THE AMINO ACID CONTENT OF EDAM CHEESE AND ITS RELATION TO FLAVOUR

L. A. M. ALI

THE AMINO ACID CONTENT OF EDAM CHEESE AND ITS RELATION TO FLAVOUR

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE WND. RECTOR MAGNIFICUS,
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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 WOENSDAG, 6 APRIL 1960 TE 16 UUR

DOOR

L. A. M. ALI

H. VEENMAN EN ZONEN N.V. – WAGENINGEN – 1960

Vanaf deze plaats wil ik een woord van dank richten aan allen, die tot het welslagen van mijn studie in Nederland hebben bijgedragen. In het bijzonder betuig ik mijn dank aan mijn promotor, Prof. Dr. H. Mulder, onder wiens leiding dit werk tot stand kwam. Zijn voortdurende aanmoediging, opbouwende kritiek en adviezen zijn mij tot grote steun geweest.

THEOREMS

I

Differences in the flavour bouquet of various types of cheeses cannot be to an appreciable extent attributed to differences in their amino acid contents.

This Thesis.

II

Suggestions of KEENEY & DAY (1957) *J. Dairy Sci.* **40**, 874., that the aldhydes formed from the Strecker degragation of amino acids play an important role in cheese flavour, cannot be accepted.

GALESLOOT, TH. E. (1956) Proc. XIV Int. Dairy Congr. Rome, II (2) 849.

III

In a cheese factory, bacteriophage attack, being a virus infection, cannot be overcome by pasteurisation or disinfection.

MEANWELL, L. J. & THOMPSON, N. (1956) J. appl. Bact., **19**, 284.
REITER, B. (1957) J. Soc. Dairy Techn., **10**, 202.

IV

In Egypt, a decision has not yet been taken to apply either milk pasteurisation or milk sterilization as a method for processing consumption milk. The former is recommended as more suitable under Egyptian conditions.

V

The selective action of hydrogen peroxide, as a bactericide, suggests investigations on the replacement of high salt concentrations in Domiati cheese by an initial treatment of the milk with H_2O_2.

ROUNDY, Z. D. (1958) J. Dairy Sci., **41**, 1460.

VI

Antibiotic residues in milk due to the infusion or injection of antibiotics in mastited udders cause more problems to the dairy industry than does the use of mastited milk.

MARTH, E. H. & ELLICKSON, B. E. (1959) J. Milk Fd Techn., **22**, 266.

VII

Contrary to the statement of Seelemann, (*Dairy Sci.* Abstr. (1957) **19**, 530.) chlorides show considerable increases in milk drawn from udders infected with mastitis streptococci, bacteria cocci and in the case of a real catarrh.

ESPE, D. & SMITH, V. R. (1952) Secretion of milk. The Iowa State College Press, Ames, Iowa. p. 186.

VIII

The so called soya „milk" cannot, from a nutritional point of view, completely replace animal milk, and should not be called „milk".

IX

Appreciable losses in the biological value of milk proteins would not arise, in manufacturing dairy products, if proper heat treatment and storage conditions were applied.

HENRY, K. M. (1957) Dairy Sci. Abstr., **19**, 603.

X

„Stamping out" as a method for the eradication of Foot and Mouth disease outbreaks in Egypt is not recommended.

XI

Relatively high cost of milk production in Egypt as compared with low purchasing power, results in low consumption.

Overdruk

637.333.92:637.127.3:
637.36

MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN,
NEDERLAND 60 (4) 1–64 (1960)

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(met een samenvatting in het Nederlands)

by

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(Received/Ontvangen 15.1.'60)

CONTENTS

1. GENERAL OUTLINE

1.1. INTRODUCTION

The ripening of cheese is a complex process that has been studied since cheese was made. It involves several biochemical changes including the fermentation of lactose, the degradation of the proteins, and the hydrolysis of fat. All these processes result in a gradual change in the cheese curd from toughness to mellowness and in the development of the aroma and taste that together constitute the typical cheese flavour. In this study the decomposition of protein is especially concerned.

A survey of earlier work shows that the main change occurring in the proteins, during the ripening of cheese, is a gradual degradation of casein under the influence of rennet enzymes, bacterial enzymes and possibly the enzymes present in the original milk. A complex mixture of proteoses, peptones, polypeptides, amino acids, amines, ammonia and other substances is formed as a result of these processes. Up till now, these substances are not yet fully explored and still much work has to be done particularly on substances that are found in minute quantities and may have an important role in cheese flavour.

The analysis of the protein breakdown products was in the past, if possible time consuming and difficult. However, in recent years, a more precise determination of some of the casein breakdown products has been made possible by the application of microbiological assays and by different chromatographic techniques.

Owing to the attention paid to the part played by amino acids as flavour promoting substances, much information is now available on many varieties of cheese concerning their amino acid contents and the variations that can be expected at some stages of ripening. Unfortunately, most of these studies were largely qualitative and only cheeses of relatively similar flavour intensities were compared; no attention was paid to any factor that may have an influence upon the cheese ripening process. Imitation products were often used in these studies. Cheeses resembling the Netherlands Edam or Gouda, the Swiss Emmenthal, the British Cheddar or the French Roquefort are made elsewhere in the world. They may lack some or most of the characteristic properties of the genuine cheese. The divergent properties of such cheeses, with the conditions under which they are produced should be the prime considerations when they are compared with the genuine cheeses. Frequently it was not mentioned how the cheese was made or what milk was used. Sometimes it was thought sufficient for carrying out an investigation to buy, from a shop, samples of cheese the history of which was completely unknown; it was even left out of consideration whether the cheese was made from raw milk or from pasteurised milk.

Accordingly, there is much confusion among the conclusions drawn by various investigators. Aspartic acid content, for instance, which was found to be constant in Swiss cheese (40), increased in Cheddar cheese (13, 18) and decreased in Tilsit cheese (68) during the ripening period. Also, threonine content, while it was reported present by almost all workers, was found absent in Italian cheese (38). An increase in the threonine-serine content was observed in Cheddar cheese (13, 18, 77) and Tilsit cheese (68), while a decrease was reported in Swiss cheese (40). The steady increase in the glutamic acid content of Cheddar cheese, during ripening (13, 18), was met by a decrease in Swiss cheese (40). In most

types of cheese studied, lysine was noticed to increase with progressive ripening except in Soviet cheese (30) in which it was found to decrease.

Testing the few quantitative data mentioned in the literature, one can find extensive variations in the concentration of an amino acid in samples of cheese even if they were of the same age. In eight months old Cheddar cheese (39), figures for aspartic acid were found to vary from 48 to 75, isoleucine from 5 to 13, glutamic acid from 37 to 98, glycine from 4 to 13 and valine from 3 to 34 mg/10 g cheese solids. Another example is the nine months old Provolone cheese (38) in which the glutamic acid content was found to vary from 2.9 to 14.9, valine from 0.1 to 6.8, and alanine from 1.5 to 8.1 mg/g cheese solids. In commercial Swiss cheese of three months old (41) aspartic acid content varied from 0.0 to 1.8, threonine + serine from 1.7 to 5.7, glutamic acid from 1.9 to 6.1, glycine from 0.9 to 2.4, tyrosine + phenylalanine from 1.6 to 5.4, tryptophane from 0.0 to 3.0 and histidine from 0.53 to 3.5 mg/g cheese.

It seems probable that the wide variations in the findings of these workers may be explained to a large extent by the nature of the cheese used for analysis (since most of cheeses were made from different milk under widely divergent conditions), by the methods of analysis and by the methods of collecting the material for analysis. Also the fact that many of the liberated amino acids are subjected to further fermentative changes, may have lead to disagreement among different results. Serine and threonine are fermented to α-alanine and α-amino butyric acid (81). The decarboxylation of glutamic acid, tyrosine, lysine, arginine, histidine and tryptophane was noted to occur in Cheddar cheese (85, 88) at various stages and at different rates of speed. The decomposition of serine at pH 5.4 was reported by KRISTOFFERSEN & NELSON (54). Also the fermentation of free proline in Cheddar cheese was suggested by SILVERMAN & KOSIKOWISKI (87).

As regards cheese flavour, although much work has been done on the chemical changes involved in cheese ripening, no one succeeded in defining the substance or substances that confer flavour to cheese. MULDER (67) drew the attention to the fact that although the ripening of cheese was almost always judged according to the protein breakdown, it is apparent that no research worker tasted the breakdown products. In this simple manner, it would have been possible to conclude whether these products have influence upon the cheese taste. He divided the substances contributing to the flavour of the cheese into substances forming the basic taste and others responsible for the bouquet of the cheese. As amino acids possess no penetrating taste or smell, they were considered to contribute to the basic taste; they cannot give rise to any important differences in flavour. MULDER emphasized the necessity of a complete quantitative knowledge of these substances.

In other types of cheese, there is no more agreement on the part played by amino acids in cheese flavour. The characteristic sweet taste of Emmenthal cheese was attributed to proprionates and proline (52, 108; 109). On the other hand, this taste was obtained by the addition of a mixture of amino acids not containing proline to a fresh curd (86). In a third investigation, the characteristic flavour was attributed to its higher content of glutamic acid and aspartic acid (97). Also in Cheddar cheese the contradiction was apparent. In some investigations (39, 58) the addition of a mixture of amino acids to a fresh curd imparted Cheddar flavour and no flavour in others (5, 18).

As no quantitative work has been published before on the individual amino

acids content of genuine Edam cheese, it seemed that a study on the quantitative changes that occur in the amino acid content of Edam cheese was necessary for a better understanding of the taste and flavour of this type of cheese.

With this in mind, cheeses with different properties made under known controlled conditions, and representing different cheese flavour intensities were intentionally produced. Using different pH conditions, different moisture contents of cheese, raw & pasteurised milk and aseptically drawn & infected milk, the amino acids of casein were determined in the cheese. Other amino acids, amines, ammonia and amino acids decomposition products were estimated as they appear during the curing period. For an easy comparison with the work of others, the total solids, fat content, salt content, total nitrogen, soluble nitrogen and total amino acid nitrogen were determined. The acidity of the cheese fat was determined for some of the cheeses when it was thought necessary to draw a conclusion regarding cheese flavour as it was previously found by MULDER (67) and by STADHOUDERS & MULDER (92) that the hydrolysis of fat in cheese affects its flavour very much.

1.2. SCOPE OF THE PRESENT STUDY

The study is designed to give a more basic knowledge of the general amino acid content of genuine Edam cheese and to provide more basic information on the amounts of amino acids present and their possible contribution to the flavour. The use of cheeses with different properties favoured the possibility of gaining a knowledge of the influence of some factors, that are known to affect cheese ripening, on the amino acid content of the cheese.

2. MATERIALS AND METHODS

2.1. THE CHEESE

As it was necessary to use only cheese produced under well known controlled conditions, all the cheeses investigated were made in the technological section of the Laboratory under the expert supervision of Mr. E. de Vries. Cheeses made for a comparative study, were always manufactured from the same milk and ripened under the same conditions.

In all experiments, the whole milk of the Friezian cattle of the Animal Breeding Department of Wageningen University was used. Only morning milk was utilized. In each experiment, the milk was mixed and divided into two or three equal parts as required in the experiment. The temperature was adjusted to 31 °C and the following substances were added to every 100 liters of milk in all experiments except where mentioned:

$K NO_3$ 20 g, starter 0.8 liter, $Ca Cl_2$ 10 ml of 35% solution and rennet 30 ml.

Cheeses produced in all experiments, being about 2 kg each, were stored in the same room in order to insure the same conditions of humidity and temperature. During the whole period of ripening, the temperature and the relative humidity were automatically controlled, being 15 °C and 95% respectively.

2.1.1. *Cheese differing in pH*

In producing this cheese, the curd was cut after 18 minutes of renneting at 30 to 31 °C. The first stirring (10 minutes duration) was followed by the decantation

of whey in the proportions of 20%, 33%, and 40% for low, for middle and high pH cheeses respectively. The water added was respectively 0%, 20% and 40% in the first experiment, and 5%, 30% and 50% in the second experiment. The curd was stirred to 20 to 25 minutes at a final temperature of 36 °C. After hooping and dressing the curd, the cheeses were pressed and brined for equal intervals.

The pH, determined in the three series of cheese before brining, showed considerable differences, whereas the difference in the moisture content was, after two months storage, less than 1.5%.

2.1.2. *Cheese from raw and pasteurised milk*

In the two experiments made, the milk was mixed and divided into three parts. One was left raw, the second was pasteurised by a H.T.S.T. plate heat exchange pasteuriser at 72 °C for 15 seconds and the third was pasteurised by the same apparatus at 94 to 95 °C for 15 seconds. The heated milk was cooled to 30 °C while the raw milk was warmed to the same temperature. Renneting was as mentioned before except that the quantity of calcium chloride used was doubled in the low temperature pasteurised milk and eight times as much in the 95 °C pasteurised milk. As it is known that the curd of the pasteurised milk retains more moisture than does the curd of raw milk, more water was used for the dilution of the pasteurised milk curd than for the raw. Differences in manufacture are mentioned in table I.

TABLE I. Differences in manufacture between raw and pasteurised milk cheese

Process	Raw milk	72 °C pasteurised	95°C pasteurised
Decanted whey %	37.5	33	40
Added water %	12.5–15	25	40
Third stirring temp. (°C)	34.5–35	35	36–37
Third stirring time in minutes	25	40–43	47–48

After hooping and dressing, the cheeses were pressed and brined for equal intervals, time being 4 and 48 hours respectively. The pH of the finished cheese before brining was almost always the same.

2.1.3. *Cheeses differing in moisture content*

To make high moisture cheese, the whey was removed after cutting and the first stirring, at a percentage equivalent to 33% of the initial milk. Water was added at a rate of 30 to 35% of the remaining curd and whey. After mixing and stirring, the temperature was raised only to 33 °C and the third stirring was much reduced lasting only for 16 to 20 minutes.

As for the cheese with low moisture content, the first stirring was followed by the decantation of 16 to 20% of the initial milk. No water was added to the remaining curd in one experiment, while only 5% was added in the case of the other. The curd was intensively stirred in order to further the expulsion of the whey from the curd. This took place at a temperature of 37 °C for 37 minutes and 38 °C for 53 minutes in the first and second experiments respectively. After hooping and dressing the curd, all the cheeses were pressed for 4.5 hours. Owing to the fact that high moisture cheese takes up more salt during brining than low moisture content cheese, the former was brined for a shorter time than the latter;

time being 40 hours for higher moisture content cheese in both experiments and 48 & 64 hours in low moisture content cheese of the first and second experiments respectively. The pH of the two series, as measured before brining, proved to be practically the same.

2.1.4. *Cheeses from aseptically drawn and from infected milk*

Two experiments were made. In each experiment, 40 liters of milk almost free from bacteria, were utilized. Milk was produced as described by STADHOUDERS and MULDER (93). Milking pails, after being thoroughly cleaned, were covered with paper and sterilized. Hands of the milkers and the udders of the cows were washed with warm water and soap, then disinfected with a hypochlorite solution. Before milking both were dried with a sterilized udder cloth. The foremilk was discarded. Twenty liters of the aseptically drawn milk were intentionally infected by pouring it twice into a milk can which had been washed in the usual method of the farm and certainly not with extra attention. Infected in this way, the milk was left at the laboratory temperature (about 18–20 °C) for two hours before it was manufactured to cheese. Bacterial counts of the two milks were carried out on tryptone glucose extract agar having the formulae:

Beef extract 3 g, glucose 1 g, tryptone 5 g and agar 15 g in 1 liter destilled water and adjusted to pH 7. Results showed that the aseptically drawn milk contained 20 and 60 bacteria per ml while the infected milk contained 970,000 and 640,000 bacteria per ml in the first and second experiments respectively.

The two parts of milk were made into cheese under the same conditions of time and temperature. Whey was decanted in a proportion of 5 to 8%, while water was added at 15% of the quantity of curd and remaining whey. Stirring and scalding were performed for 27 to 39 minutes at a final temperature of 36.5 to 37.5 °C.

2.2. PREPARATION OF CHEESE SAMPLE FOR CHROMATOGRAPHIC ANALYSIS

A section of the cheese equal to about one eighth of it, made to represent as near as possible the whole cheese, was taken. The rind of cheese (about 3 mm) was removed and the sample was milled. After the milled cheese had been thoroughly mixed in a mortar, 20 g were weighed in a 300 ml capacity conical flask. 150 ml of distilled water and 0.5 ml of 50% alcoholic phenol solution, to stop further biological changes, were added. The flask was shaken for 16 hours in a thermostatically controlled water bath maintained at 25 °C according to SIRKS (90). The flask was warmed to 40 °C and its contents were quantitatively transfered to a 250 ml volumetric flask. It was then cooled to solidify the fat then filtered through Whatman no. 12 filter paper.

In order to free the cheese extract from almost all protein breakdown products, other than amino acids, phosphotungstic acid was added in preliminary experiments. As this acid is known to partially precipitate the basic amino acids, we tried to find out the percentage of recovery of each of the basic amino acids by adding a fixed quantity of the phosphotungstic acid to different amounts of the basic amino acids in constant end-volume solutions. It was found that the percentage of recovery of the basic amino acids was not stable, being higher when the concentration of the basic amino acid was lower. This was previously found by THIMANN (100, 101). Also the recovery of these acids was lower when added

to a young cheese extract, than when added to a ripe cheese extract, although the same concentration of phosphotungstic acid was used.

As the addition of 95% alcohol to a cheese extract in the proportion of 5 to 1 precipitates proteins and peptides (3, 13), we used alcohol in preparing the sample when we found that it caused no precipitation of amino acids found in solution. A full recovery of the amino acids in the cheese extract could be obtained in this way. The small amounts of peptides that may remain in the alcohol filtrate did not interfere in the determination of the amino acids as they appear on the chromatogram as separate peaks.

To 50 ml of the filtered cheese extract, 250 ml of 95% ethanol were added. The mixture was shaken, allowed to settle for about 15 minutes to precipitate any remaining protein and then centrifuged. Of the clear solution, 150 ml were evaporated under suction over a boiling water bath. The residue was quantitatively transfered to a volumetric flask using a 0.2 N sodium citrate buffer pH 2.2. One or two ml equivalent to 80 and 160 mg of the cheese were used in the chromatographic analysis.

2.3. Methods of analysis

2.3.1. *Amino acids*

In the past, when the separation of amino acids was not well possible, it was necessary to discover a specific reaction for each individual amino acid before it could be determined. Kossel and Kutscher (51) using the technique of precipitating amino acids as their silver salts, were the first to give approximately accurate results for the diamino acids. Fischer (25) introduced the method of fraction distillation of the ethyl esters under very low pressure. This method modified by Abderhalden (1) was the first satisfactory quantitative method for the determination of monoamino acids in proteins. Van Slykes' (106) nitrogen distribution method depended on the determination of the chemical groups characteristic of amino acids. These methods and many others were reviewed by Mitchell & Hamilton (61).

Several methods have been used to determine the amino acids in cheese. In 1903 Van Slyke & Hart (107), in an effort to relate CO_2 production to proteolysis in the ripening of Cheddar cheese, found six amino acids by chemical analysis. By the fractional distillation of the amino acid ethyl esters, Winterstein (111) found 10 amino acids in 3 months old Emmenthal cheese. In another investigation on the same type of cheese, Winterstein & Bissegger (112) found proline and phenylalanine in large quantities and isoleucine & oxyproline in low concentrations by the same technique. Grimmer *et al* (32) determined the basic amino acids in the alcohol soluble fraction of 'Backstein' cheese by precipitating the amino acids as their silver salts. In 1938, Tuckey *et al* (102) identified four amino acids by means of an X ray deffraction analysis. Later Harper & Swanson (39) and Reihard & Garey (77) determined the amino acids by microbiological methods.

The foregoing methods of analysis are not well suited for the examination of a complex mixture of amino acids. They require much work and are very time consuming. They are suitable only for the identification of a few amino acids and at the same time they lack accuracy.

A notable advance was made in identifying the amino acids in cheese when

paper partition chromatography, based on the two dimensional method of CONSDEN *et al* (17) was applied to the study of Cheddar cheese by KOSIKOWISKI (49). Later LINDQVIST *et al* (56) analysed cheese with a modification of the method of MC FARREN (59) involving a one-dimensional buffered filter paper chromatogram. Quantitatively, paper chromatographic determinations do not give reliable results. KIURU *et al* (48) using this method in the study of Emmenthal cheese, mentioned that the recovery differed from one amino acid to the other, the error being ± 15 to 30%.

In 1955 MABBIT (58) used the column chromatographic method of MOORE & STEIN (63) in the quantitative estimation of the amino acids in Cheddar cheese. Inferior separations were obtained particularly for aspartic acid, threonine and serine on one hand and glutamic acid and proline on the other hand. During the course of this investigation, a paper on the examination of the amino acid content of some Danish cheeses was published (62). Column chromatography was used.

In the present study, the quantitative estimation of amino acids was performed in the following steps;

a. Separation of the mixture of amino acids found in the cheese extract into small fractions.
b. Quantitative estimation of the amino acids in these fractions.
c. For the identification of the amino acid peaks and for calculations, synthetic mixtures were analysed.

a. Separation The sulphonated polystyrene ion exchange chromatographic method of MOORE & STEIN (64) involving 150 cm column, a resin of 4% cross-linkage and an eluent of continuously increasing pH and normality, was used as a fundamental procedure in this investigation. Slight modifications were applied with the intention of making the method more reliable under the conditions of this work. It was necessary to shorten the length of time required for the completion of an experiment, to obtain better separation of the amino acids of the cheese extract especially with the acidic amino acids, tyrosine-phenylalanine and lysine-histidine.

When the method of MOORE & STEIN (64) was applied, using Dowex 50 x 4 200 – 400 mesh and the first buffer of 3.1 pH at 50 °C, it was almost impossible to avoid an unexpected overlap between the threonine – serine peaks especially in the cheese extract. This was caused by a large amine peak that was always eluted from the column before the serine. Also a considerable overlap between the proline and the glutamic acid peaks was observed. The separation of tyrosine from phenylalanine was never complete shown by a marked overlap, frequently till the top of the tyrosine peak; also lysine overlapped histidine. Moreover, the elution of arginine required from 8 to 10 days.

To avoid the difficulties mentioned, the experiments of this study were carried out in the following way:

1) *Preparation of the ion exchange column.* Chromatographic tubes (170 × 0.9 cm) pyrex glass, with ground joints and jacketed with 4 cm diameter glass tubing, were used. Dowex 50 x 4 and Dowex 50 x 5 (200–400 mesh) as supplied in the hydrogen form were utilized. It was found that different batches of this resin did not give exactly the same chromatographic performance. Three lots of Dowex 50 x 4 were tested for the separation capacity. One gave unsatisfactory results, the second showed inferior separation, while the third was very efficient.

Preliminary sieving of wet resin through a 200 mesh sieve did not give reasonable amounts of resin. Also ground resin proved to give very broad peaks with much lower flow rate.

Dowex 50 x 4 and x 5 were blended in the proportion of 2 to 1. This was found essential for the separation of tyrosine-phenylalanine on one hand and lysine-histidine on the other. The conversion of the mixture of the two resins was practised as described by MOORE & STEIN (64). Special care was taken to make the blended resin free from very fine particles and to have particles of almost one size. This increased the rate of flow and gave reproducible results when the resin was reused.

The resin was suspended in ten times its volume of distilled water and left to settle for three hours. The water, above the settled resin containing the fine particles, was decanted. This was repeated twice. The resin was suspended in twice its volume 0.2 N buffer and shaken in a graduated cylinder. It was allowed to settle for one hour before the upper one-third was removed and replaced by an equal volume of the buffer. Suspension of the resin, settling and removal of the upper part was performed several times, each time with a progressive decrease in the settling time. In this way, a settled resin, almost free from fine particles, under a two volume as much of a supernatant was obtained in about 20 minutes. Large particles were separated using the same technique.

The resin prepared in this way, and poured into a column, gave a flow rate of about 12 ml per hour under a slight pressure of 15 cm mercury without broadening the peaks and with almost the same capacity of separation shown by MOORE & STEIN (64). It was realised by these workers in their new technique of amino acid separation on an Amberlite column (66) that it was very important to get rid of the fine particles in order to speed up the elution. They described a method for the fractionation of the resin particles based on the method of HAMILTON (34).

In pouring the column, the two blended resins were suspended in a volume of 0.2 N buffer acetate citrate of pH 5 containing no detergent, such that when the resin settled, the supernatant liquid above the resin was twice the bottom layer. This lowered the probability of forming air bubbles during pouring the column which was done in sections of about half meter high. After each section had settled to a constant height, the supernatant buffer was withdrawn by suction through glass tubing 3 mm in diameter. After pouring the next section, the upper part of the settled resin is mixed with it to avoid a compact zone of the relatively smaller particles that settle last. Such zones decrease the elution rate and cause inferior separation especially when high pressure is used. As the fritted glass filter at the bottom of the column often became clogged, it was replaced by a small piece of glass wool.

