THE CALCIUM-PARACASEINATE-PHOSPHATE-COMPLEX UNDER CONDITIONS SIMILAR TO THOSE IN CHEESE

A. M. M. F. MONIB

THEOREMS

I

The cheese extracting methods of VAN SLYKE and BOSWORTH (1907) as well as the one of SIRKS (1943) used for studying the degradation of protein during the ripening of cheese are not correct.

II

The conclusion of VAN DAM that 5 per cent salt in cheese serum is necessary to make a cheese soft and mellow has no sound experimental basis.

Mulder and Monib (1962) XVIth Int. Dairy Congr. IV, 539

III

In warm-climate countries the sterilization of milk at ultra-high temperatures is to be preferred to pasteurization.

IV

The fat and protein percentages are not the only factors responsible for the inferior quality of hard cheeses made from buffaloes milk. The part played by the paracasein-complex should also be investigated.

V

To completely utilize the whey it is recommended to salt the curd and not the milk in manufacturing Egyptian 'white' soft cheese. Further information should be collected on the effect of such treatment.

VI

Preparing the milk and its by-products in various palatable forms is essential to promote the habit of drinking milk and milk by-products.

VII

There is no objection from a nutritional point of view to standardize pasteurized milk to contain 2 or 3 per cent fat.

VIII

Encouraging the development of agricultural co-operative societies is the best policy for improving agricultural production in Egypt.

IX

Friesian cows are more suitable than many other foreign breeds as well as native cows in Egypt.

El-Itriby and Asker – Empire J. of Exp. Agr. 26 (1958) 314

X

In order to increase milk production in Egypt, the dairy cattle should mainly consist of Friesian cows and selected buffaloes.

A. M. M. F. MONIB
Wageningen, december 1962

THE CALCIUM-PARACASEINATE-PHOSPHATE-COMPLEX UNDER CONDITIONS SIMILAR TO THOSE IN CHEESE

(MET EEN SAMENVATTING IN HET NEDERLANDS)

Dit proefschrift met stellingen van

AHMED MONIB MOHAMMED FARID MONIB,

geboren te Cairo (Egypte), 3 september 1924, is goedgekeurd door de promotor, Dr. H. MULDER, hoogleraar in de zuivelbereiding en de melkkunde.

De Rector Magnificus der Landbouwhogeschool
W. F. EIJSVOOGEL

Wageningen, 30 oktober 1962

THE CALCIUM-PARACASEINATE-PHOSPHATE-COMPLEX UNDER CONDITIONS SIMILAR TO THOSE IN CHEESE

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS, IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING, DE WEG- EN
WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 7 DECEMBER 1962 TE 16 UUR

DOOR

A. M. M. F. MONIB

H. VEENMAN EN ZONEN N.V. – WAGENINGEN – 1962

THE CALCIUM-PARACASEINATE-PHOSPHATE-COMPLEX UNDER CONDITIONS SIMILAR TO THOSE IN CHEESE

THESIS

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WAGENINGEN, HOLLAND
ON FRIDAY, 7 DECEMBER 1962 AT 16 HOURS

BY

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H. VEENMAN & ZONEN N.V. - WAGENINGEN - 1962

To my parents
To my wife
To my children

Dit proefschrift werd bewerkt onder leiding van Prof. Dr. H. MULDER in het laboratorium voor zuivelbereiding en melkkunde van de Landbouwhogeschool. De auteur betuigt hierbij zijn diepe erkentelijkheid jegens zijn promotor.

Overdruk

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(met een samenvatting in het Nederlands)

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CONTENTS

1. GENERAL OUTLINE

1.1. Introduction

Cheese is usually made by adding rennet to milk. The rennet converts the casein of the milk into paracasein and this is precipitated as a complex of calcium paracaseinate and calcium phosphate. The curd is formed at the same pH as that of milk for some kinds of cheese, while for others the milk is made more acid, or 'ripened', before renneting. This is usually brought about by adding the proper amount of cheese starter of lactic acid bacteria. In hard cheeses all the lactose present is converted into lactic acid during the first few days causing a rapid drop in the pH-value of the cheese.

Cheese is salted at an earley stage during its processing. This is done either by mixing the salt with the curd before pressing, by soaking the cheese in a brine solution after it has been pressed, or again by rubbing the salt on the surface of the cheese.

The main components of cheese are fat, calcium-paracaseinate-calcium phosphate-complex, lactic acid, sodium chloride and water. The acid and the salt greatly influence the paracaseinate-complex and they determine many of the characteristics of the finished product. The chemical reactions between lactic acid, salt and calcium-paracaseinate-phosphate-complex are usually accompanied by important physico-chemical changes in properties of calcium paracaseinate such as its water binding capacity and swelling.

Different enzymes hydrolyze about 25 per cent of the paracasein (22) to proteoses, polypeptides and amino acids during the ripening of the cheese. The products of hydrolysis are partially soluble in water in contrast with the unhydrolyzed paracasein. The latter which represents about 75 per cent of the cheese proteins, would be the main factor determining the consistency and the other physical properties of the ripened cheese. A greater knowledge of the changes that take place in the undissolved fraction under the effect of acid and salt is therefore important.

This complex of changes affects the cheese amongst other things by transforming it from a tough, rubber-like material to a product with a mellow, smooth and plastic consistency.

1.2. Review of literature

The influence of lactic acid and salt on the properties of cheese has long been known. Van Slyke and Hart (1902, 1905) and van Slyke and Bosworth (1907) had examined this aspect in Cheddar cheese as early as the beginning of this century. Few years later van Dam (1910, 1911, 1912) studied this extensively in Edam cheese. They were followed by many others. A survey of the available literature reveals that the various investigators approached the complicated chemical, physical and biological reactions in cheese by conducting their experiments in three different ways, viz. cheese extracting methods, paracasein-complex suspensions and on cheese juice expelled by pressing.

1.2.1. *Studies on cheese extracts*

Van Slyke and Hart (1902) and van Slyke and Bosworth (1907)

developed an extraction method, using 25 grams of cheese and extracting with water warmed to 55 °C until the extract amounted to one litre. The water soluble nitrogen was determined in the filtrate while the residue from the extraction was similarly treated with a 5 per cent solution of sodium chloride to yield what they called, the 'salt soluble' fraction.

In Cheddar cheese, VAN SLYKE and BOSWORTH (1907) found that within about ten hours from the start of pressing about 90 per cent of the total nitrogen was in the form of a 'salt soluble fraction'. Thereafter, the salt soluble fraction decreased rapidly, while the water soluble nitrogen increased. They also observed that the calcium and phosphate compounds of the freshly prepared cheese were insoluble, then gradually became soluble until after two weeks about 80 per cent of the calcium and all the phosphates were found in the extracting solution. After this period the composition appeared to remain constant.

SIRKS (1943) observed a lack of uniformity in the methods used in the preparation of the cheese extracts by the many other investigators. He modified the extraction method in order to improve the reproducibility of the results. In his method 40 grams of cheese were extracted with three portions of water of 50 °C and the extract was then made up ot one litre. Thymol in alcohol was used as a preservative. The flasks were then shaken in a water bath at 25 °C for 16 hours. He used this extraction method for studying the effect of pH on the protein degradation of Edam cheese and its relation to the consistency and the development of flavour.

NAUDTS and DE VLEESCHAUWER (1959) studied the retention of calcium in fresh soft cheese. In order to estimate what they called soluble calcium, they mixed 20 g of cheese with 100 ml of water, centrifuged and filtered twice, and then determined the calcium in the filtrate. The undissolved calcium was estimated after washing the residue twice and centrifuging it. Their results showed that in the case of soft cheese almost all the calcium was found in the extract.

1.2.2. *Studies on paracasein-complex suspensions*

VAN DAM (1911) was the first to examine the effect of the hydrogen-ion and salt concentration on the swelling and solubility of paracasein-complex. He used in his study a dry preparation of a well washed rennet curd. One gram of this preparation was suspended in 50 ml solution of lactic acid containing different salt percentages. He found that at pH 5.5 and at 5 per cent salt concentration, nearly all the calcium paracaseinate was in the extracting solution. He was led to the conclusion that the behaviour of the calcium paracasein in his experiments could be considered similar to conditions actually present in Edam cheese with respect to best consistency and swelling.

The results obtained by VAN DAM aroused much interest and several investigators repeated his work in different ways in an attempt to elucidate some of the many problems concerning cheesemaking and curing.

ORLA-JENSEN, MEYER and ORLA-JENSEN (1914-1915) studied the solubility of casein and paracasein-complex in a salt solution. They conducted their investigations on three per cent casein and paracasein-complex suspensions. They also observed a decrease in the dissolved paracasein when a six per cent suspension of what they called 'monocalcium-paracaseinate' was used.

SHARP and MCINERNEY (1936) used suspensions of one gram of dry casein and dry rennet curd in 100 ml solution of different salts, viz. sodium fluoride, sodium chloride and sodium iodide at different pH values in order to study the effect of different sodium halogens on the solubility of casein and paracasein-complex. They found that these salts differed in the degree to which they peptized casein and paracasein. This varied also with pH. The maximum peptization effect of sodium chloride on paracasein-complex was between pH 5.5 and 6.0. They concluded that the texture of Cheddar cheese is affected favourably by the peptizing action of sodium chloride on paracasein in the pH zone of 5.5 and 6.

KIERMEIER and SCHATTENFROH (1958) in their study on the slime-rind defect of Camembert cheese investigated the effect of pH and sodium chloride on the swelling and solubility of casein and paracasein-complex. When using four per cent casein and calcium paracasein suspensions in solutions containing different concentrations of salt and lactic acid, they found that the maximum quantity of the paracasein (about 80 per cent) was dissolved at a salt concentration of three per cent and pH values between 5.0 and 5.3. These results led them to conclude that in order to avoid 'deswelling' in hard cheese, the pH-value should not drop below 5.0. They also observed a decrease in the quantity of paracasein dissolved when they increased the paracasein-complex concentration. It should be noticed that these two authors did not measure the swelling directly. They used the figures for the quantity of paracasein which dissolved in their experiments as an indication for the swelling. They based this on results obtained by LOEB and LOEB, and cited by SUTTERMEISTER (1927). In these experiments a number of granules of isoelectric casein of a known diameter were suspended in 50 ml of water containing different acids at different concentrations. The swelling was determined by measuring the increase in diameter of the granule with a micrometer under the microscope at different time intervals. They observed a gradual increase in the diameter of the granules at different time intervals. This was then followed by the complete dispersion of the casein. The figures indicate a relationship between swelling and solubility of a few grams of pure casein suspended in a large volume of an acid solution. In such a situation the swelling is unlimited and the passing into solution of the casein at this point indicates that the 'gel limit' of the casein has been exceeded. However, these results do not represent the swelling taking place in cheese, where the conditions are quite different and the swelling is of a limited nature.

BELOUSOV (1959) investigated the changes in the composition of the calcium-paracaseinate-complex under the influence of lactic acid and its relation to the consistency of Edam cheese. He ignored however the effect of salt. Ten to twelve grams of air dried preparation freshly isolated from a rennet curd were mixed with 200 ml water, acidified with lactic acid, and kept at 5-7 °C for 10-15 days to allow an equilibrium between the lactic acid and calcium-paracaseinate-complex to be reached. After removal of the supernatant liquid the undissolved part of the preparation was washed and then used for analysis. In order to calculate the calcium bound to the protein, either in the fresh preparation or in the preparation suspended in lactic acid solution, BELOUSOV assumed that the inorganic phosphate occured in the form of tricalcium phosphate.

Apart from the studies made on the cheese extracts and the paracasein-complex suspensions, a few investigations have been carried out directly on the cheese serum.

BARTHEL, SANDBERG and HAGLUND (1928) developed a method in which the cheese juice was expelled by applying high pressure to a mixture of the finely divided cheese and fine sand. They found no difference in the nitrogen content of portions of the serum successively obtained in the course of pressing the cheese. They also showed that the cheese serum still contained active rennet enzyme.

1.2.3. *Studies on cheese juice*

SANDBERG, HAGLUND and BARTHEL (1930) used cheese serum to study the degradation of protein and its relation to the maturity of different types of cheese after varying periods of ageing. DE VLEESCHAUWER and HEYNDRICKX (1948) used cheese serum to examine the nitrogen compounds produced in cheese during ripening.

Neither the hydrogen ion concentration of the cheese nor its salt content were reported in the two latter investigations and, so far as the present investigation is concerned, little value can be attached therefore to the results.

1.2.4. *Effect of acid and salt on the physical properties of cheese*

Although much work has been done on the way acid and salt influence the chemical changes of the calcium-paracaseinate-phosphate-complex, little has been reported on their effect on the physical properties of paracasein, in spite of the enormous practical importance.

BOEKHOUT and OTT DE VRIES (1909) and also VAN DAM (1912) found that an excess of free lactic acid caused a hard, short and brittle texture in Edam cheese. RAADSVELD and MULDER (1949, a, b) investigated the effect of temperature, moisture and pH on the chemical, rheological and organoleptic properties of Edam cheese during its ripening. They reported that the pH and salt content exercise considerable influence on the rheological properties of cheese. The elasticity, viscosity and rigidity which increased as ripening progressed in a cheese were always considerably higher with a higher acidity. WATSON (1929) studied the relation between the hydrogen-ion concentration and the texture of Emmenthal cheese. He found that the production of a desirable soft texture did not necessarily coincide with an increase in water soluble nitrogen and amino nitrogen. Quite the opposite was in fact the case. He also obeserved that cheeses with different pH values at the same moisture content had different texture properties. He considered it probable that variations in the colloidal structure of the cheese as shown by difference in its binding of water may exert an important effect upon the texture. WATSON used differences in the freezing points of cheeses containing about the same amount of moisture as an indication for the differences in the quantity of the bound water. He also centrifuged ground cheese samples and measured the liquid expelled. The depression of the freezing point and the quantity of liquid expelled could give him an idea of the water binding capacity of the cheese. WAUSHKUHN (1953) noted that subjecting the curd to different temperatures after renneting affects the ability of the cheese produced to bind water accordingly. He also found that the swelling capacity of protein and especially the water-binding capacity of the cheese were important factors in the devel-

opment of the bacterial flora, consistency and flavour during ripening. Cheeses with similar pH-values and salt content but with a poor ability to bind water showed serious texture and flavour defects. PEJIC (1955) measured the swelling capacity of Kachkaval cheese by weighing the samples before and after 24 hours soaking in different concentrations of sodium chloride solutions. He reported swelling capacities ranging from 5 to 45 per cent and increasing as ripening advanced. He concluded that the swelling of Kachkaval cheese could be a useful measure to evaluate the changes in its colloidal properties during ripening.

1.2.5. *Discussion on the literature cited*

The survey of literature revealed that the results obtained with both extraction methods and paracasein suspension experiments were far from representing the actual conditions found in cheese.

In the extraction methods the cheese sample is diluted twentyfive to forty times. They serve well to study quantitatively the water-soluble products of protein decomposition during the different stages of cheese-ripening. Where, however, this method is used to estimate the water-soluble fractions of the casein, calcium and phosphates in cheese, it must be borne in mind that soluble and dissolved are not the same. The soluble substances as estimated in an extract are not necessarily present in solution in the cheese. Furthermore, many secondary or reverse reactions may take place during extraction mainly as a result of changes in the pH of the extract. This in turn affects the chemical and colloidal character of the components in the cheese sample.

This applies also to paracasein suspensions where these are used to study the percentage dissolved of different substances and their relation to the chemical and physical properties of the cheese.

The calcium-paracasein-calcium-phosphate-complex is the mother substance in cheese and the changes it undergoes determine many of the characteristics of the finished product. All the experiments described were made on a few grams of calcium-paracasein-complex suspended in 100 ml of lactic acid solution. This gives a calcium-paracasein to water ratio of about (1 : 100 to 5 : 100) while the same ratio in semi-hard cheese is about 1 : 2.5 and in hard cheese (Dutch cheese) about 1 : 2.

These studies of the paracasein-complex suspensions are certainly of value. They do not however reflect the actual situation in cheese especially with regard to consistency and other physical properties. Although it was stated by some investigators that a decrease in the dissolved paracasein-complex occured when its concentration was increased, surprisingly enough no experiments were conducted with paracasein-complex at the same concentrations and under the same conditions as found in cheese.

1.3. SCOPE OF THE PRESENT STUDY

The present work was done taking into consideration the background discussed in the foregoing section. It is an extension of an unpublished study by MULDER.

The investigation followed two directions.

I. Experiments were conducted in which the ratio of calcium paracasein-complex to water was the same as that of cheese. Two ratios were mostly

used, firstly a ratio of 1 : 2.5 and secondly a ratio of 1 : 2. The former represents semi-hard cheese and the latter represents hard cheese. Since the data in the literature on the protein, calcium and inorganic phosphorus dissolved in the cheese at different pH values and salt concentrations were incomplete, it was found necessary to conduct some orientation experiments. To complete the picture similar experiments were also carried out on soft cheese.

II. Experiments were designed in which the ratio of paracasein-complex to water was similar to that reported by VAN DAM, as well as experiments of intermediate ratio between that used by VAN DAM and that of hard cheese. This would illustrate definitely the influence of the different ratios of paracasein-complex to water on the physical and chemical properties of the paracasein-complex under similar concentrations of hydrogen ion and salt.

Water is the important dispersing medium in cheese and the extent to which it is bound influences many of the cheese properties. It was thus decided to include some experiments on low and moderate concentrations of paracasein-complex suspensions as well as on reconstituted cheese to determine its swelling properties. Water binding capacity and the consistency were determined for reconstituted cheese only as this investigation mainly concerned with these properties under conditions applying to cheese. By using freeze-dried preparations and choosing appropriate experimental conditions, it might be possible to obtain comparable results.

Furthermore the quantity of N-substances, calcium and inorganic phosphorus dissolved in Edam cheese with and without salt was also estimated. The determinations were made on the cheese serum obtained by pressing. It must be borne in mind that the properties of cheese are not exactly identical with characterisation of paracasein-complex mixtures (reconstituted hard cheeses), but this study tried to approximate these properties as closely as possible.

2. MATERIALS AND METHODS

It was not found practicable to use a freshly prepared and washed curd for all the experiments of the present investigation. The water content of a fresh curd cannot be rapidly reduced to the level corresponding to those in hard or semi-hard cheeses. Working with a wet curd also exposes it to a greater risk of bacterial contamination. Moreover it is difficult to achieve a constant and evenly distributed moisture content in the curd after pressing. Therefore it was decided to work with a dry calcium-paracaseinate-calcium phosphate-complex prepared from a washed rennet curd. This can be used to make up the desired combinations for reconstituted semi-hard and hard cheeses. In this way comparable conditions could be obtained for all the different experiments.

2.1. PREPARING THE WASHED RENNET CURD

During the period from January 1960 to September 1961, nine preparations of calcium-paracaseinate-phosphate-complex were prepared in different months. For each preparation 80 litres of raw, fresh skim milk were drawn from the bulk milk supply at the local dairy. This quantity yielded approxim-

ately two kilograms of freeze-dried product and it was sufficient for at least one series of experiments. In addition a few wet preparations were prepared for studies on reconstituted soft cheese.

The skim milk was renneted at 30 °C by adding 40 ml of a commercial rennet extract (1 : 12000) per 100 litres of skim milk. It was then allowed to stand for about ten minutes until the casein started to coagulate. The resulting curd was stirred vigorously for ten minutes to obtain finely divided particles and to facilitate the separation of the whey. Eighty litres of water at 30 °C were then added and vigorously stirred for another ten minutes. The curd was then left to settle and the supernatant liquid was siphoned off. Washing was repeated three times until the supernatant liquid was clear. Portions of the precipitated curd were then pressed in a cheese press for about five minutes to remove as much of the water as possible. The curd was milled, then transferred to covered stainless steel containers and held at a temperature of —20 °C.

2.2. Drying the washed rennet curd

Different methods were tried to dry a suitable quantity of the washed rennet curd.

a. Drying with alcohol and ether. In this method the finely divided curd was successively mixed with an excess of 55 per cent, 75 per cent and 95 per cent alcohol followed by ether. The ether was then removed by evaporation in a current of air. This method, although a fine and dry product in sufficient amount and in a reasonable time was obtained, required large quantities of alcohol and ether. Furthermore, it involved the risk that a small part of the casein might be removed in the course of washing with alcohol. Hipp *et al.* (1950) reported that γ-casein is soluble in 50 per cent alcohol. However, the danger of removing this fraction is not of great importance because it is relatively very small especially because calcium-paracaseinate-calcium phosphate-complex was used and not pure paracasein. Drying with alcohol could moreover alter the physical properties of the calcium paracasein-complex.

b. Drying in a vacuum oven at 58-60 °C for about six hours. This method yielded best results when using a fine curd obtained by squeezing the curd through a sieve before drying. It was not suitable for preparing large quantities. Moreover, it might be possible that the high temperatures involved would to some extent change the chemical and physical properties of the paracasein-complex. Schipper (1961) observed a change in the proportion of the calcium to the inorganic phosphorus present in the complex brought about by raising the temperature, as a result of the transformation into hydroxy apatite.

c. By spray drying. In this method a suspension of very fine curd particles was obtained by using a special mixer 'Ultra-Turrax'. This suspension was spray-dried in a drying tower as used for preparing milk powder. By using this method, it was possible to obtain a large quantity of a fine powder in a short time. Its main drawback was, however, that this powder would not easily wet when mixed with lactic acid solution to prepare reconstituted cheese and it was difficult to obtain a homogeneous reconstituted cheese sample.

d. By freeze-drying.

Of the several methods tried freeze dehydration proved to be the most suit-

able, and all the material used in this study was dried according to this method. Changes in the chemical and physical properties of the paracasein-complex caused by applying this method are supposed to be insignificant.

In order to prepare a stock quantity of freeze-dried paracasein-complex, successive portions of the washed rennet curd previously kept in the deep freeze were dried by this method. The dried portions were pulverized in a mortar, passed through a 100-mesh sieve and then thoroughly mixed together.

2.3. Preparation of reconstituted cheese

The experiments were conducted on a calcium paracaseinate-calcium phosphate-complex, reconstituted to hold the same amount of water as in cheese. Semi-hard cheese was represented by adding 100 ml of water acidified with lactic acid to 50 grams of freeze-dried preparation giving a paracasein-complex to water ratio of about 1 : 2,5 and 100 ml water acidified with lactic acid added to 60 grams of the preparation gave a ratio of about 1 : 2, which is equivalent to that of normal hard cheese (about the same as in Dutch cheese). Different salt concentrations about 3, 5 and 7 per cent in the cheese moisture were obtained by adding 3, 5 and 7 grams of fine pure salt respectively to each 100 ml solution of lactic acid. The salt was completely dissolved then a preservative was added and all were mixed well with the dry preparation. Similar samples without salt were prepared for every series of experiments to serve as a control. The samples were kept in closed sterilized glassware at room temperature (about 20 °C) for two weeks or for one month depending on the experiment.

In addition to the reconstituted cheese, other samples of lower concentrations, with two or ten grams of the freeze-dried preparation suspended in 100 ml of water acidified with lactic acid were also used. These were kept in 100 ml stoppered Erlenmeyer flasks. The pH-values were varied by changing the ratio of water to lactic acid. The proper amount of lactic acid to be added to distilled water to give the required pH of the suspension, after equilibrium had been established, was obtained from preliminary tests. The pH-values used in the different experiments ranged mostly from 4.6 to 6.2. This represents the range of pH-values met during processing and ripening in different types of cheese. In some of the experiments different amount of calcium chloride solution were added to study its effect on restricting the peptization of calcium paracasein by sodium chloride. In order to help in establishing the equilibrium, the suspensions were daily agitated in a mechanical shaker for three hours, for the first three days. The samples containing 2 g of preparation were allowed to stand for seven days and those containing 10 g of preparation were left for 9 to 10 days before analysis. A preservative was always added to inhibit the growth of micro-organisms which would cause a serious breakdown of the protein. Sirks (1943) had been found to use thymol in alcohol as a preservative for this purpose and was applied afterwards by many investigators.

Preliminary experiments showed 5 per cent thymol in chloroform to be more convenient to work with as a preservative than toluene. Where an excess of toluene was used, the enormous increase in viscosity made it difficult to obtain the cheese juice. 3 ml of 5 per cent thymol in chloroform per 100 ml diluted lactic acid were quite sufficient. This kept bacterial numbers in the

suspensions and in the cheese juice to less than 100 bacteria per one ml by applying the plate count method as described in Ned. Norm. blad, No. V 1507 (1959).

2.4. Measurement of consistency, water binding capacity and swelling of the reconstituted cheese

A simple penetrometer with 0.01 mm units was used to measure the effect of different factors on the consistency of the reconsituted cheese samples. It consists of a dial micrometer gauge combined with a cone tipped cylinder (plate 1). The penetrometer needle was adjusted so that its tip touched the surface of the sample; then it was released to penetrate into the reconstituted cheese sample for five minutes and the reading was recorded. A weight of 10 g was then added and left for another five minutes and the new reading recorded. This simple method provided comparable values for the 'consistency' of different reconstituted cheese samples. The penetrometer readings do not directly measure the factors determining the consistency of cheese but they related to them and they offer a convenient numerical means of comparison.

The swelling and the water binding capacity of the reconstituted cheese were measured by transferring the whole sample to a weighed centrifuge tube and centrifuging it at 5,000 rev/min for 20 minutes. The supernatant fluid was then quickly decanted into a graduated cylinder and the volume of the solution expelled measured.

The volume of the supernatant liquid was noticed to decrease gradually if left on the surface of the sample after centrifuging. This could be due to the elastic properties of the paracasein particles. The degree of swelling was expressed as the percentage of the increase in weight of the freeze-dried preparation originally used. This can be calculated from the weight of the reconstituted cheese after centrifuging and decanting.

2.5. Preparing the samples for analysis

1. In case of suspensions of 2 and 10 g paracasein-complex in 100 ml solution, the samples were first centrifuged at 5,000 rev/min for 20 minutes to measure the swelling if necessary. The supernatant liquid was filtered. If the filtrate was not clear, it was centrifuged once more at 15,000 rev/min for 20 min to remove any undissolved material which might have remained in suspensions at the lower centrifugal speed.

2. In case of reconstituted semi-hard or hard cheese, the cheese juice was collected by a method similar to that described by Barthel, Sandberg and Haglund (1928) and Stadhouders and Mulder (1957). The cheese was mixed with sand and pressed. The cheese juice was then centrifuged and filtered in the same manner as described above.

Ultrafiltration

Some experiments were carried out with ultrafiltration to estimate the calcium and inorganic phosphorus in the protein-free solution obtained from the reconstituted cheese. At first the ultrafiltrate was obtained by means of a small apparatus (maximum capacity 10 ml) using a collodion membrane. The

apparatus was connected to a cylinder containing compressed nitrogen. In order to test the membrane for consistent filtration, 10 ml cheese serum was put in the apparatus and then a steady pressure of 3 kg/cm² applied. The ultrafiltrate was collected in five separate successive samples each of about 2 ml. The calcium and the inorganic phosphorus contents of each sample were determined and compared with that of the original serum. Table I shows

TABLE 1. The concentration of Ca and inorg.P in the cheese-serum (pH 4.8) and in 2 ml successive portions of the ultrafiltrate 'collodion membrane'.

