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Cheese ripening studied in model systems

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A. Noomen graduated on 17 May 1978 and L. de Jong graduated on 31 May 1978 as Doctor in de Landbouwwetenschappen at the Agricultural University, Wageningen, the Netherlands, on a thesis containing Chapter II and Chapter III, respectively. The supervisor was Professor Dr H. Mulder.

Abstract

Mulder, H., A. Noomen & L. de Jong (1978) Cheese ripening studied in model systems. I. Introductory part (H. Mulder, A. Noomen & L. de Jong). II. Proteolysis in soft cheese, studied on Meshanger cheese and cheese models (A. Noomen). III. Proteolysis and consistency of Meshanger cheese (L. de Jong). Agric. Res. Rep. (Versl. landbouwk. Onderz.) 875, ISBN 90 220 0666 2, (viii) + 43 p, 4 figs, 55 refs, Eng. and Dutch summaries. Chapter II and III also published as doctoral thesis Wageningen, both with and without articles.

Enzymes predominantly responsible for the primary degradation of protein in soft cheese and for the related changes in consistency were studied. Reconstructed Noordhollandse Meshanger cheese and preserved simulated soft cheeses of different composition were used as models in the investigation. Results for proteolysis in the simulated cheeses were comparable and also comparable to those observed with normal Meshanger cheese. Protein breakdown was studied by estimating the amount of nitrogen soluble in the moisture of cheese and by quantitative polyacrylamide gel electrophoresis. Results of the two methods were well correlated. Proteolysis, which is primarily responsible for changes in consistency of soft cheeses, was caused mainly by calf rennet enzymes. The activity of rennet at different pH and concentrations of NaCl in the moisture of cheese also revealed a major role of rennet enzymes in protein breakdown in soft cheeses with an initially very low pH, ripening under the influence of a surface flora. The role of the surface flora is merely to regulate pH and so to soften the cheese body, and to give the cheese a specific flavour. Milk protease activity in soft cheese was studied in relation to pH, concentration of NaCl in moisture, ripening time and ripening temperature of the cheese. Its contribution to soft cheese ripening is minor, perhaps except for certain cheeses with a surface flora. Milk protease showed considerable activity in milk at favourable temperatures; proteolysis increased when cheese milk was subjected to low-temperature pasteurization.

Proteolysis in Meshanger cheese, estimated by quantitative polyacrylamide gel electrophoresis is discussed. The conversion of α_{s1}-casein was proportional to rennet concentration in the cheese. Changes in consistency, after a maximum, were correlated to breakdown of α_{s1}-casein. The changes in structure of the cheese during ripening, studied by electron microscopy, looked similar to those reported for Camembert cheese. Softening of cheese with high moisture content was due to rennet breakdown of α_{s1}-casein. Tests with substrates of sodium paracaseinate, calcium paracaseinate–calcium phosphate complex and a synthetic complex of casein, calcium hydroxide and phosphoric acid revealed that Na^+ and Ca^{2+} both influenced rennet proteolysis in those systems. Their interaction was considerable and depended on the type of substrate. In cheeses of lower moisture contents than Meshanger cheese, firmness was primarily regulated by volume fraction of protein in the fat-free cheese. Differences in protein breakdown cannot simply be attributed to differences in moisture content.

Free descriptors: proteolysis, cheese varieties, Noordhollandse Meshanger cheese, soft cheese, consistency, cheese model substrates, enzymes, rennet, milk protease, chymosin, gel electrophoresis of cheese, rheology, extraction method for soluble N, effect of pasteurization of milk on milk protease activity, light microscopy, electron microscopy, cheese structure, cheese ripening.

Contents

I Introductory part

H. Mulder, A. Noomen and L. de Jong

1 Methods to study cheese ripening

The ripening of cheese is a complex of many complicated processes, in which especially protein, lactose and fat are transformed. The result is a product with the desired appearance, consistency and flavour. Together with the physical, chemical and microbial properties of the milk, the method of making and the ripening conditions determine the sort of cheese that is formed. The intricacy of all this is demonstrated by the great number of cheese varieties, so that research on cheese ripening is no simple matter. Most workers in this field study the ripening of one particular cheese; hence they are tied to the whole complex of conditions that leads to a cheese of the quality desired for the sort. Therefore every kind of cheese must be investigated separately.

Nevertheless, cheeses that are quite different still have much in common in ripening and we believe that a general, but simple model would be useful to study the main ripening processes, from which the ripening of special cheeses can be deduced. Among possible approaches to such a model are simplifying the preparation of a known cheese, resulting in a cheese with a less complicated ripening (stripped cheese), and synthesizing cheese-like systems from the necessary components (simulated cheese).

2 Stripped cheese as model

As basis we chose the soft Noordhollandse Meshanger cheese. This cheese ripens in about 2 weeks; the preparation is simple; its flavour is very mild. The stripped Meshanger cheese ripens without a surface flora. If desired it can be acidified with a lactone instead of with lactic acid bacteria; the milk is pasteurized. At first sight such a simple stripped cheese may seem ideal. However it has restrictions. Its composition depends on the properties of the milk; a possible influence for instance of whey components on ripening is not yet clear. Furthermore such stripped cheeses contain rennet enzymes, enzymes like milk protease etc.. The action of lactic acid bacteria may cause complications. A stripped cheese that contains no active rennet enzymes and no milk protease can be prepared, but its structure can differ significantly from that of normal cheese because of the severe treatment necessary to inactivate enzymes. This treatment involves high pasteurization temperatures and manipulation with the calcium ion concentration.

Another way to prepare such a model cheese is not to work the cheese curd into cheese in the normal way, but to add ingredients like lactic acid and salt to curd (artificially composed stripped cheese). However there still are uncertainties, such as the influence of the whey enclosed in the curd.

3 Models composed from pure ingredients

The more radical way for making a model is to compose it from the individual components. The most important are calcium paracaseinate–calcium phosphate complex, lactic acid, NaCl and water. Enzymes or other substances, whose influence on ripening is to be studied, can be added. The attraction of these simulated cheeses is that their composition is exactly known. However they also have restrictions, for instance whey is left out. Using whey instead of water may reintroduce unknowns. Of course the structure of these systems differs from that of normal cheese.

One could continue, as many investigators did, and investigate the conversion of pure components of cheese, for instance by studying the proteolytic activity of enzymes in solutions of casein salts. We found however that one cannot extrapolate to cheese as, for instance, calcium has a considerable influence on casein breakdown. So the composition and properties of model systems should resemble those of cheese as closely as possible. All models, however, have some restrictions. The choice depends on the problem to be studied.

4 Motivation and approach

The motivation of our study was to gain knowledge on cheese ripening, especially on proteolysis and its consequences for cheese consistency. The factors thought to be most important were studied in the types of models mentioned. The influence of rennet (chymosin) was studied in all models whereas to study the action of milk protease the preferred system was simulated cheese. The relation between consistency and proteolysis was studied in the stripped Meshanger cheese, as was the relation between structure and consistency.

To study protein breakdown two methods were adapted. For estimation of the soluble nitrogen components, this was an extraction method and the unattacked proteins were estimated by quantitative electrophoresis.

Further details about motivation, results and conclusions of the work are reported in full in:

II – Proteolysis in soft cheese, studied on Meshanger cheese and cheese models, reported by A. Noomen

and

III – Proteolysis and consistency of Meshanger cheese, reported by L. de Jong.

II Proteolysis in soft cheese, studied on Meshanger cheese and cheese models

A. Noomen

Introduction

1 Purpose of the investigation

Proteolysis in cheese is a major process in cheese ripening. In our laboratory the crucial importance of the process initiated several studies on aspects of protein breakdown in cheese and phenomena related to that (1, 2, 3, 4).

During the ripening of cheese, the original rubbery coarse curd is converted into a smoother mass. These changes in consistency influence markedly the sensoric quality of cheese, and are most quickly perceptible in cheeses with a high moisture content. The study mainly deals with the ripening of soft cheese with particular reference to the question which enzymes are predominantly involved in the primary breakdown of protein and that may contribute to changes in the cheese consistency. Rheological properties of cheese as such were studied by de Jong (5).

