progressive proteolysis, the paracasein and the lesser proteins are slowly converted to simpler nitrogenous compounds, including proteoses, peptones, amino acids and ammonia (Lee, 1975).

The requirement is that the body of Cheddar cheese be firm, smooth and pliable as judged by actual feeling of the cheese by the hand. A plug of cheese removed by means of a trier should be full and free from openings and when bent, the plug should be break sharply but exhibit some flexibility. When worked between thumb and forefinger, the cheese should break down with some resistance into a smooth, cohesive mass which still possesses substance. Experienced cheese judges rely heavily on observation of body and texture (Tobias, 1976).

Regarding the effect of cold storage of milk on the quality of cheese, Mabbitt (1980) reported that at $5^{o}C$ the population of bacteria is doubled very approximately every 8 h in milk. Unfortunately the transport of milk from farm and subsequent storage at the creamery results inevitably in further contamination and also the temperature may rise and provide conditions for an increase in the bacterial growth state. It is also reported that the quality of Cheddar cheese decreased with milk storage time (Hicks *et al.*, 1980).

Little information is available on the effect of cold storage

especially lower than 4°C on the quality of Cheddar cheese. Therefore, this experiment was undertaken to follow the effect of cold storage of milk prior to cheesemaking on the organoleptic qualities of the resultant cheese during curing.

## EXPERIMENTAL

### 1. Presentation of cheese samples

Cheese samples were presented for grading in the curing room at 10°C (50°F). Cheese samples were also presented before a group of eleven members of staff of the Department of Dairy Technology, The West of Scotland Agricultural College. A well known grader (a member of staff of the Company of Scotland Cheddar Ltd.) took part in the quality assessment of the cheese. Two blocks of 4.53 kg (10 lb) from each vat of cheese made from non-stored milk were used as a control beside the cheese samples which were made from the same milks which had been held either at 2°C or 6°C for 2, 4 and 7 days prior to pasteurization and cheese production. Each sample was presented in duplicate. Cheese samples were positioned on the table in random manner (O'Mahony, 1979).

The nylon/polythene pouch was cut from one end of each block and a slice of about 1 cm thickness was cut and discarded. Another two slices were cut, one to show the openness and colour of the cheese and the other slice was cut into about 2 cm cubes for examining the flavour, taste, texture and body. The cheese samples were prepared in the curing room at 10°C (50°F) and then transferred into a well lit room at a temperature of around 16°C where the cheese samples were tested organoleptically. All of the blocks and slices of cheeses were presented on a white paper on a stainless steel table. Cheese blocks were identified by random numbers on a uniform white label.

### 2. Quality assessment

Cheese assessments were carried out on the cheese samples after 2, 4, 8 and 12 months of curing for each of the four trials. The people involved in cheese assessment were asked to grade the samples using a scale of 0 to 10 for each characteristic of the cheese as follows:-

1. Flavour/smell: 0 very poor 10 excellent
2. Taste: 0 very poor 10 excellent

| | | | |
|---|---|---|---|
| 3. | Body/firmness: | 0 very weak | 10 very firm |
| 4. | Texture/fusion: | 0 very crumbly* | 10 very smooth** |
| 5. | Openness: | 0 very open e.g. gas holes | 10 very close texture e.g. no gas holes |
| 6. | Colour: | 0 very discoloured | 10 very uniform and normal colour |

*not typical of top quality Cheddar cheese

**typical of top quality Cheddar cheese.

The panel judges were asked to identify any observable off-flavour whether it was: acid, high acid, sour, bitter, fermented, fruity, flat, garlic, onion, leek, weed, heated-whey, cooked, malty, metallic, mouldy, musty, rancid, lipase, putrid, sulphide, unclean, utensil, whey-taint, sour-whey and or yeasty. Pieces of apple were available for the panel members when grading 8 and 12 months old cheese samples. These pieces of apple were used at these stages due to the presence of some distinct flavour in those cheeses made from milks held for 4 and 7 d at $6^{o}C$. If the samples had been tasted before some of the good cheeses, it would make it difficult for the judges to grade the cheese samples which are presented randomly.

## 3. Definition of terms

Flavour may be described as the composite sensation of taste and odour. The generally accepted meaning of true taste is the gustatory sensation transmitted through the taste buds located on the tongue and the soft palate. Odour stimuli, are registered by the olfactory epithelium, to which they may gain entrance either through the nose or through the mouth. There are certain other flavour characteristics which are neither true taste nor odours but which, nevertheless, contribute to flavour (Tobias, 1976).

The term body (firmness), refers to the physical properties of consistency which include firmness, elasticity and plasticity (Al-Obaidi, 1980). Firm cheese should feel hard and offer resistance to pressure. It should also be free from rough particles of curd and the plug from such cheese should bend before breaking slowly.

Texture means the mass feel whether the piece of cheese is "smooth,

silky, waxy and fine or whether it is sticky, pasty, meaty or crumbly" (Nelson and Trout, 1965). Openness refers to the nature and extent of opening in the cheese. Whether they are regular, angular, rounded, large or small. And whether the lustre or shine of their inner surface is dry or wet.

The importance of colour and appearance of dairy products is not limited to the very obvious and important aesthetic reasons but must be considered in relation to other quality criteria. A faded colour in Cheddar cheese may not in itself be highly objectionable except for its relation to excessive acidity, which is the original cause of the difficulty (Tobias, 1976).

RESULTS

Analysis of variance of the organoleptic assessment of cheese manufactured from bulk silo milk on delivery from a commercial dairy and after cold storage is presented in Table 5:1. Significant variations ($p < 0.001$) were observed between trials in the flavour, taste, means of the "flavour and taste" scores, openness, colour and on the total scores. Cheeses made in different trials also varied in body and texture scores ($p < 0.05$). Curing time had a significant effect on the scores awarded ($p < 0.001$) for every characteristic of the cheese. All the interactions involved in this experiment are significantly important as shown (Table 5:1). The highest variations were due to the interaction between temperature X storage. This indicates that the effect of storage at $2^{o}C$ has a different effect than the storage at $6^{o}C$.

The interaction between curing time X storage of milk X temperature proved that the length of period of storage at $2^{o}C$ had a different effect compared to the length of period of storage of $6^{o}C$ during ripening of cheese. This indicates that the changes which affected milk quality are important for the ripening of cheese.

The results obtained by the eleven panelists for the grading of the duplicated samples were subjected to examination of the consistency of the assessment made by the individual panelists at each grading time (i.e. at 2, 4, 8 and 12 months). The statistical analysis indicated that two panelists gave inconsistent judgements and their results were

TABLE 5:1

Analysis of variance of the scores awarded by the members of a grading panel for the different criteria of Cheddar cheese after 2, 4, 8 and 12 months of curing

| | DF | Variation ratios | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Flavour | Taste | Flavour and taste | Body | Texture | Openness | Colour | Total scores |
| Trial | 3 | 29.464*** | 23.577*** | 26.805*** | 3.617* | 3.263* | 17.324*** | 57.825*** | 30.903*** |
| Time | 3 | 251.597*** | 115.276*** | 201.582*** | 83.036*** | 123.251*** | 66.372*** | 169.589*** | 294.388*** |
| Sampler | 8 | 119.922*** | 71.736*** | 91.187*** | 129.741*** | 101.811*** | 182.529*** | 145.595*** | 134.764*** |
| Invstor | 1 | 124.694*** | 129.120*** | 156.644*** | 48.583*** | 29.297*** | 8.992** | 82.348*** | 162.937*** |
| Trial time | 9 | 16.970*** | 13.802*** | 16.737*** | 9.584*** | 16.163*** | 17.946*** | 16.623*** | 21.169*** |
| Trial sampler | 24 | 9.656*** | 5.505*** | 6.465*** | 8.512*** | 9.696*** | 11.027*** | 5.877*** | 10.338*** |
| Time sampler | 24 | 15.513*** | 12.585*** | 14.885*** | 13.248*** | 15.531*** | 12.540*** | 12.769*** | 15.414*** |
| Trial invstor | 3 | 13.905*** | 13.851*** | 17.221*** | 2.142 | 4.340** | 12.554*** | 22.804*** | 21.352*** |
| Time invstor | 3 | 3.196* | 4.688** | 4.531*** | 4.364** | 2.636 | 0.655 | 1.047 | 2.772** |
| Sampler invstor | 8 | 4.425*** | 4.528*** | 5.155*** | 1.148 | 0.720 | 0.614 | 2.357* | 3.735*** |
| Storage | 2 | 267.007*** | 232.812*** | 299.662*** | 163.518*** | 100.632*** | 50.744*** | 151.140*** | 365.565*** |
| Temperature | 1 | 823.794*** | 817.157*** | 999.207*** | 288.071*** | 30 192*** | 81.279*** | 992.752*** | 1052.820*** |
| Trial time sampler | 68 | 6.148*** | 6.517*** | 6.600*** | 4.674*** | 7.308*** | 6.097*** | 7.631*** | 6.665*** |
| Trial time invstor | 9 | 3.348*** | 2.915** | 3.513*** | 2.754** | 2.781** | 3.543*** | 2.132* | 4.856*** |
| Trial sampler | 24 | 1.101 | 1.270 | 1.290 | 0.819 | 1.678* | 0.693 | 1.453 | 1.496 |
| Time sampler | 24 | 1.327 | 1.083 | 1.293 | 0.923 | 1.463 | 0.866 | 0.723 | 1.169 |
| Trial storage | 6 | 21.927*** | 22.732*** | 27.346*** | 16.269*** | 17.670*** | 10.168*** | 35.190*** | 39.482*** |
| Time storage | 6 | 2.308* | 2.880** | 3.196** | 1.566 | 3.075** | 5.880*** | 3.303** | 2.962** |
| Sampler storage | 16 | 7.304*** | 5.333*** | 6.612*** | 4.555*** | 3.414*** | 2.453** | 3.077*** | 6.478*** |
| Trial temp | 3 | 8.030*** | 6.461*** | 7.506*** | 6.288*** | 4.081** | 5.232** | 2.863* | 3.571* |
| Time temp | 3 | 3.601* | 1.113 | 2.042 | 8.295*** | 10.407*** | 4.348** | 25.092*** | 15.534*** |
| Sampler temp | 8 | 16.239*** | 10.620*** | 15.238*** | 7.517*** | 3.738*** | 2.208* | 7.160*** | 11.797*** |
| Storage temp | 2 | 140.198*** | 126.671*** | 163.202*** | 73.278*** | 74.837*** | 31.612*** | 123.165*** | 220.416*** |
| Trial time sampler | 68 | 1.023*** | 0.969 | 1.042*** | 0.737 | 0.964 | 0.645 | 0.797 | 0.926 |
| Trial time storage | 18 | 2.197** | 1.914** | 2.272** | 2.356** | 1.698* | 7.151*** | 6.695*** | 5.240*** |
| Trial sampler storage | 48 | 1.840*** | 1.897*** | 2.006*** | 1.112*** | 1.727*** | 1.581*** | 1.780*** | 2.088*** |
| Time sampler storage | 48 | 1.352*** | 1.130*** | 1.301*** | 1.325*** | 1.151*** | 0.849 | 1.323*** | 1.132*** |
| Trial time temp | 9 | 1.129 | 2.649** | 2.315* | 0.621 | 1.014 | 6.262*** | 5.249*** | 2.576*** |
| Trial sampler temp | 24 | 2.286*** | 1.462 | 1.872** | 1.875** | 1.544** | 1.229 | 3.589*** | 2.611*** |
| Time sampler temp | 24 | 1.038 | 0.908 | 0.932 | 1.673** | 1.128 | 1.186 | 1.076 | 0.687 |
| Trial storage temp | 6 | 25.085*** | 22.952*** | 28.838*** | 20.359*** | 15.916*** | 10.584*** | 19.484*** | 38.780*** |
| Sampler storage temp | 16 | 1.907* | 1.881* | 1.987** | 2.165 | 1.695 | 1.264 | 1.779 | 2.117** |
| Residual | 594 | 1.397 | 1.365 | 1.441 | 1.382 | 1.346 | 1.276 | 1.522 | 1.363 |
| Total | 1119 | 8.106 | 6.463 | 8.124 | 5.531 | 5.455 | 5.152 | 8.055 | 9.581 |

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

ignored in further examination of quality aspects.

Table 5:2 shows the number of observations for each characteristic of cheese during ripening. The flavour scores (Table 5:3) increased with the increase of time of storage of milk at $2^{o}C$ (up to 4 d) prior to cheese production ($p < 0.001$). Further storage at $2^{o}C$ (up to 7 d) decreased the flavour scores but this decrease was not significantly important. On the other hand, storage of milk at $6^{o}C$ for 2 d caused a slight decrease in the flavour of the resulting cheese. The further storage of milk for a total of 4 and 7 d from delivery prior to cheese production led to dramatic decreases in flavour scores ($p < 0.001$). The effect of cold storage of milk prior to use on flavour scores of cheese is presented in Fig. 5:1. Significant differences were observed between different milk storage temperatures ($p < 0.001$) and between different lengths of storage. During curing all cheese samples including the cheese produced from non stored milk were awarded lower scores ($p < 0.001$) for flavour after 8 and 12 months of curing compared to examination at 2 and 4 months. The decrease in grading points for flavour was greater in the cheeses produced from milk held for 4 and 7 d at $6^{o}C$ before cheese production. Indeed, lower flavour scores were awarded to these samples after 4 months of curing and these values affected the total mean of the 4 months old cheese. These low mean scores for flavour of cheese at 4 months old was due to the effect of very low scores given to the cheese after 2 months of curing for those cheeses which were produced from milks held at $6^{o}C$ for 4 and 7 d.

The scores awarded for taste (Table 5:4) showed the same pattern as for the flavour scores (Fig. 5:2).

Due to the similarity between the meanings of flavour and taste, the means between these two characteristics were calculated and the results were presented in Table 5:5. The analysis of variance is presented in Table 5:1. The same pattern of changes in these figures was observed as for flavour + taste (Fig. 5:3). Storage of milk up to 4 d at $2^{o}C$ prior to cheese production gave the highest of scores, while the lowest values were observed for the cheeses made from milks held for 7 d at $6^{o}C$.

Some panelists gave interesting comments on the off flavours of the

TABLE 5:2

The number of observations for flavour, taste, texture, body, colour, and openness of cheese during organoleptic assessment of Cheddar cheese

| Storage of milk | Curing time | | | | | |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | Total | Total |
| (Initial) | 144 | 144 | 144 | 144 | 576 | 576 |
| 2°C 2 d | 72 | 72 | 72 | 72 | 288 | 864 |
| 4 d | 72 | 72 | 72 | 72 | 288 | |
| 7 d | 72 | 72 | 72 | 72 | 288 | |
| Means | 216 | 216 | 216 | 216 | 216 | |
| 6°C 2 d | 72 | 72 | 72 | 72 | 288 | 864 |
| 4 d | 72 | 72 | 72 | 72 | 288 | |
| 7 d | 72 | 72 | 72 | 72 | 288 | |
| Means | 216 | 216 | 216 | 216 | 216 | |
| Means | 576 | 576 | 576 | 576 | 2304 | 2304 |

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Trial means | 576 | 576 | 576 | 576 |
| | Initial | 2 Days | 4 Days | 7 Days |
| Storage time of milk (means) | 576 | 576 | 576 | 576 |
| Curing time | | | | |
| 2 Months | 144 | 144 | 144 | 144 |
| 4 Months | 144 | 144 | 144 | 144 |
| 8 Months | 144 | 144 | 144 | 144 |
| 12 Months | 144 | 144 | 144 | 144 |

TABLE 5:3

Means of flavour scores awarded by the 9 members of grading panel for Cheddar cheese (manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C) after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage (Initial) | 2 Months | 4 Months | 8 Months | 12 Months | | |
| | 6.71 | 6.30*** | 5.13*** | 4.92*** | 5.77 | 0.1306 |
| 2°C 2 d | 6.64 | 6.40 | 5.53*** | 5.42*** | 5.99*** | |
| 4 d | 6.60 | 6.62 | 6.03** | 5.53*** | 6.20 | 0.1847 |
| 7 d | 6.37 | 5.97* | 5.04*** | 5.08*** | 5.61 | |
| 6°C 2 d | 6.59 | 6.33 | 4.99*** | 4.52*** | 5.61 | |
| 4 d | 5.46 | 5.10* | 3.83*** | 3.84*** | 4.56*** | 0.1847 |
| 7 d | 3.67 | 3.28*** | 2.65 | 2.61*** | 3.05*** | |
| Means at 2°C | 6.54 | 6.33* | 5.53** | 5.35* | 5.94 | 0.1066 |
| at 6°C | 5.24* | 4.90** | 3.82*** | 3.66*** | 4.41 | |
| Means | 6.10 | 5.7 *** | 4.79*** | 4.61*** | 5.32 | 0.06653 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 5.38 | 4.95 | 5.46 | 5.49 | 0.0653 |
| | Initial | 2 Days | 4 Days | 7 Days | SED |
| Storage tim of milk (means) | 5.77 | 5.80 | 5.38 | 4.33 | 0.0653 |
| Curing time | | | | | |
| 2 Months | 6.71 | 6.6 | 6.03 | 5.02 | 0.1306 |
| 4 Months | 6.30 | 6.36 | 5.86 | 4.62 | |
| 8 Months | 5.13 | 5.25 | 4.93 | 3.85 | |
| 12 Months | 4.92 | 4.97 | 4.69 | 3.85 | |

SED initial vs stored 0.1599

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

Fig. 5:1 Effect of cold storage of milk prior to cheesemaking at 2°C (——) and 6°C (- - -) on the mean flavour scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 individual grade points)

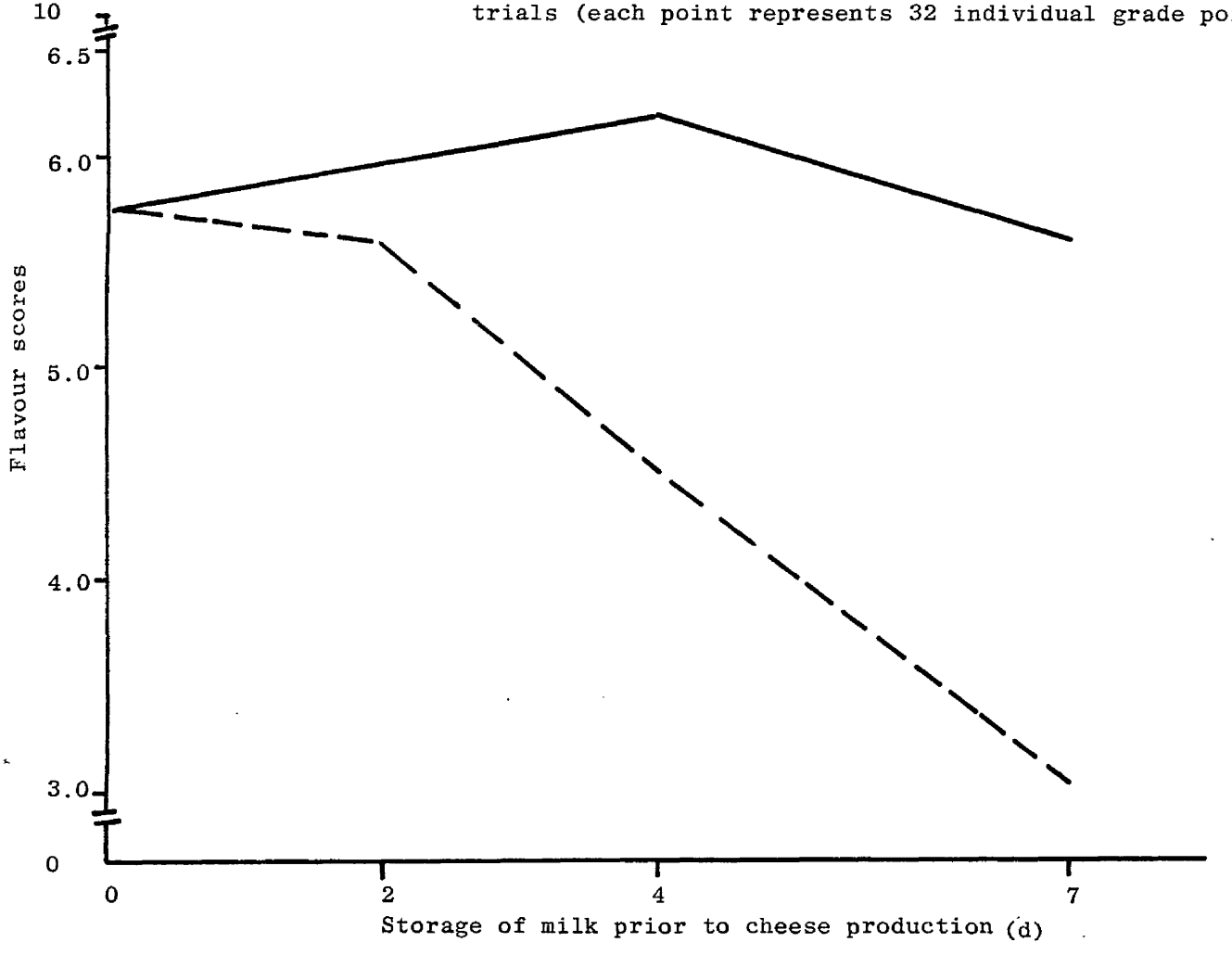


TABLE 5:4

Means of taste scores awarded by the 9 members of the panel for the Cheddar cheese (manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C) after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | Mean |
|---|---|---|---|---|---|---|
| Storage (Initial) | 2 Months | 4 Months | 8 Months | 12 Months | | |
| | 6.16 | 5.79* | 4.65*** | 4.60*** | 5.30 | 0.155 |
| 2°C 2 d | 5.22 | 5.71* | 5.24 | 5.09*** | 5.56 | |
| 4 d | 6.12 | 6.11 | 5.47** | 5.28*** | 5.75* | 0.219 |
| 7 d | 5.83 | 5.32* | 4.60*** | 4.83*** | 5.14 | |
| 6°C 2 d | 5.85 | 5.74 | 4.54*** | 4.15*** | 5.07 | |
| 4 d | 4.30 | 4.24*** | 3.33*** | 3.16*** | 3.76*** | 0.219 |
| 7 d | 2.53 | 2.29 | 1.89** | 2.09* | 2.19*** | |
| Means at 2°C | 6.06 | 5.71** | 5.10*** | 5.07*** | 5.48 | 0.127 |
| at 6°C | 4.23 | 4.09 | 3.25*** | 3.12** | 3.67 | |
| Means | 5.40 | 5.12*** | 4.29*** | 4.22*** | 4.76 | 0.078 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 4.629 | 4.450 | 5.021 | 4.937 | 0.0776 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means) | 5.30 | 5.32 | 4.75*** | 3.67* | 0.0776 |

| Curing time | | | | | |
|---|---|---|---|---|---|
| 2 Months | 6.16 | 6.03 | 5.21 | 4.18 | 0.1552 |
| 4 Months | 5.79 | 5.72 | 5.17 | 3.81 | |
| 8 Months | 4.65 | 4.89 | 4.40 | 3.24 | |
| 12 Months | 4.60 | 4.62 | 4.22 | 3.45 | |

SED initial vs stored 0.1901

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

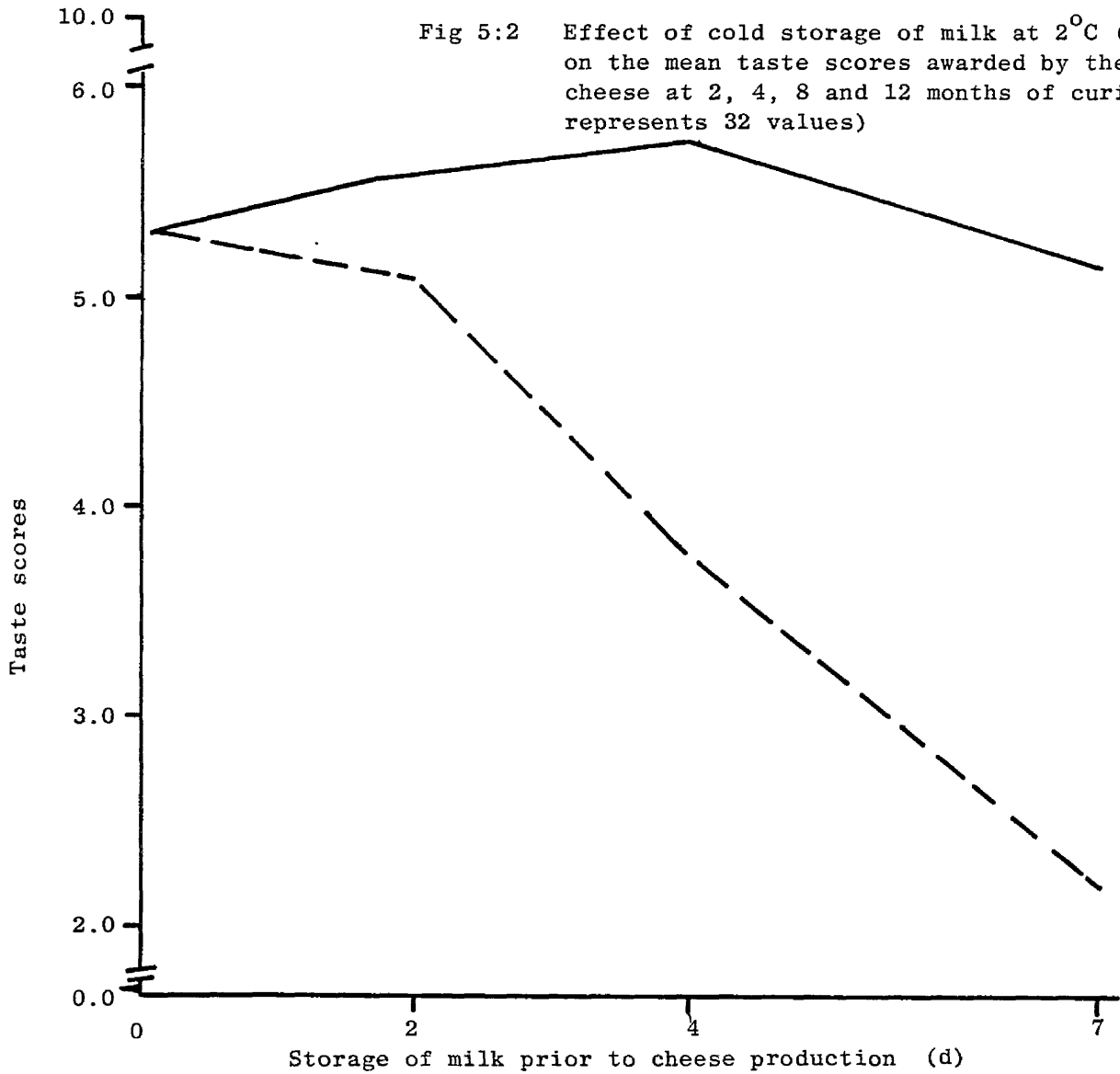



Fig 5:2 Effect of cold storage of milk at $2^{o}C$ (——) and at $6^{o}C$ (- - -) on the mean taste scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 values)

TABLE 5:5

Means of combined flavour and taste scores awarded by the 9 members of a panel for the Cheddar cheese manufactured from milk on day of delivery and after storage for 2, 4 and 7 d at $2^{o}C$ and $6^{o}C$, after 2, 4, 8 and 12 months of curing

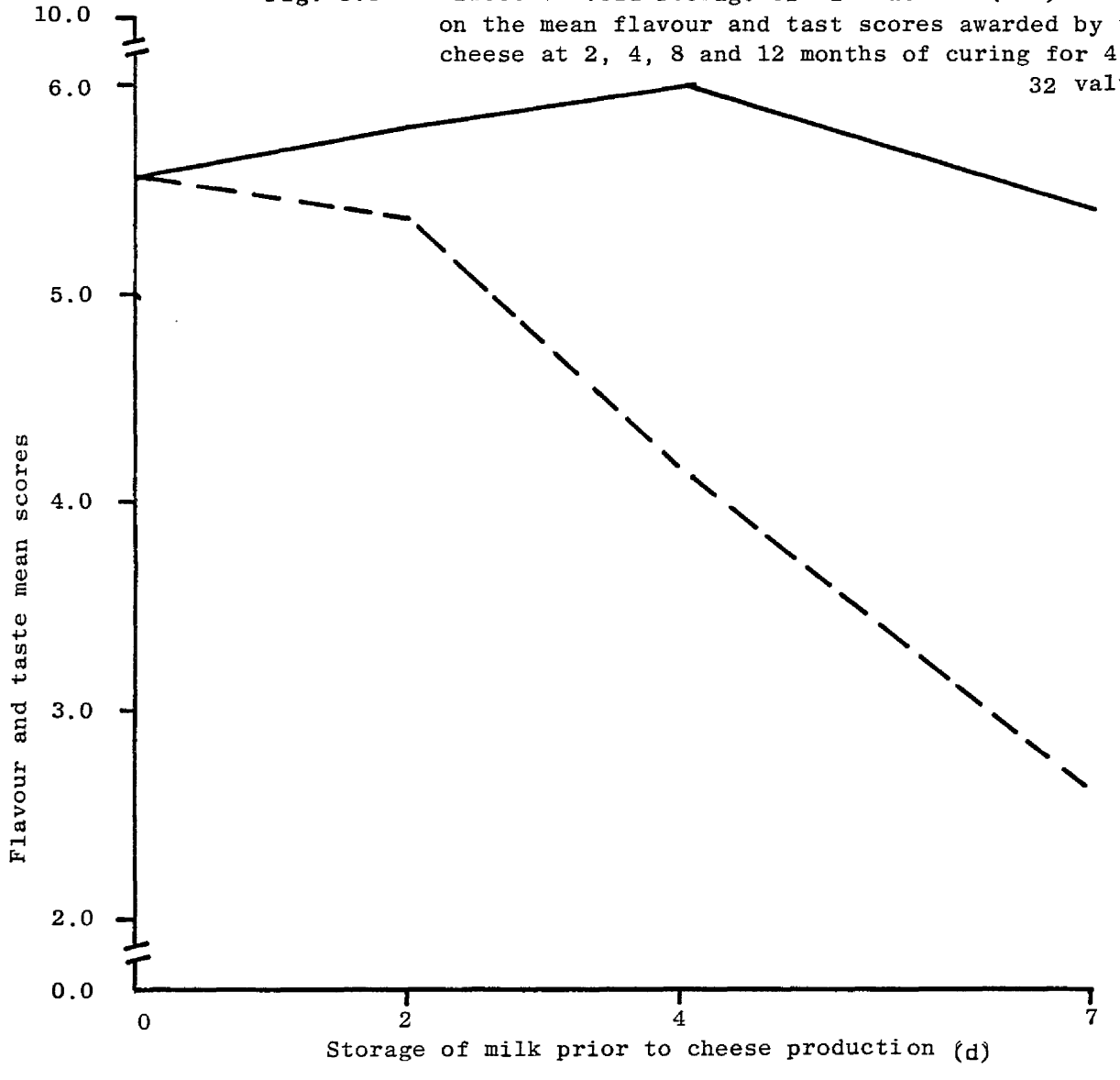
| Storage of milk | Curing time | | | | | |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | Means | SED |
| (Initial) | 6.43 | 6.04** | 4.89*** | 4.81*** | 5.54 | 0.1297 |
| $2^{o}C$ 2 d | 6.42 | 6.05** | 5.38*** | 5.28*** | 5.78 | |
| 4 d | 6.36 | 6.37 | 5.75*** | 5.21*** | 5.97** | 0.1834 |
| 7 d | 6.10 | 5.65* | 4.82*** | 5.01*** | 5.39 | |
| $6^{o}C$ 2 d | 6.22 | 6.89*** | 4.76*** | 4.39*** | 5.34 | |
| 4 d | 4.89 | 6.03*** | 3.58*** | 3.50*** | 4.16*** | 0.1834 |
| 7 d | 3.09 | 2.78 | 2.27*** | 2.35*** | 2.62*** | |
| Means at $2^{o}C$ | 6.30 | 6.02** | 5.32*** | 5.23*** | 5.71 | 0.1059 |
| at $6^{o}C$ | 4.73 | 4.49* | 3.54*** | 3.41*** | 4.04 | |
| Means | 5.74 | 5.45*** | 4.54*** | 4.44*** | 5.05 | 0.0648 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 5.00 | 4.73 | 5.24 | 5.21 | 0.0648 |
| | Initial | 2 Days | 4 Days | 7 Days | SED |
| Storage time of milk (means) | 5.54 | 5.56 | 5.06*** | 4.01*** | 0.0648 |
| Curing time | | | | | |
| 2 Months | 6.43 | 6.32 | 5.62 | 4.60 | 0.1297 |
| 4 Months | 6.04 | 6.04 | 5.52 | 4.21 | |
| 8 Months | 4.89 | 5.07 | 4.67 | 3.54 | |
| 12 Months | 4.81 | 4.82 | 4.45 | 3.68 | |

SED: initial vs stored 0.1588

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

Fig. 5:3 Effect of cold storage of milk at $2^{o}C$ (——) and $6^{o}C$ (- - -) prior to cheesemaking on the mean flavour and tast scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 values)



cheese. Some of them could detect a flat flavour or other defects such as mustiness and slight bitterness in the cheeses produced from milks held at 2°C for 7 d before use, after 8 months of curing some of these cheeses were considered to have sour, unclean and bitter flavours. These off-flavours were also observed when the cheese had been cured for one year. Some off-flavours were also detected in the cheeses made from non-stored milks.

Cheeses made from milks held at 6°C developed off-flavours more rapidly. Milk held for 2 d at 6°C resulted in the production of cheese with sour, unclean and metallic off flavours when two months old. Cheeses made from milks held for 4 d at 6°C had unacceptable bitter, rancid, fruity, musty and unclean flavours when 2 months old. As the cheese curing was lengthened these bad flavours became more severe and whey taint, bitterness, sour, rancid and high acid off flavours were also observed. Milks held for 7 d at 6°C prior to use led to cheese with more distinct off flavours. These flavours were described by panelists as yeasty, very rancid, fruity, sour, whey taste in addition to the other off flavours observed in the earlier stages of curing.

Body scores (Table 5:6) showed no significant variation in the cheeses manufactured from milks held at 2°C, although the peak value was given to the cheeses made from milks held for 4 d at 2°C. The storage of milk at 6°C however, decreased the scores awarded for the body. The longer the storage time of milk at 6°C, the lower the body scores (Fig. 5:4). During curing, significant decreases ($p < 0.001$) were observed after one year of curing in the body scores for cheeses produced from non-stored milks and milks held for 2 and 4 d at 2°C, while with 8 months old cheese greater decreases were observed in body scores where the milk had been held at 6°C for 2 and 4 d before use. Cheeses produced from milks held at 6°C for 7 d before use had the lowest scores awarded throughout the whole curing period.

Scores awarded for the texture of the cheeses are presented in Table 5:7. No significant variations in texture scores were evident from analysis of the panel awards for the cheeses made from milks held at 2°C before use but still the highest scores were given to the cheeses made from milks held for 4 d at 2°C (Fig. 5:5). Lower scores were given for the cheeses made from milks held at 6°C for 2 d (not significant) and

TABLE 5:6

Means of body scores awarded by the 9 members of the panel for the Cheddar cheese, manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C, after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | | |
| (Initial) | 6.76 | 7.00* | 6.44* | 5.86*** | 6.52 | 0.1130 |
| 2°C 2 d | 6.82 | 6.93 | 6.74 | 6.23*** | 6.68 | |
| 4 d | 6.83 | 6.72 | 6.99 | 6.23*** | 6.69 | 0.1598 |
| 7 d | 6.35 | 6.64 | 6.51 | 6.06 | 6.39 | |
| 6°C 2 d | 6.82 | 6.72 | 6.17*** | 5.76*** | 6.37 | |
| 4 d | 6.59 | 6.64 | 6.03*** | 5.63*** | 6.22* | 0.1598 |
| 7 d | 5.17 | 4.86 | 4.76* | 4.50*** | 4.82*** | |
| Means at 2°C | 6.67 | 6.76 | 6.74 | 6.18*** | 6.59 | 0.0922 |
| at 6°C | 6.20 | 6.07 | 5.65*** | 5.30*** | 6.80 | |
| Means | 6.51 | 6.56 | 6.26*** | 5.77 | 6.28 | 0.0565 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 6.16 | 6.33 | 6.30 | 6.32 | 0.0565 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means) | 6.52 | 6.82 | 6.71 | 6.76 | 0.1130 |

Curing time

| | | | | | |
|---|---|---|---|---|---|
| 2 Months | 6.76 | 6.82 | 6.71 | 5.76 | 0.1130 |
| 4 Months | 7.00 | 6.83 | 6.68 | 5.75 | |
| 8 Months | 6.44 | 6.45 | 6.51 | 5.64 | |
| 12 Months | 5.86 | 6.00 | 5.93 | 5.28 | |

SED initial vs stored 0.1384

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

Fig. 5:4 Effect of cold storage of milk at $2^{o}C$ (——) and $6^{o}C$ (- - -) on the mean body scores awarded by a panel (9 members) for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 values)

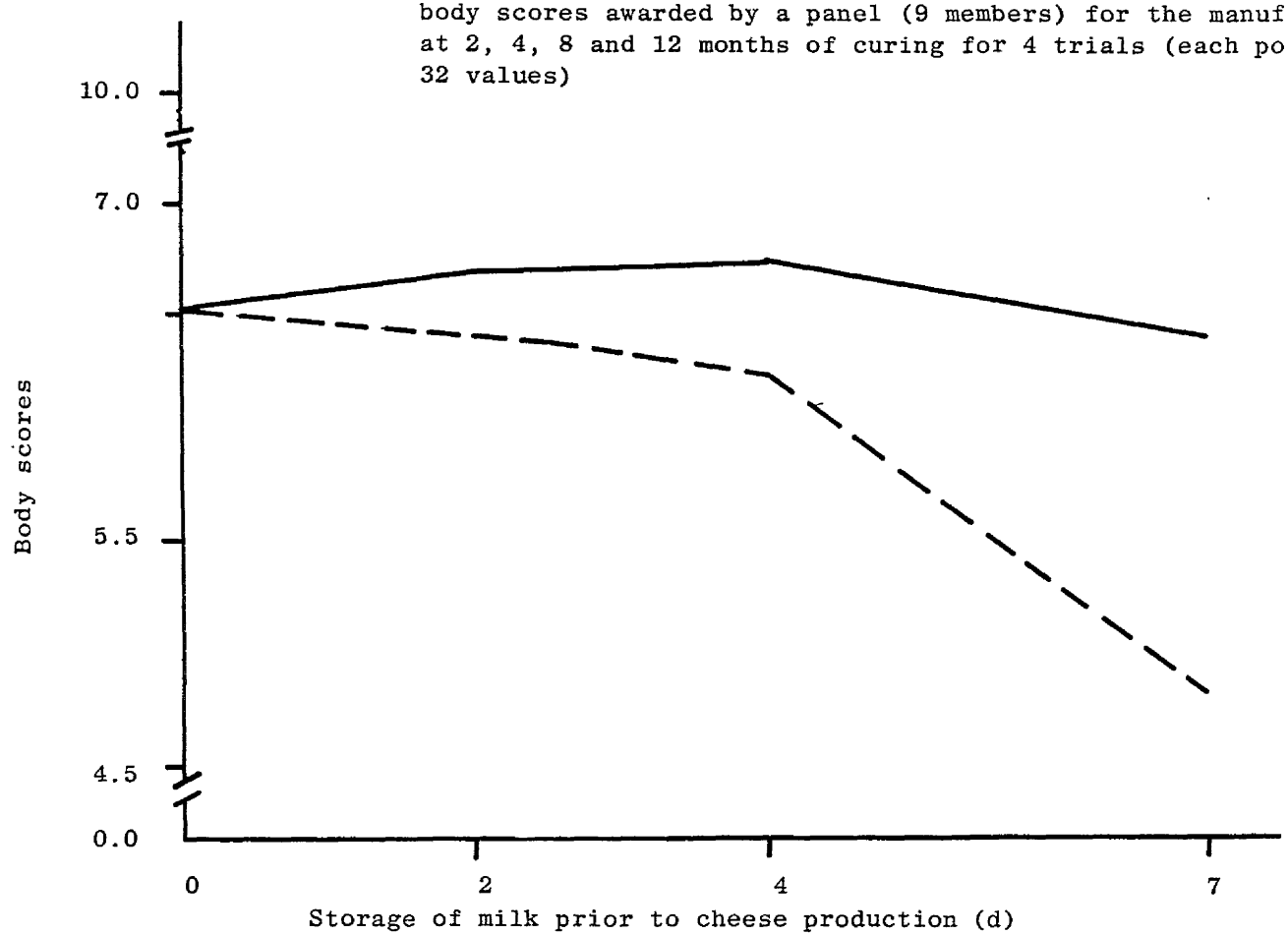


TABLE 5:7

Means of texture scores awarded by the 9 members of the panel for the Cheddar cheese, manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C, after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | | |
| (Initial) | 6.78 | 6.71 | 5.85*** | 5.74*** | 6.27 | 0.1165 |
| 2°C 2 d | 6.74 | 6.79 | 6.19*** | 6.02*** | 6.44 | |
| 4 d | 6.81 | 6.69 | 6.53 | 5.89*** | 6.48 | 0.1648 |
| 7 d | 6.48 | 6.49 | 6.44 | 6.00** | 6.35 | |
| 6°C 2 d | 6.69 | 6.64 | 5.78*** | 5.51*** | 6.15 | |
| 4 d | 6.58 | 6.39 | 5.37** | 5.35*** | 5.93* | 0.1648 |
| 7 d | 5.38 | 4.72*** | 4.42*** | 4.37*** | 4.72*** | |
| Means at 2°C | 6.68 | 6.66 | 6.39** | 5.97*** | 6.42 | 0.0951 |
| at 6°C | 6.22 | 5.92** | 5.19*** | 5.08*** | 5.60 | |
| Means | 6.53 | 6.39* | 5.81*** | 5.58 | 6.08 | 0.0583 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 6.00 | 6.02 | 6.15 | 6.13 | 0.0583 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means) | 6.27 | 6.29 | 6.20 | 5.54*** | 0.0583 |

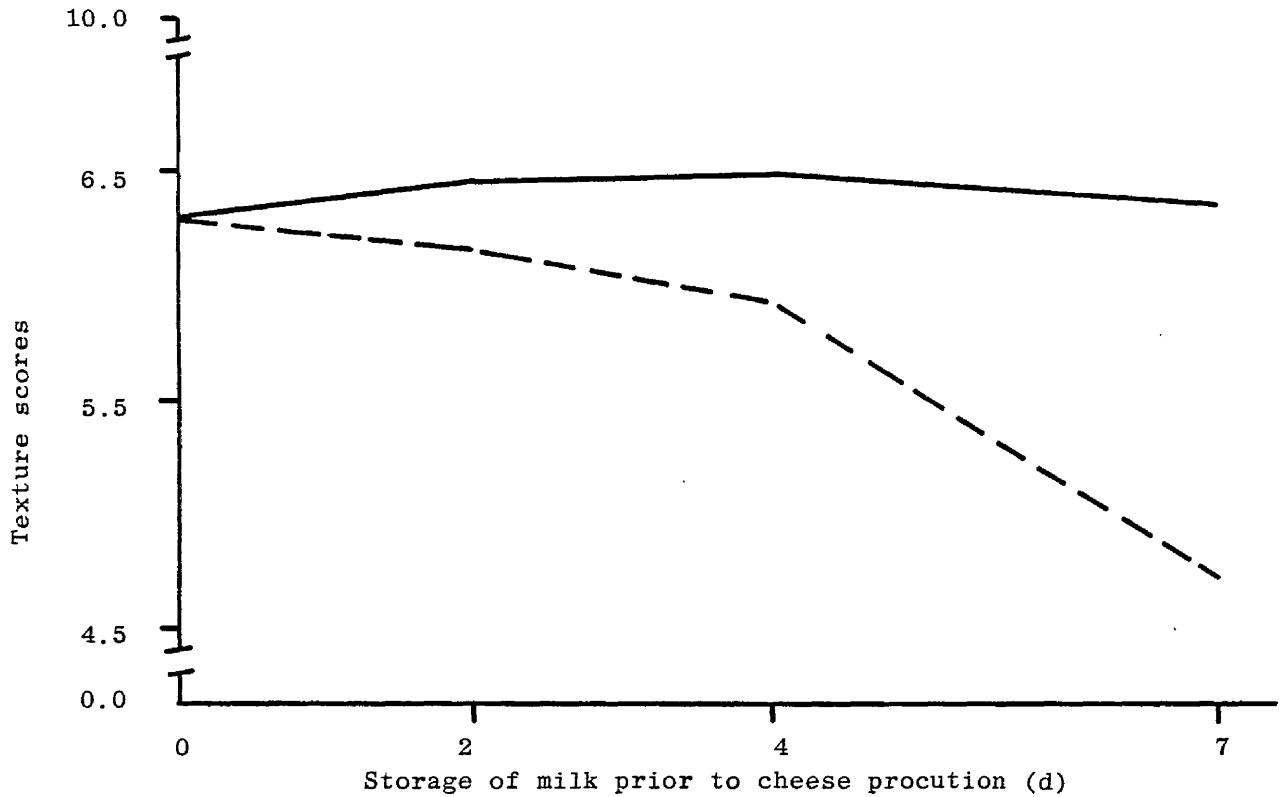
Curing time

| | | | | | |
|---|---|---|---|---|---|
| 2 Months | 6.78 | 6.71 | 6.70 | 5.93 | 0.1165 |
| 4 Months | 6.71 | 6.71 | 6.54 | 5.60 | |
| 8 Months | 5.85 | 5.99 | 5.95 | 5.43 | |
| 12 Months | 5.74 | 5.76 | 5.62 | 5.19 | |

Initial vs stored 0.1427

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

Fig. 5:5 Effect of cold storage of milk at $2^{o}C$ (——) and $6^{o}C$ (- - -) on the mean texture scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months curing for 4 trials (each point represents 32 values)



more especially for cheese from milks held for 4 d ($p < 0.05$) and 7 d ($p < 0.001$). Cheese made from milks held for 4 and 7 d at $2^{o}C$ showed slight decrease at 8 months (not significant). After a curing period of one year all cheeses were given lower scores. The lowest scores were given for cheeses made from milks held at $6^{o}C$.

Storage of milks at $2^{o}C$ for up to 7 d and at $6^{o}C$ for up to 4 d had no significant effect on the openness scores (Table 5:8) awarded for the manufactured cheeses. However, there is a slight increase in the scores awarded for cheeses made from milks held for 2 and 4 d at $2^{o}C$ (Fig. 5:6). The lowest scores were given for cheeses made from milks held at $6^{o}C$ for 7 d ($p < 0.001$). During curing, cheeses made from non-stored milks and milks held for 2, 4 and 7 d at $2^{o}C$ and for 4 and 7 d at $6^{o}C$ were scored higher ($p < 0.001$) for openness at 4 months old than at 2 months old. Higher scores were given after 8 months of curing for cheeses made from non-stored milks and milks held at $2^{o}C$ for 2, 4 and 7 d and at $6^{o}C$ for 7 d. Other cheeses were awarded lower scores at 8 months than at 2 months old. Increasing the age of the cheese to one year decreased the scores awarded for the openness except for samples made from milks held for 7 d at $6^{o}C$ ($p < 0.001$).

Storage of milk for up to 7 d at $2^{o}C$ and 2 d at $6^{o}C$ before use had no significant effect on the scores awarded for colour (Table 5:9). But the highest scores were given for cheeses made from milks held at $2^{o}C$ for 4 d (Fig. 5:7). Cheeses made from milks held for 4 and 7 d at $6^{o}C$ were scored lower for colour ($p < 0.001$). In the case of non-stored milk the resultant cheese had lower scores at 8 months old than at 2 months old ($p < 0.001$). Also, lower scores were given for cheese made from milks held at $2^{o}C$ for 4 d ($p < 0.05$) and 7 d ($p < 0.01$) at 8 months old than 2 months. AT 8 months old, cheese made from milks held at $6^{o}C$ for 2, 4 and 7 d were scored lower than they were at 2 months. After 12 months the scores were almost steady compared to the awards for the same cheese after a curing period of 8 months.