	% Ca	% Inorg.P
Original sample	0.86	0.094
1st portion of ultrafiltrate	–	0.028
2nd portion of ultrafiltrate	0.69	0.068
3rd portion of ultrafiltrate	0.87	0.094
4th portion of ultrafiltrate	0.85	0.096
5th portion of ultrafiltrate	0.84	0.090

that the calcium and inorganic phosphorus values for the first two portions are smaller than in the third and fourth portions of the ultrafiltrate. This might be due to an initial absorption or retention of the calcium and inorganic phosphorus on the collodion membrane. It could also be caused by dilution with water retained in the small pores of the membrane after it was washed with distilled water, even with the two surfaces of the membrane dried before use. Therefore, the first 4 or 5 ml have to be discarded in order to obtain reliable results. It is worth mentioning that the difference in the composition of the last three portions and of the original cheese serum was insignificant. This apparatus has the following disadvantages.

1. Limited capacity and slow filtering speed.
2. Insufficient serum for a complete analysis especially at low hydrogen ion concentration when the soluble calcium and inorganic phosphorus are low.

Another apparatus for ultrafiltration was tried in which a cellophane tube was used. The apparatus and the method are those described by KOOPS (1957). A similar test on the cellophane membrane for consistent filtration was made on 5 ml portions of serum samples at intervals during filtration.

The analytical results were compared with those of the original sample. Table II shows that the values for the calcium and inorganic phosphorus

TABLE II. The concentration of Ca and inorg.P in the cheese-serum (pH 5.5) and in 5 ml successive portions of the ultrafiltrate 'cellophane membrane'.

	% Ca	% Inorg.P
Cheese serum (after centrif. 15,000 rev./min)	0.255	0.080
1st portion (5 ml) ultrafiltrate	–	–
2nd portion (5 ml) ultrafiltrate	0.212	0.071
3rd portion (5 ml) ultrafiltrate	0.218	0.073
4th portion (5 ml) ultrafiltrate	0.217	0.072

obtained from the third and fourth portions are nearly the same. These results indicate that first 10 ml should be discarded before collecting a sample for analytical purposes (in this experiment a cellophane tube of about 80 ml capacity was used). The difference between the composition of cheese serum

and that of the ultrafiltrate, after eliminating the first 10 ml, was relatively small. This indicates that high speed centrifuging (15,000 rev/min for 20 min) almost completely removes the colloidal particles containing calcium phosphate.

2.6. Edam cheese

For purpose of comparison a few experiments were also made on Edam cheese. The cheese was made of standardized raw milk and was manufactured and brined in the normal way in the laboratory by Mr E. de Vries. In order to obtain a number of cheeses with a high salt content, they were kept for 24 hours longer in the brine (19 per cent salt) than usual. In every experiment unsalted cheese made from the same batch of milk served as a control. The serum of the Edam cheese was collected in the same manner as in reconstituted cheese. A pressure of 40,000 pounds per square inch, applied with a hydraulic press, was necessary to obtain the cheese juice for samples kept for one month or longer. It was always more difficult to obtain the cheese juice from samples containing no salt than those containing salt. It was impossible to collect any juice from unsalted cheese at an age of two months. The cheese juice was centrifuged at 15,000 rev/min for 20 minutes and filtered to obtain a clear serum.

2.7. Analytical methods

The pH of the cheese serum was measured with a glass electrode. The moisture content of the freeze-dried preparation was determined by drying about 5 g at 104 °C in a vacuum oven until constant weight was reached. In the case of Edam cheese, about 5 g of cheese were mixed well with dry sand and dried at 100 °C until constant weight.

Total nitrogen

a. The values of the T.N. in the dry preparations were obtained by the Kjeldahl method as mentioned in (Ned. Norm. blad, Nov. 1961) using a sulphuric acid and phosphoric acid mixture for the digestion, with mercury as catalyst.

b. In the cheese serum or paracasein suspensions, the total nitrogen was estimated by the micro-Kjeldahl method (sulphuric acid, mercury, potassium sulphate and titrated with 0.01 N- HCl).

Calcium and inorganic phosphorus

a. In the dry preparation, 0.3 g preparation was dissolved in 10 ml of 5 per cent ammonia solution and continually stirred on a hot plate until the preparation was completely dispersed. It was then cooled and transferred to a 50 ml volumetric flask and the protein precipitated with 15 per cent trichloroacetic acid solution. The calcium and inorganic prosphorus were estimated on the clear filtrate.

b. In the cheese serum or paracasein-complex suspensions, a suitable aliquot of the filtrate depending upon the concentration of calcium and inorganic phosporus was weighed in a 25 ml volumetric flask. The protein was precipitated in the same manner as described before.

Calcium was determined by the 'complexon' method which is used in this laboratory and was described by VAN DER HAVE (1954) and the inorganic phosphorus colorimetrically with the method described by SCHEEL (1936).

In the determination of the inorganic phosphorus in the reconstituted cheese and also in Edam cheese samples, the solution (TCA serum) was found to turn turbid with the addition of the ammonium molybdate solution. This made it impossible to estimate the phosphorus colorimetrically. It was established that neither the sodium chloride (with the maximum concentration used) nor any silicates which might have originated from the sand used when pressing, caused this turbidity or affected colour development. A similar turbidity was also noted by FISKE and SUBBAROW (1925). They ascribed it to traces of protein present in the solution and recommended reprecipitation of the protein substances with trichloroacetic acid. They also reported that turbidity, not due to proteins, could possibly be caused by substances having a very low phosphate content.

Reprecipitation of the proteins with 15 per cent TCA (end concentration) did not succeed in getting rid of the turbidity described in the present investigation. This seems to be due to nitrogenous substances (peptides) soluble in 12 per cent TCA, which are always present as a degradation product of the paracasein. It was possible to overcome this difficulty by adding 2 ml of standard solution of monobasic potassium phosphate, containing 100 γ phosphorus to one or two ml of TCA filtrate of the sample (depending upon its inorganic phosphorus content) to give the best colorimetric reading on the Kipp colorimeter. This is usually between extinction readings of 0.200 and 0.400. This kept the quantity of the sample to a minimum and increased at the same time the level of its phosphorus content to an ideal concentration for colorimetric determination.

Sodium was determined in the cheese serum with the flame photometer using the method described by VAN DER HAVE and MULDER (1957).

Lactose was estimated by the colorimetric method published by BARNETT and TAWAB (1957). The concentration of the phenol solution used was however slightly modified. The six drops of 80 per cent solution were replaced by one ml of 5 per cent phenol solution.

Lactic acid was estimated by the colorimetric method of DAVIDSON (1949).

2.8. PAPER ELECTROPHORESIS

Electrophoresis was performed with barbital buffer of 0.05 ionic strenght at pH of 8.6 at about 10 °C and using Whatman No. 1 paper. The apparatus used in this investigation was the same as that employed by KAMPEN and ZONDAG (1955). In all the experiments with casein and paracasein-phosphate-complex an electrical current of about 0.4 ma/cm, over a period of 4 hr. was used. In all experiments with cheese serum a similar current was used for 16 hours. Usually 0.02 ml of cheese serum or 5 per cent solution of paracasein-complex or 4 per cent casein in 1 N ammonia was applied to the apex of the paper. Staining was done with Bromophenol blue according to the method published by DURREUM (1950). This gave better results than azocarmine B, recommended by KAMPEN and ZONDAG (1955).

3. RESULTS AND DISCUSSIONS

3.1. COMPOSITION OF THE CALCIUM-PARACASEINATE-CALCIUM PHOSPHATE-COMPLEX

3.1.1. *Prepared from fresh milk*

Table III-A shows the composition of a number of preparations obtained at different months from January 1960 to September 1961. The figures ob-

TABLE III. Composition of calcium-paracaseinate-calcium-phosphate-complex.

	Percentage in dry matter				Total Ca g equiv./g casein × 10^{-5}	Calcium bound to casein			
	T.N.	Casein	Ca	Inorg. P		g equiv. Ca/g casein × 10^{-5} if Mol. Ca:P is		expressed as % of T. Ca, if Mol. Ca:P is	
						1.4	1.5	1.4	1.5
A.									
January '60	13.80	87.91	2.99	0.98	170	69	62	40.8	36.5
February '60	14.00	89.18	2.97	1.00	166	65	58	39.0	34.7
March '60	14.10	89.82	2.98	1.04	166	61	53	36.8	32.3
April '60	14.10	89.82	3.22	1.10	179	68	60	38.1	33.7
July* '60	14.24	90.71	3.00	0.93	165	72	66	43.9	39.9
December '60	14.10	89.82	2.98	1.07	166	58	50	35.0	30.4
February '61	14.26	90.84	2.99	0.97	164	68	60	41.3	37.1
May '61	13.88	88.42	3.11	1.04	176	69	62	39.5	35.1
September '61	14.09	89.75	3.04	1.10	169	58	50	34.5	29.8
Average . . .	14.06	89.59	3.03	1.026	169	65	58	38.8	34.4
BELOUSOV (1959)	13.20	84.08	2.71	0.90	160	—	57	—	35.7
B.									
May I (pH 6.6)	13.88	88.42	3.11	1.03	176	70	63	40.0	35.8
May II (pH 6.5)	13.90	88.54	3.09	1.00	174	72	65	41.4	37.2
May III (pH 6.3)	13.87	88.35	2.84	0.95	160	63	56	39.4	35.1
C.									
July '61	13.41	85.42	2.87	1.03	168	—	—	—	—
August '61	13.61	86.70	2.78	0.97	160	—	—	—	—
Average . . .	13.51	86.06	2.83	1.00	164	—	—	—	—

A. All preparations were made from fresh skim milk.
B. Preparations May II and May III were prepared from milk to which lactic acid was added.
C. Samples from Edam cheese before brining. All percentages are in fat free dry matter.
* The skim milk was treated with 0,05% H_2O_2 before renneting.

tained for nitrogen, total calcium and inorganic phosphorus vary mostly no more than 5 per cent from the mean values obtained for these respective constituents. Treating the milk with hydrogen peroxide did not seem to affect significantly the composition of the paracasein-complex. The table also shows that the values for the total calcium ranged from a minimum of 165×10^{-5} to a maximum of 179×10^{-5} g-equivalent calcium per g casein with an average of 169×10^{-5} g-equiv/g casein. In general these values were a little higher than

the corresponding values reported by BELOUSOV (1959). The latter ranged from 149×10^{-5} to 178×10^{-5} with an average of 160×10^{-5} g-equiv. calcium per g casein.

The molecular ratio of calcium to inorganic phosphorus of the complex in milk is 1.4 as determined by MULDER and SCHIPPER (1959) and SCHIPPER (1961). Using this ratio, to calculate the calcium bound to paracasein the average value for the bound calcium expressed as a percentage of the total calcium was 38.8, with a maximum of 43.9 and a minimum of 34.5. In general this range agreed with that found by SCHIPPER (1961). When the ratio 1.5 as reported by BELOUSOV (1959) was used, the average values for the gram equivalent calcium per gram paracasein were the same as those obtained by BELOUSOV.

3.1.2. *Prepared from milk treated with lactic acid*

The rennet curds used in the present investigation were prepared for the greater part from raw fresh skim milk. A few other preparations resembling those kinds of hard cheeses, as the English ones, where the milk is ripened before renneting were prepared. This ripening considerably decreases the pH of milk before renneting. In Dutch cheeses the decrease in the pH of the milk before renneting is usually negligible. The raw skim milk used in May 1961, was divided into three portions. The first with a pH-value of 6.6 was prepared as usual to represent the fresh milk. The second was acidified with an appropriate quantity of lactic acid to a pH of 6.5, while in the third portion the pH was decreased to 6.3 before adding the rennet. The latter value would represent the highest acidity met in the milk used for cheese manufacture.

The composition of these three different preparations (from the same sample of milk) is shown in Table III-B. The figures indicate that the slight decreases in milk pH had little effect on the composition of the resultant paracasein-complex.

There was a little less calcium bound to the paracasein (63×10^{-5} g equiv/g casein) in preparation May III than in preparation May I (70×10^{-5} g equiv/g casein), if it is considered that the calcium phosphate becomes more soluble as a result of the decrease in the pH, as reported by SCHIPPER. When the molar ratio of Ca:P of 1.35 as reported by SCHIPPER for the calcium phosphate at pH 6.3 was applied to calculate the calcium bound to paracasein a figure of 67×10^{-5} g equiv/g paracasein was obtained. On this basis the value for the protein bound calcium expressed as percentage of the total calcium comes to 41.6 per cent of the total calcium instead of 39.4 as reported in Table III B. This further supports that the changes in the composition of the complex are very small.

3.1.3. *Paracasein-complex as present in green Edam cheese*

The composition of freeze-dried preparations was also compared with that of green Edam cheese, i.e., after pressing but before brining. This cheese was manufactured in the standard way from two different milk samples. The results shown in Table III C give an indication of the general composition of the cheese and provide a basis for comparison. The values were not corrected for the whey solids retained in the cheese, the correction would give a slight increase in all the values listed. The values obtained for the total nitrogen, total calcium and inorganic phosphorus, calculated on a fat free dry matter

basis, compared well with those found for the different curd preparations. They differed only in a slight decrease in the total nitrogen and total calcium. This decrease would probably be due to the removal of a small part of the dissolved calcium and paracasein with the whey during the processing of the cheese. The figures for the total calcium expressed in g/equiv/g paracasein of the Edam cheese were of the same order as those obtained for the different preparations. At the same time the correct value for the calcium bound to the paracasein could not be calculated, with the much lower pH value as (5.1) where the paracasein-complex was separated from the cheese after pressing. For this calculation BELOUSOV assumed erroneously that the calcium was in the form of tricalcium phosphate. SCHIPPER showed in this connection that with a decreasing pH the composition of the calcium phosphate is gradually changed and a secondary calcium phosphate formed at a pH of about 5.7.

The results shown in Table III can be summarized as follows:

1. The composition of the calcium-paracaseinate-calcium phosphate-complex prepared from cows bulk skim milk was nearly constant during the period of the investigation. The figures compare well with those reported by other investigators for preparations prepared in the same manner.

2. A small decrease in pH of milk before renneting does not effect the composition of the paracaseinate-complex to any important degree.

3.2. THE EFFECT OF LACTIC ACID AND SALT ON THE COMPOSITION OF RECONSTITUTED CHEESE (ORIENTATION EXPERIMENTS)

For this series of experiments the freeze-dried paracasein-complex was mixed with water, lactic acid and salt to give a reconstituted cheese similar in composition to semi-hard or hard cheese. The reconstituted cheese was left for two weeks.

The juice was obtained from the mixtures by pressing after mixing with sand. After centrifuging, the juice was passed through a filter paper. In the filtrate (serum) total nitrogen, calcium and inorganic phosphorus were determined.

3.2.1. *Dissolved nitrogen substances*

The values for total nitrogen in table IV and V and figures 1 and 2 show that a relatively small proportion of the paracasein-complex was dissolved. In unsalted mixtures this came to between 4 and 5 per cent of the total nitrogen substances present. About one third of these nitrogen substances could not be precipitated by a 12 per cent solution of trichloroacetic acid and therefore were not protein. They always showed a positive reaction to the biuret test, indicating they were peptides, most probably formed by the action of enzymes from the milk and rennet on the paracasein. This will be discussed further in section (3.3). With one-third of the soluble nitrogen substances produced by enzyme activity, one can conclude that no more than 3 per cent at most of the nitrogen of the casein went into solution.

Adding 3 to 5 per cent sodium chloride in the cheese serum increased the quantity of the total nitrogen dissolved. This rose to between 6 and 9 per cent in the pH range of 5.3 to 5.6. About one-fourth of the nitrogen in the serum was non protein nitrogen (NPN). When substracting the NPN, the dissolved paracasein nitrogen amounted to about 5 to 7 per cent.

TABLE IV. The effect of acid and salt on reconstituted (A) semi-hard and (B) hard cheese (preparation April '60). Orientation experiments.

	% Salt in cheese serum	Lactic acid 10% added ml.	In the cheese serum						Percentage dissolved of the total prep.		
			pH	T.N. %	N.P.N. %	Ca %	Inorg. P %	Na %	T.N.	Ca	Inorg. P
A.	0.0	5.0	5.86	0.26	0.10	0.141	0.017	–	4.1	9.6	3.5
		8.0	5.70	0.20	0.08	0.192	0.018	–	3,1	13.1	3.8
		14.0	5.50	0.25	0.10	0.327	0.018	–	3.9	22.2	3.8
		18.0	5.40	0.28	0.10	0.423	0.021	–	4.4	28.8	4.4
		24.0	5.12	0.24	0.10	0.559	0.032	–	3.8	38.0	6.7
		32.5	4.84	0.16	0.09	0.742	0.055	–	2.5	50.4	11.5
	3.0	5.6	5.96	0.42	0.21	0.148	0.018	0.999	6.6	10.1	3.8
		9.0	5.73	0.36	0.12	0.205	0.024	1.001	5.7	13.9	5.0
		15.0	5.47	0.48	0.12	0.321	0.024	0.995	7.6	21.8	5.0
		19.0	5.34	0.47	0.12	0.383	0.027	0.997	7.4	26.1	5.6
		25.0	5.13	0.40	0.12	0.491	0.039	0.997	6.3	33.4	8.1
		33.0	4.86	0.20	0.09	0.615	0.056	1.008	3.1	41.8	11.7
	5.0	6.0	5.92	0.36	0.16	0.156	0.021	1.71	5.7	10.6	4.4
		10.0	5.66	0.32	0.13	0.212	0.029	1.60	5.0	14.4	6.0
		16.0	5.46	0.56	0.10	0.325	0.026	1.82	8.8	22.1	5.4
		20.0	5.20	0.32	0.09	0.405	0.030	1.83	5.0	27.6	6.3
		26.0	5.00	0.29	0.09	0.520	0.044	1.71	4.6	35.4	9.2
		34.0	4.76	0.18	0.07	0.609	0.061	1.64	2.8	41.4	12.7
B.	0.0	3.6	6.08	0.26	0.13	0.096	0.011	–	3.4	5.4	1.9
		10.0	5.72	0.23	0.10	0.223	0.017	–	3.0	12.6	3.0
		16.0	5.50	0.28	0.10	0.359	0.016	–	3.7	20.4	2.8
		20.0	5.40	0.34	0.10	0.450	0.012	–	4.5	25.5	2.1
		24.0	5.22	0.32	0.10	0.540	0.025	–	4.2	30.6	4.3
		29.0	5.06	0.30	0.09	0.660	0.032	–	3.9	37.4	5.6
		36.0	4.90	0.22	0.10	0.800	0.044	–	2.9	45.4	7.6
		42.0	4.72	0.18	0.09	0.910	0.069	–	2.4	51.6	12.0
	3.0	4.0	6.00	0.30	–	0.143	0.016	1.08	3.9	8.1	2.8
		8.0	5.88	–	–	0.185	0.024	1.06	–	10.5	4.2
		11.0	5.72	0.38	0.15	0.221	0.030	1.00	5.0	12.5	5.2
		14.0	5.65	0.40	0.12	0.256	0.024	1.01	5.2	14.5	4.2
		17.0	5.52	0.51	0.11	0.337	0.024	1.03	6.7	19.1	4.2
		21.0	5.40	0.52	0.12	0.352	0.025	1.05	6.8	20.0	4.3
		26.0	5.22	0.37	–	0.500	0.034	1.02	4.9	28.3	5.9
		32.0	5.10	0.28	0.10	0.610	0.038	–	3.7	34.6	6.6
		38.0	4.82	0.17	0.09	0.770	0.049	1.02	2.2	43.7	8.5
	5.0	5.0	5.90	0.32	0.15	0.157	0.022	1.63	4.2	8.9	3.8
		9.0	5.80	0.27	0.12	0.179	0.027	1.82	3.5	10.1	4.7
		12.0	5.64	0.23	0.10	0.216	0.028	1.72	3.0	12.2	4.9
		15.0	5.54	0.34	0.10	0.284	0.026	1.70	4.5	16.1	4.5
		18.0	54.0	0.57	0.10	0.363	0.026	1.70	7.5	20.6	4.5
		22.0	53.0	0.39	0.09	0.404	0.030	1.86	5.1	22.9	5.2
		27.0	51.5	0.32	0.09	0.471	0.037	1.69	4.2	26.7	6.4
		33.0	4.98	0.27	0.08	0.608	0.048	1.68	3.5	34.5	8.3
		42.0	4.78	0.11	0.05	0.760	0.061	–	1.4	43.1	10.6

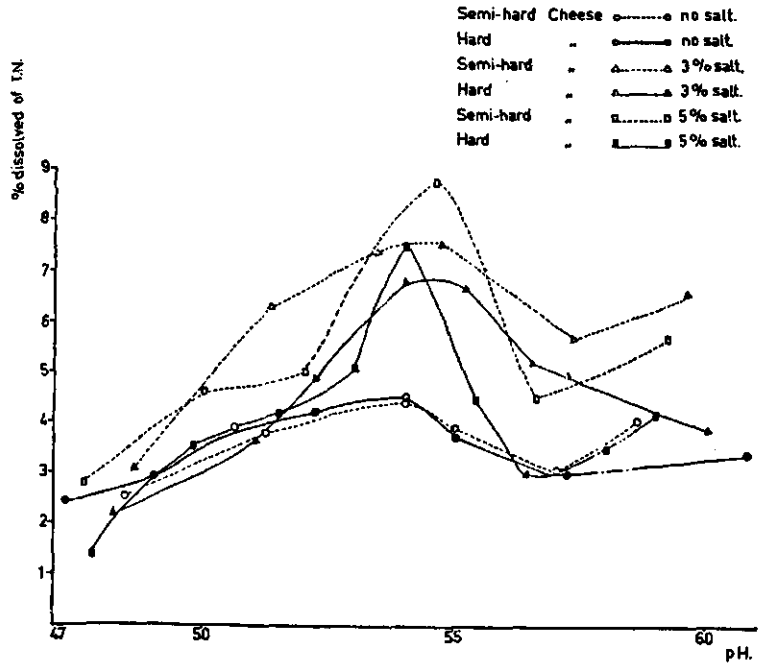

FIG. 1. Effect of pH and salt on the T.N. dissolved in reconstituted semi-hard and hard cheese (orientation exp.) – April 1960 material.

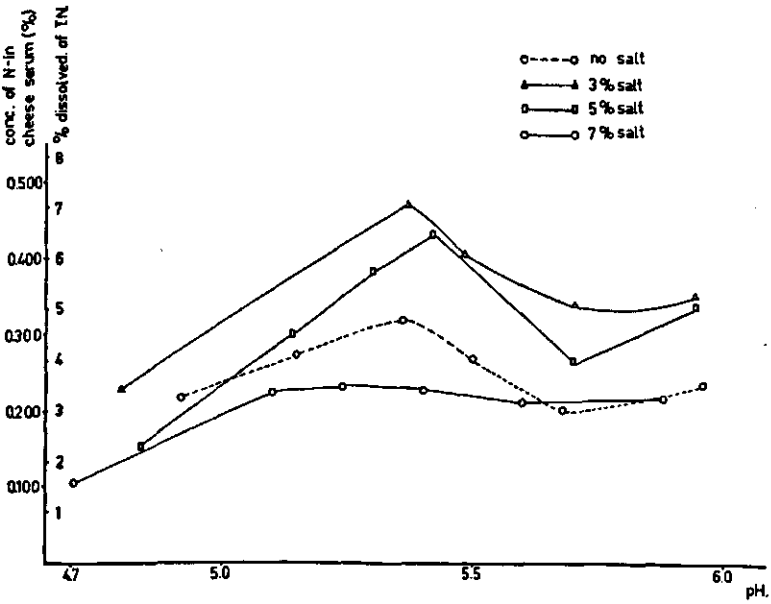

FIG. 2. Effect of pH and salt on the T.N. dissolved in reconstituted semi-hard cheese (orientation exp.) – July. 1960 material.

TABLE V. The effect of acid and salt on reconstituted semi-hard cheese (preparation july* '60). Orientation experiments.

% Salt in cheese serum	Lactic acid 10% added ml.	In the cheese serum					Percentage dissolved of the total prep.		
		pH	T.N. %	Ca %	Inorg. P %	Na %	T.N.	Ca	Inorg. P
No salt	4.8	5.96	0.234	0.128	0.016	–	3.55	9.1	3.6
	8.0	5.68	0.200	0.181	0.023	–	3.03	12.9	5.1
	14.0	5.50	0.266	0.329	0.021	–	4.04	23.5	4.7
	18.0	5.36	0.320	0.395	0.021	–	4.86	28.2	4.7
	24.0	5.15	0.268	0.550	0.031	–	4.05	39.3	6.9
	32.0	4.92	0.217	0.696	0.049	–	3.29	49.7	10.9
3% salt	5.0	5.94	0.382	0.139	0.022	0.978	5.80	9.9	4.9
	8.6	5.70	0.338	0.189	0.035	0.998	5.13	13.5	7.8
	14.6	5.48	0.408	0.302	0.026	0.990	6.19	21.6	5.8
	18.6	5.37	0.470	0.403	0.032	–	7.13	28.8	7.1
	26.0	5.10	–	0.496	0.046	0.977	–	35.4	10.2
	35.0	4.80	0.227	0.644	0.070	1.022	3.44	46.0	15.6
5% salt	5.2	5.94	0.337	0.144	0.022	1.479	5.11	10.3	4.9
	9.0	5.70	0.267	0.196	0.034	1.528	4.05	14.0	7.6
	15.0	5.42	0.433	0.289	0.029	1.468	6.57	20.6	6.4
	19.0	5.30	0.380	0.358	0.032	1.512	5.77	25.6	7.1
	27.0	5.14	0.297	0.492	0.049	1.582	4.51	35.1	10.9
	36.0	4.84	0.156	0.599	0.072	1.527	2.37	42.8	16.0
7% salt	5.6	5.88	0.221	0.155	0.025	2.078	3.35	11.1	5.6
	10.0	5.60	0.215	0.207	0.036	2.055	3.26	14.8	8.0
	16.0	5.40	0.227	0.305	0.032	2.086	3.44	21.8	7.1
	20.0	5.24	0.230	0.375	0.037	2.035	3.49	26.8	8.2
	28.0	5.10	0.225	0.443	0.044	2.178	3.41	31.6	9.9
	38.0	4.70	0.108	0.633	0.090	2.026	1.64	45.2	20.0

* The milk used for this preparation was treated with 0,05% H_2O_4 before renneting.

The high ratio of NPN would be due to enzymatic reaction during the two weeks adjusting period. In later experiments (on reconstituted soft cheese) using 'Ultra-Turrax' to come to an equilibrium much more rapidly, the NPN content amounted only to about 4 per cent of the total dissolved nitrogen as will be shown in Table XXVII.

The effect of hydrogen ion concentration on unsalted samples was very small, both for the reconstituted semi-hard and hard cheeses especially after discounting the protein degradation products resulting from enzyme action. The peptizing effect of sodium chloride on the paracasein-complex (in reconstituted cheese) was much smaller than could be expected. The literature gives the impressions that the salt has an important effect (values of 80 per cent or even more were reported).