2 Meshanger cheese as a model

Cheeses with a high moisture content can be classed into those which have a surface flora and where softening usually proceeds from the outside inwards (e.g. Camembert and Brie cheese), and those which may lack a surface flora and become soft throughout (e.g. Kernhem, Butterkäse, Bel Paese and St. Paulin cheese).

Before this study some work had already been done on Noordhollandse Meshanger cheese, a soft cheese formerly made on some farms in the Province of Noord-Holland. It became extinct about 1940, and knowledge of its production was almost completely lost. A surface flora, mainly of yeasts and moulds, seemed to develop spontaneously on its surface.

Unlike most other soft cheeses, softening was said to proceed from the centre outwards. The cheese had been simple to make and ripened in about two weeks, it had a mild flavour. It therefore seemed attractive as a model. The method of producing the cheese was reconstructed and then adjusted it to the needs of the investigation. Some parts of the original process were modified to suit modern methods of cheesemaking, and could be used if the cheese is ever produced industrially

3 Estimation of protein breakdown

The study required a choice of methods for observing protein degradation in cheese. Results from the traditional methods, based on solubility of products of protein breakdown in liquids of different composition, do not necessarily reflect the total amount of nitrogenous substances soluble in cheese moisture. Since the undissolved, and hence the dissolved nitrogen compounds are associated with the consistency of cheese, a method was needed that reflected total soluble nitrogen. A simple method was developed.

During the work with model substrates, the degradation of protein was also estimated by polyacrylamide gel electrophoresis (6).

4 Proteolysis in Meshanger cheese

For proteolysis, surface flora, lactic acid bacteria, calf rennet used in making the cheese and native milk protease were all sources of proteolytic activity. The contribution of each source was tested by comparing ripening of a normal cheese with that of a cheese without surface flora and without either surface flora or bacteria. Native milk protease was present in all types. Protein breakdown and changes in consistency during ripening were largely attributable to calf rennet.

5 Rennet activity in simulated cheese

Conditions for action of enzymes may vary considerably between varieties of cheese. Even in a single variety conditions change during the ripening, for example in soft cheeses that have an initially very low pH and that ripen under the influence of a surface flora. An important question was how far proteolytic activity of calf rennet was influenced by different conditions, in particular pH and concentration of NaCl, which may be present in soft cheeses. In theory, cheese itself must be used as substrate. In our laboratory, an aseptic technique for making cheese with only rennet and milk protease as active proteolytic agents was developed (7, 8). However, the technique is laborious and it would be difficult to vary a single factor, particularly in soft cheeses. Since the study did not include sensory tests, model substrates were considered more convenient.

Results obtained with model substrates showing conditions far remote from those in cheese are difficult to interpret for the actual progress of proteolytic processes in cheese (e.g. 9, 10, 11, 12, 13, 14). Therefore, the model substrates should as closely as possible resemble conditions in cheese, at least in the essential points. With this in mind, cheese was simulated with a m i fitted as closely as possible the situation in Mesh

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cheeses were made up from the most essential components of interest in the study: calcium paracaseinate–calcium phosphate complex, water, lactic acid and NaCl. For comparison, simulated cheeses were also prepared with cheese curd. Proteolytic activity of microbial sources was prevented. The cheeses were effectively preserved by the use of thimerosal – ((carboxyphenyl) thio) ethylmercury sodium salt – at a concentration of 100 mg per litre of moisture, in combination with anaerobic storage. The preservative hardly influenced the proteolytic activity of the rennet investigated.

6 Activity of milk protease in simulated cheese and milk

Milk protease is a natural component of cow's milk (e.g. 15, 16, 17). Its activity was generally assumed to be low. The enzyme is closely associated with casein micelles and with acid-precipitated casein (18, 19, 20), and will therefore finish up in cheese. Its contribution to protein breakdown in cheese has received little attention (4, 21, 22).

Preliminary experiments revealed a considerable activity of milk protease in Meshanger – like systems of pH 6.6, preserved with thimerosal. So activity of milk protease was investigated in simulated soft cheeses at different pH and concentration of NaCl. The cheeses were prepared either with rennet-free cheese curd or with a rennet-free calcium paracaseinate–calcium phosphate complex, made by the rennet-free technique developed at our laboratory (23). The 'curd' and the complex contained the milk protease associated with the casein micelles of the milk from which they were made.

Estimation of proteolytic activity of milk protease has most frequently been based on the estimation of the amount of tyrosine liberated in certain substrates. This method is not sensitive (18, 24). The reported weak activity of milk protease in milk could thus be partly due to the low sensitivity of formerly applied methods. With modification, the method developed to measure the amount of soluble nitrogen compounds in cheese was also suitable for study of protein breakdown in milk. With this method and by electrophoresis, proteolysis was studied in preserved aseptically drawn cow's milk (7), containing less than 30 bacteria per millilitre. Protein breakdown thus could be exclusively attributed to milk protease. In former experiments a contribution to protein breakdown from proteolytic bacteria was possible.

For reasons of public health and avoidance of cheese defects, Dutch cheese manufacturers usually pasteurize cheese milk at 72 °C for about 15 s. Milk protease is considerably heat resistant and at least partially survives moderate heat treatment of milk (e.g. 16, 17,). However, there are no clear data in the literature on how far its proteolytic activity in milk, and thus possibly in cheese, is influenced by pasteurization. To gain more information, protein breakdown was studied in well preserved raw and pasteurized samples of

aseptically drawn milks. The raw milks contained less than 30 bacteria per millilitre. Any contribution to proteolysis by sources other than milk protease was thus excluded.

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Summaries of the articles

Noordhollandse Meshanger cheese: a model for research on cheese ripening. 1. Reconstruction of the cheese
A. Noomen and H. Mulder, Neth. Milk Dairy J. 30 (1976): 230-241.

The article, the first part of a series of three, deals with Noordhollandse Meshanger cheese, a soft cheese made in former times on one or two farms in the province of Noord-Holland (the Netherlands). The method of making the cheese, which was almost completely forgotten, was reconstructed not only for professional interest but mainly for the purpose of fundamental research on cheese ripening.

The making of the cheese had many points in common with that of the earlier farmmade Edam cheese. The cheese was made from cow's milk as freshly drawn as possible, no starter was used and the production conditions were aimed at a high moisture content in the cheese. The consistency of the cheese was soft to very soft. In contradiction to most known soft cheeses with a surface flora the softening of the body proceeded from the centre to the outside of the cheese. The taste was very mildly acid and somewhat yeasty.

Noordhollandse Meshanger cheese: a model for research on cheese ripening. 2. The ripening of the cheese
A. Noomen, Neth. Milk Dairy J. 31 (1977): 75-102.

After the method of making the cheese had been reconstructed several important properties of the manufacturing process were studied. From these investigations it appeared that: 1) the moisture content of the cheese was of decisive importance; 2) the surface flora was not of crucial importance to pH regulation or to protein breakdown in the cheese; 3) the pH of the cheese was regulated by the growth of very slow acid-producing lactic acid bacteria, whose activity depended on their salt resistance and the storage temperature of the cheese, the speed of penetration of salt into the cheese which was influenced by the moisture content of the cheese, and the final salt concentration in the cheese moisture; 4) protein breakdown in the cheese could be attributed to the calf rennet used; 5) the weakening of the cheese body was very closely related to the protein breakdown under the existing conditions in the cheese.

The crucial importance of the proteolytic activity of calf rennet in cheese to the consistency of the cheese is discussed.

Noordhollandse Meshanger cheese: a model for research on cheese ripening. 3. Manufacture of the cheese on a small scale
A. Noomen, Neth. Milk Dairy J. 31 (1977): 103-108.

