A STUDY OF THE USE OF COAGULANTS IN CHEDDAR CHEESE MAKING

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SUMMARY

The aim of this work was to study the properties of different coagulants, and to find the effect of their use on the yield and quality of Cheddar cheese.

Different coagulants were found to have different clotting and proteolytic activities. Calf rennet was found to possess the best ratio of clotting activity to proteolytic activity,and other coagulants i.e., Mucor miehei rennet, a 1:1 mixture of calf rennet and porcine pepsin, and porcine pepsin had different ratios of clotting activity to proteolytic activity from that of calf rennet. It is considered that despite differences between coagulants the ratios of clotting activity to proteolytic activity of the coagulants mentioned above are of an acceptable level for Cheddar cheese production.

The clotting activity of the above coagulants was found to be affected in different ways by different levels of milk temperature and pH. Different levels of calcium in milk were found to have a smaller effect on clotting activity than different levels in the temperature and pH of milk. The hydrolysis of lactose in the milk was found to have no effect on the clotting activity of calf rennet.

Cheddar cheese was made on two different scales - laboratory (up to 1 l of milk) and small scale (180-360 l (40-80 gal) of milk) using the four coagulants referred to above. No significant differences were found in the yield of Cheddar cheese through the use of different coagulants. Cheese yield was found to be affected in the first place by the composition of the milk (in particular the content of fat and total solids). Storage of milk at low temperature (4°C) for 72 h did not affect the yield of Cheddar cheese made with the use of calf rennet or Mucor miehei rennet.

The four different coagulants used in making Cheddar cheese were found to have no effect on cheese composition except porcine pepsin which resulted in a Cheddar cheese having a significantly lower fat content (see Table 4:18) than the cheese made with other coagulants. The reason for that was the production of softer curd by porcine pepsin than by the other

three coagulants, and the consequent higher fat losses in the whey when the curd was cut.

The composition of whey from Cheddar cheese making was affected by the type of coagulant used. Higher amounts of fat in whey were associated with the use of porcine pepsin, and a higher content of non protein nitrogen in whey was found when Mucor miehei rennet was used.

During the period of cheese curing, variation was noticed in the moisture content of cheese but with no indication that it related to the type of coagulant used. Change in cheese acidity measured as pH, during the curing period occured ina definite pattern and the use of different coagulants in cheese making were found to have a significant effect on the pattern of change of pH.

The use of the four different coagulants mentioned above in cheese making were found to have a significant effect on the levels of soluble nitrogen in cheese and also to affect the rate of soluble nitrogen increase during curing. The type of coagulant was found to have a small affect on the level of free fatty acids in cheese and on their rate of increase during curing.

The results of an electrophoretic study of Cheddar cheese made with the above coagulants showed that each of the four coagulants had a specific electrophoretic pattern which could under certain circumstances be used to identify the type of coagulant used in making the cheese. The rates of hydrolysis of casein fractions were found to vary with the use of the different coagulants.

Quality assessment of the cheese made with the four coagulants after 10 to 12 weeks curing at $10^{o}C$ showed that the type of coagulant used had no adverse effect on the quality of cheese, and that factors such as the use of strains of starter culture reputed to produce bitterness, and processing techniques such as variation in salt addition had more effect on the quality of Cheddar cheese than the type of coagulant.

ABBREVIATIONS

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

CR = Calf Rennet

CR:PP = mixture of Calf Rennet and Porcine Pepsin

DF = Degree of Freedom = n-1

LSD = Least Significant Difference = SED x t value (using DF of Residual)

MM = *Mucor miehei* rennet

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

OD = Optical Density in nm = 10^{-9}m

PP = Porcine Pepsin

REP = Replicates

SE = Standard Error = V Residual MS

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

TU = Turbidity Units

VR = Variance Ratio = MS (individual variants) /MS (Residual)

THE USE OF ENZYMES IN CHEESEMAKING

INTRODUCTION

Of the many factors involved in the cheesemaking process, the coagulant used is considered to be one of the most important. The very small amount of coagulant added to the milk acts on the casein and converts the liquid milk into a strong gel holding most of the milk solids. This gel will later be converted as the result of certain treatments into different varieties of cheese. But the role of the coagulant in cheese making is not only that of turning the milk into a gel, it also contributes to cheese quality, general characteristics, and yield.

History of cheese production

It is known that cheese was first made accidentally thousands of years ago. Cheese is mentioned in the Holy Books and some cheese moulds and illustrations of how to make cheese have been found in Ancient Babylon, Assyria and Egypt. And it is possible that the same people who discovered how to make cheese had noticed the importance of the animal stomachs on turning the liquid milk into solid cheese, so, presumably they started to use a whole or a piece of the animal's stomach to make cheese. Probably they selected the young or milk fed animal's stomach because they noticed it contained some milk curd when they removed it from the newly slaughtered animals (Cheke, 1959). It is possible that plant coagulants were also discovered in an accidental way i.e., when a piece of fig fell into a jar of milk and made it curdle, and so people in different locations started to use whatever coagulants they found available to them, and, with the increased demand for the cheese, and with the improvements on the methods of cheese making, cheese producers tried to increase the shelf life of the animal's stomach, by sun drying or by extracting the juice from it either by pressing or by soaking in salt solution, and the dried vells or the liquid extracted from them, is what we now call Rennet (Cheke, 1959). Davis (1965) listed the different names for rennet in Europe and how all the names derived from Latin words such as coagulum or prender (to

grasp), in Arabic the word (Manfeha) which means (the abomasum) is also used for rennet.

In Europe, references to rennet extraction and preparation are found in the literature of the sixteenth century, but it is obvious that the methods of extraction mentioned were known for centuries before that, Cheke (1959) listed different recipes to prepare rennet, and showed how the earlier methods improved from the sixteenth century until the method used now was first established.

In the eighteenth and early nineteenth century, some farmers tried to market their home-made rennet, but they did not succeed, because cheese makers stuck to their own rennet preparation as they thought it gave them better cheese with good yield (Cheke, 1959).

The first rennet preparation marketed on a large scale was by a Danish chemist, Chr. Hansen, who established the first laboratory to make rennet in Denmark in 1874, and it was first introduced into Britain by Joseph Harding in 1875 (imported by his son-in-law A. Jenkins) whose family remained the sole importer and south western agent until the establishment of Chr. Hansen's laboratory at Shinfield, Reading in 1918 (Davis, 1965). In 1878 Chr. Hansen opened a large factory in New York, USA, and so commercial rennet production started on a large scale.

Definition

In the first supplement to the food chemicals codex, rennet was defined as the aqueous extracts made from the fourth stomach of calves, kids or lambs in the form of clear amber to dark brown liquid preparations, or white to tan powders, the major active principle is protease (chymosin), typical application; the manufacture of cheese (Nelson, 1975).

Extraction method

The early methods for extracting rennet from the vells of calves, kids and lambs, involved the addition of various flavouring, colouring and preserving materials, Cheke (1959) gave detailed recipes for the

preparation of rennet, some of those recipes including the addition of nettles, eggs, cloves, mace, reddish of blackthorn, salad burnet (Poterium sanguisorla), and finally sweet marjoram. Other recipes used egg yolk, sweet cream, powdered saffron, clove and mace and, in addition, always salt had been used in the recipes.

But with the increased knowledge of the principles of hygiene, more care was taken to prepare the rennet, and, by the end of the eighteenth century, a well established method was used by all cheese makers. The method consisted of drying the vells by one of two methods, either by air drying (Europe and New Zealand), or by the flat-salting method which was used in the USA (Webb et. al., 1974). After the collection of a large number of dried vells, the extraction was done by soaking the vells in brine solution for a week with daily stirring and weekly removal of the liquid for up to four weeks. The liquid extract was then filtered through clean straw, charcoal and sand, and an additional amount of salt was then added to preserve it (Decker, 1905).

Sammis (1918) mentioned the addition of boric acid as a preservative, and he suggested that the rennet should be held (aged) in a cool dark place before it was made to a definite strength for sale.

Leitch(1932), gave a similar procedure for extracting the rennet and he mentioned that the method was used in the Dairy Research Department of The West of Scotland Agricultural College, Auchincruive. The same method of extraction was referred to by Davis (1965), Webb et. al., (1974), and Kosikowski (1977). Hansen (1978) gave a detailed procedure for the method currently used by Chr.Hansen's Laboratorium for the production of their standard rennet. The basis of their extraction is the same as the old method, except that it is mechanised with computer control of the equipment. It seems that the time required for the extraction is shortened, but the extract is weaker and needs to be concentrated by reverse osmosis.

Here are some points to be considered for making rennet preparation:-

1) Calves should be young (10-30 days old) and milk fed only (Green,

1977).

2) Vells should be removed immediately after slaughtering and cleaned, dried, or deep frozen (Hansen, 1978).

3) The extract will contain Prochymosin (the precourser for chymosin) and it should be activated, the best activation range is at pH 5.0 in the presence of 1.5-2.0 M NaCl (Rand and Ernstrom, 1964).

4) The extract should be clarified - by alum - then filtered and preserved using a high conc. of NaCl (14 to 20 per cent) Webb et. al. 1974) or 18 per cent (Hansen, 1978).

5) Other preservatives can be added such as - Sodium benzoate or Propylene glycol (Davis, 1971).

6) Rennet extract can be dried to a powder, or made into tablets or pastes. Davis (1965) and Webb et. al. (1974) listed some advantages with the powdered rennet and suggested where it can be used, also rennet pastes were mentioned and their application in some Italian cheese varieties.

Rennet composition

The crude vell extraction contains a lot of organic materials, which need to be removed to make the rennet as pure as possible, the pure enzyme makes up only about 10 per cent of the dry matter of the crude extraction, and it consists of chymosin 94 to 100 per cent and pepsin 0-6 per cent, the rate of chymosin to pepsin depends on the age of the calf (Webb et. al., 1974). Hansen (1978) gave the following figures for the composition of different rennet preparations.

	Chymosin %	Pepsin %
Calf stomachs (Avg.)	about 80	about 20
Italian calf stomachs	" 40	" 60
Ox " "	" 20	" 80

Normal standard rennet contains 80 ± 5 per cent chymosin, as the calf

grows older, the proportion of pepsin to chymosin increases until from about five months there is an absence of chymosin (Webb et. al., 1974).

Chymosin

This is an enzyme, secreted by the fourth stomach, or abomasum, of the young calf discovered by Heinz in 1851 (Sumner and Somers, 1943)). Since that time it has been crystallized and extensively characterised. Chymosin is a proteinase also called Rennin* produced only by milk fed calves, it has a molecular weight of 31,000 (Web et. al., 1974) or 30,700 (Boyer, 1971), it is present in the stomach as Prochymosin (molecular weight 36,000), and it is converted to the active form chymosin by the addition of acid,its highly clotting activity makes it important in the cheese industry. Some other clotting enzymes are associated with high proteolytic activity which excludes them from the cheese making field. The optimum pH for chymosin is between 5.3-6.3 (Webb et. al., 1974 and Davies, 1936). Boyer (1971) gave the value of 5.5, and Davis (1965) gave 5.4 as the optimum pH. Maximum clotting activity is obtained at 40^{o} to $45^{o}C$ (Boyer, 1971), $45^{o}C$ (Davies, 1936), 42 to $48^{o}C$ (Jennes and Patton, 1959), and $41^{o}C$ by Davis (1965).

Chymosin can clot milk at temperatures between $10^{o}C$ and $65^{o}C$ depending on the milk reaction (pH) and the level of Ca^{++} (Jennes and Patton, 1959), Kosikowski (1977) reported that the activity of liquid rennet was destroyed at $55^{o}C$.

Coagulation of milk by rennet

When curd is formed by the action of rennet, the clotting does not change the chemical composition of milk components, and the presence of lactose and whey proteins in the curd is just due to occlusion (Webb et. al., 1974), the early theory of Hammarstin (1877) and of Van Slyke and Bosworth (1914) was based on the belief that casein was

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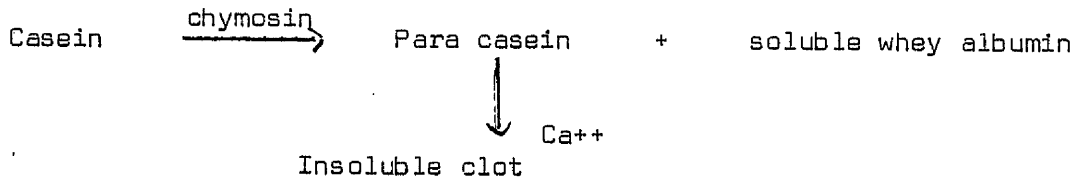
*The name Rennin was replaced by Chymosin due to the confusion between Rennin and Renin (The pancreatic enzyme).

a homomolecule and the action of rennet was expressed as follows:-



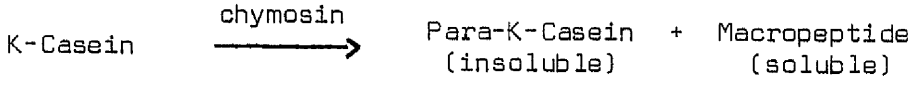
Since the discovery of the heterogeneity of casein, this theory became invalid. The term - para - used as a prefix to casein or any casein fraction, means that after treatment with rennet. But now, it is known that clotting of milk by the action of rennet happens in two distinct phases, the primary or enzymatic phase, when chymosin attacks K-casein and destroys its stabilizing capacity, and the secondary (or non-enzymatic) phase, when the destabilized system clots in the presence of Ca^{++} (Webb et. al., 1974).

The primary (enzymatic) phase

Casein consists of the following components (Rose et. al., 1970)

αS_1 Casein	45-55%
β Casein	25-35%
K Casein	8-15%
γ Casein	3-7%

Waugh and Hippel (1956) demonstrated that the function of K-casein is to stabilize the caseinate micelles, and that this stability is destroyed by chymosin action. Zittle (1961) and Zittle and Walter (1963) showed the same function for K-Casein. Wake (1959) and Garnier(1958) showed that the soluble whey albumin described by Hammerstin was liberated from K-Casein. Mackinley and Wake (1971) modified the scheme to be -



Webb et. al., (1974) reviewed the work concerning the way that K-Casein was destabilized, and reached the final conclusion, that a phenylalanine-methionine bond in K-Casein is susceptible to the attack of proteolytic enzymes. This will produce para-K-Casein (insoluble) and macropeptides

(soluble) fractions and the rest of the casein micelles are sensitive to Ca^{++} and precipitate forming a gel-like material.

The secondary (non enzymatic) phase

This phase is not fully understood, and the problem arises from the uncertainty of the structure of casein micelles and how they aggregate. Webb et. al. (1974) gave a detailed description of the most accepted models for casein micelles and discussed the evidence for each model. The recent application of electron microscopy in the examination of casein micelles has made the model of Parry and Carrol (1969) the most accepted one (Kosikowiski, 1977).

The destruction of the stabilizing effect of K-Casein by the action of chymosin makes the caseinate micelles (in the presence of Ca^{++} ions) more susceptible to clotting or to aggregate (Webb et. al., 1974), and since chymosin does not make any change in the shape, structure, size, surface, or in the general appearance of the micelles (Green et. al., 1978), aggregation of caseinate micelles starts immediately and continues at a rate dependent on the temperature. Berridge (1942) found that aggregation proceeded even at $0^{o}C$ but at a reduced rate, and he found that raising the temperature quickly to the normal clotting temperature made the curd form instantly. Green et. al. (1978) used the electron microscope to scan the aggregation process at different temperatures and times and she came to the conclusion that there was no sudden change, but micelles aggregate first in small groups which linked together in chains. Contraction in the chains made the aggregated micelles come together and fuse into each other forming a network or strands, and when this reaches a certain proportion of the total micelles, the whole system clots.

Clotting of the milk is very essential in the cheese making process, but the clot formed will need to go through a series of treatments to eventually become cheese.

The need to find substitutes of calf rennet

It is possible that rennet and plant coagulants were discovered at the

same time, but availability, convenience in use, and religious reasons made some people use rennet while others preferred the use of plant coagulants. However, plant coagulants were not considered as a substitute for animal rennet especially for cheese manufacturers who were accustomed to use calf rennet for the production of their cheese, and the situation remained so for centuries until the beginning of this century, or just after the first world war, when there was a shortage in the supply of calf rennet stomachs (Sammis, 1918). Some people in the cheese industry felt that this was the first warning of a general rennet shortage and so, thereafter some limited work was done on the purification of plant coagulants. During the second world war from 1939 to 1945, the rennet shortage became more acute, and the reason to find a substitute was not only the shortage in the supply of calves stomachs but also was due to the fact that:-

1) The number of calves slaughtered started to fall gradually due to the increased demand for meat, and farmers preferred to raise the calves to produce beef.

2) The large expansion in the cheese making industry through population increase and demand for cheese, more investment in the dairy industry, and increased consumption of cheese world wide.

3) The cost of producing calf rennet increased.

So it was necessary to find cheaper and readily available coagulants for cheese making, and research and development was started in institutes and commercial factories to find a suitable substitute for calf rennet. The criteria set for a suitable substitute were:-

1) It should be cheaper than calf rennet.

2) It should be capable of being produced simply with relatively standard strength.

3) The rate of clotting activity to proteolytic activity should be high.

4) It should resemble calf rennet in respect of the conditions required for

action (optimum temperature, pH and sensitivity to Ca^{++}).

5) It should not give any off flavour, bad taste, irregularity in colour diffusion and loss in yield in cheese making and it should not have any effect on milk fat or inhibitory effect on starter organisms.

6) The enzyme itself should be safe to use and not pathogenic or toxic.

Davis (1971) added some points to be considered in the use of new coagulants for cheese making:-

(i) The suitability of the new coagulant for different cheese varieties with different maturing periods.

(ii) The possibility of mixing the new coagulant with other milk clotting enzymes.

(iii) The distribution of the new coagulant between the cheese and whey, especially if the enzyme is heat-resistant which could cause problems when whey is processed.

Other requirements for new milk clotting enzymes have been given (Sardinas, 1969; Scott, 1973; Nelson, 1975; Sardinas, 1976; Phelan, 1977; and Green, 1977).

Even now there remain arguments over the use of new coagulants, between people in favour of them and people who oppose the use of them. The following section is a review of the work published in relation to the new milk clotting enzymes and their application in cheese making.

Milk clotting enzymes

1) Plant coagulants

In history, Varro (36 B.C.) writes "Some people use instead of rennet, the milk from a fig branch and vinegar", and the same was mentioned by Columella (60 A.D.) (Davis, 1965). Cheke (1959) listed some of the plants used in early times as coagulants such as Fig (Ficus carica), melon tree (Carica papaya), seeds and berries of a wild Indian plant

(Withania coagulans), ladies bedstraw (Calium venum), Butterwort (Pinguicula vulgaris and P. alpina), Artichoke (Cynara scolymus), Teasel (Dipsacus sylvestris), Spear Wort (Ranunculus flammula), and the thistle of (Carlina corymbosa and C. acalis).

Veringa (1961) listed several types of plant coagulants which had been used only in India for cheese making, and also some other plant coagulants which had never been used i.e., Solanum indicum, S. daeagnifolium, Calatropas gigantica, C. procera, Pinguicula vulgaris, Withania somnifera, and Castiolla elastica. Lists of plant coagulants are also given by Davis (1965), Scott (1973), and Sardinas (1972).

So while plant coagulants were known a long time ago, they had no importance to people in Europe except those who object to the use of animal coagulants, and also to make some special types of cheese like the native ripened Serra cheese in Portugal which is prepared using coagulant from the flower of the Thistle (Cynara cardunculus) (Kosikowski, 1977). In India and other Far East countries, the use of plant coagulants is a daily practice to them. Chodat and Rouge (1906) studied the cheese making procedure of the Majork islanders and gave the name Sycochymase to the preparation of the leaves and twigs of Fig the islanders used. Later Gerber (1908) succeeded in extracting the enzyme Ficin from the latex of Fig. It was only during and since the second world war (1939-1945), when a great interest in trying plant coagulants in cheese making was developed (Davis, 1965), that a lot of trials were done to extract, isolate, and purify enzymes from crude plant preparations.

The preparation from the flowers of the prickly artichoke (Cynara cardunculus)were used by Christen and Virasora (1935), Pereira de Matos and Vieira de Sa (1948), Vieira de Sa and Barbosa (1970), Vieira de Sa and Barbosa (1972), and Barbosa et. al., (1976) to make the following cheese varieties - Cammbert, Edam, Geuyere, Roquefort, Serpa, Serra, and soft and cooked cheeses. In most cases, lower yield and inferior quality were noticed with the cheeses made using the plant

coagulant in comparison to those made with calf rennet.

The plant coagulant from the berries of (Withania coaguluns) was used by Kothavalla and Khubchandani (1940), Dastur et. al., (1948), Babel (1967), and Singh et. al.(1973) to make Cheddar cheese and other hard and soft cheeses, and in all cases a bitter taste was noticed associated with the use of this coagulant.

The third plant coagulant reported to have been used was 'Ficin' from the edible fig (Ficus carica), which was used by Krishnamurti and Subrahmanyan (1948), Krishnaswammy et. al., (1961), El-Shibiny et. al., (1974), and Rice and Lantero (1975) to make Cheddar and Domiati cheeses. Good quality Cheddar cheese was produced with the 'Ficin', but the quality of Domiati cheese was not as good as that made with calf rennet, and in both cheeses i.e., Cheddar and Domiati, lower yield was noticed associated with the use of 'Ficin'.

Other plant coagulants e.g., 'Papain' from the leaves of papaya (Carica papaya), a preparation from Ash gourd (Benincasa cerifera), a preparation from the fruit of pumpkin (Cucurbita pepo), and a preparation from a wild shrub in India (Strebles asper) were reported to have been used for cheese manufacturing by Krishnamurti and Subrahmanyan (1948), Dastur (1949), Windlen and Kosikowski (1956), Ramamurti and Johar (1964), Berkowitz - Hundart et. al., (1964), and Gupta and Eskin (1977), but none of those preparations were found to be a suitable substitute for calf rennet.

2) Microbial coagulants

Milk clotting enzymes from microorganisms had been reported firstly by Conn and Gorini independently in 1892 (Sardinas, 1972), and since that time, individual attempts have been reported of isolating milk clotting enzymes from microorganisms (bacteria and moulds) and many patents have been issued in different parts of the world for the isolation of such enzymes from microorganisms. Since 1940 the number of research papers published concerning microbial enzymes and their application in cheese making has increased dramatically. The reason for this increased interest in the microbial rennets is the convenience in

their isolation and use and obviously the world shortage of animal rennets. Davis (1965) reported that microbial enzymes had not yet achieved any success in cheese making.

In 1963 a French patent was granted to Meito Sangyo K.K. of Japan for "the production and use of microbial rennet produced by Mucor pusillus var. Lindt" (Sardines, 1972) and a year later Arima and Iwasaki (1964) were granted a patent for "the production of microbial rennet from Mucor pusillus var. Lindt". The following year they were granted a second patent for "the application of Mucor pusillus Lindt rennet in cheese making". In 1966, Sardinas (Pfizer and Co. Inc.) discovered a microbial rennet from Endothia parasitica which was produced commercially in 1967 and named Sure Curd or Suparen. Later Mucor pusillus Lindt rennet (Noury) was first marketed in Europe, and in 1969 it was allowed to be used in the USA (Nelson, 1975). In 1970 Charles et. al., were granted a patent for another microbial enzyme from Mucor miehei and the enzyme was produced commercially in 1972 by Miles Laboratories Ltd., USA under the name Marzyme (Christensen, 1972). At a later date other varieties of the same species were used for the production of similar clotting enzymes which were marketed under different trade names such as Rennilase and Fromasa (Sardinas, 1972).

Since the first microbial enzyme was produced commercially, all of the research was aimed at trying the new coagulants in cheese making. Although some workers are still looking for new microbial rennets, it seems that from the thousands of organisms screened for clotting enzymes, only those three mentioned above have proved to be successful in cheese making. A review is given below of published work concerning the microbial rennets from Endothia parasitica, Mucor pusillus var. Lindt, and Mucor miehei.

I) Mucor pusillus var. Lindt rennet:- The milk clotting enzyme from this mould was first discovered by Meito Sangyo K.K. of Japan in 1963 (Sardinas, 1972) and now it is sold by different companies

under different names such as Noury, Novadel and Emporase+.

It has been reported that Cheddar cheese has been manufactured, producing a good quality and yield using rennet from Mucor pusillus var. Lindt (Robertson and Gillies, 1966; The C.S.I.R.O. of Australia Report, 1966; The Annual Report of the New Zealant Dairy Research Institute, 1966; Shovers and Bavisotto, 1967; Nelson, 1969; Robertson and Gillies, 1969; Sardinas, 1969; Czulak, 1972; Sardinas, 1972; Phelan, 1973; Nelson et. al., 1974; and Wong et. al., 1977). Cheddar cheese was reported by the above authors to have been manufactured on experimental, small and commercial scales. Other reports indicated that the use of Mucor pusillus var. Lindt rennet in Cheddar cheese manufacturing resulted in lower yield and inferior quality in comparison with the cheese made using calf rennet (Babel, 1967; Babel and Somkuti, 1968; Kikuchi et. al., 1968; Kikuchi and Toyoda, 1970; Labuschagne and Jaarsma, 1970; Wigley, 1974; and Green and Stackpoole, 1975).

Cheeses of other varieties i.e., Aseiago, Belpaese, Beri, Blue, Brick, Buttercheese, Cammbert, Colby, Cottage, Crescenza, Edam, Emmental, Finnish, Gouda, Grana, Italico, Jezioranski, Kortowski, Limburger, Minas, Montery, Mozzarella, Munster, Parmesan, Port du Salut, Prato, Provolona, Ras, Robiola, Romano, Swiss, Taleggio, and Tilsit cheeses were all reported to be manufactured with good quality and idential yield to those made with calf rennet (Tsugo et. al., 1964; Annual Reports from Belgium of 1966 and 1967; Richardson et. al., 1967; Shovers and Bavisotto, 1967; Schulz et. al., 1967*; Mann, 1967; Praprotnick, 1968; Annibaldi and Nozzola, 1969; Huig, 1969; Nelson, 1969; Swagniga et. al., 1969; Kyla Siurola and Antila, 1970; Labuschagne and Jaarsma, 1970; Organon Laboratories Ltd., 1971; Arima, 1972; Aapola et. al., 1972; Brinkman, 1972; Sandoval et. al., 1972; Sardinas, 1972; Zonji, 1972; Alberini and Nizzola, 1974; Carini et. al., 1974; Morvai, 1974; Phelan, 1975; Pien, 1975; Reps et. al., 1975; Hofi et. al., 1976; Mahran et. al., 1976; and Southward and Elston, 1976).

Other reports for the use of Mucor pusillus var. Lindt rennet in the

manufacturing of cheese varieties i.e., Cottage, Danbo 30^{+} and 45^{+} (fat in dry matter is 30 and 45 per cent), Domiati, Edam, Emmental, Fontina 50^{+}, Gouda, Herrgardsost 45^{+} (fat content), Italico, Lujta, Samsoe 45^{+} (fat in dry matter), Taleggio, Trappist, and white ewe's milk cheeses revealed differences from those made with calf rennet either in having quality defects such as body defects or bitterness, or in having lower yield than the cheese made with calf rennet.

Finally, Mucor pusillus var. Lindt rennet, like most of the other microbial coagulants is not so pH dependent as calf rennet, and it was found to survive cooking and only 3 per cent of it could be recovered in the cheese (Ernstrom, 1976).

II) Endothia parasitica rennet:

A milk clotting enzyme from Endothia parasitica was first described by Sardinas 1965, and in 1967 it was the first microbial rennet to be used on a large commercial scale. Sardinas (1968) reported on a complete study of the general characteristics of this rennet. He has at a more recent date reported that the ratio of its milk clotting activity to the proteolytic activity is among the highest of microbial rennets (Sardinas, 1972)*.

Cheddar cheese has been reported to be produced successfully using Endothia parasitica rennet in reports by Sardinas (1966), Mann (1967), Shovers and Bavisotto (1967), and Morris and McKenzie (1970).

Other reports indicate that the use of Endothia parasitica rennet in the manufacturing of Cheddar cheese resulted in the reduction of yield or in the production of defects in the cheese such as bitterness and body defects (The Annual Reports for 1967 of Research Institutes in Australia; Sardinas, 1969; Labuschagne and Jaarsma, 1970; Czulak, 1972; Phelan, 1973; Sardinas, 1976; and Emmons et. al., 1977).

It has been reported that cheeses of other varieties i.e., Asiago, Brie, Buttercheese, Cammbert, Colby, Crescenza, Domieti, Edam, Emmental, Granulated Greuyers, Limburger, Minas, Montery, Mozzarella, Munster, Soft, Palpusztei, Prato, Provolone, Ras, Romano, Swiss types, Tilsit, Trappist, Washed

curd and White brine cheeses have been produced with good quality and yield using rennet from Endothia parasitica (Mann, 1967; Shovers and Bavisotto, 1967; Alais and Novak, 1968; Prap otink, 1968; Antila and Aapola, 1969; Bolliger and Schiff, 1969; Ramet et. al., 1969; Kiss, 1969; Sandoval et. al., 1969; Sardinas, 1969; Thomasow et. al., 1970; Aapola et. al., 1972; Nadassky, 1972; Carini et. al., 1973; Carini et. al., 1974; Reps et. al., 1974; Jedrychowski et. al., 1975; Kiss et. al., 1975; Abdou et. al., 1976; Hofi et. al., 1976; Mahran et. al., 1976 and Sardinas, 1976).

Other authors reported that the use of Endothia parasitica rennet in making cheese varieties i.e., Cammbert, Danbo, Domiati, Emmental, Gouda, Palpuszlai, Parmigiano Reggiano, Samsoe, Soft white cheese, Taleggio, Trappist, and Tea cheese resulted in having a lower yield than when calf rennet used, and in having flavour and body defects associated with the use of Endothia parasitica rennet (Annibaldi and Nizzola, 1969; Edelsten et. al., 1969; Maubois and Mocquot, 1969; Birkkjaer and Thomsen, 1970; Labuschagne and Jaarsma, 1970; Resmini et. al.,1971a; Resmini et. al., 1971b; Carbone et. al., 1971; Carbone et. al., 1973; Stavland and Kiermeier,1973; and Vamos et. al., 1975).

III) Mucor miehei rennet

This rennet is unlike that derived from Mucor pusillus var. Lindt and Endothia parasitica , because a number of strains of Mucor miehei have been used for the production of coagulants. The fact that a number of strains of Mucor miehei have been used makes it difficult to assess the value of Mucor miehei rennet in cheese making (Sardinas, 1972), and most of the work published did not refer to the strain of Mucor miehei used to produce the coagulant and so I did on reviewing on the published work with this specific microbial rennet.

Aunstrup (1968) was granted a patent for the production of a milk clotting enzyme from a submerged culture of Mucor miehei. It was claimed that the enzyme had been used in cheese making and that

it produced a satisfactory cheese yield. Charles et.al., (1970) were granted a patent for an extraction method for the production of a milk clotting enzyme from Mucor miehei. They stated that cheese produced with it had an agreeable flavour and satisfactory structure and consistency.

Cheddar cheese was reported to have been made using Mucor miehei rennet as a coagulant, and reported to have good quality with no differences from the cheese produced using calf rennet in respect of yield and organoleptic qualities (Labuschagne and Jaarsma, 1970 ; Christensen, 1972; Phelan 1977; and Wong et. al., 1977). Other cheese varieties have been reported to have been produced with good quality and yield similar to those produced with calf rennet, varieties such as, Asiago, Blue, Brie, Bryndza, Buttercheese, Corgonzola, Crescenza, Domiati, Edam, Emmental, Gouda, Grana, Jarlsberg, Jazioranski, Kachkaval, Kortowska, Limburger, Mozzarella, Parmesan, Port du Salut, Provolone, Romano, St. Paulin, Swiss, Tilsit, Tollesnur, and White cheeses (Hamdy, 1970; Labuschagne and Jaarsma, 1970; Aarnes, 1971; Mayer,1971; Aapola et. al., 1972; Christensen, 1972; Hamdy, 1972; Lawrence et. al., 1972; Ramet and Alais, 1972; Thompson et. al., 1972; Botel et. al., 1973; Carini et. al., 1973; Martens, 1973; Phelan, 1973; Prins, 1973; Ramet and Alais, 1973; Sipka et. al., 1973; Carini et. al., 1974; Stefanova et. al., 1974; Dennien, 1975; Anonymous, 1975; Reps et. al., 1975; Abdou et. al., 1976; Carini et. al., 1976; Nizzola and Fantuzzi, 1976; Carini and Todesco, 1978; Dolezalek et. al., 1978; Larsen, 1978; and Reps. et. al., 1978).

Other reports indicated that the use of Mucor miehei rennet caused a reduction in the yield or the production of body and flavour defects in the following cheese varieties. Cheddar, Edam, Gouda, Herrgardsast 45^{+}, Italico, and Ras (Carbone, et. al., 1971; Carbone et. al., 1973; Dinesen et. al., 1975; Resmini et. al., 1975; Hofi et. al., 1976; Mahran et. al., 1976; Mattsson, 1976; Emmons and Beckett, 1977; and Minarik et. al., 1978).

It has been suggested that the reduction of the quantity of Mucor

miehei rennet used for making the cheese, together with the addition of calcium chloride to the milk, reduces the proteolytic activity of this coagulant but improves the quality and yield of the cheeses made with it (Botel et. al., 1973; and Prins, 1973).

Commercial preparations of Mucor miehei rennet i.e., 'Hannilase', 'Rennilase', 'Fromase', and 'Marzyme' are used widely in the cheese industry all over the world, and considered to be the most acceptable microbial substitutes for calf rennet (Phelan, 1975; Weiss, 1975; and Hansen's Laboratory, 1978).

3. Animal coagulants other than calf rennet

It has been known for a very long time, that the stomach of some animals as well as calves can be used to make cheese. The stomachs from young sheep and goats have been used for centuries in some Asian and European countries for making cheese. Studies about the use of such preparations in cheese making started to be reported around the beginning of this century.

The review by Veringa (1961) was the first for rennet substitutes - including those from animal origin - and later reviews (Sardinas, 1969; Sardinas, 1976; Scott, 1973; Green, 1976; Green, 1977; and Phelan, 1977) discussed the use of all available substitutes in cheese making, and from the many animal enzymes tried in cheese making only two were commercialised . A review is given below of the use of bovine pepsin and porcine pepsin and their mixture with calf rennet in cheese making.

1) Porcine pepsin

The first use to which pepsin was put was as a medicine to aid digestion (Sammis, 1918 and Boyer, 1971). Pepsin was first used in the cheese industry by Van Dam (1915) who reported that good quality cheese could be made using pepsin as a coagulant with the addition of 0.0025 per cent hydrochloric acid to the milk (Veringa, 1961). Shortly thereafter Graber (1917) and Stevenson (1917) achieved the same results with the use of lactic acid instead of hydrochloric acid.

Barr (1917) noticed the importance of the coagulation temperature, the amount of pepsin, and the coagulation time on the quality of cheese.

Sammis (1918) gave information about the use of pepsin in the cheese industry and mentioned the importance of ripening the milk to 0.2 per cent lactic acid before the addition of pepsin. In an attempt to determine the role of chymosin and pepsin in the ripening process Davies et. al., (1934) made Cheddar cheese with the following coagulants: calf rennet (after destroying the chymosin), calf rennet (after destroying the pepsin), normal commercial calf rennet, and a commercial pepsin solution. The cheese produced showed no differences between coagulants except that the one made with pepsin had a little bitterness.

Sherwood (1935) made Cheddar cheese using two types of pepsin preparations and calf rennet in various amounts. The use of pepsin resulted in less protein degradation than occurred with calf rennet, but no other differences were observed up to the time when the cheeses were 13 weeks old.

Tsugo (1953) compared calf rennet and porcine pepsin by measuring the clotting activity, proteolytic activity, the relation between activity and temperature and pH, and coagulation of milk by the two coagulants. Sasaki et. al., (1956) reported that pepsin decomposed casein in a similar way to chymosin. Veringa (1961) mentioned the regulations imposed by the Research Committee of the National Cheese Institute of the USA which limit the use of pepsin to cheese to be stored for long periods and then to its use in a mixture with rennet. Melachouris and Tuckey (1963) and Fox (1969) compared the proteolytic activity and clotting activity of calf rennet, bovine pepsin, and porcine pepsin and found that porcine pepsin has the highest rate of proteolytic acitivity. O'Keeffe et. al. (1977) reported that under the normal conditions of cheese making, porcine pepsin was equally as stable as calf rennet. To inactivate the porcine pepsin, modifications, such as increasing the pH of the curd to 6.6 and rapid cooking of the curd were used.

Porcine pepsin has been used successfully to produce Cheddar cheese

with equal yield and similar quality to that produced by using calf rennet (Yamamoto et. al. 1955; Maragoudakis et. al., 1961; Melachouris and Tuckey, 1964; Raadsveld, 1964; Fox, 1969; and Green, 1972). Sardinas (1969) advised that it was more satisfactory to use pepsin in a mixture with calf rennet, because pepsin alone needs a longer setting time and produces a softer curd, which causes more fat to be lost in whey and also causes some bitterness in cheese. Eino (1975) also noticed that Cheddar cheese made with calf rennet was of better quality than cheese made with bovine or porcine pepsins. The cheese yield was higher when calf rennet was used.

Cheese of other varieties i.e., Kortowski, Mozzarella, Quarg, Samsoe 45+, Telemea, Trappist, Uglich, and white pickled cheese were reported to have been made with a resultant good quality and similar yield to those made with calf rennet (Motoc et. al., 1963; Cerna-Eva et. al., 1966; Sosina et. al., 1966; Mann, 1967; Statens Forsogsmeyere of Hillerod, Denmark Report, 1968; Penev and Gruev, 1970; and Micketts and Olson, 1974). Dolgikh et. al., (1972) made Kostroma cheese using calf rennet, bovine pepsin (purified and unpurified), porcine pepsin, and a mixture of porcine pepsin with calf rennet. They noticed that porcine pepsin, whether it was used singly or in a mixture with calf rennet, produced cheese with bitterness due to a high content of bitter polypeptides.

2) Bovine pepsin

This enzyme was first prepared by Northrup (1933) and remained of little importance in cheese manufacturing until 1961 when Linklater reported that calf rennet contained a quantity of bovine pepsin which varied depending on the particular preparation.

Fox (1969) compared the milk clotting activity and proteolytic activity of calf rennet, bovine pepsin, and porcine pepsin and concluded that bovine pepsin more closely resembled calf rennet than the other coagulants and was the best substitute for calf rennet. Sardinas (1972) mentioned that bovine pepsin was superior as a rennet substitute to porcine pepsin, but he recommended that bovine pepsin should be

used in a mixture of equal quantities with calf rennet.

Cheddar cheese has been produced with good quality and yield using bovine pepsin (Fox and Walley, 1971; Phelan, 1973; and Phelan, 1977). The authors concluded that bovine pepsin is suitable to be used as the sole coagulant in the manufacture of Cheddar cheese.

In some other reports, bovine pepsin was observed to cause a reduction in the yield or the production of bitterness and body defects when used in Cheddar cheese manufacturing (Green, 1972; Green, 1976; Kim and Kim, 1976; and Emmons et. al., 1978).

Cheese of other varieties i.e., Cammbert, Kostroma, Samsoe 45^{+}, and Tilsit were reported to have been made successfully using bovine pepsin (Dolgikh et. al., 1972; Brikkjaer and Thomsen, 1974; and Thomasow and Brams, 1975).

3) Mixtures of calf rennet and pepsin

Studies started in 1960 when the Research Committee of The National Cheese Institute of the USA prohibited the use of pepsin as a complete substitute for calf rennet, and so, in 1960, Marschall Dairy Laboratory developed a commercial mixture of calf rennet and pepsin ('50/50' brand) consisted of equal parts from both coagulants. In 1964 Chr. Hansen's Laboratory Ltd. put a similar product on the market (Davis, 1971) but it was only in 1967 that reports on the use of this new preparation began to appear.

Babel (1967) reported that experiments to make cheese with porcine pepsin, a mixture of porcine pepsin and calf rennet (3:1 ratio), and calf rennet showed that the mixture gave better results than porcine pepsin alone, and trials with a (1:1 ratio) mixture gave identical results to that obtained with calf rennet. Babel also reported that 75 per cent of all Cheddar cheese made in the USA at that time was made with this mixture.

Sardinas (1969) recommended the use of calf rennet and porcine pepsin mixture of (1:1) ratio ('50/50' brand) for almost all types of hard cheeses, but not for low acid cheeses such as Swiss and certain Italian varieties. Nelson (1972) reported that a mixture of procine pepsin

with calf rennet is the best to use in the cheese industry. Sardinas (1972) stated that a (1:1) mixture of porcine pepsin and calf rennet produced satisfactory cheese and the low cost of the mixture compared with the standard calf rennet made the mixture very attractive to commercial cheese making dairies. Sardinas reported that in 1967, 75 per cent of the cheese in the USA was manufactured with the mixture and by 1970 it had reached 80 per cent. Thomasow (1972) compared the chief rennet substitutes available for their effects on the proteolysis of casein and on the yield, consistency, and flavour of the cheese, and concluded that the best results could be obtained with a misture of calf rennet and porcine pepsin. Emmons et. al., (1977) found that a 1:1 ratio of calf rennet to porcine pepsin gave higher protein nitrogen levels (by 0.0034 per cent) than the calf rennet.

The mixture of calf rennet and porcine pepsin (1:1 ratio) has been reported to have been used successfully in making Cheddar cheese (Emmons, 1968; Emmons et. al., 1971; Phelan, 1973; Poznanski et. al., 1973; Nelson et. al., 1974; Nelson, 1975; and Wong et al., 1977).

Chapman and Burnett (1968) concluded that the mixture was suitable for use in cheese making although it gave a lower yield of Cheddar cheese than calf rennet gave.

The mixture also has been reported to have been used successfully in the production of cheese varieties e.g., Bulgarian white cheese, Butter kase, Cammbert, Domiati, Danbo, Edam, Gouda, Grana, hard Brik cheese, Kachkaval, Kortowski, Kostroma, Kriske, Mazursko, Mozzarella, Parmigiano-Reggiano, Ras, Rokpol, Samsoe 45^{+}, Taleggio, Tilsit, Trappist, and white pickled cheese (Thomasow et. al., 1968; Birkkjaer and Thomsen, 1969; Martens, 1969; Poznanski et. al., 1969; Zwaginga et. al., 1969; Birkkjaer and Thomsen, 1970; Delforno and Gruev, 1970; Denkov and Kr" stev, 1970; Penev and Gruev, 1970; Zonji 1970; Thomasow, 1971; Dolgikh et. al., 1972; Carbone and Emaldi, 1971; Carbone and Emaldi, 1973; Poznanski et. al., 1973; Birkkjaer

and Thomsen, 1974; Bottazzi et. al., 1974; Corradini et. al., 1974; Ramazanov and Makhlevskaya, 1974; Khorshid et. al., 1975; Berg and Vries, 1976; Antila and Witting, 1976; Amer et. al., 1977; and Minarik et. al., 1978).

Nebert et. al., (1976) used a mixture of calf rennet and chicken pepsin in the ratio of 1:1 to make Kostroma cheese. Calcium chloride was added to the milk and high quality cheese was produced with low costs.

Antila and Aapola (1970) and Antila and Aapola (1971) reported that the use of a mixture of calf rennet and porcine pepsin in (1:1 ratio) ('50/50' brand) to make Finnish Edam cheese, resulted in the production of a softer curd. The authors also noticed a lower yield with the mixture, but the quality of the cheese was considered to be good, and no differences were detected in the taste and flavour between the cheeses made with the mixture and calf rennet.

The commercial use of rennet substitutes

Although the numerous efforts to find a rennet substitute over more than thirty years have resulted in the discovery of large numbers of new milk clotting enzymes, some of which are being used in the cheese industry, those efforts have also created some probems for people engaged in cheese making (Phelan, 1977), because until now, there is not a single coagulant which can satisfy all cheese makers, and with every coagulant one can find advantages and disadvantages.

Perhaps only the traditional calf rennet has remained the same and free from such criticism. Even so, the new coagulants have achieved the aim of stabilizing the price of rennet and overcoming a shortage.

During the years of searching for a rennet substitute, plant coagulants had been used for a short period as a substitute for calf rennet in Europe and the USA, but there were replaced by animal coagulants i.e. bovine pepsin, porcine pepsin and a mixture of calf rennet and pepsin. The main difficulty was the purification of these plant coagulants and their application in the cheese industry.

Animal coagulants gave a suitable substitute for calf rennet but the

discovery of some microbial rennets i.e. the Mucor rennets, which were cheaper than the animal rennets, and apparently had better properties than bovine or porcine pepsins led to people working in cheese industry to show a preference for microbial rennets. At a later date, when companies produced improved bovine and porcine rennets, cheese manufacturers reverted to their use.

The situation now is not fully known because statistical data is not fully available. In the United Kingdom during the period 1976/1977 the situation was as follows:

84 per cent of the cheese was made with calf rennet, 11 per cent was produced with a mixture of calf rennet and pepsin (either bovine or porcine) and 5 per cent with microbial rennets (mostly Mucor miehei rennet) (Hansen Laboratory, 1978). On the Continent of Europe, the use of microbial rennet was approved in France in 1973 but no figures were available for its use (Phelan, 1977) In Germany 50 per cent of the coagulants used in 1975 were of microbial origin, and in Switzerland 10-15 per cent of the cheese made in 1974 was from microbial rennets. In Ireland, the situation in 1976 was as follows:

64 per cent of the cheese was made with calf rennet, 28 per cent with a mixture of calf rennet and porcine pepsin and 8 per cent with Mucor derived rennets (Phelan, 1977).

In Canada 90 per cent of the coagulants used was calf rennet and the remaining 10 per cent was a mixture of calf rennet and pepsin.

In the United States of America, 40 per cent of the cheese was produced with calf rennet, 15 per cent with bovine rennet, and 40 per cent with microbial derived rennets, the remaining 5 per cent made from other sources and blends (Nelson, 1975).

Cheese makers all over the world are still looking for a cheaper and better substitute for calf rennet, but, improvements in the diary industry such as factors affecting cheese quality, e.g. milk quality, starter purity and activity, and cheese making techniques being more effectively controlled, will make the application of any new clotting

enzyme to be easier and safer. With the availability of a larger number of coagulants, one can assume that in the future, there will be no risk of rennet shortage again such as happened twenty years ago.

CHAPTER ONE

MATERIALS AND METHODS

SECTION 1 - MILK ANALYSIS

1. Determination of fat in milk

Throughout the study, the fat content of the milk used in the cheese making experiments or for any other purposes was determined according to B.S. 696, part 2; 1969 (British Standards Institution, 1969).

2. Determination of total nitrogen in milk

The improved micro - Kjeldahl method of the Association of Official Agricultural Chemists (1965) was used to determine total nitrogen, protein, or non protein nitrogen in milk using standard acid solution as receiver and titrating the excess of the acid with standard alkali solution.

3. Determination of solids not fat of milk

Milk density was determined according to B.S. 734, part 2; 1959 (British Standards Institution, 1959) and from the density value, the solids not fat were calculated using a special calculator supplied by Astell Laboratory Service Co. Ltd., Catford, London.

4. Determination of lactose in milk

The determination of lactose in milk was carried out using the modification by Nickerson et. al. (1975) of the method prepared by Fearon (1942). The method depends on the reaction of lactose with methylamine in hot alkaline solution to form a red compound with maximum absorbance at 540 nm.

5. Determination of freezing point of milk

Freezing point of milk was determined throughout the study using the Advanced Milk Cryoscope (Model 4L) provided by Advanced Instruments Inc., Needham Heights, Massachusetts. This instrument meets the requirements specified by the Association of Official Agricultural Chemists.

6. Determination of calcium concentration in milk

Throughout the study, calcium ion (Ca^{++}) concentration in milk was

determined according to the International Dairy Federation official method number 36 (1966). The principle of the method is to separate the calcium by dissolving it and precipitating the protein with trichloroacetic acid, the calcium is then precipitated as calcium oxalate which is dissolved in sulphuric acid, and the liberated oxalic acid is titrated with standard potassium permanganate solution.

7. Determination of hydrogen ion concentration (pH) in milk

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

8. Determination of titratable acidity of milk

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 made until the end point - a faint pinkish colour - was reached, the volume of NaOH solution used divided by 10 is the figure for acidity expressed as per cent lactic acid.

9. Determination of antibiotics residues in milk

Before the milk can be used for starter or cheese making, it should pass the test for antibiotic residues i.e. the Disc Assey method, based on the procedure of Galesloot and Hassing (1962) as used by the Scottish Milk Marketing Board.

This test detects antibiotics or other inhibitory substances at levels of 0.01 to 0.02 international units of penicillin per ml of milk. A small disc of filter paper is dipped into the milk sample and placed on the surface of an agar medium contained in a petri dish and inoculated with a sensitive test organism Bacillus calidolactis, the plate is then incubated at 55^{o}C for 2½ hours, normal growth of the test bacteria is rapid and by the end of the incubation period has caused the medium to become cloudy.

Antibiotics or other inhibitory substances, if present in the milk sample, pass out into the agar medium round the disc,prevent the growth of the test organism and so result in the formation of a circular clear zone, the size of this clear zone is related to the type and concentration of the antibiotic or other inhibitory material present in the milk.

SECTION 2 - COAGULANTS ANALYSIS

1. Determination of clotting activity

Determination of the clotting activity (or rennet strength) of coagulants was carried out using one of two methods to determine the clotting time of milk after the addition of a specific quantity of a coagulant solution, and then to compare this time with the time required for solution of a standard calf rennet to clot the same quantity of milk under the same conditions. The methods used to determine the clotting time were:-

(a) The B.S. 3624, 1963 (British Standards Institution, 1963) method which is a modification of the method of Berridge (1952), is based on the determination of the clotting time of 10 ml milk (reconstituted low heat spray dried milk powder in 0.01 M solution of $CaCl_2$) after the addition of 1 ml rennet solution at $30^{o}C$ $\pm 0.05^{o}C$ and with the tubes containing the milk and rennet rotating at the rate of 2-4 rpm at an angle of $30^{o}C$.

(b) A Dutch method developed by Eisses (1977) which differs from the previous method using a 50 ml quantity of milk. The milk must be fresh raw morning milk from at least 20 healthy cows excluding very early or late lactation milk, obtained under hygienic conditions, or a milk of such a quality pasteurized at $<74^{o}C$ for <30 sec., or a reconstituted low heat skim milk powder from low pasteurized milk. Bottles are used instead of test tubes and are rotated at 16-18 rpm instead of 2-4 rpm. Three pH levels i.e. 6.6, 6.5 and 6.3 are used and the temperature for the coagulation is the same i.e., $30^{o}C$ $\pm 0.05^{o}C$.

2. Determination of the ratio of chymosin to pepsin in rennets using ion - exchange column chromatography

The method of Garnot et. al. (1972) was used to determine the ratio of chymosin to pepsin in rennets.

The principle of the method is to dialyse the rennet first against a suitable buffer (0.025 M piperazine buffer, pH 5.30) to remove small ions or molecules - salts and small peptides - which could interfere

with the separation of the enzymes, and then to let a certain quantity of the dialysed rennet run through a column filled with activated microgranular DEAE (diethylaminoethyl cellulose DE 32) supplied by Whatman Ltd. - Maidstone, Kent. The elution was then carried out by a salt gradient - made of 0.15 M and 0.80 M NaCl buffer solution - the two solutions connected by a -U-bridge, the two branches of the bridge should reach the bottom of the containers, and the 0.15 M NaCl buffer should be stirred continuously while the intake tube dipped into the bottom of the container and the other end are connected to a peristaltic pump with a flow rate made up to 80 ml/h.

The exit tube from the pump was connected to the top of the column and fractions were then collected and tested for clotting activity using the Berridge method. By applying the following formula the activity of each fraction could be calculated and the ratio of the fractions may be derived:-

$$F = \frac{100(V1)}{(T - a)V2} \times \frac{t2}{t1}$$

Where - F = Activity (in rennet units).
T = Clotting time in seconds.
a = Constant (zero value was applied).
V1 = Volume of fraction collected (ml.).
V2 = Volume of dialysed rennet used.
t1 = Clotting time of rennet before dialysis.
t2 = Clotting time of rennet after dialysis.

3. Determination of proteolytic activity

The ability of the coagulants to break down casein components α, β, and K caseins and the extent of this breakdown was measured using the following methods:-

(a) Measuring the non-protein nitrogen by micro - Kjeldahl

Sodium caseinate, buffered in 0.1 M sodium phosphate was used as substrate throughout the study, the method was carried out as follows - 1 ml of the solution of coagulant was added to 10 ml of 1 per cent substrate (which is sufficient to give clotting

time of about 6 min. in milk at pH 6.6 and 30°C). The changes in the value of non-protein nitrogen with the time was then measured by precipitating the casein - using 2 ml of 15 per cent trichloroacetic acid - and taking 3 ml of the filtrate and determining the non-protein nitrogen using the micro-Kjeldahl method (as described in section1.2).

(b) Measuring the non-protein nitrogen by spectrophotometer

Miller's (1959) modification of the procedure by Lowry et. al., (1951) for protein determination was used to determine the increase in the level of non-protein nitrogen after the addition of the coagulant. The principle of the method is to dissolve 50 mg of copper sulphate ($CuSO_4 . 5H_2O$) in 10 ml of 1 per cent (w/v) sodium tartarate ($(CHOHCOONa)_2 . 2H_2O$) and 1 ml of this mixture is added to 10 ml alkaline sodium carbonate solution (prepared by dissolving the solute in 0.5 N NaOH solution). One ml aliquote of this solution are mixed with 1 ml of the substrate filtrate (in 3(a)) using a rotary mixer (Hook and Tucker Ltd.) and the materials are allowed to stand for 10 min. at room temperature, at the end of this period 3 ml of a 1 in 10 dilution of Folin and Ciocalteu's reagent were added and mixed and then incubated at 37°C for 10-15 min. After the mixed reagent and test materials have been cooled and held at room temperature for 30 min. the optical density was measured at 650 nm using ultraviolet spectrophotometer (PYE - Unicam SP 1800), the non-protein nitrogen present in the sample was then calculated from a standard curve prepared by serial dilutions of tyrosine (BDH Chemicals Ltd.).

The values obtained for non-protein nitrogen by using this method were compared with those of the previous method.

SECTION 3 - STARTER ANALYSIS

1. The revival of freeze dried cultures

Starter cultures of different strains were obtained from the National Collection of Dairy Organisms maintained at the National Institute of Research in Dairying, Shinfield, Reading. The cultures have been freeze dried and sealed under vacuum, and to revive the cultures, ampoules were first wiped with a sterilizing solution of 200 ppm hypochlorite solution. The pointed end of the ampoule was then removed by making a mark on the glass with a sterilized file and then the end of the ampoule was broken using a hot glass rod. Around 0.5 ml of sterilized milk was then added to the contents of the ampoule and after the dried culture had dissolved, the milk containing the culture was then mixed with 10 ml of sterilized milk in a test tube and incubated at $22^{o}C$ for 24 h.

The coagulated milk was then used to inoculate further tubes of sterilized milk (sub culturing) for use as a mother culture for starter preparation.

2. Growth media for starter culture

Throughout the study of activity of different starter cultures, two kinds of media were used.

(a) M16 Plating medium

This is a solid medium made as follows:- 5 g of lactose are dissolved in 200 ml distilled water and mixed with 800 ml of hot distilled water in which the following materials have been dissolved:-

10 g	Polypeptone (BBL)
5 g	Beef Extract (BBL or Oxoid)
2.5 g	Yeast Extract (BBL)
5 g	Phytone (BBL)
0.5 g	Ascorbic acid
2.8 g	Sodium acetate

The pH is adjusted to 7.1 using approximately 4 ml 2N NaOH, and 1 per cent Davies agar is added to the mixture and autoclaved at 1.1 kg/cm^2 (15 lb/sq.in.) prior to sterilization.

(b) Yeast dextrose broth

This medium was used for the growth of cultures prior to freeze drying, and also to study the activity of starter cultures using turbidity measurement. The broth was prepared as follows:-

20 g	Peptone
10 g	Lab Lemco
5 g	Dextrose
5 g	Sodium hydroxide
3 g	Yeastrel

were dissolved in 1000 ml distilled water and the pH was adjusted to 7.0, the broth was then filtered and sterilized by autoclaving at 1.1 kg/cm^2 (15 lb/sq.in.) for 20 min.

3. Turbidity measurement

Turbidity measurement was used as an indication of the growth rate of a starter culture grown in a medium such as yeast dextrose broth. It was also used to investigate the effect of bacterial concentration in the broth on the acidity development of the milk inoculated from the culture. The nephelometer used was an EEL nephelometer manufactured by Evans Electroselenium Ltd., Halstead, Essex, and it was used with two standards, (a) a tube containing uninoculated yeast dextrose broth to give zero turbidity value, and (b) a standard turbid glass tube to give the maximum value. Yeast dextrose broth was filled in to test tubes 150 mm x 16 mm matched to have the same wall thickness, colour, and base configuration and sealed with black rubber stoppers. Tubes containing sterilized yeast dextrose broth were then inoculated with the pure starter cultures, and turbidity was measured after different periods of incubation at 30^{o}C until a maximum turbidity value was reached. Sterilized milk was also inoculated from the yeast dextrose broth cultures with different levels of turbidity and the rate of acidity development was measured. Finally plates of M16 medium were inoculated from yeast dextrose broth culture contain different levels of turbidity and the total number of colonies were counted after a selected period of incubation.

4. Preparation of freeze dried cultures

Cultures for freeze-drying were grown first in yeast dextrose broth overnight, and 0.5 ml of the broth was then transferred into sterile 100 mm x 6 mm glass ampoules and covered with a loose filter cloth hood and placed in the centrifuge part of the freeze-drying apparatus (Edwards freeze-drier model EFO 3, manufactured by Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex). The vacuum pump of the freeze-drier was then operated and the chamber exhausted at the same time. The ampoules were centrifuged for 4 minutes at which time the material in the ampoule had been frozen into a wedge in the ampoule. When the culture appeared to be dry (after 18 h.), the vacuum was turned off and the ampoules were removed, plugged with sterile cotton wool prior to construction of the ampoule and secondary drying for 2 h. The ampoules were then sealed, while still under vacuum, by applying flame to the fine bore neck and breaking off with a twisting action.

5. Starter preparation

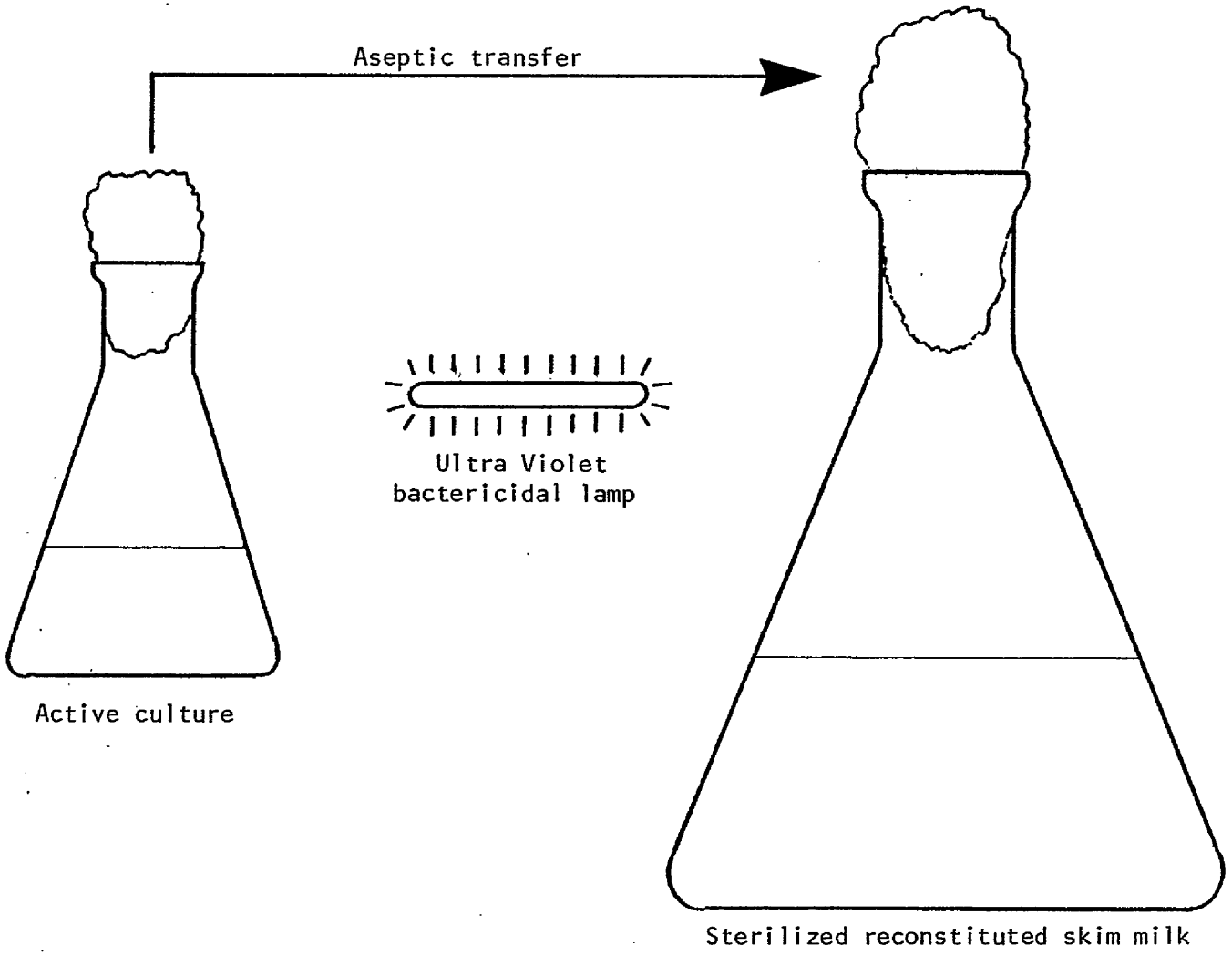
Throughout the study, starter was prepared on two scales:- a) laboratory scale, where the need for starter was to study its characteristics or to make experimental cheese by using it, the quantity of starter prepared on this scale being in the order of 250 ml or less, b) small scale starter preparation where the starter was used to make cheese on small scale, the amount required being quantities of about 9 to 18 l. (2 to 4 gal.). The technique used in preparing the starter was as follows:-

(a) Standard bacteriological method for starter preparation

Fresh raw milk, or reconstituted skim milk containing 10 per cent total solids, in conical flasks plugged with non-absorbent cotton wool was sterilized by steaming, first at 100°C for 30 minutes and then by autoclaving at 115°C [0.7 kg/cm^2 (10 lb/sq. in.)] for a further 10 min. When cooled to 22°C, the milk was inoculated aseptically with 1 per cent of an active culture grown in sterilized milk or in yeast dextrose broth in a cabinet fitted with an absolute air filter and equipped with an ultra violet bactericidal lamp. The inoculated flasks were then incubated at 22°C for 16 to 18 h.

DIAGRAM 1:1

Standard bacteriological method for starter preparation



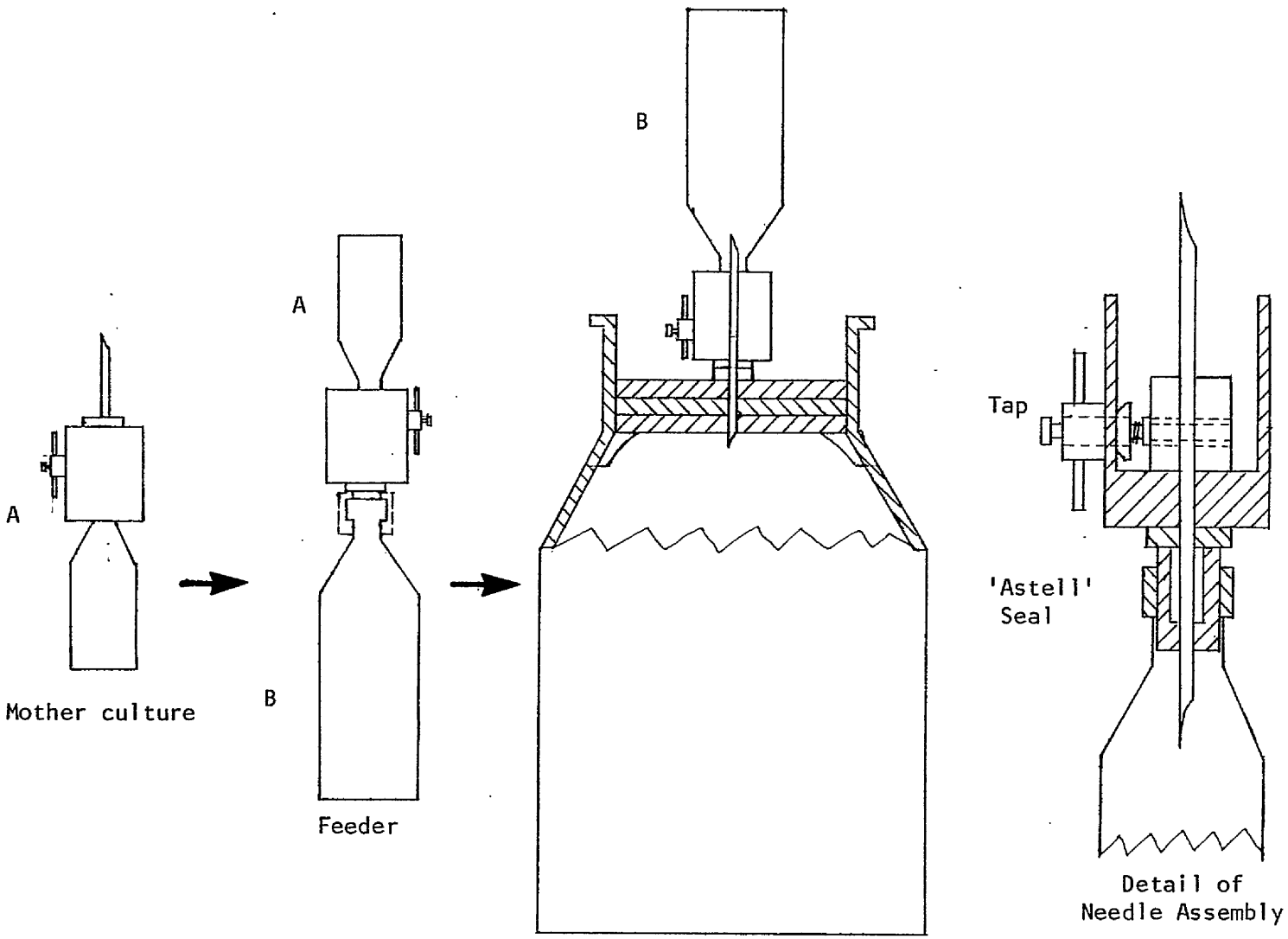
(b) Lewis method for starter preparation

Starter culture was prepared in modified polythene bottles with a range of capacity from 2 to 40 oz - which had been cleaned and sterilized by steaming for 5 min. on a steamer and filled with a 0.1 per cent solution of hypochlorite (equal to 100 ppm) for a period of not less than 10 min. Sterilized milk, steamed at 100°C for 60 min., was added to the bottles which were then sealed with 'Astel' seals and covered with a polythene cap. The milk was repasteurized for 1 h. by submerging the bottles in a water bath controlled at 74°C. The milk was then cooled to 22°C and held at this temperature for another hour before it was inoculated. The inoculation process was done by flooding the 'Astell' seal first with hypochlorite solution for both mother culture and milk bottles, and then by inserting one end of a sterile needle assembly, which had been boiled and stored in a weak hypochlorite solution, through the 'Astell' seal into the milk and inserting the other end into the mother culture bottle. By opening the tap at the middle of the needle assembly, inoculation was done by squeezing the culture bottle and allowing the required amount of the inoculum to drop into the milk. The tap was then closed and the complete needle assembly withdrawn, cleaned and sterilized for use later. The milk bottles were then incubated at 22°C for 16 to 18 h.

Milk cans of aluminium alloy with a capacity of 22.5 or 45 l. (5 or 10 gal.), which have the requirements specified in B.S. 3291; 1960 (British Standards Institution, 1960), were fitted with special lids (Reflex) to effect complete sealing of the vessels. The cans were filled with milk at a temperature below 20°C and sealed and then submerged in the heat-treatment tank for 90 min. at 85°C. Thereafter, the milk was cooled to 22°C with cold water and kept at this temperature for another 90 min. The 'Astell' seal in the centre of the special lid and the area above the lid were filled with hypochlorite solution. Inoculation was then done using the procedure mentioned above. The cans of inoculated milk were incubated at 22°C for 16 to 18 h.

DIAGRAM 1:2

Lewis method for starter preparation



6. Tests applied to starter

(a) Activity test

The test was done by inoculating sterilized, reconstituted skim milk containing 10 per cent total solids with the starter under test at a level of 1 per cent. The milk was then incubated at 30°C for 5½ h. The titratable acidity at the end of incubation indicates the activity of the starter.

(b) Scald check on starter activity

The method is based on simulating the cheese making process to find the amount of acidity produced at the end of the test. Two per cent of the starter under test is added to a pasteurized milk prior to incubation at 30°C for 2½ h. The titratable acidity is then measured and recorded as the first acidity. Rennet is added at the ratio of 1 part coagulant to 5000 parts milk, and the renneted milk is reincubated at 30°C for a further 30 min. The curd is then broken by shaking the container.

The temperature of the curds and whey is raised to 40°C and held at this temperature for 1 h.

The curd and whey mixture is then cooled to 30°C and incubated for a further 2 h., when the acidity is again taken and recorded as the second acidity. The difference between the first and the second acidity should be greater than 0.09 if the starter is suitable for cheese making.

(c) Tests for microbiological contamination

This includes the visual examination of the starter to detect any gas bubbles or syneresis or slime production, and any off flavours which are all indications of contamination of the starter.

Microscopic examination can detect heavy contamination with yeasts and moulds. Other tests applied to starter are:-

(i) Test for coliforms - which is done by inoculating three tubes of MacConkey bile salt broth with 0.5 ml from the culture under test and incubating the tube at 37°C. Production of acid

(indicated by a change in the colour of the indicator) and production of gas means the presence of coliforms.

(ii) Test for yeasts and moulds - which is done by streaking a loop-ful of culture on the surface of a suitable medium (i.e. malt extract agar) in a petridish. The presence of yeasts and moulds in the culture is indicated by their growth on the surface after one day's incubation at $30^{o}C$.

SECTION 4 - CHEESE MAKING SYSTEM

Throughout the study, Cheddar cheese was made on two different scales; experimental and small scale. Although the principle of the two methods is the same, alterations were required in the processing technique for the experimental Cheddar cheese making because of the use of different equipment in the production of the cheese.

1. Experimental Cheddar cheese making

(a) Milk pasteurization - Milk was pasteurized at 72°C for 15 sec. using a 'Safgard' pasteurizer with a capacity of 9 l. (2 gal.) manufactured by The Schluter company, Janesville, Wisconsin. At the end of the holding time (15 sec.), milk was cooled to 30°C by running cold water through the jacket of the pasteurizer.