The maximum peptization was obtained when using a salt concentration between 3 and 5 per cent within a pH range of 5.3 and 5.6. At pH about 5 or lower, the salt had only a very small effect on the amount of nitrogen dissolved. This could be explained by the high calcium level in the serum at such low pH values.

The lower values for dissolved nitrogen in the samples with 7 per cent salt than in the unsalted ones might be due to a salting out effect.

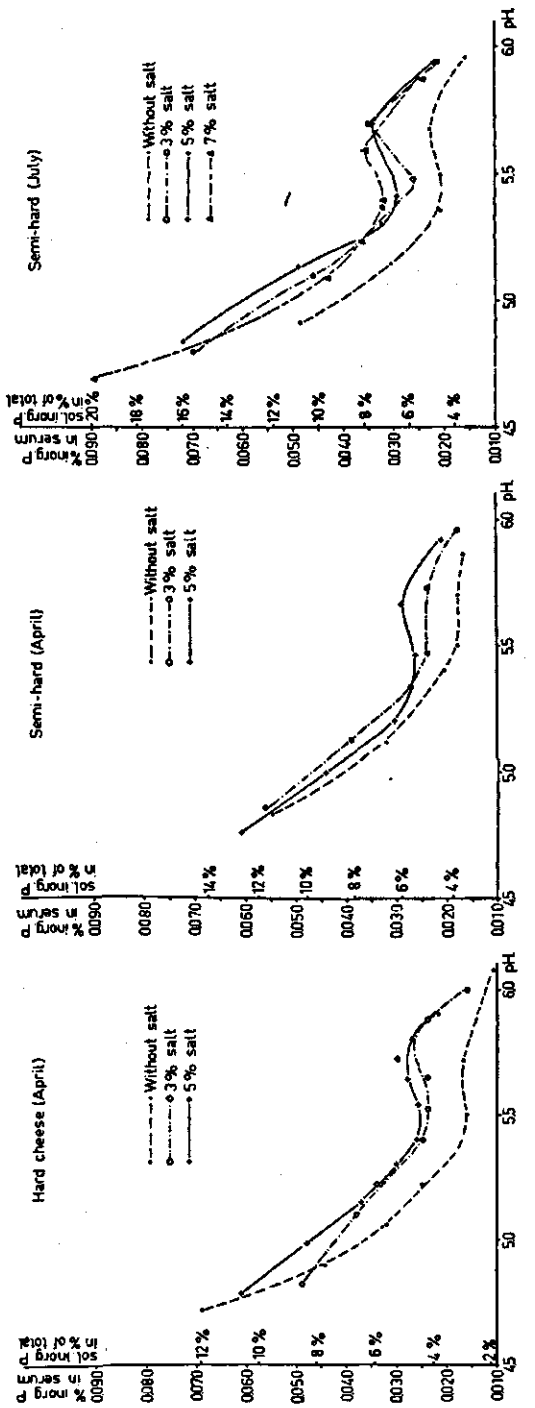

FIG. 3. Effect of pH and salt on the inorganic P dissolved in reconstituted cheese (orientation exp.).

3.2.2. Dissolved inorganic phosphorus

The results in Tables IV and V and in Figure 3 show that with a decreasing pH increasing quantities of inorganic phosphorus are found in the serum of the cheese. Adding salt raises the percentage of dissolved inorganic phosphorus. The increase was obvious between pH 5.3 and 6. The increase in inorganic phosphorus in the serum with more salt might be due to an exchange of sodium ions of the salt with calcium of the phosphate. Mulder and Schipper (1959) have proved such an exchange possible. The figures in the same tables also show that the proportion of inorganic phosphorus dissolved was generally small. At pH 4.9 only about 10 per cent of the inorganic phosphorus in the preparation was dissolved in the absence of salt. This quantity increased to about 15 per cent when salt was added. Unfortunately the literature gives no comparable figures either for the concentration of phosphorus in the serum or for the percentage dissolved of the total amount in different kinds of cheese. Other investigators found much higher values for soluble inorganic phosphorus either in cheese or in paracasein-complex suspensions. However, they used much more diluted mixtures in which the ratio of cheese or paracasein-complex to water ranged from 1 : 40 to 1 : 20 against 1 : 2 for the present experiments and in hard cheese. Therefore, it is not fair to compare the figures obtained in the present investigation with those reported by van Slyke and Bosworth (1907) who found that all the phosphate was in the extract of Cheddar cheese (5 g cheese in 100 ml water). In case of paracasein-complex suspensions (5 g/100 ml) Belousov (1959) reported that all the calcium phosphate was in solution at pH 4.8.

Salt formation of the dissolved inorganic phosphorus with the paracasein-complex at pH lower than, or near its isoelectric point, would explain its relatively low levels in the serum at pH values lower than 5. The paracasein and the phosphorus ion would be capable of forming salt linkages over a wide zone of pH values. These extend across the isoelectric point according to Lloyed and Shore (1938). At higher pH-values the phosphorus and the calcium in the cheese serum might probably form poorly soluble calcium phosphate with a ratio of Ca : P depending upon the pH of the solution. This mechanism would partially explain the relatively small amounts of inorganic phosphorus as well as of calcium in the cheese serum at pH-values higher than 5.

3.2.3. Dissolved calcium

The results in Tables IV and V and in Figure 4 show that with decreasing pH values an increasing quantity of calcium dissolves. This increase is small between pH 5.7 and 6 while it becomes markedly larger at pH lower than 5.7. Belousov (1959) supposed that it was chiefly the calcium of the phosphate which dissolved at pH-values above 5.3, while the calcium bound to the paracasein dissolved in a more acid medium. The explanation of Belousov is not soundly based. There is no reason at all to suppose that the paracasein looses no calcium at pH higher than 5.3. Schipper (1961) proved in this laboratory that at pH 6 or even higher, the calcium could separate from both the casein and the phosphate components of the complex.

The trend of the curves representing the dissolving calcium was the same in both reconstituted semi-hard and hard cheeses made from two different preparations. Almost constant values of dissolved calcium were obtained in

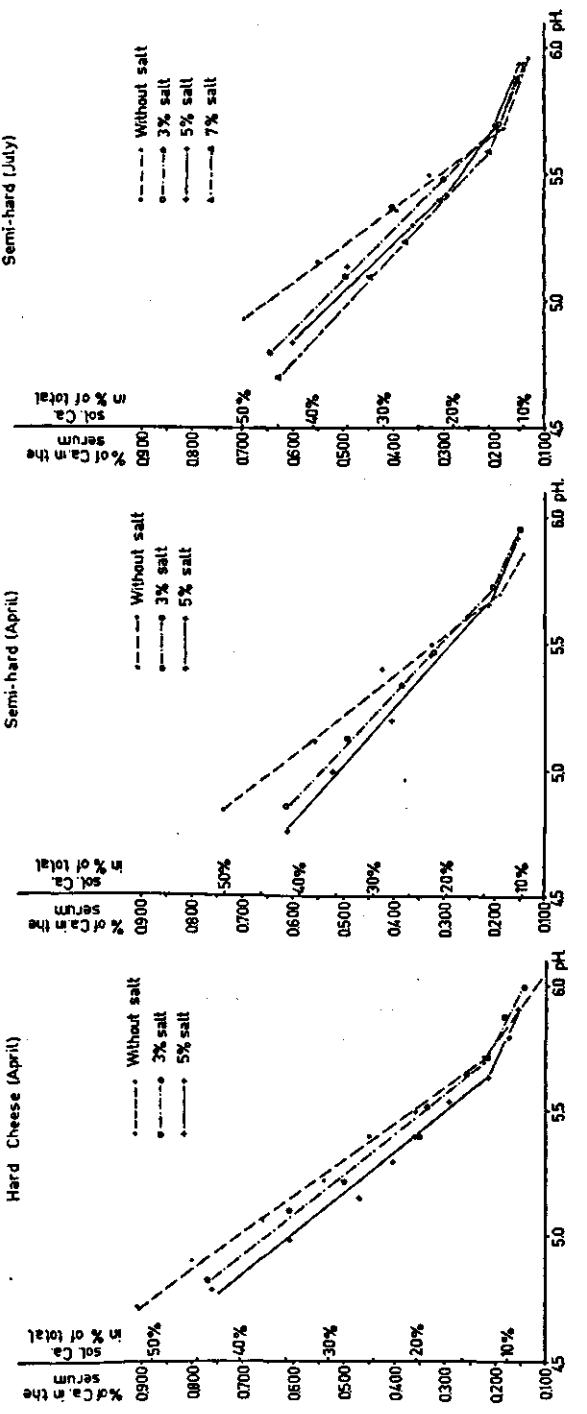

FIG. 4. Effect of pH and salt on the calcium dissolved in reconstituted cheese (orientation exp.).

different samples at the same pH and salt concentration. At pH 6 always less than 10 per cent of the total calcium dissolved, while at pH 5.5 about 20 per cent were dissolved in the samples each with and without salt. At pH 5, however, about 35 per cent of the total calcium dissolved in samples having 5 per cent salt and about 40 to 45 per cent in samples without salt. It was interesting to observe that between pH 5.7 and 6 the samples with 3 and 5 per cent salt contained more calcium in their serum than those without any salt, while at pH-values below 5.7 the calcium in solution decreased when higher quantities of salt were added. The lower the pH value the greater was the difference between unsalted and salted samples. The higher level of calcium in the serum from salted samples than in unsalted ones at pH values higher than 5.7 might be explained to be due to an exchange of sodium of the salt with calcium of the paracasein-complex. This exchange can take place with both the calcium of the paracasein as well as of the phosphate (MULDER and SCHIPPER, 1959). While it is difficult to explain why the opposite occurs at lower pH-values. This aspect will be discussed in more detail at the end of this section (3.2.).

There are no data available in the literature on the concentration of the calcium in cheese serum or on the percentage dissolved of the total calcium in cheese. All the data are either for dilute cheese extracts or for dilute paracasein-complex suspensions. In Cheddar cheese, VAN SLYKE and BOSWORTH (1907) found 80 per cent of the total calcium in the dilute cheese extract. In soft cheese, NAUDTS and DE VLEESCHAUWER (1959) reported more than 90 per cent of the total calcium in soluble form (in the diluted cheese extract). Their results say little about the condition of the calcium in cheese under actual conditions. It is worth mentioning here that BELOUSOV (1959) reported the calcium content of the paracasein-complex in suspensions (5 g in 100 ml water acidified with lactic acid) to be lower than that of green Edam cheese at similar pH-values. He attributed this to the fact that the different constituents of the cheese had not yet reached an equilibrium (with the lactic acid), while this was the case with the paracasein suspensions which had been left for two weeks. He did not consider the possibility that this difference could be due to a different paracasein-complex to water ratio in both cases.

The relativeley small quantity of the calcium found in the serum of reconstituted cheese with pH-values h'gher than 5 could be explained as due to the fact that some of the soluble calcium recombines with some of the soluble inorganic phosphorus to form calcium phosphate in which the ratio of Ca : P depends upon the pH value of the solution. The relatively small quantity of the calcium that has dissolved at pH lower than 5 (near the isoelectric point of paracasein) would suggest that either the paracasein-complex at such a low pH was still combined with calcium in the form of e.g. primary and secondary phosphates and was not entirely free of calcium as it was sometimes considered. It could also be that most of the calcium bound to the paracasein-complex goes into solution then to be reprecipitated due to the saturation of the solution in the form of some other less soluble calcium salt. Perhaps both may possibly exist.

From the data in Tables IV and V concerning the quantity of lactic acid added and the concentration of calcium in the cheese serum, it was possible to calculate that at pH 4.9 about 5.5 g calcium lactate having five molecules water of crystallization combined with the lactic acid present. In this case the

cheese serum would be nearly saturated with calcium lactate. DORN and DAHLBERG (1942) cited that the solubility of calcium lactate with 5 molecules water of crystallization varies from 3 per cent at 0 °C to 7.3 per cent at 30 °C. The situation is, however, not so simple, especially as it is probable that the calcium lactate can form in combination with one or two molecules of lactic acid, more acid compounds, which are reported in 'Beilstein' (1921) to be more soluble than the normal calcium lactate.

The problem becomes more complicated still when we consider also the possibility of the calcium lactate combining with calcium phosphate, which was reported by MARZAT (1949) to be more soluble, as well as the formation of double salts of calcium lactate with sodium salts or other salts present in the medium.

SONNTAG (1952) found that amino acids or the products of hydrolysis of proteins render calcium lactate more soluble. All these factors apply to cheese. They indicate how complicated the situation is, and how difficult it is to come to a conclusion about the conditions of the calcium salts in cheese.

3.2.4. *Crystals in cheese and reconstituted cheese*

Many investigators have reported the presence of crystals in cheese. TUCKEY *et al.* (1938) who examined the white specks isolated from old Cheddar cheese by means of X-ray analysis, found them to have the same crystal spacings as calcium lactate. MCDOWALL and MCDOWELL (1939) used chemical analysis for investigating the white particles in mature Cheddar cheese and found them to be calcium lactate. Recently, FARRER and HOLBERG (1960) reported the presence of calcium lactate on the surface of rindless cheese wrapped in flexible packaging. On the other hand DORN and DAHLBERG (1942) proved by chemical analysis that the white material which they found in ripened Cheddar cheese was principally tyrosine. They suggested that calcium phosphate salts were present as impurities. HARPER, SWANSON and SOMMER (1953) found a mixture of calcium lactate and tyrosine to be the main components of the white particles found in different samples of Cheddar cheese. Quite recently BOOY (1961) reported hard white particles in old Gouda cheese and by means of chromatography confirmed the presence of tyrosine and phenylalanine. He also added that calcium salts were found in these particles. SWIATECK and JAWORSKI (1959) made histochemical studies on the distribution of mineral matter in different types of hard and semi-hard cheese. They reported the presence of calcium phosphate kernels, their number depending on the calcium content of the cheese.

Even in young cheese, MULDER (unpublished work) also observed crystals of different shapes in young Edam cheese examined under the microscope with polarized light.

Formation of crystals was followed by regularly examining several samples of unsalted reconstituted cheese at different pH values after two weeks in the same way as MULDER did. Crystals appeared in the samples after 3 to 4 weeks. They differ in shape and number with the pH value of the sample.

These results combined with those reported in the literature indicate that crystals of mineral salts are present in cheese. It is quite possible that these crystals are mixtures of different components. The crystals will not necessarily affect the quality of the cheese especially when they are small. They

only appear as white particles or specks when they accumulate for one reason or another on the surface or inside the cheese.

One further point requires explanation. This concerns the effect of salt on the amount of the calcium dissolved in reconstituted cheese at different pH-values. Tables IV and V show that the salted samples needed more lactic acid especially at lower pH-values than the unsalted ones, to produce the same final pH-value. Besides the influence of lactic acid several factors can be expected to increase the calcium in solution when salt was added. There is firstly the ion exchange between the sodium of the salt and the calcium from the complex. Secondly, the calcium lactate may form a double salt with sodium chloride or with the acid calcium phosphate; thirdly compounds of calcium lactate and lactic acid may be produced. The last two factors as mentioned already at the end of section 3.2.3., cause the calcium lactate to form more soluble compounds. Instead of the increase of the concentration of the calcium in the serum of the salted samples, as expected, the opposite was in fact always the case, as the figures for the orientation experiments (tables IV and V) as well as the subsequent experiments show.

If we assume that the concentration of soluble calcium decreasing with the addition of salt was the result of its precipitation as calcium lactate by some mechanism, the concentration of lactate in solution should also decrease.

A small experiment carried out with reconstituted semi-hard cheese without and with 7 per cent salt at two different pH-values (5 and 4.8) showed this assumption to be baseless (see Table VI).

TABLE VI. The effect of salt on the concentration of calcium and lactate in the cheese serum and on the liberation of bound water (semi-hard cheese – preparation September 1961).

% Salt	Lactic acid (10%) added ml.	pH	Percentage in the cheese serum			γ-Lactose (in 2 ml serum) diluted 1:50	Decrease in concent. of lactose
			Lactic acid	Ca	Inorg. P		
0.0	28	4.99	2.7	0.646	0.053	140	–
0.0	32	4.83	3.3	0.732	0.065	140	–
7.0	30	4.97	3.2	0.543	0.063	122	12%
7.0	35	4.82	3.7	0.597	0.088	112	20%

3.2.5. *Effect of salt on liberation of bound water in cheese*

MCDOWELL and DOLBY (1936) also found a pronounced drop in the concentration of calcium after salting Cheddar cheese. To a lesser extent this was also the case with phosphate and lactate in the whey. They attributed this to the osmotic diffusion of water from the curd.

MULDER (personal communication) used lactose and sucrose as reference substances for estimating the bound water in Edam cheese. He found a considerable decrease in the bound water after the cheese was brined.

This release of a part of the bound water may contribute to the relatively low concentration of the calcium in the presence of salt in the cheese serum.

To confirm this explanation; the experiment mentioned before (Table VI) was repeated. Two ml. of a solution of one per cent lactose were mixed well

with every sample when it was two weeks old. Half an hour after mixing with lactose, the samples were centrifuged and the amount of lactose was determined in the supernatant liquid. The results are also shown in Table VI. The lactose concentration in the samples with 7 per cent salt at pH 4.97 was about 12 per cent less than in those containing no salt. At pH 4.80 the decrease amounted to about 20 per cent. The decreases in the concentration of calcium in the serum were 16 and 19 per cent respectively. Although large sugar molecules can hardly be compared with small ions such as calcium, yet these results would partially explain the situation. Salt releases a considerable amount of bound water and this increases as the pH is decreased. Its release would partially dilute the system. This would give a good explanation for the lower concentration of the calcium, were it not that the concentration of the inorganic phosphate and the lactic acid did not decrease in the same way.

In the foregoing experiments the values for the total nitrogen calcium and inorganic phosphorus were expressed as a percentage by weight both in the samples with and without salt, which made the comparison between the serum of salted and unsalted samples not fair. As salt would increase the density of the serum, an increase in the concentration of calcium and inorganic phosphorus could be expected if the values were expressed in grams per 100 ml serum. This is shown in Table VII, where the concentration of

TABLE VII. The effect of acid and salt on the concentration of Ca and inorg. P in the cheese serum of reconstituted soft cheese (calculated in % w/w and % w/v).

g Salt per 100 ml solution	pH	Calcium			Inorg. P		
		g/100 g	g/100 ml	Difference	g/100 g	g/100 ml	Difference
0.0	6.38	0.048	0.048	0.000	0.014	0.014	0.000
	5.64	0.183	0.183	0.000	0.056	0.056	0.000
	5.48	0.238	0.237	0.000	0.077	0.076	0.000
	5.25	0.350	0.351	0.001	0.116	0.117	0.001
	4.90	0.524	0.529	0.005	0.169	0.170	0.001
5.0	6.34	0.088	0.090	0.002	0.025	0.025	0.000
	5.60	0.205	0.215	0.010	0.069	0.072	0.003
	5.44	0.253	0.263	0.010	0.088	0.091	0.003
	5.24	0.335	0.345	0.010	0.125	0.130	0.005
	4.85	0.457	0.476	0.019	0.173	0.180	0.007
7.5	6.32	0.094	0.099	0.005	0.024	0.025	0.001
	5.52	0.216	0.225	0.009	0.076	0.079	0.003
	5.26	0.319	0.334	0.015	0.127	0.133	0.006
	4.83	0.438	0.460	0.022	0.165	0.173	0.008

calcium has been calculated in grams per 100 ml of serum instead of grams per 100 grams of serum. There was only a difference in samples containing salt. At the same time the serum of samples with 5 and 7 per cent salt (at a pH value of 4.9) held 13 and 16 per cent less calcium respectively than the unsalted samples when calculated as percentage by weight. Expressed in grams per 100 ml serum, the differences were 10 and 13 per cent respectively. The differences, however, are small, and this makes a correction for the results of salted samples hardly necessary.

The foregoing indicates that more than one factor plays a role in decreasing the level of calcium in the cheese serum where salt is added. The release of bound water due to the addition of salt is one factor. Secondly, expressing the results in percentage by weight (g/100 g serum) would cause a drop in the figures of about 4 per cent at the maximum.

Those two factors were not enough to give a definite explanation. It may be that the salt has some further effect.

3.2.6. *Conclusions:*

The orientation experiments permitted the following conclusions:

1. The experiments showed a high degree of reproducibility.

2. The figures for total nitrogen, calcium and inorganic phosphorus in the serum of reconstituted cheese were smaller than could be expected when compared with the results reported by other investigators. This discrepancy was due to the different methods used.

3. Only about 8 per cent of the N-substances at the maximum were in solution. A considerable amount of the total nitrogen to dissolve was in the form of non-protein nitrogen. This would be produced by the breakdown of proteins by enzymes.

4. The peptizing effect of salt in reconstituted cheese was quite small.

5. At a pH lower than 5 only about 50 per cent of the total calcium and less than 20 per cent of the inorganic phosphorus were dissolved in the serum of reconstituted cheese. This may be due to two important factors: first, a considerable amount of the calcium of both the caseinate and the phosphate may be still combined with the paracasein-complex at such a low pH. This complex might be an acid calcium paracaseinate combined with primary or secondary calcium phosphate. Secondly, some of the calcium and inorganic phosphorus could precipitate in one form or other, while at low pH-values some phosphorus ions might form salt linkages with paracasein. The presence of different forms of crystals is a good indication for the precipitation of salts and may contribute to the low levels of calcium and inorganic phosphorus in cheese serum.

3.3. The influence of the ratio of paracasein-complex to water on the percentage dissolving of the complex

The orientation experiments described in the preceding section 3.2 showed that the percentage of the total of nitrogen, calcium and inorganic phosphorus that dissolved differed markedly from the results reported in the literature. This has been explained by differences in the ratio of paracasein-complex to water.

The experiments discussed below were designed to provide more information on the effect of the ratio of paracasein-complex to water on the dissolving amount of the main components of the complex.

Two, ten and fifty grams of the freeze-dried preparation were brought into 100 ml solution of lactic acid, either with 5 grams salt or without salt and at various pH-values. The first concentration corresponded to that used by van Dam (1911). The last concentration represents semi-hard cheese.

3.3.1. *Dissolved nitrogen substances*

The results for nitrogen in Table VIII show that generally at the same pH, with increasing concentration of the complex both with or without salt, more

TABLE VIII. The effect of the concentration of ca-p-casein-complex* on the solubility of its main components.

	g Salt in 100 ml solution	Lactic acid add. ml	pH	g/100 g solution			Percentage dissolved of the total prep.		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
A. 2 g prep./ 100 ml sol.	0,0	0,25	6.62	0.008	0.012	0.005	3.2	23	29
		0.55	6.03	0.006	0.023	0.009	2.4	43	52
		1.05	5.72	0.008	0.037	0.015	3.2	70	87
		1.50	5.38	0.005	0.048	0.017	2.0	91	99
		2.00	4.97	0.004	–	0.018	1.6	–	105
		2.50	4.14	0.007	0.056	0.016	2.8	106	93
	5.0	0.35	6.36	0.050	0.037	0.011	19.7	70	64
		0.60	6.15	0.089	0.044	0.014	35.2	83	81
		0.70	6.06	0.096	0.047	0.016	37.9	89	93
		0.80	5.98	–	0.047	–	–	89	–
		0.90	5,83	0.116	0.049	0.017	45.8	92	99
		1.00	5.76	0.092	0.050	0.018	36.3	94	105
		1.10	5.68	0.076	0.051	0.018	30.0	96	105
		1.20	5.58	0.060	0.050	0.016	23.7	94	93
		1.40	5.26	0.013	0.051	0.017	5.1	96	99
		1.60	5.04	0.006	–	0.017	2.4	–	99
		1.90	4.78	0.003	0.050	0.018	1.2	94	105
		2.10	4.57	0.003	0.051	0.016	1.2	96	93
		2.30	4.50	0.003	0.050	0.017	1.2	94	99
B. 10 g prep./ 100 ml sol.	0.0	0.60	6.40	0.028	0.026	0.009	2.2	10	10
		1.20	6.10	0.022	0.043	0.012	1.7	16	14
		1.80	5.94	0.021	0.058	0.018	1.7	22	21
		2.20	5.84	0.020	0.072	0.022	1.6	27	26
		2.80	5.68	0.021	0.091	0.027	1.7	34	31
		3.40	5.60	0.019	0.104	0.033	1.5	39	38
		4.20	5.45	0.023	0.137	0.042	1.8	52	49
		5.00	5.33	0.023	0.161	0.046	1.8	61	53
		5.80	5.22	0.020	0.184	0.056	1.6	69	65
		7.40	5.10	0.020	0.223	0.071	1.6	84	83
		8.40	4.86	0.013	0.251	0.078	1.0	95	91
	5.0	0.80	6.24	0.049	0.065	0.016	3.9	25	19
		1.40	6.06	0.060	0.078	0.021	4.7	29	24
		2.00	5.92	0.073	0.093	0.027	5.8	35	31
		2.60	5.80	0.075	0.106	0.033	5.9	40	38
		3.20	5.66	0.071	0.122	0.039	5.6	46	45
		3.80	5.56	0.046	0.138	–	3.6	52	–
		4.40	5.46	0.043	0.151	0.051	3.4	57	59
		5.00	5.38	0.040	0.167	0.058	3.2	63	67
		6.00	5.24	0.033	0.188	0.066	2.6	71	77
		7.00	5.10	0.027	0.207	0.073	2.1	79	85
		8.20	4.90	0.017	0.226	0.078	1.3	85	91
C. 50 g prep./ 100 ml sol.	0.0	8.00	5.83	0.215	0.180	0.018	3.4	14	4
		16.00	5.52	0.220	0.362	0.021	3.5	27	5
		26.00	5.20	0.166	0.584	0.040	2.6	44	9
		34.00	4.76	0.167	0.834	0.085	2.6	63	20
	5.0	9.00	5.75	0.240	0.194	0.028	3.8	15	7
		15.00	5.51	0.302	0.300	0.026	4.8	23	6
		19.40	5.30	0.283	–	–	4.5	–	–
		28.00	5.02	0.243	0.528	0.050	3.8	40	12
		36.00	4.78	0.129	0.637	0.079	2.0	48	18

* Preparation February 1961.
A. After one week with the aid of mechanical shaking.
B. After ten days with the aid of mechanical shaking.
C. After two weeks.

of the nitrogenous substances were found in solution. The data in the same table and fig. (5) show that in samples without salt at different ratios of para-

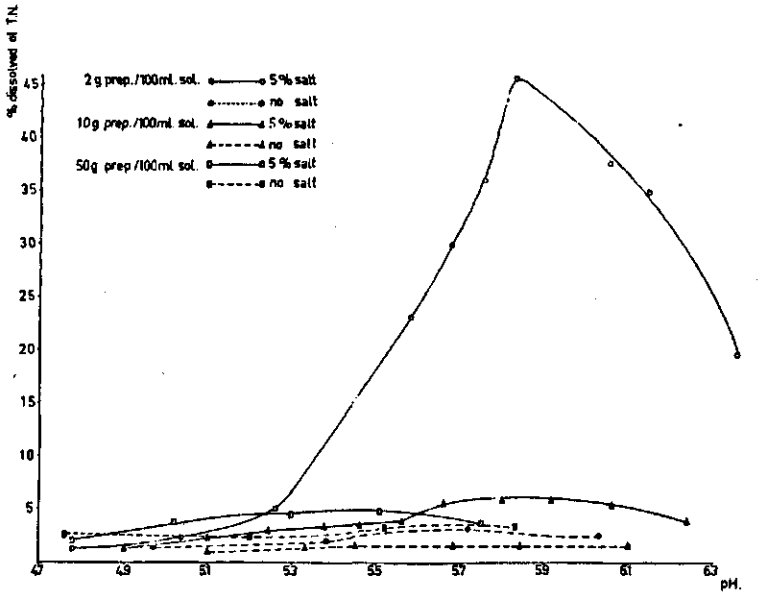

FIG. 5. The effect of the ratio of paracasein-complex to water on the T.N. dissolved.

casein-complex to water the percentage of the nitrogenous substances to dissolve was quite small. In case of the ratio 1 : 50 the peptizing effect of the salt was quite obvious within a certain range of pH-values (5.3–6.3) and ten times more of the nitrogenous compounds went into solution than in those without any salt. The higher the concentration of the paracasein-complex the smaller was the peptizing effect of the salt. At the ratio of 1 : 2 (reconstituted cheese) the effect of salt was quite small, e.g., at pH 5.5 where maximum peptization occurred about 5 per cent of the total nitrogen was dissolved, while the corresponding value was 3.5 per cent for unsalted samples.

