Rheological properties of rennet-induced skim milk gels

Promotor:	dr.ir. P. Walstra, hoogleraar in de Zuivelkunde
Co-promotor:	dr.ir. T. van Vliet, universitair hoofddocent

P. Zoon

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op woensdag 30 november 1988
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

ABSTRACT

Zoon, P. (1988). Rheological properties of rennet-induced skim milk gels. Ph.D. thesis, Wageningen Agricultural University (139 pp, English and Dutch summaries).

Key words: rheology, casein, pH, temperature, calcium, phosphorus, sodium chloride, milk gels, artificial micelles.

The rheological properties of rennet-induced skim milk gels, which are viscoelastic materials, were studied under various conditions.

Dynamic and stress relaxation experiments were performed at small deformations of the gel network, whereas constant stress (creep) experiments were performed at large deformations. Stress relaxation moduli calculated from the dynamic moduli agreed fairly well with stress relaxation moduli determined by means of stress relaxation experiments, implying that true material properties were determined.

The effects of important variables, such as casein concentration, temperature, pH, calcium, phosphorus and ionic strength on the mechanical properties of the gels were studied. The results are discussed in relation to the types of bond present in the network. Special attention was paid to the time scale at which processes, especially relaxation of bonds, occurred.

STELLINGEN

1. In het temperatuurtrajekt tussen 20 en 40°C relaxeert een aan een lebgel opgelegde spanning sneller bij een hogere temperatuur.
   Dit proefschrift.

2. In het trajekt tussen pH 6,3 en 6,7 resulteert een lagere pH in een hogere endogene synaeresedruk. Dit moet waarschijnlijk worden toegeschreven aan een grotere reaktiviteit van de caseïnedeeltjes (Van den Bijgaart) en niet aan een geringere neiging tot relaxeren van de in het netwerk opgebouwde spanning (dit proefschrift).
   H.J.C.M. van den Bijgaart, Syneresis of rennet-induced gels as influenced by cheesemaking parameters, Ph.D. thesis, Wageningen Agricultural University, the Netherlands (1988).

3. Pilosof et al. gaan eraan voorbij dat voor de bepaling van de schijnbare viscositeit de afschuifsnelheid een goed gedefinieerde parameter moet zijn.
   A.M.R. Pilosof, M.C. Lopez de Ogara & G.B. Bartholomai, J. Texture Stud. 17(1986)347.

4. Bij het verrichten van wetenschappelijk onderzoek aan levensmiddelen dient er voldoende rekening mee te worden gehouden dat de afzonderlijke komponenten elkaars werking vaak beïnvloeden.

5. De veronderstelling van Lindahl dat aan het afvalwater van de zetmeel-glutenindustrie dezelfde eigenschappen toegeschreven kunnen worden als aan het door hem uit tarwe geïsoleerde wateroplosbare materiaal is aan bedenkingen onderhevig.
   L. Lindahl, J. Dispersion Science and Technology 8(1987)309.

6. Het optreden van konvektiestroming in yoghurtkweektanks als gevolg van een verschil in temperatuur tussen de omgeving en de inhoud van de tank kan kluitjesvorming in yoghurt veroorzaken.

7. Bij de door Chevalier et al. uitgevoerde experimenten wordt onvoldoende rekening gehouden met mogelijke interakties tussen immunoglobuline en de carrageen- en alginaatmatrix.
   P. Chevalier, G.P. Cosentino, J. de la Noüe & S. Rakhit, Biotechnology Techniques 1(1987)201.

8. Nu universiteiten afhankelijker worden van de derde geldstroom dient ervoor te worden gewaakt dat er voldoende achtergrondsonderzoek plaatsvindt en dat niet slechts bestaande wetenschappelijke inzichten te gelde worden gemaakt.

9. Wettelijke regelingen ten aanzien van ouderschapsverlof voor zowel vrouwen als mannen, zoals bijvoorbeeld reeds van kracht in Zweden, zouden een wezenlijke bijdrage kunnen leveren tot een gelijkwaardiger positie van beide partners.

Stellingen behorende bij het proefschrift "Rheological properties of rennet-induced skim milk gels" door P. Zoon. Wageningen, 30 november 1988.

VOORWOORD

Aan het tot stand komen van dit proefschrift hebben velen direkt of indirekt een bijdrage geleverd. Bij deze wil ik allen hiervoor bedanken. Mijn gedachten gaan dan in de eerst plaats uit naar mijn ouders, die mij altijd hebben gestimuleerd om mij zo goed mogelijk te ontplooien.

In het bijzonder gaat mijn dank uit naar Pieter Walstra, mijn promotor, en Ton van Vliet, mijn co-promotor, voor het nemen van het initiatief voor dit onderzoek en voor hun enthousiasme en opbouwende kritiek. Aliza de Groot-Mostert wil ik bedanken voor de experimentele ondersteuning.

In het kader van hun doktoraalonderzoek hebben Gabby Limpens, Annemarie Spaargaren, Marc Maste en Annemiek Beers een belangrijke bijdrage geleverd.

Mijn ex-kamer genoten Hannemieke Luyten en Hans Westerbeek hebben er in sterke mate aan bijgedragen dat mijn promotietijd zowel gezellig als leerzaam was.

Medewerkers van de tekenkamer, met name de heer Rijpma, en van de fotografische afdeling van het Biotechnion wil ik bedanken voor hun bijdrage aan de figuren in dit proefschrift.

Buiten de universiteit heb ik hulp gekregen van D.G. Schmidt (NIZO), D.G. Dalgleish, C. Holt (Hannah Research Instituut, Schotland) en B. de Cindio (Universiteit van Napels).

Het LEB-fonds wil ik bedanken voor de financiële ondersteuning bij de afronding van dit proefschrift.

De collega's in mijn nieuwe werkkring bij het NIZO ben ik erkentelijk voor de gelegenheid die zij mij hebben geboden dit proefschrift af te ronden.

Harry heeft mij veel steun gegeven, vooral tijdens de laatste fase van de promotie.

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Chapter

PREFACE

1 Objectives and motivation

The present study is part of a large research project on the structure and properties of casein networks. Casein networks are present in many dairy products, e.g. in cheese. Cheese is a very important product in the Netherlands. More than 40% of the Dutch milk is processed into cheese and the demand for cheese is still increasing. Control of the production as well as the development of new products and manufacturing technologies require a basic understanding of the principles that underlie cheesemaking. Several researchers have worked or are still working on the project mentioned. Van Hooydonk (1) studied the renneting properties of milk. Van Dijk (2) developed a method to measure and describe the syneresis of rennet-induced milk gels and he did some preliminary work on rheological properties; in addition he initiated the study of the permeability of gels, giving information on the structure of the gels. Roefs (3) studied the structure and rheological behaviour of acid casein gels. Van den Bijgaart (4) investigated the influence of cheesemaking parameters, e.g. external pressure, on the syneresis of rennet-induced milk gels. Akkerman is still working on the drainage of (rennet-induced) curd and Luyten (5) studied the rheological and fracture properties of (Gouda) cheese.

The objective of the present study was to investigate the effect of variables, known to be important for cheesemaking, on the mechanical behaviour of milk gels formed after rennet addition and to gain a more fundamental understanding of the mechanisms involved. Special attention was paid to the time scale at which processes, especially relaxation of bonds, occurred.

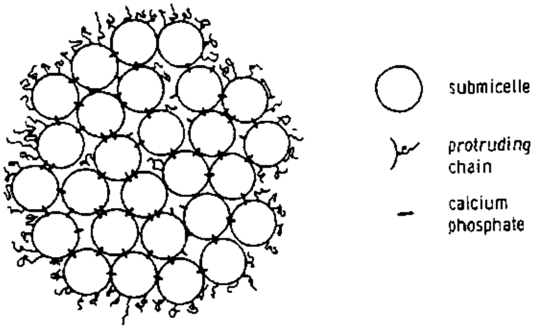

Fig. 1. Model of a casein micelle; highly schematic (From: Walstra and Jenness (6)).

2 Renneting of milk as an essential step during cheesemaking

2.1 Milk

Milk contains about 3.3% protein. About 80% of the milk protein consists of casein. Almost all casein in uncooled milk is present in roughly spherical particles, with sizes ranging from 20 to 300 nm. They are called casein micelles. These micelles are thought to be composed of small aggregates, called submicelles, with a size of 10 to 20 nm (Fig. 1)(6). Each of these submicelles contains different casein molecules, i.e. α_s, β- and κ-casein. Their composition is probably not identical, because κ-casein is almost exclusively located at the surface of the casein micelle (6,7). The C-terminal part of κ-casein is very hydrophilic. Presumably this part of the molecule sticks partly out into the serum. The protruding chains give the micelles colloidal stability due to steric repulsion (6). Casein micelles also contain water (≈ 3 g/g casein) and inorganic matter (≈80 mg/g casein), particularly calcium and phosphate, which plays an essential role in maintaining the integrity of the micelles (e.g. 8).

2.2 Cheesemaking

The production of Gouda type cheese is roughly as follows. Starter and a calcium chloride solution are added to milk of about 30.5°C. Calcium chloride accelerates the renneting due to a combined effect of an increase of the calcium concentration and a lowering of pH (1, 9). The lactic acid bacteria of the starter produce lactic acid during cheesemaking, thereby lowering the pH. A few minutes after starter addition, rennet is added to the milk. Rennet is an extract from calves' stomachs, containing two proteinases, chymosin and pepsin. The major part of the proteolytic activity during renneting is due to chymosin. At the pH of milk (≈6.6-6.7) it hydrolyzes a certain peptide bond (Phe-Met, residues 105-106) of κ-casein. The hydrophilic C-terminal part of κ-casein is thus split off and the casein micelles lose their stability. After sufficient κ-casein has been cleaved, the micelles start to aggregate (e.g. 10) and a three dimensional network forms, a rennet-induced milk gel. At a well-defined firmness, the gel, usually called curd, is cut into small pieces to promote syneresis, i.e. the expulsion of whey. Part of the whey is removed and warm water is added. The increase of temperature to about 35°C causes an acceleration of the syneresis. Then the curd particles are separated from the whey. The curd is pressed into a coherent mass, then salted in a brine bath and eventually stored for ripening.

3 Outline of the thesis

This thesis consists of six parts, five of which (1-5) have already been published or have been submitted for publication. The contents of Chapter 6, together with part of the work performed and described by Roefs (2), will be used for another paper. Parts 1 to 4 deal with the effect of important variables, such as casein and rennet concentration, calcium, phosphate, pH, NaCl and temperature on the rheological properties of rennet-induced skim milk gels at small deformation. The first paper gives an introduction to the formation and mechanical behaviour of the gels. Some background information on dynamic rheological measurements is given. In addition to dynamic experiments, stress relaxation experiments were performed, the method being described in Part 2. In Part 6 the relaxation modulus is calculated from the dynamic moduli and it is compared to the relaxation modulus determined with stress relaxation measurements. Part 5 deals with the rheological properties of the gels at very large deformations as measured with constant stress experiments.

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Rheological properties of rennet-induced skim milk gels.
1. Introduction

P. Zoon, T. van Vliet and P. Walstra

Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, the Netherlands

Received 13 May 1988; accepted 15 June 1988

Key-words: casein concentration, milk gel, proteolysis, rennet concentration, rheology.

Summary

The structure and mechanical properties of rennet-induced milk gels were studied by determination of the dynamic moduli G′ and G″ under such conditions that primarily the number of bonds between the protein particles in the gel network was varied rather than the type of interactions.

Most experiments were performed with reconstituted skim milk. By selecting proper conditions for preparation of the reconstituted milk, the results were comparable to those obtained with fresh whole milk. Protein breakdown caused by rennet and the milk proteinase plasmin did not significantly influence the rheological properties of the milk gels during the time of the dynamic measurements (usually up to 25 hours after dispersing the skim milk powder). Concentrating the milk by means of ultrafiltration resulted in an increase in the moduli. An exponential dependence between the moduli and the casein concentration factor was found, the exponent being 2.4. A higher rennet concentration resulted in an increase in the rate of gelation and an increase in the values of G′ and G″ after a long ageing time.

1 Introduction

Rennet-induced coagulation of milk is an important step during cheesemaking. Several papers have appeared on this subject. Recently a review on properties of biopolymer gels including casein aggregation and gelation was published by Clark & Ross-Murphy (1). In addition, they discussed the rheological properties and the application of gel theories on milk gels. Walstra & Van Vliet (2) made a review of the physico-chemical aspects of the transformation of milk into a loaf of cheese. A recent review and comprehensive study on rennet-induced clotting of milk under various conditions has been published by Van Hooydonk (3). Extensive studies on the structure and mechanical properties of milk gels have been performed by Van Dijk (4), Roefs (5) and Tokita et al. (6-10). Van Dijk has paid attention to the structure and syneresis behaviour of rennet milk gels made at the natural pH of milk. Roefs has studied the structure and rheological properties of casein gels formed by acidification in the cold and subsequent heating of skim milk or caseinate dispersions, both with and without rennet addition, by rheology, permeability measurements and electron microscopy. Tokita et al. (6-10) have investigated the rheological behaviour of rennet-induced milk gels at the natural pH and they fitted gelation models to their experimental data.

Formation of the gel structure. The first step in the formation of a rennet-induced milk gel is the addition of renneting enzymes. They split off the caseinomacropeptide (CMP) part of the κ-casein molecules, thereby diminishing steric and electrostatic repulsion (e.g. 11). Microscopically, it is observed that the converted micelles form irregular aggregates that grow in size until they touch and form a continuous network. The network consists of strands, being, for instance, 4 micelles in thickness and 10 micelles long, and of thicker agglomerates of micelles; there is, however considerable variation in length and thickness of the strands and in the size of the pores between them (e.g. 2, 12-15). The permeability of a rennet milk gel increases with time, indicating that the network changes (4, 16, 17). Dangling strands, that are connected to one end of the network only, become attached at more sites. Moreover strands can break and reform at other places, resulting in thicker and stronger strands and larger pores. Within the strands the contact region between the micelles extends and the micelles start to fuse (see Fig. 1). Together, these changes result in a coarsening of the spatial distribution of the casein network and an increase in stiffness of the strands (2).

To obtain information on the firmness of the gel, it is necessary to perform rheological experiments in which the relation between force and deformation as a function of time is studied.

Much attention has been paid to monitor the course of gelation by measuring the firmness of the curd, which for most instruments used, is not a well-defined parameter. In 1977 Thomasow & Voss (18) published a review article on this subject and recently Van Hooydonk & Van den Berg (3, 40) discussed several commercially available instruments.

To find causal relations it is necessary to use instruments that yield well-de-

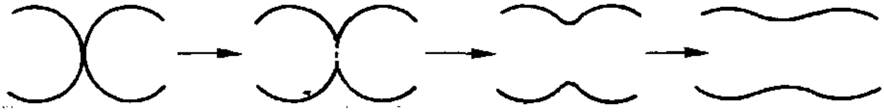

Fig. 1. Schematic picture of the fusion of flocculated paracasein micelles during ageing of the gel (after Walstra & Van Vliet (2)).

fined parameters. Because a milk gel is a viscoelastic system (e.g. 19, 20) and therefore shows time-dependent rheological behaviour, methods yielding information about these characteristics should be used, such as dynamic, creep or stress relaxation measurements. To avoid damaging of the gel structure during the measurement the applied deformation must be small.

In 1958 Scott-Blair & Burnett (19) described creep measurements using a U-tube gelometer. A kind of shear modulus and the so-called Burgers parameters could be calculated. Later this type of gelometer was also used by Culioli & Sherman (21) and by Johnston (22).

A kind of stress relaxation measurements have been done by Johnston (23) and Johnston & Murphy (24) using an Instron Universal Tester. Their method has been criticized by Van Vliet & Walstra (28).

Dynamic measurements on rennet-induced milk gels are well suited to obtain the viscoelastic properties of the gel as a function of time after rennet addition and as a function of the time scale of deformation (e.g. 4-6). The storage modulus G′, which is a measure of the energy stored, and the loss modulus G″, which is a measure of the energy dissipated during periodical application of a mostly sinusoidally varying stress or strain, can be calculated from these experiments. Several papers on dynamic measurements on milk gels have been published by Tokita et al. (6-10). They used a torsion pendulum apparatus. In 1982 Van Dijk (4) described measurements with a 'Den Otter' rheometer. Later Bohlin et al. (25) and Dejmek (26) used a Bohlin universal viscometer for this kind of work. Korolczuk et al. (27) reported on dynamic measurements with a Contraves low shear oscillatory viscometer, but they applied very large deformations on their gels. All the above-mentioned apparatus for dynamic measurements consisted of two coaxial cylinders of which the inner one (torsion pendulum apparatus and Den Otter rheometer) or the outer one (Bohlin and Contraves viscometers) could be oscillated in a sinusoidal mode. In the Appendix some background information on dynamic rheological measurements is given.

The dynamic measurements show that during gel formation and ageing the storage and loss modulus increase with time, reaching a plateau value after several hours. The moment at which the modulus starts to increase and the rate of increase depend on conditions such as rennet concentration, pH and additions such as $CaCl_2$ (e.g. 4, 25). The ratio of loss modulus G″ to storage modulus G′, tan δ, decreases very fast when the moduli start to increase and becomes constant when the moduli are just a few per cent of their final value (25, 26). The decrease in tan δ is a result of the transition from a fluid into a gel.

The values of G′ and G″ are both proportional to the number of effective bonds. The network of rennet-induced milk gels is inhomogeneous, which implies that the number of effective bonds will be considerably lower than the total number of bonds. The moduli will depend on the density and homogeneity of the gel network and on the character of the bonds. Assuming only one type of bond or a constant proportion between the numbers of bonds of various types, this implies the ratio of G″ to G′ (= tan δ) to be independent of the number of bonds and only related to the types of bond (28).

It has been suggested (2) that the nature of the bonds that contribute to the modulus probably does not change very much after the onset of gelation, because tan δ remains constant. However, one has to be cautious since tan δ was mostly measured at one angular frequency and the situation may be different at other frequencies. Besides, it is possible that a shift in the type of interaction does not result in a change of tan δ because the relaxation behaviour of the interactions is similar.

The results of various investigators on the rheological properties of milk gels are fairly similar, but remarkable differences, for instance in the temperature or concentration dependence of milk gels, are also found. This is mainly due to the type of measuring apparatus and the design of the experiments. In this article the results of a study on the influence of rennet concentration and casein concentration, which are thought to alter mainly the number of interactions and not the type of interaction, on the rheological properties of rennet milk gels is given. In later papers of this series the effect of factors such as temperature, pH, calcium and phosphate content and ionic strength, which probably influence the type of interactions, will also be studied.

2 Materials and methods

2.1 Milk

Standard reconstituted skim milk was prepared by bringing 10.4 g of a commercial low-heat skim milk powder (Krause, Heino) into 100 g of demineralized water containing 0.015 % thiomersal (BDH Chemicals LTD) or 0.02 % sodium azide (Merck) to prevent bacterial growth. The composition of the skim milk powder is given by Roefs (5). To allow equilibration the dispersion was stirred for about 16 hours at 30 °C. To some of the samples 0.02 % aprotinin (Sigma Chemical Company; strength 19.8 TIU/ml) was added to retard plasmin action.

Fresh milk was obtained from the dairy herd of the university. It was not cooled.

Cheese milk was fresh milk which had been cooled and stored at 4 °C. Just before cheesemaking it was heated at 72 °C for 20 s and subsequently cooled to 30 °C. Then starter (0.7 %), $CaCl_2$ (0.00875 %) and KNO_3 (0.02 %) were added to the milk just before rennet addition.

2.2 Renneting

Commercial calf rennet (10 800 SU, from CSK, Leeuwarden, Netherlands) was used; about 80 % of its activity originated from chymosin. The time after rennet addition required for the formation of visible clots was taken as clotting time.

2.3 Ultrafiltration

Standard reconstituted skim milk was ultrafiltered at 30 °C using an Amicon concentrator. The molecular cut-off of the membrane was about 10 000 Daltons. The protein concentration of the concentrated milk was determined by Kjeldahl analysis.

2.4 Gel electrophoresis

The proteolytic action of rennet and of milk proteinase was studied by polyacrylamide gel electrophoresis (PAGE) according to the procedure described by De Jong (29). An LKB 2001 Vertical Electrophoresis System was used.

2.5 Dynamic measurements with the 'Den Otter' rheometer

The rheometer was developed and described by Duiser (30) and Den Otter (31). Only a brief description will be given here.

The apparatus consists of two coaxial cylinders. The inner cylinder, made of stainless steel, is suspended between a torsion wire fixed to a driving shaft and a strain wire. The outer cylinder is made of glass. The temperature is controlled within 0.1 °C. The length of the inner cylinder is 15 cm, the radius is 3.75 mm. The radius of the outer cylinder is 4.5 mm.

Directly after rennet addition, the milk was brought between the inner and outer cylinder; the gel was thus formed in the apparatus itself. During the measurements a sinusoidal oscillation was applied to the driving shaft, which was transferred to the inner cylinder by the torsion wire. To avoid disturbance of the gel network the measurements were started only after a weak gel was formed ($G' \approx 2\ N \cdot m^{-2}$). The maximum amplitude of the driving shaft and of the inner cylinder were measured as well as the phase difference. From these the storage modulus G', the loss modulus G'' and $\tan \delta$ ($= G''/G'$) were calculated. The amplitude of the driving shaft was kept sufficiently low to ensure linear behaviour of the rennet milk gel, which appeared to exist if the maximum strain was smaller than 0.03. This corresponds well with the linear region of 0.03 as given by Van Dijk (4) and of 0.05 according to Dejmek (26).

During gel ageing studies, the angular frequency of the oscillation was kept at 1 $\text{rad}\cdot\text{s}^{-1}$. The influence of the angular frequency was studied after such an ageing time that the moduli did not change greatly (<3 % per hour) any more. The frequency was varied within a range from 10^{-3} to 10 $\text{rad}\cdot\text{s}^{-1}$. Some background information on dynamic rheological measurements is given in the Appendix.

3 Results and Discussion

3.1 The use of reconstituted skim milk

For most experiments reconstituted skim milk from the same batch of low heat skim milk powder was used in order to avoid variation in composition. Van Vliet & Dentener-Kikkert (32) found that there was no significant difference in the absolute value of the modulus nor in the frequency dependence of the modulus between gels made by acidification of whole milk or skim milk. To check whether the results of reconstituted skim milk were comparable with those of fresh milk, the mechanical properties of rennet milk gels made of the following 4 types of milk were followed in the Den Otter rheometer:

I) standard reconstituted skim milk (see 2.1)
II) reconstituted skim milk stirred for 1 h at 45 °C, then kept for 17 h at 4 °C and finally stirred for 1 h at 30 °C
III) bulk collected fresh whole milk (see 2.1)
IV) cheese milk (see 2.1). Here, the pH was continuously decreasing with time because of the added starter culture. At the moment of rennet addition the pH was 6.63, after 3 h it was 6.20 and after 5 h 5.60.

As can be seen in Fig. 2 the increase of G' in time for standard reconstituted skim milk was not as fast as for fresh whole milk, but in the end about the same value of the modulus was found. Similar curves were found for G'' (not shown). The same frequency dependence of G' and G'' (Fig. 3) was observed for both types of milk. The slower increase of G' in time for milk I as compared to milk III may have been caused by differences in natural composition of the milk or because of treatments during processing of the skim milk powder such as heating. The absolute values of G' and G'' were by coincidence the same: some variation in, for instance, protein content could give deviations and, moreover, differences of about 10 % were found between duplicates. Most important is the close similarity in the shape of the curves. This means that gels made of reconstituted (skim) milk and of fresh (whole) milk show the same time-dependent behaviour and probably the same type of

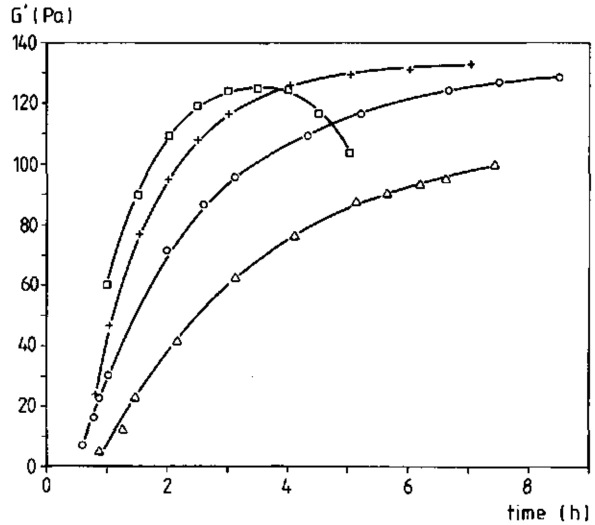

Fig. 2. Storage modulus G' as a function of time for: standard reconstituted skim milk I (○), reconstituted skim milk II (△), fresh whole milk III (+) and cheese milk IV (□). 0.025 % rennet was added to I and III, 0.05 % to II and 0.02 % to IV. Angular frequency (ω) was 1 rad/s. T = 30 °C, pH = 6.65.

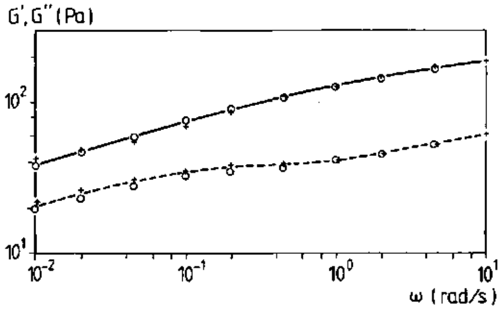

Fig. 3. Storage modulus G' (——) and loss modulus G'' (- - - -) as a function of the angular frequency for fresh whole milk III aged for 22 hours (+) and standard reconstituted skim milk I aged for 10 hours (○). T = 30 °C, pH = 6.65.

interactions are involved in the network. So relations or effects found for standard reconstituted milk gels are probably also valid for fresh milk.

The curve of the cheese milk (Fig. 2) deviates, probably caused mainly by the continuous decrease in pH. The effect of pH on the physical properties was studied, but the results will be given and discussed in the fourth paper of this series.

Reconstituted milk II showed a very different behaviour. In spite of the high rennet concentration, the clotting time was longer and the gel firming was very slow. Skim milk II also showed abnormal syneresis behaviour (42): initially no endogenous syneresis pressure could be detected. The difference in treatment as compared to standard reconstituted milk was that milk II was kept at a low temperature during most of the time. Van Hooydonk et al. (33) describe that retardation of the renneting process after cold storage of milk has also been observed for fresh milk and it has been attributed to the dissociation of casein, to the solubilization of the colloidal calcium phosphate at low temperature and to irreversible increase of the pH due to cooling. Although it appears that heating cold-stored milk to the renneting temperature, virtually re-establishes the original partition, the renneting process is still greatly affected. Stirring the reconstituted milk II during one hour at 30 °C was probably not enough to establish equilibrium, although the pH of milk II did not differ significantly from milk I. It is clear that although reconstituted milk always had the same composition its gelling behaviour could be different if not prepared in a standardized way.

3.2 Protein breakdown

Casein is the main material of which the network of a milk gel is built up. Proteolysis may influence the moduli by splitting the casein molecules in such a way that dangling strands are formed or even that the formed fragments do not remain in the gel network but go into solution resulting in a decrease of the number of effective bonds. In a milk gel the action of chymosin and of the milk proteinase, plasmin, may be important with respect to degradation of casein (20). At the natural pH of milk chymosin only splits the bond between Phe 105 and Met 106 of κ-casein at a reasonable rate. The optimal pH for overall proteolysis is about 3.8. Plasmin, on the other hand, has maximum activity at slightly alkaline pH and 37 °C. In fresh milk only a small portion is in the active form, but it can be slowly activated. Plasmin fully survives pasteurization and increased activity has even been observed after heating milk at 72 °C for 15 s (20). β-casein and α_{s2}-casein are most susceptible to plasmin and also α_{s1}-casein is attacked. Plasmin action is responsible for the production of γ-caseins and some proteose peptone fractions, from β-casein. Plasmin is a trypsin-like enzyme and its action can be retarded by aprotinin, which contains a trypsin inhibitor.

For standard reconstituted skim milk the influence of rennet and aprotinin on the degradation of protein was studied as a function of time. Fig. 4 shows a typical example of the results obtained by gel electrophoresis. Two main

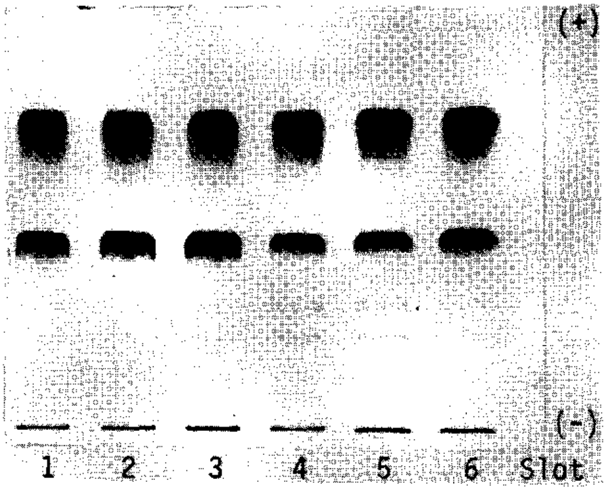

Fig. 4. Gel electrophoresis of reconstituted skim milk. Time after dispersing milk powder was 24 h for slots 1-3 and 40 h for slots 4-6. Slots 2, 3, 5, 6: 0.025 % rennet was added 16 hours after dispersing milk powder. Slots 3 and 6: 1 % aprotinin solution was added. Thiomersal was added as a preservative.

bands can be seen: the upper one for α_{s1}-casein, the lower one for β-casein. The two smaller bands beneath β-casein are from γ-casein. The β-casein band was well measurable and the degradation could be followed quantitatively. Breakdown of α_{s1}-casein could not be detected in this way. The band was rather broad and whey proteins as well as degradation products of β-casein were found just before and after the α_{s1}-casein. To get an impression of α_{s1}-casein breakdown renneted milk was centrifuged (at 11 000 *g* for 10 minutes) and the pellet was used for further investigation. Then the soluble degradation products and serum proteins did not disturb the electrophoresis pattern any more. In Table 1 a summary of the results is given.

Rheological measurements at 30 °C were usually performed within 24-30 hours after dissolving the milk powder; after more than 40 hours microbial deterioration became noticeable. From the results in Table 1 it appears that, within 24 hours and under the circumstances used, rennet did not cause β-casein breakdown, so the degradation of β-casein was mainly caused by plasmin. Also some α_{s1}-casein breakdown was found. In Fig. 5 the storage modulus G′ is depicted as a function of time for two gels made of reconstituted skim milk with and without aprotinin. For G″ similar results were found, only the values were lower. Initially the curves in Fig. 5 are nearly equal, but after a longer ageing time (4-10 h) G′ was on average somewhat lower for samples without aprotinin compared to samples containing aprotinin, although the

Table 1. The residual relative concentration of β-casein and α_{s1}-casein as a function of time for standard reconstituted skim milk with or without rennet or aprotinin added. Time (t) was measured from the moment that the powder was dissolved. Aprotinin (0.02 %) was added at t = 0 and rennet (0.025 %) at t = 16h. * Results from pellet experiment. At time t = 0 the concentration was set to be 1.

Time (h)	Residual fraction of:					
	β-casein					α_{s1}-casein
rennet	yes	yes	yes	no	yes*	yes*
aprotinin	no	no	yes	yes	no	no
1	1	1	1	1	1	1
4	1	1	1	1	0.94	1
16	0.93	0.94	0.93	0.95	0.88	0.93
24	0.86	0.86	0.94	0.95	0.86	0.93
40	0.67	0.68	0.84	0.88	0.74	0.86

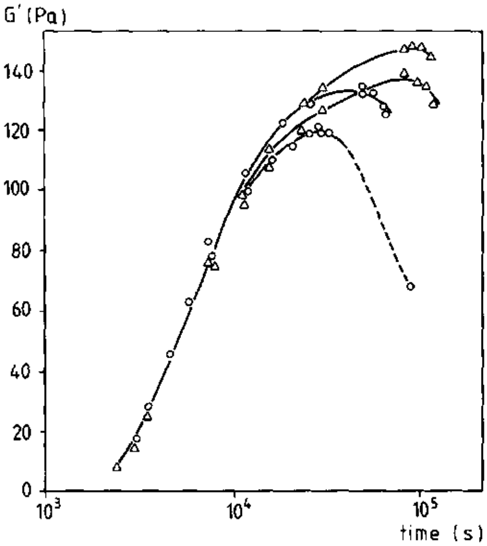

Fig. 5. Storage modulus G' as a function of time for gels made of reconstituted skim milk with (△) and without (○) aprotinin added. T = 30 °C, pH = 6.65, 0.025 % rennet.

differences between duplicates were of the same magnitude as the differences between both types of samples. The protein concentration in the whey of gels made of milk with and without aprotinin was determined about 6 hours after rennet addition (t ≈ 22 h). No significant differences were found, implying that the degradation products of α_{s1}- and β-casein remained in the gel net-

Table 2. Increase of the protein concentration of the whey due to neutrase action and the storage modulus G′ of a gel containing neutrase, 8 hours after rennet and neutrase addition. For further explanation, see text.

