Methods for strictly aseptic making of cheese and effect of some bacteria on its ripening



Dit proefschrift met stellingen van Gijsbertus Kleter, landbouwkundig ingenieur, geboren te Hoogland op 20 juni 1940, is goedgekeurd door de promotor, ir. E. A. Vos, hoogleraar in de zuivelbereiding en de melkkunde.

De rector magnificus van de Landbouwhogeschool, H. C. van der Plas

Wageningen, 26 september 1977

G. Kleter

MO 1701 700

Methods for strictly aseptic making of cheese and effect of some bacteria on its ripening

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 11 november 1977
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen



Centre for Agricultural Publishing and Documentation

Wageningen - 1977

Abstract

Kleter, G. (1977) Methods for strictly aseptic making of cheese and effect of some bacteria on its ripening. Doctoral thesis, Wageningen. (viii) + 24 p., 1 fig., 1 table, 53 refs, Eng. and Dutch summaries, Neth. Milk Dairy J. 28 (1974): 212-219, 220-237; 29 (1975): 295-302; 30 (1976): 254-270; 31 (1977): 177-187.

Also: Agric. Res. Rep. (Versl. landbouwk. Onderz.) 870, p. I-1 - 1-24.

To study the influence of different enzymes on ripening of cheese, such as Gouda, a method was developed for making cheese strictly aseptically, including equipment for aseptic cheesemaking and aseptic milking of selected cows. In aseptically drawn milk only heat-labile micrococci and coryneforms were present. Nutrients needed for their growth were determined. Starter bacteria proved essential for the ripening process of Gouda cheese, including breakdown of protein, and production of taste and flavour substances, but strains differed widely. Lactobacilli proved to be not necessary for ripening and the strains tested caused off-flavours.

Free descriptors: udder, aseptically drawn milk, micrococci, staphylococci, Corynebacterium bovis, heat-resistance, bacterial growth, cheesemaking equipment, Gouda cheese, enzymes, rennet, starter, lactobacilli, proteolysis, lipolysis, taste and flavour, bitterness.

This thesis will also be published in Agricultural Research Reports 870.

e Centre for Agricultural Publishing and Documentation, Wageningen, 1977.

No part of this book may be reproduced and/or published in any form, by print, photoprint, microfilm or any other means without written permission from the publishers.

NN 8201

700

Stellingen

1. Bij het bepalen van het kiemgetal van aseptisch gewonnen melk met 'plate count milk agar' is een verrijking van dit medium noodzakelijk, bijvoorbeeld met Tween 80.

R.W. Cobb, J. med. Lab. Technol. 20 (1963) 199. Dit proefschrift.

- 2. Volgens 'Bergey's Manual' vormt Corynebacterium bovis geen zuur uit koolhydraten. Dit is onjuist.
 - R. S. Breed, E. G. D. Murray and N. R. Smith, Bergey's Manual of determinative bacteriology. The Williams and Wilkins Company, Baltimore, 1957.

W. F. Harrigan, J. appl. Bact. 29 (1966) 380.

Dit proefschrift,

- 3. Door verschillende onderzoekers, zoals Fabian en Agenjo, wordt ten onrechte vermeld, dat het inwendige van de uier een bron is van thermoresistente micrococcen. De opname van de thermoresistentie bij de indeling van de Micrococcaceae volgens 'Bergey's Manual' zou dergelijke onjuistheden helpen voorkomen.
 - F. W. Fabian, J. Milk Technol. 5 (1942) 237.
 - C. Agenjo, Proc. 13th int. Dairy Congr., Den Haag, 1953, II, p. 153.
 - R. S. Breed, E. G. D. Murray and N. R. Smith, Bergey's Manual of determinative bacteriology. The Williams and Wilkins Company, Baltimore, 1957.
- 4. Bij het onderzoek omtrent het afweermechanisme van de koe tegen het binnendringen van bacteriën in het tepelkanaal wordt, naast een mogelijke bacteriostatische of bacteriocide werking van het keratinemateriaal, te weinig aandacht besteed aan spiercontractie.
 - E. W. Adams and C. G. Rickard, Am. J. vet. Res. 24 (1963) 122.
 - K. G. Hibbitt and Margaret Benians, J. gen. Microbiol. 68 (1971) 123.
 - W. H. Giesecke, W. H. Gerneke and I. B. J. van Rensburg, J. S. Afr. vet. Ass. 43 (1972) 351.
- 5. Dat de onderzoekingen omtrent de oorzaken van mastitis en de middelen ter preventie vaak tegenstrijdige conclusies opleveren, wordt veelal veroorzaakt doordat deze conclusies gebaseerd zijn op statistische verwerking van cijfermateriaal dat verkregen is onder omstandigheden met oncontroleerbare factoren. Dit pleit voor voortgezet fundamenteel onderzoek in deze.
 - A. J. Dijkman, Off. Org. F.N.Z. 61 (1969) 375.
 - G. Grootenhuis, S. Brandsma en A. M. G. Flamand, Tijdschr. Diergeneesk. 99 (1974) 691.
 - F. H. Dodd, T. K. Griffin and R. G. Kingwill (Ed.), 1DF Seminar on mastitis control. Reading, 1975, W.N. Philpot, p. 155.