Storage of milk at $2^{o}C$ prior to use increased slightly the mean total organoleptic scores awarded by the grading panel. The increase was greater where the milk had been held at $2^{o}C$ for 4 d before use ($p < 0.01$). On the other hand, storage of milk for 2 d before cheese production at $6^{o}C$ decreased the scores slightly and significantly ($p < 0.001$) after

TABLE 5:8

Means of openness scores awarded by the 9 members of the panel for the Cheddar cheese, manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C, after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage (Initial) | 2 Months | 4 Months | 8 Months | 12 Months | | |
| | 7.05 | 7.65*** | 7.22 | 6.87 | 7.20 | 0.1022 |
| 2°C 2 d | 6.98 | 7.50*** | 7.34* | 7.01 | 7.21 | |
| 4 d | 7.34 | 7.89*** | 7.52 | 6.97* | 7.43 | 0.1446 |
| 7 d | 6.58 | 7.63*** | 7.26*** | 7.08** | 7.14 | |
| 6°C 2 d | 7.40 | 7.52 | 7.28 | 6.90*** | 7.28 | |
| 4 d | 6.93 | 7.53*** | 6.66 | 6.72 | 6.96 | 0.1446 |
| 7 d | 6.15 | 6.66*** | 6.53** | 6.30 | 6.41*** | |
| Means at 2°C | 6.97 | 7.67*** | 7.38*** | 7.02 | 7.26 | 0.0835 |
| 6°C | 6.83 | 7.24*** | 6.83 | 6.64* | 6.88 | |
| Means | 6.93 | 7.51 | 7.13 | 6.84 | 7.10* | 0.0511 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 7.18 | 6.89 | 7.22 | 7.12 | 0.0511 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means) | 7.20 | 7.24 | 7.20 | 6.78*** | 0.0511 |
| Curing time | | | | | |
| 2 Months | 7.05 | 7.19 | 7.14 | 6.36 | 0.1022 |
| 4 Months | 7.65 | 7.51 | 7.71 | 7.14 | |
| 8 Months | 7.22 | 7.31 | 7.09 | 6.90 | |
| 12 Months | 6.87 | 6.96 | 6.84 | 6.69 | |

SED Initial vs stored 0.1252

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

Fig. 5:6 Effect of cold storage of milk prior to cheese making at 2°C (●——●) and 6°C (o- - -o) on the mean openness scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 values)

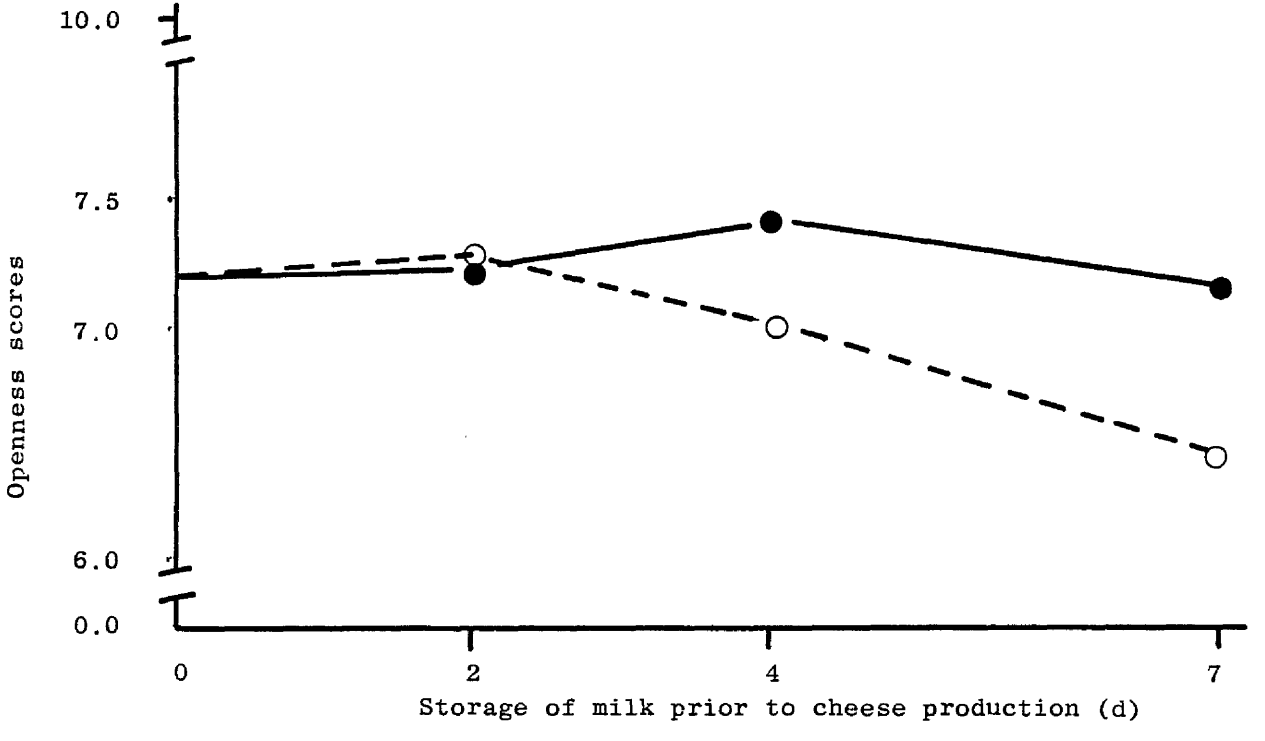


TABLE 5:9

Means of colour scores awarded by the 9 members of the panel for the Cheddar cheese manufactured from unstored milk and milks stored for 2, 4 and 7 d at 2°C and 6°C, after 2, 4, 8 and 12 months of curing

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | | |
| (Initial) | 7.54 | 7.45 | 6.71*** | 6.77*** | 7.12 | 0.1076 |
| 2°C 2 d | 7.50 | 7.44* | 6.86 | 6.95 | 7.19 | |
| 4 d | 7.50 | 7.61 | 7.12* | 7.00** | 7.31 | 0.1522 |
| 7 d | 7.33 | 7.42 | 6.86** | 6.90** | 7.13 | |
| 6°C 2 d | 7.50 | 7.40 | 6.60*** | 6.56*** | 7.01 | |
| 4 d | 7.41 | 7.25 | 5.4 *** | 5.54*** | 6.41*** | 0.1522 |
| 7 d | 5.90 | 5.75 | 4.83*** | 4.60*** | 5.27*** | |
| Means at 2°C | 7.44 | 7.49 | 6.95*** | 6.95*** | 7.21 | 0.0878 |
| at 6°C | 6.93 | 6.80 | 5.63*** | 5.57*** | 6.23 | |
| Means | 7.28 | 7.22 | 6.40*** | 6.39*** | 6.82 | 0.0538 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 6.81 | 6.42 | 6.95 | 7.10 | 0.0538 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means) | 7.12 | 7.10 | 6.86*** | 6.20*** | 0.0538 |

Curing time

| | | | | | |
|---|---|---|---|---|---|
| 2 Months | 7.54 | 7.50 | 7.45 | 6.61 | 0.1076 |
| 4 Months | 7.45 | 7.42 | 7.43 | 6.58 | |
| 8 Months | 6.71 | 6.73 | 6.29 | 5.85 | |
| 12 Months | 6.77 | 6.76 | 6.27 | 5.75 | |

SED initial vs stored 0.1318

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

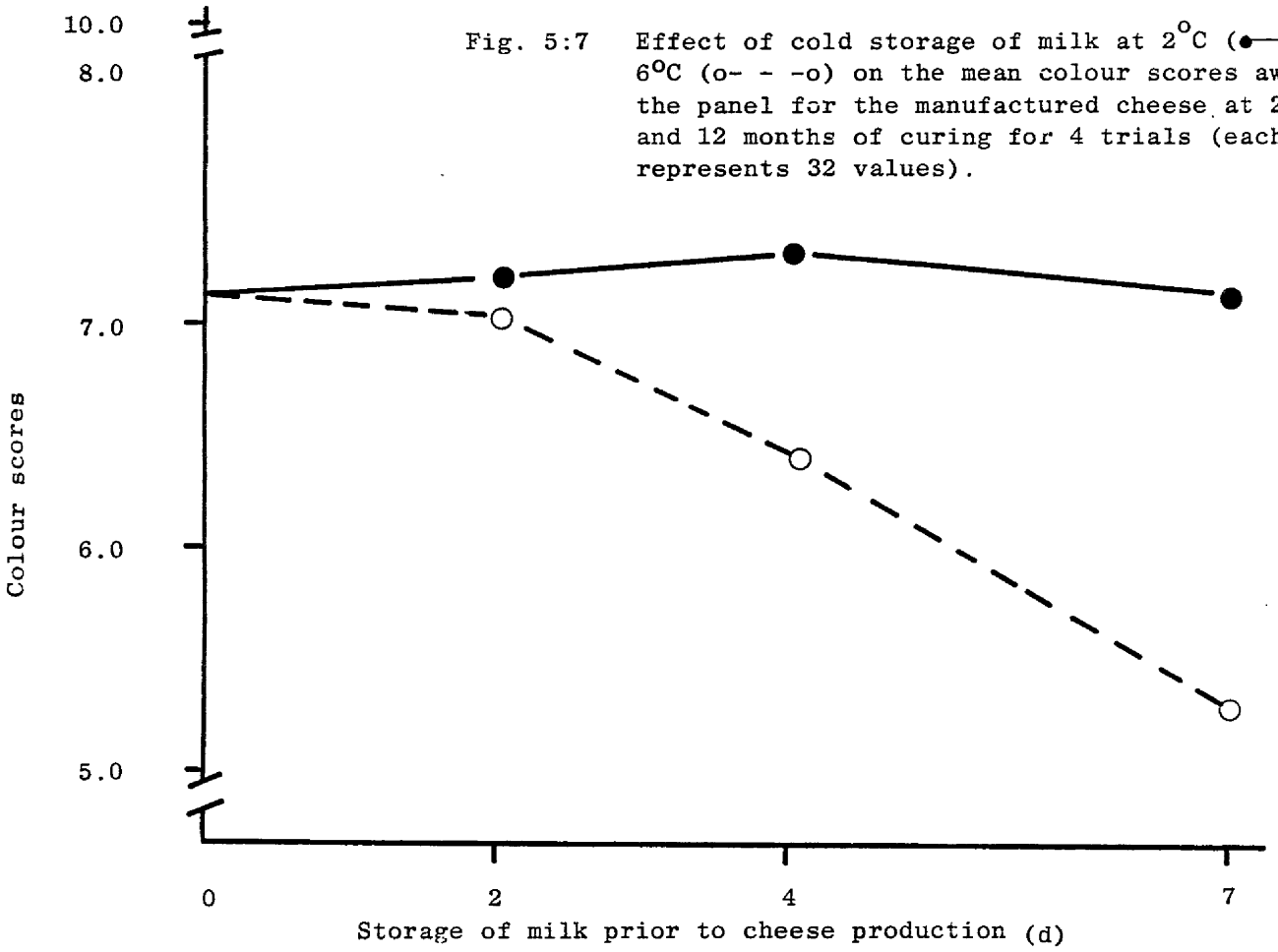



Fig. 5:7 Effect of cold storage of milk at 2°C (●——●) and 6°C (o- - -o) on the mean colour scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 4 trials (each point represents 32 values).

TABLE 5:10

Means of total organoleptic scores awarded by the 9 members of the panel for Cheddar cheese manufactured from initial milk and milks stored for 2, 4 and 7 d at 2°C and 6°C, after 2, 4, 8 and 12 months of curing (calculated as a mean of the six characteristics of cheese)

| Storage of milk | Curing time | | | | Means | SED |
|---|---|---|---|---|---|---|
| Storage | 2 Months | 4 Months | 8 Months | 12 Months | | |
| (Initial) | 6.91 | 6.43*** | 6.05*** | 5.81*** | 6.40 | 0.0780 |
| 2°C 2 d | 6.85 | 6.77 | 6.33*** | 6.13*** | 6.52 | |
| 4 d | 6.94 | 6.96 | 6.65** | 6.15** | 6.67** | 0.1104 |
| 7 d | 6.59 | 6.60 | 6.14*** | 6.01*** | 6.34 | |
| 6°C 2 d | 6.88 | 6.72 | 5.94*** | 5.59*** | 6.28 | |
| 4 d | 6.34 | 6.21 | 5.14*** | 5.05*** | 5.61*** | 0.1104 |
| 7 d | 4.94 | 4.63** | 4.20*** | 4.07*** | 4.46*** | |
| Means at 2°C | 6.79 | 6.76 | 6.37*** | 6.10*** | 6.51 | 0.0637 |
| at 6°C | 6.06 | 5.86** | 5.09** | 4.90*** | 5.48 | |
| Means | 6.55 | 6.44** | 5.81*** | 5.58*** | 6.10 | 0.0390 |

| | 1 | 2 | 3 | 4 | SED |
|---|---|---|---|---|---|
| Trial means | 6.13 | 5.87 | 6.19 | 6.18 | 0.0390 |

| | Initial | 2 Days | 4 Days | 7 Days | SED |
|---|---|---|---|---|---|
| Storage time of milk (means | 6.40 | 6.40 | 6.18*** | 5.40*** | 0.0390 |
| Curing time | | | | | |
| 2 Months | 6.91 | 6.87 | 6.64 | 5.77 | 0.0780 |
| 4 Months | 6.83 | 6.75 | 6.59 | 5.62 | |
| 8 Months | 6.03 | 6.14 | 5.89 | 5.17 | |
| 12 Months | 5.81 | 5.86 | 5.60 | 5.04 | |

SED initial vs stored 0.0956

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

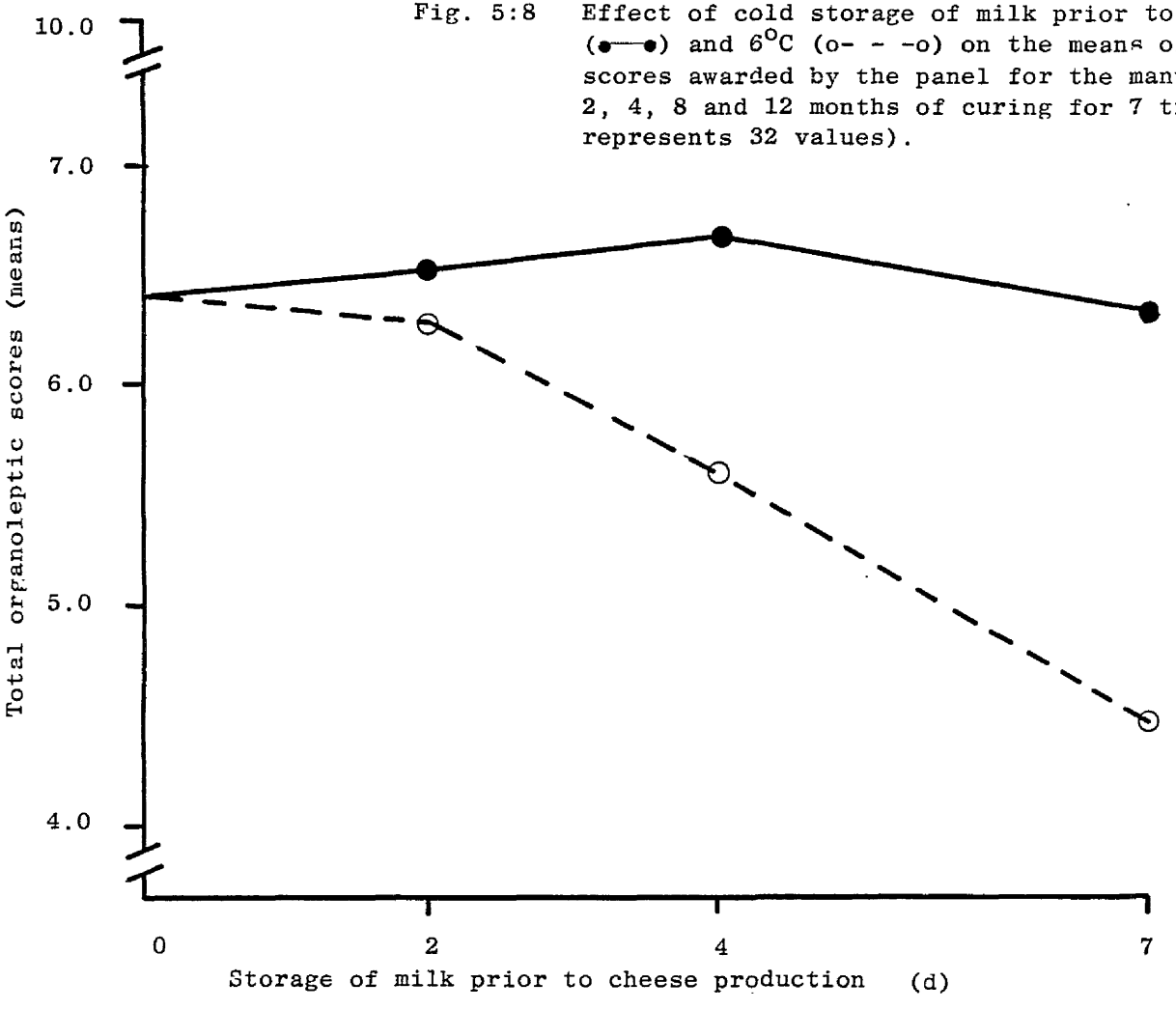



Fig. 5:8 Effect of cold storage of milk prior to cheesemaking at 2°C (●—●) and 6°C (o- - -o) on the means of total organoleptic scores awarded by the panel for the manufactured cheese at 2, 4, 8 and 12 months of curing for 7 trials (each point represents 32 values).

4 and 7 d (Fig. 5:8). After 4 months of curing cheeses made from non-stored milks and milks held for 7 d at 6°C were given lower scores than when 2 months. At 8 and 12 months all cheeses were scored lower than when they were examined after a curing period of 2 months. (Table 5:10)

DISCUSSION

There is a great concern over the storage of milk at temperatures which increase the population of micro-organisms enough to cause deterioration of the milk and thereafter affect the quality of the cheeses produced from such milks. The low quality scores awarded for the cheeses manufactured in this study from milks held at 6°C would prove this assumption. Mabbitt (1980) reported that if milk with a total bacterial count of 10,000 ml and psychrotrophs count of 1,000/ml is stored at 5°C the psychrotrophic organisms will become dominant on storage by multyplying approximately as follows:-

| Time (h) | 0 | 24 | 48 | 72 |
|---|---|---|---|---|
| No/ml | 1,000 | 8,000 | 64,000 | 512,000 |

It is not therefore surprising that milk, initially of excellent bacteriological quality, may have over $10^6$ bacteria/ml mainly psychrotrophs by the time it is processed at the creamery. From the above very approximate calculation for good quality ex-farm milk it can be expected that in practice the maximum storage period may be 2 d at about 5°C.

Some people recommend the storage of milk at lower temperatures because of the effect of temperature on bacterial growth. But, there is concern that low temperature storage could dissociate casein and affect the quality of products. In this study, the storage of milk at 2°C did not lower the quality of the produced cheese. This would indicate that the storage of milk at 2°C, prior to cheesemaking, decreases the rate of multiplication of psychrotrophs and the velocity of enzymatic reactions. In fact, cheeses made from milks held for 2 and 4 d at 2°C were higher scored than the unstored milks.

The body and texture of Cheddar cheese is often closely correlated with flavour, because the same factors may affect both (Tobias, 1976). Thus excessive acidity will pruduce an acid flavour and may cuase a short

and soft body. The presence of gas holes is associated with undesirable fermentation which may lead to fruity, yeasty or unclean flavours. This condition may also be associated with a high moisture content which may give the cheese a weak or even pasty body. A hard body, on the other hand may be associated with a lack of flavour development.

Deeth and Fitz-Gerald (1976) reported that typical Cheddar cheese flavour is due to a balance between fatty acids produced in low amounts during normal ageing and other flavour constituents. Excessive levels of fatty acids impart unclean, butyric or rancid flavours to the cheese. Rancidity in Cheddar cheese arises in most cases from lipolysis during storage, by lipase originating from microbial contamination of cheese or cheesemilk.

Chapman et al. (1978) found that storage of milk at 5$^{o}$C for 72 h resulted in good Cheddar cheese. Cheeses (6 weeks old) made from milk stored in this way were awarded for flavour 41-40 (maximum 45) and for body and texture 35-30 (maximum 40) by the grade panel. In respect of flavour the grading panel found that with the 6 months old cheese lipolytic rancidity was evident in cheese made from milks stored for 72 h. Rancidity was confirmed at higher intensity when the cheeses were re-tasted at 10 months and by then it was also present in cheeses made from milks stored for 48 h. In more recent work, Ali et al. (1980) found that when cheese was made from milk stored for up to 3 d at 4$^{o}$C the quality was virtually unaffected. Higher temperature (10-15$^{o}$C) of milk storage prior to cheesemaking led to cheese being down graded, largely for body and texture defects but also for flavour after 3 d.

In this study, the storage of milk for up to 4 d at 2$^{o}$C had not affected the organoleptic quality of the resultant cheese. Further storage of milk to 7 d at 2$^{o}$C resulted in cheeses which scored slightly lower than those cheese made from unstored milks. Scores awarded for cheeses made from milk held at 2$^{o}$C for 7 d were similar to those awarded for cheeses made from milk held for 2 d at 6$^{o}$C. On the other hand, cheeses made from milk held for 4 and 7 d at 6$^{o}$C were scored lower than other cheeses.

## CONCLUSION

1. Cheeses manufactured from milks held at $2^{o}C$ for 4 d were given higher scores (for flavour, taste, body, texture, openness and colour) than the cheeses made from milks used for cheesemaking on the day of delivery, but this increase was not significant.

2. Cheeses made from milks held at $6^{o}C$ had dramatically lower scores for all characteristics than the cheeses made from unstored milks.

3. The bad flavours were not observed in the cheeses made from milks held at $2^{o}C$ before 8 months of ripening. But, cheeses made from milks held for even 2 d at $6^{o}C$, showed off-flavour when 2 months old.

4. With increasing curing time, the off-flavour of the cheeses were more distinct.

5. Significant decrease in the body and texture scores were not observed (significantly) in the cheeses before 8 months old, except for the cheese made from milks held for 7 d at $6^{o}C$.

6. The most common characteristics of cheeses made from milks held at $6^{o}C$ were high acid, very rancid, unacceptable bitterness, fruity, musty and unclean flavour and taste. The body was weak, crumbly and wet. Cheeses made from milks held at $2^{o}C$ showed some flat, bitter, sour and unclean flavours at 8 and 12 months old. Some cheeses were smooth in body.

# CHAPTER SIX

## SLAB GEL ELECTROPHORETIC STUDIES OF THE EFFECT OF COLD STORAGE OF MILK ON THE BREAKDOWN OF CASEIN FRACTIONS OF MILK AND CHEESE MADE FROM IT

### INTRODUCTION

Electrophoresis in a polyacrylamide gel separates proteins according to their charges and molecular size (Bio Rad, 1982). The use of slab gel virtually eliminates instrument variability as a source of inconsistency.

The extent of proteolysis in raw milk during storage and in cheese produced from the stored milks was determined by the detection of NPN and NCN in milk and soluble N in cheese. These methods of study are unable to show the casein fraction involved in the proteolysis. So a method of tracing the individual casein fractions was necessary.

When Juffs (1975a) examined stored milk by starch gel electrophoresis, he reported that the first evidence of proteolysis in raw milk stored at $5^{o}C$ was the formation of para-κ-casein. However, this fraction could not be detected until the total bacterial count exceeded $10^{7}$/ml.

On the basis of the gel electrophoresis mobility of caseins in alkaline urea media with and without merceptoethanol, caseins can be divided into the following groups according to their mobilities: $\alpha_s$-casein, β-casein, κ-casein and γ-casein. Their approximate percentages in skim milk protein are 45-55, 25-35, 8-15 and 3-7 per cent, respectively, (Whitney et al., 1976). Marcos et al. (1979) recommended PAGE as a method capable of high resolution and which could give quantitative results in studies of casein hydrolysis and which indicate the type of proteolysis in cheese.

Annan and Manson (1969) isolated $\alpha_s$-casein of bovine milk by cation-exchange chromatography. Three main fractions were separated from each preparation. Two behaved as single homogeneous proteins as judged by starch gel electrophoresis; they were the main constituents of the complex $\alpha_{s_1}$ and a closely related phosphoprotein, designated $\alpha_{s_0}$-casein, which was present only in small amounts. The third fraction also made up only a small part of the total complex. It was hetero-

geneous on starch gel electrophoresis and contained 2 major and 2 minor components.

In their report Kaminogawa et al. (1980) found that the acid protease of bovine milk converted $\alpha_{s1}$-casein into a fragment with mobility equal in both disc and urea-SDS electrophoresis to that of $\alpha_{s1}$-I casein produced by the action of chymosin. New bands with mobilities equal in disc and urea-SDS electrophoresis to those of β-I and β-II fractions produced by chymosin action, appeared by the action of acid protease on β-casein. Furthermore, a para-κ-casein-like protein was also formed from κ-casein by the acid protease.

The breakdown of casein fractions during ripening of cheese is widely investigated by the electrophoresis method. However, little information is available on the effect of cold storage of milk on the behaviour of proteolysis of caseins during ripening of the resultant cheese.

Rennet enzymes appear specifically to alter $\alpha_{s1}$-casein after curd formation in Cheddar cheese manufacture. β-casein evidently is unaltered by the rennet enzymes (Ledford et al., 1966). Gripon et al. (1977) reported that several enzyme systems (rennet, microbial peptide hydrolases) work together and it is very difficult to know the action of each system separately in traditional cheeses. O'Keeffe et al. (1978) produced Cheddar cheese free of non-starter bacteria, acidified with starter or glucono-lactone and containing active coagulant (chymosin or pepsin) or inactivated coagulant (pepsin). In these cheeses the coagulant was primarily responsible for the formation of large peptides while small peptides and free amino acids were produced principally by the starter, possibly from coagulant-produced peptides.

## EXPERIMENTAL

Standard κ-, β-, $\alpha_{s2}$- and $\alpha_{s1}$- caseins were produced by the National Institute for Research in Dairying (NIRD). These standards were prepared using a method similar to the procedure described by Davies and Law (1977) but with slight modification (Andrews, 1982). The modified procedure was as follows:

Total casein was precipitated at pH 4.6 from bulk milk and washed 5

times with distilled water by dissolving and re-precipitation to remove all traces of whey proteins. Twenty-five grams of the moist casein was then centrifuged (200 g for 15 min). The casein pellet was dissolved in about 1 l of 0.05 M Tris-imidazole buffer (pH 7.0) containing 6 M urea and 0.05 M 2-mercaptoethanol and applied to a column of DEAE-cellulose. The Whatman DE-52 was previously washed and equilibrated with 0.05 M Tris-imidazole pH 7.0-urea. The column was made up in a litre parallel sided separating funnel plugged with cotton wool. After applying the sample the column was washed with 2 l of 0.05 M Tris-imidazole pH 7.0 containing 3.3 M urea and 0.01 M 2-mercaptoethanol and then eluted with 10 l of salt gradient going from 0 to 0.3 M NaCl in this same Tris-urea-mercaptoethanol buffer. Fractions of 25 ml (the biggest that could be managed) were collected and since there were over 400 of these it was decided that only every fifth one should be examined by PAGE in order to monitor the column ($OD_{280}$ readings are not much use, as with these quantities readings are off-scale most of the time) and fractions containing the individual caseins were pooled, dialysed vs $H_2O$ and lyophilised.

To avoid possible breakdown of the casein fractions (of β-casein particularly) by milk proteinase during subsequent storage, Andrews (1982) (personal contact) suggested that it would be useful to heat the casein sample (after removal of whey proteins) at 80°C for 5-10 min before applying it to the column in order to inactivate the proteinase. Heating should be done at about pH 7.0 before addition of any urea or mercaptoethanol.

On this scale the column run takes several days even at a flow rate of 50-100 ml/h and since there are a lot of fractions to handle so samples and fractions should be stored at 4°C. The column was run at room temperature.

The slab gels were photographed before scanning using 2202 ultro Scan Laser Densitometer supplied by LKB Instruments Ltd., 232 Addington Road, S. Croydon, Surrey, CR2 8YD, England. This densitometer is rather new so it is of interest to describe some of its features and applications. In the case of an ordinary scanner the gels have to be chopped before scanning which leads to the possible variation in the thickness from

one track to another. Furthermore the width of the slit from which the light beam goes through into the gel is rather wide and this did not give good separation in the elution diagram especially for the fractions of $\alpha_s$ casein. With the laser densitometer there was no need to chop the gel as there is a path where the gel (off 20 track in the author's work) and the laser beam is programmable to go through each track subsequently. The laser beam is very narrow so giving better isolation for casein fractions than the ordinary scanner. Due to the last fact there was slight variation in the scanning trace if the position at which the laser goes through within the same band is changed. So it was necessary to make sure that the laser beam was positioned to go through the middle of each band or to take more than one reading for each band. It was also possible to scan the gels at different absorbance range for the same gel. The necessity for this depends on whether the minor or the major bands are of interest to the user.

The following conditions were used with the laser densitometer:-

| | |
|---|---|
| Scan speed | 50 mm/min |
| Start position | Mostly from the start of the separating gel. Some gels were scanned from the beginning of the stacking gel. |
| End position | The end of all peaks. |
| Absorbance range | 0.5 OD some gels were scanned at 1.0 or 2.0 OD. (The absorbance range specifies the optical density range which is represented by the full scale on the chart paper). |
| Integration factor | 1.0<br>The integration factor determines the length of the gel (in mm), which is scanned during a full-scale deflection of the integration pen according to the following equation:<br>$L = K \times (IF) \times (AR)/a$<br>where |

L = Length for full scale deflection

K = constant, 2.5

IF = entered integration factor

AR = entered absorbance range

A = actual absorbance being measured.

The laser densitometer was connected to 3390 A Integrator supplied by Hewlett-Packard Ltd., Analytical Instrumentation Group, King Street Lane, Winnersh, Wokingham, Berks., RG11 5AR.

The following conditions were used for integrating:-

ATT 27 = 10 (This step is performed to set the height scale for plotting peaks).

PK WD value = 0.04 (this value tells the integrator the kind of peaks to be expected, matching its response to widths of peaks to be detected and measured).

THRSH integrator value = 8 (THRSH's function is to set a discrimination level for the integrator to ignore signal changes to be regarded as noise).

CHT SP = 6.0 (chart speed in cm/min)

AR REJ = 200,000 (Area rejected).

Under these conditions for the laster densitometer and the integrator the peaks obtained were within the limit at which the intensity and light bands can be determined. However, some very faint bands could not be detected as peaks because they had smaller area than the limit used for the integrator.

## RESULTS

The results of this Chapter could be divided into three sections:-

### Section 6:1

#### Purified standard casein fractions

To ensure that the procedure of slab gel electrophoresis separation

under the conditions used in these experiments was efficient to separate the different fractions of casein and to detect the position of each of these fractions on the gel for the precipitated casein and the casein present in cheese samples, standard κ-, β-, $\alpha_{s_2}$- and $\alpha_{s_1}$-casein fractions were applied beside commercially supplied standard casein, acid-precipitated casein and cheese samples (Plate 6:1). The electrophoretic mobility was determined as started from the beginning of the separating gel.

All the samples applied on each gel were started in a standard way to ensure a uniform straight line. Fig. 6:1 shows the scan diagram of the electrophoretic patterns of each of the purified standard caseins, acid-precipitated casein, and cheese samples. The percentages of area of each of these peak in each pattern are presented in Table 6:1. The results of these patterns are described in the following paragraphs:-

1. κ-casein

These fractions appeared as one big band occupying the position from the starting point to just before the β-casein position on the gel. The area of κ-casein band represents 93.28 per cent of the total area of the purified κ-casein pattern. Other little peaks were observed as a contaminant with κ-casein. These contaminants occupy the same positions of β- and $\alpha_s$-caseins.

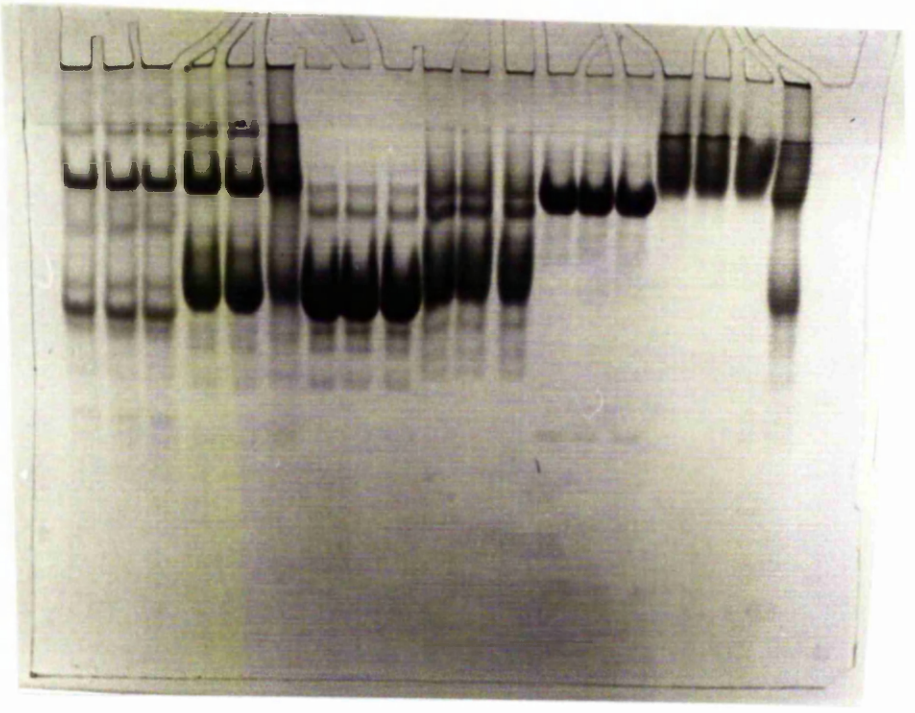
2. β-casein

This band is present at the expected position on the gel and amounts to 89.28 per cent of the total area of peaks present in this pattern. However, other impurities were also present which occupied the positions of κ- and $\alpha_s$-casein fractions. But, the β-casein fraction appeared to have a sharp single clear band.

3. $\alpha_{s_2}$-casein

Four bands were observed between the positions of β- and $\alpha_{s_1}$-caseins. Davies and Law (1977) classified these bands in order of increasing mobility as $\alpha_{s_6}$-, $\alpha_{s_4}$-, $\alpha_{s_3}$- and $\alpha_{s_2}$- caseins.

Track No. 1



Track No. 20

PLATE 6:1

The electrophoretic patterns for the polyacrylamide slab gel electrophoresis at pH 8.6 of the purified κ-, β-, $\alpha_{s2}$- and $\alpha_{s1}$-casein, acid precipitated casein, commercial casein and Cheddar cheese at 2 months old

| Pattern | Number of Tracks |
|---|---|
| κ-casein | 16, 17, 18 |
| β-casein | 13, 14, 15 |
| $\alpha_{s_2}$-casein | 10, 11, 12 |
| $\alpha_{s_1}$- casein | 7, 8, 9 |
| Commercial casein (acid pH) | 6, 19 |
| Acid precipitated casein | 4, 5 |
| Cheese at 2 months old | 1, 2, 3 |

Fig 6:1 The scan diagram of the electrophoretic patterns of κ-, β-, $\alpha_{s2}$- and $\alpha_{s1}$-caseins along with whole milk casein (acid precipitated), commercially supplied casein (acid precipitated) and cheese at 2 months old.

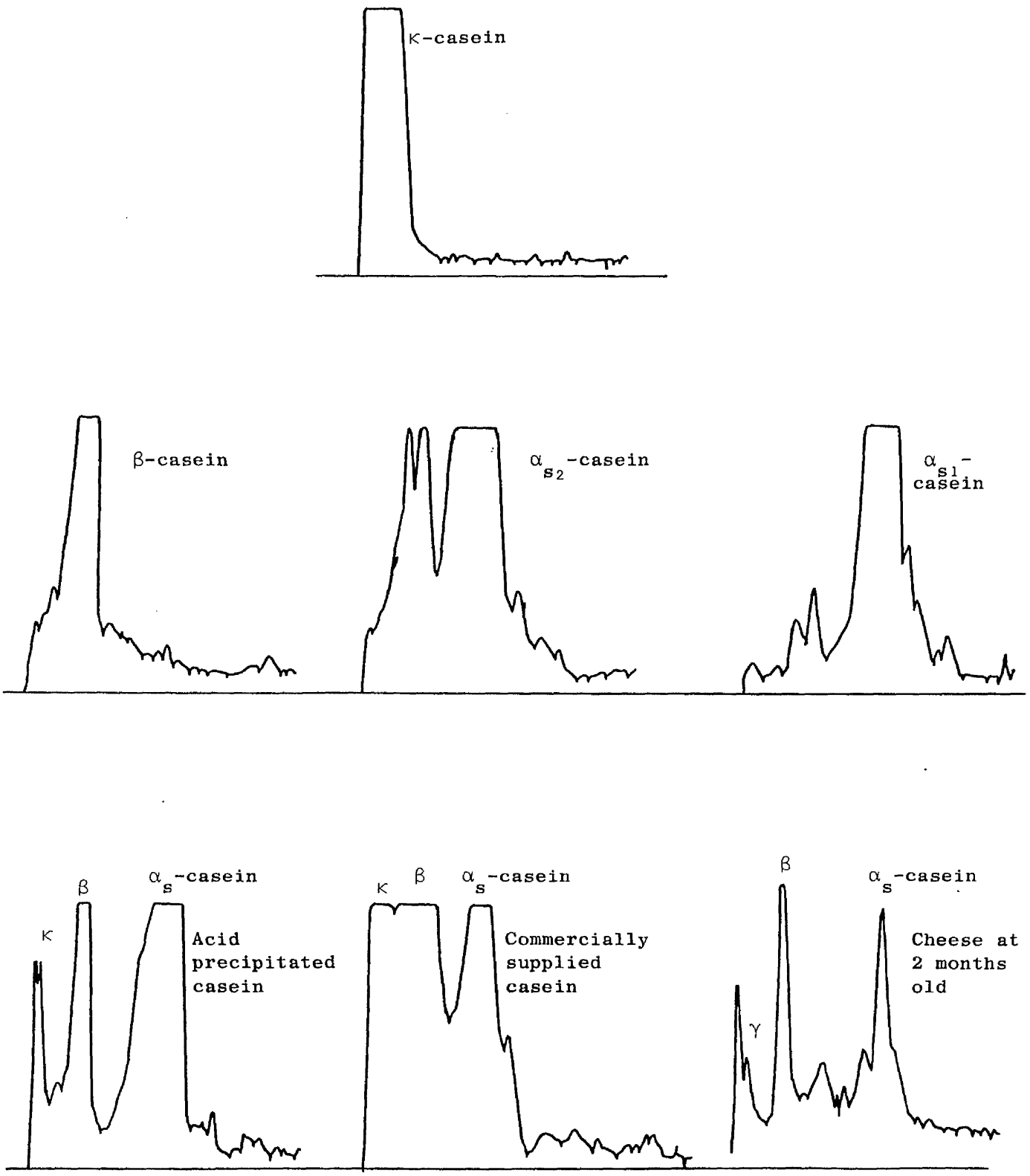


TABLE 6:1

Area of individual casein fractions expressed as a percentage of the total area of the bands formed in electrophoretic gels from purified κ-, β-, $\alpha_{s2}$- and $\alpha_{s1}$-caseins along with whole acid-precipitated casein, cheese casein at 2 months old and a commercially supplied standard casein

| Standard casein | κ-casein | β-casein | $\alpha_{s2}$-casein | $\alpha_{s1}$-casein | Whole acid-precipitated casein | Cheese casein (at 2 months) |
|---|---|---|---|---|---|---|
| 11.85 | 93.28 | 1.27 | 0.26 | 0.40 | 0.38 | 2.94 |
| - | - | 3.03 | - | - | 0.86 | 0.58 |
| - | - | - | - | 0.27 | - | |
| 31.76 | - | 89.28 | 18.48 | 3.96 | 24.37 | 47.85 |
| - | 0.73 | 1.12 | 15.05 | 6.63 | - | 0.34 |
| - | - | - | - | - | - | 4.53 |
| 39.32 | 1.51 | 1.22 | 62.09 | - | - | 0.82 |
| - | 1.17 | 1.01 | - | 74.26 | 71.02 | 10.32 |
| - | 1.37 | - | - | - | - | - |
| 2.49 | - | - | 2.43 | - | - | 31.30 |
| - | - | - | - | 7.63 | - | - |
| 3.65 | - | 0.70 | 0.64 | 4.37 | 0.36 | - |
| - | 1.05 | - | 0.49 | 2.22 | 0.15 | 0.55 |
| 4.55 | 0.69 | 0.36 | 0.15 | - | 1.27 | - |
| - | - | - | - | - | 1.02 | - |
| 1.47 | - | 2.02 | 0.39 | - | 0.30 | 0.61 |
| 1.45 | - | - | - | 1.07 | 0.27 | 0.34 |
| 0.45 | 0.25 | - | - | - | - | - |
| 1.43 | - | - | - | - | - | - |
| 1.87 | - | - | - | - | - | - |

Whitney et al.,(1976) reported that the components of the $\alpha_s$-casein fraction from milk of a homozygous cow may be identified according to their decreasing electrophoretic mobility on alkaline starch or polyacrylamide gel in the presence of urea as: $\alpha_{s_0}$-, $\alpha_{s_1}$-, $\alpha_{s_2}$-, $\alpha_{s_3}$-, $\alpha_{s_4}$- and $\alpha_{s_5}$- casein. Another two bands occupying the β-casein position were observed.

4. $\alpha_{s_1}$-casein

$\alpha_{s_1}$-casein moves slightly faster than $\alpha_{s_2}$-casein. As well as $\alpha_{s_1}$-casein band another band is expected to be present just ahead of it and is referred to as $\alpha_{s_0}$. It is not possible to see this band as a separate band from $\alpha_{s_1}$. Davies and Law (1977) observed the same results. Three other bands which moved faster than $\alpha_{s_0}$ were probably produced as a result of breakdown of the major casein fractions. These bands were similar to those bands observed with $\alpha_{s_2}$-casein.

5. Acid-precipitated casein

In this pattern the κ-casein position was occupied by two bands. One was observed close to the start point due to either high molecular weight or low electrophoretic mobility. The other which was observed slightly ahead of it and before β-casein showed a smaller peak.

β-casein appeared in one major peak. An unseparated peak could also be observed in the scan diagram on the top of the left shoulder of β-casein. This could be fraction $\beta_1$- of β-casein.

$\alpha_s$-casein appeared in the acid-precipitated casein pattern as one major band which occupies the position of $\alpha_{s_2}$- and $\alpha_{s_1}$-caseins. Other fast moving peptides were also observed.

6. Commercially supplied standard casein (acid-precipitated)

The pattern of these standards was similar to those of acid-precipitated caseins, but the separation was clearer with the acid-precipitated caseins.

7. Casein fractions of Cheddar cheese when 2 months old

The electrophoretic pattern of cheese when 2 months old was different from that of acid-precipitated casein. These variations were due to the modification and breakdown of the major casein fractions during cheesemaking and curing of the resultant cheese. One band was observed at a short distance from the start point. This fraction occupied the κ-casein position. Al-Obaidi, (1980) suggested that this fraction probably belonged to κ-casein and consisted mainly of para-κ-casein and what is left from κ-casein after clotting. Another small peak was observed just after the major κ-casein. This might be another fraction of κ-casein.

β-casein occupied the same position as in the pure fraction and in the acid-precipitated casein patterns. In cheese, β-casein showed another two bands which moved a little bit faster than the main fraction of β-casein. These two bands could not be detected by the scanner but later on after further curing they were detected as separate fractions of β-casein (see mention of this point in later section).

After the β-casein fractions, group of 8-10 bands were observed. These bands will be referred to as $\alpha_s$-casein fractions. The biggest band is $\alpha_{s_1}$-casein. A thinner band was observed which moved more slowly than $\alpha_{s_1}$-casein which is $\alpha_{s_2}$. The area occupied by these bands were 31.30 and 10.37 per cent of the total area of the peak of cheese peptides. The other peptides observed resulted from the breakdown of $\alpha_s$-casein and probably from β-casein. These bands moved faster due to the high charges and low molecular weights.

Section 6:2

Effect of cold storage of raw milk at $2^{o}C$ and $6^{o}C$ (and the resultant pasteurized milk) on the evidence of proteolysis of the fractions of casein

In the course of this study the acid-precipitated casein from raw milks on the day of delivery and after storage of 2, 4 and 7 d at $2^{o}C$ and $6^{o}C$ were applied to one slab gel. An example of these slabs is

presented in Plate 6:2. The electrophoretic pattern of acid-precipitated casein prepared from the pasteurised milks made from the above raw milks is presented in Plate 6:3. The scan diagrams of these separations are presented in Figs 6:2 and 6:3. The area of individual casein fractions is presented in Table 6:2. The bands observed in the casein pattern could be divided into two groups:

Group 1. Slow-mobility bands

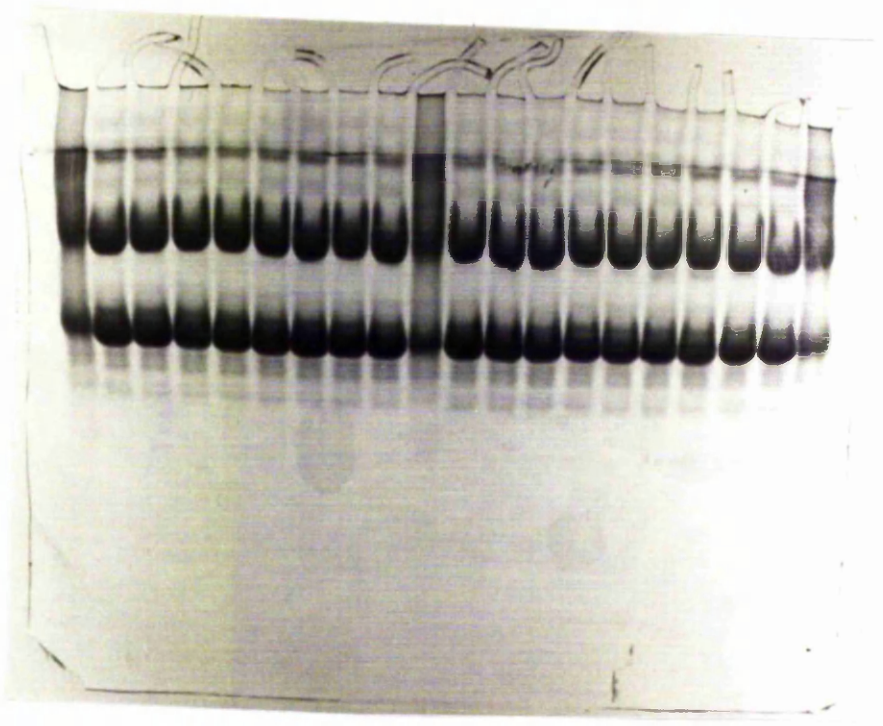
This includes all the bands from the origin of application point to the β-casein fractions. In Plate 6:2, two minor bands were observed occupying the positions of S and Ts fractions in the order of increasing mobility. One of these bands did not appear in the casein prepared from pasteurized milk. It is more likely that the band of Ts had disappeared since Ts is designated as temperature sensitive casein. These two bands (S and Ts) are fractions of γ-caseins (McKenzie, 1970).

In the position of κ-casein, one major band, observed beside two minor bands, might be another variant of κ-casein. These two bands were observed in the raw milks in each trial but not in the pasteurized milks in any trial. Another band ($\beta_1$) was detected before the major β-casein ($\beta_2$) band in the raw milk. β-casein increased in the raw milks with increasing period and temperature of milk storage. β-casein was increased in the raw milks after 2 d of storage at $2^{o}C$ but otherwise its concentration decreased as the period of storage lengthened at both temperatures. The decrease in concentration of β-casein was greater in milks held at $6^{o}C$ than at $2^{o}C$. κ-casein was the only fraction which occurred in a concentration less in the pasteurized milk than in the raw milks. Some of the peaks in the positions of γ- and κ-casein were not completely separated so they were not calculated as separate peaks. In the pasteurized milk the bands of γ- and κ-caseins were mixed and not clearly separated; the κ-casein was found in a lower concentration than that in the raw milks.