Increase in protein concentration of the whey (g protein/100 ml whey):		
* determined		0.37
* estimated,	based on the assumption that all casein degradation products directly go into solution	0.88
Storage modulus G′ (Pa):		
* determined		50
* estimated,	based on the total protein content of the network	79
* estimated,	based on the content of intact α_s- and β-casein	32

work, but they probably contributed less effectively to the modulus. We obtained comparable results (not shown) with another proteolytic enzyme 'neutrase'. Within two hours after addition of 0.15 % of neutrase-containing Novozym 257 (Novo Industri AS Copenhagen, 0.015 AU/g) a decrease of about 35 % in the concentration of α_s- and 55 % of β-casein was measured, and during the next 6 hours another 10 % of both caseins disappeared. However, the protein concentration in the whey of gels formed of this milk increased less and more gradually than might have been expected from the measured α_s-and β-casein breakdown, implying that initially the degradation products remained in the network. The moduli of these gels containing neutrase were higher than expected from the residual concentration of α_s- and β-casein and lower than expected from the protein concentration in the whey (Table 2). The degradation products remaining in the gel network were assumed to contribute to the modulus, although less effectively than α_s- and β-casein.

3.3 Development of dynamic moduli with time for rennet milk gels and comparison with acid milk gels.

The change of the moduli with time will now be discussed. As can be seen from Fig. 2 and Fig. 5 until about 3 hours after rennet addition G′ increased very fast with time, followed by a slower increase and after about 9 hours a plateau value (≈125 Pa) was reached. At a given moment G′ decreased again and the time at which this happened was not very reproducible. For acid skim milk gels (pH = 4.6) the modulus tended to increase linearly with the logarithm of time and even after 7 days the moduli still increased without any sign of reaching a plateau value (5). A big difference between both systems is that for the acid milk gel bacterial problems do not play an important role.

However, in the rennet milk gels bacterial deterioration usually started to become important 1 day after rennet addition so this cannot explain that already after 3 hours G′ increased less than linearly with the logarithm of time. Taking into account the results of Section 3.2, protein breakdown cannot be the reason either.

Probably a more important and very essential difference between the two types of gels is that 24 hours after rennet addition the permeability of rennet milk gels still increases, whereas the permeability of acid milk gels does not increase between 2 and 20 hours after onset of gelation (4). This implies that in acid milk gels rearrangements only take place at a very local scale e.g. within the strands of the network, whereas in rennet milk gels strands break and reform at another place.

The increase of the moduli with time after rennet addition must be due mainly to an increase in the number of bonds caused by the network formation mentioned in Section 1, viz. incorporation of renneted micelles and dangling strands and by the network ageing, viz. rearrangement of strands and fusion of micelles. The permeability is thought to increase because the network tries to shrink, but because it sticks to the inner and outer cylinder of the measuring apparatus only local shrinkage can take place resulting in thicker strands and larger pores (e.g. 17). This process is called micro-syneresis. After a certain ageing time these changes probably do not result any more in an increase of the number of bonds that bear stress during deformation and the moduli then remain constant, and later it may even result in a decrease of the moduli. A considerable decrease of the modulus, for instance as seen for one of the samples in Fig. 5, is probably due to syneresis causing the gel network to come loose from the wall of the rheometer.

3.4 Protein concentration

From the literature (e.g. 4, 7-10) it is known that the modulus of rennet milk gels increases more than proportionally with increasing protein concentration. A power law dependence of the moduli on the casein concentration (c) was found: $G \propto c^x$, with x higher than 1. This implies that the casein network is not uniform (5, 34), which is in accordance with microscopical observations. Van Dijk (4) and Culioli & Sherman (21) found for the instantaneous shear modulus $x = 2.7$ and 2.6, respectively. Tokita et al. wrote several articles (6-10) about the dependence of the dynamic moduli on the concentration, but their results are not always clear. For G′ they gave in (9) $x = 3.8$ at casein concentrations below 2 % and $x = 2.4$ at concentrations over 2 %, while they gave in (8) $x = 2$ above 2.5 % casein. From a graph of the logarithm of the modulus as a function of the logarithm of the concentration in

(9) x = 4 could be calculated at casein concentrations of about 1 to 8 %. From a graph in (10) of the modulus versus the concentration in the range from 0.6 to 2.5 % casein no constant exponent could be calculated: the exponent decreased with increasing concentration and its value between 2 and 2.5 % casein was about 2.6. The differences in exponent found in the various experiments may be due to different ways of varying the casein concentration, the amount of rennet and the time after rennet addition at which the modulus was measured. If the casein concentration is varied by dissolving different amounts of milk powder, as e.g. in ref. (8), then also other factors, such as the ionic strength, will vary, which may influence the results. Probably more important in the experiments of Tokita et al. is that the ratio between rennet and casein was kept constant. As already is clear from some of their figures, this implies that gelation started sooner after rennet addition for increasing casein (and enzyme) concentration. When the moduli are measured at a constant time after rennet addition, the results will be influenced in such a way that the moduli at the higher casein concentrations will be relatively too high, resulting in too high an exponent. This effect will be more serious if the measurements are done sooner after the gelation point. This may largely explain the variation in their results.

In the present study the relation between the dynamic moduli and the casein concentration was investigated varying the casein concentration by ultrafiltration of the milk, using a constant amount of rennet and measuring the moduli after a long ageing time.

The dependence of the dynamic moduli on the casein concentration was measured as a function of time after rennet addition. The temperature was 30 °C and 0.05 % of rennet was added. The casein concentration factor c_f, which is the ratio between the casein concentration of the sample and the casein concentration of non concentrated milk, was varied from 1 to 2.21. The clotting time increased slightly with increasing casein concentration, but the difference between the sample with the lowest and with the highest casein concentration was only about one minute. This is about 8 % in clotting time. The moduli were followed with time. For all samples the ratio between G' at a given moment and G' 7 hours after rennet addition was calculated (G_r'). After 7 hours the moduli hardly changed any more (<2 %/h). At any moment after the onset of gelation, G_r' was the same for all samples within experimental accuracy (Fig. 6). This implies that at any moment the same relation between the modulus and the casein concentration exists.

Fig. 7a shows the storage modulus G' and loss modulus G'' as a function of the casein concentration factor c_f for gels that had been aged for about 7

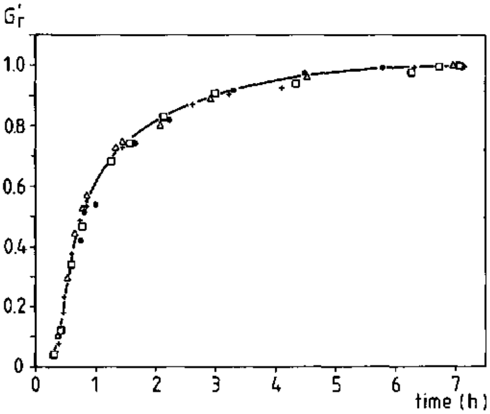

Fig. 6. Relative storage modulus G'_r as a function of time after rennet addition at 4 different casein concentrations of the milk; casein concentration factor c_f : 1 (●), 1.43 (△), 1.68 (□), 2.21 (+). Angular frequency was 1 rad/s. T = 30 °C, pH = 6.65, 0.05 % rennet.

hours. A clear power law dependence of the moduli on the concentration factor was found, the exponent being 2.4 for G′ as well as for G″. Tan δ (= G″/G′) was 0.31 ± 0.01 at all concentrations.

At three concentrations the moduli were measured as a function of the angular frequency ω. At $\omega = 0.1$ and 0.01 rad.s^{-1} x was calculated and in both cases x = 2.4 for G′ and 2.5 for G″ so the exponent appears to be independent of ω over the frequency range studied (0.01-1 rad/s).

To check whether the relation between the dynamic moduli and c may de-

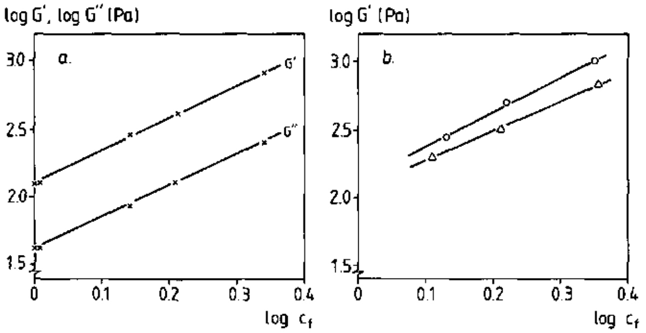

Fig. 7. Dynamic moduli as a function of the casein concentration factor c_f. Angular frequency was 1 rad/s. Fig. 7a: (×) pH = 6.65, T = 30 °C, ageing time was 7 hours. Fig. 7b: (○) pH = 6.33, T = 30 °C, ageing time was 7 hours and (△) pH = 6.65, T = 34 °C, ageing time was 5 hours.

pend on slight variations in conditions, x was also determined at a pH of 6.33 and at a temperature of 34 °C for a limited number (=3) of concentrations (Fig. 7b). For the experiments at lower pH the milk had been acidified with HCl to pH 6.33 before ultrafiltration and the temperature was 30 °C. In the temperature experiment the milk was clotted at 30 °C and 20 minutes after clotting the temperature was raised to 34 °C. At pH 6.33 $x = 2.4$ and at 33 °C $x = 2.2$, which is not significantly different from the results at pH 6.65 and 30 °C.

The exponent of about 2.4 in the relation between the dynamic moduli and the casein concentration was in agreement with the results of Van Dijk (4) and Culioli & Sherman (21) for the instantaneous shear modulus of rennet-induced milk gels and with the results of Roefs (5) for the storage modulus of acid milk gels ($x = 2.6$). But this does not imply that the exponent is fully independent of the concentration range. The values mentioned here were all measured for concentrated milk. For lower or much higher casein concentrations the exponent could be different from 2.4.

Tokita et al. (10) fitted their data of the moduli as a function of the casein concentration near the critical concentration to a modern scaling (lattice percolation) model. Gordon (35) fitted the same data to 4 variants of classical polycondensation models. These variants were random f-functional polycondensation with micelles or submicelles as 'monomer' units and classical vulcanization theory with submicelles or polypeptides as monomer units. All five models fitted the data almost equally well. This means that a good fit to experimental data does not necessarily give insight in the gelation mechanism of the studied material. Besides, although it was possible to make good fits of the data to the models, some of the assumptions were obviously incorrect. Gordon considered the casein gel as an ideal rubber, the modulus being proportional to the temperature. As will be further discussed in the second paper of this series, the modulus is not at all proportional to the temperature and a casein gel behaves like a particle gel rather than a rubber gel. Besides, in three of the four models of Gordon, polypeptides and submicelles are considered to be the 'monomer' units. As is described in the introduction, the (partly) renneted micelles coagulate and form aggregates that grow until they touch and form a network. So the 'monomer' units must be micelles or maybe even aggregates of micelles. In a later stage micelles fuse and the submicelles and polypeptides may be considered as 'monomer' units. In the models used by Tokita et al. (10) and Gordon (35) the network structure is thought to be fairly uniform whereas the structure of the casein network is not uniform. A fractal growth model probably would be better as far as the structure is concerned, although the exponent (x) has been predicted to be about 4.4 for a slow coagulating system (36). More research on gel formation in dilute caseinate systems and comparison with network models is under way in our laboratory.

3.5 Rennet concentration

As is known from the literature a higher enzyme concentration causes not only a shorter clotting time but also a higher rate of curd firming (e.g. 6, 37-39). Van Hooydonk & Van den Berg (40) supposed the latter to be partly due to the amount of CMP still to be released after gelation: the percentage of CMP released at the onset of gelation decreased with decreasing rennet concentration. Hossain (37), Garnot (38) and McMahon et al. (39) did not find differences in gel strength after a few hours of ageing. But when the latter calculated the ultimate gelstrength using a Scott-Blair and Burnett-like equation the ultimate strength appeared to increase with decreasing rennet concentration. McMahon et al. followed the gel formation in a Formagraph and only part of the curve of the curd firmness versus time was used for the curve fitting. At longer times the values of the firmness calculated were higher than those measured. They ascribed the difference to the properties of the gel network itself and to the effect of the measurement on the behaviour of the gel. Van Hooydonk & Van den Berg (40) showed that the increase in firmness as measured with the Formagraph, already lags behind that measured with the Instron 5-10 minutes after the onset of gelation. So in fact neither the measured nor the calculated values may be correct. On the other hand Van Dijk (4) and Van Hooydonk & Van den Berg (40) reported an increase in ultimate gel strength with increasing rennet concentration. These conflicting results may be due to differences in the accuracy of and the maximum strain occurring in the measuring equipment used. In this study the influence of the rennet concentration on the gelation and the ageing of skim milk gels is investigated by following the dynamic moduli in the Den Otter rheometer.

The results for three rennet concentrations are shown in Fig. 8, where the moduli are given as a function of ageing time. It is clear that at higher rennet concentration a gel is formed sooner after rennet addition and that the increase of G' as a function of time is faster. In Table 3 the rate of increase of G' is given at two values of G'. It is evident that even after a rather long period of ageing the rate of increase in G' is higher at a higher rennet concentration, although the differences are becoming smaller with time. The results for G'' (not shown) were similar and tan δ was independent of the rennet concentration.

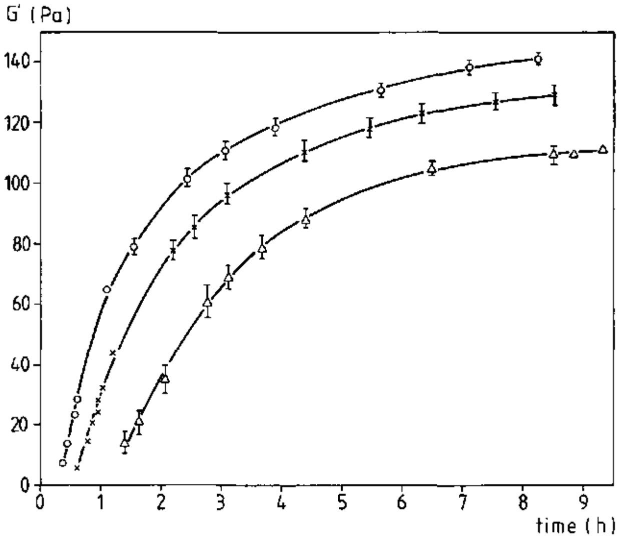

Fig. 8. Storage modulus G′ as a function of time for reconstituted skim milk gels. Rennet concentration was varied: 0.05 % (○), 0.025 % (×), 0.01 % (△). Angular frequency was 1 rad/s. T = 30 °C, pH = 6.65.

Table 3. dG′/dt at two values of G′ for gels differing in rennet concentration.

Rennet concentration (%)	dG′/dt (Pa.h^{-1})	
	G′ = 25 Pa	G′ = 75 Pa
0.010	35	17
0.025	71	25
0.050	89	29

Fig. 8 shows that even after an ageing time of more than 8 hours the moduli are higher with higher rennet concentration. Van Hooydonk & Van den Berg (40) suggest that the reason for this increase is that at a higher rennet concentration more rapid aggregation takes place, leading to a coarser network with fewer junctions but with more bonds per junction. The idea that the building of the network is influenced by the concentration of rennet seems reasonable, but it is questionable whether a coarser network is formed at higher rennet concentration. Van den Tempel (34) suggests that if aggregation proceeds more slowly a more inhomogeneous structure will be formed. Also for networks formed of fractals a higher exponent in the relation between modulus and fractal concentration is used for slow compared to fast coagulating systems (36), implying the formation of a more inhomogeneous network. However, in permeability measurements, performed after about 4 hours of ageing, no significant differences were found for gels made with rennet concentrations between 0.01 and 0.06 % (4). This does not necessarily imply that the network structure is equal at all rennet concentrations. The inhomogeneity of the network at a smaller scale than perceived in permeability experiments may be different, for instance the extent of fusion of micelles may vary. So it is very difficult to give an unambiguous explanation.

Appendix. *Dynamic rheological measurements, a brief survey (e.g. 5, 41).*

In apparatus like the Bohlin rheometer or the Den Otter rheometer the type of deformation applied to the gel is simple shear. The principle of simple shear is illustrated in Fig. 9. The force per unit surface area, called the shear stress σ (Pa), causes a deformation, called the shear strain γ, which is equal to tan α in Fig. 9.

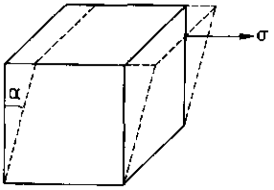

Fig. 9. Illustration of simple shear. σ is the shear stress and γ is the shear strain (= tan α).

In a dynamic experiment the sample is usually deformed in a sinusoidal way at an angular frequency ω. The shear strain is then given by:

$$\gamma(t) = \gamma_0 \sin(\omega t) \tag{1}$$

where γ_0 is the maximum shear strain. The strain is associated with a sinusoidally varying shear stress (σ):

$$\sigma(t) = \sigma_0 \sin(\omega t + \delta) = \sigma_0(\sin(\omega t)\cos\delta + \cos(\omega t)\sin\delta) \tag{2}$$

where σ_0 is the maximum stress and δ is the phase angle between the deformation and stress. This phase difference originates from the viscous properties of the material. For an ideally elastic solid, σ is in phase with γ, because σ is at maximum when γ is at maximum, and δ equals zero. For an ideally viscous

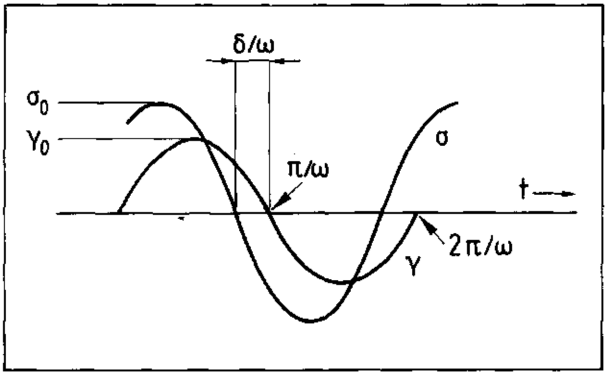

Fig. 10. Sinusoidal variation of strain and stress as a function of time for a viscoelastic material (for further explanation, see text).

fluid σ is $\pi/2$ radians out of phase, because σ is at maximum when strain rate ($d\gamma/dt$) is at maximum which is the case when γ is at minimum. Then δ equals $\pi/2$. For a viscoelastic material like a milk gel, δ has a value between 0 and $\pi/2$ (see Fig. 10).

Within the linear region σ_0 is by definition proportional to γ_0. Equation (2) can be written as:

$$\sigma(t) = \gamma_0[\sigma_0/\gamma_0(\cos\delta.\sin(\omega t)) + \sigma_0/\gamma_0(\sin\delta.\cos(\omega t))] \quad (3)$$

The elastic part of the stress, which is the part of the stress in phase with the strain, corresponds to the storage modulus G′, which is defined as:

$$G'(\omega) = (\sigma_0/\gamma_0)\cos\delta \quad (4)$$

It is a measure of the energy stored and subsequently released per cycle of deformation. The viscous part of the stress, which is part of the stress out of phase with the strain, corresponds to the loss modulus G″, which is defined as:

$$G''(\omega) = (\sigma_0/\gamma_0)\sin\delta \quad (5)$$

It is a measure of the energy dissipated as heat per cycle of deformation. The ratio of G″ to G′ is tan δ:

$$\tan\delta(\omega) = G''(\omega)/G'(\omega) \quad (6)$$

An increase in tan δ means that the relaxation of bonds increases and the material behaves relatively in a more viscous and less elastic manner.

Acknowledgements

We thank A.E.A. de Groot-Mostert for expert technical assistance and M.C.L. Maste for his work on neutrase.

Samenvatting

P. Zoon, T. van Vliet en P. Walstra, *De reologische eigenschappen van met leb gestremde ondermelkgelen. 1. Inleiding*

De opbouw en de mechanische eigenschappen van met leb gestremde ondermelkgelen zijn bestudeerd door de dynamische moduli G′ en G″ te meten. Dit gebeurde onder zodanige omstandigheden dat voornamelijk het aantal bindingen varieerde, terwijl de aard van de bindingen gelijk bleef.

De experimenten werden voornamelijk met gereconstitueerde ondermelk uitgevoerd. Indien de bereiding van deze melk op de juiste wijze en onder gestandaardiseerde omstandigheden plaats vond, waren de resultaten vergelijkbaar met die van verse melk. De invloed van eiwitafbraak, als gevolg van stremsel- en plasmine-inwerking, op de reologische eigenschappen was gering tijdens de duur van de experimenten. Een toename in de hoeveelheid toegevoegd stremsel resulteerde in een snellere toename van de moduli en tevens in hogere moduli na een lange verouderingstijd van de gelen. Het concentreren van melk met behulp van ultrafiltratie resulteerde in een toename van de moduli. Er werd een exponentieel verband gevonden tussen de moduli en de concentratiefactor van het eiwit met een exponent van 2,4.

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Rheological properties of rennet-induced skim milk gels.
2. The effect of temperature

P. Zoon, T. van Vliet and P. Walstra

Department of Food Science, Wageningen Agricultural University,
P.O. Box 8129, 6700 EV Wageningen, the Netherlands

Received 30 May 1988; accepted 15 June 1988

Key-words: casein micelles, milk gels, renneting, rheology, temperature.

Summary

The influence of temperature on the mechanical behaviour of rennet-induced skim milk gels was studied. Dynamic and stress relaxation measurements were performed. Increasing the temperature during gel formation and ageing mainly resulted in an increase of the firming rate. The moduli measured after a long ageing time decreased with increasing temperature. Gels formed and aged at 25, 30 and 35 °C all gave at 30 °C similar results for the moduli. On the other hand, gels formed and aged at 30 °C gave at increasing measuring temperature decreasing values for the moduli, and the relaxation of bonds was shifted to a shorter time scale. After a sudden temperature change it was observed that the lower the temperature to which the gel was brought, the longer it was before a constant value of the moduli was reached. A possible explanation for the results is given in terms of interaction forces and structure of the gel network.

1 Introduction

Temperature is a very important variable during cheesemaking. It affects, for instance, the rate of renneting, gel formation and ageing and syneresis. Temperature has little effect on the enzymic reaction (1, 2) but a very strong effect on the flocculation reaction (3). The rate of flocculation becomes almost zero below 15 °C and syneresis virtually stops below 18 °C (4).

A common method for studying gel formation and ageing is by measuring the rheological properties as a function of time after rennet addition (e.g. 5). Often the modulus (stress/relative deformation) is determined after a gel is formed (e.g. 5-9). It has been shown repeatedly (6-9) that the rate of increase of the moduli directly after the onset of gelation also increases with temperature. Van Hooydonk & Van den Berg (9) and Hossain (6) reported measurements up to 35 °C and they found a steady increase, Tokita et al. (7) measured up to 40 °C and also found a continuous increase, while Scott Blair & Burnett (8) found a maximum rate at 32 °C.

About the dependence of the modulus after a long ageing time, often called ultimate modulus, on the temperature, conflicting results exist. Dejmek (10) formed gels at one temperature and varied the measuring temperature in a range between 15 and 45 °C. With increasing temperature a decrease in the storage modulus was found at a frequency of 0.01 Hz, whereas at higher frequencies a shallow maximum was found at about 25 °C. For the loss modulus a shallow maximum at 25 °C was found at all frequencies. Hossain (6) and Tokita et al. (7) formed and aged gels at a certain temperature and measured the moduli at the same temperature. Hossain did not find a difference in ultimate modulus between 28 and 31.6 °C and a lower value at 35 °C. On the other hand Tokita et al. (7) found a linear increase of the ultimate shear modulus with temperature. Based on this observation, they concluded that milk gels show rubber-like behaviour, although the increase with temperature was stronger than expected for such behaviour. Van Vliet & Walstra (11) have already commented upon this approach and they explained that milk gels behave like gels of aggregated particles rather than rubber gels.

In this work the influence of the formation-and-ageing temperature and of the measuring temperature on the rheological behaviour of milk gels is studied. For this purpose dynamic and stress relaxation measurements were done. Dynamic measurements give information about the behaviour at relatively short time scales, whereas relaxation measurements give information at longer time scales. A (periodic) dynamic experiment at angular frequency ω is qualitatively equivalent to a transient experiment at time $t = \omega^{-1}$ (12). The results from both types of measurement will be compared and discussed.

2 Materials and methods

2.1 Skim milk

Reconstituted skim milk was used. It was prepared by dispersing a commercial low-heat skim milk powder (Krause, Heino) in demineralized water. Its composition is given by Roefs (13). Initially 12 g of milk powder in 100 g of water was used, milk A, but later 10.4 g of powder was used, milk B, which is in better accordance with the solids-not-fat content of fresh milk. The dispersions were stirred for 16 to 20 h at the gel formation-and-ageing temperature before use. To prevent bacterial growth 0.015 % thiomersal (BDH Chemicals LTD) or 0.02 % sodium azide (Merck) was added.

Skim milk with a pH of 5.75 and 5.95 was made by addition of HCl one hour after the powder was dispersed. The pH was measured just before renneting.

2.2 Renneting

Commercial calf rennet (10 800 SU from CSK, Leeuwarden, Netherlands) was used; about 80 % of its activity originated from chymosin. The time after rennet addition required for the formation of visible clots was taken as the clotting time. In all experiments 0.025 % of rennet was used.

2.3 Rheological measurements

Dynamic measurements with the 'Den Otter' rheometer. The method has been described before (5). Gel ageing was studied at an angular frequency of 1 $\mathrm{rad \cdot s^{-1}}$. The strain was kept below 3 %.

Stress relaxation measurements. A Weissenberg R18 Rheogoniometer was used. Only a brief description will be given here. A more extensive description is given in (14). With this instrument a sample can be deformed at a constant (shear) rate and after that the sample can be kept at constant strain. The stress is measured.

The measuring geometry consisted of a cone (top) and a plate (bottom) both having a radius of 37.52 mm. The stainless steel surfaces had been blasted with corundum to a roughness of about 4 μm. The angle between the cone and the plate was 0.0549 rad. The cone was truncated over a length of 157 μm of its axis. Temperature was controlled by pumping water through a double walled chamber enclosing the cone and plate. To prevent drying of the sample two troughs with water were placed between the sample and the inner wall of the chamber.

After rennet addition, about 10 ml of milk (B) was brought on the plate, which had been preheated for half an hour at 30 °C. Subsequently the distance between the top of the truncated cone and the plate was set at 157 μm. All gels were aged for 6 h at 30 °C. For gels tested at 30 °C stress relaxation measurements were started directly after this ageing time. For measurements at 25 and 20.5 °C stress relaxation was determined 1.5 h after the water temperature was adjusted, since such a time was needed for the sample to obtain the desired temperature. A constant shear strain of about 3-4 % was obtained by moving the plate at a constant rate (0.0143 $\mathrm{s^{-1}}$) for 2-3 seconds. The resulting stress was recorded until two hours after deformation.

3 Results

3.1 Dynamic measurements

3.1.1 Effect of equal ageing and measuring temperature on dynamic moduli. Dynamic measurements were performed on gels, made and tested at 20, 25,

Table 1. Clotting time of skim milk at five temperatures. Milk A was used.

Temperature (°C)	Clotting time (s)
20	4055
25	1691
30	1175
35	1078
40	1114

30, 35 and 40 °C. In the range from 20 to 35 °C clotting times (Table 1) decreased with increasing temperature. At 40 °C the clotting time was longer than at 35 °C, probably because of heat inactivation of chymosine.

Gel formation and ageing was followed by measuring the storage (G′) and loss (G′′) modulus as a function of time at an angular frequency of 1 rad/s. In Fig. 1 results are given for G′. Those for G′′ (not shown) show the same behaviour, only the values are lower. At 20 °C the moduli increased very slowly with time. Even after 72 h they were still increasing. Between 20 and 30 °C a strong temperature effect on the increase of the moduli with time was found while above 30 °C the initial rates were nearly equal. As has been described and discussed before (5), at 30 °C the moduli reach a plateau value after about 9 h and later on the moduli decrease again. At 35 and 40 °C the plateau value as well as the subsequent decrease of the moduli was reached sooner than at 30 °C. The plateau value decreased with increasing temperature. At

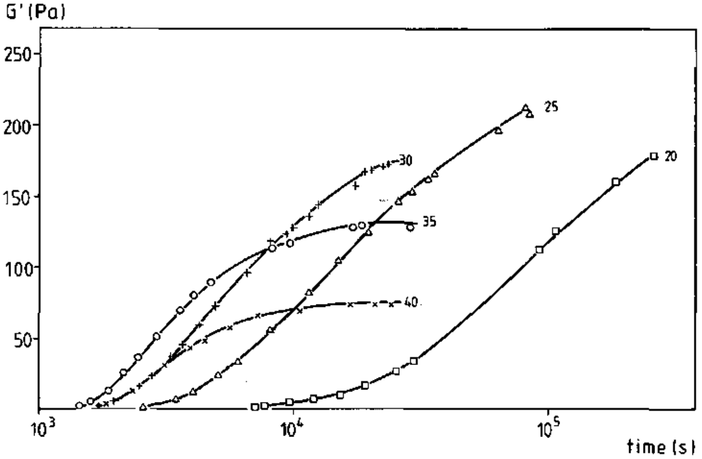
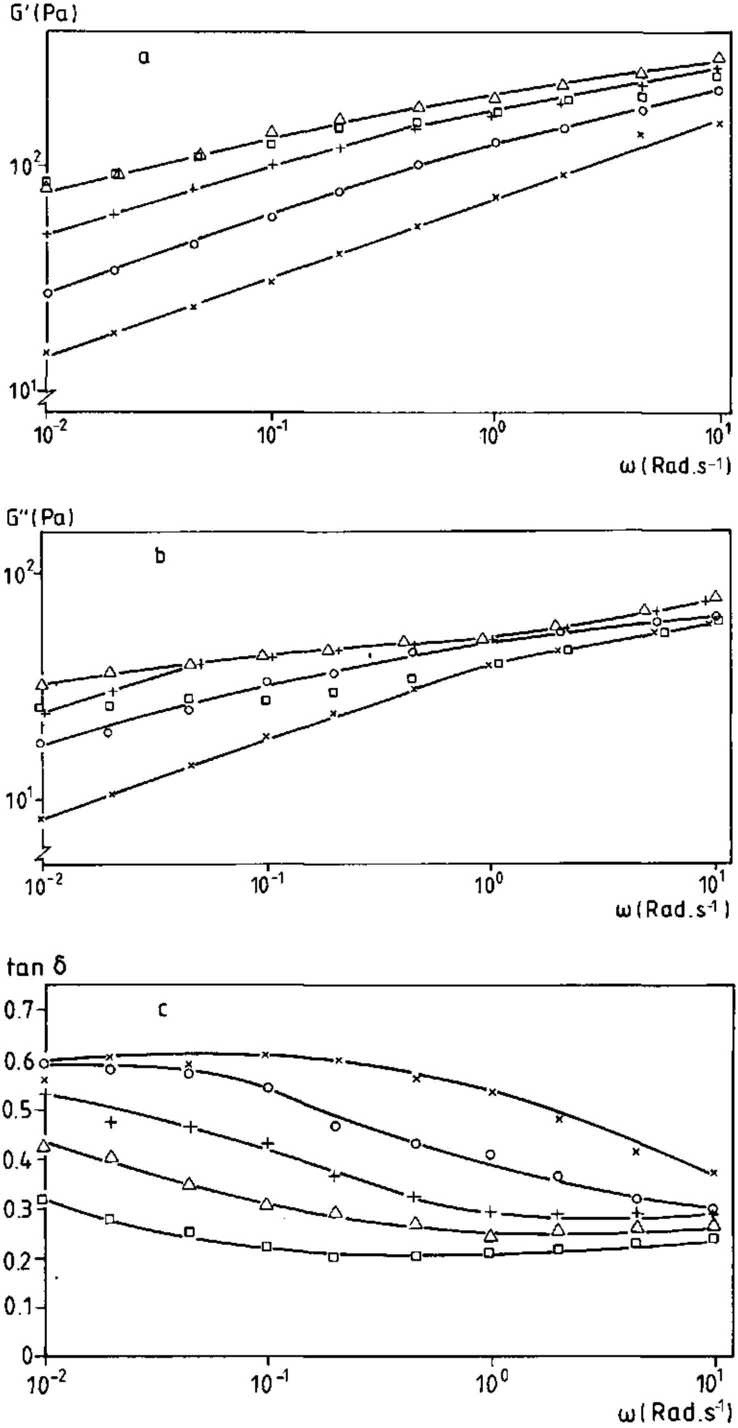


Fig. 1. Storage modulus G′ as a function of time after rennet addition for milk gels made and measured at the temperatures as indicated. Milk A was used. Angular frequency was 1 rad/s.