This article, the last one of the series, deals with the production of Noordhollandse Meshanger cheese on a small scale and describes the characteristics of the cheese when it is ripe and ready for eating.

A rapid method for the estimation of the dissolved and the undissolved nitrogen compounds in cheese
A. Noomen, Neth. Milk Dairy J. 31 (1977): 163-176.

The consistency of cheese is related to the undissolved N components of the cheese. This article deals with the estimation of the undissolved N compounds in cheese, based on an extraction method for estimating the dissolved N compounds, products of protein degradation and milk serum proteins. Difficulties were encountered with the clarification by centrifugation and filtration of extracts, prepared by extracting the cheese with a liquid with a composition comparable with that of cheese moisture. For that reason the possibility of an alternative clarification method was studied.

The aim was satisfactorily achieved by homogenizing the cheese under standardized conditions at 30 °C in a 0.037 *M* $CaCl_2$ solution with the aid of an Ultra Turrax. The pH of the extraction mixture was adjusted to 7.5. After centrifugation for 10 min at 40 000 *g* at 30 °C and filtration through paper, the quantity of nitrogen in the filtrate was estimated.

The method is simple and rapid, so that any further degradation of protein during the extraction is prevented. The results obtained with the method give a fair representation of the amount of N compounds soluble in cheese moisture. Moreover, they compare well with those obtained on the overall breakdown of α_{s1} and β casein in cheese as measured by quantitative polyacrylamide gel electrophoresis.

Activity of proteolytic enzymes in simulated soft cheeses (Meshanger type). 1. Activity of milk protease
A. Noomen, Neth. Milk Dairy J. 32 (1978): 26-48.

The activity of milk protease in soft cheese was studied with simulated cheeses, wherein milk protease was acting under conditions (ratio protein to water, pH, salt content, etc.) which approached as nearly as possible those existing

in Noordhollandse Meshanger cheese during its ripening. The cheeses were prepared with 1) rennet-free cheese curd, or with 2) a rennet-free calcium paracaseinate–calcium phosphate complex. Both preparations contained the milk protease already present in the milk from which they were made. Protein breakdown was followed by quantitative polyacrylamide gel electrophoresis and by the estimation of the amounts of soluble nitrogenous compounds liberated. In almost all tested conditions, the results of both methods for the total degradation of protein were well correlated.

Apart from differences in a quantitative sense, in all cheeses milk protease acted in a similar manner in corresponding test conditions. Quantitative differences may have been caused by e.g. differences in protease activity, protease content, the substrates, etc.

Enzyme activity appeared to be strongly influenced by the pH, the NaCl concentration in the cheese moisture and the ripening temperature of the cheese.

Protein breakdown was most extensive in cheeses with a low acidity. Whereas at a high pH (e.g. pH 6.2) β casein was much more quickly degraded than α_{s1} casein, at a low pH (e.g. pH 5.4) α_{s1} casein was attacked somewhat more than β casein. It is suggested that in addition to alkaline milk protease an acid protease, preferably acting on α_{s1} casein, under favourable conditions contributes to protein breakdown in certain soft cheeses.

Enzyme activity against both caseins was stimulated by low concentrations of NaCl in the cheese moisture, but was reduced by high concentrations. Under the conditions tested, maximum breakdown was found at about 2 % NaCl.

At pH 6.0, both α_{s1} and β casein were increasingly degraded at higher temperatures within the range 5 - 37 °C. At pH 5.2, the degradation of α_{s1} casein showed a similar tendency, whereas β casein degradation remained at a constant and low level at temperatures above 20 °C.

In view of the conditions in a ripening Meshanger cheese and the activity of milk protease observed under comparable conditions in the simulated cheeses, the contribution of milk protease to protein breakdown in this cheese is considered to be of little importance for normal ripening (see also Part 2 of the article). This contribution may possibly be more important in soft cheeses with a surface flora, showing ripening conditions which favour in particular the activity of alkaline milk protease.

Activity of proteolytic enzymes in simulated soft cheeses (Meshanger type). 2. Activity of calf rennet
A. Noomen, Neth. Milk Dairy J. 32 (1978): 49-68.

The proteolytic activity of calf rennet in soft cheese was studied with simulated cheeses under various conditions. The cheeses were prepared with 1) a calcium

paracaseinate–calcium phosphate complex, made from milk which had been freed from milk protease activity by a heat treatment, or with 2) a calcium paracaseinate–calcium phosphate complex prepared from low-temperature pasteurized milk, or with 3) rennet-free cheese curd. The latter two cheeses contained milk protease. The cheeses contained about 2.2 ml of rennet per kg of protein, being approximately 50 % more than the concentration in Meshanger cheese in a ripe condition. Protein breakdown was studied by quantitative polyacrylamide gel electrophoresis and by the estimation of the amount of soluble nitrogenous compounds formed. The results of both methods for the total degradation of protein were well correlated.

Apart from differences in a quantitative sense, rennet acted in a corresponding manner in the different cheeses in comparable test conditions and degraded the protein very extensively under favourable circumstances. For example, in cheeses of pH 5.0 and containing 4 % NaCl in the moisture, 35 to 50 % of the total protein (65 to 90 % of the α_{s1} casein) became degraded in two weeks at 13 °C. Under all conditions, rennet degraded α_{s1} casein much more than it did β casein.

The maximum activity of rennet against α_{s1} casein was found at a pH near to 5.0; optimum pH for β casein breakdown became not clear. Total breakdown was maximal at pH 4.9 - 5.0.

The degradation of α_{s1} casein was stimulated by NaCl concentrations in the moisture up to about 4 %, and retarded by higher salt contents. The breakdown of β casein was maximal in the absence of NaCl and was already considerably reduced at low salt contents. This may explain our observations that the total breakdown of protein decreased with increasing salt content.

Under the conditions tested, rennet did not liberate amino acids in any detectable amount.

Under the conditions of a ripening Meshanger cheese, rennet degrades α_{s1} casein so extensively that it causes the main ripening of the cheese. Since it has been also established that the changes of consistency of the cheese are strongly related to the degree of degradation of α_{s1} casein, the characteristic and complicated way of softening of the cheese, which proceeds from the inside to the outside, can be explained. During the ripening of the cheese the most favourable conditions for the action of rennet against α_{s1} casein (pH, NaCl content) are those which are most rapidly present in the centre of the cheese. β casein is only slightly degraded in Meshanger cheese.

The results of the investigation strongly supported our conception that rennet is just as crucial in the ripening of other cheeses with a high moisture content. This concerns cheeses without a surface flora such as Butterkäse and St. Paulin cheese as well as those with a surface flora such as Kernhem Brie, Camembert and Limburger cheese.

Proteolytic activity of milk protease in raw and pasteurized cow's milk
A. Noomen, Neth. Milk Dairy J. 29 (1975): 153-161.

A sensitive method is described for the estimation of proteolytic activity of milk protease in cow's milk. Samples of aseptically drawn milk with colony counts of less than 30/ml showed considerable proteolysis when incubated anaerobically for 3 or 6 days at 37 °C. Proteolytic activity was increased by 30 - 40 % when milk was pasteurized for 15 s at 72 °C and by 8 - 24 % when a heat treatment for 30 min at 63 °C was applied. Results obtained with the method always correlated very well with those found by PAE analysis. Both α_s and β casein were attacked by milk protease; β casein was degraded two to three times faster than α_s casein. It is suggested that the contribution of milk protease to protein breakdown in cheese is of much more interest than it is frequently thought to be. Experiments are continuing in this direction.

Discussion and conclusions

1 Regulation of pH in Meshanger cheese

In traditional production of Meshanger cheese the curd was not washed. So the freshly prepared cheese would contain much lactose. However, lactic acid fermentation did not result in an acid and crumbly cheese core, except as a defect. Therefore, in reconstructing the making of the cheese, primary attention was paid to the mechanism of pH regulation.