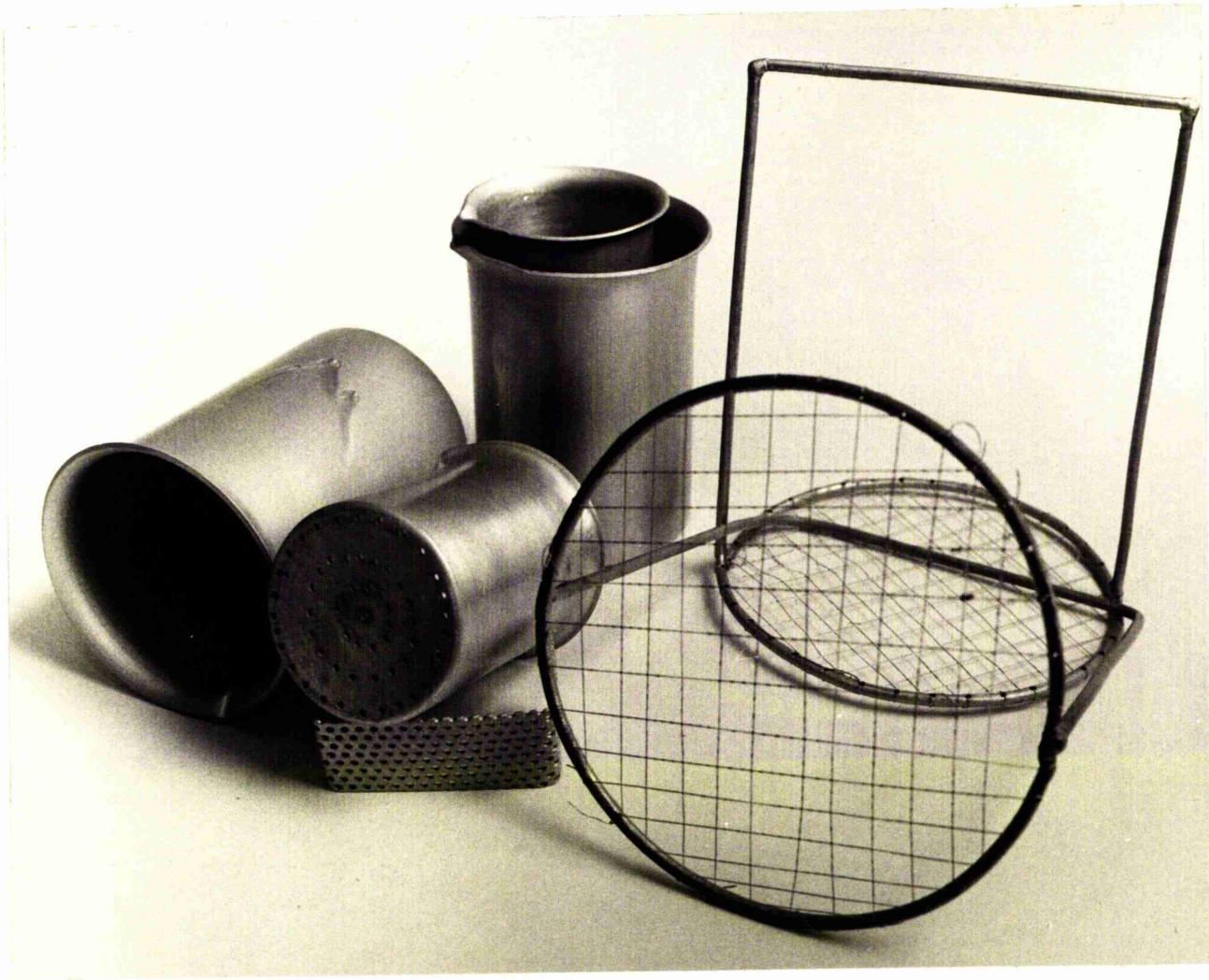
(b) Starter addition - Two per cent (w/w) of a single strain starter of Streptococcus lactis (ML8) was added to the milk at 30°C. After mixing in the starter the milk was then weighed into pyrex beakers in 1 kg amounts using a Mettler P 1210 electrical balance accurate to the second decimal place. The beakers containing the milk were then placed in a temperature-controlled water bath at 30°C ± 0.05°. The milk was stirred continuously for 30 min. using a special device designed to stir the milk at 30 rpm.

(c) Coagulant addition - The coagulant was added 30 min. after the addition of starter. The concentration of the coagulant had been adjusted so that the same clotting time was obtained with the various coagulants. The milk was stirred for two min. after the addition of the coagulant before being left undisturbed during the clotting period which took 45 min.

(d) Cutting the curd - When the curd was suffiently firm, cutting was performed using a special cutting device consisting of a metal ring (diameter 110 mm) with holes drilled in it 10 mm apart, and a crossed network made with nylon fishing line covering the entire area of the metal ring. The ring was held by a metal handle fixed over it, and cutting was completed by pushing the

PLATE 1

Laboratory scale cheese making equipments



ring down to the bottom of the beaker, twisting it twice on the way down, and then lifting it up again. This procedure was sufficient to give the desired size of curd particle.

(e) Cooking the curd (scalding) - Stirring was started immediately after cutting was completed and the temperature was raised using a thermostatically controlled water bath from 30°C to 39°C in 45 min.

Thereafter the curd and whey mixture was held at 39°C for one h. with the stirrer in operation continuously during this time.

(f) Draining the whey and cheddaring - Stirring was stopped and the whey was drained off into another container in which it was weighed. The curd which was left in the original covered beaker was maintained at a temperature of 30°C for two h. during which time it was turned over once every 30 min.

(g) Final stage of production - At the end of cheddaring, the curd was lifted out of the beaker and weighed. The curd was cut into small pieces (around 10 x 10 mm), mixed with salt added at the rate of 2 per cent (w/w) and thereafter filled into small aluminium beakers of 45 mm diameter and 70 mm height. The base of the beaker had been drilled by a series of holes (0.5 mm) to let whey pass through. The beaker, filled with curd, was then placed over a metal support inside another larger aluminium beaker of 63 mm diameter and 90 mm height and covered with a plastic bottle of 42 mm diameter and 75 mm height. Pressure of 2.8 kg f/cm^2 (38 lb/sq.in.) was applied to the curd by a vertical manual press for 18 h. Cheese was then taken out of the mould and sampled for subsequent analysis. The remainder of the cheese was placed in nylon polythene laminate vacuum pouches (145 mm x 235 mm) and sealed under vacuum by an 'Autovac' vacuum packing and sealing machine supplied by Kramer and Grebe KG, Wallan, Lahn. The cheese was stored in the curing room at 10°C for a period of more than a year during which time analyses were carried out monthly.

2. Small scale Cheddar cheese making

Throughout the study Cheddar cheese was made using either 180 or 360 l.

(40 or 80 gal.) quantities of milk.

The following points summarize the processing steps used:-

(a) Milk reception and pasteurization - Milk for cheese making was usually received the day before use and was pumped from the road tankers into the reception tank of 9000 l. (2000 gal.) capacity. From this tank, milk was immediately pumped to a storage tank and held overnight at 4°C. Samples of milk were taken at this stage for chemical analysis and to test for the presence of antibiotics. Pasteurization at 72°C for 15 sec. was carried out in the morning immediately before cheese making, the milk being first agitated for 10 min. while in the storage tank.

(b) Milk weighing and vat filling - Pasteurized milk at 31°C was pumped to the cheese room where it was filled into sterilized 45 l. (10 gal.) capacity milk cans made of aluminium alloy. The milk in the cans was then weighed to the nearest 5 g using an Avery single pan balance (accurage to 5 g) and then added to each of two stainless steel cheese vats. Depending on the quantity of milk, either vats of 225 or 450 l. (50 or 100 gal.) capacity were used. All vats were heated by steam, injected into a jacket, the temperature of the milk was maintained at 31°C by filling the vat jacket with water at the correct temperature. The milk in the vat was stirred with a special stainless steel vat paddle.

(c) Starter and coagulants addition - Two single strain starters were prepared separately using the method described in Section 3 (5b) of this chapter, and were then weighed and mixed in the proportion of one-third of fast strain and two-thirds of slow strain just before 2 per cent (w/w) of the mixture was added to the milk. Coagulants were measured out in quantities sufficient to give the same clotting time and added to the milk at the same time as the starter. The milk in the vat was then stirred for a further 5 min. before being left undisturbed during the coagulation period.

(d) Cutting the curd and stirring of the curd and whey mixture - When the coagulation was completed and the curd reached the

required firmness in 45 to 55 min., curd was then cut manually, with cheese knives, consisting of a frame with blades 1.27 cm (0.5 in.) apart, first vertically - once each way - and then horizontally - also once each way. A paddle was placed in its position in the vat and stirring was begun.

(e) Scalding the curd - Ten min. after cutting was complete the scalding process was started and the temperature was taken up to 39°C in 45 min., and maintained at 39°C for another hour.

(f) Draining the whey - Stirring was stopped and the curd was allowed to settle down five min. before the removal of the whey. Whey was collected in milk cans and weighed.

(g) Cheddaring - The fusing curd in the vat was then channelled down the centre to promote rapid and even drainage of whey. The curd was cut into uniform sized blocks which were turned over 15 min. after starting the draining of the whey. After a further 15 min. the blocks were halved in size and were piled two deep. The blocks of curd were thereafter turned and piled every 15-30 min., depending on the rate of acidity development.

(h) Milling - When the titratable acidity reached the required level and curd could be torn in strips resembling the meat from the breast of a chicken, the curd was milled into chips [(pieces around 5.1-7.6 cm (2-3 in.) long by 1.25 x 1.25 cm (½ x ½ in.)] using an electrically operated curd-milling machine (Damrow Brothers Company, Wisconsin, USA). Cheese salt (Dendritic brand) was added to the milled curd at the rate of 2.5 per cent (w/w), and the curd and salt were mixed for 5 min.

(i) Moulding and pressing - The salted curd was then placed in standard rectangular 18-14 kg (40 lb.) aluminium alloy moulds lined with disposable polypropylene cheese nets (365 x 1118 mm in two pieces) supplied by Smith and Nephew Plastics Ltd., Welwynn Garden City, Herts.). Moulds were weighed before and after filling with curd, and were then placed in a pneumatically operated press under a total thrust of 1814.4 kg (4000 lb) or 2177.2 kg (4800 lb).

(j) Packaging and storing - The cheese blocks were weighed and

wrapped in a double ply waxed cellulose laminate ('Pukkafilm' brand 660 mm x 990 mm supplied by DRG Flexible Packaging, Bristol) and sealed in a Crockatt heat sealing press, alternatively the block was cut into four smaller blocks each weighing around 4.54 kg (10 lb) which were packed in cellulose/nylon pouches and vacuum sealed by the Autovac vacuum packing and sealing machine. The packaged block of cheese was placed in a fibreboard case and stored at 10°C (50°F).

SECTION 5 - CHEESE ANALYSIS

1. Determination of moisture in cheese

Approximately 3 g of the grated cheese sample was weighed accurately in an aluminium foil container (Foilpak 10335, supplied by R.R. Brodie Ltd., Glasgow) with a diameter of 8.26 cm (3.25 in) and a depth of 1.91 cm (0.75 in). The container and the sample were placed in a fan ventilated hot air oven at 102°C. Final weight readings were taken when the weight of container and the dried sample was constant. The amount of moisutre in the sample was then calculated and the percentage of moisture in the cheese was determined B.S. 770; 1963 (British Standards Institution, 1963). The determination was made in duplicate on each sample and the mean value calculated.

2. Determination of fat in cheese

Approximately 1 g of the grated cheese sample was weighed accurately and placed in a 'Gerber' tube containing 10 ml of sulphuric acid with a 6 mm layer of distilled water over the acid.

Fat was then determined according to B.S. 696, part 2; 1969 (British Standards Institution, 1969). The average of two determinations was recorded.

3. Determination of Hydrogen ion concentration (pH) in cheese

Ten grams of the grated cheese sample were weighed and placed in a small plastic container along with 10 g distilled water. The cheese and water were mixed thoroughly by means of a Silverson mixer until a paste was obtained. The pH value was then determined by means of a PYE 290 pH meter - using a combination glass electrode (Activion Glass Ltd., Scotland). The average of two readings was recorded.

4. Determination of salt in cheese

The method described in B.S. 770; 1963 (British Standards Institution, 1963) was used, the principle of the method is the reaction of silver nitrate with the sodium chloride to form silver chloride in hot acid solution. The excess of silver nitrate will then be titrated with potassium thiocyanate, and the difference between this figure and the

one obtained from titrating potassium thiocyanate with a blank is equal to the amount of sodium chloride in the cheese, and is calculated, using the basis that each 1 ml of 0.05 N potassium thiocyanate = 0.00292 g salt.

5. Determination of total nitrogen in cheese

Sodium citrate cheese extract was prepared according to the Mogensen (1948) method described by Vakaleris and Price (1959) in which 10 g of grated cheese sample added to 40 ml of a 0.5 M solution of sodium citrate and 80 ml of distilled water were mixed thoroughly by means of a Silverson mixer. The homogenous mixture was then transferred accurately into a 200 ml volumetric flask and filled to the mark with distilled water.

The total nitrogen content of this mixture was determined by the improved micro-Kjeldahl method (Association of Official Agricultural Chemists, 1965).

6. Determination of soluble nitrogen in cheese

The method of Vakaleris and Price (1959) was used for preparing a hydrochloric acid filtrate from the sodium citrate cheese extract. Ten ml of 1.41 N hydrochloric acid was mixed with 100 ml of sodium citrate cheese extract (see the previous paragraph) and the volume was made up to 125 ml by the addition of distilled water (the final pH of the mixture should be 4.4 $\pm$ 0.05).

The mixture was then filtered through Whatman No. 42 filter paper, and the nitrogen determined in the filtrate of cheese extract using the micro-Kjeldahl method mentioned above, and expressed as per cent soluble nitrogen.

7. Determination of free fatty acids in cheese

The method described by Frankel and Tarassuk (1955) was used for the determination of free fatty acids in cheese. The method is based on the extraction of lipid materials with fat solvents and the subsequent titration of free fatty acids in the solvent extract. The procedure is to add 10 ml of a 95 per cent neutralized ethanol to 1 g of grated cheese sample in a centrifuge tube and shake vigorously for 1 min.,

15 ml of a mixture consisting of 4 parts ethyl ether and 6 parts petroleum spirit is then added and the tube is shaken vigorously for another 1 min. The tube is then centrifuged for 3 min. at 1500 rpm and 5 ml of the supernatant is added to 15 ml of 95 per cent ethanol containing five drops of 1 per cent (w/v) solution of phenolphthalin in alcohol (the 15 ml of ethanol was neutralized to a pink colour before the addition of the 5 ml supernatant). Titrate the mixture with 0.025 N alcoholic potassium hydroxide to the same pink colour using a 10 ml burette graduated to 0.01 ml. The volume of alkali used is multiplied by three to obtain the volume required to neutralize the total ether layer, the results are expressed as free fatty acids and as a per centage of the fat content in cheese.

8. Polyacrylamide gel electrophoresis

Electrophoresis is a method that utilizes charge differences for the separation and purification of protein (Haschemyer and Haschemyer, 1973). Polyacrylamide gel electrophoresis was used in the identification and comparison of casein breakdown on cheese making and to study the subsequent hydrolysis of the casein component during cheese ripening.

The gel used was prepared by dissolving 12 g of cyanogum 41 (a commercial mixture of acrylamide and N,N' - methylenebisacrylamide, supplied by BDH Chemicals Ltd., Poole, England) in 150 ml of tricitrate buffer which is made as follows.

4.59 g tris (hydroxymethyl) methylamine + 0.53 g citric acid + 270.270 g urea were dissolved in distilled water and made up to 1 litre.

To the cyanogum and tris buffer, 0.2 ml of dimethylamine propionitride (DMAPN) and 0.15 g of ammonium persulphate were then added and the solution was mixed very gently and deareated and poured into a perspex mould 180 x 110 x 5 mm and covered with a perspex lid carrying five slot formers, care must be taken to exclude all air bubbles, and the mould left until polymerisation was completed (about 40 min.).

Samples were prepared by weighing 100 mg of grated cheese into a centrifuge tube with 0.1 ml distilled water and 3 ml of gel buffer.

The mixture was then warmed to 42°C and stirred for 4 minutes to disperse the cheese particles. The tube was then centrifuged at 3000 rmp for 10 minutes. Casein samples were prepared as standard by dissolving 80 mg of casein (light white soluble) supplied by BDH Chemicals, Poole, England, in 10 ml gel buffer and centrifuged at 3000 rpm for 10 minutes.

After the samples and casein standards had been centrifuged, 'Pasteur' pipettes were used to fill the gel slots with the supernatant from the cheese samples and casein standards to 1 mm from the top of the slot. Melted vaseline was then used to cover the filled slots.

To run the gel, the mould with the slots nearest to the cathode was placed in the electrophoresis tank which contained buffer solution made up as follows.- 18.5 g boric acid and 2 g of sodium hydroxide dissolved in 600 ml distilled water. The pH was then adjusted to 8.6 and the volume was made up to 1 l with distilled water. Absorbent lint was placed at the two ends to make contact between the buffer and gel, polythene film was used to cover the mould, and electrophoresis was carried out at 4°C by applying a constant voltage of 150 volts.

When the fast moving bands - indicated by a brown line - had travelled more than 10 cm (the time taken is between 4½ and 5h), the current was stopped and the gel was removed from the mould and stained either by immersing overnight in a solution of 0.1 per cent (w/v) amido black 10B (BDH Chemicals) in 10 per cent acetic acid (v/v), or immersing for 15 min.in 1 per cent (w/v) amido black 10B in 10 per cent acetic acid (v/v). Destaining the gel was carried out by immersing the gel in a tank containing 5 per cent acetic acid solution (v/v) and applying electrical current of 12 volts (0.6 - 1.0 AMP) through the acid for 4 h. using a battery charger, 'Popular', supplied by F.C. Heayberd and Co. Ltd., London. Acid was changed once during destaining.

When the gel became clear, it was preserved by vacuum sealing in a celulose polythene laminate pouch and photographed.

Identification of the separated bands in the gel was done after scanning the gel using scanning accessories fitted on the ultra violet

spectrometer

9. Sensory evaluation of cheese

Cheese made with different coagulants were evaluated after certain periods of storing in the curing room. The evaluation process was carried out with the help of a quality assessment panel consisting of 8 to 11 persons all of them with experience in dairy technology. Cheese samples were presented in a well illuminated room at 18°C. A total of 12 cheese samples presented each time represented duplicates of four cheeses made by four different coagulants, and four standards for texture body, flavour, and bitterness. From each cheese sample, a 450 g (1 lb) piece was presented for the assessment of texture, and also cheese fingers (10 mm x 10 mm x 80 mm) from the same sample were presented for the assessment of the other cheese characteristics.

All the samples were presented at the same time, numbered from 1 to 12 and the quality assessment panel asked to evaluate the following cheese characteristics on a special form provided to them -

(a) Texture (openness)- Cheese texture can be evaluated by careful visual examination of the cheese sample, a value should be given on a scale of 0 = open to 10 = very compact.

(b) Body (firmness) - Cheese body can be evaluated by taking a portion - from the cheese finger sample - working it between the thumb and finger to determine resistance and thereafter awarding a value on the scale of 0 = very soft cheese and 10 = very firm bodied cheese.

(c) Flavour (smell) - Smell is determined by holding a portion of the cheese sample, used to evaluate the body, very close to the nose and inhaling deeply. A full Cheddar cheese flavour is given 10 points and a very bad off flavour given 0 . Flavours not accepted as typical of Cheddar cheese such as fruity, mellow, and fermented flavours are considered as off flavours.

(d) Bitterness (taste) - A small portion of the cheese sample was chewed very well and swallowed, and a very bitter taste given 0 and cheese with no bitterness given 10 .

(e) General acceptability - An award for general acceptability depends on the grader and what he thinks about the cheese sample. he examines, a very poor cheese is given 0 and a very good cheese given 10 .

SECTION 6 - WHEY ANALYSIS

1. Determination of fat in whey

Fat in the whey was determined using the Rose-Gottlieb method described in B.S. 1741; 1951 (British Standards Institution, 1951). The method is based on the precipitation of protein by alcohol and the digestion of the precipitated protein by ammonia. Fat is then extracted with diethyl ether and petrolium spirit. The ether layer is transferred into another container and dried in a vacuum oven at 70°C and thereafter on a hot air oven at 102°C until constant weight is reached. The average of two determinations was recorded.

2. Determination of total nitrogen in whey

The improved micro-Kjeldahl method (Association of Official Agricultural Chemists, 1965) was used employing the technique for total nitrogen determination in milk as described in section 1:2.

3. Determination of non protein nitrogen in whey

Nitrogen soluble in 12 per cent trichloroacetic acid was determined as follows. Ten g of whey were weighed into a 50 ml volumetric flask and the volume made up to the mark with 15 per cent trichloracetic acid making the final concentration of the trichloro-acetic acid 12 per cent. The precipitate was then filtered through a Whatman No. 42 filter paper and the total nitrogen in the filtrate was determined using the improved micro-Kjeldahl method described previously.

CHAPTER TWO

STUDY OF STARTER

INTRODUCTION

It is possible that natural souring of milk was the only method used in the past to produce the required acidity in the milk for cheese making, and at a later time, the addition of sour whey or buttermilk to the milk for cheese making and also the use of home made starter was known (Crawford, 1958). The starter prepared in the past was not on a scientific basis, and it was only at the end of the last century when the nature of the starter organisms, responsible for acidity development became known in 1889, that Conn succeeded in preparing starter from pure cultures of Straptococcus cremoris and Streptococcus lactis. In 1895, starter prepared from a pure culture of lactic acid bacteria was first used in Scotland (Crawford, 1958) and commercial cultures were marketed by Chr. Hansen's laboratory towards the end of the nineteenth century (Davis, 1965) and first introduced in England in 1905 (Davis, 1965).

Starter used for Cheddar cheese making is simply a pure culture of lactic acid bacteria, mostly two species of Streptococcus - Str. lactis and Str. cremoris - named first by Lloyd in 1899 as the two organisms responsible for acid production in Cheddar cheese (Davis, 1965). They play a most important role in Cheddar cheese making, and without their action, Cheddar cheese could never be made. Their action produces acidity development, assists in rennet action, and in curing plays a part in the formation of cheese flavour (Reiter and Moller-Madsen, 1963).

There are many strains of the two lactic acid bacteria - Str. lactis and Str. cremoris - used to prepare starter for Cheddar cheese making. The strains differ from each other by some minimal biochemical and biological differences, they also differ in their susceptibility to a range of bacteriophage (Singleton and Sainsbury, 1978).

Collins (1962) defined the different strains of Str. lactis and Str. cremoris as isolates from different sources which are sufficiently alike to be classified as belonging to the same genus and species. Crawford (1972) classified starters for cheese production according

to the Reiter and Moller-Madson pattern into the following:-

1. Single strain starters:-
    a. Str. lactis or Str. cremoris
    b. Str. diacetilactis
2. Mixed strain starters:-
    a. Str. lactis or Str. cremoris or both
    b. Str. lactis, Str. cremoris and Leuconostoc cremoris
    c. Str. lactis, Str. cremoris, Str. diacetilactis and Leuconostoc cremoris
    d. Str. lactis, Str. cremoris and Str. diacetilactis
    e. several strains of Str. diacetilactis.

The type of starter used as well as many other factors i.e., milk composition, heat treatment of milk, cheese making process, and contamination affect the development of acidity during cheese making and the development later of other characteristics of the cheese i.e., body, texture, flavour, and taste.

The aim of this chapter is to study the characteristics of some starter strains, and to find the best way of making starter with good properties and constant activity, also to see the effect of such starter on the quality and probably the yield of cheese.

EXPERIMENTAL

1. Maintenance of pure culture

Freeze dried preparation of the following strains were obtained from the National Collection of Dairy Organisms - National Institute for Research in Dairying, Shinfield, Reading.

Catalogue number	Code name (strain)	Name of organism
1218	K	Str. cremoris
1241	972	"
1995	P_2	"
509	C_{10}	Str. lactis
920	11	"
1994	ML8	"

Additional strains selected from the collection of the Dairy Technology Department of The West of Scotland Agricultural College, Auchincruive were:- AM_1, AM_2, SK_{11}, E_8 and HP (all strains of Str. cremoris), and C_2 and B_1 (both strains of Str. lactis).

Cultures were obtained in the freeze dried state in glass ampoules. The recovery of the cultures was performed following the procedure described in Chapter one - section 3:1.

Pure cultures were maintained throughout the study for use in the activity study and for starter preparation by preparing freeze dried cultures from the active cultures following the procedure described in Chapter one - section 3:4.

Purity of starter cultures was checked regularly by applying biological tests such as the arginine dihydrolase activity test and catalase test to starter cultures grown in solid medium (M16) prepared as described in Chapter one - section 3:5. Microscopic examination of culture colonies was also used as a check for the purity of starter cultures.

2. Study of acidity development

a. Acidity development in relation to cell mass

Tubes of yeast dextrose broth medium containing lactic acid bacteria which had been incubated to different levels of turbidity, 20, 30,

40 and 50 per cent, were used to inoculate reconstituted skim milk containing 10 per cent (w/v) total solids distributed in 100 ml quantities in conical flasks. The inoculated milk was then incubated at 22°C and the acidity development rate was measured by measuring the titratable acidity (Chapter one - section 1:8) at regular intervals up to 20 h.

b. Acidity development in relation to oxygen availability

To study the effect of the extent of the surface area (in relation to the oxygen availability in the milk) on the rate of acidity development by different lactic acid bacteria, milk was distributed in conical flasks of three different sizes to give a surface area to depth ratio of ½, 2 and 5 respectively. In all flasks, the quantity of milk used was the same i.e., 200 ml.

Lactic acid bacteria grown in yeast dextrose broth to the value of 40 per cent turbidity was used to inoculate the milk. For each strain three flasks were incoulated using 1 per cent inoculum. Flasks were then incubated at 22°C and the acidity developed was checked regularly during a period of 20 h.

3. Preparation of starter for cheese making

a. Starter for experimental cheese making

Small quantities of starter, around 200 ml, were prepared for use in experimental Cheddar cheese making. The method used for preparing the starter was the standard bacteriological method described in Chapter one - section 3:5e in which a single strain of Str. lactis (ML8) was used. The titratable acidity of the starter prepared, ranged from 0.75 per cent lactic acid.

b. Starter for small scale cheese making

Starter for making Cheddar cheese on a small scale i.e., using 180 or 360 l (40 or 80 gal.) quantities of milk, was prepared using the 'Lewis' method described in Chapter one - section 3:5b.

Two single strains of lactic acid bacteria were usually used together, following pairs being selected for the study:-

Fast strain (1 part)	Slow strain (2 parts)
P_2	SK11
B_1	SK11
ML8	AM_2
C_2	E8

A pair of two fast strains - HP and ML8 - known to produce bitterness in Cheddar cheese (Crawford, 1977) was used for the production of Cheddar cheese to study the effect on cheese flavour and taste in the study of organoleptic quality of Cheddar cheese made with different coagulants (Chapter seven).

RESULTS

1. Maintainance of pure culture

A reserve of pure culture was prepared by growing the culture on M16 solid medium or in yeast dextrose broth, and using them to inoculate tubes containing sterilized milk.

Immediately after inoculation, the tubes were stored in a deep freeze at a temperature of -20°C. Cultures stored by this method were found to remain active after two years' storage under frozen conditions. It was possible to use the frozen culture to prepare starter for cheese making after thawing the culture in warm water (45°C) and incubating it at 30°C for 24 h.

Microscopic examination of starter cultures was found to be useful to give a primary idea of contamination, and it was also possible to detect contamination by examining petri dishes of M16 medium streaked with a growing culture. Biological tests i.e., catalase and arginine dihydrolase tests were useful to differentiate between starter cultures and other species of Str. i.e., Str. faecalis and Str. thermophilus.

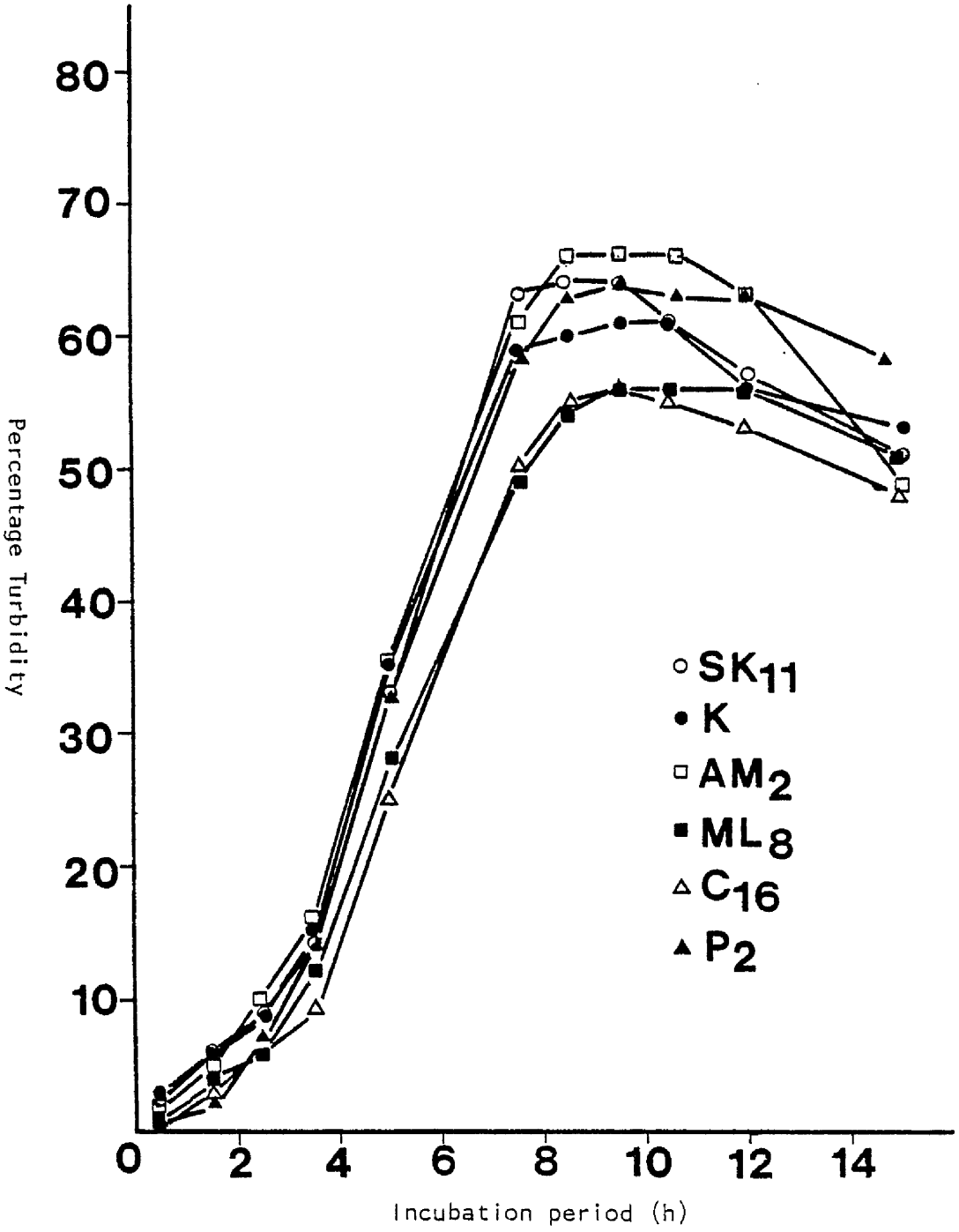
2. Study of acidity development

Different strains of starter cultures were found to differ in their ability to grow in the same media graph (2:1), and it was noticed that the growth rate of starter cultures as indicated by the turbidity measurment could be divided into four distinct phases - lag, logarithmic, stationary, and death phases. Lag phase for starter cultures lasted for about 1 to 2 h. During that time the number of organisms as indicated by turbidity measurement was constant, followed by the logarithmic phase which lasted for about 5 h, during which the number of organisms was increasing logarithmically in relation to the time. The other two phases could not be differentiated clearly because the linear relation between turbidity and time no longer existed. The formation of sediment and aggregation of cells made the suspension irregular and it was not possible to measure the turbidity value for it.

On studying the rate of acidity development in relation to cell mass,

GRAPH 2:1

Growth of different strains of starter culture in Yeast Dextrose Broth, expressed as percentage turbidity, after various periods of incubation at 30°C



different strains of lactic acid bacteria showed a similarity in their acidity development pattern graphs (2:2, 3, 4, 5 and 6).

The rate of acidity development did not always correspond with the rate of growth of the culture as determined by the turbidity level of the medium. The acidity after 20 h for all treatments was similar except for strain K which was found to produce a lower acidity after this period of growth.

The effect of aeration as determined by the ratio of the surface area of the growth medium to its depth was considerable, graphs (2:7, 8 and 9). The rate of acidity development and acidity after 20 h incubation was in the order $\frac{1}{2} > 2 > 5$.

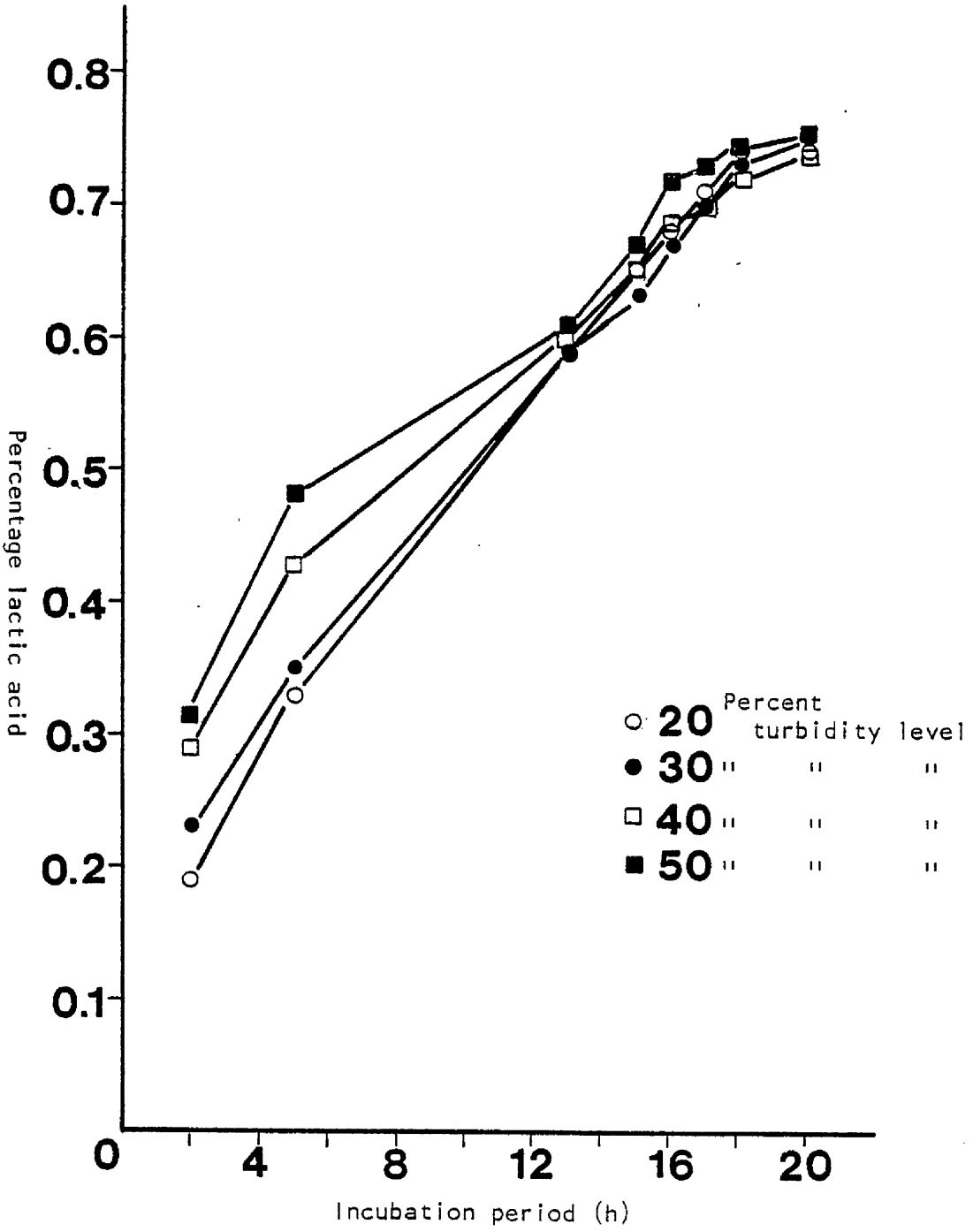
3. Preparation of starter for cheese making

For making experimental Cheddar cheese in yield trials, the single strain starter coded ML8 was used, and although measurements of acidity development during cheese making was not possible because of volume restrictions, the pH value of the finished cheese was within the recommended levels, i.e., 4.9 to 5.2, thereby indicating that the action of the starter was normal.

In the small scale Cheddar cheese making, different pairs of lactic acid bacteria gave different rates of acidity development - graph 2:10, which have made the final acidity at milling to be different from batch to batch of cheese. When strains HP and ML8 were used in a mixture to make Cheddar cheese, bitterness and bad flavour was detected during the cheese curing period, Chapter seven.

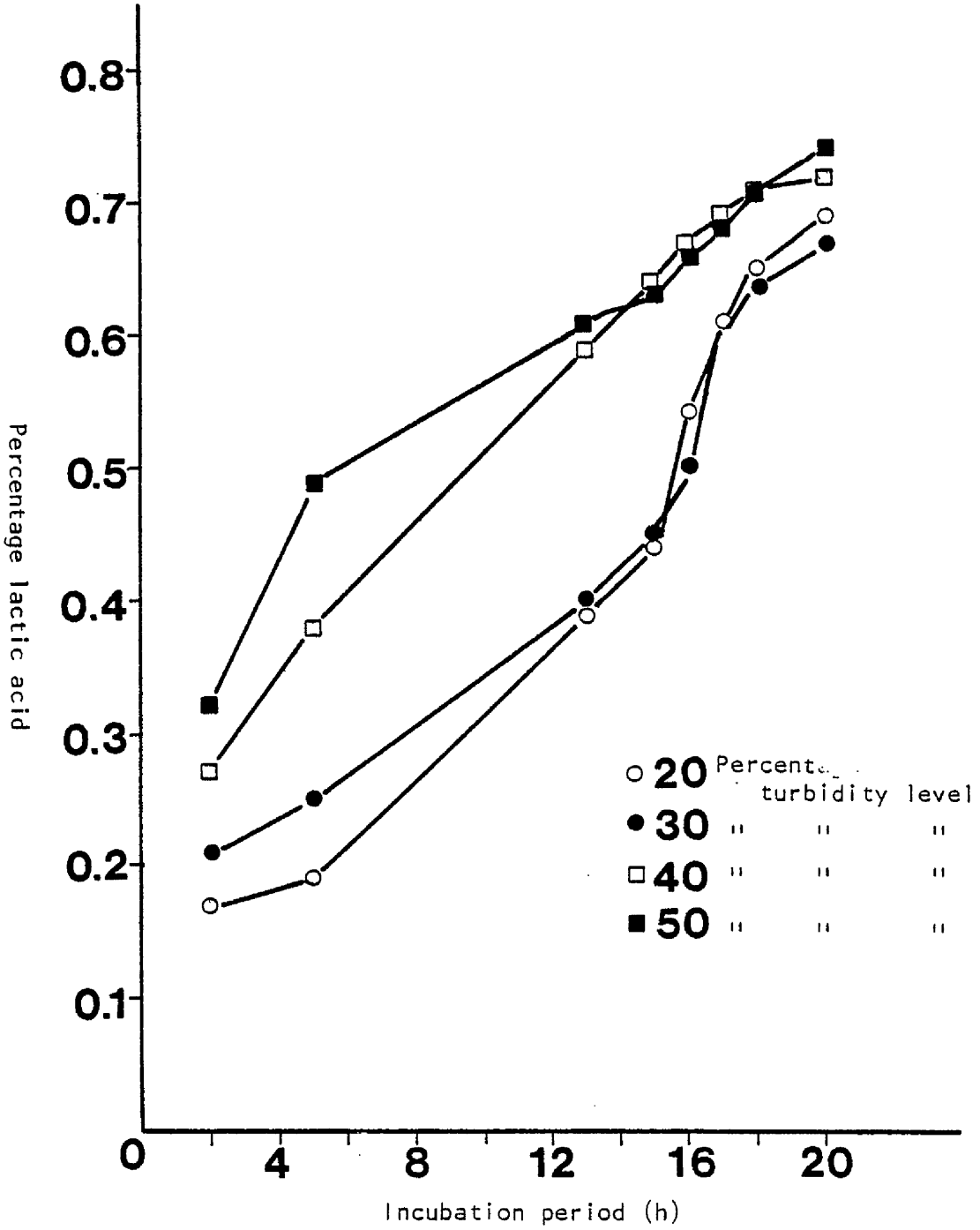
GRAPH 2:2

Acidity development after different periods of incubation at 22°C, expressed as percentage lactic acid, in milk inoculated with starter culture (strain P2) grown in YDB to different levels of turbidity (20, 30, 40 and 50)



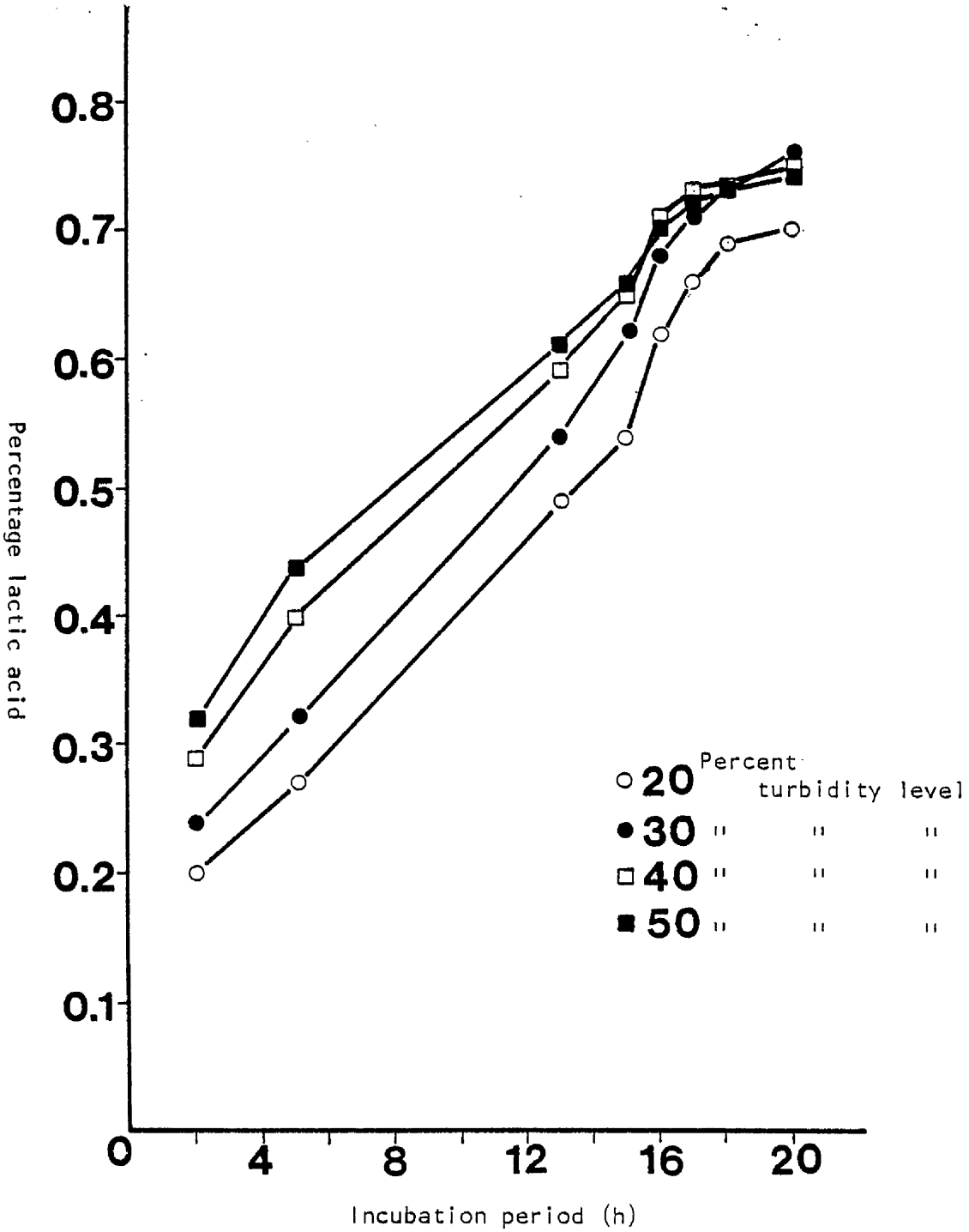
GRAPH 2:3

Acidity development after different periods of incubation at 22°C, expressed as percentage lactic acid, in milk inoculated with starter culture (strain K) grown in YDB to different levels of turbidity (20, 30, 40 and 50)



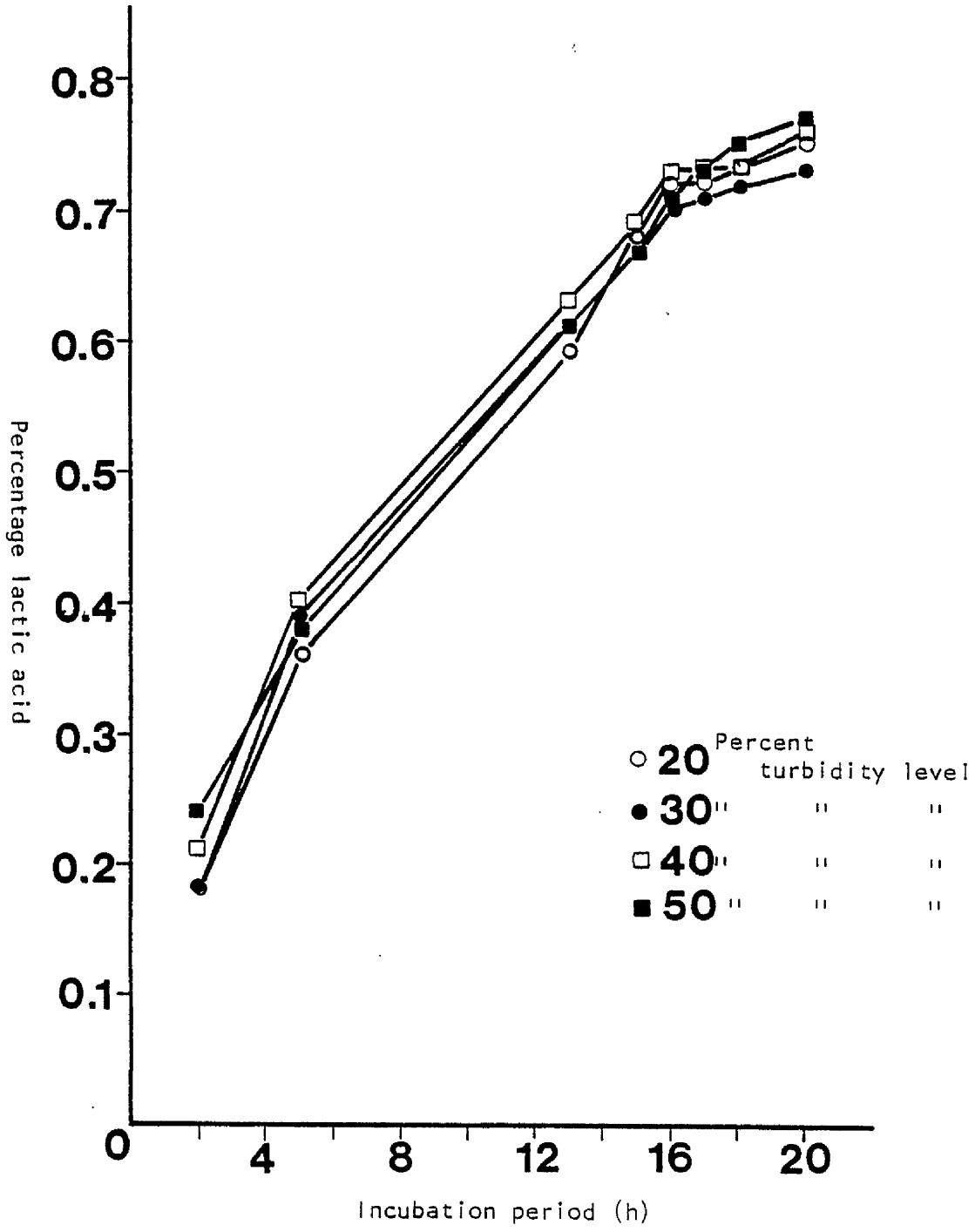
GRAPH 2:4

Acidity development after different periods of incubation at 22°C, expressed as percentage lactic acid, in milk inoculated with starter culture (strain SK11) grown in YDB to different levels of turbidity (20, 30, 40 and 50)



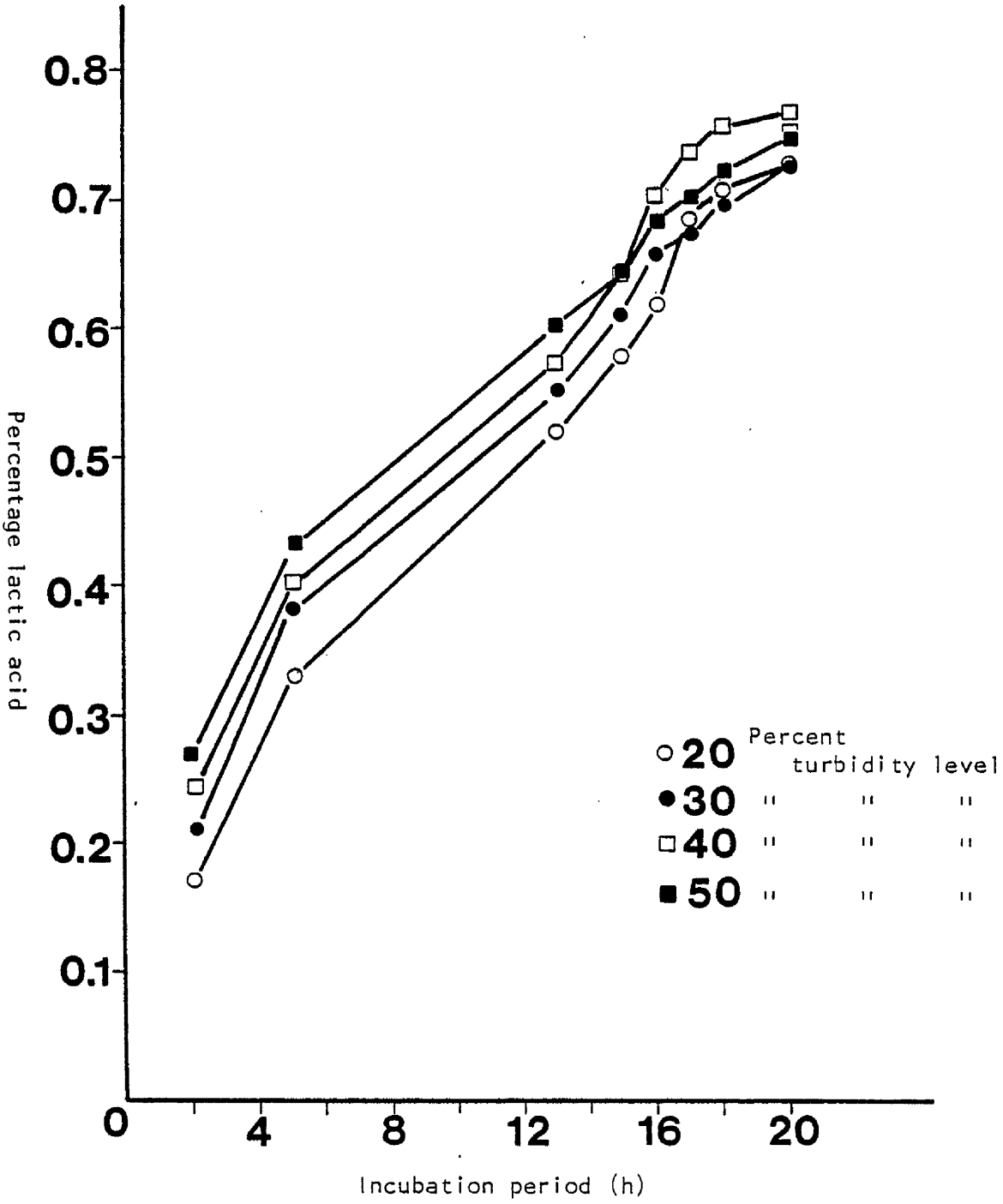
GRAPH 2:5

Acidity development after different periods of incubation at 22°C, expressed as percentage lactic acid, in milk inoculated with starter culture (strain AM_2) grown in YDB to different levels of turbidity (20, 30. 40 and 50)



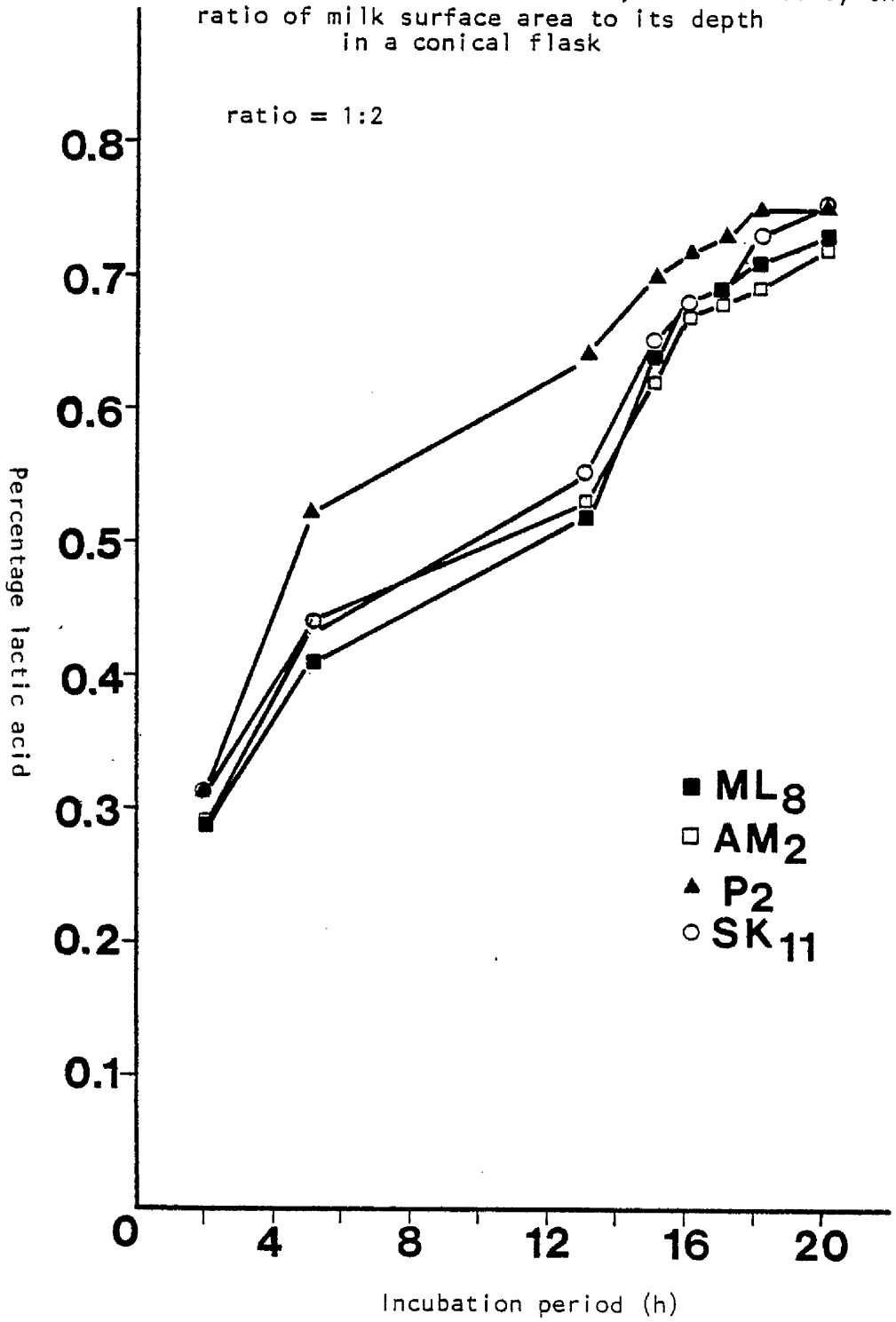
GRAPH 2:6

Acidity development after different periods of incubation at 22°C, expressed as percentage lactice acid, in milk inoculated with starter culture (strain ML8) grown in YDB to different levels of turbidity (20, 30, 40 and 50)



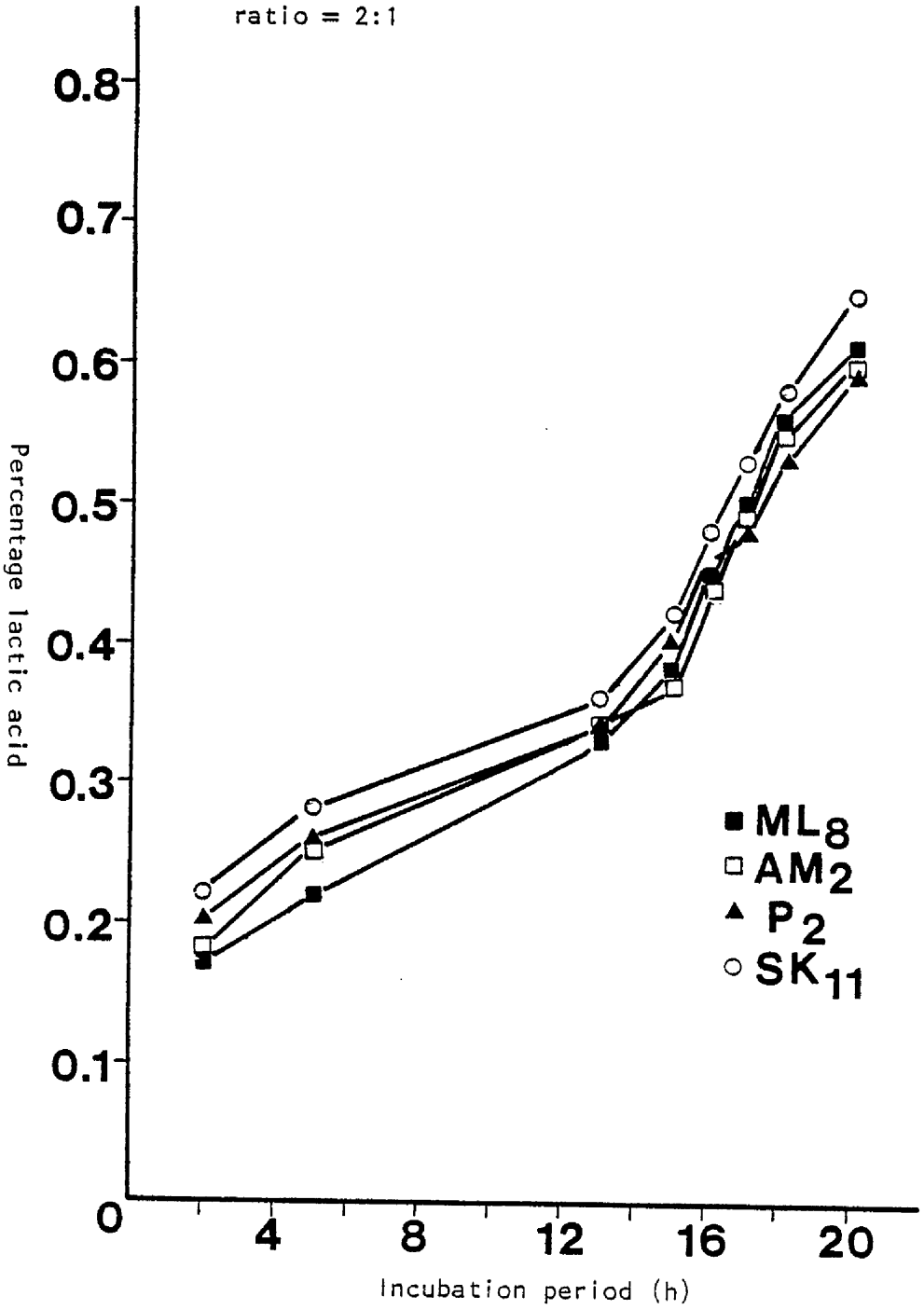
GRAPH 2:7

Acidity development in milk inoculated with different strains of starter culture expressed as percentage lactic acid, after different periods of incubation at 22°C, as affected by the ratio of milk surface area to its depth in a conical flask



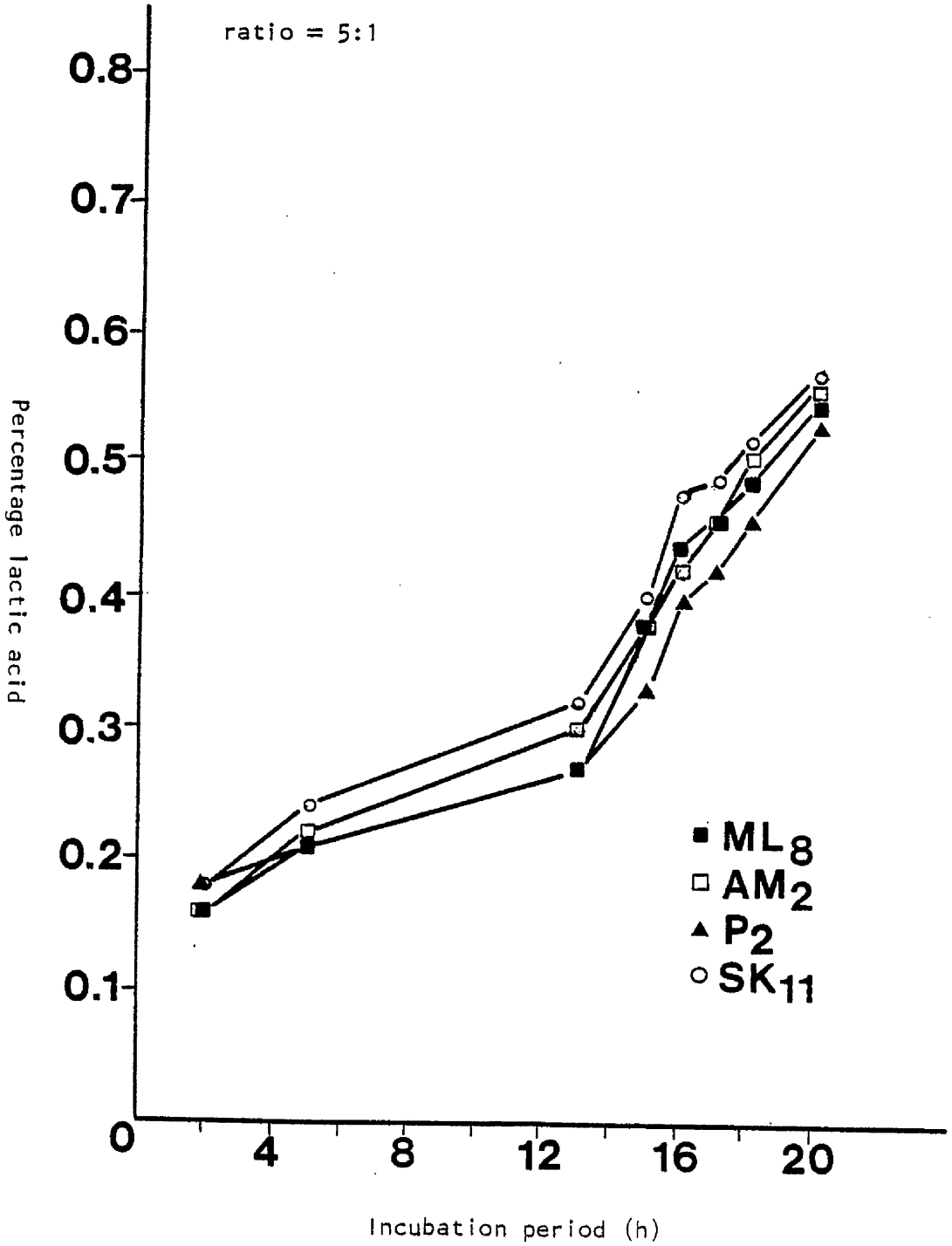
GRAPH 2:8

Acidity development in milk inoculated with different strains of starter culture expressed as percentage lactic acid, after different periods of incubation at 22°C, as affected by the ratio of milk surface area to its depth in a conical flask



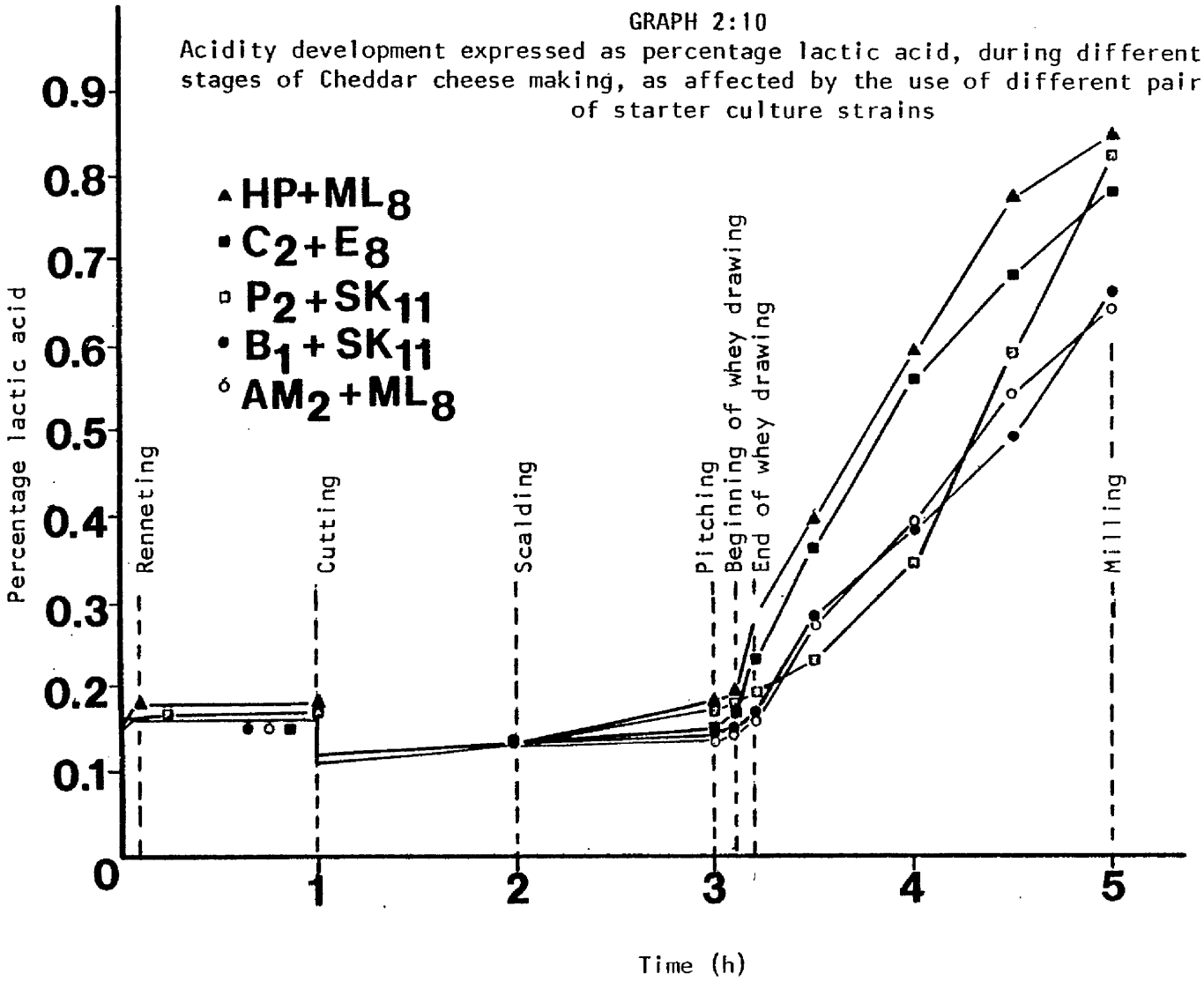
GRAPH 2:9

Acidity development in milk inoculated with different strains of starter culture expressed as percentage lactic acid, after different periods of incubation at 22°C, as affected by the ratio of milk surface area to its dept in a conical flask



GRAPH 2:10

Acidity development expressed as percentage lactic acid, during different stages of Cheddar cheese making, as affected by the use of different pairs of starter culture strains



DISCUSSION

1. To prepare deep frozen cultures successfully, it is important to store the culture immediately after inoculation in the deep freeze, a delay in doing that will cause the production of lactic acid by the culture and cause the pH of milk to drop below 6.6 which is not appropriate for starter culture (Kosikowski, 1977), a storage temperature of -20°C is acceptable although lower temperature is recommended by Davis (1965).

2. When different strains of lactic acid bacteria were inoculated into yeast dextrose broth, the suspension of the cells formed in the broth after growth makes it appear turbid, and, as the concentration of the cells increased up to a certain limit, the turbidity of the broth increased.

The nephelometer measures the amount of light scattered by the cell suspension and gives an indication of the increase in cell concentration, or in other words, an indication of the extent of growth.

Stainer et. al. (1972) stated that turbidity measurement provide the most accurate and easy method of determining the cell mass of unicellular microorganisms provided certain precautions are observed i.e. all cells in the suspension should be at the same phase of growth. They also stated that it was necessary to determine the region over which linearity between turbidity and cell mass is maintained.

In graph 2:1, it was clear that linearity between turbidity value and time of incubation was maintained for five hours. During this period the number of organisms was increasing logarithmically from around two to three hours from the time of inoculation. From the end of the logarithmic phase, the relation was lost due to the formation of sediment and aggregation of cells in the broth.

In measuring the effect of cell mass on the rate of acidity development, it was important to inoculate the milk used in the experiment with lactic acid bacteria at maximum activity i.e., during their logarithmic phase of growth. The levels of turbidity used i.e., 20, 30, 40 and 50 TU (a turbidity unit is equivalent to

600 million cells of lactic acid bacteria per ml of broth (Harrigan and McCance, 1976)) were all within this stage, graph 2:1. Differences found in the rate of acidity development in the milk inoculated with the same strains of lactic acid bacteria but with different levels of turbidity was suspected, and it is mostly due to the variation in the cell mass on the total number of organisms. The variation noticed between the different strains in the trials corresponds to previous reports on starter activity and is the basis for starter selection.

Lactic acid bacteria are all facultative anaerobes, and for the production of lactic acid from lactose, they convert the glucose - which results from the hydrolysis of lactose - into lactic acid through a metabolic sequence known as the Embden - Meyerhofpathway (Stainer et. al., 1972). The reaction can be written as follows:-

$$C_6H_{12}O_6 \longrightarrow 2CH_3COCOOH \xrightarrow[\text{reduction}]{+2} 2CH_3CHOHCOOH$$

glucose pyruvic acid lactic acid

The lactic acid bacteria need anaerobic conditions for this reaction. The presence of oxygen at a high level in the milk will be harmful to them.

Davis (1965) stated that the introduction of oxygen into the milk could result in the production of hydrogen peroxide which is poisonous to lactic acid bacteria, and it will also affect the content of carbon dioxide which is essential for lactic acid bacterial growth. The ratio of the surface area of milk to its depth in a culture vessel could have an effect on the oxygen level in the milk. With a large surface to depth ratio, more air will be sucked into the milk after sterilization and so there will be an increased level of oxygen in the milk. When milk with three different ratios of surface area to depth were used, the rate of acidity development and the final acidity reached were higher when the ratio of surface area to depth of medium was least.

3. The aim of the Cheddar cheese making experiments was to study the effect of the coagulant on cheese quality and yield. The use

of starter in the experiment was necessary to give the usual functions of starter in cheese manufacture i.e., the formation of lactic acid and its subsequent effects during cheese processing and the contribution of bacterial enzymes later in cheese curing to the development of flavour and taste (Crawford, 1958).

In cheese making trials, starter was used in all vats under the same conditions, i.e., the same starter was used at the same rate of inoculation and it was added to the milk at the same temperature, and ripened the milk for the same length of time. All manufacturing procedure and curing conditions were the same for all cheeses made in one trial. Since the conditions affecting the starter were standardised, it may be assumed that if a starter affects the cheese in any way, it will be the same for all cheeses in the trial.

Two single strains of lactic acid bacteria (HP and ML8) known to produce bitterness in Cheddar cheese (Crawford, 1977), were used to make starter. The starter was used to make standard bitter Cheddar cheese which was used in the quality assessment experiment in Chapter seven.

CONCLUSION

The experiments provided evidence that the methods adopted for preparing starters for experimental cheese making were successful and would result in the production of the required acidity within the usual time of cheese making.

The different rates of acidity development which were found to be associated with the use of different strains of lactic acid bacteria emphasised the importance of selecting suitable strains of acid producing organisms. The selection should be based on the rate of acidity development achieved by the use of the specific strain under conditions similar to those of cheese making, and on the compatibility of the strains with each other if used in pairs.

The experiments confirmed earlier reports that cheese flavour and taste may be affected by the use of specific strains of lactic acid bacteria e.g., HP and ML8 which are associated with bitter flavour.

CHAPTER THREE

STUDY OF COAGULANTS

INTRODUCTION

The conversion of milk from the liquid state to a gel or semi-solid state holding most of the milk solids is the key step in cheese making, and this step can be achieved by one of two methods depending on the cheese variety. In fresh curd cheeses, e.g., cottage, lactic curd and quarg, the separation of milk solids from milk serum is brought about as a result of acidity development in milk due to lactic acid formation, while in the case of most other well-known cheese varieties, the separation is obtained as a result of the action of milk clotting enzymes added to the milk which act on the casein micelles and make them aggregate in a network together with the other milk constituents e.g. milk fat. The separation happens when the network begins to shrink and causes the whey to be expelled.

The mechanism of milk coagulation by milk clotting enzymes is affected, as a chemical reaction, by several factors related to the milk, coagulants, or the reaction conditions. Milk composition changes after storage, pasteurization, sterilization, or homogenization, and the changes affect milk proteins, fat or minerals. It is known that coagulants from different sources i.e., from animals, plants, and microbes vary in their composition and properties and affect coagulation. And finally, the temperature and acidity of milk at the time of addition of the coagulant will have a great effect on coagulation.

Coagulation of the milk is the obvious function of coagulants, but they also hydrolyse milk proteins to a certain extent during the curing of the cheese. The aim of this chapter is to study the clotting and proteolytic activities of some milk coagulants, and the factors affecting milk coagulation.