20 and 25 °C a plateau value had not been reached during the time gel ageing was followed, but it is clear that in both cases after a long ageing time the modulus would become higher than the plateau value at 30 °C. It is not sure whether the value at 20 °C would become higher than that at 25 °C. The increase of the moduli proceeded so slowly that it would take weeks to reach a plateau value and bacteriological deterioration was to be expected. Taking into account the results of Section 3.1.4 it seems likely that after a very long ageing time the modulus at 20 °C would be higher than at 25 °C.

The observed decrease in plateau value of the modulus with increasing temperature was opposite to the results of Tokita et al. (7,15). They measured the moduli of the gels ($T = 17.5–40$ °C) until five h ($1.8\ 10^4$s) of ageing and extrapolated the results to a plateau value (7). The values they reported for the moduli were very low compared to ours. They found, for instance, 350 dyn/cm^2 (35 Pa) at 25 °C and 550 dyn/cm^2 (55 Pa) at 35 °C, whereas we found for samples with about the same casein concentration 145 and 90 Pa, respectively, for the plateau values. This implies that the moduli in (7) cannot be used to calculate plateau values. As can be seen in Fig. 1 the time after which the moduli are determined is very important. If the moduli have not yet reached a plateau value not only a temperature effect but also a time effect will be involved. As Tokita et al. remarked themselves (15), the results were unreliable because of the extrapolations. In (15) they repeated measurements at various temperatures ($T = 15–33$ °C). The gels were made of three times the normal concentration of skim milk powder and they measured up to an ageing time of 18 h ($6.5\ 10^4$ s). Again an increase of the moduli with increasing temperature was found. Eighteen hours of ageing seems to be a long time, but they worked at rather low temperatures. As can be seen in Fig. 1 we also observed an increase of the moduli with increasing temperature after such an ageing time for temperatures lower than 25 °C. They did not publish the curves of the moduli as a function of time so it is not possible to check whether the moduli were still increasing with time. The values of the moduli at a given temperature are difficult to compare with ours at the same temperature, because of the very high concentration of milk powder they used.

Hossain (6) tested milk gels in a Lactodynamograph, which is similar to a Formagraph, giving arbitrary units. The plateau value found at 28 °C was equal to that at 31.6 °C and at 35 °C a lower value was found. In this type of apparatus the apparent modulus usually reaches a plateau value faster than in an apparatus like the Den Otter (9), probably because the deformation is too strong. Differences found may be real but their magnitude is probably incorrect. Anyway, the results do not show an increase in gel firmness with temperature as found by Tokita et al (7, 15).


G'(Pa)
a
10^2
10^1
10^-2
10^-1
10^0
10^1
ω (Rad.s-1)
G"(Pa)
b
10^2
10^1
10^-2
10^-1
10^0
10^1
ω (Rad.s-1)
tan δ
c
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10^-2
10^-1
10^0
10^1
ω (Rad.s-1)

At all temperatures G''/G' (= tan δ) reached a constant value soon after the onset of gelation. The final values of tan δ increased with temperature at the angular frequency used of 1 rad·s^{-1} (Fig. 2c).

The frequency-dependence of the moduli was studied after such an ageing time that the moduli increased less than 1 Pa per hour except for 20 °C. At 20 °C the moduli increased more than 1 Pa per hour and the absolute values of the moduli at this temperature may not be compared with those at the other temperatures. In Fig. 2, G', G'' and tan δ are depicted as a function of the angular frequency, respectively. As can be seen from Figures 2a and b the moduli decreased with increasing temperature. The shape of the different curves in each of the Figures 2a-c seems to be rather similar at first sight. Roughly taken, the curves are shifted in a horizontal direction. This is most evident from Fig. 2c, where tan δ is depicted as a function of the frequency. At all temperatures there was a transition in tan δ from about 0.3 to about 0.6 and the frequency at which this happened became higher with increasing temperature. An increase in tan δ means a relatively less elastic and more viscous-like behaviour: more spontaneous breaking of bonds takes place (26). Because the time scale of a (periodic) dynamic measurement is roughly the inverse of the angular frequency, this implies that with increasing temperature bonds spontaneously break within a shorter time.

To find out whether the influence of the temperature on the moduli was due to the temperature during formation or during testing, three kinds of experiments were performed.

1. The effect of a sudden increase or decrease in temperature on the moduli and the reversibility of temperature effects were studied (3.1.2).
2. The formation-and-ageing temperature was varied while the measuring temperature was kept constant (3.1.3.).
3. The measuring temperature was varied while the formation-and-ageing temperature was kept constant (3.1.4).

3.1.2 The effects of a sudden temperature change on the dynamic moduli. Gels with pH 6.65, 5.95 and 5.75 were made and aged for several hours at 30 °C in the Den Otter rheometer. When the increase in the storage modulus, measured at an angular frequency of 1 rad/s, had become less than 2 Pa/h the temperature was decreased instantaneously to 25 °C (Fig. 3a, e, i). When the

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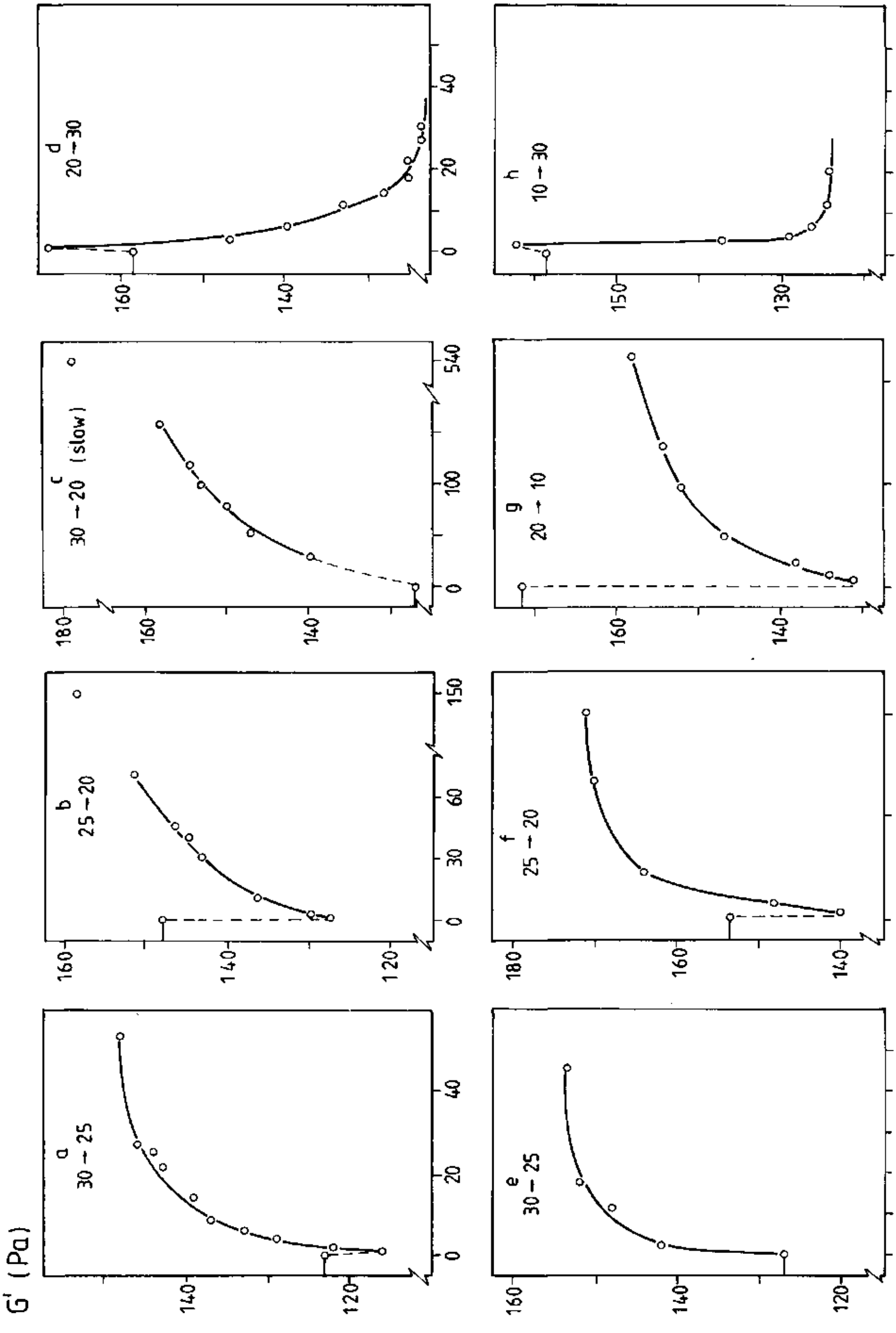
←

Fig. 2. Storage modulus G'(2a), loss modulus G''(2b) and tan δ (= G''/G')(2c) as a function of the angular frequency ω for milk gels formed and measured at 20(□), 25(△), 30(+), 35(o) and 40 °C (×). Milk A was used.


G' (Pa)
a
30 → 25
b
25 → 20
c
30 → 20 (slow)
d
20 → 30
e
30 → 25
f
25 → 20
g
20 → 10
h
10 → 30
120
140
160
180
150
130
0
20
40
30
60
150
50
100
540

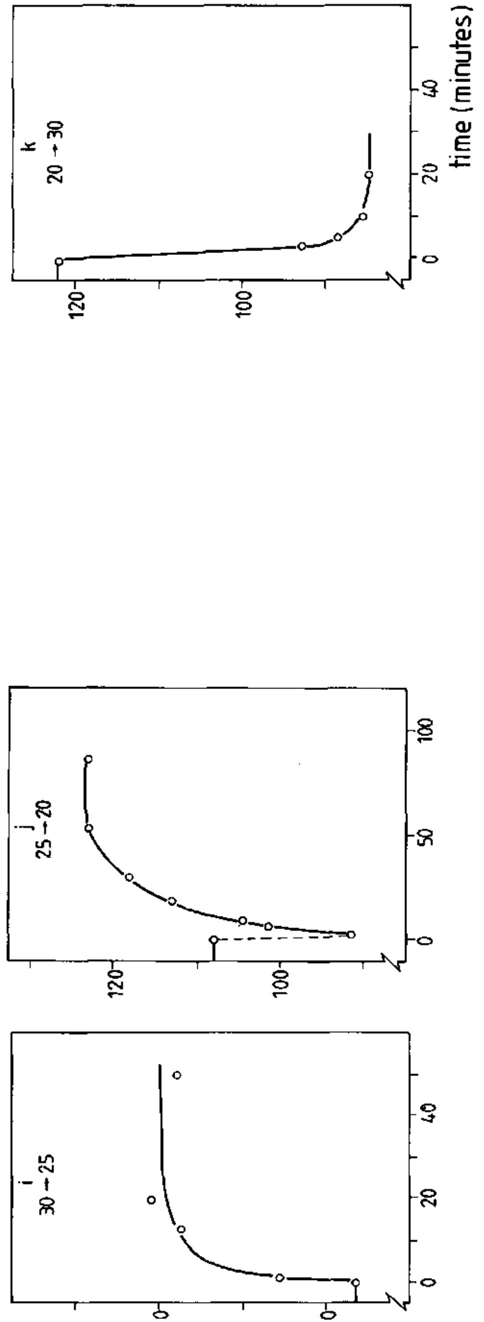


Fig. 3. Storage modulus G′ of skim milk gels at pH 6.65 (a-d), 5.95 (e-h) and 5.75 (i-k) as a function of time after a sudden temperature change. In 3c the temperature was slowly decreased (· · · ·). The temperature changes are indicated in the figures. Angular frequency was 1 rad/s; milk B was used.

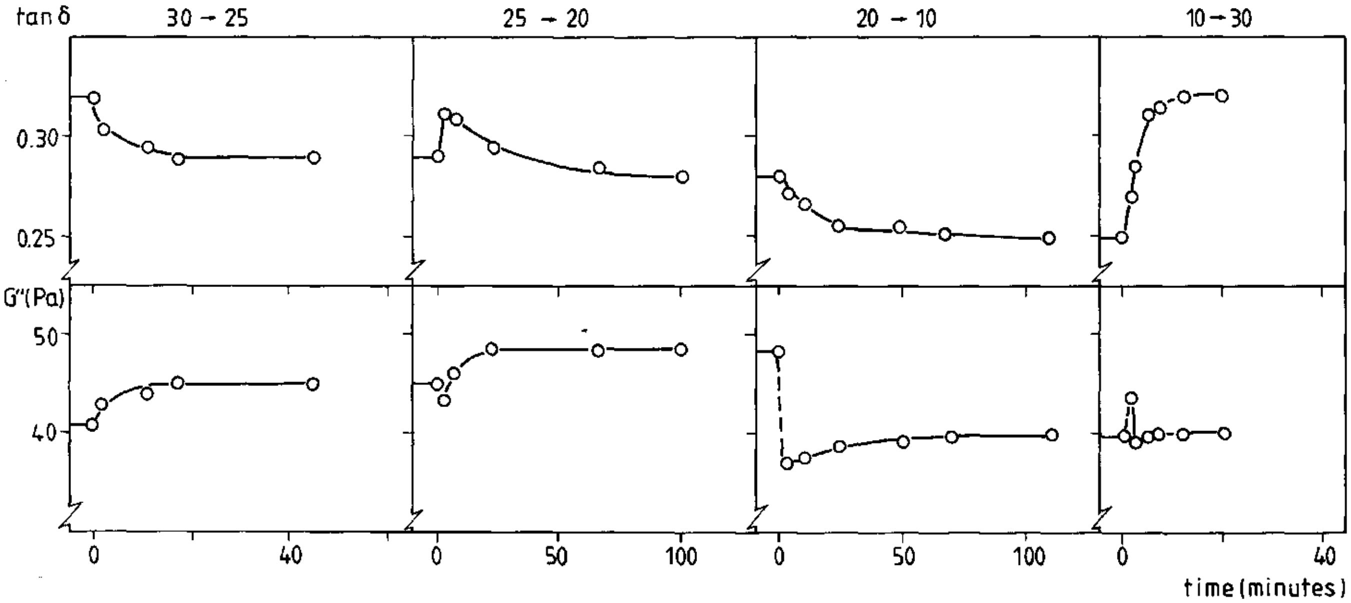


Fig. 4. The effect of a sudden temperature change on the loss modulus G'' and tan δ as a function of time after the temperature change for a gel of pH 5.95. The applied temperature changes are indicated. The same sample as in Fig. 3 e-h. Angular frequency was 1 rad/s.

moduli had reached a constant value, the temperature was lowered instantaneously to 20 °C (Fig. 3b, f, j). Afterwards at pH 6.65 and 5.75 the temperature was raised from 20 to 30 °C (Fig. 3d, k). At pH 5.95 the temperature was first lowered to 10 °C (Fig. 3g) and then raised to 30 °C (Fig. 3h). On another gel at pH 6.65 the effect of a slow decrease of the temperature, 1 °C per three minutes, was studied (Fig. 3c).

In Fig. 3 the effect of temperature changes on storage modulus G′ is shown as a function of the time after the change. In Fig. 4 the loss modulus G″ and tan δ are depicted as a function of time for the gel of pH 5.95. For the gels of pH 6.65 and 5.75 similar behaviour was found. Because the absolute values of G″ were smaller and in general the accuracy of the measurement of G″ was less than of G′, the effect of a temperature change was less clear for the loss modulus than for the storage modulus. When the temperature was lowered, tan δ decreased slowly with time. The final decrease was about 0.02 per 5 °C between 10 and 30 °C.

From the results shown in Fig. 3 it follows that after a sudden decrease of the temperature two opposite effects can be distinguished. Directly after the decrease in temperature the modulus decreased. It is not sure whether the measured moduli were reliable within the first two minutes after a change in temperature. A calculation of the heating rate of the gel showed that it took about 1-2 minutes to reach the desired temperature in the gel. Besides, it was observed for a gelatin gel that during the first two minutes after a sudden decrease in temperature the moduli measured in the Den Otter rheometer were somewhat too low. However, especially at 20 and 10 °C it is clear that the decrease of the modulus was real. The instantaneous decrease of the modulus was followed by an increase. The rate of increase was very much dependent on temperature: the lower the temperature the longer it lasted before a new steady state, viz. a constant value of G′ was reached. At pH 5.95 and pH 5.75 a constant value was reached faster than at pH 6.65 (Table 2).

After the temperature was raised again to 30 °C the modulus first increased somewhat and then decreased. At pH 5.75 the first measurement after the in-

Table 2. Time after a sudden decrease of the temperature at which the rate of increase of the modulus became relatively small (less than 4 Pa/h) for gels at a pH as indicated.

pH	Time (minutes)			
	Temp. change	30 → 25	25 → 20	20 → 10 °C
5.75		≈20	≈45	
5.95		≈25	≈45	>120
6.65		≈45	≈150	

crease of the temperature was performed after a relatively long time and no increase of the modulus was observed. At all pHs a constant value of the modulus was reached within about 20 minutes and this value did not differ significantly from the initial value at 30 °C. Therefore it may be concluded that changes in the rheological behaviour induced by a variation in temperature between 10 and 30 °C are reversible.

The results shown in Fig. 3 and 4 will be discussed further in Section 4 and a possible explanation of the temperature-dependent behaviour will be given.

The reversibility of the changes in the dynamic moduli of rennet-induced skim milk gels caused by variation in temperature were checked further for two other gels: one aged for 25 h at 25 °C and one for 5 h at 40 °C. The moduli had reached constant values after such an ageing time. The temperature was brought to 30 °C and after the gel had been at this temperature for an hour the moduli were measured. The temperature was then brought back to the ageing temperature and after half an hour at 40 °C and after one hour at 25 °C the moduli were measured again (Table 3).

The moduli before and after the temperature change to 30 °C did not differ from each other, indicating that the rheological behaviour of the gels had not changed irreversibly. The different values of the moduli and tan δ at 30 °C for gels formed at 25 °C compared to gels formed at 40 °C will be discussed further in Section 3.1.3.

Table 3. The reversibility of temperature-induced changes of the dynamic moduli; $\omega = 1$ rad/s, milk A was used.

	Temp. change	25 → 30 → 25	40 → 30 → 40
G′ (Pa)		213 → 175 → 212	76 → 145 → 74
G″ (Pa)		53 → 51 → 53	43 → 52 → 42

3.1.3 Effect of formation-and-ageing temperature on the dynamic moduli. To study the influence of the formation-and-ageing temperature gels were made at 25, 30, 35 and 40 °C. After such an ageing time that the moduli increased less than 1 Pa per hour the temperature was brought to 30 °C and after half an hour the moduli were determined as a function of the angular frequency. For gels formed at 25, 30 and 35 °C the values of G′, G″ and tan δ did not significantly differ from each other after the temperature change to 30 °C. The gels

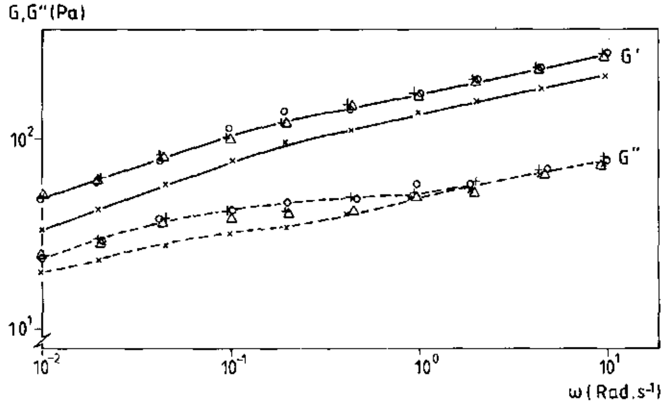


Fig. 5. Storage modulus G′ (full curve) and loss modulus G″ (dashed curve) as a function of the angular frequency ω for milk gels formed at 25(△), 30(+), 35(o) and 40 °C (×) and all tested at 30 °C. Milk A was used.

formed at 40 °C gave somewhat lower values of the moduli (Fig. 5) and about 10 % higher values of tan δ than those formed at the other temperatures. In general it was difficult to perform reliable experiments at 40 °C, probably because of the increase of the endogenous syneresis pressure with temperature (18, 24), which results in rapid micro-syneresis. The gel network then tries to contract, but as long as it sticks to the walls of the measuring apparatus this leads to relatively wide pores in the gel. It can also result in (macro-) syneresis leading to slip and release of the gel from the cylinders. It is thus not clear whether at 40 °C the structure of the network was somewhat different or the observed effect was an artefact.

From the results given here it is clear that the effect of the temperature on the moduli found in 3.1.1. cannot be mainly due to differences in the formation temperature except for its effect on the rate of gel formation and ageing.

3.1.4 Effect of measuring temperature on the dynamic moduli. To study the effect of the measuring temperature on the moduli, gels were made at 30 °C and after ageing for 10 h at 30 °C the temperature was changed to 20, 25 or 35 °C. At 25 and 35 °C the moduli reached a constant value within one hour after the temperature change. At 20 °C even after 2.5 h the value of the moduli was not yet constant. The frequency dependence of the moduli was determined at least one hour after the temperature change to 25 and 35 °C and 2.5 h after it was changed to 20 °C (Fig. 6). It took about 9 h to measure the moduli over the whole frequency range from 0.001 to 4.5 rad·s^{-1}. In the end the moduli were determined again at 1 rad·s^{-1}. The values at 25 and 35 °C had not changed, but the value of the modulus at 20 °C had increased by about 10 % within these 9 h. The moduli at the other frequencies were recalculated as if they were measured 11 h after the temperature change, assuming a linear increase with time. As can be seen from Fig. 6 the same effects of temperature are observed as shown in Section 3.1.1, where the measuring temperature was equal to the formation-and-ageing temperatures.

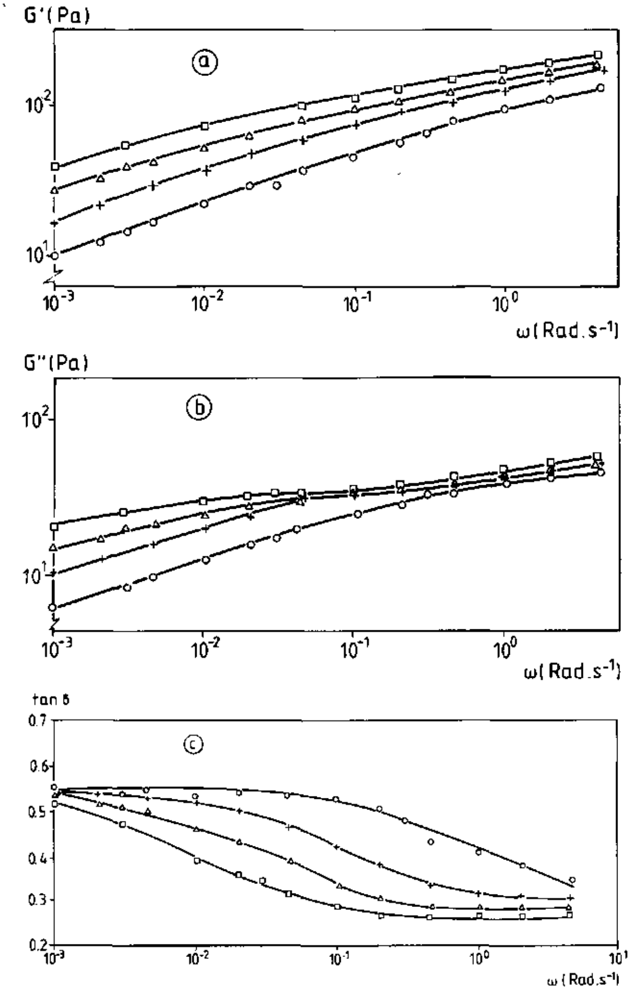


Fig. 6. Storage modulus G' (6a), loss modulus G'' (6b) and tan δ (6c) as a function of the angular frequency ω for gels aged for 10 h at 30 °C and measured at 20(□), 25(△), 30(+) and 35 °C (o). Milk B was used.

It is possible to superpose the curves of tan δ versus ω for the various temperatures on the curve at 30 °C, by shifting each curve along the vertical and horizontal axes. To superpose a curve at a 5 °C higher temperature the curve has to be shifted 0.02 units over the tan δ axis. In the horizontal direction the shifts decrease with increasing temperature. The value for the horizontal shift (in log ω units) is 0.80 between 20 and 25 °C, 0.48 between 25 and 30 °C and 0.27 between 35 and 30 °C. When the same horizontal shifts are applied to the curves of G′ and G′′ versus ω for the various temperatures the curves can practically be superposed on the curve for 30 °C.

The absolute values of the moduli from 3.1.3 cannot be compared directly with those of 3.1.1, because the quantity of skim milk powder dispersed in 100 gram water was different. It is known (5) that the dependence of the moduli on the concentration is given by: $G \propto c_f^{2.4}$ in which c_f is the concentration factor (here: 10.4/12 = 0.87). This gives a correction factor of 0.7. In Table 4 the recalculated moduli of G′ at 1 rad·s^{-1} shown in Fig. 2 are compared with those from Fig. 6.

Table 4. Storage modulus G′ for gels determined at the indicated temperatures. T_f = formation and ageing temperature, T_m = measuring temperature. * Results obtained from Fig. 2 after correction; ** Results from Fig. 6.

T_m (°C)	G′ ($T_f = T_m$ (*)) (Pa)	G′ (T_f = 30 °C (**)) (Pa)
20	120	175
25	144	145
30	120	125
35	89	90

Except for the gel formed at 20 °C the moduli found in both types of experiments are nearly equal. As mentioned in Section 3.1.1, gel formation at 20 °C is very slow, which will be the reason for the differences in Table 4. From the shape of the curves as well as from the absolute values of the moduli it can be concluded that the effects of temperature on the dynamic moduli found in 3.1.1, after correction for differences in the rate of gel formation and ageing, are mainly caused by the measuring temperature and not by the formation-and-ageing temperature.

Dejmek (10) suggests that the storage and loss moduli have a maximum at 25 °C at frequencies higher than 0.01 Hz. He changed the temperature at a rate of 0.5-1 °C per minute and measured immediately afterwards. A cycle of 8 measurements at different temperatures was performed within 1 h, implying that the gel was on average less than 5 minutes at each measuring temperature. When the temperature is decreased moduli are lower if the samples are kept at that temperature for a short time before measurement (see Figs. 3 and 4), as compared to a longer time. This is probably the reason for the different results found for the temperature effect.

3.2 Effect of measuring temperature on stress relaxation.

Milk gels were made in the Weissenberg Rheogoniometer (see 2.1-2.3). A constant strain was imposed and the resulting change in stress with time was followed. Experiments were performed at: 20.5, 25, 30 and 35 °C. At 35 °C the gel did not stick well to the cone, probably because of syneresis, and deformation of the gel did not result in a measurable stress.

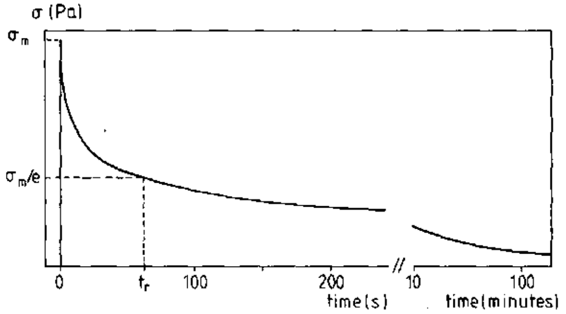


Fig. 7. Stress (σ) as a function of time during a stress relaxation experiment of a milk gel aged and tested at 30 °C. For further explanation, see text.

In Fig. 7 the change of stress with time is depicted for a gel at 30 °C. Similar curves were found for gels at the other temperatures. During the brief period in which the plate of the Weissenberg Rheogoniometer was moved, the stress increased. After the plate was stopped, the strain remained constant and the stress decreased with time. The relaxation modulus at a given time G(t) was calculated by dividing the stress at that time $\sigma(t)$ by the strain γ:

$$G(t) = \sigma(t)/\gamma \quad (1)$$

In Fig. 8 the relaxation modulus is depicted as a function of time for the gels tested at 20.5, 25 and 30 °C in a log-log plot. For simple homogeneous systems with only one type of interaction force, the decrease of stress in time may be described by a simple exponential decay function (12):

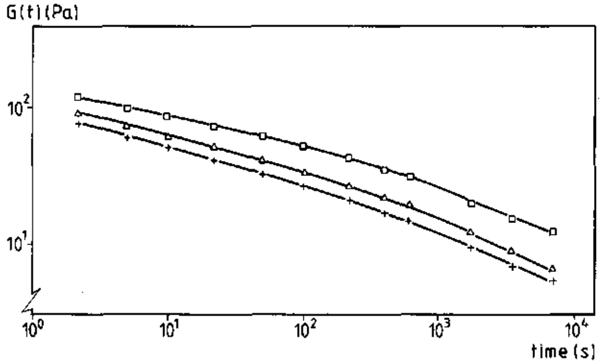


Fig. 8. Relaxation modulus G(t) as a function of time for milk gels aged at 30 °C and tested at various temperatures: 20.5(□), 25(△) and 30 °C (+). Milk B was used.

$$G(t) = G_m.\exp(-t/\tau) \tag{2}$$

where G_m is the initial value of G(t) and τ is the relaxation time.

For rennet-induced milk gels the decrease of stress could not be described by such a simple decay function. This implies that the overall relaxation is due to several relaxation processes, each with its characteristic relaxation time. In formula for n processes (12):

$$G(t) = \sum_{i=1}^{n} G_i.\exp(-t/\tau_i) \tag{3}$$

After two hours only 5-10 % of the maximum stress remained and the stress relaxation was still going on. It is therefore rather unlikely that permanent bonds in the network contributed to the stress.

Johnston (16) also reported about stress relaxation in rennet milk gels. He measured the force on a plunger that was used to compress the gel locally in a cup. He found a faster relaxation of the stress especially after a time longer than 10 seconds from the start. However, as mentioned by Van Vliet & Walstra (11), compression tests in a cup are not suitable to measure stress relaxation of milk gels. In such a test the network is probably changed locally during the compression and the locally high stress may enhance syneresis (18, 19). Local shrinkage and fracture of the network and release of the network from the plunger will result in a faster stress relaxation. In (22) values of G(t) are calculated from dynamic experiments and they are very close to the values given in Fig. 8 so we are fairly sure that the values obtained with the Weissenberg Rheogoniometer are correct.

As can be seen from Fig. 8, the relaxation modulus decreased with increasing temperature. This is the same temperature effect as discussed in Section 3.1.4, where a decrease in storage modulus with increasing measuring temperature was found.

From the curves in Fig. 8 it is difficult to judge whether temperature influences the velocity of stress relaxation. Therefore an apparent mean relaxation time t_r was calculated for the three different gels. It was defined as the time at which the stress $\sigma(t)$ was σ_m/e ($e = 2.718..$), where σ_m is the stress at the time that the plate was stopped and the stress relaxation measurement started. It is called an apparent relaxation time, because its value will depend on the time during which the plate was moved. Normally a real relaxation time is defined for the case that strain is imposed instantaneously. This is impossible because of inertial effects. If it takes a certain time to impose the strain, the bonds that live shorter than this time already relax before the stress relaxation measurement is started. This results in a longer relaxation time: only the bonds that live longer than the time needed for deformation will contribute to σ_m and the stress decay. It is called a mean relaxation time because, as was mentioned before, the observed relaxation behaviour is a summation of various relaxation processes. Therefore t_r may only be used to compare the velocity of stress relaxation at the different temperatures. In Table 5 t_r is given as a function of the measuring temperature.

Table 5. The apparent mean relaxation time t_r for gels tested at 20.5, 25 and 30 °C; milk B was used.

T_m (°C)	t_r (s)
20.5	217
25	100
30	64

From Table 5 it is clear that the stress relaxed faster at a higher temperature. A similar temperature effect is obtained if for σ_m the stress at $t = 10$ or 100 s is taken rather than at the moment the plate was stopped. This observation is in accordance with the results of the dynamic measurements reported in Section 3.1.4, where increasing measuring temperature led to an increase in tan δ at a higher frequency; this implies that relaxation of bonds occurred already at higher frequencies, which is equal to a shorter time in static experiments such as stress relaxation.

4 Discussion

The formation-and-ageing temperature probably affects the moduli, irrespective of the measuring temperature effect, in the following ways. For a higher temperature:

- the renneting reaction is faster (9), resulting in a measurable value of the moduli sooner after rennet addition (especially between 20 and 30 °C).
- the fusion of micelles within the strands proceeds faster (17). Initially this causes a higher modulus, because more bonds will be formed, but the fusion will also be finished earlier, so a plateau value is reached sooner.
- the rate of change in permeability with time is higher (absolutely as well as relatively) and the maximum in the endogenous syneresis pressure is reached sooner after rennet addition. The maximum in the endogenous syneresis pressure increases with increasing temperature (19). These factors imply a more rapid coarsening of the network and will thus result in lower moduli.

It may be concluded that a higher temperature results in a higher rate of gel formation and ageing.