After the resin had reached 150 cm height, it was washed with 0.2 N NaOH containing 0.5% BRIJ 35. A quantity not less than 150 ml was passed through the column. The greater the volume of alkaline solution used, the better the separation of the peaks and the lower the blank readings, especially on both sides of the ammonia peak and the basic amino acids. The last washing of the column with 0.2N buffer pH 3 was performed the day before the column was used to avoid the absorption of ammonia from the surroundings by the acidic buffer.

2) *Buffers.* Buffers were prepared as described in the method from reagent grade chemicals in volumes of 10 liters of 0.2N buffer pH 3 and 5 liters of the 2N

buffer pH 5.1 and stored at 2°C. Buffers were renewed monthly and were preserved by adding 0.05% thymol. The pH of the buffer was determined by a Cambridge glass electrode pH meter and proved to be stable. A saturated solution of potassium bitartrate of pH 3.57 was used as a standard. The 0.2N buffer pH 3.1 was adjusted to pH 3 in order to delay slightly the emergence of the amine peak and the glutamic acid peak which were found to overlap the respective peaks of serine and proline when the pH 3.1 buffer was used. Before starting an experiment, one liter of each buffer was boiled and cooled to avoid the release of air bubbles when the buffer was heated to 55°C during the operation of the column.

3) *Thiodiglycol.* Two samples of the thiodiglycol gave a yellow colour with ninhydrin. These were purified by redistillation according to the method described by EASTOE & EASTOE (22).

4) *Detergent.* As the 50% BRIJ 35 in water did not remain fully in solution at room temperature, the amount of water was increased by one third and the stated quantities added to the buffers were also increased by one third.

5) *The fraction collector* (plate 1). During the course of the experiments, two types of fraction collectors were used. Both were constructed in the laboratory workshop by the laboratory technician Mr. A. v. CAPELLEVEEN. The collection of fractions was, in both, based on giving a measured volume. This was done in the first apparatus by collecting a predetermined number of droplets by means of a photocell. In order to ensure an entirely constant volume, the quantity of the detergent added to the 2N buffer pH 5.1 was doubled. The second apparatus worked with two platinum contacts (Figure 1), one of which could be adjusted to a certain volume by a screw. When the upper contact was touched by the surface of the fixed volume, an electromagnetic valve opened and the effluent ran into the tube underneath. As the electromagnetic valve shut, it activated electronically the mechanical part of the collector which moved the tray of tubes into a new position. The tray consisted of three rows of 50 tubes each. By two steel wires, fixed in the tray between the end and the beginning of two successive rows, the metal arm which carried the effluent fraction from the column to the tubes was simply directed to the next row and all the tubes were used without interruption.

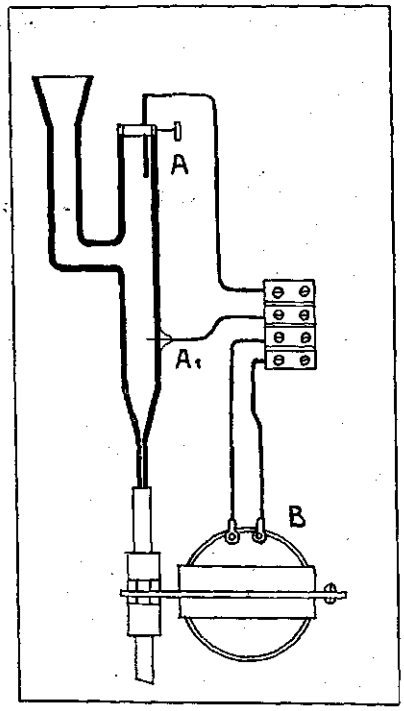


FIG. 1. An automatic device for a constant volume.
A–A_1 are platinum contacts
B is an electro magnetic valve

6) *Operation of the column.* After the column had been placed in a position above the automatic fraction collector, water at 30°C was circulated through the jacket of the column by a circulating water bath of a constant temperature (± 0.5°C). This was performed half an hour at least before the sample was added to the column. The

sample, as previously prepared, was delivered on the top of the column by means of a bent tip pipette. The volume used was 1 or 2 ml (80 and 160 mg of cheese) depending on the age of the cheese. This was chosen after preliminary experiments in order to obtain low readings on the optical density scale without further dilution of most fractions. When the sample was completely absorbed under gravity, small portions (0.5 ml) of 0.2N buffer pH 2.2 were used to wash the surface of the resin.

For the application of a buffer of gradually increasing pH and normality, the apparatus described by HUISMAN & SCHAAF (44) was used in our experiments. Glass tubing 6 mm outer diameter and 2 mm inside diameter was used to join the two reservoirs and to introduce the buffers into the top of the column. Polythene tubes were used to connect the glass tubing. The reservoirs were raised as high as possible thus the pressure which had to be applied to bring the buffer to the column was at a minimum. A 500 ml capacity flask replaced the 750 ml flask used by HUISMAN & SCHAAF (44) in order to shorten the time needed for the elution of the monoamino monocarboxylic acids.

The two reservoirs were filled with the buffer 0.2N pH 3 and connected to the column by a polythene tube; pressure from a nitrogen bomb was applied at 15 cm mercury to give an initial rate of flow of about 12 ml per hour. The effluent was collected in 2 ml fractions in pyrex glass tubes of about 20 ml capacity. After the emergence of the alanine peak, the temperature was raised to 55 °C to improve the separation of tyrosine and phenylalanine. Half an hour later, the first reservoir was filled with the second buffer 2N pH 5.1 and the second reservoir which was used as a mixing chamber was set over a magnetic stirrer. A light pressure was applied (4 to 5 cm mercury) to permit only a small stream of the 2N buffer to be mixed with the 0.2N buffer. The pressure was not raised to its previous level until the next 25 fractions were collected. This practice completed the separation between methionine, isoleucine and leucine. Owing to the application of a high temperature, the flow rate of the effluent was raised to 8 – 10 fractions per hour. The experiment was continued at the same temperature until the emergence of histidine (340 fractions) when it was raised to 75 °C to facilitate the elution of the strongly basic amino acid arginine at about fractions 390 to 400.

The starting of the elution at 30 °C and the continuation at this temperature till the alanine ran off the column together with the use of pH 3 buffer, was found to reduce the overlap between the threonine-serine and the proline-glutamic acid peaks. Also the two wings appearing on both sides of the ammonia peak were reduced. This, when occurred gave error in the determination of the basic amino acids because of the differences in blank readings.

Under these conditions, a complete experiment including the collection of 425 fractions was performed in less than three days. However, it was not possible when using unfractionated resin and a 750 ml mixer, to make a full analysis in less than 7 to 8 days.

Owing to the considerable shrinkage in the Dowex 50 as a result of using two buffers of different normality, it was not possible to use the column over again. Columns were emptied after each experiment simply by turning them upside down and leaving the resin to descend by gravity. Small portions of water added through the glass wool to avoid drying of the top of the descending resin. For pouring a new column, the used resin was washed with large quantities of distilled water under suction until it was almost free from the detergent which was found to encourage the formation of air bubbles during the pouring of a

new column. Gases were frequently formed inside the resin bed after it had been used several successive times; thus it was found advisable to boil the resin for one hour first with 4N HCL and then with 2N NaOH before preparing it in the usual way.

b. Quantitative analysis of effluent fractions. The concentration of the amino acids in the effluent fractions was determined by the modified photometric ninhydrin reagent method of MOORE & STEIN (65). Readings were taken on the optical density scale of a Beckman Spectrophotometer. As the ninhydrin reagent is strongly buffered, only 1 ml was required for each effluent fraction (2 ml). As the hydrindantin purchased gave higher blanks up to 0.15, it was freshly prepared in the laboratory by the reduction of ninhydrin with ascorbic acid (65). Excessive washings with distilled water were performed after precipitation on a gouch crucible to free the hydrindantin from impurities.

As the ninhydrin solution gave higher blanks when stored, even under nitrogen, the colour yields ran low with storage and the concentrated hydrindantin frequently precipitated, thus the reagent was freshly prepared for each experiment in 300 ml quantities and stored in the dark.

To each fraction 1 ml of the ninhydrin reagent was added. Tubes were covered with aluminium caps and shaken by hand for about 10 seconds. The tubes were heated in a boiling water bath for 20 minutes then cooled under running tap water. Addition of 5 ml 50% ethanol was enough to give a reading less than 1.0 on the optical density scale except for the top peaks of glutamic acid, leucine, phenylalanine and lysine. To determine these amino acids, dilution was necessary. Before being read at 570 mμ (440 for proline), the tubes were again shaken to mix the contents with the diluent and to oxidize the reddish colour that remained after boiling. This colour was attributed to the high concentration of the hydrindantin in the modified reagent. By this technique, the blank readings during the course of a whole experiment were always less than 0.1 except in the ammonia region where it rose to about 0.15 and sometimes more.

c. Quantitative analysis of a synthetic mixture. A standard curve was plotted for leucine at eight concentrations varying from 2.5 to 30.0 mg/liter. Leucine solutions were made in 0.1M citrate buffer pH 5. The curve as seen in figure 2 follows Beers' law with all concentrations up to an optical density of 1.2. No deviation from the straight line relationship was observed. From the graph, a table was prepared giving the relation between the optical density reading (from 0.01 to 1.00 in 0.01 steps) and the concentrations of amino acids expressed as mg/liter. These concentrations were multiplied by $\frac{13}{8}$ or $\frac{18}{8}$ to give concentrations corresponding to the readings obtained after the dilution of the 8 ml with one or two additional 5 ml aliquots of the diluent. When used for other amino acids, these concentrations were divided by the colour yield of the amino acid relative to leucine. No factor was required for the negligible loss by evaporation which might have occurred during the heating of solutions.

The relationship between the optical density and the leucine concentrations was determined by the following procedure.

Two ml leucine in 0.1M buffer pH 5 were mixed with 1 ml ninhydrin reagent and heated for 20 minutes at 100°C, and 5 ml ethanol water were added. Results are shown in table II.

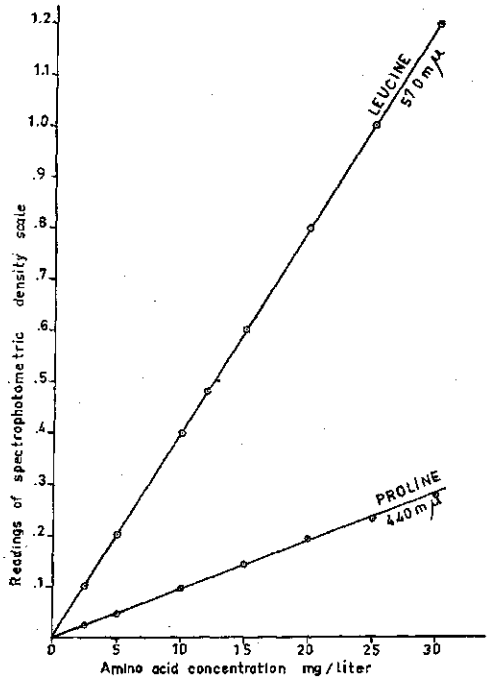


FIG. 2. Relationship between leucine and proline concentrations in 0.1 M pH 5 citrate buffer and the colour yields, (readings of the optical density scale of a Beckman Spectrophotometer).

TABLE II. Relationship between leucine concentrations in 0.1 M citrate buffer pH 5 and the optical density scale

Concentration of leucine mg/l	Desity scale reading – blank			Average
	Exp. I	Exp. II	Exp. III	
Blank	0.070	0.090	0.060	0.073
2.5	0.100	0.100	0.100	0.100
5.0	0.200	0.200	0.200	0.200
10.0	0.405	0.395	0.400	0.400
12.0	0.480	0.470	0.490	0.480
15.0	0.600	0.600	0.610	0.603
20.0	0.800	0.800	0.800	0.800
25.0	1.010	0.990	1.010	1.003
30.0	1.200	1.195	1.205	1.200

TABLE III. Relationship between proline concentrations in 0.1 M citrate buffer pH 5 and the optical density scale

Concentration of proline mg/l	Density scale reading – blank			Average
	Exp. I	Exp. II	Exp. III	
Blank	0.060	0.060	0.080	0.066
2.5	0.024	0.024	0.024	0.024
5.0	0.046	0.045	0.045	0.045
10.0	0.095	0.093	0.097	0.095
15.0	0.140	0.140	0.145	0.141
20.0	0.195	0.195	0.190	0.193
25.0	0.225	0.225	0.240	0.230
30.0	0.280	0.270	0.275	0.275

The colour yield of other amino acids relative to the colour yield of leucine proved to be reproducible within $\pm$ 2% and to be similar in most cases to the value cited by MOORE & STEIN (65). Proline was the Exception and a special curve was made under the same conditions and measured at 440 mμ. Measurements up to 0.2 density followed Beers' law, while a slight deviation less than 4% from the straight line with concentrations above 20 mg/liter was observed. Figure 2 shows the curve, while table III gives the relation between the optical density and the proline concentrations in mg/liter.

Mixtures containing 0.05 to 0.1 mg of each of the amino acids studied, in 0.2N buffer pH 2.2 were separated and quantitatively determined by this ninhydrin reagent method. Replicate trials were conducted and representative results are shown in figure 3. Mixtures added to the cheese extract did not shift the threshold volume of the amino acids. Also no noticeable increase or decrease occurred in the amino acid concentration in the sample used for analysis. Differences in the threshold volume of an amino acid and in its position on a chromatogram, were due only to differences in pH of the sample, pH of the buffer and the compactness of the column when the same resin was used.

The average recovery of the amino acids studied is given in table IV. The data reveal that all the amino acids can be quantitatively recovered with the exception of tryptophane, which may be due to decomposition during the experimental work. The method was found to give reproducible results when repeated with the same resin and the same technique. Values of replicates did not vary from the means given in table IV by more than $\pm$ 3%. The low recovery of glutamic acid and methionine was consistent and corrections for the respective 7 and 9% recovery loss, which may be due to decomposition, were calculated.

A reasonable recovery was, to a great extent, dependent on the correct choice of the blanks against which the amino acid peaks were read during the spectrophotometric determinations. The choice of a proper blank was often very difficult especially for the basic amino acids. In this study the eluate after the emergence of the aspartic acid peak was chosen as a blank for the acidic amino

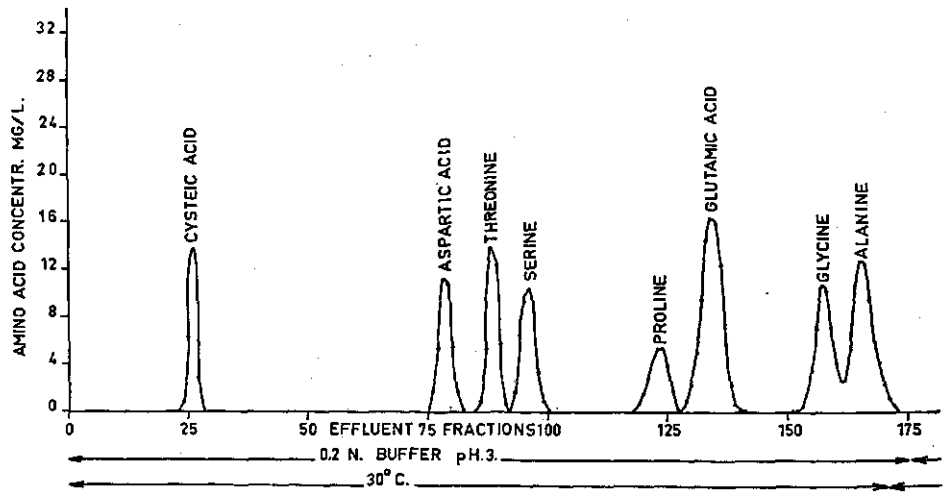


FIG. 3. Separation of an amino acid synthetic mixture on an ion exchange column of a mix-

acids. For the monoamino monocarboxylic acids, two blanks were chosen; the first, the blank for glycine alanine and valine was the eluate after the emergence of alanine from the column. The second, after the elution of leucine was used as a blank for methionine, isoleucine and leucine readings. For the ammonia and ornithine, the eluate just after the ammonia peak was chosen as the blank.

Also the blank just after the elution of histidine peak was found to give reliable results when read against the lysine and histidine. Arginine values were read against the blank after its peak.

TABLE IV. Recoveries of amino acids from synthetic mixtures (av. 3 Exp.)

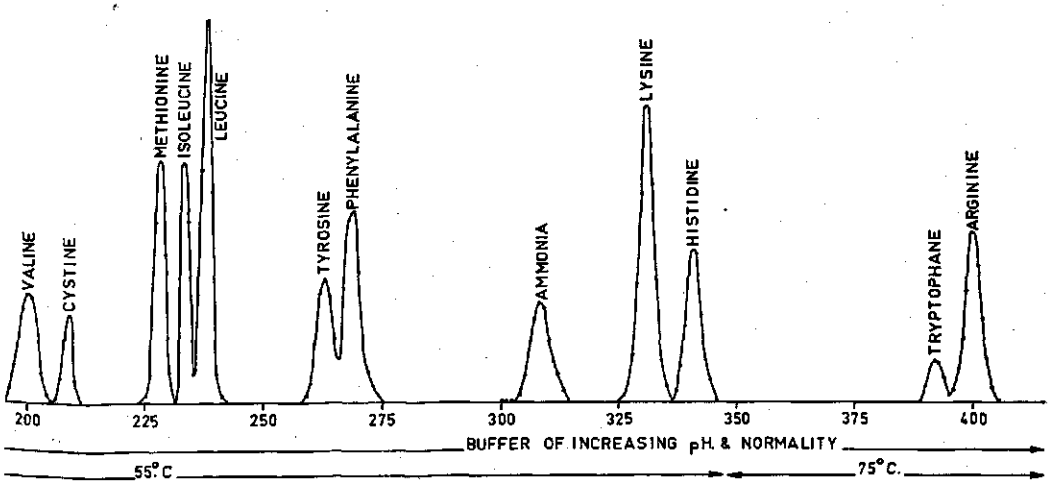
Amino acid	Recovery %	Amino acid	Recovery %
Cysteic acid	99.5	Cystine	96.5
Aspartic acid	98.0	Methionine	91.0
Threonine	96.0	Isoleucine	102.0
Serine	101.0	Leucine	103.0
Proline	97.0	Tyrosine	97.0
Glutamic acid	93.0	Phenylalanine	100.5
Glycine	97.0	Lysine	101.0
Alanine	97.5	Histidine	99.5
Valine	99.0	Arginine	97.0
		Tryptophane	36.0

2.3.2. *Methods used in cheese analysis*

a. Moisture. About 5 g of cheese were thoroughly mixed with previously washed and heated sand in a wide aluminium dish, then dried till constant weight at 100 °C.

b. Fat. This was determined by the S.B.R. method.

c. Total nitrogen. 1 g of cheese was digested with 10 ml sulphuric acid, 2 g potassium sulphate and 0.3 g copper sulphate, followed by steam distillation. N/10 solutions were used.



ture of Dowex 50 X4 and X5.

d. Soluble nitrogen. 20 g of cheese were extracted according to the method of SIRKS (90). 10 ml of this cheese extract were used in the estimation according to the method described by ROWLAND (76).

e. Amino acid nitrogen. It was determined according to SIRKS (90). 50 ml of the cheese extract equivalent to 4 g of cheese were treated with 2.5 g of phosphotungstic acid after the addition of 30 cc of H_2SO_4 diluted 1 to 3. A 40 ml aliquot of the phosphotungstic acid filtrate was used in the determination. Phosphotungstic acid was chosen for the determination of the total amino acid nitrogen because it was used in all previous studies on Edam cheese. A comparison was possible between the present results and the previous ones.

f. Salt. This was determined by the method of DAVIES (19). 2 g of cheese were digested using 25 cc HNO_3 + 10 cc saturated potassium permanganate in the presence of silver nitrate. Excess Ag NO_3 was back titrated with potassium thiocyanate using iron alum as an indicator.

g. Fat acidity. The fat of cheese was obtained by pressing equal parts by weight of ground cheese and pure sand at 50 °C and the fat acidity was determined as described by STADHOUDERS and MULDER (93).

h. The pH. This was determined with a Cambridge glass electrode pH meter directly by inserting the two electrodes into the cheese. A saturated solution of potassium bitartrate of pH 3.57 was used as a standard.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF AGE

Up till now, no attempt has been made to study quantitatively the individual amino acids set free in genuine Edam cheese during the ripening period. In a study on the taste and flavour forming substances in cheese, MULDER (67), compared the amino acid content of well ripened cheese with the amino acids in casein and concluded that very probably all the amino acids of casein are present in cheese. For a better judgement, it would certainly be essential to get a good quantitative insight into the proportions of amino acids in the cheese. As the amino acids have no strong flavour, MULDER mentioned that a study of the amino acids content of cheese, cannot give a complete picture of the flavour forming substances. The amino acid pattern of 'Edam cheese', not made in The Netherlands, was published by STORGARDS and LINDQVIST (98). Working with cheese made in U.S.S.R. DOLZALEK (20) mentioned that hard cheeses scalded at lower temperatures such as Edam generally contained the same amino acids as Emmenthal cheese but in lower concentration. Recently CHEBOTAREV *et al* (15) published one dimensional paper chromatographic determination of the amino acids of a cheese made in the same country. The cheese was analysed over a short period, all the amino acids were not separated and the composition and properties of the cheese analysed were not mentioned.

In our experiments, cheeses were made as stated in Section 2.1 to represent real Edam cheese. Two experiments each including nine cheeses were performed for orientation. A whole cheese was analysed at an arbitrary interval in the ripening period which lasted for about a year. Cheeses with different properties were all analysed at three different stages in the ripening period but cheeses made from asceptically drawn and infected milk were examined only twice.

Analytical data for the cheese composition as well as for the organoleptic examination are mentioned in tables V, VII, IX, XI and XIII while tables VI, VIII, X, XII and XIV include the quantitative results of the content of amino acids that were found in the experimental cheeses during the course of the ripening period. It was found advisable to present the data of each experiment separately. Averages, in this case, cannot give a true picture of the results since the variations in the experiments were considerably wide. Even the trends in the concentration of some amino acids, at certain stages in the ripening period, were sometimes different. This may be considered as a result of the influence of the cheese flora together with pH and other factors.

The quantitative data obtained in this investigation, made it possible to study the trends in the variations of the free amino acids in the cheese during the ripening period.

3.1.1. *Properties of the cheese*

As it is known that the pH of the cheese, the moisture content and the salt/water ratio in the cheese affect the protein breakdown, these items together with the protein degradation products, were involved in the discussion. However, as these factors which control the ripening process, together with the changes occurring during this process, were previously studied in Edam cheese, there is no need to rediscuss them here. Results obtained in this work will be treated generally just to give a picture of the cheeses used in the analysis.

The pH of the cheeses studied increased gradually with ripening up to 6.5 months when the rate of increase became much slower. A decrease in pH values was observed at the end of the ripening period. Also, the pH in the cheeses with different properties showed the same trend though the course was not always equal. These variations are in agreement with previously published investigations.

The moisture content of the cheese decreased as ripening progressed. The decrease was remarkable in the first stages of ripening. In the orientational experiments (table V), the cheese lost from 8.04 to 10.01 % of its moisture in the first 4.5 months. In the next 7 months, the loss was only 2.92–3.26 %. The same trend was to a large extent perceptable in other experiments. This is what can be expected.

Salt percentage and salt/water ratio increased with the age of cheese, the increase being relative to the loss in moisture. Also, the fat and total nitrogen percentages increased with increase in age of cheese. Calculated as a percentage of the total solids, their values remained practically constant.

As stated in the literature, there was an increase in the protein breakdown products with ripening. Values for soluble nitrogen and total amino acid nitrogen increased with the age of the cheese. This increase did not occur at the same rate during different stages of ripening, the greatest change being observed in young cheese. After only 1.5 months of ripening, the soluble nitrogen was more than two thirds of the soluble nitrogen of the cheese at 11.5 months of age. The rate of amino acids formation was a little slower. The total free amino acids content, found after the same period was less than 45 % of the total amino acids content of the cheese at the end of 11.5 months of ripening. This difference may be explained by the fact that soluble nitrogen is produced by the action of milk proteases, rennet enzymes and bacterial enzymes while the formation of amino acids in the cheese depends on bacterial enzymes (91).