- 6. Bij de bestudering van de vetsplitsing in kaas dient men rekening te houden met de beschikbaarheid van het substraat, aangezien deze de vetsplitsing sterk kan beïnvloeden.
 - H. A. Morris, J. J. Jezeski, W. B. Combs and S. Kuramoto, J. Dairy Sci. 46 (1963) 1. J. E. Kinsella and D. H. Hwang, Critical Reviews in Food Science and Nutrition, 8 (1977) 191.
- 7. Het zou op bezwaren stuiten, om ter verklaring van de resultaten van de proeven van Law e.a., aan te voeren dat enzymen die zich niet in een intacte bacteriecel bevinden of niet aan de celwand daarvan zijn gebonden, dientengevolge in kaas onwerkzaam zouden zijn onder invloed van een te hoge ionsterkte in het kaasvocht.
 - B. A. Law, Marisi J. Castañón and M. Elisabeth Sharpe, J. Dairy Res. 43 (1976) 301.
- 8. Een beperking van de invoer van kaas uit Nederland, op grond van bezwaren tegen het gebruik van nitraat bij de bereiding ervan, zou, gelet op de jongste onderzoekresultaten in deze, volstrekt ongegrond zijn.
 - K. Goodhead, F. A. Gough, K. S. Webb, J. Stadhouders and R. H. C. Elgersma, Neth. Milk Dairy J. 30 (1976) 207.
- 9. Het is onduidelijk welke factoren de groei van lactobacillen in kaas, zoals Goudse en Cheddar, bepalen. Met name dient nader te worden onderzocht of gedurende de rijping een C-bron beschikbaar is of komt.
 - L. A. Mabbitt, J. Dairy Res. 28 (1961) 303 (Review).
 - T. F. Fryer, Dairy Sci. Abstr. 31 (1969) 471 (Review).
 - K. R. Nath and R. A. Ledford, J. Dairy Sci. 56 (1973) 710.
- 10. Door de mogelijke aanwezigheid van pathogene micro-organismen in rauwe melk is de E.G.-verordening nr. 1411/71, waarbij de verkoop van deze melk vanaf de boerderij aan consumenten wordt toegestaan, niet in het belang van de volksgezondheid.
- 11. De controle op de bacteriële hoedanigheid van melkpoeder wordt extra bemoeilijkt doordat eventueel aanwezige pathogene micro-organismen mogelijk in subletale toestand verkeren.
 - E. H. Kampelmacher, M. Ingram and D. A. A. Mossel (Ed.), The microbiology of dried foods. Proc. 6th int. symp. food microbiol., Bilthoven, 1968. M. Woodbine, E. B. Reid and E. J. Rolfe, p. 69.
 - F. A. Skinner and W. B. Hugo (Ed.), Inhibition and inactivation of vegetative microbes. Soc. appl. Bact., Symp. series no. 5, 1976. M. van Schothorst, p. 317.
- 12. Slechts een gering deel van de door de koe opgenomen hoeveelheid aflatoxinen B_1 en B_2 wordt uitgescheiden in de melk in de vorm van de aflatoxinen M_1 en M_2 , en bij dierproeven bleken de M-aflatoxinen vaak minder toxisch dan de B-aflatoxinen. Niettemin is het voor een verantwoorde bereiding van zuivelprodukten van belang de huidige E.G.-richtlijn, nr. 74/63, voor de toelaatbare hoeveelheden aflatoxine in veevoeders te verlagen.
 - F. Kiermeier, The significance of aflatoxins in the dairy industry. Int. Dairy Fed., Brussel, F-Doc 51, 1976.

- 13. Bij het tot sporuleren brengen van Clostridium perfringens (welchii) volgens de methode van Duncan en Strong, is het van belang om na te gaan of de behandeling van het sporulatie-medium met actieve kool ook vooraf kan worden uitgevoerd, aangezien het scheiden van de toch al vaak geringe aantallen sporen en de actieve kool, zeer veel problemen kan opleveren.
 - C. L. Duncan and Dorothy H. Strong, Appl. Microbiol. 16 (1968) 82.
 - H. G. Lambers, Versl. doct. ond. Lab. Zuiveltechnologie en Melkkunde, Wageningen, 1976.
- 14. Het gebruik van de proceswaarde, of F-waarde, ter karakterisering van een verhittingsproces is aan beperkingen onderhevig.
 - C. R. Stumbo, Thermobacteriology in food processing, Ac. Press, New York, 1973.

Stellingen behorend bij het proefschrift van Ir. G. Kleter, getiteld 'Methods for strictly aseptic making of cheese and effect of some bacteria on its ripening'.