Group 2. Fast-mobility bands

These include the bands occupying the position of the $\alpha_s$-casein

Track No. 1



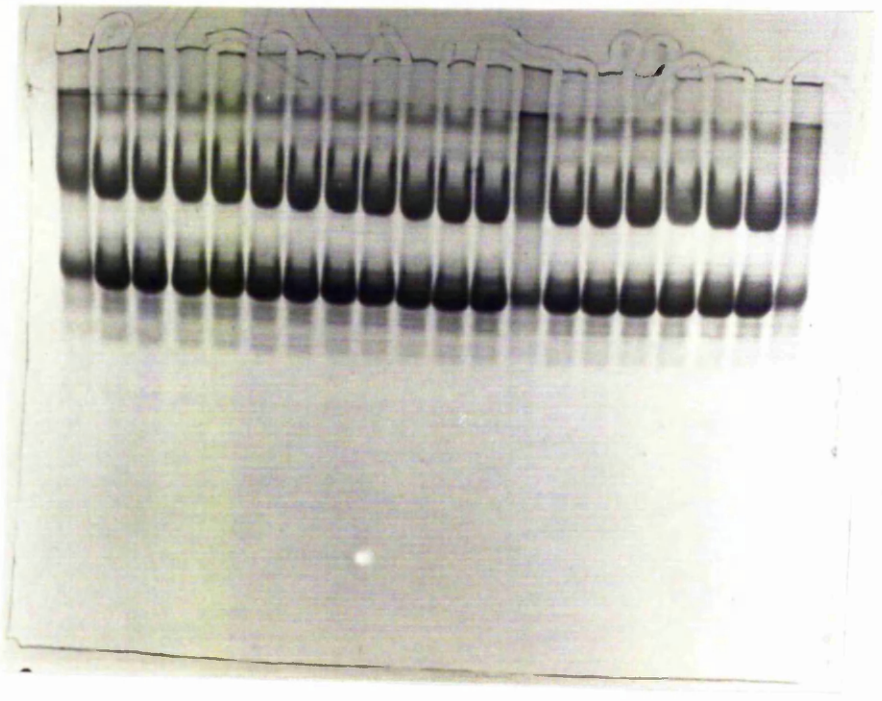
Track No . 20

PLATE 6:2

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis (PAGE) at pH 8.6, of acid-precipitated caseins of raw milk on the day of delivery and after 2, 4 and 7 days storage at $2^{o}C$ and $6^{o}C$

| Pattern of raw milk | Number of track |
|---|---|
| On the day of delivery | 2, 3 |
| On the day of delivery | 4, 5 |
| After storage for 2 days at $2^{o}C$ | 6, 7 |
| After storage for 4 days at $2^{o}C$ | 11, 12, 13 |
| After storage for 7 days at $2^{o}C$ | 18, 19 |
| After storage for 2 days at $6^{o}C$ | 8, 9 |
| After storage for 4 days at $6^{o}C$ | 14, 15 |
| After storage for 7 days at $6^{o}C$ | 16, 17 |
| Standard casein | 1, 10, 20 |

Track No. 1



Track No. 20

PLATE 6:3

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of acid-precipitated casein of pasteurized milk made from raw milk on the day of delivery and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

| Pattern for pasteurized milk made from raw milk | Number of track |
|---|---|
| On the day of delivery | 18, 19 |
| On the day of delivery | 16, 17 |
| After storage for 2 days at $2^{o}C$ | 14, 15 |
| After storage for 4 days at $2^{o}C$ | 11, 12 |
| After storage for 7 days at $2^{o}C$ | 9, 10 |
| After storage for 2 days at $6^{o}C$ | 7, 8 |
| After storage for 4 days at $6^{o}C$ | 5, 6 |
| After storage for 7 days at $6^{o}C$ | 2, 3, 4 |
| Standard casein | 1, 13, 20 |

Fig. 6:2 The scan diagrams of the electrophoretic pattern of raw milks (a) on the day of delivery, and after storage (b) at 2°C and (c) at 6°C for 2, 4 and 7 days

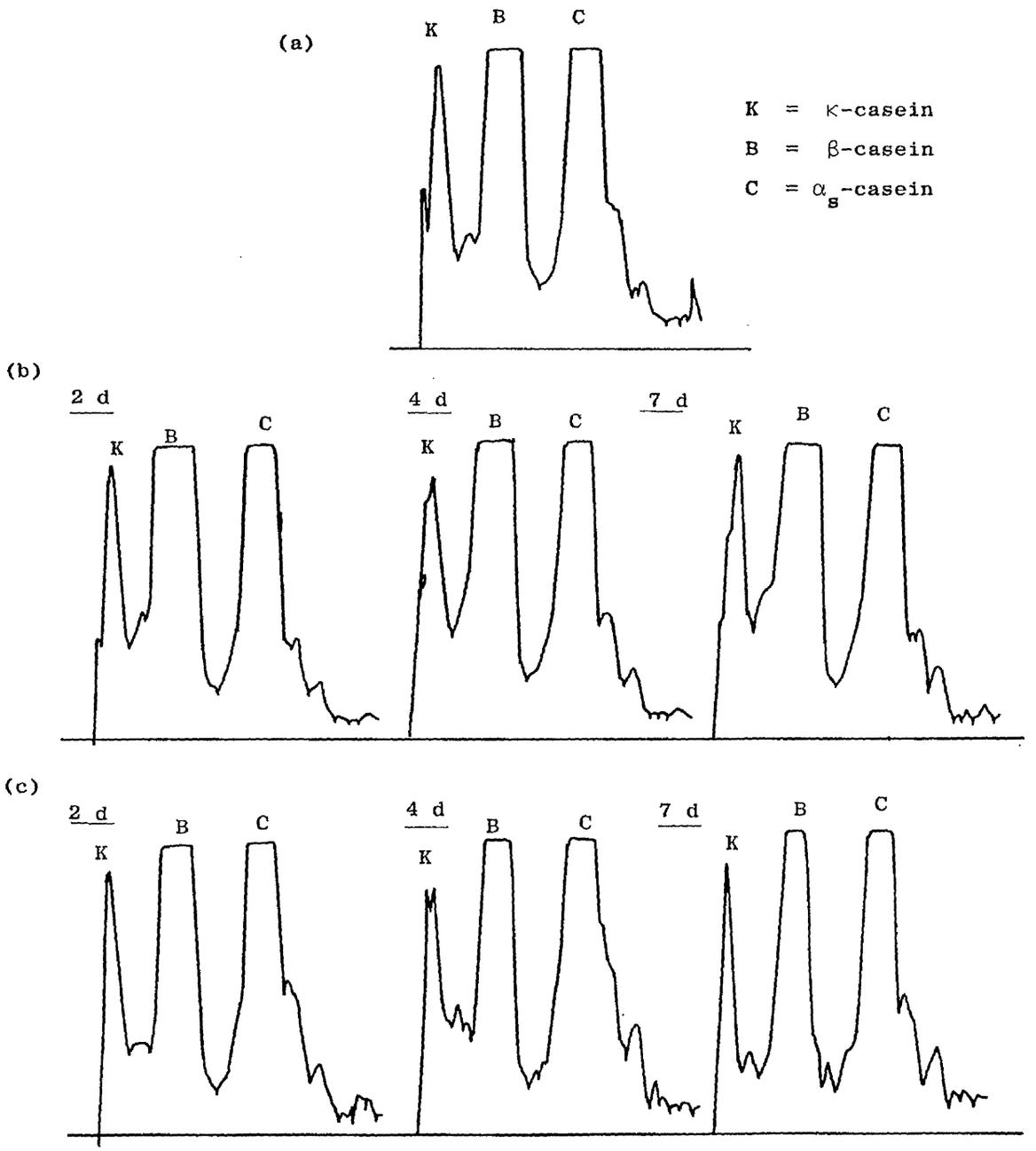


Fig. 6:3 The scan diagrams of the electrophoretic pattern of pasteurized milks made from raw milk (a) on the day of delivery, and after storage (b) at 2°C and (c) at 6°C for 2, 4 and 7 days

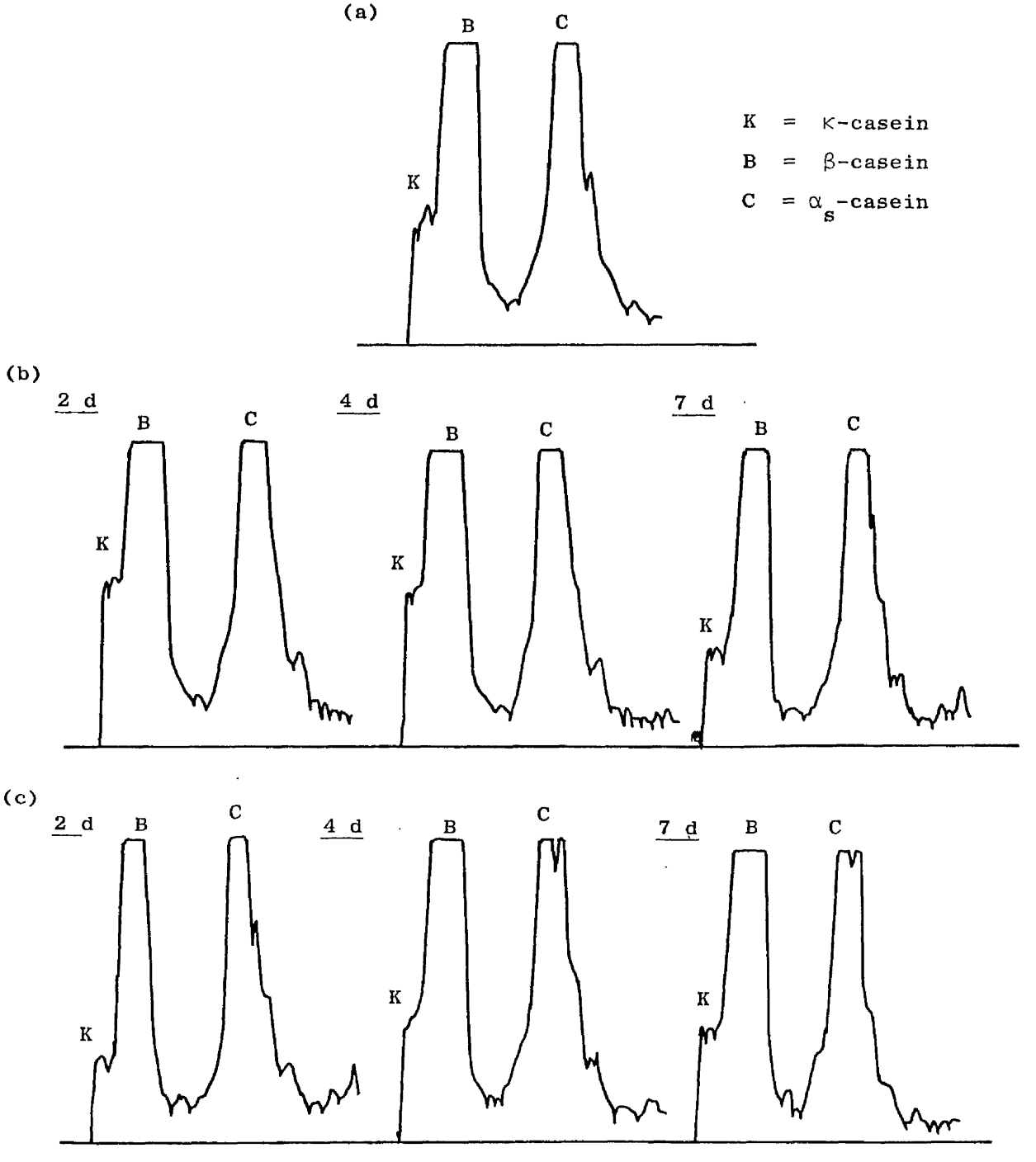


TABLE 6:2

The area of individual casein fractions expressed as a percentage of the total area of the bands formed in electrophoretic gels from raw milks on the day of delivery, raw milks held for 2, 4 and 7 days at 2°C and 6°C, and the pasteurized milk prepared from these milks and used for cheesemaking

| Casein fraction | Type of milk | On the day of delivery | Storage at 2°C (d) | | | Storage at 6°C (d) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 7 | 2 | 4 | 7 |
| S- | Raw | 0.22 | 0.69 | 0.41 | - | 0.46 | 0.91 | 0.04 |
| | Past. | - | - | - | - | - | - | - |
| Ts- | Raw | 0.62 | 0.28 | 0.60 | 0.64 | 0.61 | 0.36 | 0.64 |
| | Past. | 0.27 | 1.40 | 0.29 | 1.07 | 0.57 | - | 0.34 |
| κ- | Raw | 7.17 | 5.19 | 8.64 | 11.02 | 8.42 | 11.26 | 15.29 |
| | Past. | 3.27 | 1.66 | 1.36 | 1.63 | 1.47 | 0.41 | 2.31 |
| | Raw | 1.57 | 0.99 | 1.47 | 2.42 | 2.10 | 1.61 | 2.25 |
| | Past. | - | - | - | - | - | - | - |
| | Raw | - | - | 1.82 | 4.06 | 1.18 | 2.14 | 0.23 |
| | Past. | - | - | - | - | - | - | - |
| $\beta_1$- | Raw | - | 4.90 | - | 4.52 | - | 1.46 | 1.76 |
| | Past. | - | - | - | - | - | - | - |
| $\beta_2$- | Raw | 40.93 | 42.02 | 37.20 | 31.38 | 40.72 | 33.63 | 29.85 |
| | Past. | 35.32 | 39.66 | 44.06 | 40.47 | 39.81 | 41.76 | 38.34 |
| $\alpha_{s1}$- | Raw | - | 0.22 | 0.37 | - | - | 0.32 | 0.14 |
| | Past. | 0.24 | 0.26 | 0.34 | 1.24 | 0.37 | 0.20 | 2.01 |
| $\alpha_{s2}$- | Raw | 45.94 | 43.39 | 43.45 | 40.75 | 42.55 | 45.58 | 42.88 |
| | Past. | 53.87 | 52.66 | 49.00 | 50.38 | 45.34 | 43.67 | 45.65 |
| $\alpha_{s3}$- | Raw | 1.31 | 0.76 | 2.93 | 2.82 | 2.32 | 0.61 | 3.26 |
| | Past. | 6.33 | 1.86 | 4.09 | 1.43 | 7.95 | 12.86 | 10.42 |
| $\alpha_{s4}$- | Raw | 0.55 | 0.65 | 0.44 | 1.05 | 0.40 | 0.26 | 0.21 |
| | Past. | 0.35 | 1.33 | 0.46 | 1.66 | 0.36 | 0.69 | 0.33 |
| $\alpha_{s5}$- | Raw | 0.73 | 0.36 | 1.95 | 0.04 | 0.19 | 0.34 | 1.67 |
| | Past. | 0.32 | 0.27 | 0.30 | 0.77 | 1.81 | 0.41 | 0.36 |
| $\alpha_{s6}$- | Raw | 0.50 | 0.44 | 0.48 | 0.98 | 0.70 | 1.22 | 0.82 |
| | Past. | - | 0.88 | 0.14 | 0.46 | 1.44 | - | 0.16 |
| $\alpha_{s7}$- | Raw | 0.34 | 0.12 | 0.14 | 0.36 | 0.32 | 0.27 | 0.54 |
| | Past. | - | 0.10 | 0.12 | 0.72 | 0.88 | - | 0.07 |

fractions. These fractions may be numbered from 1 to 7; the second band was the major one. $\alpha_s$-casein fractions showed fluctuation in their concentration in raw milk during storage. These bands did not show a consistent pattern in the pasteurized milks made from stored milks. In Fig. 6:2, the shape of the $\alpha$-casein peak of milks held at $2^{o}C$ showed slight variation during storage.

These variations were more severe in the casein of milks held at $6^{o}C$. The pattern of the casein of milks held at $6^{o}C$ showed more intense small fast-moving bands than those of the milks on the day of delivery and milks held at $2^{o}C$. The amount of $\alpha_s$-casein was higher in the pasteurized milks than in the raw milks. The major $\alpha_s$ fraction showed a reduction in its concentration in the raw milk with an increasing length of storage at both $2^{o}C$ and $6^{o}C$.

The analysis of variance of all of the casein fractions did not indicate that the storage of milk at either $2^{o}C$ or $6^{o}C$ had a significant effect. The analysis of variance for only one fraction ($\alpha_s$-casein fraction) is presented in this section (Table 6:3), other fractions showed similar variations, although there are some significant variations due to the trial and the type of milk (raw or pasteurized).

The summations of the areas of all peaks appearing in each of $\gamma$-, $\kappa$-, $\beta$- and $\alpha_s$-casein position were calculated and the analysis of variance is presented in Table 6:4. The significant variations were due to the type of milk (pasteurized or raw) and to individual trial. $\kappa$-casein was the fraction most affected in the pasteurization.

Section 6:3

Proteolysis of casein fractions during ripening of Cheddar cheese made from milk pasteurized on the day of delivery and after storage for 2, 4 and 7 days at $2^{o}C$ and $6^{o}C$

The photographs of the slab gels are presented in Plates 6:4 to 6:9. The corresponding scan diagrams for these gels are presented in Figs 6:7 to 6:10. The scan diagrams are presented as they were recorded

TABLE 6:3

The analysis of variance for $\alpha_s$-casein for the effect of cold storage of milk on the casein fractions of raw and pasteurized milks

| | DF | Raw milk | | Pasteurized milk | |
|---|---|---|---|---|---|
| | | MS | VR | MS | VR |
| Trial | 3 | 148.45 | 5.414** | 108.16 | 1.262 |
| Treatment | 6 | 12.81 | 0.467 | 60.97 | 0.712 |
| Residual | 17 | 27.42 | | 85.68 | |
| Total | 26 | 38.01 | | 80.51 | |

Area of the $\alpha_s$-casein peak as a percentage of the total area of the peaks+

| Type of milk | On the day of delivery | Storage at 2°C d | | | Storage at 6°C d | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 7 | 2 | 4 | 7 |
| Raw | 45.94 | 43.80 | 43.45 | 40.75 | 42.55 | 45.58 | 42.88 |
| Pasteurized | 53.90 | 52.70 | 49.00 | 50.4 | 45.3 | 43.70 | 45.60 |

+overall three-trial figure was used

**significant at 1 per cent level

TABLE 6:4

The analysis of variance for γ-, κ-, β- and $\alpha_s$-caseins (in the order of increasing electrophoretic mobility) of raw milks on the day of delivery, after 2, 4 and 7 days storage at 2°C and 6°C, and the corresponding pasteurized milks used for cheesemaking after these periods of storage.

| | | On the day of delivery | Storage at 2°C d | | | Storage at 6°C d | | | Means | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 7 | 2 | 4 | 7 | | |
| Gamma | Raw | 0.88 | 0.41 | 0.60 | 0.28 | 0.52 | 0.06 | - | 0.39 | 0.49 |
| | Past. | 0.27 | 1.40 | 0.29 | 1.20 | 0.57 | - | 0.34 | 0.58 | |
| Kappa | Raw | 7.24 | 8.80 | 11.01 | 13.46 | 10.29 | 13.49 | 18.79 | 11.87 | 6.79 |
| | Past. | 3.27 | 1.66 | 1.36 | 1.63 | 1.47 | 0.41 | 2.31 | 1.73 | |
| Beta | Raw | 39.25 | 41.77 | 34.25 | 33.78 | 37.13 | 32.86 | 31.97 | 35.86 | 38.22 |
| | Past. | 35.56 | 39.92 | 44.40 | 41.70 | 40.18 | 41.96 | 40.35 | 40.58 | |
| Alpha | Raw | 52.60 | 47.55 | 52.36 | 48.67 | 50.55 | 51.60 | 49.24 | 50.37 | 53.75 |
| | Past. | 60.87 | 57.10 | 54.11 | 55.44 | 57.78 | 57.63 | 57.00 | 57.13 | |

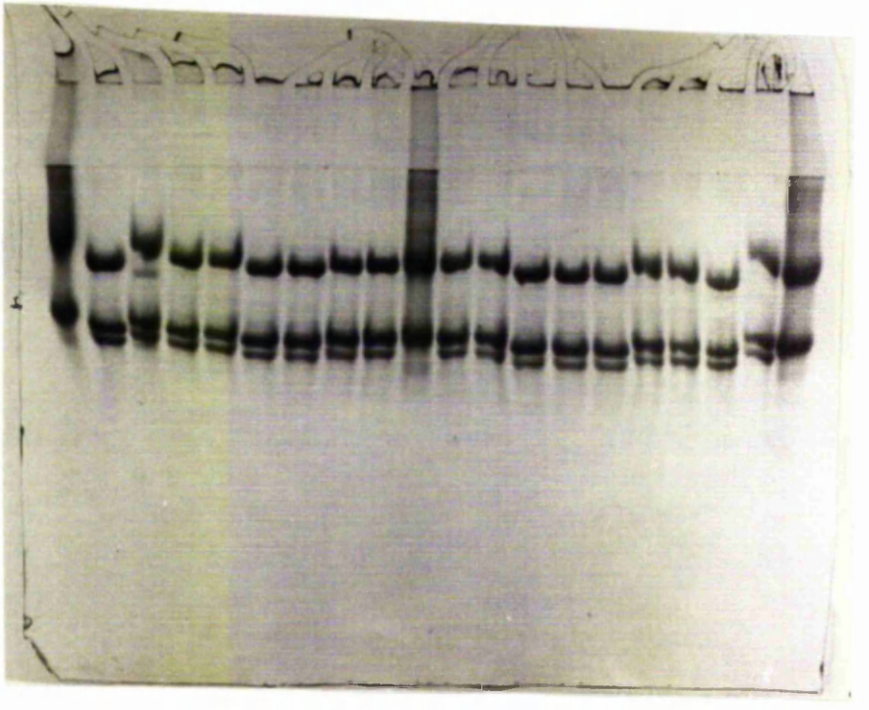
| | DF | Gamma | | Kappa | | Beta | | Alpha | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | VR | MS | VR | MS | VR | MS | VR |
| Type[1] | 1 | 0.3458 | 1.243 | 1077.44 | 87.449*** | 234.23 | 6.707** | 480.40 | 15.990*** |
| Trial | 2 | 3.9576 | 14.224*** | 128.89 | 14.844*** | 89.62 | 2.566 | 110.86 | 3.690 |
| Treatment[2] | 6 | 0.5787 | 2.080 | 20.83 | 1.690 | 14.11 | 0.404 | 15.41 | 0.513 |
| Type x Trial | 2 | 0.3893 | 1.399 | 121.27 | 9.842** | 187.02 | 5.355* | 12.58 | 0.419 |
| Type x Treat | 6 | 0.5211 | 1.873 | 25.29 | 2.053 | 47.22 | 1.352 | 9.23 | 0.307 |
| Trial x Treat | 12 | 0.2733 | 0.982 | 10.62 | 0.862 | 13.10 | 0.375 | 7.49 | 0.249 |
| Residual | 11 | 0.2782 | | 12.32 | | 24.92 | | 30.04 | |
| Total | 40 | 0.5495 | | 55.63 | | 42.42 | | 32.38 | |

[1]Type of milk

[2]Storage of milk for 2, 4 and 7 days at 2°C and 6°C

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

Track No. 1



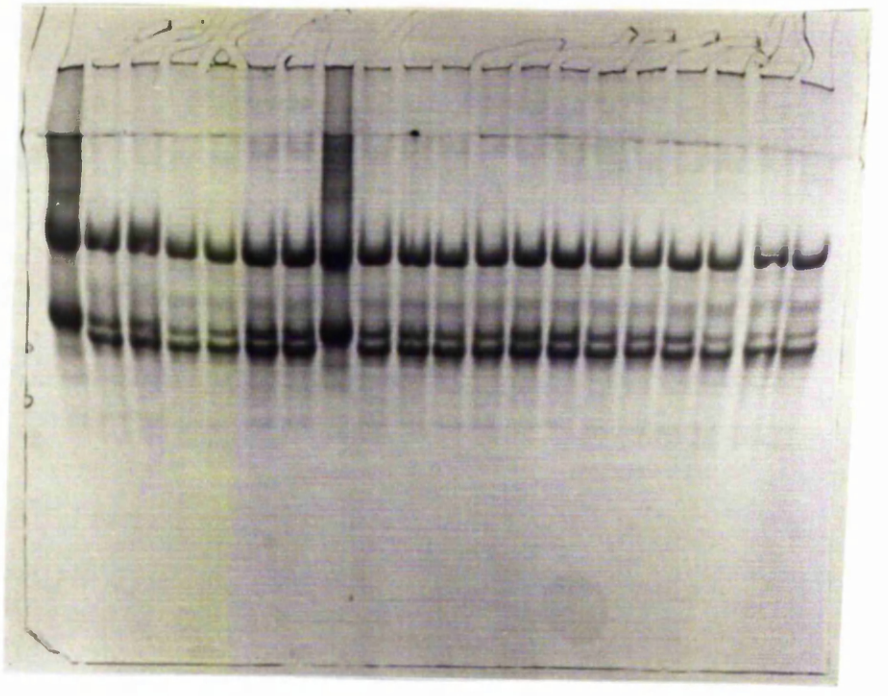
Track No. 20

PLATE 6:4

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6 of Cheddar cheese at 1 week old made from milk pasteurized on the day of delivery and after storage for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization.

| Pattern for cheese made from: | Number of tracks |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6, 7 |
| Milk pasteurized after storage raw for 4 days at 2°C | 8, 9 |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16 |
| Milk pasteurized after storage raw for 7 days at 6°C | 17, 18, 19 |
| Standard casein | 1, 10, 20 |

Track No. 1



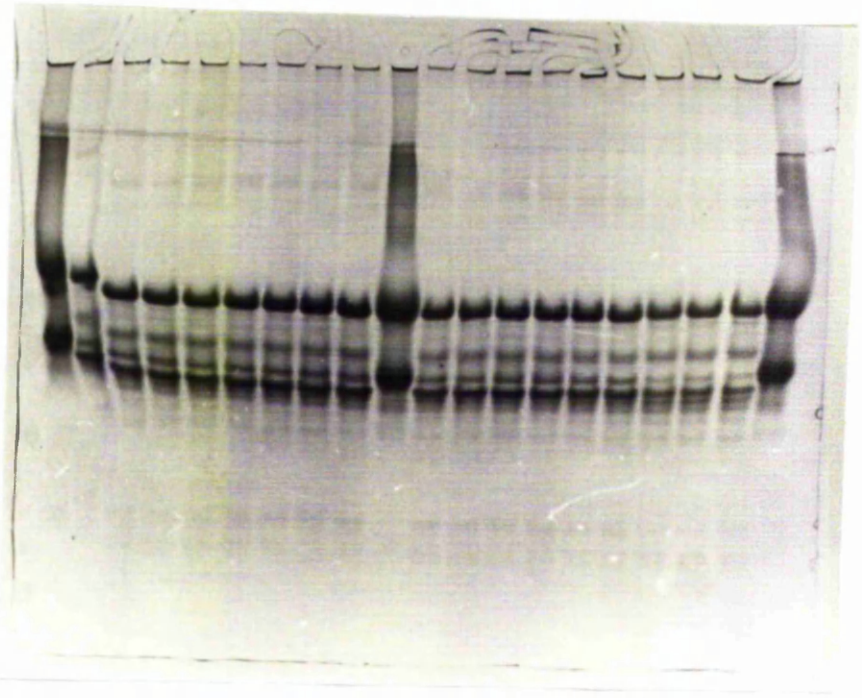
Track No. 20

PLATE 6:5

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of Cheddar cheese at 1 month old made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization

| Pattern for cheese made from: | Number of track |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6, 7 |
| Milk pasteurized after storage raw for 4 days at 2°C | 9, 10 |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16. 17 |
| Milk pasteurized after storage raw for 7 days at 6°C | 18, 19, 20 |
| Standard casein | 1, 8 |

Track No. 1



Track No. 20

PLATE 6:6

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of Cheddar cheese at 2 months old made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization.

| Pattern for cheese made from: | Number of track |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6, 7 |
| Milk pasteurized after storage raw for 4 days at 2°C | 8, 9 |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16 |
| Milk pasteurized after storage raw for 7 days at 6°C | 17, 18, 19 |
| Standard Casein | 1, 10, 20 |

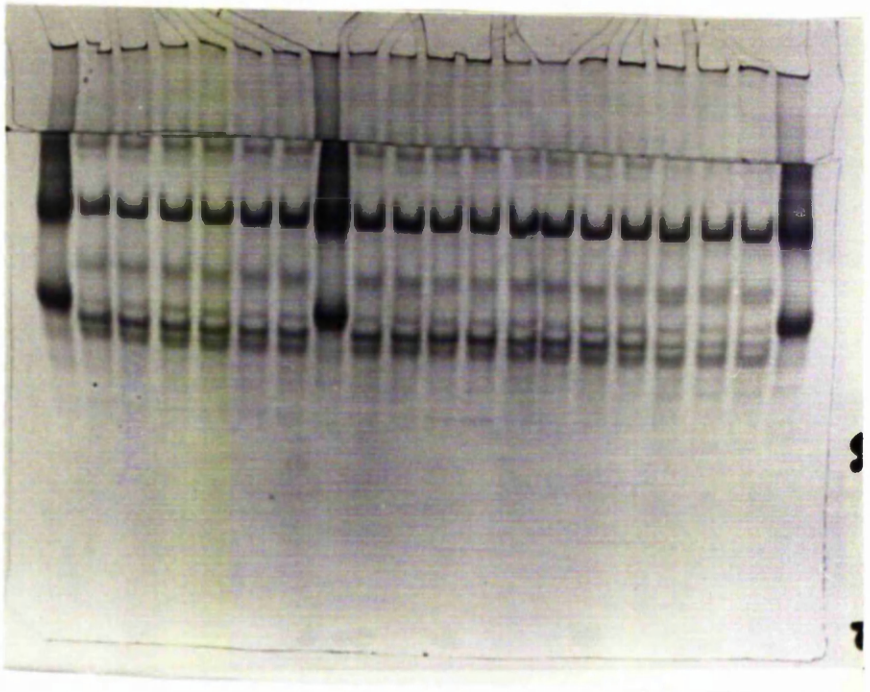
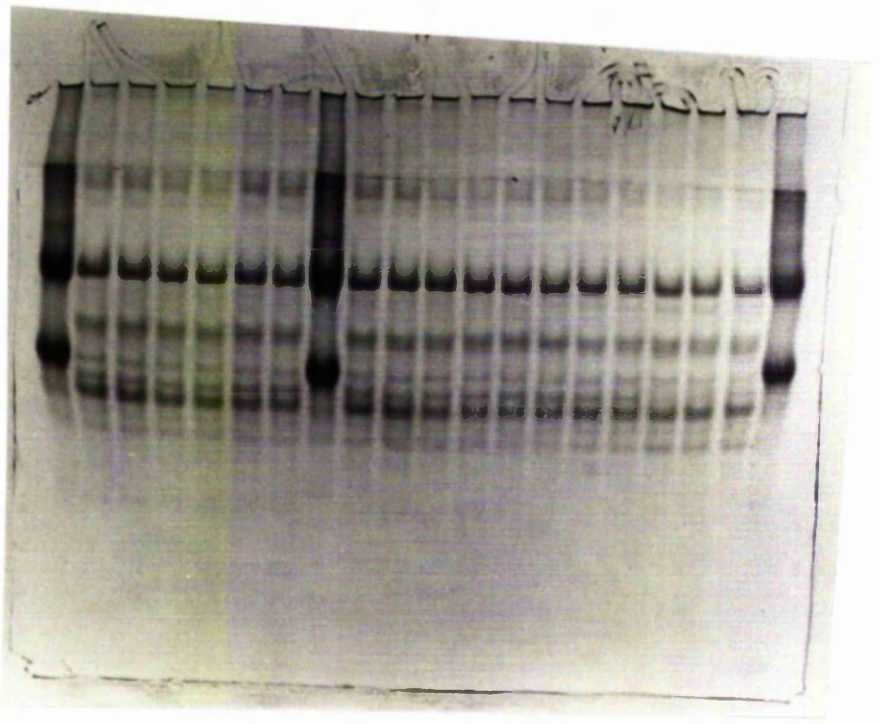



PLATE 6:7

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of Cheddar cheese at 4 months old made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization.

| Pattern for cheese made from: | Number of track |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6, 7 |
| Milk pasteurized after storage raw for 4 days at 2°C | 9, 10 |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16 |
| Milk pasteurized after storage raw for 7 days at 6°C | 17, 18, 19 |
| Standard casein | 1, 8, 20 |

Track No. 1



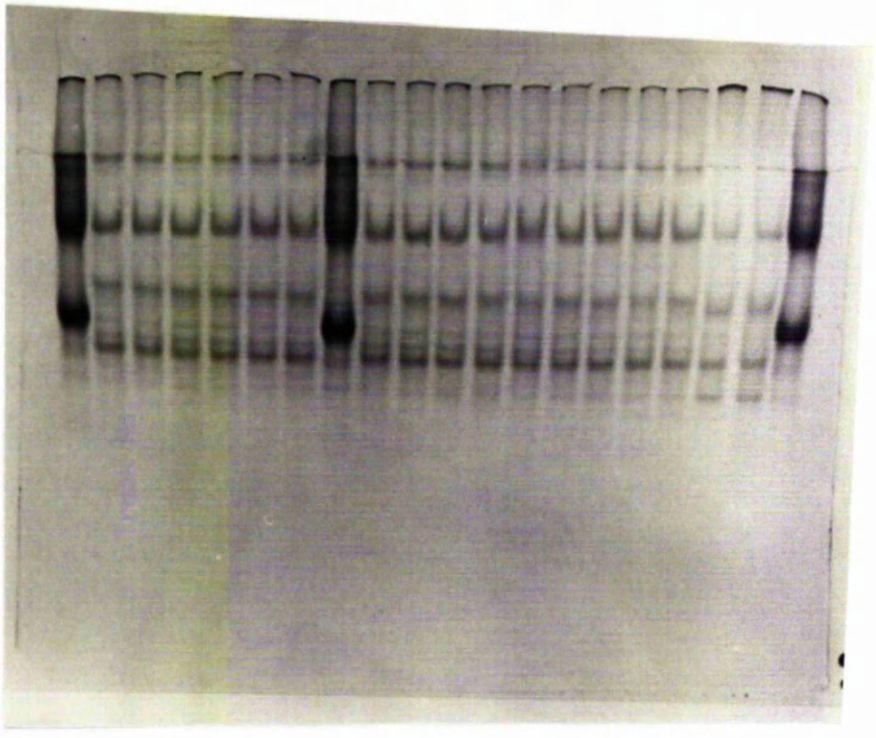
Track No. 20

PLATE 6:8

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of Cheddar cheese at 8 months old made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization.

| Pattern for cheese made from: | Number of track |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6,7 |
| Milk pasteurized after storage raw for 4 days at 2°C | |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16 |
| Milk pasteurized after storage raw for 7 days at 6°C | 17, 18, 19 |
| Standard casein | 1, 8, 20 |

Track No. 1



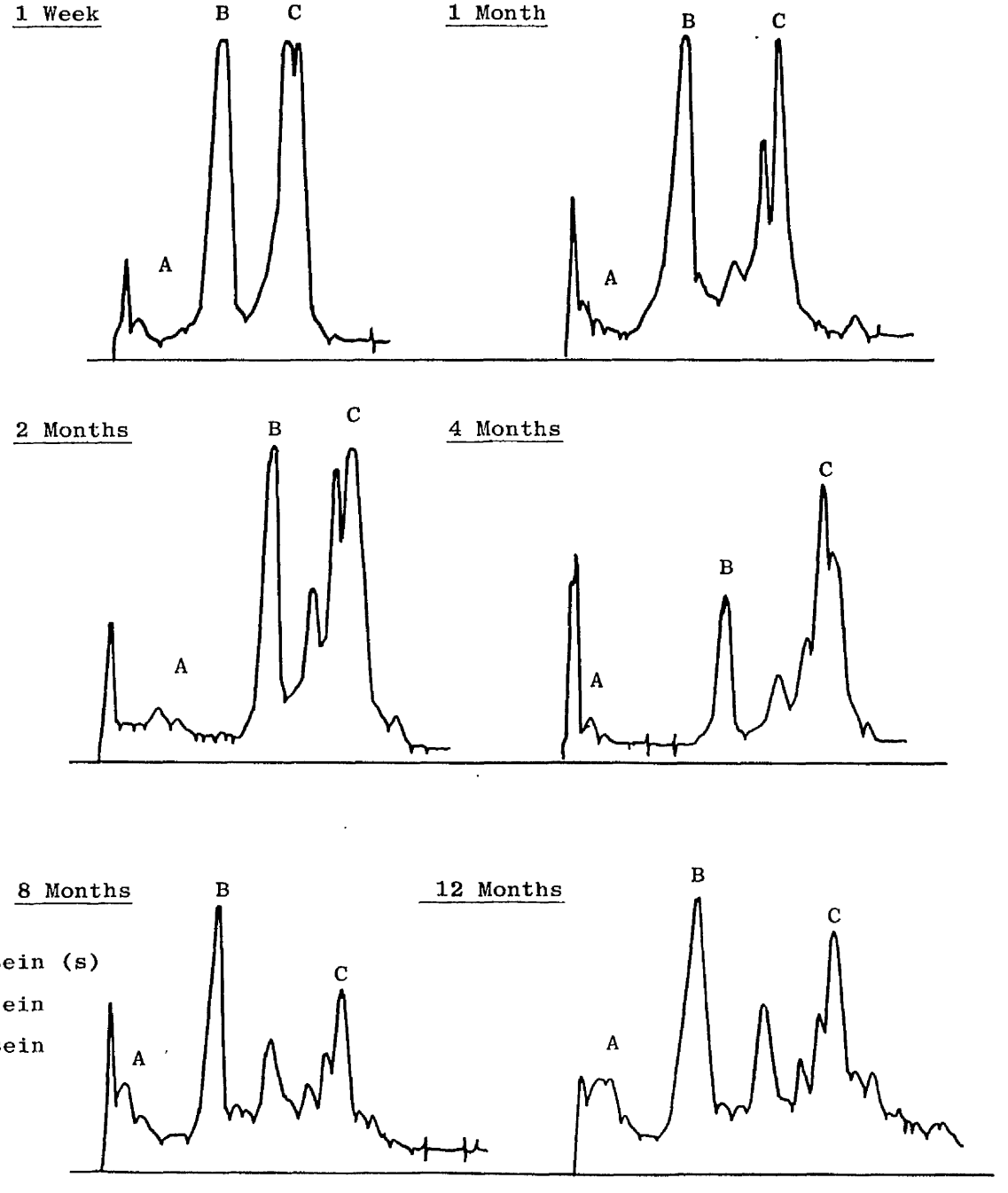
Track No. 20

PLATE 6:9

The electrophoretic pattern of the polyacrylamide slab gel electrophoresis at pH 8.6, of Cheddar cheese at 12 months old made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C and 6°C. Cheese production took place immediately after milk pasteurization.

| Pattern for cheese made from: | Number of track |
|---|---|
| Milk pasteurized on day of delivery | 2, 3 |
| Milk pasteurized on day of delivery | 4, 5 |
| Milk pasteurized after storage raw for 2 days at 2°C | 6, 7 |
| Milk pasteurized after storage raw for 4 days at 2°C | 9, 10 |
| Milk pasteurized after storage raw for 7 days at 2°C | 11, 12 |
| Milk pasteurized after storage raw for 2 days at 6°C | 13, 14 |
| Milk pasteurized after storage raw for 4 days at 6°C | 15, 16, 17 |
| Milk pasteurized after storage raw for 7 days at 6°C | 18, 19 |
| Standard casein | 1, 8, 20 |

Fig. 6:4 The scan diagrams of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized on the day of delivery




A = $\gamma$ casein (s)
B = $\beta$ casein
C = $\alpha_s$ casein

Fig. 6:5 The scan diagrams of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized after storage for 2 days at 2°C

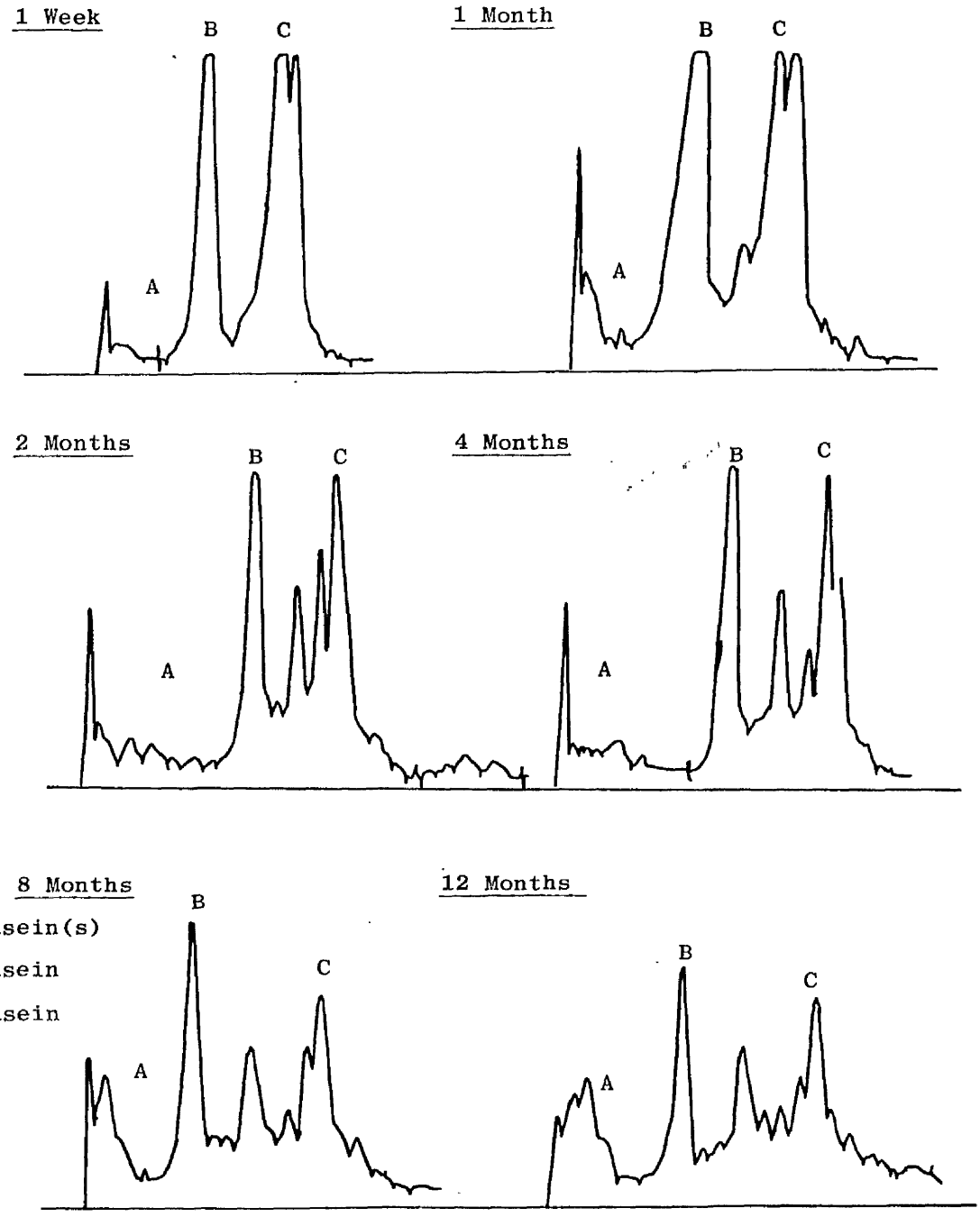


Fig. 6:6 The scan diagrams of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized after storage for 4 days at 2°C

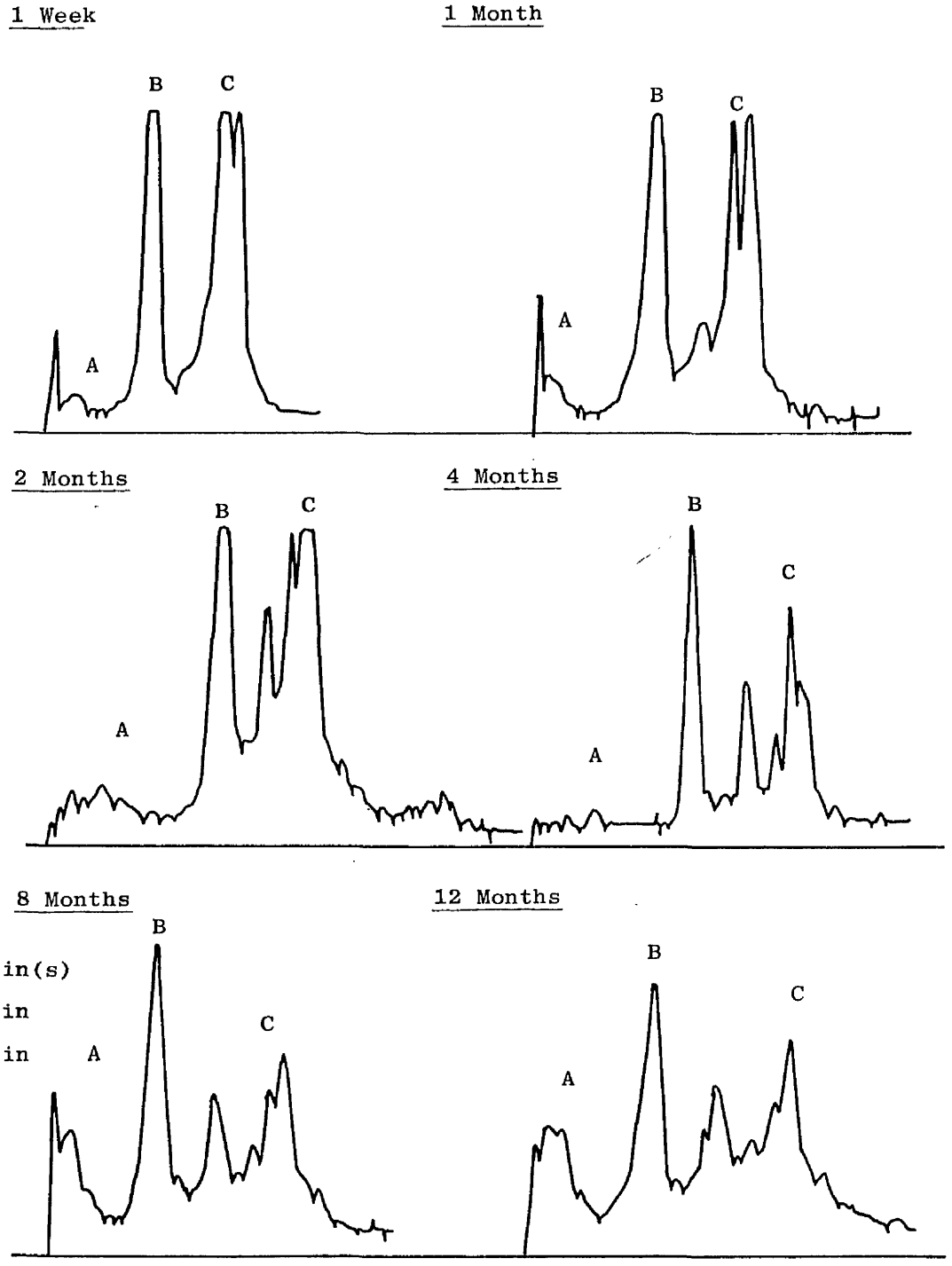
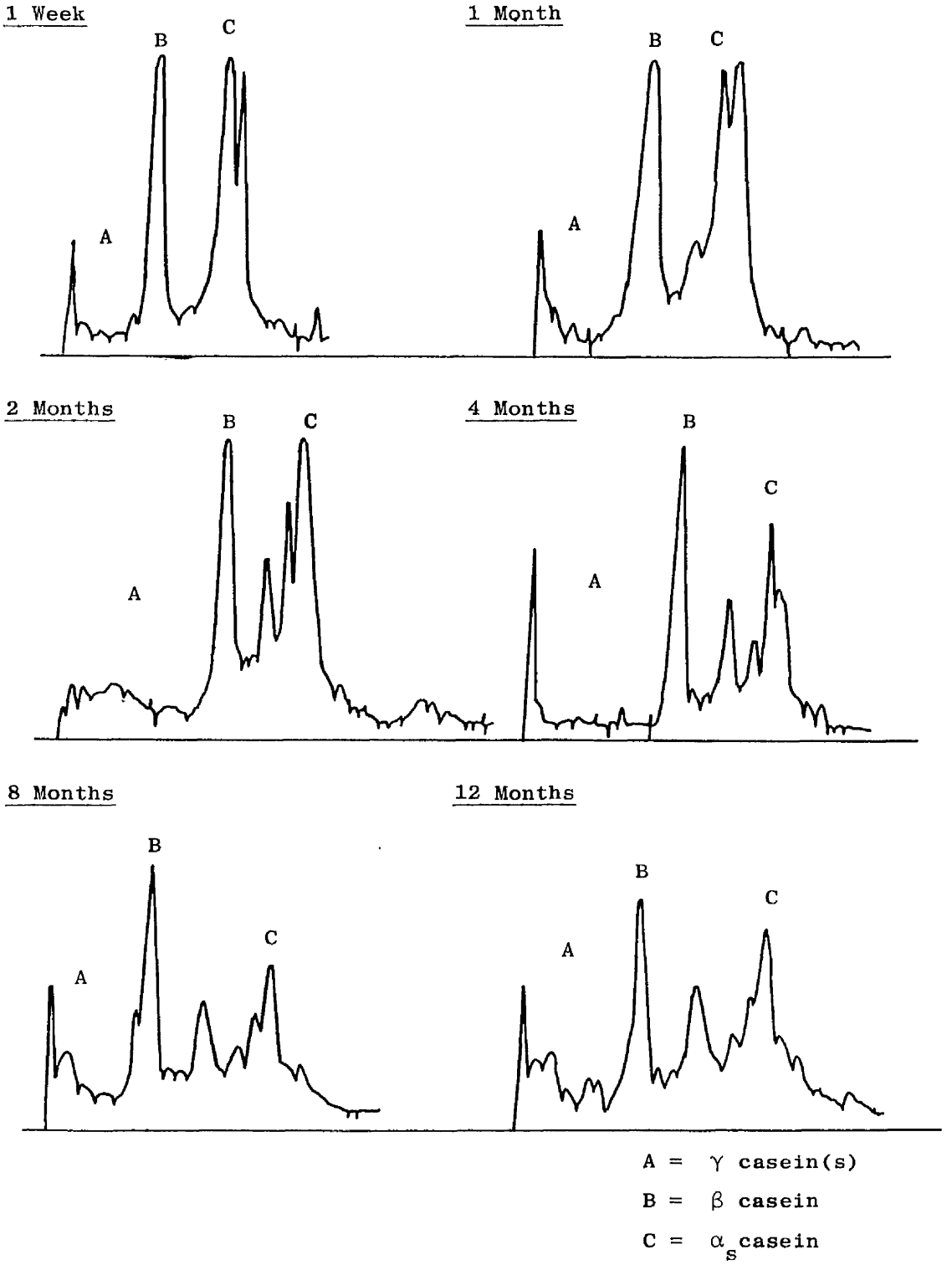


Fig. 6:7 The scan diagrams of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized after storage for 7 days at 2°C




A = γ casein(s)

B = β casein

C = $\alpha_s$ casein

Fig 6:9 The scan diagram of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized after storage for 4 days at 6°C

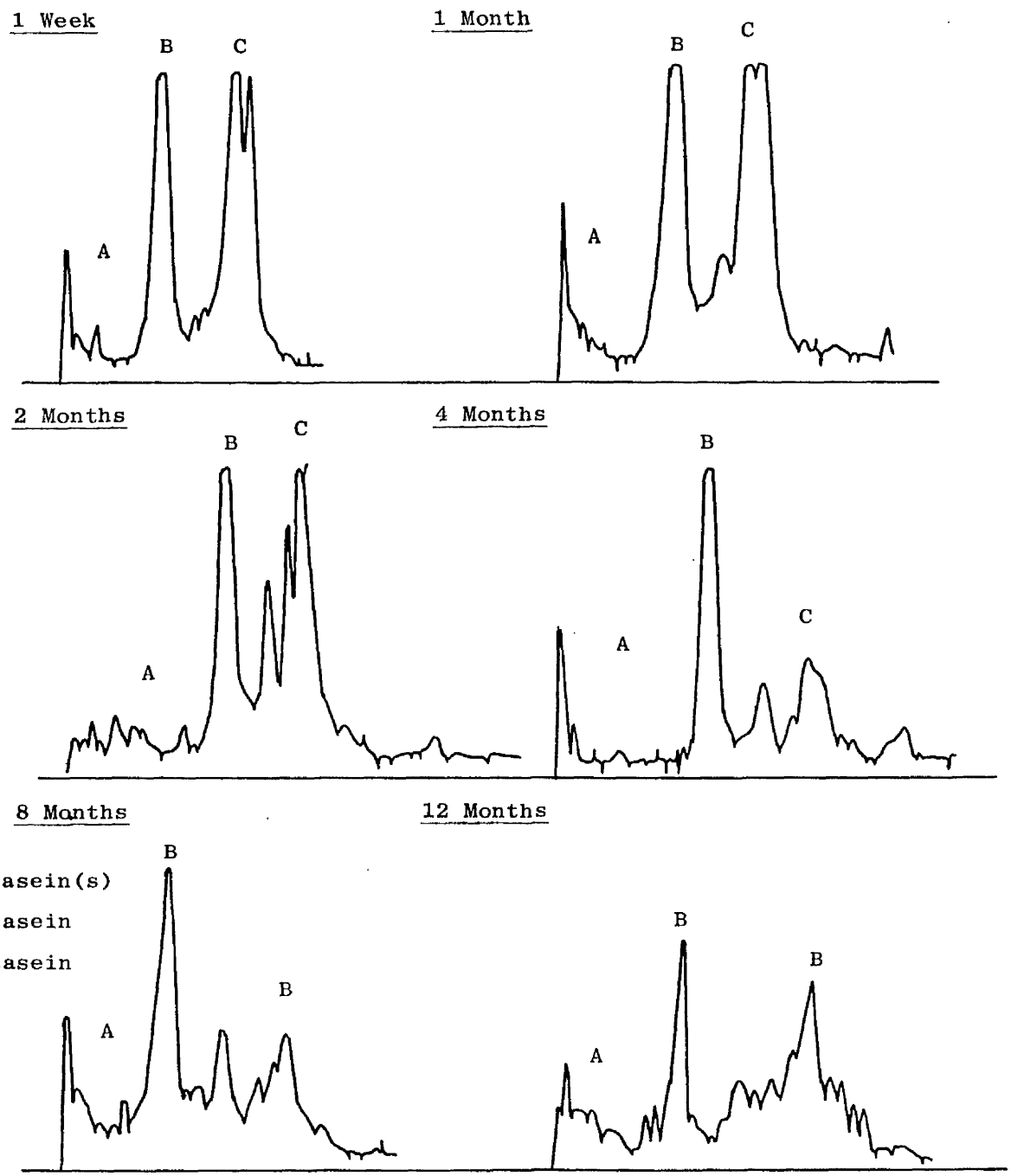
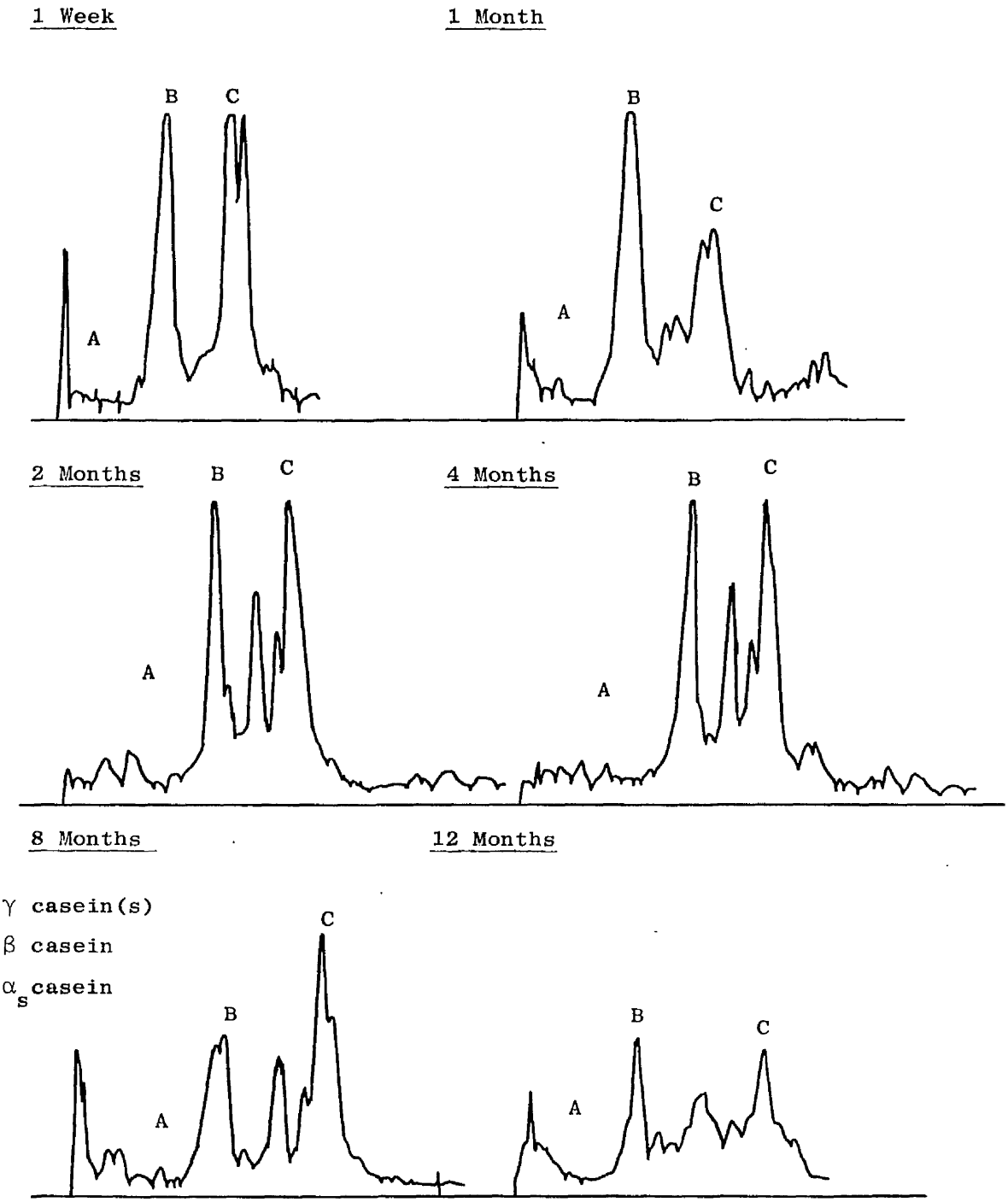


Fig. 6:10 The scan diagrams of the electrophoretic pattern of Cheddar cheese (of varying age) made from milks pasteurized after storage for 7 days at 6°C




A = γ casein(s)

B = β casein

C = $\alpha_s$ casein

after scanning.