The hydrogen ion concentrations where maximum peptization occurred using 1 : 50 suspensions were in general agreement with those reported by SHARP and MCINERNEY (1936). The figures of the present investigation were lower than those reported by VAN DAM (1911) and KIERMEIER and SCHATTENFROH (1958). In the present investigation the maximum was about 50 per cent at a pH value of about 5.8 and containing 5 per cent salt. VAN DAM reported most of the preparation in solution at 5.5 and 5 per cent salt, while KIERMEIER and SCHATTENFROH found 80 per cent of the paracasein-complex dissolved at a pH value of about 5.3 and at a 3 per cent salt concentration.

The high values mentioned in these cases might be due to the activity of micro-organisms, as the authors did not mention the use of any preservative. The high speed centrifuge (15,000 rev/min) used in the present investigations may have contributed to eliminating all undissolved protein particles, while this might not be the case for the experiments made by the previous investigators. This effect will be discussed later in chapter 3.6.

3.3.2. *Dissolved calcium*

The data in table VIII and in Fig. 6 shows the effect of the ratio of the paracasein-complex to water on the concentration of Ca in the serum Fig. 6 A. In Fig. 6 B this is expressed as a percentage of total calcium dissolved at different pH values.

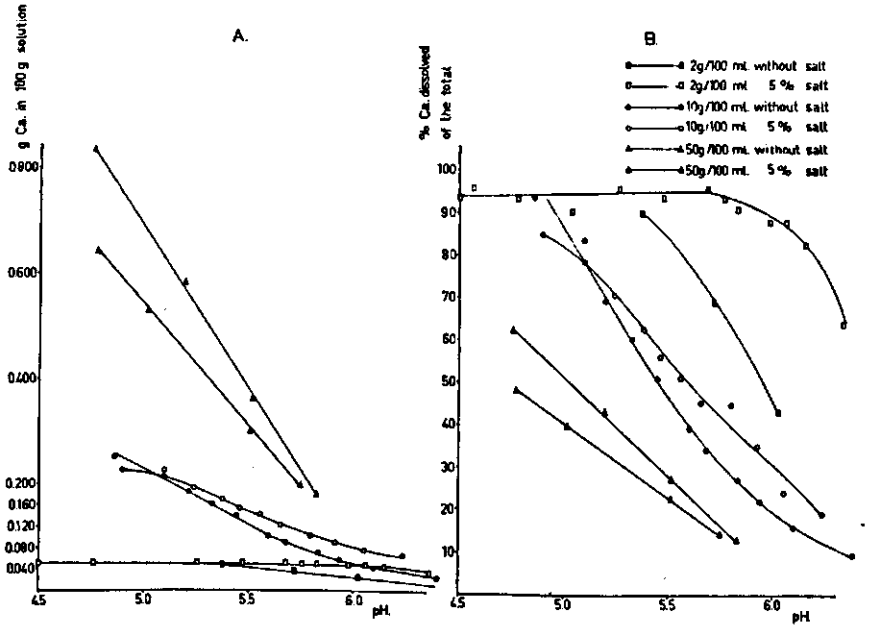

FIG. 6. The effect of the ratio of paracasein-complex to water on the calcium dissolved.

The more paracasein-complex to water, the higher the concentration of the calcium in the serum at the same pH value. This was to be expected since more lactic acid was necessary to produce the same pH in samples with higher concentration.

Adding salt increased the concentration of the calcium in the serum in the case of the low and medium ratios. This increase would be caused by the ion exchange between the sodium of the common salt and the calcium of both the paracasein and the phosphate. The effect of salt is clear at pH-values higher than 5.4. Below this pH-value and in the (1 in 50) suspensions about 90 per cent of the total calcium is dissolved when no salt is added and 95 per cent in samples with 5 per cent salt. In the samples of 1 : 10 ratio and at the same pH, the amount of calcium to dissolve in unsalted and 5 per cent salt samples were about 55 per cent and 60 per cent respectively. At a pH value of 4.9 nearly all the calcium was found in the serum of the intermediate ratio (1 : 10).

These results accord with those reported by VAN SLYKE and BOSWORTH (1907) on Cheddar cheese and those of NAUDTS and DE VLEESCHAUWER (1959) on soft cheese. These investigators used diluted cheese extracts for their determinations. These results also compare well with the results of BELOUSOV's ex-

periments done on 5 grams of paracasein-complex suspended in 100 ml water to which lactic acid was added.

The percentage of dissolved calcium in reconstituted cheese (mixtures 1 : 2) was small compared with the results mentioned above for the 1 in 50 and 1 in 10 suspensions and they were similar, in general, to those reported in the orientation experiments.

3.3.3. *Dissolved inorganic phosphorus*

The data in Table VIII and Fig. 7 shows the effect of dilution of the paracasein-complex mixtures on the concentration of the inorganic phosphorus in the serum Fig. 7 A. In Fig. 7 B this is expressed as a percentage

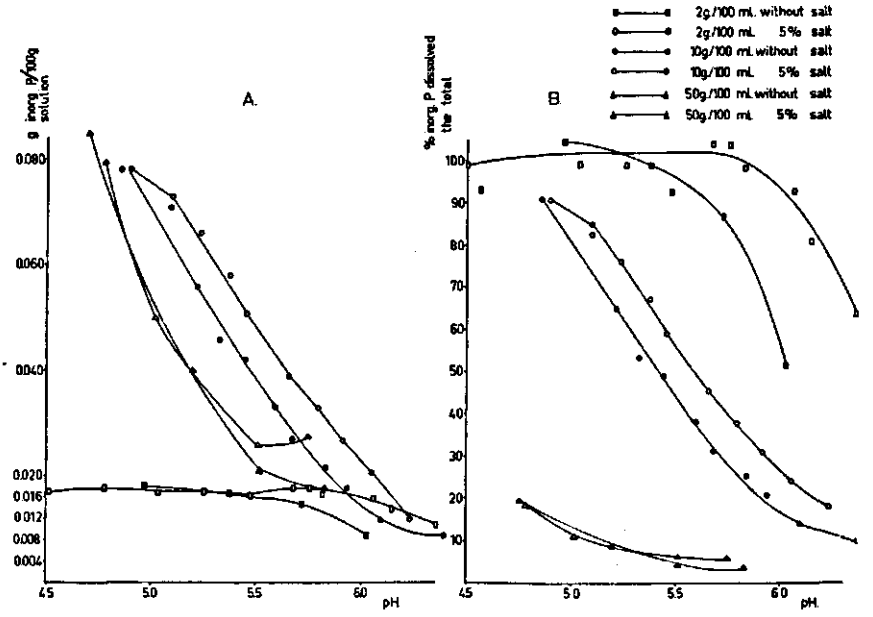

FIG. 7. The effect of the ratio of paracasein-complex to water on the inorganic phosphorus dissolved.

of the total inorganic phosphorus. Salt increased the amount of inorganic P dissolved in the serum at pH-values higher than 5.4, for all the different dilutions of the paracasein-complex. At pH 5.4 about 95 per cent of the inorganic phosphorus dissolved in the case of a ratio of 1 to 50. In the 1 in 10 suspensions, additions of salt caused the percentage of dissolved inorganic phosphorus to increase up to pH 4.9, when 90 per cent of the phosphorus was in solution. These figures correspond closely with those reported by BELOUSOV for his 1 in 20 suspensions of dried rennet curd and also with figures of VAN SLYKE and BOSWORTH for their diluted cheese extracts. In reconstituted cheese, at pH 4.8 less than 20 per cent of the inorganic phosphorus was dissolved in both samples with or without salt. Comparison therefore is only possible between figures obtained for experiments of low ratios and those reported in the literature either on the diluted suspensions of paracasein-complex, or on cheese extracts. In both cases the ratio of cheese

to water or paracasein-complex to water was almost the same and was always less than one to ten. It is wrong to compare the figures obtained under the above mentioned conditions with those in cheese. The amount of water added to the paracasein-complex in any experiment must be similar to that in cheese, i.e., as in the case of reconstituted cheese.

The above-mentioned results show clearly the effect of the paracasein-complex to water ratio on the level of the calcium and inorganic phosphorus in the serum of different mixtures. They strongly suggest that the amount of calcium in the serum influences the peptization of the paracasein-complex preparations.

Sodium chloride helps to disperse the paracasein-complex particles, mostly at pH-value higher than 5. This might be due to an exchange of sodium ions of the salt with the calcium of the paracasein-complex. At lower pH-values where the paracasein-complex has already lost an important part of its calcium, the ion exchange decreases. Although in both diluted and concentrated mixtures 5 per cent salt exists in the serum, yet the final ratio of sodium to calcium in both cases is quite different. In the first case the concentration of the calcium in the serum was relatively small and, therefore, the sodium of the salt would be easily exchanged with the calcium of the complex. In reconstituted cheese the concentration of calcium in the serum was high which would make the ion exchange quite difficult.

3.3.4. *The importance of the level of the calcium in the serum on the different properties of the paracasein-complex*

The effect of calcium ions on the chemical and physical properties of casein and paracasein has been examined by many investigators. VON HIPPEL and WAUGH (1955) mentioned that the casein micelle in skim milk was in equilibrium with dissolved casein. This also is in equilibrium with the ionic calcium and phosphate. Dilution of skim milk causes a dissociation of the casein micelle with the release of soluble casein, while addition of soluble calcium forces dissolved casein into micellar form which can be easily isolated by centrifuging.

ZITTLE, DELLAMONICA and CUSTER (1956) studied the effect of adding an increasing quantity of calcium chloride to a 2 per cent sodium caseinate solution. The sodium caseinate remained clear with concentration up to 0.005 M calcium chloride, while with higher concentrations of calcium it became opalescent. They also reported that the extent of the precipitation of sodium caseinate by calcium chloride is influenced by the pH. For example, 0.02 M calcium chloride in a 2 per cent sodium caseinate solution precipitates 30 per cent at 5.6 and 8 per cent at 7.6. At a constant pH value the extent of precipitation was proportional to the concentration of calcium.

ZITTLE, DELLAMONICA and PEPPER (1959) also investigated the effect of calcium chloride on the precipitation of casein and paracasein. They reported that at the same concentration of calcium chloride, almost equivalent amounts of α-casein and α-paracasein are sedimented in 45 minutes in the ultracentrifuge. With casein and paracasein the amounts sedimented are of the same order, but somewhat further apart. With low speed centrifuging the paracasein was easily separated, in a somewhat smaller amount than with the ultracentrifuge. However, almost none of the casein could be isolated with low-

speed centrifuging. They concluded that, although casein and paracasein are both highly aggregated in the presence of calcium ions, the casein contains a protective colloid that prevents the aggregates exceeding a certain size.

TABLE IX. The effect of the concentration of calcium on the peptization of the calcium-p-caseincomplex.*

Lactic acid added ml.	mg Ca** added	pH	g/100 g solution			Percentage dissolved T.N.
			T.N.	T.Ca	Inorg. P	
0.35	0	6.36	0.050	0.037	0.011	19.7
0.60	0	6.15	0.089	0.044	0.014	35.2
0.70	0	6.06	0.096	0.047	0.016	37.9
0.80	0	5.98	–	0.047	–	–
0.90	0	5.83	0.116	0.049	0.017	45.8
1.00	0	5.76	0.092	0.050	0.018	36.3
1.10	0	5.68	0.076	0.051	0.018	30.0
1.20	0	5.58	0.060	0.050	0.016	23.7
1.40	0	5.26	0.013	0.051	0.017	5.1
1.60	0	5.04	0.006	–	0.017	2.4
1.90	0	4.78	0.003	0.050	0.018	1.2
2.10	0	4.57	0.003	0.051	0.016	1.2
2.30	0	4.50	0.003	0.050	0.017	1.2
0.30	50	6.15	0.024	0.072	–	9.5
0.50	50	6.00	0.029	0.079	–	11.5
0.70	50	5.86	0.032	0.088	–	12.6
0.80	50	5.74	0.026	0.090	–	10.3
0.90	50	5.68	0.023	0.088	–	9.1
1.00	50	5.58	0.015	0.092	–	5.9
1.10	50	5.48	0.011	0.095	–	4.3
1.20	50	5.39	0.009	0.096	–	3.6
1.40	50	5.20	0.007	0.096	–	2.8
1.60	50	5.02	0.005	0.098	–	2.0
1.80	50	4.82	0.004	–	–	1.6
0.20	100	6.16	0.008	0.113	–	3.2
0.40	100	6.00	0.012	0.121	–	4.7
0.60	100	5.86	0.013	0.125	–	5.1
0.70	100	5.78	0.013	0.129	–	5.1
0.80	100	5.72	0.012	0.134	–	4.7
0.90	100	5.64	0.010	0.136	–	3.9
1.00	100	5.54	0.010	0.137	–	3.9
1.20	100	5.32	0.008	0.142	–	3.2
1.40	100	5.14	0.006	0.143	–	2.4
1.60	100	4.97	0.005	–	–	2.0
0,30	100	5.92	0.011	0.113	0.006	4.3
0,50	150	5.62	0.007	0.162	0.008	2.8
0.80	225	5.30	0.006	0.236	0.011	2.4
0.90	275	5.20	0.007	0.289	0.011	2.8
1.00	300	5.06	0.006	0.319	0.011	2.4
1.10	325	5.00	0.006	0.343	0.011	2.4
1.30	375	4.84	0.005	0.394	0.011	2.0
1.40	525	4.68	0.005	0.541	0.013	2.0
1.60	575	4.54	0.004	0.558	0.013	1.6
1.80	625	4.43	0.003	0.659	0.018	1.2

* All experiments were on 2 g prep./100 ml sol. with 5 g salt.
** The calcium was added in the form of calcium chloride.

The following experiments were mainly designed to clarify further this aspect of the study. Calcium chloride was added in increasing quantities to suspensions with two grams of preparation in 100 ml of lactic acid solution containing 5 g of salt. The dilute suspensions were chosen because of the high peptizing effect of sodium chloride on the paracasein-complex at this low paracasein-complex to water ratio. This would allow any suppression of the peptizing effect of the salt to be easily detected, and the data in Table IX

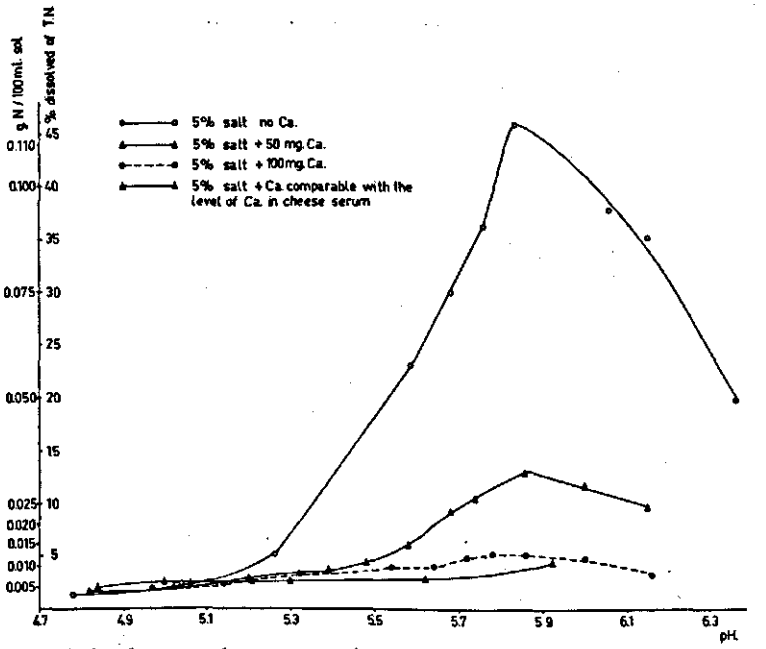

FIG. 8. The relation between the concentration of calcium and the peptization of paracasein (at 5% NaCl content).

and Fig. 8 shows this to be very clearly the case. When the concentration of calcium reached about the same level as that in the serum of reconstituted semi-hard cheese the percentage of nitrogen substances dissolving was very small and was about equal to that in a salt free suspension Fig. 5.

TABLE X. The effect of adding different amounts of calcium chloride on the solubility of calcium-p-caseinate-complex. (Conc. 2 g prep./100 ml sol., with 5 g salt).

Lactic acid added ml.	mg Ca added	pH	Percentage in solution			% Dissol. T.N.	Ca* released mg
			T.N.	Ca	Inorg. P		
1.0	0	5.78	0.089	0.051	0.017	35.2	51
0.9	25	5.80	0.047	0.070	0.016	18.6	45
0.9	50	5.78	0.030	0.090	0.016	11.8	40
0.8	75	5.78	0.022	0.109	0.014	8.7	34
0.7	100	5.78	0.013	0.125	0.012	5.1	25
0.6	150	5.80	0.010	0.142	0.011	3.9	00

* Value determined by difference of the calcium added at the start of the experiment and that found in solution after one week.

This experiment was repeated at a pH-value of about 5.8, i.e., approximately the pH for the maximum peptization. The pH was kept constant by adding the proper amount of lactic acid solution. The results are shown in Table X and Fig. 9. They confirm the above mentioned experiments.

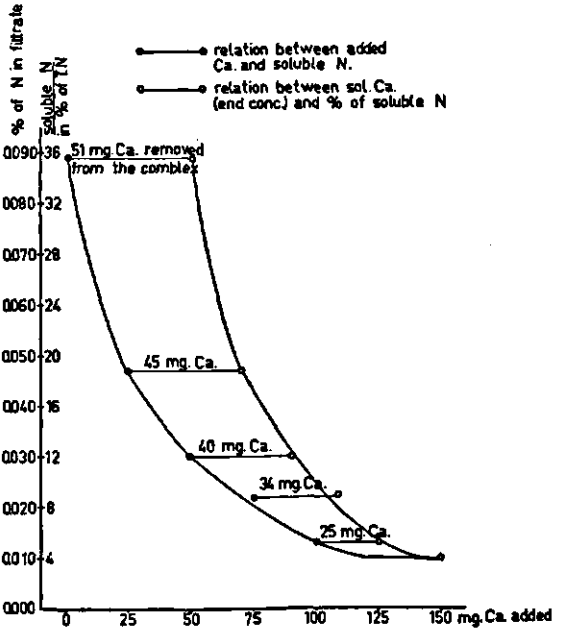

FIG. 9. Effect of raising the level of soluble calcium on peptization of paracasein-complex. (ratio 2/100, and with 5% NaCl).

Determining the concentration of calcium in the solution at equilibrium made it possible to calculate the amount of the calcium released from the preparation. The more calcium was added, the smaller the amount released from the preparation, and the less the percentage of the total nitrogen that dissolved. Similar results were also obtained by adding increasing quantities of calcium chloride to reconstituted semi-hard cheeses containing 5 per cent salt at different pH-values so as to raise the level of the calcium to be about 700 mg in 100 g serum. The data is shown in Table XI and Fig. 10. It is observed that the concentration of total nitrogen in the cheese serum was less than the values obtained when no salt was added.

In all these experiments adding the calcium chloride always decreased the concentration of inorganic phosphorus in the serum relating to the inorganic phosphorus in the samples without added calcium. This might be due to the precipitation of one or more forms of calcium phosphate on the one hand and on the other to the high level of calcium ions in the serum suppressing the exchange of sodium ions of the common salt with the calcium phosphate.

The calcium probably renders the system unstable, and thus causes the soluble paracasein to aggregate and precipitate.

This would happen when a calcium chloride solution was added to the samples after the paracasein had already been dissolved. The presence of soluble calcium (at the start) would counterbalance the peptizing effect of

TABLE XI. The effect of salt and Ca on the solubility of T.N. and inorg. P of reconstituted semi-hard cheese (prep. Febr. 1961).

Treatment	Lactic acid added ml.	mg Ca added	pH	Percentage in solution			Percentage dissolved of the total prep.		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
No salt	8.0	0	5.83	0.215	0.180	0.018	3.4	14	4.0
	16.0	0	5.52	0.220	0.362	0.021	3.5	27	5.0
	26.0	0	5.20	0.166	0.584	0.040	2.6	44	9.0
	34.0	0	4.76	0.167	0.834	0.085	2.6	63	20.0
5% salt	9.0	0	5.75	0.240	0.194	0.028	3.8	15	7.0
	15.0	0	5.51	0.302	0.300	0.026	4.8	23	6.0
	19.0	0	5.30	0.283	–	–	4.5	–	–
	28.0	0	5.02	0.243	0.528	0.050	3.8	40	12.0
	36.0	0	4.78	0.129	0.637	0.079	2.0	48	18.0
5% salt + $CaCl_2$	3.0	450	5.52	0.140	0.704	0.006	2.2	–	1.4
	5.0	400	5.33	0.094	0.709	0.014	1.5	–	3.3
	7.0	330	5.22	0.115	0.709	0.014	1.8	–	3.3
	9.0	290	5.10	0.166	0.716	0.019	2.6	–	4.4
	12.0	120	5.02	0.197	0.620	0.032	3.1	–	7.4

sodium chloride to a certain extent, depending upon the concentration of the calcium in solution, as well as its pH, until a concentration has been reached which would make the ion exchange between the sodium of the salt and the calcium of either the paracasein or the phosphate nearly impossible. However, in both cases, the results will be the same and it is obvious that the peptizing of the paracasein-complex is influenced strongly by the calcium concentration.

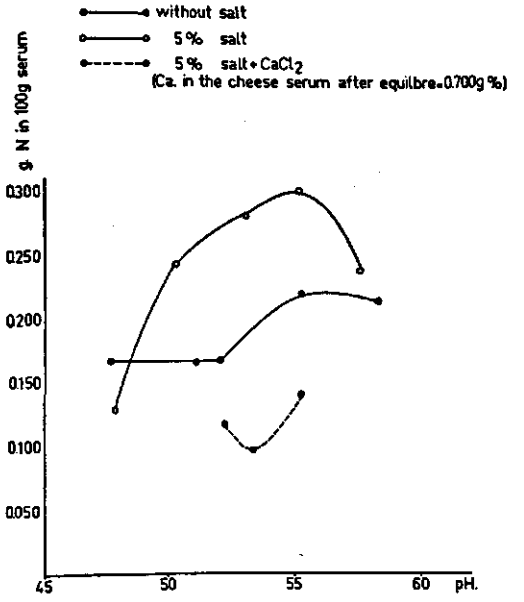

FIG. 10. Effect of adding $CaCl_2$ to reconstituted semi-hard cheese on the concentration of dissolved N-substances in the serum.

As mentioned before this aspect was not considered by many investigators who conducted similar experiments and apparently forget that they were using not pure paracasein but calcium paracaseinate-calcium-phosphate-complex. Here again their ratios of paracasein-complex to water were much lower than those applying to different kinds of cheese, and therefore the level of calcium in the serum of the latter was much higher. Their experiments, therefore, led to erroneous conclusions on the chemical or physical properties of cheese.

3.3.5. *Electrophoretic investigation of the serum of reconstituted cheese*

The previous experiments showed that adding increasing quantities of paracasein-complex to a given volume of lactic acid solution released more nitrogen substances in solution. This might be due, for instance, to fractionation of the paracasein. This aspect was investigated by comparing the electrophoretic patterns of the serum of reconstituted cheese with those of casein and paracasein dispersions.

Plate (2) summarises some of these results. Pattern (A) shows the fractions of casein and Pattern (B) is for paracasein-complex. Both were obtained under identical electrophoretic conditions. It appeared that the casein separated into two fractions at pH 8.6, while the paracasein-complex was separated into three or more indistinct fractions. It also seems that the fraction of the paracasein-complex appearing as a sharp peak in the scanning curve did not migrate in the electric field at pH 8.6 during the time of the experiment. Comparing the patterns of paracasein-complex (the mother substance) with that of the different serum samples of reconstituted semi-hard cheese after two weeks (C and F) and reconstituted hard cheese after 40 days (G and H) reveals that the components of the cheese serum are generally not related to the paracasein-complex fractions. Pattern (C) for the serum of reconstituted cheese without salt at pH 5.36 showed two bands, one at each side of the apex (shown by an arrow) which have migrated toward the cathode and one other toward the anode. All the other patterns of cheese serum samples at different pH values and with or without salt, as well as the ultrafiltrate of reconstituted hard cheese of 40 days old (pattern H) showed components that have migrated toward the cathode. This is the reverse of what is found in the case of either the casein or paracasein-complex.

The investigations showed that under these conditions none of the peaks for paracasein fractions could be detected in the serum of reconstituted cheese. The fractions appeared in the serum might represent an enzymatic cleavage of the protein.

LINDQUIST and STORGARDS (1959) found similar fractions in their electrophoretic investigations of the effect of pH on the degradation of α-casein treated with rennin.

3.3.6. *The presence of rennin in the paracasein-complex*

The preceding paragraphs indicated the possibility of the presence of rennet enzymes in the freeze-dried preparation. This was simply demonstrated by incubating 3-4 grams of the preparation in 100 ml of raw skim milk at 35 °C. After a few hours a firm coagulum was obtained, while this was not the case when another portion of the same milk was treated in the same way with 5-6

grams of preparation in which the enzyme was inactivated by drying at 60 °C for about five hours.