Several factors are involved in the regulation of pH in the reconstructed cheese:

- lactic acid is formed slowly in the cheese, because of slow acid-producing properties of the starter bacteria, their sensitivity to salt, the amount of starter added and the ripening temperature of the cheese
- the cheese must be salted immediately after pressing
- the moisture content of the cheese must be high, allowing rapid diffusion of salt through the cheese, so that even in the centre the concentration of salt in the moisture phase soon retards growth of starter bacteria
- the final salt concentration in the moisture phase
- the size of the cheese
- the continuously decreasing pH during ripening, which influences the rate of growth of starter bacteria.

The contribution of the surface flora to pH regulation during normal ripening was negligible.

Starter bacteria develop under continuously changing and locally different concentrations of salt in the moisture phase. By diffusion, the initially high concentration at the outside decreases and that in the core increases until the salt is uniformly distributed. Because of the sensitivity of the starter bacteria to salt, lactic acid fermentation proceeds most rapidly at the centre during the first days of ripening. The pH at the centre most quickly reaches values at which growth of starter bacteria is checked. At the end of ripening, pH tends to become uniform (Fig. 1).

2 Extraction method to estimate protein breakdown

In principal, an extraction method to estimate nitrogen compounds soluble

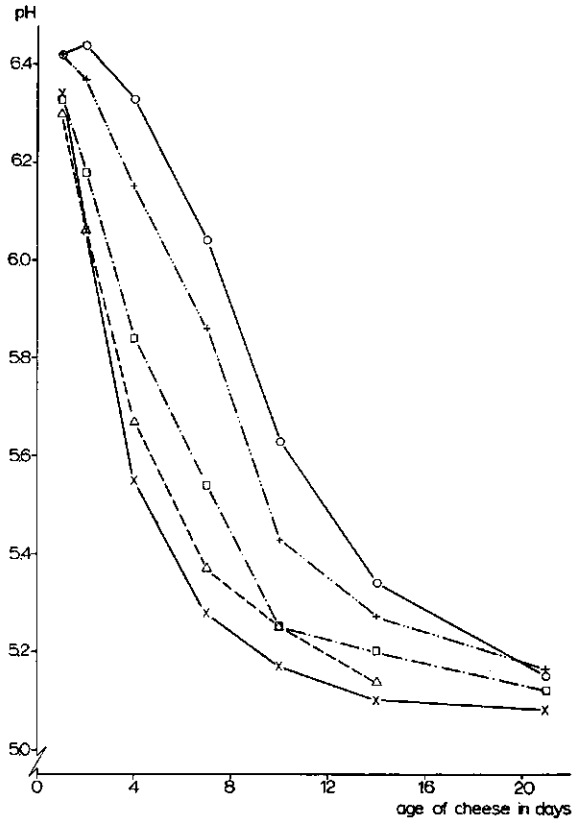

Fig. 1. Development of pH in horizontal layers of Meshanger cheese during its ripening. (○——○): outer 2 mm of the cheese; (+— . . —+), (□— . —□) and (△- - -△): successive layers of each 5 mm of cheese; (×——×): centre of the cheese.

in the moisture phase of cheese requires that the conditions of the extract correspond as closely as possible with those in cheese moisture. So extraction conditions were adapted in the most essential points (pH, temperature, calcium and sodium concentrations). However, the extracts obtained this way were turbid, because of the presence of dispersed intact protein and, to a lesser extent, insoluble products of protein breakdown. The extracts had to be clarified. The method finally chosen was selected because equal amounts of soluble nitrogen compounds are found in a particular cheese at any pH above 7. The method was applicable to all varieties of cheese. Results for total breakdown of protein obtained with the extraction method were well correlated with those of the electrophoretic method.

3 Rennet in the ripening of soft cheese

Protein breakdown in Meshanger cheese was predominantly due to calf ren-

net. Changes in consistency were related to protein breakdown, but also to physical and chemical conditions in the cheese. In soft cheeses with an initially very low pH ripening proceeds inwards, supposedly under the influence of extracellular proteolytic enzymes of the surface flora. These enzymes would enter the cheese by diffusion (1, 2). However, in our laboratory, it was observed that the diffusion of enzymes in cheese, even in Meshanger cheese with its very high content of moisture (60 %), proceeds slowly (3). The tests with simulated soft cheese revealed that calf rennet degrades caseins in cheese with a low pH and a crumbly consistency, as in softer cheese with a higher pH. The physico-chemical conditions apparently determine whether cheese softens or not. The ripening, for instance, of Camembert cheese may therefore be due to regulation of the pH by the surface flora, which also gives the cheese a specific taste and flavour. Protein breakdown in the cheese would merely be due to rennet and proteolytic enzymes of lactic acid bacteria.

4 pH and NaCl in rennet activity

The simulated cheeses, made up from calcium paracaseinate–calcium phosphate complex, water, lactic acid and NaCl or with chemically acidified and salted aseptic cheese curd, proved convenient to study the proteolytic activity of calf rennet at acidities and salt concentrations that may prevail in Meshanger cheese. Electrophoretic patterns of the different cheeses were comparable and also resembled the picture of proteolytic processes in normal Meshanger cheese. Optimum pH for rennet action was close to 5.0 for α_{s1} casein; for β casein it became not clear. Total breakdown was maximum at pH 4.9 - 5.0. These results differed markedly from those observed by other workers in tests under conditions far remote from those in cheese and with other methods of estimation of proteolytic activity (4, 5, 6, 7, 8, 9). Breakdown of α_{s1} casein was stimulated by low concentrations of NaCl and retarded by high concentrations, whereas the breakdown of β casein was retarded considerably, even by low concentrations. Total breakdown of protein decreased with increasing salt content.

During most of the ripening of Meshanger cheese, conditions in the simulated cheeses would favour extensive breakdown by rennet of α_{s1} casein, with which changes in consistency were strongly related (10).

Extrapolation of the results to conditions (pH, NaCl) in different parts of the normal ripening cheese indicated that rennet would act against α_{s1} casein most quickly in the centre of the cheese, so explaining its characteristic way of softening from the centre outwards. Rennet degrades β casein weakly in Meshanger cheese.

5 Activity of milk protease in simulated cheese and milk

As studied with simulated soft cheeses made up with rennet-free cheese curd and a rennet-free calcium paracaseinate–calcium phosphate complex, the contribution of milk protease to protein breakdown in certain soft cheeses may be far from negligible (Fig. 2). It depended on pH, concentration of NaCl in the moisture phase, time and temperature of ripening, and will also be determined by the protease activity of the cheese milk. Activity of alkaline milk protease may be considerable in soft cheeses ripening with a surface flora, and showing a high pH in the outer layers for some weeks of ripening.

The method developed to estimate protein breakdown in milk proved much more sensitive than the tyrosine method and was also superior to the occasionally used method at which protein breakdown by milk protease was estimated by the increase in nitrogen in TCA filtrates (11, 12, 13).

Contrary to the generally assumed weak activity of milk protease in milk, our experiments revealed that activity of milk protease may be considerable in milk stored at 37 °C. The activity varied between milks of different cows. Surprisingly, protein breakdown in milk was stimulated in milk subjected to a vat pasteurization for 30 min at 63 °C and, even more so, in milk pasteurized for 15 s at 72 °C. Perhaps enzyme activity was stimulated. Alternatively, the activity of milk protease as such could be partly destroyed by pasteurization

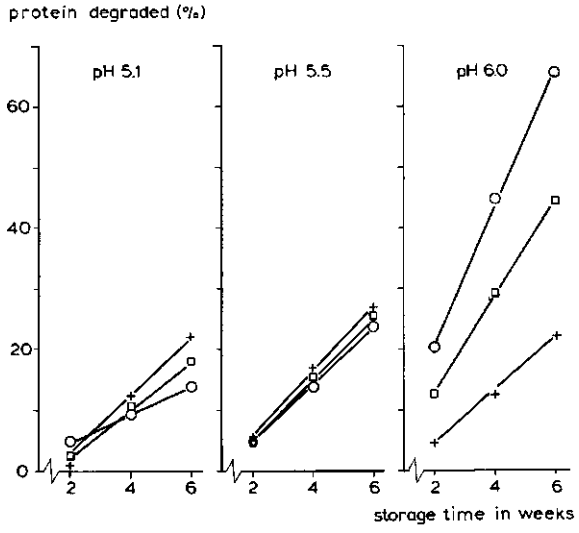

Fig. 2. Densitometric evaluation of protein breakdown by milk protease in simulated soft cheeses, made up with a rennet-free calcium paracaseinate-calcium phosphate complex, depending on the pH and the storage time at 13 °C. The cheeses contained 4% NaCl in the moisture. (+): α_{s1} casein; (○): β casein, and (□): $\alpha_{s1} + \beta$ casein.

but protease inhibitors in the milk (e.g. 14, 15, 16) could be inactivated even more, so that more protein would be degraded.