EXPERIMENTAL

The following coagulants were obtained for the study:-

Liquid calf rennet (Hansen's 'Standard' brand).
" porcine pepsin 100 per cent preparation.
" 1:1 mixture of calf rennet and porcine pepsin (Hansen's '50/50' brand).
" $2\frac{1}{3}$: 1 blend of calf rennet and porcine pepsin (Hansen's '70/30' brand).
" *Mucor miehei* rennet (Hansen's 'Hannilase' brand).

All coagulants were supplied by Chr. Hansen's Laboratory, Reading, England.

1. Determination of the clotting activity of chymosin and pepsin in rennets

Chymosin and pepsin in these rennets (calf rennet, 1:1 mixture of calf rennet and porcine pepsin, and $2\frac{1}{3}$: 1 blend of calf rennet and porcine pepsin) were separated first using column chromatography following the method of Garnot *et. al.* (1972) described in chapter one - section 2:2. The clotting activity of each enzyme was then determined using the modified method of Berridge (1952) described in Chapter one - section 2:1a.

2 Determination of clotting activity

The clotting activity of calf rennet was determined using 12 g skim milk powder reconstituted in 100 ml of 0.01 M calcium chloride solution and the rennet was used at the ratio of 1:5000 in the method used for determining clotting activity described by Berridge (1952).

Four other coagulants i.e., *Mucor miehei* rennet ('Hannilase' brand), two mixtures of calf rennet and porcine pepsin (1:1 and $2\frac{1}{3}$: 1), and porcine pepsin solution were used in different dilutions to measure their clotting activity and to find the concentration at which they would have the same activity as calf rennet.

Samples of several different rennets were received from the Government Dairy Station, The Netherlands (NI20) in the course of a cooperative trial, agreed by the International Dairy Federation, of the Dutch method developed by Eisses (1977) (described in chapter one - section 2:1b).

The rennets received were:-

1. Standard calf rennet.
2. Mucor miehei rennet code number R100S.
3. Mucor miehei rennet code number R150S.
4. Mucor miehei rennet code number F100.
5. Mucor miehei rennet code number F150.
6. Mucor pusillus rennet code number MP100.
7. Endothia parasitica rennet 200.000 mcu/g.
8. Bovine rennet code number 78B8261.
9. Porcine/calf 1:1 rennet code number 78B8262.
10. Chicken pepsin rennet.

3. Determination of non protein nitrogen released by coagulants

To study the coagulants proteolytic activity, the non protein nitrogen released from a casein substrate after the addition of coagulant was measured using either the micro Kjeldahl method described in chapter one - section 2:3a, or Miller's (1959) modification of the procedure by Lowry et. al. (1951) described in chapter one - section 2:3b. This method depends on measurement of the optical density of the casein solution filtrate. The coagulants used were - calf rennet (Hansen's 'Standard' brand), Mucor miehei rennet (Hansen's 'Hannilase' brand), mixture of calf rennet and porcine pepsin 1:1, and porcine pepsin rennet.

4. Study of factors affecting the clotting activity of coagulants

The four coagulants referred to above were used in studies on the effect of the following factors on clotting activity which was determined according to the modified method of Berridge (1952) described in chapter one - section 2:1a.

a) The effect of milk temperature on clotting activity

Clotting acitvity was determined in milk at 30°C, and at a series of higher temperatures (2°C steps) until a temperature was reached where the coagulant lost its clotting activity. The milk used for the experiment was a skim milk powder reconstituted in 0.01 M calcium chloride solution and with the pH adjusted to 6.3 with lactic acid. The temperature was controlled at the selected temperature using a water bath fitted with a sensitive thermostat (sensitivity ± 0.05°C).

b) The effect of milk pH on clotting activity

Clotting activity was determined in skim milk prepared by reconstituting powder in 0.01 M calcim chloride solution at 32°C. The pH of the milk had been adjusted by the addition of lactic acid or sodium hydroxide to nine levels from 6.0 to 6.8.

c) The effect of calcium addition on clotting activity

Skim milk powder reconstituted in distilled water was used first to determine the clotting time for the four coagulants referred to above at 32°C and pH 6.3. Clotting time was then determined in skim milk containing different levels of calcium (from 40 mg to 220 mg calcium per 100 ml milk) by reconstituting the powder in solutions of calcium chloride of different concentrations.

d) The effect of lactose hydrolysis on clotting activity

The clotting time for calf rennet was determined in skim milk (prepared by reconstituting powder in a 0.01 M calcium chloride solution) at 30°C and pH 6.6 before and after hydrolysing the milk lactose to different levels of hydrolysis. The enzyme lactase ('Maxilact 20.000' brand supplied by Gist Brocades, Delft, Holland) was used at two levels 50 mg and 75 mg/100 ml of milk at 32°C. The extent of hydrolysis was determined by measuring lactose levels using the modification by Nickerson et. al., (1975) of the method developed by Fearon (1942) described in chapter one - section 1:4. Freezing point depressions were measured at the same times when the lactose was measured, using the method described in chapter one - section 1:5 and correlated to the hydrolysis of lactose.

RESULTS

1. Determination of chymosin and pepsin clotting activity in rennets

It was noticed that the clotting time of the dialysed rennets was increased due to the dilution of the rennets during dialysis. When preparing the separation column, packing of the column with the activated cellulose to the exact height specified in the method (8 cm) was found to be important in obtaining complete separation of the chymosin and the pepsin with the specific flow rate of the salt gradient (80 ml/h).

Packing of the column to heights of more than 10 cm did not work well and separation of the chymosin and the pepsin was not achieved using a flowrate of the salt gradient of 80 ml/h. Attempts to increase the flow rate of the salt gradient above 90 ml/h was not successful and resulted in incomplete separation of the chymosin and the pepsin. Table 3:1 shows the clotting time for rennets before and after dialysis and gives the clotting time and per cent activity of the chymosin and the pepsin fractions.

2. Determination of clotting activity

A solution of 0.2 per cent (v/v) from each one of five different coagulants in distilled water was used to determine clotting time. The coagulants used were calf rennet, Mucor miehei rennet ('Hannilase' brand), 1:1 and $2\frac{1}{3}$: 1 mixtures of calf rennet and porcine pepsin, and porcine pepsin rennet. One ml from the solution of each coagulant was found to clot 10 ml milk in 490 s., 552 s., 420 s., 442 s., and 370 sec. respectively. Solutions of various concentrations were prepared, from the coagulants referred to above, in distilled water in an attempt to find the concentration at which different coagulants will have the same clotting time (graph 3:1). From the graph, it is evident that the concentrations of coagulants which gave clotting time equal to that obtained with calf rennet i.e., 490 s. are:-

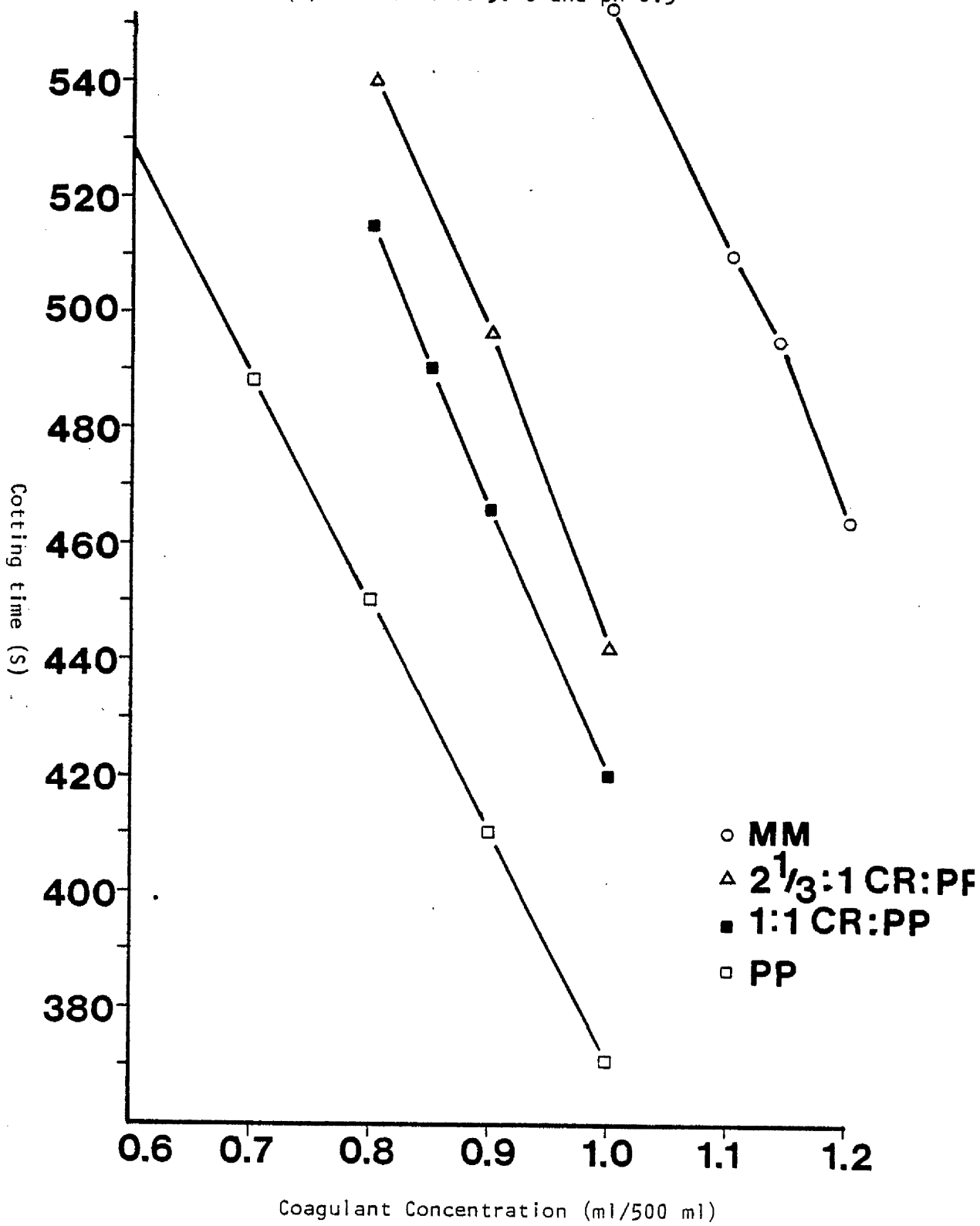
TABLE 3:1

Amount of chymosin and pepsin expressed as percentage from different rennets as determined by separating the enzymes using column chromatography, in addition to clotting time expressed in seconds and clotting activity expressed in rennet units (RU) of the two enzymes

	Calf rennet	1:1 mixture CR/PP	$2\frac{1}{3}$: 1 mixture CR/PP
Clotting time of the rennet before dialysis (0.2 per cent solution of rennet in distilled water)	506 s	428 s	496 s
Clotting time of the rennet after dialysis (0.2 per cent solution)	735 s	722 s	780 s
Volume of the first fraction	90 ml	87.1 ml	87 ml
Clotting time of the first fraction (25ml to 50ml with dis. water)	316 s	897 s	543 s
Volume of the second fraction	105 ml	113 ml	112 ml
Clotting time of the second fraction (without any dilution)	2877 s	895 s	1338 s
Clotting time of the intermediate fraction	90 min	150 min	100 min
Activity (RU) of the first fraction	82.74	32.75	50.39
Activity (RU) of the second fraction	5.30	42.59	26.33
Per cent activity of the first fraction (chymosin)	94.0	43.5	65.68
Per cent activity of the second fraction (pepsin)	6.0	56.5	34.32

GRAPH 3:1

Relation between coagulant concentration (ml coagulant made up to 500 ml with distilled water) and clotting time (S) in milk at 30°C and pH 6.3



Coagulant	Concentration as number of millilitres of of coagulant in 100 ml solution
CR	0.20 ml
MM	0.23 ml
1:1 CR/PP	0.17 ml
$2\frac{1}{3}$:1 CR/PP	0.18 ml
PP	0.14 ml

High quality skim milk powder should be used to obtain a milk with a constant pH. Furthermore the reconstituted milk should not be held in the water bath for more than 90 min, as the effect of a longer holding time on milk constituents will affect the clotting time. In the experiments, the temperature of the milk had reached the temperature of the water ($30^{o}C \pm 0.05$) in the bath within 15 min and there was no need to wait for the 30 to 60 min suggested in the procedure. The use of a syringe to add the coagulant to the milk gave sufficient mixing of the coagulant with the milk and there was no need for shaking and inverting the tube before starting rotation of the tube in the bath.

From graph 3:1, it is apparent that the relation between concentrations of the coagulant and the clotting time is similar for Mucor miehei rennet ('Hannilase' brand) and the two mixtures of calf rennet and porcine pepsin (1:1 and $2\frac{1}{3}$:1), as the relation for porcine pepsin was different, being steeper.

When coagulant strength was measured by the Eisses (1977) method it was found (table 3:2) that Mucor pusillus Lindt rennet had the highest clotting activity of all samples of coagulants, almost double the clotting acitivity of calf rennet. The sample of Endothia parasitica rennet and the 1:1 mixture of calf rennet and porcine pepsin was found to give the next highest clotting activity. Each of the four samples of Mucor miehei rennets had a lower clotting activity than the calf rennet except the R1505 preparation which had about the same clotting activity as calf rennet.

Bovine rennet had a low strength, and when mixed with calf rennet in the ratio of 1:4 the clotting activity improved to about the same level of

TABLE 3:2

Coagulant clotting activity expressed as a percentage of the clotting activity of a commercial brand of calf rennet

Coagulant	Solution	Clotting activity @ pH 6.6	Clotting activity @ pH 6.5	Clotting activity @ pH 6.3
(1) Standard calf rennet	1 g/100 ml	100.000	100.000	100.000
(2) Mucor miehei rennet R1005	1 g/100 ml	73.806	73.606	74.136
(3) Mucor miehei rennet R1505	1g/100 ml	99.695	106.474	113.187
(4) Mucor miehei rennet R 100	1 g/100 ml	53.838	64.481	57.222
(5) Mucor miehei rennet R150	1 g/100 ml	75.136	101.724	85.152
(6) Mucor pusillus Lindt RENNET MP 100	1 g/100 ml	192.700	219.380	188.991
(7) Endothia parasitica rennet 200.000mcu/g	1 g/100 ml	186.550	167.822	187.500
(8) Bovine rennet 78B 8261	1 g/100 ml	54.555	60.319	78.794
(9) Calf rennet/porcine pepsin mixture (1:1) 78B8262	1 g/100 ml	169.077	213.333	211.008
(10) Chicken rennet	1 ml/100 ml	25.284	34.629	41.488
(11) Calf rennet/bovine pepsin mixture (4:1)	0.8g(1) + 0.2g (8)/100 ml	85.154	90.439	99.229
(12) Calf rennet/bovine pepsin mixture (1:1)	0.5g(1) + 0.5g (8)/100 ml	72.935	83.113	94.299

TABLE 3:9

Comparison of results for the determination of the clotting activity of coagulants expressed as a percentage of the clotting activity of a commercial brand of calf rennet, obtained by the author with those of other Laboratories (in an IDF collaborative study)

Coagulant	*	The author	Clotting activity as determined by other laboratories	
			Mean	Standard deviation
MM R1005	1	73.806	69.400	8.900
	2	73.606	68.200	6.800
	3	74.136	68.500	6.800
MM R1505	1	99.695	101.100	15.100
	2	106.474	95.700	9.300
	3	113.187	97.500	9.400
MM F100	1	53.838	55.100	8.600
	2	64.481	52.500	5.700
	3	57.222	51.600	5.600
MM F150	1	75.136	81.500	12.100
	2	101.724	81.900	11.900
	3	85.152	79.800	9.700
MP	1	192.700	208.300	17.200
	2	219.380	217.600	16.400
	3	188.991	266.100	19.900
EP	1	186.550	211.800	51.400
	2	167.822	184.900	50.900
	3	187.500	158.200	37.800
BR	1	54.555	58.300	9.300
	2	60.319	60.400	16.200
	3	78.794	70.700	20.800
CR:PP 1:1	1	169.077	166.300	22.000
	2	213.333	192.100	27.700
	3	211.008	245.200	30.700

Table 3:9 (cont'd.)

Coagulant	*	The author	Clotting activity as determined by other laboratories	
			Mean	Standard deviation
Ch.r	1	25.284	23.100	4.500
	2	34.629	26.500	4.200
	3	41.488	41.600	6.700
CR:BR	1	85.154	101.200	12.100
	2	90.439	89.600	11.700
	3	99.229	97.900	5.800
CR:BR	1	72.935	78.100	4.500
	2	83.113	76.900	8.900
	3	94.299	81.900	19.500

* 1 Clotting activity at pH 6.6
2 " " " " 6.5
3 " " " " 6.3

calf rennet (table 3:2).

Chicken rennet had the lowest clotting activity of all coagulants. The preparation was crude and contained a high amount of a muddy precipitate, it also had an unpleasant smell.

The clotting activity of the coagulants increased as the pH of the milk decreased for most of the samples (table 3:2). The use of whole milk in the determination of clotting activity was difficult and mistakes could arise from the cream line developed on holding the milk in the water bath, also the adjustment of milk pH was difficult and required a long time (2 h) to stabilize.

3. Determination of non protein nitrogen released by coagulants

A 1 per cent solution (w/v) of casein in 0.1 M sodium phosphate was used as substrate for the coagulants. Ten ml of the coagulant solution in distilled water (0.2 per cent calf rennet, 0.23 per cent Mucor miehei rennet, 0.17 per cent 1:1 mixture of calf rennet and porcine pepsin, and 0.14 per cent of porcine pepsin) was added to 100 ml of the casein solution at 32°C and pH 5.5. Non protein nitrogen (soluble in 2 per cent trichloroacetic acid) was determined after certain periods by micro-Kjeldahl method and by measuring the optical density at 690 mm using Miller's (1959) modification of the procedure described by Lowry et. al., (1951).

The increase in non protein nitrogen liberated from casein as indicated by the increase in optical density is given in table 3:3 and graphs 3:2 and 3:3. Determination of non protein nitrogen by micro-Kjeldahl measurements for the same experiment are given in table 3:4 and graphs 3:4 and 3:5.

The results of the tests indicated that Mucor miehei rennet released non protein nitrogen from casein more rapidly than the other coagulants. Calf rennet gave the slowest rate of non protein nitrogen release, and the 1:1 mixture of calf rennet and porcine pepsin released nonprotein nitrogen at about the same rate as porcine pepsin. The results of the two methods correlated significantly as shown by graph 3:6.

TABLE 3:3

Measurement of optical density for casein solution filtrate at wave length 690 nm after different periods of incubation at 32°C as an indication of proteolytic activity of different coagulants

Incubation period (h)	Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	Porcine pepsin	Casein control
0.08	0.87	0.78	0.87	0.85	0.20
0.50	0.95	0.90	0.90	0.88	0.20
2.00	0.95	1.25	1.00	1.04	0.23
24.00	1.43	2.09	1.68	1.71	0.29
48.00	2.64	3.71	3.03	3.13	0.58
Mean 14.916	1.368	1.746	1.492	1.522	0.300
Cor. coeff.	0.980***	0.991***	0.991***	0.990***	0.961***
Y intercept	0.851	0.895	0.850	0.846	0.191
Slope	0.035	0.057	0.043	0.045	0.007
SE of slope	0.010	0.016	0.012	0.013	0.002
Cor. coeff. with NPN	0.875*	0.958**	0.953**	0.958**	0.950**
Y intercept with NPN	0.041	0.018	0.031	0.031	0.010
Slope	0.033	0.064	0.047	0.048	0.067
SE of slope	0.009	0.018	0.012	0.013	0.019

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 3:4

Measurement of non protein nitrogen (calculated as protein) and expressed as a percentage liberated from casein solution after different periods of incubation at 32°C as an indication of the proteolytic activity of different coagulants

Incubation period (h)	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin	Casein control
0.08	0.059	0.066	0.060	0.060	0.021
0.50	0.061	0.067	0.069	0.072	0.022
2.00	0.079	0.085	0.080	0.079	0.026
24.00	0.108	0.193	0.133	0.137	0.036
48.00	0.121	0.241	0.165	0.173	0.048
Mean 14.916	0.086	0.130	0.101	0.104	0.031
Cor. coeff.	0.941**	0.978**	0.980**	0.983**	0.991***
Y intercept	0.067	0.074	0.070	0.070	0.023
Slope	0.001	0.004	0.002	0.002	0.001
SE of slope	0.0004	0.0011	0.0006	0.0007	0.0002

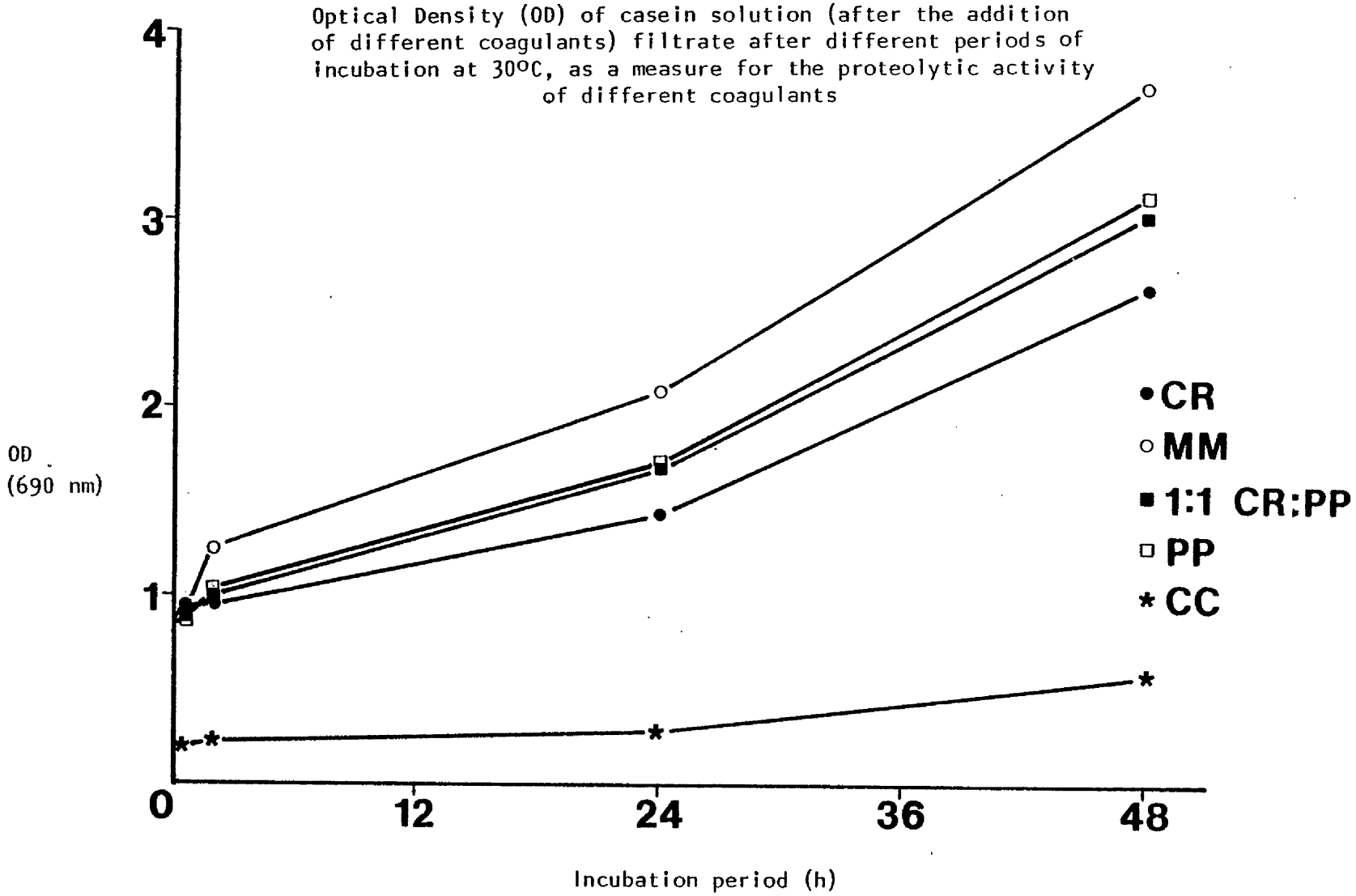
* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

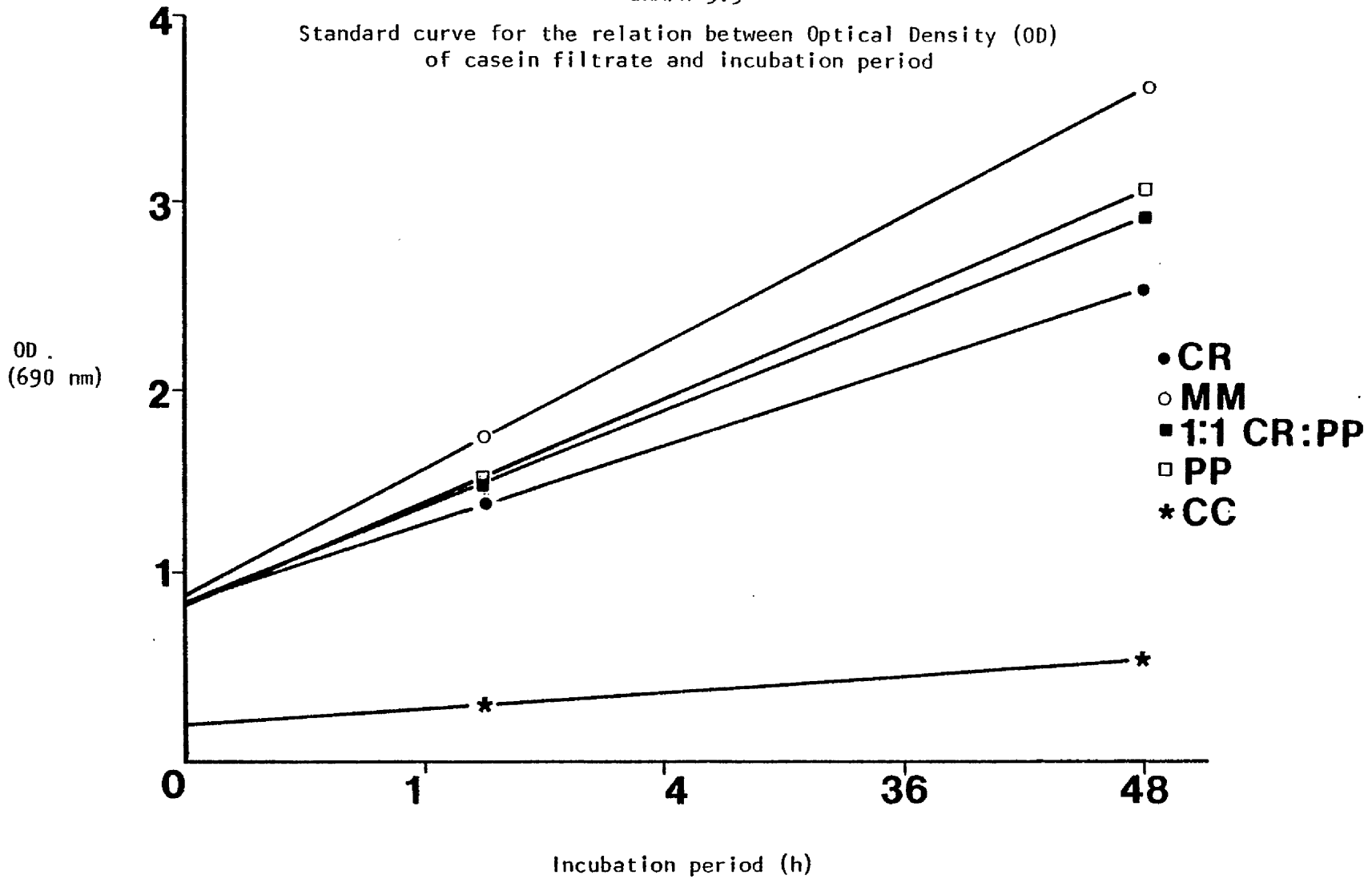
GRAPH 3:2

Optical Density (OD) of casein solution (after the addition of different coagulants) filtrate after different periods of incubation at 30°C, as a measure for the proteolytic activity of different coagulants



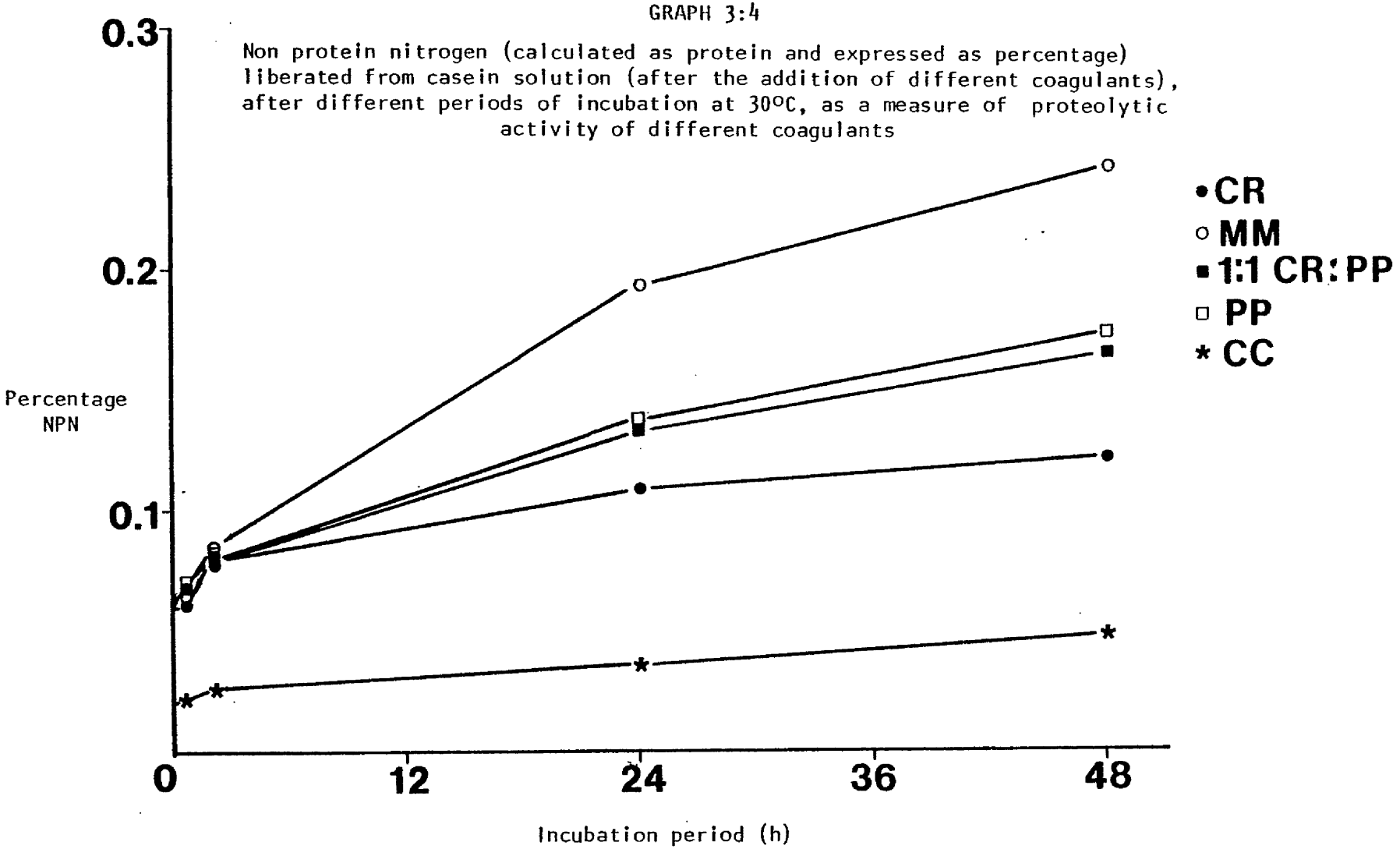
GRAPH 3:3

Standard curve for the relation between Optical Density (OD) of casein filtrate and incubation period



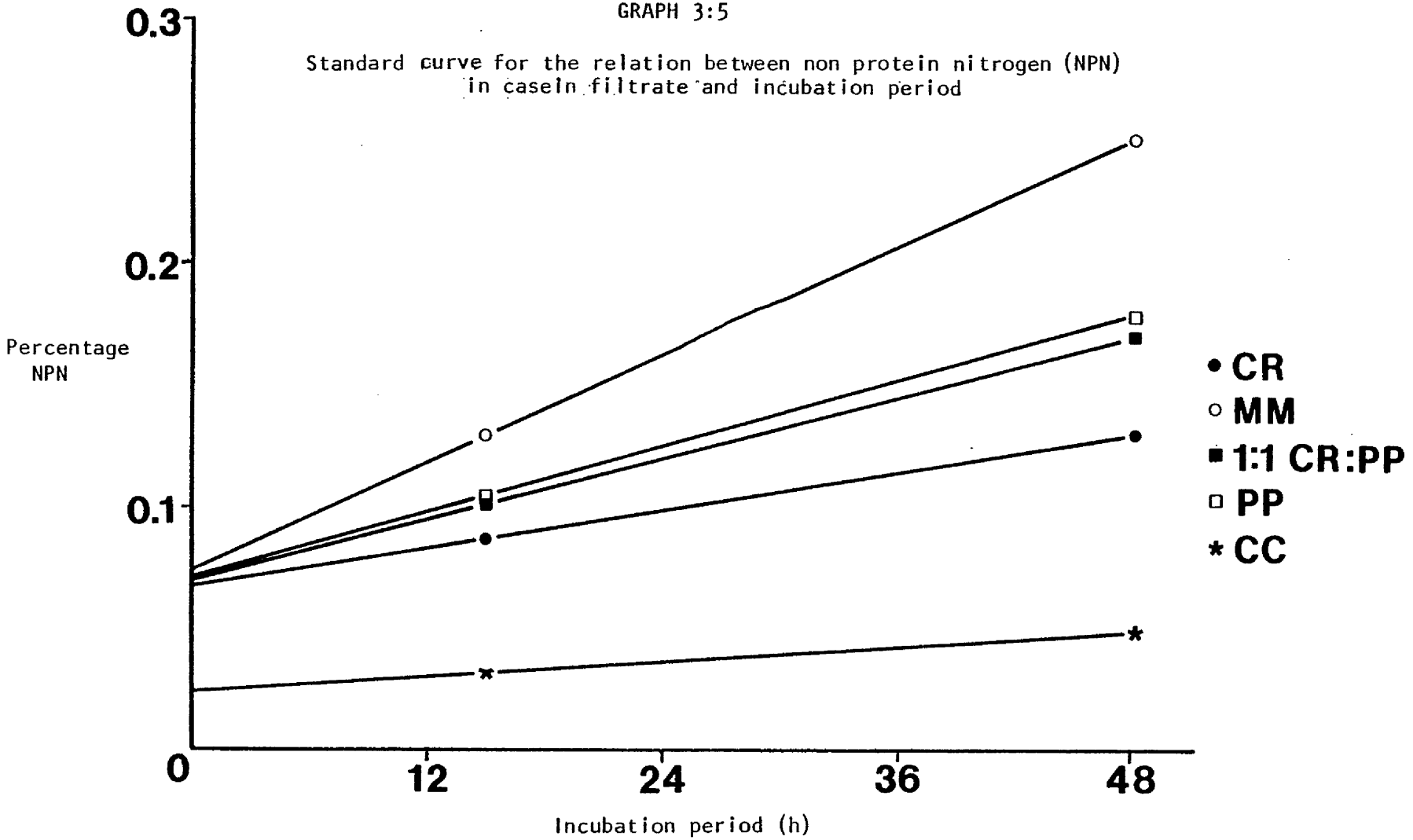
GRAPH 3:4

Non protein nitrogen (calculated as protein and expressed as percentage) liberated from casein solution (after the addition of different coagulants), after different periods of incubation at 30°C, as a measure of proteolytic activity of different coagulants

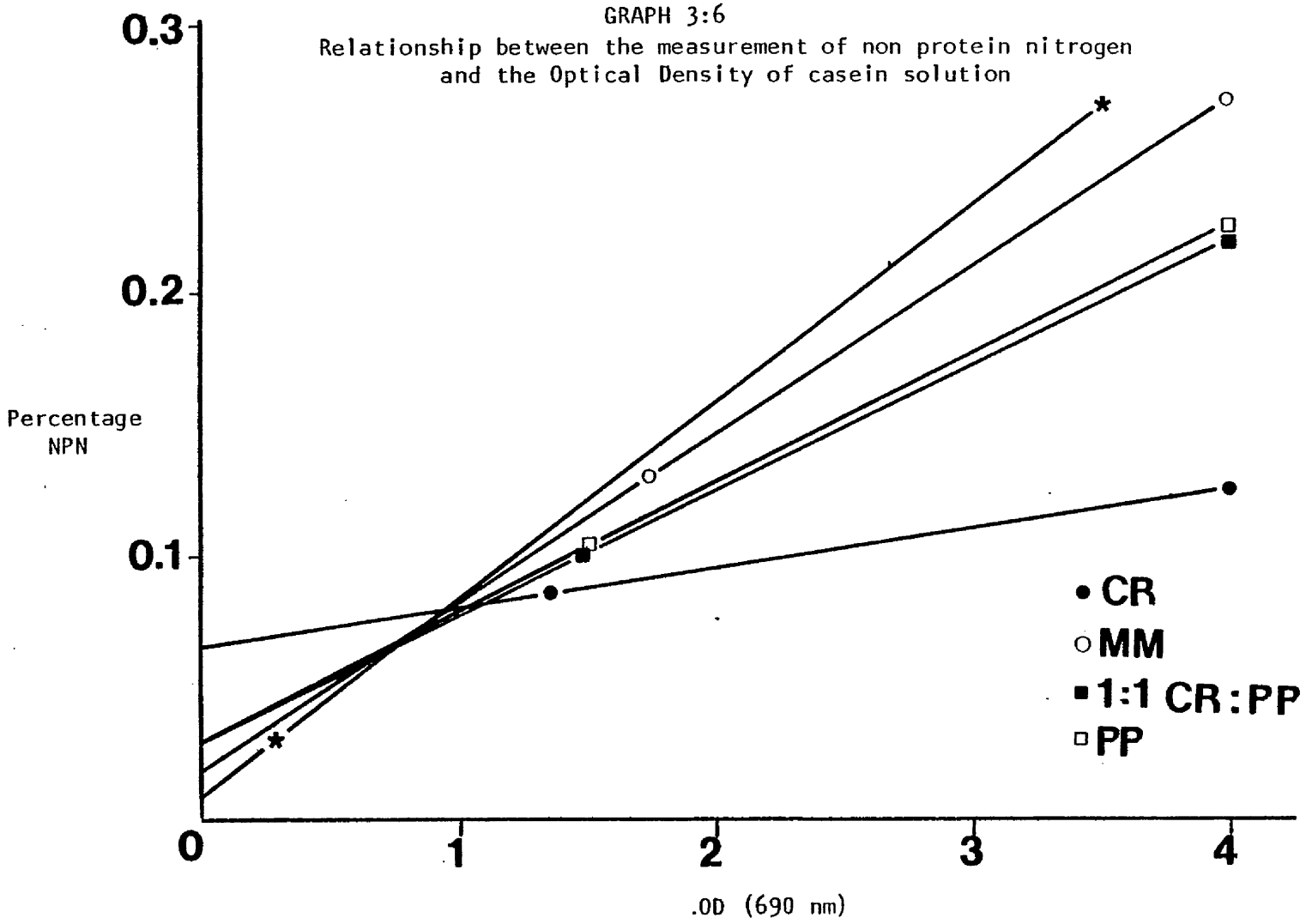


GRAPH 3:5

Standard curve for the relation between non protein nitrogen (NPN) in casein filtrate and incubation period



GRAPH 3:6
Relationship between the measurement of non protein nitrogen
and the Optical Density of casein solution



Doubling of the concentration of coagulant did not materially affect the amount of non protein nitrogen released by each of the four coagulants but did bring about release in a shorter time (graph 3:7).

Graph 3:8 shows the standard curve prepared by using serial dilutions of tyrosine.

The pasteurization of the casein solution and the addition of preservative to it as suggested by Fox (1969) was considered to be unnecessary as indicated by the minimal increase in the non protein nitrogen values for the control. The use of a higher wave length - 690 nm instead of 650 nm as specified in the method - was found to be necessary to give better absorbance. The dilution of the filtrate (after the addition of trichloroacetic acid) was necessary and otherwise the intensity of the colour produced gave absorbance values beyond the scale of the instrument.

4. a) The effect of milk temperature on clotting activity

The results given in table 3:5 and graph 3:9 indicate that porcine pepsin was the coagulant most affected by temperature level, this coagulant showed its highest activity at 40°C, lost its activity at 48°C, and at temperatures between 30 and 40°C had the highest activity of all coagulants. The 1:1 mixture of calf rennet and porcine pepsin had its highest activity at 42°C, lost its activity at 56°C, while calf rennet showed its highest activity at 52°C but no clotting activity was evident at 58°C. Mucor miehei rennet ('Hannilase' brand) was active within a wider range of temperature, started with the lowest activity among other coagulants at 30°C, and activity increased gradually with an increase in temperature, at 42°C it was more active than calf rennet and the 1:1 mixture of calf rennet and porcine pepsin. At 66°C, the coagulant had its highest activity and maintained the same high activity up to 70°C, and at 72°C the coagulant was inactive.

b) The effect of milk pH on clotting activity

The results given in table 3:6 and graph 3:10 show that, in the pH range of 6.0 to 6.4, porcine pepsin had the highest activity of all four

TABLE 3:5

The effect of milk substrate (pH 6.3) temperature on the clotting activity of different coagulants

Milk temperature (°C)	Clotting time in seconds Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	Porcine pepsin
30	571	623	562	424
32	534	541	527	346
34	465	483	425	315
36	388	401	365	259
38	367	372	343	235
40	304	320	317	214
42	300	288	300	218
44	293	261	341	239
46	269	241	422	454
48	259	217	465	no activity
50	245	201	534	" "
52	243	185	645	" "
54	255	162	900	" "
56	326	157	no activity	" "
58	no activity	137	" "	" "
60	" "	126	" "	" "
62	" "	118	" "	" "
64	" "	111	" "	" "
66	" "	102	" "	" "
68	" "	111	" "	" "
70	" "	135	" "	" "
72	" "	no activity	" "	" "

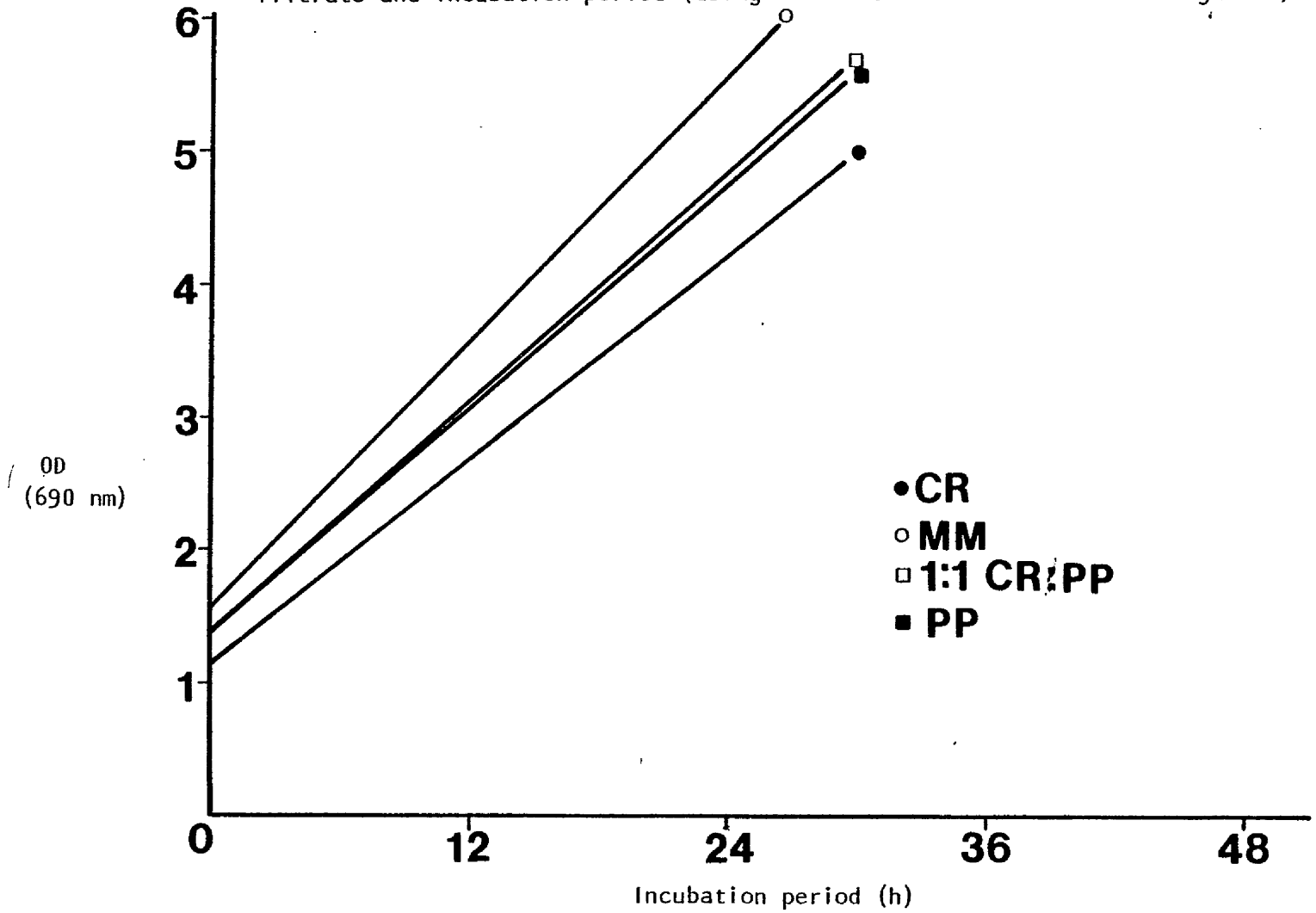
TABLE 3:6

The effect of milk substrate (at 32°C) pH on the clotting activity of different coagulants

Milk pH	Clotting time in seconds			
	Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	Porcine pepsin
6.0	246	276	203	150
6.1	254	301	217	161
6.2	441	483	406	281
6.3	527	541	534	346
6.4	588	600	606	458
6.5	786	775	991	995
6.6	1146	1079	2243	4200
6.7	1608	1404	no activity	no activity
6.8	3188	3191	" "	" "
6.9	no activity	no activity	" "	" "

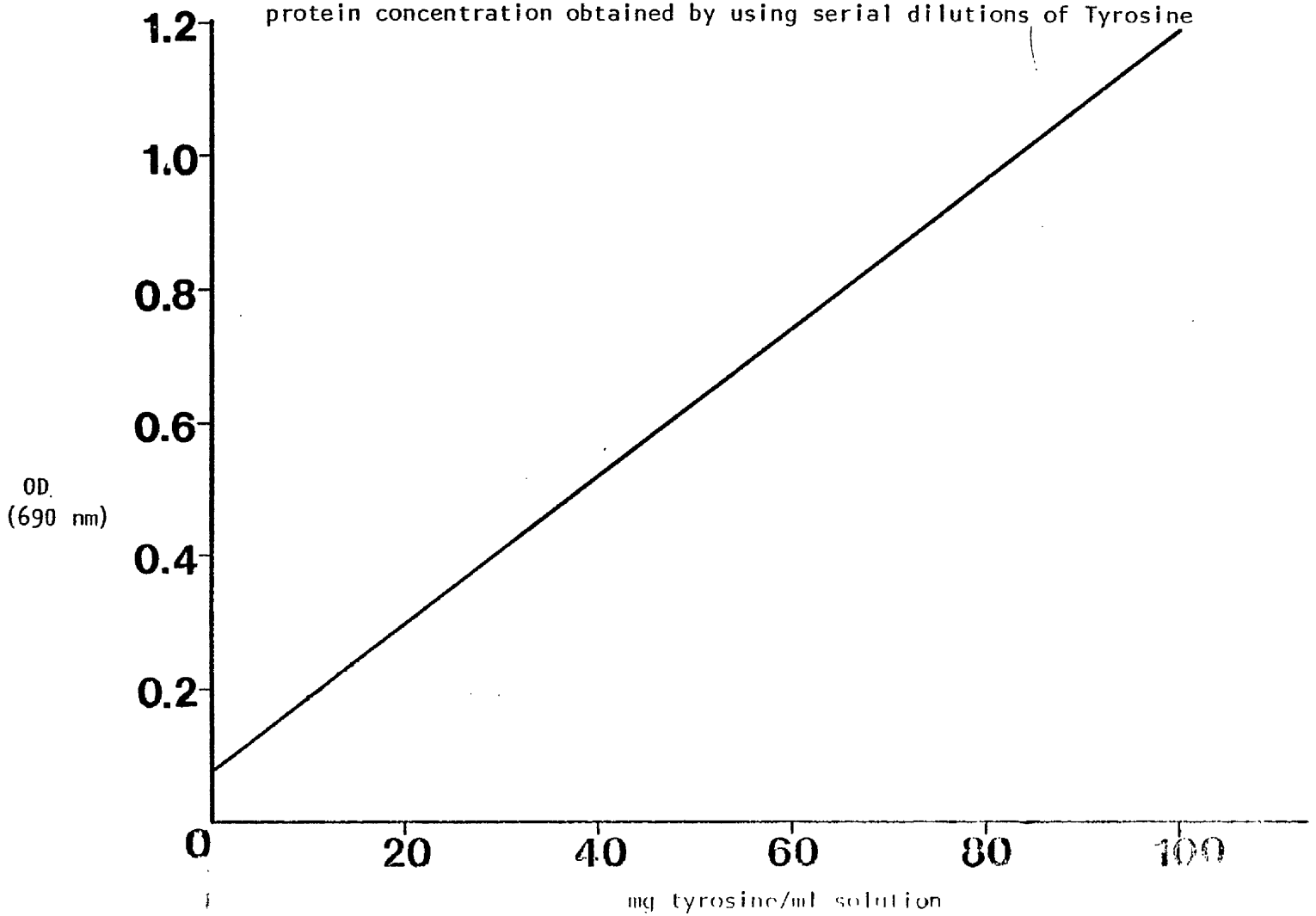
GRAPH 3:7

Standard curve for the relation between Optical Density (OD) of casein filtrate and incubation period (using double the normal amount of coagulant)



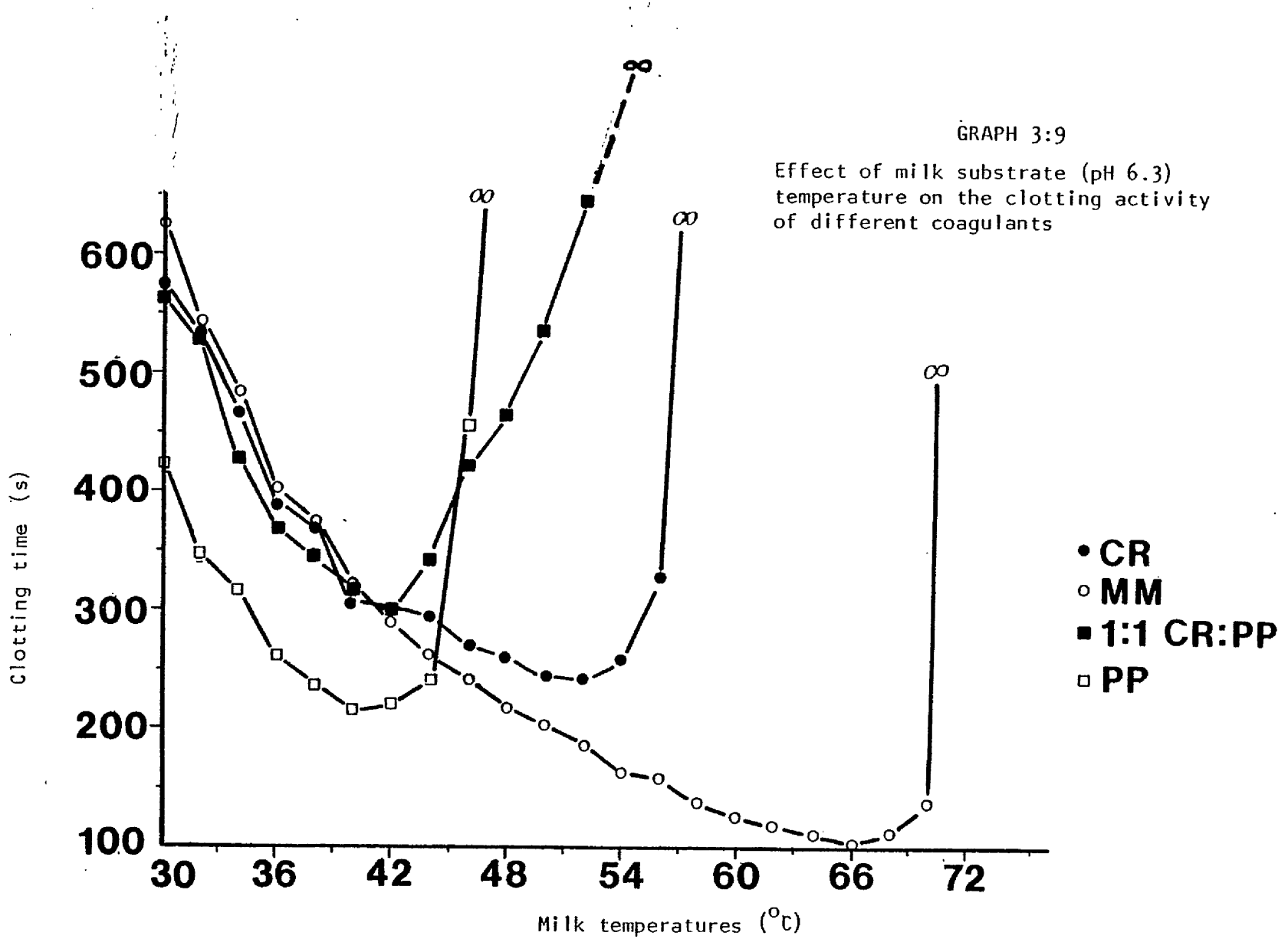
GRAPH 3:8

Standard curve for the relation between Optical Density and protein concentration obtained by using serial dilutions of Tyrosine



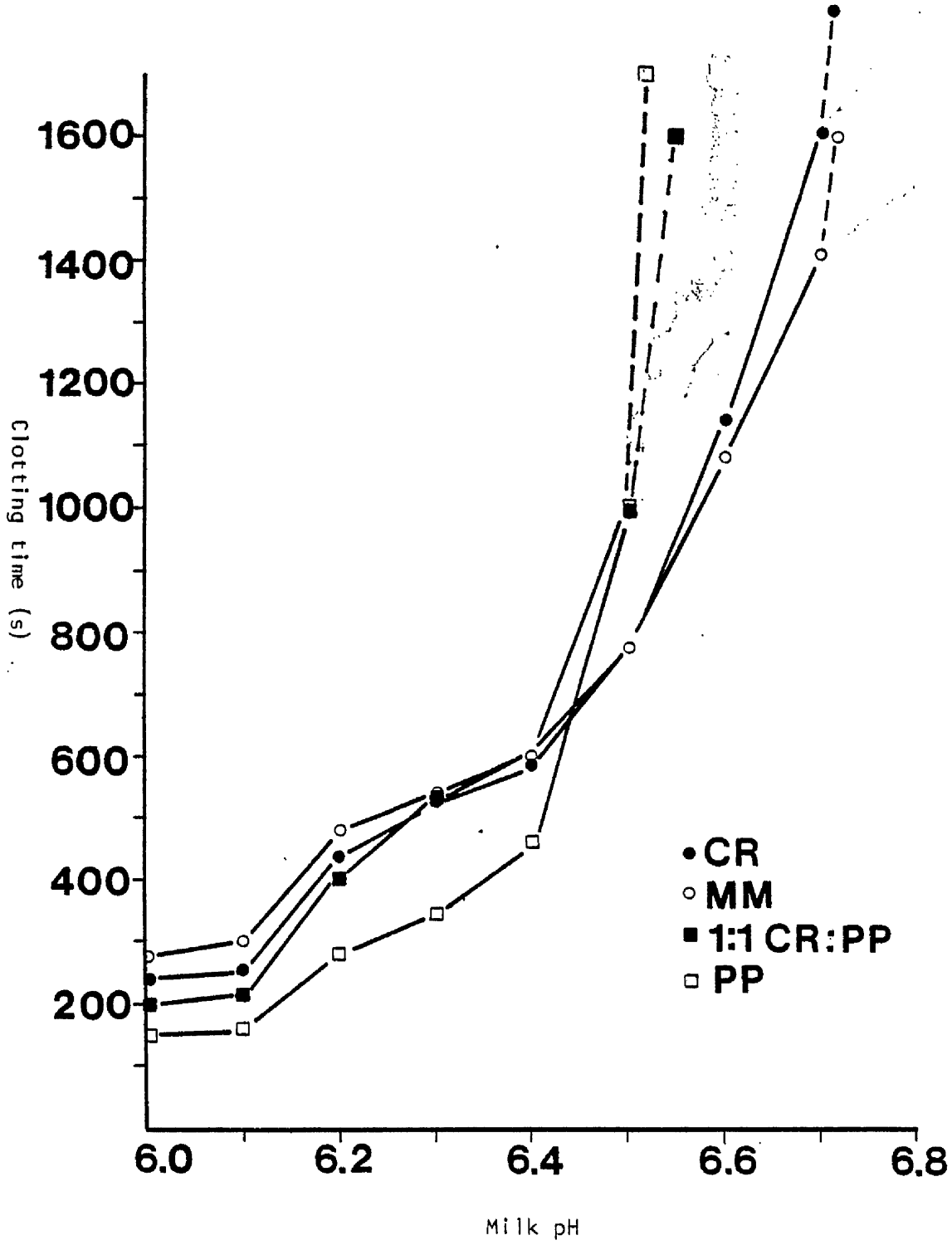
GRAPH 3:9

Effect of milk substrate (pH 6.3) temperature on the clotting activity of different coagulants



GRAPH 3:10

Effect of milk substrate pH (at 32°C) on the clotting activity of different coagulants



coagulants. It lost about 50 per cent of its activity between pH 6.4 and pH 6.5. At pH 6.6, porcine pepsin required 70 min. to clot the milk, and at pH 6.7 porcine pepsin was unable to clot the milk after 5 h. The activity of the 1:1 mixture of calf rennet and porcine pepsin was next to that of porcine pepsin and showed a similar activity change with pH. It clotted the milk at pH 6.6 after 45 min. and was inactive at pH 6.7.

Calf rennet and Mucor miehei rennet ('Hannilase' brand) showed similar behaviour in relation to pH. The clotting activity of both coagulants was low in comparison to the other two coagulants up to pH 6.3. Thereafter clotting activity decreased gradually with increase in pH, and at pH 6.8 both clotted the milk in about 52 min. Both coagulants were inactive at pH 6.9.

c) The effect of calcium on clotting activity

Milk made by reconstituting skim milk powder in distilled water and with the pH adjusted to 6.3, was found to contain 25 mg calcium/100 ml. On using this milk with the four coagulants referred to above, clotting time was found to be double that obtained in section 4b at pH 6.3. Milk powder was then reconstituted in solutions with a different concentration of calcium chloride to give levels of calcium in milk from 40 mg to 240 mg/100 ml. Increasing the level of calcium in milk from 25 mg to 40 mg/100 ml caused sharp reduction in the clotting time of the four coagulants. Further increase in the level of calcium in milk i.e. up to 240 mg/100 ml did not have much effect on the clotting activity. It was found that the calcium level in milk of 140 to 180 mg/100 ml is the best for the four coagulants to give the highest clotting activity (table 3:7 and graph 3:11).

d) The effect of lactose hydrolysis on clotting activity

Lactose hydrolysis by the enzyme lactase was measured either by measuring the optical density or measuring the depression in the freezing point of the milk.

The amount of lactose which had been hydrolysed was calculated using the

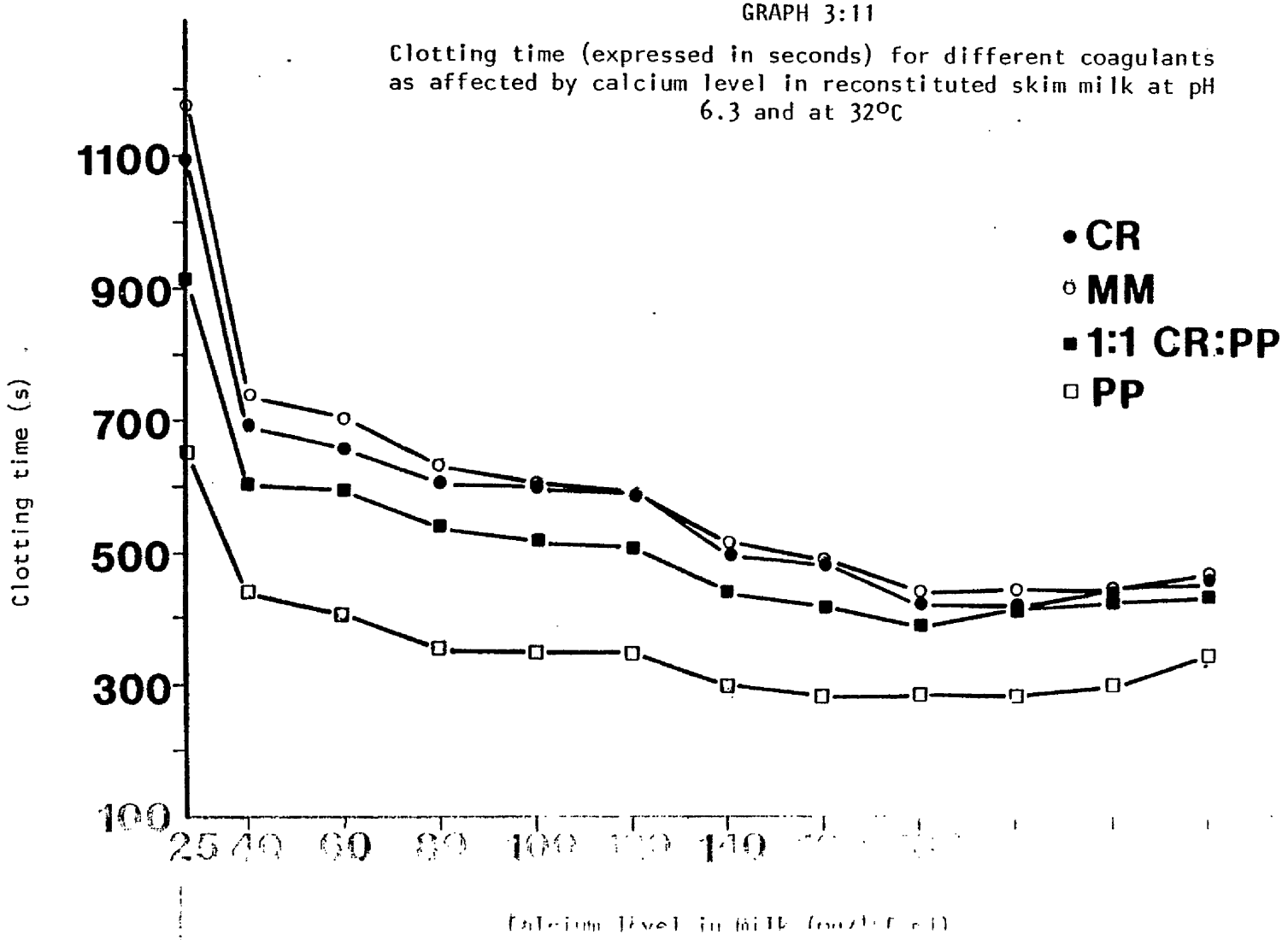
TABLE 3:7

The effect of the calcium level of milk substrate (reconstituted skim milk at 32°C and pH 6.3) on the clotting activity of different coagulants

Calcium level (mg/100 ml)	Clotting time in seconds			
	Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	Porcine pepsin
25	1096	1174	909	652
40	688	732	600	438
60	655	703	595	406
80	604	631	535	353
100	595	603	516	349
120	588	589	505	346
140	493	514	433	295
160	480	481	415	283
180	423	442	391	282
200	421	444	418	280
220	448	445	427	295
240	454	466	433	340

GRAPH 3:11

Clotting time (expressed in seconds) for different coagulants as affected by calcium level in reconstituted skim milk at pH 6.3 and at 32°C



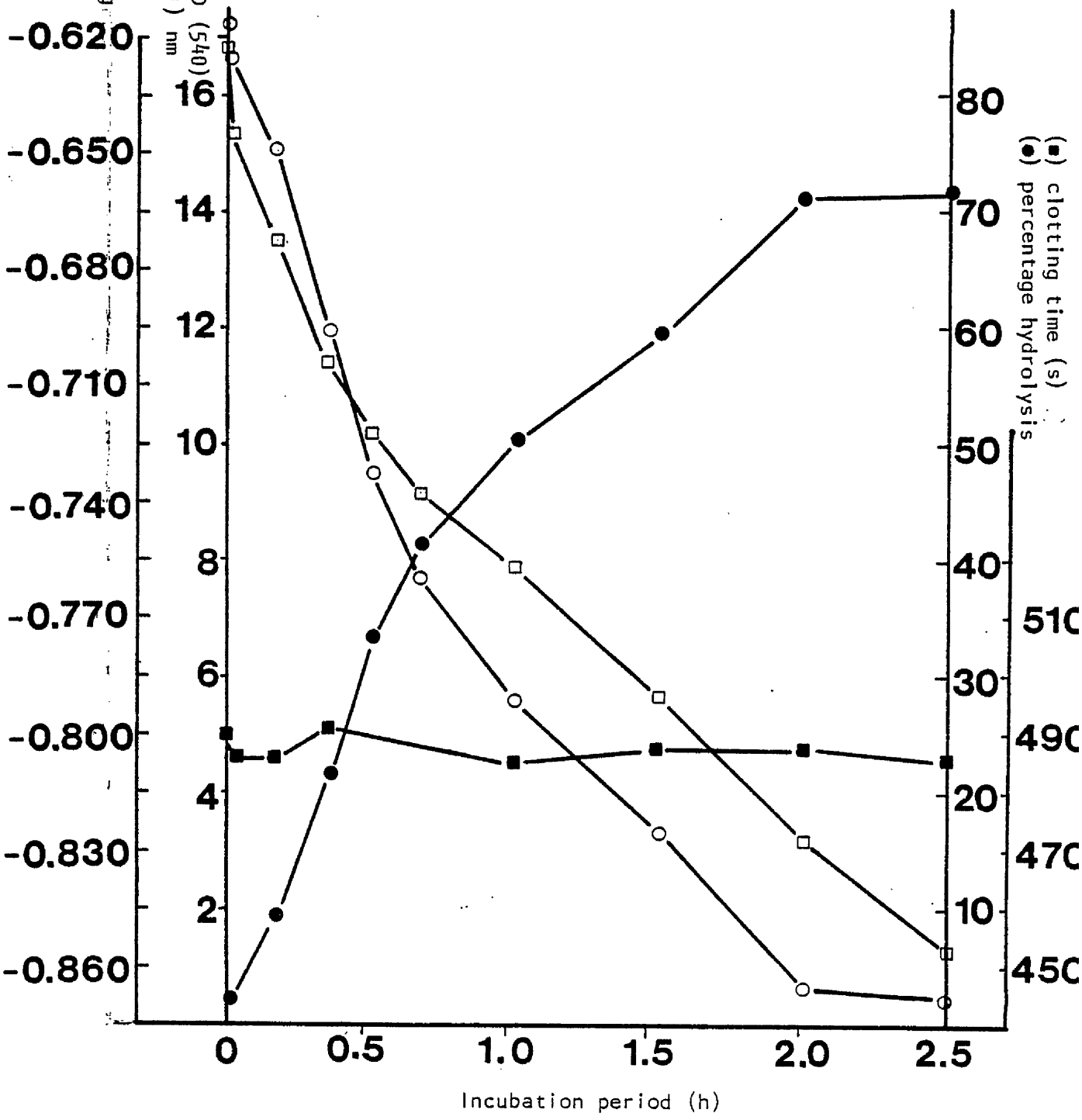
standard curve obtained with a standard solution of lactose (graph 3:14). The extent of lactose hydrolysis (expressed as a percentage) in relation to optical density and freezing point depression is given in graph 3:12 together with the clotting time for the different levels of lactose hydrolysis.

The figures indicate that there is no relation between clotting time and level of lactose hydrolysis.

Graph 3:13 shows the correlation between the freezing point depression and optical density.

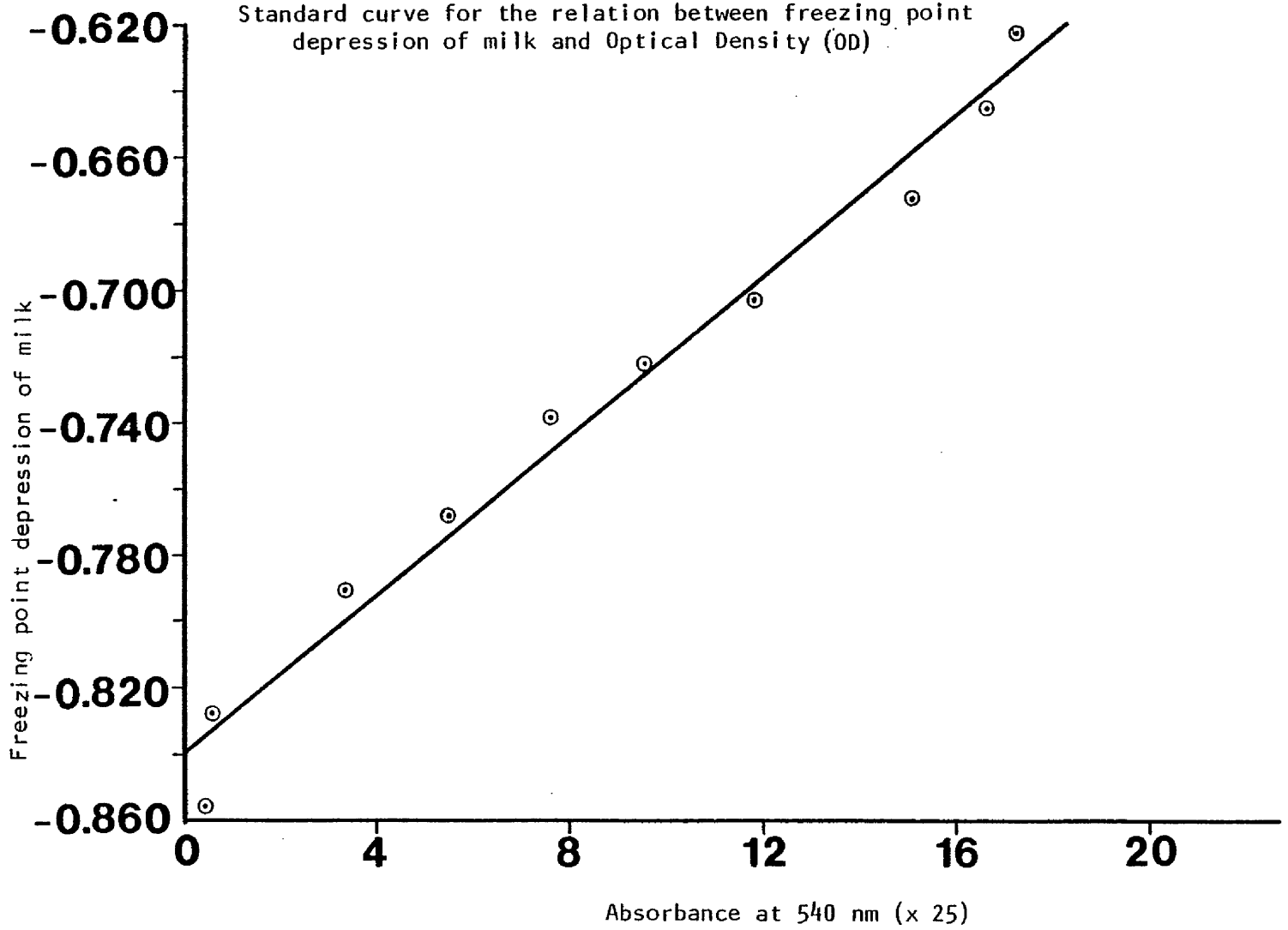
GRAPH 3:12

The effect of hydrolysing milk lactose (using Maxilact 20,000) to different levels, on the clotting activity of calf rennet at 32°C



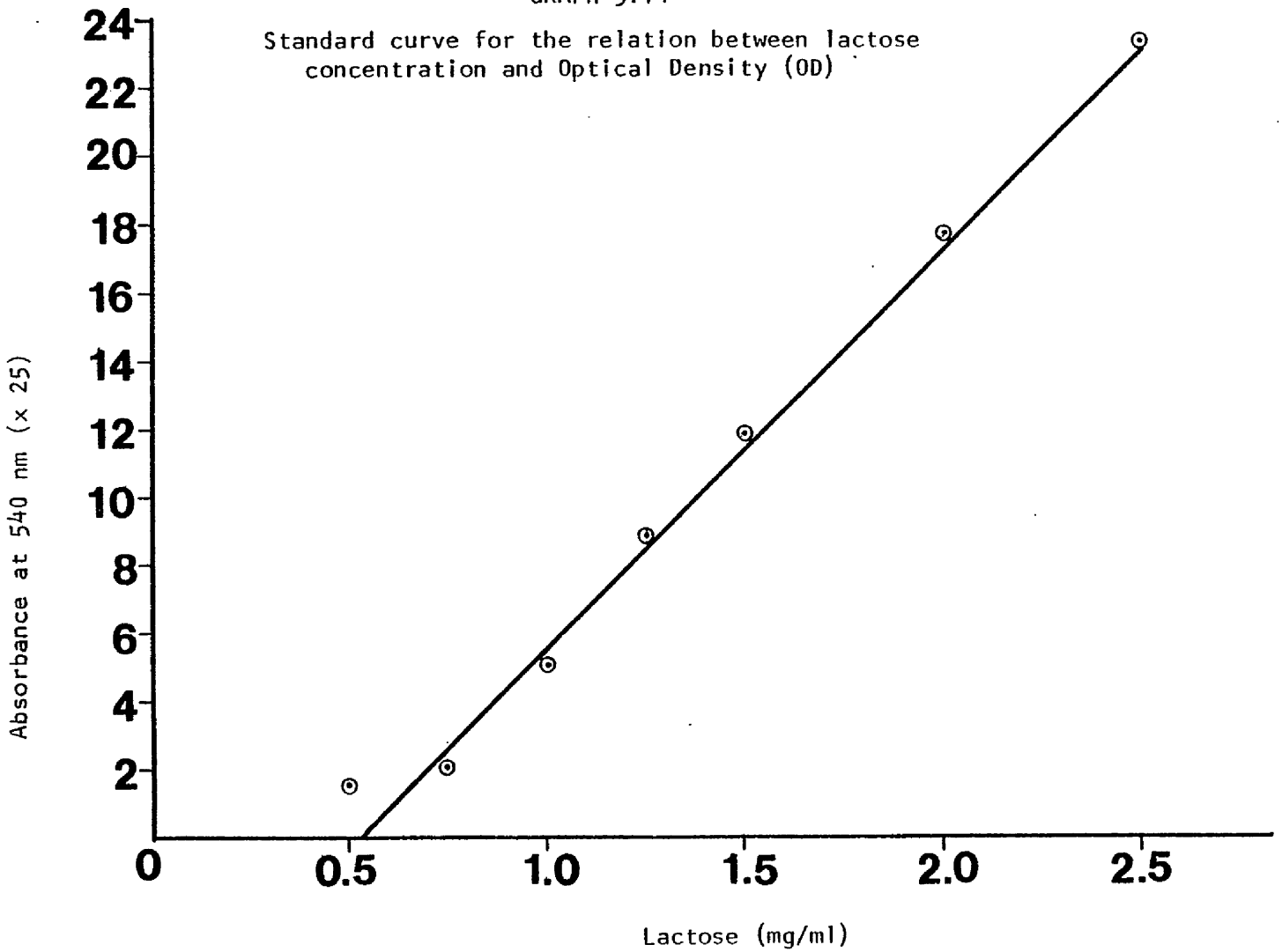
GRAPH 3:13

Standard curve for the relation between freezing point depression of milk and Optical Density (OD)



GRAPH 3:14

Standard curve for the relation between lactose concentration and Optical Density (OD)



DISCUSSION

The international consultation on coagulating enzymes used for cheese manufacture held in Brussels in 1977, under the aegis of the International Dairy Federation considered the different levels of bovine pepsin present in so-called 'calf' rennet - and suggested maximum levels for rennets so designated.