After changing the temperature to 30 °C similar values of the moduli were found for gels formed and aged at different temperatures (up to 35 °C). It is not sure whether the structure of the gels was also similar. Permeability of the gels can give some information on the large scale structure of the network. Van den Bijgaard (19) performed permeability experiments at ageing temperatures between 20 and 34 °C. Based on his results the permeability at 30 °C was estimated for gels aged at 25, 30 and 34 °C after an ageing time of 20, 8 and 4.5 h respectively (about the times at which the temperature was changed to 30 °C in the dynamic experiments). Van den Bijgaart (19) tested up to 3 h after rennet addition. Linear extrapolation was applied to attain the permeabilities at longer ageing times. Besides, it was supposed that the factor with which the permeability increased after an increase of the temperature was independent of the ageing time. For ageing temperatures of 25, 30 and 35 °C permeability coefficients were estimated to be (in 10^{-13} m^2): 6.2, 7.4 and 7.7, respectively. The permeability seems to increase somewhat at higher ageing temperature, but the differences are rather small, implying that the structure of the network did not differ strongly.

The measuring temperature had a large effect on the observed rheological behaviour of the milk gels. In 3.1.2 it was shown that after a sudden decrease in temperature the moduli decreased instantaneously and then slowly increased. The lower the temperature the longer it lasted before a new steady state was reached, indicating that structural changes take place. It is supposed that this occurs at least at two levels, probably that of the protein molecules and that of the submicelles. Because of the reversibility of the effect, changes at the level of the spatial structure of the network are unlikely. Also other types of experiments point to structural changes. An increase in the voluminosity of casein micelles (20) even of coagulated micelles (21) has been reported with decreasing temperature. Van den Bijgaart (27) measured the apparent viscosity of skim milk in an Übbelohde-type capillary viscometer. After a sudden change in temperature from 30 to 20 °C the apparent viscosity increased, but even after 4.5 h no steady state had been attained. He also found that, after a sudden temperature change from 30 to 20 °C, the permeability of gels, which were aged for 3.5 h, decreased and it took nearly 2 h to attain a constant value.

In general protein conformation is determined by a competition between different intra- and intermolecular interaction forces as well as entropic contributions to the free energy. Different types of forces will act, such as electrostatic attraction and repulsion, including bonding via the colloidal calcium phosphate, hydrogen bonding, Van der Waals attraction, hydrophobic interaction, Born repulsion and deformation of covalent bonds. Conformational entropy of the peptide chain and most interaction forces are not strongly temperature dependent, except for hydrophobic interactions: below about 45 °C, the strength of these will decrease with decreasing temperature (23). It is thought that hydrophobic interaction is mainly acting within the submicelles, and a decrease in hydrophobic interaction may lead to a swelling of the submicelles. This agrees with the already mentioned increase in voluminosity of casein micelles with decreasing temperature. The instantaneous decrease of the moduli, especially of G′, after a sudden decrease of the temperature, may be caused by a decrease in the strength of bonds due to hydrophobic interaction. This decrease will also result in a swelling of the submicelles, but it seems reasonable to assume that swelling does not take place instantaneously, because some rearrangement of the protein molecules must be involved. The submicelles, which are thought to be the largest entities in the strands of the network after some time (e.g. 24), are connected to each other and a swelling of the submicelles will cause a closer approach and a bigger contact area between them. This will probably induce a rearrangement of the bonds between the submicelles leading to a new state with a minimum Gibbs energy. Besides the swelling of the submicelles, the rearrangement of the bonds may take some time. Probably the final result is an increased interaction between the swollen submicelles, leading to a higher G′ at a lower temperature.

For the shift in G′, G″ and tan δ along the frequency axis (Section 3.1.4) only a speculative and qualitative explanation can be given. This stems largely from the complications arising from the structural changes at molecular and submicellar level by a change in temperature, and because different interaction forces are involved. A decrease in temperature causes somewhat smaller conformational freedom of the peptide chains and maybe this results in a longer lifetime of some interactions, such as entanglements. Van Dijk (18) supposed entanglements to play a part for large deformations. It is uncertain whether this is also the case for small deformations. Probably more important is that, with a decrease in temperature, a shift in interactions takes place: from inside submicelles to between submicelles. Because of the observed shift to lower frequencies or longer time scales, this would imply that the interactions with a relative long relaxation time increase relative to the interactions with a shorter relaxation time with decreasing temperature. Because of the slower relaxation of bonds at low temperatures, rearrangements will last longer and the eventually higher value of the modulus at a lower temperature may also be ascribed, at least partly, to the slower relaxation of bonds, since it implies that a larger proportion of the total number of bonds is elastically effective at the considered time scale.

In polymer science (e.g. 12) a shift in the direction of the logarithmic frequency axis caused by a temperature change is known as the time-temperature superposition. It is used to obtain values of the moduli in frequency ranges not covered by the frequency range of the apparatus used. Conditions for the validity of this method are that the shape of the measured curves must fit one another well, that the value of the shift factor along the logarithmic frequency axis is independent of the viscoelastic function considered and that the temperature dependence of the shift factor must have a reasonable form (12). The Williams, Landel and Ferry equation has proved to be widely applicable in this respect. One of the prerequisites for application of the time-temperature superposition is that all relaxation times should have the same temperature dependence. The shift factor found in this study was much more temperature-dependent than observed for polymers (e.g. 12, 25), which is not surprising, because with a change in temperature the relative importance of the contributing interaction forces may well change and the relaxation times cannot be expected to have the same temperature dependence. Therefore we feel that the fit is probably fortuitous and that the time-temperature superposition may not be applied to rennet milk gels. Roefs (13) came to the same conclusion for acid milk gels. The impossibility of time-temperature superposition for these gels follows, among other things, from the behaviour of tan δ versus ω as function of the temperature (Fig. 9, after Roefs (13)).

For rennet-induced skim milk gels the temperature dependence was found to be partly different from that of acid skim milk gels (13). A similarity is the decrease of the moduli with increasing measuring temperature, but especially

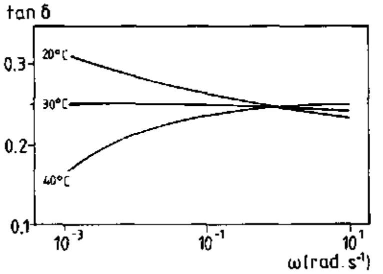


Fig. 9. Loss tangent, tan $\delta = G''/G'$, as a function of the angular frequency for acid skim milk gels. Measuring temperature is indicated (after Roefs (13)).

at frequencies below 0.5 rad/s the acid skim milk gels became more liquid-like at low temperature and more elastic at high temperature (see Fig.9, after Roefs (13)). Roefs (13) stated that since the elastic character of the acid casein gels increased with the measuring temperature, it seemed logical that hydrophobic binding was one of the interaction forces that were involved in acid milk gels. On the other hand he assumed, just as for rennet-induced milk gels, that the effect of the measuring temperature on the values of the dynamic moduli was at least partly due to an indirect effect of hydrophobic interactions involving protein rearrangement. This does not seem to be unreasonable. In a kind of stress relaxation experiment it was found (13) that for acid milk gels the decrease of the pseudo-stress relaxation modulus $G^*(t)$ with time was more pronounced at lower measuring temperatures. However, after 10^4 s $G^*(t)$ seemed to be almost independent of measuring temperature and $G^*(t)$ still had a considerable value, indicating that a kind of permanent (elastic) network was present in acid skim milk gels. As was explained in Section 3.2, in rennet-induced milk gels such a permanent network is probably not present, i.e. rearrangement may occur more easily. This may, at least partly, explain the difference in the development of permeability with time between both types of milk gels, as mentioned in the preceding article of this series (5).

Several workers have assumed that a milk gel may be described as a rubber gel (e.g. 7, 16), among other things because the moduli would increase with temperature (7, 15). In this study it is shown that the moduli decrease with increasing temperature. Moreover, there are other reasons why a milk gel should be considered as a particle gel rather than as a rubber gel (11).

Acknowledgements

The Institute for Cereals, Flour and Bread TNO, Wageningen, kindly offered us the opportunity to perform measurements on the Weissenberg Rheogoniometer and we are indebted to J.M.P. Wokke and H.M. van de Haar for their help. We thank A.E.A. de Groot-Mostert for technical assistance.

Samenvatting

P. Zoon, T. van Vliet & P. Walstra, *Reologische eigenschappen van met leb gestremde ondermelkgelen. 2. Effect van de temperatuur*

De invloed van de temperatuur op de mechanische eigenschappen van met leb gestremde ondermelkgelen werd onderzocht. Daartoe werden dynamische en spanningsrelaxatie-metingen uitgevoerd. Verhoging van de temperatuur tijdens de vorming en veroudering van het gel had vooral een versnelling van de gelering tot gevolg, terwijl de moduli na een lange verouderingstijd lager waren. Voor gelen die bij 25, 30 en 35 °C gevormd waren, werden bij een meettemperatuur van 30 °C gelijke waarden voor de moduli gevonden. Anderzijds gaven gelen die bij 30 °C gevormd waren en vervolgens bij verschillende temperaturen onderzocht werden een toename van de moduli bij een afname van de meettemperatuur te zien. Tevens verschoof de relaxatie van bindingen naar een langere tijdschaal. Na een plotselinge temperatuurverandering duurde het enige tijd voordat de moduli weer een konstante waarde hadden en de benodigde tijd was langer naarmate de temperatuur lager was. Voor de gevonden resultaten werden mogelijke verklaringen gegeven met betrekking tot interaktiekrachten en de struktuur van het gelnetwerk.

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Neth. Milk Dairy J. 42 (1988) 295-312

Rheological properties of rennet-induced skim milk gels.
3. The effect of calcium and phosphate

P. Zoon, T. van Vliet and P. Walstra

Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, the Netherlands.

Received 15 June 1988; accepted 12 August 1988

Key-words: artificial casein micelles, calcium, casein gels, phosphate, renneting, rheology

Summary

The effect of the calcium ion activity and the concentration of micellar calcium phosphate (MCP) on the dynamic rheological properties of rennet-induced gels made of skim milk and artificial micelle dispersions has been studied.

A minimum amount of calcium was needed for clotting to occur. At constant calcium ion activity, a lower MCP concentration resulted in a longer clotting time and lower moduli. For MCP concentrations higher than in milk the moduli tended to decrease again. A slight decrease of the MCP concentration did not affect tan δ (= ratio of the loss modulus G'' to the storage modulus G'). A higher tan δ was observed if the MCP concentration was lower than about half the concentration in milk. At such low MCP concentrations, enhanced visible syneresis occurred. MCP was thought to be directly involved in bonds between micelles and, moreover, to enhance indirectly the formation of bonds by affecting the micellar structure.

The calcium ion activity influenced the clotting time and the moduli, but not significantly tan δ in the range studied. Probably, the calcium ion activity is related to shielding of negative charges of the casein and to enhancing a positive charge on the MCP.

1 Introduction

Cheese milk is normally enriched with calcium cloride to accelerate the renneting process. The acceleration is due to a combined effect of the increased calcium concentration as such and a drop of pH (1). Addition or removal of calcium does not influence the enzymic reaction if the pH is kept constant. The influence of calcium on the aggregation reaction is much more pronounced. A minimum calcium concentration is needed to initiate gelation of rennet-converted casein micelles. Above this minimum level the rennet coagulation time of milk decreases with the calcium concentration (1). At very high concentrations of added $CaCl_2$ (>50 mM) the coagulation time increases again if the pH is kept constant (2, 3). If small amounts of $CaCl_2$ are added to the milk at constant pH the gel strength after a long ageing time (ultimate gel strength) increases. Hossain (4) added up to 4.5 mM $CaCl_2$ and he found a continuous increase in ultimate gel strength. McMahon et al. (2) found a maximum in the gel strength for 50 mM added $CaCl_2$. Not only calcium but also the presence of inorganic phosphate is essential for the production of a visibly homogeneous gel at the casein concentration observed in milk (7, 8).

For a better understanding of the mechanism behind the effects of Ca and inorganic phosphorous (P_i) on gel formation and ageing, the partition of Ca and P_i in milk between serum and micelles must be taken into account. Milk contains on average about 30 mM of Ca and 22 mM of P_i (5). About 10 mM of Ca and 11.5 mM of P_i is present in the serum, the remainder is associated with the casein micelles. It is believed (e.g. 6) that the micellar calcium exists partly ($\approx$15 mM) as a calcium phosphate salt, called micellar calcium phosphate (MCP), whose concentration is independent of the calcium ion activity and partly as 'bound' Ca^{2+} ($\approx$5 mM), whose concentration is dependent on the calcium ion activity. In this respect 'bound' Ca^{2+} is meant to include counterions. All of the inorganic phosphorus associated with the micelles is thought to be present in the MCP. Holt et al. (6) found that MCP as well as 'bound' Ca^{2+} were important with regard to the integrity of the micelles.

Removal of MCP in such a way that serum composition remained unaltered markedly prolonged the renneting process and this appeared to be mainly due to structural changes in the micelles (3).

Experiments with (partly) dephosphorylated casein showed that the casein phosphoserine residues were also important with regard to coagulation and gel formation. (e.g. 9, 10, 11). The action of chymosin was not much influenced by dephosphorylation (9), but coagulation of renneted micelles was slower and the gel strength, which in these experiments was not a well defined parameter, at a given ageing time was lower (9-11). Yun et al. (10) concluded that gel formation and ageing involves interaction between calcium ions and serine phosphate groups of the casein. But Yamauchi & Yoneda (9) and Reimerdes & Roggenbuck (11) remark that dephosphorylation alters the structure of the micelles and this is supposed to be correlated to the physical properties.

In the present study the effect of the calcium ion activity and the concentration of micellar calcium phosphate on the mechanical properties of rennet-induced gels made of skim milk and artificial micelle dispersions was investigated.

2 Materials and methods

2.1 Skim milk

Reconstituted skim milk was prepared by dispersing a commercial low-heat skim milk powder (Krause, Heino) in demineralized water. Its composition is given by Roefs (12). For the experiments in Section 3.1, 12 g of milk powder was used per 100 g of water. To obtain samples with elevated calcium level 6 mM of $CaCl_2$ was added to the water. The reconstituted milk was stirred about 20 hours at 30 °C prior to renneting. Three hours after dispersing the milk powder the pH of the samples containing $CaCl_2$ was adjusted to 6.6. Just before renneting the pH was checked again to be 6.6. For the experiments in Section 3.2, 9.5 g of milk powder was used per 100 g of water. To prevent bacterial growth 0.01 % of thiomersal (BDH Chemicals LTD) was added.

Bulk collected milk was obtained from the dairy herd of the university.

2.2 Caseinate

A spray dried sodium caseinate powder was used. Its composition is given by Roefs (12).

2.3 Dialyzed milk

Micellar calcium phospate (MCP) was removed by the method of Pyne & McGann (13). Reconstituted skim milk, which was stirred for 5 hours at 30 °C, was chilled and acidified to pH 4.9, followed by dialysis at 4 °C for 4 days against an excess of bulk skim milk (about 20 times the volume of the reconstituted milk). The bulk milk was renewed twice during this period.

The concentration of calcium was increased subsequently by dialysis of the acidified reconstituted skim milk for 2 days at 4 °C against an excess of bulk skim milk enriched with 1.5 to 6 g of $CaCl_2 \cdot 2H_2O$ per litre of bulk milk. The pH of the bulk milk was set at 6.6. To some of the samples a $CaCl_2 \cdot 2H_2O$ solution (18 %) was added after these dialysis steps. The temperature was raised to 30 °C, the pH was checked to be 6.6 and another 0.01 % of thiomersal was added. The milk was then stirred for about 16 hours at 30 °C; if necessary the pH was set at 6.6 again and the milk subsequently was renneted.

2.4 Calcium phosphate caseinate dispersions

Sodium caseinate (2.8 %) was added to a calcium chloride solution containing 0.01 % thiomersal. After stirring for 16 hours at 45 °C the temperature was lowered to 30 °C and a sodium phosphate solution (0.5 M) was added slowly while continuously stirring. The pH was set at 6.6 and after about 25 hours rennet was added.

2.5 Artificial micelle dispersions

Artificial micelle dispersions were made by the method of Schmidt et al. (14). Only a short description will be given here. Appropriate volumes of solutions of sodium caseinate, calcium and magnesium chloride, sodium and potassium phosphate and potassium citrate were slowly and simultaneously added to a vat containing distilled water while vigorously stirring. The pH was kept at 6.7 (±0.02) and the temperature at 37 °C (±0.1).

Two different types of artificial micelles were made. Type X was most similar to 'real' milk and contained all the above mentioned components. Type Y only contained sodium caseinate, sodium and potassium phosphate and calcium chloride. The content of micellar calcium phosphate and the calcium ion activity in artificial micelle dispersion type Y was varied by adjusting the concentration of calcium and phosphate in the solutions for preparation of the micelles. To some of the samples NaCl was added to increase the ionic strength.

To prevent bacterial growth 0.02 % of sodium azide was added to the artificial micelle dispersions. They were stirred for about 20 hours at 30 °C and subsequently renneted.

2.6 Renneting

Commercial calf rennet (10 800 SU from CSK, Leeuwarden, Netherlands) was used. The time after rennet addition required for the formation of visible clots was taken as the clotting time. In all experiments 0.025 % of rennet was used.

2.7 Calcium plus magnesium content

Calcium plus magnesium content was determined by direct complexometric titration. The titration was carried out at pH 10 with 0.01 M Titriplex (Merck) in the presence of magnesium Titriplex. Erichrome black T (Merck) was used as indicator.

2.8 Inorganic phosphorus content

To estimate the inorganic phosphorus content 5 ml of milk or whey were pipetted into a volumetric flask. About 20 ml of water and 20 ml of a 30 % trichloroacetic acid solution were added. The volume was adjusted to 50 ml with water. The solution was mixed and after one hour it was filtered through a fine filter paper. In the clear filtrate the phosphorus content was measured according to International Standard FIL/IDF 33A (1971).

2.9 Calcium ion activity

Calcium ion activity was determined with a calcium ion selective electrode (Orion, model 93-20) at 30 °C. The calibration was performed with three $CaCl_2$ solutions containing 1, 2 or 10 mM $CaCl_2$ and 80 mM KCl. The calcium ion activity of these solutions was calculated by multiplying the calcium concentration with the activity coefficient of Ca^{2+} (=0.40), as calculated with the Debye-Hückel equation (25). Association between Ca^{2+} and Cl^- was not taken into account, because no reliable association constants were available at this ionic strength. Linear extrapolation from data at high ionic strength (>0.36 M) reported by Johnson & Pytkowicz (26), gave a stoichiometric association constant of 2.82 l/mol at an ionic strength of 0.08 M. This would imply that about 18 % of the calcium is not present as free ion, but associated with chloride. The calcium ion activity of the solutions would then be 18 % lower than assumed. Therefore the calcium ion activities given in this report probably will be somewhat too high. However, other investigators who used a calcium ion selective electrode (e.g. 27) for determining the calcium ion activity, did not take the association between Ca^{2+} and Cl^- into account.

2.10 Rheological measurements

Dynamic measurements were performed with the 'Den Otter' rheometer. The apparatus and the method followed have been described before (15). Gel ageing was studied at an angular frequency of 1 rad/s. The strain was kept below 3 %. Temperature during the measurements was 30 °C (±0.1 °C).

3 Results

3.1 $CaCl_2$ addition

Gels were made of reconstituted skim milk to which 6 mM $CaCl_2$ was added. The rheological properties were compared with those of gels made of reconstituted skim milk without calcium addition. The pH was kept at 6.6. The clotting time of the sample with and without added calcium was 16.8 and 20 minutes, respectively. The shorter clotting time is due to the faster coagulation of renneted micelles (1). The gel formation and ageing was followed by determining the storage modulus G' and the loss modulus G'' as a function of time. In Fig. 1 the graph of G' is depicted. The results for G'' (not shown) were similar, only the values were lower. After a long ageing time the moduli of the samples with $CaCl_2$ were about 10 % higher than of those without $CaCl_2$. The frequency dependence, measured after 7 hours of ageing, gave graphs that were similar in shape for both types of samples, but the moduli of

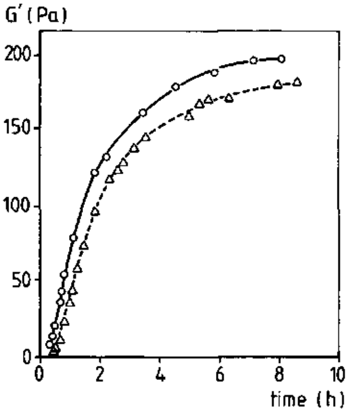

Fig. 1. Storage modulus G' as a function of time after rennet addition for skim milk gels; (○) 6 mM $CaCl_2$ added, (△) no $CaCl_2$ added; pH = 6.6, T = 30 °C, angular frequency = 1 rad/s.

the samples with added calcium were on average 10 % higher. The values of tan δ were not affected by the calcium addition.

Van den Bijgaart (16) found that the permeability and the change of permeability in time were not significantly affected by addition of 4.5 mM $CaCl_2$ if the pH was kept constant. The syneresis rate was slightly higher and the maximum rate of syneresis was reached sooner after rennet addition, which was ascribed to the somewhat faster aggregation.

Addition of $CaCl_2$ causes the calcium ion activity as well as the concentration of MCP to increase, so it is not possible to judge which factor plays the more important part with respect to the variation observed.

3.2 Gels made of dialyzed milk

MCP was removed from the milk and subsequently the Ca concentration was raised (see Section 2.3).

After removal of MCP the milk had a translucent green appearance indicating that the original casein micelles did not exist any longer. The calcium plus magnesium concentration was about 18.5 mM, the inorganic phosphorus concentration was about 12.0 mM and the calcium ion activity was about 0.80 mM. The dialyzed milk did not coagulate after rennet addition although the calcium ion activity was slightly higher than that of the original skim milk (0.73 mM).

Increasing the calcium content until a calcium plus magnesium concentration of 22 mM was measured, gave the milk a white, non-translucent appearance, but still no renneting took place. This only happened at a calcium plus magnesium concentration of about 25 mM or higher.

In Table 1 a survey of the compositional data and clotting time is given of

Table 1. Compositional data and rennet clotting time of dialyzed reconstituted skim milk. Ca = concentration of calcium (mM), P = concentration of inorganic phosphorus (mM). Subscripts: m = concentration in the milk, w = concentration in the whey, d = concentration in the milk minus (0.97×) concentration in the whey. $a(Ca^{2+})$ = calcium ion activity (mM). t_c = clotting time (min). *estimated value (not determined).

Sample	Ca_m	Ca_w	Ca_d	P_m	P_w	P_d	$a(Ca^{2+})$	t_c
D1	38.0	–	–	6.3	–	≈3*	3.9	14
D2	26.2	–	–	8.1	–	≈3*	1.5	14
D3	24.9	14.3	11.0	10.2	6.7	3.7	1.2	18
D4	27.5	15.6	12.4	10.2	6.2	4.2	1.4	12
D5	32.9	18.7	14.8	9.9	4.9	5.1	1.9	12
D6	38.7	22.0	17.4	10.1	4.2	6.0	2.7	12
D7	41.9	22.2	20.4	10.2	3.5	6.8	3.1	12
skim milk	32.3	12.3	20.4	22.0	12.6	9.8	0.73	20

samples of dialyzed milk, all having a low MCP content and different concentrations of calcium. The concentration of inorganic phosphorus associated with the micelles (P_d), is best related to the concentration of MCP, because it is all thought to be part of the MCP.

The more calcium a sample contained at a certain phosphate concentration, the higher the amount of MCP and the calcium ion activity. A relatively long clotting time was found for samples with a relatively low calcium ion activity (skim milk) and/or low MCP concentration (D1 - D3). The clotting time of sample D1, with a high calcium ion activity, but a low concentration of P_i and therefore also a low MCP concentration, was longer than expected for a sample with such a high calcium ion activity. This implies that besides the calcium ion activity, also the amount of MCP is important with respect to the clotting time. In Section 4 this will be further discussed.

The formation and ageing of the gels was followed by measuring the storage modulus G′ and loss modulus G″ of the gels as a function of time. In Fig. 2, G′ is depicted for the samples mentioned in Table 1. Similar results were found for G″ (not shown), only the values were lower. The sample with the lowest concentration Ca (D3) showed syneresis 1.5 hours after rennet addition. For the other samples no severe syneresis was observed within the time of the experiments. With increasing Ca concentration the value of the moduli after a long ageing time increased, except for sample D1 which has a low P_i concentration. This sample also had a longer clotting time than expected from its calcium ion activity. Presumably, a minimum amount of MCP is needed to give casein particles that exhibit 'normal' gel formation and ageing.

The frequency-dependence of the moduli was determined. G′ and G″ ver-

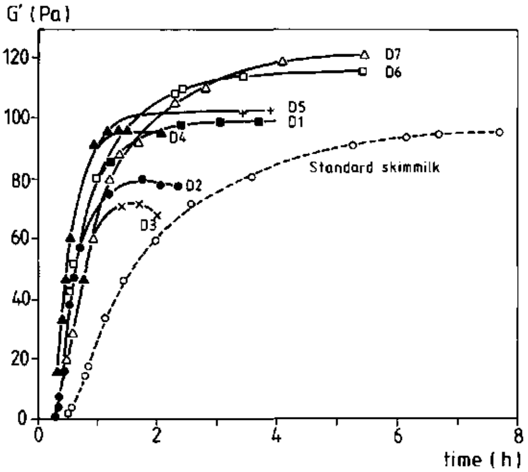

Fig. 2. Storage modulus G′ as a function of time after rennet addition for gels made of reconstituted skim milk (dashed curve) and of dialyzed milk (full curves). The sample numbers are indicated; same samples as in Table 1. pH = 6.6, T = 30 °C, angular frequency = 1 rad/s.

sus angular frequency gave about the same shape of the graphs for all samples, only the absolute values of the moduli were different. Graphs of samples D2, most probably containing the lowest concentration of MCP, and D7, containing the highest MCP concentration, are given in Fig. 3a. The ratio of G″ to G′, tan δ, appeared to be highest for D2 (Fig. 3b), having the lowest Ca content of the samples of which the frequency dependence could be measured, followed by D4 with a somewhat higher Ca content and D1 with the low P_i content. Tan δ of samples D5 to D7 was similar to tan δ of skim milk within experimental error. Because both calcium ion activity and MCP content were lowest in the sample with the highest tan δ, it is not clear which is the more important factor. Taking into account the high value of tan δ for sample D1 having a low phosphorus content and the low value of tan δ of skim milk, having the lowest calcium ion activity, the MCP content seems to be more important with respect to the value of tan δ in these experiments. In the next section this is further considered.

3.3 Gels made of caseinate dispersions

In these experiments the possibility was tested to form a gel of a dispersion made by mixing sodium caseinate, calcium chloride and sodium phosphate solutions (see Section 2.4). The size of the formed casein particles, about 1 μm as measured with a light microscope, was much bigger than that of casein

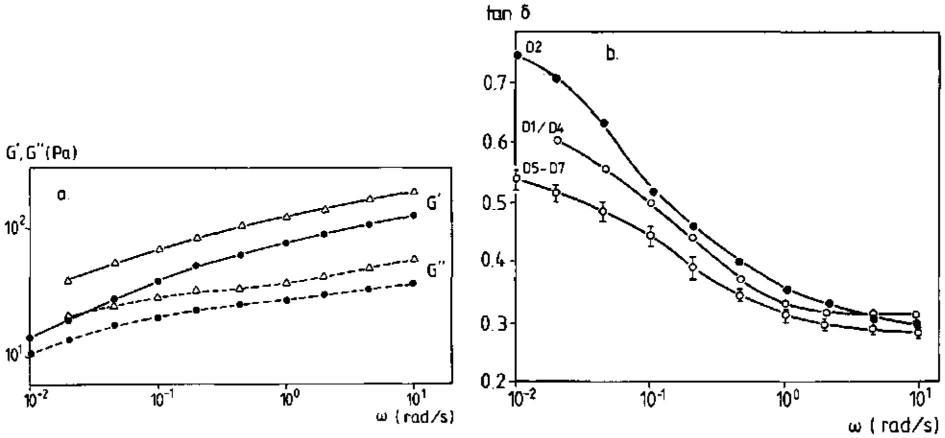

Fig. 3. Storage modulus G' and loss modulus G'' (Fig. 3a) and tan δ (Fig. 3b) as a function of the angular frequency. Fig. 3a: samples D2 (●) and D7 (△); Fig. 3b: the sample numbers are indicated. Same samples as in Fig. 2.

micelles (≈30-200 nm).

In introductory experiments it was found that at least 13 mM of calcium was needed for gel formation after rennet addition (if no phosphate was present). Increasing the concentration of P_i from 0.2 to 5 mM at a constant concentration of calcium (15 mM) resulted in a decrease of the rate of visible syneresis.

Gel formation and ageing was followed by determining the dynamic moduli. The moduli of the gels made of calcium phosphate caseinate dispersions were much lower than those of skim milk gels with a similar casein concentration (Fig. 4). Probably this was caused by the much bigger size of the casein particles in the caseinate dispersions compared to those in skim milk. Roefs (12) got comparable results for acid caseinate gels formed from sodium caseinate dispersions with varying calcium content: addition of 20 mM calcium to sodium caseinate dispersions led to an increase in the size of the casein particles and a strong decrease of the dynamic moduli of the gels formed.

The rennet-induced caseinate gels containing 20 or 25 mM P_i (Fig. 4) showed less visible syneresis than skim milk gels within the time of the experiments, but on top of a gel made of a casein solution containing 30 mM calcium and only 5 mM phosphate a layer of expelled whey appeared after several hours. The value of tan δ of this sample ws also higher than that of the other two samples (Fig. 5), probably because of a low MCP content. Compared with skim milk gels the values of tan δ at low frequencies were somewhat lower for the caseinate gels containing 20 and 25 mM phosphate. The lesser ten-

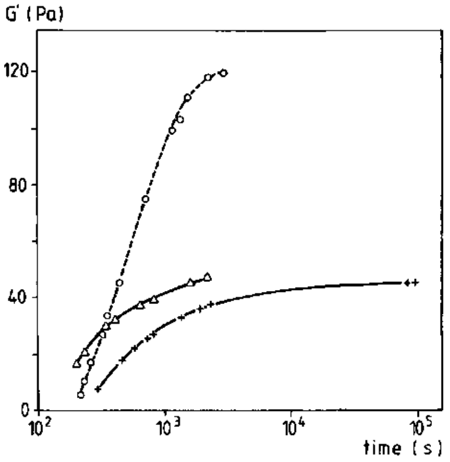

Fig. 4. Storage modulus G′ as a function of time of gels made of reconstituted skim milk (dashed curve) and gels made of calcium-phosphate-caseinate dispersions (full curves) containing 30 mM calcium and 20 (△) or 25 (+) mM phosphate; pH = 6.6, T = 30 °C, angular frequency = 1 rad/s.

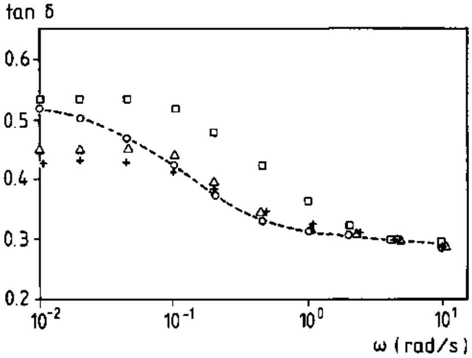

Fig. 5. Tan δ as a function of the angular frequency for gels made of reconstituted skim milk (○) and gels made of caseinate dispersions containing 30 mM Ca and 5 (□), 20 (△) and 25 (+) mM phosphate; partly the same samples as in Fig. 4.

dency for visible syneresis may be explained by the somewhat lower value of tan δ together with the, because of the bigger structural elements (casein particles), probably thicker strands in the network (17).

3.4 *Gels made of artificial micelle dispersions*

3.4.1 Artificial micelle dispersions resembling skim milk. Artificial micelles were made according to the method of Schmidt et al. (14), see Section 2.5. From electron microscopic studies (14) it appeared that the internal structure of artificial micelles, made of whole casein and having about the same composition as natural micelles, was similar to that of natural micelles within experimental error. Also the size distribution of the artificial and natural micelles

Table 2. Compositional data and clotting time of artificial micelle (AM) dispersions. Symbols are as in Table 1. I = ionic strength (mM). Concentrations are in mM. * = samples made of AM-dispersions type X; the other samples are made of type Y. Ca = calcium concentration for type Y and calcium plus magnesium concentration for type X.