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Summary

The ripening of soft cheese was studied with particular reference to the question which proteolytic enzymes were predominantly involved in the primary degradation of protein and the changes in cheese consistency related to that.

Noordhollandse Meshanger cheese, an extinct Dutch cheese with a high moisture content, served as a model in the investigation. The method of production of the cheese was reconstructed and its ripening was studied. The proteolytic activity of calf rennet and of milk protease under different physical and chemical conditions was studied with preserved simulated soft cheeses, composed with either calcium paracaseinate–calcium phosphate complex, water, lactic acid and NaCl or with chemically acidified and salted cheese curd. Milk protease activity in raw and pasteurized cow's milk was also investigated.

Protein breakdown was estimated by an extraction method, which gives a good estimate of the amount of N compounds soluble in the moisture of cheese, and by quantitative polyacrylamide gel electrophoresis. Results for total breakdown of protein obtained with both methods were well correlated.

The regulation of pH in Meshanger cheese was found to be decisive to the way the cheese body softened from the centre outwards. It was mainly determined by the growth of starter bacteria producing lactic acid slowly, the high moisture content and the salting process of the cheese.

Results for proteolysis in the simulated cheeses were comparable and differed only quantitatively. The picture of proteolytic processes resembled that in normal Meshanger cheese.

Protein breakdown in Meshanger cheese, which was closely related to the development of consistency, was caused by calf rennet. The rennet degraded α_{s1} casein far more than β casein. Its maximum activity against α_{s1} casein was found at a pH near to 5.0; against β casein it became not clear. The total degradation of protein was maximum at pH 4.9 - 5.0.

Concentrations up to about 4 % NaCl in the cheese moisture stimulated the degradation of α_{s1} casein, whereas higher concentrations had an inhibitory effect. The breakdown of β casein by rennet was maximum in the absence of NaCl and was reduced even at low salt contents. The total breakdown of protein decreased with increasing NaCl concentrations. It is postulated that the primary degradation of protein in soft cheeses with an initially very low

pH that show visual ripening from the outside to the inside under the influence of a surface flora, are caused by rennet enzymes and by proteolytic enzymes of the internal flora of lactic acid bacteria. The role of the surface flora is to lower the acidity of the cheese and so make the cheese body soft, and to give the cheese a specific taste and flavour.

The contribution of milk protease to protein breakdown in soft cheese is determined by pH, NaCl concentration in the cheese moisture, ripening temperature and ripening time of the cheese, and by protease activity of the cheese milk used. Results suggested that besides alkaline milk protease an acid protease may be active in cheese, which contrary to the former degrades α_{s1} casein preferably to β casein. The degradation of both caseins was stimulated by low concentrations of NaCl, maximum breakdown being found with 2 % NaCl in the moisture under the conditions tested. At a high pH both α_{s1} and β casein were increasingly degraded at higher temperatures up to 37 °C (the highest temperature tested). At a low pH, the breakdown of α_{s1} casein showed a corresponding behaviour, whereas β casein was no further degraded at temperatures above 20 °C. Milk protease is of minor importance to proteolysis in soft cheeses, except perhaps certain cheeses with a surface flora and with conditions favourable to the activity of alkaline milk protease.

Milk protease may considerably degrade the protein in milk at a favourable temperature (e.g. 37 °C). The proteolytic activity varies between milks of different cows. Compared to that in raw milk, protein breakdown by milk protease is increased in low-temperature pasteurized milk (30 min at 63 °C; 15 s at 72 °C).

III Proteolysis and consistency of Meshanger cheese

L. de Jong

Introduction

1 Introduction and motivation

In our laboratory Noordhollandse Meshanger cheese, a Dutch soft cheese serves as a model for studying cheese ripening (1, 2, 3). The cheese is characterized among other things by its fast ripening (about 14 days), not complicated by the action of a surface flora, and a soft, smooth, consistency, salient for the mature cheese. Prolonged ripening leads to the ultimate liquefaction of the cheese.

The interest for Meshanger cheese in our laboratory has been stimulated by the necessity to have a system available to study the processes involved in the protein ripening of cheese traditionally experienced as important. During the investigations it became clear that it could very well serve as model to study the relation between protein breakdown and cheese consistency.

The salient softening of Meshanger cheese differs in an important and principal way from that of well known soft cheeses as Camembert and Brie cheese. The latter have a change in consistency from the outer parts to the inner part of the cheese, whereas Meshanger starts to soften in its centre, proceeding to the rind.

The consistency of cheese can be expected to be regulated by its moisture content and the properties of the only solid continuous phase in cheese, the protein matrix. The discontinuous globular fat, which has some influence on the consistency of Meshanger cheese just after preparation, is not likely to contribute to its softening.

The preparation of cheese, in essention a process in which the larger part of the milk dry matter is concentrated, involves the conversion of $\varkappa$-casein by chymosin. Due to this conversion and in the presence of sufficient Ca^{2+} the protein matrix mentioned above is constructed from the paracasein micelles. A three dimensional protein network forms in which the milk fat globules are embedded. The mass contracts and extrudes whey. The conditions during these steps of cheesemaking determine the properties that develop during ripening. The properties of the constructional components, the paracasein micelles, composed of submicelles consisting mainly of α_{s1}-, β-, and para-$\varkappa$-casein molecules (4), largely determine the properties of the matrix. Those properties depend on physico-chemical conditions which start to change

markedly as soon as the cheese is formed.

The first drastic change for the paracasein micelles is a drop in pH, from 6.7 to 5.2 – in some cases even to 4.6 – by conversion of lactose by starter bacteria into lactic acid. This reduces the charge of the protein molecules and causes the solution of calcium and calciumphosphate complexly bound to the protein (5). Despite those drastic changes the system remains intact as a viscous gel, which even contracts further. Neither does the increase in the concentration of Na^+ and Cl^- in the moisture phase by salting of the cheese cause visible changes.

These changes in pH and ion concentration occur mainly in the first week of ripening of Meshanger cheese; we still have a green cheese. This green cheese, however, turns in the next week, from a stiff salty rigid clump to a soft creamy mass with a slightly acid taste.

With this information in mind the purpose of the research was formulated: collect data from Meshanger cheese and other model systems to explain the changes in consistency during ripening. It shall be understood that this assumes a close relation between the breakdown of the components of the protein matrix, viz the different casein molecules, the structure of this protein gel and its outward appearance, the cheese consistency.

2 Method of assessing consistency

A criterion was needed to estimate consistency in an easy and reproducible way. Penetrometer methods seemed attractive but proved impracticable because the cheese was not uniform. A gauge that could measure the force needed to extrude a rather large piece of cheese through a grid consisting of parallel bars proved more practical: the Allo Kramer shear press (6). It indicated not only the softening of the cheese during ripening but also the differences in consistency between the inside and outside.

3 Electrophoresis

To establish the relation between proteolysis and consistency, it seemed better to estimate protein breakdown from the decrease in the content of the original components of the protein matrix of the cheese rather than by the increase in soluble nitrogen compounds. The latter method was usable for other purposes. Therefore a polyacrylamide gel electrophoresis method was elaboreted with which the caseins and their decomposition products are separated. Stained protein in the bands was estimated from absorption of radiation at wavelength 600 nm.