In the USA a 12 per cent level of bovine pepsin was considered the maximum, and in France a 25 per cent level was mentioned as maximum. One manufacturer of commercial rennet gave the level of 30 per cent bovine pepsin as the maximum in calf rennet. Emmons et. al., (1978) reported that, in Canada, analyses of commercial calf rennets on sale indicated the presence of large quantities of bovine pepsin in calf rennet. The bovine pepsin could account for more than 50 per cent of the rennet's clotting activity. The importance of knowing the composition of a rennet or other coagulant is important because the differences in the properties of chymosin and pepsin could affect the quality and probably the yield of cheese. Davies et. al., (1934) was the first to investigate the effect of chymosin and pepsin on Cheddar cheese ripening, by using selective denaturation in calf rennet to chymosin or pepsin which enabled him to separate the two enzymes. The principle of denaturation used was that of employing different pH values and different temperatures to inactivate one enzyme while allowing the continued activity of the other. The differences in the optimum temperature and pH for the activity of milk clotting enzymes remained the principle of all separation methods until other physical and chemical properties of the enzymes were identified. Nelson (1975) summarized ten differentiation procedures for milk clotting enzymes and at a later date, other workers were developing more methods for the identification of milk clotting enzymes. A summary of all available methods is given in table 3:8.

In the method developed by Garnot et. al. (1972) a good separation of chymosin from pepsin was obtained and both enzymes can be quantified precisely. The only disadvantage with the method occurs with mixtures

TABLE 3:8

Summary of methods for differentiating milk clotting enzymes

Author(s)	Principle(s)	Coagulants differentiated(*)	Sensitivity
de Koning et.al. (1969)	pH stability, amylase activity	CR, PP, EP, MP from other MCE	Not specified
Richardson (1970)	Casein-agar gels	CR, PP, EP, MP	0.25-1.0% MP or EP in CR PP in CR/PP
Richardson & Choudhary (1970)	Casein-agar gels, pH stability	CR, BR	1% BR
Douillard (1971)	Urea inactivation	C, BPI, BPII	No specified
Elliot & Emmons (1971)	Serological	C, EP, MP	ca 1 mu/g cheese
Garnot et.al. (1972)	DEAE cellulose chromatography	C, BPII, PP	Not specified
Shovers et.al. (1972)	Electrophoresis	CR, BR, PP, EP, MM MP	5% of any MCE's except Pepsins (C) (D)
de Koning & Draaisma(1973)	Isoelectric focusing	CR,BR,PP,EP,MM,MP	Not specified
Lim and Dinesen (1973)	Temp. & pH stability	CR, BR, PP, MM, MP	1%
Shovers et.al. (1973)	pH stability	PP from other MCE's	2% PP
Rothe et. al. (1976)	Immunological	C, BPI, BPII	Not specified
Mulvihill & Fox (1977)	Urea inactivation, pH stability	C, BP, PP, MM, MP	Not specified
Takahashi et. al. (1977)	Enzyme inhibitor (pepstalin), casein-agar gels.	C, BP	5% BP or 1 g/ml

*Coagulant code -

MCE	=	milk clotting enzyme(s)
CR	=	calf rennet
C	=	chymosin
BR	=	Bovine rennet
BP	=	Bovine pepsin
PP	=	Porcine pepsin
EP	=	*Endothia parasitica* rennet
MP	=	*Mucor pusillus* Lindt rennet
MM	=	*Mucor miehei* rennet

of bovine pepsin and porcine pepsin obtained as one fraction. This was noticed in the separation of 1:1 and $2\frac{1}{3}$: 1) mixtures of calf rennet and porcine pepsin. The two fractions obtained were one for chymosin and the other one for both pepsins.

The method can be improved using different salt gradients and flow rates to separate bovine pepsin from porcine pepsin. The alternative is to use another method to separate bovine pepsin from porcine pepsin such as the Mulvihill and Fox (1977) method which appears to be easier and needs a shorter time.

The results of fractionation indicated that calf rennet contained only 6 per cent pepsin, and because commercial calf rennets usually contain about 20 per cent pepsin (Hansen, 1978) the calf rennet used in the study must be prepared from vells of very young calves. Commercial calf rennets are extracted from a collection of a large number of vells obtained from calves of different ages and for that reason they vary in their composition. The 1:1 mixture of calf rennet and porcine pepsin could also vary in composition depending on the calf rennet used in the mixture. Møller-Madson and Hansen (1969) used a DEAE column to separate chymosin and pepsin and reported that a commercial 1:1 mixture of calf rennet and porcine pepsin contained 75 per cent porcine pepsin.

The use of coagulants in the exact amount to give, upon the addition to milk, a clotting time of 45 min ($\pm$ 15 min) with the required curd firmness, is very important in cheese making. The use of higher than normal amounts may result in defects in the cheese such as bitterness (Davis, 1965) and also cost more money. The use of lower than the normal requirement will result in incomplete clotting and lowered cheese yield.

Commercial preparations of calf rennet are normally used in the ratio of 1:4000 (i.e., one part of the liquid coagulant to 4000 parts of milk).

This ratio of addition depends on the strength of the preparation and on its age. The ratio of 1:5000 was found to be sufficient to give the

required firmness in the specified time. This reduction in the amount of rennet is of financial significance and means a saving of about 22.5 l (5 gal.) of calf rennet a day for a large commercial cheese factory using 455,000 l (100,000 gal) of milk daily.

The results obtained using the method of Eisses (1977) for the determination of coagulant clotting activity were in agreement with those obtained by other laboratories (table 3:9). The strength of Mucor miehei rennet which was found to be about the same as calf rennet was reported by Reps et. al. (1975) and Larsen (1977). Mucor pusillus Lindt rennet which was found to have double the activity of calf rennet was reported with similar results by Kikuchi et. al. (1968).

During the action of rennet on milk casein, limited proteolysis of K casein takes place during the primary phase of its action. This is followed by the secondary phase in which the milk gradually coagulates, and during the tertiary phase an unspecific proteolytic decomposition of casein occurs.

All coagulants hydrolyse casein at different speeds, and the rate of hydrolysis by a particular coagulant should be slow for the coagulant to be considered suitable for cheese making.

Methods for measuring the proteolytic activity of coagulants by determining the rate of casein hydrolysis were categorized by Green (1977) as follows:-

1. Measurement of the distribution of nitrogen: In which the release of trichloroacetic acid soluble nitrogen is measured (Richardson et. al., 1967; Fox, 1969; Green, 1972; and Phelan, 1977). The disadvantage of this method is that it is not clear what is being measured.

2. Measurement of the number of bonds hydrolysed: Using a spectrophotometer after the reaction of coagulant with 2, 3, 4-trinitrobenzene sulphoric acid (TNBS) (Tam and Whitaker, 1972).

3. Gel electrophoresis: The bands can be measured and identified

(Creamer et. al., 1971; and Creamer, 1975).

4. Gel filtration: The hydrolysed casein components are separated according to their molecular size (Green and Foster, 1974).

5. Measurement of the total amount of amino acids and their identification in relation to the peptide bonds cleaved (Minamiura et. al., 1972; Hill et. al., 1974; and Creamer, 1976).

6. More than one of the above methods.

The results obtained in the experiments described above of measuring non protein nitrogen (soluble nitrogen in 2 per cent trichloroacetic acid) are in agreement with those obtained by other workers. Fox (1969) found that porcine pepsin was very active initially and continued to liberate non protein nitrogen at a faster rate than calf rennet. The same observation about porcine pepsin was reported by Thomasow (1971) and Itoh (1972).

The results for the 1:1 mixture of calf rennet and porcine pepsin was not in agreement with some workers e.g. Thomasow et. al. (1968) and Martens (1969) who reported that the mixture had less proteolytic activity than calf rennet, and Antila and Witting (1976) who found no difference between calf rennet and the mixture as regards proteolytic activity. The results were in agreement with those of Zonj (1970) and Bottazzi et. al. (1974). The differences between the results concerning proteolytic activity of the mixture could be from differences in the composition of the mixture.

The results of Mucor miehei rennet proteolytic activity were in agreement with those of Hansen (1970), Thomasow et. al. (1971), Vanderpoorten and Weckx(1972), Alais and Lagrange (1972), Stefanova-Kondratenko et. al. (1974), Reps et. al (1975), Philippos and Christ (1976), and Reps et. al. (1978).

The highly significant correlation found between the levels of non protein

nitrogen as determined by micro-Kjeldahl and optical density methods indicated that optical density measurement could be used as indication for proteolysis.

The finding that the temperature of 30-52^{o}C gives the highest clotting activity for calf rennet is in agreement with that of Fox (1969) and Prins and Nielsen (1970). Other workers however reported the optimum temperature to be 44-45^{o}C (Tsugo et. al., 1964; Iwasaki et. al., 1967; Su et. al., 1971; and Bottazzi et. al., 1974). The reason for this reported difference in optimum temperature is likely to be the milk used and its pH. The temperature of inactivation of calf rennet was found to be just below 58^{o}C and agreed with the results of Fox (1969). Ibrahim et. al. (1973) reported that calf rennet lost activity at 60^{o}C.

In the case of Mucor miehei rennet, the optimum temperature was 66^{o}C and the inactivation temperature found to be between 70-72^{o}C. The results agree with those of Prins and Nielsen (1970) and Houins et. al. (1973) who reported 65^{o}C as the optimum temperature. Hamdy and Edelsten (1970) reported 42^{o}C to be the optimum temperature for Mucor miehei rennet. This figure differs considerably from other reports and the results obtained by the author. Ibrahim et. al. (1973) reported that Mucor miehei rennet lost its activity at above 75^{o}C. The results of the effect of temperature on the clotting activity of porcine pepsin agree with those of Fox (1969).

The clotting activity of porcine pepsin is affected by pH change more than that of other coagulants. Results indicating the inability of porcine pepsin to clot milk at pH 6.7 agreed with those of Ernstrom (1961) and Fox (1969). They differ from those of Green (1972) who reported activity for porcine pepsin at pH 6.81 and who found that by using higher concentration of the enzyme at high pH levels.

The activity of the 1:1 mixture of calf rennet and porcine pepsin was affected by pH level in a similar way to porcine pepsin. Phelan (1973) reported that 1:2 mixture of calf rennet and porcine pepsin did not clot milk at pH 6.8 in 60 minutes from the time of addition. The low

activity of the mixture at pH values above 6.6 was expected due to the high proportion of pepsin in the blend.

Mucor miehei rennet and calf rennet showed a similar relationship between clotting activity and pH change, and this result agreed with those of Prins and Nielsen (1970), Thomasow et. al. (1971), Alais and Lagrange (1972), Hounis et. al. (1973), Ibrahim et. al. (1973), and Stefanova-Kondratenko et. al. (1974). The results obtained differ from those reported by Phelan (1973) and Dolezalek and Havlova (1974) who reported that Mucor miehei rennet is less sensitive to pH variation than calf rennet.

The similarity between calf rennet and Mucor miehei rennet in relation to the effect of pH level of the milk substrate on clotting activity made the latter coagulant appear to be a good substitute for calf rennet.

By increasing the calcium content in reconstituted skim milk from 25 mg/100 ml to about 40 mg/100 ml, clotting time was reduced by one third for all coagulants, but further increases in the content of calcium had little effect on clotting time. These results agreed with those of Ibrahim et. al. (1973), Reps et. al. (1975), and Reps et. al. (1978). Davis (1965) reported that the addition of calcium chloride to milk is not common in the United Kingdom, but it is common on the Continent of Europe where milk is usually pasteurized at 80^{o}C for a few seconds a treatment which causes some of the ionic calcium to precipitate.

The hydrolysis of milk lactose had been reported by Nijples (1976) to accelerate acid formation and shorten the time required for coagulation by calf rennet. The reduction in the clotting time could be due to the fast drop in pH resulting from the high rate of acidity development, and from some other changes in milk affecting the clotting process. The results obtained in the experiment described above and shown in graph 3:12 did not indicate any relation between lactose hydrolysis and clotting activity. Thus, lactose hydrolysis did not change the factors affecting clotting activity i.e. casein composition, calcium distribution and milk pH.

The relationship between milk pH and the clotting activity of different coagulants was found to be significant. It has also been shown by Kowalchyk and Olson (1977) that the acidity level of milk affects curd firmness. The commercial significance of these points should be further considered in the light of developments within the past twenty years or so in the production and handling of milk on farms in Scotland and in cheese making techniques. The view has been expressed (Crawford, 1980) that the natural titratable acidity of milks received at cheese factories has decreased with the advent of bulk collection (since 1954 in Scotland).

Furthermore, the introduction of single strain starters in Scotland in 1956 and the use of very much shorter ripening times has led to lower acidity levels at rennet addition.

CONCLUSION

Of the coagulants examined, calf rennet was found to possess the best ratio of clotting activity to proteolytic activity. This is the most important characteristic to be examined in assessing the suitability of a coagulant for the production of Cheddar cheese.

The clotting activity of calf rennet at high pH values (6.7 to 6.8) is important in ensuring successful clotting and a firm curd if milk has a high pH e.g. where the cheese making process does not include a ripening time before rennet addition and where low acidity starters are used.

Mucor miehei rennet showed a ratio of clotting activity to proteolytic activity which is considered by the author to be satisfactory for Cheddar cheese making. The high rate of proteolytic activity associated with this rennet in experiments involving casein substrates arose from the use of higher amounts of this coagulant (the amount of the coagulant used in the experiments was determined on the basis that it would clot the milk in a similar time to that of calf rennet).

The amount of Mucor miehei rennet in cheese making can be reduced if a higher renneting temperature is employed e.g. 32°C instead of 30°C.

The experiments confirmed reports of the activity of Mucor miehei rennet up to 72°C. Since whey from cheese made with this coagulant, may contain up to ninety per cent of the original amount used in cheese making, notice should be taken of the heat stability of the enzyme in case of its continued activity in whey by-products.

A commercial 1:1 mixture of calf rennet and porcine pepsin was found to have a ratio of clotting activity to proteolytic activity similar to calf rennet. Other characteristics of this mixture were found to be similar to those of calf rennet and it is concluded that it is a suitable substitute for calf rennet for Cheddar cheese making.

Porcine pepsin was found to have a high clotting activity and a high proteolytic activity. The inactivation of procine pepsin at a pH of about 6.6 will prevent milk clotting and hence cheese making. And from that point of view, care must be taken to ensure a milk pH of less than 6.6 when porcine pepsin is used in cheese making.

CHAPTER FOUR

MAKING CHEDDAR CHEESE USING DIFFERENT COAGULANTS

INTRODUCTION

When a coagulant is added to milk for the production of cheese, it will not only clot the milk but it will also contribute to the cheese quality and may affect the cheese yield. The action of the coagulant on milk proteins i.e., the breakdown of casein (α or β) to peptides of various sizes and also the liberation of certain amino acids will effect the quality of cheese. In addition, the strength of the curd formed may have an effect on yield especially if a weak curd is formed and a large quantity of the milk proteins and milk fat are lost in the whey.

For several centuries calf rennet has been considered to be the ideal coagulant to produce Cheddar cheese with a typical flavour, good quality, and a satisfactory yield. The shortage of supply of calf rennet and the increase in its price have made cheese producers look for an alternative coagulant which should be cheaper, have no restriction on its availability, and have no adverse effect on cheese quality and yield.

But, with the introduction of substitutes to calf rennet, problems in cheese production began to develop. Many research studies have resulted in much new information and also in various opinions about the new coagulants. Some reports indicated that a particular new coagulant used, gave either a higher or lower cheese yield than calf rennet. Some reports indicated no difference in the yield of cheese made with different coagulants. Other effects of using the new coagulants were reported in relation to cheese compostion, flavour, and keeping quality.

In this chapter, investigations on the effect of some coagulants on cheese yield and cheese composition are reported.

EXPERIMENTAL

Throughout the experiments referred to in this chapter, all milk used for making cheese or for starter preparation was analysed for fat content, total nitrogen content, solids not fat content, and for residual antibiotics using the methods described in chapter one (sections 1:1, 2, 3, and 9). Milk for starter preparation was obtained from the College farm, and milk for cheese making was supplied by the Scottish Milk Marketing Board. Cheddar cheese was made in four series of experiments described below.

1. Laboratory scale cheese making: Cheddar cheese was made on a small scale using 1 kg quantities of milk following the technique described in chapter one (section 4:1). The resultant cheese was analysed for moisture and fat using the methods described in chapter one (section 5:1, 2). The pH of the whey and cheese were measured during the cheese making process using the method described in chapter one (section 5:3).

Cheese was made on this scale in three experiments:-

(a) Series 1: The six coagulants used in this experiment were:- calf rennet ('standard' Hansen's brand), Mucor miehei rennet ('Hannilase' Hansen's brand), (1:1) and ($2\frac{1}{3}$: 1) mixtures of calf rennet and porcine pepsin, 100 per cent porcine pepsin (Hansen's brand), and Endothia parasitica rennet ('Suparen' brand).

Cheese was made using each of the above coagulants on six separate days and the coagulants were used according to their clotting activity.

(b) Series 2: Four coagulants (calf rennet, Mucor miehei rennet, and (1:1) and ($2\frac{1}{3}$: 1) mixtures of calf rennet and porcine pepsin) chosen from the above six were each used in a further series of six cheese making trials.

(c) Series 3: Four coagulants were selected from series 1, calf rennet, Mucor miehei rennet, (1:1) mixture of calf rennet and porcine pepsin, and a 100 per cent porcine pepsin rennet, were each tested in duplicate on five further cheesemaking trials.

2. Small scale cheese making: Twenty four vats of milk [each of 180 l. (40 gal.) quantity] were made into Cheddar cheese using the four coagulants chosen for the third series of the laboratory scale cheese making referred to above. Cheese was made in the following sequence:-

	Coagulant used	
Week	Vat 1	Vat 2
1	Mucor miehei rennet	porcine pepsin
2	calf rennet	Mucor miehei rennet
3	porcine pepsin	calf rennet
4	Mucor miehei rennet	(1:1) mixture of CR and PP
5	(1:1) mixture of CR and PP	calf rennet
6	porcine pepsin	(1:1) mixture of CR and PP
7	(1:1) mixture of CR and PP	Mucor miehei rennet
8	calf rennet	porcine pepsin
9	porcine pepsin	Mucor miehei rennet
10	calf rennet	(1:1) mixture of CR and PP
11	Mucor miehei rennet	calf rennet
12	(1:1) mixture of CR and PP	porcine pepsin

The design of the experiment was a balanced incomplete block pattern (Cochran and Cox, 1950) and the coagulants were placed in the above sequence using a random numbers table (Fisher and Yates, 1974).

The cheese was made during the period February to June 1978. Cheese was analysed for moisture content, fat content, pH value, and salt content using the methods described in chapter one (sections 5:1, 2, 3 and 4). Whey was analysed for fat, total nitrogen, and non protein nitrogen using the methods described in chapter one (section 6:1, 2 and 3).

3. Small scale cheese making: Twenty four vats of milk [each of 360 l. (80 gal.)] were made into Cheddar cheese using calf rennet and Mucor miehei rennet. Two vats were made into cheese on three days each week during April 1979. All cheese was analysed for moisture, fat, pH, and salt content using the methods described in chapter one (section 5:1,

2, 3 and 4).

4. Small scale cheese making: To find the effect of milk storage on cheese yield, Cheddar cheese was made using milk which had been stored at 4°C for periods of 0, 24, 48 and 72 hours.

Two 180 l (40 gal.) quantities of the milk stored at 4°C were made into Cheddar cheese on each of the four days of the experiment using the original milk.

The experiment was performed twice (two successive weeks in October 1979).

Two different coagulants were used throughout the experiment (one coagulant in each vat) to investigate the effect of their use on the yield of Cheddar cheese and to find if there is an interaction between the period of milk storage and coagulant type in relation to cheese yield.

The milk used was obtained from a creamery silo containing between 22,500 l and 33,750 l (5000 and 7500 gal.) milk. On receipt at the College the milk was divided into two parts, the first portion of 495 l (110 gal.) being pasteurized immediately at 72°C for 15 s and delivered to the cheese room where its freezing point depression was measured using the method described in chapter one (section 1:5). The pasteurized milk was collected in a sterilized 360 l (80 gal.) vat and after it had been thoroughly mixed, it was weighed into sterile milk cans 45 l (10 gal.) capacity and divided into two 180 l (40 gal.) cheese vats and made into Cheddar cheese using the method described in chapter one (section 4:2).

The remainder of the milk (around 1530 l (340 gal.) was held at 4°C in a refreigerated milk storage tank until after 24, 48 and 72 h. 495 l (110 gal.) portions were drawn off and pasteurized prior to being made into Cheddar cheese using the technique described above.

RESULTS

1. Laboratory scale cheese making: Tables 4:1 to 4:12 contain the results of the three experiments and related statistical analysis.

The results include the actual yield, the yield calculated assuming a moisture value of 35 per cent, moisture content of the cheese, and fat content of the cheese.

In the case of the actual cheese yield, the results showed no overall significant difference in the cheese yield when different coagulants were used (tables 4:1, 5 and 9). The variance ratio (VR) obtained when different coagulants were used was very low and not significant by comparison with the tabulated F-values for the particular degree of freedom of each experiment. There were no significant differences between the yield obtained with different coagulants in the first and second series, while in the third series a significant difference (at 5 per cent level) was present between the mean yield by Mucor miehei rennet or by the 1:1 mixture of calf rennet and porcine pepsin and by calf rennet (table 4:9).

Day to day variation in the cheese yield due to variation in milk compostion or some experimental differences was highly significant in each of the three experiments. The high value of variance ratio indicates a highly significant difference in the yield from one day to another.

In the third series, cheese making trials with coagulants were conducted in duplicate on the same day, and the possibility of an interaction between milk and coagulants was checked. The values obtained of the variance ratio indicates the absence of such a relation.

When the cheese yield was recalculated assuming the cheese contained 35 per cent moisture (tables 4:2, 6 and 10), the results obtained in the first and second series again showed no overall significant differences in the yield between individual coagulants and the overall mean or between the coagulants themselves. In the third series, however, the

Table 4:1

The effect of using different coagulants in experimental cheddar cheese making on cheese yield (expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP	Porcine pepsin	*Endothia parasitica* rennet
13.00	11.83	12.09	11.82	12.08	11.76
12.97	12.65	12.91	13.12	12.96	13.24
11.85	12.02	11.88	11.97	11.97	12.50
11.31	11.58	11.40	11.52	11.25	11.00
10.21	10.16	10.12	10.18	10.04	10.25
10.77	10.40	10.43	10.60	10.64	10.47
Mean 11.685	11.441	11.472	11.535	11.490	11.538

Overall mean 11.527

	DF	MS	VR
Milk	5	5.497	18.634***
Coagulant	5	0.044	0.149
Residual	25	0.295	
Total	35	1.002	

	Milk	Coagulant
REP	6	6
SED	0.314	0.314

* Significant at 5 per cent level

** " " 1 " " "

*** " " 0.1 " "

Table 4:2

The effect of using different coagulants in experimental Cheddar cheese making on cheese yield (adjusted to moisture level of 35 per cent and expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP	Porcine pepsin	*Endothia parasitica* rennet
10.44	9.96	10.04	9.89	9.99	9.85
9.94	9.95	10.28	10.15	10.23	10.21
9.89	9.86	9.62	9.76	9.77	10.09
9.47	9.46	9.51	9.55	9.24	9.33
9.38	9.39	9.32	9.12	9.31	9.44
9.54	9.08	9.23	9.23	9.17	9.15
Mean 9.618	9.616	9.668	9.619	9.616	9.681

Overall mean 9.636

	DF	MS	VR
Milk	5	0.887	33.598***
Coagulant	5	0.005	0.192
Residual	25	0.026	
Total	35	0.146	

	Milk	Coagulant
REP	6	6
SED	0.094	0.094

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:3

The effect of using different coagulants in experimental Cheddar cheese making on cheese moisture (expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP	Porcine pepsin	*Endothia parasitica* rennet
47.79	44.28	46.00	45.60	46.27	$45\frac{1}{2}$56
50.16	48.85	48.23	49.70	48.70	49.86
45.76	46.71	47.36	46.98	46.96	47.51
45.57	46.93	45.76	46.13	46.63	44.85
40.33	39.94	40.18	41.75	39.73	40.12
42.45	43.23	42.50	43.38	44.00	43.20
Mean 45.343	44.990	45.002	45.590	45.382	45.183

Overall mean 45.248

	DF	MS	VR
Milk	5	37.924	86.016***
Coagulant	5	0.330	0.490
Residual	25	0.673	
Total	35	8.803	

	Milk	Coagulant
REP	6	6
SED	0.474	0.474

* Significant to 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:4

The effect of using different coagulants in experimental Cheddar cheese making on cheese fat (expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP	Porcine pepsin	*Endothia parasitica* rennet
27.25	30.13	28.90	29.55	28.90	28.85
26.97	27.80	27.51	26.97	26.97	26.70
27.70	27.15	27.80	28.35	28.60	28.15
27.63	27.82	28.01	27.53	27.61	27.35
30.49	31.65	30.25	29.98	30.67	30.06
28.79	28.67	28.96	28.12	28.14	28.32
Mean 28.138	28.870	28.573	28.417	28.482	28.238

Overall mean 28.453

	DF	MS	VR
Milk	5	8.462	27.817***
Coagulant	5	0.403	1.325
Residual	25	0.304	
Total	35	1.484	

	Milk	Coagulant
REP	6	6
SED	0.318	0.318

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:5

The effect of using different coagulants in experimental Cheddar cheese making on cheese yield (expressed as percentage)

Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP
11.89	12.10	12.12	12.31
12.40	12.60	12.48	12.41
13.41	12.47	12.93	13.26
12.27	11.92	11.57	11.82
13.21	12.30	13.00	12.49
11.25	11.06	11.39	11.50
Mean 12.406	12.076	12.248	12.298

Overall mean 12.257

	DF	MS	VR
Milk	5	1.548	19.469***
Coagulant	3	0.114	1.429
Residual	15	0.079	
Total	23	0.403	

	Milk	Coagulant
REP	4	6
SED	0.199	0.163

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:6

The effect of using different coagulants in experimental Cheddar cheese making on cheese yield (adjusted to a moisture level of 35 per cent and expressed as a percentage)

Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP
10.44	10.36	10.54	10.74
10.69	10.79	10.64	10.58
9.63	9.45	9.60	9.64
8.46	8.57	8.37	8.40
10.69	10.73	10.78	10.64
9.99	9.98	10.04	9.98
Mean 9.981	9.980	9.997	9.996

Overall mean 9.989

	DF	MS	VR
Milk	5	3.039	273.537***
Coagulant	3	0.001	0.047
Residual	15	0.011	
Total	23	0.668	

	Milk	Coagulant
REP	4	6
SED	0.075	0.060

* Significant at 5 per cent level

** " " 1 " "

** " " 0.1 " "

Table 4:7

The effect of using different coagulants in experimental Cheddar cheese making on cheese moisture (expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP
42.98	44.36	43.45	43.29
44.00	44.36	44.59	44.56
53.34	50.76	51.72	52.76
54.15	53.24	52.94	53.85
47.40	43.27	46.10	44.63
42.29	41.36	42.71	43.57
Mean 47.360	46.225	46.918	47.110

Overall mean 46.903

	DF	MS	VR
	5	89.091	95.183***
	3	1.410	1.506
	15	0.936	
	23	20.162	

	Milk	Coagulant
REP	4	4
SED	0.684	0.559

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:8

The effect of using different coagulants in experimental Cheddar cheese making on cheese fat (expressed as a percentage)

Calf rennet	*Mucor miehei* rennet	1:1 mixture CR:PP	$2\frac{1}{3}$: 1 mixture CR:PP
31.80	30.20	32.70	33.50
32.65	32.65	32.90	31.83
25.60	24.50	25.00	25.00
25.50	25.40	27.30	26.80
27.15	27.80	26.75	27.80
30.37	30.98	30.16	30.15
Mean 28.845	28.588	29.135	29.180

Overall mean 28.937

	DF	MS	VR
	5	39.656	59.957***
	3	0.457	0.691
	15	0.661	
	23	9.112	

	Milk	Coagulant
REP	4	6
SED	0.575	0.470

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:9

The effect of using different coagulants in experimental Cheddar cheese making on cheese yield (expressed as a percentage)

Calf rennet		*Mucor miehei* rennet		1:1 mixture CR:PP		Porcine pepsin	
10.41,	10.41	10.40,	10.37	10.33,	10.35	10.40,	10.40
10.19,	10.19	9.92,	10.18	10.03,	10.32	10.42,	10.05
10.61,	10.16	10.14,	10.26	9.93,	10.28	10.36,	10.17
10.18,	10.36	9.91,	9.74	10.05,	9.69	9.80,	9.97
11.03,	11.03	11.12,	10.83	10.67,	11.18	11.03,	10.92
Mean 10.475		10.286		10.282		10.351	

Overall mean 10.344

	DF	MS	VR
Milk	4	1.18109	38.026***
Coagulant	3	0.06661	2.144
Milk coagulant	12	0.01648	0.530
Residual	20	0.03106	
Total	39	0.14726	

	Milk	Coagulant	Milk coagulant
REP	8	10	2
SED	0.088	0.079	0.176

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:10

The effect of using different coagulants in experimental Cheddar cheese making on cheese yield (adjusted to a moisture level of 35 per cent and expressed as a percentage)

Calf rennet		Mucor miehei rennet		1:1 mixture CR:PP		Porcine pepsin	
9.49,	9.49	9.47,	9.47	9.48,	9.48	9.47,	9.47
9.41,	9.41	9.35,	9.33	9.38,	9.37	9.36,	9.35
9.34,	9.34	9.32,	9.32	9.32,	9.31	9.31,	9.31
9.53,	9.52	9.48,	9.44	9.51,	9.51	9.51,	9.50
9.87,	9.87	9.84,	9.83	9.87,	9.87	9.88,	9.87
Mean 9.525		9.487		9.511		9.504	

Overall mean 9.507

	DF	MS	VR
Milk	4	0.3611	1919.202***
Coagulant	3	0.0025	13.270***
Milk coagulant	12	0.0002	1.124
Residual	20	0.0002	
Total	39	0.0374	

	Milk	Coagulant	Milk coagulant
REP	8	10	2
SED	0.0068	0.0061	0.0137

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:11

The effect of using different coagulants in experimental Cheddar cheese making on the cheese moisture (expressed as a percentage)

Calf rennet		Mucor miehei rennet		1:1 mixture CR:PP		Porcine pepsin	
40.76,	40.76	40.82,	40,64	40.32,	40.48	40.81,	40.78
40.00,	40.00	38.70,	40.40	39.20,	40.95	41.65,	39.50
42.78,	40.24	40.23,	40.93	38.95,	41.08	41.60,	40.50
39,10,	40.30	37,80,	37.00	38.50,	36,20	36.93,	38.06
41.85,	41.85	42.50,	40.98	39.90,	42.60	41.75,	41.20
Mean 40.76		40.00		39.82		40.28	

Overall mean 40.21

	DF	MS	VR
Milk	4	14.783	14.122***
Coagulant	3	1.697	1.621
Milk coagulant	12	0.576	0.551
Residual	20	1.047	
Total	39	2.361	

	Milk	Coagulant	Milk coagulant
REP	8	10	2
SED	0.512	0.458	1.023

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

Table 4:12

The effect of using different coagulants in experimental Cheddar cheese making on the cheese fat (expressed as a percentage)

Calf rennet		*Mucor miehei* rennet		1:1 mixture CR:PP		Porcine pepsin	
30.45,	30.45	31.55,	33.20	29.90,	29.90	31.00,	31.00
31.00,	31.00	31.50,	30.30	30.20,	30.10	29.90,	30.45
27.25,	28.90	28.62,	28.07	29.65,	28.35	29.15,	29.65
31.00,	31.00	31.00,	31.55	32.10,	33.20	32.60,	31.30
29.90,	29.90	31.00,	30.45	31.55,	29.90	29.90,	29.90
Mean 30,085		30.724		30.485		30.485	

Overall mean 30.445

	DF	MS	VR
Milk	4	9.8328	25.104***
Coagulant	3	0.7021	1.793
Milk coagulant	12	1.0613	2.710*
Residual	20	0.3917	
Total	39	1.5899	

	Milk	Coagulant	Milk coagulant
REP	8	10	2
SED	0.3129	0.2799	0.6259

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

variance ratio obtained from using different coagulants was significant, but the difference between the mean yield obtained with the use of porcine pepsin and the 1:1 mixture of calf rennet and porcine pepsin was not significant.

In all three series, the daily variation in the cheese yield was highly significant, and again no interaction was found between milk composition and coagulants in relation to yield.

The analysis of the moisture content of cheese revealed no significant differences due to the use of different coagulants in the three series (tables 4:3, 7 and 11). Furthermore there were no significant differences between the individual coagulants themselves in each series. The daily variation in the moisture content of cheese was significant in the three series, and no interaction was found between milk composition and coagulants in relation to the moisture content of cheese.

The analysis of the results for fat content of cheese showed no overall significant differences due to the use of different coagulants in the three series (tables 4:4, 8 and 12), the differences between the individual coagulants were not significant, except the difference in the mean fat value for Mucor miehei rennet and calf rennet in the first and third series, where, in both trials, the use of Mucor miehei rennet gave higher values for fat in cheese than were found when calf rennet was the coagulant.

The variance ratio for the interaction of milk and coagulant (table 4: 12) was significant at > 1 per cent level, which means a significant interaction between variation in the fat content of cheese and changes in milk composition and coagulants. The variation in fat content of the cheese due to the different milks was significant in the three series. In addition, no appreciable differences were noticed during the cheese making trials with different coagulants. Curds of similar strength were formed by the different coagulants, and the stage of cutting the curd, scalding and cheddaring showed no visible differences between all coagulants. When the cheddard curd was cut and salted, the texture

appeared to be the same for all cheeses. Finally, no differences in the taste and flavour of all cheeses were noticed after the pressing.

2. Small scale cheese making: From the three series of cheese making experiments described above, it was found that using different milk every day had a great effect on cheese yield and on cheese composition. The effect was confirmed by the results obtained in this series of experiments and it was possible to relate the yield and cheese composition to the variation in the individual components of milk.

Because of the experiment design, a different method of statistical analysis was followed from that used in the previous experiment. In the case of each variate, the mean value for each coagulant is an estimated mean calculated to reduce the error which could happen if an ordinary mean, was calculated. The method of statistical analysis was from Cochran and Cox (1950).

Results revealed that coagulants had no significant effect on yield. Variation in the yield arising from the use of different milk every week was significant at 1 per cent level (table 4:13), and similar results were obtained from the analysis of the calculated yield figures (i.e., on the basis that cheese contains 35 per cent moisture (table 4:14)).

The analysis of the results of the moisture content of the cheese showed no significant effect for coagulants in the variation in the moisture, and no significant differences between the mean value of moisture for each coagulant (table 4:15), variation in the moisture due to milk composition was less significant than that in cheese yield, which means that the effect of variation in milk composition on cheese moisture content is not as definite as that for cheese yield. Different results were obtained from the analysis of the fat content of cheese. Although the overall effect of coagulants on fat content of cheese was not significant, the differences between the mean value for each coagulant were significant (table 4:16).

The estimated mean for the fat content of cheese made by calf rennet was higher than all the means of other coagulants, and the difference is

TABLE 4:13

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on cheese yield (expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	9.30	-	9.26
1 February	9.26	9.89	-	-
8 February	10.62	-	-	9½98
15 February	-	10.57	10.47	-
22 February	10.69	-	10.81	-
1 March	-	-	10.56	9.97
7 June	-	10.07	10.35	-
14 June	10.16	-	-	10.41
21 June	-	10.00	-	9.91
28 June	10.17	-	10.18	-
5 July	10.11	10.09	-	-
19 July	-	-	10.40	10.43
Estimated mean	10.110	10.224	10.257	10.018

Overall mean 10.152

	DF	MS	VR
Milk	11	0.31761	5.478**
Coagulant	3	0.04806	0.829
Residual	9	0.05798	
Total	23	0.18086	

	Milk	Coagulant
REP	4	6
SED	0.1390	0.1703

* significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:14

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on cheese yield (adjusted to moisture level of 35 per cent and expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	9.31	-	9.38
1 February	9.01	9.34	-	-
8 February	10.11	-	-	9.70
15 February	-	10.33	10.09	-
22 February	10.46	-	10.32	-
1 March	-	-	10.06	9.49
7 June	-	9.78	10.05	-
14 June	9.91	-	-	9.94
21 June	-	10.10	-	9.79
3 June	9.98	-	10.11	-
5 July	9.82	9.77	-	-
19 July	-	-	10.02	10.22
Estimated mean	9.884	9.959	9.916	9.717

Overall mean 9.869

	DF	MS	VR
Milk	11	0.22852	4.942**
Coagulant	3	0.04462	0.965
Residual	9	0.04624	
Total	23	0.13321	

	Milk	Coagulant
REP	4	6
SED	0.1242	0.1521

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:15

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on the cheese moisture content (expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	34.91	-	34.13
1 February	36.76	38.65	-	-
8 February	38.10	-	-	36.80
15 February	-	36.50	37.33	-
22 February	36.42	-	37.95	-
1 March	-	-	38.07	38.09
7 June	-	36.89	36.89	-
14 June	36.60	-	-	37.95
21 June	-	34.36	-	35.79
28 June	36.23	-	35.43	-
5 July	36.87	37.07	-	-
19 July	-	-	37.31	36.27
Estimated mean	36.36	36.80	37.85	36.68
Overall mean		36.72		

	DF	MS	VR
Milk	11	2.2453	3.205*
Coagulant	3	0.3202	0.457
Residual	9	0.7005	
Total	23	1.3897	

	Milk	Coagulant
REP	4	6
SED	0.483	0.592

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:16

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on the cheese fat content (expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	33.20	-	34.03
1 February	32.65	31.00	-	-
8 February	33.20	-	-	32.65
15 February	-	35.13	34.30	-
22 February	35.40	-	34.85	-
1 March	-	-	34.30	34.30
7 June	-	33.20	33.20	-
14 June	34.03	-	-	32.38
21 June	-	34.85	-	34.03
28 June	32.65	-	32.65	-
5 July	33.20	32.65	-	-
19 July	-	-	31.55	31.55
Estimated mean	33.992	33.200	33.200	33.099

Overall mean 33.373

	DF	MS	VR
Milk	11	2.4780	10.460***
Coagulant	3	0.6897	2.911
Residual	9	0.2369	
Total	23	1.3678	

	Milk	Coagulant
REP	4	6
SED	0.2810	0.3442

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

more than the LSD value at 5 per cent probability, which means a probable significant difference. Furthermore the variation in fat content because of milk was highly significant, which means a strong relation between milk composition and cheese fat content.

Moisture in fat-free cheese (MFFC) and fat in dry matter (FDM) are two values which are derived from the moisture and fat contents of the cheese, and are useful in giving a better understanding of the relation between different cheese components, and of the consistency of the cheese legislation concerning cheese composition (i.e., The Cheese Regulations (1970) specify FDM and maximum moisture levels for cheese sold in the United Kingdom).

The statistical analysis of those two values (tables 4:17 and 18) showed no effect for coagulants on the MFFC and no significant difference in the mean value obtained for the use of each coagulant. In the case of the FDM value however there was a probable significant effect for coagulants.

The variance in this value, because of coagulant type,, is significant at 5 per cent level, and also the difference between the mean value of FDM for each coagulant is significant. The effect of milk on the variation of MFFC and FDM value was significant (highly significant in the case of FDM).

The statistical analysis of the values of cheese pH (table 4:19) showed no significant effect for coagulants on cheese pH, and also no significant differences between cheese pH value for cheese made with individual coagulants.

Variation in cheese pH from week to week was highly significant and the reason for this variation lies, not in variation in milk composition, but in variation in the rate of acidity development during cheese making.

The variation from week to week in the salt content of the cheese was highly significant. The reason for this variation was the differing amounts of salt added to the curd after milling since variation was made because of different levels of acidity of the curd, and because of

TABLE 4:17

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on the moisture content in fat free cheese (MFFC) expressed as a percentage)

Cheese making date = 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	52.26	-	51.74
1 February	54.58	56.01	-	-
8 February	57.04	-	-	54.64
15 February	-	56.27	56.82	-
22 February	56.38	-	58.25	-
1 March	-	-	57.95	57.98
7 June	-	55.20	55.20	-
14 June	55.48	-	-	56.12
21 June	-	52.74	-	54.25
28 June	53.79	-	52.61	-
5 July	55.20	55.04	-	-
19 July	-	-	54.51	52.99
Estimated mean	55.10	55.09	55.47	54.84

Overall mean 55.13

	DF	MS	VR
Milk	11	6.1618	6.630**
Coagulant	3	0.2628	0.283
Residual	9	0.9294	
Total	23	3.3449	

	Milk	Coagulant
REP	4	6
SED	0.557	0.682

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:18

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on the fat content in cheese dry matter (FDM) expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	51.01	-	51.62
1 February	51.63	50.53	-	-
8 February	53.63	-	-	51.66
15 February	-	55.32	54.73	-
22 February	55.68	-	56.16	-
1 March	-	-	55.39	55.46
7 June	-	52.60	52.60	-
14 June	53.67	-	-	52.18
21 June	-	53.09	-	52.99
28 June	51.54	-	50.56	-
5 July	52.59	51.88	-	-
19 July	-	-	50.33	49.51
Estimated mean	53.482	52.546	52.726	52.293

Overall mean 52.782

	DF	MS	VR
Milk	11	6.9817	30.621***
Coagulant	3	1.0491	4.601*
Residual	9	0.2280	
Total	23	3.5651	

	Milk	Coagulant
REP	4	6
SED	0.276	0.338

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:19

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on cheese hydrogen ion concentration (pH)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	5.30	-	5.25
1 February	5.20	5.25	-	-
8 February	5.40	-	-	5.25
15 February	-	5.45	5.35	-
22 February	5.40	-	5.35	-
1 March	-	-	5.10	5.05
7 June	-	4.90	4.90	-
14 June	5.10	-	-	5.05
21 June	-	5.15	-	5.15
28 June	5.11	-	5.12	-
5 July	5.18	5.11	-	-
19 July	-	-	5.05	5.06
Estimated mean	4.959	4.947	4.922	4.894
Overall mean		4.930		

	DF	MS	VR
Milk	11	1.559892	884.293***
Coagulant	3	0.003300	1.876
Residual	9	0.001764	
Total	23	0.747157	

	Milk	Coagulant
REP	4	6
SED	0.0242	0.0297

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

variation in the physical condition of the curd at this stage of the process. The variation in salt content, because of using different coagulants, was not significant (table 4:20).

During cheese making, whey was collected and weighed, the first whey, (whey at running) collected after scalding the curd/whey mixture accounted for 93 per cent of the total whey (the values ranged from 90 to96 per cent). The second whey, (whey at cheddaring) which was collected during cheddaring accounted for about 6 per cent of the total whey (individual values ranged from 4 to 8 per cent). The third whey, (whey at pressing) collected during the overnight pressing of the cheese represents about 1 per cent of the total whey.

The analysis of each of the whey samples showed that, in general the first whey contained the normal amount of nitrogen, non protein nitrogen, and fat given in the literature (i.e., 0.841, 0.259 and 0.359 per cent respectively). The second whey contained higher amounts of nitrogen and non protein nitrogen than the first whey (i.e., 0.884 and 0.316 per cent) but contained smaller amounts of fat (0.184 per cent). The third, or pressing whey, contained higher amounts of nitrogen and non protein nitrogen than the other wheys (i.e., 1.016 and 0.377 per cent), and by comparison to the other wheys contained very high amounts of fat (2.503 per cent).

The statistical analysis of the results of the overall whey composition (derived from the results of individual wheys composition) showed that the type of coagulant used had no significant effect on the total nitrogen content of whey and also showed no significant difference between the individual coagulants. The effect of different milks on the total nitrogen content of whey was highly significant (table 4:22), and the reason may be variation in the age of the milk or protein content.

The variation in the non protein nitrogen content of whey due to different coagulants or milks was not significant. The difference between the mean value of non protein nitrogen in the whey resulting from the use of Mucor miehei rennet or calf rennet was significant, and the reason for this difference could be the high proteolytic activity

TABLE 4:20

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) vats] on the salt content in cheese (expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	2.20	-	2.24
1 February	1.79	1.67	-	-
8 February	1.67	-	-	1.56
15 February	-	1.99	1.81	-
22 February	1.75	-	1.66	-
1 March	-	-	1.47	1.57
7 June	-	1.67	1.76	-
14 June	1.78	-	-	1.66
21 June	-	2.04	-	1.98
28 June	2.14	-	1.18	-
5 July	1.62	1.81	-	-
19 July	-	-	1.92	1.92
Estimated mean	2.104	2.096	2.039	2.055
Overall mean		2.074		

	DF	MS	VR
Milk	11	1.905863	293.526***
Coagulant	3	0.003992	0.615
Residual	9	0.006493	
Total	23	0.914561	

	Milk	Coagulant
REP	4	6
SED	0.0465	0.0570

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:21

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on fat content in overall whey (expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	0.394	-	0.456
1 February	0.328	0.280	-	-
8 February	0.383	-	-	0.446
15 February	-	0.536	0.523	-
22 February	0.457	-	0.499	-
1 March	-	-	0.428	0.458
7 June	-	0.321	0.278	-
14 June	0.308	-	-	0.379
21 June	-	0.323	-	0.395
28 June	0.292	-	0.294	-
5 July	0.351	0.356	-	-
19 July	-	-	0.209	0.282
Estimated mean	0.357	0.359	0.360	0.419
Overall mean		0.374		

	DF	MS	VR
Milk	11	0.014289	31.037***
Coagulant	3	0.0036405	7.908**
Residual	9	0.0004604	
Total	23	0.007489	

	Milk	Coagulant
REP	4	6
SED	0.01239	0.01517

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:22

The effect of using different coagulants in small scale Cheddar cheese making (180 l (40 gal.) cheese vats) on the total nitrogen content in the overall whey (calculated as protein and expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine ppesin
24 January	-	0.820	-	0.834
1 February	0.829	0.867	-	-
8 February	0.843	-	-	0.806
15 February	-	0.809	0.791	-
22 February	0.818	-	0.819	-
1 March	-	-	0.815	0.787
7 June	-	0.839	0.867	-
14 June	0.793	-	-	0.823
21 June	-	0.884	-	0.864
28 June	0.894	-	0.888	-
5 July	0.905	0.907	-	-
19 July	-	-	0.813	0.894
Estimated mean	0.839	0.850	0.845	0.846

Overall mean 0.845

	DF	MS	VR
Milk	11	0.002568	5.606**
Coagulant	3	0.000074	0.161
Residual	9	0.000458	
Total	23	0.001417	

	Milk	Coagulant
REP	4	6
SED	0.01236	0.01513

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

of Mucor miehei rennet (table 4:23).

The variation in the fat content of whey, due to the use of different coagulants, was significant, the reason for this difference was porcine pepsin which gave whey containing a high amount of fat (table 4:21).

The variation in fat content of whey due to milk composition was highly significant which could have resulted from variation in the milk fat content and type.

Graph (4:1) shows the variation in the milk components during the weeks of cheese making. The variation found corresponds to published figures for seasonal variation in milk composition. The relation between milk components and the yield of cheese obtained with the different coagulants was calculated and it was shown that the fat content of the milk correlated highly significantly with the overall yield (table 4:24). The correlation coefficient between milk fat and the yield obtained with different coagulants was significant except where porcine pepsin was used and where there was no correlation. Content of fat in the milk showed a highly significant correlation with the overall fat in cheese dry matter (FDM), except in the case of porcine pepsin where there was no correlation (table 4:24).

A similar result was obtained in the correlation between fat in milk and fat in whey where an overall highly significant correlation was found.

The relation between the content of milk protein and overall cheese yield was highly significant, but different correlations were found between the individual coagulants and milk protein from those obtained for the same coagulants and milk fat. Porcine pepsin showed a significant correlation with milk protein (table 4:24).

Less significant correlation to that found between cheese yield and milk content of fat and protein was found between cheese yield and milk solids not fat content (table 4:24).

The total solids content of the milk showed a highly significant

TABLE 4:23

The effect of using different coagulants in small scale Cheddar cheese making [180 l (40 gal.) cheese vats] on the non protein nitrogen content in the overall whey (calculated as protein and expressed as a percentage)

Cheese making date - 1978	Calf rennet	Mucor miehei rennet	1:1 mixture CR:PP	Porcine pepsin
24 January	-	0.261	-	0.241
1 February	0.277	0.342	-	-
8 February	0.231	-	-	0.255
15 February	-	0.253	0.268	-
22 February	0.213	-	0.233	-
1 March	-	-	0.276	0.236
7 June	-	0.302	0.270	-
14 June	0.235	-	-	0.249
21 June	-	0.299	-	0.255
28 June	0.265	-	0.262	-
5 July	0.275	0.315	-	-
19 July	-	-	0.223	0.323
Estimated mean	0.243	0.286	0.249	0.274

Overall mean 0.263

	DF	MS	VR
Milk	11	0.001405	2.393
Coagulant	3	0.001653	2.814
Residual	9	0.000587	
Total	23	0.001117	

	Milk	Coagulant
REP	4	6
SED	0.01399	0.01713

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:24

Correlation coefficient found between the yield (adjusted to a moisture level of 35 per cent and expressed as a percentage) of Cheddar cheese made with different coagulants, and the components of cheese and whey

Relation between	Calf rennet [1]	Mucor miehei rennet [1]	1:1 mixture CR:PP [1]	Porcine pepsin [1]	Overall [2]
Cheese yield and milk fat	0.956**	0.804*	0.694	-0.121	0.701***
Cheese yield and milk protein	0.595	0.657	-0.162	0.871*	0.632***
Cheese yield and milk SNF	0.806*	0.715	0.297	-0.490	0.486*
Cheese yield and milk TS	0.938**	0.784	0.639	-0.257	0.665***
Cheese yield and whey fat	0.563	0.576	0.590	-0.946**	0.153
Cheese yield and whey TN	-0.028	-0.076	-0.292	0.688	0.069
Cheese yield and whey NPN	-0.780	-0.400	-0.131	0.841*	-0.285
Milk fat and FDM	0.751	0.833*	0.943**	0.462	0.688***
Milk fat and whey fat	0.611	0.706	0.930**	0.436	0.728***

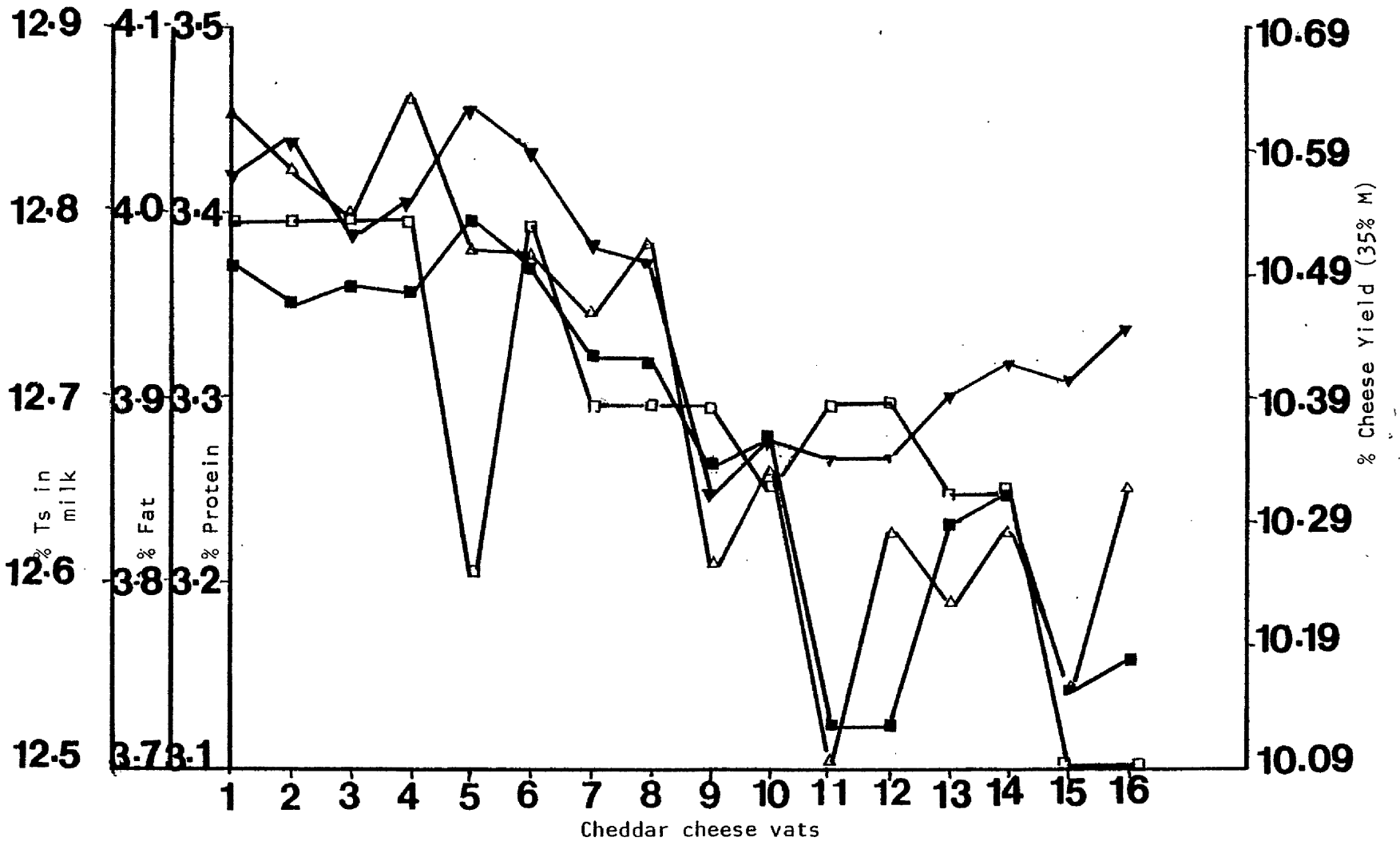
(1) DF = 4

(2) DF = 22

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

GRAPH 4:1

Variation in cheese yield (△), contents of milk from fat (□), protein (▲), and total solids (■) during Cheddar cheese making experiment (4)



correlation with cheese yield except for porcine pepsin which showed no correlation.

3. Small scale cheese making: Mucor miehei rennet ('Hannilase' brand) and calf rennet were selected for a further investigation of the effect of type of coagulant on cheese yield. The results of this experiment and their statistical analysis indicated the following points.

There was no sifnigicant difference in the cheese yield from using either of the two rennets. The variation in yield from one day to another was significant at 5 per cent level (table 4:26). On calculating the yield, assuming a constant moisture content of 35 per cent, less variation in the yield than that found with the actual yield, results was noticed (table 4:27), and again the difference in the yield because of coagulant type was not significant, whereas the difference in the yield because of milk was significant at 5 per cent level.

No significant differences were found between the cheese made by calf rennet or by Mucor miehei rennet in respect of their moisture and fat contents. The variance in moisture content or fat content due to milk was also not significant (tables 4:28 and 29).

The calculated values for moisture in fat free cheese (MFFC) and for fat in dry matter (FDM) showed no significant difference between the two coagulants (tables 4:30 and 31). The variance in the MFFC and in the FDM, because of using different milk every day, is significant.

The analysis of the pH values of cheese showed no significant difference due to the use of different coagulants in making the cheese (table 4: 32).

A highly significant difference was obtained in the pH of cheese made on different days.

Variation in the salt content of cheese made with different coagulants was not significant (table 4:33), but variation in the salt content of cheese made on different days was significant at 1 per cent level.

On checking the mass balance of the experiment, there was no significant

TABLE 4:25

The effect of using different coagulants in small scale Cheddar cheese making 360 l (80 gal.) cheese vats on the mass recovery (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	99.76	99.23
6 April	98.85	99.74
10 April	99.17	99.24
11 April	99.26	99.35
18 April	99.70	99.39
19 April	99.87	99.67
20 April	99.22	99.36
24 April	99.20	99.28
25 April	99.32	99.38
26 April	98.95	99.43
2 May	99.33	99.28
3 May	99.66	99.75
Mean	99.359	99.425

Overall mean 99.392

	DF	MS	VR
Milk	11	0.075350	1.154
Coagulant	1	0.026136	0.400
Residual	11	0.065300	
Total	23	0.068400	

	Milk	Coagulant
REP	2	12
SED	0.2555	0.1043

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:26

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the cheese yield (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	10.54	10.24
6 April	10.58	10.67
10 April	10.54	10.45
11 April	10.59	10.58
18 April	10.56	10.08
19 April	10.32	10.00
20 April	10.24	10.42
24 April	10.18	9.80
25 April	10.07	10.27
26 April	9.92	10.02
2 May	10.46	10.24
3 May	10.52	10.34
Mean	10.376	10.258

Overall mean 10.317

	DF	MS	VR
Milk	11	0.08932	3.359*
Coagulant	1	0.08331	3.133
Residual	11	0.02659	
Total	23	0.05906	

	Milk	Coagulant
REP	2	12
SED	0.1631	0.0666

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:27

The effect of using different coagulants in small scale Cheddar cheese making (360 l (80 gal.) cheese vats) on the cheese yield (adjusted to a moisture level of 35 per cent and expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	10.15	9.74
6 April	9.93	10.32
10 April	10.04	9.99
11 April	10.18	10.06
18 April	9.77	9.72
19 April	9.86	9.58
20 April	9.58	9.62
24 April	9.87	9.53
25 April	9.71	9.69
26 April	9.48	9.65
2 May	9.89	9.78
3 May	9.89	9.95
Mean	9.867	9.801
Overall mean	9.834	

	DF	MS	VR
Milk	11	0.07373	2.969*
Coagulant	1	0.02620	1.055
Residual	11	0.02483	
Total	23	0.04828	

	Milk	Coagulant
REP	2	12
SED	0.1576	0.0643

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:28

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the cheese moisture content (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	37.45	38.17
6 April	38.99	37.10
10 April	38.08	37.84
11 April	37.49	38.22
18 April	39.86	37.38
19 April	37.87	37.76
20 April	39.14	39.98
24 April	37.00	36.79
25 April	37.31	38.70
26 April	37.85	37.43
2 May	38.28	37.87
3 May	38.89	37.43
Mean	38.180	37.890
Overall mean	38.035	

	DF	MS	VR
Milk	11	0.7764	1.144
Coagulant	1	0.5092	0.751
Residual	11	0.6784	
Total	23	0.7179	

	Milk	Coagulant
REP	2	12
SED	0.824	0.336

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:29

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the cheese fat content (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	34.20	32.65
6 April	32.10	32.65
10 April	34.30	34.30
11 April	34.85	33.20
18 April	32.65	35.40
19 April	34.85	34.30
20 April	33.20	32.65
24 April	33.20	32.65
25 April	34.30	33.75
26 April	34.30	34.85
2 May	33.20	33.75
3 May	32.65	33.20
Mean	33.660	33.610
Overall mean	33.635	

	DF	MS	VR
Milk	11	1.0576	1.511
Coagulant	1	0.0126	0.018
Residual	11	0.7001	
Total	23	0.8412	

	Milk	Coagulant
REP	2	12
SED	0.837	0.342

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:30

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the moisture content in the fat free cheese (MFFC) (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	57.01	56.67
6 April	57.42	55.09
10 April	57.96	57.59
11 April	57.55	57.22
18 April	59.18	57.86
19 April	58.13	57.47
20 April	58.59	59.37
24 April	55.39	54.63
25 April	56.79	58.42
26 April	57.62	57.45
2 May	57.25	57.16
3 May	57.75	56.04
Mean	57.550	57.080
Overall mean	57.315	

	DF	MS	VR
Milk	11	2.1187	3.867*
Coagulant	1	1.3292	2.426
Residual	11	0.5479	
Total	23	1.3331	

	Milk	Coagulant
REP	2	12
SED	0.740	0.302

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:31

The effect of using different coagulants in small scale Cheddar cheese making 360 l (80 gal.) cheese vats on fat content in the cheese dry matter (FDM) (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	54.84	52.81
6 April	52.61	51.91
10 April	55.39	55.18
11 April	55.75	53.74
18 April	54.29	56.53
19 April	56.09	55.11
20 April	54.55	54.40
24 April	52.70	51.66
25 April	54.72	55.06
26 April	55.19	55.70
2 May	53.76	54.32
3 May	53.43	53.07
Mean	54.440	54.120
Overall mean	54.280	

	DF	MS	VR
Milk	11	2.8801	4.111**
Coagulant	1	0.6179	0.882
Residual	11	0.7007	
Total	23	1.7394	

	Milk	Coagulant
REP	2	12
SED	0.837	0.342

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:32

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the cheese hydrogen ion concentration (pH0

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	4.95	5.01
6 April	5.02	5.16
10 April	4.92	4.85
11 April	5.00	5.01
18 April	4.98	5.00
19 April	4.88	4.87
20 April	4.97	4.98
24 April	5.09	5.02
25 April	5.11	5.08
26 April	5.12	5.12
2 May	4.88	4.89
3 May	4.92	4.97
Mean	4.987	4.997
Overall mean	4.992	

	DF	MS	VR
Milk	11	0.014539	8.885***
Coagulant	1	0.000600	0.367
Residual	11	0.001636	
Total	23	0.007762	

	Milk	Coagulant
REP	2	12
SED	0.04045	0.01651

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:33

The effect of using different coagulants in small scale Cheddar cheese making [360 l (80 gal.) cheese vats] on the salt content in cheese (expressed as a percentage)

Cheese making date - 1979	Calf rennet	Mucor miehei rennet
5 April	2.11	2.04
6 April	2.09	2.17
10 April	2.16	2.21
11 April	2.10	2.09
18 April	1.92	1.99
19 April	2.08	2.14
20 April	2.11	2.22
24 April	2.11	2.10
25 April	2.01	2.11
26 April	1.98	1.90
2 May	1.97	1.93
3 May	2.03	1.99
Mean	2.056	2.073
Overall mean	2.065	

	DF	MS	VR
Milk	11	0.013941	5.992**
Coagulant	1	0.001584	0.681
Residual	11	0.002326	
Total	23	0.007849	

	Milk	Coagulant
REP	2	12
SED	0.04823	0.01969

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

difference between the total output materials when calculated as a percentage from the total input materials for both coagulants (i.e., 99.359 per cent for calf rennet and 99.425 per cent for Mucor miehei rennet), also no significant difference was noticed in the mass balance from one day to another (table 4:25).

4. Effect of milk storage on cheese yield: It was found at the beginning of the experiment that the amount of cheese obtained was higher than the predicted yield. The result of this was that the quantity of salt used was not sufficient to give the required salt content in the cheese i.e., 1.8 per cent. In the second trial, the quantity of salt was increased from 500 g to 600 g for each vat, and this amount was sufficient to give the required salt content in cheese, but it caused a reduction in the yield and in moisture content of the cheese.

The results indicated that the storage of milk at 4°C for 72 hours had no significant effect on cheese yield (tables 4:34 and 35). There was no interaction between the milk storage period and type of coagulant. The difference in yield,because of using two different coagulants,was not significant after moisture content had been corrected to 35 per cent. The highly significant difference in yield between the two trials was caused by changing the amount of salt used. It may also have been due to the use of different milk in the two trials.

Mass balance was normal, and milk storage was found to have no effect on it (table 4:36). The use of different coagulants had no effect on mass balance.

Results of cheese analysis (table 4:37 to 4:48) showed that milk storage had no significant effect on the content of moisture, fat, protein, non protein nitrogen, fat in dry matter, moisture in fat free cheese, and salt. It had no effect on cheese pH.

The use of different coagulants affected only the fat content of the cheese (tables 4:37 and 4:42).

The use of different amounts of salt in the two trials had a significant

TABLE 4:34

The effect of storage at 4°C on bulked milk for various periods of time prior to cheese manufacture by different coagulants on cheese yields

Storage period (h)	Cheese yield (per cent of milk)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	11.29	10.84	11.08	10.72
24 hours	11.19	10.76	11.11	10.49
48 hours	11.18	10.70	11.14	10.66
72 hours	11.41	10.72	11.03	10.44
Trial mean	11.268	10.755	11.090	10.578
Coagulant mean	11.011		10.834	
Overall mean	10.923			

	DF	MS	VR
Duplicate	1	1.051137	172.221***
Coagulants	1	0.125848	20.619**
Storage	3	0.006927	1.135
Coagulant storage	3	0.013628	2.234
Residual	7	0.006103	
Total	15	0.085427	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0391	0.0391	0.0552	0.0781

* Significant at 5 per cent level

** " " 1 " "

** " " 0.1 " "

TABLE 3:35

The effect of storage at 4°C on bulked milk for various periods of time prior to cheese manufacture by different coagulants on cheese yield (adjusted to a moisture level of 35 per cent)

Storage period (h)	Cheese yield (per cent of milk)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	10.63	10.25	10.58	10.34
24 hours	10.64	10.29	10.54	10.09
48 hours	10.52	10.22	10.51	10.29
72 hours	10.52	10.32	10.46	10.15
trial mean	10.578	10.27	10.523	10.218
Coagulant mean	10.424		10.369	
Overall mean	10.397			

	DF	MS	VR
Duplicate	1	0.373932	103.702***
Coagulants	1	0.011990	3.325
Storage	3	0.005296	1.469
Coagulant storage	3	0.008972	2.488
Residual	7	0.003606	
Total	15	0.030265	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0300	0.0300	0.0425	0.0600

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:36

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the mass recovery (expressed as a percentage from the masses used)

Storage period (h)	Mass recovery (per cent of masses produced from masses used)			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	98.86	99.10	98.94	99.25
24 hours	99.34	99.32	99.55	99.14
48 hours	99.27	99.18	99.27	99.29
72 hours	99.32	99.34	99.30	99.59
trial mean	99.198	99.235	99.265	99.318
coagulant mean	99.218		99.291	
overall mean	99.254			

	DF	MS	VR
Duplicates	1	0.00922	0.322
Coagulants	1	0.02131	0.745
Storage	3	0.09400	3.284
Coagulant storage	3	0.00259	0.090
Residual	7	0.02863	
Total	15	0.03471	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0846	0.0846	0.1196	0.1692

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:37

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the cheese fat (expressed as a percentage)

Storage period (h)	Per cent cheese fat			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	32.0, 31.9	32.2, 32.0	33.3, 33.5	32.7, 32.6
24 hours	32.9, 32.6	32.3, 32.6	33.3, 33.4	32.6, 32.9
48 hours	31.8, 32.1	32.5, 31.5	32.9, 32.9	32.6, 33.3
72 hours	32.9, 32.0	32.9, 32.9	32.7, 33.1	33.1, 33.2
trial mean	32.163	32.363	33.138	32.875
coagulant mean	32.262		33.006	
overall mean	32.634			

	DF	MS	VR
Duplicates	1	0.00039	0.029
Coagulants	1	0.22127	16.264**
Storage	3	0.01239	0.911
Storage coagulant	3	0.00743	0.546
Residual	7	0.01360	
Total	15	0.02509	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01844	0.01844	0.02608	0.03688

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:38

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the cheese moisture (expressed as a percentage)

Storage period (h)	Per cent cheese moisture			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	38.82, 38.85	38.49, 38.56	38.03, 37.83	37.31, 37.33
24 hours	38.01, 38.33	37.67, 37.97	38.21, 38.49	37.43, 37.47
48 hours	38.93, 38.77	38.03, 37.82	38.65, 38.68	37.27, 37.19
72 hours	40.06, 40.02	37.47, 37.40	38.39, 38.40	36.83, 36.87
trial mean	38.97	37.93	38.34	37.21
coagulant mean	38.45		37.77	
overall mean	38.11			

	DF	MS	VR
Duplicate	1	10.7174	29.101***
Coagulants	1	0.5644	1.532
Storage	3	0.0585	0.159
Coagulant storage	3	0.4629	1.257
Residual	7	0.3683	
Total	15	1.0283	

	Duplicates	Coagulants	Storage	Coagulant storage
REP	8	8	4	2
SED	0.303	0.303	0.429	0.607

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

effect on the moisture content of cheese (tables 4:38 and 4:41). The significant difference found in the FDM of cheese made in the two trials (table 4:42) was because of differences in the composition of the milk used in the two trials.

Analysis of whey sampled at different steps during cheese making (tables 4:45 to 4:59) gave the following results. Milk storage had no significant effect on the composition of whey at running (1st whey) in respect of the content of fat, protein, non protein nitrogen, and curd fines. The effect of using different coagulants for cheese production with milk of different ages was not significant except for the non protein nitrogen content of the whey where Mucor miehei rennet was used. The use of this coagulant resulted in significantly higher non protein nitrogen than was present in the whey from cheese made with calf rennet. Differences between the two trials in relation to the composition of whey at running were not significant except the total nitrogen content which was significantly higher in the first trial than in the second trial (table 4:45, 49, 52 and 55).

The results of the analysis of whey at cheddaring (2nd whey) shown in tables 4:46, 50, 53, and 56, indicate that milk storage had no significant effect on the composition of whey in relation to the content of fat, protein, non protein nitrogen, and curd fines. The effect of type of coagulant was significant in relation to the content of total nitrogen and non protein nitrogen, and Mucor miehei rennet gave higher figures than calf rennet. The differences between the two trials were not significant except in relation to the total nitrogen content.