Aan Christel Gijsbrecht en Margriet Bij het gereedkomen van dit proefschrift wil ik gaarne te kennen geven, dat de totstandkoming ervan slechts mogelijk is geweest door de samenwerking met en de ondersteuning van velen.

Allereerst wil ik mijn moeder danken, die het mij mogelijk heeft gemaakt te studeren.

Bij het rijpingsproces van kaas zijn enzymen, afkomstig van de melk, het stremsel en diverse micro-organismen, van groot belang. Zowel de ontwikkeling van geur en smaak, als de consistentieveranderingen in kaas worden erdoor in hoge mate bepaald. Uitgangspunt bij het onderzoek was de bestudering van de activiteit van de diverse enzymen, zowel afzonderlijk als in gewenste combinaties in kaas, zonder de activiteit van niet in onderzoek zijnde enzymen. Hooggeleerde Vos, uw voorstel om hiertoe een methode te ontwikkelen voor het bereiden van kaas onder strikt aseptische omstandigheden, heeft onmiddellijk mijn enthousiasme doen ontstaan. Het lag voor de hand om na de ontwikkeling van deze methode, in eerste instantie na te gaan wat de invloed is van enkele wellicht van belang zijnde bacteriesoorten op de rijping van Goudse kaas. Dat de methode zich vervolgens ook voor andere en verdergaande studies omtrent de werking van enzymen in kaas zou lenen, stond van meet af aan voor u vast. Zoals later zou blijken geheel terecht. Uw begeleiding tijdens de ontwikkeling van de methode van het aseptisch-melken van geselecteerde koeien en de apparatuur voor aseptische kaasbereiding, alsmede bij het kaasonderzoek, is een grote steun voor mij geweest. Uw stimulerende invloed en opbouwende kritiek tijdens het op-schrift-stellen van de resultaten heb ik als bijzonder nuttig ervaren. Zeer bijzondere dank ben ik u daarom verschuldigd.

Hooggeleerde E.G. Mulder, niet alleen heb ik bij u mijn opleiding in de Microbiologie gekregen, doch tevens heeft u mijn interesse voor deze tak van de wetenschap ten zeerste bevorderd.

Dat de bacteriële kwaliteit van de aseptisch gewonnen melk bleek te voldoen aan de bijzonder hoge eisen welke wij er aan meenden te moeten stellen, is mede te danken aan de goede en prettige samenwerking met het Melkhygiënisch Onderzoek Centrum te Wageningen. In het bijzonder wil ik ir. Tj. de Vries en de heer J. T. Nuninga mijn dank betuigen voor hun daadwerkelijke steun bij de selectie van de koeien en het aseptisch-melken. De afdeling Veeteelt van de Landbouwhogeschool bleek steeds weer bereid de benodigde koeien ter beschikking te stellen. Ik ben deze afdeling hiervoor zeer erkentelijk, alsmede de medewerkers van deze afdeling die betrokken zijn geweest bij de selectie van de koeien en het aseptisch-melken.

Mijn dank gaat zeker ook uit naar de vele medewerkers van de vakgroep Levensmiddelentechnologie, in het bijzonder de sectie Zuiveltechnologie en melkkunde, van de Landbouwhogeschool, die vaak op velerlei wijze hebben bijgedragen tot de uitvoering van het onderzoek en de totstandkoming van dit proefschrift. Heel in het bijzonder wil ik noemen de heren H. Paardekooper en C. Rijpma voor hun bijdrage bij het ontwerpen en de constructie van de apparatuur voor het aseptisch kaasbereiden. Voorts hebben de heren J. L. Uitentuis en H. J. van der Stege een ruime bijdrage geleverd aan de uitvoering van de experimenten, waarvoor mijn oprechte dank.

Tot slot wil ik ook de medewerkers van het Pudoc noemen voor wat betreft de redactionele verzorging van de artikelen en het proefschrift.

Curriculum vitae

De auteur is geboren te Hoogland op 20 juni 1940. Na het behalen van het diploma HBS-B aan het Constantijncollege te Amersfoort, begon hij in 1960 met zijn studie aan de Landbouwhogeschool te Wageningen. Als studierichting koos hij de Zuivelbereiding en melkkunde. In 1968 werd het ingenieursdiploma behaald, met als hoofdvak de Zuivelbereiding en melkkunde en als keuzevakken de Ziekte- en gezondheidsleer en de Microbiologie (verzwaard). Sindsdien is hij werkzaam bij het Laboratorium voor Zuiveltechnologie en melkkunde van de Landbouwhogeschool, eerst als wetenschappelijk assistent en vervolgens als wetenschappelijk medewerker I. Het promotie-onderzoek werd op dit laboratorium uitgevoerd onder leiding van prof.ir. E. A. Vos.