Sample	Ca_m	Ca_w	Ca_d	P_m	P_w	P_d	$a(Ca^{2+})$	I	t_c
A1*	35.7	12.4	23.7	22.5	11.7	11.2	0.64	96	17
A2	25.0	2.8	22.3	26.9	15.0	12.3	0.68	93	14
A3	22.8	2.6	20.3	34.5	24.2	11.0	0.41	82	19
A4	24.6	3.3	21.4	19.5	10.1	9.7	0.77	63	13
A5	24.9	3.5	21.5	19.7	9.8	10.1	0.93	64	11
A6	24.4	3.6	20.9	19.8	9.9	10.2	0.96	66	11
A7	25.3	4.0	21.4	19.9	8.9	11.3	1.08	93	-
A8	27.3	5.4	22.1	15.8	4.8	11.2	1.73	91	11
A9	17.4	3.1	14.4	14.5	9.3	5.5	0.78	49	20
A10	17.1	3.1	14.1	14.2	8.8	5.7	0.79	49	18
A11	21.4	3.8	17.7	17.3	9.1	8.5	1.03	92	13
A12*	36.0	12.6	23.8	21.6	11.0	10.9	0.81	97	17
A13	29.9	3.9	26.1	22.7	8.9	14.1	1.08	92	13
A14	35.8	3.3	32.6	25.8	7.9	18.1	1.02	93	13
skim milk	32.3	12.3	20.3	22.0	12.6	9.8	0.73	80	20

was nearly equal. The artificial micelles had a slightly lower stability towards dialysis and pressure than the natural ones (18); the rennetability of the artificial micelles was not investigated.

It appeared to be possible to rennet artificial micelle dispersions (AM-dispersions) that have about the same micellar calcium phosphate (MCP) content and calcium ion activity as reconstituted skim milk. This was the case for AM- dispersions type X, containing in addition to calcium and phosphate also magnesium and citrate, as well as for AM-dispersions type Y, containing no magnesium or citrate (see Section 2.5). In Table 2 some compositional data of these samples are given. The ionic strength was calculated using an ion equilibrium calculation programme of Holt et al. (19). As can be seen from Fig. 6, no great differences were found between gels made of AM-dispersions with or without magnesium and citrate. The variation in the moduli was probably caused by variation in the calcium ion activity. The clotting time was longer for dispersions of type X than for Y. It is unlikely that this was caused by the presence of magnesium. Van Hooydonk et al. (1) found a decrease in clotting time if magnesium was added to skim milk. So citrate must have been the main factor causing variation. For AM-dispersions with a composition comparable to that of the reconstituted skim milk used, the clotting time was

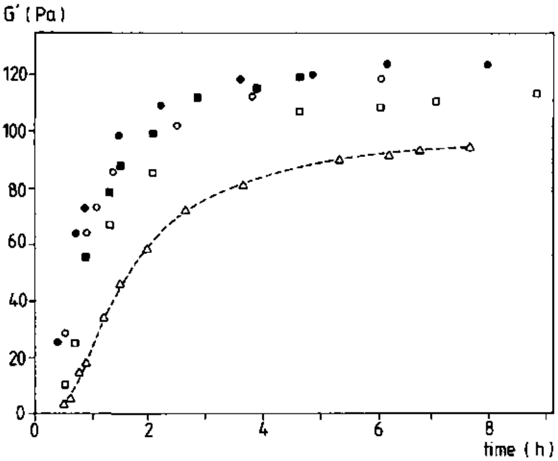

Fig. 6. Storage modulus G' as a function of time after rennet addition for gels made of reconstituted skim milk (△) and of artificial micelle dispersions: A1 (□), A12 (■), A2 (○) and A4 (●). T = 30 °C, pH = 6.6, angular frequency = 1 rad/s.

somewhat shorter, the increase of the moduli in time of the gel formed after rennet addition faster and the moduli after a long ageing time about 20 % higher (Fig. 6). The differences between AM-samples and milk samples may be caused by several factors. The temperature history of the caseinate and the skim milk powder as well as the casein composition may have been different, although the total casein concentration of the gels was equal. Probably the AM-dispersions did not contain plasmin, which may give slightly higher moduli because of less protein breakdown (15). Besides, the AM-dispersions did not contain lactose, which may slightly affect the moduli.

The frequency dependence was tested after an ageing time of about 7 hours. As can be seen from Fig. 7a, the shape of the curves of gels made of AM- dispersions were rather similar to that of gels made of reconstituted skim milk. This was also seen for tan δ-as-function of the frequency (Fig. 7b). Especially above 1 rad/s the differences were very small. The gels made of AM-solution type Y, containing no citrate or magnesium, tended to have a tan δ that was about 5-10 % lower at low frequencies (<0.1 rad/s).

3.4.2 Influence of the calcium ion activity. AM-dispersions were prepared with about the same MCP content as reconstituted skim milk. The calcium ion activity was varied from 0.4 to 1.7 mM, as measured with a calcium ion selective electrode. In Table 2 compositional data and the clotting times of these samples are given. Samples with a Ca^{2+} activity between 0.68 and 1.73 (A2,

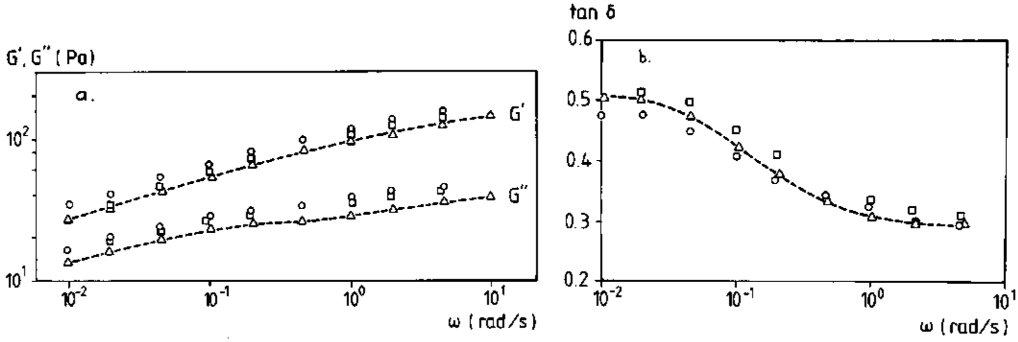

Fig. 7. Storage modulus G' and loss modulus G'' (Fig. 7a) and tan δ (Fig. 7b) as a function of the angular frequency for gels made of skim milk (△) and of artificial micelle dispersions; same samples and symbols as in Fig. 6.

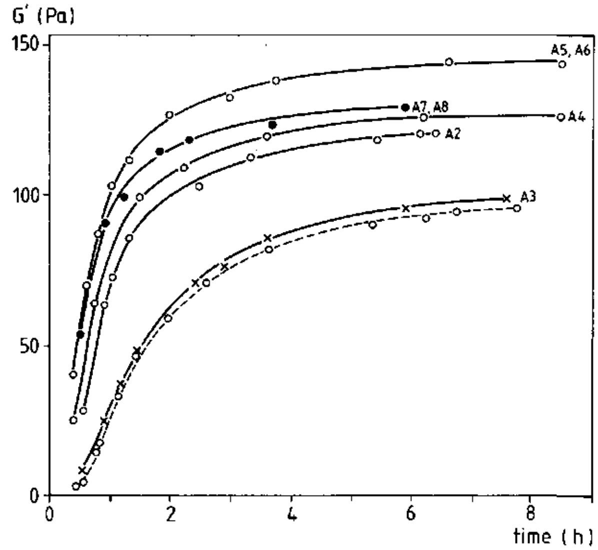

Fig. 8. Storage modulus G' as a function of time after rennet addition for gels made of reconstituted skim milk (dashed curve) and of artificial micelle dispersions (full curves) containing about the same amount of MCP, but differing in calcium ion activity. The sample numbers are indicated; same samples as in Table 2. T = 30 °C, pH = 6.6, angular frequency = 1 rad/s.

A4-A8) did not vary greatly as to clotting time and development of G' (Fig. 8) and G'' (not shown) in time. It is doubtful whether the higher values of G' in Fig. 8 for samples A5 and A6 are related to their Ca^{2+} activity. The only sample that gave an obviously different result was A3 with the lowest calcium ion activity.

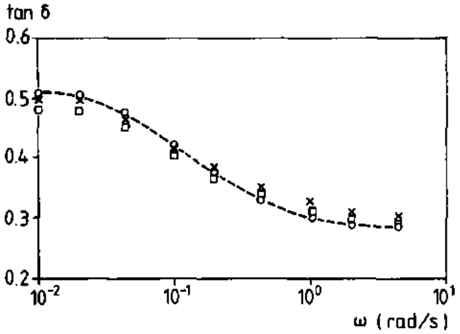

Fig. 9. Tan δ as a function of the angular frequency for gels made of skim milk (○) and of artificial micelle dispersions: × = A3, □ = A2 and A4-A8. Same samples as in Table 2 and Fig. 8.

The shape of the graphs of the dynamic moduli versus the angular frequency was equal for all samples. Only the absolute values of the moduli differed. As can be seen from Fig. 9 no significant differences in tan δ were found between the samples. The values of the sample with the lowest calcium ion activity (A3) were very similar to those of reconstituted skim milk, and they were about 3 % higher than those of the samples with higher calcium ion activities. The differences observed are not greater than differences between duplicates.

3.4.3 Influence of the micellar calcium phosphate content. AM-dispersions were made with a calcium ion activity of about 0.8 to 1 mM and a MCP content that varied from about half the quantity present in reconstituted milk to more than 1.5 times that quantity. Compositional data and clotting time for the samples (A9-A14) are given in Table 2. For samples A9 and A10 with a low MCP content relatively long clotting times were found. It is doubtful whether at higher concentrations the clotting time was much affected by the amount of MCP. The differences in time were not big and, in addition, the ionic strength varied somewhat.

Gel formation and ageing were followed in the 'Den Otter' rheometer (Fig. 10). It appeared that the modulus after a long ageing time was highest for samples containing about the quantity of MCP present in milk. No big differences were found in tan δ. Only tan δ of the samples containing about half the normal amount of MCP was significantly higher compared to the others (Fig. 11). Increasing the concentration of MCP to a higher level than that in the skim milk did not result in an increase in moduli (probably even in a decrease), nor in a decrease of tan δ. From, for instance, Ref. 20 it may be deduced that at increasing concentrations of MCP the size of the artificial micelles also increased and this may lead, just as in the case of the caseinate dis-

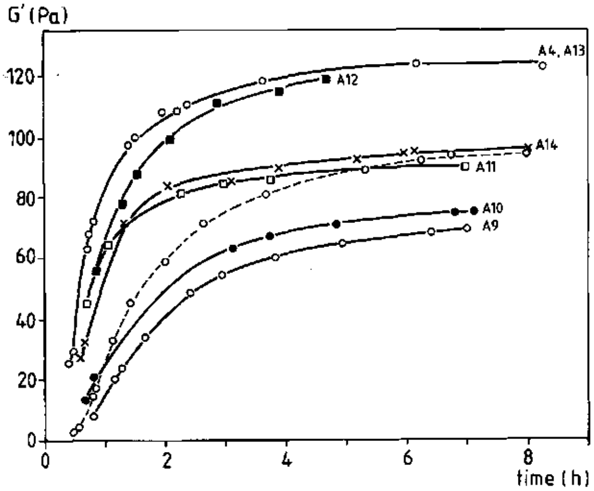

Fig. 10. Storage modulus G' as a function of time after rennet addition for gels made of reconstituted skim milk (dashed curve) and of artificial micelle dispersions (full curves), differing in amount of MCP and with a fairly constant calcium ion activity. The sample numbers are indicated; same samples as in Table 2. T = 30 °C, pH = 6.6, angular frequency = 1 rad/s.

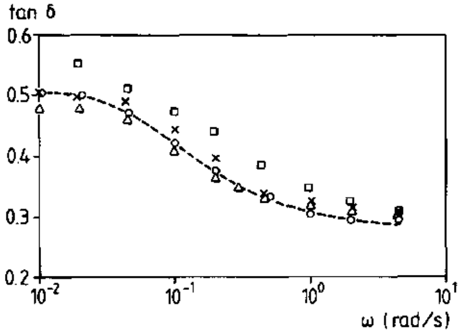

Fig. 11. Tan δ as a function of the angular frequency for gels made of reconstituted skim milk (○) and of artificial micelle dispersions: sample A9 and A10 (□), A11 (×) and A12-A14 (△). Same samples as in Table 2 and Fig. 10.

persions in Section 3.3, to lower values of the moduli. Probably the concentration of MCP in milk is at an optimal level with respect to a short clotting time and high values of the moduli.

4 Discussion

From experiments with dialyzed milk and artificial micelle dispersions it appeared that decreasing the concentration of micellar calcium phosphate

(MCP) resulted in lower moduli. Removal of a small part of the MCP did not change tan δ. Only if the concentration of MCP was decreased below about half the amount normally present in milk was tan δ increased, especially at lower frequencies i.e. over longer time scales (e.g. 15). Holt et al. (6) found that removal of more than 30 % of MCP caused dissociation of casein from the micelles. Neither in experiments with dialyzed milk nor in those with artificial micelle dispersions was a significant increase in the protein concentration of the whey found if more than 30 % of the MCP had been removed. Possibly casein was dissociated after removal of MCP, but reassociation took place during renneting. This has also been observed to happen at low pH (24). Any effect of reassociated casein on the mechanical properties of the network is unknown.

MCP is thought to connect submicelles to each other, and removal of MCP might cause a decrease in the number of bonds within the strands of the casein gel network, resulting in lower moduli. Although it is still not completely sure what the structure of the MCP is and to which groups of the casein MCP is bound, it is supposed that MCP is anyhow linked to negatively charged phosphoseryl residues (e.g. 6, 21, 22). Assuming that the MCP is positively charged, removal of MCP then leads to an increase of the negative charge of the already net negative charge of the casein, which may result in more electrostatic repulsion between caseinate building blocks, hence in lower moduli. Also interaction between MCP and casein via free NH_3^+-groups of the casein has been reported (e.g. 23), implying that the MCP is negatively charged. Removal of MCP may then cause an increase of positively charged groups which might result in more electrostatic attraction. Because no increase of the moduli was found this will play a secondary role, if it happens at all.

The increase in tan δ at low MCP content may be explained by assuming that the interaction between MCP and casein has a relatively long relaxation time. If the number of bonds with relatively long relaxation time diminishes, G' at frequencies higher than the reciprocal of the relaxation time will decrease, resulting in a higher tan δ. Another possibility is that MCP indirectly affects tan δ, e.g. by diminishing another interaction with a long relaxation time, because of the change in structure of the micelles.

From our results it appeared that the MCP content of milk is probably at an optimal level with regard to short clotting times and high values of the moduli, while the calcium ion activity of the milk used was lower than optimal. The positive effect of calcium chloride addition at constant pH must be ascribed to an increase in the calcium ion activity. The calcium ion activity influenced the clotting time and the values of the moduli. Probably an increasing calcium ion activity leads to a higher positive charge of the MCP and more shielding of negatively charged groups of the casein resulting in a faster coagulation and more interactions within the gel network. Holt et al. (6) found that at free Ca^{2+} concentrations in the milk below 1 mM, which corresponds to a calcium ion activity of about 0.4 mM, casein dissociates. This was thought to be due to a reduction in bound Ca^{2+} itself or to a reduction in the bonding of MCP with casein. Schmidt et al. (22) assumed that bound Ca^{2+} was involved in the connection between MCP and casein, and Holt et al. (6) supposed that the high P_i concentration in their samples with low free Ca^{2+} concentrations, which was essential to preserve the normal level of MCP, could displace phosphoseryl residues involved in linkages with the MCP, and thereby induce dissociation. This implies that at such low calcium ion activities an increase in tan δ can be expected, at least if the milk can be renneted. From our results it is not possible to judge whether bound Ca^{2+} is involved in the linkage between MCP and casein. The lower moduli of sample A3 in Fig. 8 may have been caused partly by a diminished interaction between MCP and casein, but the different development of G′ in time, compared to e.g. sample A11, that had a slightly reduced MCP content, implies that Ca^{2+} not only influences the gel formation and ageing via the interaction of MCP with casein.

Acknowledgements

We are indebted to C. Holt and D. G. Dalgleish (Hannah Research Institute) for the use of their ion equilibrium calculation programme and to D. G. Schmidt (NIZO) for his help with the preparation of artificial micelles.

Samenvatting

P. Zoon, T. van Vliet en P. Walstra, *Reologische eigenschappen van met leb gestremde ondermelkgelen. 3. Het effect van calcium en fosfaat*

Het effect van de calcium-ionactiviteit en van de concentratie aan micellair calciumfosfaat (MCP) op de dynamische eigenschappen van met leb gestremde gelen, gemaakt van ondermelk en van kunstmatige micel dispersies, werd onderzocht.

Een minimale hoeveelheid calcium was nodig om vlokking plaats te doen vinden. Bij een constante calcium-ionactiviteit resulteerde een lagere MCP-concentratie in een langere vloktijd en in lagere moduli. Bij MCP-concentraties hoger dan die in melk neigden de moduli weer lager te worden. Een geringe verlaging van de MCP-concentratie had geen effect op tg δ (verhouding tussen de verliesmodulus G″ en de opslagmodulus G′). Een hogere tg δ werd waargenomen indien de MCP-concentratie lager was dan ongeveer de helft van de concentratie aanwezig in melk. Bij zulke lage MCP-concentraties trad een verhoogde zichtbare synerese op. Verondersteld wordt dat MCP enige tijd na stremseltoevoeging deel uitmaakt van de bindingen tussen micellen en dat het tevens indirect de vorming van bindingen beïnvloedt door middel van de structuur van de caseïnemicellen.

De calcium-ionactiviteit beïnvloedde in het onderzochte gebied de vloktijd en de hoogte van de moduli, maar tg δ nauwelijks of niet. Waarschijnlijk leidt een verhoging van de calcium-ionactiviteit tot een verhoging van de positieve lading van het MCP en een betere afscherming van de (netto) negatieve lading van de micellen.

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Neth. Milk Dairy J. 43 (1989) 17-34

Rheological properties of rennet-induced skim milk gels.
4. The effect of pH and NaCl

P. Zoon, T. van Vliet and P. Walstra

Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, the Netherlands.

Received 15 June 1988; accepted 12 August 1988

Key-words: milk gels, pH, NaCl, renneting, rheology

Summary

The influence of pH and NaCl on the mechanical behaviour of rennet-induced skim milk gels was studied. Dynamic and stress-relaxation measurements were performed. Decreasing the pH in the range from pH 6.7 to 5.7, resulted in a maximum of the storage modulus G′ near pH 6.15. Above pH 6.0, no significant influence was found of pH on tan δ (G''/G'), whereas at pH 5.7 a higher tan δ was observed at all frequencies between 10^{-3}-4.5 rad/s. The relaxation time found for gels at pH 6.65 and pH 6.25 did not differ significantly, but at pH 5.72 the relaxation time was clearly shorter. Solubilization of micellar calcium phosphate and increase of electrostatic attraction appeared to be important effects of decreasing pH.

NaCl addition did not affect tan δ. Its influence on gel formation and ageing appeared to be dependent on the experimental conditions. If the clotting time was kept constant, additions of NaCl up to 200 mM increased the modulus at pH 6.65. At constant rennet concentration, this happened only up to 100 mM, whereas at higher NaCl concentrations gel formation-and-ageing was retarded. At pH 6.25 and constant clotting time, gel formation was slightly retarded up to 100 mM NaCl, whereas at 200 mM considerable retardation was observed, although the ultimate modulus was not much affected.

1 Introduction

The pH is an important factor in the manufacture of several types of dairy products such as yoghurt, quarg and cheese. It is an important cheesemaking parameter, affecting, for instance, the rate of renneting and syneresis (e.g. 1).

A recent study on the influence of pH on the physico-chemical changes and the clotting of milk has been published by Van Hooydonk et al. (2,3). Due to acidification, solubilization of micellar calcium phosphate (MCP) occurs; below pH 5.3 no micellar inorganic phosphorus, and only about 14 % of the micellar calcium, is still present. Also casein dissociates from the micelles, showing a peak value around pH 5.6. No dissociation is observed for rennettreated micelles, at least at 30 °C. Besides, the voluminosity of un-renneted micelles shows a minimum around pH 6.0 and a maximum around pH 5.3, whereas for renneted milk a slight decrease in voluminosity is found with decreasing pH in the range from 6.6 to 4.6, with a pronounced shoulder around pH 5.6 (2). Decreasing the pH of milk causes an increase in the rate of the enzymic stage of the renneting reaction, with a maximum in the velocity at pH 6.0. With decreasing pH, aggregation of micelles starts at a lower conversion of κ-casein and the rate of aggregation and gel formation increases (3).

Recently, Roefs (4) has published a study on the influence of pH on the rheological properties of casein gels. Gels were made by acidification (pH 4.3-4.9), or acidification combined with rennet action (pH 4.4-5.8), at low temperature and subsequent heating. Around pH 5.2, a minimum in the storage modulus G' and a maximum in the ratio of the loss modulus G'' to the storage modulus G' ($\tan \delta$) were found (4). A maximum in G' was found between pH 4.7 and 4.8 for acid casein gels made with rennet addition and around pH 4.5 for acid casein gels made without rennet addition. Another optimum pH for the curd firmness of rennet-induced milk gels has been reported, i.e. around 5.9 (5). However, these measurements were done very soon (35 minutes) after rennet addition and the increased firmness may have been due to faster gel formation at this pH. On the other hand, Hossain (6) found in the pH range from 6.36 to 6.7, that the ultimate curd firmness increased with decreasing pH. This implies, combined with the minimum of the storage modulus at pH 5.2, a maximum in the curd firmness somewhere between pH 5.2 and 6.36.

In the present study the rheological properties of rennet-induced skim milk gels were investigated in the pH range of 5.7 to 6.7, by performing dynamic and stress-relaxation measurements.

Another effect of the acidification of milk is the increase of the ionic strength. Roefs (4) calculated that by decreasing the pH of milk to 4.6, the ionic strength increased from about 80 mM to about 130 mM. The ionic strength had a marked effect on the formation of acid casein gels. A minimum salt concentration of about 100 mM NaCl was required to make a gel from sodium caseinate solutions by acidifying in the cold and subsequent heating. However, adding more than 240 mM NaCl prevented gel formation even at elevated temperatures. For acid skim milk gels, addition of NaCl always resulted in a lower storage modulus in dynamic measurements, especially if more than 50 mM was added. The upper limit for gel formation at 30 °C seemed to be around 150 mM of added NaCl.

For rennet-induced milk gels a minimum in the clotting time for an addition of about 50 mM has been reported, if the pH was not kept constant (e.g. 7-

10). NaCl addition to milk causes a decrease of pH, so it is questionable whether the reported effect was due to the NaCl addition or to the drop in pH. At constant pH a gradual increase of the clotting time as a function of the NaCl addition was observed (e.g. 9, 11). Addition of NaCl was reported to decrease the rate of the enzymic reaction (12) and also the coagulation of renneted micelles (13). However, the coagulation of the micelles was tested in a buffer solution and the increase of the ionic strength probably caused a decrease of the calcium ion activity, which would also considerably slow down the reaction. About the influence of NaCl addition on the modulus or the 'curd tension' of rennet-induced milk gels, conflicting results exist. Grufferty & Fox (11) observed that the curd tension after 2.5 times the clotting time was not affected by added NaCl up to 500 mM. Gouda et al. (14) reported a decrease in the curd firmness with increasing NaCl addition at high concentrations of added NaCl (500 - 1500 mM) one hour after the clotting time (which was kept constant at about 10 minutes). Jen & Ashworth (5) reported that addition of NaCl or KCl up to 100 mM increased the curd tension, but markedly decreased it at higher concentrations. They measured 30 minutes after rennet addition and the results must have been strongly influenced by the NaCl dependence on the clotting time.

In the present study besides the effect of pH, the effect of addition of sodium chloride, in the range of 50-300 mM added NaCl, at pH 6.65 and 6.25 on the rheological properties of rennet-induced skim milk gels was investigated by performing dynamic measurements.

2 Materials and methods

2.1 Skim milk

Reconstituted skim milk was used. A commercial low heat skim milk powder (Krause, Heino) was dispersed in demineralized water to which 0.015 % thiomersal or 0.02 % azide was added as preservative. Its composition is given by Roefs (4). The dispersion was stirred for about 16 hours at 30 °C before renneting. All samples contained 10.4 g of skim milk powder per 100 g of water or salt solution at the moment of renneting.

Skim milk was acidified with HCl or H_3PO_4. Three hours after dispersing the skim milk powder, a HCl or H_3PO_4 solution was added slowly and under continuous stirring (T = 30 °C). The acidified milk was then stirred for 10-16 hours. The pH was measured just before renneting.

Skim milk with NaCl addition: a solution of NaCl was added directly when the skim milk powder was dispersed in the water. After two hours the pH was adjusted with NaOH or HCl to the desired value. The pH was measured just before renneting.

2.2 Renneting

Commercial calf rennet (10 800 SU, from CSK, Leeuwarden, the Netherlands) was used. The time after rennet addition required for the formation of visible clots was taken as clotting time.

2.3 Chemical analyses

The same methods for determination of calcium plus magnesium and inorganic phosphorus were used as described before (15). The calcium ion activity was measured with a calcium ion selective electrode (Orion, model 93-20) at 30 °C (15).

2.4 Rheological measurements

Dynamic measurements were performed with the 'Den Otter' rheometer as described before (16). Gel ageing was studied at an angular frequency of 1 rad/s, the temperature was 30 °C (±0.1) and the strain was kept below 3 %.

Stress-relaxation measurements were performed with the Weissenberg R18 Rheogoniometer as described before (17) at 30 °C. Gels were aged for 6 hours at pH 6.65 and 6.25 or for 5 hours at pH 5.72 before they were deformed to a constant strain of about 3 % in about 2.5 seconds.

3 Results

3.1 Role of pH

3.1.1 Influence of pH on dynamic moduli. Skim milk with a pH between 5.72 and 6.75 was renneted and the gel formation-and-ageing was followed by determining G′ and G′′ as a function of time in the Den Otter rheometer. Table 1 gives a survey of some compositional and rheological data of the samples. A maximum in the moduli after such an ageing time that the moduli increased by less than 1 % per hour was found around pH 6.15 (Fig. 1). This is a somewhat higher pH than given in Ref. (5), but as mentioned in Section 1 those authors determined the curd firmness very soon after rennet addition (30 min) and this may influence the results (see below).

For all gels, the moduli were determined as a function of time and as a function of frequency, in the range from 10^{-2} to 4.5 rad/s. For samples of pH 6.65, 6.25 and 5.72, the frequency dependence was also tested between 10^{-3} to

Table 1. Compositional and rheological data of rennet-induced skim milk gels. a(Ca^{2+}) = calcium ion activity; t_c = clotting time; Ca = calcium concentration; P = inorganic phosphorus concentration; subscript d: concentration in milk minus (0.97×) concentration in whey. G'_∞ = G' after a long ageing time $t_{a,\infty}$ (h). All concentrations are in mM. *0.05 % rennet was used instead of 0.025 %; **acidified with H_3PO_4 instead of HCl.

pH	a(Ca^{2+})	Ca_d	P_d	t_c	G'_∞	$t_{a,\infty}$	tan δ
6.75	0.58	22.9	–	16'00"*	118	7	0.33
6.65	0.60	22.7	11.0	21'27"	123	7	0.32
6.54	0.72	22.0	–	16'55"	135	7	0.31
6.43	0.89	21.5	–	14'20"	142	7	0.33
6.25	1.10	19.9	9.0	8'00"	148	6	0.31
6.20	1.29	19.6	–	–	168	6	0.31
6.17	1.41	19.1	–	7'00"	168	6	0.32
6.13	1.36	18.8	8.5	6'50"	154	6	0.31
5.89	2.03	16.3	7.0	4'50"	127	5	0.34
5.72	2.74	13.3	6.2	4'10"	102	4	0.36
5.84**	1.30	17.2	7.9	4'26"	138	5	0.32
5.77**	1.39	16.5	7.3	4'14"	132	5	0.33

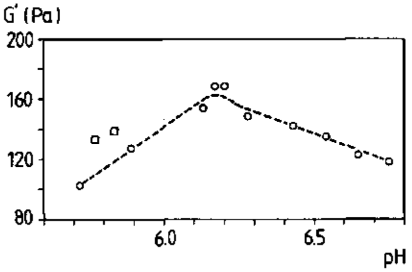

Fig. 1. Storage modulus G' as a function of the pH for rennet-induced milk gels after a long ageing time (see Table 1). (○) acidified with HCl, (□) acidified with H_3PO_4. T = 30 °C, ω = 1 rad/s, 0.025 % rennet added.

10^{-2}. For the sake of clarity, only the most interesting results will be presented in the following figures of this section. Fig. 2 shows that the shape of the ageing curves depends on pH. For a lower pH, the initial slope tended to be steeper, and a constant value and subsequent decrease of the moduli was reached sooner after rennet addition. This was especially clear for the gel of pH 5.72. G' and G'' were determined as a fuction of the angular frequency ω after the moduli had reached a constant value. The shape of the curves of log G' and log G'' versus log ω (Fig. 3) appeared to be similar for all samples. The curves were only shifted in a vertical direction.

Above pH 6 no differences in tan δ were observed in the frequency range from 10^{-2} to 4.5 rad/s. At pH 5.72 a significantly higher tan δ (Fig. 3c) and at pH 5.89 (Fig. 6) a slightly higher tan δ was found than at a pH above 6 (Fig. 3c) over the whole frequency range. At frequencies between 10^{-3} and 10^{-2} the

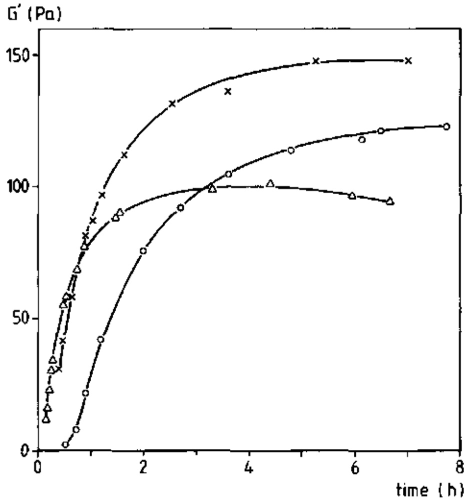

Fig. 2. Storage modulus G' as a function of time after rennet addition for skim milk gels. Same samples as in Table 1. (○) pH 6.65, (×) pH 6.25, (△) pH 5.72. T = 30 °C, ω = 1 rad/s, 0.025 % rennet added.

gels at pH 6.25 also tended to have a higher tan δ than at pH 6.65, although the differences were small.

Two samples were acidified with HCl 5 minutes instead of 16 hours before renneting. It appeared that the clotting time decreased and gel formation was observed sooner after rennet addition, as compared to the samples acidified about 16 h before renneting (Fig. 4). This implies that addition of HCl induces slow changes. The time of acidification had no effect on tan δ at frequencies between 0.01 and 4.5 rad/s.

In an effort to retain more MCP within the micelles, two samples of milk were acidified with H_3PO_4 instead of HCl. The pH of these samples was 5.84 and 5.77. The sample with pH 5.77 had a total inorganic phosphate concentration that was about 15 mM higher than normal, but only about 1 mM more inorganic phosphate was associated with the micelles as compared to acidification with HCl. Although the calcium ion activity was lower, the clotting time was about the same as for the samples acidified with HCl. The moduli of the samples acidified with H_3PO_4 appeared to be somewhat higher (Figs. 1 and 5) and tan δ somewhat lower (Fig. 6) than those of samples acidified with HCl. In Section 4 the solubilization of MCP is discussed further.

In another type of experiment, the pH of milk was decreased during gel formation-and-ageing by adding δ-gluconolactone to the milk just before rennet addition. The pH of the milk then decreased slowly with time. Gel formation started when the pH was still rather high, 6.4 at clotting time, so not much MCP was dissolved at that time. After about 7 hours the pH was 5.71 and then

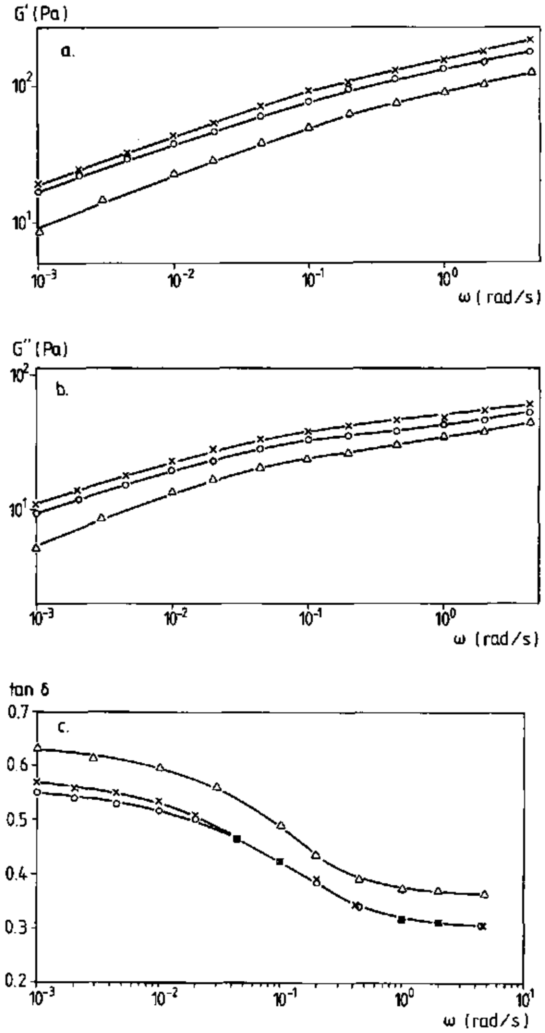

Fig. 3. Storage modulus G' (Fig. 3a), loss modulus G'' (Fig. 3b) and tan δ (Fig. 3c) as a function of the angular frequency. Same samples and symbols as in Fig. 2; the gels were aged for 7 h at pH 6.65 (○), 6 h at pH 6.25 (×) and 4 h at pH 5.72 (△).

remained constant. In Fig. 7 the pH and G' are depicted as a function of time. Initially the modulus rapidly increased. When the pH was about 6.0 the modulus started to decrease again. The optimum pH was lower than in the experiments in which samples were acidified with HCl before renneting (Fig. 1). The maximum values of G' were equal; the modulus at pH 6.0 was higher for

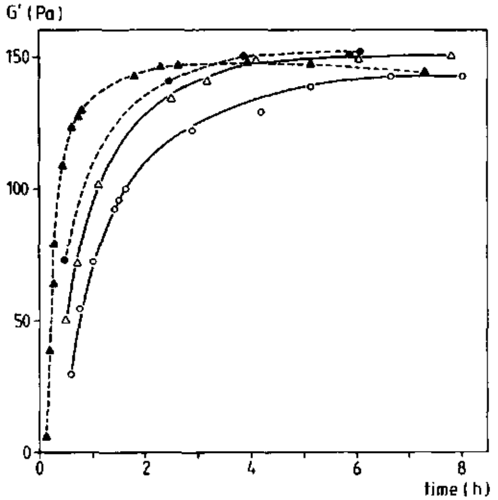

Fig. 4. Storage modulus G' as a function of time after rennet addition for skim milk gels at pH 6.43 (○, ●) and pH 6.09 (△, ▲). Acidification 16 hours before renneting (open symbols) or 5 minutes before renneting (filled symbols). T = 30 °C, ω = 1 rad/s, 0.025 % rennet added.