The juice obtained from reconstituted cheese was also examined for the presence of rennet enzymes. Five ml of this juice added to 10 ml of fresh skim milk at 39 °C produced a firm coagulum after two hours. The addition of 10 ml of the same juice after heating for 5 minutes to 80 °C to similar skim milk did not have this effect. This would mean that some enzyme was

TABLE XII. The effect of the concentration of calcium-p-caseinate-phosphate-complex on the solubility of its main components* (preparation May I).

	g Salt in 100 ml solution	Lactic acid added ml	pH	Percentage in solution			Percentage dissolved		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
2 g prep./ 100 ml solution	0.0	0.4	6.36	0.014	0.022	0.011	5.7	40.0	60.3
		0.7	6.05	0.013	0.031	0.014	5.3	56.4	76.8
		0.9	5.91	0.012	0.037	0.015	4.9	67.3	82.2
		1.2	5.58	0.009	0.045	0.018	3.7	81.8	98.7
		1.5	5.31	0.006	0.051	0.019	2.4	92.8	104.2
		1.8	4.77	0.003	0.055	0.019	1.2	100.0	104.2
	5.0	0.3	6.53	0.059	0.038	0.013	24.0	69.1	71.3
		0.5	6.29	0.062	0.044	0.016	25.2	80.0	87.7
		0.7	6.04	0.057	0.048	0.017	23.2	87.3	93.2
		0.9	5.80	0.053	0.052	0.019	21.6	94.6	104.2
		1.1	5.55	0.040	0.056	0.019	16.3	101.9	104.2
		1.3	5.34	0.027	0.053	0.019	11.0	96.4	104.2
		1.5	5.09	0.012	0.054	0.019	4.9	98.2	104.2
		1.8	4.82	0.009	0.053	0.019	3.7	96.4	104.2
10 g prep./ 100 ml solution	0.0	0.5	6.28	0.049	0.045	0.019	4.0	16.4	20.8
		1.0	5.93	0.045	0.069	0.028	3.7	25.1	30.7
		1.5	5.74	0.046	0.097	0.031	3.7	35.3	34.0
		2.1	5.46	0.047	0.125	0.039	3.8	45.5	42.8
		3.2	5.13	0.039	0.193	0.060	3.2	70.2	65.8
		4.2	4.84	0.028	0.250	0.086	2.3	90.9	94.3
	5.0	0.3	6.32	0.128	0.064	0.020	10.4	23.3	21.9
		0.7	6.06	0.119	0.083	0.028	9.7	30.2	30.7
		1.0	5.96	0.119	0.097	0.033	9.7	35.3	36.2
		1.5	5.69	0.112	0.125	0.044	9.1	45.5	48.2
		2.1	5.46	0.129	0.153	0.056	10.5	55.7	61.4
		2.6	5.32	0.092	0.176	0.068	7.5	64.0	74.6
		3.2	5.16	0.066	0.203	0.079	5.4	73.8	86.6
		4.2	4.87	0.037	0.299	0.085	3.0	83.3	93.2
60 g prep./ 100 ml solution	0.0	8.0	5.88	0.507	0.179	0.017	6.9	10.9	3.1
		14.0	5.68	0.569	0.305	0.019	7.7	18.5	3.4
		18.0	5.56	0.169	0.393	0.021	8.4	23.8	3.8
		22.0	5.43	0.739	0.473	0.028	10.0	28.7	5.1
		30.0	5.15	0.358	0.638	0.039	4.9	38.7	7.1
	5.0	8.4	5.90	0.651	0.197	0.023	8.8	11.9	4.2
		14.4	5.68	0.892	0.314	0.025	12.1	19.0	4.5
		18.6	5.55	0.861	0.379	0.027	11.7	23.0	4.9
		23.0	5.40	0.811	0.456	0.029	11.0	27.6	5.2
		31.0	5.16	0.434	0.567	0.044	5.9	34.4	8.0

* All the experiments were analysed after one month.

bound to the paracasein particles and was not removed by washing. This appears to contradict the findings of VAN STIJGEREN (1943) who proved that all the rennin enzyme went quantitatively to the whey. He may possibly have overlooked extremely small quantities of the enzyme.

In spite of the very small quantity of the enzyme remaining in the freeze-dried preparation, it may cause an increase in the hydrolysis of the protein especially in reconstituted cheese where the concentration of the substrate and with it relatively the enzyme will be high. This will be discussed later.

The proteolytic effect of the enzyme in the freeze-dried preparation was investigated in another experiment. Here, 2, 10 and 60 grams of the paracasein-complex were mixed with 100 ml of lactic acid solution, both with and without salt. All samples were left for one month. The samples containing 2 and 10 grams preparation were shaken by hand once daily.

The data are given in Table XII. The amount of calcium and inorganic phosphorus in the serum and the percentage that dissolved were generally the same as those in the previous experiments, where the suspensions of 2 and 10 g preparation had been left for 7 and 10 days respectively (Table VIII). At the same time the values for the concentration of nitrogen substances in the serum as well as the percentage dissolved of the total nitrogen differed from those obtained in the previous experiments (where the samples had been left for a shorter period). A comparison showed the following:

1. In case of the ratio 1 : 50 the amount of nitrogen substances in the serum and the percentage of the total to dissolve were slightly higher for samples without salt and generally lower for samples containing 5 per cent salt in comparison with the corresponding values in the previous experiment.

2. When the ratio was increased to 1 : 10 the amount of nitrogen substances dissolved as a percentage of the total nitrogen nearly doubled in both samples with and without salt.

3. In the month old samples of reconstituted hard cheese the results were higher again.

In another experiment on reconstituted cheese of pH 5.5 and 5 per cent

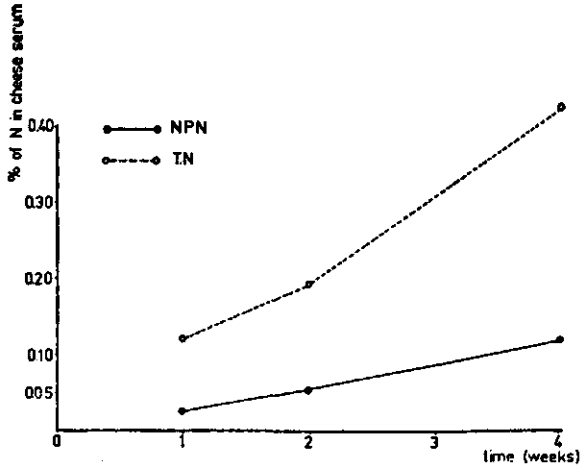

FIG. 11. The increase of the hydrolysis of proteins by enzymes in reconstituted hard cheese with time (5% salt and at pH 5.5).

salt Fig. 11 it was proved that the values obtained for both the total nitrogen and NPN in the cheese serum were nearly doubled for samples kept for four weeks when compared with the respective values for reconstituted cheese of two weeks old.

The above mentioned results support the supposition of the effect of the proteolytic enzymes on the decomposition of paracasein. In case of suspension of 2 g preparation in 100 ml solution the effect of the enzyme was not clear, because the concentration of both the enzyme and the substrate was very small. When the concentration was increased to 10 g in 100 ml solution the percentage of the total nitrogen that dissolved was nearly doubled when left for one month instead of ten days. In this case the concentration of both the enzyme and the substrate increased considerably and thus the proteolytic effect of the enzymes increased. Increasing the concentration of the preparation to 60 g in 100 ml solution, the amount of nitrogen dissolved as a percentage of the total nitrogen after one month was about 11 to 12 per cent in samples with 5 per cent salt. The corresponding value obtained for similar reconstituted cheese of two weeks old was about 4 to 6 per cent (Table IV). Here again this can be explained as due to the effect of the proteolytic enzymes. The increase in the concentration of the total nitrogen in the cheese serum due to the effect of enzymes was also accompanied by an increase in the NPN as mentioned before and which seemed to be a fraction of the paracasein splitting under the effect of the enzyme on the paracasein.

3.3.7. *The effect of heating on the properties of the paracasein-complex*

The proteolytic enzymes from the rennet still present in the freeze-dried preparations were found to have a considerable effect on the percentage of soluble nitrogen especially in reconstituted cheese kept for longer periods.

The investigation described here, studies the effect of heating the washed rennet curd on the main constituents of the reconstituted cheese serum.

The washed rennet curd of the December 1960 batch, was divided into two portions. The first was pressed and freeze-dried directly in the usual way, the second was heated for 15 minutes at 60 °C and then pressed and freeze-dried. This temperature and time combination were chosen to inactivate the enzymes and in the same time were supposed to have the least effect on denaturation of protein or otherwise alter the composition and the properties of the complex.

Table XIII indicates that the total nitrogen and its fraction soluble in 2 per cent trichloroacetic acid (end concentration) are generally lower in the heated than in the unheated samples. This difference is obvious for samples of reconstituted semi-hard or hard cheese. A similar decrease in the amount of nitrogen soluble in 12 per cent trichloroacetic acid (NPN) as well as for the nitrogenous substances present in the ultrafiltrate was noticed in reconstituted hard cheese after three weeks aging. These differences were not obvious in reconstituted semi-hard cheese which was only two weeks old.

Heating the curd under conditions similar to 'cooking' of the curd in some types of hard cheese did not produce any noticeable change in the level of calcium and inorganic phosphorus in the ultrafiltrate from both the samples with and without salt (at the same pH).

These results show therefore that a heat treatment which inactivates the proteolytic enzymes, reduces the concentration of the nitrogen compounds in

TABLE XIII. The effect of heat treatment on the solubility of calcium-p-caseinate-complex (preparation december 1960).

A. Reconstituted semi-hard cheese after 2 weeks ageing

	% Salt	pH	Percentage in the cheese serum			Percentage in the ultrafiltrate		
			T.N.	Sol. N-fractions in % TCA		N	Ca	Inorg. P
				2	12			
No heat treatment	0.0	5.50	0.310	0.284	0.060	0.086	0.318	0.019
	5.0	5.50	0.595	0.300	0.055	0.111	0.308	0.027
	0.0	5.00	0.266	0.260	0.076	0.086	0.524	0.031
	5.0	5.00	0.194	0.160	0.064	0.071	0.449	0.043
Curd heated at 60 °C for 15 min.	0.0	5.48	0.210	0.182	–	0.088	0.324	0.016
	5.0	5.43	0.213	0.130	–	0.097	0.294	0.025
	0.0	5.12	0.154	0.102	0.069	0.052	0.522	0.032
	5.0	5.08	0.101	0.084	0.048	0.040	0.440	0.041

B. Reconstituted hard cheese after 3 weeks ageing

No heat treatment	5.0	5.60	0.519	0.167	0.110	0.184	0.257	0.022
	5.0	5.48	0.432	0.172	0.102	0.188	0.306	0.022
Curd heated at 60 °C for 15 min.	5.0	5.60	0.233	0.100	0.068	0.113	0.258	0.019
	5.0	5.49	0.291	0.114	0.060	0.107	0.295	0.019

the serum of the reconstituted cheese. This reduction was in many cases about 50 per cent of the respective values of samples made from unheated curd.

3.3.8. *Conclusions*

The foregoing section (3.3) has shown that the results of earlier investigators, who studied the effect of acid and salt on diluted suspensions of paracasein-complex or on diluted cheese extracts, do not represent the actual conditions in cheese. This was mainly due to the fact that the previous research workers have forgotten that curd is not identical with pure paracasein but with calcium-paracaseinate-calcium-phosphate-complex. At the same time many investigators confused the terms 'soluble' and 'dissolved'. What is soluble in diluted cheese extracts is not necessary dissolved in the cheese serum.

The following factors are responsible for the high values reported by different investigators for the amount dissolving of nitrogen substances, calcium and inorganic phosphorus when expressed as a percentage of the total quantities either in cheese or paracasein-complex.

1. The great difference in the proportion of the paracasein-complex to water in their experiments than in cheese.

2. A lower level of calcium in the serum of the diluted cheese extract or paracasein-complex suspensions than in cheese or reconstituted cheese. The more diluted the suspension the greater the peptizing effect of the salt, in cheese therefore, this effect is much smaller. These factors cause more nitrogen to dissolve expressed as a percentage of the total nitrogen.

For the same reason mentioned under 1, the earlier results reported in the literature by different investigators concerning the percentage of calcium and inorganic phosphorus dissolved of the total amount in cheese or paracasein-complex were much higher than the actual amount dissolving in cheese.

The foregoing results also showed that the rennet enzyme retained on the paracasein particles, though small in amount, is an additional factor in this investigation. It causes an increase in the percentage dissolved of total nitrogen.

This can be corrected (see effect of heating the curd section 3.3.7.) and this may amount to about half the quantities of nitrogen dissolving compounds.

3.4. Experiments with paracasein-complex separated from slightly acidified milk

In the manufacture of some types of cheese, the milk is 'ripened' or acidified by adding a starter. This increase of acidity will release calcium and inorganic phosphorus from the paracasein-complex. The amount dissolved depends upon the extent to which the hydrogen ion concentration of the milk is increased. The chemical and physical properties of the paracasein-complex might be affected accordingly.

It has been mentioned by van Slyke and Bosworth, 1907, that the paracasein-complex becomes more soluble in lactic acid solution containing salt due to the removal of a part of its calcium. Orla-Jensen (1931) added that this solubility is diminished in the presence of excess of acid. This is supposed to be due to the fact that 'monocalcium paracaseinate' is soluble while free paracasein is insoluble in a salt solution. By this he means that removing part of the calcium from the paracasein-complex makes it more soluble in a solution of salt. Kiermeier and Schattenfroh (1958) found that slightly acidifying the milk before renneting promotes the swelling and solubility of paracasein-complex. They added that the pH of milk should not fall below 6, i.e., to avoid shrinkage and a decrease in the solubility. They came to these conclusions by experiments made on suspensions of 4 g paracasein-complex in 100 ml lactic acid solution. They considered that the percentage of paracasein dissolving in the cheese depends for the greater part on the degree of ripening of the milk.

3.4.1. *The effect of the acidity of milk on the chemical and physical properties of the paracasein-complex*

The aim of this part of investigation was to obtain more information concerning this problem. Three preparations were prepared from fresh skim milk (pH 6.6) and milk to which lactic acid was added until pH 6.5 and 6.3 respectively. The last value represents the highest acidity met in milk ripened for the manufacture of cheese. The composition of the three preparations was found to be the same as shown in section 3.1. Each of these preparations was mixed with water in the three ratios of 2 : 100, 10 : 100 and 60 : 100. The first two mixtures were mechanically shaken and left for 7 and 10 days respectively. The third representing hard cheeses was left for one month before analysis.

The results are set out in Tables XIV, XV and XVI. The different mixtures prepared from the three preparations show no significant differences in the percentages of dissolved nitrogen, calcium and inorganic phosphorus. This applied to all the concentrations used in this investigation.

The influence of acid and salt on the degree of swelling of paracasein-complex, mixed with various amounts of water was also investigated.

TABLE XIV. The effect of acidifying the milk on some chemical and physical properties of the resultant ca-p-caseinate-complex (preparation May I = fresh milk pH: 6.6).

	g Salt in 100 ml solution	Degree of swell.	pH	Percentages in sol.			Percentage dissolved of the total prep.		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
2 g prep./ 100 ml sol. [1]	0.0	430	5.97	0.008	0.026	0.012	3.3	47.3	65.8
		375	5.83	0.007	0.032	0.014	2.9	58.2	76.8
		375	5.69	0.007	0.037	0.015	2.9	67.3	82.2
		365	5.31	0.005	0.049	0.019	2.0	89.1	104.2
		360	4.70	0.003	0.054	0.018	1.2	98.2	98.7
	5.0	340	6.50	0.057	0.044	0.011	23.2	80.0	60.3
		340	6.30	0.076	0.049	0.014	30.9	89.1	76.8
		315	6.18	0.089	0.053	0.015	36.2	96.4	82.2
		310	6.11	0.100	–	0.015	40.7	–	82.2
		325	5.95	0.115	0.050	0.016	46.8	90.9	87.7
		405	5.70	0.088	0.053	0.016	35.8	96.4	87.7
		670	5.32	0.010	0.050	0.017	4.1	90.9	93.2
		510	5.06	0.004	0.050	0.017	1.6	90.9	93.2
10 g prep./ 100 ml sol. [2]	0.0	292	6.25	0.0299	0.038	0.015	2.4	13.8	16.4
		298	5.90	0.0252	0.066	0.025	2.1	24.0	27.4
		296	5.68	0.0264	0.096	0.035	2.1	34.9	38.4
		298	5.48	0.0263	0.132	0.045	2.1	48.0	49.3
		292	5.20	0.0244	0.202	0.071	2.0	73.4	77.9
		302	4.93	0.0194	0.249	0.087	1.6	90.6	95.4
	5.0	310	6.38	0.058	0.061	0.017	4.7	22.2	18.6
		364	6.18	0.062	0.073	0.024	5.0	26.6	26.3
		306	5.98	0.071	0.083	0.027	5.8	30.2	29.6
		266	5.74	0.062	0.115	0.041	5.0	41.8	45.0
		302	5.58	0.059	0.135	0.051	4.8	49.1	55.9
		342	5.40	0.042	0.162	0.062	3.4	58.9	68.0
		332	5.22	0.034	0.191	0.074	2.8	69.5	81.1
		312	5.05	0.022	0.214	0.081	1.8	77.8	88.8
60 g prep./ 100 ml sol. [3]	0.0	221	5.88	0.507	0.179	0.017	6.9	10.9	3.1
		221	5.68	0.569	0.305	0.019	7.7	18.5	3.4
		220	5.56	0.619	0.393	0.021	8.4	23.8	3.8
		220	5.43	0.739	0.473	0.028	10.0	28.7	5.1
		227	5.15	0.358	0.638	0.039	4.9	38.7	7.1
	5.0	251	5.90	0.651	0.197	0.023	8.8	11.9	4.2
		234	5.68	0.892	0.314	0.025	12.1	19.0	4.5
		236	5.55	0 861	0.379	0.027	11.7	23.0	4.9
		244	5.40	0.811	0.456	0.029	11.0	27.6	5.2
		250	5.16	0.434	0.567	0.044	5.9	34.4	8.0

[1]) After one week, with the aid of mechanical shaking.
[2]) After ten days, with the aid of mechanical shaking.
[3]) After one month.

TABLE XV. The effect of acidifying the milk on some chemical and physical properties of the resultant preparation (preparation May II; milk acidified till pH 6.5).

	g Salt in 100 ml solution	Degree of Swell.	pH	Percentages in sol			Percentage dissolved of the total prep.		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
2 g prep./ 100 ml sol. [1]	5.0	390	6.52	0.058	0.038	0.013	23.2	68.4	72.2
		365	6.27	0.093	0.044	0.015	73.2	79.2	83.3
		370	6.16	0.099	0.046	0.016	39.6	82.8	88.9
		360	6.00	0.108	0.047	0.016	43.2	84.6	88.9
		400	5.90	0.116	0.048	0.016	46.4	86.4	88.9
		510	5.62	0.076	0.048	0.017	30.4	86.4	94.4
		485	5.49	0.060	0.050	0.016	24.0	90.0	88.9
		560	5.31	0.010	0.049	0.017	4.0	88.2	94.4
		580	5.18	0.008	0.049	0.016	3.2	88.2	88.9
10 g prep./ 100 ml sol. [2]	0.0	302	6.36	0.033	0.024	0.011	2.6	8.6	12.2
		296	5.74	0.025	0.070	0.026	2.0	25.2	28.9
		306	5.55	0.024	0.097	0.033	1.9	34.9	36.7
		296	5.42	0.027	0.118	0.039	2.2	42.5	43.3
		298	5.29	0.025	0.145	0.047	2.0	52.2	52.2
		308	5.08	0.022	0.198	0.066	1.8	71.3	73.3
		296	4.96	0.019	0.237	0.081	1.5	85.3	90.0
	5.0	286	6.30	0.082	0.059	0.016	6.6	21.2	17.8
		254	5.84	0.093	0.094	0.031	7.4	33.8	34.4
		262	5.64	0.103	0.116	0.041	8.2	41.8	45.6
		296	5.52	0.058	0.135	0.051	4.6	48.6	56.7
		296	5.39	0.051	0.155	0.059	4.1	55.8	65.6
		308	5.14	0.037	0.198	0.075	3.0	71.3	83.3
		330	5.10	0.029	0.222	0.081	2.3	79.9	90.0
60 g prep./ 100 ml sol. [3]	0.0	238	5.92	0.481	0.186	0.015	6.4	11.2	2.8
		225	5.74	0.726	0.279	0.018	9.7	16.7	3.3
		235	5.61	0.705	0.361	0.020	9.4	21.7	3.7
		234	5.41	0.641	0.498	0.024	8.5	29.9	4.4
		233	5.16	0.518	0.637	0.035	6.9	38.2	6.5
	5.0	258	5.82	0.601	0.190	0.029	8.0	11.4	5.4
		264	5.74	0.682	0.260	0.026	9.1	15.6	4.8
		249	5.57	0.699	0.336	0.024	9.3	20.2	4.4
		262	5.36	0.665	0.441	0.030	8.9	26.5	5.6
		262	5.16	0.535	0.555	0.042	7.1	33.3	7.8

[1]) After one week, with the aid of mechanical shaking.
[2]) After ten days, with the aid of mechanical shaking.
[3]) After one month.

Generally, the pH did not seem to affect the degree of swelling in the 2 g suspensions without salt. However, the conditions when salt is added are quite different, because a large amount of the preparation dissolves. At most pH values higher than 5.3 a major part of the nitrogen component dissolves. If this is taken into account the degree of swelling of the undissolved part is considerably higher (before going into solution) and may amount to more than 600 (six-fold increase in weight). This complicates the situation and makes comparison of swelling under these conditions nearly impossible. Only samples at a pH less than 5.3 can therefore be compared, as no more than 5

TABLE XVI. The effect of acidifying the milk on some chemical and physical properties of the resultant preparation (preparation May III; milk acidified till pH 6.3).

	g Salt in 100 ml solution	Degree of swell.	pH	Percentages in sol.			Percentage dissolved of the total prep.		
				T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
2 g prep./ 100 ml sol. [1]	0.0	0	6.47	0.009	0.013	0.006	3.6	25.6	35.3
			6.03	0.008	0.023	0.010	3.2	45.3	58.8
			5.87	0.008	0.029	0.012	3.2	57.2	70.6
			5.70	0.007	0.035	0.014	2.8	69.0	82.4
			5.47	0.005	0.040	0.016	2.0	78.8	94.1
			5.30	0.005	0.045	0.016	2.0	88.7	94.1
			5.03	0.003	0.049	0.016	1.2	96.6	94.1
			4.62	0.003	0.049	0.016	1.2	96.6	94.1
	5.0	0	6.68	0.038	0.031	0.010	15.3	61.1	58.8
			6.44	0.067	0.039	0.012	27.9	76.9	70.6
			6.28	0.078	0.040	0.015	31.4	78.8	88.2
			6.16	0.096	0.044	0.014	38.7	86.7	82.4
			6.04	0.098	0.045	0.015	39.5	88.7	88.2
			5.94	0.113	0.046	0.016	45.5	90.5	94.1
			5.80	0.125	0.048	0.016	50.4	94.6	94.1
			5.67	0.095	0.048	0.016	38.3	94.6	94.1
			5.40	0.028	0.048	0.016	11.3	94.6	94.1
			5.18	0.006	0.047	0.016	2.4	92.6	94.1
10 g prep./ 100 ml sol. [2]	0.0	286	6.36	0.024	0.025	0.013	1.9	9.9	15.3
		292	6.08	0.020	0.039	0.018	1.6	15.4	21.2
		290	5.90	0.020	0.059	0.024	1.6	23.3	28.2
		292	5.76	0.020	0.079	0.031	1.6	31.1	36.5
		286	5.59	0.021	0.105	0.040	1.7	41.4	47.1
		284	5.45	0.022	0.138	0.053	1.8	54.4	62.4
		294	5.31	0.021	0.171	0.064	1.7	67.4	75.3
		290	5.18	0.020	0.204	0.078	1.6	80.4	91.8
		298	4.75	0.013	0.243	0.083	1.0	95.8	97.6
	5.0	322	6.24	0.085	0.062	0.018	6.8	24.4	21.2
		328	6.10	–	0.068	0.021	–	26.8	24.7
		284	5.94	0.093	0.084	0.028	7.5	33.1	32.9
		238	5.85	0.101	0.094	0 032	8.1	37.1	37.6
		312	5.75	0.091	0.107	0.037	7.3	42.2	43.5
		274	5.60	0.070	0.129	0.046	5.6	50.8	54.1
		326	5.52	0.054	0.138	0.051	4.4	54.4	60.0
		296	5.40	0.045	0.155	0.058	3.6	61.1	68.2
		376	5.26	0.038	0.173	0.064	3.1	68.2	75.3
		340	5.12	0.029	0.191	–	2.3	75.3	–
		316	5.02	0.023	0.210	0.082	1.9	82.8	96.5
60 g prep./ 100 ml sol. [3]	0.0	225	5.95	0.599	0.172	0.023	8.0	11.3	4.5
		221	5.72	0.857	0.283	0.023	11.5	18.6	4.5
		222	5.50	0.662	0.421	0.024	8.9	27.7	4.7
		225	5.29	0.697	0.522	0.037	9.4	34.3	7.3
	5.0	246	5.82	0.576	0.171	0.029	7.7	11.2	5.7
		239	5.70	0.843	0.260	0.027	11.3	17.1	5.3
		247	5.44	0.835	0.382	0.033	11.2	25.1	6.5
		244	5.26	0.751	0.498	0.046	10.1	32.7	9.0
		264	5.06	0.490	0.603	0.054	6.6	39.6	10.6

[1]) After one week, with the aid of mechanical shaking.
[2]) After ten days, with the aid of mechanical shaking.
[3]) After one month.

TABLE XVII. The effect of acid and salt on some chemical and physical properties of reconstituted hard cheese of one month old.