4 Techniques for studying microstructure

The changes in structure of the cheese protein matrix were studied by light and by electron microscopy. For the former fluorescence and interference-contrast techniques, as frequently employed in our laboratory, was used (7). The latter was done with an embedding and with a freeze-fracturing technique with assistance from the Technical and Physical Engineering Research Service (TFDL), Wageningen, the Netherlands.

5 Approach

With the techniques briefly summarized above, the ripening of Meshanger cheese was studied. In some tests the preparation of the Meshanger cheese was modified to obtain cheeses with less moisture. These trials were designed to collect data that would make the results obtained with cheeses with high moisture contents applicable to (semi)hard cheese.

Although Meshanger cheese proved suitable for study of cheese ripening processes, the system was too complicated for some purposes. For instance to get a quick impression of the influence of pH, and concentrations of Na^+ and Ca^{2+} on decomposition of caseins by rennet, suspensions of salts of the proteins incubated with enzymes were considered to be better usable as compared to whole cheeses if only because of the simplicity of the fixation of the required substrate conditions. The disadvantages of the use of suspensions to check certain aspects of casein proteolysis by rennet will be commented in the chapter 'Discussion and Conclusions'.

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Summaries of the articles

A quantitative electrophoretic method of studying cheese ripening
L. de Jong, Neth. Milk Dairy J. 29 (1975) 162-168.

Gel electrophoresis is frequently used as a qualitative method for the evaluation of cheese ripening. This paper describes a quantitative variant of polyacrylamide gel electrophoresis (PAE). It was demonstrated that, if used in the way described, PAE combined with a suitable densitometric method yields information on the proportions of non-soluble nitrogenous substances in cheese.

Protein breakdown in soft cheese and its relation to consistency. 1. Proteolysis and consistency of 'Noordhollandse Meshanger' cheese
L. de Jong, Neth. Milk Dairy J. 30 (1976) 242-253.

This paper on the relation between proteolysis and consistency of soft cheese deals with the softening of 'Noordhollandse Meshanger' cheese. The ripening of this cheese is uncomplicated, which makes it suitable to serve as a model for this study. The proteolysis during the ripening was studied with the aid of polyacrylamide gel electrophoresis. The course of the consistency during ripening is discussed as well as the relation between consistency and the breakdown of α_{s1}-casein.

Protein breakdown in soft cheese and its relation to consistency. 2. The influence of the rennet concentration
L. de Jong, Neth. Milk Dairy J. 31 (1977) 314-327.

The relation between the breakdown of α_{s1}-casein, the proportion of enclosed rennet and the consistency of Meshanger cheese was studied. It was demonstrated that a linear relation exists between the rennet concentration and the decomposition rate of α_{s1}-casein during the ripening of Meshanger cheese.

The relation between α_{s1}-casein breakdown and changes in consistency is also described. In cheese without α_{s1}-casein breakdown no softening occurs. It is emphasized that views with respect to the softening of cheese being caused by the action of proteolytic enzymes of its surface flora are very questionable.

The softening of cheeses with high moisture contents must primarily be attributed to the decomposition of α_{s1}-casein by rennet.

Protein breakdown in soft cheese and its relation to consistency. 3. The micellar structure of Meshanger cheese
L. de Jong, Neth. Milk Dairy J. 32 (1978) 15-25.

The structure of Meshanger cheese was studied using fluorescence and interference-contrast microscopy as well as electron microscopy. Structural changes in the protein matrix during the ripening are described and compared with those in other soft and in hard cheeses. It is indicated that there are no visible differences in structural changes during the ripening of soft and hard cheese. The consequences concerning cheese ripening and casein micelle structure theory are discussed.

The proteolytic action of rennet on different casein substrates under various conditions
L. de Jong & Aliza E. A. de Groot-Mostert, Neth. Milk Dairy J. 31 (1977) 296-313.

The decomposition of α_{s1}- and β-casein by rennet in a substrate containing sodium paracaseinate was determined using a quantitative gel electrophoretic method. The conditions during incubation were varied with respect to pH, and Na and Ca content. To obtain more information on the influence of added Ca, experiments were performed with calcium paracaseinate-calcium phosphate complex and a similar 'synthetic complex' prepared from acid precipitated casein. The effects of pH, $[Na^+]$ and $[Ca^{++}]$ were considerable and mutually dependent. The results obtained did not permit conclusions to be drawn on the mechanism of the stimulation and inhibition of the proteolytic reaction by Ca, although the results are important for the elucidation of the decomposition of the cheese protein.

The influence of the moisture content on the consistency and the protein breakdown of cheese
L. de Jong, Neth. Milk Dairy J. 32 (1978) 1-14.

Cheeses with moisture contents between 40 and 60 % were prepared using methods derived from the production method for Meshanger cheese. The protein breakdown during the ripening of these cheeses is discussed, as well as the change in the firmness. The relation between the volume fraction of the protein in the fat-free cheese (φ_p) and the firmness is given. Attention is paid to the possibility of the chymosin molecule diffusing in the moisture of the

cheese, which is thought to be composed of globulair fat and protein particles. It was concluded that the transport of chymosin in the cheese moisture is very small compared with that of Na and Cl ions in the same medium.

Discussion and conclusions

1 Proteolysis in Meshanger cheese

The electrophoretic separation of the different caseins in cheese on polyacryl amide gels, followed by a densitometrical estimation, proved satisfactoryly for the qualitative and quantitative reproduction of the decomposition of α_{s1}- and β-casein during cheese ripening.

After a slow start, which may be due to the high pH at the beginning of ripening of Meshanger cheese, the decomposition of α_{s1}-casein accelerated until about the 8th day. After about 2 weeks half had disappeared. Conversion into α_{s1}-I (1), the primary decomposition product of α_{s1}-casein, was completed after about 30 days. The α_{s1}-I was further decomposed at a slower rate and at 30 days still much was left in the cheese. Decomposition of β-casein was fast during the first days of ripening, becoming negligible after 5 days. The breakdown product with an electrophoretic mobility only slightly higher than that of the original β-casein is likely to be β-I (2). Significant changes in the protein fractions with an electrophoretic mobility less than β-casein were not observed.

The fastest moving breakdown products increase in amount during the ripening. These are presumably products formed by the decomposition of α_{s1}-I.

Normally electrophoresis is under alkaline conditions in the presence of urea. The caseins and their decomposition products mentioned above then have a negative charge and move towards the positive pole. Electrophoresis with reversed current yielded a slowly moving diffuse band that increased with ripening. This positive charged product was not further identified.

2 Consistency

The course of the change of the firmness of the Meshanger cheese during the ripening (Fig. 1) estimated with the Allo Kramer shear press may be remarkable at first sight; however, it is very well explainable. The increase in firmness just after preparation will be due to

- decrease in moisture content,
- salt uptake,

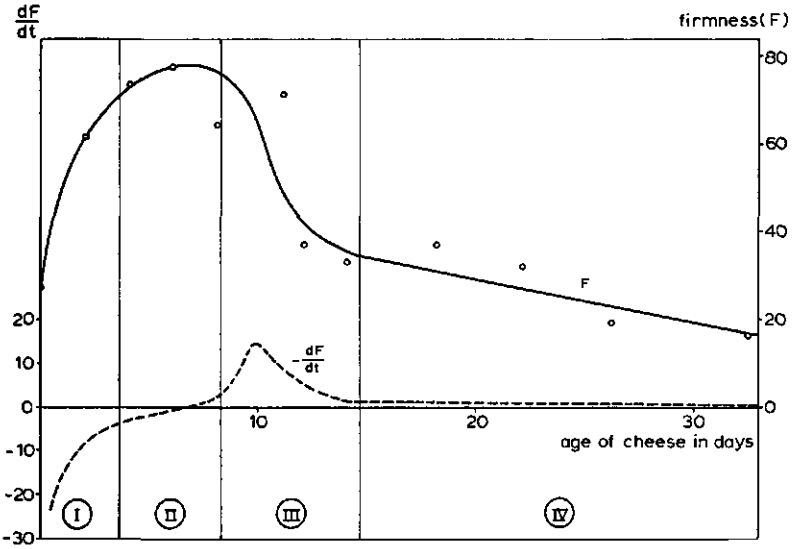

Fig. 1. The course of the firmness during the ripening of Meshanger cheese.