TABLE V. Chemical and organoleptic examinations of Edam cheese during the course of ripening (orientation

Exp. No.	Age of cheese	PH	Moisture %	Fat %	Salt %	T.N. %	Sol.N. %	Amino acid N. %	Sol.N. in % of T.N.	Amino acid N in % of T.N.	Salt/water ratio	Protein %
I	1 day	5.02	—	—	—	—	—	—	—	—	—	—
	15 days	5.02	39.88	31.08	1.89	3.469	0.653	0.067	18.83	1.95	4.74	22.1
	1 month	5.06	39.53	31.09	1.98	3.480	0.760	0.174	21.86	5.01	5.00	22.2
	1.5 months	5.07	37.52	31.91	1.99	3.610	1.025	0.218	28.39	6.03	5.30	23.0
	2.5 „	5.09	36.39	32.52	2.18	3.680	1.095	0.276	29.75	7.50	5.99	23.5
	4.5 „	5.14	31.84	34.87	2.35	3.950	1.190	0.345	30.12	8.70	7.38	25.2
	6.5 „	5.20	30.54	35.61	2.55	4.036	1.365	0.468	33.82	11.59	8.36	25.7
	8.5 „	5.19	30.35	35.80	2.58	4.088	1.457	0.506	35.64	12.37	8.50	26.1
	11.5 „	5.12	28.92	35.85	2.60	4.172	1.610	0.568	38.58	13.62	8.99	26.6
II	1 day	4.94	—	—	—	—	—	—	—	—	—	—
	15 days	5.05	43.24	28.23	2.06	3.332	0.886	0.095	26.59	2.86	4.76	21.2
	1 month	5.05	40.53	29.52	2.18	3.465	0.966	0.186	27.87	5.37	5.37	22.1
	1.5 months	5.16	37.14	31.20	2.25	3.662	1.053	0.243	28.75	6.63	6.05	23.4
	2.5 „	5.22	36.78	31.51	2.42	3.685	1.111	0.306	30.15	8.30	6.57	23.5
	4.5 „	5.30	33.21	33.26	2.54	3.892	1.375	0.380	35.32	9.76	7.62	24.8
	6.5 „	5.40	32.50	33.57	2.78	3.933	1.520	0.520	38.64	13.23	8.55	25.1
	8.5 „	5.43	30.05	34.44	2.87	4.129	1.598	0.558	38.72	13.51	9.55	26.1
	11.5 „	5.39	29.95	34.92	2.88	4.184	1.759	0.694	42.30	16.60	9.61	26.7

TABLE VI. The free amino acid content of Edam cheese of different ages, mg/100 g protein (Orientation

	First experiment							
Age of cheese	1 day[1])	15 days	1 month	1.5 m	2.5 m	4.5 m	6.5 m	8.5 m
Cysteic acid	–	–	–	–	–	–	33	28
Aspartic acid	4	38	62	77	31	142	170	182
Threonine	–	17	22	112	109	276	533	490
Serine	–	–	17	43	26	90	222	242
Amines	11	108	165	165	344	605	1033	903
Proline	–	–	–	–	24	282	720	664
Glutamic acid	19	276	336	506	984	1424	2387	2421
Glycine	–	41	47	77	138	238	542	481
Alanine	–	48	50	85	148	393	678	601
Valine	6	116	140	261	506	813	1094	1079
Cystine	–	–	7	–	9	–	–	–
Methionine	–	–	26	72	158	258	535	534
Isoleucine	–	19	25	82	141	263	501	560
Leucine	22	42	333	571	797	1231	1579	1748
Tyrosine	–	–	–	64	19	–	162	237
Phenylalanine	11	167	188	225	438	589	672	722
Ammonia	147	604	666	767	1099	1391	1697	1372
Ornithine	–	81	167	229	244	366	325	225
Lysine	–	65	206	331	591	785	1190	1176
Histidine	–	–	–	–	Lost	119	228	148
Tryptophane	–	–	–	–	–	–	–	–
Arginine	–	–	–	–	89	–	–	–

[1]) Calculated as mg/100 g cheese.
v.f.a. = volatile fatty acids

experiments)

Organoleptic tests	
Consistency	Flavour
Soft with no holes	Fresh curdy, clean after taste
Rather firm, with few gas holes	Freshly acid and curdy, very little aroma
Firm, more brittle than smooth, few holes	Beginning cheese flavour, pure taste, sl. acid
Firm, rather short, close	Sl. cheesy, no taints, sl. bitter after taste
Good, few small holes, a little short	More pronounced aroma than taste, bitter after taste
Short and brittle, few holes	Very cheesy, more v.f.a. flavour, sl. bitter
Short and brittle, few cracks	Sharp fatty acids taste, sl. rancid bitter
Short and brittle, few cracks, rare eyes	Saline, a full flavour of v.f.a. & amino acids, sl. bitter after taste
Very soft, no holes	Curdy, no objectionable taste
Rather springy, small gas holes	Curdy, clean after taste
Rather soft, many holes	Clean after taste, curdy, no off flavours
Long, very smooth, propionic gas eyes	Clean taste, sl. strong taste
Smooth, rather springy, easy to cut	Saline, strong broth-like taste, sl. heavy
Smooth and waxy, close, a little long	Strong broth-like taste, few v.f.a., sl. heavy taste of H_2S
Few wide cracks, smooth and waxy	Very strong taste, more broth-like than v.f.a.
Smooth, few wide cracks, sticky	Very strong, sl. saline, few v.f.a., sl. sweet, sl. bitter

experiments)

	Second experiment								
11.5 m	1 day[1])	15 days	1 month	1.5 m	2.5 m	4.5 m	6.5 m	8.5 m	11.5 m
37	–	–	–	–	–	–	24	29	151
217	5	43	14	55	71	161	262	276	316
580	–	50	–	129	125	334	503	584	610
364	–	–	–	98	89	300	485	652	604
928	–	48	–	40	531	735	1019	972	1027
909	–	–	125	216	314	679	869	979	1011
2445	17	221	381	735	1455	1930	2790	2816	3260
711	–	Lost	67	153	216	397	501	649	813
593	–	69	126	97	226	367	630	602	651
1256	15	154	275	310	665	804	1025	1401	1490
–	–	–	–	–	–	–	–	–	–
524	–	–	218	197	392	384	564	480	648
502	–	75	154	131	241	366	573	571	806
1511	24	334	736	731	1342	1336	1633	1698	1764
236	–	–	55	15	57	22	164	211	222
806	8	201	383	423	569	596	672	688	764
2193	107	878	1436	913	1361	1269	1721	1257	3542
344	17	151	242	303	237	432	500	487	531
1577	9	129	306	385	447	900	1060	1367	1545
398	–	–	42	90	79	157	177	253	417
–	–	–	–	–	–	43	–	–	–
149	–	–	–	–	–	50	–	–	–

TABLE VII. Chemical and organoleptic examinations of Edam cheese differing in pH

Exp. no.	Age in months	Type of cheese	pH in cheese		Moisture %	Fat %	Salt %	T.N. %	Sol. N. %	Amino acid N. %	Sol. N.	Amino acid N.
			Before brining	Before analysis							in % of T.N.	
I	2	Low pH	4.82	4.84	37.51	–	–	3.687	1.037	0.262	28.14	7.11
		Middle pH	4.94	4.96	38.94	–	–	3.615	1.026	0.283	28.38	7.81
		High pH	5.17	5.18	38.89	–	–	3.654	1.061	0.335	29.04	9.16
	5	Low pH	4.82	5.13	34.04	33.84	2.62	3.920	1.330	0.448	33.92	11.42
		Middle pH	4.94	5.19	34.10	33.68	2.44	3.940	1.400	0.506	35.53	12.84
		High pH	5.17	5.49	34.74	33.57	2.40	3.930	1.463	0.564	37.21	14.36
	8	Low pH	4.82	5.19	29.74	36.11	2.70	4.158	1.498	0.615	36.01	14.79
		Middle pH	4.94	5.28	30.38	35.77	2.55	4.106	1.562	0.633	38.10	15.41
		High pH	5.17	5.57	30.37	35.97	2.42	4.195	1.714	0.670	40.84	16.25
II	2	Low pH	4.83	4.84	38.03	32.22	2.31	3.632	0.906	0.233	24.94	6.41
		Middle pH	4.96	5.04	38.07	32.48	2.04	3.598	0.925	0.235	25.70	6.53
		High pH	5.13	5.19	37.61	32.69	1.97	3.631	0.950	0.239	26.16	6.58
	5	Low pH	4.83	4.97	32.40	35.22	2.58	3.980	1.325	0.391	33.29	9.83
		Middle pH	4.96	5.20	33.04	34.93	2.39	3.920	1.425	0.433	36.35	11.04
		High pH	5.13	5.50	31.77	35.68	2.33	4.020	1.613	0.501	40.11	12.46
	8	Low pH	4.83	5.21	28.88	36.82	2.75	4.180	1.467	0.430	35.07	10.28
		Middle pH	4.96	5.45	29.91	36.45	2.52	4.095	1.552	0.532	38.80	12.99
		High pH	5.13	5.63	28.88	36.92	2.52	4.185	1.739	0.588	41.54	14.05

TABLE VIII. The Free Amino acid content of Edam cheese from the experiments of cheese differing in pH

First experiment									
Age of cheese	Two months			Five months			Eight months		
pH of cheese	Low	Middle	High	Low	Middle	High	Low	Middle	High
Cysteic acid	–	–	–	–	–	–	–	–	26
Aspartic acid	166	201	172	307	381	524	345	554	832
Threonine	144	160	159	365	391	418	467	539	555
Serine	72	106	–	158	111	66	429	220	92
Amines	408	432	516	741	736	533	822	780	671
Proline	216	211	448	670	933	972	974	1151	2239
Glutamic acid	993	1094	1629	2353	2409	3372	2750	3728	4442
Glycine	254	207	326	452	520	681	669	823	892
Alanine	213	252	424	477	529	664	544	667	731
Valine	490	531	770	1009	1031	1513	1178	1517	1585
Cystine	–	–	–	–	–	–	–	8	–
Methionine	130	183	296	379	399	623	476	551	600
Isoleucine	170	220	290	503	572	843	641	783	1163
Leucine	960	899	1261	1612	1645	2017	1678	1802	1971
Tyrosine	50	–	40	130	Lost	55	233	137	153
Phenylalanine	241	302	380	681	707	801	798	753	839
Ammonia	N.D.	N.D.	N.D.	1016	Lost	1849	1738	2304	2231
Ornithine	N.D.	N.D.	N.D.	330	455	457	320	622	351
Lysine	N.D.	N.D.	N.D.	1080	1143	1292	1234	1756	1619
Histidine	N.D.	N.D.	N.D.	141	229	370	231	395	455
Tryptophane	N.D.	N.D.	N.D.	–	–	–	37	–	–
Arginine	N.D.	N.D.	N.D.	147	96	80	211	–	–

N.D. = not determined.

Salt/water ratio	Protein %	Organoleptic tests	
		Consistency	Flavour
–	23.56	Firm, rather brittle, no holes	Clean, no bitterness, a shade of fatty acids
–	23.10	Rather firm, a little close, no holes	Slightly bitter, freshly acid, curdy
–	23.35	Soft and springy, few small gas holes	Clean after taste, broth-like
7.69	25.05	Rather brittle and short, mechanical openings, few cracks	Salty, clean after taste, strong flavour of v.f.a.
7.15	25.24	Firm, smooth, no holes and cracks	Clean after taste, mild cheese flavour
6.90	25.19	Soft, smooth, wide eyes	Slightly bitter, tainted H_2S, off-flavour
9.06	26.58	Brittle, very short, few cracks	Very strong flavour of v.f.a., sl. bitter, sharp
8.39	26.26	smooth, less firm, many small holes	Clean after taste more amino acids than v.f.a.
7.96	26.76	Waxy, smooth, many gas eyes	Strong broth-like taste, sweet, tainted H_2S
6.07	23.21	Firm, short, few mechanical holes	Acid taste, slightly bitter
5.35	22.99	Rather firm, rather close, no holes	Clean taste, curdy
5.23	23.20	Soft, springy, large gas eyes	Slightly tainted, heavy taste, H_2S
7.96	25.43	Brittle and short, solid, no holes	Shade of bitterness after taste, strong flavour of v.f.a.
7.23	25.35	Rather short, few cracks	Very bitter
7.30	25.69	Very smooth and long, wide gas eyes	bitter, sweet, tainted H_2S
9.52	26.71	Very short, crumbly, few cracks,	Flavour of v.f.a., rather sharp, no bitterness
8.42	26.56	Firm, rather long, no cracks	Clean after taste, more broth taste than v.f.a. flavour, less sharpness, sl. bitter and saline
8.72	26.74	Smooth, waxy, gas eyes	Sl. bitter, predominent broth-like taste, sweet and tainted H_2S

(mg/100 g protein)

Second experiment								
Two months			Five months			*Eight months*		
Low	Middle	High	Low	Middle	High	Low	Middle	High
–	–	–	54	30	97	–	–	24
212	176	180	206	139	241	271	315	408
142	141	155	265	265	268	357	488	475
45	35	33	113	79	59	180	170	166
387	471	516	643	575	770	726	955	906
–	215	266	–	592	662	745	849	1176
1216	1158	1174	1884	1982	2204	2405	2688	3041
190	197	234	235	448	571	603	705	710
159	208	254	323	363	494	363	565	568
498	536	565	834	903	1046	901	1076	1110
5	–	–	–	–	–	–	–	–
157	159	179	358	633	592	454	725	576
127	198	215	314	468	505	489	564	713
545	768	870	1353	1343	1647	1419	1879	1913
27	75	37	Lost	46	166	113	170	293
310	274	336	563	585	663	588	769	747
922	940	986	Lost	1475	2182	1332	1706	1929
169	186	172	241	270	395	138	Lost	204
556	575	638	817	865	977	913	1095	959
31	–	–	65	179	326	248	220	265
–	–	–	–	–	–	–	–	–
–	–	–	113	–	–	195	–	–

TABLE IX. Chemical and organoleptic examinations of Edam cheese from raw and pasteurised milk (P.)

Exp. no.	Age in months	Type of Milk	pH		Moisture %	Fat %	Salt %	T.N. %	Sol. N. %	Amino acid N. %	Sol. N.	Amino acid N.
			Before brining	Before analysis							in % of T.N.	
		Raw	5.02	5.07	37.52	31.91	1.97	3.634	1.025	0.212	28.20	5.83
	1.5	72°C P.	5.05	5.08	40.39	30.37	2.01	3.512	0.969	0.209	27.58	5.95
		95°C P.	5.06	5.13	39.34	30.83	2.10	3.532	0.937	0.246	26.09	6.84
		Raw	5.02	5.12	31.84	34.87	2.22	3.950	1.375	0.392	34.81	9.89
I	4.5	72°C P.	5.05	5.12	33.63	34.27	2.35	3.900	1.290	0.345	33.07	8.86
		95°C P.	5.06	5.25	34.13	33.47	2.28	3.880	1.156	0.378	29.79	9.77
		Raw	5.02	5.19	30.35	35.50	2.58	4.088	1.531	0.579	37.45	13.91
	8.5	72°C P.	5.05	5.16	30.92	35.05	2.53	4.167	1.439	0.471	34.54	11.29
		95°C P.	5.06	5.37	30.37	34.91	2.65	4.208	1.269	0.512	30.15	12.16
		Raw	4.94	5.01	37.14	31.20	2.18	3.662	1.053	0.243	28.75	6.63
	1.5	72°C P.	4.97	5.09	37.87	30.67	2.05	3.648	0.950	0.227	26.04	6.22
		95°C P.	4.98	5.08	39.67	29.83	2.28	3.588	0.841	0.251	23.42	6.99
		Raw	4.94	5.30	33.22	33.26	2.37	3.910	1.290	0.413	32.99	10.55
II	4.5	72°C P.	4.97	5.30	32.97	33.27	2.40	3.918	1.180	0.390	30.11	9.95
		95°C P.	4.98	5.35	34.32	32.65	2.48	3.862	1.150	0.380	29.77	9.83
		Raw	4.94	5.43	30.05	34.44	2.57	4.129	1.538	0.558	37.28	13.51
	8.5	72°C P.	4.97	5.32	29.07	34.81	2.44	4.206	1.558	0.468	35.21	11.11
		95°C P.	4.98	5.34	30.34	34.49	2.62	4.159	1.296	0.446	31.16	10.72

TABLE X. The free amino acid content of Edam Cheese from the experiments with raw and pasteurised milk

First experiment

Age of cheese	1.5 months			4.5 months			8.5 months		
Type of milk	Raw	72°C P.	95°C P.	Raw	72°C P.	95°C P	Raw	72°C P.	95°C P.
Cysteic acid	–	23	18	–	39	16	–	16	38
Aspartic acid	88	156	104	142	88	36	322	222	223
Threonine	123	68	76	296	100	196	492	411	314
Serine	38	83	180	76	100	112	221	370	407
Amines	152	324	373	612	386	519	912	838	893
Proline	–	93	66	298	245	179	632	635	1063
Glutamic acid	612	602	793	1478	1292	1381	2465	2048	2137
Glycine	78	107	165	208	206	375	398	246	698
Alanine	93	148	148	381	253	317	598	452	596
Valine	274	Lost	429	866	611	710	1182	1002	1153
Cystine	–	–	–	–	–	–	–	–	–
Methionine	106	Lost	169	239	384	452	572	570	474
Isoleucine	93	73	123	277	243	297	567	466	513
Leucine	588	599	642	1272	1077	1162	1791	1466	1676
Tyrosine	27	36	37	–	182	174	151	299	285
Phenylalanine	252	179	292	563	527	518	822	612	620
Ammonia	882	762	1072	1482	1186	1766	1388	912	1095
Ornithine	221	150	195	366	270	294	241	159	287
Lysine	196	237	285	761	800	884	1066	1194	1229
Histidine	36	–	–	101	124	206	121	154	278
Tryptophane	–	18	–	–	–	–	–	–	–
Arginine	–	35	–	–	261	145	–	360	102

P. = pasteurised.

Salt/ water ratio	Protein %	Fat acidity	Organoleptic tests	
			Consistency	Flavour
5.25	23.22	N.D.	Rather firm, good	Clean after taste, slightly cheesy
4.96	22.44	N.D.	Soft, many holes, tough	Curdy, slightly bitter
5.33	22.95	N.D.	Rather firm, few holes, tough	Tasteless, bitter
6.97	25.24	N.D.	Firm, good, few small holes	Clean cheesy taste, sl. bitter
6.98	24.92	N.D.	No more toughness, good	Sl. cheesy, more taste than aroma
6.68	24.79	N.D.	A little tough, no holes	Flat taste, sl. bitter
8.50	26.12	3.86	Rather short, good	Very cheesy, rancid bitter of v.f.a.
8.18	26.24	1.85	Good, a little short	Less cheesy, strong broth, sl. bitter
8.71	26.88	1.07	Tough, hard rind	Neutral taste, no flavour, bitter
5.86	23.40	N.D.	Firm, good	Sl. cheesy, clean after taste
5.41	23.30	N.D.	Good, a little tough	Clean curdy taste
5.74	22.92	N.D.	Rather firm, tough	Tasteless, bitter
7.12	24.97	4.08	Smooth, rather long	Very cheesy, sharp "heavy" taste
7.27	25.04	1.08	A little tough and rubbery	No aroma, broth-like taste
7.22	24.68	0.08	Rubber-like	No aroma, insipid taste, bitter
8.55	26.38	5.52	smooth, waxy, few holes	Very strong broth, cheesy, sl. bitter
8.39	26.88	1.82	Good and smooth	More broth than v.f.a., bitter, sl. sweet
8.63	26.57	1.12	Smooth but rubbery	Poor taste, bitter after taste

(mg/100 g protein)

Second experiment

1.5 months			4.5 months			8.5 months		
Raw	72°C P.	95°C P.	Raw	72°C P.	95°C P.	Raw	72°C P.	95°C P.
–	17	21	–	21	23	27	14	–
63	30	71	198	124	131	292	239	193
111	60	90	366	320	225	588	454	362
77	117	123	315	308	258	684	485	466
396	283	490	744	750	728	963	1000	871
203	110	287	683	384	621	1222	877	1090
761	499	694	1888	1576	1443	2877	2597	2003
133	114	222	391	355	493	598	558	756
96	127	Lost	376	380	384	621	537	400
434	354	395	823	738	730	1446	1227	1156
–	–	–	–	–	–	–	–	–
198	228	110	385	314	356	488	586	411
122	167	153	372	337	302	611	580	453
763	705	815	1312	1201	1463	1709	1592	1634
17	61	66	46	188	189	68	214	279
441	392	375	617	497	537	848	684	717
988	1195	1439	1322	974	872	1396	1453	1196
292	122	321	452	187	323	461	160	233
366	489	429	873	824	978	1302	1615	1351
87	95	96	159	132	235	233	383	339
–	–	–	–	–	–	–	–	–
–	187	–	50	255	110	–	180	–

TABLE XI. Chemical and organoleptic examinations of Edam cheese with different moisture contents

Exp. no.	Age in months	Type of cheese	pH in cheese		Moisture %	Fat %	Salt %	T.N. %	Sol. N. %	Amino acid N. %	Sol. N.	Amino acid N.
			Before brining	Before analysis							in % of T.N.	
	3	High moisture	4.99	5.05	39.87	32.25	2.67	3.678	1.036	0.328	28.18	8.93
		Low moisture	4.93	4.99	37.28	33.51	2.22	3.838	0.926	0.318	24.14	8.30
I	6	High moisture	4.99	5.50	38.65	32.92	2.94	3.750	1.433	0.481	38.22	12.83
		Low moisture	4.93	5.38	35.11	34.89	2.43	4.002	1.261	0.440	31.50	11.01
	9	High moisture	4.99	5.61	30.93	37.28	2.99	4.224	1.744	0.672	41.30	15.90
		Low moisture	4.93	5.47	29.12	37.96	2.48	4.306	1.423	0.592	33.04	13.74
	3	High moisture	4.92	5.12	40.76	30.28	2.52	3.566	1.116	0.363	31.30	10.19
		Low moisture	4.92	5.10	35.83	32.94	2.66	3.869	1.029	0.341	26.61	8.82
II	6	High moisture	4.92	5.27	39.81	30.96	2.85	3.620	1.414	0.422	39.06	11.66
		Low moisture	4.92	5.13	33.27	34.08	2.80	4.043	1.366	0.407	33.79	10.07
	9	High moisture	4.92	5.46	30.25	35.74	2.89	4.192	1.702	0.595	40.60	14.19
		Low moisture	4.92	5.22	29.62	36.04	2.83	4.258	1.512	0.508	35.50	11.93

TABLE XII. The free amino acid content of Edam cheese from experiments of cheese differing in moisture conte

	First experiment					
Age of cheese	3 months		6 months		9 months	
Moisture content of cheese	High	Low	High	Low	High	Low
Cysteic acid	–	–	–	–	195	119
Aspartic acid	104	111	374	322	471	354
Threonine	122	80	297	222	396	435
Serine	–	51	–	204	–	245
Amines	370	236	727	653	676	729
Proline	–	–	974	102	1433	642
Glutamic acid	756	611	2628	1968	3257	2034
Glycine	121	102	554	515	543	564
Alanine	86	151	343	323	528	467
Valine	258	183	978	809	1523	1191
Cystine	–	–	–	–	–	–
Methionine	135	100	668	532	585	524
Isoleucine	98	108	659	589	917	714
Leucine	569	452	1569	1437	1925	1489
Tyrosine	20	11	143	82	181	173
Phenylalanine	154	178	783	654	765	691
Ammonia	424	464	1271	1042	2322	2137
Ornithine	67	66	167	106	127	–
Lysine	331	213	682	629	721	754
Histidine	–	62	231	186	406	391
Tryptophane	–	–	–	–	–	–
Arginine	–	–	–	–	–	–

Salt/ water ratio	Protein %	Organoleptic tests	
		Consistency	Flavour
6.71	23.50	Rather soft, few gas eyes	Water taste, slightly bitter after taste
5.95	24.53	Good, rather soft, few small holes	Slightly cheesy, clean after taste
7.60	23.96	Rather soft, long, no cracks	Saline, strong broth like, "heavy" taste, bitter
6.92	25.57	A little long, smooth, few cracks	Sl. saline, very strong broth like taste
9.66	26.99	Long, waxy, sticky, few cracks	Very strong taste, saline and bitter
8.51	27.51	Firm, smooth, many cracks	Strong broth-like taste, bitter after taste
6.17	22.79	Many gas eyes, soft	Water taste, bitter after taste
7.43	24.73	Rather firm, good consistency	Slightly saline, slightly bitter
7.15	23.12	Rather soft, wide cracks	Saline, intensively tainted H_2S, bitter and sweet
8.41	25.83	A little short, firm, few holes	Saline, more v.f.a., clean after taste
9.56	26.76	Very smooth, waxy, many cracks	Very bitter before and after taste
9.55	27.21	Very short and brittle, many holes	Very cheesy, sharp flavour of v.f.a., sl. saline

(mg/100 g protein)

Second experiment					
3 months		6 months		9 months	
High	Low	High	Low	High	Low
–	–	–	–	24	–
360	326	264	202	367	304
132	220	314	222	377	383
38	134	43	116	215	258
382	199	600	476	755	665
320	337	658	348	1217	796
1716	1732	2591	2385	2757	2409
348	380	656	605	741	777
295	240	513	443	491	543
618	705	919	1063	1102	1157
–	–	–	(30)	–	–
175	172	634	374	704	596
316	152	600	493	795	604
1279	812	1655	1517	1792	1521
24	–	137	113	188	Lost
483	405	782	655	776	713
1329	926	1928	1655	2094	1928
211	148	298	207	278	146
519	493	1014	960	1448	1224
–	108	180	200	373	331
–	–	–	–	–	–
–	–	159	157	175	223

TABLE XIII. Chemical and organoleptic examinations of Edam cheese from aseptically drawn and infected

Exp. no.	Age in months	Type of milk used	pH in cheese		Moisture %	Fat %	Salt %	T.N. %	Sol. N. %	Amino acid N. %	Sol. N.	Amino acid N.
			Before brining	Before analysis							in % of T.N.	
I	4	Aseptically drawn	5.00	5.06	35.67	29.74	1.42	4.221	0.928	0.311	21.97	7.34
		Infected	4.98	4.98	36.62	29.47	1.49	4.056	1.050	0.315	25.88	7.76
II	7½	Aseptically drawn	5.06	5.33	29.87	31.78	2.35	4.534	1.186	0.437	26.15	9.64
		Infected	5.04	5.27	30.70	31.37	2.52	4.488	1.304	0.521	29.07	11.60

v.f.a. = volatile fatty acids.