The position of casein fractions in different gels was different due to the variation in the length of the stacking gel. Although the slab gel separation technique minimizes the variation from one run to another there is still some variation in the different slab gels at different ages of Cheddar cheese during curing.

The procedure used by Al-Obaidi (1980) for identification of casein fractions in cheese during curing of Cheddar cheese was used.

The results presented in these studies are based on the means of the three trials. One of the main sources of variations in the pattern under study was the curing time. The results are given in Tables 6:5 to 6:8 and Figs 6:11 to 6:24. To study the effect of cold storage and curing time on the proteolysis of casein fractions it is necessary to study each fraction individually:

Group 1: The slow-mobility fractions

κ-casein fraction

This fraction either did not move at all in the separation gel or moved to only within 2 mm of the start of the separation gel of 90 mm length. There were significant variations in κ-casein as a result of curing time. The area covered by this fraction varied between 0.21 and 5.59 per cent of the total area of the peaks. This fraction was present in all cheeses at different curing times. The casein fractions of cheese made from milk pasteurized on the day of delivery did not show high variation during curing, whereas it was increased in the cheeses made from stored milk. The highest increase was found in the cheese made from milks held for 7 d at $2^{o}$C.

Ts-casein fraction

This fraction moved slightly faster than κ-casein. This fraction might be Ts, R or S casein. Al-Obaidi (1980) referred to this fraction as the Ts fraction so designated by Emmons et al. (1976) and this identity will be used in this study.

TABLE 6:5

The area of individual casein fractions of the slow-mobility bands expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made from milk pasteurized on the day of delivery and after storage raw for 2,4 and 7 days at 2°C. The results represent three trials. Cheese production took place immediately after milk pasteurization.

| Milk storage d | Cheese curing | κ | Ts | γ-casein | | | β-casein | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| 0 | 1 Week | 0.84 | 0.66 | - | 0.09 | - | 0.23 | 35.87 | - | 0 |
| | 1 Month | 1.75 | 0.41 | - | - | - | 0.29 | 42.26 | - | 2.32 |
| | 2 Months | 1.57 | 1.10 | 0.58 | 1.25 | 0.34 | 0.31 | 30.11 | - | 2.06 |
| | 4 Months | 2.07 | 1.98 | 0.37 | - | - | - | 28.13 | 1.47 | 2.55 |
| | 8 Months | 1.58 | 1.35 | - | 0.13 | - | - | 29.53 | 0.24 | 0.34 |
| | 12 Months | 1.58 | 3.16 | 3.86 | 0.13 | - | - | 29.06 | 1.44 | 0.09 |
| 2 | 1 Week | 0.81 | 0.30 | - | - | - | 0.07 | 31.14 | - | - |
| | 1 Month | 0.50 | 0.54 | 0.98 | - | - | 0.24 | 37.46 | 0.29 | |
| | 2 Months | 3.46 | 4.66 | 0.41 | 0.46 | 0.07 | 0.15 | 29.44 | - | 1.08 |
| | 4 Months | 1.66 | 2.30 | 0.67 | - | - | - | 30.04 | 3.25 | 1.13 |
| | 8 Months | 2.39 | 3.40 | - | - | - | - | 30.05 | 0.09 | 0.09 |
| | 12 Months | 0.52 | 1.46 | 2.78 | - | - | - | 25.25 | 0.08 | 6.08 |
| 4 | 1 Week | 0.51 | 0.61 | - | - | - | - | 28.17 | - | - |
| | 1 Month | 0.58 | 0.39 | 0.82 | - | - | - | 37.91 | - | 1.49 |
| | 2 Months | 3.17 | 0.66 | 2.20 | 0.84 | 0.59 | 0.30 | 38.05 | - | 0.06 |
| | 4 Months | 3.94 | 0.65 | 0.07 | - | - | - | 30.60 | 4.37 | 1.30 |
| | 8 Months | 3.60 | 0.93 | - | - | - | - | 29.32 | 0.10 | - |
| | 12 Months | 4.76 | 1.99 | 1.09 | - | - | - | 25.72 | 0.74 | 1.64 |
| 7 | 1 Week | 0.53 | 0.59 | - | 0.16 | - | 0.26 | 35.09 | - | - |
| | 1 Month | 0.96 | 0.10 | - | - | - | - | 40.39 | - | 1.11 |
| | 2 Months | 1.38 | 1.79 | 1.68 | 1.41 | 1.13 | 0.26 | 29.53 | - | 1.75 |
| | 4 Months | 1.49 | 3.10 | 0.26 | 0.22 | - | - | 26.98 | 3.99 | 1.22 |
| | 8 Months | 5.53 | 2.09 | 0.35 | 1.77 | 0.19 | - | 24.27 | 0.11 | 1.56 |
| | 12 Months | 5.59 | 0.76 | 1.04 | 0.19 | 0.73 | 0.21 | 22.30 | 0.16 | - |

TABLE 6:6

The area of individual casein fractions of the slow-mobility bands expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made from milks pasteurized after storage raw for 2, 4 and 7 days at 6°C. The results represent three trials. Cheese production took place immediately after milk pasteurization

| Milk storage d | Cheese curing | κ | Ts | γ-casein | | | β-casein | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| 2 | 1 Week | 0.46 | 0.22 | - | 0.60 | - | 0.16 | 35.98 | - | - |
| | 1 Month | 0.41 | 0.25 | 0.12 | - | - | - | 38.35 | - | 1.62 |
| | 2 Months | 1.31 | 4.06 | 1.45 | 0.81 | 0.47 | 0.27 | 26.25 | - | 3.74 |
| | 4 Months | 1.10 | 2.68 | 0.20 | 0.35 | - | - | 25.04 | 2.28 | 3.35 |
| | 8 Months | 2.17 | 4.25 | 1.03 | 0.43 | - | - | 31.23 | 1.98 | 0.09 |
| | 12 Months | 2.81 | 1.28 | 1.72 | 0.26 | 0.11 | 0.26 | 24.63 | 0.22 | 0.18 |
| 4 | 1 Week | 0.44 | 0.38 | - | - | - | 0.24 | 26.39 | - | - |
| | 1 Month | 0.23 | 0.21 | 0.19 | - | - | 0.07 | 32.28 | - | 2.70 |
| | 2 Months | 1.93 | 1.29 | 0.21 | 4.11 | 0.36 | 0.25 | 27.80 | - | 1.15 |
| | 4 Months | 0.95 | 2.67 | 0.11 | 0.20 | - | - | 35.65 | 0.09 | - |
| | 8 Months | 2.29 | 1.17 | 1.53 | 0.60 | - | - | 33,43 | 0.43 | - |
| | 12 Months | 4.24 | 0.55 | 0.43 | 0.42 | 0.52 | 0.60 | 21.56 | 1.20 | 3.47 |
| 7 | 1 Week | 0.21 | 0.56 | - | - | - | 0.50 | 33.81 | - | - |
| | 1 Month | 0.91 | 1.00 | 0.19 | 0.22 | 0.23 | 0.13 | 32.67 | - | 0.92 |
| | 2 Months | 1.85 | 1.76 | 1.09 | 0.73 | 0.93 | 0.59 | 29.86 | - | 0.82 |
| | 4 Months | 1.06 | 1.42 | 0.61 | 0.16 | 0.16 | 1.07 | 27.13 | 0.28 | 2.90 |
| | 8 Months | 1.71 | 2.80 | 2.05 | 0.34 | 0.75 | - | 24.60 | 0.27 | 0.12 |
| | 12 Months | 3.66 | 0.87 | 0.10 | 0.23 | 0.15 | - | 25.59 | 0.62 | 4.30 |

Fig. 6:11 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized on the day of delivery. Cheese production took place immediately after pasteurization.

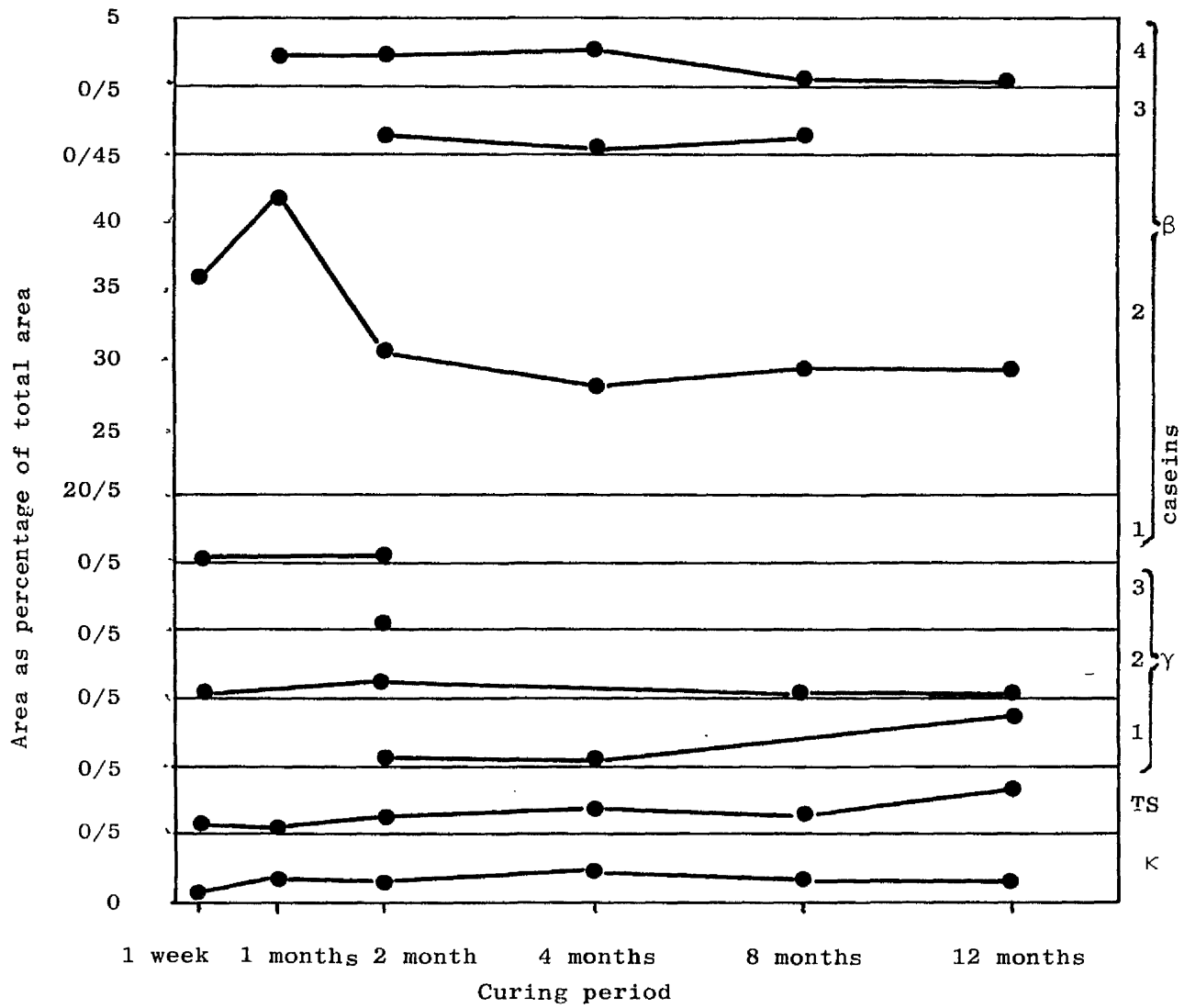


Fig. 6:12 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 2 days at 2°C. Cheese production took place immediately after pasteurization.

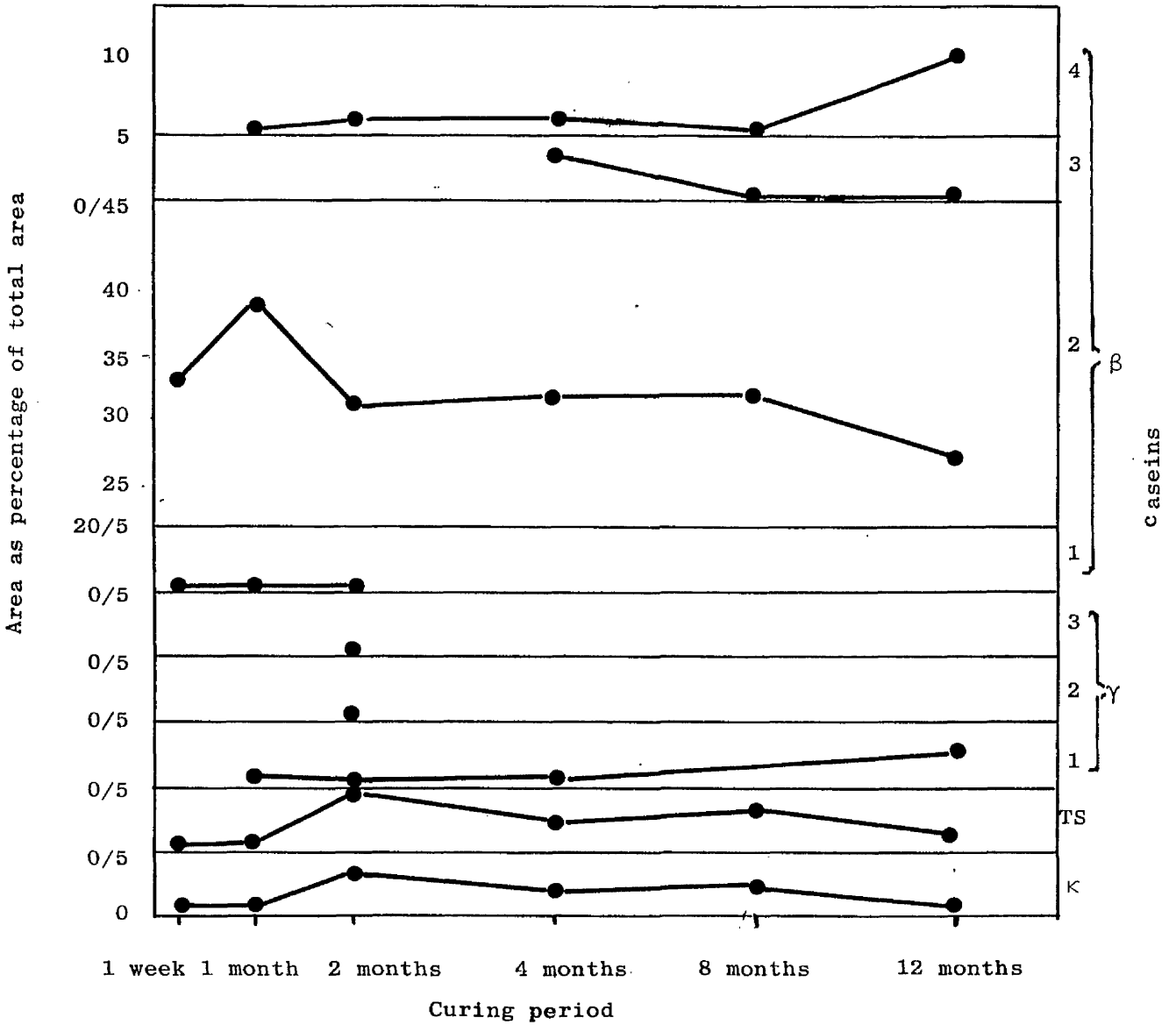


Fig. 6:13 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 4 days at 2°C. Cheese production took place immediately after pasteurization.

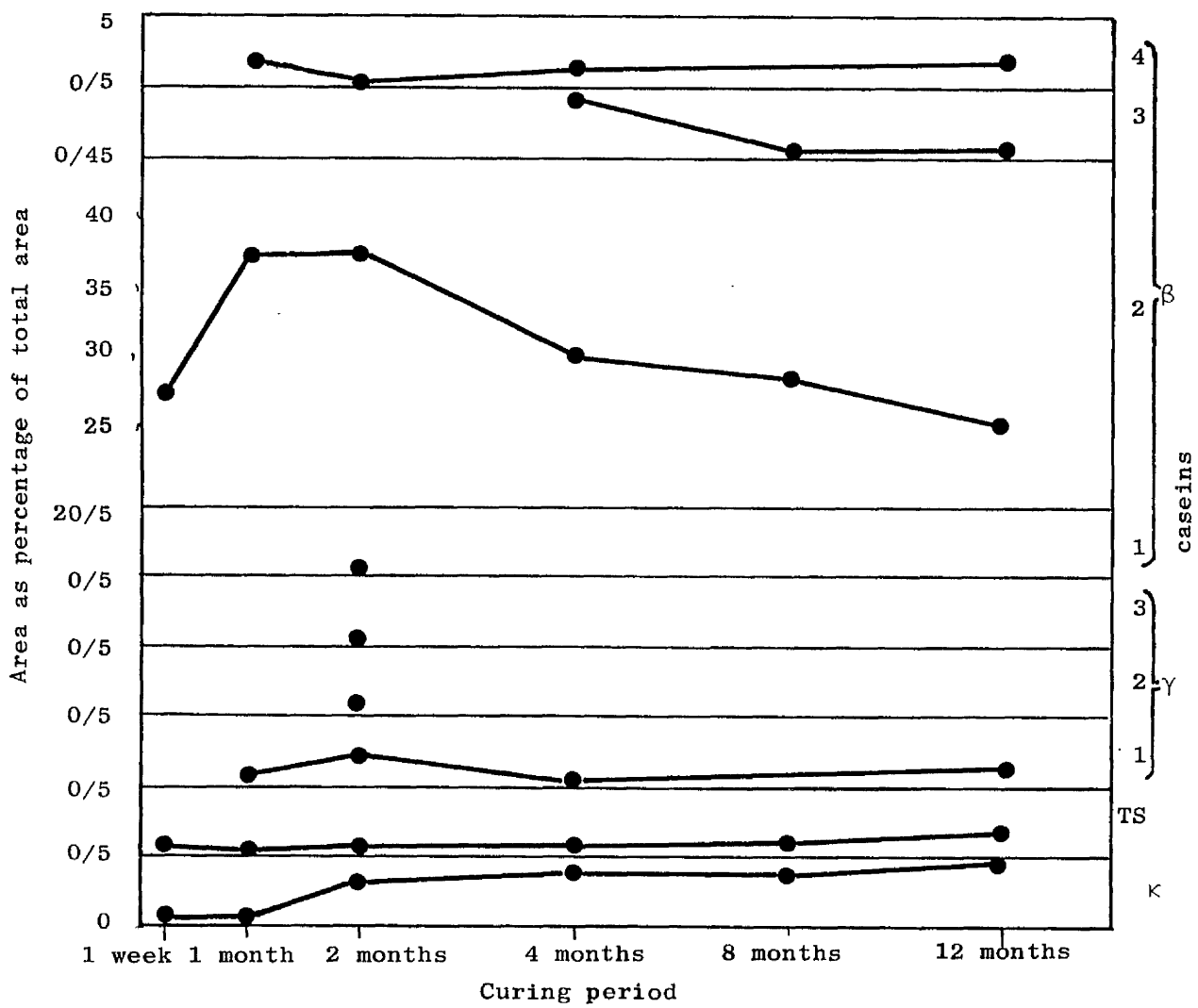


Fig. 6:13 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 4 days at 2°C. Cheese production took place immediately after pasteurization.

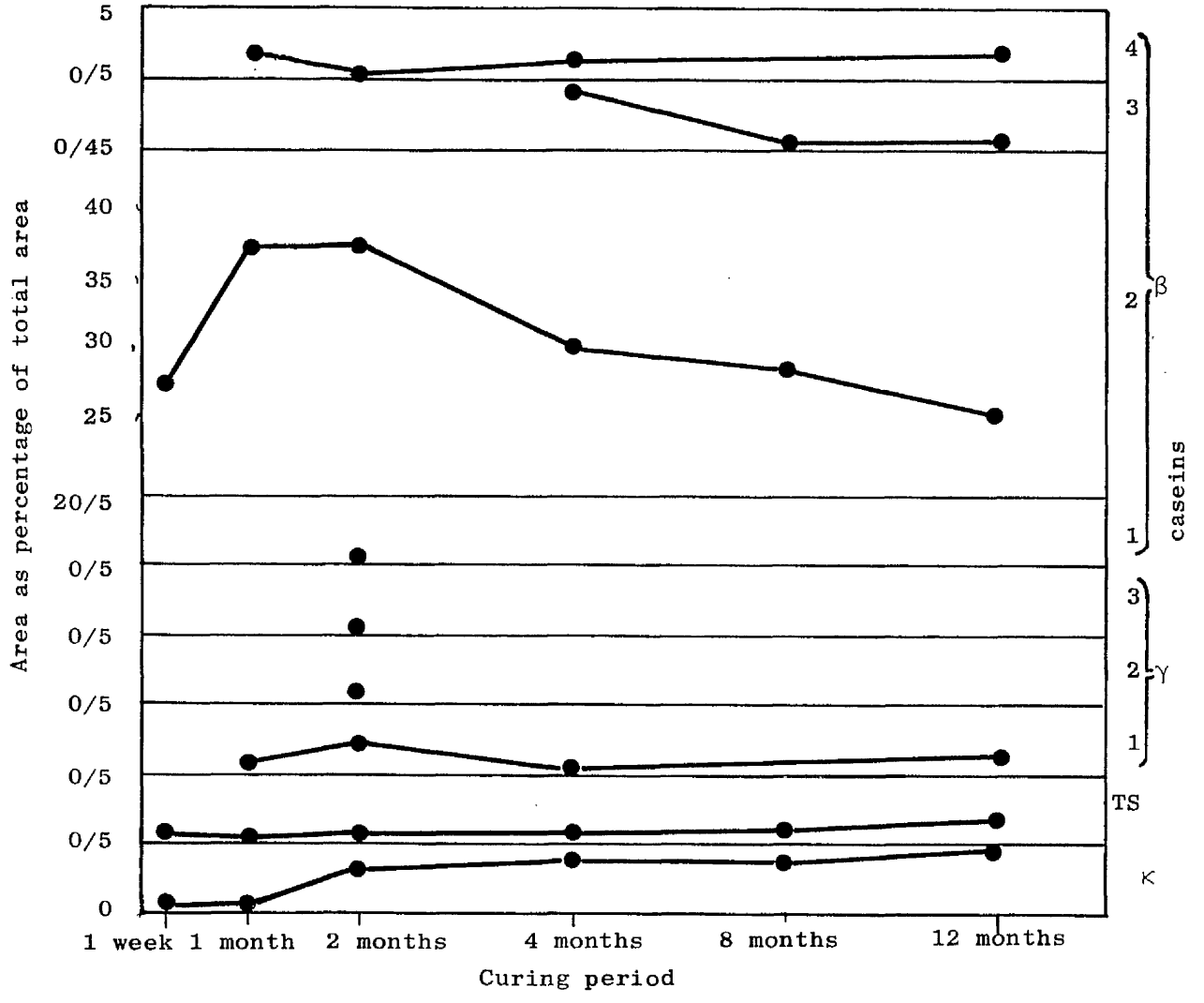


Fig. 6:14 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 7 days at 2°C. Cheese production took place immediately after pasteurization.

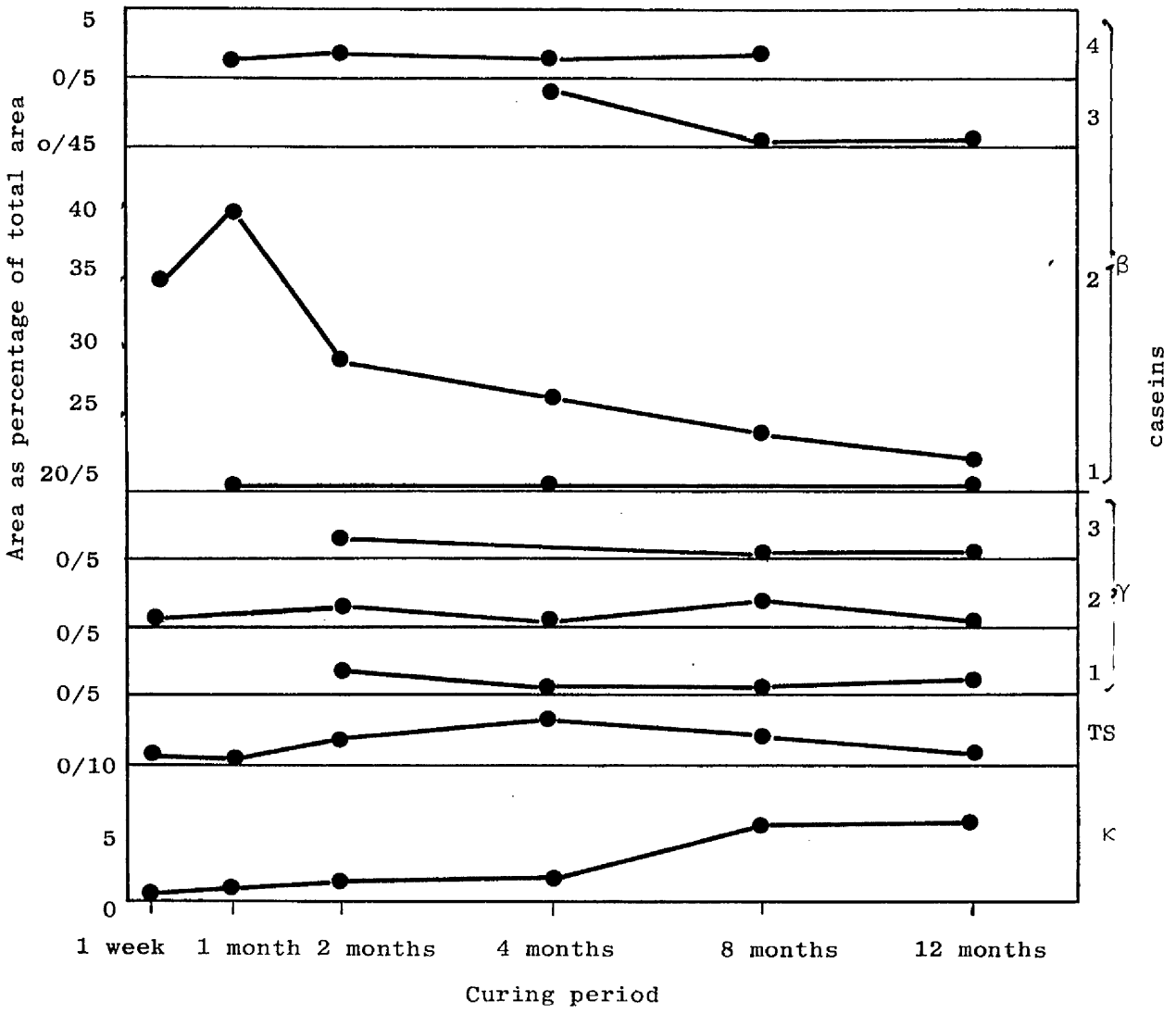


Fig. 6:15 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 2 days at 6°C. Cheese production took place immediately after pasteurization.

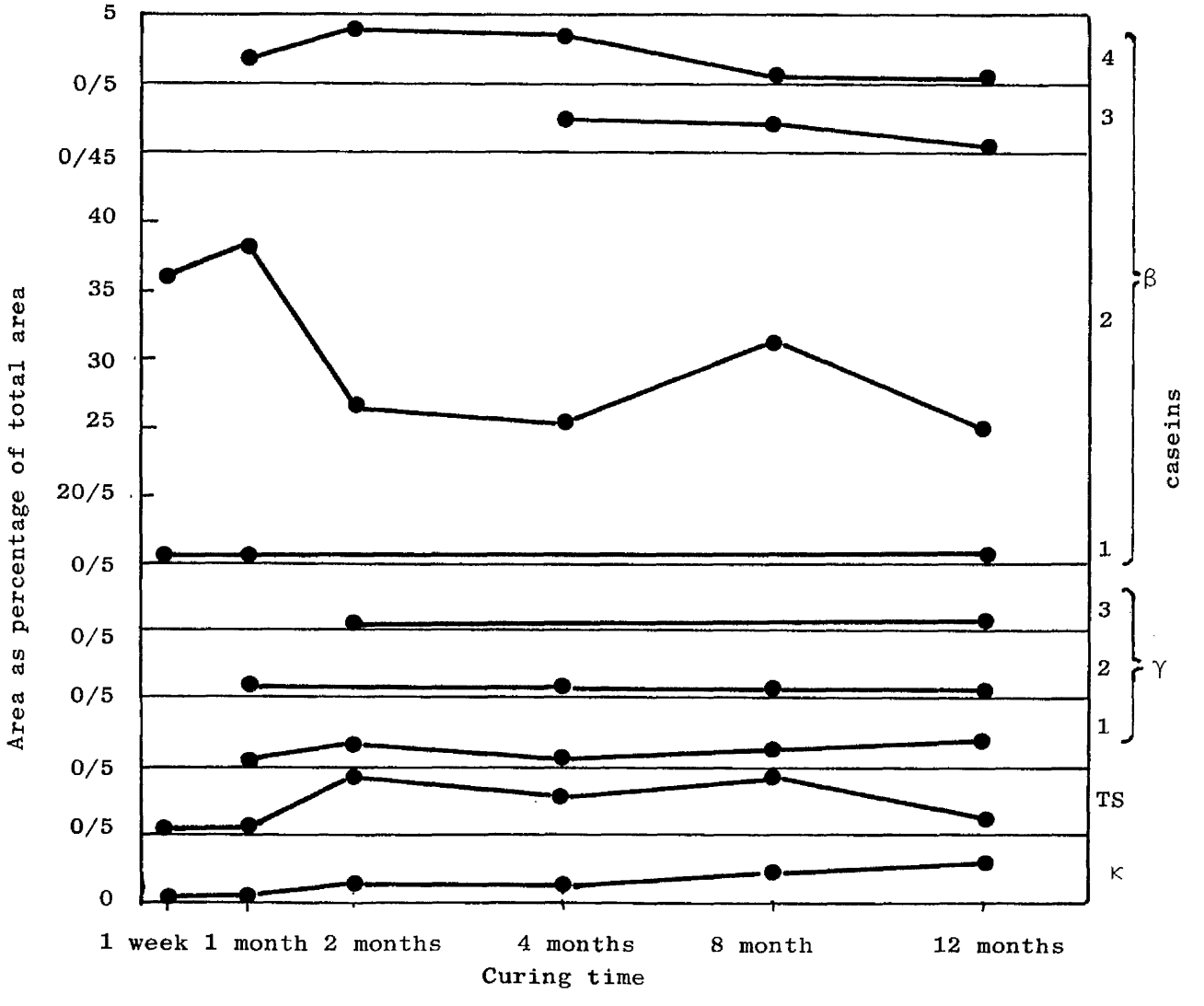


Fig. 6:16 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 4 days at 6°C. Cheese production took place immediately after pasteurization.

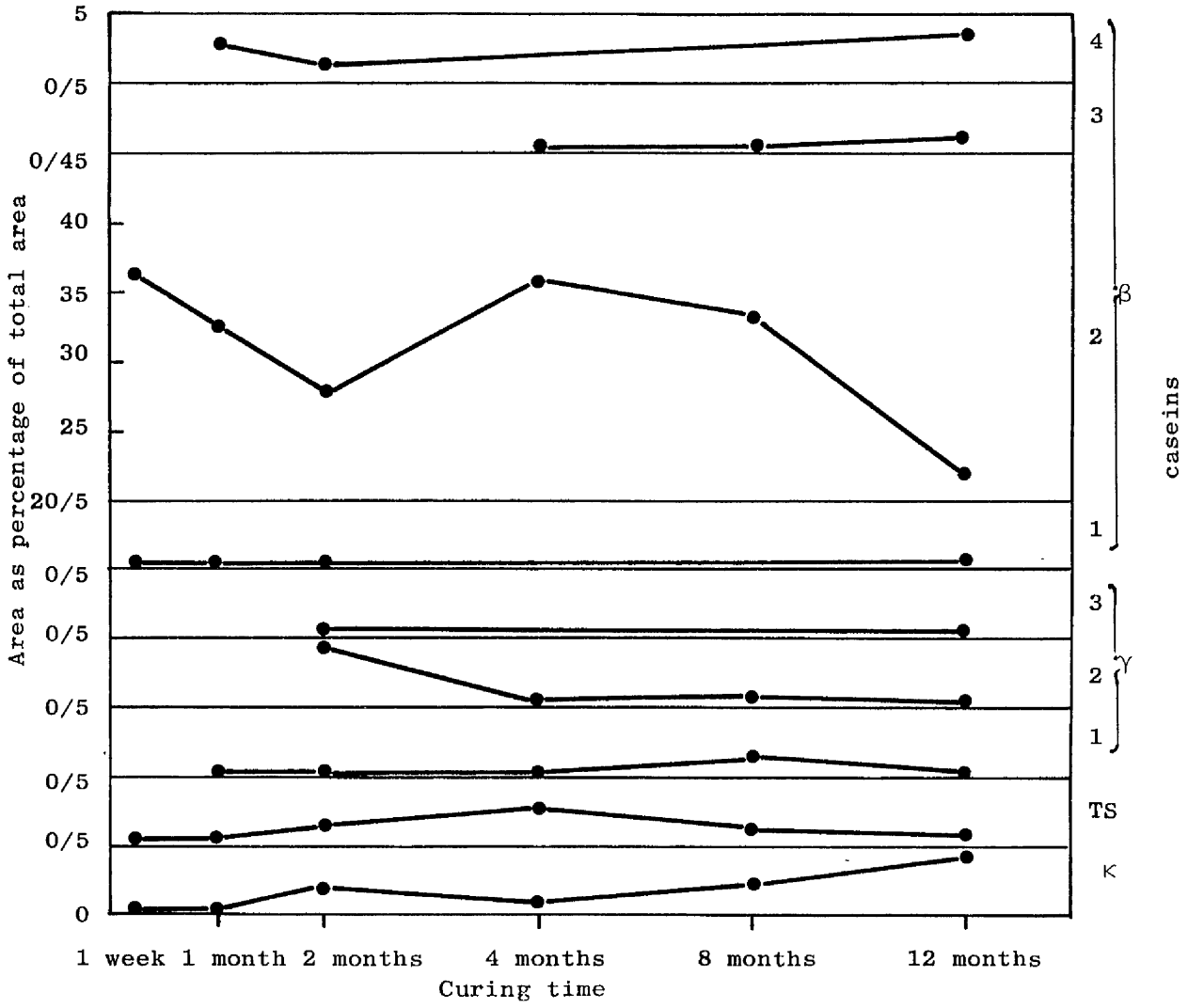
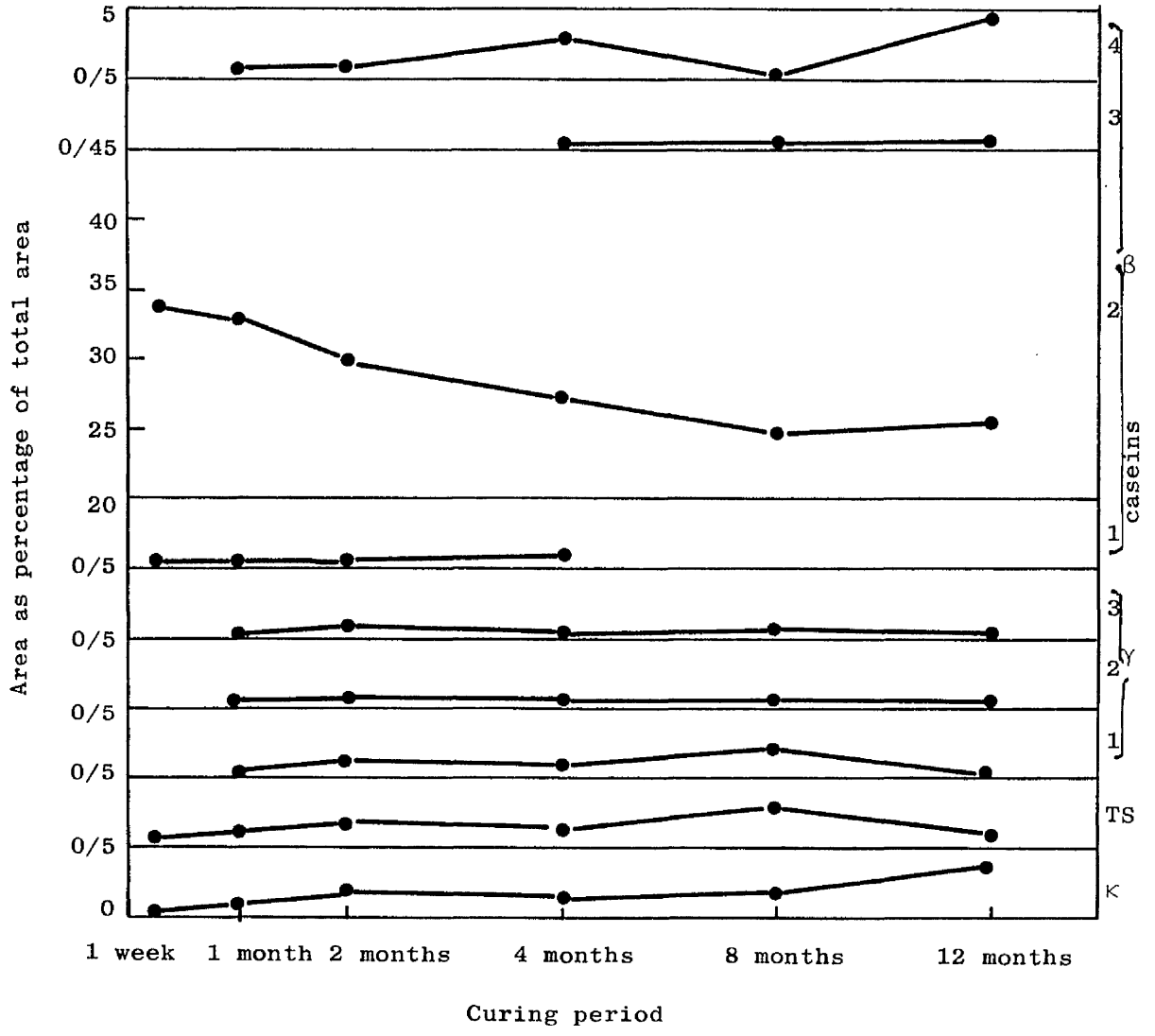


Fig. 6:17 Variations in the area of casein fractions in the slow-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 7 days at 6°C. Cheese production took place immediately after pasteurization.



This fraction was present in cheeses at different curing times but in less concentration than the κ-fraction. During curing this fraction showed some fluctuations in its concentration in the cheese.

γ-casein

This minor fraction consists of 3 bands positioned between Ts and β-caseins. γ-casein 1 was absent in all cheeses when 1 week old. It appeared after 1 month of curing in all the cheese except those made from milk on the day of delivery and from milk stored for 7 d at 2°C prior to pasteurization where it appeared after 2 months of curing. γ-casein 1 increased during ripening although it showed some fluctuation at some curing stages. Of the fractions of γ-casein that identified as 1 had the highest concentration on the scan diagrams. γ-casein 2 was present in cheeses made from milk stored at 6°C more frequently during curing than in cheeses made from milks held at 2°C.

It should be stressed that the small amounts of these fractions were sometimes outside the lower limit of determination set for the scanner.

β-casein

When cheese was 1 week old, β-casein appeared in one large band. In some cheeses another minor band appeared slower than the major band of β-casein which was identified as β-casein fraction 1. After curing of cheese β-casein 2 (the major β-casein fraction) appeared in a lower concentration while β-casein 1 showed slight increases in the early stages of curing, thereafter its concentration was decreased. In cheeses made from milk on the day of delivery, milks stored at 2°C for 2 and 7 d and milks stored for 2 d at 6°C, β-casein 2 increased from 1-week old to 1 month-old cheese and then started to decrease. In the case of cheese made from milk held at 2°C for 4 d this decrease started after two months. On the other hand, β-casein 2 in cheeses made from milks held for 4 d at 6°C decreased after 1 week of curing, but had increased when tests were made at 4 months. Thereafter the fraction decreased with

further curing. In cheeses made from milks held for 7 d at 6°C, β-casein 2 decreased after 1 week of curing.

Another two bands were also observed which moved slightly faster than β-casein 2. β-casein 3 did not appear in all cheeses before 4 months of curing. β-casein 4 appeared after 1 week of curing. These two β-casein fractions of 3 and 4 did not show a consistent pattern during the curing of the cheese.

Group 2 : The fast-moving fractions

This group contains the fast mobility bands. In the earlier stages of cheese curing, two major bands ($\alpha_{s_2}$- and $\alpha_{s_1}$-caseins) were observed in this area besides other minor bands. Further curing of Cheddar cheese resulted in breakdown in these proteins and the minor peptides concentration increased. This group contains the following fractions:-

1. $\alpha_s$ fraction 1 which moved slower than the major $\alpha_s$-casein. This band increased in concentration with the increase in curing period, in cheese made from milk on the day of delivery and milk held at 2°C prior to pasteurization. The highest increase was observed between 1 and 2 months of curing. In cheeses made from milks held at 6°C for 4 and 7 d there was the same pattern of increases up to 8 months but after 12 months of curing the concentration of this fraction had decreased.

2. Fraction 2 (or $\alpha_{s_1}$-casein)

This fraction covered the largest area of all the other bands in all gels in 1 week-old cheeses. The concentration of this fraction decreased during the ripening of the cheese. This reduction was higher in cheeses made from milks held at 2°C than at 6°C.

3. Fraction 3

This fraction was the second largest on the scan diagram after the $\alpha_{s_1}$-casein. The concentration of this fraction also decreased during curing of cheese.

4. Fractions 4, 5 and 6

Fraction 4 increased dramatically in concentration after 4 months

TABLE 6:7

The area of individual casein fractions of the fast-mobility bands expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made from milk pasteurized on the day of delivery and after storage raw for 2, 4 and 7 days at 2°C. The results represent the means of 3 trials. Chesse production took place immediately after pasteurization.

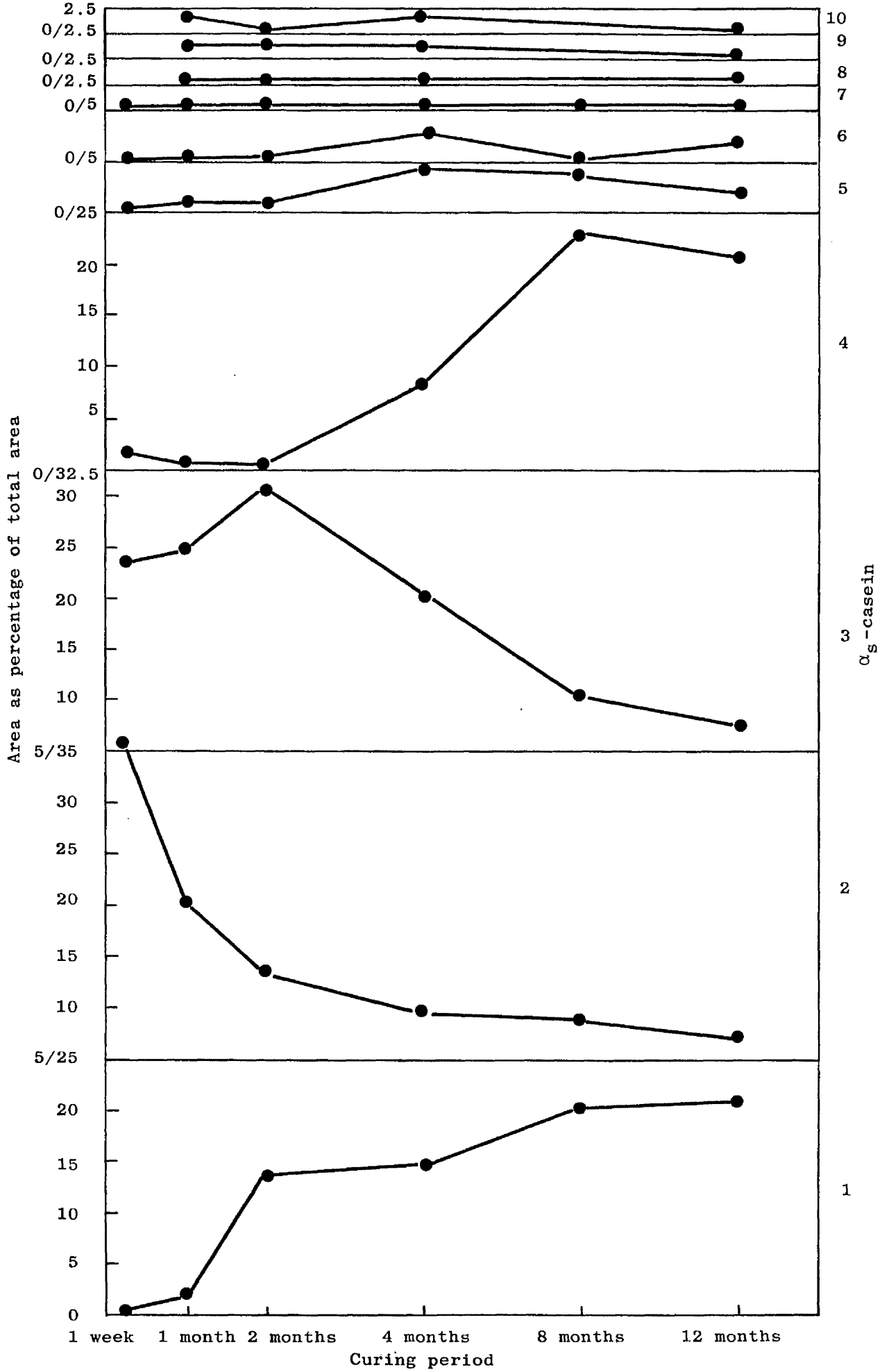
| Milk storage d | Cheese curing | $\alpha_s$-casein | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 0 | 1 Week | 0.37 | 35.94 | 23.55 | 1.50 | 0.34 | 0.37 | 0.22 | - | - | - |
| | 1 Month | 1.93 | 20.10 | 24.94 | 0.62 | 0.92 | 0.43 | 0.58 | 0.25 | 1.36 | 1.85 |
| | 2 Months | 13.82 | 13.39 | 30.94 | 0.54 | 0.68 | 0.52 | 0.51 | 0.55 | 1.38 | 0.32 |
| | 4 Months | 14.53 | 9.41 | 20.11 | 8.46 | 4.16 | 2.90 | 0.29 | 0.44 | 1.16 | 1.64 |
| | 8 Months | 20.34 | 8.73 | 10.35 | 23.15 | 3.55 | 0.46 | 0.23 | - | - | - |
| | 12 Months | 20.94 | 6.71 | 7.55 | 20.68 | 1.73 | 1.73 | 0.46 | 0.59 | 0.10 | 0.18 |
| 2 | 1 Week | 0.69 | 41.08 | 19.37 | 1.75 | 1.31 | 0.61 | 0.91 | 0.37 | 0.89 | - |
| | 1 Month | 4.26 | 25.18 | 26.10 | 1.79 | 0.56 | 1.72 | 0.11 | 0.20 | 0.08 | - |
| | 2 Months | 9.33 | 12.27 | 32.28 | 2.65 | 0.60 | 0.89 | 0.69 | 1.25 | 0.10 | - |
| | 4 Months | 11.37 | 10.89 | 16.53 | 13.39 | 3.07 | 3.09 | 0.79 | 0.45 | 0.22 | 0.88 |
| | 8 Months | 22.72 | 5.76 | 9.68 | 23.66 | 0.78 | 0.41 | 0.86 | 0.12 | - | - |
| | 12 Months | 20.65 | 2.31 | 6.72 | 27.83 | 3.84 | 1.01 | 0.80 | 0.35 | 0.32 | - |
| 4 | 1 Week | 1.76 | 38.57 | 27.95 | 1.00 | 0.32 | 0.54 | 0.35 | 0.23 | 0.25 | - |
| | 1 Month | 1.56 | 25.94 | 27.79 | 1.28 | 1.93 | 0.31 | - | - | - | - |
| | 2 Months | 7.62 | 11.61 | 27.74 | 2.59 | 0.69 | 0.78 | 1.73 | 0.95 | 0.47 | 0.10 |
| | 4 Months | 9.70 | 10.85 | 20.03 | 11.52 | 1.96 | 2.30 | 1.02 | 1.35 | 0.46 | - |
| | 8 Months | 21.15 | 2.61 | 14.88 | 23.99 | 0.94 | 1.38 | 1.10 | - | - | - |
| | 12 Months | 21.70 | 5.63 | 9.11 | 24.22 | 1.86 | 0.14 | 0.72 | 0.23 | 0.25 | 0.21 |
| 7 | 1 Week | 0.19 | 41.92 | 19.56 | 0.74 | 0.09 | 0.18 | 0.52 | 0.19 | 0.18 | - |
| | 1 Month | 5.37 | 22.79 | 28.84 | - | - | 0.43 | - | - | - | - |
| | 2 Months | 10.75 | 14.74 | 30.44 | 1.75 | 1.14 | 0.50 | 0.18 | 0.55 | 0.51 | 0.19 |
| | 4 Months | 12.28 | 8.81 | 20.82 | 12.27 | 3.22 | 2.05 | 1.24 | 0.25 | 0.46 | 1.33 |
| | 8 Months | 22.06 | 5.12 | 11.55 | 23.34 | 0.83 | 0.31 | 0.45 | 0.35 | 0.25 | 1.33 |
| | 12 Months | 25.61 | 0.35 | 7.26 | 27.75 | 6.00 | 0.44 | 0.66 | 0.41 | 0.10 | 0.10 |

TABLE 6:8

The area of individual casein fractions of the fast-mobility bands expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made from milk pasteurized after storage raw for 2, 4 and 7 days at 6°C (means of three trials). Cheese production took place immediately after pasteurization

| Milk storage d | Cheese curing | $\alpha_s$-casein | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 2 | 1 Week | 0.18 | 40.18 | 19.33 | 0.39 | 0.44 | 0.33 | 0.84 | 0.16 | 0.32 | 0.20 |
| | 1 Month | 1.38 | 23.26 | 26.72 | 0.44 | 2.74 | 1.60 | 2.09 | 0.23 | 0.47 | 0.35 |
| | 2 Months | 10.57 | 12.32 | 25.60 | 4.23 | 3.27 | 1.21 | 1.18 | 2.14 | 0.46 | 0.34 |
| | 4 Months | 13.09 | 8.67 | 25.37 | 6.52 | 3.55 | 3.95 | 0.59 | 0.60 | 0.61 | 1.94 |
| | 8 Months | 20.65 | 8.18 | 8.87 | 18.24 | 0.61 | 0.75 | 0.78 | 0.20 | 0.09 | - |
| | 12 Months | 21.11 | 8.62 | 11.37 | 23.21 | 0.56 | 0.88 | 1.53 | 0.42 | 0.21 | 0.62 |
| 4 | 1 Week | 0.33 | 34.95 | 22.92 | 1.34 | 0.83 | 0.89 | 0.24 | 0.33 | 0.30 | 0.64 |
| | 1 Month | 5.86 | 25.23 | 30.24 | 1.42 | 0.41 | 0.66 | 0.37 | 0.10 | - | - |
| | 2 Months | 13.22 | 11.46 | 34.12 | 1.39 | 0.26 | 0.38 | 0.60 | 0.51 | 0.17 | 0.46 |
| | 4 Months | 8.07 | 8.64 | 20.92 | 5.15 | 3.94 | 4.43 | 4.22 | 2.54 | 0.67 | 1.71 |
| | 8 Months | 22.30 | 6.82 | 9.01 | 19.17 | 1.82 | 0.36 | 0.59 | 0.27 | 0.09 | 0.12 |
| | 12 Months | 16.71 | 7.00 | 9.80 | 22.14 | 4.06 | 2.02 | 2.12 | 1.19 | 0.81 | 1.16 |
| 7 | 1 Week | 1.35 | 35.22 | 25.42 | 0.86 | 0.19 | 0.54 | 0.59 | 0.33 | 0.26 | 0.16 |
| | 1 Month | 11.59 | 11.94 | 32.34 | 2.45 | 1.58 | 3.00 | 0.28 | 0.19 | 0.31 | 0.24 |
| | 2 Months | 6.39 | 11.31 | 31.22 | 3.45 | 4.13 | 9.78 | 9.31 | 1.14 | 2.81 | 0.31 |
| | 4 Months | 13.61 | 8.36 | 13.00 | 15.83 | 4.93 | 2.56 | 0.63 | 3.34 | 1.78 | 1.19 |
| | 8 Months | 20.05 | 6.61 | 5.23 | 23.35 | 6.38 | 2.65 | 1.15 | 2.20 | 0.21 | - |
| | 12 Months | 15.25 | 6.21 | 8.73 | 26.86 | 2.69 | 4.46 | 0.24 | 0.14 | - | - |

Fig. 6.10 Variations in the area of casein fractions in the fast-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized on the day of delivery. Cheese production took place immediately after pasteurization.



made from milk pasteurized after storage for 2 days at 2°C.
Cheese production took place immediately after pasteurization.