– decrease in pH,
– solidification of the fat as the cheese cools from production temperature (30 °C) to the temperature of the ripening room (13 °C).

After a maximum it decreased rather rapidly. This softening of the cheese is attributable to the decomposition of the protein matrix of the cheese, and especially to the breakdown of the α_{s1}-casein component, by the chymosin present in the Dutch commercial rennet used.

3 Trials with different rennet concentrations

To collect more information on the relation between the proteolysis of α_{s1}-casein and the development of cheese consistency, Meshanger cheese was made with different amounts of enclosed rennet. Cheeses without detectable active rennet were prepared by a technique developed in our laboratory (3). In cheeses without rennet, no α_{s1}-casein was broken down and in the cheeses with increasing content of rennet breakdown increased proportionally. Except at the very beginning of the ripening when large changes in pH and NaCl concentration occur, decomposition of α_{s1}-casein was proportional to firmness.

4 Microstructure

By light microscopy at magnification 2200-3400 changes in the protein struc-

ture of soft cheese could not be distinguished from those of hard cheese. They were characterized as a transition from a network of separate particles into a smooth uniform mass without recognizable structure.

Electron-micrographs at magnification 10 500, 34 000 and 70 000 revealed a more detailed structure. The paracasein micelles remained visible from just after the preparation of the cheese until 7 days of ripening but lost their globular shape. The position of milk fat globules was clearly visible (Fig. 2). In the mature cheese, the micelles completely disappeared, only particles with a diameter an order of magnitude less than casein micelles remained visible. These structural changes are identical with those reported during the ripening of Camembert cheese (4). It seems reasonable to attribute softening of Camembert cheese to the same process as in Meshanger cheese: breakdown of α_{s1}-casein by rennet enzymes. Older opinions that the softening of Camembert cheese be due to the proteolytic enzymes from the surface flora

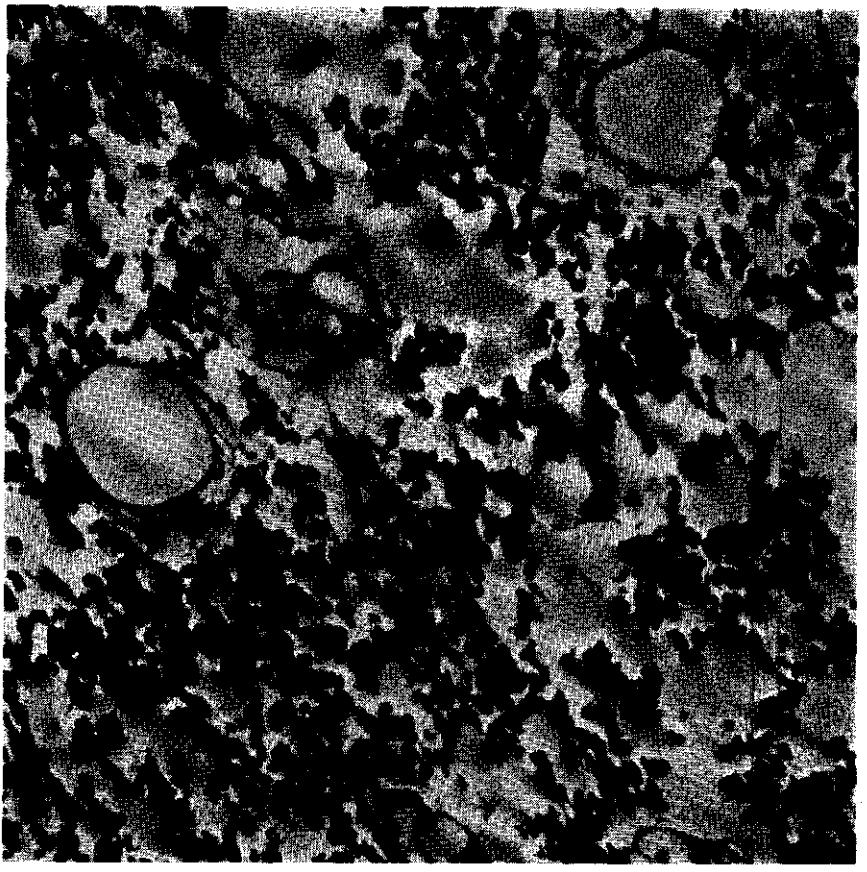

Fig. 2. Meshanger cheese after 7 days ripening. Magnification × 10 500. Electronmicrograph by TFDL.

(e.g. 4) seem questionable moreover because the diffusion of those enzymes into the cheese is very difficult as is indicated below.

5 Proteolysis in synthetic models

In substrates with sodium paracaseinate as protein constituent was no difference between proteolysis caused by Dutch commercial rennet and that caused by the enzyme preparation extracted from the abomasum of newly born calves. By adjusting the pH and the NaCl concentration of those substrates optima for the proteolytic reaction of rennet on sodium paracaseinate were found. Also the influence of Ca was tested. It affected proteolytic breakdown of both α_{s1}-casein and β-casein. To elucidate the processes involved, tests were set up with calcium phosphate-calcium paracaseinate instead of sodium paracaseinate and also with a synthetic complex of calcium caseinate and calcium phosphate (5, 6). Even then the exact mechanism of the mutual influences between Na^+, Ca^{2+} concentrations and proteolysis could not be fully explained. Proteolysis of caseins under different conditions is difficult to study in such suspensions, since the state of protein is not known exactly, so that data are difficult to interpret in connection with cheese ripening.

6 Moisture content and consistency

To see whether the results with Meshanger cheese were applicable to the ripening of other cheeses, cheeses with lower moisture contents were prepared. Proteolysis was the same in cheeses with different moisture contents and consistency of those cheeses was determined primary by the volume fraction of protein matrix in the fat-free cheese.

7 Diffusion of enzymes in cheese

To get an impression of the kinetics of the proteolysis in cheese, the diffusion of chymosin was calculated with a simplified theoretical model (7). In that model the radius of the chymosin molecule was so large relative to the pores in cheese that transport would be impeded.

In test to detect the transport of alkaline phosphatase in soft cheese (8) was revealed that compared to NaCl (7), the pseudo diffusion coefficient of NaCl (about 20 mm^2/day) was much greater than of phosphatase (0.4 mm^2/day).

As the molar mass of chymosin and alkaline phosphatase are similar, their transport equations may be assumed to be similar. This further indicates that diffusion of chymosin in cheese will be difficult and may explain the differences in breakdown rates estimated in cheese and in liquid substrates. Presumably enzymes from a surface flora could only diffuse slowly into a cheese.

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Summary

The relation was studied between proteolysis and consistency of cheese, using Noordhollandse Meshanger cheese as model system. This cheese, redeveloped in our laboratory, is a soft cheese that ripens in 2 weeks and totally liquefies in 4 weeks.

Consistency of cheese was thought to be regulated by the only solid continuous phase in cheese, the protein matrix. This matrix is formed, if sufficient Ca^{2+} is present, when $\varkappa$-casein is converted by, for instance, chymosin to para-$\varkappa$-casein. The matrix consists of paracasein micelles, constructed from submicelles, mainly composed from α_{s1}-, β- and para-$\varkappa$-casein and calcium phosphate.

The changes in this matrix during ripening were studied by the following techniques.

- By polyacrylamide gel electrophoresis, the amounts of unattacked proteins were estimated.
- By an extrusion method, consistency was estimated.
- With light and electron microscopy, the structure of the matrix was studied.