Pressing whey (3rd whey) which accounted for only 1 per cent of the total whey was found to contain a high per cent of fat, protein and non protein nitrogen. It was not possible to determine the content of whey from curd fines because of difficulties in filtering the whey due to the high content of fat. The analysis of pressing whey (tables 4:47, 51 and 54) showed no significant effect of milk storage or type of coagulant on the composition of whey. Comparison of the results of the two trials showed that there was only a significant difference in the total nitrogen of the whey.

TABLE 4:39

The effect of storage at $4^{o}C$ of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the cheese total nitrogen (calculated as protein and expressed as a percentage)

Storage period (h)	Percent of total nitrogen (calculated as protein) in cheese			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2 trial
No storage	38.82, 38.85	38.49, 38.56	38.03, 37.83	37.31, 37.33
24 hours	38.01, 38.33	27.67, 37.97	38.21, 38.49	37.43, 37.47
48 hours	38.93, 38.77	38.03, 37.82	38.65, 38.68	37.27, 37.19
72 hours	40.06, 40.02	37.47, 37.40	38.39, 38.40	36.83, 36.87
trial mean	38.97	37.93	38.34	37.21
coagulant mean	38.45		37.77	
overall mean	38.11			

	DF	MS	VR
Duplicates	1	10.7174	29.101***
Coagulants	1	0.5644	1.532
Storage	3	0.0585	0.159
Coagulant storage	3	0.4629	1.257
Residual	7	0.3683	
Total	15	1.0283	

	Duplicates	Coagulants	Storage	Coagulant storage
REP	8	8	4	2
SED	0.303	0.303	0.429	0.607

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:40

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the cheese soluble nitrogen (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of soluble nitrogen (calculated as protein) in cheese			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.92, 0.84	1.43, 1.35	0.99, 0.91	1.39, 1.41
24 hours	1.26, 1.23	1.35, 1.39	1.42, 1.35	1.28, 1.39
48 hours	1.31, 1.31	1.35, 1.35	1.39, 1.35	1.38, 1.42
72 hours	1.56, 1.53	1.16, 1.24	0.98, 0.86	1.39, 1.32
trial mean	1.245	1.327	1.156	1.372
coagulant mean	1.286		1.264	
overall mean	1.275			

	DF	MS	VR
Duplicates	1	0.08925	2.038
Coagulant	1	0.00191	0.044
Storage	3	0.03341	0.763
Coagulant storage	3	0.02023	0.462
Residual	7	0.04378	
Total	15	0.03724	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.1046	0.1046	0.1480	0.2092

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:41

The effect of storage at $4^{o}C$ of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the moisture in the fat free cheese expressed as a percentage)

Storage period (h)	Per cent of moisture in fat free cheese (MFFC)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2md tria
No storage	57.09, 57.05	56.77, 56.71	57.02, 56.89	55.44, 55.39
24 hours	56.65, 56.87	55.64, 56.34	57.29, 57.79	55.53, 55.84
48 hours	57.08, 57.10	56.34, 55.21	57.60, 57.65	55.30, 55.76
72 hours	58.91, 58.85	55.84, 55.74	57.04, 57.40	55.05, 55.19
trial mean	57.45	56.07	57.34	55½44
Coagulant mean	56.76		56.39	
overall mean	56.57			

	DF	MS	VR
Duplicates	1	10.7174	29.101***
Coagulant	1	0.5644	1.532
Storage	3	0.0585	0.159
Coagulant storage	3	0.4629	1.257
Residual	7	0.3683	
Total	15	1.0283	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.303	0.303	0.429	0.607

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:42

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on fat in cheese dry matter (expressed as a percentage)

Storage period (h)	Per cent of fat in cheese dry matter (FDM)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	52.30, 52.17	52.35, 52.08	53.74, 53.88	52.16, 52.02
24 hours	53.07, 52.86	51.82, 52.56	52.89, 54.30	52.10, 52.61
48 hours	52.07, 52.43	52.44, 50.66	53.63, 53.65	51.97, 53.02
72 hours	53.39, 53.35	52.61, 52.56	53.08, 53.73	52.40, 52.59
trial mean	52.71	52.13	53.74	52.36
coagulant mean	52.420		53.048	
overall mean	52.734			

	DF	MS	VR
Duplicates	1	3.7976	23.766**
Coagulant	1	1.5781	9.876*
Storage	3	0.2198	1.375
Coagulant storage	3	0.2435	1.524
Residual	7	0.1598	
Total	15	0.5256	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.1999	0.1999	0.2827	0.3997

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:43

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the salt in cheese (expressed as a percentage)

Storage period (h)	Per cent of salt in cheese			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	1.57, 1.58	1.75, 1.76	1.77, 1.76	1.79, 1.76
24 hours	1.46, 1.48	1.90, 1.89	1.58, 1.61	1.81, 1.81
48 hours	1.59, 1.58	1.83, 1.84	1.53, 1.52	1.76, 1.76
72 hours	1.61, 1.59	1.91, 1.91	1.55, 1.52	1.94, 1.93
trial mean	1.56	1.85	1.61	1.82
coagulant mean	1.703		1.712	
overall mean	1.708			

	DF	MS	VR
Duplicate	1	0.256289	29.752***
Coagulant	1	0.000352	0.041
Storage	3	0.003610	0.419
Coagulant storage	3	0.005343	0.620
Residual	7	0.008614	
Total	15	0.022920	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0464	0.0464	0.0656 ,	0.0928

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:44

The effect of storage at $4^{o}C$ of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the hydrogen ion concentration (pH) of the cheese

Storage period (h)	pH of cheese			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	5.24	5.05	5.24	5.12
24 hours	5.06	5.27	5.03	5.32
48 hours	5.27	5.27	5.25	5.13
72 hours	5.40	5.27	5.42	5.29
trial mean	5.24	5.22	5.24	5.22
coagulant mean	5.229		5.225	
overal mean	5.227			

	DF	MS	VR
Duplicates	1	0.00226	0.142
Coagulant	1	0.00006	0.004
Storage	3	0.02846	1.791
Coagulant storage	3	0.00269	0.169
Residual	7	0.01588	
Total	15	0.01380	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0630	0.0630	0.0891	0.1260

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:45

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the fat in they whey at running (expressed as a percentage)

Storage period (h)	Per cent of fat in whey at running (1st whey)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.488, 0.374	0.348, 0.354	0.326, 0.331	0.330, 0.336
24 hours	0.341, 0.335	0.381, 0.338	0.373, 0.359	0.385, 0.375
48 hours	0.345, 0.338	0.362, 0.368	0.361, 0.338	0.388, 0.330
72 hours	0.464, 0.405	0.377, 0.328	0.562, 0.465	0.368, 0.383
trial mean	0.386	0.357	0.389	0.361
coagulant mean	0.372		0.376	
overall mean	0.374			

	DF	MS	VR
Duplicates	1	0.003221	1.667
Coagulant	1	0.000064	0.033
Storage	3	0.003705	1.918
Coagulant storage	3	0.002752	
Residual	7	0.001932	
Total	15	0.002312	

	Duplicats	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0220	0.0220	0.0311	0.0440

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:46

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the fat in the whey at cheddaring (expressed as a percentage)

Storage period (h)	Per cent of fat in whey at cheddaring (2nd whey)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.206, 0.192	0.208, 0.212	0.299, 0.288	0.208, 0.173
24 hours	0.191, 0.186	0.189, 0.188	0.118, 0.118	0.246, 0.236
48 hours	0.236, 0.237	0.226, 0.260	0.241, 0.226	0.236, 0.236
72 hours	0.280, 0.275	0.294, 0.339	0.288, 0.297	0.271, 0.262
trial mean	0.225	0.239	0.234	0.233
coagulant mean	0.232		0.234	
overall mean	0.233			

	DF	MS	VR
Duplicates	1	0.000176	0.089
Coagulant	1	0.000009	0.005
Storage	3	0.007422	3.744
Coagulant storage	3	0.000603	0.304
Residual	7	0.001982	
Total	15	0.002542	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0223	0.0223	0.0315	0.0445

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:47

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the fat in the whey at pressing (expressed as a percentage)

Storage period (h)	Per cent of fat in whey at pressing (3rd whey)			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	2.75, 2.32	3.21, 2.99	2.79, 2.77	3.06, 2.84
24 hours	3.85, 3.93	2.55, 2.35	4.27, 4.36	2.92, 2.84
48 hours	3.41, 3.66	3.22, 3.00	4.00, 3.94	3.87, 3.82
72 hours	3.17, 3.36	3.04, 3.12	3.42, 3.10	3.18, 3.03
trial mean	3.31	2.94	3.58	3.19
coagulant mean	3.12		3.39	
overall mean	3.25			

	DF	MS	VR
Duplicate	1	0.005738	2.239
Coagulant	1	0.002862	1.117
Storage	3	0.004312	1.682
Coagulant storage	3	0.000804	0.314
Residual	7	0.002563	
Total	15	0.002792	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0253	0.0253	0.0358	0.0506

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:48

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the fat in the overall whey (expressed as a percentage)

Storage period (h)	Per cent of fat in overall whey			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.443	0.375	0.349	0.357
24 hours	0.370	0.373	0.411	0.401
48 hours	0.369	0.389	0.381	0.390
72 hours	0.451	0.379	0.532	0.400
trial mean	0.408	0.379	0.418	0.387
coagulant mean	0.394		0.403	
overall mean	0.398			

	DF	MS	VR
Duplicates	1	0.003660	2.492
Coagulant	1	0.000324	0.221
Storage	3	0.003238	2.205
Coagulant storage	3	0.002215	1.508
Residual	7	0.001469	
Total	15	0.002042	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01916	0.01916	0.02710	0.03833

* Significant at 5 per cent level

** " " 1 " "

** " " 0.1 " "

TABLE 4:49

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total nitrogen (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of total nitrogen (calculated as protein) in whey at running (1st whey)			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.958, 0.951	0.913, 0.926	0.984, 1.029	0.917, 0.929
24 hours	0.969, 0.957	0.916, 0.922	0.983, 0.982	0.868, 0.874
48 hours	0.972, 0.964	0.917, 0.918	1.011, 1.011	0.946, 0.946
72 hours	0.959	0.914, 0.923	0.980, 0.986	0.941, 0.955
trial mean	0.961	0.919	0.996	0.922
coagulant mean	0.9462		0.9589	
overall mean	0.9526			

	DF	MS	VR
Duplicate	1	0.0166409	41.191***
Coagulant	1	0.0006376	1.578
Storage	3	0.0007582	1.877
Coagulant storage	3	0.0005384	1.333
Residual	7	0.0004040	
Total	15	0.0015998	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01005	0.01005	0.01421	0.02010

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:50

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total nitrogen (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of total nitrogen (calculated as protein) in whey at cheddaring (2nd whey)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	1.032, 1.031	0.956, 0.963	1.104, 1.059	0.996, 0.994
24 hours	1.007, 0.994	0.960, 0.954	1.113, 1.106	1.010, 1.012
48 hours	1.006, 1.010	0.979, 0.972	1.055, 1.046	0.951, 0.963
72 hours	1.011, 1.009	1.007, 1.003	1.055, 1.046	1.061, 1.075
trial mean	1.013	0.974	1.073	1.008
coagulant mean	0.9934		1.0404	
overall mean	1.0169			

	DF	MS	VR
Duplicate	1	0.0107121	11.566**
Coagulant	1	0.0088360	9.541**
Storage	3	0.0008599	0.928
Coagulant storage	3	0.0008186	0.884
Residual	7	0.0009261	
Total	15	0.0020711	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01522	0.01522	0.02152	0.03043

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:51

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total nitrogen (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of total nitrogen (calculated as protein) in whey at pressing (3rd whey)			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	1.377, 1.339	1.128, 1.130	1.294, 1.309	1.097, 1.158
24 hours	1.272, 1.300	1.194, 1.212	1.442, 1.357	1.192, 1.199
48 hours	1.179, 1.248	1.138, -	1.217, 1.335	0.932, 0.979
72 hours	1.337, 1.320	1.080, 1.104	1.296, 1.456	1.076, 1.073
trial mean	1.297	1.141	1.338	1.088
coagulant mean	1.218		1.213	
overall mean	1.216			

	DF	MS	VR
Duplicates	1	0.164430	40.440***
Coagulant	1	0.000121	0.030
Storage	3	0.010825	2.662
Coagulant storage	3	0.002471	0.608
Residual	7	0.004066	
Total	15	0.015527	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0319	0.0319	0.0451	0.0638

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:52

The effect of storage at $4^{o}C$ of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total nitrogen (calculated as protein and expressed as a percentage) in the overall whey

Storage period (h)	Per cent of total nitrogen (calculated as protein) in overall whey			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.962	0.923	1.012	0.928
24 hours	0.968	0.924	0.991	0.881
48 hours	0.972	0.921	1.015	0.947
72 hours	0.965	0.923	0.990	0.954
trial mean	0.967	0.923	1.002	0.928
coagulant mean	0.945		0.965	
overall mean	0.955			

	DF	MS	VR
Duplicates	1	0.0140422	40.842***
Coagulant	1	0.0016000	4.654
Storage	3	0.0003772	1.097
Coagulant storage	3	0.0004102	1.193
Residual	7	0.0003438	
Total	15	0.0013607	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.00927	0.00927	0.01311	0.01854

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:53

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the non protein nitrogen (calculated as protein and expressed as a percentage) in the whey at running

Storage period (h)	Per cent of non protein nitrogen (calculated as protein) in whey at running			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.272, 0.272	0.265, 0.335	- , 0.320	0.310, 0.279
24 hours	0.287, 0.287	0.270, 0.272	0.331, 0.329	0.296, 0.293
48 hours	0.352, 0.367	0.261, 0.261	0.316, -	0.316, 0.313
72 hours	0.278, 0.278	0.261, 0.259	0.327, 0.324	- , 0.304
trial mean	0.299	0.261	0.323	0.302
coagulant mean	0.2798		0.3365	
overall mean	0.3082			

	DF	MS	VR
Duplicates	1	0.003615	2.518
Coagulant	1	0.012854	8.952**
Storage	3	0.007020	1.406
Coagulant storage	3	0.000666	0.464
Residual	7	0.001436	
Total	15	0.002305	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01895	0.01895	0.02679	0.03789

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:54

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the non protein nitrogen (calculated as protein and expressed as a percentage) in the whey at cheddaring

Storage period (h)	Per cent of non proten nitrogen (calculated as protein) in whey at cheddaring			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.301, 0.308	0.322, 0.317	0.308, 0.341	- , 0.382
24 hours	0.313, 0.322	0.327, 0.332	0.384, 0.382	- , 0.382
48 hours	0.339, 0.339	0.319, 0.319	0.409, 0.409	0.342, 0.342
72 hours	0.312, 0.314	0.348, 0.346	0.359, 0.357	0.402, 0.397
trial mean	0.319	0.329	0.369	0.376
coagulant mean	0.3236		0.3797	
overall mean	0.3517			

	DF	MS	VR
Duplicates	1	0.0020562	1.176
Coagulant	1	0.0126001	14.023**
Storage	3	0.0008279	0.921
Coagulant storage	3	0.0004614	0.514
Residual	7	0.0008985	
Total	15	0.005876	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01499	0.01499	0.02120	0.02997

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:55

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the non protein nitrogen (calculated as protein and expressed as a percentage) in the whey at pressing

Storage period (h)	Per cent of non protein nitrogen (calculated as protein) in whey at pressing			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.387, 0.396	0.363, 0.363	0.376, 0.380	0.398, -
24 hours	0.405, 0.403	0.413, 0.413	0.438, 0.438	0.442, 0.438
48 hours	0.356, 0.347	0.354, 0.354	0.429, 0.429	0.376, 0.370
72 hours	0.419, 0.426	0.382, 0.391	0.447, 0.445	0.755, -
trial mean	0.392	0.379	0.423	0.392
coagulant mean	0.3857		0.4071	
overall mean	0.3964			

	DF	MS	VR
Duplicates	1	0.0019802	2.757
Coagulant	1	0.0018276	2.544
Storage	3	0.0018083	2.517
Coagulant storage	3	0.0005208	0.725
Residual	7	0.0007183	
Total	15	0.0010549	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01340	0.01340	0.01895	0.02680

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

The analysis of milk (tables 4:59 to 4:65) showed a significant effect of the storage on milk fat (at 5 per cent level). The results revealed a very slight but consistent decrease in the milk fat content. Storage had no other significant effect on milk composition. The results also indicated that there was no difference in the composition of milk used in each vat, so confirming that the method employed to mix the milk before dividing it was effective.

Highly significant differences were noticed in the milk composition used in the two trials. The differences were in the content of milk fat, total nitrogen, non protein nitrogen, and total solids. Other milk characteristics i.e., content of solids not fat (SNF), pH and freezing point depression were less variable than the above characteristics.

Highly significant correlations were found between cheese yield (after adjusting the moisture to 35 per cent) and milk composition i.e., fat content, total nitrogen content, non protein nitrogen content, and total solids content.

A highly significant correlation was found between cheese yield (moisture adjusted to 35 per cent) and total nitrogen in whey (table 4:66).

TABLE 4:56

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the non protein nitrogen (calculated as protein and expressed as a percentage) in the overall whey

Storage period (h)	Per cent of non protein nitrogen (calculated as protein) in overall whey			
	Calf rennet		Mucor miehei reneet	
	1st trial	2nd trial	1st trial	2rd trial
No storage	0.274	0.254	0.321	0.299
24 hours	0.289	0.275	0.332	0.300
48 hours	0.359	0.264	0.321	0.316
72 hours	0.281	0.264	0.328	0.308
trial mean	0.301	0.264	0.326	0.306
coagulant mean	0.283		0.316	
overall mean		0.299		

	DF	MS	VR
Duplicates	1	0.0031641	8.032**
Coagulant	1	0.0043891	11.142**
Storage	3	0.0005521	1.401
Coagulant storage	3	0.0003341	0.848
Residual	7	0.0003939	
Total	15	0.0008646	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.00992	0.00992	0.01403	0.01985

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:57

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on curd fines (expressed as mg/l of whey in the whey at running

Storage period (h)	mg curd fines/l in whey at running (1st whey)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	22	27	37	45
24 hour	100	41	303	30
48 hour	170	59	139	27
72 hour	67	135	229	38
trial mean	90	65.5	177	35
Coagulant mean	78		106	
overall mean	92			

	DF	MS	VR
Duplicates	1	27639	4.358
Coagulant	1	3221	0.508
Storage	3	6528	1.029
Coagulant storage	3	2772	0.437
Residual	7	6342	
Total	15	6877	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	39.8	39.8	56.3	79.6

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:58

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on curd fines (expressed as mg/l of whey) in the whey at cheddaring

Storage period (h)	mg curd fines/l in whey at cheddaring (2nd whey)			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	26	45	54	152
24 hours	79	141	39	38
48 hours	82	109	285	107
72 hours	69	34	131	145
trial mean	64	82.25	127.25	110.5
coagulant mean	74		119	
overall mean	97			

	DF	MS	VR
Duplicates	1	1	0.000
Coagulant	1	7921	2.341
Storage	3	4715	1.394
Coagulant storage	3	6227	1.841
Residual	7	3383	
Total	15	4295	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	29.1	29.1	41.2	58.2

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:59

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the milk fat content (expressed as a percentage)

Storage period (h)	Per cent of fat in milk			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	4.0, 4.0	3.9, 3.9	4.0, 4.0	3.8, 3.9
24 hours	4.0, 4.0	3.9, 3.9	4.0, 4.0	3.9, 3.9
48 hours	3.8, 3.8	3.9, 3.8	4.0, 4.0	3.9, 3.8
72 hours	3.9, 3.9	3.7, 3.7	3.9, 3.9	3.7, 3.7
trial mean	3.925	3.837	3.975	3.825
coagulant mean	3.881		3.900	
overall mean	3.891			

	DF	MS	VR
Duplicates	1	0.056406	17.671**
Coagulant	1	0.001406	0.441
Storage	3	0.018906	5.923*
Coagulant storage	3	0.003073	0.963
Residual	7	0.003192	
Total	15	0.009740	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0282	0.0282	0.0399	0.0565

* Significant at 5 per cent level

** " " 1 " "

** " " 0.1 " "

TABLE 4:60

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total nitrogen in milk (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of total nitrogen (calculated as protein in milk			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	3.41, 3.44	3.29, 3.19	3.44, 3.45	3.32, 3.24
24 hours	3.41, 3.41	3.25, 3.29	3.38, 3.40	3.25, 3.29
48 hours	3.46, 3.46	3.33, 3.28	3.44, 3.43	3.32, 3.31
72 hours	3.36, 3.39	3.37, 3.31	3.39, 3.38	3.29, 3.33
trial mean	3.417	3.288	3.414	3.294
coagulant mean	3.353		3.354	
overall mean	3.354			

	DF	MS	VR
Duplicates	1	0.061256	50.335***
Coagulant	1	0.000006	0.005
Storage	3	0.001442	1.185
Coagulant storage	3	0.000373	0.306
Residual	7	0.001217	
Total	15	0.005015	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.01744	0.01744	0.02467	0.03488

* Significant at 5 per cent level

** " " 1 " "

** " " 0.1 " "

TABLE 4:61

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the non protein nitrogen in milk (calculated as protein and expressed as a percentage)

Storage period (h)	Per cent of non protein nitrogen (calculated as protein) in milk			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	0.209, 0.209	0.196, 0.192	0.210, 0.206	0.189, 0.195
24 hours	0.211, 0.211	0.162, 0.177	- , 0.211	0.162, 0.177
48 hours	0.213, 0.215	0.166, 0.140	0.215, 0.213	0.162, 0.160
72 hours	0.218, 0.214	0.191, 0.193	0.237, 0.239	0.195, 0.191

trial mean	0.2125	0.1771	0.2177	0.1788
coagulant mean	0.1948		0.1983	
overall mean		0.1966		

	DF	MS	VR
Cuplicates	1	0.0055131	38.014***
Coagulant	1	0.0000490	0.338
Storage	3	0.0004716	3.252
Coagulant storage	3	0.0000338	0.233
Residual	7	0.0001450	
Total	15	0.0005396	

	Duplicates	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.00602	0.00602	0.00852	0.01204

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:62

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the milk solids not fat (expressed as a percentage)

Storage period (h)	Per cent of solids not fat (SNF) in milk			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	8.75, 8.80	8.75, 8.78	8.74, 8.77	8.78, 8.78
24 hours	8.76, 8.76	8.62, 8.63	8.77, 8.76	8.62, 8.63
48 hours	9.01, 8.99	8.72, 8.85	8.79, 8.76	8.74, 8.86
72 hours	8.82, 8.83	8.85, 8.87	8.82, 8.83	8.84, 8.85
trial mean	8.84	8.76	8.78	8.76
coagulant mean	8.799		8.771	
overall mean	8.785			

	DF	MS	VR
Duplicate	1	0.009752	2.022
Coagulant	1	0.003164	0.656
Storage	3	0.019339	4.010
Coagulant storage	3	0.002642	0.548
Residual	7	0.004823	
Total	15	0.007508	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0347	0.0347	0.0491	0.0694

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:63

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the total solids in milk (expressed as a percentage)

Storage period (h)	Per cent of total solids (TS) in milk			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	12.75, 12.80	12.65, 12.68	12.74, 12.77	12.68, 12.68
24 hours	12.76, 12.76	12.52, 12.53	12.77, 12.76	12.52, 12.53
48 hours	12.81, 12.79	12.62, 12.65	12.79, 12.76	12.64, 12.66
72 hours	12.72, 12.73	12.55, 12.57	12.72, 12.73	12.54, 12.55
trial mean	12.77	12.60	12.76	12.60
Coagulant mean	12.681		12.677	
overall mean	12.679			

	DF	MS	VR
Duplicates	1	0.104816	62.719***
Coagulant	1	0.000039	0.023
Storage	3	0.007651	4.578
Coagulant storage	3	0.000018	0.011
Residual	7	0.001671	
Total	15	0.009304	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0204	0.0204	0.0289	0.0409

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:64

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on the milk pH

Storage period (h)	pH of milk			
	Calf rennet		*Mucor miehei* rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	6.54	6.65	6.71	6.62
24 hours	6.72	6.71	6.71	6.71
48 hours	6.69	6.65	6.67	6.64
72 hours	6.65	6.62	6.50	6.68
trial mean	6.65	6.66	6.65	6.66
coagulant mean	6.654		6.655	
overall mean	6.654			

	DF	MS	VR
Duplicates	1	0.000506	0.129
Coagulant	1	0.000006	0.002
Storage	3	0.007723	1.963
Coagulant storage	3	0.002390	0.607
Residual	7	0.003935	
Total	15	0.003893	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.0314	0.0314	0.0444	0.0627

* Significatn at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:65

The effect of storage at 4°C of bulked milk for various periods of time prior to cheese manufacture by different coagulants on milk freezing point (expressed in degree centigrade)

Storage period (h)	Freezing point (C°) of milk			
	Calf rennet		Mucor miehei rennet	
	1st trial	2nd trial	1st trial	2nd trial
No storage	-0.532	-0.536	-0.532	-0.536
24 hours	-0.535	-0.529	-0.535	-0.529
48 hours	-0.539	-0.534	-0.539	-0.534
72 hours	-0.534	-0.531	-0.534	-0.531
trial mean	-0.535	-0.532	-0.535	-0.532
coagulant mean	-0.5337		-0.5337	
overall mean	-0.5337			

	DF	MS	VR
Duplicates	1	0.000025000	2.869
Coagulant	1	0.000000000	0.000
Storage	3	0.000016333	1.874
Coagulant storage	3	0.000000000	0.000
Residual	7	0.000008714	
Total	15	0.000009000	

	Duplicate	Coagulant	Storage	Coagulant storage
REP	8	8	4	2
SED	0.001476	0.001476	0.002087	0.002952

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 4:66

Correlation coefficients found between cheese yield (adjusted to a moisture level of 35 per cent) and different components in milk and whey

Type of relation			Correlation coefficient
Cheese yield (35/M)	-	milk fat	0.627**
"	-	milk protein	0.856***
"	-	Milk NPN	0.726***
"	-	Milk SNF	0.274
"	-	Milk TS	0.876***
"	-	Whey fat	0.144
"	-	Whey TN	0.774***
"	-	Whey NPN	0.309
"	-	Whey curd fines	0.349

DF = 14

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

DISCUSSION

Cheese makers try to assure two things (a) to produce a high yield of good quality cheese and (b) to produce cheese at the lowest possible cost. But, with the shortage of calf rennet since around the mid sixties and the related price increase, cheese makers have not always been able to achieve these objectives. This being so, many research studies have been aimed at finding suitable alternative coagulants to calf rennet which has been regarded as the most suitable for Cheddar cheese from the points of view of quality and yield.

The impression one would have, after reviewing the published work on different coagulants, is that from the quality point of view, there are some coagulants which all workers agree produce cheese with a quality equal to that produced by calf rennet. From the point of view of yield, however, there is no one coagulant which all workers agree produces a yield equal to that produced with calf rennet. With every single coagulant other than calf rennet, there are reports in favour of using it because it has been found to give good yield, and there are also some reports against its use because it has been found to give low yield.

From the results obtained in the experiments described above and their statistical analysis, it is clear that cheese yield is not a constant thing.

Cheese yield is affected by many factors which are difficult to control, and a variation in the cheese yield may happen, even if every precaution is taken to avoid it.

It has happened, in the experiments of this chapter, that when the only variant was the coagulant and a difference in yield was obtained in all cheese making trials, even when the same coagulant was used in duplicate (table 4:9), there was still a difference in the yield between the duplicates made with the same coagulants.

The variation in the yield of cheese made from the same milk using

similar conditions i.e., starter, coagulant, and cheese making technique was very small, such variation was due to small differences in cheese making conditions such as a slight difference in the setting temperature which will affect the curd strength. Cutting the curd at a slightly different size, or stirring the curd at a slightly different speed or time, will result in variation in the yield because more curd dust will be lost in the whey. Finally, scalding the curd/whey mixture at a slightly different temperature will also affect the yield indirectly by producing cheese with different moisture contents.

From the statistical analysis of the cheese making trials described in this chapter, there was no evidence that different types of coagulants could affect the yield of cheese. In most of the experiments, the difference between the yield of cheese made with different coagulants was negligible. In most of the trials however, the difference in the yield which arose from using different milk every day was highly significant. This result means, that cheese yield is affected mostly by the variation in milk composition and not by the use of different coagulants.

The highly significant correlation found between cheese yield and milk fat, protein, and total solids, explains the variation in cheese yield from one day to another. This relation has been known for a long time and is the basis of several formulae to predict cheese yield from a knowledge of the composition of the milk used for cheese production.

Davis (1965) discussed the relation between milk composition and cheese yield and listed the different formulae developed to estimate cheese yield from milk composition. It is clear, that most of the formulae depend on using the contents of milk fat and casein to calculate the yield, but some recent formulae used also the content of solids not fat (SNF) and cheese composition to estimate the yield.

Joost et. al. (1968) developed a formula which uses the contents of milk fat and protein and the moisture in the fat free cheese (MFFC) to estimate the yield of blue-veined cheese. Another formula depending

on milk composition was developed by Mickelsen and Dayton (1974) to predict yield of Cottage cheese.

The relationship between milk composition and cheese yield is of importance to cheese producers and dairy farmers, for in some countries the price of milk is determined according to this relationship (Davis, 1965).

Davis (1965) found, from figures obtained over several years, that cheese yield correlated very well with the total solids content of milk, and he noticed a good correlation between cheese yield and fat and solids not fat in milk. Hamdy and El-Koussy (1969) found a direct relationship between the yield of Domiati cheese and fat and total solids of milk, they also noticed an inverse relationship between casein/fat ratio and cheese yield.

Angevine (1974) considered the solids not fat content of milk as the most significant factor affecting Cottage cheese yield. Boitsun (1974) Peichevski (1974), Olson (1977), and Graham et. al. (1978) reported a direct relationship between milk composition (mostly fat content) and cheese yield, and the results obtained by the author agree with these views.

The overall correlation coefficient values presented in tables 4:24 and 66 show a direct relationship between milk fat, protein, and total solids and cheese yield.

The relationship between solids not fat and overall yield (i.e. obtained in all trials) was not as definite as that obtained with milk fat and protein. The reason for this was the inverse relationship found between cheese yield and solids not fat in milk when porcine pepsin was used.

Porcine pepsin was found to give a different relationship between cheese yield and milk components compared with other coagulants. The author recommends that further studies take place to investigate more fully the relationship between milk composition and cheese yield where porcine pepsin is used. It would, for example, be worthwhile to see if the high proteolytic activity of the coagulant is the reason for the

unusual relationship.

The highly significant correlation found between cheese yield and total nitrogen in whey (table 4:66) means that cheese yield could be estimated from a knowledge of the total nitrogen in the whey. Emmons et. al. (1977) used the figures for total nitrogen in whey to develop a formula to estimate cheese losses.

Coagulant type had more effect on cheese composition than on cheese yield, especially the effect of coagulant type on the fat content of the cheese. Porcine pepsin was found to cause more fat to be lost in the whey and less fat to be recovered in the cheese than other coagulants.

The effect of porcine pepsin on the amount of fat recovered in the cheese was not observed in the laboratory scale cheese making, because the method of manufacture allowed the curd to hold more whey than in the small scale cheese making.

The reason why porcine pepsin cheese contains less fat than that made with other coagulants is the strength of curd at the normal time of cutting. Porcine pepsin was found to produce softer curd than other coagulants and more fat was lost in the first whey (whey at running).

The reason for curd produced by porcine pepsin being softer than that produced by other coagulants is the milk pH which is normally higher than the optimum for porcine pepsin activity (chapter 3).

To know that there is a difference in the fat content of two cheeses is not sufficient evidence on which to establish the quality of each cheese, because the fat and moisture contents of cheese are inter-dependent (Pearce, 1978). A comparison based on the fat in dry matter will be a better way of assessing the effect of any difference in the fat content of cheese on its quality.

The analysis of the results of the fat in dry matter (FDM) again showed a significant difference, but not because porcine pepsin gave the lowest FDM, but because calf rennet gave the highest FDM with a significant

difference from the other three coagulants used in the small scale cheese making experiment (table 4:18). The difference in the FDM between the other three coagulants is very low and not significant. The high FDM in the cheese made with calf rennet means that the use of this coagulant caused less fat to be lost than with the other three coagulants.

In comparing the moisture content of cheese and the figure for moisture in the fat free cheese with the type of coagulant used in making the cheese, no relationship was noticed, and all the observed differences were due to the use of different milk in each trial. Because of the direct relationship between cheese quality and moisture content of the cheese (Pearce, 1978), a conclusion may be reached that coagulants, per se, have no adverse effect on cheese quality, and that all defects observed in the cheese were due to the quality of milk used. The lack of influence of coagulant type on pH during cheese production means that the coagulants did not affect the rate of acidity development during the making of the cheese.

The same is true about the salt content in cheese. In fact, all variations in cheese composition, except that of fat content, were found to be related directly to the milk used and to the techniques used for making the cheese i.e. the temperature of milk at renneting, the size of curd when cut, the temperature of scalding, and the rate of acidity development during cheddaring. It is possible to control all these factors and produce cheese of good quality irrespective of the type of coagulant used.

Whey analysis revealed some effects of the type of coagulant used in the composition of the whey. Mucor miehei rennet ('Hannilase' brand) had an effect on the non protein nitrogen content of the whey, indicating that the casein or whey proteins were degraded to a greater extent with this coagulant than with the other coagulants, and this might have an effect on the characteristics of cheese during ripening.

CONCLUSION

Cheese yield is dependent on a very complicated set of factors of which the most important is the composition of the milk. The coagulant types had very little effect on cheese yield, especially when normal milk was used, and although the difference in cheese yield obtained with different coagulants was not significant statistically, it could be significant commercially. For example, a commercial brand of *Mucor miehei* rennet was found to give a lower yield than calf rennet in two series of experiments (9.801 and 10.369 per cent against 9.867 and 10.424 per cent). These differences, when calculated in financial terms, mean a loss of £172.90 and £144.10 per day for a medium sized cheese factory (i.e. around 225,000 l (50,000 gal.) of milk a day assuming that the price of cheese is £1250/metric tonne and the price of the coagulants is £2.57/litre for calf rennet and £2.10/litre for *Mucor miehei* rennet (Hansen's Laboratory, 1979). In a third series of cheese making experiments, *Mucor miehei* rennet ('Hannilase' brand) was found to give an estimated yield higher than that of calf rennet (9.959 against 9.884 per cent) indicating a gain of £228 per day for a medium sized cheese factory where *Mucor miehei* rennet is used as a replacement for calf rennet.

But because of all the above differences in cheese yield were not significant statistically, which means it only happened by chance, a large number of carefully controlled commercial scale experiments are required before one could conclude that the use of this particular coagulant will give a different yield from that obtained with the use of calf rennet.

The use of porcine pepsin in cheese making resulted in significantly higher losses in milk fat and cheese yield compared to calf rennet. This result which stems from higher fat losses in the whey can be avoided by using milk for cheese making with a pH level lower than 6.6 (chapter 3) or by using porcine pepsin in a mixture with calf rennet (i.e., 1:1 ratio mixture).

Milk storage at $4^{o}C$ for up to 72 hours will have no adverse effect on cheese composition and yield. One would not expect milk to be stored at $4^{o}C$ for longer periods before use in cheese production.

The figures for the fat content in the whey, (derived from the individual analysis of the three samples taken in each trial) corresponded with the results obtained for FDM of the cheese, and, because cheese made with porcine pepsin showed a lower FDM value, it gave a higher fat content in the whey.

Storage of milk at $4^{o}C$ for up to 72 hours had no significant effect on cheese yield, cheese composition or whey composition.

The effect of milk storage at $4^{o}C$ was only significant on the content of milk fat. This effect could be due to the lipolytic activity of some bacterial and milk enzymes resulting in the production of volatile free fatty acids and causing this slight reduction in the milk fat content.

CHAPTER FIVE

THE EFFECT OF THE TYPE OF COAGULANT ON CHEDDAR CHEESE RIPENING

INTRODUCTION

On completion of the making process, Cheddar cheese has little or no flavour related to the typical Cheedar cheese flavour, which only develops after several months. The period of curing (or the period of ripening) may last for Cheddar cheese from 4 months up to one or one and a half years. The period of curing depends on the initial quality of the cheese, but is mainly related to other factors such as marketing demands and storing expenses (Davis, 1965).

There are specific storage environments for different cheese varieties and in the case of Cheddar cheese, different temperatures of storage may be chosen between 2 and 16^{o}C (Kosikowski, 1977). In the case of bandaged (traditional) Cheddar cheese, the relative humidity of the curing room should be between 80 and 90 per cent (Davis, 1965).

Almost all Cheddar cheese is now packaged immediately after pressing in oxygen impermeable wrappers e.g. waxed cellulose laminates or nylon/ polythene laminated pouches. The quality of the ripened Cheddar cheese depends on the following factors:-

1. Quality of the cheese milk and materials used in the process.
2. Behaviour of coagulant and starter during cheese making and during the ripening period.
3. Cleanliness of equipment.
4. Storage factors i.e., temperature and duration of storage.

That the quality of the milk will affect the final quality of cheese is obvious, many factors which affect the quality of milk used for cheese making have been discussed by Davis (1965), Kosikowski (1977), Scott (1972) and Nelson (1975). The factors can be summarised into three groups, milk handling, milk contamination, and milk storage, and the damage which may happen to milk components from the previous factors will be either biochemical i.e., proteolysis and lipolysis, or mechanical i.e., rupturing the fat globule membrane, and the

limited breakdown of milk proteins which may happen during the heat treatment prior to cheese making. Those factors will affect milk quality from the time of its production until it reaches the cheese vat.

Some end products from the above factors i.e., heat resistant enzymes, will continue their action affecting milk components even during the ripening period.

During the production of cheese, milk will be clotted and casein will be separated from milk serum by the action of the coagulant used with the aid of a starter. Cutting the curd, raising the temperature of the curd, and acidity development during cheddaring, milling and salting, hooping and pressing, all those steps will control the characteristics of the green Cheddar cheese. Many of the changes in the cheese protein and fat during ripening are actually started earlier during making the cheese.

Changes in cheese components during ripening will involve the conversion of lactose to pyruvic acid and lactic acid, fat to fatty acids, esters and ketones, and proteins to peptides, amino acids, and ammonia. And all those changes will result in the production of the typical texture and flavour of the mature Cheddar cheese.

The role of the coagulant and starter culture during ripening will be the completion of the chemical changes which they started earlier during cheese making, and the extent of their influence will depend on their survival during ripening. The extent of the survival of the coagulant at the end of cheese making will depend on the type of coagulant used and on the final pH value of the cheese. Holmes and Ernstrom (1973) reported that 5 per cent of calf rennet was recovered in the cheese soon after the end of cheese making, and calf rennet remaining in the cheese was reported by Green (1976) to retain its activity for up to 7 months of curing. Green (1976) reported that Cheddar cheese made without coagulant showed slower proteolysis and no change in cheese composition after 5 months curing.

Microbial derived rennets were affected less than calf rennet by the

different conditions during cheese making, but the amounts of microbial coagulants recovered from cheese were significantly lower than that of calf rennet (Nelson, 1975). Porcine pepsin was affected to a greater extent by cheese making conditions than all the other coagulants, and this enzyme could not be recovered from the cheese soon after its production (Holmes and Ernstrom, 1973; Green and Foster, 1974).

The viable count of starter organisms was found by Green and Foster (1974) to begin falling shortly after the start of curing, and at the end of 7 months ripening was only about 1 to 10 per cent of the initial value.

The lysis of starter cells produces proteinases and peptidases which are responsible for the degradation of large peptides, produced by the action of coagulants, and the formation of smaller peptides and free amino acid (O'Keeffe et. al., 1978).

The type of coagulant used bears no relationship to the destruction of starter cells during curing (Green and Stackpoole, 1975), but the use of different coagulants in cheese making could lead to the production of different casein breakdown products which thereafter lead to the production of different flavour compounds and could affect the rate of proteolysis (Phelan, 1973; Green and Stackpoole, 1975). This view was confirmed by Wong et. al., (1977) who found that the content of free amino acids in Cheddar cheese made with four different coagulants differed quantitatively but not qualitatively.

In relation to the effect of storage conditions on the ripening of Cheddar cheese, the temperature used and the duration of storage are related to each other. The higher the temperature used the shorter will be the ripening period.

The relative humidity of the cheese curing room has no effect on the curing process since the packaging materials are impermeable to water and vapour.

Every cheese maker wishes to be sure that the use of different coagulants will have no adverse effect during cheese ripening, i.e., in

relation to the extent of proteolysis and lypolysis. The aim of the work described in this chapter was to determine the nature of the changes which take place during the ripening of Cheddar cheese made with different coagulants, and to establish the cause of any changes which were observed.

EXPERIMENTAL

Cheddar cheese which had been made in the laboratory and small scale experiments were examined by the methods described below to determine the effect of curing at 10°C on the moisture content, the acidity level (pH) and the extent of proteolysis and lipolysis (as judged by the increase in the amounts of soluble nitrogen and free fatty acids). The experiments were carried out as follows:-

1. Measurements of moisture content of cheese during curing

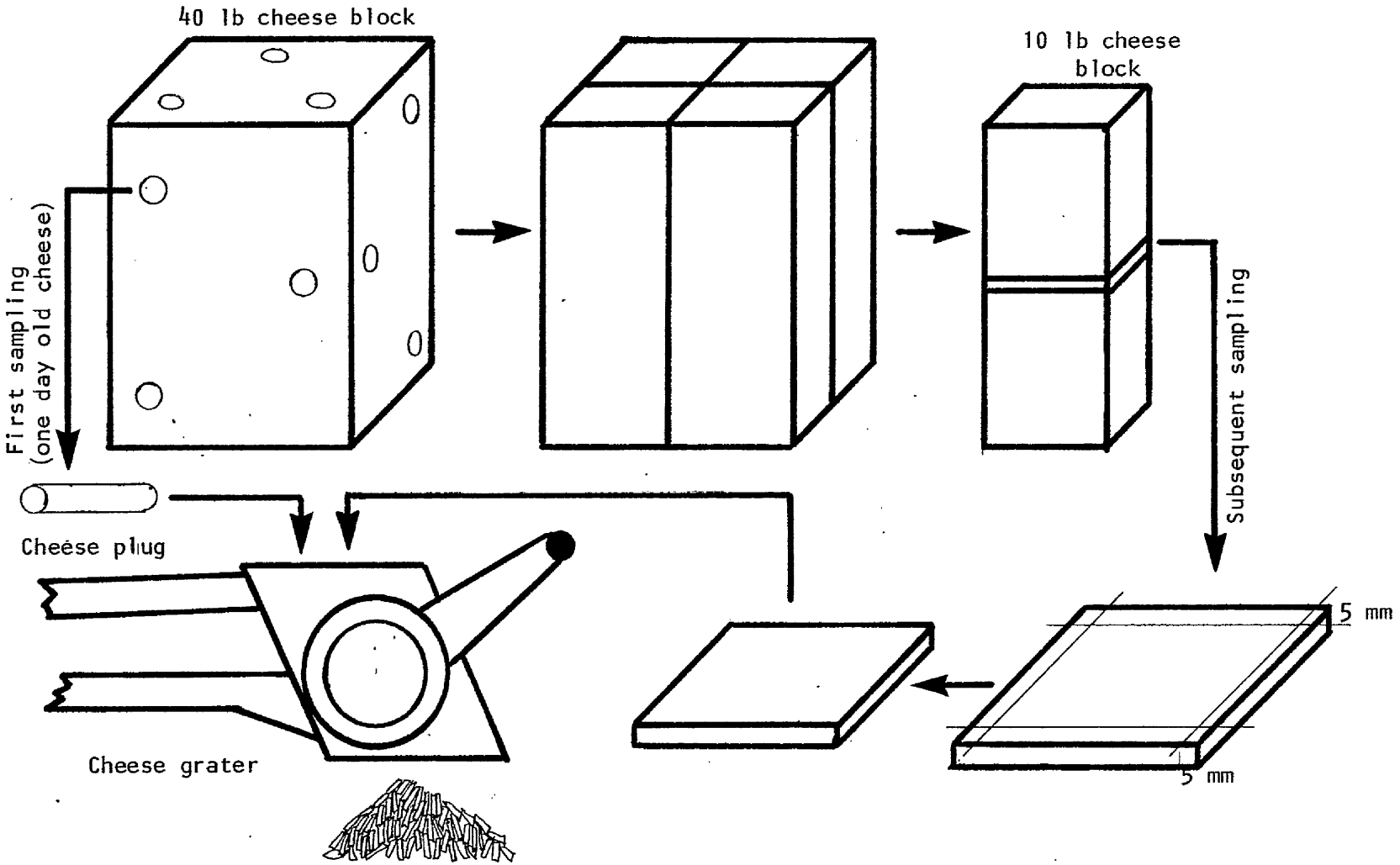
Cheddar cheese which had been made in the small scale cheese making experiment 180 l (40 gal) vat using four different coagulants e.g. calf rennet (Hansen's 'standard' brand), Mucor miehei rennet ('Hannilase' brand), 1:1 mixture of calf rennet and porcine pepsin (Hansen's '50/50' brand), and porcine pepsin rennet (Hansen's brand) were sampled after curing periods of 1 and 2 weeks, and after 1, 2, 3, 4, 5, 6 and 9 months. The first moisture determination (one day old cheese ex-press) was made on the composite of three core samples taken from each of three different sides of the whole cheese block (19 kg) using a cheese trier (Diagram 5:0).

The cheese plugs were grated using a domestic cheese grater, and the moisture content was then determined using the method described in chapter one, section 5:1.

After the samples had been taken from the block, it was cut into four smaller blocks 4.5 kg (10 lb) and placed in separate nylon/polythene pouches which were evacuated and sealed before being placed in the cheese curing room at 10°C. The next sample was taken after 1 week from one of the 4.5 kg cheese blocks. After removing the nylon/polythene pouch, the surface of the cheese was wiped with a dry tissue. Then the block was cut into two halves and a slice about 5 mm thick was taken from one half. The edges of the slice (5 mm) were removed before it was grated and the moisture content determined. The other moisture determinations during cheese ripening were made following the same procedure. In every case duplicate analyses were made.

DIAGRAM 5:0

Cheese sampling procedure



2. Acidity changes during curing were followed by making duplicate pH determinations on 10 g portions of the samples according to the method described in chapter one, section 5:3.

3. Protein degradation during cheese curing Cheddar cheese which had been made in the laboratory and small scale experiments were used in this experiment. Ten cheeses made with each of the four coagulants were used. The age of the cheese ranged from 1.5 to 15 months old. Half the samples (20 samples) were from Cheddar cheese made in laboratory scale experiments, and the other half were from Cheddar cheese made in small scale experiments.

Total nitrogen (calculated as protein) was determined using the methods described in chapter one, section 5:5 for the preparation of the cheese extract and the determination of total nitrogen in the extract. Soluble nitrogen at pH 4.4 (calculated as protein) was determined using the cheese extract and following the method described in chapter one, section 5:6. The increase in the level of soluble nitrogen was followed using Miller's (1959) modification of the Lowrey et. al. (1951) method for protein determination described in chapter one, section 2:3b. In this method the filtrate was diluted with distilled water when it was necessary to obtain a solution with colour intensity within the range of the instrument used to measure the optical density. Values of optical density obtained at 650 nm were then multiplied by their dilution factor.

4. Measuring the extent of lipolysis during cheese curing
Lipolysis in cheese during curing was estimated in the same selection of cheeses tested in the previous section for the extent of proteolysis. The increase in the free fatty acids was measured using Frankel and Tarassuk's (1955) method described in chapter one, section 5:7.

The values obtained for the content of free fatty acids were calculated as a percentage of cheese fat which was determined as described in Chapter one, section 5:2.

RESULTS

1. Measurement of moisture content of cheese during curing

The moisture content of each cheese sample was determined in duplicate, and the mean values of the duplicates are given in table 5:1. Statistical analysis of the variation in the values was carried out using the same method of analysis applied in chapter four for the incomplete block design of experiment. The results of the statistical analysis are given in table 5:2. The moisture value for each coagulant was an estimated mean for three cheese samples tested at any particular time. The source of variation in cheese moisture came in the first place from initial differences in cheese moisture arising from using different milk each time. All those effects are under the name of milk in table 5:2.

The effect of curing on the variation is significant at 5 per cent level, and less effect was noticed with the coagulants (significant at 5 per cent level). No interaction was observed between different coagulants and the period of curing.

The overall mean for moisture content for each coagulant, showed that cheese made with calf rennet had a lower moisture content than that made with the other three coagulants. The difference between the moisture content of the calf rennet cheese and that made with the other coagulants was significant at 5 per cent level. The overall effect of the curing period on the moisture content of all cheeses is summarized in graph 5:1 and shows that all cheeses had a reduced moisture content after the first week of curing. Variations in the moisture content determinations continued up to 3 months of curing. Thereafter the values for moisture remained static.

2. Acidity changes during curing The pH values obtained on the samples analysed for moisture at various times during curing were analysed. In table 5:3, the pH values of cheese made with different coagulants after various curing periods are presented, and the results of statistical analysis are presented in table 5:4. The values of variance ratios indicated that the sources of variation in cheese pH were firstly, the curing period and secondly, the initial differences

TABLE 5:1

The effect of curing period on the content of moisture in Cheddar cheese made with different coagulants

Cheese manufacturing date	Coagulant type	Curing period									
		1day	1 wk.	2 wk	1 m	2 m	3 m	4 m	5 m	6 m	7 m
7.6.78	MM	36.89	36.14	35.84	36.04	36.46	35.55	36.01	36.21	36.18	35.97
	(1:1)CR:PP	36.89	36.20	35.66	35.98	37.26	36.42	36.37	36.54	36.46	36.07
14.6.78	CR	36.60	35.07	35.03	34.76	35.96	35.53	35.67	35.80	35.42	35.62
	PP	37.95	36.95	36.52	37.11	37.36	37.45	37.39	37.37	37.10	36.85
21.6.78	MM	34.36	34.12	33.96	34.02	34.76	34.35	34.62	34.49	34.19	33.58
	PP	35.79	34.57	34.55	34.73	35.94	35.74	35.89	36.01	35.73	34.67
28.6.78	CR	36.23	35.57	35.08	36.32	36.55	36.44	36.43	36.22	36.25	36.70
	(1:1)CR:PP	35.43	35.20	35.57	36.00	36.81	36.73	36.63	36.51	36.33	36.60
5.7.78	CR	36.87	36.54	36.34	35.93	36.15	36.23	35.99	35.83	36.35	36.25
	MM	37.07	35.84	35.85	36.11	35.85	35.94	35.78	35.63	35.55	35.95
19.7.78	(1:1)CR:PP	37.31	36.01	37.25	36.54	37.58	37.55	37.01	37.35	36.67	36.83
	PP	36.27	36.11	36.87	36.71	36.35	36.50	37.14	37.23	36.49	36.48

TABLE 5:2

The effect of curing period on the moisture content of Cheddar cheese made with different coagulants

Curing period	CR	MM	(1:1)CR:PP	PP
1 day	36.32	37.00	36.27	36.30
1 wk	35.48	35.93	35.53	35.83
2 wk	35.24	35.83	35.89	35.89
1 m	35.43	36.04	35.90	36.05
2 m	35.98	36.50	36.94	36.26
3 m	35.82	36.16	36.63	36.20
4 m	35.79	36.31	36.40	36.49
5 m	35.71	36.37	36.53	36.47
6 m	35.76	36.24	36.21	36.03
9 m	35.95	35.95	36.23	35.74
Mean	35.746	36.231	36.252	36.125
Overall mean		36.088		

	DF	MS	VR
Milk	5	9.1789	23.741***
Coagulant	3	1.1041	2.856*
Curing period	9	0.9272	2.398*
Coagulant curing	27	0.1383	0.358
Residual	75	0.3866	
Total	119	0.7587	

	Coagulant	curing period	coagulant curing period
REP	30	12	3
SED	0.1966	0.2538	0.5202
except when comparing means with same levels of coagulant			0.5077

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 5:3

The effect of curing period on the acidity (pH) of Cheddar cheese made with different coagulants

Cheese manufacturing date	Coagulant type	Curing period									
		1 day	1 wk	2 wk	1 m	2 m	3 m	4 m	5 m	6 m	9 m
7.6.78	MM	4.90	4.75	4.87	4.82	4.79	4.83	4.86	4.92	4.88	5.02
	(1:1)CR:PP	4.90	4.85	4.90	4.91	4.90	4.90	4.88	4.94	4.86	5.04
14.6.78	CR	5.10	5.10	5.11	5.09	5.10	5.10	5.07	5.08	5.18	5.57
	PP	5.05	5.10	5.10	4.98	4.93	4.92	5.00	4.95	4.99	5.22
21.6.78	MM	5.15	5.11	4.98	4.93	5.10	5.11	5.07	5.03	5.19	5.57
	PP	5.15	5.11	5.00	5.00	4.95	4.99	5.00	4.99	5.00	5.10
28.6.78	CR	5.11	5.00	4.95	4.88	5.01	5.05	5.02	5.03	5.13	5.47
	(1:1)CR:PP	5.12	5.01	4.99	4.92	5.04	5.04	5.00	5.01	5.00	5.33
5.7.78	CR	5.18	4.99	4.97	4.99	5.12	5.07	5.10	5.16	5.16	5.55
	MM	5.11	5.01	4.95	4.97	5.02	5.03	5.03	5.03	5.09	5.45
19.7.78	(1:1)CR:PP	5.05	4.88	4.93	4.96	4.95	4.93	4.99	4.98	4.96	5.30
	PP	5.06	4.94	4.97	4.99	5.00	4.96	4.98	5.03	4.99	5.25

TABLE 5:4

The effect of curing period on the acidity (pH) of Cheddar cheese made with different coagulants

Curing period	CR	MM	(1:1)CR:PP	PP
1 day	5.101	5.051	5.076	5.065
1 wk	5.001	4.954	4.966	5.028
2 wk	4.981	4.938	4.993	4.995
1 m	4.958	4.928	4.983	4.945
2 m	5.048	4.918	5.016	4.988
3 m	5.045	4.948	5.009	4.975
4 m	5.035	4.961	5.009	4.995
5 m	5.061	4.978	5.029	4.981
6 m	5.128	4.988	4.993	5.035
9 m	5.501	5.188	5.276	5.325
Mean	5.086	4.985	5.035	5.033

Overall mean 5.0348

	DF	MS	VR
Milk	5	0.128173	28.345***
Coagulant	3	0.034032	7.526***
Curing period	9	0.135546	29.975***
Coagulant curing period	27	0.004969	1.099
Residual	75	0.004522	
Total	119	0.020472	

	Coagulant	Curing period	Coagulant curing period
REP	30	12	3
SED	0.0213	0.0275	0.0563
except when comparing means with same levels of coagulant			0.0549

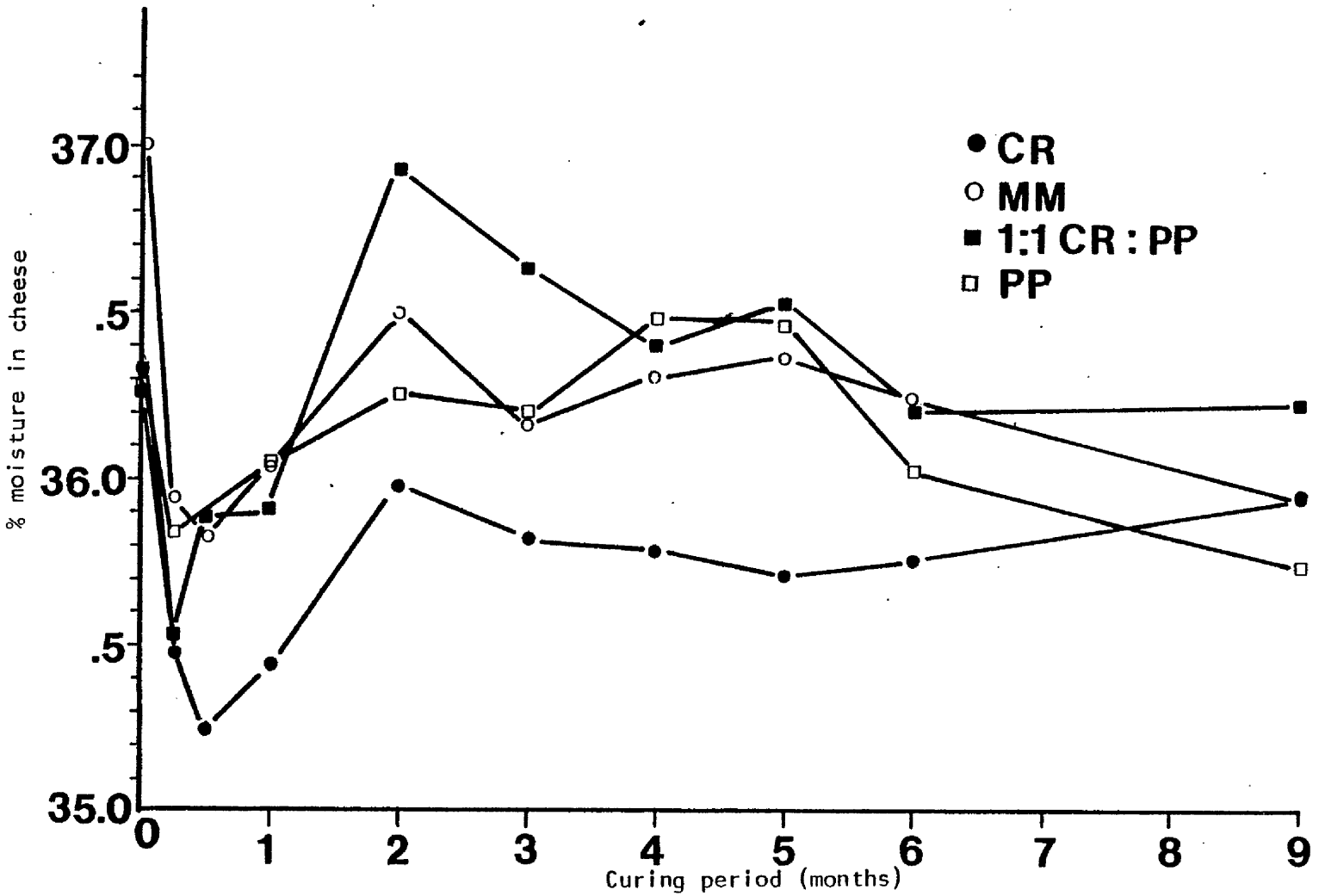
* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

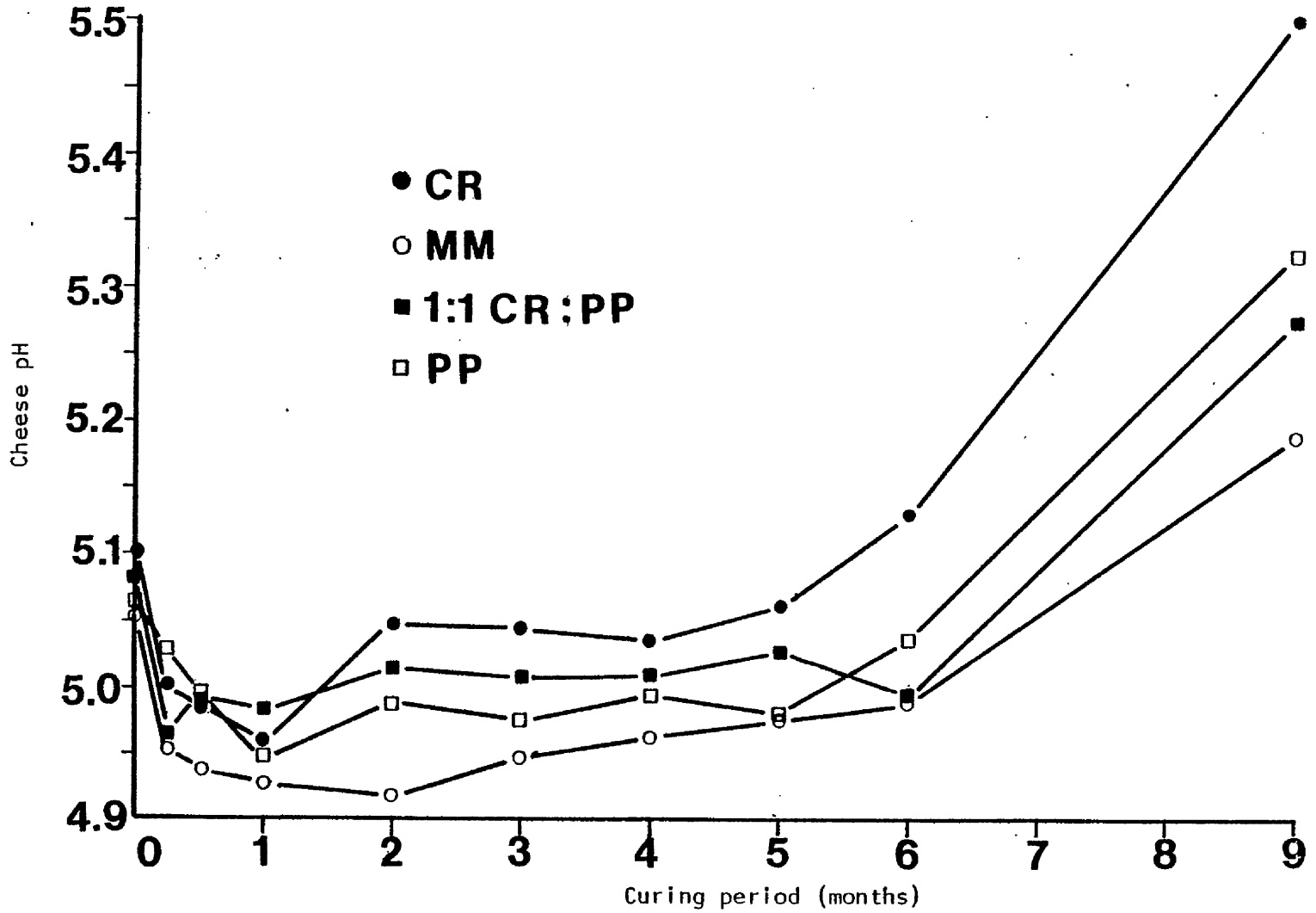
GRAPH 5:1

Variation in the moisture content of Cheddar cheese made with different coagulants during curing at (10°C)



GRAPH 5:2

Variation in the acidity (pH) of Cheddar cheese made with different coagulants during curing at (10°C).



in cheese pH arising from using different milk each time and referred to as the effect of milk. Both effects are highly significant. Cheese pH was also significantly affected by different coagulants (at 0.1 per cent level) but again as with the moisture variation, there was no interaction between the type of coagulant used and the curing period in the variation of pH.

The overall mean values for pH showed that the cheese made with Mucor miehei rennet had a lower pH than cheese made with any of the other coagulants. The difference in the values between cheese made with Mucor miehei rennet and those made with calf rennet were highly significant. Differences between the pH values of cheese made with Mucor miehei rennet and cheese made with a 1:1 mixture of calf rennet and porcine pepsin and with porcine pepsin were less significant (at 5 per cent level).

Graph 5:2 showed that cheeses made with the four coagulants followed a similar pattern of pH change during curing. The pH of all cheeses fell during the first month of ripening to a minimum value and thereafter started to increase, except for the cheese made with Mucor miehei rennet, whose pH continued to fall for another month before it started to increase to the normal pH level i.e. 4.95 to 5.05. This was maintained in all cheese until the sixth month of ripening, after which time the pH of all the cheeses started to increase and in particular to high levels after 9 months of ripening.

3. The extent of cheese protein degradation during curing

The content of total nitrogen in cheese made with different coagulants was calculated as protein using the factor 6.38 and the results are presented in tables 5:5, 6,7 and 8. In the statistical analysis, the results for all the samples of cheese made with a particular coagulant were grouped together and considered as one cheese at a particular stage of ripening. This procedure was necessary to see the effect of the curing period on the different variants measured.

In table 5:9, the results of the regression analysis of the variation in the level of total nitrogen in cheese during curing are given, and show no significant difference whether because of using different

TABLE 5:5

The effect of curing period on the contents of Cheddar cheese made with calf rennet, of percentage total nitrogen (TN), per centage soluble nitrogen (SN), percentage soluble nitrogen of total nitrogen (SN/TN), optical density (OD) measured at 650nm, and free fatty acids (FFA) as a percentage of cheese fat

Curing period in days	OD	% SN	% TN	% SN/TN	% FFA
47	2.85	3.81	23.00	16.56	5.51
69	3.44	5.88	22.43	26.20	6.30
89	3.92	5.06	23.58	21.46	6.96
176	4.04	6.43	22.38	28.72	9.07
202	4.48	6.96	23.81	29.22	9.62
251	4.80	7.45	22.27	33.45	11.12
316	4.76	6.31	25.27	24.97	13.34
337	5.92	8.32	24.68	33.72	13.95
412	6.88	9.27	22.83	40.61	15.43
427	6.56	9.06	21.62	41.91	15.56
Mean 232.6	4.77	6.85	23.19	29.68	10.69
Cor.coeff.	0.961***	0.913***	0.051	0.869***	0.998***
Y intercept	2.638	4.203	23.090	18.075	4.400
Slope	0.009	0.011	0.0004	0.050	0.027
SE of slope	0.0009	0.0018	0.0029	0.0101	0.0007

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 5:6

The effect of curing period on the contents of Cheddar cheese made with Mucor miehei rennet, of percentage total nitrogen (TN), percentage soluble nitrogen (SN), percentage soluble nitrogen of total nitrogen(SN/TN), optical density (OD) measured at 650 nm, and free fatty acids (FFA) as a percentage of cheese fat

Curing period in days	OD	% SN	% TN	% SN/TN	% FFA
56	3.95	5.00	23.40	21.36	5.77
71	4.20	5.91	23.83	24.78	6.64
85	4.07	5.38	22.51	23.92	7.85
197	5.77	8.44	23.11	36.53	9.14
211	5.75	8.67	24.08	36.00	10.42
218	5.65	8.95	25.58	34.96	10.55
314	6.27	10.33	24.90	41.47	12.56
346	6.40	9.88	24.69	40.02	13.42
423	7.65	12.23	21.48	56.94	15.39
443	6.28	9.38	22.19	42.25	16.62
Mean 236.4	5.599	8.417	23.58	35.823	10.836
Cor. coeff.	0.927***	0.906***	-0.202	0.916***	0.992***
Y intercept	3.754	4.898	24.012	19.634	4.819
Slope	0.008	0.015	-0.002	0.068	0.025
SE of slope	0.0011	0.0025	0.0032	0.0106	0.0012

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 5:7

The effect of curing period on the contents of Cheddar cheese made with (1:1) mixture of calf rennet and porcine pepsin, of percentage total nitrogen (TN), percentage soluble nitrogen (SN) percentage soluble nitrogen of total nitrogen (SN/TN), optical density (OD), measured at 650 nm, and free fatty acids (FFA) as a percentage of cheese fat

Curing period in days	OD	% SN	% TN	% SN/TN	% FFA
48	3.28	3.47	23.20	14.97	6.66
69	3.68	4.40	23.86	18.42	7.35
90	3.94	4.32	22.32	19.36	8.31
187	4.42	6.70	21.92	30.57	10.03
195	4.24	5.93	21.21	27.98	11.19
202	4.32	6.55	21.91	29.89	12.36
328	5.58	7.09	22.98	30.86	13.12
362	6.78	8.98	25.15	35.69	14.61
426	8.60	10.86	25.07	43.33	16.73
447	6.84	8.16	22.59	36.12	17.42
Mean 235.4	5.168	6.646	23.021	28.719	11.778
Cor. coeff.	0.934***	0.927***	0.374	0.934***	0.984***
Y intercept	2.591	3.265	22.226	15.442	5.843
Slope	0.011	0.014	0.003	0.056	0.025
SE of slope	0.0015	0.0021	0.0030	0.0076	0.0016

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 5:8

The effect of curing period on the contents of Cheddar cheese made with procine pepsin, of percentage total nitrogen (TN), percentage soluble nitrogen (SN), percentage soluble nitrogen of total nitrogen (SN/TN), optical density (OD) measured at 650 nm, and free fatty acids (FFA) as a percentage of cheese fat

Curing period in days	OD	% SN	% TN	% SN/TN	% FFA
54	2.60	2.39	21.89	10.90	5.71
82	2.70	3.01	23.09	13.05	6.70
89	2.41	2.93	22.84	12.83	8.15
193	3.60	5.80	22.43	25.85	10.82
215	3.48	5.42	22.65	23.92	11.13
229	4.30	5.76	24.09	23.91	11.94
334	5.90	8.02	24.40	32.84	13.22
370	6.56	8.98	22.74	39.46	13.86
434	6.70	10.58	22.41	47.24	15.57
460	8.88	11.04	20.69	53.36	18.40
Mean 246	4.713	6.393	22.723	28.336	11.550
Cor. coeff.	0.964***	0.997***	-0.193	0.986***	0.977***
Y intercept	1.211	1.160	23.058	4.180	5.109
Slope	0.014	0.021	-0.001	0.098	0.026
SE of slope	0.0014	0.0006	0.0025	0.0059	0.0020

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 5:9

The regression analysis for the effect of curing period on the total nitrogen (TN) content of Cheddar cheese made with different coagulants

	Estimate	S.E.	T
Y intercept CR	23.08863831	0.79604272	29.00
" MM	24.01333618	0.79434720	30.23
" (1:1)CR:PP	22.22573840	0.77029392	28.85
" PP	23.06051636	0.79306320	29.08
Slope CR	0.00042020	0.00297668	0.14
" MM	-0.00184411	0.00292058	-0.63
" (1:1)CR:PP	0.00338335	0.00281483	1.20
" PP	-0.00136639	0.00280060	-0.49

Analysis of variance -

	DF	SS	MS
Regression	8	21402.42	2675.303
Residual	32	49.38	1.543
Total	40	21451.80	536.295
Change	0	- 3.22	0

Percentage variance accounted for 99.7

coagulants in making the cheese or because of the curing period. Graph 5:3 indicates the effect of using different coagulants on the values for total nitrogen in cheese. All the coagulants have the same slope but different intercepts for their graphs, and in graph 5:4, different slopes were applied for each coagulant because of the effect of curing, but the overall T value indicated no significant difference between the four coagulants.

Following the measurement of soluble nitrogen at pH 4.4, the resultant values were calculated as percentage protein in the cheese and presented in tables 5:5, 6, 7 and 8. The values of soluble nitrogen were also expressed as a percentage of the total nitrogen in the cheese.

The results of the regression analysis presented in table 5:10 indicate that there were differences in the level of soluble nitrogen in the cheese made with different coagulants. Initially the cheese made with porcine pepsin contained the lowest level of soluble nitrogen and this increased during curing at the highest rate. The cheese made with Mucor miehei rennet contained initially the highest level of soluble nitrogen with a significant difference from that in the cheese made with the 1:1 mixture of calf rennet and porcine pepsin but not from cheese made with calf rennet.

During curing, the rate of soluble nitrogen increase in cheese made with Mucor miehei rennet was higher than was obtained in cheese made with calf rennet and its mixture with porcine pepsin. This high rate did not make a significant difference in the content of soluble nitrogen.

In the case of the cheese made with calf rennet and its mixture with porcine pepsin, there was no significant difference between the two cheeses whether in the initial values or in the rate of increase during curing.