TABLE XIV. The free amino acid content of Edam cheese from experiments with aseptically drawn and infected milk (mg/100 g protein)

Age of Cheese	4 months		7.5 months	
Type of milk	Aseptically drawn	Infected	Aseptically drawn	Infected
Cysteic acid	14	–	17	68
Aspartic acid	115	248	175	473
Threonine	235	264	344	400
Serine	199	106	344	403
Amines	644	407	721	556
Proline	336	332	627	682
Glutamic acid	1476	1634	1954	2828
Glycine	372	300	574	746
Alanine	284	287	460	395
Valine	685	646	853	1334
Cystine	–	–	–	–
Methionine	392	343	418	496
Isoleucine	344	349	654	789
Leucine	1154	1202	1185	1495
Tyrosine	140	–	330	224
Phenylalanine	560	587	729	971
Ammonia	1222	1297	1890	1918
Ornithine	122	359	349	298
Lysine	720	733	1270	1477
Histidine	166	124	267	255
Tryptophane	–	–	–	–
Arginine	300	–	58	–

3.1.2. *Free amino acids during cheese ripening*

A rough comparison of the data in tables VI, VIII, X, XII and XIV shows that, under the influence of factors known to affect cheese ripening, there is almost no variation in the amino acid pattern of Edam cheese of approximately the same age. Even cheeses with different properties, have almost the same amino acid peaks on the chromatogram. The concentrations of the amino acids were affected by these factors, but not to the extent that a certain amino acid disappeared from the cheese. An exception was the amino acid pattern of the cheese in the first few weeks of maturation. Under the influence of some of the factors studied, it was found that some amino acids showed late appearance. These were

milk

Salt/ water ratio	Protein %	Acidity of cheese Fat	Organoleptic tests	
			Consistency	Flavour
3.98	26.97	1.96	Very close and tough, sl. long	Insipid flat taste, no trace for v.f.a.
4.06	25.96	7.60	good, firm with few small holes	Cheesy, sl. acid, sl. bitter after taste
7.94	28.97	2.26	Rather hard and tough, many holes	Pure taste, no aroma, slightly sweet
8.20	28.69	13.22	Good consistency, smooth and easy to cut	Very cheesy, saline, sl. rancid bitter

serine, proline, histidine and arginine. It should not be forgotten, that at this period of ripening, growth and variation in the cheese microflora occur. These amino acids may be, under certain conditions, essentially utilized for metabolism by certain types of bacteria in the cheese. Except for the amino acids mentioned, the amino acids pattern of young cheeses was always the same.

Amino acids that were found in every Edam cheese and always in a large concentration were glutamic acid, leucine, lysine, valine, and phenylalanine. Others that were always found but in relatively smaller amounts were methionine, isoleucine, alanine, glycine, threonine, ornithine and aspartic acid. Amino acids that were sometimes absent were serine, proline, tyrosine, histidine, arginine and cysteic acid. Cystine and tryptophane were rarely found.

Other nitrogenous substances that were always found on the amino acid chromatograms and in considerable concentrations were amines and ammonia. Some small unknown peaks may appear during the analysis, (A, B & C figure 11). They could not be considered as contributors to the general amino acid pattern of cheese since they were detected only in some cheeses at certain stages of ripening after which they might disappear completely. Such peaks were rather frequent and larger in young cheese. They may be due either to the presence of some peptides or to amino acid decomposition products.

As shown in table VI, it is interesting to note the early appearance of some amino acids in cheese. As early as the second day of manufacture, and just after cheese was ready for brining, five to seven amino acids were detected in Edam cheese. Being found in very small amounts, their occurrence may be due to the addition of the starter. Their concentrations were expressed as mg/100 g cheese since the nitrogen content of the cheese had not been determined at that time. The presence of ammonia on the second day of manufacture in a concentration from 106 to 146 mg/100 g cheese, reported in this work, may be partly due to the absorption from the air of this nitrogenous compound by the acidic buffers during the column chromatographic analysis. SIRKS (90) found as much as 0.2% of the total nitrogen as ammonia in a week old Edam cheese. This maximum quantity is equivalent to less than 50 mg ammonia per 100 g cheese. Large amounts found here may be partly due also to early deamination of amino acids resulting in the formation of ammonia (2).

Amino acids that showed early appearance in both orientational experiments, were glutamic acid, leucine, valine, phenylalanine and aspartic acid, whereas lysine and ornithine were detected in one experiment.

The early appearance of amino acids in cheese was mentioned in the literature. CHEBOTAREV *et al* (15) reported the appearance of 9 amino acids and amino acids combinations in one day old Russian Edam cheese. The early detection of amino acids in foreign types of cheese was also previously reported. In Cheddar cheese KOSIKOWISKI (49) observed the presence of glutamic acid and aspartic acid a few hours after the curd had been produced. HONER *et al* (43) were able to detect glutamic acid and leucine at the time of milling the curd of the same cheese. DACRE (18) found that glutamic acid, lysine or arginine, leucine and tyrosine were the first group of amino acids to appear in New Zealand Cheddar cheese. On the other hand, KIURU *et al* (48) found appreciable amounts of lysine, ornithine, proline, glutamic acid, arginine, tyrosine, valine, leucine and alanine on the first day in Emmenthal cheese. In Tilsit cheese, OSWALD (68) demonstrated the presence of glutamic and aspartic acids within a few hours of manufacture, whereas FRICKER (29) was able to find most of the amino acids after salting the same cheese. WILSSENS & VLEESCHAUWER (110) found glutamic acid, aspartic acid, leucine and valine in Gouda cheese of one to two days.

After 15 days storage at 15°C, the presence of 11 amino acids, amines and

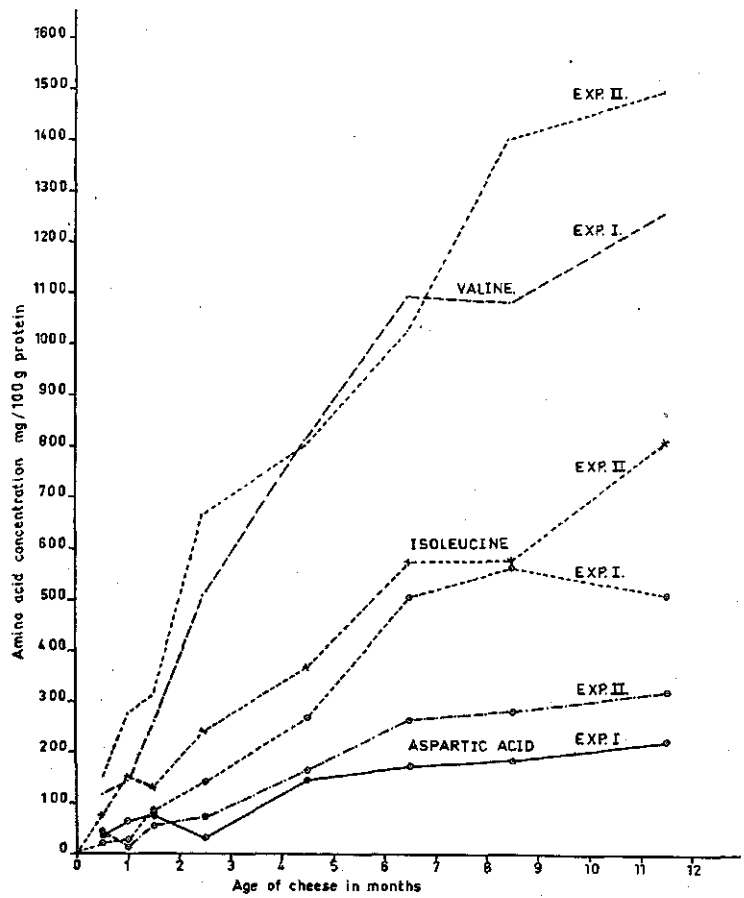


FIG. 4. Aspartic acid, isoleucine and valine contents of Edam cheese during ripening.

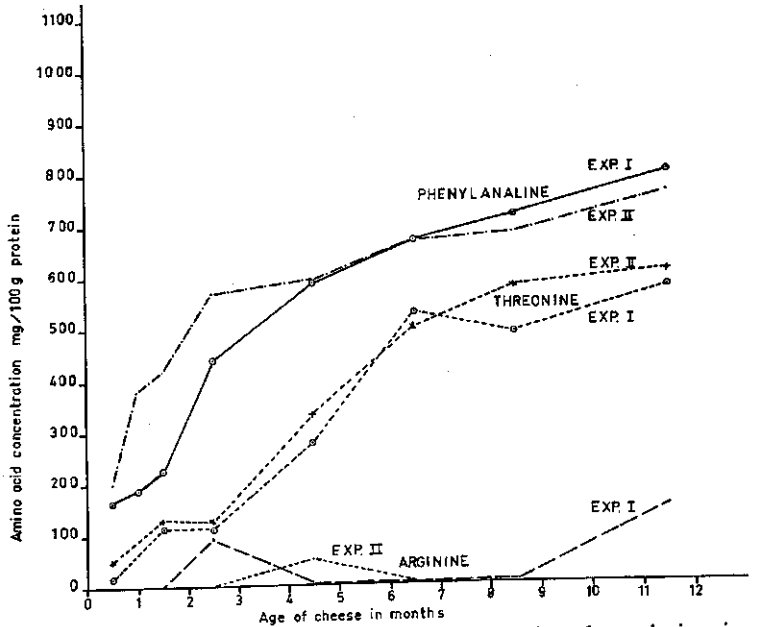


FIG. 5. Threonine, phenylalanine and arginine contents of Edam cheese during ripening.

ammonia was demonstrated. However, some amino acids appeared relatively late. Methionine, serine and tyrosine were noted only after a month, whereas proline and histidine were observed after one month in the first experiment and after 2.5 months in the case of the other. Arginine showed late appearance. It was found only after 2.5 months in the first experiment and after 4.5 months in the case of the other. The differences in the two experiments may be due either to the higher moisture content and pH of the second experiment or to the differences in the microflora of the milk used in making the cheese.

Some amino acids showed late appearance in cheeses with different properties. Serine and histidine were absent in a two months old cheese with high pH; and proline was not present in low pH cheese of the same age. In both types of cheese, cysteic acid and arginine were not found at this age (table VIII). In the low and high moisture content cheeses, histidine was still absent in high moisture cheese first analysed after three months' ripening; serine was present in one experiment but absent in the other. Also, proline was not found in low and high moisture content cheese of this age. In cheese produced from pasteurised milk histidine was absent after 1.5 months' ripening. The corresponding cheese made from raw milk did not contain cysteic acid, proline and arginine.

The late appearance of these amino acids cannot be taken as an indication that they are not liberated or that the amino acid parts of the casein were more resistant to proteolytic agents. It is probable that these amino acids were utilized for bacterial metabolism or converted by enzyme action to other substances that could or could not be detected in the cheese extract.

Late appearance of amino acids was reported in the literature. BULLOCK & IRVINE (13) noted the late appearance of arginine, histidine and proline in Cheddar cheese. SILVERMAN & KOSIKOWISKI (87) observed the late appearance

of proline, and DACRE (18) the late appearance of methionine, threonine, serine and proline. HONER and TUCKEY (43) reported the appearance in Cheddar cheese of aspartic acid and threonine after 77 days and serine & glycine after 100 days of ripening.

At the end of 2.5 months of ripening, nearly all the amino acids of casein were found in the free state in Edam cheese. About 21 peaks in the column chromatographic analysis, representing amino acids, amines and ammonia were found at this age. The number of the amino acids did not increase with progressive ripening. As shown in table VI and in figure 4 to 10, the amino acids increased in concentration from the time they were set free. All the amino acids did not increase at the same rate. In the period of ripening from 1 to 2.5 months, some amino acids such as threonine, serine, histidine and aspartic acid showed a decrease in their concentration in both experimental cheeses. Others showed a decrease in one experiment or at least a levelling off of the normal curve of

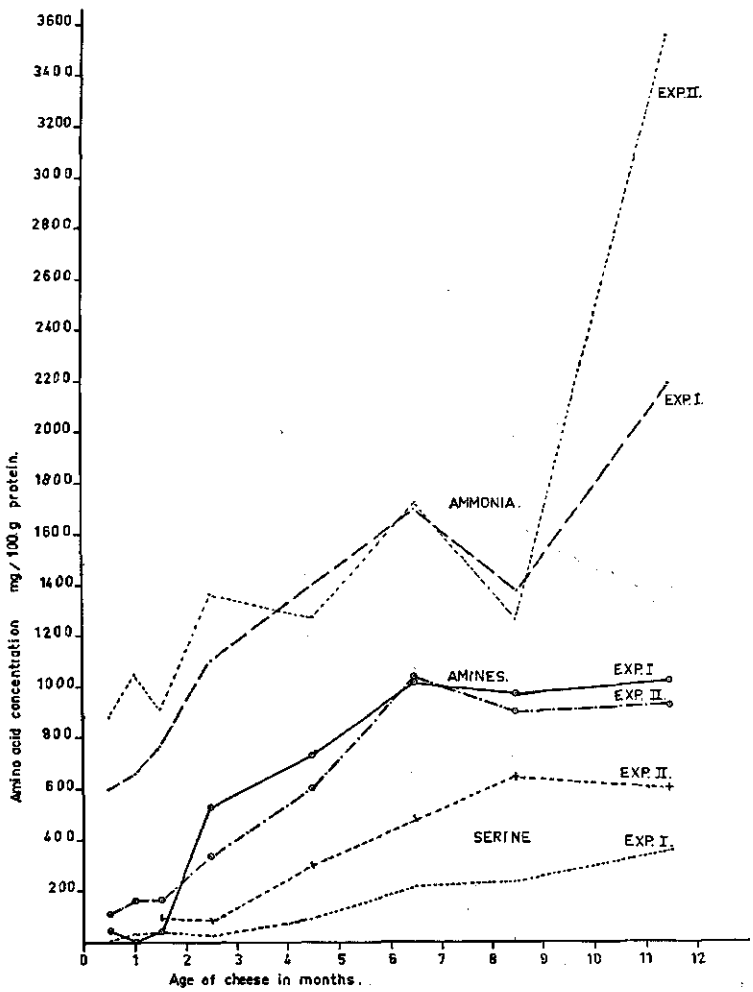


FIG. 6. Serine, amines and ammonia contents of Edam cheese during ripening.

increase. This decrease or levelling off may be due to the utilization of these amino acids for bacterial metabolism during the first stages of the ripening period. This amino acid decrease was reported by CHEBOTAREV *et al* (15) in three months old Russian Edam cheese. SALERNO & PAOLIS (80) observed diminution in all amino acids except aspartic acid in Emmenthal cheese 30 days old. In 3 months old cheese, they noticed a decline in serine, glycine, tyrosine, alanine, glutamine and threonine. Also BULLOCK & IRVINE (13) observed a decrease in the concentration of some amino acids in a 60 days old raw milk Cheddar cheese.

The most striking increase in the concentration of all amino acids, amines and ammonia (figure 4 to 10), was in the period of ripening between 2.5 and 6.5 months. At the end of this period, most of amino acids reached a high concentration and sometimes their maximum level. With progressive ripening, the trends of variations were no more consistent. It differed according to the amino acid and to the cheese. Lysine, glycine, valine, proline and phenylalanine showed

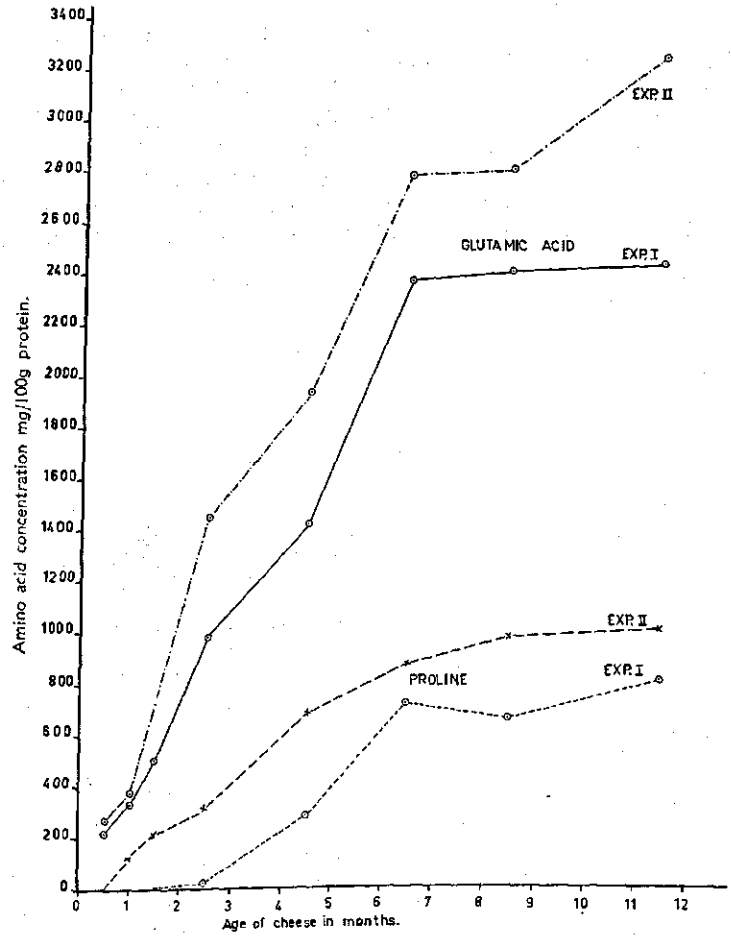


FIG. 7. Glutamic acid and proline contents of Edam cheese during ripening.

continuous increase though the rate was lower than at the period from 2.5 to 6.5 months. Aspartic acid, threonine, tyrosine and ornithine were more or less constant. These amino acids showed the same trend in both experiments. On the other hand alanine, methionine, isoleucine and leucine that tended to decrease in the first experiment, were either constant or increased in the second. In both experiments amines showed a decrease with progressive ripening.

The increase in the concentration of amino acids during ripening was reported in the literature for Cheddar cheese (13, 18, 39, 43, 49, 77), Emmenthal & Swiss type cheeses (40, 48, 80), Tilsit (29, 68), Gouda type & St. Paulin (110) and Samsoe cheese (62). Most amino acids were found to increase steadily, others were more or less constant while few others decreased.

Thus, in the orientation experiments, the general trend would be an initial increase in the concentration of amino acids during ripening followed sometimes by a slight decrease or a levelling off at the age of 1.5 to 2.5 months, then a subsequent increase as ripening progressed. With some amino acids, this increased concentration was constant after 6.5 months, whereas with others a levelling off or a decrease occurred.

In cheeses with different properties, although few were analysed, the same general trend of amino acid variation could be observed. It is worthy to note that the trend of variations in the amino acid content of cheese with different pH were almost the same throughout the ripening period, particularly in the cheese of one experiment. Most of the amino acids showed a considerable increase in the first period of ripening which lasted from the second to the fifth month. In the second period, namely from the fifth to the eighth month, the rate of increase was lower. Tyrosine and serine did not follow the trend. They showed a greater

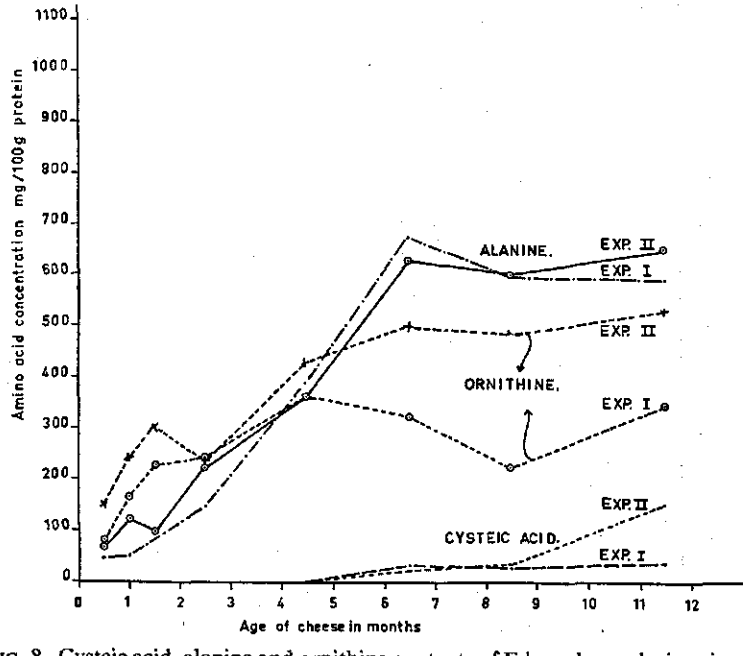


FIG. 8. Cysteic acid, alanine and ornithine contents of Edam cheese during ripening.

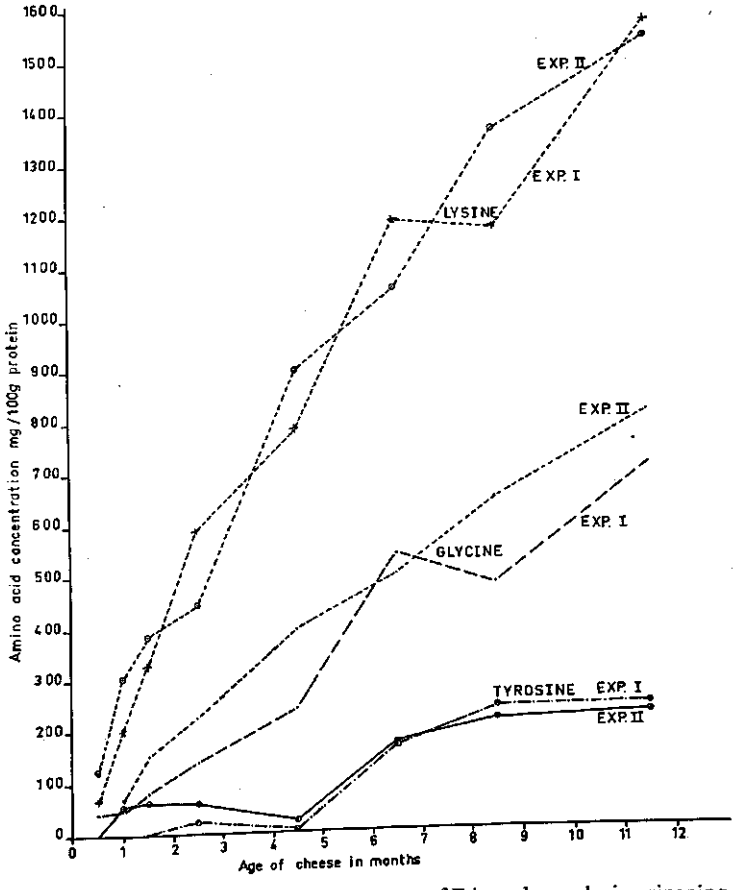


FIG. 9 Glycine, tyrosine and lysine contents of Edam cheese during ripening.

increase in the second period than in the first, probably due to more decarboxylation and to a greater bacterial activity in the early stages of ripening. Most of the amino acids showed a rise in concentration at the eighth month with the exception of ornithine that decreased in the three types of different pH cheeses and methionine, arginine, and histidine that decreased only in high pH cheese.