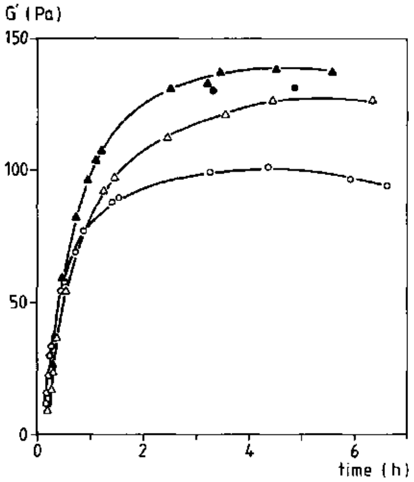

Fig. 5. Storage modulus G' as a function of time after rennet addition for skim milk gels. (○) pH 5.72, (△) pH 5.89, both acidified with HCl. (▲) pH 5.84, (●) pH 5.77, both acidified with H_3PO_4. T = 30 °C, ω = 1 rad/s, 0.025 % rennet added.

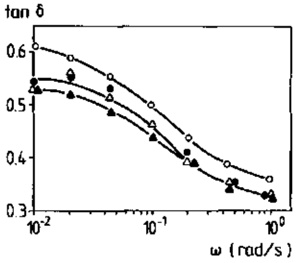

Fig 6. tan δ as a function of the angular frequency; same samples and symbols as in Fig. 5.

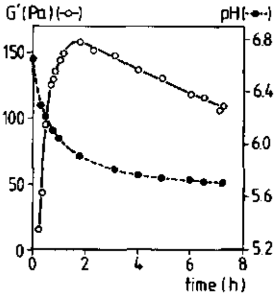

Fig. 7. Storage modulus G′ (O) and pH (●) as a function of time after rennet addition for a skim milk gel to which δ-gluconolactone had been added just before renneting. T = 30 °C, ω = 1 rad/s, 0.025 % rennet added.

the sample acidified with δ-gluconolactone. This will be discussed further in Section 4.

3.1.2 Influence of pH on stress-relaxation. Gels with pH 6.65, 6.25 and 5.72 were formed and aged in the Weissenberg Rheogoniometer (see Section 2.4). A constant strain was imposed and the resulting change in stress with time was measured. Some general aspects of the interpretation of this type of stress-relaxation measurements were considered in paper 2 of this series (17).

In Fig. 8 the relaxation modulus G(t), being the measured stress (σ) divided by the applied strain (γ), is depicted as a function of time. In accordance with the results of the dynamic measurements, G(t) was highest at pH 6.25 and lowest at pH 5.72. The stress-relaxation moduli were also calculated from the dynamic moduli and the results fitted well with each other (22). From Fig. 8 it is clear that the shape of the graphs for pH 6.65 and 6.25 did not differ much, whereas for pH 5.72 G(t) decreased faster with time, especially after a longer time. In the same way as in (17), an apparent mean relaxation time, t_r, was determined with t_r the time at which the stress $\sigma(t)$ was equal to σ_m/e (σ_m is the initial stress). As followed from the smaller t_r, the stress relaxed faster at pH 5.72 than at 6.25 and 6.65 (Table 2). This observation is in accordance

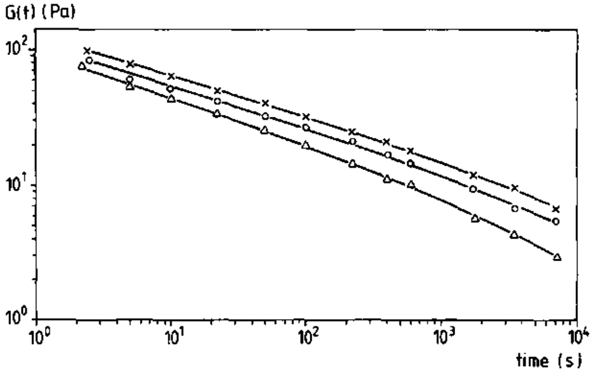

Fig. 8. Relaxation modulus G(t) as a function of time after deformation of (aged) rennet-induced skim milk gels. (○) pH 6.65, (×) pH 6.25, (△) pH 5.72. $\gamma \approx 3$ %, time needed for deformation ≈ 2.5 s. T = 30 °C, 0.025 % rennet added.

Table 2. The apparent mean relaxation time t_r of (aged) rennet-induced skim milk gels of pH = 6.65, 6.25 and 5.72.

pH	t_r (s)
6.65	63
6.25	72
5.72	37

with the results of the dynamic measurements, where significantly higher values of tan δ were found at pH 5.72 than at pH 6.25 and 6.65; an increase in tan δ implies that relatively more bonds relax within the time scale of the measurement. In the dynamic measurements slightly higher values of tan δ were found at pH 6.25 than at pH 6.65 at angular frequencies below 10^{-2} rad/s. Correspondingly, within a time scale of $1/10^{-2}$ s no differences in t_r were expected between pH 6.25 and 6.65, while at time scales longer than 100 s a somewhat faster relaxation was expected at pH 6.25. From Table 2 it appears that t_r was even a bit lower at pH 6.65 than at pH 6.25, but the differences were of the same magnitude as differences between duplicates. If for σ_m the stress at t = 100 s was taken instead of the initial stress, t_r was about 28 minutes at pH 6.25 and 31 minutes at pH 6.65. Although the trend was in accordance with the expectations, this difference was, again, of the same magnitude as differences between duplicates. The conclusion must be that no significant difference in relaxation behaviour exists between pH 6.65 and 6.25 within the time scale of the measurement.

Table 3. Compositional data and clotting time of rennet-induced skim milk gels. Symbols are the same as in Table 1. [ ]: concentration added. Subscript w: concentration in the whey.

pH	[rennet] (%)	[NaCl] (mM)	t_c	Ca_w (mM)	P_w (mM)	$a(Ca^{2+})$ (mM)
6.65	0.0250	0	21'22"	12.5	12.3	0.60
6.65	0.0350	100	22'25"	12.9	12.2	0.74
6.65	0.0700	200	21'10"	12.8	12.2	0.84
6.65	0.0500	300	45'	13.4	12.3	1.00
6.25	0.0250	0	7'30"	15.3	14.5	1.08
6.25	0.0375	50	7'27"	15.6	14.4	1.18
6.25	0.0600	100	7'43"	16.1	14.3	1.24
6.25	0.1250	200	7'40"	16.7	14.5	1.36

3.2 Addition of sodium chloride

To investigate whether the ionic strength had any effect on the mechanical properties of rennet-induced skim milk gels, sodium chloride was added to the milk. The pH was kept constant. Three types of experiments were done:

a) 0, 100 or 200 mM NaCl was added to skim milk at pH 6.65. Such a rennet concentration was chosen that the clotting time was equal for all samples.
b) 0, 50, 100, 200 or 300 mM NaCl was added to skim milk at pH 6.65 and a constant amount of rennet was added: 0.05 %.
c) 0, 50, 100 or 200 mM NaCl was added to skim milk at pH 6.25 and such an amount of rennet was added that the clotting time was equal for all samples.

If NaCl had been added to the milk, a higher rennet concentration was needed to get a similar clotting time as in milk without added NaCl, and the extra amount of rennet increased with the NaCl concentration, both at pH 6.65 and at pH 6.25 (Table 3). It appeared that at pH 6.25 more additional rennet was needed to keep the clotting time constant than at pH 6.65.

Increasing the NaCl concentration resulted in a small increase of the calcium concentration of the whey and this was more pronounced at pH 6.25 than at pH 6.65. The phosphate concentration did not change, implying that no micellar calcium phosphate dissolved, which is in accordance with results of Van Hooydonk (12) and Grufferty & Fox (11). Probably the higher concentration of calcium in the whey was caused by a substitution of calcium associated with the micelles by sodium (12).

Also an increase of the calcium ion activity was observed with increasing NaCl concentration. The increase of the calcium concentration of the whey alone seems too small to explain the increase of the calcium ion activity. Prob-

Table 4. Calcium ion activity a(Ca^{2+}) (mM) of milk, milk ultrafiltrate, a $CaCl_2$-NaCl solution and a calcium citrate-NaCl solution before and after addition of 300 mM NaCl. T = 25 °C, pH = 6.65. For further explanation, see text.

	Milk	Ultrafiltrate	$CaCl_2$	Ca-citrate
a(Ca^{2+}) before	0.69	0.67	0.40	0.041
a(Ca^{2+}) after	1.17	1.10	0.26	0.095

ably, the concentration of free Ca^{2+} was increased by a shift in the salt equilibria of the milk serum as illustrated by the next experiment. The effect of an increase of the NaCl concentration by 300 mM on the calcium ion activity was determined for milk, milk ultrafiltrate, a solution containing 1 mM $CaCl_2$ in 80 mM NaCl and a solution containing 2.5 mM $CaCl_2$, 6 mM citric acid and NaCl, all having an ionic strength of about 80 mM and a pH of 6.66 (Table 4). Addition of NaCl decreased the calcium ion activity of the $CaCl_2$ – NaCl solution. Due to the increase of the chloride concentration after addition of 300 mM NaCl the association between Ca^{2+} and Cl^- increases, resulting in a lower Ca^{2+} concentration and in a decrease in Ca^{2+} activity. A decrease in Ca^{2+} activity was also expected for the other samples unless the free calcium ion concentration became higher. NaCl addition to the solution containing $CaCl_2$ and citric acid increased the calcium ion activity by a factor of about 2.3. Such an increase can only be due to a considerable dissociation of calcium citrate. For milk and milk ultrafiltrate also an increase of the calcium ion activity was found and the increase for milk ultrafiltrate was only slightly less than for milk, where calcium was released from the casein micelles. This implies that the increase in calcium concentration of the milk serum only slightly contributes to the increase of the calcium ion activity due to NaCl addition. The dissociation of calcium citrate must then, at least partly, be responsible for the increase of the calcium ion activity after NaCl addition in milk.

After rennet addition, gel formation-and-ageing was followed in the den Otter rheometer by determining the storage modulus G′ and the loss modulus G′′ as a function of time. In Fig. 9a, G′ is depicted for the gels at pH 6.65 and constant clotting time. Initially, there were no great differences, but after longer ageing times the moduli increased faster during ageing for a higher NaCl concentration. At pH 6.25 the effect of the NaCl addition was reversed: as can be seen from Fig. 9b, an increase of the NaCl concentration resulted in a slower rise of G′ with time and after a long ageing time the modulus was lowest for the sample with the highest NaCl concentration, although the differences were relatively small. For the samples at pH 6.65 with constant ren-

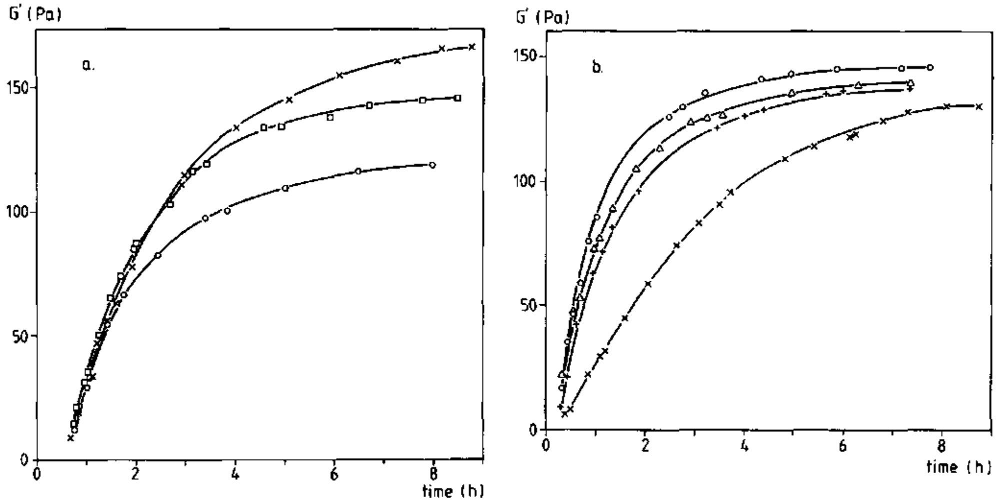

Fig. 9. Storage modulus G′ as a function of time after rennet addition for skim milk gels. T = 30 °C, ω = 1 rad/s. Fig. 9a: pH = 6.65, clotting time ≈ 21′30″, (○) 0.025 % rennet and 0 mM NaCl added, (□) 0.035 % rennet and 100 mM NaCl, (×) 0,07 % rennet and 200 mM NaCl. Fig. 9b: pH ≈ 6.25, clotting time ≈ 7′45″, (○) 0.025 % rennet and 0 mM NaCl added, (△) 0.0375 % rennet and 50 mM NaCl, (+) 0.06 % rennet and 100 mM NaCl, (×) 0.125 % rennet and 200 mM NaCl.

net concentration, addition of 50 and 100 mM NaCl did not change the initial increase of G′ with time, but the increase continued longer, eventually resulting in higher moduli (Fig. 10). If more than 100 mM NaCl was added, the onset of gelation was clearly retarded and also the increase of the moduli was less than at lower concentrations of added NaCl. The moduli after a long ageing time had about the same value as the sample to which 50 mM NaCl was added. The moduli of the samples to which 300 mM NaCl was added were well reproducible until 5 hours after rennet addition. After longer ageing times the reproducibility was very poor. Although no difference in the sticking of the gel to the walls of the measuring apparatus was observed, loosening of the gel from the cylinders may have been the reason for the poor reproducibility.

For G″ similar results were found, only the values were lower. After an ageing time of 7 to 8 hours the frequency dependence was determined between 0.01 and 4.5 rad/s. All graphs of G′ and G″ versus the angular frequency had the same shape (not shown), only the absolute values differed. Tan δ (not shown) versus the angular frequency was equal for all samples within experimental error, implying that NaCl addition did not change the relaxation behaviour of the milk gels.

The influence of NaCl addition. NaCl addition to milk caused an increase of the calcium ion activity. It has been shown (15) that this increased the modulus after a long ageing time. In addition, NaCl addition causes a better shielding of charges and because of the net (negative) charge of casein this may lead to less electrostatic repulsion. On the other hand the positive charges would also be shielded, resulting in less electrostatic attraction. Moreover, sodium is exchanged for micellar calcium and maybe some specific binding of chloride to casein occurs. It was observed (12) that the voluminosity of micelles, native and renneted, somewhat increased after addition of NaCl. It is not sure whether this leads to an increase or a decrease of the modulus. On the one hand the increase of the voluminosity points to a decrease in the interactions within the micelles. On the other hand it may lead to more interparticle interactions.

If the concentration of rennet was kept constant, a maximum in ultimate modulus occurred at about 100 mM added NaCl. Above 100 mM added NaCl the onset of gelation was retarded and the modulus obviously increased at a slower rate compared to lower concentrations of added NaCl. This can be partly ascribed to the decrease of the enzyme activity (12). As had been shown before (16), decreasing the rennet concentration resulted in a slower increase of the moduli with time and to a lower ultimate modulus.

At pH 6.25 the effect of NaCl on the modulus was different from that at pH 6.65. More rennet was needed to keep the clotting time constant, the increase of the modulus in time was slower, especially after addition of 200 mM of NaCl, and a small decrease of the ultimate modulus was found. At pH 6.25 the calcium ion activity is already high and a further increase of the calcium ion activity presumably does not decrease the clotting time or increase the modulus. Shielding of charges will have a more negative effect at pH 6.25 than at 6.65, because of a smaller net (negative) charge and a bigger number of positive charges. For acid milk gels, this effect will be even stronger and it is not surprising that for acid milk gels a decrease of the modulus was found after NaCl addition.

Acidification to pH 5.75 increases the ionic strength, as determined with an ion equilibrium programme of Holt et al. (21), with about 20 mM. Such a small increase probably does not significantly influence the rheological behaviour.

Acknowledgements

The Institute for Cereals, Flour and Bread TNO, Wageningen, kindly offered us the opportunity to perform measurements on the Weissenberg

Rheogoniometer and we are indebted to J. M. P. Wokke and H. M. van de Haar for their help. We thank A. Spaargaren and M. J. G. W. Roozen for their work on the ionic strength.

Samenvatting

P. Zoon, T. van Vliet en P. Walstra, *Reologische eigenschappen van met leb gestremde ondermelkgelen. 4. De invloed van pH en NaCl*

De invloed van pH en NaCl op de mechanische eigenschappen van met leb gestremde ondermelkgelen werd onderzocht. Indien de verouderingstijd lang genoeg was, werd bij pH 6,15 een maximum in de opslagmodulus G′ gevonden. Boven pH 6,0 werd geen invloed van de pH op de verhouding tussen de verliesmodulus G″ en de opslagmodulus G′ (tg δ) waargenomen, terwijl bij pH 5,72 een hogere tan δ gevonden werd. De relaxatietijd van gelen met pH 6,65 en 6,25 was ongeveer gelijk, maar ze was duidelijk korter bij pH 5,72. Het in oplossing gaan van micellair calciumfosfaat en de toename van de elektrostatische attractie bleken belangrijke verklarende effecten te zijn voor de invloed van een pH-daling.

Het toevoegen van NaCl aan melk beïnvloedde tg δ niet. De invloed van NaCl op de vorming en veroudering van de gelen bleek afhankelijk te zijn van de proefomstandigheden. Indien de vloktijd constant gehouden werd, resulteerde toevoeging van NaCl tot 200 mM in een verhoging van de modulus. Bij een constante stremselconcentratie gebeurde dat ook tot 100 mM toegevoegd NaCl, maar bij hogere NaCl-concentraties werd de vorming-en-veroudering van de gelen vertraagd. Bij pH 6,25 en constante vloktijd werd de gelvorming enigszins vertraagd tot een concentratie van 100 mM toegevoegd NaCl; bij 200 mM trad een aanzienlijke vertraging op, maar uiteindelijk kwam de modulus niet veel lager uit.

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Some work concerning large deformations on milk gels has been done by Van Dijk (5) and Roefs (6), who performed creep experiments. In those experiments a constant stress is applied to a sample and the deformation is measured as a function of time (7). It appeared that increasing the applied stress resulted in earlier fracture of the gels, but no clear relation with the deformation at which fracture happened was reported. Roefs (6) reported that the stress which had to be applied to fracture the gel within a certain time was higher for acid milk gels than for rennet-induced milk gels, while the deformation at fracture was smaller. Furthermore, gel formation and ageing is often monitored by methods in which a relatively large strain is applied. Applying a large strain may be done on purpose; for instance, in the viscosity measurements of Korolczuk & Maubois (8), which give some information about the rate of formation of new bonds in relation to the disruption of existing ones; or in measurements to determine the mechanical strength of curd by a plate extrusion method, as described by Jablonka & Munro (9). On the other hand, the application of a large strain may be merely inherent for the type of measuring apparatus, as in the experiments of Korolczuk et al. (11) where they determined the changes in rheological properties with a Contraves Low Shear oscillatory viscometer. In such experiments, the stress or the strain are usually not well defined and quantitative conclusions cannot be drawn.

Recently Luyten (10) made an extensive and comprehensive study on the rheological and fracture properties of cheese. Methods to measure these properties and the influence of different factors such as pH, temperature, water content and protein breakdown were studied. It was shown how, for instance, fusion of particles and eye or slit formation in Gouda cheese are influenced by the rheological and fracture properties of the cheese.

In this study the influence of ageing time and pH of rennet-induced skim milk gels, as well as the influence of the applied stress and of the measuring temperature, on the rheological behaviour of those gels was studied in creep experiments.

2 Materials and methods

2.1 Skim milk

10.4 g of a commercial skim milk powder (Krause, Heino) was dispersed in 100 g of demineralized water to which 0.02 % sodium azide was added as a preservative. The dispersion was stirred about 16 hours at 30 °C before renneting.

Acidified milk. The skim milk was acidified with HCl about one hour after dispersing the powder. The pH was determined just before renneting. The concentration of casein was the same as for non-acidified milk.

2.2 Renneting

Commercial calf rennet (10800 SU, from CSK, Leeuwarden, the Netherlands) was used; 0.025 % was added to the milk.

2.3 Rheological measurements

Creep measurements were performed with a constant stress apparatus, a Deer PDR 81 Rheometer from Deer Ltd. A stainless steel measuring body of coaxial cylinder geometry was used. The inner cylinder was constructed with a cone at the bottom, which has the advantage that the strain is nearly homogenous throughout the sample. The diameter of the inner cylinder was 12 mm and that of the outer one 15 mm; the height was 30 mm. The sample volume was 2 ml. The measuring body was immersed in a thermostatting bath and the apparatus was placed in a perspex box to avoid interference by temperature gradients and air currents. A constant stress was applied to the inner cylinder by means of an induction type electric motor. The rolling friction was reduced by using an air bearing support. The angular displacement of the inner cylinder was determined by a non-contacting electronic sensor. The stress σ (sum of stresses of coaxial cylinder and cone-plate geometry) and strain γ were calculated as follows:

$$\sigma = \left\{\frac{4\pi h R_1^2 R_2^2}{R_2^2 + R_1^2} + \frac{2\pi R_1^3}{3}\right\}^{-1} \cdot T \qquad (1)$$

$$\gamma = \left\{\frac{R_2^2 + R_1^2}{R_2^2 - R_1^2}\right\} \cdot \alpha \qquad (2)$$

where: σ = applied stress (Pa), γ = strain (–), h = height of cylinders (m), R_1 = radius of inner cylinder (m), R_2 = radius of outer cylinder (m), T = torque (Nm), α = angular displacement (rad).

The gels were formed in the measuring body. Directly after rennet addition, the milk was brought between the cylinders. A thin layer of paraffin oil was put on the gel to prevent drying of the upper part of the gel. In the ageing experiments, gels were formed and aged at 30 °C, and 0.5, 1, and 3.5 hours after rennet addition a stress was applied. In the pH experiments, the gels were formed and aged at 30 °C and the stress was applied after 3.5 hours. In the 'measuring temperature' experiments the gels were formed and aged at

30 °C; after 3 hours the temperature was changed to the desired value and half an hour later the stress was applied.

3 Results

3.1 Interpretation of the creep curves

The behaviour of rennet-induced skim milk gels at large deformations was studied by creep measurements (Section 2.3). So-called creep curves are obtained, in which the measured shear deformation is depicted as a function of time. If the stress applied was large enough for fracture to occur, the creep curve of a rennet-induced milk gels had a shape as shown in Fig. 1. Initially the strain increased instantaneously, i.e. within the response time (≈1 s) of the rheometer (up to γ_0), followed by a more gradual increase; then the increase of strain with time ($d\gamma/dt$) became more or less constant for a while, after which $d\gamma/dt$ increased again. Eventually, at time t_f, the inner cylinder started to rotate very fast, indicating that fracture of the protein network had occurred. When stopping the apparatus syneresis could be observed in a plane mostly somewhere between both cylinders. The strain at t_f was called the fracture strain γ_f.

It is likely that fracture did not occur only during the last stage of the creep experiment. Considering the stresses applied in this study, the instantaneous deformations γ_0 were already far outside the linear region (which is the range over which strain is proportional to stress) as determined in dynamic experiments, which extends up to $\gamma = 0.03 - 0.05$ (1). This implies that, besides elastic deformation, probably also some local fracture occurs immediately and that relatively soon irreversible changes in the network occur. To get some idea about the importance of the irreversible changes, a rennet-induced skim milk gel was formed and aged (for 4 h) in the Deer rheometer and the (dy-

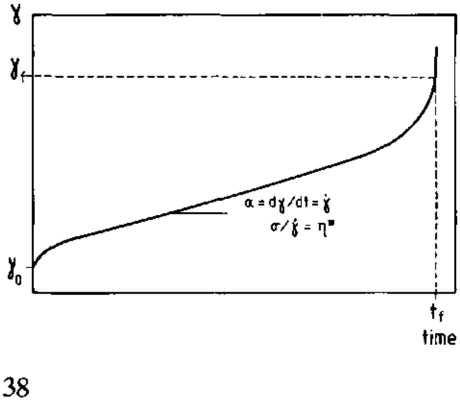


Fig. 1. Example of a creep curve. The strain γ is measured as a function of time (t) after a constant stress σ is applied. For further explanation see text.

namic) storage and loss moduli were determined (within the linear region) at various frequencies between 0.01 and 1 rad/s. Then, during 4 minutes a constant stress was applied to the gel resulting in a strain of about 1. During the next hour no stress was applied and the strain diminished more or less exponentially until about $\gamma = 0.16$. The dynamic moduli were determined again (within the linear region) and it appeared that they were about 5-10 % lower than before deformation, indicating that irreversible changes occurred. Van Dijk (5) came to a similar conclusion. He applied various constant shear strains (0.35-1.2) to rennet-induced skim milk gels that had been aged for 1 h. One hour later he started to measure the permeability of the gels. It appeared that already at a shear strain of 0.35 the permeability was higher than that of the reference gel, which was not deformed, implying that irreversible changes in the casein network had occurred.

Usually creep measurements are performed within the linear region. Then an apparent viscosity can be calculated by dividing the applied stress by the strain rate. In the present study for all samples a region was observed in which the strain increased linearly with time, hence a constant strain rate ($\dot{\gamma}$). However, the strains were far outside the linear region and no pure flow of the network occurred, because of ongoing formation of cracks and growth of cracks. It is therefore better to call it a plastic flow region characterized by a pseudoviscosity η^*.

It was difficult to judge when macroscopic fracture started, because only the top of the gels could be seen. We assume that directly after a stress is applied, straightening of the casein strands takes place during which breaking of (protein-protein) bonds at molecular scale occurs. At higher deformations whole strands will be broken at several places in the network, which may be interpreted as the formation of microscopic cracks. Then these cracks will grow and fuse to form macroscopic cracks and eventually fracture through the whole network will occur. Naturally, the mass remains continuous, because of the abundance of water.

The effect of the various factors on the rheological behaviour is dealt with separately in the following sections. In Table 1 a survey of the results is given and in Section 4 the results are discussed.

3.2 Influence of stress

The influence of the applied stress is illustrated and discussed for gels made and tested under standard conditions: t_a was 3.5 h, T_m was 30 °C and pH was 6.65. The gels made and tested under other conditions gave qualitatively the same results.

In Fig. 2a the strain is depicted as a function of the relative time t_{re} for three

Table 1. Influence of ageing time t_a, measuring temperature T_m, pH and applied stress σ on some parameters of creep experiments: γ_o = instantaneous strain, γ_f = strain at which fracture of the casein network occurred, t_f = time after stress application at which fracture of the gel occurred, η^* = pseudo viscosity, $d\gamma/dt$ = strain rate at the linear part of the creep curve. Deviations from standard conditions are indicated in the first column.

Sample	σ (Pa)	γ_o	$d\gamma/dt$ (10^{-3} s^{-1})	η^* (10^3 Pas)	γ_f	t_f (s)
t_a = 0.5 h	14	1.98 (?)	3.9	3.6	4.9	505
	16	0.93	5.6	2.9	3.8	385
	20	0.99	9.9	2.1	3.9	229
	27	1.37	31	0.85	2.8	32
	34	1.21	43	0.79	2.6	18
	41	1.23	51	0.80	2.6	14
	48	1.59	192	0.25	2.3	3
t_a = 1 h	34	0.43	3.2	11	3.2	534
	48	0.50	5.3	9.1	3.2	295
	68	0.56	9.2	7.4	2.7	131
	102	0.64	14	7.2	2.5	75
	136	0.69	27	5.1	2.2	29
	177	0.81	52	3.4	1.9	13
standard	25	0.20	0.69	36	3.8	2430
(t_a = 3.5 h)	35	0.22	1.2	29	3.3	1350
(T_m = 30 °C)	48	0.25	1.9	25	2.7	655
(pH = 6.65)	68	0.32	3.6	19	2.2	300
	75	0.32	5.0	15	2.3	264
	136	0.42	7.9	17	2.0	117
	238	0.52	24	10	1.8	30
T_m = 25 °C	68	0.35	1.2	55	2.1	847
	136	0.42	3.1	44	1.8	271
	238	0.55	9.1	26	1.6	71
	313	0.55	18	18	1.5	29
T_m = 35 °C	41	0.23	3.9	11	3.2	402
	68	0.37	11	6.2	2.7	126
	136	0.46	42	3.2	2.1	22
	177	0.56	63	2.8	2.0	15
pH = 6.25	50	0.25	1.3	38	3.1	1224
	60	0.29	1.9	32	3.1	876
	75	0.33	2.6	29	2.9	540
	100	0.36	4.7	21	2.7	318
	125	0.41	6.6	19	2.6	204
pH = 5.75	50	0.26	1.2	44	3.4	1380
	60	0.28	1.3	34	3.0	960
	75	0.31	2.3	33	2.7	606
	100	0.34	4.2	24	2.5	366
	125	0.37	6.0	20	2.4	228

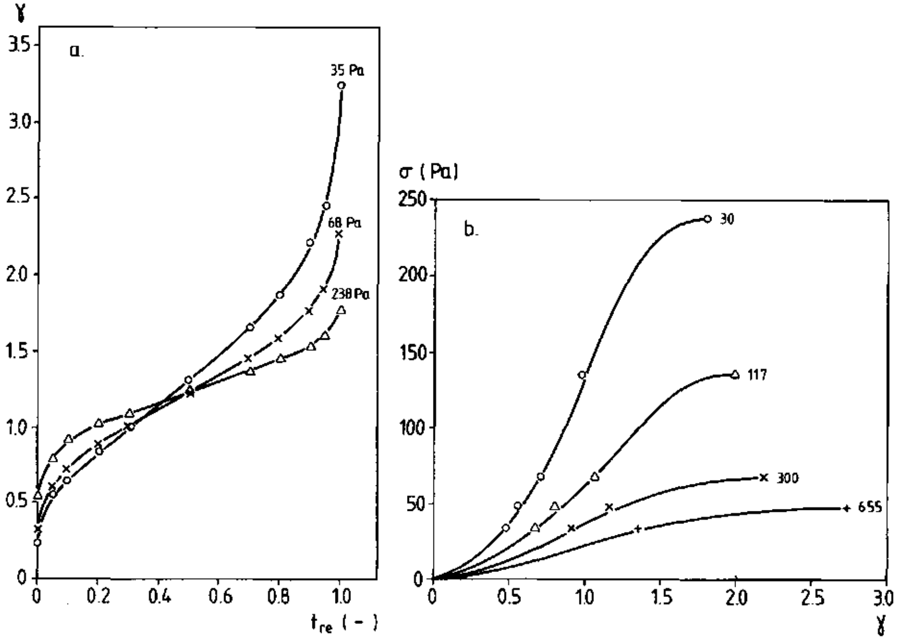


Fig. 2. a. The strain γ as a function of the relative time t_{re} for rennet-induced skim milk gels to which stresses were applied as indicated. b. Stress-strain curves; the time during which the stress was applied, t_f* is indicated. $t_a = 3.5$ h, pH = 6.65, T = 30 °C.

different stresses; t_{re} was defined as: t/t_f, where t is the time after stress application. Increasing the applied stress resulted in an increase of the instantaneous strain (γ_o) and in a decrease of the deformation due to plastic flow. This resulted in a decrease of the fracture strain (γ_f). Fracture occurred earlier after stress application. The pseudo viscosity (see Section 3.1) was calculated and depicted as a function of the strain rate (see Fig. 6a). The pseudo viscosity decreased with increasing strain rate, a kind of shear thinning; the slope was about −0.4.