	% Salt	Consistency (1)	Consistency (2)	Sol. expel. ml.	Degree of swell.	In the cheese serum (analysis after one month) pH	T.N. %	Ca %	Inorg. P %	Percentage dissolved of the total prep. T.N.	Ca	Inorg. P
A.	0.0	1.140	1.203	25.0	221.0	5.88	0.507	0.179	0.017	6.9	110.9	3.1
		1.210	1.230	26.0	220.7	5.68	0.569	0.305	0.019	7.7	18.5	3.4
		1.110	1.180	28.4	220.0	5.56	0.587	0.393	0.021	8.0	23.8	3.8
		1.015	1.080	27.0	220.0	5.43	0.514	0.473	0.028	7.0	28.7	5.1
		–	–	21.0	227.3	5.15	0.358	0.638	0.039	4.9	38.7	7.1
	5.0	0.680	0.718	12.0	251.0	5.90	0.651	0.197	0.023	8.8	11.9	4.2
		0.852	0.899	21.6	234.3	5.68	0.892	0.314	0.025	12.1	19.0	4.5
		0.798	0.849	20.0	236.0	5.55	0.861	0.379	0.027	11.7	23.0	4.9
		0.700	0.776	18.0	244.0	5.40	0.811	0.456	0.029	11.0	27.6	5.2
		–	–	12.0	250.3	5.16	0.434	0.567	0.044	5.9	34.4	8.0
	7.0	0.811	0.860	17.2	247.0	5.84	0.452	0.198	0.024	6.1	12.0	4.3
		0.630	0.680	16.8	242.0	5.64	0.644	0.294	0.021	8.7	17.8	3.8
		0.720	0.772	18.0	242.0	5.48	0.703	0.370	0.025	9.5	22.4	4.5
		0.501	0.540	16.0	250.0	5.34	0.663	0.433	0.033	9.0	26.3	6.0
		0.481	0.525	9.0	–	5.03	0.452	0.537	0.047	6.1	32.6	8.5
B.	0.0	0.967	1.018	13.0	238.3	5.92	0.481	0.186	0.015	6.4	11.2	2.8
		1.234	1.320	21.6	225.0	5.74	0.726	0.279	0.018	9.7	16.7	3.3
		1.070	1.140	14.0	235.3	5.61	0.705	0.361	0.020	9.4	21.7	3.7
		0.885	0.949	14.6	234.3	5.41	0.641	0.498	0.024	8.5	29.9	4.4
		0.929	1.000	17.0	233.0	5.16	0.518	0.637	0.035	6.9	38.2	6.5
	5.0	0.510	0.565	7.0	258.0	5.82	0.601	0.190	0.029	8.0	11.4	5.4
		0.590	0.630	–	264.0	5.74	0.682	0.260	0.026	9.1	15.6	4.8
		0.482	0.520	13.0	249.0	5.57	0.699	0.336	0.024	9.3	20.2	4.4
		0.420	0.450	5.0	262.0	5.36	0.665	0.441	0.030	8.9	26.5	5.6
		0.350	0.400	4.0	262.3	5.16	0.535	0.555	0.042	7.1	33.3	7.8
	7.0	0.320	0.375	11.2	258.7	5.74	0.454	0.187	0.031	6.1	11.2	5.7
		0.570	0.620	17.0	246.0	5.62	0.536	0.258	0.027	7.1	15.5	5.0
		0.588	0.629	22.0	239.3	5.48	0.654	0.349	0.029	8.7	20.9	5.4
		0.390	0.421	16.4	251.0	5.28	0.560	0.433	0.034	7.5	26.0	6.3
		0.425	0.465	12.0	255.3	5.02	0.422	0.552	0.046	5.6	33.1	8.5
C.	0.0	–	–	23.0	225.3	5.95	0.599	0.172	0.023	8.0	11.3	4.5
		–	–	25.0	221.0	5.72	0.857	0.283	0.023	11.5	18.6	4.5
		–	–	21.0	222.2	5.50	0.662	0.421	0.024	8.9	27.7	4.7
		–	–	22.0	225.0	5.29	0.697	0.522	0.037	9.4	34.3	7.3
	5.0	–	–	12.4	246.0	5.82	0.576	0.171	0.029	7.7	11.2	5.7
		–	–	15.6	239.3	5.70	0.843	0.260	0.027	11.3	17.1	5.3
		–	–	14.0	247.0	5.44	0.835	0.382	0.033	11.2	25.1	6.5
		–	–	14.4	243.7	5.26	0.751	0.498	0.046	10.1	32.7	9.0
		–	–	3.0	263.7	5.06	0.490	0.603	0.054	6.6	39.6	10.6

Consistency 1 = after 5 min.
Consistency 2 = + 5 min. more and 10 g/weight.
A = May I. preparation.
B = May II. preparation.
C = May III. preparation.

per cent of the paracasein-complex dissolves. The effect of salt here needs no further comment.

Increasing the ratio of paracasein-complex to water generally decreased the degree of swelling (Tables XIV, XV and XVI). Both in the 10 g suspensions in 100 ml lactic acid solutions and in the reconstituted hard cheeses, variations in the hydrogen ion concentration and in salt made little difference to the degree of swelling.

Both VAN DAM (1911) and KIERMEIER and SCHATTENFROH (1958) used solubility as a measure of swelling. This is valid for experiments on dilute suspensions of paracasein-complex (2-5 g in 100 ml water). It is well known that the swelling is the first step toward solubility, and in this case the swelling is of unlimited nature. This does not apply to cheese with its much lower water content. This will be further considered.

It has been shown already in chapter (3.3) that the percentage dissolved of the nitrogen substances at different ratios of paracasein-complex to lactic acid solutions containing salt is controlled in large part by the calcium concentration in the serum. Therefore it is to be expected that calcium has a similarly great influence on the degree of swelling of the paracasein-complex.

3.4.2. *The effect of acidity of milk on the physical and chemical properties of reconstituted cheese*

The series of experiments described in this section are concerned with the consistency, the degree to which the water is bound or held by the protein as well as the swelling in reconstituted hard cheese. They were designed in the first place to try to arrive at a simple measure for comparing the physical properties in reconstituted cheese.

3.4.2.a. Consistency of the reconstituted cheese

Paracasein-complex of the May I, May II and May III batches was used for this investigation to study the physical and chemical properties of reconstituted cheeses under different conditions. Table XVII shows the values obtained to represent the physical properties of the reconstituted cheeses as well as the composition of their serum. Table XVIII summarizes some of the results of the previous table for reconstituted cheeses at the same pH value and at different salt concentrations.

The samples without salt always gave higher penetrometer readings than the samples with 5 or 7 per cent salt, (Table XVIII). This indicates a firm body for salted samples and a weaker body for the unsalted ones. This was observed on samples from both May I and May II preparations. At the same time most of the penetrometer values obtained for reconstituted cheese made from preparation May II were relatively lower than those from preparation May I. These differences cannot however be ragarded as significant. Further investigations are needed to come to a definite conclusion. In this respect one has to consider that the penetrometer readings only indirectly measure some of the factors determining the consistency of reconstituted cheese, they offer however a convenient numerical means of comparison.

Salt obviously increases the firmness of reconstituted cheese.

At the same time in both salted and unsalted samples the penetrometer readings generally decreased with a decrease in pH.

Although more protein is hydrolyzed in cheese than in reconstituted cheese,

TABLE XVIII. The effect of pH and salt concentration on some physical and colloidal properties of reconstituted hard cheese and its relation to soluble N and Ca.*

	% Salt	Consistency	Sol. expell. ml	Degree of swell.	pH	% in the cheese serum		Sol. in % of the total	
						T.N.	Ca	T.N.	Ca
A.	0.0	1.140	25.0	221	5.88	0.507	0.179	6.9	11.0
	5.0	0.680	12.0	251	5.90	0.651	0.197	8.8	12.0
	7.0	0.811	17.0	247	5.84	0.452	0.198	6.1	12.0
	0.0	1.210	26.0	221	5.68	0.569	0.305	7.7	18.5
	5.0	0.852	22.0	234	5.68	0.892	0.314	12.1	19.0
	7.0	0.630	17.0	242	5.64	0.644	0.294	8.7	17.8
	0.0	1.110	28.0	220	5.56	0.587	0.393	8.0	23.8
	5.0	0.798	20.0	236	5.55	0.861	0.379	11.7	23.0
	7.0	0.720	18.0	242	5.48	0.703	0.370	9.5	22.4
	0.0	1.020	27.0	220	5.43	0.514	0.473	7,0	28.7
	5.0	0.700	18.0	244	5.40	0.811	0.456	11.0	27.6
	7.0	0.501	16.0	250	5.34	0.633	0.433	9.0	26.3
B.	0.0	1.234	21.6	225	5.74	0.726	0.279	9.7	16.7
	5.0	0.590	–	264	5.74	0.682	0.260	9.1	15.6
	7.0	0.320	11.2	259	5.74	0.454	0.187	6.1	11.2
	0.0	1.070	14.0	235	5.61	0.705	0.361	9.4	21.7
	5.0	0.482	13.0	249	5.57	0.699	0.336	9.3	20.2
	7.0	0.570	17.0	246	5.62	0.536	0.258	7.1	15.5
	0.0	0.885	14.6	234	5.41	0.641	0.498	8.5	29.9
	5.0	0.420	5.0	262	5.36	0.665	0.441	8.9	26.5
	7.0	0.588	22.0	239	5.48	0.654	0.349	8.7	20.9
	0.0	0.929	17.0	233	5.16	0.518	0.637	6.9	38.2
	5.0	0.350	4.0	262	5.16	0.535	0.555	7.1	33.3
	7.0	0.425	12.0	255	5.02	0.422	0.552	5.6	33.1
C.	0.0	–	25.0	221	5.72	0.857	0.283	11.5	18.6
	5.0	–	15.6	239	5.70	0.843	0.260	11.3	17.1
	0.0	–	21.0	222	5.50	0.662	0.421	8.9	27.7
	5.0	–	14.0	247	5.44	0.835	0.382	11.2	25.1
	0.0	–	22.0	225	5.29	0.697	0.522	9.4	34.3
	5.0	–	14.0	243	5.26	0.751	0.498	10.1	32.7

*) Summary of data in Table XVII.

these findings generally agree with those reported by VAN DAM (1910) and RAADSVELD and MULDER (1949) obtained from their investigations on the effect of the pH on the soluble nitrogen and the consistency of Edam cheese. It has to be borne in mind that in genuine hard cheese the influence of the hydrolysis of protein on the consistency is much greater. Moreover there is an important difference in the treatments between hard cheese and that of reconstituted cheese, in the former the paracasein-complex and the fat together form a compact mass, while in reconstituted cheese the material paracasein-complex was in a finely divided form. It is therefore hard to make valid com-

parisons between these results and that of Edam or other hard cheeses as regards consistency and other body characters.

As the concentration of the calcium in the cheese serum always increases when the pH is decreased, it is possible that when the pH is lowered, the consistency of the cheese is affected in some way by the higher concentration of the calcium. In this respect it is difficult to say wether the conclusion arrived at by BELOUSOV (1959) that the calcium content of the paracasein-complex separated from the cheese after pressing plays an important rôle in the consistency of the cheese is correct or not. It is most probable, however, that the amount of the dissolved calcium in the cheese serum is of the greatest importance.

3.4.2.b. The swelling and the water binding capacity of reconstituted cheese

It was difficult to observe any visible differences in the swelling of reconstituted cheeses with variations in acid or salt. However, it was supposed that some differences in the degree of swelling of the insoluble part of the paracasein-complex (after the sample had been centrifuged) would be obtained. By using this method a part of the liquid which is weakly held by the swollen gel will then be expelled. As already discussed in section (2.4.) it was difficult to measure accurately the actual volume of the liquid expelled.

Columns 4 and 3 in Tables XVII and XVIII respectively record the effect of salt on the volume of the solution expelled. It can be seen that the latter decreases somewhat as the salt content increases. This applied to nearly all the samples from May I, May II and May III preparations, except, in May II, for those mixtures with 5 per cent salt. Here less liquid was expelled than in others without salt and with 7 per cent salt.

The general effect of the hydrogen ion concentration at different levels of salt was not always clear. More solution was expelled between pH-values of 5.4 and 5.6 than at either higher or lower pH-values. More experiments would have to be conducted to come to definite conclusions on the effect of acid and salt on the water binding capacity of paracasein-complex in reconstituted cheese.

Preliminarily, it can be said that salt affects the amount of water held by the paracasein-complex particles, while the effect of the pH is less pronounced. It is difficult to explain, however, the figures for the volume of the solution expelled under different conditions. It has been well established that salt liberates the water bound in cheese and reconstituted cheese (section 3.2). At the same time the present results suggest that the salt caused the paracasein-complex particles to hold the water more tightly.

As the values of the degree of swelling depend (in the present investigation) on the amount of the liquid held by the paracasein-complex particles, a slight increase in the degree of swelling was generally noticed when salt was added to reconstituted cheese. This increase was mostly less than 10 per cent and it applied to both the samples with 5 or 7 per cent salt when compared with unsalted samples.

Slightly acidifying the milk used for the paracasein-complex appeared to have no special effect on the swelling of reconstituted hard cheese.

The present investigation could give no more than an idea of the physical and colloidal properties in reconstituted cheese. It provides a simple way of

measuring them. More experiments are needed to throw light on this aspect and other methods will have to be designed.

3.4.3. *Conclusions*

The results and discussion of the foregoing section indicates that acidifying the milk to pH 6.3 appeared to have no important effect on the dissolved nitrogen, calcium and inorganic phosphorus in reconstituted cheese as well as in dilute suspensions of paracasein-complex in lactic acid.

Similarly, slightly acidifying the milk has no significant effect either on the physical and colloidal properties of reconstituted cheese or on dilute suspensions of the paracasein-complex.

3.5. The effect of the pH and the salt content of Edam cheese on the composition of its serum

This investigation served to collect information on the concentration of calcium and inorganic phosphorus in the actual cheese serum. In all previous investigations mentioned in the literature the object was to determine either the total quantity of calcium and phosphorus in cheese; i.e., the soluble and insoluble constituents together, (Zahrndt, Lane and Hammer, 1944) or the parts dissolved in water extracts, e.g., for Cheddar cheese by van Slyke and Bosworth, 1907 or for soft cheese by Naudts and de Vleeschauwer, 1959.

In this study reconstituted cheese was compared with typical Edam cheese, concerning the effect of hydrogen ion and salt concentration on the composition of the serum. The Edam cheeses used were manufactured in the normal way. The cheese juice was obtained as described for reconstituted cheese. The serum was then separated by centrifuging the cheese juice at 15.000 rev/min for 20 minutes.

Tables XIX and XX show the results obtained for two lots of Edam cheese prepared in the laboratory in the standard way. One cheese in every lot was left unsalted as a control. Generally the unsalted cheese held somewhat more

Table XIX. The effect of acid and salt on the solubility of the main components of Edam cheese [1].

Age of cheese	Moisture %	pH	Percentage in the cheese serum				Percentage dissolved of the total		
			salt	T.N.	Ca	Inorg. P	T.N.	Ca	Inorg. P
One day	54.0	5.14	0.00	0.294	0.590	–	6.28	58.0	–
One week	53.3	5.28	0.00	0.636	0.569	0.086	13.40	54.2	22.9
	50.8	5.20	3.27	0.585	0.565	0.094	11.70	51.9	23.7
Two weeks	52.7	5.34	0.00	1.026	0.601	–	21.30	57.5	–
	49.7	5.28	3.34	0.817	0.566	0.103	16.00	50.9	25.4
One month	49.7	5.37*	0.00	1.490	–	0.051	29.10	–	12.6
	48.4	5.30*	3.43	1.099	0.544	0.045	20.90	47.7	10.8

[1]) Edam cheese manufactured during July 1961.
*) The pH value was determined in the cheese not in the serum.

TABLE XX. The effect of acid and salt on the solubility of the main components of Edam cheese [1]).

Age of cheese	Moisture %	pH	Percentage in the cheese serum				Percentage dissolved of the total		
			salt	T.N.	Ca	Inorg.P	T.N.	Ca	Inorg.P
One day	54.7	5.07	0.00	0.259	0.590	0.021	5.5	64.8	6.2
One week	54.1	5.24	0.00	0.729	0.625	0.101	15.3	62.8	29.2
	51.3	5.13	3.47	0.629	0.566	0.110	12.9	55.9	30.4
	50.4	5.12	4.30	0.618	0.550	0.103	12.5	55.6	28.1
Two weeks	53.7	5.20*	0.00	1.209	0.611	0.077	25.6	59.7	22.1
	50.2	5.23	3.55	0.937	0.547	0.082	18.8	52.8	22.2
	49.2	5.21	4.40	0.830	0.538	0.080	16.4	53.3	21.3
One month	50.3	5.25*	0.00	1.203	0.594	0.045	23.2	54.5	12.0
	47.3	5.33	3.76	1.198	0.491	0.055	22.7	44.8	14.0
	46.1	5.32	4.67	1.098	0.485	0.056	20.5	45.1	14.0

[1]) Edam cheese manufactured during August 1961.
*) The pH value was determined in the cheese not in the serum.

moisture than a salted one of the same age. Also, increasing the amount of salt, decreased the moisture content. This would be due to diffusion of part of the water from the cheese during brining.

3.5.1. *The effect of salt on the physical properties of Edam cheese*

It had been observed that the juice was always easier to collect from a salted cheese than from unsalted one. It was for instance, impossible to collect any juice from unsalted cheese two months old, while obtaining the juice from a salted one of the same age and pH was quite easy, and this despite a slightly higher moisture content in the former. A pressure of 40,000 pounds per square inch was always used. This indicates that the water is held very tightly in unsalted samples. This may possibly point to a higher degree of swelling.

Salt liberates bound water (section 3.2.5.) and might cause a salting out effect. This does not agree with the opinion of VAN DAM and many others who always regard that 5 per cent salt in the cheese serum promotes swelling and helps to furnish favourable conditions for optimum consistency. The unsalted Edam cheese seemed, however, to be softer and perhaps of better consistency but with rather a weak body than the salted one. The former could always flatten when left on the shelf for few weeks, while this was not the case with the salted cheeses.

The figures for the swelling and the consistency of reconstituted hard cheese in Table XVII in section 3.4 also indicate that salt in general does not increase either the swelling or the amount of nitrogen which dissolves.

3.5.2. *Dissolved calcium and inorganic phosphorus in Edam cheese*

In the unsalted samples of Edam cheese the dissolved calcium expressed as a percentage of the total calcium in cheese was slightly lower but then increased as maturity progressed in case of cheese made during July.

A slightly higher value was obtained which was then followed by a gradual decrease in the case of cheese made from the milk batch of August. These fluc-

tuations might be caused by a lack of equilibrium in the system as a result of evaporation of a part of the moisture on the one hand, or by precipitation of a part of the dissolved calcium on the other. Incomplete conversion of the lactose into lactic acid could also be considered partially responsible for these differences.

In the case of samples containing salt the dissolved calcium generally decreased as the cheese became older. It was also observed that increasing the salt content while other conditions remained the same generally decreased the dissolved calcium.

When comparing Edam cheese with reconstituted hard cheese, it was observed that the concentration of the calcium in the serum of unsalted Edam cheese of one month old and pH of about 5.3, was 0.594 g in 100 g serum (Table XX) while the corresponding values for reconstituted cheese average about 0.530 g in 100 g serum (Table XVII). When salt was added, the average values for Edam cheese and reconstituted cheese were 0.507 and 0.500 respectively. In both cases less than 50 per cent of the total calcium dissolved.

When comparing the inorganic phosphorus in the serum samples, it was observed that in the case of unsalted samples, the average values were about 0.048 and 0.035 g in 100 g serum in Edam cheese and reconstituted cheese respectively. The corresponding values for salted samples were slightly higher in both cases. At the same time the dissolved inorganic phosphorus expressed as a percentage of the total was less than 15 per cent in the case of Edam cheese and it did not reach 10 per cent in reconstituted cheese.

The results obtained for calcium and inorganic phosphorus in reconstituted cheese are comparable with those existing in cheese.

3.5.3. *The effect of salt on the concentration of T.N. in the cheese serum*

If the slight differences in moisture and pH between unsalted and salted Edam cheeses are ignored, a gradual increase in the concentration of the total nitrogen in the serum can be observed as maturity proceeds. This is well known, and is caused by hydrolysis of proteins. The values for dissolved nitrogen were always higher in samples without salt than in samples with salt, Tables XIX and XX. The nitrogenous substances to dissolve expressed as a percentage of the total nitrogen of the cheese at an age of one month averaged about 26 per cent in unsalted samples with a pH of 5.3. This average was about 21 per cent in cheese at the same pH and containing about 4 per cent salt in its serum. The difference is relatively small, yet it was interesting to calculate that it corresponded to the bound water liberated as a result of the effect of salt, (0.4 g bound water per gram casein). Although both salted and unsalted cheeses gave identical bacterial counts, the effect of salt on the activity of micro-organisms should not be overlooked. STADHOUDERS (1961) mentioned that the hydrolysis of protein in Edam cheese by streptococci depends upon the proteolytic capacity by the bacterial cell as well as on the total number of streptococci present in the cheese. However, under the present conditions this difference was very small.

It is difficult to compare the present figures and those reported by other investigators for the total nitrogen content of the juice of different kinds of cheese, as the latter make no mention either of the salt content or the pH of the cheese (SANDBERG *et al.*, 1930) and DE VLEESCHAUWER and HEYNDRICKX, 1948). For instance, SANDBERG *et al.* reported the percentage of the total

nitrogen which was dissolved in two Edam cheeses of unknown age, pH or salt content to be 26 and 27 per cent. These results are slightly higher than the results reported in Table XIX and XX for Edam cheeses which were one month old.

The total nitrogen in the serum of Edam cheese as well as the percentage dissolved of the total quantities in cheese cannot be compared with that in reconstituted hard cheese in the previous section. This is mainly due to different conditions in the former such as the microflora, and the proteolytic enzymes besides the effect of acid and salt.

3.5.3.a. Comments on the water extraction methods

It is impossible to compare results from the present investigation with those reported by SIRKS (1943). This is because he used a dilute cheese extract to study the influence of pH on the protein degradation in Edam cheese and its relation to the consistency. It is of interest however to discuss some of his results, as this method was used by many investigators. Table XXI summarizes

TABLE XXI. Summary of some of the results reported by SIRKS (1943) on Edam cheese (in a dilute extract).

Sample	Age of cheese	pH		Water soluble N in % of T.N.
		in cheese	in water extract	
AC	one week	5.19	5.81	19.3
	three weeks	5.28	5.75	23.9
AD	one week	5.35	6.01	12.7
	four weeks	5.38	5.94	18.1
HK	one week	5.28	5.56	12.5
	three weeks	5.33	5.56	14.8

some of the results reported by SIRKS. The results were chosen for cheeses with about the same pH and age as those examined in the present study (Tables XIX and XX). SIRKS did not mention the salt content of his cheeses.

The values of SIRKS were generally lower than would be expected, especially with the pH of the water extract much higher than that in the actual cheese. It is difficult to explain why this is so. It may be due to the low end concentration of the sodium chloride in the water extract compared with the 5 per cent usually found for the cheese serum. This will have the effect that the peptization of the protein components will be less pronounced. It might also be possible that the thymol in alcohol, used by SIRKS to preserve his preparations (100 mg thymol per 100 ml extracting solution) influenced the solubility of the protein (section 3.6).

Apart from the results obtained with water or salt extracting methods, a major objection to the extraction method is mainly the changes in the composition of the exracting solution compared with that actually found in cheese. Any shift in the pH of the extracting solution away from that in the original cheese, as well as in the concentration of calcium and sodium, will cause variations in the amount of nitrogenous substances to dissolve. This will make the extracting method, as it is applied, not suitable for studying the conditions

of calcium and total nitrogen occurring in cheese and its relation to the physical and colloidal properties.

Many investigators, (SANDBERG *et al.*, and DE VLEESCHAUWER and HEYNDRICKX) have pointed out the differences between the 'pressure' and the water extraction method. They recommened the former because it approximates more closely to the conditions in cheese. ORLA-JENSEN (1940) preferred the extraction method for the determination of the soluble nitrogen in cheese and proposed extracting the finely ground cheese mass at room temperature for 15 hours. This can be justified in the case of matured cheese where part of the soluble amino acids form crystals because of lack of water. With the 'pressure' method crystallized amino acids would not appear in solution although they can be regarded as part of the soluble nitrogen, and a dilute extraction method is needed where hydrolysis of proteins in cheese is wanted. This does not say anything however about the conditions in cheese.

When designing an extraction method, one has to take into account that several factors influence the results of the extraction, i.e. pH, salt content, calcium concentration, enzymes and bacteria. Until now this was not considered sufficiently by different investigators.

How the extraction method is devised depends upon the aim of the investigation, viz. hydrolysis of proteins or partially imitating the conditions in cheese. This is of great importance. Consequently an extraction with water, the method used by VAN SLYKE and also by SIRKS cannot be approved. Extracting with 5% salt solution (VAN SLYKE) will easily lead to erroneous conclusions as far as the concentration of calcium and the hydrogen ion were not controlled.

3.6. THE EFFECT OF THYMOL ON THE SOLUBILITY OF PARACASEIN

In section 3.5 it was questioned whether the thymol used as a preservative would affect the solubility of the protein. The results which SIRKS (1943) obtained for extractable total nitrogen in Edam cheese were smaller than they were expected to be.

Although thymol was used previously in similar experiments, in the literature no mention was made of any influence of thymol on the solubility of proteins. This may explain why already the greater part of the experiments were finished before the influence of thymol was considered of importance.

An investigation of the question, however, became indispensable when some doubt arose on the figures for extractable nitrogen estimated by the method of SIRKS.

Thus it became necessary to make experiments in such a short time that no micro-organisms could influence the results. This required in the first place a rapid method for establishing equilibrium between the paracasein-complex and the diluted lactic acid, to enable results obtained for samples with and without thymol to be compared under similar conditions.

A rapid equilibrium in the system would furthermore have the advantage of minimizing any enzymic action.

3.6.1. *A rapid method for establishing equilibrium between paracasein-complex and lactic acid*

This was achieved with the 'Ultra-Turrax' (a high speed mixer) which could

disperse a freshly prepared paracasein-complex in a solution of lactic acid within a few minutes. Preliminary experiments showed that four grams of the wet preparation (55% moisture) dispersed in 100 ml water to which lactic acid was added and with or without salt reached equilibrium within 8 to 10 minutes. The temperature did not exceed 25 °C during mixing.

It is important to control the temperature as a rise in temperature will cause a change in the amount dissolved of the paracasein-complex. The temperature during mixing was controlled by putting the sample in cold water and oparating with the Ultra-Turrax for two periods each of four or five minutes. It was found that dispersing the particles any longer under these conditions did not significantly change the dissolved constituents in the serum.

The Ultra-Turrax made it also possible to increase the amount of paracasein-complex in the water (acidified with lactic acid) up to a ratio of 1 : 3.5 which is similar to the ratio in soft cheese. For this purpose 60 grams of the wet preparation, holding 55 per cent moisture, were vigorously mixed with 60 ml lactic acid solution.

The equilibrium in this case was obtained within 12 to 15 minutes of vigorous mixing at about 20 °C. To hold this temperature the container with the sample was always kept in a water + ice mixture. Every sample required a total period of about one hour to attain the equilibrium.

It was unfortunately not possible to prepare a homogeneous mixture of paracasein-complex with water in ratios higher than 1 : 3.5. Therefore no experiments with reconstituted hard cheese could be made.

3.6.2. *The effect of thymol on protein in solution*

Serum freshly prepared from reconstituted soft cheese and centrifuged at 15,000 rev/min for 20 minutes was divided into two portions. The first served as a control and was analysed immediately for total nitrogen. To the other thymol in chloroform (150 mg thymol per 100 ml serum) was added and mixed vigorously with the Ultra-Turrax for a few minutes, which caused the solution to become turbid.

The samples were then centrifuged again and filtered for the determination of total nitrogen. The results are shown in Table XXII. From the figures it is

TABLE XXII. The effect of mixing thymol with reconstituted cheese serum on the concentration of the nitrogen substances

pH	Total nitrogen (g/100 ml serum)		Differences g/100 ml
	no thymol	with thymol	
6.20	0.047	0.033	0.014 = 30%
5.53	0.365	0.245	0.120 = 33%
5.08	0.273	0.107	0.166 = 61%

obvious that adding thymol to the serum of reconstituted soft cheese significantly decreases the concentration of the nitrogen substances in solution. The influence of thymol was greater at a lower pH (5.08) than at higher one (6.2). The decrease was about 60 per cent in the former case and came to about 30 per cent in the latter.

3.6.3. *The effect of thymol on dilute suspensions of paracasein-complex*

The effect of adding thymol to the paracasein-complex suspensions on the

concentration of total nitrogen in the serum was also investigated. For this, the experiment of section 3.3 (with a paracasein-complex to water ratio of 1 : 50) was repeated, but this time with the Ultra-Turrax mixer to give a rapid equilibrium. Four grams of the wet preparation (55% moisture) were suspended in 100 ml diluted lactic acid containing 5 grams salt. After stirring with the Ultra-Turrax the samples were centrifuged at 15.000 rev/min for 20 minutes and filtered. A second lot of samples had 3 ml of 5 per cent thymol in chloroform added to every 100 ml of lactic acid solution (150 mg thymol per 100 ml solution) before it was similarly mixed and treated. The results of analysis for samples with and without thymol are set out in Table XXIII.