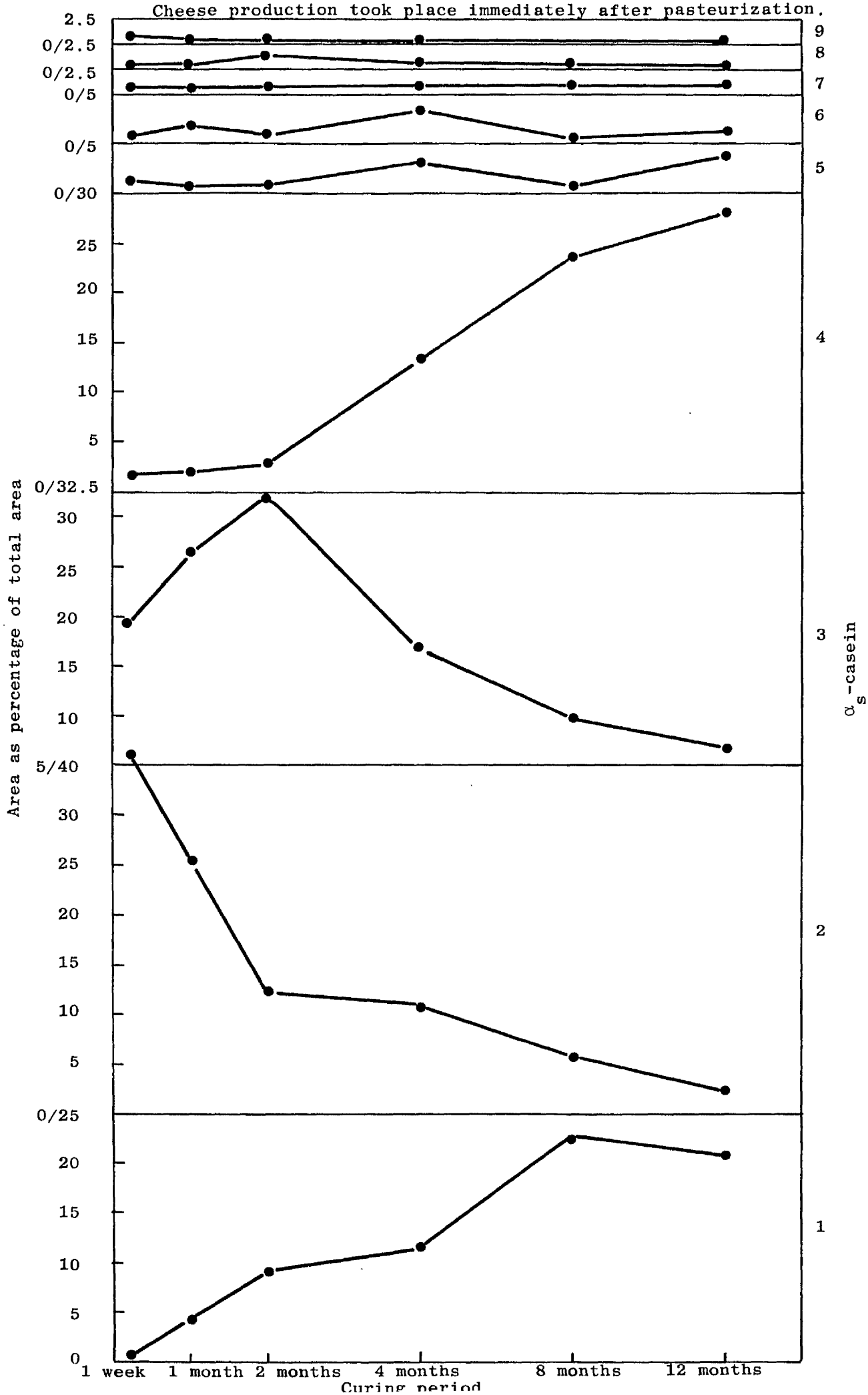


Fig. 6:20 Variations in the area of casein fractions in the fast-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 4 days at 2°C. Cheese production took place immediately after pasteurization.

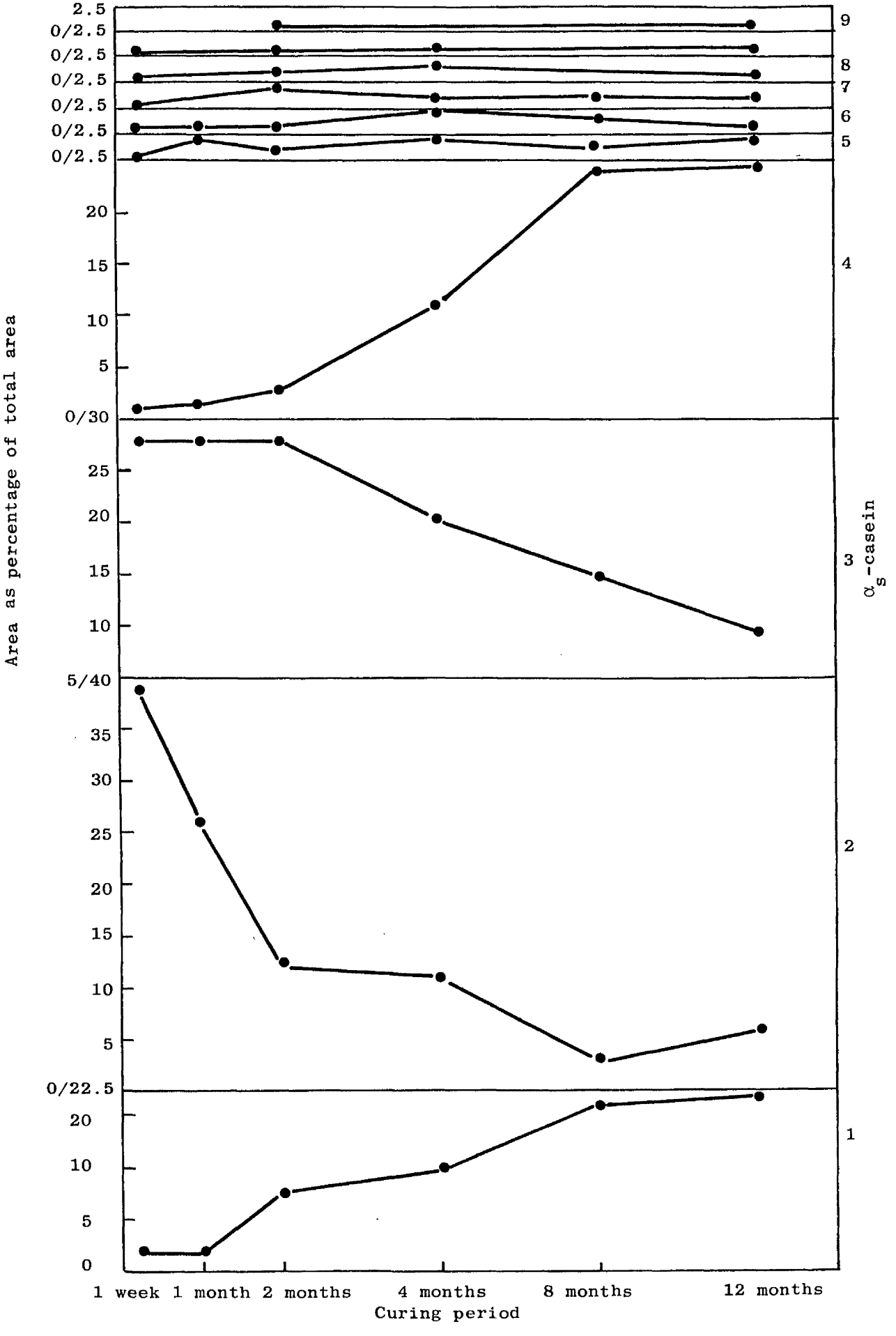


Fig. 6:21 Variations in the area of casein fractions in the fast-mobility bands (calculated as percentage of total area) in Cheddar cheese made from milk pasteurized after storage for 7 days at 2°C. Cheese production took place immediately after pasteurization.

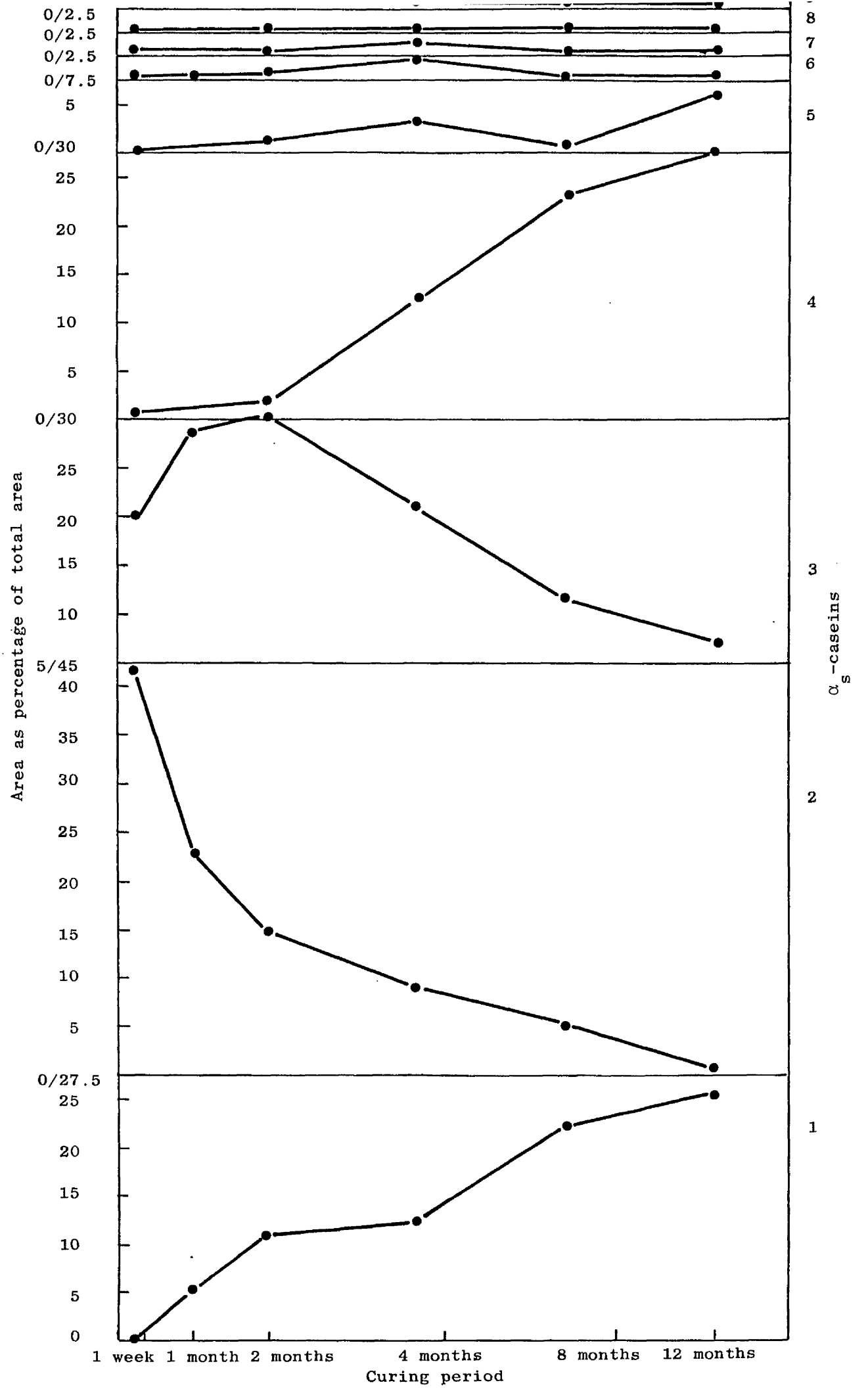
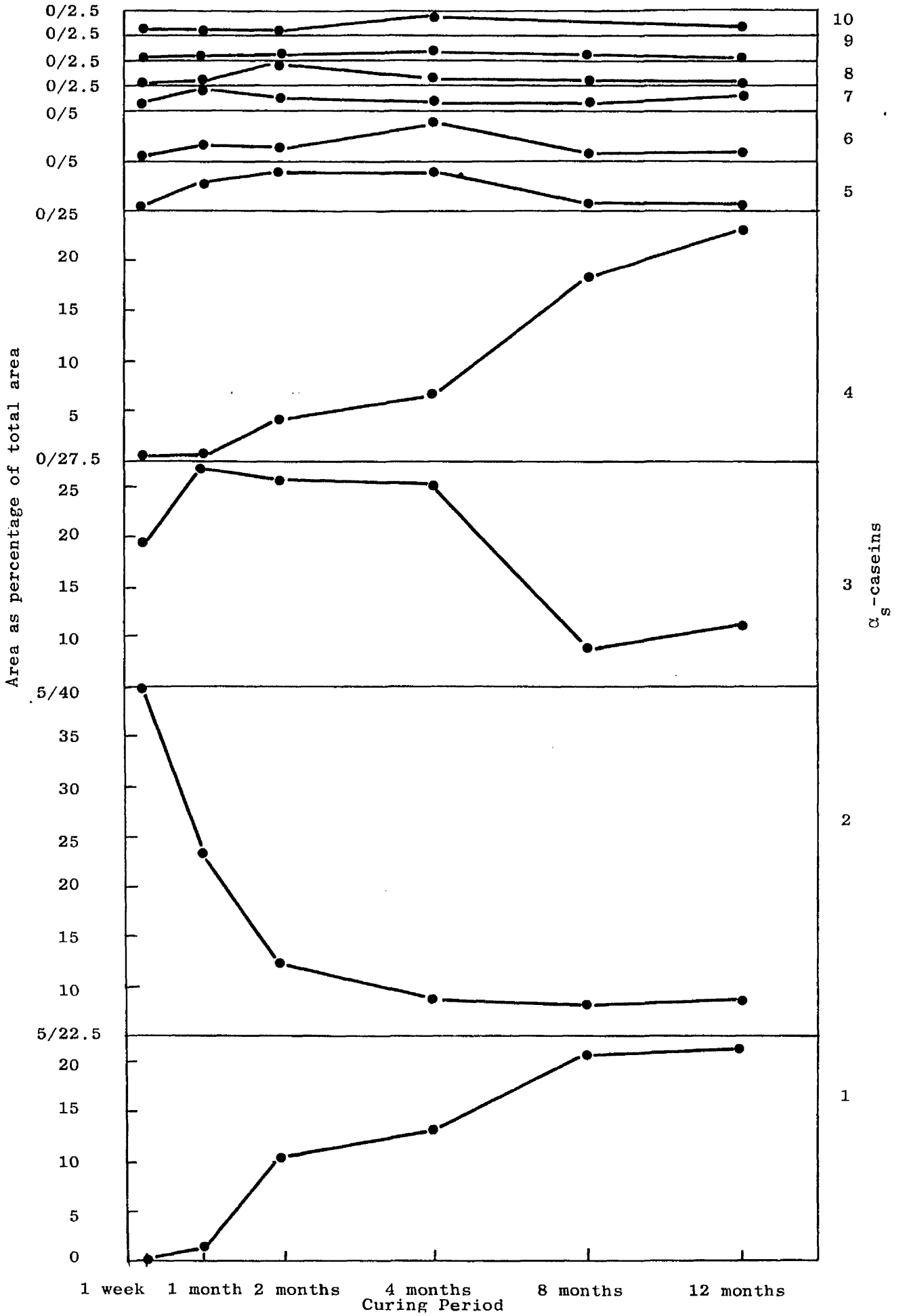

0/2.5
0/2.5
0/2.5
0/7.5
5
0/30
25
20
15
10
5
0/30
25
20
15
10
5/45
40
35
30
25
20
15
10
5
0/27.5
25
20
15
10
5
0
8
7
6
5
4
3
2
1
Area as percentage of total area
$\alpha_s$-caseins
1 week
1 month
2 months
4 months
8 months
12 months
Curing period

Fig. 6:22 Variations in the area of casein fractions in the fast-mobility band (calculated as percentage of total area) in Cheddar cheese made from milks pasteurized after storage for 2 days at 6°C. Cheese production took place immediately after pasteurization



bands (calculated as percentage of total area) in Cheddar cheese made from milks pasteurized after storage for 4 days at 6°C. Cheese production took place immediately after pasteurization.

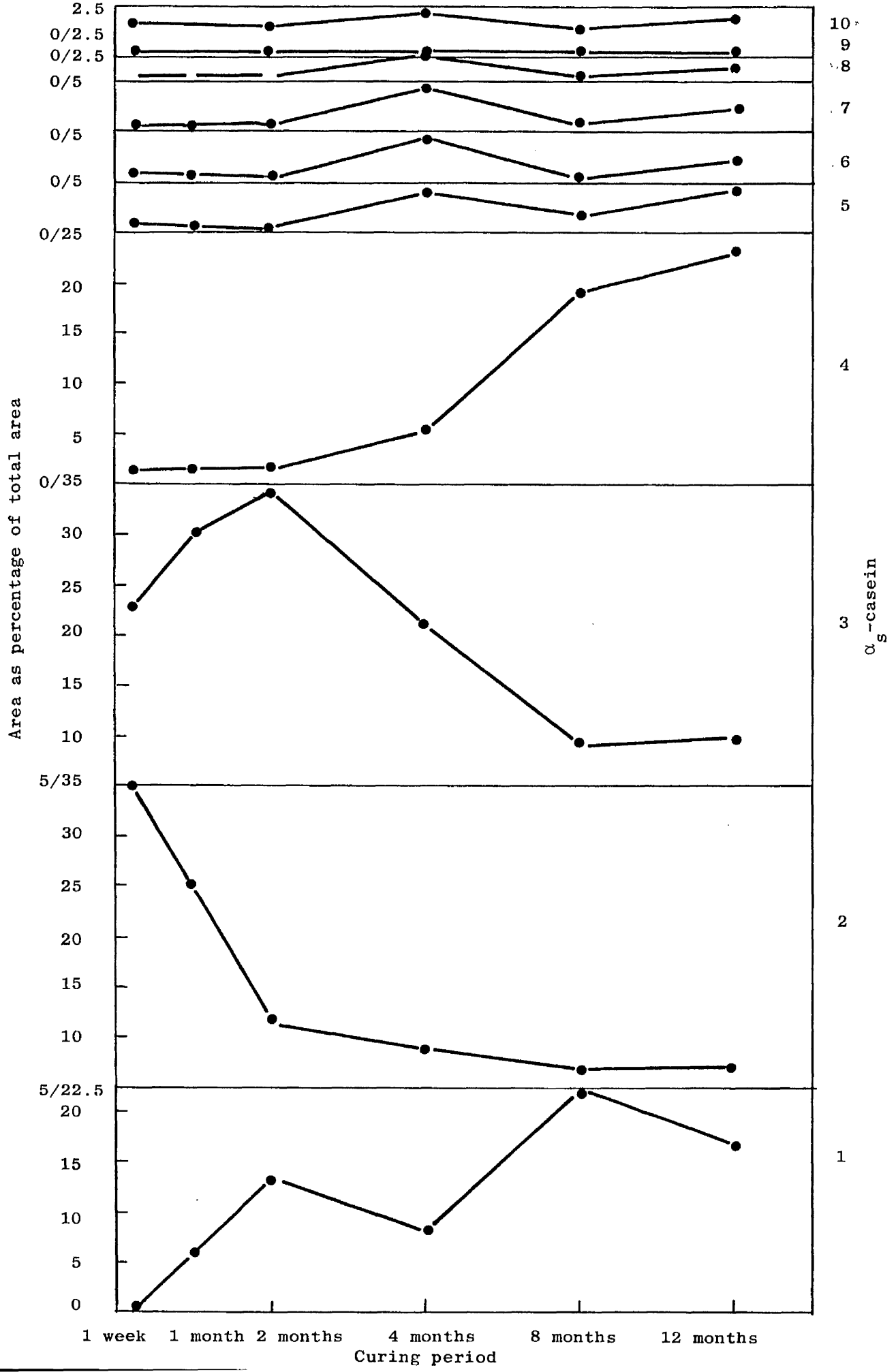
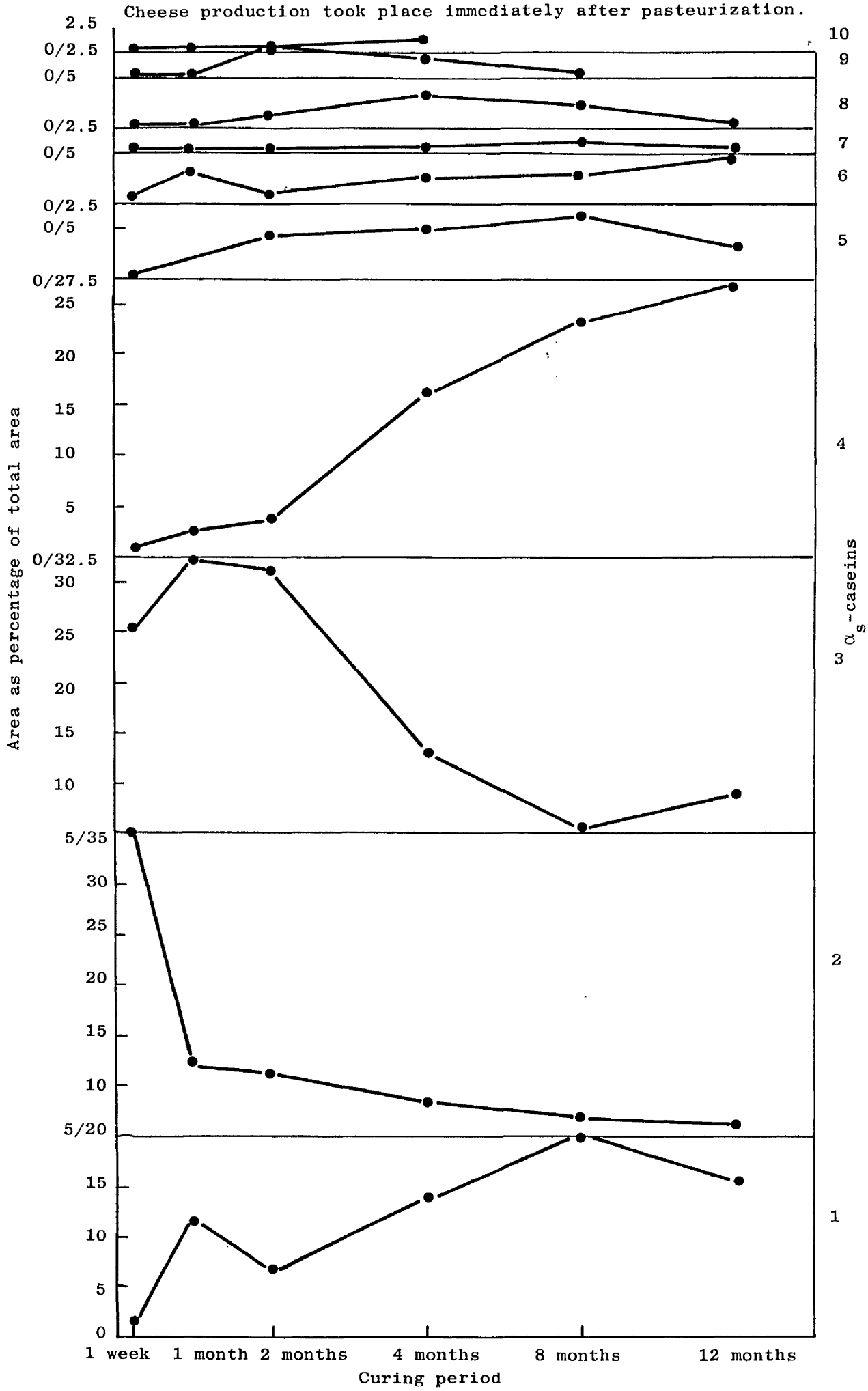


Fig. 3.21 Variation in the area of casein fractions in the fast mobility bands (calculated as percentage of total area) in Cheddar cheese made from milks pasteurized after storage for 7 days at 6°C. Cheese production took place immediately after pasteurization.



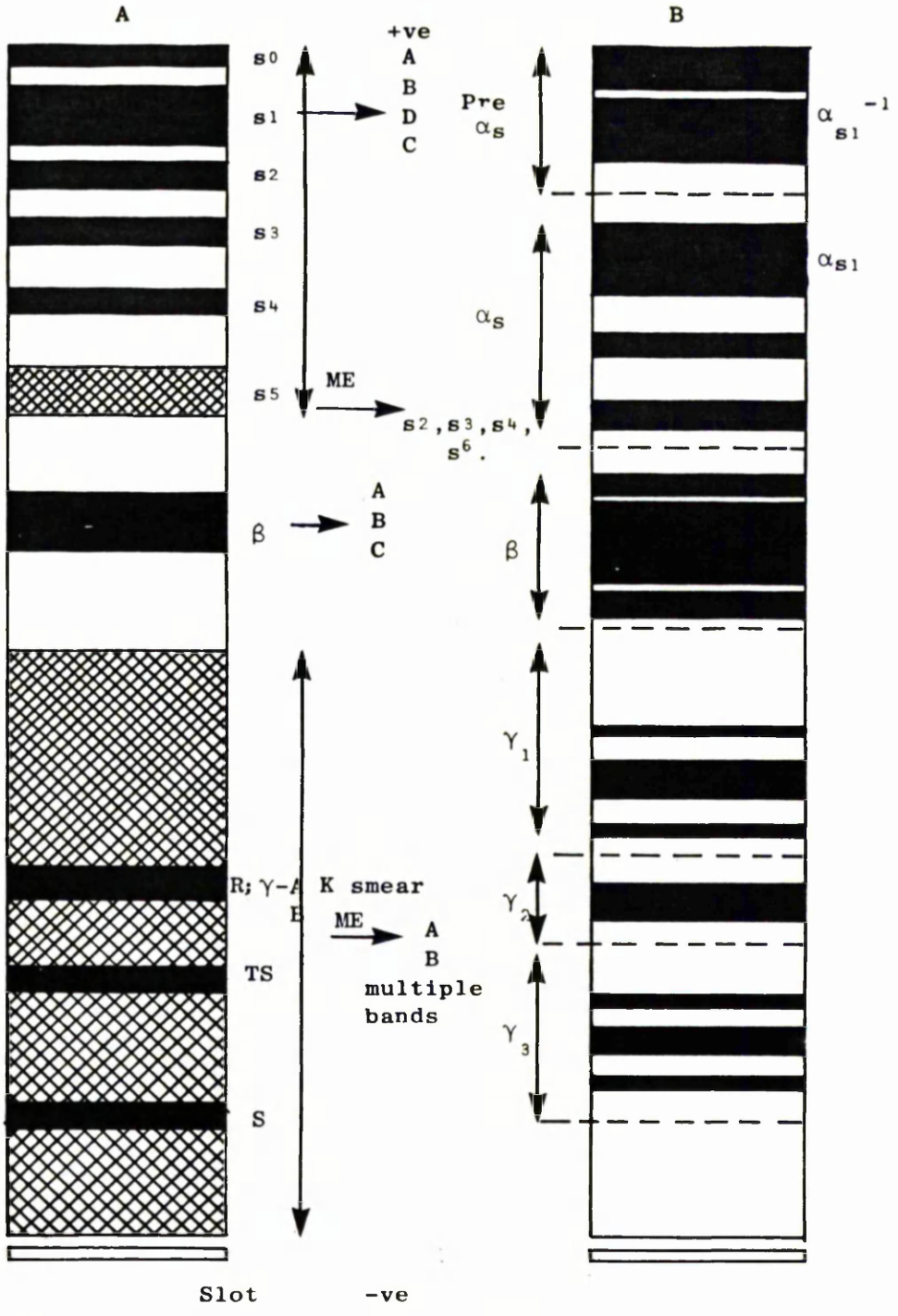



Fig. 6:25 Schematic electrophoretic patterns showing the relative positions of zones from whole bovine casein (A) and the typical components found in cheese made from cows milk clotted by calf rennet (B). From Al-Obaidi, 1980, originated from (A) McKenzie (1970) (B) Marcos et al. (1979).

of curing. There was no consistent pattern for fractions 5 and 6 during ripening of cheese, but their concentrations were highest in 4 month old cheeses made from milks on the day of delivery and milks held at $2^{o}C$ for up to 7 d prior to heat treatment and immediate cheese production. In the case of cheese made from milks held for 7 d at $6^{o}C$ prior to pasteurization these fractions continued to increase with increasing curing period.

5. Fractions 7, 8, 9 and 10

These fractions were the fastest bands in electrophoretic runs. While they were low in concentration in all cheeses, they were present in highest concentration in cheeses made from milks held at $6^{o}C$. Some of these bands were sometimes absent during curing of the cheese.

## DISCUSSION

The slab gel electrophoretic study was carried out using standardised procedures to minimize the possibility of variations from one run to another. Variations were also expected in the staining capability of different bands. de Jong (1975) reported that the quantity of amido black bound per gram of substance may differ between breakdown products and the original protein. Calculations based on those bands of breakdown products particularly when comparisons are based on the original proteins may be erroneous. El-Shibiny and Abd El-Salam (1976) found that β-casein showed higher optical densities than similar concentrations of $\alpha_s$-casein so indicating differences in the amido black capacities of β- and $\alpha_s$- casein. In the present study Coomassie brilliant blue which is Ca 10 times as sensitive as amido black (Bio Rad, 1982) was used. Despite the use of this, dye binding capability may vary.

The scanning was carried out using a scanner based on a laser beam which because it is very narrow measures the density in a very limited area of the band. The areas of the peaks were calculated by the integrator after setting a limit for the area to be calculated. Under these circumstances some of the small bands were not detected.

### Section 6:1

The purified fractions of casein contained some impurities. The

presence of these impurities depends on the method and accuracy of purification. However, proteolysis of some of milk proteins during storage and preparation may have resulted in an extra source of impurities. $\alpha_s$-casein appeared to have several fractions. These fractions are different from each other in the number of phosphorus atoms present in their structure (Andrews, 1982). Two bands appeared with $\alpha_{s_2}$- and $\alpha_{s_1}$-casein fractions in the position of β-casein. These might have resulted from the breakdown of β-casein.

κ-casein appeared to have the maximum purity. κ-casein appeared in one major fraction. Another band was observed in the whole acid precipitated casein which may be a variant of κ-casein. Davies and Law (1977) found κ-casein in A and B variants. They believed that this was probably due to the methods of preparation used in their experiment. The apparent variation in the major caseins of the milk of Ayrshire cows appears to be confined to κ-casein.

A small peak could be detected between κ- and β-casein. This may be Ts-casein. McKenzie (1970) reported that all milk samples examined had a band, designated Ts casein.

β-casein appeared in a major band in acid-precipitated casein. Before the top of the β-casein peak, a hump was present. This might be an uncompletely separated peak for another β-variant. Al-Obaidi (1980) reported that in cheese β-casein appeared in 4 bands, fraction 2 is the major band, fraction 1 is slower than 2 and fractions 3 and 4 moved faster than 2.

The commercially-supplied acid-precipitated casein did not show a very good separation. This might have been due to the long time of storage of these caseins which caused further breakdown of the major casein bands.

Schematic electrophoretic pattern of whole bovine casein and the typical components found in cheese made from cows milk clotted by calf rennet is presented in Fig. 6:25.

Section 6:2

According to amino acid analysis carried out by Groves et al. (1973) the Ts-$A^2$ and S-caseins form one pair of polymorphs and R- and Ts-B another.

These caseins are minor components of micellar caseins typed β- and $\gamma A^2$ and B. The Ts designation indicates higher solubility in aqueous solution with decrease in temperature (Gordon and Groves, 1975). These minor caseins were related genetically to the type of β- and γ-casein in the whole casein. For example, R- and Ts-A caseins were never found in homozygous type β-, γ-casein B; nor were S- and Ts-B caseins ever found associated with β-, γ-casein $A^2$. It was reported (Brunner, 1981) that γ-casein should be designated as a "derived" β-casein. Downey and Murphy (1970b) reported that $\alpha_s$-, β- and κ-caseins accounted for approximately 95 per cent of the protein present in the acid casein precipitates obtained from skim-milk.

In the present studies, the greatest variations observed were due to the heat treatment of milk (pasteurization) and to the trial. Although the effect of cold storage of milk was statistically not significant, several changes took place, the κ-casein fraction increased during the storage of milk and the increase was higher in milks held at $6^{o}C$ than at $2^{o}C$. Juffs (1975) found that the first evidence of proteolysis detected in raw milk during storage at $5^{o}C$ was the formation of para-κ-casein.

Dalgleish (1978) reported that $\alpha_{s_1}$-casein does not aggregate extensively, but the β- and κ-caseins form micelle-like aggregates, and the modifications or blocking of the sulphydryl groups inhibits aggregation, but not the stabilising ability of the κ-casein for $\alpha_{s_1}$-casein.

κ-casein was present in three bands in the raw milk. These bands might be variants of κ-casein. Brunner (1981) reported that κ-casein exists as a mixed species, varying in the carbohydrate moiety of from none to five or six.

The formation of different bands in the area of γ and κ-casein in the pasteurized milk might be due to the formation of other aggregates of these proteins with other protein. Harper (1981) stated that the interaction occurs during heat treatment where more than 50 per cent of the whey protein has been denatured. In his article, Brunner (1981) added that β-lactoglobulins undergoes time-dependent thermodenaturation and aggregation at temperatures in excess of $65^{o}C$. He also reported that β-lactoglobulin interacts with κ-caseins when milk is heated, the

exact mechanism of the reaction and role played by other whey proteins remains in doubt. Most of the available evidence which has been derived largly from model system studies, points to -SH/S-S interchange as the principle mechanism. In this study, the higher increase in the κ-casein in milks held at 6°C than at 2°C was associated with a lower increase in the free -SH groups in the milks held at 6°C than 2°C.

The major $\alpha_s$- and β-caseins were degraded with increasing periods and temperatures of storage. These degradations might provide the evidence of proteolysis since the smaller bands increased in intensity.

There are two sources of proteolytic enzymes in the milk, one the native enzymes, and the other proteolytic enzymes produced by proteolytic bacteria. Kaminogawa et al. (1980) reported that there are at least two proteases present in bovine milk. The susceptibility of whole casein to milk protease and chymosin was less than that of $\alpha_{s_1}$-casein. The higher resistance of whole casein to the two proteases may be attributed to the complete interaction among the casein components (interaction of $\alpha_{s_1}$- with κ-casein and of $\alpha_{s_1}$- with β-casein). These two enzymes brought about the appearance of new band with a higher mobility than $\alpha_{s_1}$ appeared after 24 h incubation of milk acid protease at 37°C (designated as $\alpha_{s_1}$-I). They indicated that $\alpha_{s_1}$-I originated from $\alpha_{s_1}$-casein. Visser and de Groot-Mostert (1977) found that the primary breakdown product of $\alpha_{s_1}$-casein during ripening of Gouda cheese showed the same mobility as $\alpha_{s_1}$-I. Therefore, the possibility that milk acid protease contributes to the conversion of $\alpha_{s_1}$-casein to $\alpha_{s_1}$-I during cheese ripening cannot be ignored.

During bulk milk storage, the growth of several types of psychrotrophic bacteria such as Pseudomonas fluorescens, are known to result in the production of proteinase which are thermoresistant. Bacterial proteinase predominately attacks κ-casein by which para-κ-casein-like material is formed; β- and $\alpha_{s_1}$-caseins in this order, are degraded at a lower rate. Native milk proteinase shows minimal affinity towards κ-casein (Visser, 1981).

The mechanisms of breakdown and interaction between different casein fractions are not yet fully understood. Although, a lot of work has been published on the structure of peptides, further studies are

required on the changes on these structures during cold storage of milk.

Section 6:3

Ts-casein showed some fluctuation in its concentration in the cheese during curing. Its original content in the cheese also varied in different cheeses made from unstored and stored milks.

The κ-casein was rather stable during the curing period in cheeses made from milks used on the day of delivery. In the case of cheeses made from milks held at 6°C before use the κ-casein content increased slightly and with cheese made from milks held at 2°C the amount of increase in κ-casein was greater.

It was reported by Richardson and Creamer (1973) that in Cheddar cheese, the κ-casein was converted to para-κ-casein very early in the process and the para-κ-casein was resistant to further attack. The increase in κ-casein during curing of cheese made from cold-stored milks might be related to the variations in rennet clotting time (RCT) determined in these present studies.

Changes in κ- and para-κ-casein in cheeses made from cold stored milks requires further study. Increase in the κ-casein might be due to the formation of a complex which includes κ-casein, and several recent studies by Dalgleish (1978) have referred to the formation of a complex between κ-casein and $\alpha_s$- and β-caseins. This author also reported that the electrostatic interaction occurs with polysacharides even when protein and polysaccharide are negatively charged, and κ-casein is the only casein to show such complex formation, presumably reflecting the differences in primary structures of the proteins.

γ-casein was found in three small bands occupying positions between Ts- and β-caseins. These bands were found to be more intense in cheeses made from milk held at 6°C than other cheeses. This might indicate that in cheeses made from milks held at 6°C the proteolysis of caseins was higher than in cheeses made from milks held at 2°C. These results might be due to the higher bacterial counts of milks held at 6°C but this proteolysis was not always associated with higher bacterial counts. Also, the heat-resistant enzymes and the accumulation of them by

products might have helped in such proteolysis. This increase in γ-casein fractions in the cheese was associated with the decrease in the β-casein (fraction 2) concentration. This indicated that γ-caseins are a result of degradation of β-casein. Creamer (1975) reported that β-casein is degraded to γ-caseins in very mature cheese, probably by the milk proteinase. Marcos et al. (1979) indicated that the increase in whole γ-casein derived from β-casein in cheese is unlikely to be due to chymosin action because the β-casein in cheese is resistant to chymosin attack. Milk protease, responsible for the whole γ-casein in fresh bovine milk, is thought to be the enzyme most likely responsible for the formation of these peptides in cheese.

β-casein In the present studies the β-casein was the other major band observed in the gel and was at a higher concentration in 1-month old cheesesthan in 1-week old cheeses made from milks on the day of delivery, and from milks held at 2°C for 2, 4 and 7 d and milks held for 2 d at 6°C. β-casein showed slow degradation during the ripening period of cheese. Minor β-casein (fraction 1) moved more slowly than fraction 2 (the major β-casein). This minor fraction was present more frequently in cheeses made from milks held at 6°C than at 2°C. Two other small fractions appeared which were faster than fraction 2. These bands did not appear in 1-week old cheeses, but they appeared with further curing might be as a result of degradation of the major β-casein. No significant variations were observed between different cheeses in the pattern of the proteolysis of β-casein. No appreciable increase in fractions 1, 3 and 4 occurred during ripening of the cheese.

Marcos et al. (1979) observed in the region of β-casein that a major central band of β-casein was present between two minor bands which were usually masked by the major one. One of the minor bands migrated slightly faster than the accompanying major protein and probably represent a breakdown product named β-I. These authors referred to Creamer (1971) who showe that chymosin acts on β-casein to give three N-terminal peptides designated β-I, β-II and β-III in order of appearance and of increasing electrophoretic mobility in PAGE at alkaline pH.

The $\alpha_s$-casein was found to degrade faster into smaller bands compared to β-casein which remained for a long time unaltered during the ripening

of the cheese. These findings are in agreement with the results reported by many other workers including Ledford, et al. (1966); Richardson and Creamer (1973); Marcos et al. (1979) and Visser (1981).

In cheeses at 1 week old, $\alpha_s$-casein was found degraded into two bands. The slower one was the more concentrated. These might be the degradation product of α-casein. Marcos et al. (1979) found two bands of fast electrophoretic mobility that appear in the position of pre-$\alpha_s$- are degradation products from $\alpha_{s_1}$-caseins. Of these, the slower and major one is the peptide named $\alpha_{s_1}$-I, the first product formed by the action of rennet on $\alpha_{s_1}$-casein. They suggested that the other band, of maximal electrophoretic mobility, may be a degradation by-product of $\alpha_{s_1}$-I caused by the endopeptidase acitivity of lactic acid bacteria.

With further curing of cheese, the $\alpha_s$-fractions were degraded further producting faster moving bands. Ledford, et al. (1966) reported that proteins of the vast majority of cheese are altered first by the action of rennet enzymes which, under the conditions applying to Cheddar cheese partially degrade $\alpha_{s_1}$- leaving β-casein largely unchanged. These authors suggested that the subsequent pattern of proteolysis by microbial enzymes occurring in different cheese varieties appears to be similar in that the $\alpha_{s_1}$-casein was always degraded in the cheese which they examined. This might explain the greater intensity of the fast moving bands ($\alpha_s$-fractions 7, 8, 9 and 10) of cheeses made in this present study from milks held at 6°C compared to those in cheeses made from milks processed and manufactured on the day of delivery or after storage at 2°C. These variations might be due to the higher bacterial counts of milks held at 6°C than milks held at 2°C.

During curing, $\alpha_s$-fractions 2 and 3 decreased in concentration as a result of degradation, while fractions 4, 5 and 6 increased in concentration. In cheeses made from milks held at 2°C for 7 d before use fraction 2 had almost disappeared after 12 months of curing while fractions 1 and 4 were at their maximum concentration. These results which represent the overall means of three trials were affected by the absence of fraction 2 in one trial in the case of cheese tested when 12 months old.

Gripon et al. (1977) reported that acid protease of Penicillium

roqueforti and the neutral protease of P. caseicolum play a fundamental role in the proteolysis induced by these micro-organisms. The enzymes from lactic Streptococci mainly are characterized by the release of free amino acids. Their action is both different from and complementary to that of rennet which only produces peptides. O'Keeffe et al. (1976) found that rennet was mainly responsible for the level of proteolysis detected by gel electrophoresis, by analysis for pH 4.6 soluble N and by gel filtration. However, rennet alone was capable of producing only a limited range of FAAs; only methionine, histidine, glycine, serine, and glutamic acid were produced at quantifiable level (> 0.2 μ moles/g) in chemically acidified cheese. They suggested that FAAs in Cheddar cheese are mainly the same. More recently the same authors (O'Keeffe et al., 1978) have reported that the coagulant was primarily responsible for the formation of large peptides while small peptides and FAAs were produced principally by the starter bacteria, possibly from coagulant-produced peptides. Some contribution by milk proteinase to the formation of soluble peptides cannot be excluded since the enzyme is fairly heat-stable.

Mulvihill and Fox (1979) found that in the presence of 5 per cent (w/v) NaCl in pH 5.2 buffer, $\alpha_{s_1}$-casein was hydrolysed to $\alpha_{s_1}$-I which was then hydrolysed to $\alpha_{s_1}$-VII and $\alpha_{s_1}$-VIII. Proteolytic specificity was found to be modified by NaCl. On the other hand, they found that in dilute buffer > pH 5.8, chymosin hydrolysed bovine $\alpha_{s_1}$-casein to $\alpha_{s_1}$-I, which was then hydrolysed to $\alpha_{s_1}$-V. These findings might explain the variations found in the present study in the concentration of $\alpha_{s_1}$-casein fractions in different cheeses. Although cheeses did not vary significantly in NaCl content (at 1 month old) it might be different after the course of curing especially if we consider the variations in the moisture contents of different cheeses through the loss of some of the liquids during cheese ripening.

In their article, Law et al. (1979) found that PAGE reveals small but probably significant differences in the protein breakdown patterns of cheeses made from control milk and milk inoculated with psychrotrophic strains of Pseudomonas fluorescens, Ps. putida and Acinetobacter spp. after the cheese has been stored for 22 weeks. In their work the size of the peak of $\alpha_{s_1}$-casein was rather variable and was not related to

storage time of the milk before processing or to the bacterial count, so they suggested that the variations were probably due to small differences in the extent of $\alpha_{s_1}$-casein breakdown by continued action of chymosin.

## CONCLUSION

1. The method used for the electrophoretic study was applicable for purified standard caseins, acid-precipitated casein of milks and the resultant Cheddar cheese. The use of slab gel electrophoresis gave the opportunity to use the samples of all the treatments of storage on one slab gel so that the variations in analysis could be minimized.

2. The cold storage of raw milk resulted in increased concentration of κ-casein. This increase was higher in milks held at $6^{o}C$ than milks held at $2^{o}C$. The major β- and $\alpha_s$-caseins decreased in concentration while the small band intensity was increased, so indicating the occurrence of proteolysis during cold storage.

3. β-casein was degraded faster in milks held at $6^{o}C$ than in milks held at $2^{o}C$. This finding encourages the storage of milk at $2^{o}C$ rather than at higher temperatures prior to use for cheese production in view of the frequently expressed view that cold storage of milk at temperatures near $4^{o}C$ results in loss of the β-casein into the whey.

4. Temperatures and periods of the milk storage did not show significant effect on the electrophoretic pattern of milk caseins. Most of the variation was due to the type of milk (raw or pasteurized) and to the trial.

5. Pasteurization of milk resulted in an apparent reduction in the κ-casein content and an increase in $\alpha_s$-casein. Pasteurization also seemed to affect the level of β-casein determined by PAGE. Some interaction between the casein fractions or between casein fractions and whey proteins may have taken place during pasteurization.

6. The rate of breakdown of β-casein which is an important factor in the ripening of Cheddar cheese was faster in cheeses made from milks held at 6°C than milks held at 2°C.

7. $\alpha_s$-casein showed the normal breakdown during ripening of the cheese.

# CHAPTER SEVEN

## THE CORRELATION BETWEEN THE COMPOSITION AND ORGANOLEPTIC QUALITY OF CHEESE AND THE CHEMICAL, MICROBIOLOGICAL AND ORGANOLEPTIC QUALITY OF COLD-STORED MILK USED FOR ITS MANUFACTURE

### INTRODUCTION

During the cold storage of milk prior to cheese making several types of enzymatic and microbial action take place. Senyk et al. (1981) identified the following flavours produced by different organisms when growing in milk:

1. Aeromonas hydrophila fermented, sour, bitter;
2. Hafnia alvei unclean, hay-like;
3. Pseudomonas aeruginosa slight unclean;
4. P. putida (a) slight rancid, unclean;
5. P. putida (b) rancid;
6. P. putida (c) burnt, pyrazine-like, unclean;
7. P. putida unclean, rancid.

It is not the theme of this study to go into details of the chemical background of aroma of milk and cheese so the author would like to refer to an excellent review on the recent advances in the study of aroma compounds of milk and dairy products by Badings and Neeter (1980).

The purpose of this part of the study is to correlate the chemical, microbiological and organoleptic characteristics of milk with the composition and quality of Cheddar cheese derived from the milk. Therefore, the author will make use of data obtained in another branch of this study (Al-Saltan, 1982) in particular those parts concerned with the microbiological quality of cold-stored milk since both parts of the study used the same raw milks and therefore some of the information is common. It would be interesting to do the correlation and regression analyses for the analysis of milk and cheese aiming to find out the possible explanation for the different compositional, organoleptical and microbiological changes which took place during the storage of milk

and relate this information to the effect on Cheddar cheese.

## EXPERIMENTAL

The data was extracted from the following sections:-

1. Chemical analysis of milk: Chapter Three;
2. Chemical analysis of cheese: Chapter Four;
3. Organoleptic evaluation of cheese: Chapter Five;
4. Organoleptic evaluation of milk: Al-Saltan (1982);
5. Microbiological quality of milk: Al-Saltan (1982).

The correlation coefficients between these sets of data were determined according to the methods of Snedecor and Cohran (1976).

## RESULTS

### 1. The correlation between the individual organoleptic characteristics of Cheddar cheese during curing

At 2 months old, the flavour, tast and texture were correlated significantly ($p < 0.001$) with each other. The flavour and taste correlate with the body and colour ($p < 0.001$) and openness ($p < 0.05$). However, texture, body and the colour did not correlate significantly with openness.

With cheese which was 4, 8 and 12 months old, the flavour, taste, texture, body, colour and openness correlated with each other significantly ($p < 0.001$). The values of these correlations were higher when the cheeses were 4 months old than when they were 2 months old (Table 7:1). Similar correlations were observed with older cheeses.

The regression analysis for these correlations (Table 7:2) supported the significant effect of each of these characteristics on the other organoleptic characteristics of the cheese. As an example for the correlation between organoleptic chracteristics of cheese, Fig. 7:1 shows the standard curves for the relation between the flavour and the other characteristics of cheese.