The relationship between the period of curing and the increase in the level of soluble nitrogen in cheese made with different coagulants is shown in graphs 5:5 and 5:6, these graphs indicate that the initial content of soluble nitrogen in the cheese made with porcine pepsin was

TABLE 5:10

The regression analysis for the effect of curing period on the soluble nitrogen (SN) content of Cheddar cheese made with different coagulants

		Estimate	S.E.	T
Y intercept	CR	4.203249	0.512212	8.21
"	MM	4.897315	0.511121	9.58
"	(1:1)CR:PP	3.265759	0.495644	6.59
"	PP	1.158996	0.510295	2.27
Slope	CR	0.011397	0.001915	5.95
"	MM	0.014883	0.001879	7.92
"	(1:1)CR:PP	0.014363	0.001811	7.93
"	PP	0.021274	0.001802	11.81

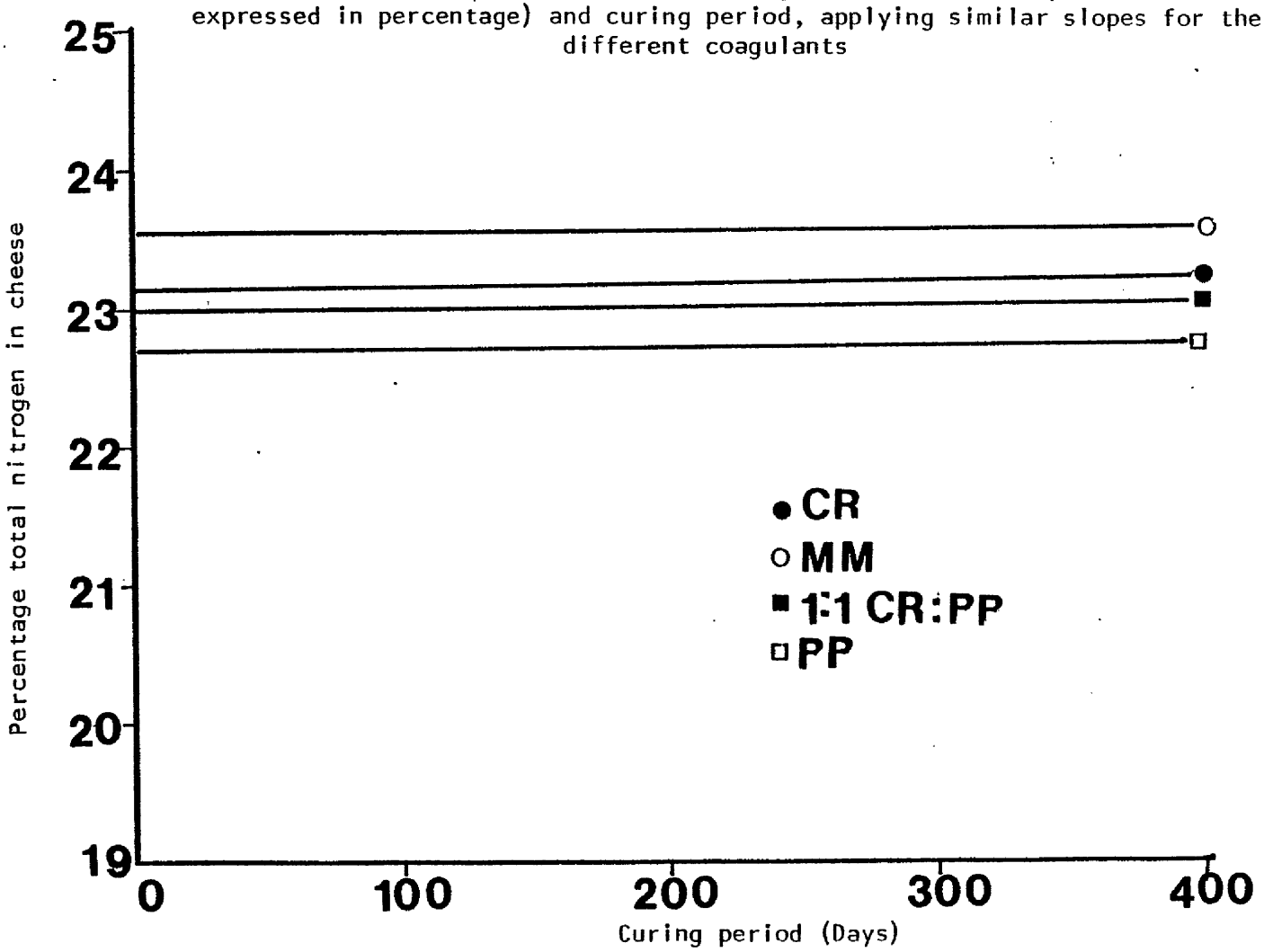
Analysis of variance -

	DF	SS	MS
Regression	8	2220.35	227.5432
Residual	32	20.44	0.6388
Total	40	2240.79	56.0198
Change	0	- 9.80	0

Percentage variance accounted for 89.5

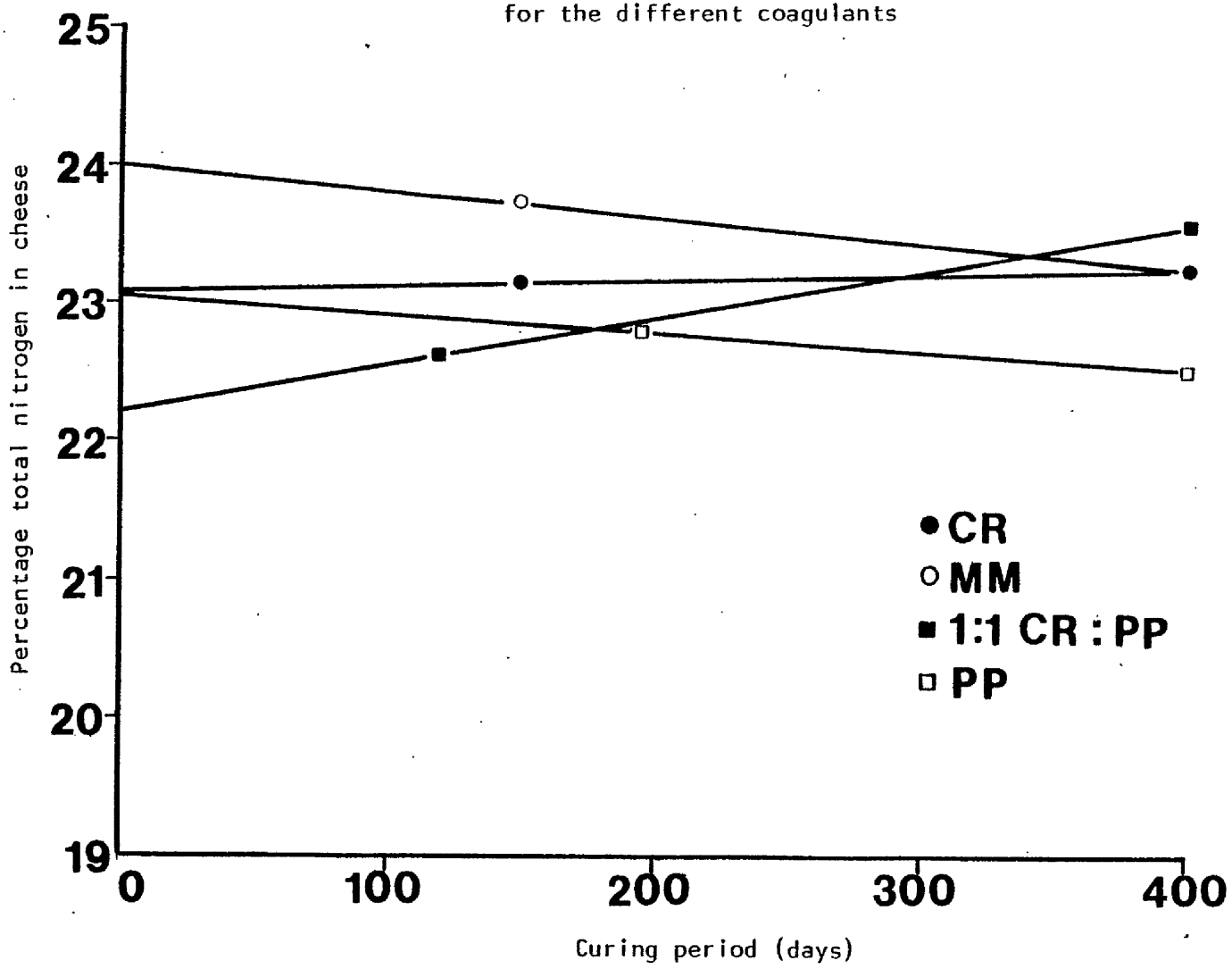
GRAPH 5:3

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of total nitrogen (calculated as protein and expressed in percentage) and curing period, applying similar slopes for the different coagulants



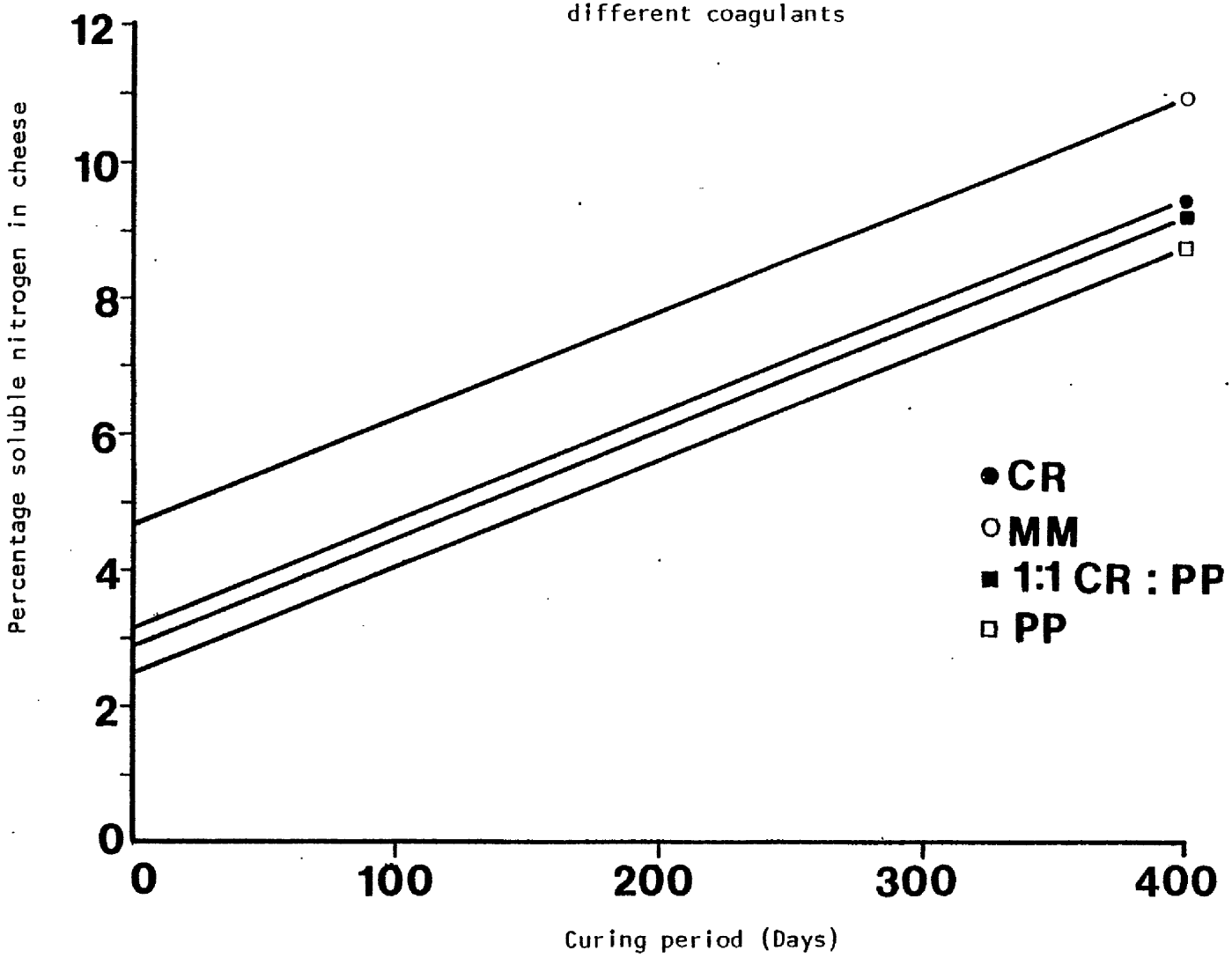
GRAPH 5:4

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of total nitrogen (calculated as protein and expressed in percentage) and curing period, applying different slopes for the different coagulants



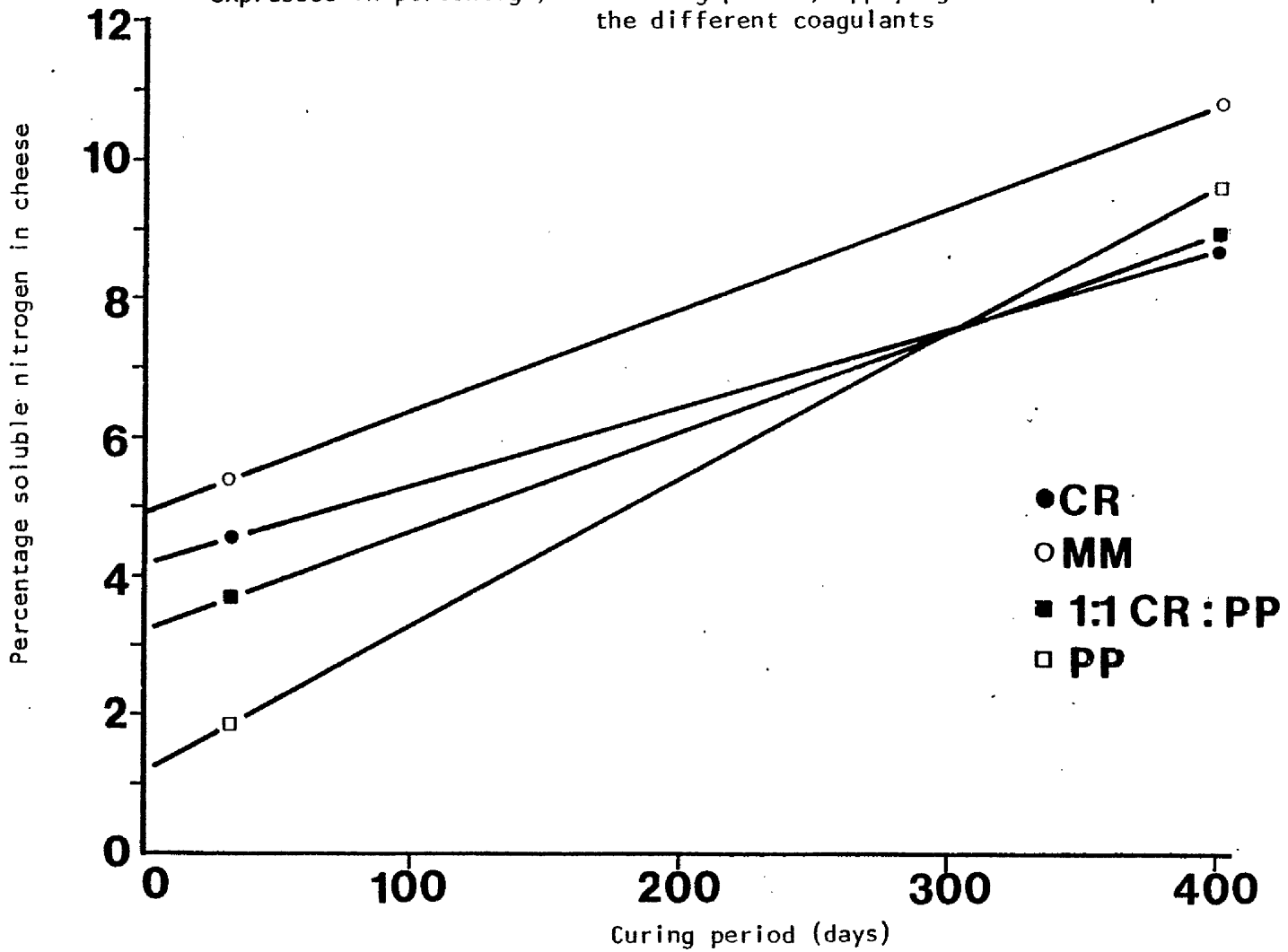
GRAPH 5:5

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of soluble nitrogen (calculated as protein and expressed in percentage) and curing period, applying similar slopes for the different coagulants



GRAPH 5:6

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of soluble nitrogen (calculated as protein and expressed in percentage) and curing period, applying different slopes for the different coagulants



significantly lower than in the cheese made with other coagulants. However, the higher rate of production of soluble nitrogen in cheese made with porcine pepsin during curing resulted in the final level of soluble nitrogen exceeding the levels of soluble nitrogen in cheese made with both calf rennet and the 1:1 mixture. In the case of the cheese made with calf rennet, the initial level of soluble nitrogen was the second highest, but because of the low rate of soluble nitrogen production with this coagulant the final level after 12 months' ripening was the lowest level of soluble nitrogen in cheese made with the four coagulants.

Similar results were obtained when the soluble nitrogen was expressed as a percentage of the total nitrogen in cheese because of the similarity in the total nitrogen content of different cheeses. Results of the regression analysis presented in table 5:11, with the graphs 5:7 and 5:8 explain the initial differences between the different coagulants and the differences which occur due to the effect of curing.

The optical density of the filtrate - pH 4.4, was measured at 650 nm in an attempt to correlate these measurements with those obtained with the micro-Kjeldahl method, and to see if there are any differences between different coagulants in this respect.

The results which are presented in the tables 5:5, 6, 7 and 8, together with the regression analysis results in table 5:12 indicate similar results to those obtained with the micro-Kjeldahl measurements. Slight differences between results obtained by the two methods are discussed below.

Graphs 5:9 and 5:10 show that the filtrate from the cheese made with Mucor miehei rennet had the highest initial optical density value compared with that of other coagulants and the lowest rate of increase during curing. The opposite was found in the case of the filtrate from the cheese made with porcine pepsin.

4. The extent of lipolysis during cheese curing

Lipolysis in cheese during curing was assessed by measuring the level of free fatty acids in a cheese extract. The amount of alcoholic 0.025 N potassium hydroxide used to neutralize the extract was then

TABLE 5:11

The regression analysis for the effect of curing period on the soluble nitrogen content (expressed as a percentage of total nitrogen) in Cheddar cheese made with different coagulants

	Estimate	S.E.	T
Y intercept CR	18.077377	2.396687	7.54
" MM	19.634933	2.391582	8.21
" (1:1)CR:PP	15.443834	2.319163	6.66
" PP	4.181494	2.387716	1.75
Slope CR	0.049892	0.008967	5.57
" MM	0.068480	0.008793	7.79
" (1:1)CR:PP	0.056392	0.008475	6.65
" PP	0.098193	0.008432	11.65

Analysis of variance

	DF	SS	MS
Regression	8	41718.9	5214.86
Residual	32	447.6	13.99
Total	40	42166.4	1054.16
Change	0	-262.2	0

Percentage variance accounted for 98.7

TABLE 5:12

The regression analysis for the effect of curing period on the optical density (OD) measured at 650 nm of filtrate from Cheddar cheese made with different coagulants

	Estimate	S.E.	T
Y intercept CR	2.6379423	0.3484335	7.57
" MM	3.7491207	0.3476913	10.78
" (1:1)CR:PP	2.5907354	0.3371631	7.68
" PP	1.2112055	0.3471293	3.49
Slope CR	0.0091447	0.0013029	7.02
" MM	0.0078125	0.0012784	6.11
" (1:1)CR:PP	0.0109484	0.0012321	8.89
" PP	0.0142349	0.0012258	11.61

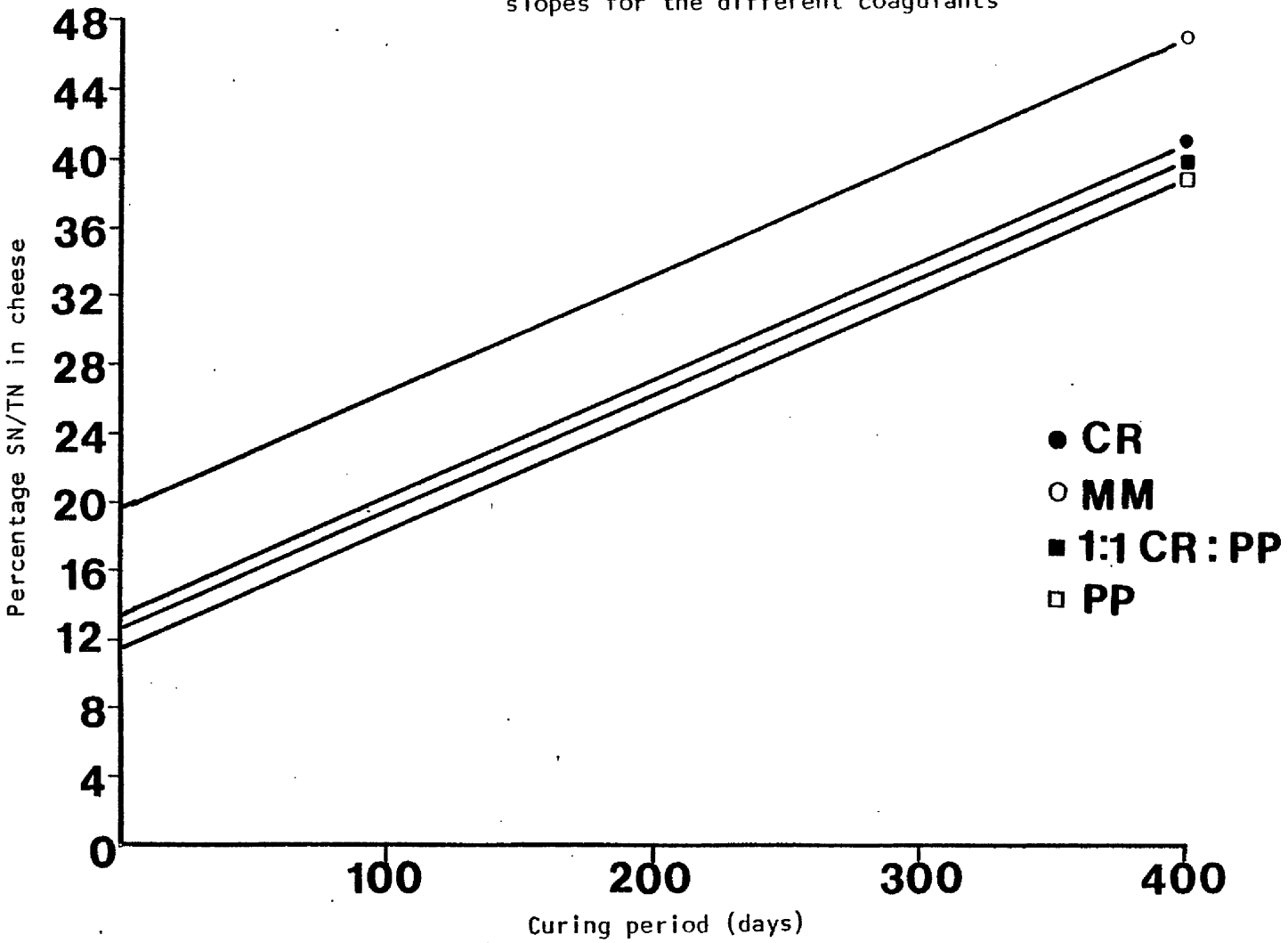
Analysis of variance

	DF	SS	MS
Regression	8	1118.221	139.7776
Residual	32	9.460	0.2956
Total	40	1127.681	28.1920
Change	0	-4.396	0

percentage variance accounted for 99.0

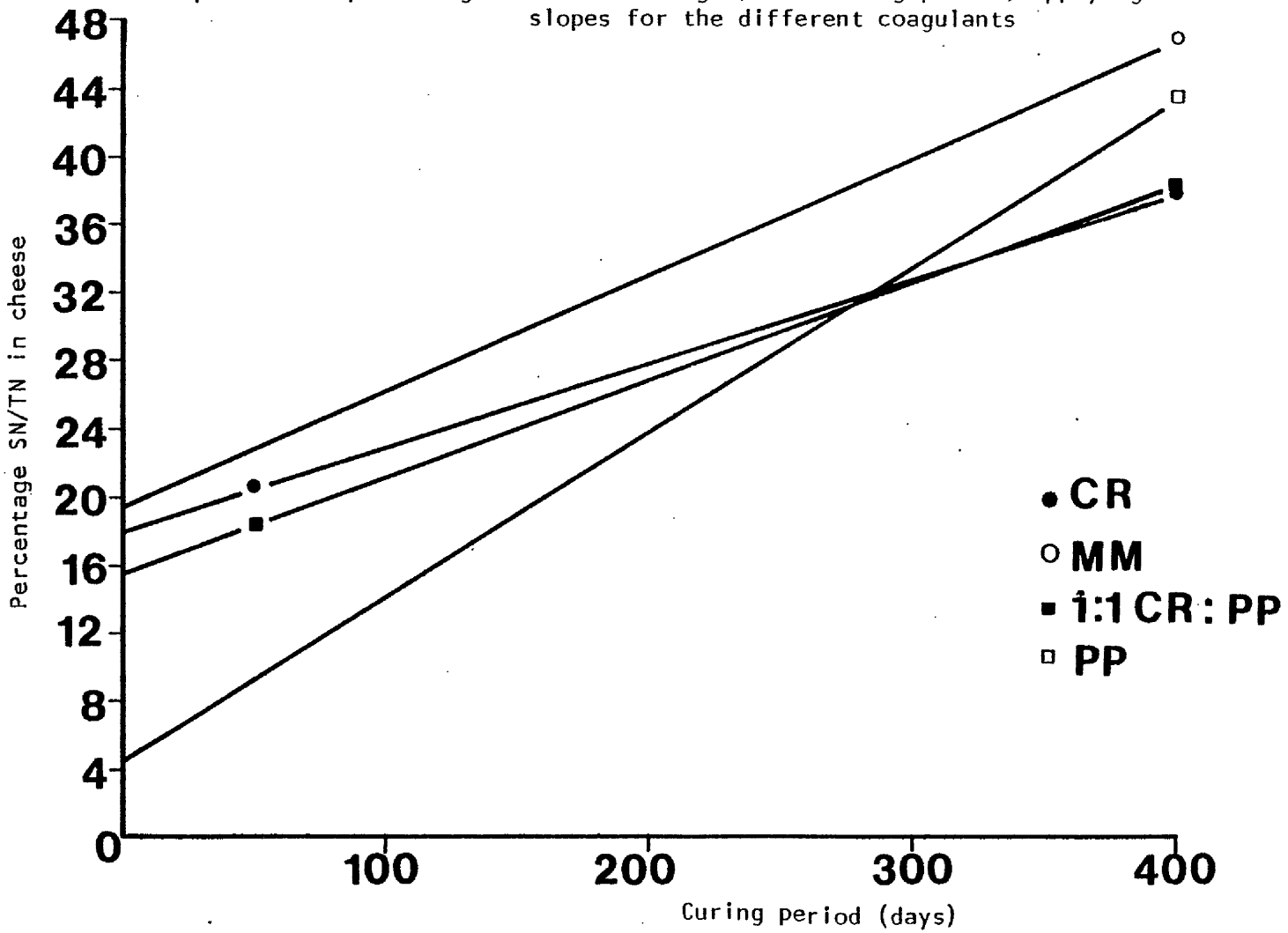
GRAPH 5:7

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of soluble nitrogen (calculated as protein and expressed in percentage of total nitrogen) and curing period, applying similar slopes for the different coagulants



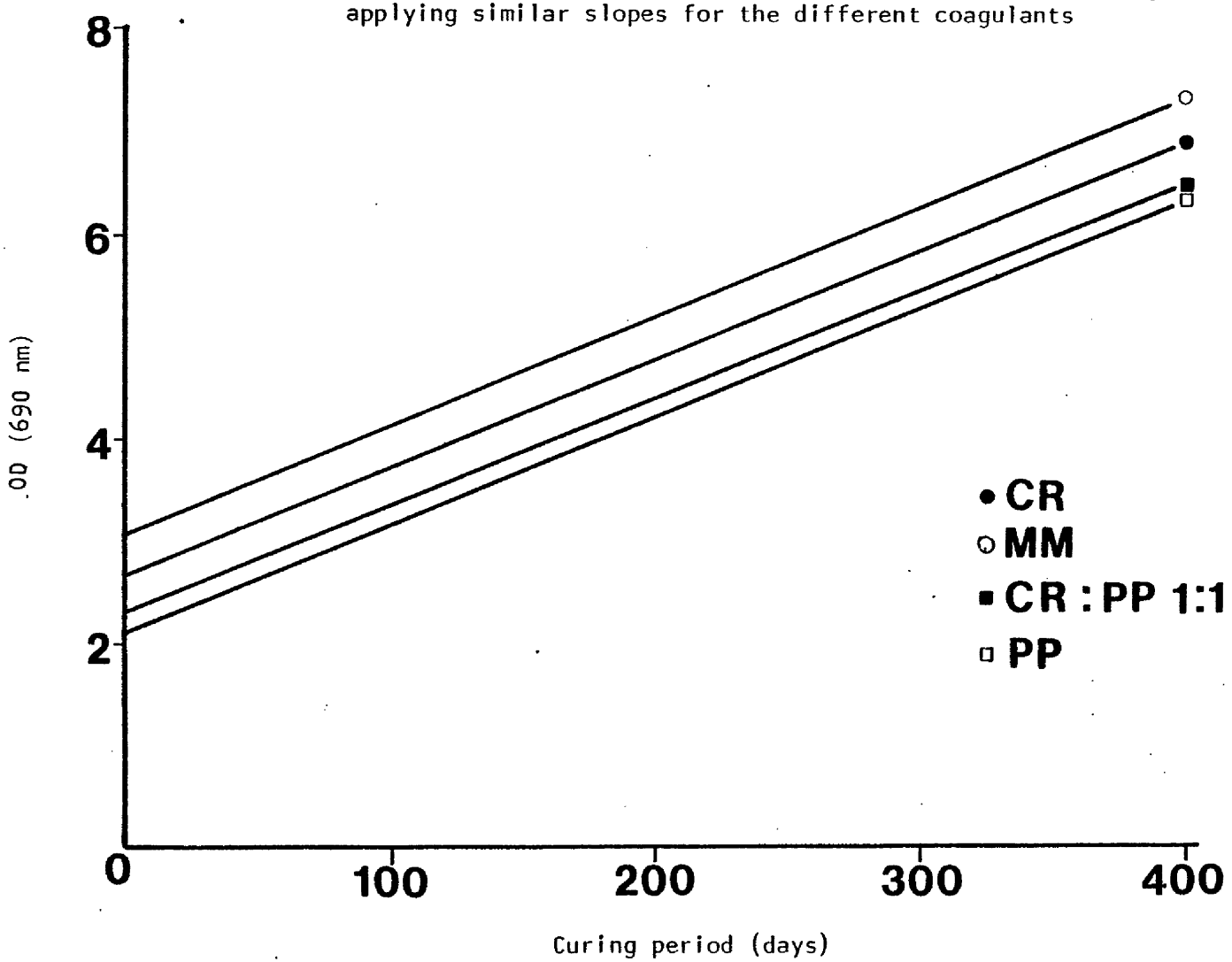
GRAPH 5:8

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of soluble nitrogen (calculated as protein and expressed in percentage of total nitrogen) and curing period, applying different slopes for the different coagulants



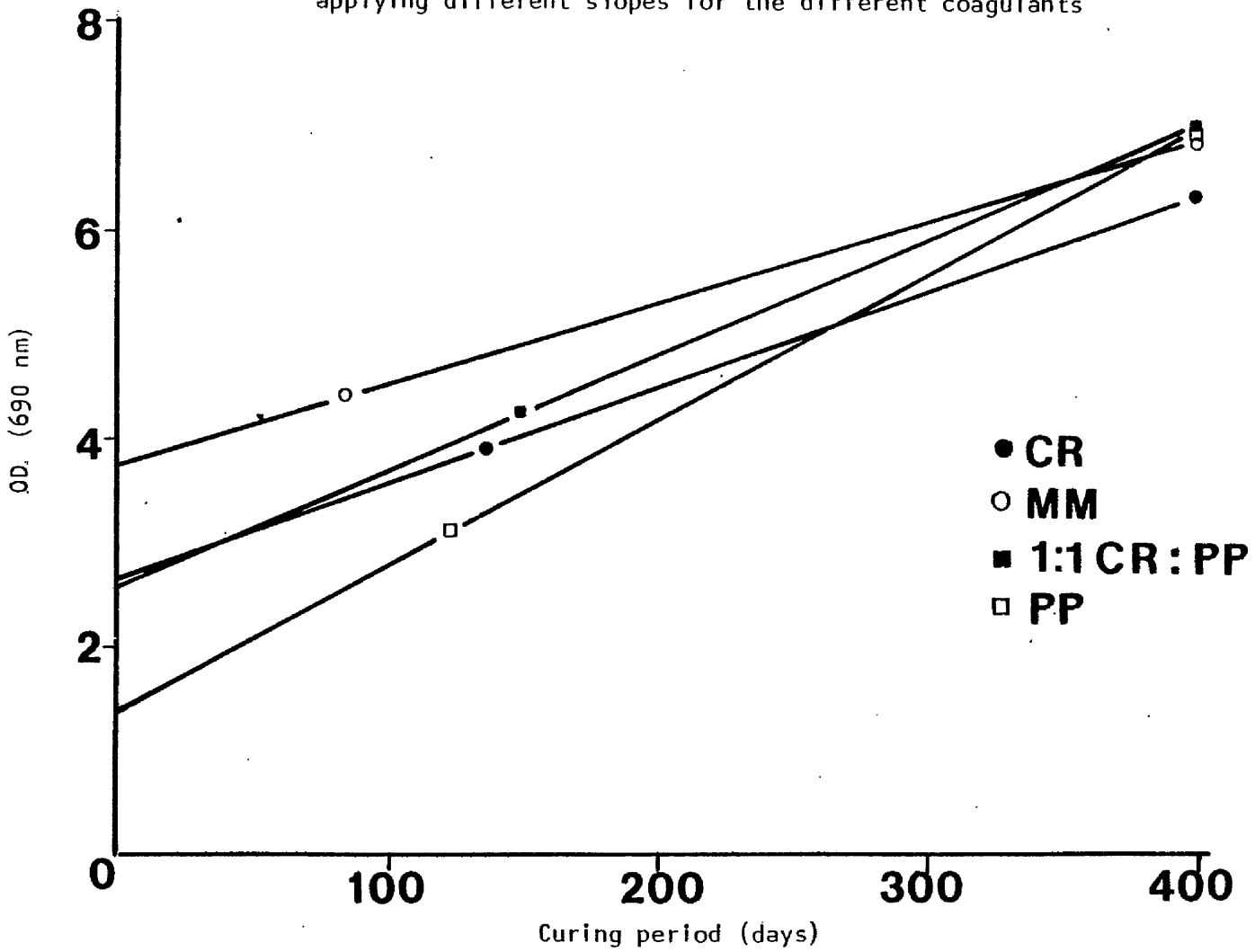
GRAPH 5:9

Standard curve for the relation between Optical Density (OD) of the extract filtrate of Cheddar cheese (made with different coagulants) and curing period applying similar slopes for the different coagulants



GRAPH 5:10

Standard curve for the relation between Optical Density (OD) of the extract filtrate of Cheddar cheese (made with different coagulants) and curing period applying different slopes for the different coagulants



calculated as a percentage of cheese fat and expressed as the per cent of free fatty acids (tables 5:5, 6, 7 and 8).

The regression analysis presented in table 5:13 indicated a strong linear relationship between the increase in the level of free fatty acids in the different cheeses and the curing period. The initial level of free fatty acids was higher in the cheese made with the 1:1 mixture of calf rennet and porcine pepsin (the difference is significant at 0.1 per cent level from calf rennet and at 1 per cent level from Mucor miehei rennet). The cheese made with porcine pepsin had the second highest level of free fatty acids but with no significant difference from other coagulants. Cheese made with calf rennet and Mucor miehei rennet showed similar levels of free fatty acids.

The level of free fatty acids in different cheese increased during curing by a different rate for each coagulant. The cheese made with calf rennet showed the highest rate of increase in free fatty acids. Cheese made with porcine pepsin gave the second most rapid increase in free fatty acid development, and the commercial 1:1 mixture of calf rennet and porcine pepsin the third most rapid. The cheese made with Mucor miehei rennet showed a very close rate of increase to that produced by the 1:1 mixture, but because of the different initial values, the overall picture for the increase in free fatty acids during curing indicates no significant differences between all the coagulants (graphs 5:11 and 12).

On calculating the correlation coefficients between the variants, age, optical density, soluble nitrogen in cheese, total nitrogen in cheese, soluble nitrogen as a percentage of total nitrogen, and the free fatty acids in cheese, highly significant correlations were obtained between most of them. Table 5:14 gives the correlation matrix of all the previous variants, and it indicates a highly significant correlation between age and all other variants except the total nitrogen in cheese. This significant correlation indicates a regular controlled change in the above variants during the curing period.

Furthermore, a significant correlation can be seen between the optical

TABLE 5:13

The regression analysis for the effect of curing period on the free fatty acids content (FFA)(expressed as a percentage of fat content) in Cheddar cheese made with different coagulants

	Estimate	S.E.	T
Y intercept CR	4.401916	0.406489	10.83
" MM	4.816031	0.405623	11.87
" (1:1)CR:PP	5.844035	0.393340	14.86
" PP	5.106770	0.404967	12.61
Slope CR	0.027009	0.001520	17.77
" MM	0.025455	0.001491	17.07
" (1:1)CR:PP	0.025208	0.001437	17.54
" PP	0.026229	0.001430	18.34

Analysis of variance

	DF	SS	MS
Regression	8	5541.89	692.7363
Residual	32	12.87	0.4023
Total	40	5554.77	138.8691
Change	0	-0.36	0

Percentage variance accounted for 99.7

TABLE 5:14

The correlation coefficient matrix for the effect of curing period on the contents of Cheddar cheese made with different coagulants, of percentage total nitrogen (TN), percentage soluble nitrogen (SN), optical density (OD), soluble nitrogen as a percentage of total nitrogen (SN/TN), and free fatty acids as a percentage of fat content

Curing period	1	1.0000					
OD	2	0.9006***	1.0000				
% SN	3	0.8714***	0.9442***	1.0000			
% TN	4	0.0138	0.0953	0.1042	1.0000		
% SN/TN	5	0.8720***	0.9316***	0.9835***	-0.0714	1.0000	
% FFA	6	0.9807***	0.8768***	0.8269***	-0.0241	0.8347***	1.0000
		1	2	3	4	5	6

DF = 38

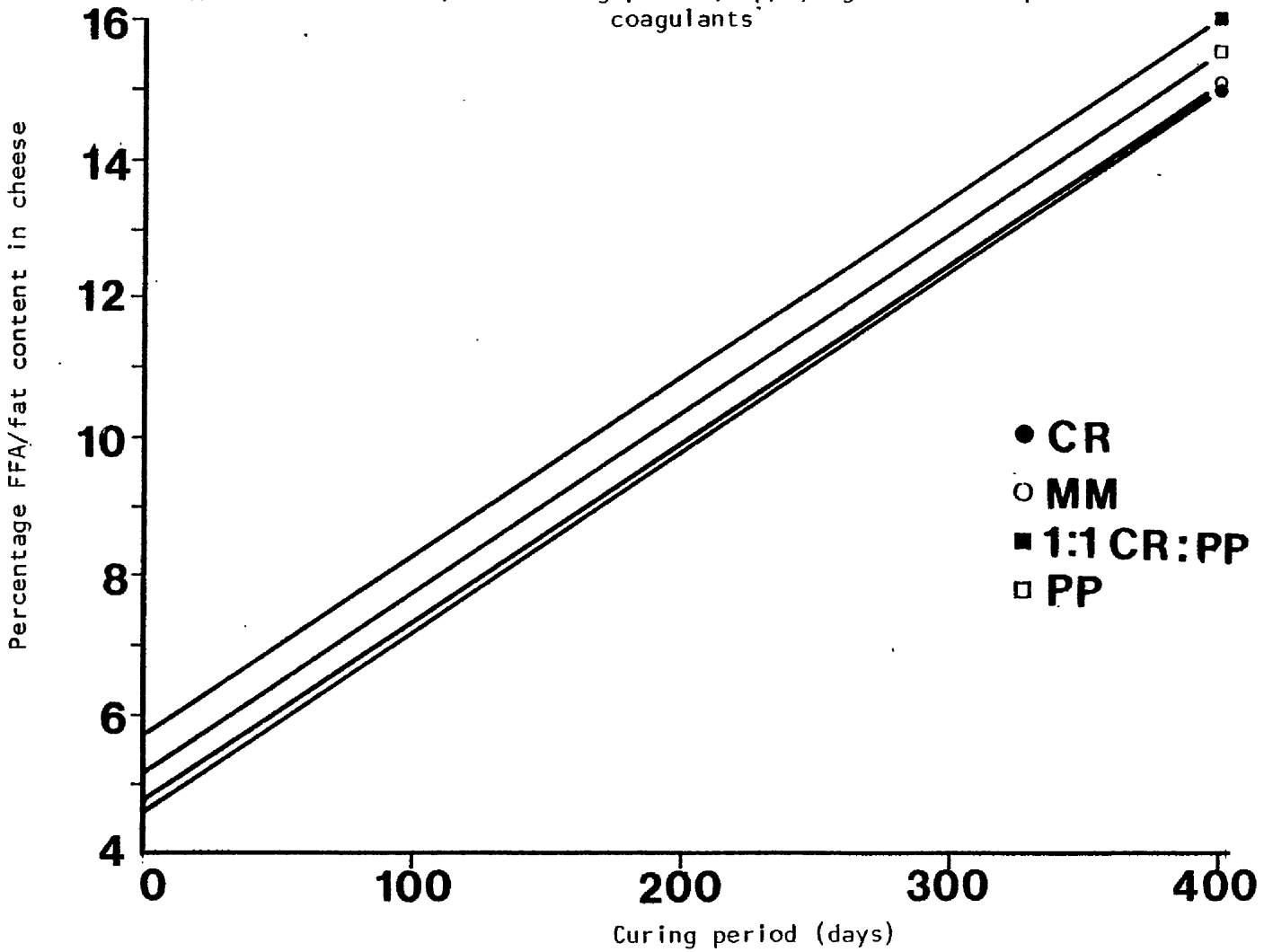
* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

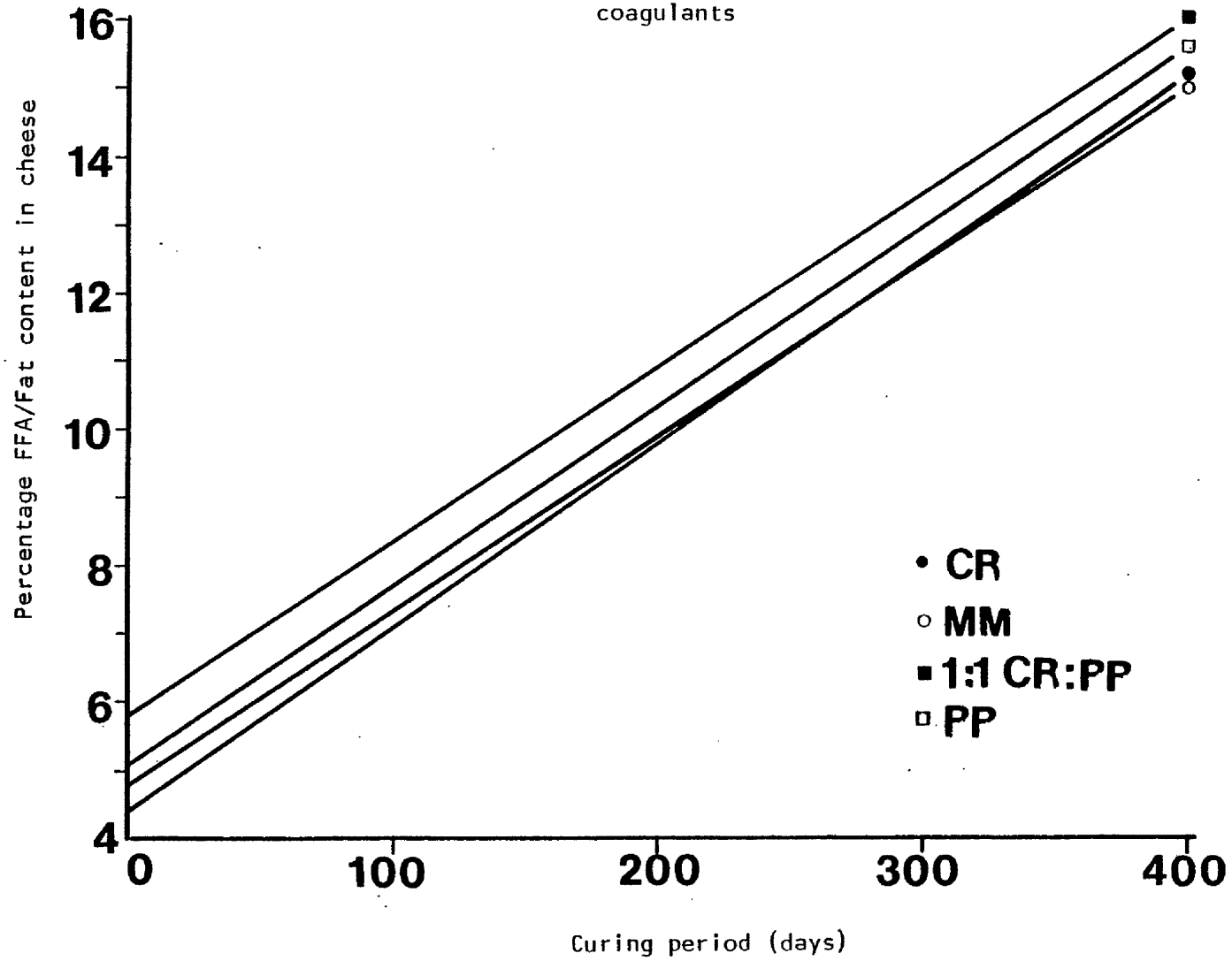
GRAPH 5:11

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of free fatty acids (expressed in percentage of cheese fat content) and curing period, applying similar slopes for the different coagulants



GRAPH 5:12

Standard curve for the relation between the content of Cheddar cheese (made with different coagulants) of free fatty acids (expressed in percentage of cheese fat content) and curing period, applying different slopes for the different coagulants



density and soluble nitrogen (expressed as a percentage of cheese or as a percentage of total nitrogen). A significant correlation can be seen between the content of free fatty acids and the previous variants - except total nitrogen. The importance of these correlations is discussed below.

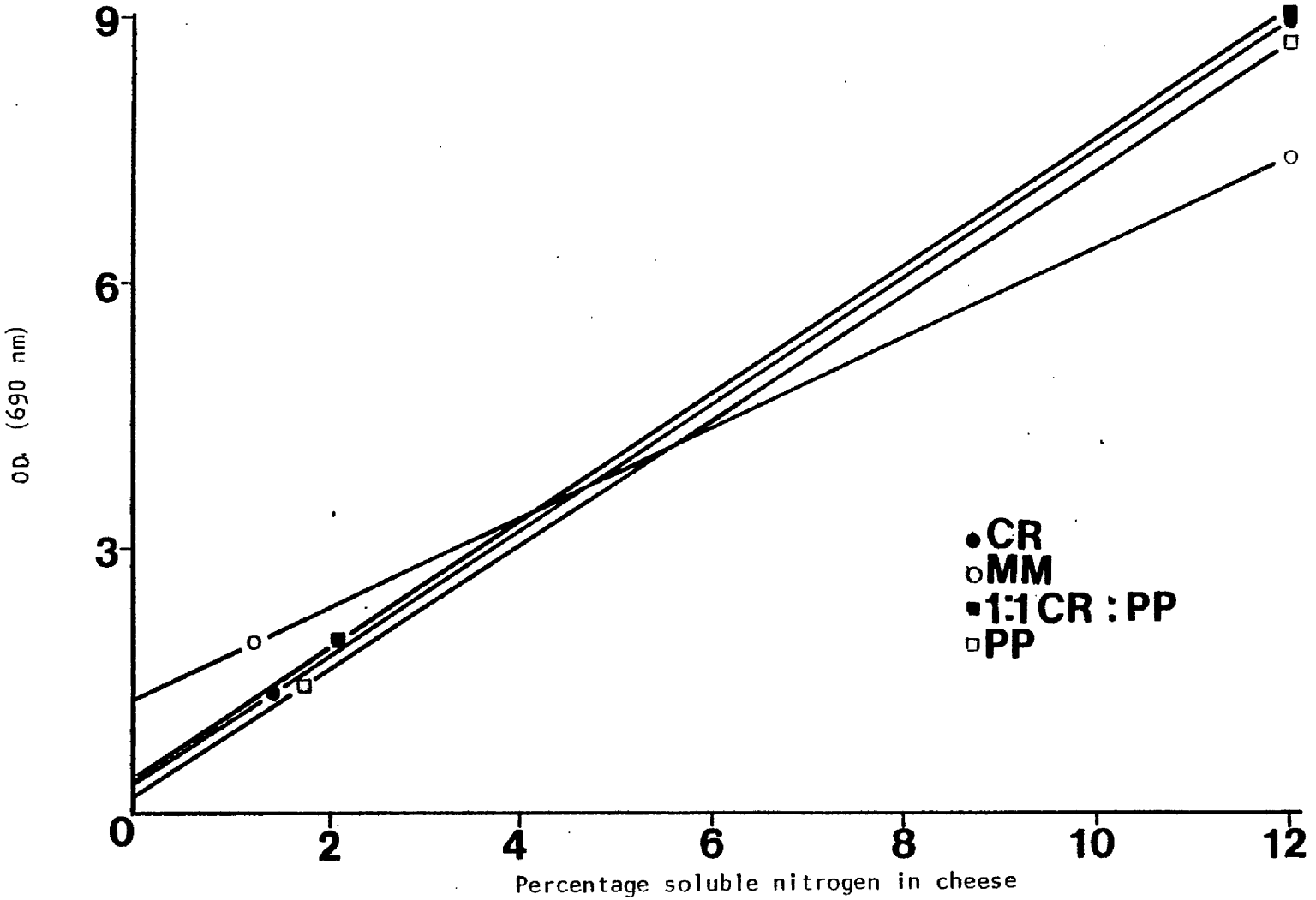
TABLE 5:15

The effect of using the mean values for the moisture content of Cheddar cheese after different periods of curing in the calculation of yield of Cheddar cheese made with different coagulants

Date	Coag.	Initial moisture	% Yield	% Yield (35% M)	Mean of moisture	% Yield (35% M)
7.6	MM	36.89	10.069	9.776	36.129	9.894
	1:1 CR:PP	36.89	10.346	10.045	36.375	10.127
14.6	CR	36.60	10.163	9.913	35.546	10.078
	PP	37.95	10.414	9.941	37.205	10.061
21.6	MM	34.36	9.998	10.096	34.245	10.114
	PP	35.79	9.913	9.559	35.362	9.858
28.6	CR	36.23	10.174	9.981	36.179	9.989
	1:1 CR:PP	35.43	10.177	10.109	36.181	9.992
5.7	CR	36.87	10.106	9.815	36.248	9.912
	MM	37.07	10.087	9.776	35.957	9.938
19.7	1:1 CR:PP	37.31	10.295	10.020	37.010	10.074
	PP	36.27	10.426	10.222	36.615	10.167
Mean of CR			9.903		9.993	
	MM		9.879		9.982	
	1:1 CR:PP		10.058		10.064	
	PP		9.907		10.029	
Overall mean			9.937		10.017	
Variance			0.370		0.112	

GRAPH 5:13

Standard curve for the relation between the Optical Density (OD) of Cheddar cheese extract filtrate and the content of Cheddar cheese (made with different coagulants) of soluble nitrogen (calculated as protein and expressed in percentage)



DISCUSSION

The variation in the moisture content of experimental cheese during curing could be caused by several factors such as sampling, salt diffusion, and the chemical and biological reactions in the cheese which all affect the balance between the amounts of free and bound water in the cheese.

In the experiments described above rindless cheese was produced in packaging materials, impermeable to air and water vapour. Under these conditions the humidity level of the atmosphere has no effect on the moisture content of the cheese.

The effect of sampling on the variation in the moisture content of cheese during curing was the factor of most influence although samples were taken initially from a different point (3 on each of 3 sides) of the cheese block and every care was taken to make all samples as representative as possible of the whole cheese block. The procedure which was adopted thereafter, of cutting the block into 4 pieces for more effective protection during curing, may have reduced the representative status of the samples.

Sutherland (1977) pointed out that wide variations in the moisture content of cheese could occur even in the 19 kg cheese block due to the uneven distribution of salt in cheese. Variation in the moisture content of cheese depends on the initial level of moisture in the cheese. Scott (1954) reported that bandaged cheese with a high moisture content will lose more of its moisture during curing than cheese with a lower moisture. In the case of the experiments described above, the moisture which came from the cheese remained in the package due to its impermeable properties and was removed prior to sampling.

The other important factor affecting the moisture variation is the salt diffusion, which starts immediately after salting of the milled curd, prior to filling the curd into the moulds. The optimum treatment at this stage is to allow the salt to come into contact with the curd and penetrate into the curd for a period of 20 to 40 minutes before filling the curd into the moulds (Al-Dahhan, 1977). This

was not possible because of the wish to avoid abnormal whey losses in the experiments carried out on the effect of coagulant type on yield. The curd was filled into the moulds soon after this addition of salt to the curd. This practice could cause insufficient distribution of salt within the cheese which might cause the variation in the moisture content at different points in the cheese. Small differences in the moisture content at different points of the cheese will not be dispersed even after 4 to 6 months ripening due to the very slow diffusion of salt (Sutherland, 1977).

The variation observed in the moisture content of cheese during curing could affect the calculation of yield (chapter four). In table 5:15 the mean value of moisture for the whole period of curing for cheese made with each coagulant was calculated and the yield was also recalculated on the basis of 35 per cent moisture. The results showed that, when this was done, the differences between the cheese yield produced with different coagulants became much less than it was, and the variance was also reduced.

The variation in the pH values of different cheeses during curing was found to be different from that of the moisture content. Because the pH values indicate the activity of the starter cutlture, the alteration in pH will be dependent on cultures, enzymes and the amount of lactose in the cheese.

During the early stage of curing of Cheddar cheese (i.e. up to 2 months) the acidity increase due to the production of lactic acid by starter culture as well as to the production of other acids such as propionic and acetic acid by non starter organisms.

During the following 3 to 4 months of curing, the action of enzymes, such as proteinases released from the cells of starter culture, and other proteolytic enzymes present in the cheese including those from the coagulants, result in the production of different compounds such as peptides, aldehydes, ketones, and volatile flavour compounds such as methanethiol, butanone, acetone, methanol, ethanol, and 2-pentanone. The concentration of the above compounds increase during curing (Manning, 1978), and change the buffering system in

the cheese, resulting in a reduction in the acidity, and causing the pH level to increase to higher values i.e. 5.6 (Kosikowski, 1977). The high level of acidity obtained during cheese curing affects the quality of the cheese, especially of that containing a high amount of moisture.

Lawrence and Gilles (1969) concluded that bitterness in cheese was related to the pH level when the cheese was 14 days old within certain salt:moisture limits. They reported that bitterness was likely to be formed when the ratio of salt:moisture (as per cent) is less than 4.30 and also between 4.3 and 4.9 when the pH at 14 days was less than 4.95. This was the case in the cheese made using Mucor miehei rennet in one vat and a 1:1 mixture of calf rennet and porcine pepsin in the other after the first week of curing (table 5:3). Both cheeses had a ratio of salt to moisture between 4.3 and 4.9 and a pH value of lower than 4.95. Bitterness was detected in both cheeses after 3 months ripening. This point is discussed in chapter 6.

The difference in the cheese pH because of using different coagulants was found to be significant and all the variance was found to be from the cheese made with Mucor miehei rennet, which had a lower pH than the other cheeses. The reason for the low pH could be because of the different products formed during curing having different buffering capacities and resulting in the low pH value.

In the experiments examining the effect of different coagulants on the pattern and extent of proteolysis during curing, many results were found in agreement with the results of other workers. The total nitrogen levels were found to be similar during curing and the slight differences found in different cheeses could be due to differences in the moisture content of cheeses.

The measurement of soluble nitrogen increase in the cheese as an indication of proteolysis is an acceptable method and has been used in other work (Linklater and Ernstrom, 1961; Ajaib Singh et.al., 1967; Scott, 1967; Puhan and Irvine, 1973; and O'Keeffe et. al., 1976). The results found in this work indicated that porcine pepsin

produced Cheddar cheese with a low initial level of soluble nitrogen, but the high rate of soluble nitrogen production during curing caused the cheese,made with this coagulant to have the second highest level of soluble nitrogen after 9 months of curing.

The cheese made with Mucor miehei rennet contained the highest level of soluble nitrogen at the same age. This finding may be related to the break down of casein in milk clotting by porcine pepsin to medium size peptides which are insoluble at pH 4.4 and which would not be determined as soluble nitrogen. This is probably the cause of the low initial level of soluble nitrogen. After a short period of curing, during which the action of the enzymes from starter culture broke down the peptides to amino acids and smaller peptides, there was a rapid increase in the levels of soluble nitrogen.

Green and Foster (1974) reported that porcine pepsin gave a slower rate of proteolysis than calf rennet. This view was not confirmed by the results obtained by the author.

The 1:1 mixture of calf rennet and porcine pepsin was intermediate in its rate of proteolysis compared with the individual coagulants and was closer to calf rennet and gave approximately the same initial level of soluble nitrogen in cheese and the same rate of increase. The results agreed with those of Babel (1967).

Cheese made with Mucor miehei rennet contained high initial levels of soluble nitrogen indicating that on milk clotting, this coagulant breaks down casein to smaller peptides to a greater extent than the other three coagulants. The rate of increase of soluble nitrogen in cheese made with Mucor miehei rennet during curing was higher than with other coagulants except porcine pepsin.

The variation in the initial levels of soluble nitrogen and the rate of increase during ripening was not significant, and even for cheese made with porcine pepsin or Mucor miehei rennet, similar levels of soluble nitrogen may be anticipated after 6 to 9 months ripening, which is the period used for the production of 'mature' Cheddar cheese.

The spectrophotometric method of Vakaleris and Price (1958) was used

to measure the increase in the level of soluble nitrogen by measuring the optical density of the sodium citrate-hydrochloric acid extract of the cheese. This technique which involves colouring the cheese extract Miller's (1959) modification of Lowry et. al. (1951) method for protein determination gave slightly different results from the micro-Kjeldhal method for measuring soluble nitrogen. This difference was not from lack of correlation between the two methods, which in fact was highly significant as is shown in tables 5:5, 6, 7 and 8, but was due to differences in the intensity of the colour produced by different cheese extracts.

Mucor miehei rennet was the only coagulant which gave a lower colour intensity (OD) for a particular value of soluble nitrogen (graph 5:13). This result can be explained if we suppose that the Cheddar cheese made with different coagulants had the same type but different quantities of free amino acids (Wong et. al., 1977).

It could be, that different amino acids give different optical densities, so the cheese made with Mucor miehei rennet may have contained higher proportions of certain amino acids which gave less colour intensity than others. In the case of each of the other three coagulants, it is clear from graph 5:13 that the same free amino acids were produced in the cheese in the same proportions. These results indicate a similar pattern of protein breakdown to that reported by Green and Foster (1974).

The extent of lipolysis during cheese curing will depend, in the first place, on the condition of the fat in the milk before cheese production.

Fat exists in milk as an aqueous suspension of globules which consist of a central core of fat surrounded by a lipoprotein membrane (Scott, 1967).

The fat globule membrane may be ruptured by mechanical action such as agitation or during heat treatment, and some micro-organisms can produce enzymes which rupture the membrane. The breakdown of the fat globule membrane allows the fat to leak out and become attacked by bacterial lipases formed in the milk by psychrotrophic bacteria

(Scott, 1972).

Before milk is made into cheese it may contain some free fatty acids. At the end of cheese making, some bacterial lipases may be present in the cheese as the result of the growth of contaminant bacteria and will attack the fat,producing more free fatty acids. The third source of free fatty acids produced in cheese is the coagulant which may contain lipases (Davis, 1965), especially crude or unpurified preparations.

Free fatty acids are important in the development of cheese flavour as they are converted into different methyl, ketones, lactones, and other volatile compounds (Scott, 1967 and Kosikowski, 1977). The rate of production of free fatty acids is also important, and should be close to the rate of proteolysis, to produce within a certain period of cheese ripening mature cheese with full flavour and good texture. Variation in the rates and patterns of proteolysis and lipolysis during ripening, could lead to differences in flavour and texture (Green and Stackpoole, 1975).

From the results obtained, it was observed that the rate of production of free fatty acids in cheese made with different coagulants was similar, but the initial levels 1 day after production varied. This finding agreed with the conclusion of Wong et. al. (1977). The differences in the initial levels of free fatty acids in cheese produced with different coagulants (incomplete block design experiments) could only have come from contamination of the milk or from the coagulant. The significant correlations obtained between the level of free fatty acids and the soluble nitrogen indicate a similar progress in proteolysis and lipolysis in cheese during curing.

CONCLUSION

Variations found in the moisture content of cheese during curing arise because of errors from sampling. The type of coagulant did not affect the level of moisture found in cheese during curing.

The period of curing has a great effect on cheese pH. The type of coagulant had no effect on the initial value of cheese pH, but affected the pattern of pH change during curing.

The use of different coagulants in cheese making caused differences in the initial levels of soluble nitrogen as well as differences in the rate of increase of soluble nitrogen during curing. This effect of a particular coagulant, in addition to its effect on pH change during curing, may cause the production of different flavour compounds and may lead to differences in the texture of cheese.

The length of the curing period affects the extent of fat lipolysis , less than it does,casein proteolysis. There was no relation between fat lipolysis and any of four different coagulants used in cheese experiments.

CHAPTER SIX

ELECTROPHORETIC STUDY OF THE ROLE OF COAGULANTS IN CHEDDAR CHEESE RIPENING

INTRODUCTION

The effect of coagulant type used in making Cheddar cheese on the extent of casein proteolysis during ripening was investigated in the previous chapter by measuring the rate of soluble nitrogen increase in the cheese as an indication of the progress of proteolysis. Such measurement does not indicate the casein fraction involved in the proteolysis, and the use of another method which allows the tracing of changes which might occur in the individual casein fractions was necessary.

Casein which is the major protein in cheese, consists of different groups in the following proportions - α casein (40 per cent of the total casein), β casein (35 per cent), K casein which represents 15 per cent of the total casein, and the minor caseins which make the remaining 10 per cent of the total casein (McKenzie, 1970). Milk clotting breaks down the casein micelles causing 50 per cent of the K casein to hydrolyse to para-K-caseinate and macropeptide fraction. Most of the macropeptides formed during the ripening stage, due to rennet action, will be lost in the whey. The fate of the remaining macropeptide in the cheese is unknown (O'Keeffe et. al., 1977). The breakdown of casein micelles will cause the loss of the protective layer of casein micelles and makes the other casein fractions susceptible to the attack of proteolytic enzymes. Casein fraction proteolysis after clotting is controlled by several factors:- (1) the activity of enzymes from milk, starter culture, and coagulants (2) cheese conditions i.e., pH, water activity (a_w), and solute concentration, and (3) by the nature of the casein fractions and their susceptibility to proteolysis.

To determine the nature of the casein fractions, and to see the effect of the coagulants on each fraction, polyacrylamide gel electrophoresis (PAGE-method) was used to follow the changes in casein fractions during cheese ripening. The procedure can be defined as a high resolution method for molecule fractionation which separates molecules on the basis

of their size, conformation and net charges (Chrambach and Radbard, 1971). In this method, charged molecules (casein fractions) are forced through a porous gel by an electric field generated in a buffer which will permeate the gel and make the molecules separate according to their electrophoretic mobilities (Bio-Rad Laboratories, 1979). Variations in the gel and buffer make it possible to separate molecules different in molecular weights, isolectric points, or in the biospecific affinity even if they have the same net charge (McKenzie, 1970).

Compositional differences between casein fractions cause differences in the properties of compounds formed from each fraction during cheese ripening, and casein variants in the cheese will be present in different ratios to which they are in milk. Marcos et. al. (1979) gave the following figures for the proportions of casein fractions in Cheddar cheese:- pre αs casein 24 per cent, αs casein 20 per cent, β casein 11 per cent, γ_1 casein 21 per cent, γ_2 casein 8 per cent, and γ_3 casein 16 per cent. The different electrophoretic patterns of milk and cheese are shown in figure (6:12).

αs casein in cheese is found to be degraded more easily and extensively than β casein, Visser and de Groot-Mastert (1977) reporting that nearly all α_s casein was degraded after one month's ripening while 50 per cent of the β casein was still intact after 6 months ripening. Ledford et. al., (1966) found no differences in the intensity of β casein and K casein after one month's ripening. O'Keeffe et. al., (1977) reported that β casein was degraded to γ casein only in very mature cheese by the effect of milk proteinase. Similar results were reported by Marcus et. al., (1979) when he proved that γ_1, γ_2, and γ_3 caseins are residues from β casein. That different electrophoretic patterns of cheese are a function of using different coagulants and proteolysis during ripening was reported by Webb et. al., (1974), Green and Faster (1974), Creamer and Richardson (1974), El -Shibiny and Abd-El- Salam (1977), and O'Keeffe et. al., (1977). Flavour formation during cheese ripening is affected by the extent of degradation of specific casein groups, and the different rate and amount of proteolysis may have no affect on flavour formation (Phelan et. al., 1973), but the type of products formed have the most important effect.

The work of this chapter was aimed at studying cheese ripening using the PAGE method, in an attempt to determine the exact effect of using different coagulants in cheese making on the extent of proteolysis and on the quality of Cheddar cheese.

EXPERIMENTAL

All Cheddar cheese made in the course of studies described earlier in this thesis were analysed for their electrophoretic patterns using the PAGE method described above (Chapter one, Section 5:8).

The resultant gels, after destaining, were photographed and then scanned as described in the method. The elution diagrams plotted during scanning were used to calculate the area occupied by the specific casein fractions which were then expressed as a percentage of the whole casein. The extent of proteolysis during ripening was followed by examining the changes observed in such casein fraction from the beginning of ripening until the cheese was 9 to 15 months old.

The relative position of each fraction in the electrophoretic patterns was used to identify the fraction using for identification purposes of the work of McKenzie (1970), McKenzie (1971), Emmons et. al., (1976), and Marcos et. al. (1979).

RESULTS

The different electrophoretic patterns of cheese made with different coagulants and changes which occurred in the casein fractions during ripening are shown in plates 2 to 5 which correspond to the elution diagrams in plates 6 to 9.

The area of the different peaks found in the elution diagrams of each gel was determined by measuring the height of the peak and its width at the middle of the height, and multiplying the two figures.

$$\text{area } (mm^2) = \tfrac{1}{2} \text{ width (mm)} \times \text{height (mm)}.$$

The position of the peaks was found by measuring the distance between the centre of the peak and the original slot. Because of differences in gel length, the peaks of the same casein fractions were found to occupy different positions in different gels. This was overcome by fixing the position of the peak of β casein at 50 and calculating the position of other peaks on that basis. The other difference noticed was in the intensity of gel staining which resulted in large variations in the area of the peaks, and this was overcome by calculating the area of each peak as a percentage of the total area of all peaks in each gel.

The 35 gels which were scanned contained 143 separate electrophoretic patterns divided as follows:- 25 patterns from Cheddar cheese made with calf rennet, 35 patterns from Cheddar cheese made with Mucur miehei rennet ('Hannilase' brand), 27 patterns from Cheddar cheese made with porcine pepsin, 32 patterns from Cheddar cheese made with a 1:1 mixture of calf rennet and porcine pepsin, and 24 patterns of casein standard solution.

To study the relationship between the different peaks of the electrophoretic patterns, and to find the possible changes which might have happened to each casein fraction during ripening, casein fractions were divided into two groups on the base of their electrophoretic mobility.