If the applied stress is depicted as a function of the strain at a given time after stress application (t_f*), a kind of stress-strain curves (Fig. 2b) are obtained. As was already described by Van Dijk (5), the stress increased initially more, but at higher deformations less than proportional to the strain. Probably the number of effective, i.e. stress carrying, bonds first increased, presumably because more protein molecules and strands become straightened, whereas at higher deformations the number of effective bonds decreased because of local fracture.

Table 2. Energy supplied up to fracture to rennet-induced skim milk gels under standard conditions for times during which stress was applied t_f* as indicated (for further explanation see text).

Time (s)	30	117	300	655
Energy (J/m^3)	216	130	83	80

In Fig. 2b the highest value of the strain on each curve is the fracture strain. From this figure it is also clear that with decreasing duration of stress application, the stress at a certain strain increases and the strain at fracture decreases. For stress-strain curves determined by measuring the stress as a function of increasing strain, the area below the curve represents the total energy per m^3 that is needed to break the sample within a certain time. The areas below the constructed curves of Fig. 2b were also calculated (Table 2). These results must be considered as a rough estimate for the energy, because the curves were not real stress strain curves and the shapes of the curves were not known exactly, because of the limited number of data. It appears that, within the range studied, the (estimated) energy to be supplied to break the casein network increased with decreasing time scale.

3.3 Ageing time

When the milk gels became older, the stress needed to fracture them within a given time increased (Table 1). In Fig. 3a the strain is depicted as a function of t_{re} of gels aged for 0.5, 1 and 3.5 hours for stresses causing about equal t_f. It is seen that the instantaneous strain decreased with increasing ageing time. This was also the case if results at equal stress were compared (Table 1, e.g. $\sigma = 48$ Pa). With increasing ageing time, the deformation due to plastic flow decreased. This, in combination with the smaller instantaneous strain, resulted in a smaller strain at fracture. For all samples a shear thinning character of the pseudo viscosity was observed (see Fig. 6a). Gels that had been aged for 0.5 hours gave a steeper slope (about −0.7) than those aged for 1 or 3.5 hours (about −0.4). The pseudo viscosity at a certain strain rate increased with ageing time. In Fig. 3b (constructed) stress-strain curves are depicted for the gels with different ageing times for stress being applied during about 30 s. The area below the curves was calculated (Table 3); increasing the ageing time increased the so determined energy to fracture a gel within a certain time.

3.4 Measuring temperature

Gels were aged at 30 °C and measured at 25, 30 and 35 °C (Section 2.3). At a certain t_f, the instantaneous strain was about constant for the various samples

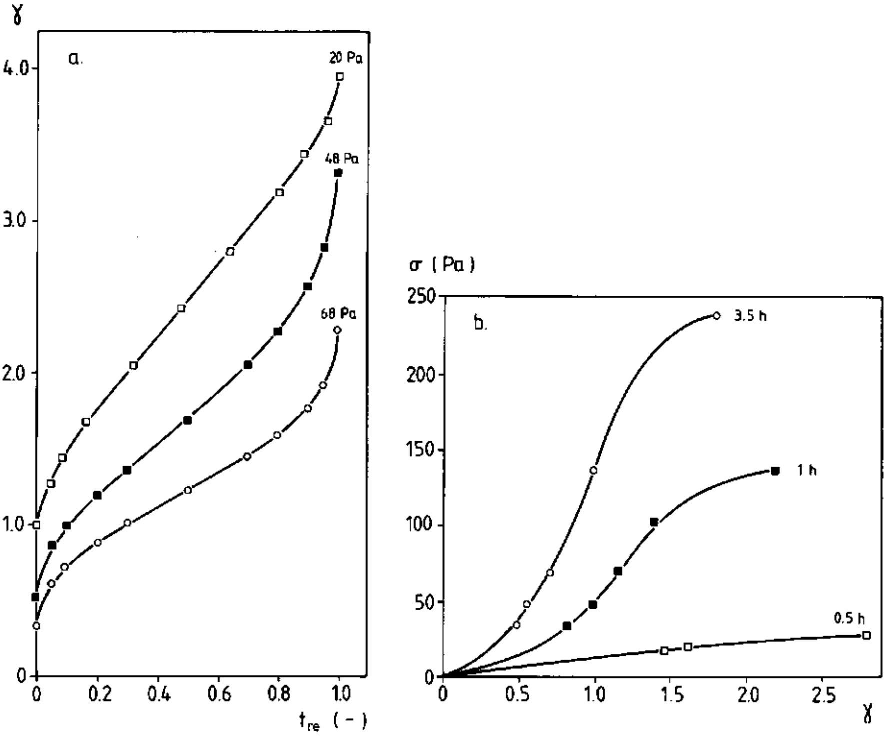
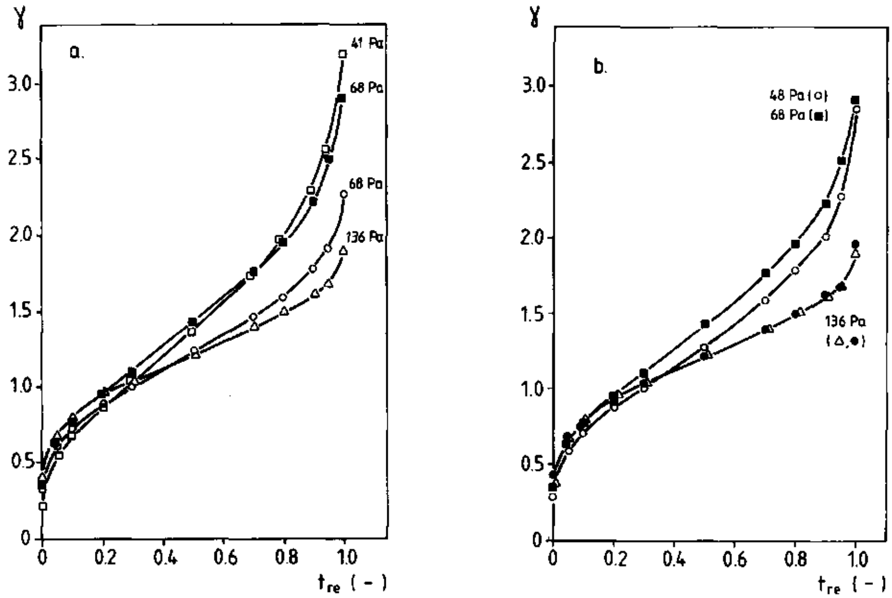


Fig. 3. Effects of ageing of rennet-induced skim milk gels: t_a = 0.5 h (□), 1 h (■) and 3.5 h (○). a: γ as a function of t_{re} for stresses (indicated) giving a t_f of about 300 s. b: σ as a function of γ for stresses applied for about 30 s; ageing time is indicated. T = 30 °C, pH = 6.65.

(Table 1), whereas the applied stress decreased and the strain at fracture increased with increasing measuring temperature (Fig. 4a, c). The higher the temperature, the larger the deformation due to plastic flow observed at constant t_f (Fig. 4a). It was possible to get about the same curves at various temperatures varying the time up to fracture t_f (see Fig. 4b). This implies that the shape of the curves is not essentially different, only a different t_f has to be chosen to get the same results at different temperatures: the higher the tempera-

Table 3. Energy supplied up to fracture to rennet-induced skim milk gels with ageing times as indicated; t_f^* was about 30 s.

Ageing time (h)	0.5	1	3.5
Energy (J/m^3)	43	156	216



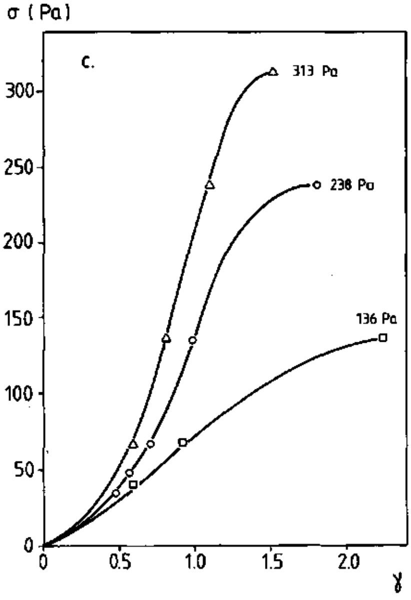


Fig. 4. Effects of testing temperature on rennet-induced skim milk gels: 25 °C (△), 30 °C (○, ●) and 35 °C (□, ■). The strain as a function of t_{re} (Figs. 4a, b) and stress-strain curves (Fig. 4c). The applied stress is indicated, $t_a = 3.5$ h and pH = 6.65. a. $t_f \approx 300$ s for 25 and 30 °C and 126 s (■) or 402 s (□) for 35 °C. b. $t_f = 271$ s for 25 °C (△), 117 s (●) or 655 s (○) for 30 °C and 126 s for 35 °C (■). c. time scale ≈30 s.

Table 4. Energy supplied up to fracture to rennet-induced skim milk gels at measuring temperatures as indicated; t_f^* was about 30 s.

Measuring temperature (°C)	25	30	35
Energy (J/m^3)	236	216	158

ture, the shorter is t_f. Increasing the measuring temperature decreased the calculated energy to break a gel in a certain time (Table 4). Also the pseudo viscosity at a certain strain rate was lower at a higher temperature, whereas the slopes of the pseudo viscosity versus strain rate were about equal for all temperatures (see Fig. 6b).

3.5 pH

Creep measurements were performed on gels with pH 5.75, 6.25 and 6.65 (standard gel) after an ageing time of 3.5 hours. The strain as a function of time appeared to be rather similar for the gels with the various pHs (Fig. 5a) as long as t_f was fairly constant. The differences between the samples differing in pH were comparable to those between duplicates. The stress-strain

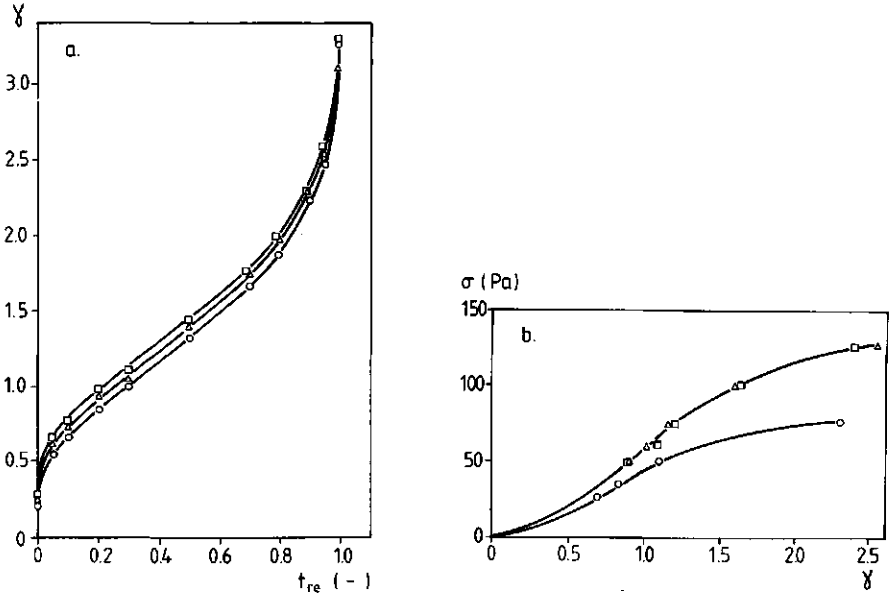


Fig. 5. The strain as a function of time t_{re} (Fig. 5a) and stress-strain curves (Fig. 5b) for rennet-induced skim milk gels with various pH: 6.65 (○), 6.25 (△) and 5.75 (□). a: $t_f \approx 1300$ s. b. time scale ≈230 s. $t_a = 3.5$ h, T = 30 °C.

calculated that the slope of log W versus log t_f^* was about −0.38. If all the energy were stored and assuming that W′ and W_f are proportional to G′, the same dependence on time would be expected for the storage modulus and for the energy. From graphs of log G′ versus log ω as given in ref. (2), at 30 °C a slope of about 0.30 was derived; hence the slope of log G′ versus log time ($\approx 1/\omega$) would be −0.3. The somewhat stronger time-dependence of the energy implies that energy-dissipating processes are also important for these types of gels. If the time up to fracture is shorter, the strain rate will be higher on average and energy dissipation per unit volume increases with increasing strain rate. Assuming η and $\dot{\gamma}$ to be constant during deformation, the following equation can be written:

$$W'' = \eta^* \cdot \dot{\gamma}^2 \cdot t_f^* \qquad (4)$$

With the present data it is not possible to calculate the energy dissipation due to friction. It is only possible to estimate the time-dependence of the plastic flow. For the curves in Fig. 2b, $\dot{\gamma}$ was calculated as the fracture strain divided by t_f^*. It was derived that the slope of log (calculated) $\dot{\gamma}$ versus log t_f^* was about −1.1. The slope of log η^* versus log $\dot{\gamma}$ was about −0.4 (Section 3.2 and Fig. 6a) The slope of log W″ versus log t_f^* would then be about −0.76, which is a stronger dependence on time than found for the total energy. This is not surprising, because also W′ and W_f, showing a weaker dependence on time than W, contributed to W.

Another effect of a shorter t_f, and therefore a higher strain rate, is that molecules or particles are for a shorter time in the vicinity of each other, which will decrease the chance that new bonds will be formed during the shearing process. This may explain the observed shear thinning behaviour (Fig. 6a, b): lower pseudo viscosity at a higher strain rate.

The ageing time of a gel is probably important with respect to the number of effective bonds present in the network and the formation of new bonds during deformation, but not for the proportion of protein-protein bonds that spontaneously break: in dynamic experiments it is observed that tan δ remains constant, at an angular frequency of 1 rad/s, soon after the onset of gelation (at standard conditions about half an hour after rennet addition), whereas the moduli are still rapidly increasing (e.g. 1) because of the increase in the number of effective bonds. This may explain the higher stress and the higher energy input needed to break a gel within a certain time, as well as the higher pseudo viscosity and the higher energy input after a longer ageing time. In dynamic experiments it is observed (1) that the rate of increase of the modulus, hence the rate of formation of new bonds, markedly decreases with time. This must be the reason for the differences in the results of the creep measurements between an ageing time of 0.5 and 1 h being larger than between 1 and 3.5 h. It also implies that after a relatively short ageing time reformation of bonds, i.e. repair of fractures at a local scale, will occur more readily, leading to a larger fracture strain (see Table 1) and a steeper slope of the pseudo viscosity versus the strain rate (see Fig. 6a).

During ageing the strands in the network become thicker and thereby less mobile, the contact region between the micelles increases, fewer dangling ends exist and the pores in the network become larger due to microsyneresis (5). The decrease of the instantaneous strain γ_0 with increasing ageing time will be due to the increase of the number of bonds and to the above mentioned changes in the large scale structure.

An increase of the *measuring temperature* was observed to result in a shift of relaxation processes to shorter time scales (2): within a certain time more bonds spontaneously break and reform, resulting in more flow and plastic flow and therefore a larger fracture strain. In dynamic experiments the modulus was found to decrease with increasing measuring temperature, implying fewer bonds or weaker bonds. Together with the higher proportion of bonds that relax within a certain time this may explain the smaller stress needed to break the gel within a certain time and the lower pseudo viscosity.

Lowering the *pH* from 6.65 to 5.75 may be expected to result in a larger deformation due to flow at a certain t_f, because tan δ increases somewhat and the relaxation time decreases (4). However, Fig. 5a and Table 1 show that the strain as a function of time is nearly equal for gels with different pHs (at a certain t_f). Possibly the increased relaxation at pH 5.75 and the decreased rate of formation of new bonds counteract each other at an ageing time of 3.5 h. After 3.5 h the modulus of the gel with pH 5.75 had already reached a plateau value, whereas the moduli of gels at the higher pHs were still increasing; the moduli at pH 6.65 and 5.72 were about equal and the modulus at pH 6.25 was higher (4). The stress to break the gels within a certain time and the pseudo viscosity of the gels with pH 5.75 and pH 6.25 were observed to be about the same, while they were lower for the gels with pH 6.65. This implies that the modulus determined at small deformation is not necessarily positively correlated to the stress needed for fracture. Also the large scale structure and its changes during deformation will be important. As was mentioned in a preceding article (4), the large scale structure of the gels becomes more unhomogeneous at a lower pH, while the micelles in the strands are already fused to a larger extent, maybe resulting in a higher stress needed to break a strand within a certain time.

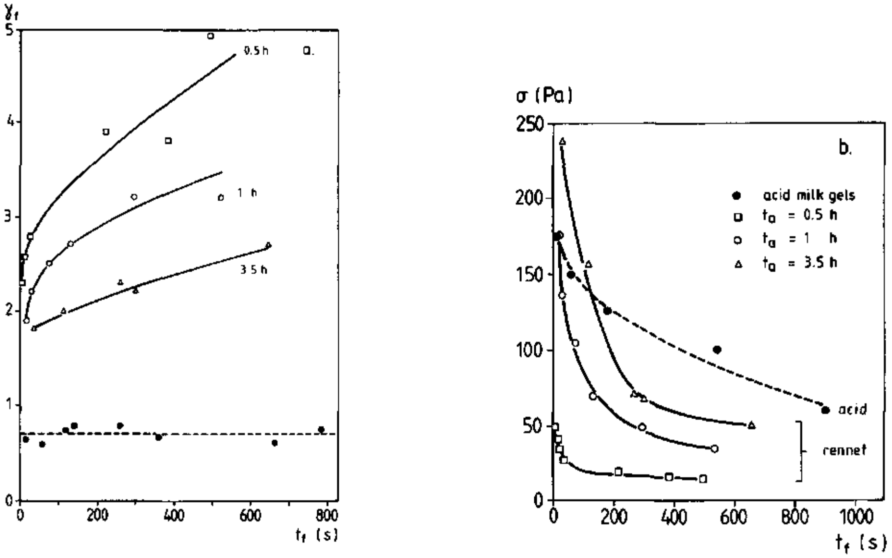


Fig. 7. The fracture strain (Fig. 7a) and the stress (Fig. 7b) as a function of the time up to fracture for an acid skim milk gel (aged for 16 hours at 30 °C, measured at 30 °C, pH = 4.6 after Roefs (6)) (●) and for rennet-induced skim milk gels aged for 0.5 h (□), 1 h (○) and 3.5 h (△); T = 30 °C and pH = 6.65.

Compared to *acid skim milk gels* it appeared that, as already mentioned by Roefs (6), the fracture strain was much larger for rennet-induced skim milk gels (Fig. 7a). Besides, the fracture strain is much more time-dependent for rennet-induced than for acid milk gels. Roefs mentioned also that a higher stress had to be applied to acid milk gels (ageing time = 16 h) to break them within a certain time as compared to rennet-induced milk gels (ageing time = 1 h). If the comparison is made with rennet-induced milk gels that had been aged for 3.5 hours, the stress needed to break a gel is indeed higher for acid milk gels if t_f was longer than about 100 s, but for shorter times up to fracture the stress was lower (Fig. 7b), implying that the rennet-induced milk gels show much more time-dependent behaviour. This was also concluded from other, earlier observations (e.g. 1, 2).

The results obtained for rennet-induced milk gels are qualitatively in accordance with the results for *young Gouda cheese* with a pH above 5.2: if the time to break a piece of this cheese by compression is longer, the fracture stress is lower, the fracture strain higher and the total energy needed lower (10). Cheese with lower pH was observed (10) to behave more like acid milk gels. Also for older cheeses, which contain such a low concentration of water that flow of the network was strongly hindered (also at pH above 5.2), no effect of the time up to fracture on the fracture strain was observed. For cheese shear-thinning behaviour also was observed (10). The slope of the graph of the pseudo viscosity versus the strain rate was about −0.8 for the first mentioned type of cheese and about −0.6 for the second type. The slope for rennet-induced milk gels was less steep than for cheese, about −0.4 (Fig. 6a, b). The difference may be due to the difference in concentration of casein. The possibility for the formation of new elastically effective bonds during the deformation process will be greater for cheese (higher casein concentration) than for milk gels.

Acknowledgements

We are indebted to A. E. A. de Groot-Mostert for technical assistance and to M. C. L. Maste and A. M. Beers for execution of most of the creep experiments.

Samenvatting

P. Zoon, T. van Vliet en P. Walstra, *De reologische eigenschappen van met leb gestremde ondermelkgelen. 5. Het gedrag bij grote vervormingen*

De reologische eigenschappen bij grote vervormingen van met leb gestremde ondermelkgelen werden bestudeerd door een zo hoge afschuifspanning op de gelen uit te oefenen dat na een zekere tijd (ongeveer 5-2000 s) breuk door het hele gel optrad. De vervorming werd gemeten als functie van de tijd (kruipproeven). De invloed van de verouderingstijd en de pH van de gelen, de meettemperatuur en de opgelegde spanning werden onderzocht.

Het bleek dat bij een kortere tijd tot breuk van het eiwitnetwerk, bij een lagere meettemperatuur en bij een langere verouderingstijd van de gelen, de spanning nodig om het gel binnen een zekere tijd te breken hoger was. De vervorming bij breuk was als gevolg van meer plastische vloei hoger voor langere tijd tot breuk en hogere meettemperatuur, terwijl de vervorming bij breuk afnam met toenemende verouderingstijd van het gel als gevolg van een kleinere momentane vervorming en relatief minder plastische vloei. Er werden voor een gegeven tijd tot breuk geen significante verschillen waargenomen in de vervorming als een functie van de tijd tussen gelen met verschillende pH's (pH 5.75-6.65), terwijl de spanning het laagst was voor de gelen met pH 6.65 en ongeveer gelijk voor gelen met pH 6.25 en 5.75. De snellere gelvorming en -veroudering bij een lagere pH heeft waarschijnlijk de resultaten beïnvloed.

De resultaten van de kruipproeven werden vergeleken met de resultaten van dynamische en spanningsrelaxatiemetingen voor met leb gestremde ondermelkgelen, zoals beschreven in de voorafgaande artikelen van deze serie, en met de resultaten van experimenten bij grote vervormingen uitgevoerd aan zure ondermelkgelen en aan kaas.

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Rheological properties of rennet-induced skim milk gels. 6. Interrelation between the dynamic moduli and the relaxation modulus

Summary

The time dependent rheological properties of rennet-induced skim milk gels were determined by two methods, viz. the stress relaxation modulus G(t) was determined in stress relaxation experiments with a Weissenberg Rheogoniometer and the dynamic moduli in dynamic experiments with a "Den Otter" rheometer. G(t) was calculated from the dynamic moduli by using a simple approximation formula and by means of a more complex procedure, via calculation of the relaxation spectrum. Both calculation methods gave the same results for G(t). G(t) measured in stress relaxation experiments was slightly lower than the calculated G(t), probably caused by inaccuracy in the stress relaxation measurements and an uncertainty in the determination of the time scale of dynamic experiments. Generally speaking, the agreement between the measured and the calculated G(t) was rather good, implying that the rheological properties determined by both methods are true material properties.

1 Introduction

Dynamic as well as stress relaxation measurements have been performed (1, 2) to obtain information on the rheological behaviour of rennet-induced milk gels over a rather large range of time scales. It is usually necessary, e.g. because the temperature-time superposition may not be applied, to combine the results of different kinds of measurements to map out the viscoelastic behaviour of a material over a relatively long time scale. The dynamic experiments give information at relatively short times and stress relaxation at relatively long times. If the rheological data may be described by a generalized Maxwell model, it is, within the linear region, in principle possible to calculate any viscoelastic function from any other, provided the latter is known over a sufficiently wide range of time or frequency (3). Moreover, if the same time dependent rheological data can be calculated from different types of experiments, it implies that real material properties have been measured. A great deal of literature on the recalculation of one viscoelastic function into another one is available for gels of flexible macromolecules or polymer melts (see e.g. 3), but not for particle gels. Roefs (7) reported about the calculation of the relaxation spectrum from the dynamic moduli for acid casein gels, which, like rennet-induced gels, behave as a particle gel.

In this study the stress relaxation modulus is calculated from the dynamic moduli in two different ways and the calculated relaxation modulus is compared to the relaxation modulus measured in stress relaxation experiments for rennet-induced milk gels.

2 Theory

The calculations are based on the idea that the rheological properties of a milk gel in the linear region may be imitated by the properties of a series of parallel Maxwell elements (Fig.1).

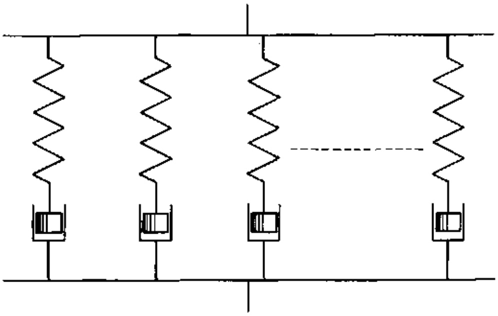

Fig. 1. Illustration of a Maxwell model consisting of several Maxwell elements in parallel.

A Maxwell element consists of a spring combined with a dashpot. The spring represents the elastic behaviour and the dashpot the viscous behaviour of the material. The spring is assigned a stiffness (force/displacement) analogous to a shear modulus G_i and the dashpot is assigned a viscosity η_i. The ratio of η_i to G_i is the relaxation time τ_i. In this study the following viscoelastic functions exhibited by a Maxwell element are used (e.g. 3):

$$G(t) = G_i \exp(-t/\tau_i) \tag{1}$$

$$G'(\omega) = G_i \omega^2 \tau_i^2 / (1 + \omega^2 \tau_i^2) \tag{2}$$

$$G''(\omega) = G_i \omega\tau_i / (1 + \omega^2 \tau_i^2) \tag{3}$$

With: $G(t)$ = relaxation modulus (Pa)
t = time (s)
G' = storage modulus (Pa)
G'' = loss modulus (Pa)
ω = angular frequency (rad/s)

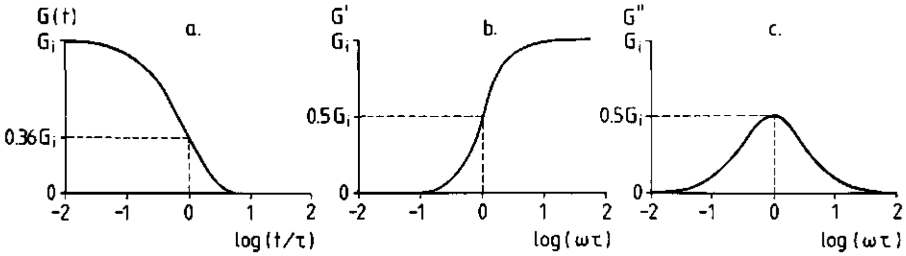

Fig. 2. Illustration of three viscoelastic functions of a Maxwell element as a function of the time scale of the experiment; a. the relaxation modulus G(t), b. the storage modulus G' and c. the loss modulus G"; τ is the relaxation time of the Maxwell element.

Fig. 2 gives an illustration of these functions. A Maxwell element contributes to G' if the angular frequency of deformation is higher than about 0.1 times the inverse of the relaxation time of this element, whereas it contributes to G" when the frequency is between 0.01 to 100 times the reverse of the relaxation time. It contributes to the relaxation modulus when the time scale of the experiment is shorter than about 10 times the relaxation time of the element.

Usually the mechanical behaviour of viscoelastic materials cannot be described by one Maxwell element (e.g. 8), but it can be imitated by the behaviour of a generalized Maxwell model with a sufficient number of elastic and viscous elements (Fig. 1).

When the Maxwell model is subjected to a stress relaxation experiment, the force on each spring-dashpot pair relaxes exponentially, each with its own rate dependent on the relaxation time of the element. The viscoelastic function G(t) is obtained by summing the expression in equation 1 over all the parallel elements; thus, if there are n elements:

$$G(t) = \sum_{i=1}^{n} G_i \exp(-t/\tau_i) \tag{4}$$

If part of the bonds in the network is permanent, one of the relaxation times must be infinite and the corresponding modulus contribution is G_e, the equilibrium modulus. G' and G" can be obtained as in eq. (4), by summing the expression in equations 2 and 3, respectively, over all the parallel elements.

If the number of elements in the Maxwell model of Fig. 1 is increased without limit, the result is a continuous spectrum in which each infinitesimal contribution to rigidity $F d\tau$ is associated with relaxation times lying in the range between τ and $\tau + \delta\tau$.

$$G(t) = G_e + \int_0^{+\infty} F(\tau)\exp(-t/\tau)d\tau \tag{5}$$

Usually, the viscoelastic function is calculated over several decades of frequency and it is more convenient to take a logarithmic time scale; accordingly the continuous relaxation spectrum is defined as $H(\tau)d\ln\tau$; hence $H(\tau) = \tau F(\tau)$. Equation 5 then becomes:

$$G(t) = G_e + \int_{-\infty}^{+\infty} H(\tau)\exp(-t/\tau)d\ln\tau \qquad (6)$$

Equation 6 permits calculation of the relaxation modulus G(t) when H(τ) is known. For G'(ω) and G"(ω) the relation with H(τ) is given by (3):

$$G'(\omega) = G_e + \int_{-\infty}^{+\infty} H(\tau)\omega^2\tau^2/(1 + \omega^2\tau^2)d\ln\tau \qquad (7)$$

$$G''(\omega) = \int_{-\infty}^{+\infty} H(\tau)\omega\tau/(1 + \omega^2\tau^2)d\ln\tau \qquad (8)$$

For the calculation of one function from the other, several methods have been developed (e.g. 3, 5). In this study two methods have been used to calculate G(t) from the dynamic moduli G' and G" as described in section 3.2.

3 Materials and methods

3.1 Experimental methods

A description of the materials and methods of the measurements used for determining G(t), G' and G" have been reported before in refs (1, 2, 6).

3.2 Mathematical procedure

The methods used to calculate G(t) from the dynamic moduli were:

I) a simple approximate equation derived by Ninomiya and Ferry (4).

$$G(t) = G'(\omega) - 0.40G''(0.40\omega) + 0.014\ G''(10\omega) \qquad (9)$$

II) calculation of the relaxation spectrum from G" and subsequently calculation of G(t), as described partly by de Cindio et al. (5). The first step is to estimate $H(\tau)$ from measured values of $G''(\omega)$; $(\tau = 1/\omega)$:

$$H(\tau) = (2/(3\pi))[G''(\omega) + G''(\omega/0.2) + G''(0.2\omega)] \qquad (10)$$

It is then checked whether the estimate of $H(\tau)$ is correct by calculating the loss modulus with equation 8, G_c''. If the difference between the calculated and measured loss modulus is on average larger than 2% a new estimate for $H(\tau)$ is made:

$$H(\tau)_{new} = H(\tau)_{old} + \Delta H(\tau)\ ,\ \text{with:} \qquad (11)$$

$$\Delta H(\tau) = (2/(3\pi))\{[G''(\omega) - G_c''(\omega)] + [G''(\omega/0.2) - G_c''(\omega/0.2)] + [G''(0.2\omega) - G_c''(0.2\omega)]\} \qquad (12)$$

This procedure is repeated until the difference between the calculated and measured loss modulus is on average equal to or smaller than 2%. Next G_c' is calculated:

$$G_c'(\omega) = \int_{-\infty}^{+\infty} H(\tau)\omega^2\tau^2/(1 + \omega^2\tau^2)\,d\ln\tau \qquad (13)$$

and G_e is calculated:

$$G_e(\omega) = G'(\omega) - G_c'(\omega) \qquad (14)$$

Subsequently G(t) is calculated with equation 6.

The integrals are calculated by a step method as will be explained for the calculation of G" (at a certain ω) with eq. 8. First an auxiliary variable $A(\tau)$ is calculated by means of the next equation:

$$A(\tau) = H(\tau)\omega\tau/(1 + \omega^2\tau^2). \qquad (15)$$

in which ω is kept constant. $A(\tau)$ is calculated for several values of τ ($=1/\omega$) within the range over which the dynamic moduli are determined; the steps between two succeeding values of τ was always $10^{0.2}$. The average value of every two succeeding $A(\tau)$'s is calculated and multiplied by $\ln 10^{0.2}$. Then the obtained values are summed, giving G" at the chosen value of ω.

If for the calculations the moduli were needed at frequencies at which they were not determined, they were estimated by linear interpolation (on log-log scale).