Contents

Introduction 1. The ripening of cheese 2. Enzymes active during cheese ripening 3. How to study the action of different enzymes 4. Purpose of the investigations 5. Elucidation of the performance of the work References	I- 3 I- 3 I- 3 I- 4 I- 5 I- 6 I- 7
Aseptic milking of cows G. Kleter and Tj. de Vries, Neth. Milk Dairy J. 28 (1974): 212-219.	I-11
The bacterial flora in aseptically drawn milk G. Kleter, Neth. Milk Dairy J. 28 (1974): 220-237.	I–12
Apparatus for making cheese under strictly aseptic conditions G. Kleter, Neth. Milk Dairy J. 29 (1975): 295-302.	I-13
The ripening of Gouda cheese made under strictly aseptic conditions. 1. Cheese with no other bacterial enzymes than those from a starter streptococcus G. Kleter, Neth. Milk Dairy J. 30 (1976): 254-270.	I-14
The ripening of Gouda cheese made under strictly aseptic conditions. 2. The comparison of the activity of different starters and the influence of certain lactobacillus strains G. Kleter, Neth. Milk Dairy J. 31 (1977): 177-187.	I-15
Discussion and conclusions 1. Selection of cows and bacteriological quality of the aseptically drawn milk 2. Bacterial flora of aseptically drawn milk from selected cows 3. Equipment for aseptic cheesemaking 4. Influence of bacterial enzymes on ripening of Gouda cheese made under strictly aseptic conditions References	I-17 I-18
Summary	I-22
Samenvatting	I-23

Introduction

1 The ripening of cheese

Cheese is a product of great economic and social importance. It keeps long, is nutritious and is enjoyed for its taste and flavour. It is consumed all over the world. Most types of cheese are ripened for some weeks to a year or even more, to develop their consistency and especially the taste and flavour characteristic for the type of cheese. Ripening of cheese is a complicated physical, enzymic and chemical process. The taste and flavour typical for a type of cheese requires a subtile balance between taste and flavour substances (1,2,3). Attempts to shorten ripening time, for instance by adding enzymes or a large number of certain micro-organisms to the milk, have met little success, resulting in imbalance of such a complicated system.

Taste and flavour, and physical changes, which govern the sensoric properties of the cheese, result from enzymic and other chemical processes during ripening. Enzymes involved in cheese ripening have always recieved much attention, since they rather than purely chemical reactions are largely responsible for the changes during ripening. Schormüller (4) has reviewed enzymes that can be involved in cheese ripening. Proteins, fats and carbohydrates in the cheese can all be attacked by the enzymes. The conversions of all three main components are important in relation to the development of characteristic taste and flavour. Decomposition of the lactose especially to lactic acid, is necessary also for the conservation of the cheese. Cheese is in fact a form of preserved milk substances. Breakdown of the proteins governs the physical state of the cheese, usually called the 'consistency'.

Cheese taste and flavour components can each result from different enzymic pathways. For example, ketones and aldehydes result from breakdown of citric acid or fatty acids, the latter especially in soft types of cheese with a surface growth of moulds (e.g. 5) and probably also in Cheddar cheese (e.g. 6). Amino acids result from protein breakdown or are synthesized from amines or amonia (4).

2 Enzymes active during cheese ripening

The raw milk itself contains many enzymes that could be involved in cheese ripening. However recent decades, it has become usual in factories to pasteurize the milk that is used for cheesemaking. This is done for the sake of public health and to avoid cheese defects. With the time-temperature combination usual in pasteurization (15 s 72 °C), certain enzymes are partly or completely destroyed in milk. An example of this is the milk lipase, which is highly active in cheese from raw milk, but not in cheese from pasteurized milk (7). So the presence of the enzymes from the milk itself will be limited in cheese from pasteurized milk. An exception is milk protease (8).

Most types of cheese are made with rennet, used to coagulate the milk. During ripening, the enzymes from rennet still show proteolytic activity (e.g. 9,10,11). The lipolytic activity of enzymes from rennet in the cheese seems negligible (7).

Although pasteurization of milk reduces the microbial flora and microbial enzymes in the milk, for instance lipolytic and proteolytic enzymes from Gramnegative rods (12,13) and also certain micro-organisms itself are heat-resistant. Further after pasteurization of the milk the cheesemaker adds micro-organisms, such as starter bacteria, propionic acid bacteria or moulds. Other micro-organisms enter the cheese milk by contamination after pasteurization. Thus in all types of cheese, even in cheese from pasteurized milk, considerable numbers of different types of micro-organisms and many different microbial enzymes can be found. These microbial enzymes are extremely important in cheese ripening (e.g. 4,14) through decomposition of carbohydrates, proteins and fats.

The conditions in the cheese determine the growth of all these micro-organisms and hence the amount and types of microbial enzymes. The activities of these enzymes, and the enzymes from rennet and milk are also influenced by conditions. There can be interactions between the activity of different enzymes. These aspects have been considered by Schormüller (4). Conditions in cheese depend on several factors such as moisture content, concentration of soluble constituents, pH and redox potential, which in turn are influenced by the composition of milk and especially by the cheesemaking process. The shape of the cheese and the keeping conditions too are important. For these reasons it is not surprising that so many types of cheese exist.