TABLE XXIII. The effect of adding thymol on the solubility of paracasein-complex (paracasein-complex:water = 2:100).

pH	No thymol		With thymol	
	T.N. g/100 ml*	% dissolved	T.N. g/100 ml*	% dissolved
6.22	0.078	29.1		
5.88	0.122	45.5		
5.56	0.175	65.3	0.069	25.8
5.27	0.181	67.5	0.038	14.2
5.16	0.089	33.2	0.008	3.0
4.93	0.066	24.6		
4.70	0.008	3.0		

* In order to obtain the soluble fraction the samples were centrifuged at 15,000 rev/min for 20 min.

Mixing thymol with the suspensions greatly decreased the concentration of the soluble nitrogen substances. This effect was greater at lower pH values.

Dispersing the paracasein-complex with the Ultra-Turrax and centrifuging it at 15,000 rev/min left in most cases turbid solutions. This turbidity will make a comparison with the suspensions used in the previous experiments (section 3.3) less valid. In the latter case the serum was relatively clear. The same experiment was therefore repeated with the samples centrifuged for 60 instead of 20 minutes to eliminate as much as possible of the turbidity. The results are shown in Table XXIV.

TABLE XXIV. The effect of centrifuging the dispersed paracasein-complex (2:100– at 15,000 rev./min for 60 min. instead of 20 min.,* on the concentration of T.N. in the solution

pH	T.N. g/100 ml	% Dissolved of the total
5.92	0.114	42.5
5.70	0.135	50.4
5.51	0.145	54.1
5.22	0.128	47.7
5.05	0.074	27.6
4.86	0.014	5.2

* See Table XXIII without thymol.

When comparing the data in Table XXIV with the respective values in Table XXIII, it is observed that increasing the time of centrifuging considerably decreases the total nitrogen values especially in the pH zone of maximum peptization of the paracasein. The extra 40 minutes of centrifuging cleared the solution sufficiently for a fair comparison of the present results (without thymol added) with the previous ones (section 3.3 Table VIII) where thymol was used as a preservative.

Table XXIV shows that at the pH point of maximum peptization for paracasein-complex about 55 per cent of the total nitrogen of the preparation was dissolved. This value was higher than that reported in the previous experiments (section 3.3) Table VIII (about 45 per cent) in which thymol was used as a preservative. The amounts of dissolved nitrogen were still markedly higher in the pH range of 5–5.5 in Table XXIV than where thymol had been used Table VIII and Fig. 5.

This indicates that thymol added to the paracasein suspensions has a considerable influence.

In another series of experiments 4 grams of wet preparation were suspended in 100 ml water containing lactic acid both with and without salt. These suspensions were used to investigate the influence of 150 and 100 mg thymol (dissolved in chloroform) added respectively per 100 ml suspension. The former concentration was used in the present investigation for all the experiments with freeze-dried paracasein-complex. The latter concentration was used by SIRKS (1943) in his experiments.

In all cases the thymol was mixed with the Ultra-Turrax during dispersion of the paracasein in the water to which lactic acid was added. The samples were then centrifuged at 15,000 rev/min for 60 minutes to obtain as clear a serum as possible. The results are shown in Table XXV. As shown in the

TABLE XXV. The effect of the concentration of thymol on the percentage dissolved of T.N. of paracasein-complex in diluted suspensions (2:100).

	No thymol			+ 100 mg thymol/ 100 ml solution			+ 150 mg thymol/ 100 ml solution		
	pH	N. subst. g/100 ml	% dissol. of T.N.	pH	N. subst. g/100 ml	% dissol. of T.N.	pH	N. subst. g/100 ml	% dissol. of T.N.
No salt	5.80	0.011	4.1	5.94	0.003	1.1			
	5.22	0.008	3.0	5.40	0.004	1.5			
	4.98	0.007	2.6	5.18	0.003	1.1			
5% salt	5.83	0.104	38.8	5.99	0.066	24.6	6.00	0.066	24.6
	5.40	0.131	48.9	5.32	0.065	24.3	5.40	0.068	25.4
	5.27	0.141	52.6	4.98	0.010	3.7	5.28	0.042	15.7

table, thymol has an obvious effect on both the samples with and without salt. Adding 150 mg thymol had about the same effect as 100 mg thymol (with similar pH). In both cases the effect was more pronounced when the hydrogen ion concentration was increased.

These experiments indicate that thymol causes a pronounced decrease in the percentage of the total nitrogen to dissolve in dilute suspensions of paracasein in water acidified with lactic acid especially at pH 5.5 or lower. It

follows also that the concentration of thymol in alcohol used by SIRKS (1943), significantly decreased the percentage of total nitrogen available in cheese for solution during extraction. Therefore, SIRKS's method cannot be accepted as reliable for studying the hydrolysis of protein in cheese. The thymol in this case would alter the results in different ways depending upon the pH of the extracting solution.

At the same time the influence of thymol does not change the general conclusions drawn in the previous sections (3.3) of the present investigation. Only the shape of the curve in Fig. 5 should be changed in the part for pH-values of 5.7 and lower where the effect of thymol was great. Both the previous and the new curves are shown together in Fig. 12.

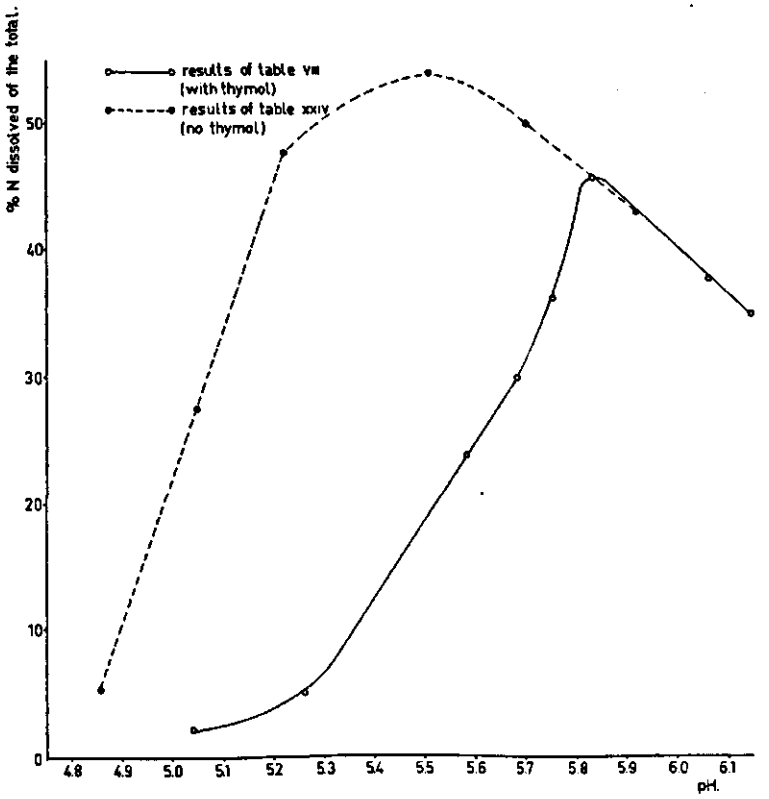

FIG. 12. Comparison between the curves obtained in the previous experiments to which thymol was added and the new experiments without adding thymol. (ratio 2/100 and with 5% salt).

It will be remembered that it was concluded from the experiments described in section 3.3 that all the work previously reported in the literature was done with dilute paracasein-complex suspensions and it provided little useful data on the physical and chemical conditions and changes in cheese.

3.6.4. *The effect of thymol on concentrated mixtures of paracasein-complex (reconstituted soft cheese)*

In the previous section (3.6.3.) it was shown that thymol in chloroform

significantly affects the percentage of the total nitrogen to dissolve in dilute suspensions of paracasein-complex. It was also necessary to find out what effect thymol had on mixtures containnig more paracasein. Reconstituted soft cheeses at different pH values were freshly prepared for this purpose, other samples were prepared in the same way, but these were treated in two different ways with thymol in chloroform (150 mg thymol per 100 ml cheese serum). In the first the preservative was added before the paracasein-complex was mixed with the Ultra-Turrax. In the second treatment the preservative was added to the samples after mixing with the Ultra-Turrax. In this case the preservative was worked into the homogeneous sample by thorough stirring with a glass rod. The samples were then left for 24 hours, and the serum was collected for analysis. This was regarded as long enough to reveal any effect of the thymol.

In all cases the cheese juice was expelled by first centrifuging the samples at 5,000 rev/min for 20 min, and then centrifuging the supernatant liquid once more at 15,000 rev/min for one hour in order to obtain as clear a serum as possible. The results of these experiments are shown in Table XXVI. They

TABLE XXVI. The effect of mixing thymol with reconstituted soft cheese on the concentration of T.N. in the serum.

	No thymol		+ thymol before mixing with Ultra-Turrax		+ thymol after mixing with Ultra-Turrax	
	pH	T.N. (g/100 ml serum)	pH	T.N. (g/100 ml serum)	pH	T.N. (g/100 ml serum)
No salt	5.72	0.090	5.61	0.060	5.70	0.097
	5.50	0.095	—	—	—	—
	5.30	0.110	5.22	0.066	5.34	0.081
	5.09	0.154	5.00	0.079	5.10	0.102
5% salt	5.79	0.138	—	—	5.64	0.164
	5.42	0.381	5.45	0.100	—	—
	5.31	0.553	—	—	5.29	0.336
	5.10	0.328	5.15	0.112	5.08	0.330
	4.94	0.157	4.95	0.100	—	—

indicate that thymol decreased the concentration of the total nitrogen in the cheese serum considerably, where it was added before mixing. This effect was greater in the case of salted samples than in the unsalted ones. Where the thymol was added after mixing with the Ultra-Turrax the effect was only small.

Thymol is very soluble in alcohol and chloroform, while it is nearly insoluble in water. This would explain why it has less effect when added to the reconstituted soft cheese after the latter has been mixed with the Ultra-Turrax. At the same time the thymol here decreased the amount of total nitrogen in the serum of reconstitued soft cheese less than in the more dilute suspensions.

It is difficult, however, to determine which type of treatment can approximate more closely to the conditions of the previous experiments in which the thymol and the diluted lactic acid solution were mixed at the same time

with the freeze-dried preparation (reconstituted cheese) and then kept for two weeks.

As mentioned before homogeneous mixtures of paracasein-complex and water corresponding with hard or semi-hard cheese could not be prepared with the Ultra-Turrax method. The effect therefore of thymol in chloroform, under the conditions of the experiments on reconstituted cheese in sections 3.2 and 3.4, cannot be accurately evaluated. This problem, as a result, had to be approached from another direction.

It was proved in section 3.3 that the level of the calcium in the serum exert a major influence on the degree of peptization of the paracasein-complex.

Raising the level of calcium in the serum of reconstituted soft cheese, therefore, offered a way of approximating semi-hard or hard cheese in this respect. An analysis of the serum of the resultant mixture will allow the results from the previous experiments for reconstituted semi-hard and hard cheese with thymol to be compared. The next section will deal with this point.

3.7. The effect of lactic acid and salt on the chemical and physical properties of reconstituted soft cheese

The method described in section 3.6 was used to prepare reconstituted soft cheese both unsalted and containing 5 per cent salt in its serum and at different pH values. All the samples were from one stock of wet preparation which in turn was separated from the same mixed bulk milk.

The water binding and the swelling capacity of these samples were measured in the same way as described in chapter 2 (p. 11).

The degree of swelling is the increase in weight (g) of 100 grams dry matter. The supernatant liquid obtained after centrifuging the samples at 5,000 rev/min was once more centrifuged at 15,000 rev/min for 60 minutes in order to obtain as clear a cheese serum as possible.

3.7.1. *The effect of pH and salt on the physical properties of reconstituted soft cheese*

From the amount of the solution expelled (Table XXVII) it appears that

Table XXVII. The effect of acid and salt on the physical and chemical properties of reconstituted soft cheese. (Paracasein complex: water = 1:3.5)

Salt in serum %	Sol. expel. ml.	Degree of Swelling	pH	In the cheese serum mg./100ml. serum.				Percentage dissolved of the total in prep.		
				T.N.	N.P.N.	Ca	inorg. P	T.N.	Ca	inorg. P
0.0	49.5	232	6.00	86	2	129	24	2.1	15.1	8.5
	54.0	222	5.72	90		172	41	2.2	20.1	14.5
	58.5	205	5.50	95	4	252	61	2.4	29.4	21.6
	59.0	202	5.30	110		303	82	2.7	35.4	29.0
	55.0	219	5.09	154	4	445	137	3.8	52.0	48.4
	54.0	259	4.95	156		540	167	3.9	63.1	59.0
5.0	53.0	253	5.88	158	4	147	33	3.9	17.2	11.7
	45.0	254	5.79	138		157	40	3.4	18.3	14.5
	–	–	5.65	217	4	193	50	5.4	22.5	17.5
	55.0	231	5.43	461		216	77	9.5	25.2	27.2
	50.0	236	5.31	553	4	288	86	13.8	33.6	30.4
	51.0	231	5.10	328		379	123	8.2	44.3	43.5
	49.0	247	4.94	157	5	460	168	3.9	53.7	59.4

adding salt slightly increases the water binding capacity of reconstituted soft cheese. As a result of this an increase in the degree of swelling was also noticed in general in samples containing 5 per cent salt in its serum than in unsalted samples. The values for expelled solution and the degree of swelling varied with different pH conditions.

The differences were not large or consistent enough to be significant. The present results agree in general with those of the previous experiments on reconstituted hard cheese (section 3.4). The values obtained for the degree of swelling were mostly slightly higher in soft cheese than the respective values in reconstituted hard cheeses.

3.7.2. *Effect of acid and salt on the composition of the serum of reconstituted soft cheese*

The data from these tests have also been included in Table XXVII. The table and Fig. 13 show the concentration of the total nitrogen in the cheese

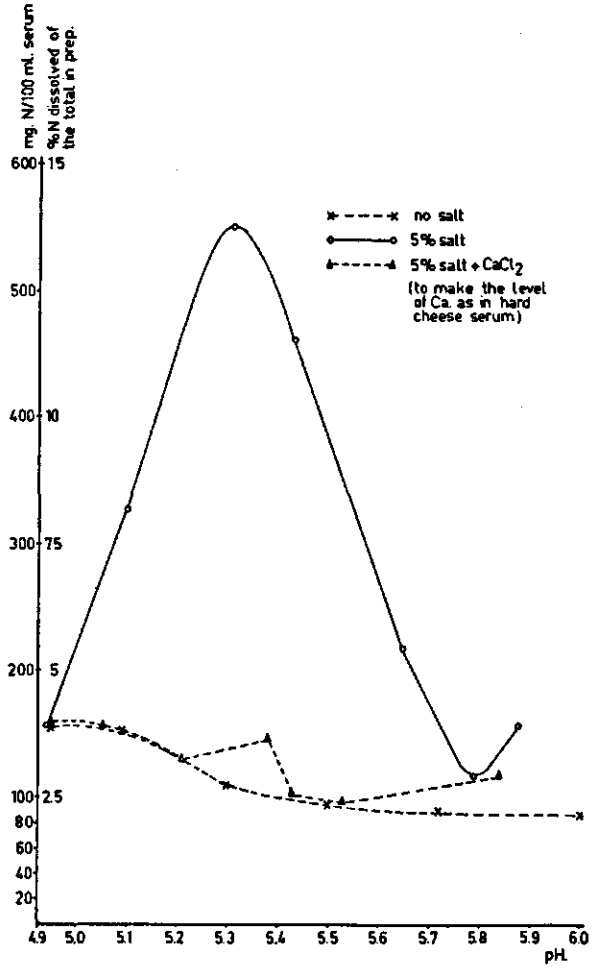

FIG. 13. The effect of pH, salt and adding calcium chloride on the concentration of N-substances in the serum of reconstituted soft cheese.

serum of unsalted samples to be relatively small, while it gradually increases as the pH decreases. At pH 5 about 4 per cent of the total nitrogen in the sample was dissolved. In case of samples containing 5 per cent salt, the concentration of nitrogen in the serum was smallest at pH values of 5.8 and 4.9. Here the amount of dissolve nitrogen were nearly the same as for unsalted samples. In between these two hydrogen ion concentrations the salt exerted an obvious peptizing effect on the paracasein-complex under conditions representing soft cheese. This was highest at about 5.3 where about 14 per cent of the total nitrogen of the preparation was dissolved. It is of interest that only a very small fraction (about 4 per cent) of the dissolved nitrogen substances was soluble in 12 per cent trichloroacetic acid (NPN). This applied to both samples with and without salt. The values for NPN in the previous experiments on reconstituted hard cheese (two weeks old) described in section 3.1. were about 20 to 30 per cent of the total nitrogen dissolved in the serum. This difference might be due to the activity of enzymes present in the freeze-dried preparation during the two weeks the samples were left standing to come to an equilibrium. In case of reconstituted soft cheeses, the samples were prepared and the serum was separated within about two hours.

The concentration of the calcium in the cheese serum (Table XXVII and Fig. 14 increased gradually as the hydrogen ion concentration increased. This applied to both salted and unsalted samples. Between pH 5.7 and 6 the concentrations of calcium were nearly the same in both treatments. At lower

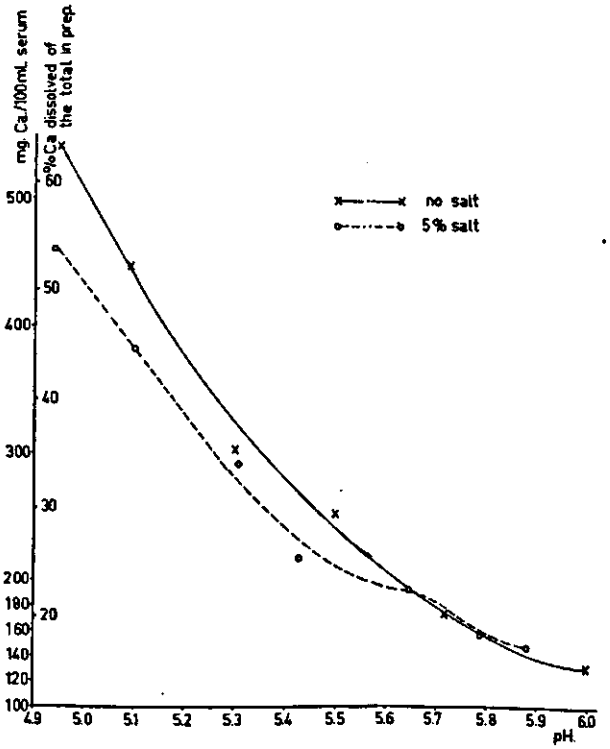

FIG. 14. The effect of pH and salt on the concentration of calcium in the serum of reconstituted soft cheese.

pH values the concentration of calcium in the unsalted samples was always higher than in samples with 5 per cent salt. The percentage of the total calcium to dissolve at pH 4.95 was about 63 per cent in unsalted reconstituted soft cheese. In a sample containing 5 per cent salt in its serum this was 53 per cent. The concentration of calcium in the serum was generally lower than in the serum of reconstituted semi-hard and hard cheeses (section 3.1) while the percentages of the total calcium which dissolved was higher in reconstituted soft cheese samples than in reconstituted hard cheeses. The curves for the samples without and with 5 per cent salt in the serum were generally similar for the different kinds of cheese. The greater proportions of the total calcium to dissolve in reconstituted soft cheese can be explained by the lower ratio of the paracasein-complex to water than in the reconstituted hard cheeses.

Table XXVII and Figure 15 give the amounts of inorganic phosphorus

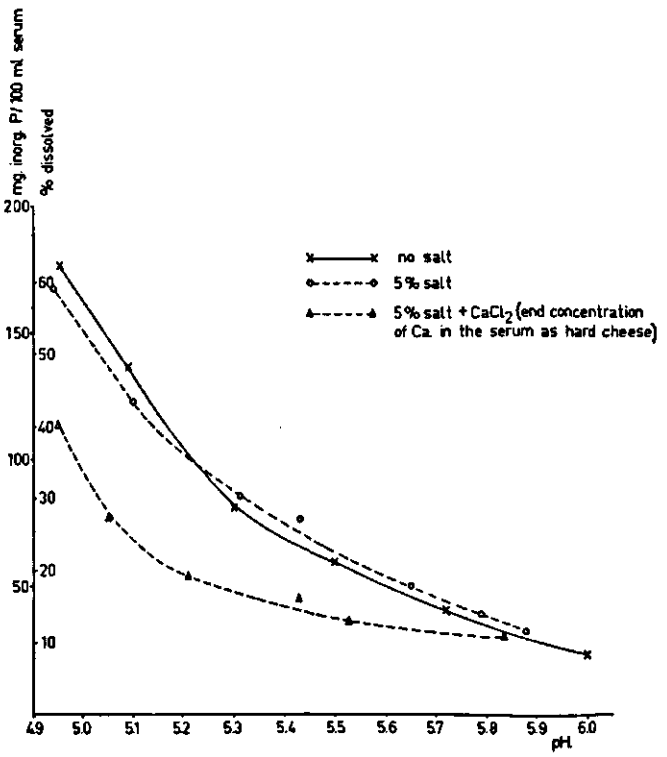

FIG. 15. The effect of pH, salt and adding $CaCl_2$ on the concentration of inorg. P in the serum of reconstituted soft cheese.

found in the serum of reconstituted soft cheese under different conditions of salt and pH. Between pH 5.3 and 5.9 the samples containing 5 per cent salt held more dissolved inorganic phosphorus than those without salt. This corresponded to the results obtained with reconstituted semi-hard and hard cheese (section 3.1). This was explained as being due to an exchange of sodium from the salt and calcium from the complex resulting in sodium phosphate. Conside-

rably more inorganic phosphorus (about 60 per cent at pH 4.9) was dissolved in reconstituted soft cheese than in reconstituted semi-hard and hard cheese (about 15 and 10 per cent respectively). This could be due to the higher ratio of the paracasein-complex to water in hard cheeses. The lower concentration of calcium in the serum of reconstituted soft cheese could also contribute to this. The last could be confirmed by increasing the level of the calcium in reconstituted soft cheese samples to equal that in the serum of reconstituted hard cheese. It resulted in a considerable decrease in the concentration of the inorganic phosphorus. The new phosphorus values attained by adding calcium came to about 23 per cent when expressed as a percentage of the total in reconstituted hard cheese (c.f. Table XXVIII and Fig. 15.

3.7.3. *The effect of raising the calcium of the serum of soft cheese*

The above results for the serum of reconstituted soft cheese indicate that calcium and inorganic phosphorus dissolved in reconstituted soft cheese and reconstituted hard cheese are related. This completes the picture and makes it clearer. The differences in the concentration of the calcium and inorganic phosphorus in the serum, as well as the percentage of the total in the preparation to dissolve, accorded with the previous conclusions concerning the importance of the ratio of the paracasein-complex to water as well as the concentration of the calcium in the serum on the behaviour of the different constituents in different kinds of cheese.

The situation is more complicated with respect the soluble nitrogen substances. This applies both to its concentration in the cheese serum as well as to the percentage dissolved of the total nitrogen of the paracasein-complex. The freshly prepared reconstituted soft cheese and the reconstituted hard cheese, as examined in the previous experiments (section 3.2), cannot be compared. This is due to a complex of factors affecting the results. The first of these is the presence of traces of the rennet enzyme. It was proved to have a considerable effect on the breakdown of the protein during the two weeks of standing needed to establish equilibrium between the lactic acid and the freeze-dried paracasein-complex. The second factor is the thymol which was used as a preservative in reconstituted hard cheese was also found to influence the concentration of the nitrogen substances in the cheese serum. In order to overcome these difficulties, the problem was approached from another direction, viz. by raising the concentration of the calcium in the serum of reconstituted soft cheese. The cheese was prepared by mixing with the Ultra-Turrax as discribed in (section 3.6), and the calcium in the serum was increased to the same level as in reconstituted hard cheese. Some preliminary experiments were necessary to determine the quantities of calcium chloride solution and lactic acid (10%) which had to be added to the wet preparation to give an end concentration of calcium in the serum similar to that in hard cheese. The nitrogen, calcium and inorganic phosphorus content of the serum were determined under the new conditions to be compared with the results previously obtained for reconstituted hard cheese. Table XXVIII shows the results of these experiments. They are all for samples containing 5 per cent salt in the serum.

The values for total nitrogen as shown in the table and in Fig. 13 illustrate that raising the level of calcium markedly decreased the concentration of nitrogen substances in the cheese serum. The soluble fraction of the total

TABLE XXVIII. The effect of raising the concentration of the calcium in the serum of soft cheese, to be similar to hard cheese, on the concentration of the total nitrogen in the cheese serum.[1])

ml Lactic acid 10%	mg Ca[2]) added	Sol. expel. ml	Degree of Swell	In the cheese serum mg/100 ml serum				Percentage dissolved of the total prep.	
				pH	T.N.	Ca	Inorg. P	T.N.	Inorg. P[3])
4	55	44	252	5.84	119	200	31	3.0	11.0 (6.3)
6	135	47	235	5.53	97	294	37	2.4	13.1 (7.5)
7	165	51	255	5.43	104	339	45	2.6	15.9 (9.1)
8	110	53	225	5.38	144	358	–	3.6	– –
8	170	53	228	5.21	125	402	52	3.0	18.4 (10.5)
11	175	54	230	5.05	157	472	78	3.9	27.6 (15.8)
14	110	52	245	4.95	160	512	114	4.0	40.3 (23.0)

[1]) All the samples contain 5 per cent salt in the serum.
[2]) Calcium was added in the form of calcium chloride solution.
[3]) The values between two brackets represent the inorg. P dissolved, expressed as a percentage of the total in hard cheese.

nitrogen in the preparation ranged from 2.5 to 4 per cent. These values were very much lower than those for reconstituted soft cheese to which no calcium chloride had been added. Only at pH values of 4.9 and 5.8 did the additions of calcium slightly affect the concentration of the nitrogen substances. At these hydrogen ion concentrations the peptizing effect of the salt was at a minimum.

In another experiment the effect of varying the level of calcium in the serum of reconstituted soft cheese with 5 per cent salt and at a pH of about 5.3 was studied by adding two different quantities of calcium chloride. The results are shown in Table XXIX and Fig. 16. They indicate, first, the

TABLE XXIX. The effect of adding increasing quantities of calcium to reconstituted soft cheese on the concentration of T.N. in its serum

Ca added mg	In the cheese serum		
	pH	(mg/100 ml serum) T.N.	Calcium
00	5.29	634	326
28	5.30	504	345
56	5.32	207	358

sensitivity of the dissolved nitrogen substances to the concentration of the calcium in the cheese serum, and secondly, that adding a given amount of calcium chloride solution will not cause an equivalent increase of the final concentration of calcium in the serum. This may be due to the nature of the equilibrium between the dissolved and undissolved calcium in cheese.