4 Results and discussion

The dynamic moduli were measured with the Den Otter rheometer over a frequency range as large as feasible, viz. from 10^{-3} to 4.5 rad/s (1, 2). The integration procedure involved in the calculation of G', G" and G(t) (equations 6-8, 13) therefore occurred over a truncated range: from $\tau = 1/4.5$ to $1/0.001$ s ($\tau = 1/\omega$). This truncation clearly affects the results near the boundaries of the τ range. In the first cycle of the recalculation of G" using equation 7, the recalculated values of G" near the boundaries of the frequency range are too low, because (part of) the contribution of the bonds with relaxation times just below 1/4.5 s or just above 1/0.001 s are not taken into account. The value of $H(\tau)$ near the boundary is then increased mathematically (equation 11) to such an extent that a correct value for G" is obtained. Then, however, the values calculated for H(τ), G_e and G(t) near the boundaries will not be correct. In order to get more reliable results for H(τ) over the whole range over which the dynamic moduli were measured (ω = 0.001 - 4.5 rad/s), the procedure was adapted somewhat. First G" (measured) was (linearly) extrapolated (in a log-log plot) for one decade at both sides of the frequency range and next H(τ) was calculated. Probably this procedure

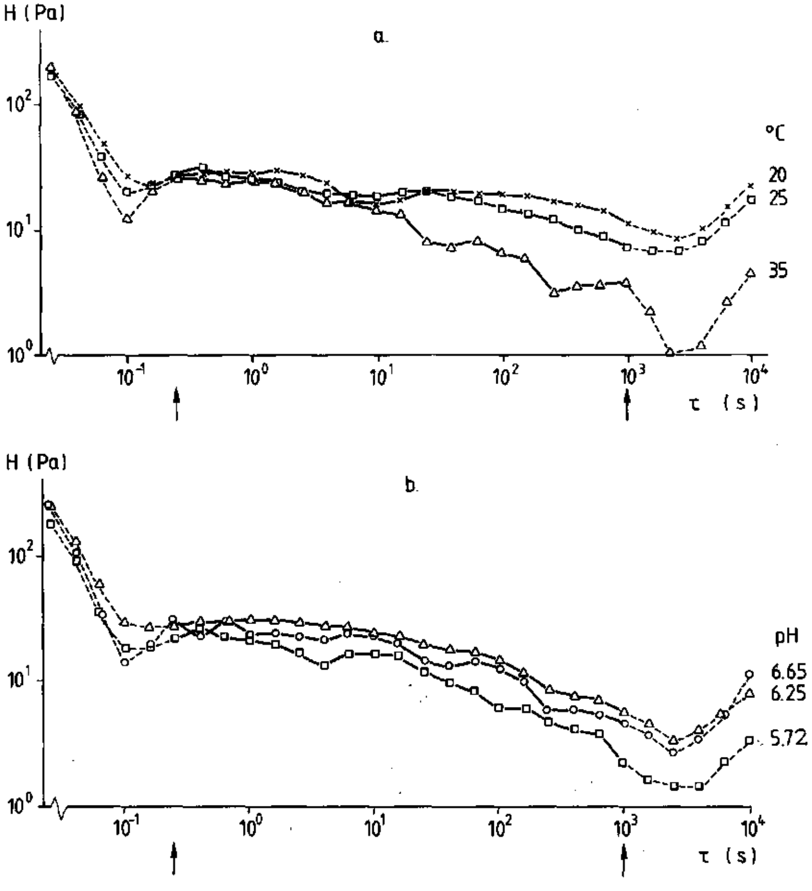

Fig. 3. The relaxation spectrum H(τ) calculated from the loss modulus G" (see text) for rennet-induced skim milk gels; a. gels with pH 6.65, formed and aged at 30°C (for 10 h) and tested at the indicated temperature. b. gels with pH as indicated, formed, aged and tested at 30°C. The arrows on the time axis indicate the range over which the loss moduli were determined. Outside this range they were estimated by extrapolation (see text).

does not introduce large errors, because if G" was measured up to 10 rad/s a straight line of log G" versus log ω was observed in the range between 1 and 10 rad/s. Moreover, at long time scales (low frequency) no irregularities were observed in the curve of G(t) versus t and therefore no big deviation from a straight line of log G" versus log ω between 10^{-3} and 10^{-4} rad/s are expected. In Fig. 3 the so calculated $H(\tau)$ is depicted as a function of the relaxation time τ in a log-log plot, with $\tau = 1/\omega$. The arrows on the time axis indicate the boundaries of the range over which the dynamic moduli had been measured. It is only in this range that the value of $H(\tau)$ may be considered more or less valid.

The curves of $H(\tau)$ versus τ are a bit wavy. This may be due to the upswing of H near the boundaries or to the shape of the graph of log G" versus log ω, which roughly is composed of two straight lines of positive slope linked by a more horizontal part (e.g. 1). Because of the rather irregular shape of log G" versus log ω it might cause a rather irregular shape of the curve of log H versus log τ. Moreover, the individually measured values of G" were not always exactly on the curve due to experimental inaccuracy.

$H d\ln\tau$ represents the contribution to the modulus of bonds with relaxation times whose logarithms lie in the range between $\ln\tau$ and $\ln\tau + d\ln\tau$. A higher temperature resulted in lower values of H at relatively long relaxation times, whereas at short relaxation times nearly no difference occurred (Fig. 3a). This implies that at a higher measuring temperature the contribution to the moduli of bonds with relatively long relaxation times is smaller than at a lower temperature. This is in accordance with the conclusion drawn in (1) that increasing the measuring temperature led to a shift of relaxation to shorter time scales. Lowering the pH from 6.65 to 6.25 resulted in somewhat higher values of H (Fig. 3b), due to the higher moduli at this pH; the slopes of the $H(\tau)$ versus τ graphs (Fig. 3b) were about equal. Lowering the pH from 6.65 to 5.72 resulted in a decrease of H and a steeper slope in the range of the highest relaxation times.

This must be caused by a decrease in the number of bonds with a relatively long relaxation time, which is probably due to solubilization of micellar calcium phosphate (2).

The limited range of frequencies over which the dynamic moduli are measured also affects G_e. G_e is defined as the equilibrium modulus, representing the contribution to the storage modulus of bonds with a infinitely long relaxation time. Due to the truncation applied in the calculations, G_e as a matter of fact represents the contribution to the storage modulus of bonds with relaxation times longer than about 1/0.001 s. This implies that the value of G_e depends on the value of the lowest frequency at which the dynamic moduli have been determined and it does not give information about the existence of bonds with infinitely long relaxation times.

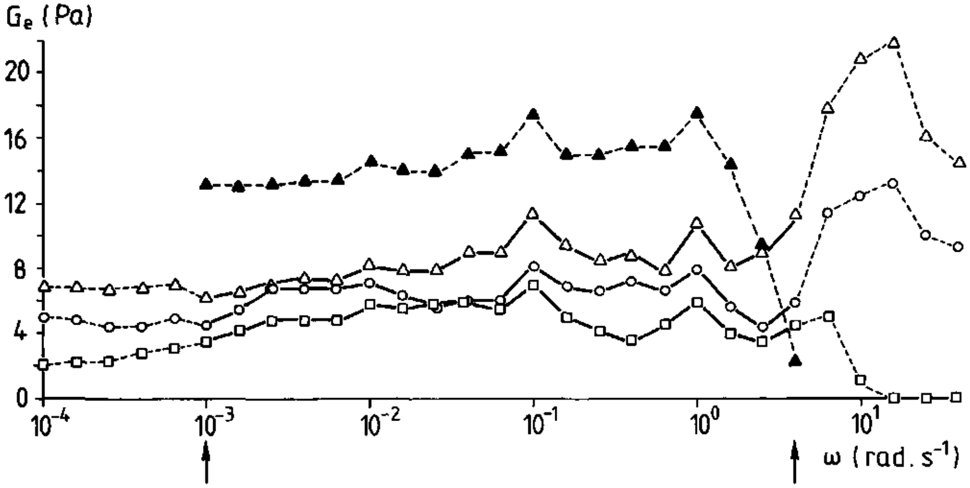

Fig. 4 The equilibrium modulus G_e calculated from the loss modulus G" (see text) for rennet-induced skim milk gels with pH 6.65 (o), 6.25 (△) and 5.72 (□). Same samples and conditions as in Fig. 3. For the sample with pH 6.25, G_e is also depicted if no extrapolation is applied (▲).

In Fig. 4 G_e is depicted as a function of the angular frequency for the gels at pH 6.65, 6.25 and 5.72. The same samples and extrapolation of G" versus ω are used as in Fig. 3b (open symbols). For the sample with pH 6.25, G_e is also depicted if no extrapolation (of G" versus ω) is applied. If the extrapolations and calculations are correct, G_e must be the same at all frequencies. From Fig. 4 it is clear that at the high frequency boundary, the calculated G_e is not at all constant. In Fig. 3 it is clear that at the low τ (high frequency) boundary $H(\tau)$ deviates most strongly from the graph ($H(\tau)$ versus τ)). The value of G_e is directly related to the value of $H(\tau)$. If $H(\tau)$ is for instance too high, G_c' will be too high and therefore G_e will be too low (eq. 14). Because at high frequencies the moduli are much higher than at low frequencies, this will probably cause larger (absolute) errors in $H(\tau)$ and therefore in G_e. Besides, for the calculation of G_e at high frequencies the difference of two relatively large numbers (G' and G_c') is calculated, resulting in a relatively inaccurate value of the difference. Extrapolation of the experimental G" versus ω does not influence the shape of the curve of G_e versus the frequency; only G_e is higher if no extrapolation is applied, because bonds with relaxation times longer than 10^3 s are already considered as bonds with infinite long relaxation times, as compared to 10^4 s in case of extrapolation.

G(t) is calculated from the dynamic moduli by methods I and II (see section 2) and it is also determined by stress relaxation measurements with the Weissenberg Rheogoniometer (1, 2). Some of the gels tested in stress relaxation measurements were aged for a shorter time than the gels tested in the dynamic measurements. Therefore a correction factor is applied to correct for the difference in ageing time. These factors are deduced from graphs of G' and G" versus ageing time (1, 2, 6) assuming that the correction was independent of the frequency. For the gels that have been aged at pH 6.65 and 30°C (including those tested at 25 and 20°C), G(t) measured with the Weissenberg is multiplied by

1.13 (G' at an ageing time of 10 h divided by G' at an ageing time of 6 h) and for gels with pH 6.25 G(t) is multiplied by 1.06. G(t) of gels with pH 5.72 is not corrected, because the ageing time was equal in the dynamic and the stress relaxation measurements. Moreover, the temperature in the Weissenberg Rheogoniometer was 20,5°C instead of 20.0°C. No correction has been made for this difference in temperature. In Fig. 5a, G(t) calculated and measured is depicted for the gels with pH 6.65, 6.25 and 5.72 and in Fig. 5b for the gels tested at 25 and 20°C. No differences were found for G(t) calculated by method I or II, which implies that the approximate formula derived by Ninomya and Ferry (4) gives good results for rennet-induced skim milk gels. The disadvantage of the use of this approximate formula is that G(t) can only be calculated over a very limited range of time, because to calculate G(t) for a certain time the dynamic moduli over a frequency range of 0.4 to 10 times the inverse of this time has to be known. However, if an extrapolation for G" versus ω is made, like for the calculation of G(t) with method II, G(t) could be calculated over the whole (measured) frequency range.

G(t) measured in stress relaxation measurements was for most samples somewhat lower than the calculated G(t). Only for samples with pH 5.72 the values were the same. For the samples tested at 25 and 20°C the measured G(t) was about 20% lower than the calculated G(t), and at pH 6.25 and 6.65 this was about 10%. There may be several reasons for the difference.

- Differences between duplicates of about 20% were found in stress relaxation measurements. The results given in Fig. 5 are mean values of 2 to 4 different measurements. Rennet-induced milk gels show rather rapid syneresis. Severe syneresis occurred at lower temperatures in the Weissenberg rheogoniometer than in the Den Otter rheometer. As was mentioned in (1), increasing the temperature to above 30°C in the Weissenberg Rheogoniometer caused such severe syneresis of the gel that nearly no stress was measured after

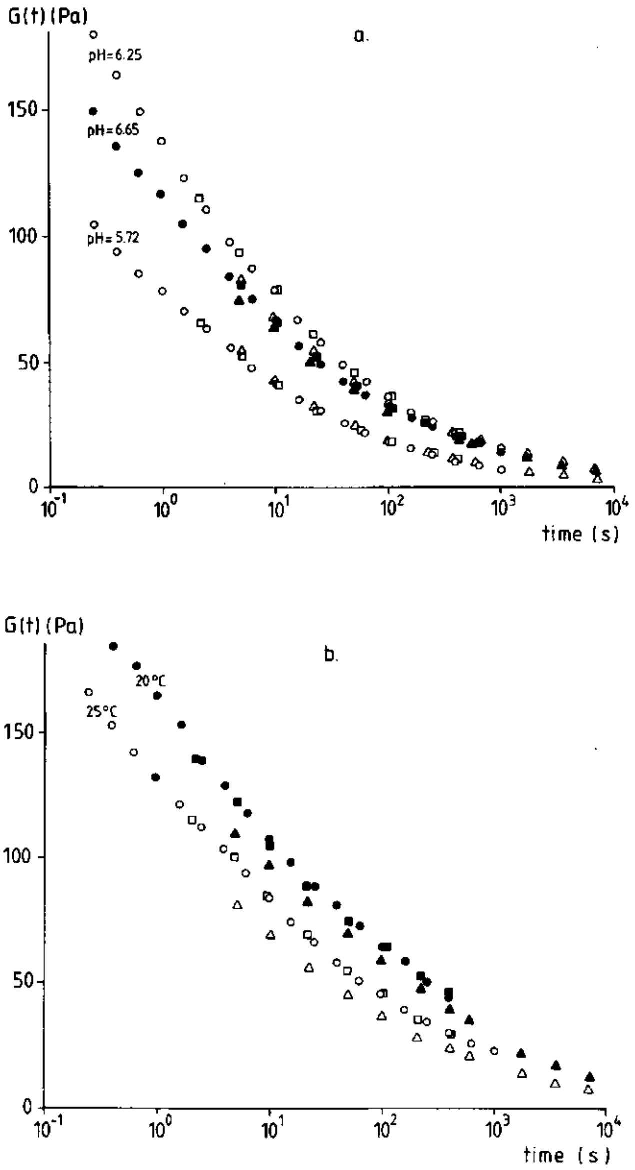

Fig. 5. The relaxation modulus G(t) as a function of time; a. gels with pH as indicated , formed, aged and tested at 30°C. b. gels with pH 6.65, formed and aged at 30°C (for 6 h) and tested at the indicated temperature. The same samples as in Fig. 4. Symbols: (△,▲) measured with the Weisssenberg Rheogoniometer; (□,■) calculated with method I (see text); (○,●) calculated with method II (see text).

deformation of the gel. In the Den Otter rheometer severe syneresis occurred only at a temperature of 40°C or higher. Maybe also at 30°C the modulus measured in the Weissenberg Rheogoniometer is somewhat lower than measured in the "Den Otter" rheometer because of syneresis.

- The correction for the difference in ageing time might have been not completely correct.
- The system of temperature control in the Weissenberg Rheogoniometer, viz. by air, which is rather poor, might be the reason for the relatively big deviation between the measured and calculated G(t) for tests at lower temperatures.
- The time scale of the dynamic experiments was calculated by the normally used relation that t is the inverse of the angular frequency $t = 1/\omega$). It is questionable whether this relation is quantitatively correct. In a dynamic experiment the modulus is determined by the ratio of the amplitude of stress to strain. The amplitude is reached in one fourth of a cycle and therefore it seems reasonable to take as time scale of a dynamic experiment 0.25 times the inverse of the frequency or $\pi/2$ times the inverse of the angular frequency. This implies that the time scale would become $\pi/2$ longer and the graphs of the calculated G(t) would shift to the right and the results of the calculated and measured G(t) would be in better accordance with each other.
- In the stress relaxation experiments the strain was not applied instantaneously, but within about 2.5 seconds. As zero time was taken the start of the strain application. It is probably more correct to take as zero time a time somewhere between the start and the finish of the strain application. The graphs of G(t) would then shift to a somewhat shorter time scale, which increases the differences between the measured and calculated G(t), especially at short times.

Taking into account the considerations mentioned above, it is concluded that the differences between measured and calculated G(t) probably are mainly due to inaccuracy of the measurements (especially of the stress relaxation measurements) and of the determination of the time scale of the experiments. The way in which G(t) is calculated from the dynamic moduli does not result in different values of G(t). But generally speaking the results of the dynamic and stress relaxation experiments are rather well in accordance with each other. This implies that the rheological properties of rennet-induced milk gels and its dependence on several factors as pH and measuring temperature as determined in this study may be considered as material properties (within the inaccuracy of the experimental errors). Moreover, it follows that the theory on the relaxation spectrum and its relation to the various moduli apply as well to viscoelastic particle gels as to gels of flexible polymers and to polymer melts.

Acknowledgements

We are indebted to B. de Cindio (Istituto di Principi di Ingegneria Chimica, Universita di Napoli, Naples (Italy)) for his help with the calculation of the relaxation modulus from the dynamic moduli.

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LIST OF SYMBOLS

a	activity	M
c	concentration	M
c_f	casein concentration factor	-
e	base of natural logarithms	-
G	shear modulus	Pa
G'	storage shear modulus	Pa
G"	loss shear modulus	Pa
G_c'	calculated storage shear modulus	Pa
G_c''	calculated loss shear modulus	Pa
G_e	equilibrium shear modulus	Pa
G_m	maximum shear modulus (stress relaxation measurements)	Pa
G_r'	relative shear storage modulus (G'/G_∞')	Pa
G_∞'	storage shear modulus after a long ageing time	Pa
G(t)	relaxation shear modulus	Pa
G*(t)	pseudo relaxation shear modulus	Pa
H	relaxation spectrum	Pa
h	length of cylinder	m
I	ionic strength	M
n	number	-
R_1	radius of inner cylinder	m
R_2	radius of outer cylinder	m
T	temperature	°C
T	torque	N.m
T_f	temperature during formation and ageing	°C
t	time	s
t_a	ageing time	s
$t_{a,\infty}$	long ageing time	s
t_c	clotting time	s
t_f	time up to fracture	s
t_f*	time-scale (of constructed stress-srain curve)	s
t_r	apparent mean relaxation time	s
t_{re}	relative time (t/t_f)	-
W	total energy input	$J.m^{-3}$
W'	stored energy	$J.m^{-3}$
W"	dissipated energy not due to fracture	$J.m^{-3}$

W_f	energy used for fracture	$J.m^{-3}$
α	angular displacement	rad
γ	shear strain	-
γ_o	maximum shear strain (dynamic measurements)	-
γ_o	instantaneous shear strain (creep measurements)	-
γ_f	fracture strain	-
$\dot{\gamma}$	shear strain rate	s^{-1}
δ	phase angle between stress and strain	rad
η^*	pseudo viscosity	Pa.s
σ	shear stress	Pa
σ_o	maximum shear stress (dynamic measurements)	Pa
σ_f	fracture shear stress	Pa
σ_m	maximum shear stress (stress relaxation measurements)	Pa
τ	relaxation time	s
ω	angular frequency	$rad.s^{-1}$

SUMMARY

The rheological behaviour of rennet-induced skim milk gels was studied under various conditions to obtain a better understanding of the main factors influencing the structure and mechanical behaviour of these gels. Moreover, it was tested whether certain relations derived for viscoelastic macromolecular gels could be applied to the milk gels, which can best be considered as particle gels. Three types of rheological measurements were performed:

- dynamic measurements with a "Den Otter" rheometer.
- stress relaxation measurements with a Weissenberg rheogoniometer.
- constant stress measurements at large deformations with a Deer rheometer.

For all three methods the gels were deformed in shear. With the dynamic measurements the gel formation and ageing was followed as well as the time dependent behaviour of the gels by determining the storage modulus G' and the loss modulus G" as a function of time and of frequency. The time dependent behaviour stems from relaxation of bonds within the time scale of the measurement. Relaxation was further investigated by means of stress relaxation measurements. Dynamic measurements give information about the behaviour at relatively short time scales and stress relaxation measurements at longer time scales. The time scales of both types of measurements partly overlapped, whereby results could be compared to each other. In the dynamic and stress relaxation experiments the strain was kept sufficiently low (< 0.03) to deform the gels within the linear region. On the other hand the constant stress measurements were performed at such high stresses that fracture of the gels occurred in the end.

In Part 1 an introduction to the subject is given. For all experiments reconstituted skim milk or artificial micelle dispersions were used. It was observed that if proper conditions were selected for preparation of the reconstituted

skim milk the results were comparable to those obtained with fresh whole milk. The casein and rennet concentrations appeared to affect the values of both G' and G", but not the ratio between them nor the time dependent behaviour of the gels.

Parts 2 to 4 are devoted to the influence of several factors on the rheological properties of the gels at small deformation. Part 2 is concerned with the influence of temperature on the dynamic and stress relaxation moduli. In the range from 20 to 40°C a higher temperature during gel formation and ageing resulted in a faster firming rate, whereas after a long ageing time the moduli decreased with increasing temperature. No differences in moduli were found if gels formed at different temperatures were tested at a certain measuring temperature. On the other hand, gels formed and aged at 30 °C gave at increasing measuring temperature decreasing values for the moduli while the relaxation of bonds was shifted to shorter time scales. It was concluded that a change in temperature caused a change in structure of the network and a change in the relative importance of the different types of interactions.

Part 3 deals with the effect of calcium and phosphate on the dynamic moduli of rennet-induced gels made of skim milk and artificial micelle dispersions. Decreasing the concentration of micellar calcium phosphate (MCP) resulted in a decrease of the moduli. A slight decrease of the MCP concentration did not affect tan δ (= G''/G'). A higher tan δ was observed if the MCP concentration was lower than about 60% of the concentration normally present in milk. At such low MCP concentrations enhanced visible syneresis occurred. MCP was thought to be directly involved in bonds and, moreover, to indirectly enhance the formation of bonds by affecting the micellar structure. The calcium ion activity influenced the clotting time and the moduli, but not significantly tan δ in the range studied.

Part 4 deals with the effect of pH and NaCl on the rheological properties at small deformation. Decreasing the pH from 6.65 to 5.72 resulted in a maximum of the modulus after a long ageing time near pH 6.15. Above pH 6.0 no significant influence was found of pH on tan δ (G"/G'), whereas at pH 5.72 a higher tan δ was observed at all frequencies between 10^{-3}-4.5 rad/s, implying that a higher proportion of the bonds present relaxed. The relaxation time found for gels at pH 6.65 and pH 6.25 did not differ significantly, but at pH 5.72 the relaxation time was clearly shorter. Solubilization of micellar calcium phosphate and increase of electrostatic attraction were thought to be important effects of decreasing pH.

NaCl addition at constant pH caused an increase of the clotting time. Its effect on the gel firming rate and the moduli after a long ageing time appeared to depend on the experimental conditions. No influence of NaCl addition on tan δ was observed.

Part 5 is devoted to the rheological properties of rennet-induced skim milk gels at large deformation. Constant (high) stresses were applied to the gels and the deformation was measured as a function of time. After an instantaneous deformation plastic flow occurred and eventually fracture of the casein netork was observed. The effect of the ageing time and the pH of the gels as well as the effects of the measuring temperature and the magnitude of the applied stress were investigated. It appeared that the stress needed to break the casein network of a gel increased if more or stronger bonds had to be broken, since such an increase was observed for a longer ageing time or if relatively little relaxation occurred, viz. at a relatively low measuring temperature or a short time up to fracture. The strain at fracture correlated with the instantaneous strain and the extent of plastic flow. The instantaneous strain decreased with decreasing stress and with increasing ageing time of the gel. The deformation caused by plastic flow decreased with decreasing time up to fracture and decreasing measuring temperature. For gels differing in pH (5.75 - 6.65) no difference in strain as a function of time was found if the time up to fracture was the same, but at pH 6.65 less stress was needed to cause fracture of the gel within a certain time. However, these results must be affected by the higher rate of gel formation and ageing at a lower pH.

In Part 6 the relaxation modulus G(t) is calculated from the dynamic moduli and it is compared to G(t) determined with stress relaxation measurements. Two different calculation methods gave the same results for G(t). G(t) determined with stress relaxation measurements was somewhat lower than the calculated G(t), probably caused by inaccuracy in the stress relaxation measurements and an uncertainty in the time scale of dynamic experiments. Upon the whole, the agreement in the measured and calculated G(t) was satisfactory, implying that the rheological properties determined by both methods were true material properties.

SAMENVATTING

Het doel van het beschreven onderzoek was meer inzicht te verwerven in de faktoren die van belang zijn voor de struktuur en de mechanische eigenschappen van met leb gestremde ondermelkgelen. Hiertoe werd het reologische gedrag van deze gelen bij verschillende omstandigheden onderzocht. Tevens werd nagegaan in hoeverre bepaalde relaties die voor viscoelastische polymeren zijn afgeleid, ook toegepast konden worden op melkgelen, die beschouwd moeten worden als deeltjes gelen. Drie soorten reologische meetmethoden werden gebruikt:

- dynamische metingen met een "Den Otter" reometer.
- spanningsrelaxatie-metingen met een Weissenberg reogoniometer.
- konstante-spanningsmetingen (kruipmetingen) bij grote vervorming met een Deer reometer.

Bij al deze methoden werd het gel in afschuiving vervormd. Met de dynamische metingen werd de gelvorming en -veroudering gevolgd en ook het tijdschaal afhankelijk gedrag van de gelen vastgesteld door de opslagmodulus G' en de verliesmodulus G" te bepalen als funktie van de tijd na stremseltoevoeging en als funktie van de frequentie. De tijdschaal bij dynamische metingen is ongeveer gelijk aan de reciproke van de (hoek)frequentie. Het gedrag is tijdschaal afhankelijk doordat er relaxatie, het breken van bindingen die spanning dragen en het vervolgens vormen van spanningsvrije bindingen, optreedt in de tijdschaal van de experimenten. De relaxatie werd nader onderzocht met behulp van de spanningsrelaxatie-metingen. De dynamische metingen geven informatie bij relatief korte tijdschalen terwijl de spanningrelaxatie-metingen informatie bij wat langere tijdschalen geven. De tijdschalen van beide metingen overlapten elkaar gedeeltelijk, zodat de resultaten met elkaar vergeleken konden worden. Bij deze metingen werd de vervorming zo klein (<0,03) gehouden dat er in het lineaire gebied werd gewerkt. Bij de konstante-spanningsmetingen werden grote spanningen opgelegd. Er trad binnen hoogstens een half uur

makroskopische breuk van het caseïne-netwerk van de gelen op.

In Deel 1 wordt een inleiding op het onderwerp gegeven. In alle experimenten werd gebruik gemaakt van gereconstitueerde ondermelk of van dispersies van kunstmatige micellen. Het bleek dat indien de juiste kondities voor de bereiding van de gereconstitueerde ondermelk werden gekozen, de resultaten hiervan vergelijkbaar waren met die van verse volle melk. De caseïne- en stremselconcentratie hadden invloed op G' en G", maar niet op tg δ (G"/G') en ook niet op het tijdsafhankelijk gedrag van de gelen.

De Delen 2 tot en met 4 zijn gewijd aan de invloed van diverse faktoren op de reologische eigenschappen van de gelen bij kleine vervormingen. In Deel 2 wordt de invloed van de temperatuur op de dynamische en spanningrelaxatie-moduli behandeld. Een hogere temperatuur tijdens de gelvorming- en veroudering resulteerde in een verhoging van de snelheid waarmee het gel steviger werd, terwijl na een lange verouderingstijd lagere moduli werden gevonden bij hogere temperatuur. Indien de gelen bij verschillende temperaturen (lager of gelijk aan 35°C) werden gevormd en na een lange verouderingstijd bij eenzelfde temperatuur werden getest, waren de moduli voor alle gelen gelijk. Anderzijds gaven gelen die bij 30°C werden gevormd en daarna bij verschillende temperaturen werden getest, bij een toename van de temperatuur lagere moduli en een verschuiving van de relaxatie naar kortere tijdschalen. Waarschijnlijk veroorzaakt een verandering in temperatuur een verandering in de struktuur van het netwerk en in de verhouding van de soorten interakties die een bijdrage leveren aan de moduli.

Deel 3 behandelt het effekt van calcium en fosfaat op de dynamische moduli van de gelen gemaakt van gereconstitueerde ondermelk en van dispersies van kunstmatige micellen. Een lagere concentratie van micellair calcium-fosfaat (MCP) resulteerde in lagere moduli. Een geringe vermindering van de MCP-concentratie had geen invloed op tg δ, maar wanneer minder dan ongeveer 60% van het oorspronkelijk in melk aanwezige MCP over was, werd een hogere tg δ waargenomen. Bij zo'n lage MCP-concentratie trad ook meer zichtbare synerese op. Er werd verondersteld dat MCP direkt betrokken was in bindingen en tevens bij de vorming van andere bindingen doordat het de struktuur van de micellen beïnvloedde. De calciumionaktiviteit had invloed op de vloktijd en op de moduli, maar binnen de onderzochte grenzen niet op tg δ.

Deel 4 behandelt de effekten van de pH en NaCl-toevoegingen op de reologische eigenschappen bij kleine vervormingen. Het verlagen van de pH van 6,65 naar 5,72 resulteerde in een maximum van de modulus na een lange verouderingstijd bij ongeveer pH 6,15. Boven pH 6,0 werd geen signifikante invloed van de pH op tg δ (G"/G') gevonden, terwijl bij pH 5,72 een hogere tg δ werd waargenomen bij alle frequenties tussen 10^{-3} en 4,5 rad/s, hetgeen inhoudt dat meer relaxatie plaatsvond. Hiermee in overeenstemming was dat de relaxatietijd voor gelen met pH 6,65 en 6,25 gelijk was en voor gelen met pH 5,72 duidelijk korter was. Het oplossen van micellair calcium-fosfaat en een verhoging van de elektrostatische aantrekking zijn waarschijnlijk verantwoordelijk voor de veranderingen in geleigenschappen bij verlaging van de pH.

Het toevoegen van NaCl bij konstante pH leidde tot een langere vloktijd. Het effekt op de snelheid van steviger worden van het gel en op de modulus na een lange verouderingstijd bleek af te hangen van de experimentele omstandigheden. Er werd geen invloed op tg δ waargenomen.

In Deel 5 zijn de reologische eigenschappen van met leb gestremde ondermelkgelen bij grote vervorming beschreven. Een konstante (grote) spanning werd opgelegd en de vervorming werd gemeten als funktie van de tijd. Na een momentane vervorming vond er plastische vloei plaats en uiteindelijk trad er breuk van het caseïne-netwerk op. De effekten van de verouderingstijd en de pH van de gelen, de meettemperatuur en de opgelegde spanning werden onderzocht. Het bleek dat de spanning die nodig was om het caseïne-netwerk van een gel te breken hoger was indien de verouderingstijd langer was of wanneer er minder relaxatie optrad, hetgeen gebeurde bij een lagere meettemperatuur of een kortere tijd tot breuk; in al deze gevallen moeten naar alle waarschijnlijkheid meer bindingen verbroken worden. De vervorming bij breuk hing af van de momentane vervorming en van de mate waarin plastische vloei optrad. De momentane vervorming was kleiner bij een lagere opgelegde spanning en bij een langere verouderingstijd van de gelen. De plastische vloei was geringer bij een kortere tijd tot breuk en bij een lagere meettemperatuur. Voor gelen met pH tussen 5,75 en 6,65 werd geen verschil gevonden in de vervorming als funktie van de tijd, maar bij pH 6,65 was minder spanning nodig om het gel in een bepaalde tijd te breken, vergeleken met gelen met pH 5,75 en 6,25. De resultaten moeten echter beïnvloed zijn door de snellere gelvorming en -veroudering bij een lagere pH.

In Deel 6 wordt de spanningrelaxatie-modulus G(t) berekend uit de dynamische moduli en vergeleken met G(t) bepaald met spanningsrelaxatie-metingen. Twee verschillende berekeningsmethoden gaven overeenkomende resultaten. De berekende G(t) kwam echter steeds iets lager uit dan G(t) bepaald met spanningsrelaxatie-metingen. Waarschijnlijk werd dit veroorzaakt door onnauwkeurigheden in de spanningsrelaxatiemetingen en door onzekerheid ten aanzien van de tijdschaal van dynamische metingen. In het algemeen was de overeenkomst tussen de berekende en de door meting bepaalde G(t) echter bevredigend, hetgeen inhoudt dat de reologische eigenschappen die met beide methoden bepaald zijn, echte materiaaleigenschappen zijn.

CURRICULUM VITAE

De auteur werd geboren op 17 maart 1960 te Maassluis. In 1978 behaalde zij het diploma gymnasium β van de scholengemeenschap prof. Casimir te Vlaardingen. Daarna begon zij de studie Levensmiddelentechnologie aan de toenmalige Landbouwhogeschool te Wageningen. In januari 1985 slaagde ze voor het doctoraalexamen, met als hoofdvak Levensmiddelenchemie en als bijvakken Melkkunde gekombineerd met Kolloidchemie, Technische Mikrobiologie en Pedagogiek. Van 1 februari 1985 tot 1 februari 1988 was zij werkzaam als wetenschappelijk assistent bij de sektie Zuivel en Levensmiddelennatuurkunde van de Landbouwuniversiteit. In deze periode werd het in dit proefschrift beschreven onderzoek uitgevoerd. Vanaf 1 mei 1988 werkt zij als wetenschappelijk medewerker bij het Nederlands Instituut voor Zuivelonderzoek (NIZO) te Ede.