The electrophoretic method, as adapted, was suitable to estimate proteolysis of caseins in cheese.

The breakdown of α_{s1}-casein during ripening of Meshanger cheese proceeded slowly during the first days. In the next 10 days it accelerated. After 30 days, all α_{s1}-casein was converted mainly into α_{s1}-I, the breakdown product characteristic for rennet proteolysis of α_{s1}-casein. The breakdown of β-casein first proceeds faster than that of α_{s1}-casein; after 5 days, however, it became nearly negligible.

The starter used showed hardly any proteolytic activity.

Cheese, prepared by a method developed in our laboratory, without active rennet enclosed showed no breakdown of α_{s1}-casein. Breakdown was proportional to rennet concentration in the cheese. Changes in consistency were related to the amount of unattacked α_{s1}-casein.

Changes in the structure of the protein matrix appeared to be identical with those reported for (semi)hard cheese. More detailed pictures by electron-microscope revealed the same, suggesting that the changes in structure were similar in Meshanger and Camembert cheese.

Softening of all cheese of high moisture content can be attributed to breakdown of α_{s1}-casein by rennet. A major role of proteolytic enzymes from the surface flora is unlikely.

Calculations suggested that the diffusion of chymosin in cheese moisture was negligible, as the radius of the chymosin molecule is of the same order as that of pores supposed to exist in cheese.

Tests with alkaline phosphatase revealed that the pseudo diffusion coefficient in cheese moisture for this enzyme (0.4 mm^2/day) was considerable smaller than that of NaCl (20 mm^2/day) in the similar system. As chymosin and alkaline phosphatase have comparable molar masses, this is further indication of the difficult transport of chymosin in cheese.

For rapid detection of the influence of sodium and calcium ions on the proteolysis by rennet, substrates tested were sodium paracaseinate, calcium paracaseinate–calcium phosphate complex and a complex made up from casein, calcium hydroxide and phosphoric acid. Sodium and calcium influenced the reaction; the mutual influence was unexpected. The influence depended on the substrate used. The actual mechanism could not be described. This type of experiment can better be performed with model systems, in which the conditions are more similar to those in cheese.

Tests with cheeses lower in moisture showed that firmness was primarily depending on the volume fraction of protein in the fat-free cheese. Differences in breakdown rate between cheeses could not simply be attributed to differences in moisture content.

Samenvatting

De bereiding en de rijping van Noordhollandse Meshanger kaas, een zachte kaas met een hoog vochtgehalte die tot omstreeks 1940 in Noord-Holland werd gemaakt, is uitgebreid bestudeerd in ons laboratorium. Nadat de bereidingstechniek was gereconstrueerd bleek deze kaas zeer geschikt om te gebruiken als modelsysteem om de vervloeiing van kaas te bestuderen. De bereidingswijze is eenvoudig en de kaas rijpt zonder oppervlakteflora in ongeveer 2 weken, na 4 weken is de kaas geheel vervloeid.

Uitgegaan werd van de gedachte dat de consistentie-eigenschappen van kaas voornamelijk bepaald zullen worden door de eigenschappen van de eiwitmatrix, de enige continu fase in de kaas die vast is. Deze eiwitmatrix wordt gevormd indien, bij een voldoende hoge concentratie van calciumionen, de $\varkappa$-caseïne in melk door bijvoorbeeld stremsel wordt omgezet in para-$\varkappa$-caseïne. De matrix bestaat uit paracaseïnemicellen die op hun beurt weer opgebouwd zijn uit submicellen (voornamelijk bestaande uit α_{s1}-, β- en para-$\varkappa$-caseïne en calciumfosfaat).

De veranderingen die deze eiwitmatrix ondergaat tijdens de kaasrijping werden op drie manieren bestudeerd.

- Met behulp van een instrument waarmee de kracht gemeten kon worden die nodig is om een tamelijk groot stuk kaas door een rooster te persen werden de consistentie-eigenschappen bepaald.
- Met behulp van een kwantitatieve polyacrylamidegel-elektroforese-methode werd nagegaan welk deel van de oorspronkelijke eiwitmoleculen nog aanwezig was.
- Ten slotte werd de structuur van de eiwitmatrix bestudeerd met zowel licht- als elektronenmicroscopie.

Het bleek dat de door ons verder ontwikkelde elektroforesetechniek heel geschikt was voor het kwantitatief weergeven van de eiwitafbraak in kaas.

De afbraak van α_{s1}-caseïne tijdens de rijping van Meshanger verloopt de eerste dagen langzaam. Daarna volgt een periode van ongeveer 10 dagen waarin de afbraak sneller gaat. Na 30 dagen is alle α_{s1}-caseïne omgezet, voornamelijk in het afbraakprodukt dat karakteristiek is voor de proteolyse door stremsel: α_{s1}-I. De afbraak van β-caseïne verloopt in het begin sneller dan die van α_{s1}-caseïne, na ca. 5 dagen echter komt de afbraak praktisch tot stilstand.

Het gebruikte zuursel heeft nauwelijks proteolytische eigenschappen.

In kazen bereid volgens een op ons laboratorium ontwikkelde techniek waarin geen actief stremsel aanwezig was, werd geen afbraak van α_{s1}-caseïne waargenomen. Het bleek dat deze afbraak rechtevenredig was met de stremselconcentratie in de kaas en dat de consistentieveranderingen, nadat eerst een bepaalde maximale stevigheid was bereikt, weer evenredig waren met de hoeveelheid niet omgezet α_{s1}-caseïne.

De veranderingen in de structuur van de eiwitmatrix tijdens de rijping bleken overeen te komen met die in harde kaas gerapporteerd worden. Uit een meer gedetailleerd beeld dat verkregen werd met elektronenmicroscopie kon dezelfde conclusie getrokken worden. Bovendien kwamen de structuurveranderingen tijdens de rijping overeen met die zoals beschreven voor Camembertkaas.

Het lijkt er op dat het vervloeien van zachte kazen toegeschreven moet worden aan de omzetting van α_{s1}-caseïne door stremsel. Opvattingen waarin de proteolytische enzymen van een oppervlakteflora een belangrijke rol spelen zijn dubieus.

Berekeningen aan de hand van een theoretisch model toonden aan dat de diffusie van chymosine in kaas zeer traag verloopt omdat de straal van het chymosinebolletje ongeveer gelijk is aan de straal van de poriën die verondersteld werden in de kaas te bestaan.

Experimenten die uitgevoerd werden met alkalische fosfatase toonden aan dat de pseudo-diffusiecoëfficient voor dit enzym in kaasvocht (0,4 mm²/dag) veel kleiner is dan die voor NaCl in een dergelijk systeem (20 mm²/dag). Aangezien alkalische fosfatase en chymosine een overeenkomstig molecuulgewicht hebben is dit een nadere aanwijzing voor de moeilijke verplaatsing van chymosine in kaas.

Om snel na te gaan wat voor invloed o.a. natrium- en calciumionen hebben op de proteolyse door stremsel, werden experimenten uitgevoerd met substraten met natriumparacaseïnaat, calciumparacaseïnaat-calciumfosfaat-complex en een dergelijk complex samengesteld uit caseïne, calciumhydroxide en fosforzuur. Afgeleid kon worden dat zowel natrium als calcium een grote invloed hebben op het verloop van de proteolyse en bovendien dat het verloop van de reactie afhankelijk was van het gebruikte substraat. Het feitelijke mechanisme van de reactie kon niet worden opgehelderd, wel werd geconcludeerd dat voor dergelijke experimenten modelsystemen die de omstandigheden in kaas beter benaderen geschikter zijn.

De experimenten met kazen met een vochtgehalte lager dan dat van Meshanger leerde ons dat de stevigheid van deze kazen in de eerste plaats bepaald wordt door de volumefractie van het eiwit in de vetvrije kaas en tevens dat de verschillen in eiwitafbraaksnelheid tussen kazen niet eenvoudig toe te schrijven zijn aan verschillen in vochtgehalte.