3 How to study the action of different enzymes

Two requirements have to be fulfilled to obtain definite information about the ripening process in cheese. First, we need a system in which the different enzymes can be studied separately and in desired amounts and combinations, without the activity of other enzymes. Secondly, the conditions in this system have to be the same as in cheese itself. These two requirements can be fulfilled by making cheese strictly aseptically.

We in the Netherlands are interested especially in Gouda cheese. Much is known about the ripening of this type of cheese (e.g. 7,15,16,17,18,19,20,21,22,23), although the tests were not under strictly aseptic conditions. A simplified model system cannot be used instead of cheese itself, if also organoleptic properties are among those to be examined. Model systems have also the danger that the conditions may differ from those in cheese. But such experiments have given valuable information (e.g. 24,25,26,27) and they have the advantage that in one experiment a single factor such as pH or NaCl content can be varied to a great extent or even several factors can be varied. However, to draw definite conclusions about the influence of the different enzymes on the ripening of cheese, aseptic cheesemaking in addition to all these researches is necessary as has also been argued by other workers such as Fryer (28) and Lawrence et al. (29).

To make cheese under strictly aseptic conditions, the bacterial count of the raw milk has to be low, preferably even nil, at least after pasteurization. An extremely low level of bacterial enzymes in the milk is necessary, since the ripening of the cheese will

take months. So we paid considerable attention to the milking of the cows and the bacterial quality of the milk. In the cheese, no growth of micro-organisms other than those being studied, is allowed. Every contamination during cheesemaking has to be prevented.

The necessity of an aseptic method has already been emphasized by von Freudenreich (30). His cows were milked under aseptic conditions and an open sterilized cheesevat was used for cheesemaking (31). This method was also used by Boekhout & Ott de Vries (32) and later by Stadhouders et al. (7,33) for Dutch-type cheese. Despite possible contamination during cheesemaking in an open vat, Stadhouders & Veringa (33) reported that some of the cheeses were usable for tests, although no full description of the bacterial quality of the cheese was given.

To study the influence of bacteria on the ripening of Cheddar cheese, aseptic cheese vats have been developed (34,35,36). Although in this way any contamination during cheesemaking could be prevented, the milk used was drawn in the normal way and was therefore not sterile, even after pasteurization. Reiter et al. (37) reported, that fully aseptic conditions were reached when the milk was drawn by cannula, but only very small cheeses of 100 g could be made, and a full description of the bacterial quality of the milk and the cheese is not given.

4 Purpose of the investigations

The purpose of our work, as reported in the five following papers, was twofold:

- 1. To develop a method for making cheese, such as Gouda cheese, under strictly aseptic conditions, including an aseptic milking technique and equipment for aseptic cheesemaking.
- 2. To examine the influence of the activity of the enzymes from certain bacteria, which might be important, on the ripening of Gouda cheese.

For the production of Gouda cheese, streptococci (Streptococcus cremoris strains), usually with aromaproducing bacteria (Streptococcus diacetylactis and/or Leuconostoc citrovorum), are used as starter. In cheese produced under normal conditions, there are also bacteria that survive pasteurization and may multiply in the cheese. There are heat-resistant bacterial enzymes, and contaminants after pasteurization of the milk, especially lactobacilli, which normally multiply to very large numbers during cheese ripening. The main question to be answered was, Can starter bacteria give a normal cheese ripening process without the activity of any other bacterial enzymes in the cheese? Besides its scientific interest, the question is also relevant to cheesemaking practice. Sanitary conditions on farms have improved, resulting in less bacteria in raw milk and little opportunity for growth. Moreover there is an increasing interest in closed cheese vats in factories, for better cleaning and disinfection, which could exclude, for instance, lactobacilli.

After completing the two tasks as mentioned above, we hoped to use the method of making cheese under strictly aseptic conditions for further studies on ripening of cheese.

5 Elucidation of the performance of the work

The bacterial count of the milk has to be low, preferably nil, at least after pasteurization. The bacteria still present in the milk have to be prevented from growing in the milk and in the cheese made from it. Antibiotics or other substances preventing bacterial growth may not be added, as the growth of added bacteria and the activity of enzymes might be altered, and sensory tests are unpermissible. The milk cannot be sterilized by heat (e.g. 10 min 120 °C or ultra high temperature treatment) as clotting of the milk and cheese ripening would be altered and a false impression would be obtained of ripening of cheese.

Comparison of samples of milk obtained by aseptic-hand-milking with those obtained by puncture of the reservoir of the udder (38,39,40) have shown that the milk in the upper parts of the udders of clinically healthy cows is usually sterile and is contaminated during milking by the typical flora of the teat canal. So the best way of obtaining milk seemed that developed by Tolle & Zeidler (41). The milk is drawn directly from the reservoir of the udder to avoid contamination in the teat canal. As yet the method has not proved as useful as first claimed (42). A cannula (37) may be used for drawing only small amounts of milk. So only aseptic milking of cows as done by von Freudenreich (30,31) and others (7,32,33) remains feasible. Disinfection of the teat canal before each milking has not been described, as far as we know. In preliminary tests (43) we in our laboratory found that disinfection of the teat canal gave variable results and proved impractical. However, teat-canal bacteria found in milk, are destroyed by pasteurization (30,44,45,46). In factories, milk for cheese is usually pasteurized, so we could do so during our experiments.