Also in cheese made from raw and pasteurised milk there was, during the ripening period from 1.5 to 8.5 months, a steady increase in the concentration of all amino acids except serine and aspartic acid. Serine showed a decrease in a cheese made from milk pasteurised at 95 °C after 4.5 months, whereas aspartic acid decreased in both types of pasteurised milk cheese. This decrease may reflect the late utilization of these two amino acids by bacteria in pasteurised milk cheese. After 8.5 months of storage, the methionine content of cheese from milk pasteurised at 95 °C levelled off while ornithine and arginine decreased in all cheeses made from raw and pasteurised milk with the exception of two cheeses. The levelling off of methionine may be due to its conversion by *streptococci* (70). No explanation can be given why there is no levelling off both in cheese from raw milk and from milk pasteurised at 72 °C. The decrease of

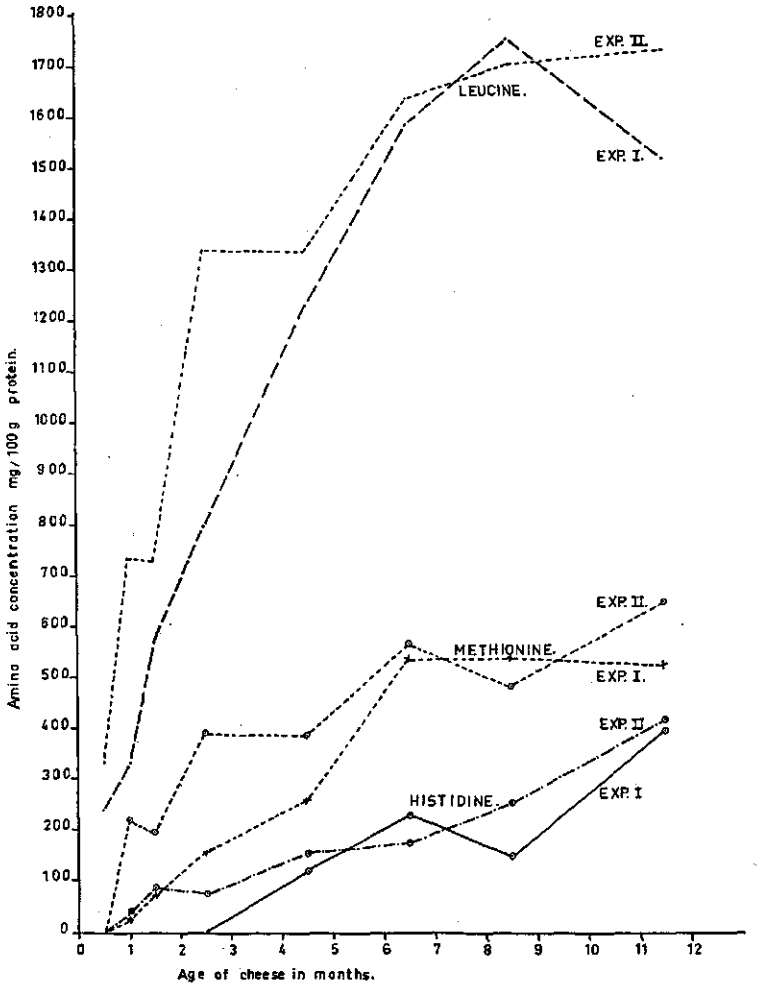


FIG. 10. Methionine, leucine and histidine contents of Edam cheese during ripening.

arginine and ornithine in cheese from raw and pasteurised milk may suggest that the *streptococci* of the starter is capable of converting the arginine to ornithine as previously reported by SILVERMAN and KOSIKOWISKI (88) and the further decomposition of the latter.

In cheeses with low and high moisture content, which were analysed at 3, 6 and 9 months, all amino acids except serine and aspartic acid showed a considerable increase in the first period. The concentration of serine decreased in a low moisture content cheese at 6 months and the aspartic acid decreased both in a low and a high moisture cheese of the same age. It may be probable that the development of some microorganisms capable of utilizing these two amino acids at a rate faster than they were being liberated was responsible for this decrease. During the second period of ripening, there was still an increase in the concentration of most amino acids, though the rate was slower than in the first period. However, few amino acids decreased after 9 months ripening particularly

in high moisture content cheese. Phenylalanine, glycine, ornithine, alanine, methionine and amines decreased in high moisture content cheese, while in low moisture cheese, the decrease was noticed only with methionine and ornithine. It should be noted that with the exception of ornithine that decreased in all cheeses, these amino acids decreased in some cheeses only.

In cheese made from aseptically drawn milk, the concentration of all the amino acids except arginine increased from the 4th to 7.5 months ripening. In the corresponding cheese made from infected milk, all amino acids with the exception of ornithine increased in the same period.

A comparative study of amino acids in different cheeses shows that at the end of the ripening period, some amino acids had not reached a high concentration. These included the aspartic acid, tyrosine and histidine. Similarly the last two acids were reported by REIHARD & GAREY (77) to remain at a low level. Also the concentrations of arginine and cysteic acid, when detected in the cheese, did not seem to be greatly affected by the stage of ripening. A conclusion drawn by BULLOCK & IRVINE (13) that the concentration of arginine and histidine did not appear to vary a great deal with the age of Cheddar cheese, agrees with the present results.

3.1.3. *Amino acid effect on cheese flavour during maturation*

Organoleptic tests of the cheese during the stage of ripening showed that the increase in flavour was accompanied by an increase in its total amino acid content. This does not necessarily mean that the amino acids are responsible for cheese flavour. The more or less flat taste of cheese made from pasteurised or aseptically drawn milk is a clear evidence that the influence of amino acids on cheese flavour is not dominating.

The fresh, curdy, insipid taste of a young cheese which contained small quantities of amino acids, changed to a tasty cheesy flavour in a ripe cheese containing larger amounts of amino acids. It is noticed that the increase in cheese flavour particularly in the period of ripening from 2.5 to 6.5 months coincided with a sharp rise in the amino acid content of the cheese. It does not mean that changes in flavour were due to high concentrations of amino acids in ripe cheese, but it is not only a matter of coincidence. Amino acids have, no doubt, a special role in cheese flavour.

When table XV, previously published by MULDER (67) is considered, it can be concluded that when the free amino acids found in the cheese are tasted individually as pure chemical compounds, they may be classified as sweet, bitter, broth-like, acidic, sulphur-like or tasteless. However, one amino acid may be characterised by two or more of these tastes. The influence of an amino acid on the taste of the cheese would be dependent on its concentration in the cheese, the intensity of its taste and the presence or absence of some other compounds that may mollify or intensify the taste. Yet, a group of amino acids having the same taste may assist in the dominance of a particular taste in the cheese under certain conditions (sweet, bitter). Glutamic acid is one of the amino acids that was found in a considerable quantity and must have influence on the cheese taste. Its strong broth-like flavour can easily be detected in the cheese. Leucine, characterised by a slightly bitter and slightly broth-like taste is found in large amounts in the cheese and may accentuate the glutamic acid flavour.

As the mixture of amino acids found in cheese is nearly odourless and possesses

no penetrating smell, it can be concluded as previously supposed by MULDER (67) that the amino acids contribute to the basic taste of the cheese. They give to the cheese almost always a broth-like slightly bitter taste and under some conditions they contribute to the sweet or bitter taste.

During the course of ripening of our cheeses some abnormal flavours appeared. At 4.5 months, a 'heavy' taste (an expression used in the Netherlands by

TABLE XV. The taste of the amino acids in casein

Amino acids	Quantity in % of total weight of casein	Taste
Glycine	3.5	sweet
Alanine	5.6	sweet
n-Valine	0.2	slightly bitter, sl. sweet
Valine	7.9	slightly sweet, sl. bitter
Leucine	12.1	slightly bitter
Isoleucine	1.4	bitter
Phenylalanine	3.9	slightly bitter
Proline	8.0	sweet
Serine	4.7	slightly sweet
Threonine	3.5	slightly sweet
Tyrosine	5.8	almost tasteless
Aspartic acid	4.1	bitter, sl. broth-like
Glutamic acid	21.6	broth-like
Hydroxyglutamic	0.3	–
Tryptophane	1.4	bitter
Arginine	4.7	bitter sweet
Histidine	2.5	bitter, sl. sweet
Lysine	6.1	bitter
Cystine	0.3	rubber-like
Methionine	3.2	slightly bitter, sl. sweet.

cheese graders) as a result of much hydrogen sulphide, could be detected. This taste disappeared with progressive ripening. Also, a slightly sweet taste was noticed in the cheese of the second experiment at 11.5 months of age. This cheese was characterised by a high moisture content, a high pH and had undergone a propionic acid fermentation at 2.5 months. This sweet taste may rather be due to the propionates formed or to the propionates, being accentuated by the sweet amino acids that were found in a large concentration in this cheese.

As amino acids contribute only to the basic taste of cheese and as their pattern in different cheeses is more or less the same, characteristic differences in the taste and flavour cannot be explained by differences in amino acids contents.

3.2. EFFECT OF PH

For many years it has been known that acidity is one of the most important factors affecting the consistency of the cheese. BOEKHOUT and OTT DE VRIES (10) found that an excessive amount of lactic acid was the direct cause of brittle texture in Edam cheese. VAN DAM (103) stated the same fact and added that the progress of ripening was dependent upon the concentration of lactic acid. Recently SIRKS (90), RAADSVELD & MULDER (74) and RAADSVELD (72) examined during different stages of ripening the effect of pH on the concentration of protein breakdown products, the consistency, taste and flavour of the cheese.

They all came to the conclusion that a high pH promotes protein degradation resulting in a higher concentration of protein breakdown products in the ripened cheese. Low pH, on the other hand, was found (67, 92) to stimulate the hydrolysis of cheese fat giving rise to the volatile fatty acids taste of cheese.

No work has been done on the effect of the pH upon the concentration of individual amino acids. In order to obtain a picture of the influence of pH on the concentration of different free amino acids in Edam cheese, we compared three series of cheeses of different pH prepared from the same raw milk as was described in Section 2.1.1. Data expressing the composition of this cheese as well as its organoleptic examination are given in table VII; the concentration of the free amino acids in the cheese, expressed as mg/100 g protein and determined from the cheese extract, are given in table VIII.

3.2.1. *Properties of the cheese*

Owing to the difference in manufacture, the pH of the cheeses differed considerably, when determined for the first time just before brining. In middle and less acid cheeses the pH was respectively 0.12 and 0.35 units higher in the first experiment than the value of low pH cheese (4.82 and 4.83) and 0.13 and 0.30 units higher in the second experiment. With progressive ripening, the difference became greater. After 5 months' ripening the increase in pH in higher pH cheeses exceeded that in low pH cheeses by 0.36 and 0.53 units; after 8 months the difference was 0.38 and 0.48 units in experiments I and II respectively. Generally the pH of all cheese increased during the ripening period although the amount of increase was not equal. The change in pH was in low pH cheese less than that in high pH cheese.

The difference in moisture content of the cheeses with different pH, when it was analysed for the first time after 2 month's storage, was less than 1.5% in both experiments. This difference did not disappear completely with progressive ripening, but in most cheeses it decreased to lower values.

The salt content of low pH cheese was slightly higher than that of high pH cheese, although all the cheeses were brined for equal periods.

Protein decomposition: it was noticed that cheese of high pH contained more protein breakdown products than the other cheese. In all cheeses, the increase during the ripening period in the soluble nitrogen and in total amino acid nitrogen was more remarkable with high pH cheese. In the three types of different pH cheese the difference in soluble nitrogen content after 2 months maturation was not so great. Calculated as a percentage of the total nitrogen, values for low, middle and high pH cheese in the first experiment were 28.14, 28.38 and 29.04%. The corresponding values in the second experiment were 24.94, 25.70 and 26.16%. The same observation was made in the total amino acid nitrogen (table VII). In the second period of ripening, namely after 5 months, both soluble and total amino acid nitrogen showed a considerable increase with pH. It averaged 7.06, 8.90 and 11.06% for soluble nitrogen and 3.86, 4.76 and 5.53% for the total amino acid nitrogen of cheeses with low, middle and high pH respectively. After 8 months' storage although the values of soluble and total amino acid nitrogen did not increase at the same rate as in the previous period, the differences between cheeses of different pH were still considerably wide. At this period, the soluble nitrogen content of cheese of high pH exceeded that of low pH cheese by 4.83 & 6.47%, while the corresponding difference in the total amino acid

nitrogen was 1.46 & 3.77% in both experiments; all results were calculated as a percentage of the total nitrogen.

3.2.2. *Amino acid content*

Studying the data in table VIII, one can generally notice no apparent principal difference in the three series of cheeses. The amino acid pattern of low, middle and high pH cheese was almost the same. Figure 11 shows the form of the elution curves and the relative position, on the chromatogram, of the different known amino acids and the unknown components. This is for the low and high pH cheese of the first experiment after 5 months ripening. The sample put to the column was equivalent to 80 mg cheese.

It can be seen in the same table that the amino acid content of the first experiment was generally higher than that of the second experiment in both young and ripe cheese. This is in accordance with the results of the total amino acid nitrogen. Although the differences showed a similar trend in both experiments, the degree of variation was much greater in the first experiment than in the second.

The first analysis of this type of cheese was made after 2 months of ripening. Almost all the amino acids of casein were present in the free state in the three types of the cheese. It was previously mentioned that at this age histidine and serine were absent in high pH cheese, proline in low pH cheese and cysteic acid and arginine in both. Comparison of the concentrations of the amino acids in the three types of cheese at this age, showed that most of the amino acids, amines and ammonia were present in higher concentration in cheese with high pH than

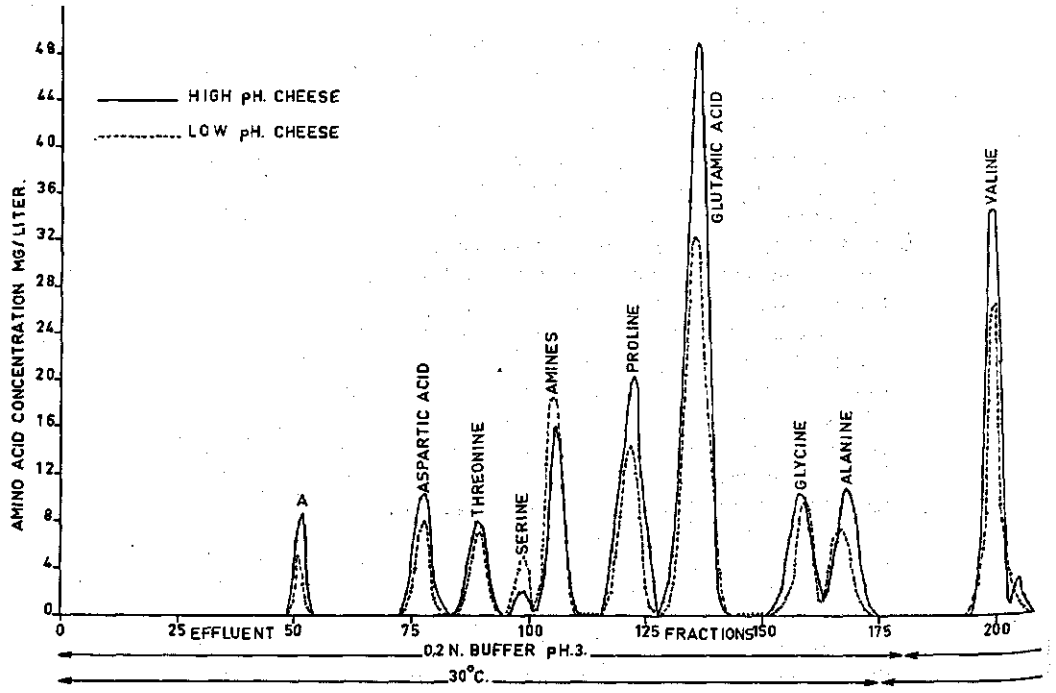
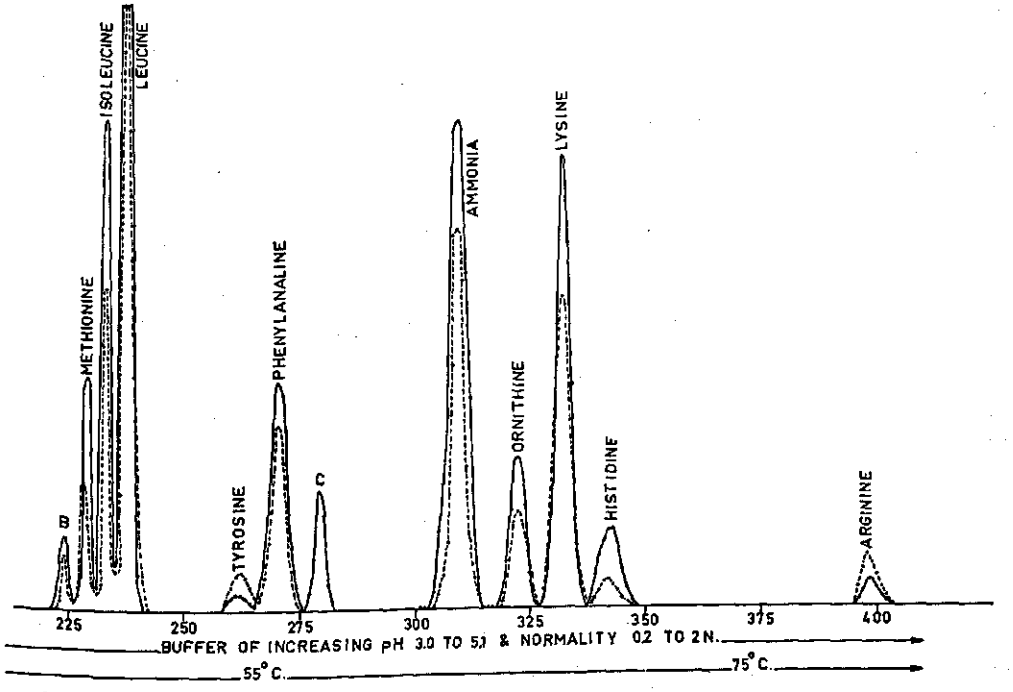


FIG. 11. Separation of free amino acids in high and low pH Edam cheese (5 months old) on an ion exchange

in low pH cheese. However, the differences in aspartic acid and tyrosine were markedly lower. Also the concentration of amino acids in middle pH cheese did not always lie between the values of low and high pH cheese at this age. This may be attributed either to differences in utilization of amino acids by the different bacteria found in the cheese in the first period of ripening under various conditions of pH, or to the different rate of liberation of these amino acids in low and high pH cheese, or to both.

At later stages of the cheese ripening, the difference in the concentrations of all amino acids, due to the difference in the pH of the cheese, were considerably great. Cheese with high pH contained larger quantities while acid cheese contained lower concentrations, with the exception of serine and arginine. These two amino acids were present in higher concentrations in cheese with low pH, while low concentrations or their complete absence was reported for high pH cheese. That low pH cheeses contain more serine than high pH cheeses may perhaps be interpreted through the findings of KRISTOFFERSEN & NELSON (54). They found that the rate of serine deamination by L. casei was affected by the pH of the media. As for arginine, previous findings of VIRTANEN *et al* (109) confirms the present results. They reported that arginine was decomposed in Emmenthal cheese (a cheese of high pH) at approximately the same rate as was casein. On the other hand arginine was found to increase in Cheddar cheese (a cheese of low pH) (49; 58).

The amino acids in cheese were not affected to the same degree by change in pH of cheese. Threonine, tyrosine and phenylalanine were less affected than other amino acids. The differences in the concentrations of these amino acids in



column of a mixture of Dowex 50 X4 and X5.

low, middle and high pH cheese were not very great. On the contrary, the proline content of the cheese seems to be closely related to its pH. Acid cheese contained low concentrations of proline, whereas high pH cheese was rich in proline. In one experiment the proline was still absent in the cheese of 5 months old. The pH of this cheese was 4.97 – markedly low for such age. The corresponding cheese of high pH contained 661 mg proline per 100 g protein. The pH of this cheese was 5.5 units. These results may explain the late appearance of proline in Cheddar cheese as reported by SILVERMAN & KOSIKOWISKI (87) and the high concentration of proline in Emmenthal cheese as found by several workers (41, 50, 108; 109).

After eight months' storage of the three types of different pH cheese, all the amino acid values showed a rise with the exception of methionine, arginine ornithine and histidine. It seems that the decrease in the concentrations of these four amino acids in ripe cheese is more characteristic of cheese with high pH. These amino acids with the exception of ornithine showed a decrease only in high pH cheese. As a high pH of the cheese favours propionic acid fermentation, some or perhaps all of these acids were decomposed by the propionic acid bacteria (2). Yet the decrease in the amino acids was previously reported in Emmenthal cheese after 180 days' ripening (48).

3.2.3. *Flavour of the cheeses*

The strong broth-like taste due to more soluble substances including amino acids was more pronounced in high pH cheese. On the other hand, the typical sharp cheese flavour due mainly to the presence of volatile fatty acids was generally more predominant in low pH cheese and appeared earlier than in cheese of higher pH. Middle pH cheese had a more balanced flavour, containing as they do considerable amounts of the products of protein breakdown and fat hydrolysis.

Generally bitterness was more frequent in cheese with high pH and was only sometimes very faint in low pH cheese. Also sweetness was detected only in high pH cheese. Undesirable flavours, due to abnormal fermentations in the cheese, occurred only in middle and high pH cheese especially at 5 months of age. Hydrogen sulphide was easily tasted in this cheese. It disappeared with progressive ripening and the taste of the cheese improved.

Although the amino acids contribute to the flavour of the cheese, the difference in flavour of low and high pH cheese cannot be attributed to any amino acid. Some amino acids, glutamic acid and leucines in particular, give to the cheese a broth-like, slightly bitter taste which is mollified in acid cheese by the penetrating flavour of fatty acids. In cheese with high pH, the lower concentration of fatty acids due to less fat hydrolysis, made the taste of amino acids, the broth-like in particular, predominant. Sweetness, frequently tasted in high pH cheese may be due to propionates, perhaps together with the accumulated sweet amino acids particularly proline which was found in high concentrations in this cheese.

3.3. EFFECT OF PASTEURISATION

Experiments for the use of pasteurised milk in the manufacture of cheese in different countries began about 1900 (105). Difficulties in manufacture due to

alterations in the physical and chemical properties of the milk were overcome by the addition of calcium chloride and by modifications in the cheese making process. After 1925 the percentage of the Netherlands cheeses made from pasteurised milk began gradually to increase (11). Though the resultant cheese was known to be inferior in quality to that of raw milk cheese, particularly in taste and flavour, the replacement of raw by pasteurised milk occurred because of sanitary, economical and technical reasons.

Research on the problem of cheese making from pasteurised milk was conducted almost from the beginning of this century. Studies were made on the changes in protein breakdown due to the use of pasteurised milk. In Edam cheese VAN DAM (104) observed that in cheese made from pasteurised milk, the protein broke down somewhat more slowly. He also found that pasteurised milk cheese was characterised by a low proportion of total amino acid nitrogen; its amount decreased with the increase in pasteurisation temperature. He added that the flavour of the pasteurised milk cheese was rather flat while that of raw milk cheese was more intense and desirable. Recently, RAADSVELD (72) found the nitrogen distribution to be almost the same after four weeks of ripening. After longer storage periods, the soluble nitrogen content of raw milk cheese was somewhat lower than in pasteurised milk cheese. In Cheddar cheese, LANE & HAMMER (55) pointed out that the formation of water soluble protein derivatives was more rapid and extensive in cheese made from raw milk. This was confirmed by the findings of SHERWOOD (82). CALL & PRICE (14) reported that the extent of protein breakdown decreased with the increasing severity of heat treatment.

Not very much work has been done regarding milk pasteurisation as a factor that may influence the amino acid composition of cheese. The use of cheeses from raw and pasteurised milk in the study of the amino acid pattern of Edam cheese, gave us an idea of the influence of this process on the concentration of the separate amino acids in the cheese and their probable effect on cheese flavour.

3.3.1. *Properties of the cheese*

Before brining, the pH of the pasteurised milk cheese was slightly higher than that of raw milk cheese. This may be due to the larger amounts of water used in diluting the whey in making the pasteurised milk cheese. This increase was constant until the cheese was 1.5 months old. The rate with which the pH of the raw milk cheese increased was faster. At 4.5 months, it was the same as that of low temperature pasteurised milk cheese, but after eight and half months, the average exceeded by 0.07 units. The pH of cheese from milk pasteurised at 95 °C was still the highest. The pH of the cheese in the two experiments, and particularly in the case of raw and low temperature pasteurised milk cheese did not increase with the same intensity. Generally the increase in pH was much greater in the case of raw and high temperature pasteurised milk cheese during the whole period of ripening than in that of low temperature pasteurised milk cheese.

There was a considerable difference in the moisture content of cheeses of 1.5 months old, the pasteurised milk cheese being on the higher side. This difference slightly decreased after 4.5 months of storage, while it disappeared almost completely after 8.5 months. This may be due to the retention of more moisture by the curd of pasteurised milk cheese. The loss in moisture content of raw milk cheese in the period from 1.5 to 8.5 months of ripening averaged 7.13% while the corresponding losses in 72 & 95°C pasteurised milk cheese were 9.13

& 9.15% respectively. The high moisture content of 95°C treated milk cheese at 1.5 months age, together with the high pH at this age, may have stimulated further protein breakdown as shown by higher total amino acid content.

Protein breakdown: It is very striking that there is greatest breakdown in raw milk cheese in all periods of ripening. The soluble nitrogen content (Table IX) of raw milk cheese was markedly higher than that of the corresponding pasteurised milk cheese. These values, when calculated as a percentage of the total nitrogen, showed the same trend. Again the soluble nitrogen decreased with the increase in the pasteurisation temperature. Soluble nitrogen was consistently higher in 72°C pasteurised milk than in the high temperature pasteurised milk cheese. This is in accordance with previous findings of VAN DAM (104) and CALL & PRICE (14). On the other hand, the total amino acid nitrogen did not follow these trends. There were no great differences in the various young cheeses of 1.5 months old. With high temperature pasteurised milk cheese, the total amino acid nitrogen content was slightly higher at this age. After 4.5 months of storage, at 15°C, the amino acid nitrogen content of raw milk cheese exceeded that of both types of pasteurised milk cheese. The difference became larger after 8.5 months of ripening. There was no definite trend in the total amino acid nitrogen content of low and high temperature pasteurised milk cheese.