TABLE 7:1

The correlation coefficient between the organoleptic characteristics of Cheddar cheese after 2, 4, 8 and 12 months of curing

| Age of cheese | | Flavour | Taste | Texture | Body | Colour | Openness |
|---|---|---|---|---|---|---|---|
| 2 Months | Flavour | 1.000 | 0.9721*** | 0.8219*** | 0.7609*** | 0.7458*** | 0.4364* |
| | Taste | | 1.000 | 0.7542*** | 0.6625*** | 0.7139*** | 0.4277* |
| | Texture | | | 1.0000 | 0.9245*** | 0.6598*** | 0.3307 |
| | Body | | | | 1.0000 | 0.5815 | 0.3171 |
| | Colour | | | | | 1.0000 | 0.5478 |
| | Openness | | | | | | 1.000 |
| 4 Months | Flavour | 1.0000 | 0.9914*** | 0.8590*** | 0.8776*** | 0.8041*** | 0.5667*** |
| | Taste | | 1.0000 | 0.8413*** | 0.8525*** | 0.7784*** | 0.5283*** |
| | Texture | | | 1.0000 | 0.9402*** | 0.8410*** | 0.5773*** |
| | Body | | | | 1.0000 | 0.8577*** | 0.6394*** |
| | Colour | | | | | 1.0000 | 0.7895*** |
| | Openness | | | | | | 1.0000 |
| 8 Months | Flavour | 1.0000 | 0.9807*** | 0.8738*** | 0.9076*** | 0.9263*** | 0.7231*** |
| | Taste | | 1.0000 | 0.8792*** | 0.9248*** | 0.8815*** | 0.7247*** |
| | Texture | | | 1.0000 | 0.9140*** | 0.8793*** | 0.7718*** |
| | Body | | | | 1.0000 | 0.8166*** | 0.7069*** |
| | Colour | | | | | 1.000 | 0.7220*** |
| | Openness | | | | | | 1.000 |
| 12 Months | Flavour | 1.0000 | 0.9537*** | 0.8648*** | 0.7630*** | 0.9355*** | 0.6730*** |
| | Taste | | 1.0000 | 0.9052*** | 0.8324*** | 0.9378*** | 0.7474*** |
| | Texture | | | 1.000 | 0.8773*** | 0.8722*** | 0.7458*** |
| | Body | | | | 1.0000 | 0.7217*** | 0.6132*** |
| | Colour | | | | | 1.0000 | 0.6985*** |
| | Openness | | | | | | 1.0000 |

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

TABLE 7:2

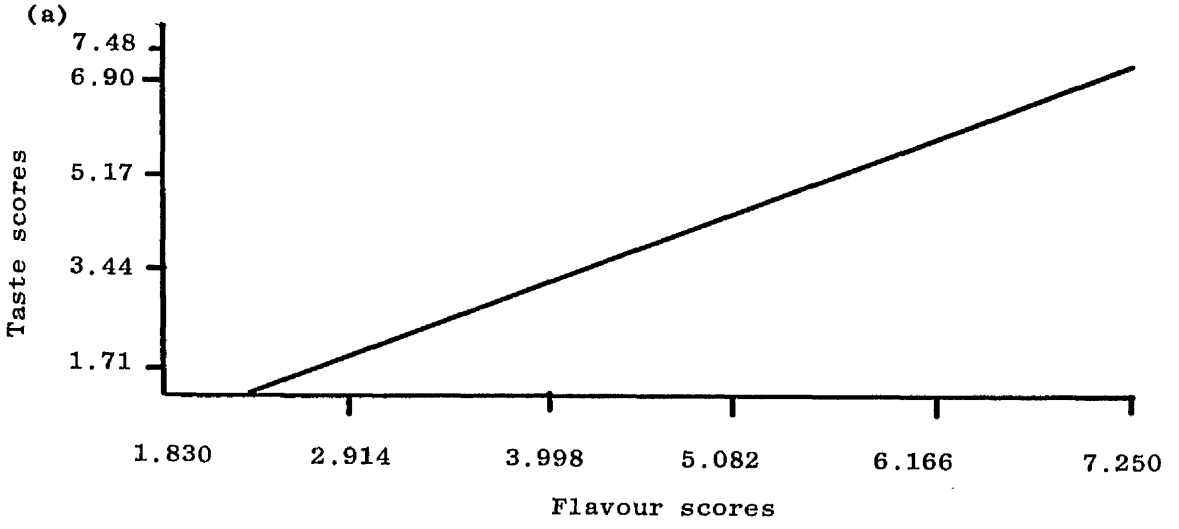
The regression analysis for the correlations between flavour and (a) taste, (b) texture, (c) body, (d) colour and (e) openness of cheese

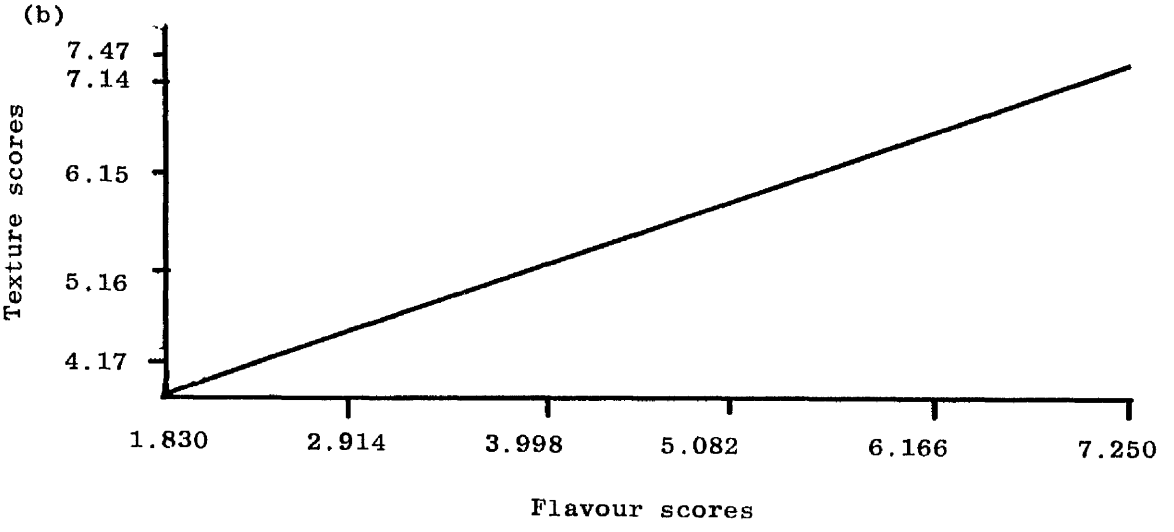
| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (flavour)<br>Slope (taste) | 1.39470<br>0.83924 | 0.22064<br>0.03919 | 6.32<br>21.41 |
| Y intercept (flavour)<br>Slope (texture) | -4.1440<br>1.5755 | 1.3361<br>0.2075 | -3.10<br>7.59 |
| Y intercept (flavour)<br>Slope (body) | -2.1730<br>1.2586 | 1.3203<br>0.2032 | -1.65<br>6.19 |
| Y intercept (flavour)<br>Slope (colour) | -3.154<br>1.2864 | 1.6178<br>0.2275 | -1.95<br>5.65 |
| Y intercept (flavour)<br>Slope (Openness) | -0.56620<br>0.93407 | 2.53087<br>0.36188 | -0.22<br>2.58 |

Analysis of variance

| | | Flavour and taste | | Flavour and texture | | Flavour and body | | Flavour and colour | | Flavour and openness | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 46.949 | 46.9488 | 34.19 | 34.1864 | 29.57 | 29.5670 | 27.36 | 27.3568 | 10.12 | 10.119 |
| Residual | 26 | 2.662 | 0.1024 | 15.42 | 0.5932 | 20.04 | 0.7709 | 22.25 | 0.8559 | 39.49 | 1.519 |
| Total | 27 | 49.611 | 1.8374 | 49.61 | 1.8374 | 49.61 | 1.8374 | 49.61 | 1.8374 | 49.61 | 1.837 |

Fig. 7:1 Standard curves for the correlations between flavour and (a) taste, (b) texture, (c) body, (d) colour and (e) openness of cheese



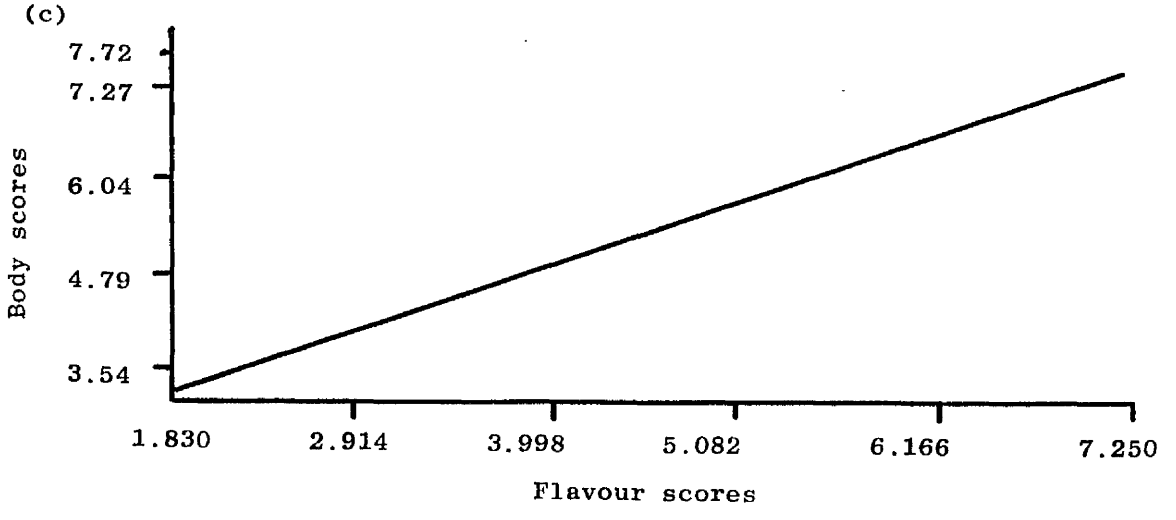
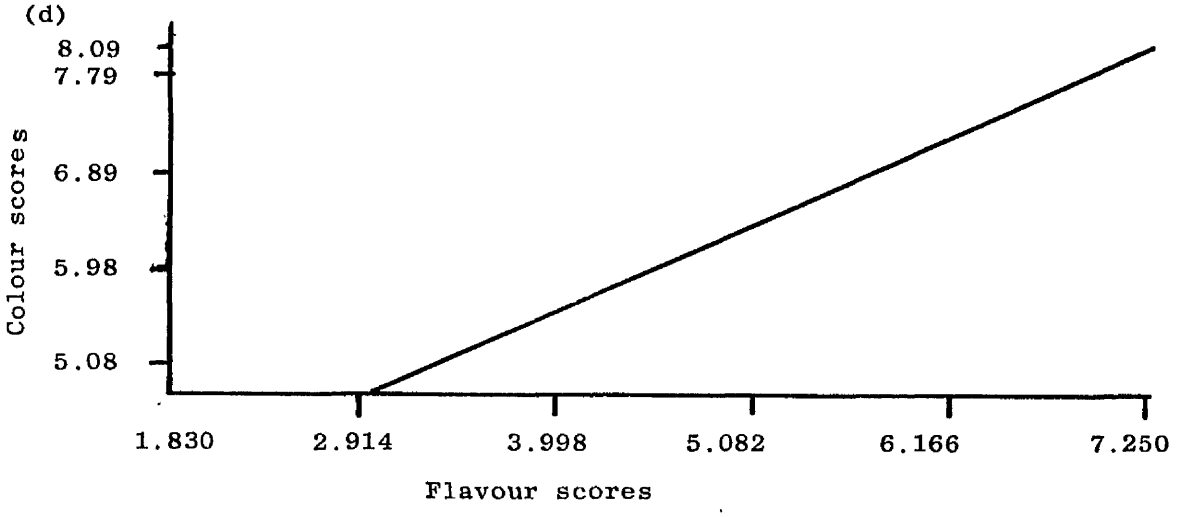
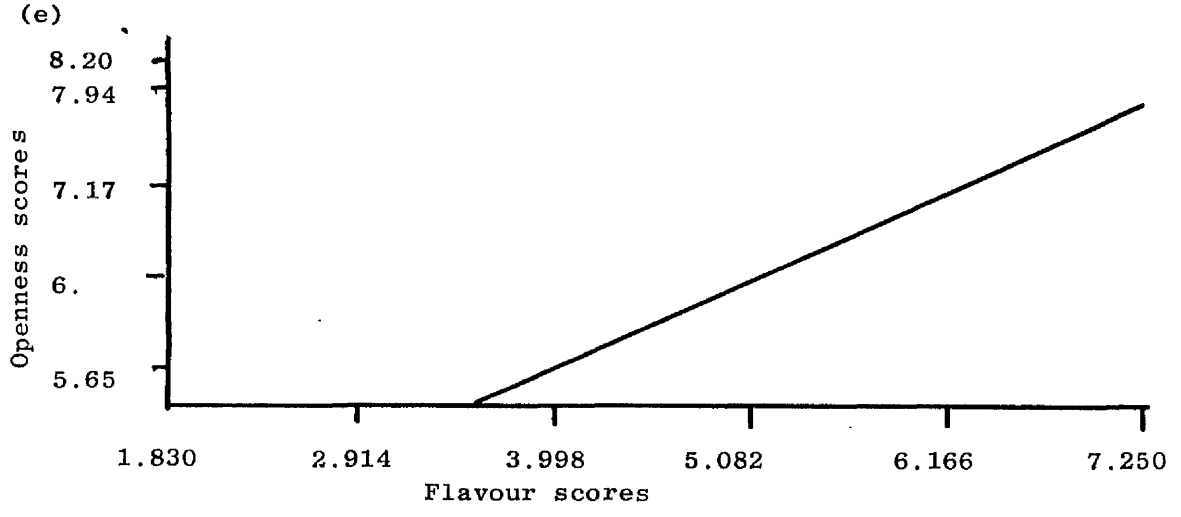



Fig. 7:1 continued .....



2. The correlation between the composition and the organoleptic characteristics of cheese when 2 months old are presented in Table 7:3

The flavour showed a significant positive correlation with calcium, and fat ($p < 0.001$); ash ($p < 0.01$), elasticity, aqueous salt and total nitrogen ($p < 0.05$). But it was negatively correlated with ADV, moisture, and MFFC ($p < 0.001$) and firmness (B.C.T) ($p < 0.01$).

Taste showed significant positive correlation with calcium and fat ($p < 0.001$); ash, aqueous salt, phosphorus and elasticity ($p < 0.05$). On the other hand negative significant correlations were observed between taste and ADV, moisture, and MFFC ($p < 0.001$), and firmness (B.C.T) ($p < 0.05$).

The texture showed significant positive correlation with calcium, ash, and total N ($p < 0.001$), fat ($p < 0.01$) and with aqueous salt, and elasticity ($p < 0.05$). Negative significant correlations were observed between texture and the ADV, firmness (B.C.T), moisture, and MFFC ($p < 0.001$).

The body scores had a positive significant correlation with calcium, and total N ($p < 0.001$); ash, and fat ($p < 0.01$) and aqueous salt ($p < 0.05$). However, negative correlations were observed between body and the firmness (B.C.T), moisture, and MFFC ($p < 0.001$); ADV ($p < 0.01$).

There were significant positive correlations between colour and calcium, and elasticity ($p < 0.001$); fat ($p < 0.01$), ash, and phosphorus ($p < 0.05$). Negative correlations were observed between colour and the ADV and moisture ($p < 0.001$) and MFFC ($p < 0.01$).

Openness showed a slight positive correlation with phosphorus and negatively correlated with ADV ($p < 0.05$).

At 4 months old (Table 7:4) the correlations between the composition and the organoleptic characteristics showed the same pattern of correlation. Openness was the only character which was different than it was in the younger cheese. It showed significant correlation with elasticity ($p < 0.001$); calcium, fat and aqueous salt ($p < 0.01$); with fat and total N ($p < 0.05$). Negative correlations were also observed between openness, ADV, and moisture ($p < 0.001$), MFFC

TABLE 7:3

The correlation coefficient between the composition of Cheddar cheese and its organoleptic assessment at 2 months old

| | Flavour | Taste | Texture | Body | Colour | Openness |
|---|---|---|---|---|---|---|
| Calcium | 0.7236*** | 0.6780*** | 0.6889*** | 0.6589*** | 0.6792*** | 0.2725 |
| Ash | 0.4789** | 0.4456* | 0.5700*** | 0.5312** | 0.4429* | 0.1537 |
| Fat | 0.5592*** | 0.5614*** | 0.4508** | 0.4667** | 0.5105** | 0.2960 |
| Aqueous salt | 0.3852* | 0.3609* | 0.3617* | 0.3882* | 0.2380 | -0.0860 |
| Salt | 0.1020 | 0.0704 | 0.1150 | 0.1530 | -0.0271 | -0.2258 |
| Phosphorus | 0.13774 | 0.3825* | 0.0972 | 0.1097 | -0.4169* | 0.4239* |
| $ADV_1$ | -0.8543*** | -0.8359*** | -0.5939*** | -0.4717** | -0.6510*** | -0.4059* |
| $ADV_2$ | -0.8232*** | -0.7912*** | -0.5763*** | -0.4588** | -0.6642*** | -0.3676* |
| Firmness (B.C.T) | -0.4532** | -0.3511* | -0.5984*** | -0.6793*** | -0.2490 | -0.2131 |
| $Elasticity_a$ | 0.4968* | 0.4429* | 0.4196* | 0.3252 | 0.7168*** | 0.3367 |
| Total N | 0.4459* | 0.3376 | 0.5972*** | 0.5752*** | 0.3253 | -0.1473 |
| Soluble N | -0.0711 | -0.0336 | 0.0628 | -0.0059 | -0.1298 | -0.2631 |
| Moisture | -0.7337*** | -0.7168*** | -0.6946*** | -0.6829*** | -0.6568*** | -0.2203 |
| MFFC | -0.5802 | -0.5543*** | -0.6050*** | -0.5766*** | -0.4743** | -0.0756 |
| pH | 0.2204 | 0.1893 | 0.3103 | 0.2858 | 0.2723 | -0.0301 |

$ADV_1$ = the ADV of cheese when cheese is 9 months old

$ADV_2$ = the ADV of cheese when cheese is 12 months old

| | | [a] |
|---|---|---|
| *significant at 5 per cent level | 0.3494 | 0.4018 |
| ** " " 1 " " " | 0.4487 | 0.5118 |
| *** " " 0.1 " " " | 0.5541 | 0.6249 |

TABLE 7:4

The correlation coefficient between the composition of Cheddar cheese and its organoleptic assessment at 4 months old

| | Flavour | Taste | Texture | Body | Colour | Openness |
|---|---|---|---|---|---|---|
| Calcium | 0.6722*** | 0.6371*** | 0.6710*** | 0.6861*** | 0.7792*** | 0.5416** |
| Ash | 0.4744** | 0.4493** | 0.6043*** | 0.5073** | 0.6504*** | 0.5136** |
| Fat | 0.5436** | 0.5342** | 0.5651*** | 0.5185** | 0.5151** | 0.3944* |
| Aqueous salt | 0.3758* | 0.3600* | 0.4165* | 0.3998* | 0.4298* | 0.5057** |
| Salt | 0.1083 | 0.0989 | 0.1489 | 0.1523 | 0.1539 | 0.3090 |
| Phosphorus | 0.4566** | 0.4445* | 0.4960** | 0.4954** | 0.3368 | 0.1520 |
| $ADV_1$ | -0.8465*** | -0.8192*** | -0.579*** | -0.6746*** | -0.6645*** | -0.5650*** |
| $ADV_2$ | -0.8377*** | -0.8049*** | -0.5987*** | -0.6926*** | -0.6883*** | -0.5228** |
| Firmness | -0.4530 | 0.5399** | -0.5397*** | -0.5428** | -0.3149 | -0.3700* |
| Elasticity | 0.5731*** | -0.4252* | 0.4825** | 0.6135*** | 0.6810 | 0.7607*** |
| Total N | 0.5978*** | 0.5722*** | 0.5148** | 0.5088** | 0.6378*** | 0.4485* |
| Soluble N | -0.1269 | -0.1389 | -0.3549 | -0.1238 | -0.0386 | 0.2473 |
| Moisture | -0.6649*** | -0.6455*** | -0.6803*** | -0.6793*** | -0.6869*** | -0.5796*** |
| MFFC | -0.4903** | -0.4670** | -0.4756** | -0.5172** | -0.5168** | -0.4932** |
| pH | 0.1393 | 0.1364 | 0.0459 | 0.0956 | 0.1315 | 0.0736 |

$ADV_1$ = acid degree value at 9 months old

$ADV_2$ = acid degree value at 12 months old

MFFC = moisture in free fat cheese

| | | |
|---|---|---|
| * | significant at 5 per cent level | 0.3494 |
| ** | " " 1 " " " | 0.4487 |
| *** | " " 0.1 " " " | 0.5541 |

($p < 0.01$) and firmness ($p < 0.05$).

The correlations between the composition of cheese and its organoleptic quality at 8 and 12 months are similar and are shown in Tables 7:5 and 7:6, respectively.

Tables 7:7 and 7:8 and Figs 7:2 to 7:5 showed the effect of RCT, SAV (by titration) and composition of cheese on its flavour and taste, respectively. With longer RCT the flavour and taste scores were higher while with higher SAV (by titration) the flavour and taste scores were lower. As expected, with increase in FFA, moisture and MFFC content in cheese, the flavour and taste scores were lower.

Increasing ball compressor readings (firmness) were associated with decreases in the body and texture scores (Tables 7:9 and 7:10, Figs 7:6 to 7:9). On the other hand the increases in the elasticity values were associated with increases in the body and texture scores. Cheeses with higher total N content were scored higher for body and texture while it was scored lower with increase in its moisture and MFFC contents.

3. The correlation between the microbiological quality and the composition of milk on delivery and after 2, 4 and 7 days of storage at $2^{o}C$ and $6^{o}C$

Close positive correlations ($p < 0.001$) were observed between the total counts and the contents of soluble calcium, soluble phosphorus, soluble ash and titratable acidity, freezing point depression (FPD) and the titratable acidity of the milk inoculated with starter and held at $30^{o}C$ for $5\frac{1}{2}$ h (SAV). A correlation between total count and NPN ($p < 0.01$, Table 7:11) and ADV ($p < 0.05$) of the milk was found.

On the other hand, negative correlations ($p < 0.001$) were observed between total count and pH value ($p < 0.001$) ash content, and RCT ($p < 0.05$). Psychrotrophic, coliform, lipolytic and proteolytic counts showed similar patterns of correlations as total counts with milk constituents.

The regression analysis and standard curves for the correlation between NPN and ADV of milk and its microbiological quality are presented in Tables 7:12 and 7:13 and Figs 7:10 and 7:11. These figures show the degree of increases in NPN and ADV as bacterial numbers increased during storage which indicated further breakdown in the milk protein and fat.

TABLE 7:5

The correlation coefficient between the composition of Cheddar cheese and its organoleptic assessment at 8 months old

| | Flavour | Taste | Texture | Body | Colour | Openness |
|---|---|---|---|---|---|---|
| Calcium | 0.5638*** | 0.5865*** | 0.5930*** | 0.6810*** | 0.4262* | 0.3997* |
| Ash | 0.4723** | 0.5239** | 0.5700*** | 0.6094*** | 0.3421 | 0.3207 |
| Fat | 0.3684* | 0.3724* | 0.2145 | 0.4223* | 0.1634 | 0.3010 |
| Aqueous salt | 0.3958* | 0.4617** | 0.4002* | 0.4978** | 0.3188 | 0.243 |
| Salt | 0.1890 | 0.2467 | 0.2269 | 0.2599 | 0.1932 | 0.0889 |
| Phosphorus | 0.3281 | 0.3339 | 0.0986 | 0.3324 | -0.1688 | 0.0292 |
| $ADV_1$ | -0.8530*** | -0.7934*** | -0.6774*** | -0.7371*** | -0.8223*** | -0.6073*** |
| $ADV_2$ | -0.8240*** | -0.7676*** | -0.6630*** | -0.7367*** | -0.7862*** | -0.5314** |
| Firmness | -0.0901 | -0.1383 | 0.0746 | -0.1837 | 0.1840 | -0.0156 |
| Elasticity | 0.6849*** | 0.7246*** | 0.6988*** | 0.8061*** | 0.5642*** | 0.6447*** |
| Total $N_{(a)}$ | 0.6626*** | 0.7223*** | 0.7280*** | 0.7970*** | 0.5338* | 0.7125*** |
| Soluble $N_{(b)}$ | 0.4082* | 0.4220* | 0.6047*** | 0.4934** | 0.5304** | 0.5993** |
| Moisture | -0.5508** | -0.6036*** | -0.5266** | -0.6608*** | -0.3698* | 0.5713*** |
| MFFC | -0.4829** | -0.5445** | -0.5742*** | -0.5939*** | -0.3992 | -0.5653*** |
| pH | 0.3037 | 0.2986 | 0.2242 | 0.2303 | 0.3557 | 0.0410 |

$ADV_1$ = acid degree value at 9 months old

$ADV_2$ = acid degree value at 12 months old

MFFC Moisture in free fat cheese

| | | a | b |
|---|---|---|---|
| *significant | at 5 per cent level | 0.3494 | 0.4227 | 0.3651 |
| ** " | " 1 " " " | 0.4487 | 0.5368 | 0.4678 |
| *** " | " 0.1 " " " | 0.5541 | 0.6524 | 0.8744 |

TABLE 7:6

The correlation coefficient between the composition of Cheddar cheese and its organoleptic assessment at 12 months old

| | Flavour | Taste | Texture | Body | Colour | Openness |
|---|---|---|---|---|---|---|
| Calcium | 0.4857** | 0.5288** | 0.6365*** | 0.7159*** | 0.4573** | 0.4259* |
| Ash | 0.4120* | 0.5060** | 0.5877*** | 0.6995*** | 0.3367 | 0.4570** |
| Fat | 0.2343 | 0.2972 | 0.2799 | 0.4255* | 0.2475 | 0.3222 |
| Aqueous salt | 0.4738** | 0.4914** | 0.5327** | 0.5544** | 0.4268* | 0.3797* |
| Salt | 0.3483 | 0.3344 | 0.3503* | 0.3272 | 0.2961 | 0.2373 |
| Phosphorus | 0.4110 | 0.3786* | 0.2620 | 0.3976* | -0.3164 | 0.2766 |
| $ADV_1$ | -0.8538*** | -0.8482*** | -0.7462*** | -0.6828*** | -0.8807*** | -0.5681*** |
| $ADV_2$ | -0.8649*** | -0.8533*** | -0.7616*** | -0.7079*** | -0.8608*** | 0.2027 |
| Firmness | 0.1750 | 0.0644 | -0.0656 | -0.3635 | 0.1672 | 0.0473 |
| Elasticity | 0.1236 | 0.2447 | 0.4099* | 0.6413*** | 0.1171 | 0.2027 |
| Total N | 0.3993 | 0.4271 | 0.4612** | -0.3635* | 0.3635* | 0.2845 |
| Soluble $N_{(a)}$ | 0.5032** | 0.5042** | 0.4872** | 0.3442 | 0.4745** | 0.2287 |
| Moisture | -0.3247 | -0.4629** | -0.5273** | -0.5915*** | -0.3512 | -0.4052* |
| MFFC | -0.1929 | -0.3222 | -0.4366 | -0.3697* | -0.2664 | -0.2143 |
| pH | 0.5134 | 0.5272 | 0.5553 | 0.5457 | 0.5741 | 0.1984 |

$ADV_1$ = Acid degree value at 9 months old

$ADV_2$ = Acid degree value at 12 months old

MFFC = Moisture in free fat cheese

| | | a |
|---|---|---|
| *significant at 5 per cent level | 0.3494 | 0.3651 |
| ** " " 1 " " " | 0.4487 | 0.4678 |
| *** " " 0.1 " " " | 0.5541 | 0.8744 |

TABLE 7:7

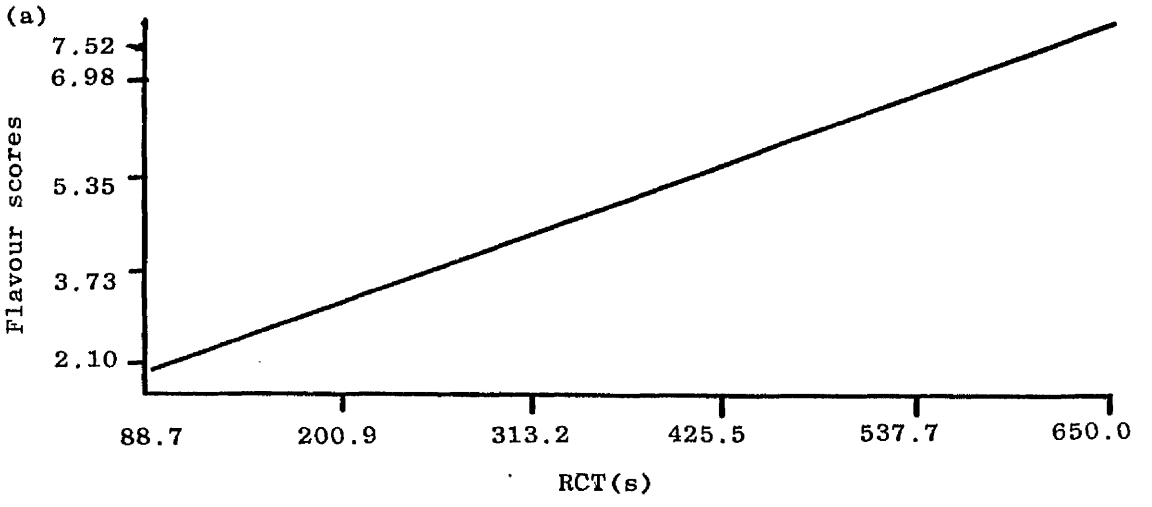
The regression analysis for the effect of (a) RCT (b) SAV by titration, (c) FFA of cheese (d) Moisture and (e) MFFC content on flavour

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (RCT) | -182.88 | 65.13 | -2.81 |
| Slope (flavour) | 109.81 | 10.70 | 10.26 |
| Y intercept (SAV by titration) | 0.854121 | 0.056306 | 15.17 |
| Slope (flavour) | -0.069478 | 0.009252 | -7.51 |
| Y intercept (FFA of cheese) | 22.6521 | 2.6812 | 8.45 |
| Slope (flavour) | -3.1943 | 0.4406 | -7.25 |
| Y intercept (Moisture | 42.40089 | 0.91234 | 46.47 |
| Slope (flavour) | -0.89909 | 0.14991 | -6.00 |
| Y intercept (MFFC) | 59.28769 | 1.15732 | 51.23 |
| Slope (flavour) | -0.78553 | 0.19016 | -4 13 |

Analysis of variance

| | DF | RCT | | SAV by titration | | FFA of cheese | | Moisture | | MFFC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 598248 | 598248 | 0.2395 | 0.239480 | 506.2 | 506.206 | 40.10 | 40.104 | 30.61 | 30.613 |
| Residual | 26 | 147747 | 5683 | 0.1104 | 0.004247 | 250.4 | 9.629 | 28.99 | 1.115 | 46.65 | 1.794 |
| Total | 27 | 745995 | 27629 | 0.3499 | 0.012959 | 756.6 | 28.021 | 69.09 | 2.559 | 77.26 | 2.861 |

Fig. 7:2 Standard curves for the correlations between the flavour of cheese and the (a) rennet clotting time (RCT) and (b) SAV (by titration)



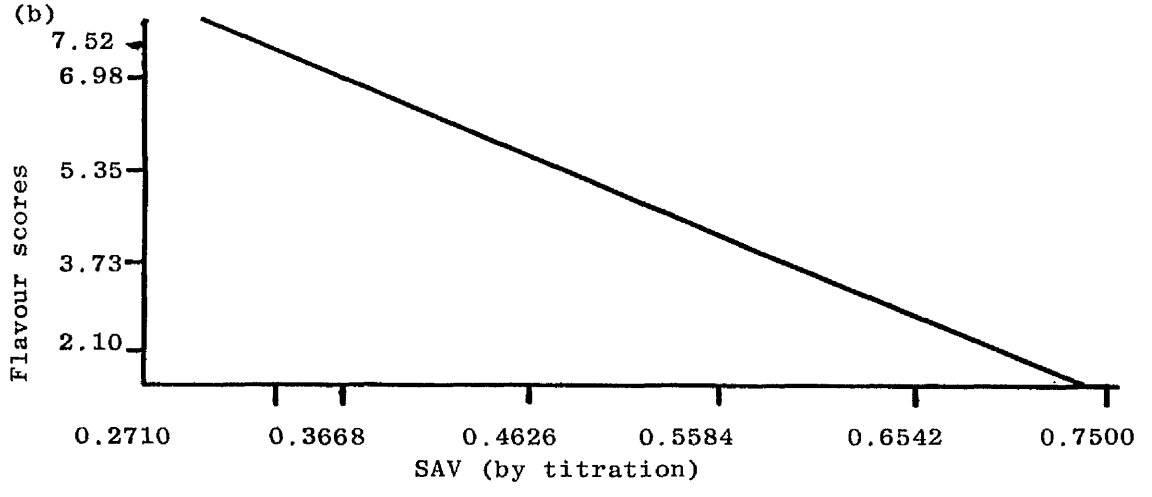
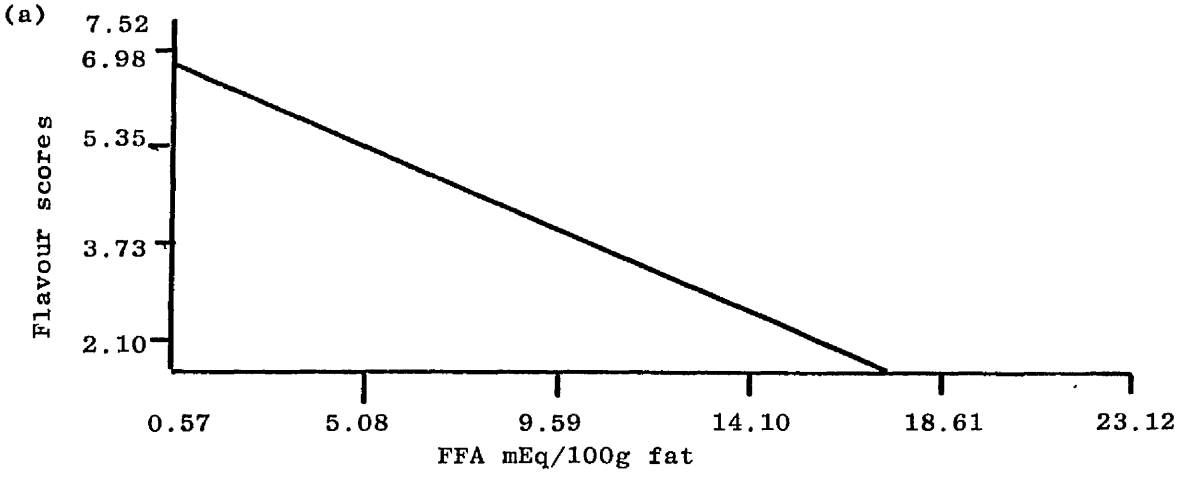
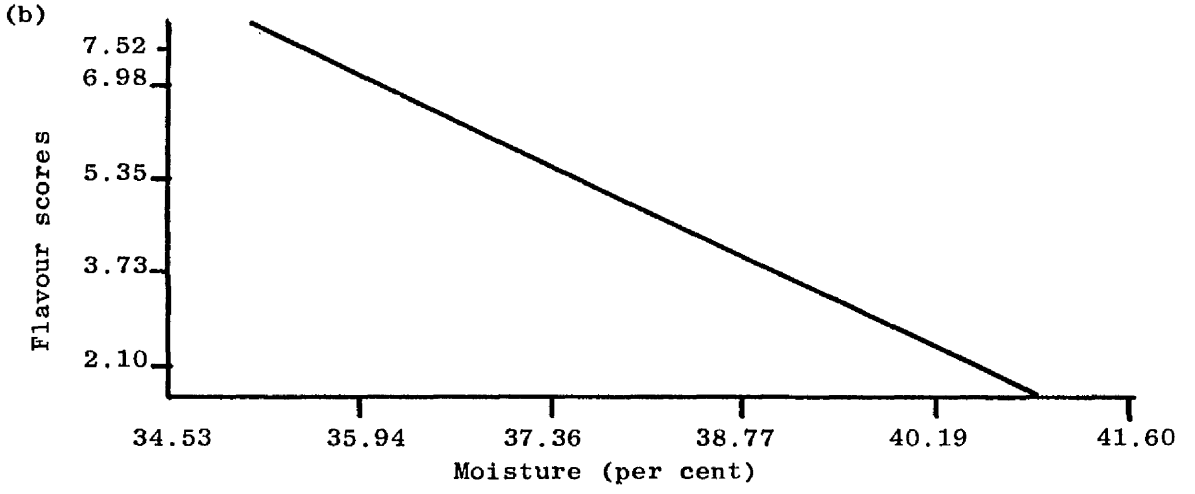



Fig 7:3 Standard curves for the correlations between flavour of cheese and its content of (a) free fatty acids (FFA) (b) moisture, and (c) MFFC.



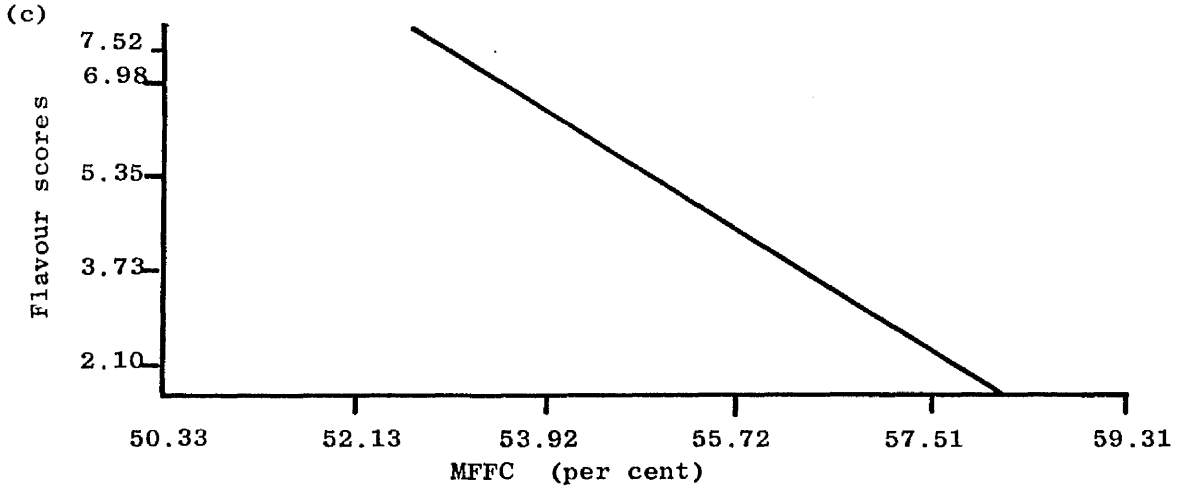



TABLE 7:8

The regression analysis for the correlations between taste of cheese and (a) the RCT, (b) SAV by titration, (c) the FFA, (d) moisture and (e) MFFC contents of cheese

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (RCT) | -14.143 | 62.654 | -0.23 |
| Slope (taste) | 89.281 | 11.129 | 8.02 |
| Y intercept (SAV by titration) | 0.750896 | 0.049179 | 15.27 |
| Slope (taste) | -0.057141 | 0.008735 | -6.54 |
| Y intercept (FFA of cheese) | 6.23328 | 0.22819 | 27.50 |
| Slope (taste) | -0.23398 | 0.03582 | -6.53 |
| Y intercept (moisture) | 41.15302 | 0.75326 | 54.63 |
| Slope (taste) | -0.75567 | 0.13380 | -5.65 |
| Y intercept (MFFC) | 58.12646 | 0.94994 | 61.19 |
| Slope (taste) | -0.64712 | 0.16873 | -3.84 |

Analysis of variance

| | DF | RCT | | SAV by titration | | FFA of cheese | | Moisture | | MFFC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 531341 | 531371 | 0.2176 | 0.217642 | 41.42 | 41.4178 | 38.06 | 38.065 | 27.91 | 27.915 |
| Residual | 26 | 214654 | 8256 | 0.1322 | 0.005006 | 25.24 | 0.9708 | 31.03 | 1.193 | 44.34 | 1.898 |
| Total | 27 | 745995 | 27629 | 0.3499 | 0.012959 | 66.66 | 2.4688 | 69.09 | 2.559 | 77.26 | 2.861 |

Fig. 7:4 Standard curves for the correlations between the taste of cheese and the (a) rennet clotting time (RCT), and (b) SAV (by titration).

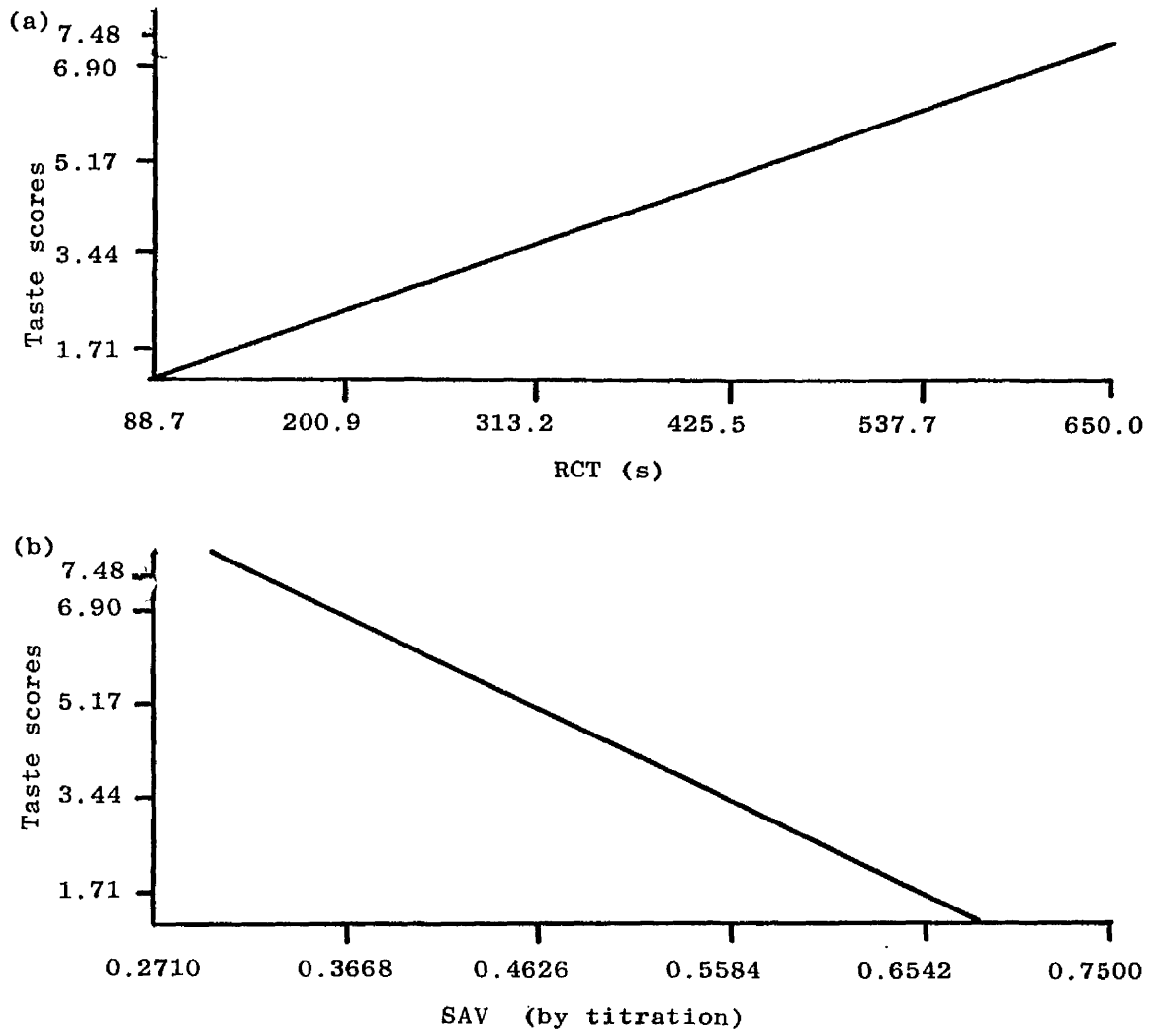
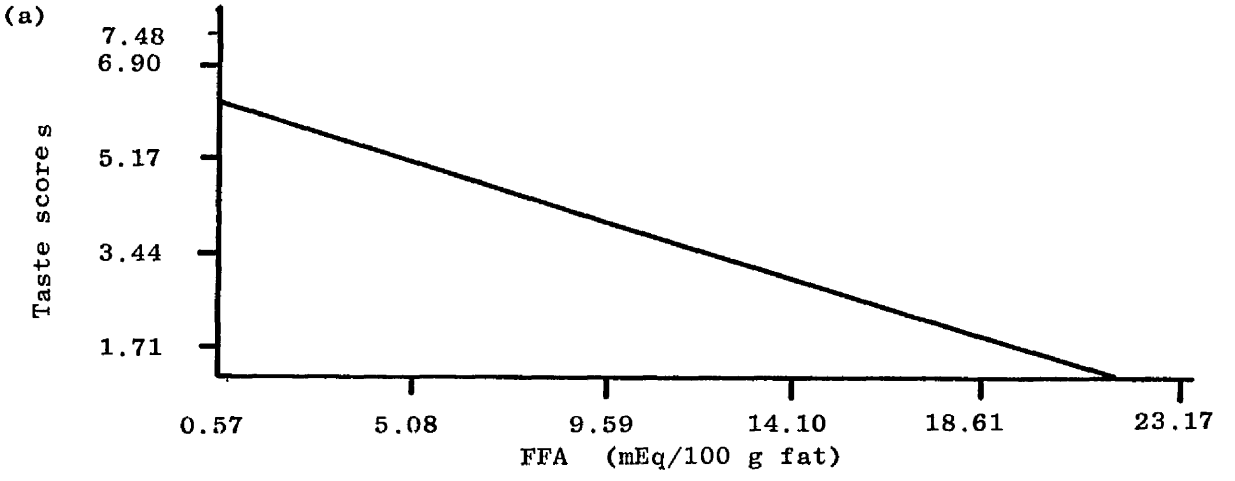
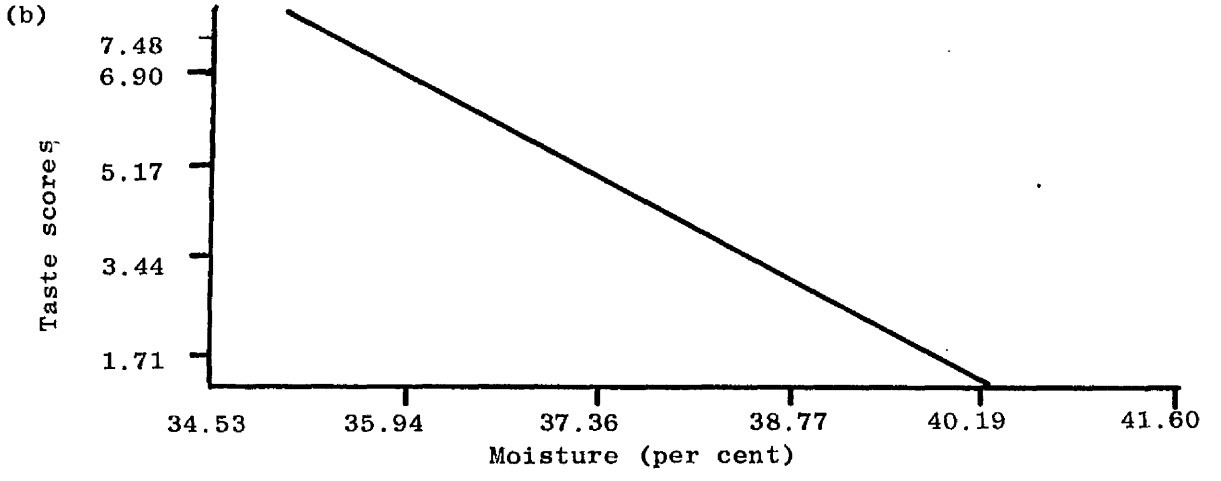


Fig. 7:5 Standard curves for the correlation between the tast of cheese and its content of (a) free fatty acids (FFA) (b) moisture, and (c) MFFC.



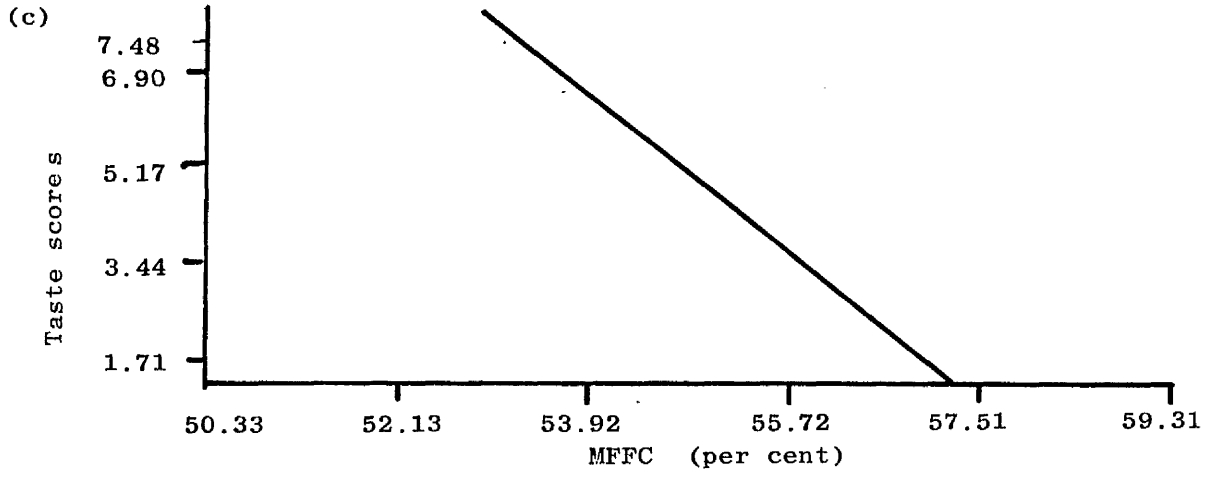



TABLE 7:9

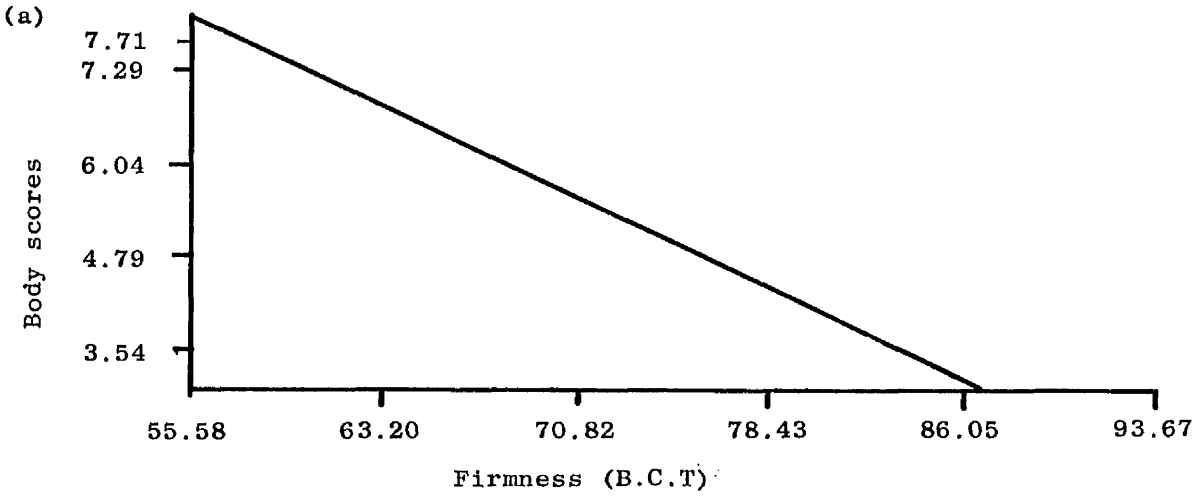
The regression analysis for the correlations between body of cheese and (a) ball compressor readings (firmness), (b) elasticity, (c) total N (protein), (d) moisture, (e) MFFC contents of cheese

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (firmness) | 105.2184 | 8.2575 | 12.74 |
| Slope (body) | -6.1000 | 1.2711 | -4.80 |
| Y intercept (elasticity) | 31.5495 | 5.7866 | 5.45 |
| Slope (body) | 6.2905 | 0.8907 | 7.06 |
| Y intercept (total N) | 17.45341 | 1.23627 | 14.12 |
| Slope (body) | 0.76406 | 0.19030 | 4.02 |
| Y intercept (moisture) | 45.9706 | 1.7049 | 26.96 |
| Slope (body) | -1.3823 | 0.2624 | -5.27 |
| Y intercept (MFFC) | 62.9072 | 2.0091 | 31.31 |
| Slope (body) | -1.2854 | 0.3093 | -4.16 |

Analysis of variance

| | DF | Firmness | | Elasticity | | Total N | | Moisture | | MFFC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 694.5 | 694.54 | 738.6 | 738.60 | 10.90 | 10.8967 | 35.67 | 35.666 | 30.84 | 30.841 |
| Residual | 26 | 784.1 | 30.16 | 385.0 | 14.81 | 17.57 | 0.6759 | 33.42 | 1.286 | 46.42 | 1.785 |
| Total | 27 | 1478.6 | 54.76 | 1123.6 | 41.62 | 28.47 | 1.0545 | 69.09 | 2.559 | 77.26 | 2.861 |

Fig. 7:6 Standard curves for the correlations between the body and ball compressor reading (firmness) and the elasticity of cheese.