For our requirements, the bacterial count of the raw milk obtained by aseptic milking is rather high (47,33). The bacterial count of the milk from individual cows with clinically healthy udders had to be examined in order to obtain raw milk with an extremely low bacterial count. Also the bacterial flora in the aseptically drawn raw milk, the heat-resistance and growth of these bacteria had to be examined, and a method to monitor the cheese made from this milk for absence of these bacteria – in the presence of starter bacteria – had to be found.

For selection of the cows and for aseptic milking, help was given by the Milk Hygiene Research Centre at Wageningen and one of the staff of that centre was coauthor of the paper on aseptic milking of cows (Kleter & de Vries, 1974).

To prevent any contamination during cheesemaking, an aseptic cheesemaking equipment had to be designed and constructed. In particular, contamination by lactobacilli must be prevented, since they multiply to very large numbers in cheese. If the action of rennet and possibly heat-resistant milk proteases are studied without the activity of any bacterial enzymes, even an extremely small contamination with any type of micro-organism would risk bacterial growth in the cheese, as the lactose is still present. Therefore the equipment for making cheese under strictly aseptic conditions had to be completely closed and had to withstand sterilization by steam before each cheesemaking. To allow simultaneous experiments with the same milk supply, for instance with inoculation of milk with only starter bacteria and inoculation also with lactobacilli, two aseptic vats were made. The chemical properties of the cheese from the two vats, for instance pH and moisture content, had to be exactly the same at the

beginning of the ripening period.

During ripening, the cheese was monitored microbiologically and chemically, and was submitted to a panel for sensory assessment. The cheese had to be monitored for microbes for two reasons. First, to check for undesired micro-organisms whether strictly aseptic conditions were achieved. Secondly, to count micro-organisms that were being investigated, during ripening in the cheese. The cheese had to be monitored chemically, also for two reasons. First, to check the cheese for the right composition. Secondly, to monitor the ripening process. Development of the technique for making cheese strictly aseptically, including selection of cows, aseptic milking and development and construction of equipment for aseptic cheesemaking, was laborous and time-consuming. Therefore, as a first approach, chemical monitoring of cheese was by simple methods, but had to give as complete a picture of the ripening process as possible.

References

- 1. F. V. Kosikowski & G. Mocquot. Advances in cheese technology. F.A.O., Rome, 1958, p. 141.
- 2. H. Mulder. Taste and flavour forming substances in cheese. Neth. Milk Dairy J. 6 (1952) 157.
- 3. L. A. Mabbitt. The flavour of Cheddar cheese. (Review). J. Dairy Res. 28 (1961) 303.
- 4. J. Schormüller. The chemistry and biochemistry of cheese ripening. (Review). Adv. Food Res. 16 (1968) 231.
- 5. J. E. Kinsella & D. H. Hwang. Enzymes of Penicillium roqueforti involved in the biosynthesis of cheese flavor. (Review.) Crit. Rev. Food Sci. Nutr. 8 (1977) 191.
- 6. J. R. L. Walker. Some volatile compounds in New Zealand Cheddar cheese and their possible significance in flavour formation. J. Dairy Res. 28 (1961) 1.
- 7. J. Stadhouders. The hydrolysis of cheese fat in relation to the flavour of the cheese. Thesis. Agricultural University Wageningen. Mededelingen Landbouwhogeschool 56, 1956.
- A. Noomen. Proteolytic activity of milk protease in raw and pasteurized cow's milk. Neth. Milk Dairy J. 29 (1975) 153.
- 9, N. J. Berridge. Rennin and the clotting of milk. Adv. Enzymol. 15 (1954) 423.
- R. B. O'Keeffe, P. F. Fox & C. Daly. Contribution of rennet and starter proteases to proteolysis in Cheddar cheese. J. Dairy Res. 43 (1976) 97.
- 11. F. M. W. Visser. Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. III. Protein breakdown: analysis of the soluble nitrogen and amino acid nitrogen fractions, Neth. Milk Dairy J. 31 (1977) 210.
- F. M. Driessen & J. Stadhouders. Thermal activation and inactivation of exocellular lipases of some Gram-negative bacteria common in milk. Neth. Milk Dairy J. 28 (1974) 10.
- F. M. Driessen. Eiwitafbraak in stromend gesteriliseerde melk en melkprodukten. Voedingsmiddelentechnologie 9 (1976) No. 21 p. 12.
- 14. E. F. Gale. The chemical activities of bacteria. University Tutorial Press, London, 1951.
- 15. W. van Dam. Onderzoekingen over het Edammerkaasrijpingsproces. Versl. Landbouwk. Onderz. 7 (1910) 56.
- H. A. Sirks. Eenige proeven over den invloed van den reëelen zuurheidsgraad op de rijping van Edammer kaas. Versl. Landbouwk. Onderz. 49 (1943) 221.
- C. W. Raadsveld & H. Mulder. The influence of the temperature on the ripening of Edam cheese. Neth. Milk Dairy J. 3 (1949) 117.
- C. W. Raadsveld & H. Mulder. The influence of pH on the ripening of Edam cheese. Neth. Milk Dairy J. 3 (1949) 222.
- C. W. Raadsveld. Ripening phenomena in cheeses of the Edam type with different water contents and different salt concentrations. Neth. Milk Dairy J. 6 (1952) 342.
- P. J. de Koning. Papierchromatografisch onderzoek van peptiden en aminozuren geïsoleerd uit kaas.
 Neth. Milk Dairy J. 14 (1960) 34.