An explanation of the difference in the protein breakdown in raw and pasteurised milk cheese may be given by the work of STADHOUDERS (91). He found that milk proteases, rennet enzymes and species of *Pseudomonas* and *Flavobacterium* increased the soluble nitrogen but produce no amino acids in Netherlands cheese. The *streptococci* of the starter and the *enterococci* increased the amino acid nitrogen of the cheese, considerably; the *Beta cocci* and the *lactobacilli* also, but to a much lower extent. Since the *enterococci* survive pasteurisation temperatures (35), they had with the starter streptococci the same influence on pasteurised milk cheese. The high total amino acid nitrogen content of 95°C pasteurised milk cheese cannot be explained from these findings. However, high temperature heated milk is known to be more favourable for producing higher numbers of streptococci (23, 27).

The salt/water ratio of all cheeses was almost the same. In raw milk cheese it was slightly higher in young cheese, whereas in 95°C pasteurised milk cheese it was higher in ripe cheese.

The acidity of fat as determined in some cheeses, was much higher in raw milk than in pasteurised milk cheese, indicating more fat hydrolysis in the former cheese than in the latter. Raising the temperature of pasteurisation up to 95°C had a pronounced effect, the fat being less hydrolysed at the higher temperature of milk pasteurisation. Up to 4.5 months of age, there was almost no fat hydrolysis in high temperature pasteurised milk cheese. Low figures indicating slight fat hydrolysis, in this cheese at 8.5 months of age, may be due to the effect of the surface microflora of the cheese or to bacterial thermoresistant lipases. Present results agree with the previous findings in this laboratory (65, 93).

3.3.2. *Animo acids*

Peaks representing the amino acids, amines and ammonia of raw and both types of pasteurised milk cheese were almost the same after 1.5 months ripening when the cheese was first analysed. About 19 peaks were found. No further

increase in the number of the amino acids was noted till the cheese was 8.5 months old. This is confirmed by previous results with Cheddar cheese (49).

A quantitative comparison of the amino acid content of raw and pasteurised milk cheeses shows that the amino acids were not present in the same concentration at a given age. A general trend could be observed for some amino acids including glutamic acid, alanine, valine, isoleucine, phenylalanine, threonine, leucine, ornithine and amines. They were found in higher concentrations in 95°C pasteurised milk cheese at 1.5 months of age, while in the next periods, namely at 4.5 and 8.5 months' ripening, the concentration was higher in raw milk cheese. However, it is possible that at a certain age, a certain amino acid of this group may have not followed the same trend. Also the pattern of variation between low and high temperature pasteurised milk cheese was not constant at 4.5 and 8.5 months of ripening.

It could be noticed from table X that some amino acids were present in higher concentrations in pasteurised milk cheese. They seem to be characteristic of these cheese. Glycine in the first place, was found in much higher concentration in all cheese of 95°C pasteurised milk cheese than in both low temperature pasteurised milk and raw milk cheese. It was reported by KRISTOFFERSEN *et al* (53) that Cheddar cheese made from commercial pasteurised milk contained more than ten times the glycine concentration in raw milk cheese. It should be noted that commercial milk pasteurisation is always carried out at temperatures higher than 72°C. High levels of histidine and arginine were also noticed in both types of pasteurised milk cheese, whereas low concentrations or complete absence of these acids were noted for raw milk cheese. Lysine was also more concentrated in pasteurised milk cheese, although the differences were not great. Tyrosine was demonstrated in larger amounts in pasteurised milk cheese. However, the free tyrosine in this cheese was still far less than in casein. Decarboxylation of tyrosine might have occurred in the three types of cheese, being greater in raw milk cheese.

Some of the present results were previously reported in the literature. Low levels of histidine and tyrosine in raw milk Cheddar cheese and high level in the pasteurised were noticed by KRISTOFFERSEN *et al* (53).

These observations cannot be fully explained. Though tyrosine decarboxylase was found to be heat resistant (75), its activity will be destroyed at such a temperature as 95°C. Five bacterial amino acid decarboxylases other than tyrosine decarboxylase were noted by RAIBAUD *et al* (75). They include the decarboxylases of histidine, lysine, arginine, glutamic acid and ornithine. Higher values reported in this study for the first three amino acids in pasteurised milk cheese may be due to the destruction of these enzymes by the pasteurisation temperature.

Also, the isolation of amino acid decarboxylases from bacteria which are known to be common in raw milk, is reported in the literature. SHER & MALLETTE (83) were able to prepare lysine and arginine decarboxylases from *Escherichia coli*. This microorganism does not survive pasteurisation (35a). It was also found to contain l-cysteine desulfhydrase (60). The presence of cysteic acid in both low and high temperature pasteurised milk cheese and its absence in raw milk cheese up to the age of 4.5 months may be attributed to this enzyme. Also the *lactobacilli* were found to possess histidine and arginine decarboxylases (78).

Other amino acids did not show a regular trend. They were sometimes higher in pasteurised milk cheese and somtimes higher in raw milk cheese. Proline

for example, seems not to be affected by milk pasteurisation. Its concentration in the cheese was more dependent on the pH of the cheese.

The high aspartic acid content of raw milk cheese reported in the literature (13, 49; 84) is not in complete agreement with the present results. Only raw milk cheese of 4.5 months contained markedly higher quantities of aspartic acid. This trend held true only with the ripe cheese and not with the young cheese. On the contrary serine was previously found in higher concentration in pasteurised than in raw milk Cheddar cheese (13, 84; 88). These observations are in full agreement with the present results of the first experiment. However, it held true in the young cheese of the second experiment. It is worthy to note that results of the second experiment showed abnormally higher concentrations of serine, if compared with the first. Since in both experiments, the cheeses were made and stored under similar conditions, it is possible that a difference in the bacteriological conditions, under which the milk of both experiments was produced, is responsible for these variations. It will be shown in section 3.5.2. that the microflora of the milk has a marked effect on the serine content of the cheese.

3.3.3. *Cheese flavour*

It could be seen from the organoleptic tests in table IX that young cheeses from pasteurised milk were characterised by a tough and a rubber-like consistency. This was more evident in 95 °C pasteurised milk cheese. This toughness began to disappear in low temperature pasteurised milk cheese at 4.5 months, while it still existed in high temperature pasteurised milk cheese even after 8.5 months ripening when the cheese had a hard rind.

Cheese flavour began to develop in raw milk cheese at 1.5 months, while after 4.5 months' ripening, a cheesy flavour occurred. Also a 'heavy' taste which was not noted in the pasteurised milk cheeses was present at this age. This heavy taste may be due to the presence of large amounts of hydrogen sulphide. Significance of this compound in the flavour of Netherlands cheese was discussed by MULDER (67). Recently KRISTOFFERSEN *et al* (53) suggested that the difference of the H_2S content of raw and pasteurised milk cheese is one of the factors responsible for their difference in flavour. However, we are of the opinion that the H_2S may assist in giving a stronger background to the taste of raw milk cheese.

Pasteurised milk cheese examined at 1.5 months were only curdy and tasteless while bitterness was predominant in all cheese. After 4.5 months storage, low temperature pasteurised milk cheese was slightly 'cheesy' with slight bitterness and less odour, while the high temperature pasteurised milk cheese still had flat flavour and left a bitterness after tasting. In ripe cheese of 8.5 months, many flavouring substances could be tasted in the raw milk cheese, giving the cheese a full bouquet cheesy taste. Low temperature pasteurised milk cheese was less cheesy and had less odour due to a lower concentration of fatty acids, while high temperature pasteurised milk cheese had not only little and almost no odour but also a pronounced bitterness.

Comparison between the amino acid concentration and the flavour of the cheese shows that there is no striking quantitative difference between the amino acid content of raw and pasteurised milk cheese that can be directly responsible for the completely different taste of both cheeses. Although the amino acid

content of raw milk cheese of 4.5 months was much lower than that of 8.5 months old 95 °C pasteurised milk cheese, the taste and flavour of the former was more cheesy and palatable than that of the latter. Bitter tasting amino acids may probably assist in the appearance of the bitter taste generally detected in pasteurised milk cheese. These amino acids are found in larger quantities in raw milk cheese and bitterness was less frequent. This may be explained by the presence of free fatty acids which mollify or completely nullify the bitter taste. These free fatty acids were found in raw milk cheese in much greater amounts as shown by the higher fat acidity reported here and in previous investigations in this laboratory. However, when large amounts of bitter peptones and other substances (73) are found in the cheese, the influence of fatty acids in nullifying bitterness is too small to counterbalance this defect in taste and the cheese is characterised by a pronounced bitterness.

There is still another factor that may mask the amino acids taste in pasteurised milk cheese. MULDER (67) observed that a tough consistency prevents the display of the full cheese bouquet and that the flavour forming substances can be better tasted in the cheese if it is previously teriturated.

From the foregoing observations, it may be concluded that the differences in the taste and flavour between raw and pasteurised milk cheese cannot be attributed to differences in the amino acid content of the cheese.

3.4. EFFECT OF THE MOISTURE CONTENT OF CHEESE

It is already known that the high moisture content of a cheese results in extensive protein breakdown. As early as 1903, VAN SLYKE & HART (107) concluded that a cheese of higher moisture content generally contains larger amounts of soluble nitrogen, especially after the early stages of ripening. Covering half the cheese as a method of avoiding dryness was done in view of this observation. SAMMIS & GERMAIN (79) pointed out that bacterial growth and chemical changes are rapid in cheese of high moisture content. FREEMAN & DAHLE (28) did not succeed in obtaining series of cheeses with considerable differences in moisture content. Recently, RAADSVELD (71) found that the soluble nitrogen content of Edam cheese was always higher in cheese with high moisture content.

By comparing the amino acid composition of cheese of high and low moisture content, we tried to form a preliminary idea of the influence of moisture content of the cheese on its contents of individual amino acids.

3.4.1. *Properties of the cheese*

The initial pH in high and low moisture content cheeses was almost the same. With progressive ripening, the increase in the pH of high moisture content cheese was more pronounced and the pH of this cheese became considerably higher than that of low moisture curd cheese. Protein cleavage products may have been partly responsible for this differences.

The moisture content of the cheese was not determined after manufacture. However, the difference between the water content of high and low moisture cheese at 3 months of age, when the cheese was first analysed was obviously great. It should not be forgotten that, during the first three months of ripening,

high moisture content cheese dry out more quickly than do low moisture cheeses (71).

The salt/water ratio in low moisture content cheese was still lower than that in higher moisture content cheese, when the former was brined for only 8 hours more than the latter. When this period was prolonged to 24 hours, the salt water ratio was higher in lower moisture content cheese. However, the difference was not sufficient to change the trend of protein breakdown.

Concerning protein degradation in this cheese, the data in table XI shows that the high moisture content cheese exhibited greater protein breakdown than the relatively drier curd cheese. The respective differences in soluble nitrogen of the first and second experiments, after three months were 4.04 & 4.69 % of the total nitrogen. This difference became greater with progressive ripening particularly at 6 months.

Also the total amino acid nitrogen was higher in high moisture content cheese, the difference being quantitatively less than in soluble nitrogen, especially in cheese of 3 months in the first experiment.

These results are in agreement with previous findings (72). Only the high salt concentration in high moisture cheese in RAADSVELDS' investigation (72) made the difference in the amino acid nitrogen due to differences in moisture probably less evident.

3.4.2. *Amino acids of the cheeses*

After 3 months of ripening, when the cheese was first analysed, all amino acids were present in both low and high moisture content cheeses with the exception of proline, histidine, serine and arginine which were absent in some cheeses. Comparison of the data in table XII showed that there was no constant trend in the difference between the amino acid content of low and high moisture content cheese at this age. In both experiments, only a few amino acids were definitely more concentrated in high than in low moisture content cheese. These were glutamic acid, leucine, lysine, methionine, ornithine and also the amines. With other amino acids the trend was not consistent. An amino acid might have been more concentrated in one experiment in high moisture content cheese and lower in the cheese of the other experiment. These variable trends in the first periods of ripening have already been discussed under other headings. It cannot be concluded that the low concentration of some amino acids in high moisture cheese at this period was due to less liberation of these amino acids; it is more probable that these amino acids were utilized to a greater extent by bacteria under favourable conditions of high moisture content during the early stages of ripening.

After 6 months storage, the amino acids were considerably greater in concentration in high than in low moisture content cheese with the exception of serine in all experiments, and valine in low moisture cheese of a higher salt/water ratio. That the amino acids were found in higher concentration in high than in low moisture cheese did not occur with the same intensity. Proline, glutamic acid, methionine, isoleucine, leucine, ornithine and also ammonia showed the widest differences. Other amino acids were affected to a somewhat lower extent.

After 9 months of ripening, the analysis of amino acids showed that there was still an increase in most of them in both types of cheese. However the decrease of some amino acids particularly in high moisture content cheese may be considered as evidence for a greater amino acid decomposition in this cheese when it is

ripe. In the high moisture content cheese of the first experiment, threonine, glycine, methionine, phenylalanine, lysine, ornithine and also amines showed a decrease. The pH of this cheese was extremely high (5.61 units). In the second experiment, a decrease was less frequent. It was observed in threonine, glycine, alanine, phenylalanine and ornithine. In low moisture content cheese of the same age, fewer amino acids showed a decrease. A decline in methionine and ornithine was observed in one cheese.

It can be seen from the organoleptic tests in table XI that high moisture content cheese had undergone propionic acid fermentation and was evident in 3 months old cheese. Propionic acid bacteria were found by ANTILA (2) to possess a decarboxylase system. However, several amino acids were found to decrease in ripe Emmenthal cheese at 180 days (48). This decrease of the amino acids may be due either to the blocking of synthesis while the amino acids were still being utilized or rather to the action of enzymes. Amino acid decarboxylases were reported in the literature for several amino acids as previously mentioned.

When the quantities of the amino acids in high and low moisture content cheese at 9 months of age were compared, it was found that the amounts of some amino acids were slightly lower in the former than in the latter. This was probably due to more intensive decomposition in high moisture cheese. Decomposition of amino acids occurred in both low and high moisture content cheeses as mentioned, but in high moisture cheese, the conditions were more favourable and there was greater decomposition.

It was surprising to observe, in the analysis of these cheeses, the absence of serine in a high moisture content cheese even at 9 months. Complete deamination of this amino acid in this cheese is probable.

3.4.3. *Flavour of the cheeses*

Low moisture content cheese was always firmer than high moisture cheese, the latter often being soft. In young cheese, the difference in consistency of the two types of cheese was very apparent. With progressive ripening, when the cheese lost moisture, the former cheese became hard and firmer while the high moisture cheese remained rather mellow.

Young high moisture cheese was characterised by a watery taste, and left bitterness after tasting. With prolonged storage, bitterness became more intense while a heavy taste due to large amounts of hydrogen sulphide was predominant. also sweetness was sometimes detected. With the exception of the bitter taste these defects disappeared in older cheese of 9 months and the cheese had a very sharp taste.

The above mentioned undesirable tastes due to abnormal fermentations in high moisture content cheese could not be detected to any appreciable extent in low moisture content cheese. The flavour of this cheese was more balanced as it contained considerable amounts of amino acids and volatile fatty acids; or perhaps its pure clean taste made the taste and odour of these compounds more apparent.

The presence of an amino acid cannot be correlated with the difference in flavour between high and low moisture content cheese. However the broth-like taste which was more pronounced in high moisture content cheese may be due to a higher amino acid content, glutamic acid and leucine contents in particular. Sweetness is rather to be related to the presence of propionates. Here, proline concentration was not very high in the cheese with a sweet taste.

3.5. Effect of microflora of milk

It is known that it is impossible to get milk from the udder of a cow free from bacteria. However, aseptically drawn milk usually contains only a low number of bacteria. Evans (24), Steck (95, 96), Breed (12), Dorner (21) and Graber (31) studied the flora of this milk. They found that the *staphylococci* are the most prevalent organism in aseptically drawn milk.

In this laboratory, Stadhouders & Mulder (94) studied the microorganisms involved in the hydrolysis of fat in the interior of the cheese. They came to a conclusion that the *micrococci* of the aseptically drawn milk are not fat-splitters, while species of *Serratiae*, *Pseudomonas* and the *Achromobacteriaceae* occurring in large numbers in infected milk are important in fat hydrolysis.

Because of the results gained by Stadhouders & Mulder (94) and because we obtained different amino acid concentrations in cheese produced under the same conditions of manufacture and storage, we aimed at comparing cheeses from aseptically drawn and from infected milk to form an idea whether the microflora of infected milk has an influence upon the amino acid content of the cheese.

As it was almost impossible to get enough aseptically drawn milk at one time to make more than two cheeses, two lots of aseptically drawn milk were used and the cheese of each lot was ripened for a different period. Composition of the cheese and the organoleptic examination are presented in table XIII while the amino acid content at two stages in the ripening period are shown in table XIV.

3.5.1. *Properties of the cheese*

The pH of the cheeses in both experiments was, before brining, practically equal. Though the milk used in the first and second experiment was from different lots, the pH of the aseptically drawn milk cheese changed more rapidly than did the pH of the infected milk cheese at 4 & 7.5 months of age. The accumulation of fatty acids as a result of fat hydrolysis which occurred intensively in infected milk cheese, might have counterbalanced the effect of the alkaline products of protein breakdown.

The difference in the moisture content of the two types of cheese was less than 1%, the infected milk having slightly more. Also the salt content of the infected milk cheese was slightly higher than that of the aseptically drawn milk cheese.

On the other hand, it was surprising to find that there exists almost no difference in the total amino acid nitrogen of both aseptically drawn milk and infected milk cheeses after 4 months of ripening. It may be suggested that under the conditions of this experiment, the microflora of the milk, mainly contaminants from milk utensils has, up to the age of 4 months, no effect on amino acid production. Although it is dangerous to make such a suggestion from the results of one experiment, Stadhouders (91) found that *Pseudomonas* and *Flavobacterium* of the raw milk microflora, though strongly proteolytic, caused no amino acid increase when added to pasteurised milk. However, after storage for 7.5 months at 15°C, the total amino acid nitrogen content of infected milk cheese was markedly higher than that of aseptically drawn milk cheese, the difference being about 2% of the total nitrogen.

Fat hydrolysis occurred to a far greater extent in the infected milk cheese than in the aseptically drawn milk cheese. Expressed as the concentration of

free fatty acids in the cheese, there occurred 4 times as much fat hydrolysis in the infected milk cheese at 4 months. At the age of 7.5 months, the concentration of free fatty acids in infected milk cheese was about 6 times as much as in the aseptically drawn milk cheese. The small acidity that occurred in the latter, may be due to the effect of milk lipase, since the microflora of this aseptically drawn milk is known to be non lipolytic (94). These results are in agreement with previous results reported by STADHOUDERS & MULDER (94) from this laboratory. They found that strains from the bacteria found in milk utensils are important in fat hydrolysis.

3.5.2. *Amino acids and milk microflora*

Although quantitatively the total amino acid nitrogen of aseptically drawn milk cheese was almost equal to that of infected milk cheese of 4 months, yet there was some difference in the amino acid composition of the two types of cheese. As individuals, some amino acids differed in concentration.

Generally, most of the amino acids were slightly higher in concentration in the infected milk cheese probably as a result of deeper proteolysis. On the other hand, some amino acids were surprisingly much higher in aseptically drawn milk cheese and two others were extremely low. The concentration of aspartic acid in aseptically drawn milk cheese was less than half that in the infected milk cheese while the amines content of the former was much higher than that of the latter which may indicate more asparagine content in aseptically drawn milk cheese. Also the occurrence of arginine in a considerable quantity in aseptically drawn milk cheese and its absence in infected milk cheese, together with the presence of about three times as much ornithine concentration in infected milk cheese may be interpreted as due to complete transformation of arginine to ornithine in infected milk cheese; with hygienic milk cheese, there was only slight conversion of arginine to ornithine. Moreover, the presence of tyrosine in aseptically drawn milk cheese and its absence in infected milk cheese may indicate that dicarboxylation of the liberated tyrosine was less intense in the former than in the latter. Other amino acids that were more concentrated in aseptically drawn milk cheese include cysteic acid, glycine, serine, and histidine. Lower contents of these amino acids, reported in infected milk cheese may be due either to their greater utilization in the first stages of ripening by the high bacterial population or to their decomposition by one or more biological reactions.

At 7.5 months in the course of ripening most of the amino acid contents were markedly higher in infected milk cheese. Almost the same variations as observed in 4 months old cheese still existed at 7.5 months. However, the greater liberation of amino acids in infected milk cheese together with the inhibition of their decomposition, might have caused the rise of most amino acids in infected milk cheese at the age of 7.5 months. Cysteic acid, serine and glycine concentrations were no more higher in aseptically drawn milk cheese at this age. Also the low content of ornithine in infected milk cheese at this age may be due to further decomposition.

As both aseptically drawn and infected milk cheeses were made in such a way as to have the most similarity in composition, the only possible cause of the differences in the concentrations of the mentioned amino acids under similar conditions of ripening can be the microflora of the milk.

Results obtained here reveal that the differences in the microflora of milk

result in a difference in the quantitative amounts of some of the free amino acids of Edam cheese. Further studies may be necessary for the better understanding of the nature and origin of these characteristic differences.

3.5.3. *Milk microflora and cheese flavour*

A comparison of the data from aseptically drawn and infected milk cheeses shows that the differences in flavour of the cheeses cannot be related to differences in their amino acid contents. The amino acids that were found in a greater or lower concentration in aseptically drawn milk cheese cannot change the flat insipid flavour of this cheese to the full bouquet cheesy flavour of the infected milk cheese. Although the 7.5 months ripened hygienic milk cheese contained a higher concentration of amino acids than the 4 months ripened infected milk cheese, the flavour of the latter was more piquant and cheesy than the former.

It is worthy to note that the consistency of the aseptically drawn milk cheeses was more tough than mellow. Though the cheese contained a considerable quantity of amino acids, the taste of the cheese was flat. Taste forming substances including the amino acids may have been bound up in the texture of the cheese and so could not easily come into contact with the taste organs. This was evident from the fact that the taste of the amino acids, particulary the broth-like taste, was more apparent if the cheese was teriturated.

No special amino acid can be responsible for the difference in taste between these types of cheese. However, amino acid decomposition products that occurred undoubtedly in infected milk cheese may contribute to the full flavour of this cheese. On the other hand, the presence of considerable amounts of volatile fatty acids in infected milk cheese, due to lipolysis (almost absent in aseptically drawn milk cheese) is probably the most important factor that can be related to the differences in the flavour of the cheeses.

4. GENERAL DISCUSSION AND CONCLUSIONS

4.1 Free amino acids in matured cheese

4.1.1. *General amino acid pattern of Edam cheese*

The results of this study have shown that almost all the amino acids occuring in casein and previously stated in the literature to be found in the free state in cheese are found in Edam cheese. In some cases, particularly in very young cheese, not all the amino acids of casein were found in the free state. Some showed late appearance while some others may disappear after a short time.

In ripe Edam cheese the amino acid pattern was, in a general way, almost the same in our experiments. This made it possible to calculate an average (table XVI) for the amino acid content of this cheese. For calculations, we used the analytical results of 20 ripe cheeses of the same age (7.5 to 9 months). These cheeses were intentionally made with different properties. The preparation and the final contents of this table lead to the conclusion which could of course be expected that the amino acids in the free state in Edam cheese are not found in the same concentration.

Amino acids that are always found in relatively higher concentration (more

TABLE XVI. Difference between the free amino acids content in ripe Edam cheese and in casein hydrolysates

Free amino acid content in cheese mg/100 g protein			Amino acid % in cheese	Amino acid % in casein hydrolysates	
Amino acid	Range	Average		Average from literature	Present results
Cysteic acid	000–195	37	0.28	0.00	0.50
Aspartic acid	175–832	329	2.53	6.65	7.24
Threonine	314–610	459	3.53	4.44	4.31
Serine	000–684	333	2.48	6.40	5.63
Proline	627–2239	982	7.55	12.65	11.51
Glutamic acid	1954–4442	2698	20.75	23.69	21.78
Glycine	246–892	652	5.06	2.17	2.19
Alanine	240–731	523	4.02	3.23	4.21
Valine	853–1585	1223	9.40	7.20	5.03
Cystine	000–8	00	0.00	0.40	0.00
Methionine	411–725	545	4.19	3.36	1.93
Isoleucine	454–1163	657	5.05	6.72	4.02
Leucine	1185–1971	1671	12.85	10.22	8.66
Tyrosine	55–330	205	1.57	6.27	6.60
Phenylalanine	588–971	745	5.73	5.75	6.01
Lysine	721–1756	1268	9.73	8.29	7.80
Histidine	121–455	298	2.29	3.01	2.28
Tryptophane	000–37	2	0.01	1.52	0.00
Arginine	000–360	75	0.58	4.10	3.65
Ornithine	127–622	285	2.19	0.00	0.00
Amines	557–1027	830	–	–	–
Ammonia	912–3542	1787	–	–	–

than 1000 mg/100 g protein) are glutamic acid, leucine, lysine, valine and proline. Amino acids present at a concentration of about 500 to 1000 mg/100 g protein include phenylalanine, isoleucine, glycine, alanine, and methionine. The last group of amino acids generally present in a concentration less than 500 mg/100 g protein are threonine, aspartic acid, histidine, serine, ornithine, tyrosine, arginine and cysteic acid.