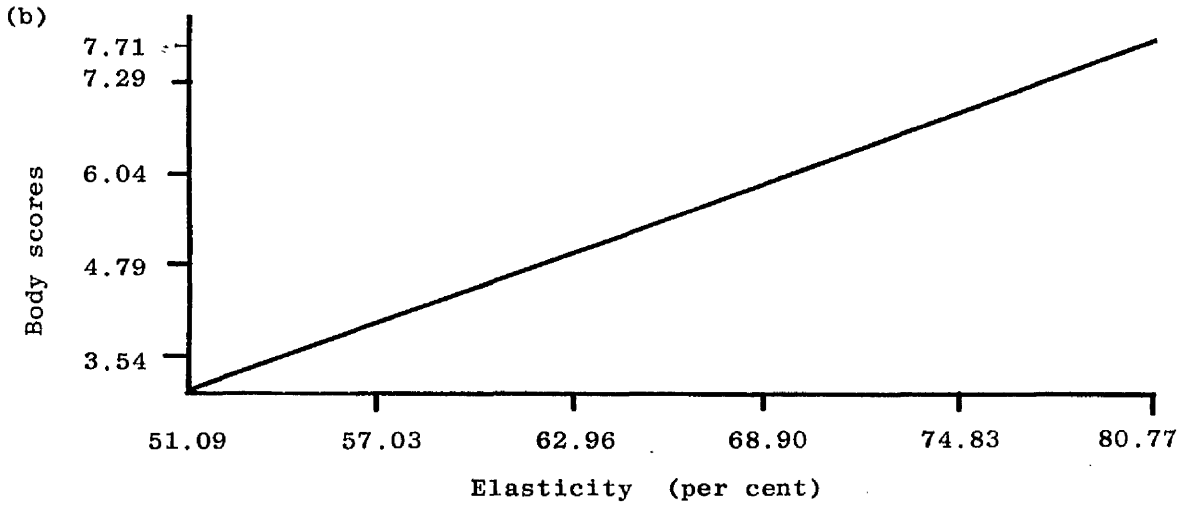



TABLE 7:10

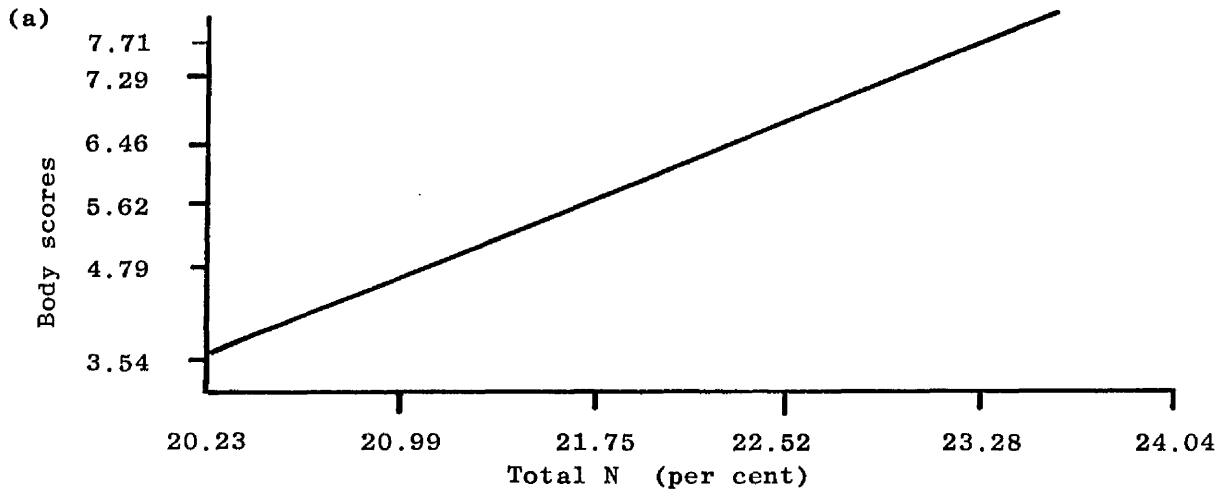
The regression analysis for the correlation between texture of cheese and (a) ball compressor reading (firmness) (b) elasticity, (c) total N (protein), (d) moisture and (e) MFFC contents of cheese

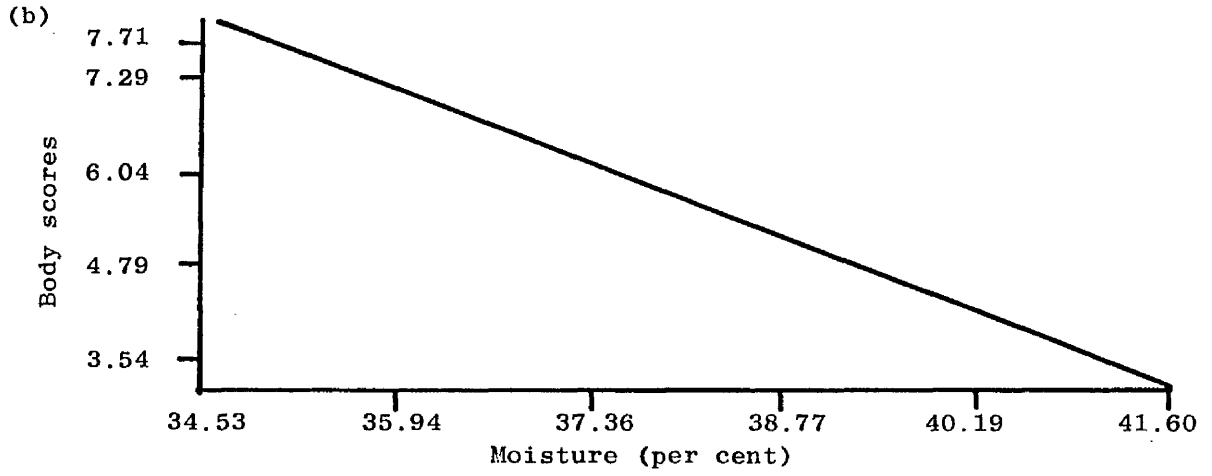
| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (firmness) | 105.9484 | 10.4271 | 10.16 |
| Slope (texture) | -6.2573 | 1.6196 | -3.86 |
| Y intercept (elasticity) | 25.6650 | 6.7939 | 3.78 |
| Slope (texture) | 7.2546 | 1.0553 | 6.87 |
| Y intercept (total N) | 16.40919 | 1.38158 | 11.88 |
| Slope(texture) | 0.93264 | 0.21460 | 4.35 |
| Y intercept (moisture) | 47.1937 | 2.0002 | 23.59 |
| Slope (texture) | -1.5832 | 0.3107 | -5.10 |
| Y intercept (MFFC) | 64.3150 | 2.2991 | 27.97 |
| Slope (texture) | -1.5145 | 0.3571 | -4.24 |

Analysis of variance

| | DF | Firmness | | Elasticity | | Total N | | Moisture | | MFFC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 539.3 | 539.26 | 724.9 | 724.85 | 11.98 | 11.9799 | 34.52 | 34.524 | 31.59 | 31.591 |
| Residual | 26 | 939.4 | 36.13 | 398.8 | 15.34 | 16.49 | 0.6343 | 34.57 | 1.330 | 45.67 | 1.756 |
| Total | 27 | 1478.6 | 54.76 | 1123.6 | 41.62 | 28.47 | 1.0545 | 69.09 | 2.559 | 77.26 | 2.861 |

Fig. 7:7 Standard curves for the correlations between the body of cheese and its content of total N (protein) , moisture and MFFC.



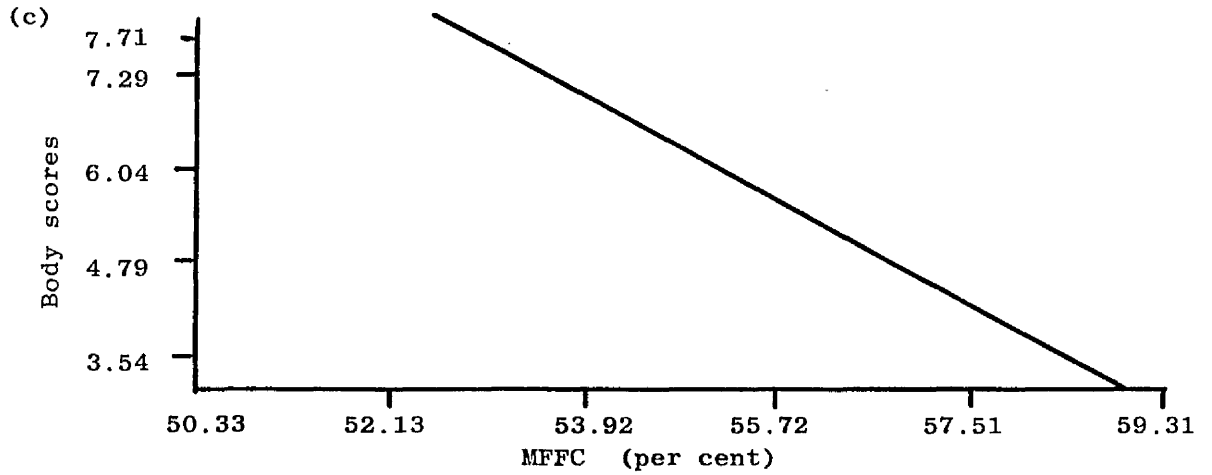



Fig. 7:8 Standard curves for the correlations between texture and ball compressor total (firmness) and the elasticity of cheese.

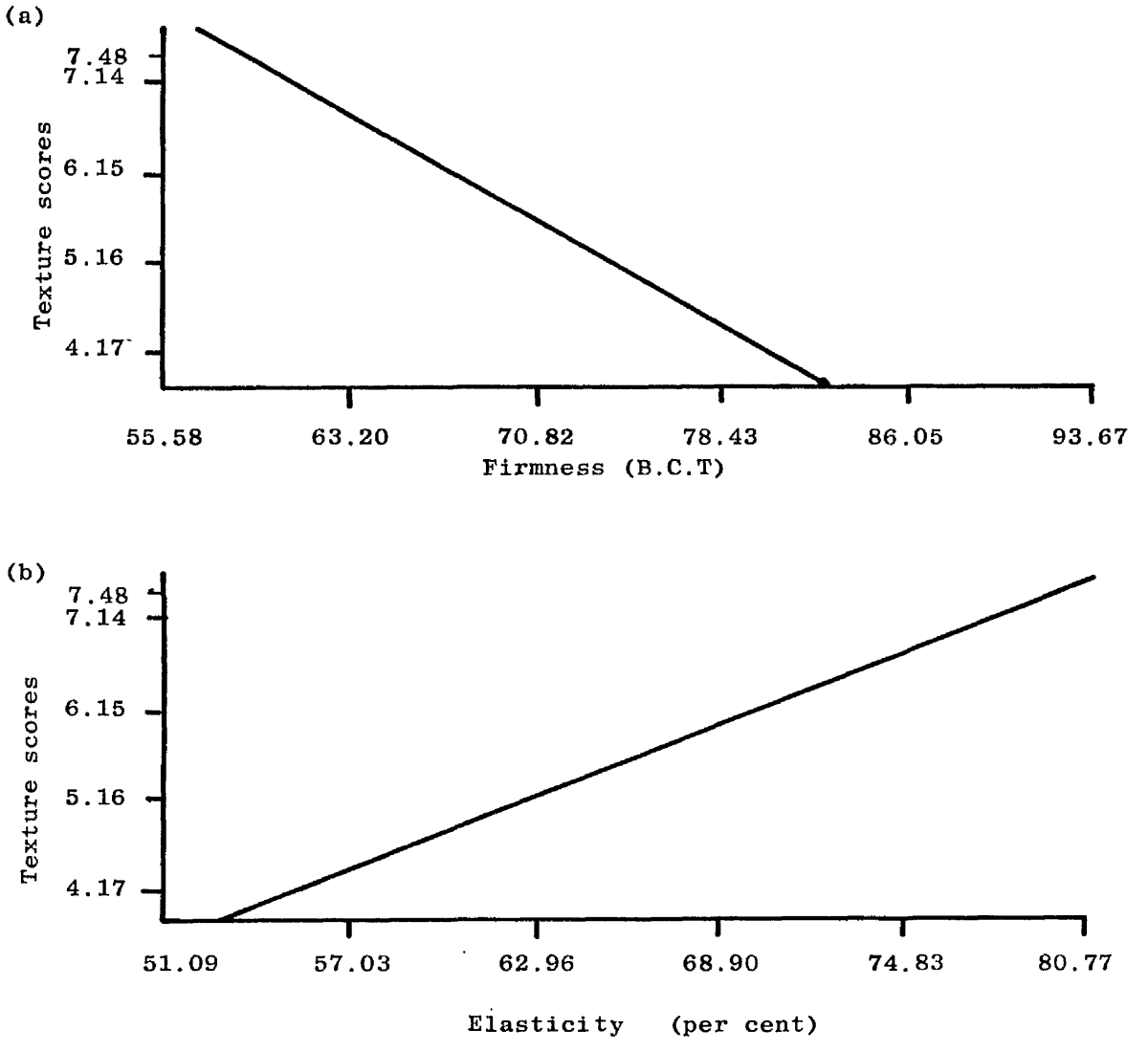


Fig. 7:9 Standard curves for the correlations between texture and total N (protein), moisture and MFFC content of cheese.

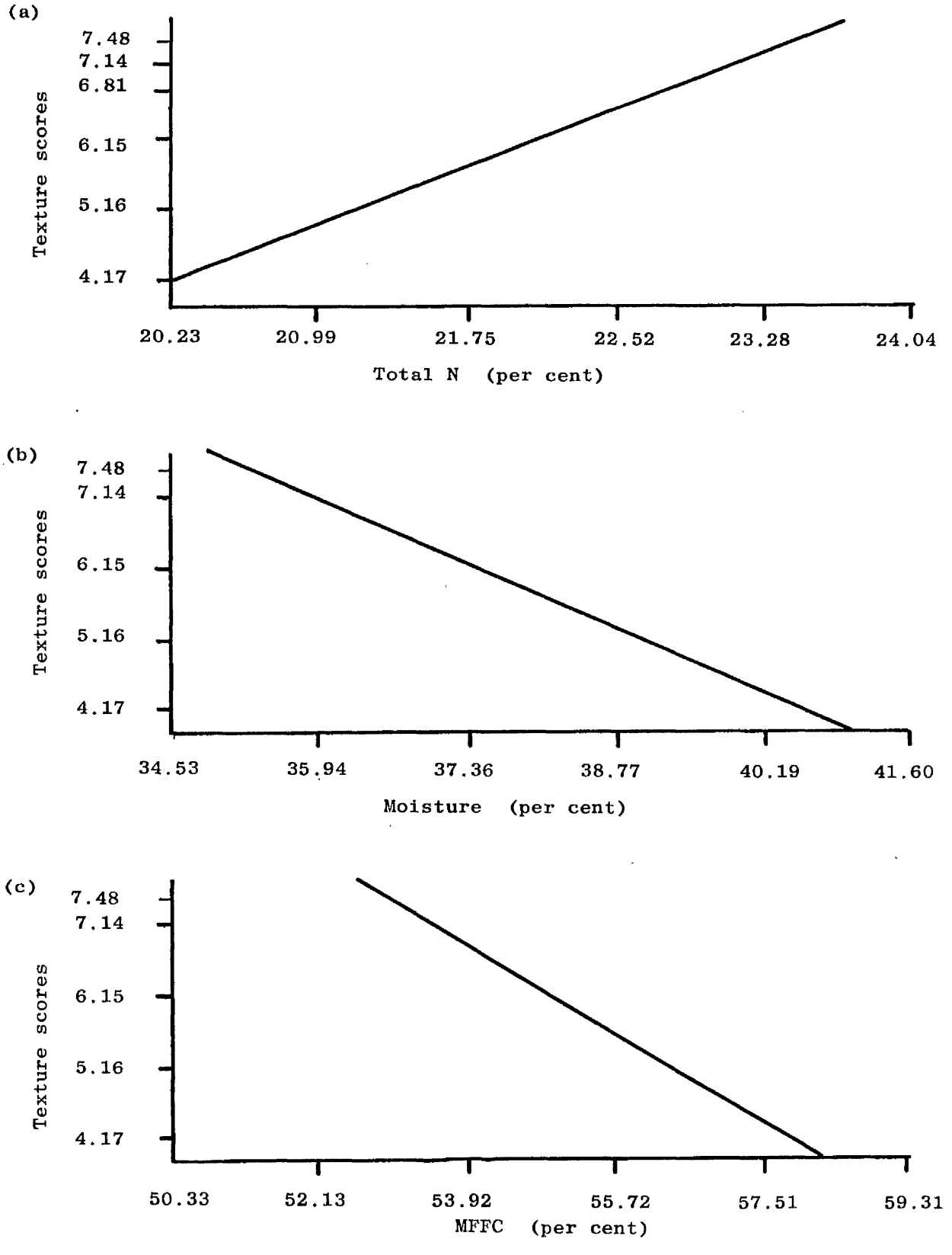


TABLE 7:11

The correlation coefficient of the microbiological quality and composition of milk on delivery and after storage

| Microbiological / Compositional | Total count ($log_e$) | Psychrotrophic count ($log_e$) | Coliform count ($log_e$) | Lipolytic count ($log_e$) | Proteolytic count ($log_e$) | Thermoduric count ($log_e$) |
|---|---|---|---|---|---|---|
| Protein | 0.1427 | 0.0199 | 0.0930 | -0.0326 | 0.0449 | 0.4171 |
| Non-casein N | 0.3919** | 0.1871 | 0.5065** | 0.4168 | 0.4133 | 0.3602 |
| Non-protein N | 0.6108*** | 0.6424*** | 0.6434*** | 0.6643*** | 0.5775** | -0.1379 |
| Calcium | 0.0322 | -0.0425 | 0.0285 | -0.0331 | -0.0993 | 0.6989*** |
| Phosphorus | -0.1116 | 0.0140 | -0.0361 | 0.0818 | -0.1113 | 0.2535 |
| Ash | -0.6030** | -0.4243 | -0.4951* | -0.4341* | -0.5225* | 0.0305 |
| Fat | -0.1117 | -0.0425 | -0.0823 | -0.0452 | 0.0221 | -0.6801*** |
| ADV | 0.5367* | 0.5471* | 0.5786* | 0.6622** | 0.6588** | -0.3996 |
| Total solids | 0.3516 | -0.1860 | -0.3543 | -0.2940 | -0.2379 | -0.4598* |
| Soluble calcium | 0.6823*** | 0.5604** | 0.7443*** | 0.6318** | 0.6070** | -0.0025 |
| Soluble phosphorus | 0.6896*** | 0.5817** | 0.7448*** | 0.6386** | 0.6704*** | -0.1406 |
| Soluble ash | 0.6862*** | 0.5333* | 0.6567** | 0.6563** | 0.6357** | -0.2503 |
| SH groups | -0.1172 | -0.1049 | -0.1027 | -0.1831 | -0.1038 | 0.3127 |
| Titratable acidity | 0.7319*** | 0.5721** | 0.8328*** | 0.6097** | 0.6455** | 0.0554 |
| pH | -0.7988*** | -0.6336** | -0.8841*** | -0.6735*** | -0.7122*** | -0.4530* |
| FPD | 0.7256*** | 0.5392* | 0.8082*** | 0.5453* | 0.6050** | 0.1509 |
| RCT | -0.6370** | -0.4463* | -0.7447*** | -0.4303* | -0.5543** | -0.1102 |
| SAV (by titration) | 0.6814*** | 0.5251* | 0.7811*** | 0.6045* | 0.6722*** | -0.0377 |
| SAV (by pH) | -0.3604 | -0.1322 | -0.4834* | -0.3336 | -0.3601 | -0.0757 |

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

TABLE 7:12

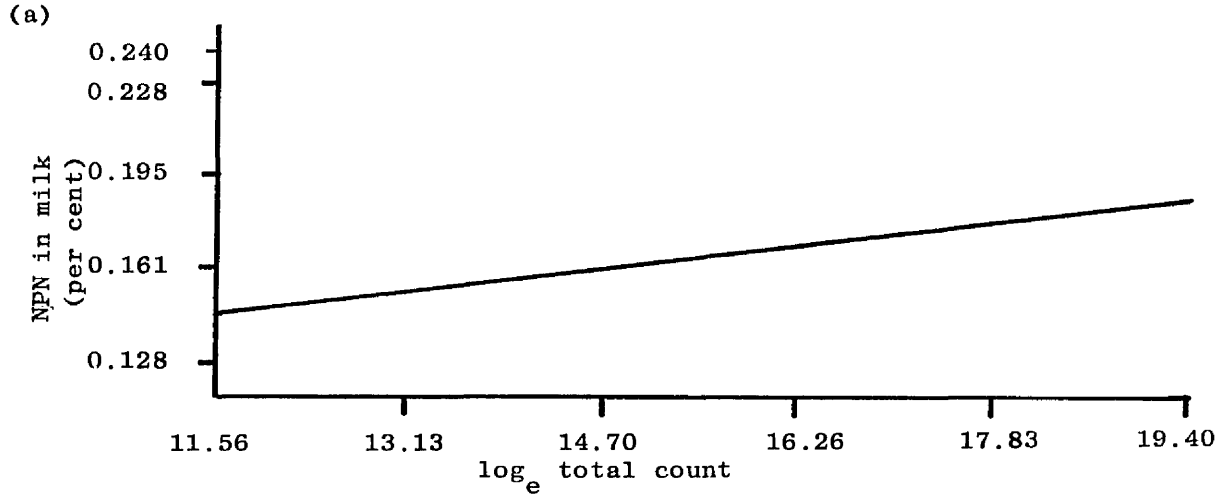
The regression analysis for the effect of total, psychrotrophic, proteolytic, lipolytic and coliform counts on the level of NPN in milk

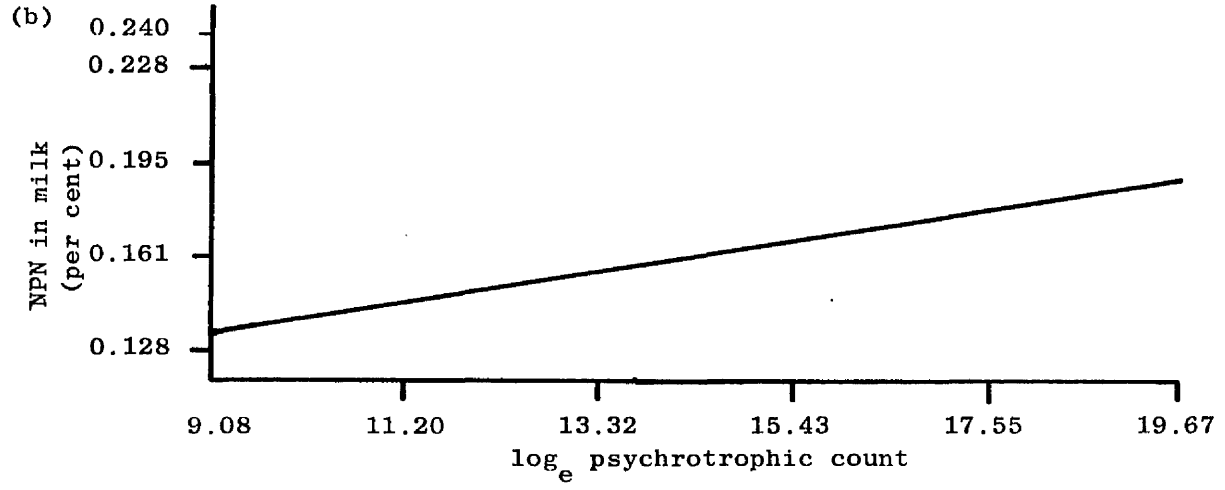
| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (NPN) | 0.06508 | 0.0299023 | 2.19 |
| Slope ($\log_e$ total count) | 0.006618 | 0.0019661 | 3.36 |
| Y intercept (NPN) | 0.0847717 | 0.0223896 | 3.79 |
| Slope ($\log_e$ psychrotrophic) | 0.0052804 | 0.0014452 | 3.65 |
| Y intercept (NPN) | 0.0693011 | 0.0313536 | 2.21 |
| Slope ($\log_e$ proteolytic) | 0.0060961 | 0.0019770 | 3.08 |
| Y intercept (NPN) | 0.0799966 | 0.0223550 | 3.58 |
| Slope ($\log_e$ lipolytic) | 0.0056677 | 0.0014631 | 3.87 |
| Y intercept (NPN) | 0.1003528 | 0.0181956 | 5.52 |
| Slope ($\log_e$ coliform) | 0.0057955) | 0.0015817 | 3.66 |

Analysis of variance

| | DF | $\log_e$ total count | | $\log_e$ psychrotrophic count | | $\log_e$ proteolytic count | | $\log_e$ lipolytic count | | $\log_e$ coliform count | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 0.005720 | 0.0057200 | 0.006326 | 0.0063263 | 0.00511 | 0.0051131 | 0.006765 | 0.0067649 | 0.006347 | 0.0063472 |
| Residual | 19 | 0.009610 | 0.0005058 | 0.009004 | 0.0004739 | 0.01022 | 0.0005377 | 0.008565 | 0.0004508 | 0.008983 | 0.0004728 |
| Total | 20 | 0.015330 | 0.0007665 | 0.015330 | 0.0007665 | 0.01533 | 0.0007665 | 0.015330 | 0.0007665 | 0.015330 | 0.0007665 |

Fig. 7:10 Standard curves for the correlation between NPN level in milk and (a) $\log_e$ total count, (b) $\log_e$ psychrotrophic count, (c) $\log_e$ proteolytic count, (d) $\log_e$ lipolytic count, and (e) $\log_e$ coliform count during the cold storage of raw milk.



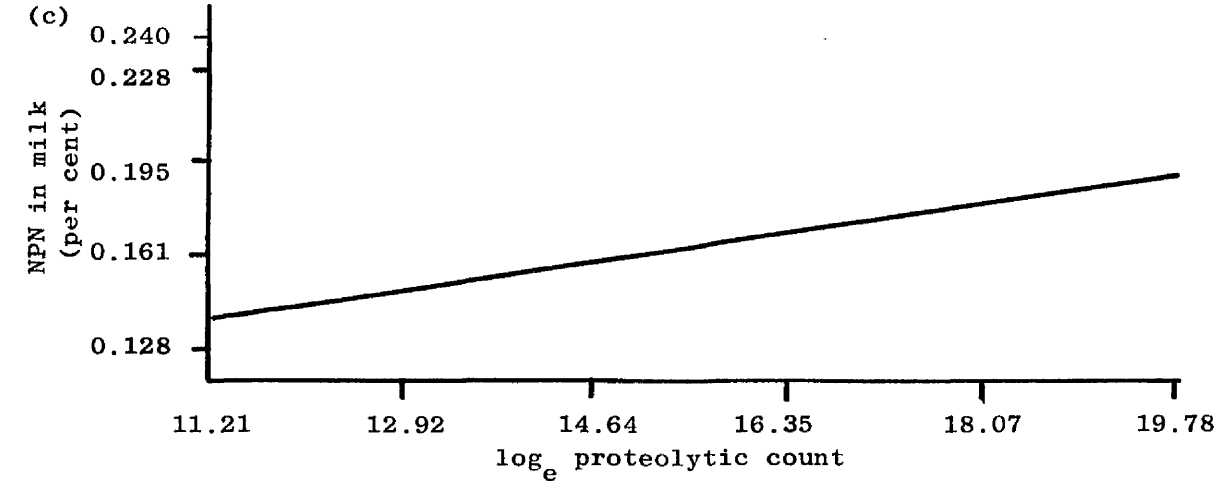
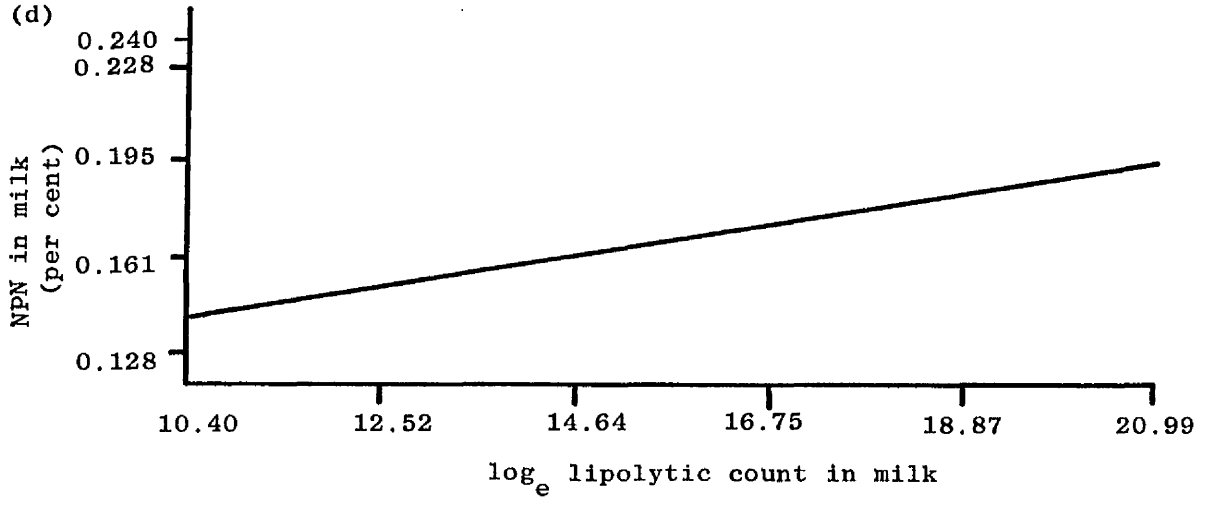



Fig. 7:10 continued .....



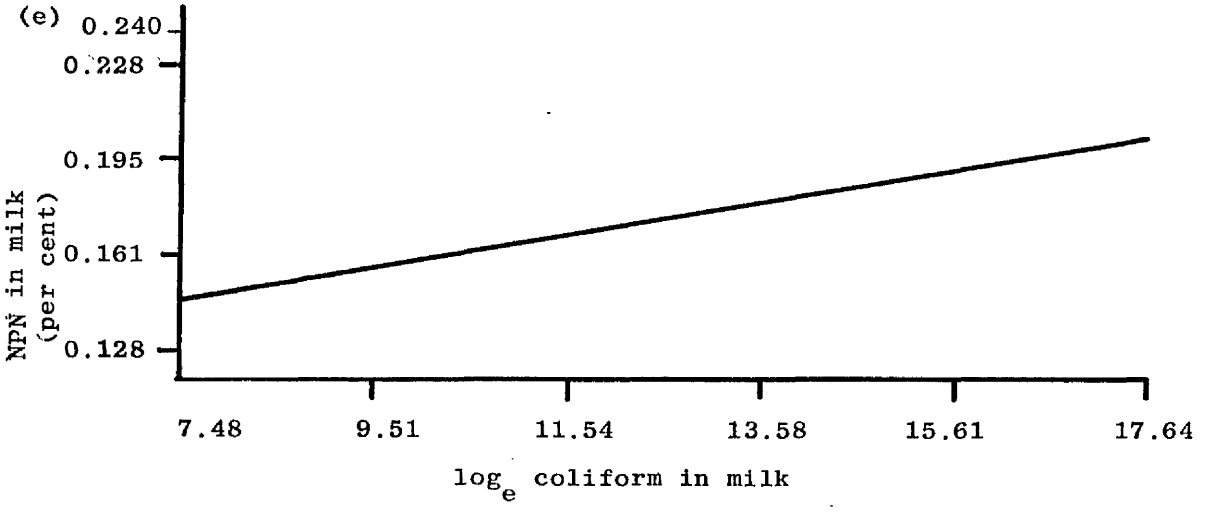



TABLE 7:13

The regression analysis for the effect of total, psychrotrophic, proteolytic, lipolytic and coliform counts on the ADV of milk

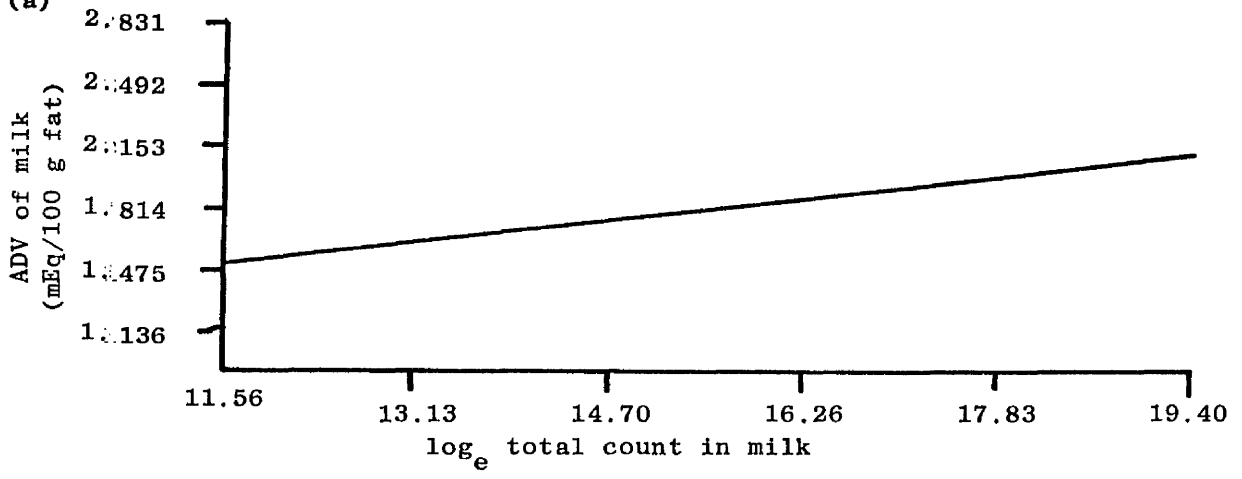
| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (ADV) | 0.509004 | 0.460955 | 1.11 |
| Slope ($\log_e$ total count) | 0.084027 | 0.030309 | 2.77 |
| Y intercept (ADV) | 0.785663 | 0.353728 | 2.22 |
| Slope ($\log_e$ psychrotrophic) | 0.065052 | 0.022833 | 2.85 |
| Y intercept (ADV) | 0.19610 | 0.41793 | 0.47 |
| Slope ($\log_e$ proteolytic) | 0.10059 | 0.02635 | 3.82 |
| Y intercept (ADV) | 0.548916 | 0.324154 | 1.69 |
| Slope ($\log_e$ lipolytic) | 0.081727 | 0.021215 | 3.85 |
| Y intercept (ADV) | 0.933430 | 0.280438 | 3.33 |
| Slope ($\log_e$ coliform) | 0.075377 | 0.024379 | 3.09 |

Analysis of variance

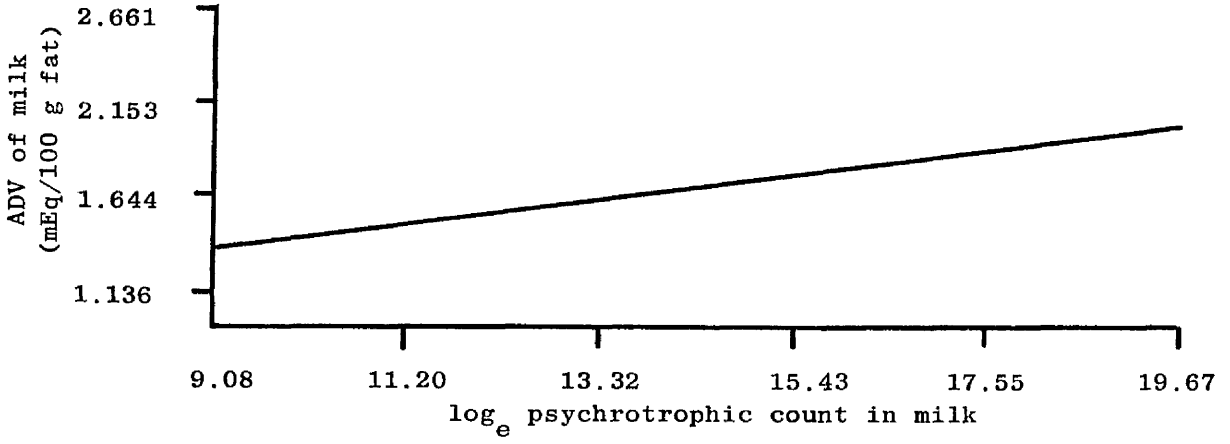
| | DF | $\log_e$ total count | | $\log_e$ psychrotrophic count | | $\log_e$ proteolytic count | | $\log_e$ lipolytic count | | $\log_e$ coliform count | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 0.924 | 0.9238 | 0.960 | 0.9602 | 1.392 | 1.39222 | 1.407 | 1.40662 | 1.074 | 1.0737 |
| Residual | 19 | 2.284 | 0.1202 | 2.247 | 0.1183 | 1.815 | 0.09554 | 1.801 | 0.09479 | 2.134 | 0.1123 |
| Total | 20 | 3.208 | 0.1604 | 3.208 | 0.1604 | 3.208 | 0.16038 | 3.208 | 0.16038 | 3.208 | 0.1604 |

Fig. 7:11 Standard curves for the correlation between ADV level in milk and (a) $\log_e$ total count, (b) $\log_e$ psychrotrophic count, (c) $\log_e$ proteolytic count, (d) $\log_e$ lipolytic count, and (e) $\log_e$ coliform count during the cold storage of raw milk

(a)




(b)




(c)

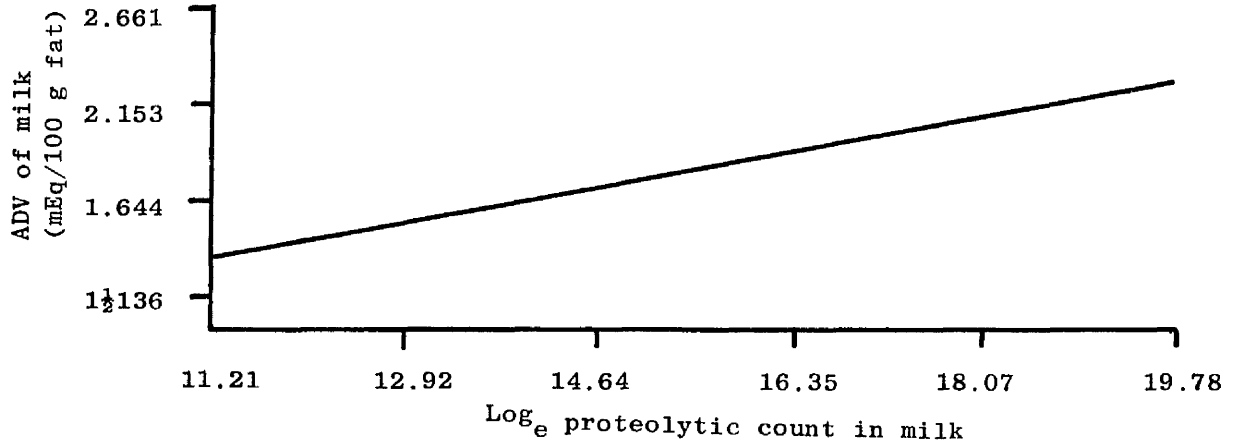
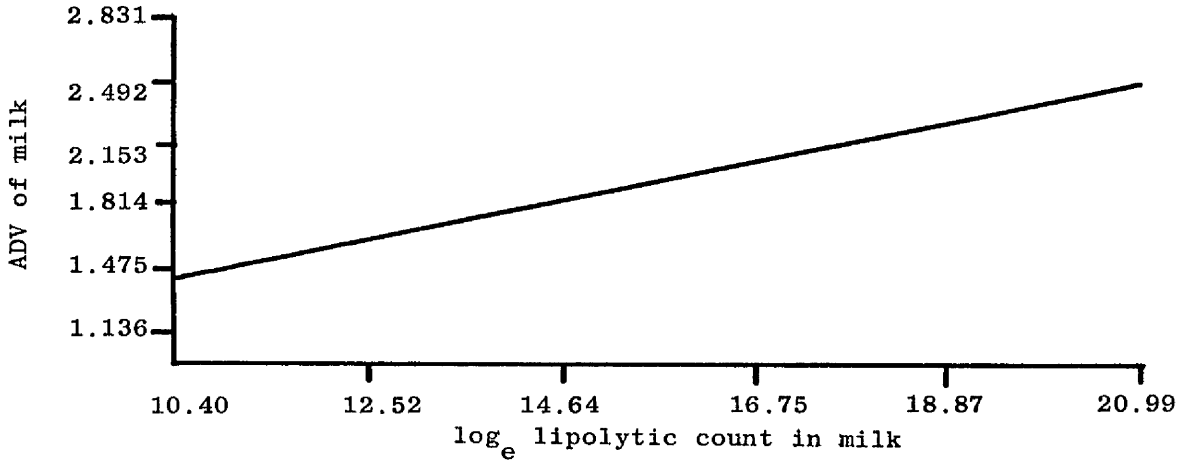
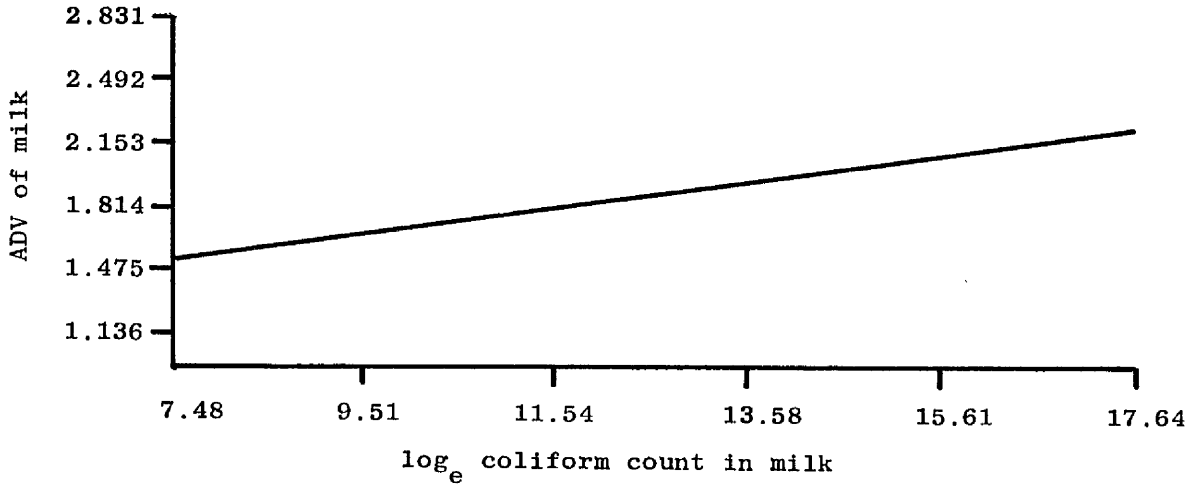


Fig. 7:11 continued .....

(d)




(e)



The thermoduric counts were correlated positively with calcium ($p < 0.001$) and negatively with fat ($p < o.00$), total solids, and pH ($p < 0.05$).

4. The correlation between the different bacteriological counts

Highly significant positive ($p < 0.001$) correlations were observed between the different microbiological counts under study (Table 7:14) except for thermoduric count which showed no significant correlation with any of the other bacterial counts. The regression analyses for these correlations were carried out by Al-Saltan (1982).

5. The correlation between the composition of cheese and the composition of milk from which it was made

Significant positive correlations were observed between the total protein retained in the cheese and the phosphorus content of the milk ($p < 0.001$, Table 7:15). Slight positive correlations were also observed between the total protein of the cheese and the RCT, and the protein content of the milk ($p < 0.05$). But, significant negative correlation was observed between the protein contents of the cheese and the fat contents of the milk ($p < 0.001$).

The soluble N content of the cheese was correlated positively with (a) the protein, NPN, soluble Ca, and FPD ($p < 0.01$) and (b) calcium, and soluble ash ($p < 0.05$) in the milk. Negative correlations were observed between the soluble N, and fat ($p < 0.001$); ash, total solids, and pH ($p < 0.05$) of milk.

The moisture content of the cheese showed positive correlations with titratable acidity, FPD, and SAV (by titration) ($p < 0.01$), soluble ash ($p < 0.05$). Negative correlations were also observed between the moisture content of the cheese and the pH, RCT, and the SAV (by pH) ($p < 0.001$) and ash ($p < 0.05$). The MFFC showed correlations with composition of milk which were similar to those found between the moisture content of cheese and the various parameters. However, MFFC was also positively correlated ($p < 0.05$) with the phosphorus content of the milk.

The pH of the cheese was correlated significantly with the ADV of the milk ($p < 0.001$). The pH of cheese did not correlate significantly

TABLE 7:14

The correlation coefficient between the microbiological counts of raw milk

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total count ($log_e$) | 1 | 1.0000 | | | | | |
| Psychrotrophic count ($log_e$) | 2 | 0.8668*** | 1.0000 | | | | |
| Coliform count ($log_e$) | 3 | 0.9521*** | 0.8305*** | 1.0000 | | | |
| Lipolytic count ($log_e$) | 4 | 0.9102*** | 0.8770*** | 0.8761*** | 1.0000 | | |
| Proteolytic count ($log_e$) | 5 | 0.9580*** | 0.8957 | 0.9154*** | 0.9362*** | 1.0000 | |
| Thermoduric count ($log_e$) | 6 | 0.0230 | -0.1489 | 0.0715 | -0.1653 | -0.0749 | 1.0000 |
| | | 1 | 2 | 3 | 4 | 5 | 6 |

***significant at 0.1 per cent level

TABLE 7:15

The correlation coefficients between the composition of Cheddar cheese (at 1 month old) and the composition of the cold-stored milk from which the cheese was made

| Milk composition | Protein (total N | Soluble N | Moisture | MFFC | pH | Fat | FFA at 9 month | FFA at 1 year | Calcium | Phos-phorus | Asn | Aqueous salt | Salt | Firmness (B.C.T) | Elas-ticity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total N (protein) | 0.4732* | 0.5475** | 0.1869 | 0.9738 | -0.2280 | -0.2911 | -0.1134 | -0.0909 | -0.1029 | -0.3804* | -0.1651 | -0.0916 | 0.0188 | 0.0353 | 0.3047 |
| Non-casein N | 0.0995 | 0.1327 | 0.2512 | 0.1936 | 0.2358 | -0.2405 | 0.3829* | 0.4023* | -0.2154 | -0.2876 | -0.2459 | -0.029 | 0.0501 | 0.2767 | -0.1172 |
| Non-protein N | 0.3678 | 0.5688** | 0.1459 | 0.0035 | -0.1885 | -0.3086 | 0.1600 | 0.2424 | -0.1457 | -0.5362 | -0.1582 | -0.0668 | 0.0308 | 0.0193 | 0.1320 |
| Calcium | 0.0543 | 0.4357* | -0.3531 | -0.3868* | -0.1533 | 0.2238 | -0.1253 | -0.0201 | 0.0667 | -0.0741 | -0.0685 | 0.0819 | -0.0967 | -0.5081** | 0.1447 |
| Phosphorus | 0.6397*** | 0.3806 | -0.2791 | -0.4672* | 0.1584 | -0.0453 | -0.2909 | -0.2343 | 0.2496 | -0.2077 | 0.3638 | 0.5261** | 0.5034** | -0.1845 | -0.2509 |
| Ash | -0.0657 | -0.4581* | -0.4665* | -0.5040** | 0.2847 | 0.3002 | -0.3849* | -0.3613 | 0.4459* | 0.4713* | 0.4572* | 0.4165* | 0.2714 | -0.3119 | 0.2963 |
| Fat | -0.7482*** | -0.8497 | 0.0882 | 0.2420 | 0.1973 | 0.1466 | 0.2920 | 0.2183 | -0.0383 | 0.4605* | 0.0765 | -0.1844 | -0.1842 | 0.2605 | -0.7707*** |
| Total solids | -0.1292 | -0.4415* | 0.0997 | 0.0926 | 0.0252 | -0.1617 | -0.2075 | -0.2866 | 0.1299 | 0.3186 | 0.0760 | -0.2320 | -0.1932 | 0.2527 | -0.1424 |
| Soluble calcium | 0.0485 | 0.5286** | 0.5665** | 0.4478* | -0.2075 | -0.5873** | 0.4615* | 0.4266* | -0.7090*** | -0.5307** | -0.3350 | -0.0247 | 0.2958 | 0.3178 | -0.3759 |
| Soluble phosphorus | -0.1849 | 0.3346 | 0.5630** | 0.5211** | -0.1578 | -0.4957* | 0.6129*** | 0.5640** | -0.6623*** | -0.4453* | -0.3492 | -0.2216 | 0.0652 | 0.4924* | -0.6324*** |
| Soluble asn | 0.0955 | 0.4313* | 0.4616* | 0.3894* | -0.2091 | -0.4228* | 0.4866* | 0.4231* | -0.5280** | -0.5551** | -0.2599 | -0.1086 | 0.1293 | 0.3566 | -0.4157* |
| SH groups | 0.1396 | -0.0087 | 0.1774 | 0.1538 | 0.2455 | -0.1642 | -0.1818 | -0.2204 | -0.1448 | 0.2458 | 0.0512 | 0.1250 | 0.1880 | 0.2367 | 0.0498 |
| Titratable acidity | -0.1976 | 0.3759 | 0.6624*** | 0.5924** | -0.1440 | -0.6036*** | 0.6567*** | 0.6613*** | -0.8403*** | -0.4958* | -0.4009* | -0.1276 | 0.2141 | 0.4559* | -0.5271** |
| pH | 0.1455 | -0.4151* | -0.6798*** | -0.5948** | 0.0828 | 0.6312*** | -0.6993*** | -0.7130*** | 0.8079*** | 0.5643** | 0.3971* | 0.1606 | -0.1798 | -0.5367** | 0.5046** |
| FPD | -0.1144 | 0.5153** | 0.7301*** | 0.6301*** | -0.2178 | -0.6854*** | 0.6150*** | 0.6135*** | -0.8713*** | -0.5839** | -0.4726* | -0.2074 | 0.1536 | 0.5012** | -0.4965** |
| RCT | 0.4510* | -0.1743 | -0.7112*** | -0.7177*** | 0.1512 | 0.5405** | -0.7185*** | -0.7087*** | 0.8692*** | 0.3357 | 0.5095** | 0.2506 | -0.0761 | -0.5065** | 0.6663*** |
| SAV (by titration) | -0.2014 | 0.2163 | 0.7157*** | 0.5818** | 0.1627 | -0.7150*** | 0.6991*** | 0.6170*** | -0.7208*** | -0.5552** | -0.3488 | -0.1581 | 0.2059 | 0.5710** | -0.5977*** |
| SAV (by pH) | -0.0438 | -0.1969 | -0.6230*** | -0.4929* | -0.2296 | 0.6532*** | -0.4964** | -0.4092* | 0.5191** | 0.4099* | 0.2853 | 0.0260 | -0.2985 | -0.5130** | 0.3360 |
| ADV | -0.3663 | -0.2559 | 0.2085 | 0.1354 | 0.5113*** | -0.2010 | 0.5375** | 0.5003** | -0.1436 | -0.1081 | 0.0790 | -0.1485 | -0.0548 | 0.3771 | -0.6490*** |

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

with other milk constituents.