- J. Stadhouders. De eiwithydrolyse tijdens de kaasrijping. De enzymen die het eiwit in kaas hydrolyseren. Neth. Milk Dairy J. 14 (1960) 83.
- 22. J. Stadhouders. De eiwitasbraak in kaas gedurende de rijping. Enige methoden om de rijping te versnellen, Neth. Milk Dairy J. 15 (1961) 151.
- L. A. M. Ali & H. Mulder. Taste and flavour-forming compounds in cheese. The amino acid content of Edam cheese. Neth. Milk Dairy J. 15 (1961) 377.
- F. A. Exterkate. An introductory study of the proteolytic system of streptococcus cremoris strain HP. Neth. Milk Dairy J. 29 (1975) 303.
- F. A. Exterkate. The proteolytic system of a slow lactic-acid-producing variant of streptococcus cremoris HP. Neth. Milk Dairy J. 30 (1976) 3.
- F. A. Exterkate. Comparison of strains of Streptococcus cremoris for proteolytic activities associated with the all wall. Neth. Milk Dairy J. 30 (1976) 95.
- 27. A. Noomen. Personal communication.
- T. F. Fryer. Microflora of Cheddar cheese and its influence on cheese flavour. (Review). Dairy Sci. Abstr. 31 (1969) 471.
- 29. R. C. Lawrence, T. D. Thomas & B. E. Terzaghi. Cheese starters. (Review). J. Dairy Res. 43 (1976) 141.
- E. von Freudenreich. Ueber einen neuen, in geblähten Käsen gefundenen Bacillus (Bacillus schafferi).
 Landw. Jb. Schweiz. 4 (1890) 17.
- 31. E. von Freudenreich. Über die Erreger der Reifung der Emmenthaler Käse Landw. Jb. Schweiz. 11 (1897) 85.
- 32. F. W. J. Boekhout & J. J. Ott de Vries. Over de Edammerkaas-rijping. Landbouwk. Tijdschr. 9 (1901) 356.
- J. Stadhouders & H. A. Veringa. Fat hydrolysis by lactic acid bacteria in cheese. Neth. Milk Dairy J. 27 (1973) 77.
- 34. L. A. Mabbitt, Helen R. Chapman & M. Elisabeth Sharpe. Making Cheddar cheese on a small scale under controlled bacteriological conditions. J. Dairy Res. 26 (1959) 105.
- 35. K. D. Perry & W. A. McGillivray. The manufacture of 'normal' and 'starter-free' cheddar cheese under controlled bacteriological conditions. J. Dairy Res. 31 (1964) 155.
- Helen R. Chapman, L. A. Mabbitt & Elisabeth M. Sharpe. Apparatus and techniques for making cheese under controlled bacteriological conditions. Proc. 17th Int. Dairy Congr. (München, 1966) D-1: 55.
- B. Reiter, Y. Sorokin, A. Pickering & A. J. Hall. Hydrolysis of fat and protein in small cheeses made under aseptic conditions. J. Dairy Res. 36 (1969) 65.
- 38. J. A. Beech & D. Forbes. Pathogens in the streak canal. Vet. Rec. 77 (1965) 1086.
- 39. H. J. Heidrich, D. Grossklaus & M. Mülling. Vergleichende bakteriologische Untersuchungen an durch Handmelken und durch Venülenpunktion der Drüsenzisterne gewonnene Milchen. I. Untersuchungen an Vernülenmilchen. II. Vergleichende Untersuchungen an Handmelk- und Vernülenmilchen. Berl. Münch. tierärzti. Wschr. 77 (1964) 83.85.
- 40. W. Trautmann. Vergleichende bakteriologische Untersuchungen über die primäre und sekundäre Milchflora an durch Handmelken und durch Punktion der Drüsenzisterne gewonnene Proben. Schweiz. Landw. Forsch. 12 (1973) 125.
- 41. A. Tolle & H. Zeidler. Der kontinuierliche Milchentzug ein experimentell-chirurgisches Verfahren zur Gewinnung steriler Rohmilch, Milchwissenschaft 24 (1969) 590.
- 42. H. Grossberger. Zur experimentellen Gewinnung steriler Rohmilch über ein implantiertes Kathetersystem. Thesis. Freie Universität Berlin, Journal-Nr. 759, 1971.
- 43. R. J. M. le Rütte. Internal report. Agricultural University of Wageningen, Dept. Dairy Science and Technology.
- 44. T. Gibson & Y Abd-El-Malek. The effect of low-temperature pasteurisation on the bacterial flora of milk. Proc. Soc. agric. Bact. (1940) 19.
- 45. T. Gibson & Y. Abd-El-Malek, The micrococci of milk. Proc. Soc. agric. Bact. (1941) 31.
- 46. Y. Abd-El-Malek & A. H. Kirdany. Studies on the bacterial flora of buffalo's and cow's udder milk. Cairo University, Faculty of Agriculture. Bulletin No. 86, 1956.
- 47. J. Stadhouders & H. Mulder. Fat hydrolysis and cheese flavour. I. The enzymes responsible for the hydrolysis of fat in cheese, Neth. Milk Dairy J. 11 (1957) 164.