PLATE 2

The electrophoretic pattern of Cheddar cheese made with calf rennet, after different periods of curing

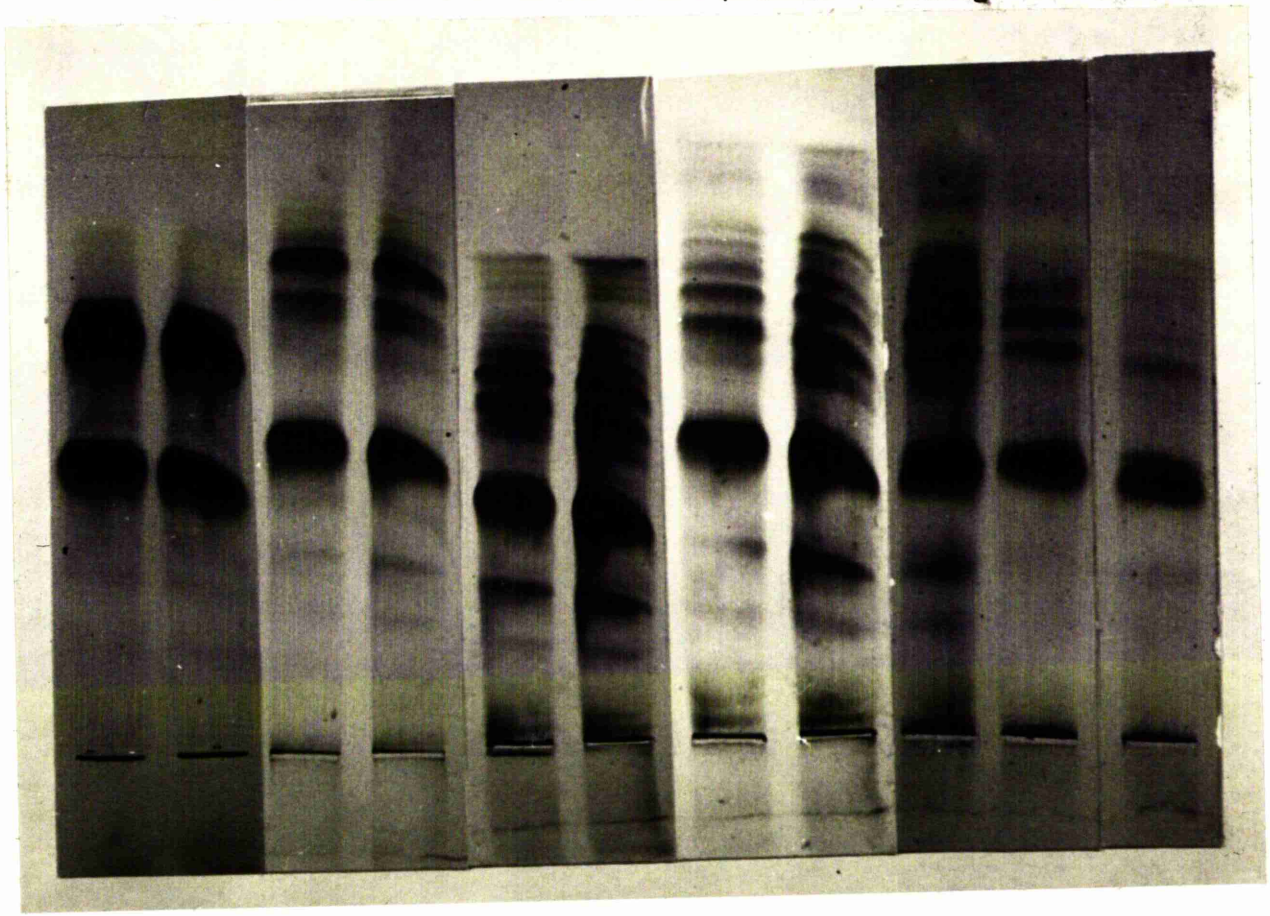


PLATE 3

The electrophoretic pattern of Cheddar cheese made with *Mucor miehei* rennet, after different periods of curing

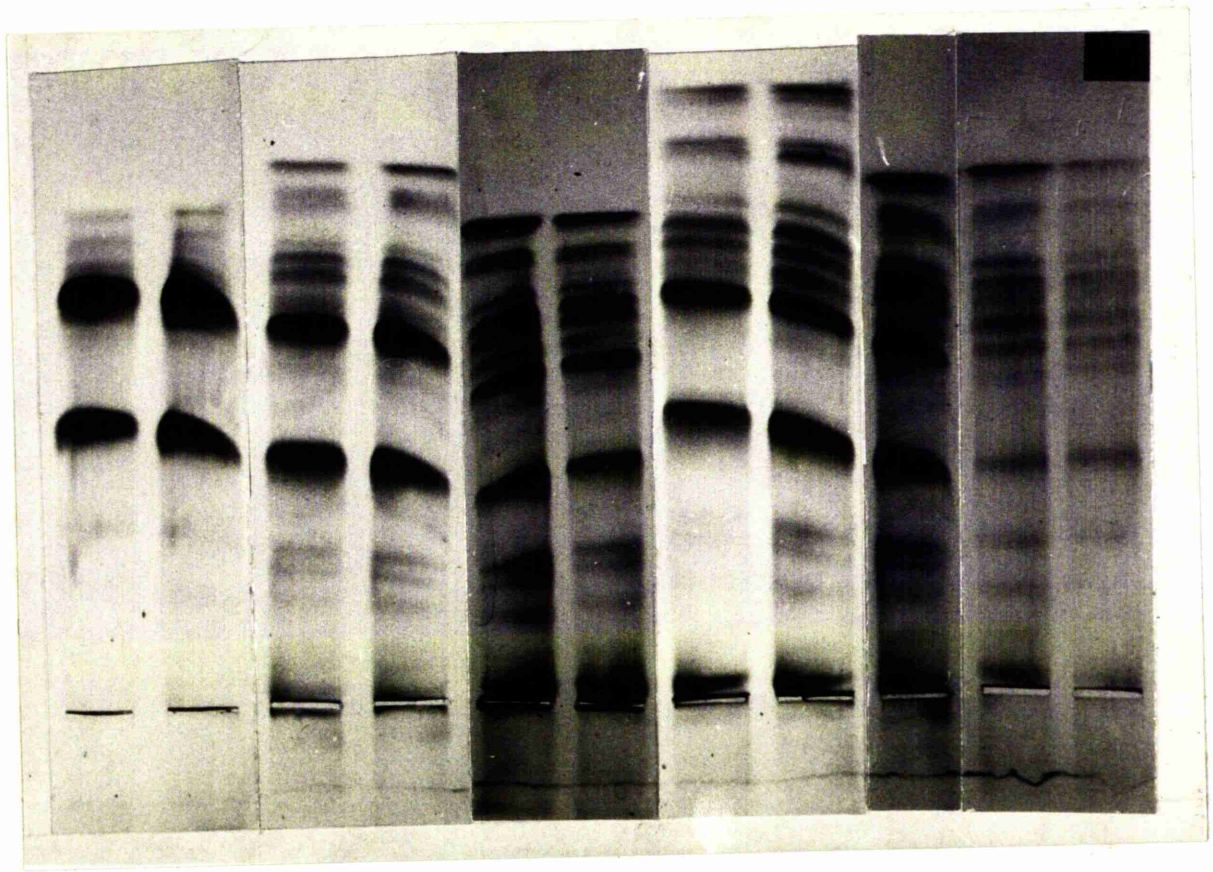


PLATE 4

The electrophoretic pattern of Cheddar cheese made with a 1:1 mixture of calf rennet and porcine pepsin, after different periods of curing

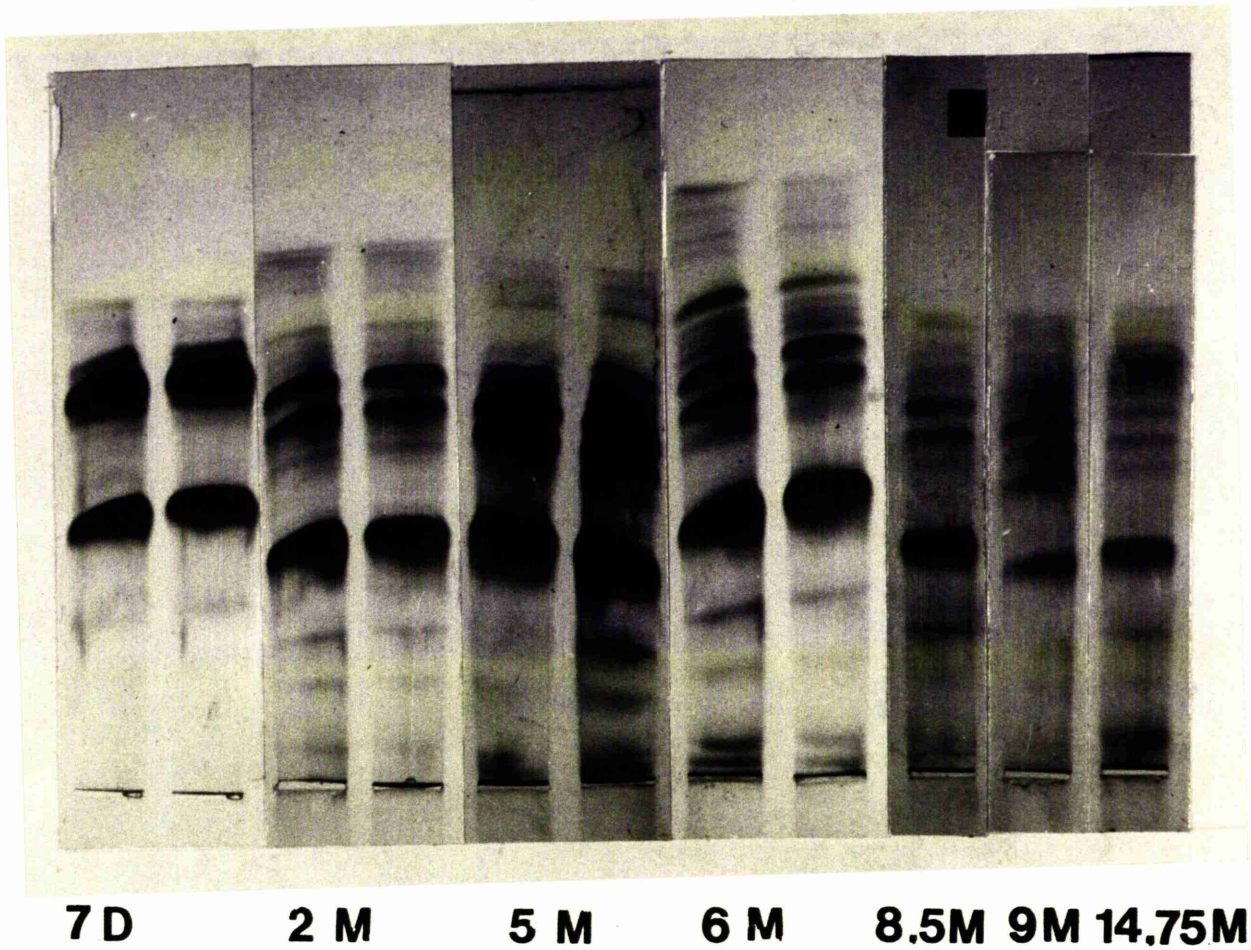
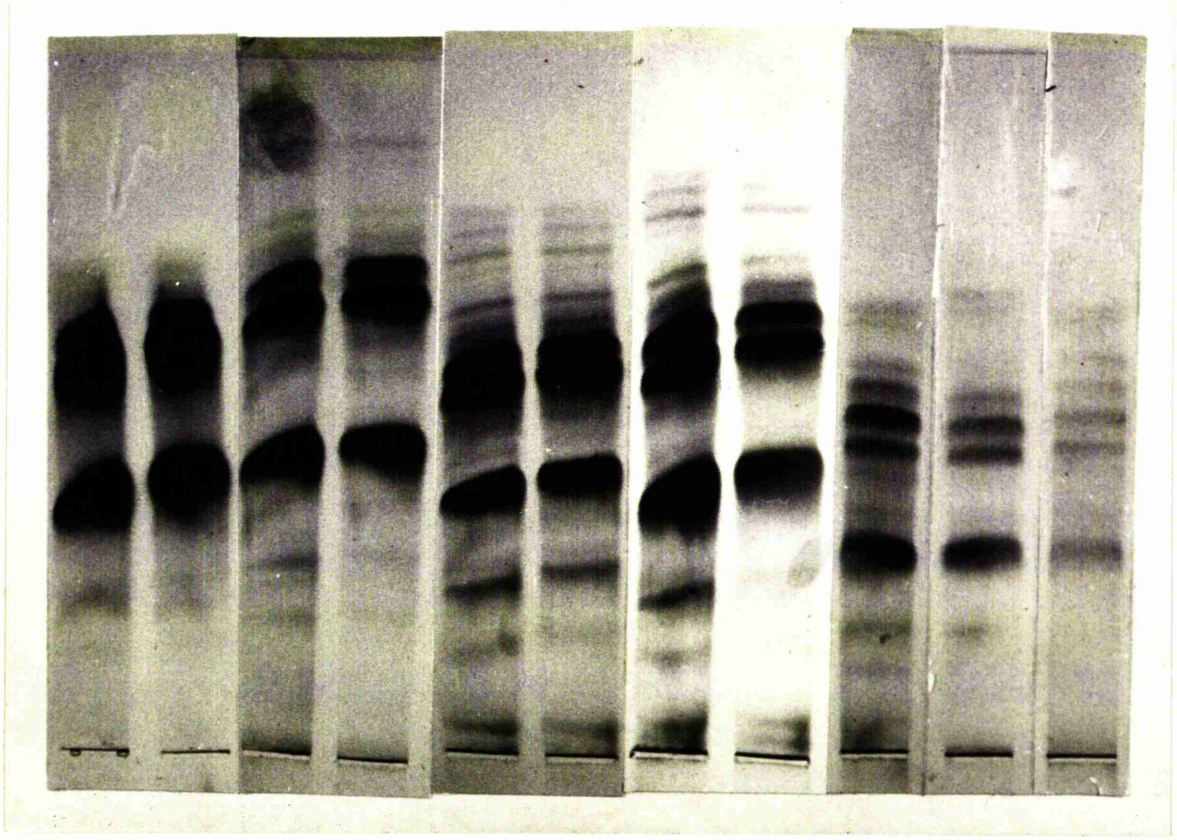


PLATE 5

The electrophoretic pattern of Cheddar cheese made with porcine pepsin, after different periods of curing



5D 2 M 5 M 6M 7M 9M 15M

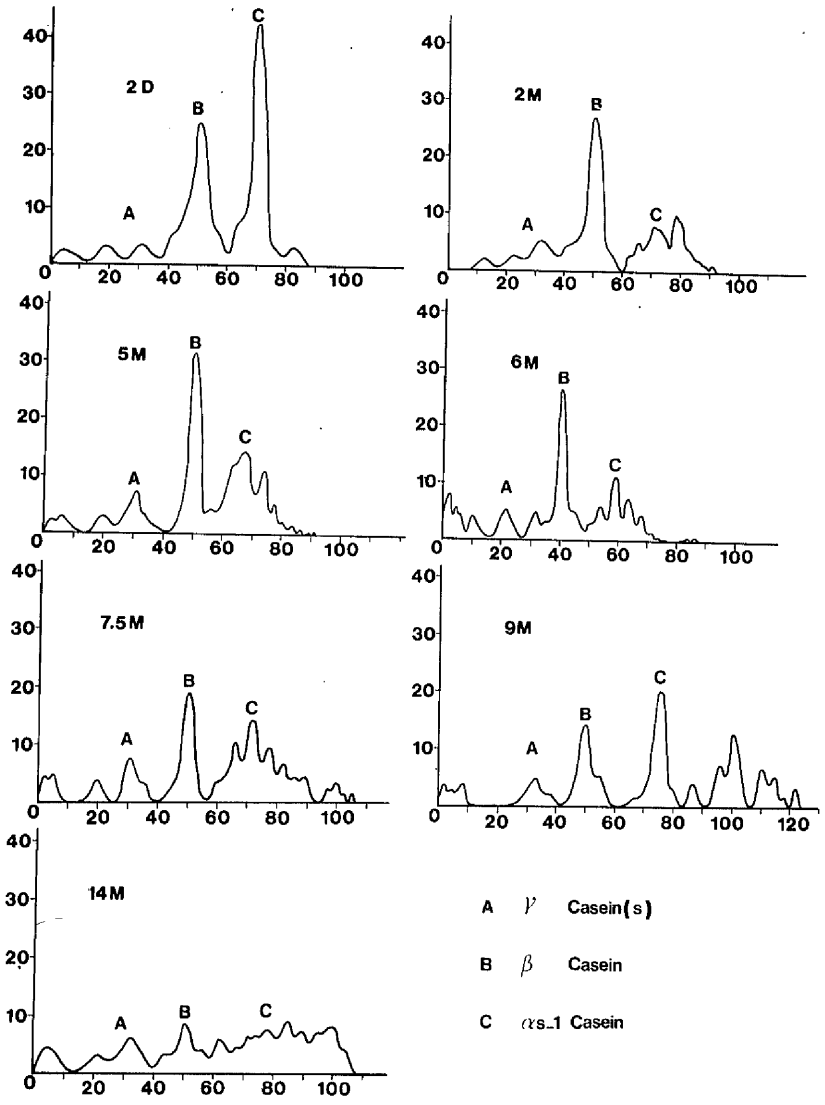


PLATE 6

The Elution diagrams of the electrophoretic patterns of Cheddar cheese made with calf rennet, after different periods of curing

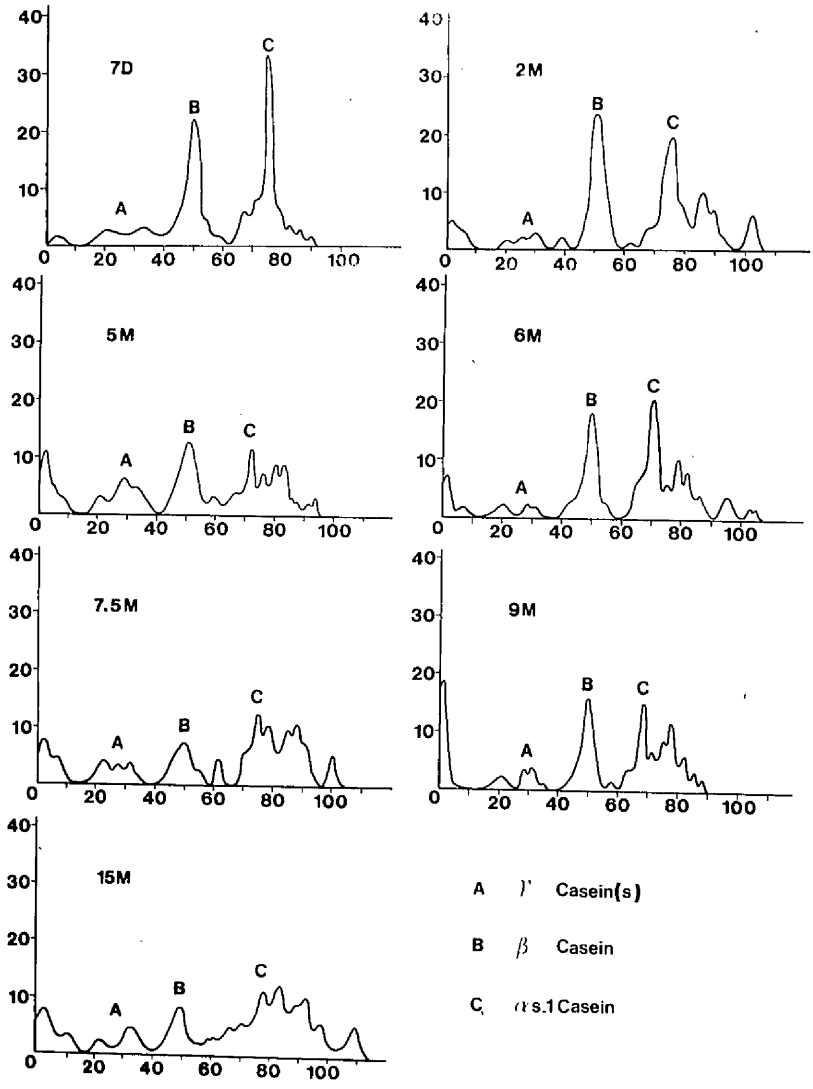


PLATE 7

The Elution diagrams of the electrophoretic patterns of Cheddar cheese made with Mucor miehei rennet, after different periods of curing

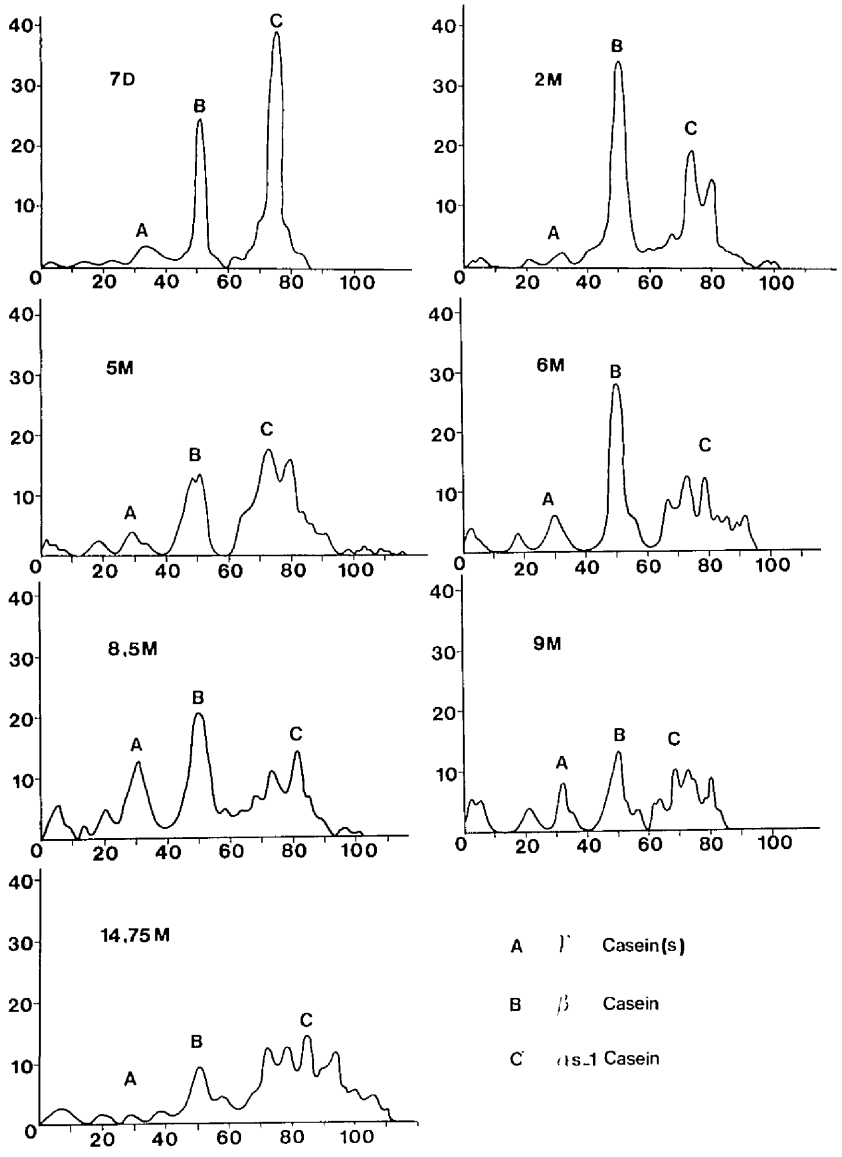


PLATE 8

The Elution diagrams of the electrophoretic patterns of Cheddar cheese made with a 1:1 mixture of calf rennet and porcine pepsin, after different periods of curing

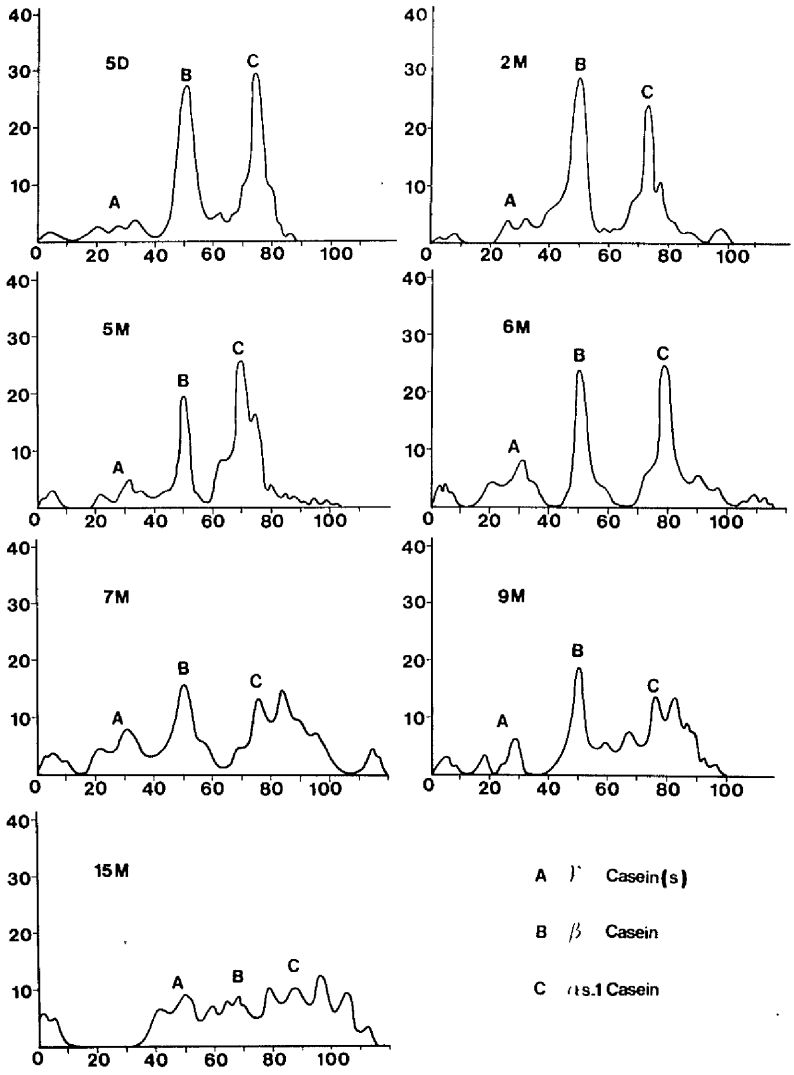


PLATE 9

The Elution diagrams of the electrophoretic patterns of Cheddar cheese made with porcine pepsin, after different periods of curing

Group 1 - which contains the slow mobility casein fractions and falls between the original slot and β casein are included in the group, the group contains the following casein fractions:-

(1) casein fractions which remained very close to the original slot and whose position on the gel never altered during the ripening period, because of their extremely slow electrophoretic mobility or their large molecular weight. The fractions probably belong to K casein and consist mainly of para-K-caseinate and what is left from K casein after clotting. Peaks in this position will be referred to as K-fraction throughout the study.

(2) a casein fraction which had a slightly faster mobility than the K fraction or had a lower molecular weight which allowed its movement to a position above the K fraction and below the γ casein fractions. This fraction may consist of a minor casein designated T_s (temperature sensitive casein) (McKenzie, 1971). Although it is possible that this fraction could be R or S casein and not T_s casein, the opinion of Emmons et. al. (1976) is taken and this fraction will be referred to as T_s casein.

(3) γ casein fractions which were noticed as three separate bands between T_s casein and β casein.

(4) β casein fractions, which were seen as three or four bands, the most intense being referred to as fraction 2, was in the middle of the β casein fractions and had one fraction below it termed fraction 1 and the two other fractions above it being referred to as fractions 3 and 4.

Group 2 - which contains the fast mobility casein fractions appears between α_s casein and the top of the gel. The group comprises:-

(1) α_s casein fractions 1 and 2, which were noticed to have slower electrophoretic mobility than αs_1 casein and occupied positions below αs_1 casein.

(2) αs_1 casein fraction which was the largest band in all the gels and was termed fraction 3 in the α casein complex.

(3) αs casein fractions which were noticed to have faster electrophoretic mobility than αs_1 casein and consisted of four fractions termed fractions 4, 5, 6, and 7.

(4) αs casein fractions which were noticed to have the fastest electrophoretic mobility of all fractions and occupied the very top positions in all gels, and consisted of two or three fractions which were numbered 8, 9, and 10.

The results revealed differences between different coagulants in their electrophoretic patterns. The differences were in the initial area of some of the casein fractions as well as in the rate of their degradation during ripening.

To see the differences between coagulants, it is necessary to study the results of each casein fraction individually.

K-fraction

Bands noticed in this region were very close to the original slot during the whole period of ripening and did not move within the gel more than 5 mm from the slot (in a gel of 110 mm length). The area of this fraction at the beginning of the ripening was between 0.8 to 1.5 per cent of the total band area of all the cheeses except in the case of the cheese made with Mucor miehei rennet which did not have this fraction until after 35 days of ripening. During ripening the K fraction showed a significantly slight increase (significant at 0.1 per cent level). The rate of increase in the fraction was similar in calf rennet and in Mucor miehei rennet cheese but slower with the other two coagulants, with no significant difference between all coagulants. The mean value for the K fraction was about the same in all coagulants except with Mucor miehei rennet which showed about doublt the value of other coagulants. The results are given in Tables 6:1, 2, 3 and 4, and graphs 6:1, 2, 3 and 4.

T_s casein

As in the case of K casein this fraction of casein was very close to the original slot which means it had little electrophoretic mobility.

TABLE 6:1

Area of individual casein fractions of group 1 (slow mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with calf rennet, after various periods of curing

Cheese curing period (days)	K casein	T_s casein	γ casein			β casein			
			1	2	3	1	2	3	4
2	0.7	-	0.3	-	-	4.9	32.8	-	-
5	0.8	-	2.4	-	-	5.0	32.7	-	-
60	2.0	-	3.2	5.2	-	4.4	25.5	4.7	1.8
120	2.2	3.2	4.5	11.0	-	-	21.2	-	-
150	2.9	2.6	2.9	6.4	3.3	3.2	20.6	2.2	1.8
180	3.6	6.2	2.4	6.9	3.3	-	19.9	3.0	2.9
270	6.1	8.5	1.9	14.1	5.0	1.6	19.8	3.2	-

Mean 112.429	2.614	2.929	10.400	30.171
Cor.coeff.	0.974***	0.955***	0.941***	- 0.811***
Y intercept	0.532	-0.758	2.519	36.635
Slope	0.019	0.033	0.070	- 0.057
SE of slope	0.002	0.005	0.011	0.019

Cor. coeff.	- 0.886 ++
Y intercept	38.947
Slope	- 0.844
SE of slope	0.197

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 6:2

Area of individual casein fractions of group 1 (slow mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with Mucor miehei rennet ('Hannilase' brand), after various periods of curing

Cheese curing period (days)	K casein	T_s casein	γ casein			β casein			
			1	2	3	1	2	3	3
5	-	1.0	2.3	-	4.9	-	26.6	9.4	-
10	-	1.6	2.6	3.7	4.0	-	20.1	4.2	2.8
35	1.8	1.7	2.1	2.1	4.4	2.5	21.1	3.2	-
60	5.1	4.6	2.2	3.2	3.5	4.1	21.0	-	-
70	4.4	4.6	2.2	3.4	3.3	3.5	18.1	3.2	1.2
90	5.5	4.8	4.2	3.3	3.0	3.6	13.3	-	-
150	12.9	5.3	3.9	3.5	5.7	3.4	11.4	-	-
180	7.6	4.0	3.5	3.2	4.7	3.6	10.0	3.6	-
210	7.0	4.6	4.6	3.8	4.4	4.2	8.0	3.8	-
270	5.8	5.1	4.1	3.3	3.8	3.7	9.5	3.3	-
340	10.5	4.4	3.6	3.4	3.8	1.5	7.6	2.9	3.0
450	7.0	3.1	2.7	5.7	4.8	3.4	7.4	3.2	3.0

Mean 155.833	5.633	3.733	10.575		21.200
Cor. coeff.	0.620*	0.379	0.713**		- 0.720**
Y intercept	2.973	3.101	9.058		26.617
Slope	0.017	0.004	0.010		- 0.035
SE of slope	0.007	0.003	0.003		0.011
Cor. coeff.				- 0.865***	
Y intercept				15.760	
Slope				- 0.245	
SE of slope				0.045	

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 6:3

Area of individual casein fractions of group 1 (slow mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with 1:1 mixture of calf rennet and porcine pepsin ('50/50' brand), after various periods of curing

Cheese curing period (days)	K casein	T_s casein	γ casein			β casein			
			1	2	3	1	2	3	4
8	1.2	1.2	2.1	2.5	3.2	-	28.6	2.0	2.2
37	1.8	1.9	2.1	3.3	3.8	-	26.8	4.7	-
60	2.9	3.1	2.6	4.6	4.5	-	26.1	3.6	-
90	1.5	1.5	3.2	7.3	3.1	-	27.1	3.4	-
150	3.1	2.6	2.6	5.3	7.0	-	19.2	3.2	-
180	3.8	3.4	2.7	6.4	5.1	-	19.5	4.4	-
263	4.6	3.9	3.3	9.3	3.0	-	16.7	4.5	3.5
360	4.5	2.5	2.9	.65	3.4	-	15.7	2.9	4.9
Mean 143.500		2.925	2.513	12.475				27.500	
Cor. coeff.		0.896***	0.559	0.648*				- 0.774**	
Y intercept		1.510	1.888	10.353				31.208	
Slope		0.010	0.004	0.015				- 0.026	
SE of slope		0.002	0.003	0.007				0.009	
Cor. coeff.							- 0.830**		
Y intercept							28.072		
Slope							- 0.567		
SE of slope							0.156		

* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

TABLE 6:4

Area of individual casein fractions of group 1 (slow mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with porcine pepsin, after various periods of curing

Cheese curing period (days)	K casein	T_s casein	γ casein			β casein			
			1	2	3	1	2	3	4
7	1.5	1.7	3.1	4.0	1.4	4.2	26.0	-	-
45	2.0	2.7	3.4	5.6	2.6	-	25.7	-	-
63	2.0	2.2	1.9	3.7	2.8	-	27.4	2.5	-
80	2.1	3.0	2.6	4.3	4.2	4.8	21.0	-	-
150	2.0	2.7	2.1	4.4	1.6	1.9	18.2	-	-
180	3.7	3.7	3.3	5.1	1.9	1.5	17.8	2.9	2.2
210	5.0	4.5	4.2	7.1	2.3	-	16.4	-	-
270	2.5	4.7	2.8	11.7	4.5	1.5	14.9	-	-
330	3.5	4.3	4.5	3.8	4.7	4.0	9.3	6.4	4.9

Mean 148.333	2.700	3.278	11.511		23.789
Cor. coeff.	0.644*	0.901***	0.656*		- 0.668*
Y Intercept	1.709	1.969	8.440		28.535
Slope	0.007	0.009	0.021		- 0.032
SE of slope	0.003	0.002	0.009		0.013
Cor. coeff.				- 0.685*	
Y intercept				22.258	
Slope				- 0.452	
SE of slope				0½181	

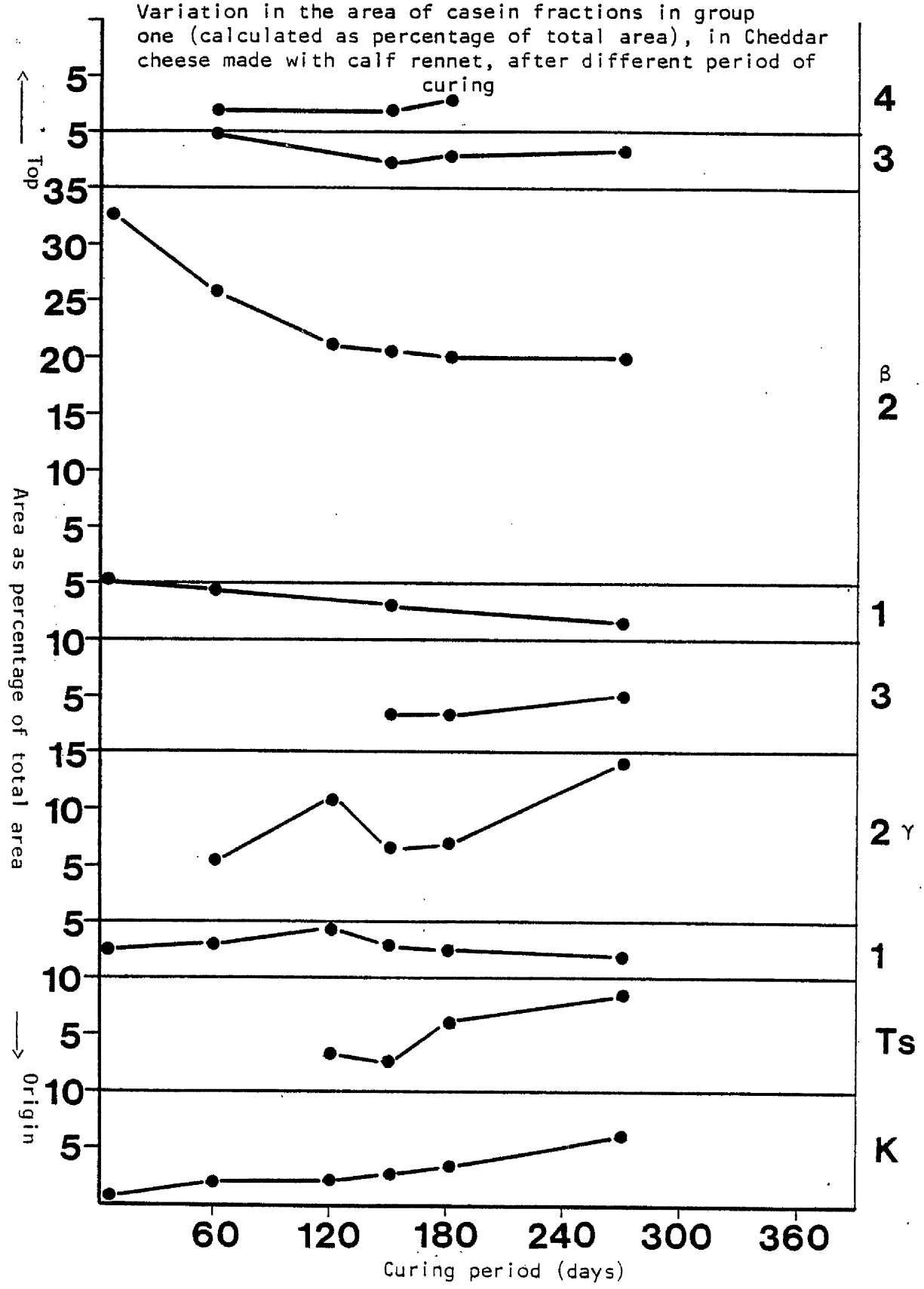
* Significant at 5 per cent level

** " " 1 " "

*** " " 0.1 " "

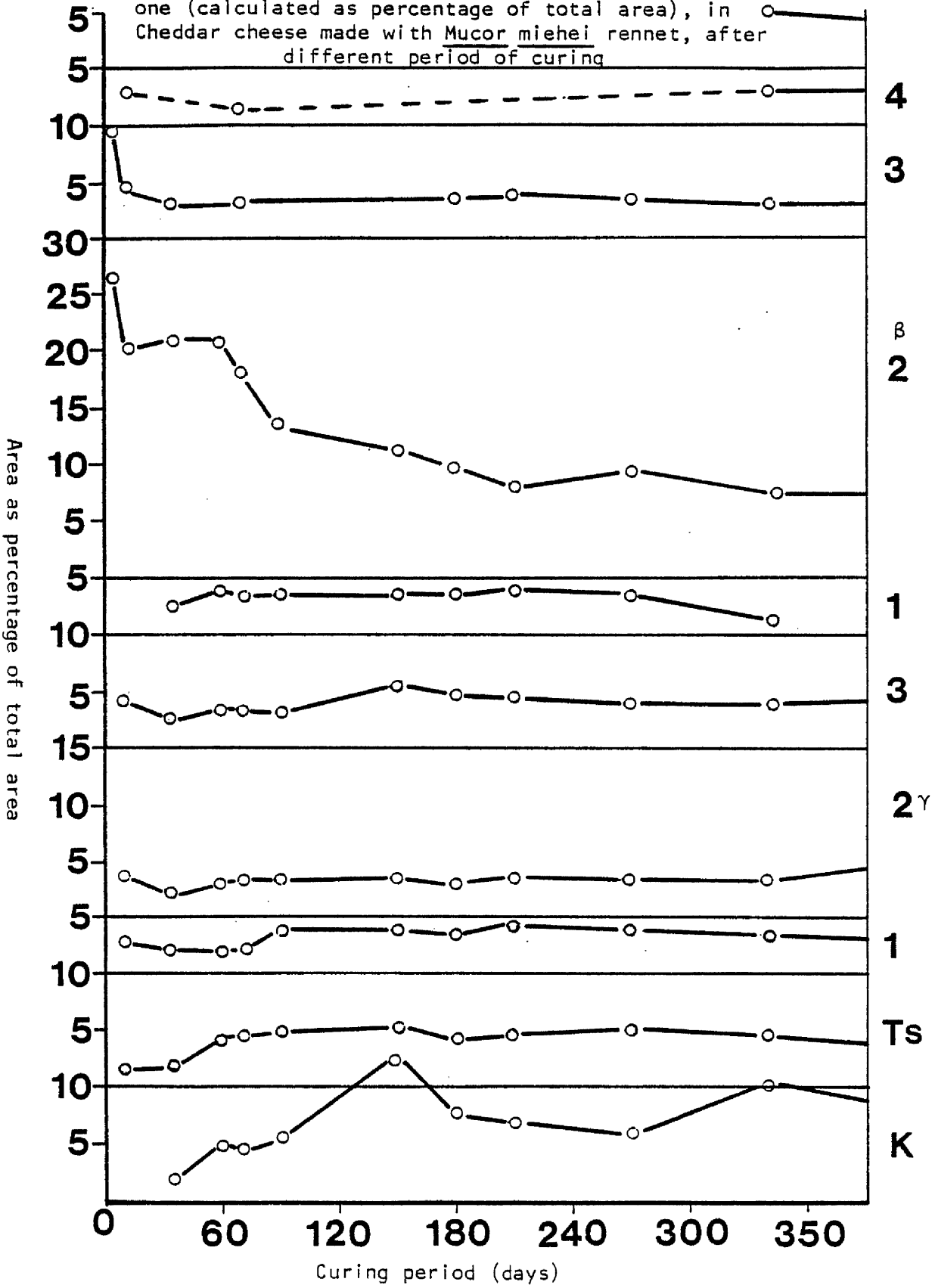
GRAPH 6:1

Variation in the area of casein fractions in group one (calculated as percentage of total area), in Cheddar cheese made with calf rennet, after different period of curing



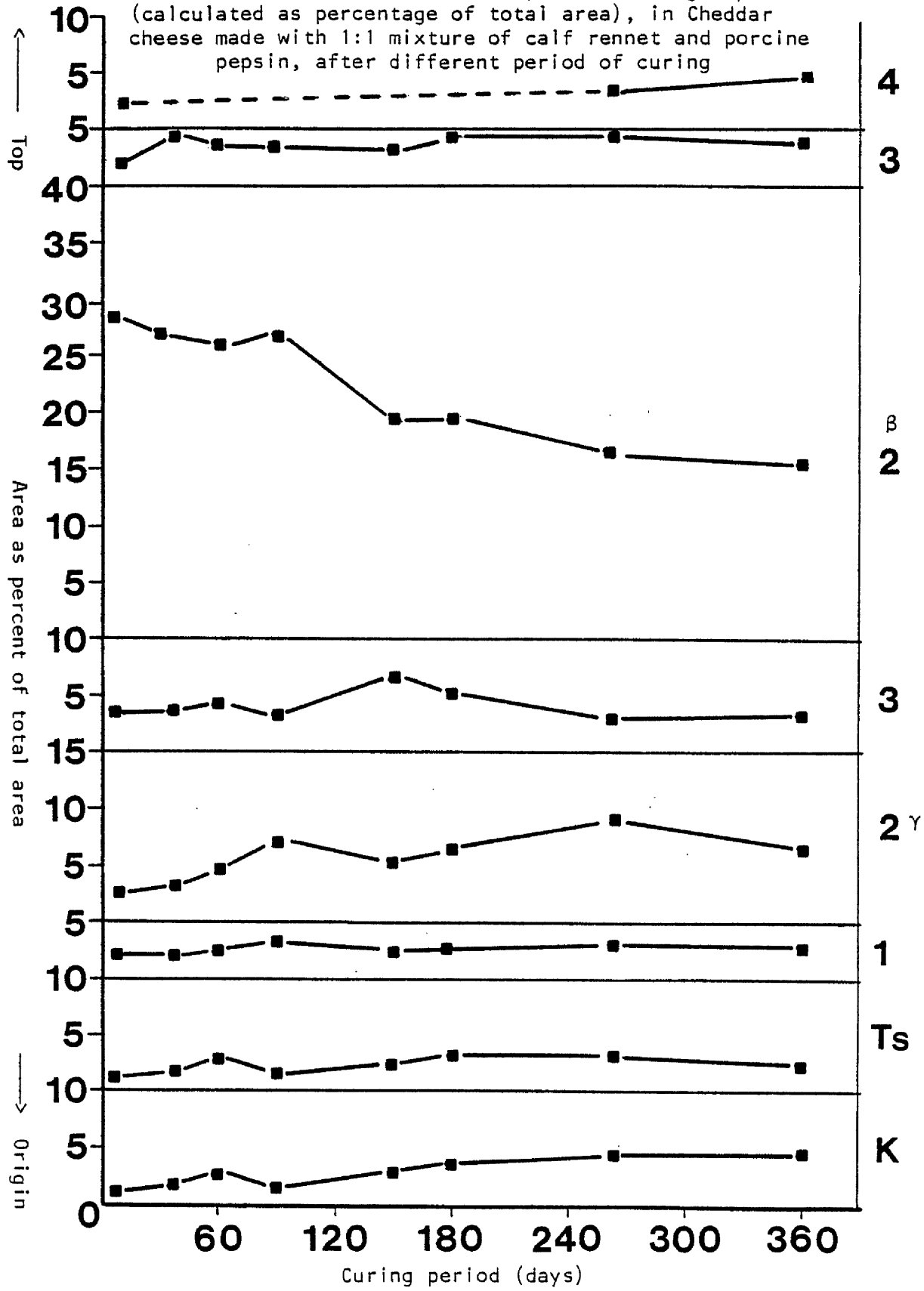
GRAPH 6:2

Variation in the area of casein fractions in group one (calculated as percentage of total area), in Cheddar cheese made with Mucor miehei rennet, after different period of curing



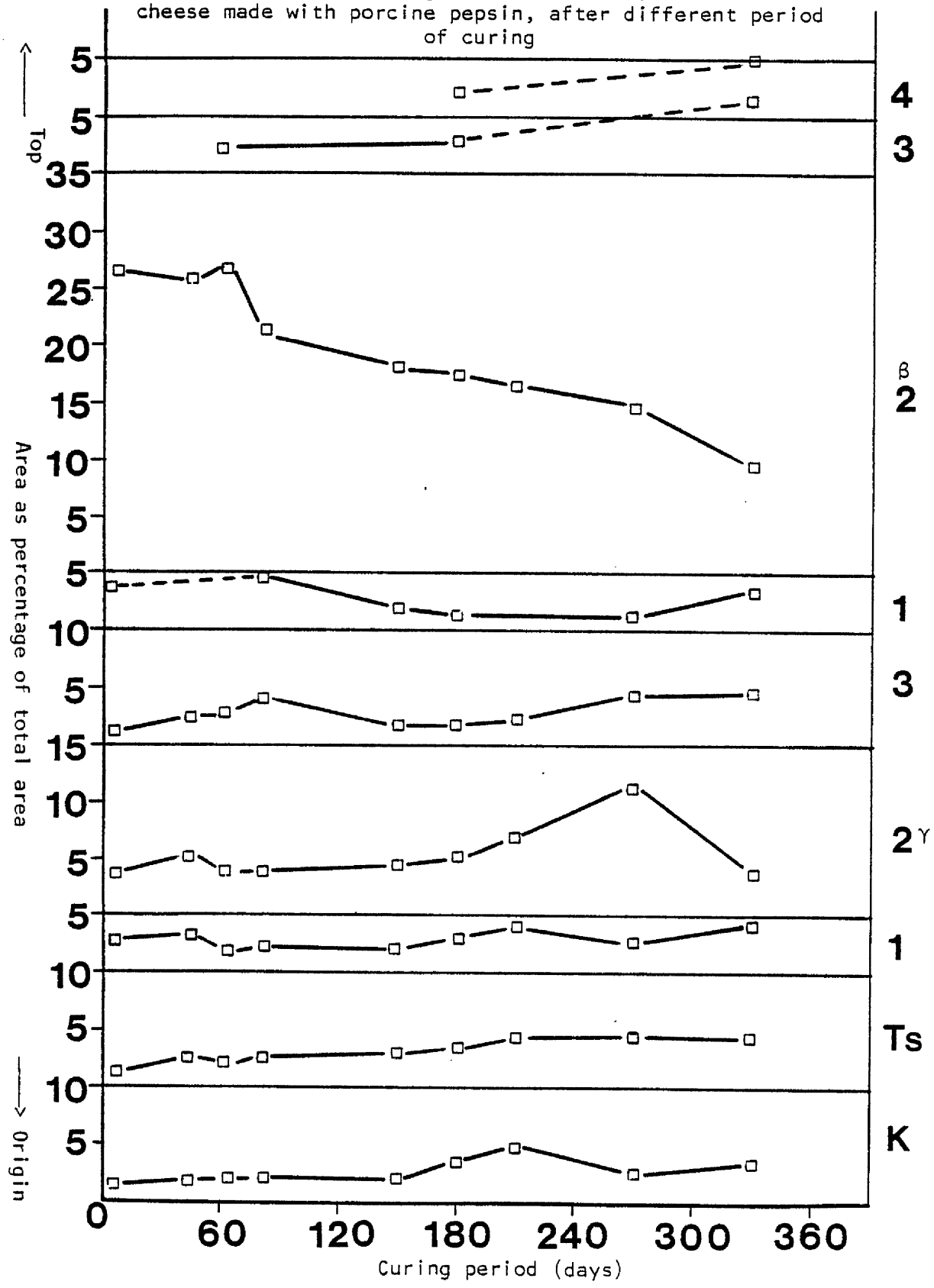
GRAPH 6:3

Variation in the area of casein fractions in group one (calculated as percentage of total area), in Cheddar cheese made with 1:1 mixture of calf rennet and porcine pepsin, after different period of curing



GRAPH 6:4

Variation in the area of casein fractions in group one (calculated as percentage of total area), in Cheddar cheese made with porcine pepsin, after different period of curing



The area of this fraction was less than that of the K fraction. The T_S casein was present in the gels of all cheeses from the beginning of ripening except in the cheese made with calf rennet where it started to appear after 4 months ripening. The amount of this casein fraction remained approximately constant during ripening but the overall relationship between the ripening period and T_S casein showed a very slight increase (significant at 0.1 per cent level), with the rate of increase about half that noticed with the K fraction. No significant difference was noticed between the T_S casein content of all coagulants.

γ casein

Three separate bands belonging to γ casein were noticed between β casein and T_S casein. The bands were present from the beginning of ripening of all cheeses except where the cheese was made with calf rennet - in this case the γ casein 1 had the lowest mobility compared with the other two bands and was produced first, followed after 2 months ripening by γ casein 2 and after 5 months ripening by γ casein 3. Another difference noticed between the coagulants was in the position of the γ casein fractions (Figure 6:13). The different positions of γ casein 2 and 3 between Mucor miehei rennet on the one hand and the other three coagulants on the other are shown in this diagram. The area of each γ casein fraction showed a difference between Mucor miehei rennet producing the three γcasein fractions in approximately equal quantities while the other three coagulants produced a high quantity of γ casein 2 and about equal quantities of γ casein 1 and 3 as shown in table 6:10.

The total area of γ casein fractions showed a similarity between the coagulants except for calf rennet which had a higher quantity of γ caseins. The relationship between the ripening period and the γ casein quantity was significant for all the coagulants (tables 6:1, 2, 3 and 4). A different rate of increase was noticed for the different coagulants. Calf rennet had the highest rate and Mucor miehei rennet had the lowest rate (graph 6:9).

The overall relationship between ripening period and increase in γ casein

was significant at a level of 0.1 per cent (table 6:9) and graph (6:10).

β casein

At the beginning of the ripening period, β casein was the second largest casein fraction after αs_1 casein in all cheeses, and it appeared as one large fraction, β casein 2 with two or three small fractions just started to separate from it. It was noticed during ripening, that β casein 2 was decreased gradually and that there was no appreciable increase in the quantity of the other fractions i.e., β casein 1, 3 and 4. The initial quantity of β casein 2 was similar, in the case of all coagulants, except Mucor miehei rennet which, shortly after the beginning of the ripening period, produced a large fraction 3 which causes a lower initial quantity of β casein 2, but the rate of β casein 2 degradation was slower than that of the other three coagulants. The 1:1 mixture of calf rennet and porcine pepsin cheese showed a difference in not having the β casein 1 fraction which was noticed with the other three coagulants.

The mean of the areas of β casein 2 band for the different coagulants showed a similar value for the calf rennet and the 1:1 mixture of calf rennet and porcine pepsin cheeses, a slightly lower mean area for porcine pepsin cheese, and a significantly lower area for a Mucor miehei rennet cheese. The per centage of each fraction of the total area of β casein showed that Mucor miehei rennet produced two large fractions 1 and 3 from casein 2. This could be the reason for the low mean area of β casein 2, in Mucor miehei rennet (table 6:11).

The relationship between ripening the period and the decrease in the quantity of β casein 2 was significant for all coagulants, and showed the highest rate of decrease with porcine pepsin. The three other coagulants had a lesser but similar rate of reduction (tables 6:1, 2, 3 and 4), and graphs (6:1, 2, 3, 4 and 9). The overall relationship between the ripening period and the reduction in β casein 2 was significant at 0.1 per cent level (table 6:9) and graph 6:10). A significant relationship also was noticed between the gradual reduction in the total β casein and the gradual increase in total γ casein (tables 6:1, 2, 3 and 4), the slope of the

curves formed indicating a lower rate for γ casein production from β casein by Mucor miehei rennet and a higher rate for calf rennet (graph 6:10).

α_s casein

Cheddar cheese at the end of its manufacture would have one large fraction (αs_1 casein) and a few other small α_s casein fractions, but with the beginning of the ripening period, αs_1 casein started to degradate producing additional fractions of αs casein, and it was noticed that these different patterns resulted from the use of the four coagulants in cheese making (Figures 6:12 and 13). Because of the large number of fractions beloning to this group and due to the presence of three different patterns, the results will be discussed in four parts.

(1) In the fractions of αs casein noticed below αs_1 casein, calf rennet produced only one fraction number 2, while the other three coagulants each produced two fractions referred to as fraction 1 and 2. While the two fractions (1 and 2) remained fairly stable during ripening, in the case of calf rennet, fraction 2 showed a gradual but slight decrease. The mean area of fraction 2 showed no differences between all the coagulants, and there was no difference in the mean area of fraction 1 of the coagulants Mucor miehei rennet, the 1:1 mixture of calf rennet and porcine pepsin, and porcine pepsin.

(2) Fraction 3 or αs_1 casein showed a similar mean area with the four coagulants used, but the relationship between the ripening period and the decrease in the quantity of αs_1 casein showed another difference in cheese made with calf rennet compared with the cheese made with other coagulants. The difference was in the rate of degradation of αs_1 casein which, in the cheese made with calf rennet, was more than double that in cheese made with the other coagulants. The addition of fractions 1 and 2 to αs_1 casein did not alter the significance of the correlation but it increased the rate of degradation of αs_1 casein in the cheese made with calf rennet (tables 6:5, 6, 7 and 8) and graphs (6:5, 6, 7, 8 and 9).

TABLE 6:5

Area of individual casein fractions of group 2 (fast mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with calf rennet, after various periods of curing

Cheese curing period (days)	αS_1 casein									
	1	2	3	4	5	6	7	8	9	10
2	-	5.9	49.3	-	3.0	3.1	-	-	-	-
5	-	8.6	37.8	-	8.7	4.0	-	-	-	-
60	-	9.4	14.9	-	15.1	4.4	3.7	1.8	4.0	-
120	-	4.7	12.0	9.8	10.9	9.2	8.5	1.7	1.2	-
150	-	5.1	10.8	11.4	8.8	5.8	4.9	3.1	4.4	-
180	-	2.7	11.0	12.5	8.4	6.7	4.5	1.9	4.2	-
270	-	3.8	7.0	12.7	6.5	4.6	1.7	3.3	-	-
Mean 112.429		5.743	20.400	6.629	8.771	5.400	3.329	1.686	1.964	
Cor.coeff.		-0.737*	-0.830**	0.910**	-0.024	0.385	0.351	0.887**	0.198	
Y intercept		7.821	35.972	0.064	8.875	4.493	2.101	0.346	1.481	
Slope		-0.018	-0.139	0.058	-0.001	0.008	0.011	0.012	0.004	
SE of slope		0.008	0.042	0.012	0.017	0.009	0.013	0.003	0.010	
Mean			26.143				27.779			
Cor.coeff.			-0.876**				0.668			
Y intercept			43.793				17.360			
Slope			-0.157				0.093			
SE of slope			0.039				0.046			
Cor.coeff.					-0.925**					
Y intercept					46.494					
Slope					-0.716					
SE of slope					0.131					

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

TABLE 6:6

Area of individual casein fractions of group 2 (fast mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with Mucor miehei rennet ('Hannilase' brand) after various periods of curing

Cheese curing period (days)	αs_1 casein 1	2	3	4	5	6	7	8	9	10
5	-	10.1	40.5	5.1	-	-	-	-	-	-
10	3.8	9.2	33.5	6.5	4.4	2.9	1.7	-	-	-
35	4.7	7.1	31.2	7.2	5.6	3.6	1.8	1.9	-	-
60	3.6	4.0	20.4	7.1	7.9	5.7	3.4	3.0	2.0	3.3
70	3.4	4.8	18.8	7.3	9.1	6.0	2.9	3.8	1.9	3.1
90	3.7	6.2	15.0	7.6	8.8	7.0	4.5	3.1	2.7	3.7
150	3.2	3.5	13.4	6.9	8.6	7.8	3.2	1.9	2.8	2.6
180	4.8	6.7	10.8	8.3	9.3	9.4	5.2	2.5	2.1	1.7
210	4.5	5.1	9.6	8.2	9.7	8.5	5.8	3.3	5.2	-
270	2.9	4.5	13.5	8.4	9.0	10.9	6.3	4.2	1.7	-
340	4.9	3.6	9.6	11.2	16.0	8.4	7.4	4.1	4.1	-
450	5.5	5.5	8.6	9.8	9.1	7.3	6.9	6.9	-	-
Mean 155.833	3.750	5.858	18.742	7.800	7.292	6.458	4.092	2.892	1.875	1.200
Cor. coeff.	0.543*	-0.525	-0.776**	0.870***	0.470	0.680**	0.899***	0.840***	0.280	-0.334
Y intercept	2.894	7.029	27.938	6.278	5.799	4.155	1.791	1.132	1.348	1.776
Slope	0.005	-0.008	-0.059	0.010	0.010	0.015	0.015	0.011	0.003	-0.004
SE of slope	0.003	0.004	0.015	0.002	0.006	0.005	0.002	0.002	0.004	0.003

Mean	28.383	31.608
Cor. coeff.	-0.744**	0.717**
Y intercept	37.947	22.279
Slope	-0.061	0.060
SE of slope	0.017	0.018

Cor. coeff.	-0.973***
Y intercept	59.584
Slope	-0.986
SE of slope	0.072

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

TABLE 6:7

Area of individual casein fractions of group 2 (fast mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with a 1:1 mixture of calf rennet and porcine pepsin ('50/50') brand, after various periods of curing

Cheese curing period (days)	αs_1 casein 1	2	3	4	5	6	7	8	9	10
8	3.9	6.6	29.9	7.9	3.0	2.1	2.1	1.5	-	-
37	4.6	6.7	22.8	14.2	3.1	1.5	1.5	2.2	-	-
60	3.1	6.0	15.8	14.9	4.0	2.6	2.0	2.0	2.2	-
90	5.7	6.8	15.6	12.0	2.7	3.0	1.4	1.2	4.5	-
150	3.7	7.7	15.6	11.1	6.9	3.2	2.0	2.1	4.7	-
180	3.9	8.1	11.1	12.7	5.3	5.6	3.6	3.0	1.4	-
263	4.4	5.8	10.4	12.0	7.1	4.0	5.4	1.9	0.6	-
360	4.6	4.4	8.2	11.5	9.4	6.5	3.7	3.8	4.8	-
Mean 143.500	4.238	6.513	16.175	12.038	5.188	3.563	2.713	2.188	2.275	
Cor. coeff.	0.161	-0.494	-0.848**	-0.005	0.931***	0.886**	0.769*	0.701	0.431	
Y intercept	4.089	7.189	23.358	12.051	2.507	1.751	1.441	1.540	1.195	
Slope	0.001	-0.005	-0.050	0.000	0.019	0.013	0.009	0.005	0.008	
SE of slope	0.003	0.003	0.013	0.007	0.003	0.003	0.003	0.002	0.006	

Mean	26.925	27.963
Cor. coeff.	-0.872**	0.918**
Y intercept	34.636	20.484
Slope	-0.054	0.052
SE of slope	0.012	0.009

Cor. coeff	-0.954***
Y intercept	51.617
Slope	-0.879
SE of slope	0.113

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

TABLE 6:8

Area of individual casein fractions of group 2 (fast mobility bands) expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with porcine pepsin, after various periods of curing

Cheese curing period (days)	αs_1 casein 1	2	3	4	5	6	7	8	9	10
7	4.5	7.0	34.1	6.9	3.2	1.6	-	-	-	-
45	-	2.5	28.1	13.6	4.1	3.1	2.4	1.4	2.8	-
63	2.5	3.0	25.4	13.4	4.4	3.0	2.2	1.2	2.4	-
80	4.2	5.5	19.6	11.4	4.8	2.3	1.7	3.6	4.9	-
150	5.6	6.0	22.2	17.7	5.6	2.8	1.8	1.6	3.8	-
180	3.7	5.1	18.3	14.8	6.8	3.4	2.3	1.9	1.6	-
210	4.9	5.6	12.7	13.7	8.6	6.0	3.6	2.9	2.5	-
270	4.0	7.1	15.4	13.5	7.6	5.1	3.0	1.7	-	-
330	4.5	6.0	10.7	8.8	8.0	4.5	5.0	4.3	2.8	-
Mean 148.333	3.767	5.311	20.722	12.644	5.900	3.533	2.444	2.067	2.311	
Cor. coeff.	0.415	0.424	-0.912***	0.100	0.928***	0.785**	0.848**	0.641*	-0.078	
Y intercept	2.836	4.389	30.105	12.206	3.476	2.034	0.854	0.917	2.481	
Slope	0.006	0.006	-0.063	0.003	0.016	0.010	0.011	0.008	-0.001	
SE of slope	0.005	0.005	0.011	0.011	0.002	0.003	0.003	0.004	0.006	

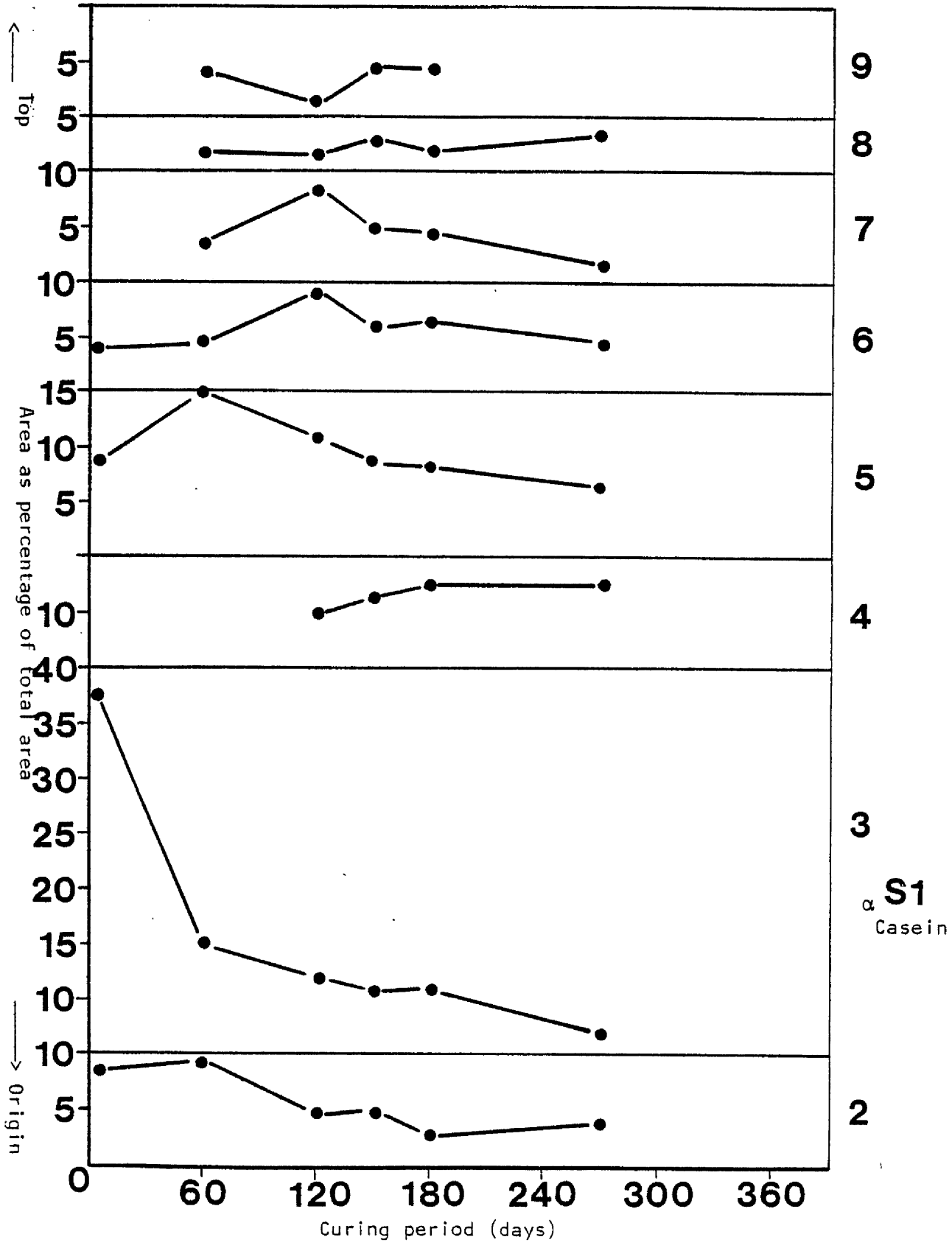
Mean	29.800	28.900
Cor. coeff.	-0.779**	0.702*
Y intercept	37.329	21.968
Slope	-0.051	0.047
SE of slope	0.015	0.018

Cor. coeff.	-0.882***
Y intercept	55.762
Slope	-0.901
SE of slope	0.182

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

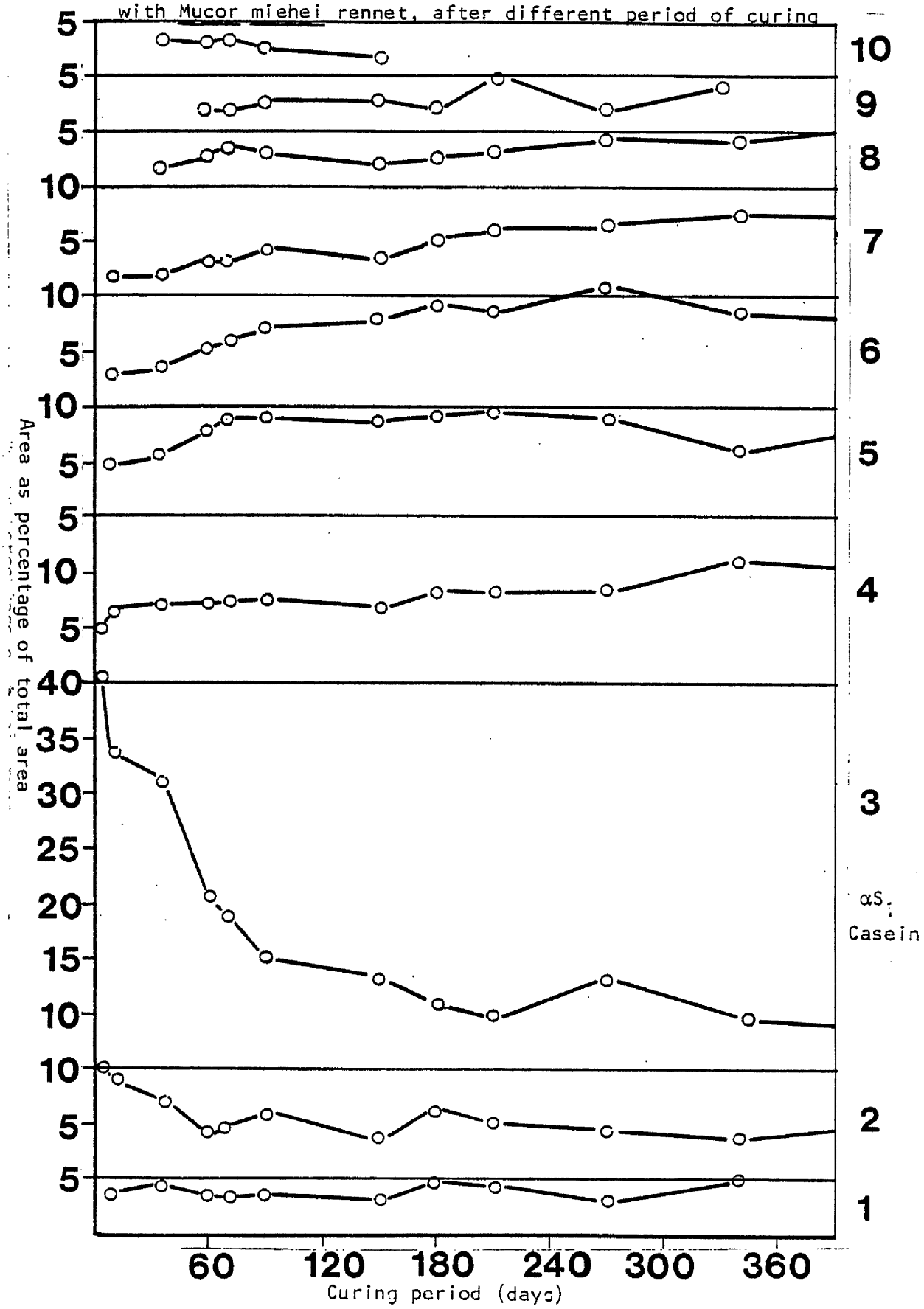
GRAPH 6:5

Variation in the area of casein fractions in group two (calculated as percentage of total area), in Cheddar cheese made with calf rennet, after different period of curing



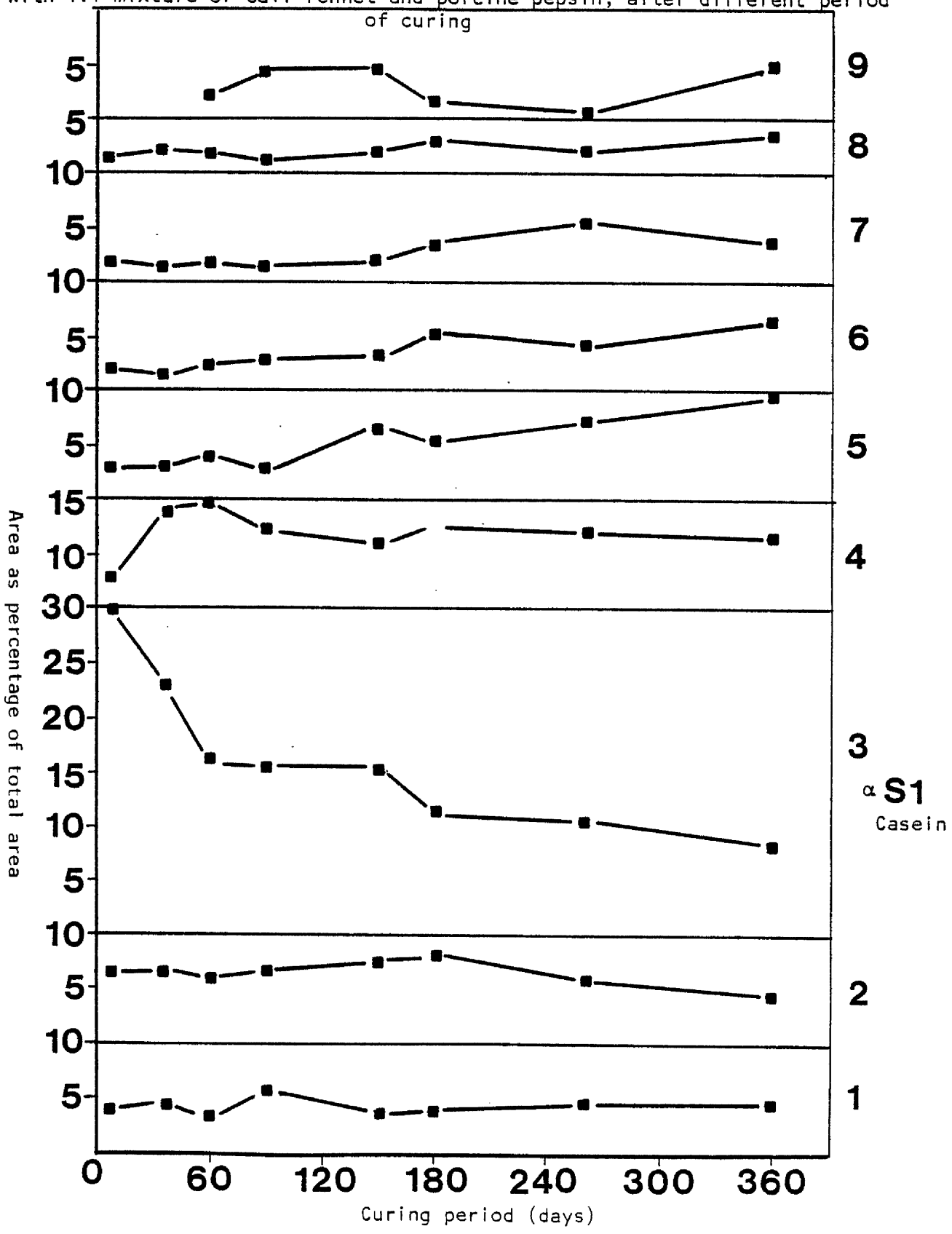
GRAPH 6:6

Variation in the area of casein fractions in group two (calculated as percentage of total area), in Cheddar cheese made with Mucor miehei rennet, after different period of curing



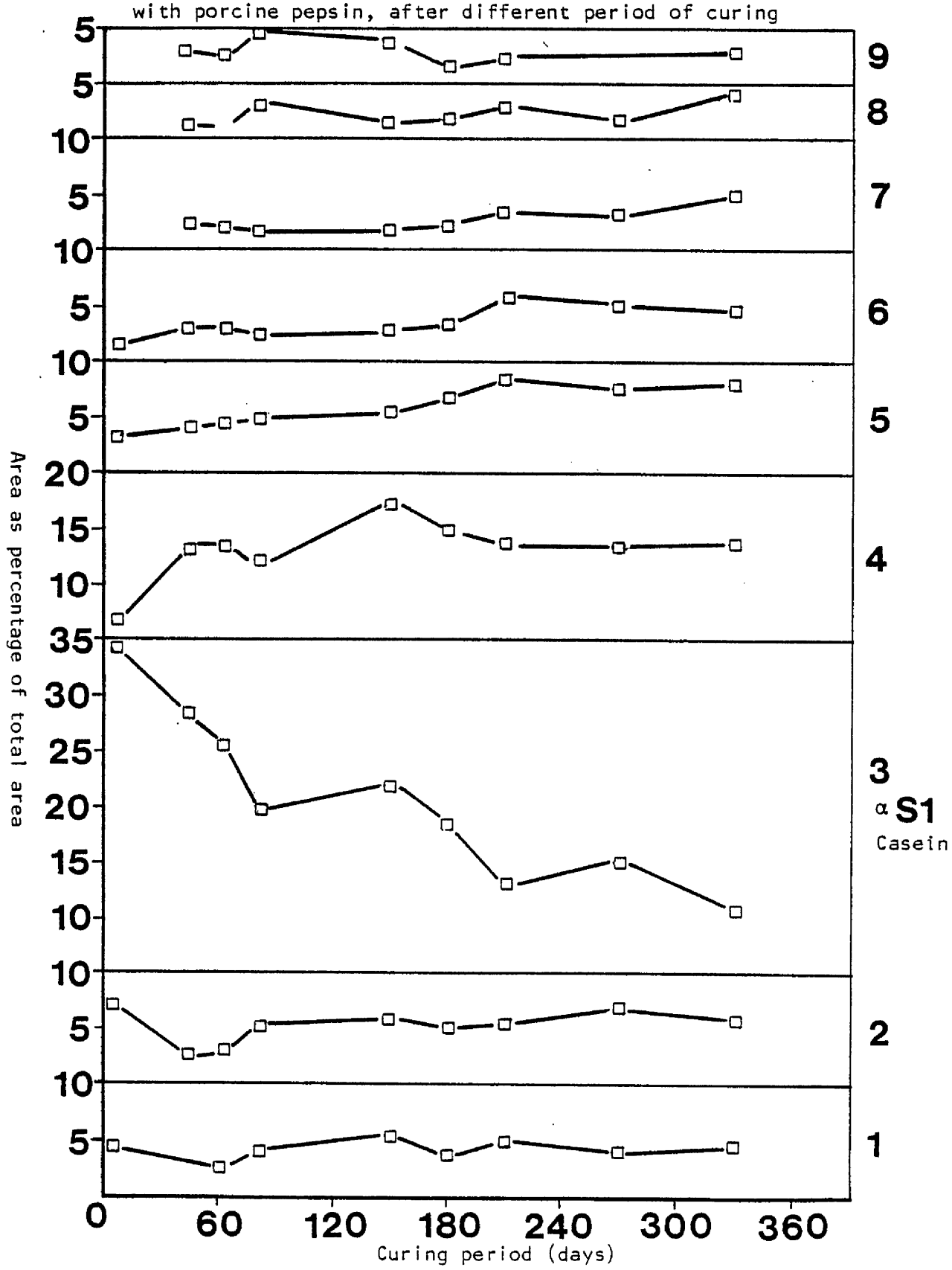
GRAPH 6:7

Variation in the area of casein fractions in group two (calculated as percentage of total area), in Cheddar cheese made with 1:1 mixture of calf rennet and porcine pepsin, after different period of curing



GRAPH 6:8

Variation in the area of casein fraction in group two (calculated as percentage of total area), in Cheddar cheese made with porcine pepsin, after different period of curing



The overall relationship between the ripening period and the sum of fractions 1, 2 and 3 was significant at the 0.1 per cent level table (6:9) and graph (6:10).