Tryptophane and cystine were rarely found to be present. Since very low recoveries of tryptophane were sometimes obtained by the methods used in the analysis, it could not be concluded from the observations that tryptophane is not a component of the cheese extract. Though it was always reported absent in the literature, (50, 69, 89), yet LINDQVIST *et al* (56) usually found it present in some varieties of cheese including a cheese bearing the name Edam. It is suggested that tryptophane should be estimated by a specific chemical reaction.

Cystine, known to form a small part of the casein molecule (table XVI), was not found in the free state in the cheeses analysed. Its absence may be due either to methods of analysis or to its decomposition. Decomposition of cystine was previously reported (11). Its decomposition product, cysteic acid was sometimes found in small quantities. Relatively higher concentrations reported in the literature for this acid are probably due to some positive ninhydrin substances that are eluted with the cysteic acid peak. These substances form about 4 to 6% of the total ninhydrin positive substances in the cheese. Its concentration increased with the age of the cheese. This was found true for phosphotungstic acid filterates of the cheese extract. As these substances were completely

precipitated by alcohol in preparing our sample for chromatographic analysis (described in section 2.2), it could never be considered to be cysteic acid. MABBIT (58) previously stated that to account for the presence of such a quantity of cysteic acid in the cheese, the whole of the cystine of the casein would have to be oxidized.

4.1.2. *Comparison of the amino acid composition of cheese and casein*

To compare the amino acid composition of Edam cheese with that of casein, we made use of the data published in the literature on the composition of casein. There is a considerable difference in the values for the amino acids content of casein from one investigation to the other, and a comparison of one of these analysis with the composition of casein may result in a high degree of error; it was found advisable, therefore to calculate an average of the different published data on the amino acid composition of cows' milk casein. An average of 28 recent analyses in the literature reviewed by BLOCK & WEISS (9) is given in table XVI.

Furthermore, we hydrolysed a sample of casein (prepared in this laboratory) with 6 N HCl for 5 hours and determined the amino acids in the hydrolysate by the new method of MOORE & STEIN 1958 (66). Results obtained (same table) were then compared with the above average. An agreement in the percentages of some amino acids and a more or less important difference in that of others can be noted. As our hydrolysis lasted only for a short time as compared with that used in the literature, it is probable that the low figures reported for some amino acids in our analysis were due to incomplete hydrolysis rather than to destruction. It is well recognised that the rate of hydrolysis of some peptide links is faster with some than with others (8). Some linkages require prolonged hydrolysis (36, 42), while again others are easily broken (2, 26). When we increased the time of hydrolysis, to 10 hours, we noticed a considerable increase in histidine, arginine, valine, methionine, leucine and lysine, with a marked decrease in tyrosine and phenylalanine and to a smaller extent aspartic acid. Further experiments on the influence of the conditions of hydrolysis on the amino acid content of casein hydrolysates are still under research in this laboratory.

Comparison of the average amino acid composition of casein with the average amino acid composition of ripe Edam cheese (figure 12) shows that while at the first sight, the percentages are almost the same, there are, however, interesting differences.

Nearly all the amino acids of the casein are found in the free state in Edam cheese. They are not necessarily found in the free state in cheese relative to their occurrence in casein. This was previously reported, and examples could be found in the papers of HARPER & LONG (38) and STORGARDS & LINDQVIST (98).

The concentration of a number of amino acids are to a certain extent related to their concentration in the milk casein. Glutamic acid, lysine, phenylalanine, methionine, histidine, isoleucine and threonine are present in almost the same amounts in the free state in the cheese and in casein. Such a relationship does not hold true for all amino acids. Some are found in considerable quantities, forming together a high percentage of casein; yet they either form a small portion of the amino acid content of the cheese or even show complete absence. Serine, tyrosine, aspartic acid, arginine and proline contents decrease considerably in the cheese extract while the decrease in the concentration of other amino

acids is much lower. A few amino acids are, on the contrary, present in relatively smaller amounts in casein than in cheese. These include glycine especially and alanine, valine and leucine to a smaller extent.

It is worthwhile to note that the relative proportions of some amino acids are affected by different properties of the cheese. Proline and histidine, for instance, have a relatively higher percentage in cheese with high pH and high moisture content, whereas serine is lower. Also, arginine, histidine, and glycine are relatively higher in both pasteurised and aseptically drawn milk cheeses. Also, the quoted concentration of amino acids in Edam cheese (table XVI) are more liable to variations in very young cheese probably due to differences in the utilization of the various amino acids by bacteria. It should not be forgotten that the relatively low concentration of some amino acids in the cheese extract may result in an increase in the concentration of others. Lower values for some amino acids are possible because of decarboxylation and deamination systems. However the formation of amino acids from sodium citrate by certain cultures (6) and by ground emulsified cheese from radioactive citric acid (37) may perhaps explain the relatively high percentages of some amino acids in the cheese.

The presence of all the amino acids of the casein in the free state in the cheese, may be explained by the complete breakdown of the casein molecule or by the supposition that the amino acids have had the same chance to be liberated. Yet the difference in the concentration of amino acids in casein and in cheese gives rise to the question of whether the difference is due to the decomposition of

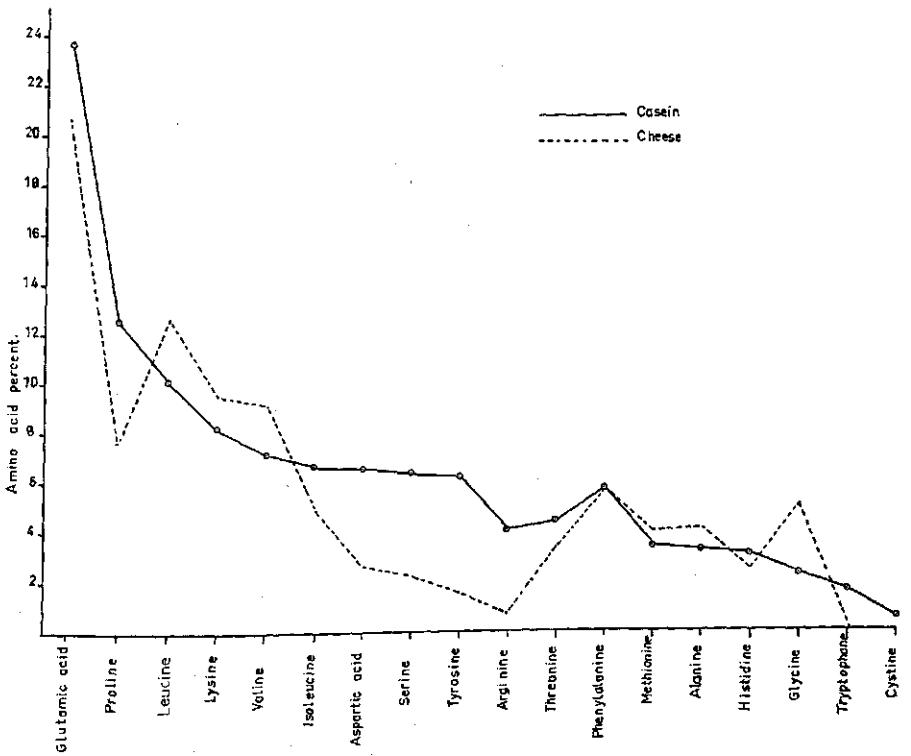


FIG. 12. Comparison between the free amino acids in Edam cheese and in casein hydrolysates.

these amino acids by different biological agents or to differences in the rate of liberation of different amino acids from the casein molecule.

Amino acids that showed a considerable decrease are, as mentioned before, aspartic acid, tyrosine, arginine and serine. As the decomposition products of the first three amino acids are found in the cheese, according to the results of this study and previous others, and as serine has a special significance in bacterial metabolism, it is rather probable that some of the liberated amino acids are partially or completely transformed during cheese ripening.

4.1.3. *Amino acids and cheese maturation*

It was previously mentioned that as early as the second day of manufacture some amino acids were detected in Edam cheese. Results in this investigation may give an explanation of the observation mentioned in the literature on the liberation of lysine and arginine. These two acids were previously determined together (18, 49). As BULLOCK & IRVINE (13) detected arginine only after 60 days storage and lysine could be detected early in ripening, they attributed the early appearance of this basic amino acid group to the presence of lysine. LING (57) found it interesting to investigate at what stage in the ripening process, these amino acids are set free, since it is claimed that the terminal amino acids in α & β casein are arginine and lysine. Mind full of the results of BULLOCK & IRVINE (13) and that the more probable NH-P link in α-casein is phospho-arginine, he concluded that the arginine region of the casein molecule may be resistant to attack by hydrolytic agents in cheese. In this study, ornithine, the precursor of arginine (88) was detected as early in ripening as lysine and therefore, it is suggested that both amino acids may be set free at almost the same stage of ripening with the difference that arginine is decomposed in the cheese at approximately the same rate as casein is split. This decomposition was previously noted in other types of cheese (47, 99; 109)

Under all conditions studied, all the free amino acids in Edam cheese were found to increase in concentration with progressive ripening. Most of them reached high concentrations at the end of the ripening period if compared with their concentration in young cheese. However, a few others, forming together a considerable part of the casein molecule, remain at a low level or may even disappear.

The decrease of the amino acid concentration at the end of the ripening period was found to vary under the different conditions. Factors that were found to encourage amino acid liberation in a cheese encouraged the decomposition of the amino acids in late stages of ripening. The decrease was more frequent in cheese with high pH, with high moisture content and raw milk cheese than in cheese with low pH, low moisture content, made from pasteurised milk and from aseptically drawn milk. Low levels of some amino acids and differences in trends during the ripening may be interpreted by making use of the 'biochemical metabolic pool' concept. Synthesis and catabolism of amino acids in the cheese may be dependent on the cheese microflora which is constantly changing from the moment of manufacture until the cheese is ripe.

Comparison of the amino acid content, of the cheeses produced under the different conditions studied reveals the following;

1. The occurrence of greater amounts of amino acids in raw milk cheeses, high pH cheese, and high moisture content cheese, than in cheeses with low

pH, low moisture content, from pasteurised and from aseptically drawn milk. This indicates that the former cheeses were subjected to deeper protein breakdown.

2. The higher serine content in low pH cheese, low moisture content cheese, cheese from pasteurised milk and from aseptically drawn milk. Relatively low levels of serine in all cheeses. These 2 facts may show that serine is utilized by most types of bacteria, its concentration in the cheese being related to bacterial counts of the cheese.

3. The presence of cysteic acid in both aseptically drawn milk and pasteurised milk cheese together with its absence in raw and infected milk cheese. These suggest the decomposition of this amino acid by the microflora of milk.

4. Low aspartic acid content of both aseptically drawn and pasteurised milk cheese. This can be attributed only to the capacity of the starter organisms to catabolise this amino acid by decarboxylation or some other biological reaction especially because the amount found in the cheese was always far lower than its concentration in the casein. On the other hand, the presence of larger quantities of this amino acid in raw and in infected milk cheese suggests the presence of a bacterial enzyme that may convert asparagine or some amino acid to aspartic acid. The lower amine content of raw and infected milk cheese may confirm the suggestion. Moreover, aspartic acid was found to be one of the most actively transaminated among 22 amino acids studied (7).

5. High levels of glycine and histidine in aseptically drawn and pasteurised milk cheese. This may be attributed to decomposition of these two amino acids in raw and in infected milk cheese.

6. Low levels or the complete absence of arginine in raw and in infected milk cheese, with higher concentrations in pasteurised and aseptically drawn milk cheese. This suggests that the milk microflora is one of the agents responsible for arginine decomposition. However, the decrease of this amino acid with advancing age, particularly in aseptically drawn milk cheese suggests that the streptococci of the starter form a second agent causing this decomposition. Also high levels of ornithine found always in cheese containing low arginine content may be an indication of the decomposition of arginine to ornithine in the cheese.

It could be suggested from the above items that the milk, as it is drawn from the cow, contains no agents that can be responsible for important changes in the amino acids in the cheese. The microflora of milk especially those microorganisms which are contaminants from milk utensils, and the starter streptococci should be considered responsible for differences in the amino acid content of cheese, just as was found for fat hydrolysis in this laboratory.

The differences in the amino acid content of cheeses with low and high pH, low and high moisture content, from raw and pasteurised milk and from aseptically drawn & infected milk cannot be explained by differences in proteolytic activity. It is partly due to the subsequent utilization of certain amino acids by microorganisms and to their decomposition due to the action of bacterial enzymes. An investigation under conditions occurring in cheese into the reaction on different amino acids of the microflora of both raw and pasteurised milk and of the starter streptococci would be important for the better understanding of the origin of the variations in the amino acid contents of cheese.

4.2. AMINO ACIDS AND CHEESE FLAVOUR

Although the role of amino acids in cheese flavour has been one of the main considerations in cheese research, yet as was mentioned before, a conflict concerning their importance in imparting flavour to different types of cheese is still apparent. MULDER (67) tasted the amino acids of casein separately and added mixtures of these amino acids to very young tasteless cheeses. In connection with this simple experiment, he attributed the broth-like after taste of cheese to glutamic acid and aspartic acid. He mentioned that mature cheese often has a slightly sweet flavour although no propionic acid fermentation had not occurred. This sweet taste, as well as a slight bitter taste may possibly be caused by amino acids. MULDER (67) laid stress upon the contribution of amino acids only to the basic taste of cheese and not to the bouquet which is very important for high quality cheese. HARPER & SWANSON (39) found that the addition of amino acids to fresh curd Cheddar cheese gave flavours reminiscent of cheese. This was confirmed by MABBIT (58). A contrary experience was obtained by BAKER & NELSON (5) and DACRE (18). They concluded that no specific amino acid could be related to the development of the typical flavour of cheese. Also the typical flavour could not be reproduced by the addition of an equivalent amino acid mixture to fresh curd. VIRTANEN *et al* (108, 109) attributed the sweet taste of Emmenthal cheese to the presence of proline and propionates, whereas SILVERMAN & KOSIKOWISKI (86) were able to obtain an Emmenthal like flavour with a mixture of amino acids lacking proline. This sweetness was attributed either to the propionates or to proline content by KRETT *et al* (52). STORGARDS & HIETARANTA (97) stated the importance of glutamic acid and aspartic acid while ZEILINGER & OSWALD (113) and JAGER (45, 46) were not able to relate the amino acid contents in this cheese to its flavour. In other types of cheese. CLEMENS (16) found that the flavour of Dutch, Tilsit and Limburger cheese was not affected by any specific amino acid.

The importance of amino acids in cheese flavour depends to a great extent on their concentration in the cheese and on the properties of the cheese itself. Under high pH and high moisture conditions that promote greater protein degradation, the taste of the amino acids is sufficient to counterbalance the penetrating flavour of the volatile fatty acids, which are relatively low in concentration in such cheeses and a broth-like taste is the net result. When the influence of the free fatty acids on the flavour of the cheese is almost absent as in pasteurised and aseptically drawn milk cheese, where little fat hydrolysis has occurred and when almost no H_2S can be tasted, the taste of teriturated cheese is due mainly to the amino acids present. The broth-like taste of glutamic acid can easily be detected. Also, a bitter taste, which may be partly due to the bitter amino acids and partly due to other compounds (73) was frequently detected. However, the flavour of such cheese could not be considered only as a mixture of the sweet, bitter and broth-like tastes of amino acids, It is possible that amino acid decomposition products probably contribute to the taste of this cheese. To mention an example, the starter culture was found by PATRICIA & MORGAN (70) to be capable of forming aldhydes from amino acids. These compounds are known to be aromatic and if present they would have an influence upon the cheese flavour. In connection with this idea, we added a mixture of aldhydes prepared according to the Strecker degradation reaction, to the milk before cheese manufacture began. It was found that after 6 weeks' storage, the strongly penetrating taste and smell of the added aldhydes could not to any extent be

traced in the cheese. The control cheese had exactly the same flavour. Importance given to the influence of such substances on cheese flavour is no doubt due to the addition of such flavourable substances to a fresh, flat, tasteless curd. Two facts were always forgotten (a) that any taste in a fresh curd is preferable to a tasteless one (b) and that the chance of aldhydes to be formed in cheese is very remote owing to the low oxidation-reduction potential in cheese.

Considering the results of this study, we are of the opinion that the amino acids are important in cheese flavour. They form a base for the taste of the cheese. As the concentration of some amino acids decrease in ripe cheese, their decomposition products may have an indirect effect on cheese flavour. Amino acids that were always found in high concentration in Edam cheese e.g. glutamic acid with its broth-like taste and leucine with a slightly bitter and a slightly broth-like taste may contribute to the flavour of Edam cheese under most ripening conditions. Under high pH conditions when abnormally large amounts of proline are found, this amino acid together with other sweet amino acids may contribute to the sweet taste of the cheese. Further, no other amino acid could be directly related to the typical Edam cheese flavour. An equilibrium between the amino acids and the free fatty acids may be necessary

Results reported here confirm completely the opinion of MULDER (67) and it has already been supported by other investigations in this laboratory. Now the first step has been made, and neither the amino acids of the cheese nor the differences in their concentrations can explain flavour differences. The next step should be taken and the amino acid reaction products formed in the cheese should be studied.

SUMMARY

In this investigation the amino acid composition of Edam cheese with different properties was studied. Results reveal that the general amino acid pattern of a ripe Edam cheese includes the following amino acids;

1. Glutamic acid, leucine, lysine, valine and proline in a concentration more than 1000mg/100 g protein.
2. Phenylalanine, glycine, alanine, isoleucine and methionine generally present in a quantity differing from 500 to 1000 mg/100 g protein.
3. Aspartic acid, serine, histidine, tyrosine in a concentration usually lower than 500 mg/100 g protein.
4. Cysteic acid and arginine in a very low concentration.
5. Ornithine was always found in the cheese and could be added to the amino acid pattern of the cheese.

Considerable amounts of amines and ammonia found in every cheese analysed may be related as amino acid decomposition products.

In very young cheese, the absence of some amino acids was significant. Amino acids that were sometimes absent in this cheese were proline, arginine, serine, tyrosine, histidine, and cysteic acid.

A comparison between the composition of the free amino acids in cheese and the amino acid composition of casein showed that, generally speaking, there is almost no qualitative difference. The cheese contained all the amino acids of casein except cystine and tryptophane. It is probable that their absence was due to methods of analysis. Aspartic acid, serine, tyrosine and arginine quantitatively form a small part of the amino acid content of cheese while they are found in a

high concentration in casein. Also there is a considerable deficit in the free proline content of the cheese if compared with its concentration in casein. In contrast, glycine, valine, leucine and alanine were found in a higher concentration in cheese than in casein, the first to a greater extent and the three others to a lower one. Other amino acids are in more or less, the same concentration.

Also, under the influence of high and low pH, high and low moisture content, pasteurised and raw milk and aseptically drawn and infected milk, there was no important difference in the amino acid pattern of different cheeses. Quantitatively, differences can be summarised as follows:

1. Cheese of high pH contained higher concentrations of all amino acids except serine and arginine if compared with low pH cheese. Proline was the amino acid showing most change with change in pH.
2. The decrease in the amino acid content of ripe cheese was less frequent in cheese of low than of high pH. Methionine, Arginine, ornithine and histidine decreased in the latter cheese.
3. The concentration of all amino acids with the exception of serine were higher in high than in low moisture content cheese.
4. The decrease in amino acid concentration in ripe cheese was more frequent in high moisture content cheese. A decrease was noticed in its threonine, glycine, methionine, phenylalanine, lysine, ornithine and also the amine contents.
5. Pasteurised milk cheese contained higher amounts of glycine, arginine, histidine, tyrosine, and cysteic acid. Lower values in raw milk cheese were attributed to amino acid decarboxylases.
6. The higher serine content of pasteurised milk cheese and the high aspartic acid in raw milk cheese, previously reported in Cheddar cheese held true only in some cheeses.
7. Although there was almost no difference between the total amino acid nitrogen in aseptically drawn milk cheese and infected milk cheese of four months, a difference occurred in the amounts of some individual amino acids. Aspartic acid and ornithine were considerably higher in infected milk cheese, while on the other hand, serine, arginine, histidine, glycine, tyrosine and also amines were higher in aseptically drawn milk cheese. Their presence in lower concentrations in infected milk cheese may have been due to a greater utilization of these acids by the microflora of the infected milk or as a result of bacterial decarboxylation.

Amino acids showed early appearance in Edam cheese. As early as the second day of manufacture, glutamic acid, leucine, valine, phenylalanine, aspartic acid, lysine and ornithine were detected. The presence of ornithine in one day old cheese may indicate an early decomposition of free arginine.

On the other hand, some amino acids showed late appearance. Low pH retarded the appearance of proline in cheese whereas in high moisture content and high pH cheeses serine and histidine were detected late in ripening. Other amino acids that showed late appearance in young cheese were threonine, methionine, tyrosine and arginine.

From the time the amino acids appear in the free state in cheese, their concentrations increase with progressive ripening. In young cheese of 1.5 to 2.5 months there occurred a decrease in the amount of some amino acids, and a levelling off in the increase of some others. The increase proceeded until the age of 6.5 months. This increase remained with some acids as ripening progressed but at a

slower rate, while the values of some others showed a decrease after this age. In all cheeses it was noticed that the rate of increase in the concentrations of some amino acids was more pronounced in the early than in the later periods of ripening. Aspartic acid and serine contents were the exceptions. All amino acids with the exception of aspartic acid, tyrosine, serine, histidine, cysteic acid and arginine reached higher concentration in the cheese.

The decrease noticed in the concentration of amino acids at the end of the ripening period was attributed to the inhibition of synthesis and to further decomposition of these amino acids through the action of bacterial enzymes.

Amino acids were found to contribute to the basic taste of Edam cheese. They impart a broth-like, slightly bitter taste to the cheese, mostly due to glutamic acid and leucines. The intensity of this taste in the cheese depends on the degree of protein degradation, on the presence or absence of other breakdown products and on the consistency of the cheese. However, the presence of high amounts of amino acids in a cheese should not be considered as indication of a piquant and desirable cheesy flavour. Under high pH conditions and high moisture content, when the cheese contained large amounts of amino acids, the broth-like taste was stronger, while the flavour of the cheese was frequently abnormal as a result of abnormal fermentations. Also, the taste of amino acids due to the presence of high amounts of amino acids in pasteurised milk cheese (particularly that pasteurised at a high temperature) or in aseptically drawn milk cheese, did not appear completely in such cheese. Owing to the tough consistency of this cheese, the amino acids cannot come easily in contact with the taste organs. They are closed up in the texture of the cheese. The flat taste of such cheese was mostly attributed to lower fat hydrolysis.

Differences in the flavour bouquet between cheeses of low and high pH, with low and high moisture content, from raw and pasteurised milk and from aseptically drawn and infected milk cannot be related to the quantitative differences in the concentration of certain amino acids, the tastes of which differ from broth-like, sweet, bitter or tasteless.

The results of this ion exchange column chromatographic study of the amino acid composition of Edam cheese confirm completely the views of MULDER (67) on the flavour forming substances of cheese which were based on very simple qualitative experiments.

SAMENVATTING

Het hier beschreven onderzoek had tot doel het voorkomen van vrije aminozuren in kaas en de invloed van deze verbindingen op de smaak van kaas te bestuderen.

Hiertoe werd een iets gewijzigde analysemethode volgens MOORE & STEIN (64), waarbij gebruik wordt gemaakt van ionenuitwisselaars (Dowex 50 x 4 en x 5), toegepast.

Het aminozuurpatroon bleek voor alle onderzochte kazen, in grote trekken, gelijk te zijn. Daar de kazen zeer veel in eigenschappen verschilden, zou men hier uit kunnen concluderen dat het aminozuurpatroon voor gerijpte Edammer kazen, in het algemeen, weinig variatie zal vertonen.

De vrije aminozuren verschijnen reeds vroeg in Edammer kaas. In een dag oude kaas konden al glutaminezuur, leucine, valine, phenylalanine, asparaginezuur, lysine en ornithine worden aangetoond; (deze aanwezigheid van ornithine in kaas van slecht één dag oud, kan wijzen op een vroegtijdige afbraak van arginine). Ander aminozuren zijn daarentegen pas later aanwezig; proline, arginine, serine, tyrosine, histidine en cysteine. Bij lage pH is de verschijning van proline traag, terwijl in kazen met hoge pH of een hoog vochtgehalte, serine en histidine eerst laat konden worden aangetoond. Andere aminozuren, die laat in de jonge kaas optreden, zijn: threonine, methionine, tyrosine, en arginine.