The fat content of cheese had a positive correlation with the pH of milk, and the SAV (by pH) and the RCT ($p < 0.01$) and negatively with titratable acidity, FPD and, SAV (by titration) ($p < 0.001$); with soluble calcium ($p < 0.01$) and with soluble phosphorus, and soluble ash contents of the milk ($p < 0.05$).

The level of lipolysis in the cheese as determined by measuring the free fatty acids values of nine and twelve-months-old cheeses correlated positively with the level of soluble phosphorus, titratable acidity, FPD, and SAV (by titration) ($p < 0.001$) and with the ADV of milk ($p < 0.01$), and the NCN, soluble Ca, and soluble ash ($p < 0.05$). It correlated negatively with pH, and RCT ($p < 0.001$) with ash and SAV (by pH) ($p < 0.05$). Patterns of lipolysis of the 9 and 12 months-old cheeses were similar.

The calcium retained in the cheese was positively correlated with the milk pH, and RCT ($p < 0.001$), and SAV (by pH) ($p < 0.01$). While it is negatively correlated with soluble Ca, soluble P, titratable acidity, FPD, the SAV (by titration) ($p < 0.001$) and soluble ash ($p < 0.01$). The phosphorus content of the cheese was correlated positively with the pH of milk ($p < 0.001$); and with the ash, fat, and SAV (by pH) ($p < 0.05$). On the other hand it was correlated negatively with soluble Ca, soluble ash, FPD, and SAV (by titration) ($p < 0.01$) and with protein, soluble phosphorus, and titratable acidity of milk ($p < 0.05$). The ash content was correlated positively with RCT ($p < 0.01$) and with ash, and pH ($p < 0.05$) and negatively with titratable acidity, and FPD ($p < 0.05$).

The aqueous salt level showed positive correlation with phosphorus ($p < 0.01$) and ash ($p < 0.05$) contents of the milk. The total salt retained in the cheese was positively correlated only with the phosphorus content of the milk ($p < 0.01$).

The firmness of cheese is positively correlated with FPD, and the SAV (by titration ($p < 0.01$); and with soluble phosphorus, and titratable acidity of milk ($p < 0.05$) it was negatively correlated to calcium, pH, RCT, and SAV (by pH) ($p < 0.01$).

The elasticity was correlated positively with the RCT ($p < 0.001$) and pH ($p < 0.01$) of the milk. It is correlated negatively with fat, soluble phosphorus, SAV (by titration), and ADV ($p < 0.001$); and milk TA, and FPD ($p < 0.01$).

Regression analysis was carried out for the correlation between some milk components and cheese composition (Tables 7:16 and 7:17, Figs 7:12 and 7:13). It is obvious that by using milk with higher concentrations of protein the resultant cheese would have higher contents of protein and soluble N. Increases in the NPN in the milk resulted in higher soluble N contents in the cheese.

Milks with higher ADV resulted in cheeses with higher level of FFA. On the other hand, milks with higher soluble calcium resulted in cheeses with lower calcium and phosphorus contents. These results made it possible to predict the composition of the cheese provided the chemical analysis of the milk was known and provided the conditions of cheese-making remained similar.

6. The correlation between the microbiological quality and composition of milk and the yield of Cheddar cheese

The yield of Cheddar cheese was found to correlate positively (Table 7:18) with milk fat, and SAV (by titration) ($p < 0.05$). However, it was correlated negatively with the phosphorus content of the milk, and RCT ($p < 0.05$); with SAV (by pH) ($p < 0.05$); and with calcium.

In correlating the yield of cheese as calculated on a moisture content of 35 per cent (w/w) some of the water soluble components of milk showed different correlations from the previously mentioned. When this principle was used the yield of cheese was correlated positively with total solids ($p < 0.01$); and fat ($p < 0.05$); and negatively with NPN ($p < 0.05$); soluble calcium ($p < 0.01$); and soluble ash ($p < 0.05$).

When the correlation coefficients were calculated excluding the results of milk held at $6^{o}C$ for 7 d, most of the previously observed correlations diminished in significance.

TABLE 7:16

The regression analysis for the correlation between (a) milk protein and cheese protein, (b) milk protein and soluble N of cheese and (c) NPN in milk and soluble N in cheese

| | Estimate | E.S. | T |
|---|---|---|---|
| Y intercept (cheese protein) | 14.4206 | 8.8501 | 1.63 |
| Slope (milk protein) | 2.6112 | 2.9843 | 0.87 |
| Y intercept (soluble N) | -3.2627 | 1.3760 | -2.37 |
| Slope (milk protein) | 1.5614 | 0.4585 | 3.41 |
| Y intercept (soluble N) | 0.56713 | 0.27657 | 20.5 |
| Slope (NPN) | 4.98438 | 1.59640 | 3.12 |

| | DF | Milk protein and cheese protein | | Milk protein and soluble N of cheese | | NPN of milk and soluble N in cheese | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 0.61 | 0.6052 | 0.536 | 0.53633 | 0.474 | 0.47419 |
| Residual | 19 | 15.02 | 0.7905 | 1.203 | 0.04625 | 1.265 | 0.04864 |
| Total | 20 | 15.62 | 0.7812 | 1.739 | 0.06440 | 1.739 | 0.06440 |

Fig. 7:12 Standard curves for the correlation between (a) milk protein and cheese protein, (b) milk protein and soluble N in cheese and (c) NPN in milk and soluble N in cheese

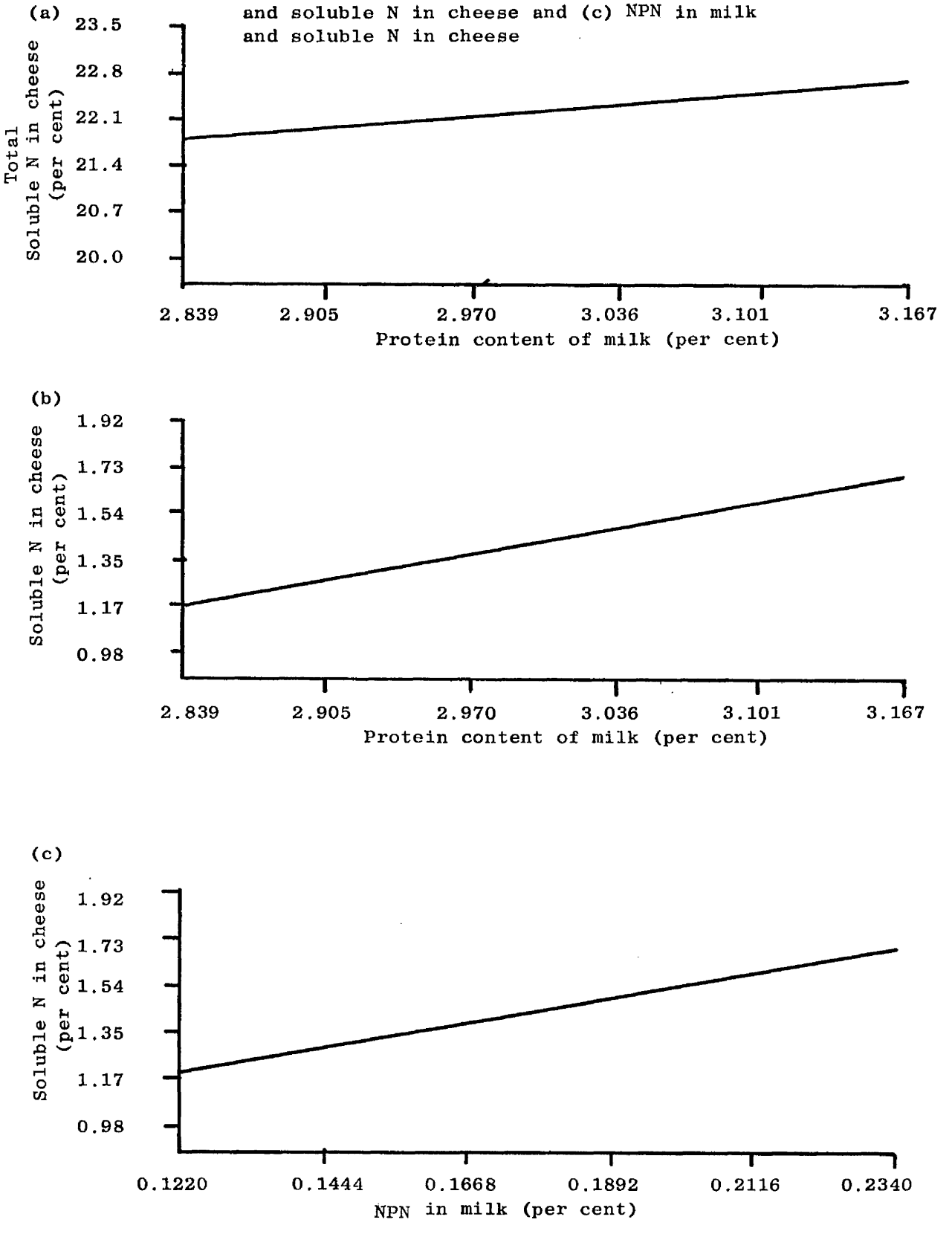


TABLE 7:17

The regression analysis for the correlation between the (a) FFA of cheese and the ADV of milk, (b) soluble calcium of milk and calcium content in cheese and (c) phosphorus content of cheese and soluble calcium in milk

| | Estimate | S.E. | T |
|---|---|---|---|
| Y intercept (FFA of cheese) | -8.9670 | 5.0732 | -1.77 |
| Slope (ADV of milk) | 7.4707 | 2.7980 | 2.67 |
| Y intercept (cheese calcium) | 207.5043 | 5.8663 | 35.37 |
| Slope (soluble Ca) | -3.6481 | 0.8885 | -4.11 |
| Y incercept (cheese phosphorus) | 506.4949 | 19.1694 | 26.42 |
| Slope (soluble Ca) | -8.5047 | 2.6640 | 3.19 |

Analysis of variance

| | DF | FFA of cheese and ADV of milk | | Cheese Ca and and soluble Ca | | Cheese phosphorus and soluble Ca | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | SS | MS | SS | MS |
| Regression | 1 | 179.0 | 179.02 | 1954 | 1954.2 | 21000 | 21000 |
| Residual | 19 | 477.1 | 25.11 | 2202 | 115.9 | 53574 | 2061 |
| Total | 20 | 656.1 | 32.81 | 4157 | 207.8 | 74563 | 2762 |

Fig. 7:13 Standard curves for the correlations between (a) FFA of cheese and ADV of milk, (b) soluble calcium in milk and calcium content of cheese, and (c) soluble calcium content of milk and phosphorus content of cheese

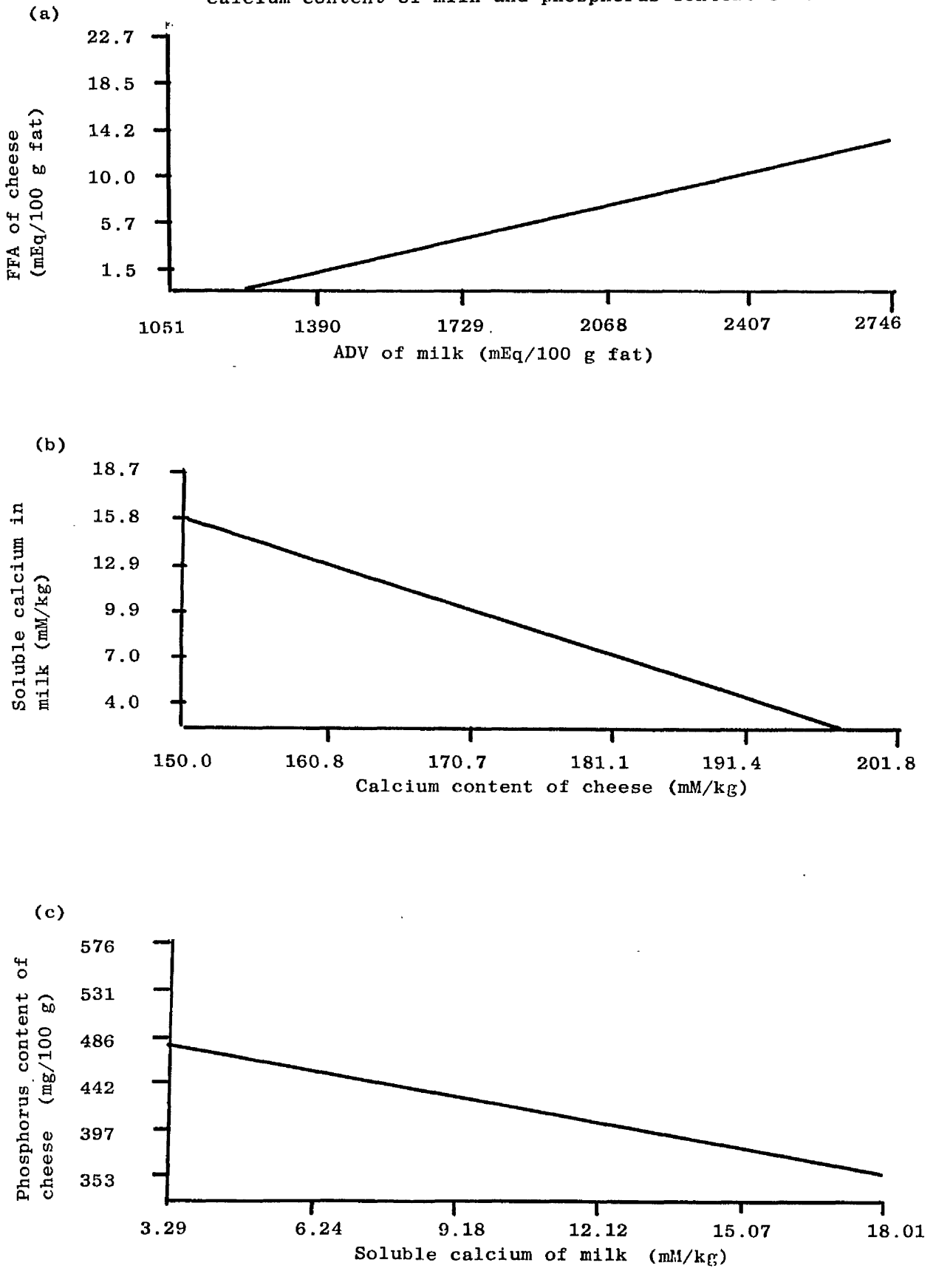


TABLE 7:18

The correlation coefficient between the microbiological quality and composition of milk and the yield of Cheddar cheese

| | Yield for overall the experimental storage conditions (DF = 25) | | Ignoring the 7th day storage of milk at 6°C (DF = 21)+ | |
|---|---|---|---|---|
| | Cheese yield | Yield calculated on 35% moisture basis | Cheese yield | Yield calculated 35% (w/w) moisture in cheese |
| Protein | 0.0604 | -0.0391 | 0.0832 | 0.0301 |
| Non-casein N | 0.3167 | 0.0817 | 0.3754 | 0.1536 |
| Non-Protein N | -0.1669 | -0.4347* | -0.1848 | -0.3033 |
| Calcium | -0.4992** | -0.4596* | -0.6706*** | -0.6021** |
| Phosphorus | -0.4458* | -0.3103 | -0.0793 | -0.0553 |
| Ash | -0.2332 | 0.1904 | 0.3316 | 0.4155 |
| Fat | 0.4089* | 0.4748* | 0.3731 | 0.3924 |
| ADV | 0.2258 | -0.0100 | 0.0153 | -0.0454 |
| Total solids | 0.4162* | 0.5851** | 0.5876** | 0.5864** |
| Soluble calcium | 0.1175 | -0.5033** | -0.2703 | -0.2554 |
| Soluble phosphorus | 0.2752 | -0.3468 | -0.1345 | -0.2624 |
| Soluble ash | 0.1001 | -0.4078* | -0.2384 | -0.2684 |
| SH groups | 0.2248 | 0.2482 | 0.1455 | 0.3838 |
| Titratable acidity | 0.2865 | -0.3681 | -0.2581 | -0.3344 |
| pH | -0.3327 | 0.3168 | 0.0393 | 0.1764 |
| Freezing point depression | 0.3254 | -0.3502 | -0.1931 | -0.3221 |
| RCT | -0.4677* | 0.1539 | -0.2265 | -0.2141 |
| SAV (by titration) | 0.4455* | -0.1428 | 0.3657 | 0.2694 |
| SAV (by pH) | -0.4392 | -0.0487 | -0.4204 | -0.4267* |
| Total count ($\log_e$) | 0.2324 | -0.2191 | -0.3763 | -0.4682 |
| Psychrotrophic count($\log_e$) | 0.0903 | -0.2455 | -0.3380 | -0.3736 |
| Coliform count ($\log_e$) | 0.2914 | -0.1911 | -0.2577 | -0.3678 |
| Lipolytic count ($\log_e$) | 0.0210 | -0.3522 | -0.3650 | -0.4628 |
| Proteolytic count ($\log_e$) | 0.2331 | -0.1432 | -0.2017 | -0.2843 |
| Thermoduric count ($\log_e$) | -0.0930 | -0.1400 | -0.1831 | -0.0792 |

Note: The degree of freedom for microbiological characteristics is 16.

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

+because of abnormal cheesemaking with milk held at 6°C for 7 days.

TABLE 7:19

The correlation coefficient between the organoleptic quality of milk and its composition and quality

| Organoleptic quality of milk / Composition of milk | Flavour | Odour | Appearance |
|---|---|---|---|
| Protein | 0.4333 | 0.4042 | -0.6310 |
| Non-casein N | -0.4431 | -0.3836 | 0.2638 |
| Non-protein N | -0.9510*** | -0.8615* | -0.1799 |
| Calcium | 0.3551 | 0.4107 | 0.1686 |
| Phosphorus | 0.2214 | 0.0785 | -0.3955 |
| Ash | 0.3467 | 0.4581 | -0.1316 |
| Fat | -0.4422 | -0.4278 | 0.2438 |
| ADV | -0.4574 | -0.3674 | 0.6045 |
| Total solids | 0.2198 | 0.4365 | 0.0570 |
| Soluble calcium | -0.6081 | -0.7204 | -0.1306 |
| Soluble phosphorus | -0.5394 | -0.6246 | -0.3472 |
| Soluble ash | -0.4271 | -0.4586 | 0.2384 |
| SH groups | 0.3102 | 0.3086 | 0.5472 |
| Titratable acidity | -0.7605* | -0.8942** | -0.2805 |
| pH | 0.8383* | 0.9355** | -0.2805 |
| Freezing point depression (FPD) | -0.7818* | -0.8830** | -0.3045 |
| Total count ($\log_e$) | -0.7246 | -0.8111* | 0.1737 |
| Psychrotrophic count ($\log_e$) | -0.7414 | -0.8271* | 0.1929 |
| Coliform count ($\log_e$) | -0.8852** | -0.9229** | -0.0445 |
| Lipolytic count ($\log_e$) | -0.6765 | -0.7761* | 0.2639 |
| Proteolytic count ($\log_e$) | -0.6307 | -0.7596* | 0.2496 |
| Thermoduric count ($\log_e$) | -0.8505* | -0.8447* | -0.0499 |

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

TABLE 7:20

The correlation coefficient between the organoleptic quality of milk and cheese

| Organoleptic quality of milk / Cheese characteristics | Flavour | Odour | Appearance |
|---|---|---|---|
| Flavour | 0.9621*** | 0.9479** | 0.3154 |
| Taste | 0.9727*** | 0.9414** | 0.3009 |
| Texture | 0.8497* | 0.8967** | 0.2403 |
| Body | 0.8639* | 0.7605* | 0.2524 |
| Colour | 0.8974** | 0.8855** | 0.2772 |
| Openness | 0.3766 | 0.2700 | 0.0323 |

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

The cheese yield (at 35 per cent moisture level) correlated positively with SH groups of milk and negatively with total and lipolytic counts when the yield of the 7th day of storage at 6°C was ignored. These correlations were almost significant.

The results involving the use of milk held at 6°C for 7 d were ignored because of the abnormality of cheesemaking with such deteriorated milk.

7. The correlation between organoleptic quality of milk and its composition and microbiological quality

The flavour of milk showed positive correlation with pH ($p < 0.05$, Table 7:19). It showed negative correlations with NPN ($p < 0.001$); coliform ($p < 0.01$); titratable acidity, FPD, and thermoduric count ($p < 0.05$).

The odour of milk showed positive correlations with pH ($p < 0.01$) and negative correlations with titratable acidity, FPD, and coliform ($p < 0.01$); NPN, total psychrotrophic, lipolytic, proteolytic, and thermoduric counts ($p < 0.05$). The appearance of milk did not show any significant correlation with the milk composition and its microbiological quality.

8. The correlation between the organoleptic quality of milk and cheese

The flavour and odour scores of the raw milk showed significantly positive correlations with the organoleptic characteristics of the cheese except the openness of cheese (Table 7:20). Milk appearance did not show any correlations either with the organoleptic quality or the composition of cheese.

## DISCUSSION

1. The correlation between the individual organoleptic characteristics of cheese during curing

The results of this study showed a very close correlation between the different characteristics of cheese during the curing period. This

provides evidence concerning the assumption of Tobias (1976) that the body and texture of Cheddar cheese is often closely correlated with flavour because the same factors may affect both. Excessive acidity will produce an acid flavour, and at the same time give a short and mealy body. The presence of gas holes signifies an undesirable fermentation which may give rise to fruity, yeasty or unclean flavour. Such defects in quality may also be associated with a high moisture content of the cheese and be also related to weak or pasty body.

Most trained graders in Scotland rely on the body and texture to judge the cheese quality. The grader of the cheese gave some comments on cheese characteristics. It was observed that weak, crumbly and sticky body is associated with high acid and unclean flavour. The high acid cheese were faint in colour due to the discolouration which occurred at lower pH. Cheeses with high moisture content were compact and sticky. The regression analysis supported the significant relation between moisture content of the cheese and its body and texture scores.

The openness of cheese did not show a high correlation with other characteristics of the cheese at 2 months old. But, later when the cheese was from 4 to 12 months old the correlation between openness and other properties were higher and in many cases were significant. This might be due to the bacterial and enzymatic activity which took place on further curing and resulted in changes in the texture and body of cheese to a degree sufficient to change the openness of the cheese. Hence, the correlations between openness and other cheese characteristics were higher than during the earlier stages of cheese ripening. The colour of the cheese at 2 months did not correlate significantly with the body but later when the cheese was 4 months or older a correlation was evident.

## 2. The correlation between the composition and the organoleptic assessment of cheese

Cheese can be defined as "the fresh or matured product obtained by the drainage (of liquid) after the coagulation of milk, cream, skimmed or partly skimmed milk, butter milk or a combination thereof" (Scott, 1981). Cheese is composed of the casein and usually almost of the fat, insoluble salts, and colloidal materials, along with part of the whey

solids of the milk used. This whey contains lactose, whey protein, soluble salts, vitamins and other milk solids (Lee, 1975).

During the ripening of Cheddar cheese, the insoluble nitrogenous components undergo change, which convert them (in whole or part) to soluble forms (Lee, 1975). In the course of this progressive proteolysis, the paracasein and the lower molecular weight proteins are slowly converted to simple nitrogenous compounds including proteoses, peptones, amino acids and ammonia.

It is well knownthat the calcium ion is an important binding agent for the casein after the action of rennet during cheesemaking. The balance between calcium and phosphorus is also of importance during this process.

The ash constituents include potassium, sodium, calcium and magnesium which are present in larger amounts as well as smaller quantities of aluminium, iron, copper, manganese and zinc and arsenic, iodine, fluorine and other elements present in these amounts (Joslyn, 1970). While the overall amounts of these constituents in cheese are small they are nevertheless of great importance in relation to their role in the structure of cheese components. Typical examples are:- cobalt which forms the centre of the vitamin B12 complex; zinc in carbonic anhydrase; magnesium in arginase; iron in both xanthine oxidase and lactoperoxidase (Scott, 1981).

Salt (sodium chloride) is added to cheese curd for two main purposes: to control ripening and to improve flavour. Lee (1975) reported that salt also has a desiccating effect so increasing firmness. Too little salt in Cheddar cheese causes a weak and pasty body, abnormal ripening, and increased shrinkage in curing, while too much salt causes a dry, brittle, body. The correlation observed between the salt in aqueous phase and the organoleptic scores was higher than the correlation between total salt and the organoleptic scores. This might have been due to the retention of higher moisture in the cheese manufactured from milk stored at $6^{o}C$ compared to the unstored milks. O'Connor (1968) reported that acid bitterness decreased with increased salt, "fruity" flavours usually accompanied insufficient acid development, and cheese containing over 2 per cent salt failed to mature normally. O'Connor (1968) found a good relationship between salt content of cheese and the

total organoleptic score. He reported that the best average overall score was obtained for cheese with an average salt content of 1.18 per cent (w/w) while the lowest overall score was obtained for cheese with an average salt content of 2.58 per cent (w/w). He also observed that the pH values show a definite increase with increase in salt content but these differences in pH values had disappeared in the cheese at 8 weeks old when the maximum difference was 0.09 pH unit compared with 0.32 pH unit in the cheese at 1 d old.

Of all the characteristics measured, the FFA had the highest correlations with the organoleptic quality of the cheese. It is believed (Deeth and Fitz-Gerald, 1976) that excessive levels of free fatty acids impart unclean, butyric or rancid flavours to cheese. However, the same authors stated that the grading results do not always agree well with the chemical results because of the presence of numerous other flavours unrelated to the chemical analysis performed. The free fatty acids aid in the development of oxidized flavour in three ways:

1. the acids themselves oxidize more readily when they are free than when they are combined in the glycerides of normal fat,

2. they catalyse the oxidation of the fat by decomposing the peroxides, the first formed, flavourless substances of the oxidation reaction, to the smaller compounds responsible for the oxidized flavour, and

3. they retard the action of natural antioxidant which help to prevent oxidation.

The ADV of good Cheddar cheese is around 1.2 - 1.8, whereas rancid or butyric flavours are evident at ADVs over about 3 and values as high as 15 may be encountered in extremely poor cheese (Deeth and Fitz-Gerald, 1976). Law et al. (1976) found no evidence that free fatty acids had a direct beneficial effect on Cheddar cheese flavour. High concentration of FFA produced by lipases from heat-resistant psychrotrophs, were distinguished organoleptically as rancid and soapy off-flavours. Forss (1979) reported that cheesiness and characteristic cheese flavours may originate from lipid and non-lipid components. Certain conditions are required to control the flavour development in cheese. These conditions are:- a supply of flavour precursors derived from the enzymatic breakdown

of lactose, protein and fat; a high acidity which prevents most enzyme-catalyst reactions from proceeding too rapidly and suppresses the growth of spoilage organisms; a low redox potential (-150 to - 200 mV) which is important not only in controlling general reaction equilibria but also in maintaining compounds such as methanethiol in their reduced form.

Recently, Law (1981) stated that the relationship between fat or protein breakdowns and flavour development are used as indices of ripening and these products probably contribute to the development of back-ground taste in all cheeses by releasing FFAs and amino acids. The close correlations found in this study are in agreement with the last mentioned theory.

Firmness (B.C.T) of cheese showed a significant negative correlation with the organolpetic assessment of the cheese at 2 and 4 months. The highest correlation was observed between firmness and body and texture as detected by hand feel. These correlations were present but not significant at 8 and 12 months. On the other hand, elasticity showed slight positive correlation with organoleptic assessment and these correlations became stronger at the later stage of cheese ripening. The author has no further explanation for these correlations but it is well known that the sensory grading by subjective means does not always agree with objective measurements obtained by instruments.

As expected, significant correlations were observed between total protein and the organoleptic data. Increasing levels of protein in the cheese correlated with higher grade scores.

Soluble N showed a slight negative correlation which was not significant with the flavour and taste of cheese at 2 and 4 months. Further ripening until cheese was 8 and 12 months old resulted in a significant positive correlation between soluble N and the sensory characteristics of the cheese.

These correlations can be understood if we consider that the first negative correlation is due to bitter peptides which might have developed as a result of protein breakdown in some cheeses. On further ripening and due to the breakdown of these bitter peptides into non-bitter compounds, the acceptability of the cheese was higher. These findings are in agreement with the steps of breakdown of caseins

reported by Crawford (1977):

1. degradation of casein by rennet to produce a pool of high molecular weight peptides which are mostly non-bitter;

2. some of these peptides are hydrolysed by protease of starter streptococci to low molecular weight peptides which are bitter;

3. the third stage concerns further degradation to non-bitter peptides and amino acids by peptidases of starter streptococci.

Forss (1979) reported that amino acids and proteins, while not volatile themselves, contribute in many ways to flavour. Apart from being precursors of many aroma compounds they may completely change the flavour of a food through reaction of the α-amino group of lysine with carbonyl compounds and lower fatty acids resulting from lipid oxidation.

The close correlations observed between degradation of fat and protein and sensory characteristic may be attributed to the close correlation between these sensory characteristics. In other words, if the flavour of the cheese is affected, the other sensory characteristics will be affected. These findings are in agreement with the recent results of Cheeseman (1981) who reported that the hydrolytic changes occurring during maturation are not only important in regard to flavour development but also influence changes in body and texture of the cheese.

Moisture was observed to be a very important factor in controlling the organoleptic quality of the cheese during ripening. The higher the moisture content of the cheese the worse the quality would be. The amount of moisture present controls the concentration of solubles which are metabolites for bacteria and control their growth by the osmotic effect on the cell walls of the organisms. Thus the growth of micro-organisms is more prolific in high moisture curd than in low moisture curd, and the ripening rate of high moisture cheese is faster than for low moisture cheese (Scott, 1981).

Positive correlations were observed between the pH of the cheese and its organoleptic quality. But, these correlations were not significant until at 12 months of age. Pearce and Gilles (1979) found close correlation between the pH at 14 day old cheese and cheese quality.

Scott (1981) reported that the gradual change in pH value from stage to stage enables ripening to proceed somewhat stepwise, and at any one time the flavour and aroma compounds reach a specific level of intensity. As the ripening proceeds, some of the earlier flavour (or aroma) compounds may be used up, and the more strident flavours disappear and are replaced by more acceptable mellow flavours.

3. The correlation between the microbiological quality and the composition of milk on delivery and after 2, 4 and 7 days of storage at 2°C and 6°C

The close positive correlations between the microbiological quality of milk and the protein breakdown during storage of milk are in agreement with the results of the preliminary experiments of this study (Chapter Two). The correlations between the microbiological quality of milk and its NPN content are closer than that of the microbiological quality and NCN. This might indicate that the breakdown which occurred in the casein is more likely to be due to the natural occurring enzymatic action rather than microbiological activity. On the other hand the breakdown of the other milk proteins is mainly due to the microbial activity in the milk.

Significant correlations were also observed between ADV and the microbiological quality of milk, particularly the lipolytic and proteolytic counts. This indicates that the lipolytic micro-organisms play an important role in the breakdown of milk fat. Due to the importance of NPN and ADV levels in indicating the level of proteolysis and lipolysis respectively in milk, further regression analysis was carried out to investigate the effect of increasing bacterial numbers on these two characteristics of the milk. Increased numbers of bacteria in the milk were associated with increases in the level of ADV and NPN contents in milks.

There were significant correlations between NPN and ADV on one hand and between the lipolytic and proteolytic counts on the other hand ($r = 0.936$). It might be useful to examine whether or not the products of growth of one group of organisms affected the other. As expected, close correlations were also observed between all the microbiological counts under study except the thermoduric count. Titratable acidity,

pH and FPD (after 4 d at 6°C) are closely related to the microbiological counts. Acid-producing bacteria are the main reason for such correlations and the counts of the coliform organisms was most correlated with the titratable acidity, pH and FPD of the milk.

The findings of this study are in good agreement with those of other authors. Arima et al. (1965) found that abnormality in chemical composition, heat stability, alcohol test and flavour were found when the total bacterial count exceeded 4 million/ml.

In studies involving storage of milk for up to 3 days at 5°C Kaczorek et al. (1973) reported no relationship between the chemical characteristics e.g. methylene blue reduction, $NH_3$ content, residual formol value, and proteose and peptone content, and the numbers and ratios of caseolytic, psychrotrophic and coli-aerogenes bacteria except for a relationship between the caseolytic count and the $NH_3$ content in fresh and 24 h old milk.

Juffs (1973) investigated some aspects of quality of bulk refrigerated raw milk supplied to Brisbane in Australia for the production of pasteurized milk. Significant positive relationships between the tyrosine value (TV) and the total bacterial count for supplies from some sources, but in no instance was a significant relationship found between TV and psychrotrophic or proteolytic counts. Significant positive relationships were found between the TV of bulk milk supplies from some sources and atmospheric temperature. The lack of relationship of TV with either the psychrotrophic count or the numbers of proteolytic psychrotrophs was somewhat unexpected in view of refrigerated storage of the milk. Juffs (1973) was of the opinion that this might indicate that the proteinase responsible for any increase in TV of bulk milks was produced by the general contaminant microflora growing on farm or factory equipment rather than by bacteria actually growing in the refrigerated milk during storage. On the basis of this evidence, Juffs concluded that the TV estimation is of limited value as a quality test for bulk raw refrigerated milk stored for 2 d or less. In later work the same author (Juffs, 1975a) found relationships between total bacterial count, TV and organoleptic quality of cold-stored milk where the storage time was intended to 7-10 days at 5°C. Carini et al.

(1977) reported that high counts (numbers not stated) were found for total bacteria, lactic acid bacteria and thermoduric, proteolytic and coliform organisms in milk samples stored for 6-48 h at 5-6°C. The pH and NPN were affected and there were changes in the electrophoretic properties of casein and whey proteins (time not stated).

Hicks et al. (1978) found that the NCN and FFA contents of Grade A raw milk increased with storage time up to 10 d at 5°C and as the psychrotrophic and total counts increased. In the same year, Muir et al. (1978) found no statistically significant correlation between FFA in stored milk (up to 48 h at 6°C) and the mesophilic count. The correlations between the 'Gram-negative' count and FFA almost reached significance. However, both the psychrotrophic count ($p < 0.05$) and the water agar test counts ($p < 0.01$) did correlate significantly with the concentrations of FFA after storage. The lipolysis which occurred on storage was significantly correlated with the total counts of psychrotrophic bacteria and the counts by water agar test on the milks before storage. Luhtala et al. (1970) reported a slight positive correlation (not significant) between the somatic cell count and the glyceride synthesis. There was no demonstrable correlation between the original lipase activity of milk and the glyceride synthesis.

In commercial trials Ledford et al. (1981) studied the correlation between the chemical characteristics of milk and microbiological changes on storage of up to 13 d (temperature is not stated). They reported the following r values: 0.98 for standard plate count vs rapid psychrotrophic count; 0.72 for standard plate count vs pyruvate value; 0.71 for rapid plate count vs pyruvate value; 0.61 for standard plate count vs ADV; 0.60 for rapid psychrotrophic count vs ADV; 0.58 for standard plate count vs tyrosine value and 0.59 for rapid psychrotrophic count vs tyrosine value. The 7 samples (out of 24) having flavour scores of 7.00 or better at 13 days had average log standard plate count and rapid psychrotrophic count of 5.16 and 5.04, respectively. Shipe et al. (1981) found that the average daily increase in ADV of commercial milks (used in previous trials by Ledford et al. (1981) was 0.06 during the first 6 d of storage and 0.10 during the last 7 d. The corresponding average daily increase in tyrosine values was 1.2 and 1.9 mg respectively and the pyruvate values increased by 0.4 mg/l and 1.4 mg/l for the first 6 d and last 7 d, respectively. Inhibition of microbial growth by the

addition of merthiolate suppressed the rate of change in the values.

In the present work the significant positive correlations between the microbiological counts and level of soluble calcium, soluble phosphorus and soluble ash may be due to the fact that these components are present in the soluble phase. It is well known that bacterial growth is dependent on the absorption of nutrient from the growth medium. It is not surprising then to see the correlation between the microbial numbers and the level of these components which are necessary for the metabolic activity of the bacterial cell. The importance of phosphorus is due to a component of nucleic acids, phospholipids and coenzymes, (Berkeley and Campbell, 1979). Calcium acts in the stability of some extracellular enzymes, and in bacterial sporulation. The soluble ash which includes phosphorus and calcium, as well as sulphur, potassium, sodium, iron, copper, zinc etc. is also necessary for some bacterial metabolic activities.

The significant negative correlations between the microbiological counts and RCT looks to be an indirect effect. Milks with higher bacterial counts are associated with lower pH which help the renneting process proceed more rapidly. In these milks the amount of phosphorus and NPN soluble calcium and soluble phosphorus and NPN were greater than those found in milks with a lower bacterial count. This may indicate that the colloidal state of casein micelles is rather disturbed and hence the RCT would be shorter. These findings are in agreement with the results of Cousin and Marth (1977a) who found that raw milks (held for up to 8 d at $7^{\circ}C$) inoculated with Lactobacillus spp. and Pseudomonas spp. coagulated more slowly with rennet than uninoculated milk. They suggest that the added bacteria may have retarded activity of the normal flora. In the same article, Cousin and Marth (1977a) compared the RCT of stored and unstored milks. They reported that after 2 d of refrigerated storage, raw milk inoculated with all psychrotrophic bacteria (Flavobacterium spp., Lactobacillus spp., Pseudomonas spp.) required less time for coagulation than it did initially. This was also true for the uninoculated raw milk. It is possible that these bacteria interacted with the native microflora causing a greater decrease in RCT than when the pure cultures were not added.

As expected, significant positive correlation existed between the SAV (by

titration) and the microbiological counts, the highest correlations being with the coliform counts. These correlations may be due to the higher titratable acidity of milk before inoculation of the starter culture. There is also the possibility of an interaction between the starter cultures and the native microflora causing the significance of these correlations. On the other hand, the SAV (by pH) correlated negatively with the bacterial counts.

4. The correlation between the different microbiological counts

The strong correlations between the different bacterial counts are to be expected if we consider that under the refrigeration conditions the most active lipolytic and proteolytic organisms are the psychrotrophs. The strong correlation between lipolytic and proteolytic counts may be due to the action of the proteolytic bacteria on the fat globule membrane which helps to free more fat for use as a nutrient by the lipolytic bacteria. Mergl and Cerna (1969) found the following count/ml in milk initially (milk obtained under hygienic condition from 6 cows of one producer) and after storage at $5^{o}C$ for 49 and 126 h: total count, 7000, 14500 and 178000; coliform 10, 340 and 3250; psychrophils 4,400, 10,000 and 2,100,000; proteolytic organisms 500, 450 and 7,800; lipolytic organisms 225,305 and 2,100. Counts of other organisms (enterococci, aerobic and anaerobic spore-formers, thermodurics, yeasts and moulds) showed little or no change. Titratable acidity, pH and organoleptic characteristics did not change throughout the period of storage and the alcohol test remained negative. Kiuru et al. (1971) found that 53 per cent of 300 strains of psychrotrophic micro-organisms isolated from samples of farm tank milk were proteolytic and were composed of Pseudomonas spp. 84 per cent, Enterobacter spp. 5 per cent and Flavobacterium spp. 5 per cent and other strains 6 per cent.

The mean total colony count for the raw milk used in this study were (a) on the day of delivery by the road tanker, $203 \times 10^{3}$ per ml, (b) after 7 d storage of the raw milk at $2^{o}C$ the count had increased to $18 \times 10^{6}$ per ml, (c) the storage of the same milk at $6^{o}C$ for 7 d resulted in a count of $224 \times 10^{6}$ per ml. The mean counts for (a), (b) and (c), for coliform were $3 \times 10^{3}$, $291 \times 10^{3}$ and $3 \times 10^{6}$ per ml; for psychrotrophic organisms, $175 \times 10^{3}$, $22 \times 10^{6}$ and $246 \times 10^{6}$ per ml; for lipolytic count, $40 \times 10^{3}$, $87 \times 10^{6}$ and $501 \times 10^{6}$ per ml; for

proteolytic count, 200 x $10^3$, 47 x $10^6$ and 258 x $10^6$ per ml; and for thermoduric count 8 x $10^3$, 866 x $10^3$ and 893 x $10^3$ per ml respectively (Al-Saltan, 1982).

5. The correlation between the composition of cheese and the composition of milk from which it was made

The cheese solids, mainly fat and casein, are held in suspension in colloidal solution in milk and are mechanically held in rennet coagulum, which forms the cheese curd (Chapman, 1981). Fat and casein form over 90 per cent of the solid portion of cheese. The other cheese solids are made up of calcium salts of phosphoric, lactic, and citric acids, the salt added during cheesemaking, a small amount of albumin, and some lactose which mostly disappears in a few days. The protein content of the cheese is positively correlated with the phosphorus and protein contents of milk. This indicates that the higher the phosphorus and protein content of the milk, the higher will be the protein content of the cheese.

The total protein of cheese made in these experiments was correlated positively with RCT which indicates that an increase in the RCT during storage of milk (at 2°C) might allow more time for the protein to be retained in the curd. But, in the case of a reduction in RCT as a result of storage of the milk for more than two days at 6°C this action was due to the decrease in pH which increased the milk proteins lost into whey. As expected, the fat content of milk correlated negatively with total protein of cheese. This might be due to the change in casein/fat ratio which is important in cheesemaking and which might be altered as a result of cold storage at higher temperature for longer time.

The increase in soluble N in cheese was associated with higher protein, NPN, calcium, soluble calcium and soluble ash content in the milk.

The negative correlation between the pH and soluble N might be due to the greater loss of nitrogenous components into the whey when the pH of the milk is low, particularly when the milk was stored for 7 d at 6°C.

The positive correlations between moisture and MFFC values of the

cheese and the soluble calcium, soluble phosphorus and soluble ash might be due to the higher content of cold-stored milk of these soluble components. Cheese produced from these milks retained more moisture. The correlation between the composition of milk and the composition of cheese might be influenced by other factors which affected the statistical correlations indirectly. The correlations observed in these studies need to consider the overall effect of cold storage of milk on its composition and the resultant effect on the cheesemaking procedure.

The shorter RCT was associated with less moisture lost into the whey during cheesemaking compared to that lost with the longer RCT. The loss of moisture into the whey may be associated with the loss of some water soluble components (e.g. calcium and phosphorus).

Chapman and Sharpe (1981) stated that as the rigidity of the rennet coagulum increases, its water holding capacity decreases. The starter bacteria are trapped in the coagulum at renneting, and the majority are retained in the curd particles after cutting (Chapman and Sharpe, 1981). These ferment lactose in the curd moisture producing lactic acid which cannot immediately escape into the whey, so the concentration of lactic acid is greater within the curd particles. The acid brings about certain chemical changes in the casein, and some calcium phosphate is dissolved.

There was no significant correlation between the pH value of cheese and the composition of milk except the ADV. This might be due to the high buffer capacity which is against change in pH values in the curd in the early stages, but the breakdown of proteins, reduce their buffer capacity (Scott, 1981).

The fat content of the cheese seems to depend on the titratable acidity of milk. In the more acid milks, the fat lost into whey was more, so the resultant cheese had less fat.

Increase in the titratable acidity, soluble calcium, soluble phosphorus, soluble ash, FPD of milk and SAV by titration caused a reduction in the calcium and phosphorus content of the resultant cheese. This might be due to the higher loss of these components into whey where acidic milks were used for cheesemaking. The use of milks with higher ash content,

produced cheese which retained more calcium and phosphorus.

As expected, when the milk had higher calcium, lower soluble phosphorus, lower TA, higher pH and or higher FPD the resultant cheese was firmer (lower B.C.T). With longer RCT the firmness was lower.

Elasticity of cheese is correlated negatively with fat. This may be due to the function of the fat in the development of the body of the cheese where the fat has a lower density than the other components.

Green et al. (1981) found that the measurements made on the whole cheese with the ball compressor confirmed that the firmness of the cheese increased approximatley linearly with increase in the solid content (concentration factor) of the milk, but there tended to be a decrease in the elasticity.

6. The correlation between microbiological quality and composition of milk and the yield of Cheddar cheese

Chapman (1981) reported that the yield of cheese from milk depends upon three factors:- the content of fat and casein in the milk, the milk constituents especially fat and casein lost in cheesemaking, and the amount of water retained in the cheese. The same author added that any comparison of yields should be based on the yield of cheese that contains a uniform amount of moisture if the results are to have any relation to the milk constituents.

In the present studies a significant correlation was established between the yield of cheese (calculated on a cheese moisture of 35 per cent) and the fat and total solids contents of the milk. These results are in agreement with the findings of Chapman (1981) who stated that as the cheese-producing solids increase in the milk there is an increase in the yield of cheese. When the ratio of casein to fat in milk varies the ratio of milk fat to cheese yield must also vary.

The NPN showed high negative correlation coefficient with the cheese yield. But, the NCN showed a very slight negative correlation (not significant) with cheese yield. It was reported (Chapman, 1981) that changes in the fat and casein take place at different rates; a rise of 1 per cent fat being accompanied by a rise of only 0.4 per cent

casein. As casein increases more slowly than fat, high fat milk usually contains less casein in proportion to fat than does milk less rich in fat. In other words, it has a lower C/F ratio, and the amount of cheese made per kg of milk fat decreases as the fat level increases.

The increase in the level of soluble calcium, soluble phosphorus, soluble ash, and titratable acidity were associated with a decrease in the cheese yield. These might be due to the higher losses of milk constituents into whey during cheesemaking as a result of cold storage at higher temperature.

The free SH groups in the milk correlate positively with the cheese yield. These constituents had never been studied before in correlation to cheese yield. It was found in this study that the level of free SH groups increased in milks held at $2^{o}C$. The cheese yield was slightly higher for these milks.

The first step in rennet action of milk is the destruction of the protective colloidal nature of κ-casein and para-κ-casein is formed within the casein micelles (Chapman and Sharpe, 1981). Both half-cystine residues produced in this process are located in the para-portion and present as SS, none as SH (MacKenzie, 1970). This might indicate that the availability of free SH groups during cheesemaking is involved in the coagulation of the milk.

The bacterial counts of milk correlated negatively with the yield of cheese. This might be due to the resultant breakdown of fat and proteins of the milk used for cheesemaking. The correlation coefficients were lower when they were calculated including the results of the abnormal milks (held at $6^{o}C$ for 7 days).

7. The correlation between the organoleptic quality of milk and its composition and microbiological quality

The negative correlation observed between NPN and the flavour and odour of milks might be due to the presence of some bitter peptides which were produced from the proteolytic action of enzymes and micro-organisms.

As expected the more acidic milk gave lower scores for flavour and odour. The negative correlations between flavour and odour scores of milk and

its bacterial counts indicate that milks with higher bacterial counts were awarded lower scores for flavour and odour. These findings may be due to the production of unacceptable flavour compounds which arise from the partial breakdown of protein and fat of milk during storage.

Nelson (1981) reported that organoleptically detectable levels of change commonly involve bacterial populations in excess of $10^6$/ml, and frequently $10^7$/ml. Occasionally even populations of $10^9$/ml or more, will not cause an organoleptically-detectable change, because of the relatively low level of relevant biochemical activity.

8. The correlation between the organoleptic quality of milk and cheese

The close correlation between the flavour and odour of milk and the organoleptic quality of cheese indicates the importance of acceptability of cheese milk on the organoleptic quality of Cheddar cheese. This indicates that it might be possible to predict the quality of cheese from the acceptability of raw milk provided the cheesemaking procedure remained unchanged and no contamination of the cheese milk or cheese occurred during processing. The use of milk of bad flavour resulted in cheese of bad flavour. These bad flavours of the resultant cheese are not only due to retention of the bad flavour compounds from the milk, but are also due to the further action of heat resistant enzymes which may produce breakdown in fat and protein during cheesemaking and curing of the cheese. These findings are in agreement with the assumption of Downey (1980a) who stated that the quality in various dairy products is attributed to the heat-resistant enzymes such as flavour impairment of cheese.

CONCLUSION

1. Close correlations were observed between the organoleptic characteristics supporting the view that the good grader of cheese relies on hand feeling and smell to judge the quality of cheese.

2. It is possible now to grade the cheese depending on its composition. Moisture, ADV and calcium are the major factors in this judgement provided that the acidity levels are within the limits for Cheddar cheese.

3. Close correlations were observed between NPN, ADV, soluble calcium, solbule phosphorus, soluble ash, titratable acidity, pH, RCT and SAV (by titration) and the bacteriological quality of the milk.

4. The cheese yield was positively correlated with the level of fat, total solids, SH and pH of the milk. The cheese yield decreased when the milk levels of NPN, soluble calcium, soluble phosphorus, soluble ash, titratable acidity, FPD and bacterial counts increased.

5. The most important characteristics of milk correlated with the composition of cheese are:- soluble calcium, soluble phosphorus, titratable acidity, pH, FPD and RCT.

6. The higher scored milks for flavour and odour are associated with higher pH and lower NPN, titratable acidity and lower bacterial counts.

7. Using milk stored for up to 4 d at $2^{o}C$ and the unstored milks for cheesemaking resulted in cheeses awarded higher scores than cheeses produced for milks stored at $6^{o}C$. Lower scored cheeses were associated with higher FFA, moisture and lower calcium and ash content.

8. Some of the significant correlations observed between milk and cheese characteristics are due to an indirect effect of storage of milk on its constituents.

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