(3) Fractions 4, 5, 6 and 7 were produced in different amounts by the different coagulants. In the case of calf rennet, fractions 5 and 6 were produced first with fraction 5 being larger than fraction 6 and both fractions showed a gradual increase up to the second month of ripening when it started to decrease gradually. Fraction 4 was produced after four months ripening and fraction 7 was produced after two months ripening. Mucor miehei rennet produced the four fractions at the beginning of the ripening period, and all the fractions showed a small gradual increase as ripening progressed, with similar mean areas of fractions 4 and 5. Fraction 8 was slightly less than fraction 4 or 5 and fraction 7 was about half that of fraction 4 (table 6:12). By comparison with calf rennet, only fraction 5 was different between the two coagulants. The 1:1 mixture of calf rennet and porcine pepsin, and procine pepsin, both produced a third distinctive pattern for those four fractions. Both produced the four fractions at the beginning of the ripening period. Fraction 4 was in large quantity and remained approximately stable during ripening. Fractions 5, 6 and 7 showed a significant gradual slight increase during ripening . The mean area of fraction 4 produced by the 1:1 mixture of calf rennet and porcine pepsin, and the porcine pepsin (table 6:12) was about double that of fraction 4 in both calf rennet and Mucor miehei rennet. The area of the the other three fractions in the 1:1 mixture of calf rennet and porcin pepsin, and the procine pepsin were lower than that in calf rennet and Mucor miehei rennet.

(4) The remaining fractions 8, 9 and Mucor miehei rennet 10, showed no significant difference between the coagulants, nor did they show a significant relationship with the length of ripening period due to their small area in comparison with the other fractions.

The sum of all the fractions produced above αs_1 casein showed significant correlation with the ripening period and with the

TABLE 6:9

Relationship between the area of individual casein fractions (expressed as a percentage of the total area of the bands formed in electrophoretic gels from Cheddar cheese made with different coagulants) with the cheese curing period

Casein fractions	Mean	Cor. coeff.	Y intercept	Slope	SE of slope
K casein	3.711	0.600***	1.664	0.014	0.003
T_s casein	3.192	0.562***	1.947	0.009	0.002
γ caseins	11.197	0.609***	8.312	0.020	0.005
β caseins	24.992	-0.669***	30.419	-0.038	0.007
αs caseins	27.978	-0.717***	37.517	-0.067	0.011
pre αs caseins	29.376	o.704***	20.840	0.060	0.010
Curing period	142.778				
DF	34				

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

TABLE 6:10

Area of individual casein fraction of γ casein expressed as a percentage of the total area of γ casein bands formed in electrophoretic gel from Cheddar cheese made with different coagulants

Fraction number	CR	MM	1:1 CR:PP	PP
1	24.18	29.94	21.54	26.93
2	59.89	30.42	45.29	47.97
3	15.93	39.64	33.17	25.10

TABLE 6:11

Area of individual casein fractions of β casein expressed as a percentage of the total area of β casein bands formed in electrophoretic gel from Cheddar cheese made with different coagulants

Fraction number	CR	MM	1:1 CR:PP	PP
1	9.04	13.17	-	8.36
2	81.68	68.44	81.68	82.81
3	6.20	14.47	13.50	5.51
4	3.08	3.93	4.82	3.32

TABLE 6:12

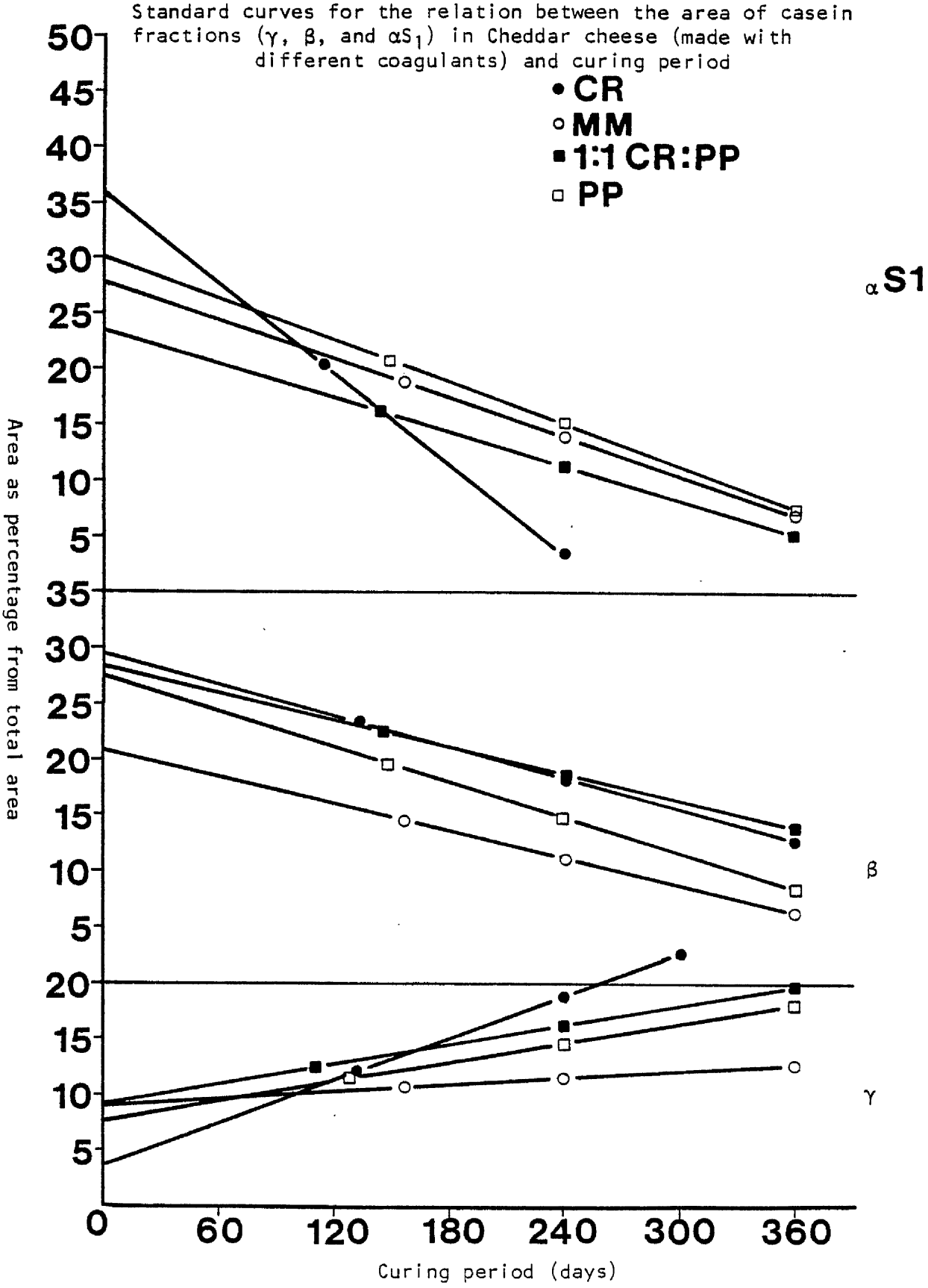
Area of individual casein fractions of group 2 (fast mobility bands) expressed as a percentage of the total area of group 2 bands formed in electrophoretic gels from Cheddar cheese made with different coagulants

Fraction number	CR	MM	1:1 CR:PP	PP
1	-	6.25	7.72	6.42
2	10.65	9.76	11.84	9.05
3	37.84	31.21	29.48	35.30
4	12.30	12.99	21.93	21.54
5	16.27	12.14	9.46	10.05
6	10.02	10.83	6.49	6.01
7	6.18	6.83	4.94	4.16
8	3.13	4.83	3.99	3.53
9	3.64	3.16	4.15	3.94
10	-	2.00	-	-

decrease in the sum of fractions 1, 2 and αs_1 casein (tables 6:5, 6, 7, 8 and 9, and graphs 6:5, 6, 7, 8, 10 and 11).

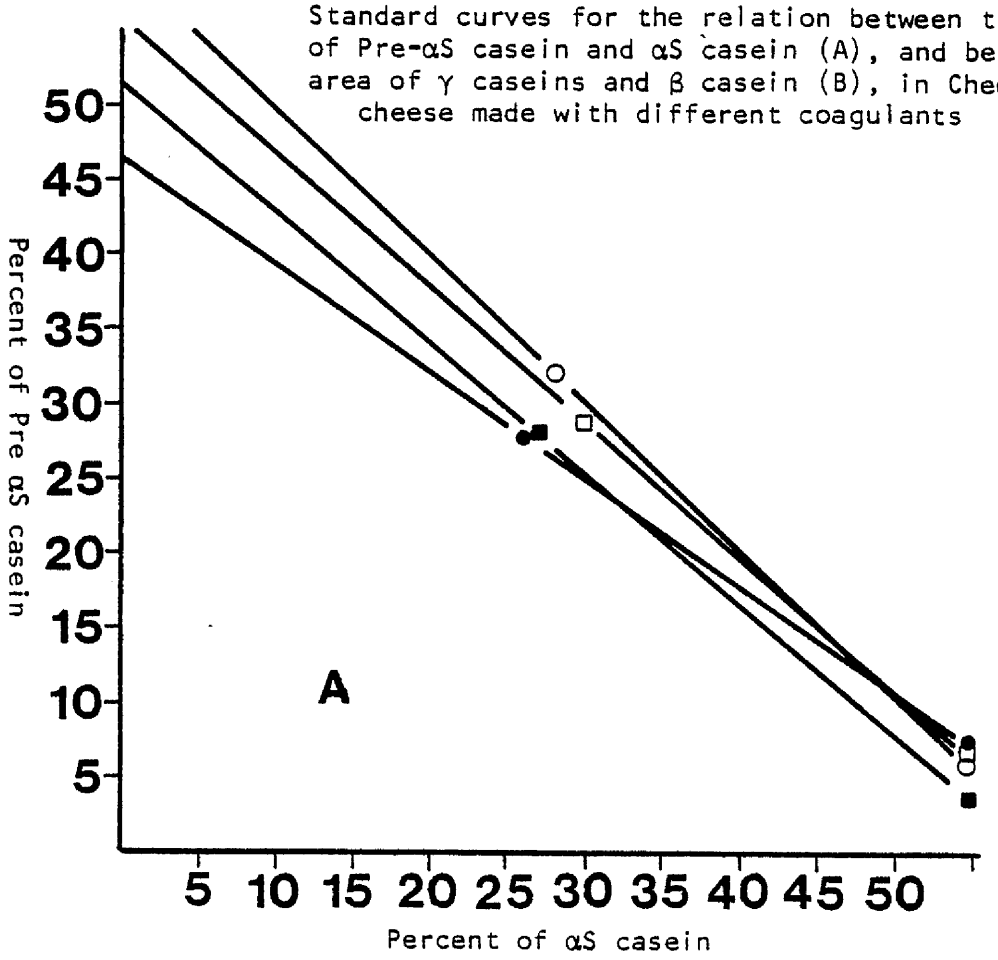
GRAPH 6:9

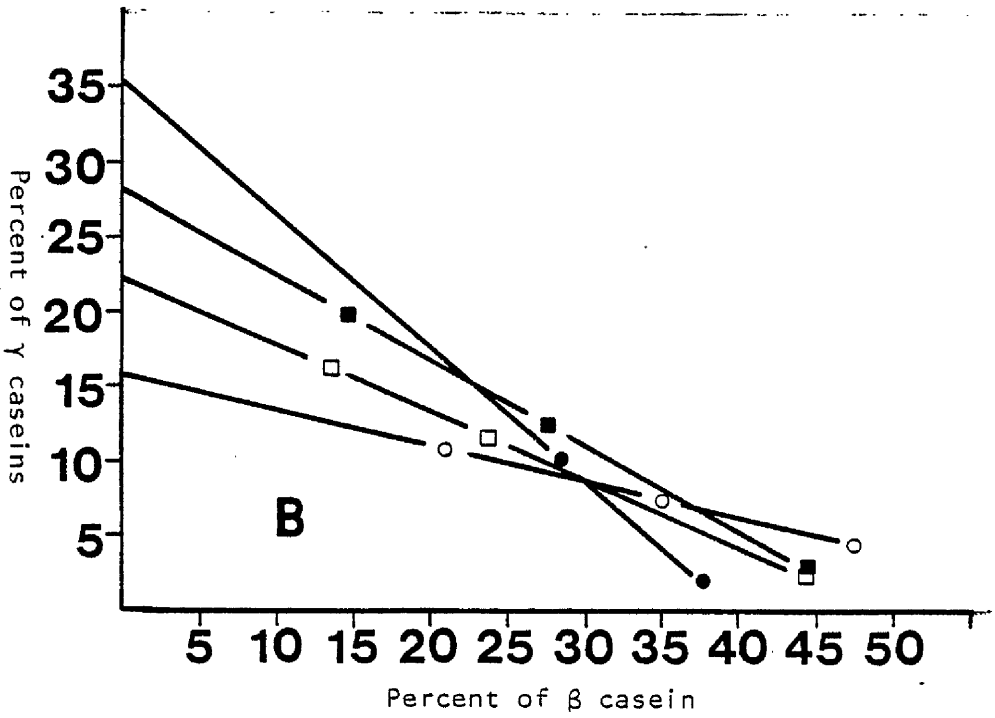
Standard curves for the relation between the area of casein fractions (γ, β, and αS_1) in Cheddar cheese (made with different coagulants) and curing period



GRAPH 6:10

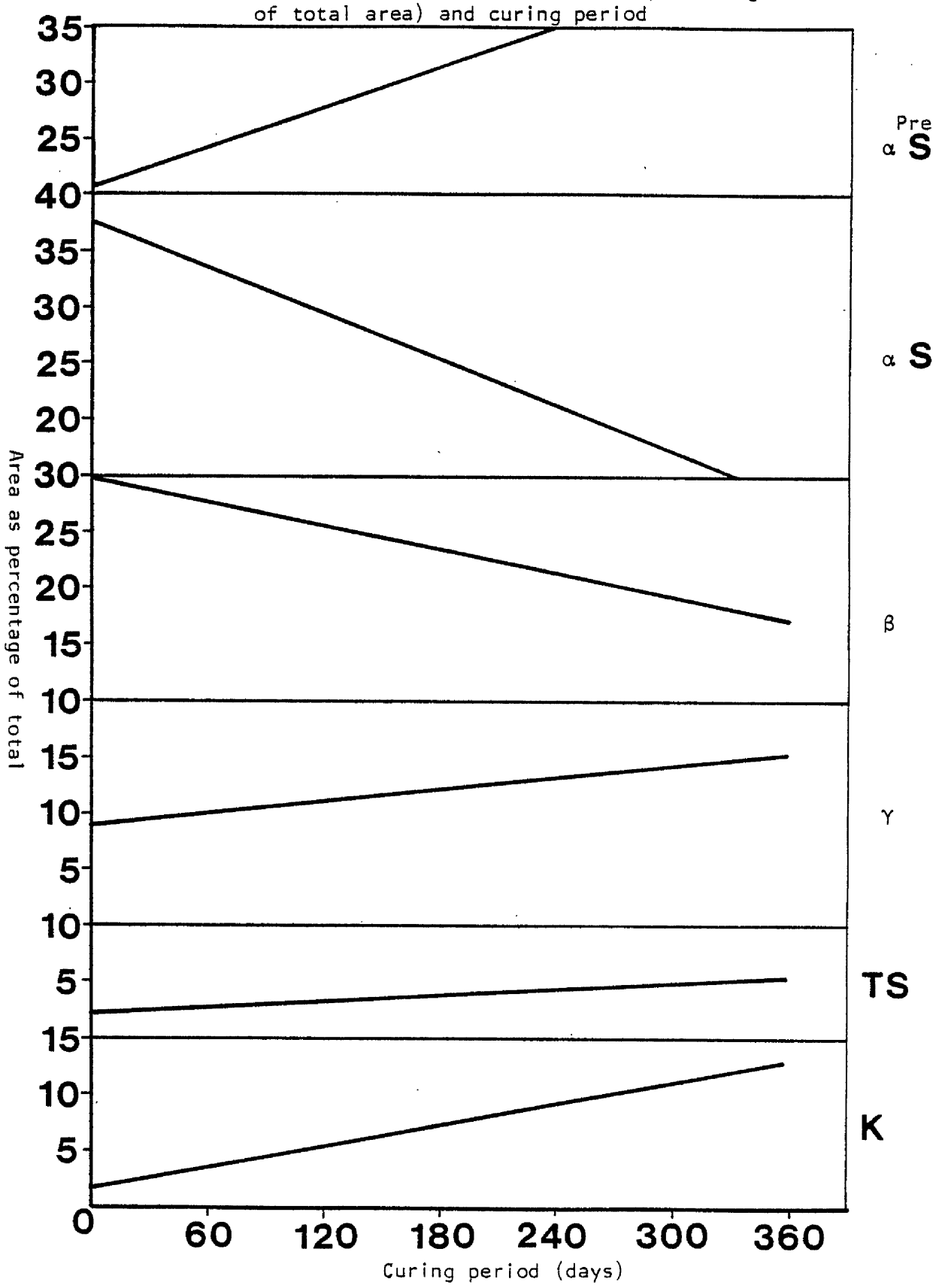
Standard curves for the relation between the area of Pre-αS casein and αS casein (A), and between the area of γ caseins and β casein (B), in Cheddar cheese made with different coagulants





GRAPH 6:11

Standard curves for the relation between the area of different casein fractions (calculated as percentage of total area) and curing period



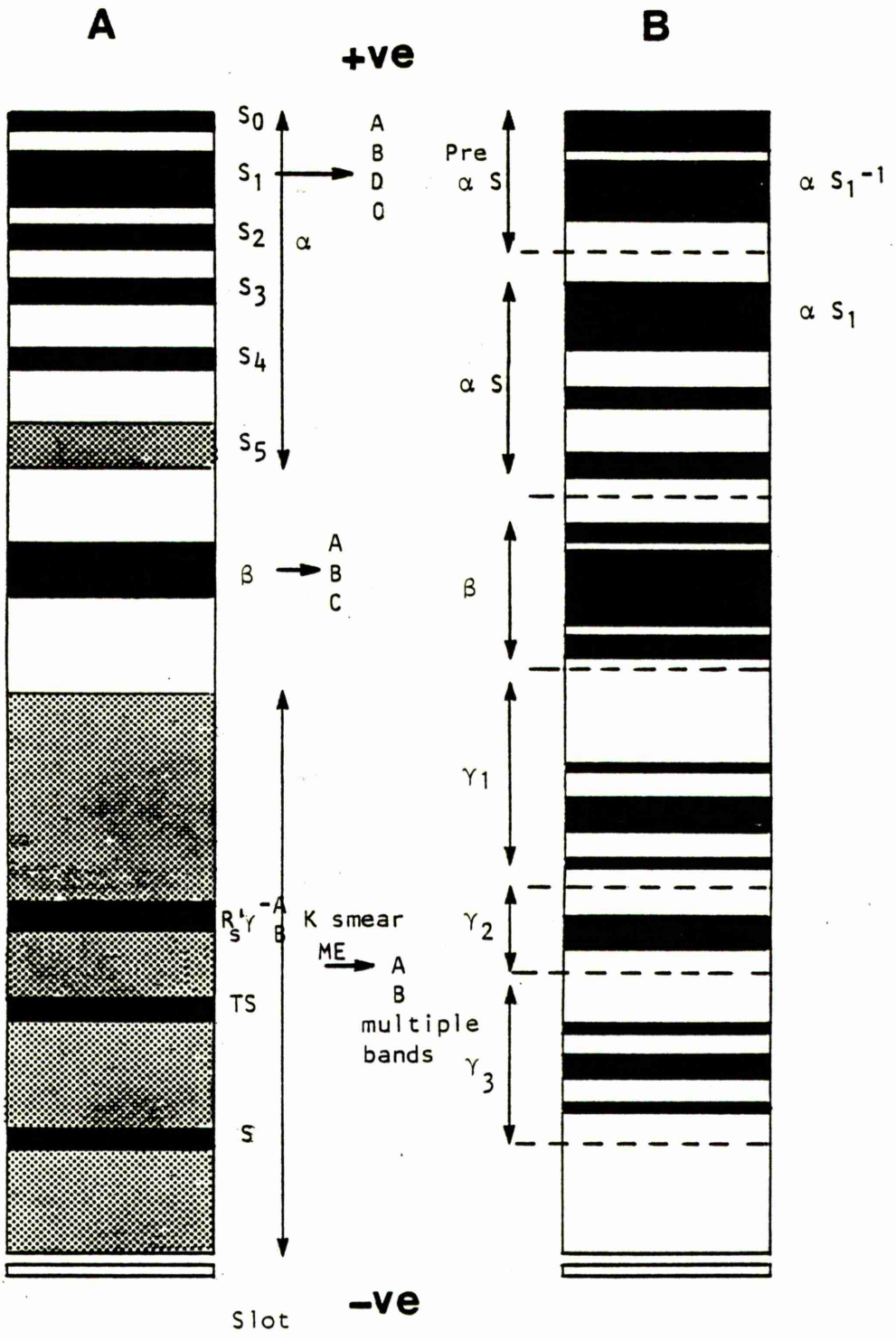


FIGURE 6:12

Schematic electrophoretic patterns showing the relative positions of zones from whole bovine casein (A) and the typical components found in cheese made from cow's milk clotted by calf rennet (B).

(A) from McKenzie (1970) (B) from Marcos et.al.(1979)

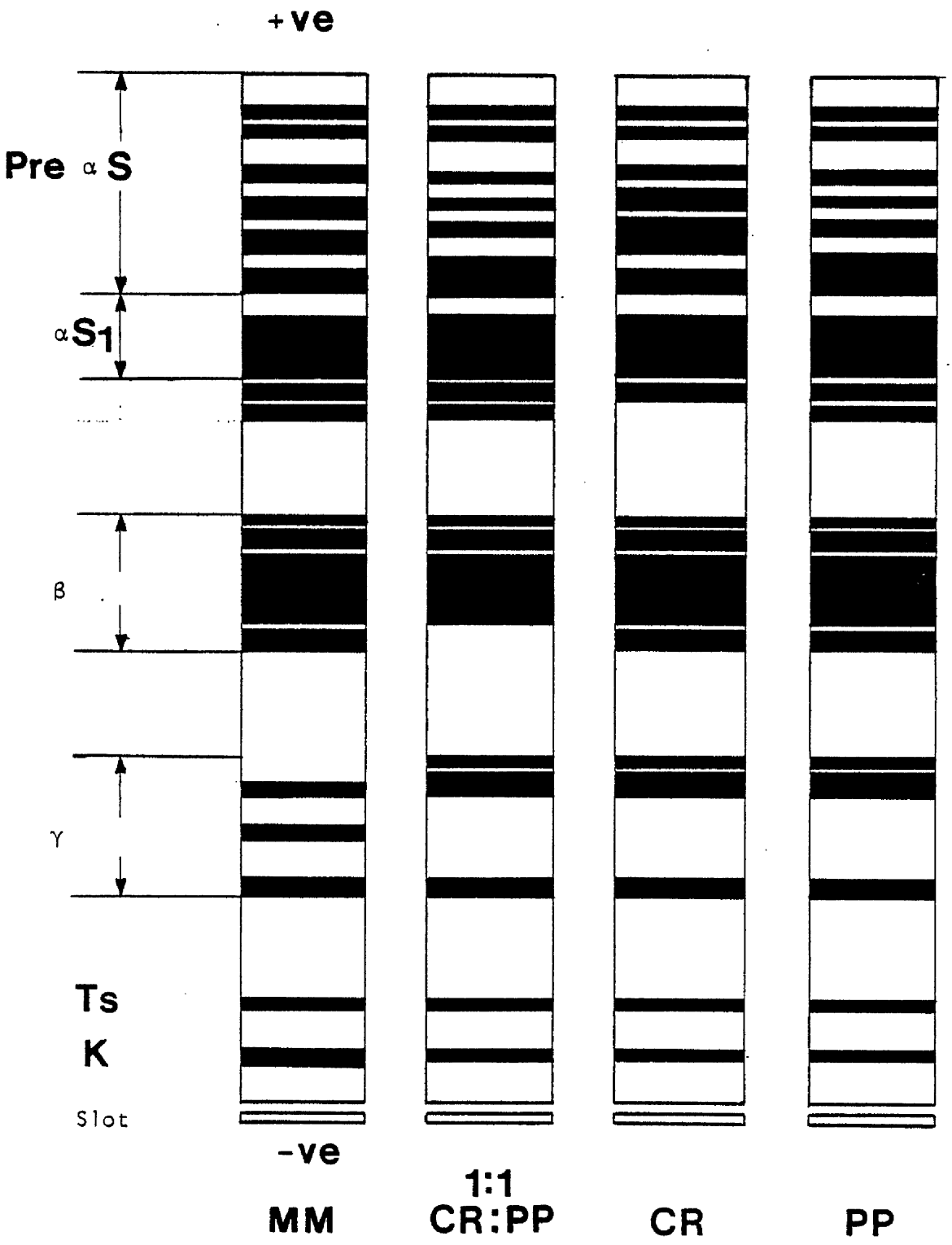


Figure 6:13

Schematic electrophoretic patterns showing the relative positions of casein fractions in Cheddar cheese made by using Mucor miehei coagulant (MM), mixture of CR and PP (1:1), calf rennet (CR), and porcine pepsin (PP)

DISCUSSION

Throughout this electrophoretic study, many problems arose concerning the quality of resolution achieved in relation to gel composition, buffer composition, running conditions, and gel staining.

Although the method used was standardised from the beginning of the study, small unavoidable changes such as buffer ionic strength, maintainance of constant electrical current during running the gel, the temperature of the gel during running, stain concentration and period of staining, affected the resolution power of the gel and resulted in gels having different staining intensity and in the disappearance of certain fractions from thecasein. Also, defects such as smearing and tailing of the bands, happened frequently due to impurities in the gel or the incomplete separation of the fat from cheese samples.

The effect of the above factors on the study was in the reduction of the number of gels which could be used to investigate the effect of coagulants or ripening period on casein fractions.

Scanning of the gels resulted in elution diagrams containing a number of unseparated peaks as well as other well-formed peaks depending on the quality of the gel and on the state of separation. Therefore to calculate the area of each peak, estimations and approximations were required in the measurement of peaks dimensions and in the identification of separate peaks.

The repositioning of casein fractions after fixing the β casein position solved the problem of having different lengths of gels. Peaks belonging to the same casein fraction were found, after repositioning, to occupy the same position in all gels in spite of the type of coagulant used or the length of ripening period.

The division of the electrophoretic pattern into two groups on the base of electrophoretic mobility was the obvious choice because of the well known fact that β casein produces fractions with slower electrophoretic mobility and αs casein produces fractions with faster electrophoretic mobility than the intact β and αs casein (Green and Foster,

1974, Green and Stackpoole, 1975; and Marcos et. al., 1979). It is also well known that the rate of degradation is different between the β casein group which has a low degree of proteolysis and the αs casein group which has a high degree of proteolysis during ripening (Creamer, 1970); Creamer, 1971; Fox and Walley, 1971; Phelan et. al., 1973) Green and Foster, 1974; Green and Stackpoole, 1975; and Visser and de Gresot-Mostert, 1977).

The study of individual casein fractions makes it possible to find the relationship between the fractions themselves on one hand and between them and the coagulant type and ripening period on the other hand. In the case of the K fraction, the resistance of the fraction to the applied current through the gel resulted in an unchanged position during ripening. The resistance could only come from the very low net charge carried by this fraction, and the only casein fraction having such a very low net charge is identified as K casein (McKenzie, 1971). The stability of this fraction during ripening which was noticed throughout the work corresponds to that of K casein reported by Ledford et. al. (1968), Green and Foster (1974), and Emmons et. al. (1977) which adds further evidence to the identity of this fraction.

The higher mean area of the bands of the K fraction in the cheese made with Mucor miehei rennet could have happened because Mucor meihei rennet required a different quantity of K casein to clot the milk than the quantity of K casein required by calf rennet.

T_s casein showed more stability than K casein did, and that was possibly because of the unimportance of this minor casein in the clotting process or during ripening. The exact identification of the fraction requires to be isolated, the measurement of its molecular weight observed and its amino acids content established. The results for the stability of T_s casein during ripening agreed with the report of Emmons et. al. (1977).

The significant relationship found between changes in γ casein and β casein indicates that γ casein originated from β casein. This finding agreed with that of O'Keeffe et. al. (1977) and Marcos et. al. (1979)

Mucor miehei rennet which was found to produce γ casein in different positions and the rate of production possibly means a different mechanism of β casein degradation by Mucor miehei rennet, and if we consider the low mean area of β casein in Mucor miehei rennet cheese, the assumption of a different mechanism for β casein degredation is justified, and possibly a number of low molecular weight, fast mobile fractions were produced by Mucor miehei rennet from β casein during ripening.

Because of the importance of a slow regular hydrolysis of β casein in the formation of Cheddar cheese flavour (Green and Stackpoole, 1975) the differences in hydrolysis of β casein caused by some of the coagulants i.e. Mucor miehei rennet with irregular hydrolysis, and porcine pepsin with rapid hydrolysis, could lead to the production of small peptides having a detrimental effect on the flavour of ripened cheese, such as bitter peptides, and although there are many references which declare that the rate of β casein degradation determined by the cheese composition specially pH value and moisture content of the cheese (Creamer, 1970; Creamer, 1971; Fox and Walley, 1971; and Phelan et. al., 1973), the effect of coagulant type on the rate of degradation of β casein is important and showed significant differences between the different coagulants.

The overall rate of β casein degradation by all coagulants is in agreement with the results of Emmons et. al. (1977). The conclusion of O'Keeffe et. al. (1977) that milk proteinases are the cause of β casein degradation is possible if we suppose that the coagulants initiated the degradation of β casein, and after their inactivation during cheese making or during ripening, milk and starter proteinases continued the degradation.

The αs casein region showed more differences between the different coagulants used and also showed faster changes in the different fractions noticed. The fractions below αs_1 casein were identified by Ledford et. al. (1966) and Marcos et. al. (1979) as αs_2 and αs_3 caseins. Also it is possible that the fractions are variants D and C of αs_1 casein (McKenzie, 1971). The relationship found through the course

of this study between the two fractions (termed 1 and 2) and αs_1 casein (termed 3) proved that the two fractions are αs_2 and αs_3, because they were detected in all cheeses since the beginning of ripening, and their quantity remained approximately stable during the ripening period. In the same time, casein fractions above αs casein were produced gradually during ripening and showed slight but regular increase during ripening. This means that the fractions above αs_1 casein are variants and degradation products from αs_1 casein but not the fractions below αs_1 casein. The addition of fractions 1 and 2 to αs_1 casein showed a strong correlation with the ripening period and increase the rate of degradation of αs_1 casein which proves that the fractions are separate αs caseins and they also degradated during ripening.

The significant high rate of αs_1 casein degradation associated with the use of calf rennet is due to the specificity of chymosin (the major active enzyme in calf rennet) on attacking αs_1 (McKenzie, 1971).

The different patterns found through the course of this study in the production of αs_1 casein degradation products (fractions 4, 5, 6 and 7) were a significant indication and could be used in the identification of coagulant types used in cheese making. The four fractions were identified as αs_1 - I, αs_1 - II, αs_1 - V, and αs_1 - III taking the opinion of O'Keeffe *et. al.* (1977).

Fraction 4 or αs_1 - I, provides the greatest difference between the different coagulants. When it was produced in a large quantity in both porcine pepsin and in the 1:1 mixture of calf rennet and porcine pepsin cheeses, it was stable during ripening. In both calf rennet and *Mucor miehei* rennet cheeses, αs_1 - I fraction was produced in a small quantity at the beginning of the ripening period and showed a small regular increase during ripening.

The reason for the different rate of proteolysis observed for the different coagulants in the fifth chapter could be because of their different pattern of αs_1 casein degradation. The different quantities and molecular weights of the previous four fractions could be the reason for the slow or fast rate of proteolysis noticed associated with the four coagulants used.

CONCLUSION

The use of different coagulants in Cheddar cheese making, resulted in the production of different electrophoretic patterns of casein. The different patterns found were produced as a result of differences between the coagulants in the clotting mechanism (as for Mucor miehei rennet and in the proteolysis mechanism.

The high rate of β casein degradation is not favourable during cheese ripening because most of the defects in cheese flavour come from the excessive hydrolysis of β casein, and because two coagulants - Mucor miehei rennet and porcine pepsin - showed ability to produce low molecular weight peptides from β casein, bitterness and other flavour defects which were most likely to be produced in their cheeses and which can be avoided if cheese composition is controlled i.e. contains low moisture and a moderate pH.

The rate of the coagulant in cheese ripening will be effective whether the coagulant remains active during ripening, such as calf rennet and Mucor miehei rennet, or inactivated soon after the end of cheese making, like porcine pepsin. Their effect will be in the production of certain peptides early in the ripening process which will be degraded into further smaller peptides by the action of active milk and starter proteinases.

CHAPTER SEVEN

QUALITY ASSESSMENT OF CHEDDAR CHEESE MADE WITH DIFFERENT COAGULANTS

INTRODUCTION

The most important factor limiting the use of new coagulants in cheese making in addition to their effect on cheese yield, is their effect on the quality of the cheese produced. There should be no harmful effect of the new coagulant on cheese flavour and body and texture, because a coagulant which causes defects in the cheese quality will be rejected by all cheese producers even if high cheese yield will result from its use.

Quality of cheese, unlike the compositional properties of the cheese, cannot be determined quantitatively or assessed easily by applying ordinary chemical and physical methods, and, within limits, knowing cheese composition gives no indication of the quality of the cheese (Nelson and Trout, 1965). From the consumer's point of view, cheese quality is the impression gained from the sensation of seeing, feeling, smelling, and tasting the cheese, and such sensations can only be measured or determined by an organoleptic assessment of the cheese.

Cheese 'grading', which is an organoleptic assessment of cheese quality, is a normal practice in the developed export oriented countries, used to establish a uniformity of quality of their produce and to assist in determining the most suitable market for their products (Al Dahhan, 1977). Grading of cheese is usually carried out about eight weeks after the cheese has been made and that is important in determining the disposal of the cheese. In Scotland, a system of three standards of quality is used by the Company of Scottish Cheesemakers Ltd. Cheese of '1st Grade' which conforms closely to the accepted features of the variety, will bring a maximum price on selling it fully matured, 'Graded' cheese which is deficient in some respects requires to be sold soon after grading, otherwise its quality will deteriorate more during curing, and 'No Stamp' cheese which is of very poor quality and normally used in the manufacturing of processed cheese.

Cheese 'grading' which is usually carried out by a professional grader, depends mainly on the assessment of flavour, texture and body, colour, and finish, and the grader will be able to allocate one of the previous standards to the cheese under test by taking a cheese sample (plug) from the whole cheese block using a two edged, curve-blade instrument known as a cheese trier or iron. The grader examines the cheese plug and assesses the texture after careful visual examination of the plug, then the body or firmness of the cheese is assessed by taking a small piece from the cheese plug and working it between the thumb and fingers into a uniform mass. The body or firmness is related to the moisture and fat contents as well as the extent of curing. Finally, the flavour is assessed by placing the worked mass under the nose and observing the flavour.

Professional graders in Scotland do not taste the cheese. The main deficiency in this system is that bitterness is not detected.

Cheese manufacturing procedures affect cheese composition and quality, and most of the defects in texture and body are related to the moisture and fat content of the cheese, and also to the acidity of the cheese. Cheese which is too acid has a crumbly texture. Cheese flavour is affected by the starter culture and the active proteinases released from their dead cells.

Coagulants used in making the cheese have been reported to have an effect on the development of texture and flavour of Cheddar cheese (Emmons et. al., 1976; Green and Foster, 1974; Green and Stackpoole, 1975; Koning, 1978; and Phelan, 1977).

The work of this chapter was aimed at finding the differences in texture, body, flavour and taste of Cheddar cheese which would result from the use of different coagulants.

EXPERIMENTAL

1. Preparing cheese standards

To assist the panel members to know the different properties used in the assessment of cheese quality, four cheese standards were prepared to give the following:-

(a) Standard soft cheese:- This standard cheese was made by adding a low amount (1.5 per cent w/w) of salt to the milled curd. The resultant cheese had a high moisture content (about 39 per cent) and low salt content (1.2 per cent).

(b) Standard firm cheese:- This cheese was made by adding a high amount of salt to the milled curd (3 per cent w/w) allowing 10 minutes after mixing the salt with curd (mellowing) to permit more whey to be expelled. The resultant cheese had a low moisture content (about 34 per cent) giving it a firm body. The salt content was 2.2 per cent.

(c) Standard non-bitter cheese:- This standard was made by adding 2 per cent (w/w) of an active single strain starter (AM_2 and ML8) to the milk. This mixture would not produce bitterness in cheese under the normal condition of cheese making (Crawford, 1977).

The starter is mixed in the rate of 1/3 of the fast acid producing strain (ML8) and 2/3 of the slow acid producing strain (AM_2) just before addition to the milk, and the cheese was then made, following the normal procedure of making Cheddar cheese.

(d) Standard bitter cheese:- A bitter cheese was made by using 3 per cent (w/w) of active single strain starter known to produce bitterness in cheese (HP and ML8) (Crawford, 1977) mixed in equal parts, and by following the normal cheese making procedure.

A solution of 0.005 per cent(w/v) quinine sulphate was also used as a standard for bitter taste (Crawford, 1977).

2. Selecting cheese samples for quality assessment

Cheddar cheese which had been made with different coagulants were selected for sensory evaluation taking into consideration cheese composition and age. All cheese was the same age at examination.

3. Presentation of cheese samples

Cheese samples were set out for examination in a well illuminated and ventilated large room, free from smells of chemicals, and at a temperature of around 16°C. The cheese samples (unknown and standards) were placed on a clean table in the room after they had been tempered to the same temperature of the room. Cheese standards were identified by lables, a code being given to each of the unknown cheeses. Samples of cheese from the standards were also presented as unknowns with codes similar to the other samples.

All cheese samples, whether they were standards or unknown, were presented in identical shape and quantity, and from each cheese sample a 1 kg piece was reserved for texture assessment in addition to a number of cheese fingers from each sample which were made available for the assessment of other properties.

4. Qualtiy assessment

A panel consisted of 8 to 11 persons, all of them having an interst in testing dairy products, acted as graders in the assessment of cheese quality. The panel was asked to assess the cheese samples on the score sheet (table 7:1) supplied, for the following properties:-

(a) Texture (openness):- The term refers to the compactness of the particles of cheese and to the manner of union of the curd particles. A good textured cheese (referred to as very compact and awarded 10 points) is one which has very good fusion between the curd particles and is free from openings or gas holes. On the other hand, an open textured cheese is one which has a large number of holes of various sizes and shapes, and cheese of this quality is awarded 0 on the grading scale.

(b) Body (firmness):- The term refers to the physical properties of consistency which include firmness, elasticity and plasticity. Firm cheese should feel solid and offer a slight resistance to pressure. It should also be free from rough particles of curd and the plug from such cheese should bend before breaking slowly. A cheese with those characteristics is awarded 10 points while weak (soft) spongy, sticky or crumbly cheese should be awarded 0 on the grading scale.

TABLE 7:1

A scoring sheet used by the panel on assessing the quality of Cheddar cheese

GRADER:- _____ DATE:- _____

Sample	Texture (openness) 0 open 10 very compact	Body (firmness) 0 soft 10 firm	Flavour (smell) 0 very bad 10 very good	Bitterness (taste) 0 very bitter 10 not bitter	General Acceptability 0 very poor 10 very good	General Comments
1						
2						
3						
4						
5						
6						
7						
8						

(c) Flavour (smell):- The term refers to the sensation perceived via the olfactory organ in sniffing certain volatile substances. The flavour of Cheddar cheese is derived from a blend of volatile organic compounds resulting from the breakdown of protein and milk fat, and full flavoured cheese should have a clean, fine, nutty and pleasantly sweet smell. A cheese with such flavour should be awarded 10 points, while cheese with unclean, rancid, mouldy, yeasty or with any flavour uncharacteristic of good quality Cheddar cheese flavour should be awarded 0 in the scoring sheet.

(d) Bitterness (taste):- The term refers to the sensation perceived via the taste buds resulting from the presence of certain soluble substances.

Bitter taste inccheese is due to the presence of bitter low molecular weight peptides resulting from the incomplete breakdown of peptides. A cheese free from bitter taste should be awarded 10 points, while cheese with very pronounced bitter taste should be awarded 0 in the scoring sheet.

(e) General acceptability:- The term is not concerned with a specific cheese property, and the scoring depends completely on the judgement of each member of the panel. A cheese which is thought to be very good by any member of the panel is awarded 10 points by that member on the other hand a very poor cheese gains 0 points.

(f) General comments:- In addition to awarding points, the panel members were asked to give their comments and observations on the cheese samples on the specific characteristics given above. Also their preference of the samples they tested.

The quality assessment of Cheddar cheese was carried out on three successive weeks (one trial each week). In the first and second weeks, a total of 12 unknown samples were presented consisting of 4 standard cheeses and 2 experimental cheeses made with different coagulants, all samples were duplicated.

In the third week, 8 unknown samples were presented. Duplicate samples from 4 experimental cheeses made with different coagulants

were examined.

In all three weeks, identified cheese standards as described above were presented to the panel to assist in their quality assessment.

RESULTS

1. First trial:- Results of the quality assessment of 10 week old Cheddar cheese made with calf rennet and with porcine pepsin and four cheese standards, are presented in tables 7:2, 3, 4, 5, 6, 7 and 8.

(a) Texture:- The results showed a highly significant difference between the cheese made with calf rennet (the highest quality) and the cheese made with porcine pepsin (the lowest quality). The difference between their mean values (1.312) is more than the LSD value at 0.1 per cent.

In comparison with the standards, cheese made with calf rennet was found to have a similar texture to the standards for firmness and taste. The standard soft cheese was found to have an extremely open texture, and the mean value for its texture is lower than that of cheese made with porcine pepsin, with highly significant differences for all cheeses. The analysis of the results also revealed a highly significant difference between the graders and a highly significant interation between graders and cheese which means that certain graders from the panel persisted in giving scores to a particular cheese which were different from the scores given by other graders.

(b) Body:- Less differences were noticed in the firmness of the different cheeses and the cheese made with porcine pepsin had a slighly firmer body than that made with calf rennet, but no overall significant difference was noticed between all cheeses.

(c) Flavour:- A highly significant difference was found between the graders in their assessment of flavour, and a less significant difference was noticed between the different cheeses (> 5 per cent). The standard bitter cheese was found to have an unpleasant flavour described as bitter and fruity.

(d) Bitterness:- A highly significant difference was found between the graders, and also between the different cheeses. The difference between the cheeses was because of the bitter standard cheese which had very low scores in comparison to the other cheeses.

TABLE 7:2

Scores awarded by the members of panel for the texture of 10 wk old Cheddar cheese, where a 0 score means open texture and a 10 score means very compact texture

Grader	Standard soft		Standard firm		Standard no Bit		Standard Bitter		Exp. CR		Exp. PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	7	8	8	8	8	8	8	9	8	8	8	7
2	5	7	7	8	8	8	8	9	8	9	8	8
3	4	4	7	6	8	7	6	8	7	7	4	4
4	1	3	9	7	7	5	6	6	4	3	4	6
5	3	5	6	8	9	8	7	8	7	8	6	4
6	4	5	6	8	9	8	6	8	6	8	7	7
7	4	5	5	6	6	5	6	5	6	5	4	4
8	3	4	6	7	7	8	7	8	9	8	5	4
Mean	4,500		7.000		7,437		7.187		6.937		5.625	

TABLE 7:3

Scores awarded by the members of panel for the body of 10 wk old Cheddar cheese, where a 0 score means soft body and a 10 score means very compact body

Grader	Standard soft		Standard firm		Standard no Bitter		Standard Bitter		Exp. CR		Exp. PP	
	1	1	1	2	1	2	1	2	1	2	1	2
1	4	8	4	8	8	8	4	8	8	8	9	4
2	6	8	7	9	7	8	8	7	7	8	8	8
3	5	6	6	5	6	6	5	7	6	5	6	6
4	7	4	5	8	6	5	8	7	6	5	7	4
5	3	3	9	7	5	7	7	5	3	3	8	7
6	8	5	5	4	8	6	7	4	3	8	10	10
7	8	7	7	7	6	8	9	8	8	6	8	9
8	1	8	4	4	9	6	6	8	8	8	6	5
Mean	5.69		6.19		6.81		6.75		6.25		7.19	

TABLE 7:4

Scores awarded by the members of panel for the flavour of 10 wk old Cheddar cheese, where a 0 score means very bad flavour and a 10 score means very good flavour

Grader	Standard soft		Standard firm		Standard no Bitter		Standard Bitter		Exp. CR		Exp. PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	9	6	7	7	8	7	6	8	8	8	6	6
2	7	8	8	8	7	8	8	8	7	8	8	8
3	7	6	6	7	5	6	5	5	7	7	6	7
4	3	7	9	9	5	6	6	0	8	7	8	2
5	4	5	7	8	7	6	2	2	6	7	8	8
6	3	9	4	8	3	1	7	1	8	5	2	2
7	8	6	7	8	8	8	8	8	7	7	7	7
8	6	8	8	9	9	9	6	8	7	7	8	9
Mean	6.37		7.50		6.44		5.50		7.12		6.37	

TABLE 7:5

Scores awarded by the members of panel for the bitterness in 10 wk old Cheddar cheese, where a 0 score means very bitter taste and a 10 score means no bitter taste

Grader	Standard soft		Standard firm		Standard no Bitter		Standard Bitter		Exp. CR		Exp. PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	10	8	9	8	9	8	8	9	9	8	9	8
2	8	10	8	9	9	10	4	7	10	10	8	10
3	8	7	9	7	4	4	5	2	7	7	5	7
4	3	8	10	10	5	5	7	0	10	7	10	2
5	3	5	5	3	5	4	0	0	6	5	8	8
6	0	8	3	7	1	1	7	0	8	6	5	5
7	8	8	5	8	7	8	5	7	5	7	9	9
8	3	8	8	8	9	6	3	6	8	9	9	10
Mean	6,56		7.31		5.94		4.38		7.62		7.62	

TABLE 7:6

Scores awarded by the members of panel for the general acceptability of 10 wk old Cheddar cheese, where a 0 score means very poor quality and a 10 score means very good quality

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. CR		Exp. PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	9	8	7	8	8	8	8	9	9	8	8	8
2	7	8	7	7	7	8	5	6	7	8	6	8
3	7	6	7	7	4	5	5	3	7	7	6	7
4	3	5	9	8	5	5	7	3	6	6	7	3
5	5	5	8	6	7	6	5	4	6	6	8	8
6	3	8	4	7	3	2	7	2	7	6	4	5
7	5	5	7	6	5	5	7	6	6	6	5	5
8	3	6	5	9	8	7	7	6	8	8	6	8
Mean	5.81		6.94		5.81		5.62		7.00		6.37	

TABLE 7:7

Total scores awarded by members of panel for 10 wk old Cheddar cheese, where the maximum score is 50

Grader	Standard soft		Standard firm		Standard no bitter		Standard Bitter		Exp. CR		Exp. PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	39	38	35	39	41	39	34	43	42	40	40	33
2	33	41	37	41	38	42	33	37	39	43	38	42
3	31	29	35	32	27	28	26	25	34	33	27	31
4	17	27	42	42	28	26	34	16	34	28	36	17
5	18	23	35	32	33	31	21	19	28	30	38	35
6	18	35	22	34	24	18	34	15	32	33	27	29
7	33	31	31	34	32	34	35	34	32	31	33	34
8	16	34	31	37	42	36	29	36	40	40	34	36
Mean	28.94		34.94		32.44		29.44		34.94		33.19	

TABLE 7:8

Analysis of variance of the scores awarded by the members of panel for the different criteria of 10 wk old Cheddar cheese

	DF	Texture (openness)		Body (firmness)		Flavour (smell)		Bitterness (taste)		General acceptability		Total scores	
Grader MS,VR	7	13.641	17.939***	7.256	2.721*	17.046	7.084***	33.701	8.108***	12.844	7.658***	249.14	9.521***
Cheese MS,VR	5	20.935	27.531***	4.642	1.741	7.710	3.204*	25.585	6.156***	5.835	3.480*	109.50	4.185**
Grader cheese MS,VR	35	1.802	2.370***	3.199	1.200	3.439	1.429	4.233	1.018	2.140	1.276	30.32	1.159
Residual MS	48	0.760		2.667		2.406		4.156		1.677		26.17	
Mean	95	6.45		6.48		6.55		6.57		6.26		32.31	
SEDM Grader	12	0.356		0.667		0.633		0.832		0.529		2.088	
SEDM cheese	16	0.308		0.577		0.548		0.721		0.458		1.809	
SEDM Grader cheese	2	0.872		1.633		1.551		2.039		1.295		5.115	
SE Grader sample	48	0.872		1.633		1.551		2.039		1.295		5.115	
CV % Grader sample	48	13.5		25.2		23.7		31.0		20.7		15.8	

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

The taste values for cheeses made with calf rennet or porcine pepsin were the best among all cheeses and both sets of cheese were scored equally by the panel.

(e) General acceptability:- A highly significant difference was observed between the graders regarding their different preferences. The difference between the cheeses was less significant (> 5 per cent). The results showed that cheese made with calf rennet had the highest scores of all cheese. The standard bitter had the lowest scores followed by the soft cheese and the non bitter standard.

The analysis of total scores showed a highly significant difference between the graders and a significant difference between the different cheeses (> 1 per cent). The cheese with the highest score was one made with calf rennet and the standard firm cheese, and that made with porcine pepsin also had high total scores close to that of calf rennet. The lowest score cheese was the standard soft and bitter cheeses.

2. Second trial:- Results of the quality assessment of 10 week old Cheddar cheese made with Mucor miehei rennet ('Hannilase' brand) or with a 1:1 mixture of calf rennet and porcine pepsin (commercial '50/50' brand) and cheese standards are presented in tables 7:9, 10, 11, 12, 13, 14 and 15.

(a) Texture:- A highly significant difference was found between the graders in their assessment of texture, and between the different cheeses. The best texture was observed with the standard cheese for firmness, bitterness, and non bitter cheeses. Cheese made with the 1:1 mixture of calf rennet and porcine pepsin also had a good texture but was awarded slightly lower scores than the above standard cheese. Cheese made with Mucor miehei rennet and the standard soft cheese were found to have very open texture and both differed significantly from other cheeses in respect of texture.

(b) Body:- A highly significant difference was found in the firmness of different cheeses due to the highly significant difference between the graders and between the cheeses. As was noticed in the texture, the highest scores for body were awarded to the standard cheese, for firmness, bitterness, and non-bitter cheeses.

TABLE 7:9

Scores awarded by the members of panel for the texture of 10 wk old Cheddar cheese, where a 0 score means open texture and a 10 score means very compact texture

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp (1:1) CR:PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	5	4	9	10	8	10	9	8	4	4	6	5
2	5	2	7	8	7	9	9	7	7	5	7	6
3	8	7	8	9	8	9	9	8	7	9	8	8
4	5	5	7	9	7	9	9	9	5	5	7	6
5	5	4	7	6	8	8	8	9	4	4	7	8
6	7	3	6	7	6	7	8	7	5	5	6	6
7	7	2	7	9	3	9	8	7	3	4	6	7
8	7	6	8	9	8	9	9	9	7	7	8	8
9	8	6	10	10	9	10	10	9	8	7	9	10
10	8	6	9	10	9	10	10	10	9	9	9	9
11	6	4	8	8	7	8	8	9	5	6	7	9
Mean	5.636		8.227		8.091		8.591		5.864		7.364	

TABLE 7:10

Scores awarded by the members of panel for the body of 10 wk old Cheddar cheese, where a 0 score means soft body and a 10 score means very compact body

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp.(1:1) CR:PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	9	6	9	5	8	7	6	7	9	5	6	4
2	8	0	5	8	9	9	9	9	6	8	7	4
3	8	7	8	7	8	8	8	8	7	8	8	7
4	7	9	8	9	9	8	7	9	9	8	9	7
5	6	6	9	7	7	8	8	8	6	5	7	6
6	6	5	6	6	7	6	7	6	6	5	6	5
7	7	8	8	8	7	9	9	8	6	9	8	8
8	5	5	8	8	8	8	9	9	7	8	9	7
9	6	6	7	7	8	8	8	9	7	7	8	6
10	7	7	8	9	8	9	9	8	7	8	7	9
11	6	6	7	8	8	7	7	8	7	7	7	8
Mean	6.36		7.50		7.91		8.00		7.09		6.95	

TABLE 7.11

Scores awarded by the members of panel for the flavour of 10 wk old Cheddar cheese, where a 0 score means very bad flavour and a 10 score means very good flavour

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp.(1:1) CR:PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	6	6	5	8	7	8	8	4	5	5	5	7
2	7	7	8	7	7	8	6	8	8	8	8	7
3	8	7	8	8	8	8	8	8	8	8	8	8
4	7	9	8	8	8	9	8	8	7	8	9	7
5	7	5	9	9	7	8	6	8	7	6	8	6
6	7	7	5	7	6	6	5	5	4	5	6	6
7	6	8	8	8	6	8	7	8	6	4	7	6
8	9	9	8	9	9	9	9	9	6	8	9	9
9	9	8	9	9	9	9	8	9	9	9	8	9
10	8	8	7	8	8	8	9	8	9	7	8	8
11	7	6	6	7	7	7	8	8	6	6	7	7
Mean	7.318		7.682		7.727		7.500		6.773		7.409	

TABLE 7:12

Scores awarded by the members of panel for the bitterness in 10 wk old Cheddar cheese, where a 0 score means very bitter taste and a 10 scores means no bitter taste

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp.(1:1) CR:PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	6	6	5	8	7	8	8	2	5	5	4	3
2	8	10	8	9	3	9	7	8	3	7	4	5
3	8	7	8	8	8	8	3	6	8	7	6	6
4	6	8	9	7	9	7	4	4	8	7	8	9
5	4	5	5	9	6	4	8	5	4	6	6	8
6	7	4	7	7	6	6	5	4	3	3	7	6
7	8	7	7	8	7	8	9	3	5	4	7	7
8	8	8	7	9	8	9	4	7	5	4	8	8
9	8	7	9	9	8	8	5	7	4	6	6	8
10	8	8	7	7	10	8	6	7	9	7	8	7
11	7	7	6	7	8	7	7	6	7	7	6	7
Mean	7.05		7.55		7,36		5.68		5.64		6.55	

TABLE 7:13

Scores awarded by the members of panel for the general acceptability of 10 wk old Cheddar cheese, where a 0 score means very poor quality and a 10 score means very good quality

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp.(1:1) CR:PP	
	1	2	1	2	1	2	1	3	1	2	1	2
1	6	5	6	8	7	8	8	2	3	4	4	5
2	6	4	7	7	4	9	8	8	5	6	7	5
3	8	7	8	8	8	8	4	7	7	7	7	7
4	7	8	9	8	9	9	7	7	8	7	9	8
5	4	3	8	7	8	6	7	8	3	6	7	7
6	7	6	6	7	6	6	5	4	3	4	7	7
7	7	7	7	8	7	8	9	3	4	5	7	7
8	8	8	7	9	9	9	7	8	6	5	8	8
9	8	7	8	9	8	8	7	8	6	7	7	8
10	8	8	7	8	9	8	8	7	8	7	7	7
11	7	7	6	6	8	7	7	6	6	7	6	7
Mean	6,636		7.455		7.682		6.591		5.636		6.909	

TABLE 7:14

Total scores awarded by the members of panel for 10 wk old Cheddar cheese where the maximum score is 50

Grader	Standard soft		Standard firm		Standard no bitter		Standard bitter		Exp. MM		Exp.(1:1) CR:PP	
	1	2	1	2	1	2	1	2	1	2	1	2
1	32	27	34	39	37	41	39	23	26	23	25	24
2	34	23	35	39	30	44	39	40	29	34	33	27
3	40	35	40	40	40	41	32	37	37	39	37	36
4	32	39	41	41	42	42	35	37	37	35	42	37
5	26	23	38	38	36	34	37	38	24	27	35	35
6	34	25	30	34	31	31	30	26	21	22	32	30
7	35	32	37	41	30	42	42	29	24	26	35	35
8	37	36	38	44	42	44	38	42	31	32	42	40
9	39	34	43	44	42	43	38	42	34	36	38	41
10	39	41	38	42	44	43	42	40	42	38	39	40
11	33	30	33	36	38	36	37	37	31	33	33	38
Mean	33.00		38.41		38.77		36.36		31.00		35.18	

TABLE 7:15
Analysis of variance of the scores awarded by the members of panel for the different criteria of 10 wk old Cheddar cheese

	DF	Texture (openness)		Body (firmness)		Flavour (smell)		Bitterness (taste)		General acceptability		Total scores	
Grader MS, VR	10	14.823	11.716***	5.705	3.765***	10.980	14.942***	6.138	3.092**	8.814	6.844***	177.67	15.07***
Cheese MS, VR	5	35.141	27.776***	8.539	5.636***	2.626	3.573	15.145	7.631***	11.618	9.021***	204.64	17.36***
Grader cheese MS,VR	50	1.321	1.044	1.123	0.741	0.766	1.042	2.609	1.314	1.528	1.187	15.34	1.30
Residual MS	66	1.265		1.515		0.735		1.985		1.288		11.79	
Mean	131	7.295		7.30		7.402		6.64		6.818		35.45	
SEDM Grader	12	0.459		0.503		0.350		0.575		0.463		1.402	
SEDM cheese	22	0.339		0.371		0.259		0.425		0.342		1.035	
SEDM Grader cheese	2	1.125		1.231		0.857		1.409		1.135		3.433	
SE Grader sample	66	1.125		1.231		0.857		1.409		1.135		3.433	
CV % Grader sample	66	15.4		16.9		11.6		21.2		16.6		9.7	

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

Cheeses made with Mucor miehei rennet and the 1:1 mixture of calf rennet and porcine pepsin were found to have good body but were awarded slightly lower scores than the above standards. The softest body was found in the standard soft cheese.

(c) Flavour:- A highly significant difference was obtained between the graders in their assessment of flavour, and a significant difference was found between the flavour scores of different cheese. The difference was mainly because of the cheese made with Mucor miehei rennet which had the lowest flavour scores, and differed significantly from all cheeses. The flavour defects noticed were described as yeasty, acid, or fruity flavour.

(d) Bitterness:- A significant difference was found between the graders in their assessment of cheese for bitterness, and a highly significant difference was found between the different cheeses. The standard cheese for softness, firmness, and non bitter cheeses were the least bitter. Cheese made with the 1:1 mixture of calf rennet and porcine pepsin came after the above standards but with significant difference from them (> 5 per cent). Cheese made with Mucor miehei rennet and the standard bitter cheese were both equal in the degree of bitterness and differed significantly from the cheese made with the 1:1 mixture (> 5 per cent).

(e) General acceptability:- A highly significant difference was found in the general acceptability of the different cheeses, and that was because of the differences between graders and between cheeses. The cheese given the highest grade score was the non bitter standard cheese followed by the firm standard cheese and the 1:1 mixture of calf rennet and porcine pepsin cheese. The scores for the bitter and soft standard cheeses followed the above two cheeses with significant difference. The least acceptable cheese was the one made with Mucor miehei rennet whose grade scores differed significantly from those for all other cheeses.

The analysis of total scores showed a highly significant difference because of differences between graders and between cheeses. The best cheeses were the standards for firmness and non-bitter taste and they differed significantly from other cheeses. The standard bitter

cheese and 1:1 mixture of calf rennet and porcine pepsin cheese were similar and came in the second place. The lowest total scores were awarded to the soft cheese standard and Mucor miehei rennet cheese and both differed significantly from other cheeses.

3. Third trial:- In this trial, 8 samples of 12 week old cheese made with four different coagulants were assessed for their quality. The results are presented in tables 7:16, 17, 18, 19, 20, 21 and 22.

(a) Texture:- The results revealed a highly significant difference in the scores due to graders. The variance due to the different coagulants was not significant. The texture of the cheese made with calf rennet was the best among the cheeses and differed significantly (> 5 per cent) from the texture of cheese made with porcine pepsin.

(b) Body:- A highly significant difference was found between the graders in their assessment of the firmness of cheese. The difference between the firmness of different cheese was less significant (> 5 per cent). Cheese made with porcine pepsine had the highest score for firmness and this differed significantly from the three other cheeses, and especially from the cheese made with Mucor miehei rennet which had the weakest body.

(c) Flavour:- A highly significant difference was found between the graders in their assessment of flavour, but no significant difference was found due to the use of different coagulants in cheese making. Cheese made with porcine pepsin had the highest scores for flavour followed by the cheese made with the 1:1 mixture of calf rennet and porcine pepsin, calf rennet, and Mucor miehei rennet cheeses. The differences between all cheeses were not significant except between the cheese made with porcine pepsin and those made with Mucor miehei rennet which was significant at >5 per cent.

(d) Bitterness:- A highly significant difference was obtained between the graders in their assessment of bitterness in cheese samples, but there was no significant difference between the different cheeses in relation to bitterness. Cheese made with porcine pepsin was the least bitter followed by the cheese made with the 1:1 mixture

TABLE 7:16
Scores awarded by the members of panel for the texture of 12 wk old Cheddar cheese made with different coagulants: where a 0 score means open texture and a 10 score means very compact texture

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	10	10	10	10	9	10	10	10
2	9	10	10	9	9	10	9	10
3	9	10	9	9	8	9	8	10
4	7	8	8	7	7	8	7	7
5	8	9	8	8	7	8	7	8
6	8	9	9	8	8	8	7	8
7	9	9	9	9	9	9	9	9
8	8	9	6	7	8	8	6	8
9	7	9	8	7	7	9	7	8
10	8	9	8	8	7	8	8	8
Mean	8.750		8.350		8.300		8.200	

TABLE 7:17

Scores awarded by the members of panel for the body of 12 wk old Cheddar cheese made with different coagulants, where a 0 score means soft body and a 10 score means very compact body

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	6	7	6	6	7	8	8	7
2	6	6	6	6	5	7	5	7
3	9	6	7	8	8	9	9	9
4	6	6	6	6	6	6	6	6
5	8	7	7	8	7	8	9	9
6	8	7	7	8	8	8	8	8
7	8	7	7	8	7	7	9	8
8	7	8	6	5	7	7	8	8
9	5	8	4	7	4	7	6	9
10	7	7	8	6	7	8	7	7
Mean	6.95		6.60		7.05		7.65	

TABLE 7:18

Scores awarded by the members of panel for the flavour of 12 wk old Cheddar cheese made with different coagulants, where a 0 score means very bad flavour and a 10 score menas very good flavour

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	9	7	9	8	9	9	9	9
2	9	8	6	8	8	9	9	9
3	7	8	7	7	8	8	8	8
4	8	7	7	7	6	8	7	6
5	7	9	7	8	8	8	8	8
6	8	8	8	8	7	7	8	8
7	7	8	8	8	8	8	9	8
8	8	8	8	8	8	8	8	8
9	7	7	8	9	8	8	8	9
10	8	7	8	7	8	7	7	8
Mean	7.759		7.650		7.900		8.100	

TABLE 7:19

Scores awarded by the members of panel for the bitterness in 12 wk old Cheddar cheese made with different coagulants, where a 0 score means very bitter taste and a 10 score means no bitter taste

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	5	4	8	7	8	5	5	4
2	8	3	6	7	10	4	10	9
3	8	7	7	8	9	9	10	9
4	7	4	4	7	7	7	7	6
5	8	8	7	8	8	9	9	9
6	9	8	8	8	8	8	8	8
7	9	8	7	8	8	8	8	8
8	8	7	8	8	8	8	8	8
9	8	6	8	8	8	6	7	9
10	8	7	7	7	6	8	7	8
Mean	7.00		7.30		7.60		7.85	

TABLE 7:20

Scores awarded by the members of panel for the general acceptability of 12 wk old Cheddar cheese made with different coagulants where a 0 score means very poor quality and a 10 score means very good quality

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	7	7	8	8	7	7	7	6
2	8	5	6	7	8	5	7	8
3	8	8	7	8	8	9	9	9
4	8	6	5	7	7	8	7	7
5	8	8	7	8	8	9	8	9
6	9	8	8	8	8	8	8	8
7	7	8	7	8	8	8	8	9
8	8	7	8	8	8	8	8	8
9	7	8	7	8	7	6	6	9
10	8	7	7	7	7	8	7	8
Mean	7.5000		7.350		7.600		7.800	

TABLE 7:21

Total scores awarded by the members of panel for 12 wk old Cheddar cheese made with different coagulants, where the maximum score is 50

Grader	CR		MM		(1:1) CR:PP		PP	
	1	2	1	2	1	2	1	2
1	37	35	41	39	40	39	39	36
2	40	32	34	37	40	35	40	43
3	41	39	37	40	41	44	44	45
4	36	31	30	34	33	37	34	32
5	39	41	36	40	38	42	41	43
6	42	40	40	40	39	39	39	40
7	40	40	38	41	40	40	43	42
8	39	39	36	36	39	39	38	40
9	34	37	36	38	34	36	34	44
10	39	37	38	35	35	39	36	39
Mean	37.95		37.25		38.45		39.60	

TABLE 7:22

Analysis of variance of the scores awarded by the members of panel for the different criteria of 12 wk old Cheddar cheese made with different coagulants

	DF	Texture (openness)		Body (firmness)		Flavour (smell)		Bitterness (taste)		General acceptability		Total score	
Grader MS, VR	9	6.133	12.912***	4.924	5.396***	1.522	4.059***	6.424	4.247***	2.451	3.566**	42.924	7.894***
Cheese MS, VR	3	1.167	2.456	3.813	4.178*	0.767	2.044	2.712	1.793	0.713	1.036	19.579	3.601*
Grader cheese MS, VR	27	0.278	0.585	0.535	0.586	0.415	1.106	1.453	0.961	0.444	0.646	4.542	0.835
Residual MS	40	0.475		0.913		0.375		1.512		0.688		5.437	
Mean	79	8.400		7.06		7.850		7.44		7.563		38.31	
SEDM Grader	8	0.3446		0.478		0.306		0.615		0.415		1.166	
SEDM cheese	20	0.2179		0.302		0.194		0.389		0.262		0.737	
SEDM Grader cheese	2	0.6892		0.955		0.612		1.230		0.829		2.332	
SE Grader sample	40	0.6892		0.955		0.612		1.230		0.829		2.332	
CV % Grader sample	40	8.2		13.5		7.8		16.5		11.0		6.1	

* Significant at 5 per cent level

of calf rennet and porcine pepsin, Mucor miehei rennet, and calf rennet in that order,but the difference between all cheeses was not significant except the scores for cheese made with porcine pepsin and calf rennet which were significantly different at > 5 per cent.

(e) General acceptability:- A significant difference was found between the graders, but no significant difference was found between the different cheeses. Cheese made with porcine pepsin was awarded the highest scores followed by cheese made with 1:1 mixture of calf rennet and porcine pepsin, calf rennet, and Mucor miehei rennet cheeses in that order with no significant difference between all of them.

Analysis of the total scores showed the same highly significant difference between the graders as was found in relation to their scoring of individual criteria. A significant difference at 5 per cent level was observed between the cheese due to the use of different coagulants in the cheese making process. The results indicated that cheese made with porcine pepsin was most acceptable followed by 1:1 mixture of calf rennet and porcine pepsin, calf rennet and Mucor miehei rennet cheese. The difference between porcine pepsin cheese and both calf rennet and 'Hannilase' cheeses is significant at > 5 per cent.

DISCUSSION

The organdeptic evaluation of the quality of cheese is a difficult task especially for persons who do not have full time official grading duties, it requires a complete knowledge of the typical characteristics of the cheese variety under test, as well as the ability of the individual to detect and diagnose early signs of defects in the cheese. During the three trials for quality assessment a highly significant difference (> 0.1 per cent) was always found because of the graders and their differences in the assessment of the defined characteristics of the cheese. The differences between the graders could be because of one or more of the following reasons:-

1. The number of cheese samples to be tested each time.
2. The properties which the panel members were asked to assess.
3. The diagnostic ability of the individual members of the panel in relation to cheese quality.

The total number of samples presented each time has a great effect on the accuracy of grading, and large number of samples (above 10) will affect the ability of graders to give the right decision especially if the test will involve tasting of samples.

The properties of cheese which the panel were asked to judge could affect the results especially when some members of the panel were not able to judge a specific cheese property because either (a) they did not understand the property well, or (b) the property was not clear. The first reason seems to be the true reason for the differences especially in the case of texture and body assessment, where it was noticed that most of the panel members were confused by the two properties, where on one hand low scores were awarded for a cheese body with open texture while on the other, low scores were awarded for cheese texture because of dry or crumbly body.

Differences between the panel members themselves were very wide and it could be the sole reason behind the significant differences noticed. Members of the panel were found to have four different attitudes:-

1. Members knew exactly the different cheese properties and gave reasonable scores,

2. Members knew exactly the different cheese properties but gave extreme scores (i.e. either very high or very low within the scale of points),

3. Members knew exactly the different cheese properties but always gave low scores,

4. Members did not know exactly the different cheese properties and always gave similar scores for all kinds of qualities.

The presence of the above attitudes in the panel is the source of most of the variation in the results.

In the first two trials, the significant differences found between the cheese were because of the presence of 'standard' cheese with defined characteristics e.g. firm body, along with the experimental cheese.

The results showed that most of the differences were between the 'standard' cheese and very little difference was observed between the experimental cheeses. In certain cases when defects were noticed in the experimental cheeses i.e. bitterness in the cheese made with Mucor miehei rennet,or body defects in the cheese made with porcine pepsin, the reason might have been caused by the inclusion of grade scores from 'standard' cheese or incorrect judgement associated with one or more of the factors mentioned above.

The differences found between the experimental cheeses in the first two trials were checked in the third trial by eliminating the 'standard' cheese from the samples for quality assessment. The results showed the same significant difference between the panel members, but there was no significant difference between the four experimental cheeses, and that proves the effect of the presence of cheese standards on the quality assessment of the experimental cheeses.

The trials of quality assessment conducted in this work can not be considered as 'Grading', because in cheese grading, an official grader

must examine the cheese, and allocate to the cheese one of three grades as applied in Scotland (1st Grade, Graded, and No stamp), or one of four standards as applied in the USA (AA, A, B, and C grades) (Davis, 1965; Herschdoerfer, 1968; Nelson and Trout, 1965; and Kosikowski, 1977). In both systems, the flavour is considered of great importance and makes up 40 to 45 per cent of the total available score points. Second in importance is the body and texture of cheese and these quality aspects make up about 30 per cent of the total available scores. The remaining 25 per cent will be for the colour and finish of the cheese. The range of scoring will be as follows (Nelson and Trout, 1965).

	No defects	Slight defects	Definite defects	Pronounced defects
Flavour	40	36 - 39	34 - 38	31 - 37
Body and Texture	30	28 - 29	27 - 28	25 - 27

The effect of detecting a defect in flavour is more than the effect of detecting a defect in the body and texture although it depends on the type of defect. In the case, however, of the scoring applied on the quality assessment trials described above, all cheese properties were considered equal in their importance and wider limits were applied to scoring i.e. a cheese with pronounced bitterness could be awarded 0 out of 10 points in the quality assessment, but it might be awarded 34 out of 45 points in the grading system. The wider limit of scoring increased the variance of the results, but it was important to detect even slight differences in the qualities of the different cheeses.

The results of quality assessment indicate an overall similarity for the experimental cheeses which means that the use of Mucor miehei rennet, 1:1 mixture of calf rennet and porcine pepsin, or porcine pepsin rennets in cheese making will not result in the production of poor quality Cheddar cheese. This opinion agrees with the reults of Sherwood (1935), Yamamoto et. al. (1955), Maragoudakis et. al. (1961), Melachouris and Tucky (1963 and 1964), Emmons (1968), Fox (1969) , Emmons et. al. (1971), Sardinas (1972), Phelan (1973), Carini et. al. (1974), Dennien (1975), Phelan (1975), Phelan (1977), and

Wong et. al. (1977).

The formation of defects in Cheddar cheese such as bitterness or fruity flavour which were noticed in the trials as a result of using porcine pepsin were reported by Davies et. al. (1934), Green (1972) and Eino (1975).

Mucor miehei rennet was reported by Dinesen et. al. (1975) to cause the production of inferior flavour in Cheddar cheese (compared with the flavour of cheese made with calf rennet) from the age of 3 weeks to 3 months, but the flavour of cheese made with Mucor miehei rennet had improved after 6 months ripening and continued to be good after 15 months ripening.

CONCLUSION

Standard cheese for use in grading trials were successfully made by the use of strains reputed to produce bitterness, and by processing techniques such as variation in salt addition.

Comments are made on the inclusion of standard cheese samples in grading trials.

The quality of Cheddar cheese made with four different coagulants differed very little. The likelihood of defects occurring in cheese made with Mucor miehei rennet is greater than in cheese made with calf rennet.

The different rates of proteolysis obtained with different coagulants had no adverse effect on the quality of cheese after it had been cured for 12 weeks.

The quality assessment of Cheddar cheese by non-official graders can provide a satisfactory indication about the quality of cheese provided that certain properties of cheese e.g. bitterness, softness, and openness are well defined and explained to the members of the panel.

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