Adding calcium chloride solution to reconstituted soft cheese with 5 per cent salt in its serum (Table XVIII) decreases the values obtained for the nitrogen in the serum. These values were about half those previously obtained in reconstituted hard cheese (section 3.2). The higher values in the latter case can be easily explained as being due to the effect of enzymes. This is supported by the fact that in reconstituted hard cheeses where the enzymes in the curd had

been inactivated by heat treatment, the amount of dissolved nitrogen was about half that in cheese made from unheated curd (the effect of thymol was not considered in both cases). This indicates firstly that the enzymes which

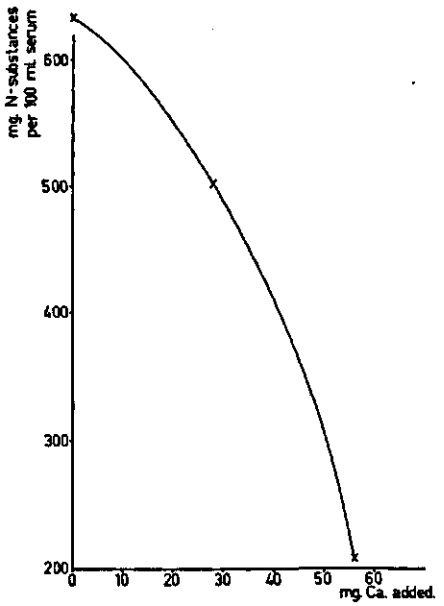

FIG. 16. The effect of varying the level of calcium in the serum of reconstituted soft cheese on the concentration of N-substances. (5% salt and at pH 5.3).

are present double the total nitrogen to dissolve under the effect of pH and salt at higher paracasein-complex to water ratios (reconstituted hard cheese – orientation experiments).

Secondly, the thymol in chloroform used for the series of experiments of reconstituted semi-hard and hard cheese (sections 3.2 and 3.4) did not principally alter the amount of the soluble nitrogen in the serum of reconstituted cheese.

Table XXVIII also shows that raising the level of calcium in the serum of reconstituted soft cheese has little influence on the degree of swelling.

4. SUMMARY

The calcium-paracaseinate-calcium-phosphate-complex separated from milk by renneting is the mother substance of all the different kinds of cheese. The state of this paracasein-complex has a dominant influence on the consistency and other properties of cheese.

The paracasein-complex was obtained by renneting fresh skim milk drawn from a bulk supply and washing the curd out thoroughly. The washed curd was freeze-dried in batches of several kilograms, to provide a starting material of constant composition.

The composition of the paracasein-complex was found to vary very little in different batches. The values calculated for the calcium bound to the paracasein compare well with those reported in the literature for the calcium bound to casein. Slightly acidifying the milk to produce conditions corresponding with those of the 'ripening' process in the cheese manufacture did not cause important changes in the composition of the paracasein-complex, nor did it seem to affect the physical or the chemical properties of the resultant reconstituted cheese.

A small part of the rennet enzyme was found to be retained in the freeze-dried preparation.

Reconstituted cheeses were prepared from the paracasein-complex preparations by mixing them with water (acidified with lactic acid) in ratios similar to those in hard and semi-hard cheese. Salt levels in the reconstituted cheese were varied from nil to 7 per cent and the hydrogen ion concentration from pH 4.8 to about pH 6. In the majority of the experiments thymol in chloroform was used as a preservative. The reconstituted cheeses were kept (in sterilized glass containers) for two weeks to establish the equilibrium between the liquid and the paracasein-complex.

The amount of nitrogen substances, calcium and inorganic phosphorus to dissolve in the reconstituted cheese were studied on the juice expelled from the cheese by pressure after mixing with sand. The juice was further clarified by centrifuging it at 15,000 rev/min.

Only a relatively small percentage of the total nitrogen substances was found dissolved after two weeks. The maximum which was found with mixtures of reconstituted cheeses containing 5 per cent salt, was about 8 per cent. Part of the serum protein subsequently was found to be the product of enzymatic hydrolyses of the paracasein. About one fourth of the nitrogen substances in the serum was soluble in 12 per cent trichloroacetic acid which indicated that it was non-protein nitrogen (NPN). This might be liberated from the paracasein by the rennet enzymes. The real maximum percentage of paracasein nitrogen which dissolves for mixtures with 5 per cent salt, therefore amounted only to less than 6 per cent.

Other investigators had reported quite different figures for soluble protein, calcium and inorganic phosphorus in the serum. This was the result of using a dilute extract of cheese or suspensions of few grams of paracasein-complex in a large volume of water (acidified with lactic acid), instead of cheese or reconstituted cheese.

The peptizing effect of salt on the paracasein-complex is enormously influenced by the ratio of paracasein-complex to water. In dilute suspensions (1 in 50 ratios) the percentage of nitrogen substances to dissolve was very high (50 per cent or higher), and it was greatly influenced by pH and salt concentration. In the various reconstituted cheeses this percentage was only very small and the pH and the salt had only little influence. This indicated that all the earlier work reported in the literature, either on cheese extracts, or on dilute suspensions of paracasein-complex in diluted lactic acid, did not represent conditions in actual cheese, and would lead to wrong conclusions.

The investigators who had used paracasein-complex and not pure paracasein had not taken into account that:

1. The ratio of paracasein-complex to water in their experiments was about 1 : 50, while this ratio is only about 1 : 2 in hard cheese.
2. The concentration of the calcium in the serum from a dilute suspension is very much lower than in cheese.
3. In using 5 per cent sodium chloride in their suspensions to approximate the concentration of salt in the cheese serum, they did not consider the great influence of calcium which was shown here to play an important rôle in diminishing the peptization of paracasein-complex by sodium chloride.

Extraction methods are essential for studying the progress of the degradation of proteins in cheese during its maturing. The methods for obtaining the extract should however, be standardized.

It is proposed that this is best achieved by adjusing the pH of the extracting solution and the temperature so that they are similar to that of the cheese. No further breakdown of the proteins by micro-organisms or enzymes should be allowed during extraction. This can be easily achieved by rapidly extracting the cheese by using the 'Ultra-Turrax'.

If the extracting method, however, is used to give some information about the conditions in cheese, then it is quite necessary to modify the method completely.

In this case pH, calcium and sodium should be adjusted in the extracting solution to be similar to the actual condition in cheese. Besides, the temperature during extraction and the time should be controlled.

At pH 5 about 50 per cent of the total calcium and less than 20 per cent of the inorganic phosphorus were dissolved in the serum of reconstituted hard and semi-hard cheese. Similar results were also obtained for Edam cheese.

At this low pH a considerable amount of calcium and inorganic phosphorus could still be combined with the paracasein in cheese. The precipitation of some of the dissolved calcium and inorganic phosphorus in the form of crystals would also contribute to the relatively small percentage of the total components to dissolve in the cheese.

These results could not be compared with earlier work, as other workers had determined only the total quantity (soluble + insoluble) of calcium and phosphorus present in cheese, or else they had worked with dilute extracts of the cheese.

The present investigation also showed that the swelling of reconstituted cheese was limited. It was not greatly affected by the level of salt or the pH under the experimental conditions applied. It was very much harder to expell the cheese juice with the same hydraulic pressure from unsalted Edam cheese samples than from otherwise similar salted ones. This would indicate that salt is not the most important factor for promoting the swelling and water binding capacity in cheese.

Thymol has been widely used as a preservative for similar experiments. Thymol in chloroform was proved to be a quite effective preservative compared with other preservatives. It was found, however, in the course of this study that thymol markedly affects the solubility of the nitrogen components (section 3.6).

Study of this effect demanded a method to establish rapidly an equilibrium between the paracasein-complex and lactic acid solution, i.e., one which did away with thymol but at the same time prevented the micro-organisms from interfering. This was achieved with a special mixer 'Ultra-Turrax' which made it possible to mix a semi-dry paracasein-complex preparation (50% moisture) with water at the required ratio.

Adding thymol (in chloroform), either at the concentration used in the present investigation or as that used by SIRKS (1943), to dilute suspensions of paracasein-complex in water (ratio 1 : 50) followed by vigorous mixing, markedly altered the figures obtained for the dissolved nitrogen especially at pH values between 5 and 5.7 (Table XXV). This influence did not however change the general conclusions initially arrived at (ref. sections 3.3, 3.6 and 3.7).

Adding the same quantity of thymol in chloroform to paracasein-complex and water mixtures corresponding to soft cheese was found to have a smaller effect on the nitrogen substances to dissolve.

It was not possible to prepare reconstituted hard cheese by using the 'Ultra-Turrax' method and the effect of thymol on the figures initially obtained (sections 3.2 and 3.4) could not be checked this way. This last was done indirectly by adding calcium chloride to reconstituted soft cheese during its preparation to raise the calcium concentration in the serum to about that in hard cheese.

The method of preparing homogeneous reconstituted soft cheese at about one hour, using the 'Ultra-Turrax', made it possible to study also the effect of lactic acid and salt on the chemical composition of the serum of reconstituted soft and hard cheeses, without interference by the proteolytic enzymes, and without need to add preservatives. It helped to provide a more complete and accurate picture on the effect of pH and salt on the total nitrogen dissolving in reconstituted cheeses.

The same experiments also indicate that adding salt raises the concentration of dissolved nitrogen substances in reconstituted soft cheese (between pH 5 and 5.7), while its effect is much less pronounced in reconstituted hard cheese.

The results for calcium and inorganic phosphorus in reconstituted soft cheese, generally accorded with those previously obtained for reconstituted hard and semi-hard cheeses.

The results of this investigation allow the following conclusion on the changes taking place in the rheological properties of cheese with maturing.

The green hard cheese is a rigid gel with high elasticity (rubber-like consistency). This elasticity disappears gradually in the course of maturing as a result of hydrolysis of the protein as well as changes in the hydrogen ion and salt concentrtaion.

The salt seems to act in two directions:
a. by causing a salting-out effect, and b. by liberating a part of the bound water.

This does not agree with the theory of VAN DAM and many other investigators who assumed that the protein of the green cheese was peptized by the salt. The conclusions which were drawn by VAN DAM (1911) on the influence of pH and salt concentration on the consistency of cheese, were criticized in section 3.3.

Unsalted cheese (Edam cheese), in fact becomes smooth and of a rather good consistency, although it is somewhat weak. The salt is not necessary to make the cheese soft. It helps to form the right consistency, not by a peptizing effect as always considered, but by a salting out effect. Near the iso-electric point the protein precipitates. This makes it easy to understand why cheese is hard and brittle at a low pH value.

SAMENVATTING

Het calciumparacaseïnaat-calciumfosfaat-complex, door stremming uit melk gewonnen, is het uitgangsmateriaal voor de verschillende soorten kaas. De toestand, waarin dit paracaseïne-complex verkeert, heeft een alles overheersende invloed op de consistentie en de andere eigenschappen van de kaas. In het op de voorafgaande pagina's beschreven werk zijn verschillende aspecten onderzocht.

Het paracaseïne-complex werd verkregen door verse ondermelk van gemengde melk te stremmen en de wrongel goed uit te wassen. De gewassen wrongel werd gevriesdroogd in porties van enkele kilogrammen, om een uitgangsmateriaal met een constante samenstelling te verkrijgen.

De samenstelling van het paracaseïne-complex varieerde zeer weinig in de verschillende porties. De waarden, die berekend werden voor het aan de paracaseïne gebonden calcium komen zeer goed overeen met de waarden, die in de literatuur aangegeven worden voor het aan caseïne gebonden calcium. Een weinig aanzuren van de melk, teneinde omstandigheden te verkrijgen welke overeenkomen met die tijdens het rijpingsproces bij de gewone kaasbereiding, bracht geen belangrijke veranderingen in de samenstelling van het paracaseïne-complex te weeg. Ook scheen dit geen invloed te hebben op de fysische of chemische eigenschappen van de verkregen „kunstkazen".

Een klein gedeelte van het leb-enzym werd in het gevriesdroogde preparaat teruggevonden.

Uit de paracaseïne preparaten werden „kunstkazen" gemaakt, door deze te mengen met water (aangezuurd met melkzuur) in verhoudingen overeenkomend met die in harde en half-harde kaassoorten. Het zoutgehalte van de gereconstitueerde kazen werd gevarieerd van 0 tot 7% en de pH van 4,8 tot ca. 6. Bij het merendeel van de proeven werd een oplossing van thymol in chloroform als conserveermiddel gebruikt. De „kunstkazen" werden twee weken (in gesteriliseerde glazen potten) bewaard om een evenwicht tussen de paracaseïne en het vocht te verkrijgen.

De hoeveelheden stikstof, calcium en anorganisch fosfaat, die in de gereconstitueerde kazen in oplossing gingen, werden bepaald in het vocht, zoals dit verkregen werd door de kaas, na menging met zand, uit te persen. Het verkregen perssap werd verder geklaard door het te centrifugeren met een snelheid van 15000 omwentelingen per minuut.

Slechts een betrekkelijk klein gedeelte van de totaal aanwezige hoeveelheid stikstof bleek na 2 weken in oplossing te zijn gegaan. Bij de kazen die 5% zout bevatten, bedroeg het maximum ongeveer 8%. Een deel van de opgeloste stikstof bleek het product te zijn van een enzymatische hydrolyse van de paracaseïne. Ongeveer een vierde deel van de stikstof in het perssap bleek namelijk oplosbaar te zijn in 12% trichloorazijnzuur en moet dus tot de z.g. niet-eiwit-

stikstof (NPN) gerekend worden. Deze niet-eiwit-stikstof zou door het stremsel uit de paracaseïne afgesplitst kunnen zijn. Het werkelijke maximale gehalte van de paracaseïne-N, die bij kazen met 5% zout oplost, bedroeg daarom hoogstens 6%.

Andere onderzoekers hebben voor de gehalten aan opgelost eiwit, calcium en anorganisch fosfaat in het kaasvocht geheel andere waarden gevonden. Dit wordt veroorzaakt doordat deze inplaats van kaas of gereconstitueerde kaas, een verdund extract of een suspensie van enkele grammen kaas in een grote hoeveelheid water (aangezuurd met melkzuur) gebruiken.

Het peptiserend effect van zout op het paracaseïnaat-complex wordt zeer sterk beïnvloed door de verhouding paracaseïne : water. In verdunde suspensies (verhouding 1 : 50) bleek het gehalte aan opgeloste stikstof zeer hoog te zijn (50% van de totale hoeveelheid stikstof of meer), en dit gehalte bleek sterk beïnvloed te worden door de pH en de zoutconcentratie. Bij de verschillende gereconstitueerde kazen bleek het gehalte daarentegen slechts laag te zijn en slechts weinig beïnvloed te worden door pH en zoutgehalte. Dit wijst er op, dat alle vroegere publicaties die gebaseerd zijn op proeven met kaasextracten of met verdunde suspensies van het paracaseïne-complex in verdund melkzuur, geen juist beeld geven van de werkelijke toestand in kaas en aanleiding kunnen geven tot verkeerde gevolgtrekkingen.

De onderzoekers, die gebruik maakten van het paracaseïne-complex en niet van zuivere paracaseïne, hebben geen rekening gehouden met het volgende:

1°. De verhouding paracaseïne-complex tot water was ongeveer 1 : 50 bij hun proeven, terwijl bij harde kaas deze verhouding ongeveer 1 : 2 is.
2°. De Ca-concentratie in het vocht van een verdunde suspensie is veel kleiner dan in kaas.
3°. Bij het gebruik van 5% keukenzout in de suspensies, teneinde de zoutconcentratie in kaas te benaderen, werd geen rekening gehouden met de grote invloed van calcium.

Bij onze proeven bleek de peptisatie van het paracaseïne-complex in belangrijke mate verminderd te worden door calcium.

Om het voortschrijden van de eiwitafbraak in kaas gedurende de rijping te bestuderen zijn extractie-methoden van groot belang. De wijze, waarop het extract verkregen wordt, behoort echter gestandaardiseerd te zijn.

Voorgesteld wordt, dat dit het beste bereikt wordt door de pH en de temperatuur van de extractie-vloeistof zodanig te regelen, dat deze overeenkomen met die in kaas. Gedurende de extractie dient geen verdere afbraak van het eiwit door bacteriën of enzymen op te treden. Dit kan gemakkelijk bereikt worden door de kaas snel te extraheren door middel van een „Ultra-Turrax".

Indien de extractie-methode echter gebruikt wordt om gegevens te verkrijgen omtrent de toestand in de kaas, dan is het noodzakelijk de methode geheel aan te passen. In dit geval dienen de pH en de calcium- en natrium concentratie overeen te komen met de werkelijke toestand in kaas. Bovendien behoren de temperatuur en de duur van extractie in de hand gehouden te worden.

Bij pH 5 bleek ongeveer 50% van de totale hoeveelheid Ca en 20% van het anorganisch fosfaat in het sap van de gereconstitueerde harde en half-

harde kazen opgelost te zijn. Voor Edammer kaas werden dezelfde resultaten verkregen.

Bij deze lage pH kunnen dus nog aanzienlijke hoeveelheden calcium en anorganisch fosfaat aan de paracaseïne gebonden blijven. Het precipiteren van een deel van het opgeloste calcium en het opgeloste anorganisch fosfaat in kristalvorm zou ook nog kunnen bijdragen tot het betrekkelijk lage percentage van de totaal aanwezige bestanddelen die in de kaas oplossen.

Deze resultaten kunnen niet vergeleken worden met vroegere publikaties, daar de betreffende onderzoekers slechts de totale hoeveelheid (opgelost + niet-opgelost) calcium en fosfor bepaalden, dan wel met verdunde extracten van de kaas hebben gewerkt.

Bij dit onderzoek bleek ook dat de zwelling van de kaas slechts beperkt is. Bij de door ons gekozen proefomstandigheden bleek deze niet erg beïnvloed te worden door de zoutconcentratie of de pH. Het was veel moeilijker om bij dezelfde persdruk sap te verkrijgen uit ongezouten Edammer kaas dan uit, overigens gelijke, gezouten kaas. Dit wijst er op dat voor de bevordering van de zwelling en het waterbindend vermogen, zout niet de belangrijkste factor is.

Thymol werd veelal als conserveermiddel bij soortgelijke proeven gebruikt. Vergeleken met andere middelen bleek thymol in chloroform een zeer effectief conserveermiddel te zijn. Tijdens dit onderzoek werd evenwel gevonden, dat thymol een merkbare invloed uitoefent op de oplosbaarheid van het eiwit (paragraaf 3.6).

Om dit effect te bestuderen was een werkwijze nodig, waarbij in korte tijd een evenwicht verkregen werd tussen het paracaseïne-complex en de melkzuuroplossing, d.w.z. een methode, waarbij geen thymol gebruikt werd, maar waarbij desondanks de bacterie-werking uitgeschakeld werd. Dit werd bereikt met een speciale mixer, „Ultra-Turrax" genaamd, waarmee het mogelijk was om een semi-droog paracaseïne-complex preparaat (50% vocht) te mengen met water in de vereiste verhouding.

Het toevoegen van thymol in een concentratie, zoals die toegepast is bij dit onderzoek of zoals die toegepast is door SIRKS (1943), bij verdunde suspensies van paracaseïne-complex in water (verhouding 1 : 50), gevolgd door een krachtige menging, gaf een merkbare verandering in de waarden, die verkregen werden voor de opgeloste stikstof, vooral bij pH-waarden tussen 5,0 en 5,7 (tabel XXV). Deze invloed bleek evenwel geen verandering met zich te brengen ten aanzien van de reeds getrokken conclusies (zie paragraaf 3.3, 3.6 en 3.7).

Werd dezelfde hoeveelheid thymol in chloroform toegevoegd aan paracaseïne-water preparaten, overeenkomend met zachte kaas, dan bleek dit een geringere invloed te hebben op de oplosbaarheid van het eiwit.

Het was niet mogelijk om met de Ultra-Turrax methode gereconstitueerde harde kaas te bereiken en het effect van thymol op de oorspronkelijk verkregen cijfers (paragraaf 3.2 en 3.4) kon op deze manier niet nagegaan worden. Daarom is dit laatste indirect gedaan door tijdens de bereiding van gereconstitueerde zachte kaas zoveel calciumchloride toe te voegen, dat het calciumgehalte in het kaasvocht ongeveer even hoog was als dat in harde kaas.

De methode om met behulp van de Ultra-Turrax homogene gereconstitu-

eerde zachte kaas te bereiden in ongeveer één uur, maakte het ook mogelijk het effect van melkzuur en zout op de chemische samenstelling van gereconstitueerde zachte en harde kaas te bestuderen zonder inwerking van proteolytische enzymen en zonder toevoeging van conserveermiddelen. Het heeft bijgedragen tot een meer volkomen en nauwkeurig beeld van het effect van pH en zout op de oplosbaarheid van het eiwit in gereconstitueerde kaas.

Bij dezelfde proeven werd ook aangetoond, dat het toevoegde zout de hoeveelheid opgelost eiwit deed toenemen in gereconstitueerde zachte kaas (tussen pH 5,0 en 5,7), terwijl dit effect veel minder uitgesproken is in gereconstitueerde harde kaas.

De verkregen cijfers voor calcium en anorganisch fosfaat in gereconstitueerde zachte kaas waren over het algemeen in overeenstemming met die, welke vroeger reeds gevonden werden voor gereconstitueerde harde en halfharde kaas.

Uit de resultaten van dit onderzoek kunnen de volgende conclusies getrokken worden met betrekking tot de veranderingen in de rheologische eigenschappen van de kaas, die plaats vinden bij de rijping.

Jonge kaas is een zeer stevig gel met een grote elasticiteit (rubberachtige consistentie). Deze elasticiteit verdwijnt geleidelijk gedurende de rijping als gevolg van eiwit-hydrolyse en ook van veranderingen in de waterstofionen- en zoutconcentratie.

Het zout schijnt op twee wijzen werkzaam te zijn:
a. door een uitzoutingseffect en b. door het gedeeltelijk vrij maken van het gebonden water.

Dit is niet in overeenstemming met de theorie van VAN DAM en van vele andere onderzoekers, die aannemen dat het eiwit van jonge kaas gepeptiseerd wordt door het zout.

De tekortkomingen in de proeven, uitgevoerd door VAN DAM zijn reeds besproken (paragraaf 3.3).

Ongezouten kaas wordt inderdaad zacht en krijgt een tamelijk goede consistentie, ofschoon een enigszins week zuivel. Het zout is niet noodzakelijk om een smedig zuivel te krijgen. Het draagt wel bij tot een goede consistentie, maar dat gebeurt niet door een peptisatie-effect zoals veelal gedacht wordt, doch door een uitzoutingseffect. In de buurt van het iso-electrisch punt wordt het eiwit geprecipiteerd. Hierdoor is het gemakkelijk te begrijpen waarom kaas hard en brokkelig is bij een lage pH.

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Addendum:

46a. STADHOUDER, J. and MULDER, H. (1957) Neth. Milk and Dairy J. **11**, 164.

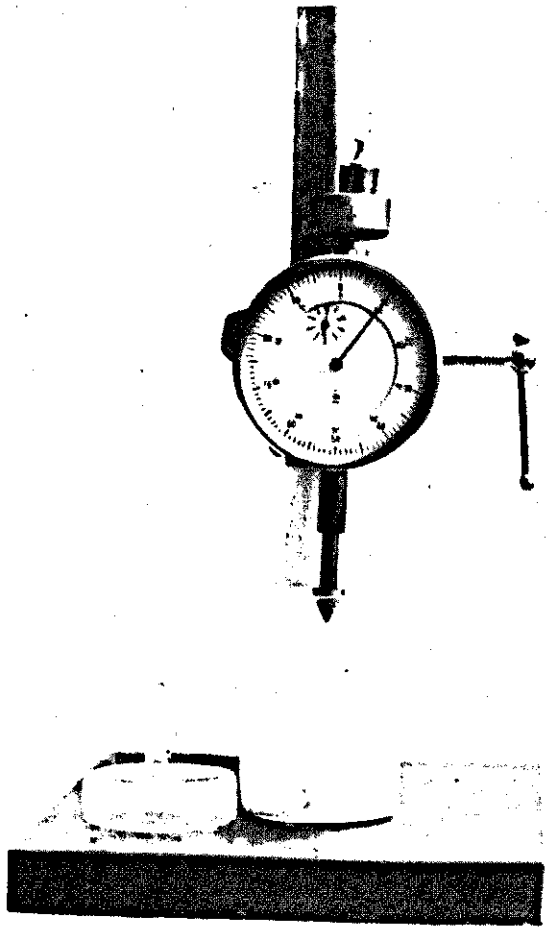

PLATE 1. The penetrometer used for measuring the 'consistency' of reconstituted cheese.

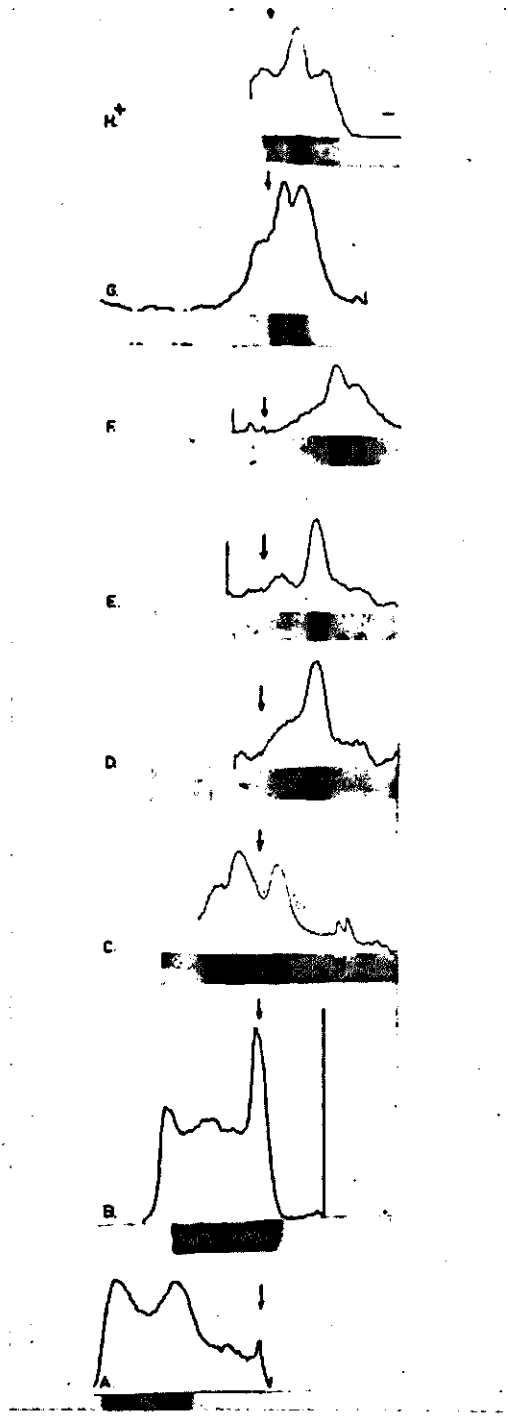

PLATE 2. Differences in the electrophoretic patterns obtained for: A = casein (4% solution), B = paracasein-complex (5% solution) – C, D, E and F, serum of reconstituted semi-hard cheese of 2 weeks old and at different pH values. G and H, serum of reconstituted hard cheese of 40 days old.