Vanaf het ogenblik dat de vrije aminozuren in de kaas verschijnen, neemt hun concentratie toe met het voortschrijden van de rijping. Tegelijkertijd moet echter een afbraak plaats vinden. Na ca. 6 tot 10 weken nemen nl. bepaalde aminozuren in hoeveelheid af, terwijl van enkele andere de toeneming kleiner wordt. Daarna zet de stijging weer door, tot een ouderdom van ca. 6½ maand. Na deze rijpingstijd vertonen sommige aminozuren nog een geringe toeneming bij verdere rijping; andere laten daarentegen een afneming in concentratie zien. Bij alle kazen werd geconstateerd, dat de toeneming van de concentratie aan aminozuren in de eerste rijpingsperiode groter is dan in de latere, uitgezonderd voor asparaginezuur en serine.

In de regel komen in gerijpte Edammer kaas de volgende aminozuren voor:

1. glutaminezuur, leucine, lysine, valine, en proline in concentratie groter dan 1000 mg/100 g eiwit.
2. phenylalanine, glycine, alanine, isoleucine, en methionine gewoonlijk in hoeveelheden van ongeveer 500 tot 1000 mg/100 g eiwit.
3. asparaginezuur, serine, histidine, en tyrosine in concentratie, die in het algemeen kleiner dan 500 mg/100 g eiwit zijn.
4. cysteine en arginine in zeer lage concentraties.
5. Er werd een belangrijke hoeveelheid ornithine in de kaas gevonden, hoewel dit aminozuur niet in caseïne voorkomt. Het is waarschijnlijk een afbraakproduct van arginine.

De onderlinge verhouding van de hoeveelheden vrije aminozuren in Edammer kaas blijkt in grote trekken overeen te komen met de samenstelling van caseïne.

In kaas werden alle aminozuren van caseïne gevonden, behalve cystine en tryptophaan. De gevolgde analysemethode is echter minder geschikt voor het aantonen van deze twee aminozuren, zodat het mogelijk lijkt dat toch wel een weinig van deze zuren in kaas kan voorkomen.

Bij een meer gedetailleerde beschouwing blijkt dat asparaginezuur, serine, tyrosine en arginine slechts in kleine concentratie in kaas voorkomen, hoewel ze in caseïne in veel grotere hoeveelheden aanwezig zijn. Er is tevens een duidelijk tekort aan proline in de kaas, indien men het gehalte vergelijkt met dat van caseïne. Daarentegen komen glycine, valine, leucine en alanine in relatief hogere concentratie voor dan in caseïne; en wel glycine in hoge mate en de drie andere minder duidelijk. De andere aminozuren komen in kaas en in caseïne in ongeveer dezelfde verhouding voor.

De lage concentraties van bepaalde aminozuren, zowel als het afnemen in concentratie ervan gedurende een bepaalde periode van de rijping, kan worden toegeschreven aan vermindering van de hydrolyse van caseïne gecombineerd met verbruik van de aminozuren door bacteriën, en/of afbraak door enzymen. De relatief grote hoeveelheden van enkele aminozuren kunnen wellicht worden

toegeschreven aan afbraak van andere stoffen en aan synthese van aminozuren.

Zoals reeds werd opgemerkt hadden alle onderzochte kazen, hoezeer ook verschillend van smaak en consistentie, in pH, vochtgehalte en bacteriologische gesteldheid, in grote trekken, hetzelfde aminozuren-patroon. Bij een meer gedetailleerde quantitatieve vergelijking treden de volgende verschillen naar voren:

1. Bij hogere pH is de concentratie van alle aminozuren groter, behalve die van serine en arginine. Het gehalte aan proline ondervindt het meest invloed van pH.
2. Bij lage pH neemt in de gerijpte kaas het aminozuurgehalte minder af dan bij hoge pH.
3. Met uitzondering van serine, bereiken alle aminozuren een hogere concentratie, naarmate het vochtgehalte van de kaas hoger is.
4. In kaas met een hoog vochtgehalte nemen de gehalten aan threonine, glycine, methionine, phenylalanine, lysine, ornithine en aminen bij de verdere rijping af. Dit is bij een lager vochtgehalte met minder aminozuren het geval.
5. Kaas uit gepasteuriseerde melk bevat relatief hogere gehaltes aan glycine, arginine, histidine, tyrosine en cysteïne, dan kaas uit rauwe melk. De lage gehaltes in de laatste kaas worden toegeschreven aan afbraak door aminozuurdecarboxylasen. Het relatief hoge gehalte aan serine bij kaas uit gepasteuriseerde melk en het relatief hoge gehalte aan asparaginezuur in kaas uit rauwe melk, zoals dit eerder werd vastgesteld bij Cheddar kaas, werd slechts bij enkele kazen bevestigd.
6. Tussen kaas van 4 maanden oud, bereid uit aseptisch gewonnen melk en die uit geïnfecteerde melk, is vrijwel geen verschil in de totale hoeveelheid aminozuur-stikstof. Daarentegen is er wel enig verschil voor sommige individuele aminozuren. De gehaltes aan asparaginezuur en ornithine zijn belangrijk hoger in de kaas uit geïnfecteerde melk, terwijl de concentraties aan serine, arginine, histidine, glycine, tyrosine, en aminen lager zijn, dan in de kaas uit aseptisch gewonnen melk. Deze lage gehaltes kunnen waarschijnlijk worden toegeschreven aan een groter verbruik door microörganismen, of aan enzymatische decarboxylatie.

Vrije aminozuren hebben alle een zekere smaak en zullen dus bijdragen tot de smaak van Edammer kaas. Toch zijn er nog andere belangrijke smaakstoffen aanwezig, want er treden evenwel dikwijls grote verschillen in smaak op tussen kazen met vrijwel hetzelfde aminozuren patroon.

De voornaamste smaken van de individuele aminozuren zijn bouillonachtig (b.v. glutaminezuur), bitter (b.v. lysine, isoleucine) en zoetachtige (b.v. proline). De mate waarin deze smaken bijdragen tot de smaak van kaas is allicht afhankelijk van de hoeveelheid en de onderlinge verhouding der aminozuren, maar ook van de aanwezigheid van andere afbraakprodukten en van de consistentie van de kaas. Men dient het voorkomen van grote hoeveelheden aminozuren die alle weinig krachtig van smaak zijn in de kaas niet te beschouwen als een noodzaak voor een pikante smaak.

In kaas van hoge pH en hoog vochtgehalte – zulke kaas bevat grote hoeveelheden aminozuren – is de bouillonachtige smaak vaak uitgesproken duidelijk. Maar dikwijls is de smaak dan tevens afwijkend, tengevolge van abnormale rijpingsprocessen. Veelal hebben kazen met een hoge pH ook een zoete smaak, mede tengevolge van (propionzure zouten) en een hoog gehalte aan vrije proline.

Het hoge gehalte aan vrije aminozuren van kaas bereid uit (hoog) gepasteuriseerde of aseptisch gewonnen melk, correspondeert niet geheel met de smaak van deze kaas. Deze soorten kaas zullen dus een tekort hebben aan andere belangrijke smaakstoffen (vrije vetzuren). Ook is de consistentie vaak nogal taai, zodat de reuk- en smaakstoffen niet gemakkelijk in contact komen met de smaakorganen.

De aanwezige vrije aminozuren zullen bijdragen tot de bittere smaak (zeer waarschijnlijk zullen nog andere en sterker bittere stoffen aanwezig kunnen zijn), maar deze komt vooral tot uiting bij afwezigheid van produkten die ontstaan bij de vetsplitsing. In kaas uit rauwe melk bereid, worden deze bittere smaakstoffen in normale gevallen waarschijnlijk grotendeels bedekt door de aanwezigheid van grotere hoeveelheden vrije vetzuren en treedt dus de bittere smaak niet op de voorgrond. Indien er echter zeer veel bittere stoffen (doorgaans peptonen) aanwezig zijn, kunnen de vrije vetzuren de bittere smaak niet meer bedekken en zal de kaas bitter gaan smaken.

Verschillen in smaak tussen kazen met lage en hoge pH, laag en hoog vochtgehalte, of bereid uit melk met verschillende bacteriologische gesteldheid, kunnen dus niet worden verklaard met quantitatieve verschillen in de hoeveelheden van bepaalde vrije aminozuren.

De resultaten van dit onderzoek bevestigen de inzichten van MULDER (67) aangaande de smaakstoffen in kaas, welke inzichten gebaseerd waren op zeer eenvoudige, qualitatieve experimenten.

AKNOWLEDGEMENTS

This investigation has been carried out at the Laboratory of Dairying, Agriculture University in Wageningen, Netherlands.

I have the pleasure in expressing my great indebtedness to Professor Dr. H. MULDER for his helpful suggestions and encouragements, his stimulating criticism and constant advice throughout the course of the work, and for his keen observations during the preparation of this manuscript.

Special thanks are due to Miss L. Williams, Queensland University, Australia, for the correction of the English text.

Thanks to all who contributed, in any way, to my achievements in Netherland.

LITERATURE CITED

1. ABDERHALDEN, E. (1910). – Handbuch der Biochemschen Arbeits Methoden, Band **II**, 470.
2. ANTILA, M. (1958). – Meijerit Aikakausk. **18/19**, 3.
3. AWARPA, J. (1948). – Arch. Biochem. **19**, 172.
4. BAILEY, K. (1937). – Biochem. J. (London) **31**, 1396.
5. BAKER, R. J. and NELSON, F. E. (1949). – J. Dairy Sci. **32**, 769.
6. BASSET, E. W. and HARPER, W. J. (1956). – Proc. XIVth Int. Dairy Congr. Rome **II**, (2) 38.
7. BIGGER-GEHRUG, L. (1955). – J. Gen. Microbiol. **13**, 45.
8. BLOCK, R. J. and BOLLING, D. (1951) – The amino acid composition of proteins and foods. Springfield, Illinois, Thomas, p. 576.
9. BLOCK, R. J. and WEISS, K. W. (1936). – Amino acid handbook. Springfield, Illinois, Thomas, p. 266.
10. BOEKHOUT, F. W. J. and OTT DE VRIES, J. J. (1909). – Rév. Gén. Lait **7**, 385.
11. BALEN WALTER, B. C. VAN (1951). – Landbouwk. Tijdschr. **63**, 522.

12. BREED, F. A. (1928). – New York State Agr. Expt. Sta. Techn. Bull. 132.
13. BULLOCK, D. H. and IRVINE, O. R. (1956). – J. Dairy Sci. **39**, 1229.
14. CALL, A. C. and PRICE, W. V. (1944). – J. Dairy Sci. **27**, 681.
15. CHEBOTAREV, A. J. and MARIA V. VELTSONA (1959). – Proc. XVth Int. Dairy Congr. London **II**, 691.
16. CLEMENS, W. (1954). – Milchwissenschaft **9**, 195.
17. CONSDEN, R., GORDON, A. H. and MARTIN, A. J. P. (1944). – Biochem. J. **38**, 224.
18. DACRE, J. C. (1953). – J. Sci. Fd. Agric. **4**, 604.
19. DAVIES, W. L. (1932). – Analyst **57**, 79.
20. DOLEZALEK, J. (1956). – Prumysl Potravin **7**, 175. c.f. Dairy Sci. Abstr. **18**, 778, (1956).
21. DORNER, W. (1930). – New York State Agric. Expt. Sta. Techn. Bull. 165.
22. EASTOE, J. E. and EASTOE, B. (1954). – Biochem. J. **57**, 453.
23. ELLIKER, P. R. and FRAZIER, W. C. (1939). – J. Dairy Sci. **22**, 821.
24. EVANS, A. C. (1916). – J. Inf. Dis. **18**, 437.
25. FISCHER, E. (1901). – Z. physiol. Chem. **33**, 151.
26. FOSTER, G. L. (1945). – J. Biol. Chem. **159**, 431.
27. FOSTER, E. M. (1952). – J. Dairy Sci. **35**, 988.
28. FREEMAN, TH. R. and DAHLE, C. D. (1938). – Penna. Agr. Exp. St. Techn. Bull. 362. c.f. J. Dairy Sci. Abstr. **21**, 237 (1938).
29. FRICKER, A. (1956). – Kieler Milchw. Forsch. ber. **8**, 89.
30. GONASHVILI, SH. (1955). – Mol. Prom. **16**, 22 c.f. Dairy Sci. Abstr. **18**, 86 (1956).
31. GRABER, H. (1943). – Die Bedeutung der Euterinfektion und der Milchkontamination für die Keimzahl und die Haltbarkeit der Milch, Schaffhausen.
32. GRIMMER, W., BODSCHWINNA, W. and SCHUTZLER, K. (1929). – Milchw. Forsch. **7**, 595.
33. GUIRARD, B. M. and SNELL, E. E. (1954). J. Am. Chem. Soc. **76**, 4745.
34. HAMILTON, P. B. (1958). – Anal. Chem. **30**, 914.
35. HAMMER, B. W. and BABEL, F. J. (1957). – Dairy Bacteriology 4th edN. ew York, John. Willey & Sons, p. 213 (a) p. 218.
36. HARFENIST, E. J. (1953). – J. Am. Chem. Soc. **75**, 5528.
37. HARPER, W. J. (1959). – J. Dairy Sci. **42**, 212.
38. HARPER, W. J. and LONG, J. E. (1956). – J. Dairy Sci. **39**, 129.
39. HARPER, W. J. and SWANSON, A. M. (1949). – Proc. XIIth Int. Dairy Congr. Stockholm, **II**, 147.
40. HINTZ, P. C., SLATTER, W. L. and HARPER, W. J. (1954). – J. Dairy Sci. **37**, 639.
41. HINTZ, P. C., SLATTER, W. L. and HARPER, W. J. (1956). – J. Dairy Sci. **39**, 235.
42. HODSON, A. Z. and KREUGER, G. M. (1947). – Arch. Biochem. **12**, 435.
43. HONER, C. J. and TUCKEY, S. L. (1951). – J. Dairy Sci. **34**, 475.
44. HUISMAN, T. H. S. and SCHAAF, P. C. VAN DER (1955). – Chem. Weekbl. **51**, 3.
45. JAGER, H. (1955). – Milchwiss. Ber. Wolfpassing **5**, 93.
46. JAGER, H. (1958). – Milchwiss. Ber. Wolfpassing, **8**, 19.
47. KIURI, V. J. (1949). Proc. XIIth Int. Dairy Congr. Stockholm, V, 30.
48. KIURI, V. J., TYBECK, E. and VIRTANEN, A. I. (1953). – Suomen Kem. **26B**, p. 55.
49. KOSIKOWSKI, F. V. (1951). – J. Dairy Sci. **34**, 228.
50. KOSIKOWSKI, F. V. and DAHLBERG, A. C. (1954). – J. Dairy Sci. **37**, 167.
51. KOSSEL, A. and KUTSCHER, F. (1900). – Z. physiol. chem. **31**, 165.
52. KRETT, O. J., LE BOYER, A., DAUME, H. E. and STINE, J. B. (1952). – J. Dairy Sci. **35**, 480.
53. KRISTOFFERSEN, T., GOULD, I. A. and HARPER, W. J. (1959). – Milk Prod. J. **50** (5) 14.
54. KRISTOFFERSEN, T. and NELSON, F. E. (1955). – J. Dairy Sci. **38**, 1319.
55. LANE, C. B. and HAMMER, B. W. (1955). – Iowa State Agr. Exp. St. Res. Bull. **183**, 353.
56. LINDQVIST, B., STORGARDS, T. and GORANSSON, B. (1953). – Proc. Int. Dairy Congr. The Hague **III**, 1254.
57. LING, E. R. (1958). – J. Dairy Res. **25**, 138.
58. MABBIT, L. A. (1955). – J. Dairy Res. **22**, 224.
59. MACFARREN, E. F. (1951). – Anal. chem. **23**, 168.
60. METAXAS, M. A. and DELWICHE, E. A. (1955). – J. Bacteriol. **70**, 735.
61. MITCHELL, H. H. and HAMILTON, T. S. (1929). – The Biochemistry of amino acids. The Chem. Catalog. Com. Inc. New York. p. 104.
62. MOLLER-MADSEN, A. (1959). – Proc. XVth Int. Dairy Congr. London. **III**, 1454.
63. MOORE, S. and STEIN, W. H. (1951). – J. Biol. chem. **192**, 663.
64. MOORE, S. and STEIN, W. H. (1954). – J. Biol. chem. **211**, 893.
65. MOORE, S. and STEIN, W. H. (1954). – J. Biol. chem. **211**, 907.
66. MOORE, S. and STEIN, W. H. (1958). – Anal. Chem. **30**, 1185.

67. MULDER, H. (1952). – Neth. Milk & Dairy J. **6**, 157.
68. OSWALD, W. (1954). – Milchwiss. Ber. Wolfpassing. **4**, 3.
69. PAOLIS, P. DE and SALERNO, A. (1956). – Latte **30**, 658.
70. PATRICIA MACLEOD and MORGAM, M. E. (1958). – J. Dairy Sci. **41**, 908.
71. RAADSVELD, C. W. (1952). – Neth. Milk & Dairy J. **6**, 342.
72. RAADSVELD, C. W. (1953). – Proc. XIIIth Int. Dairy Congr. The Hague **II**, 671.
73. RAADSVELD, C. W. (1953). – Proc. XIIIth Int. Dairy Congr. The Hague **II**, 676.
74. RAADSVELD, C. W. and MULDER, H. (1949). – Neth. Milk & Dairy J. **3**, 222.
75. RAIBAUD, P., MOCQUOT, G. and KOSIKOWISKI, F. V. (1956). – Proc. XIVth Int. Dairy Congr. Rome **II** (2), 454.
76. ROWLAND, S. J. (1938). – J. Dairy Res. **9**, 42.
77. REIHARD, D. G. and GAREY, J. C. (1949). – Soc. Am. Bact. abstr. of papers p. 52
78. RODWELL, A. W. (1953). – J. Gen. Microbiol. **8**, 224.
79. SAMMIS, J. L. and GERMAIN, L. (1929). – Nat. Butt. Cheese J. **20**, 30.
80. SALERNO, G. J. and PAOLIS, P. DE (1956). – Latte **30**, 663.
81. SCHORMÜLLER, J., GLATHE, M. and HUTH, H. Z. (1956). – Z. f. Lebensm. Unt. und Forsch. **14**, 103.
82. SHERWOOD, I. R. (1936). – J. Dairy Res. **7**, 271.
83. SHER, I. H. and MALETTE, M. F. (1954). – Arch. Biochem. & Biophys. **53**, 354, 370.
84. SILVERMAN, G. J. (1955). – Diss. Abstr. **15**, 968.
85. SILVERMAN, G. J. (1955). – Ph. D. Thesis Cornell University Ithaca, N.Y. c.f. KOSIKOWISKI, F. V. and MOCQUOT, G. (1958). – "Advances in cheese technology". F.A.O. Rome, p. 128.
86. SILVERMAN, G. J. and KOSIKOWISKI, F. V. (1953). – J. Dairy Sci. **36**, 574.
87. SILVERMAN, G. J. and KOSIKOWISKI, F. V. (1956). – Proc. XIVth Int. Dairy Congr. Rome II (2) 532.
88. SILVERMAN, G. J. and KOSIKOWISKI, F. V. (1956). – J. Dairy Sci. **39**, 1134.
89. SIMONART, P. and MAYAUDON, J. (1952). – Neth. Milk & Dairy J. **6**, 1.
90. SIRKS, H. A. (1943). – Versl. Landbouwk. Onderz. **49**, 221.
91. STADHOUDERS, J. (1959). – Proc. XVth Int. Dairy Congr. London **II**, 703.
92. STADHOUDERS, J. and MULDER, H. (1953). – Proc. XIIIth Int. Dairy Congr. The Hague **II**, 681.
93. STADHOUDERS, J. and MULDER, H. (1957). – Neth. Milk & Dairy J. **11**, 164.
94. STADHOUDERS, J. and MULDER, H. (1958). – Neth. Milk & Dairy J. **12**, 237.
95. STECK, W. (1921). – Landw. Jahrb. Schweiz **35**, 511, 629.
96. STECK, W. (1930). – Die latente Inf. der Milchdruse, Hannover.
97. STORGARDS, T. and HIETARANTA, M. (1949). – Proc. XIIth Int. Dairy Congr. Stockholm **II**, 227.
98. STORGARDS, T. and LINDQVIST, B. (1953). – Milchwissenschaft **8**, 5.
99. STORGARDS, T., HIETARANTA, M. and HEIKINHEIM, J. (1950). – Karjantuote No. 19, Helsinki.
100. THIMANN, K. V. (1926). – Biochem. J. **20**, 1190.
101. THIMANN, K. V. (1930). – Biochem. J. **24**, 368.
102. TUCKEY, S. L., RUEHE, H. A. and CLARK, G. L. (1938). – J. Dairy Sci. **21**, 777.
103. VAN DAM, W. (1910). – Rév. Gén. Lait **8**, 169.
104. VAN DAM, W. (1928). – Versl. Landbouwk. Onderz. **33**, 187.
105. VAN DER BURG, B. and HARTMANS, S. (1952). – Zuivelbereiding IV. De kaasbereiding in de fabriek. Kon. Ned. Zuivelbond. p. 172.
106. VAN SLYKE, D. D. (1910). – Ber. chem. Ges. **43**, 3170.
107. VAN SLYKE, L. L. and HART, E. B. (1903). – New York Agr. Exp. St. Geneva N.Y. Bull. 236.
108. VIRTANEN, A. I. and KREULA, M. S. (1948). – Meijerit. Aikakausk. **10**, 13.
109. VIRTANEN, A. I., KREULA, M. S. and NURMIKKO, V. T. (1949). – Proc. XIIth Int. Dairy Congr. Stockholm. **II**, 268.
110. WILSSENS, A. and VLEESCHAUWER, A. (1954). – De Meded. Landb. Hogesch. Gent **19**, 689.
111. WINTERSTEIN, E. (1904). – Hoppe Sylers Zeitschr. physiol Chem. **41**, 485.
112. WINTERSTEIN, E. and BISSEGGER, W. (1907). – Hoppe Seylers Zeitschr. physiol. Chem. **47**, 28.
113. ZELLINGER, A. and OSWALD, W. (1955). – Milchwiss. Berichte. Wolfpassing **5**, 1.

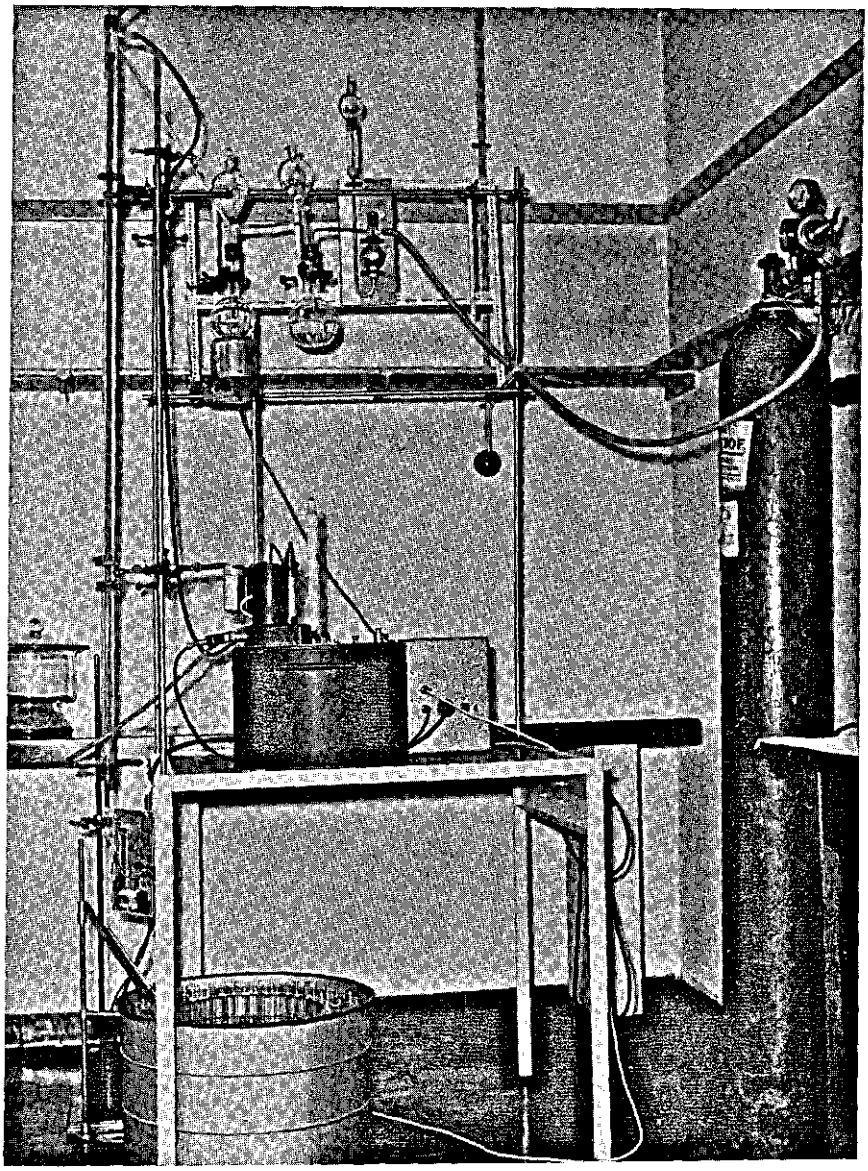


PLATE 1. Apparatus, with fraction collector used in the separation of amino acids.