Aseptic milking of cows

G. Kleter¹ and Tj. de Vries²

- ¹ Dairying Laboratory, Department of Food Science, University of Agriculture, Wageningen, the Netherlands
- ² Milk Hygiene Research Centre (MOC), Wageningen, the Netherlands

Received: 7 November 1974

Summary

To study the influence of certain special bacteria upon the ripening of Gouda cheese, without the activity of all other micro-organisms, aseptic milking of cows is necessary. Since the bacterial count of the milk from individual cows with clinically healthy udders was found to vary considerably (Table 1), the cows were selected so as to obtain milk which was suitable for our purpose.

With unselected cows, we had an average bacterial count of 138 per ml. This is lower than figures taken from literature, which normally quote some hundreds per ml. Using selected cows, we were able to obtain quantities of about 100 litres of raw milk with a bacterial count which was nearly always < 100, on average 46 and sometimes even only 6 per ml.

In the raw milk no coliforms, other Gram-negative rods or lactobacilli were found. After pasteurization at 72°C for 15 s, no bacteria could be detected, even when portions of 100 ml of pasteurized milk were kept at 20°C for weeks.

1 Introduction

In order to study the influence of certain special bacteria on the ripening of Gouda cheese without being confused by the activities of other bacteria, we needed milk which was suitable for this special purpose. Other types of bacteria could influence the results by their growth in the cheese or by their enzymes which resist pasteurization or are released after their death in the cheese. A very low level of these bacterial enzymes is quite significant, since the ripening period of the cheese will extend for months.

Until about 1900 it was generally believed that milk at the time of leaving the teats is sterile (1, 2). However, Schulz (3) found that this is not true, which was soon confirmed by other workers (4). From that time it even became generally accepted that the milk in all parts of the udder of every cow contains bacteria (5, 6, 7, 8, 9, 10, 11). Therefore, it seemed rather contradictory that the bacterial counts of aseptically drawn samples of milk

were nearly always very low, especially those obtained at the end of the milking (3, 9, 12, 13). More recently it has been shown (14, 15, 16) by the comparison of samples of milk obtained by aseptic hand milking with those obtained by puncture of the cistern, that the milk in the upper parts of the udder of clinically healthy cows is, as a rule, sterile and is contaminated during milking by the typical flora of the teat canal.

In view of these findings, the best way to obtain milk for our purpose seems to be the method developed by Tolle & Zeidler (17). By means of a catheter system which is operatively implanted into the udder, the milk is obtained directly from the cistern to avoid contamination in the teat canal. However, until now the method did not prove to be as useful as it first appeared, because until some days after the operation the milk could obtain antibiotics, and soon after the udder often became infected by different types of bacteria (18). Milking by canula (19) is only suitable for small portions of milk, and the likelihood of obtaining sterile milk is small, since contamination of the canula may occur by passing through the teat canal.

Therefore, the method already used by von Freudenreich (20, 21) and Boekhout & Ott de Vries (2, 4) and later by Stadhouders & Mulder (22), remains for the moment as the only possibility. The hands of the milker and the udder of the cow are disinfected and sterilized milk equipment is used. Although, as a result of contamination of the milk in the teat canal, the milk obtained in this way is not sterile, these bacteria are reported (20, 23, 24, 25) to be killed by pasteurization. Disinfection of the teat canal before each milking has not been described before as far as we know.

In relation to our requirements mentioned above, the bacterial count of the raw milk obtained in this way is still relatively high (22), even when the cows are machine-milked (26). However, there are indications (9, 13, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37) that the rate of infection of the milk in the teat canal varies considerably from cow to cow, which was confirmed by us in a preliminary experiment. This brought us to the idea of reducing the bacterial count of aseptically drawn milk by selection of the cows.

On some occasions the milk was cooled immediately after leaving the udder. This will be discussed further in a subsequent publication.

2 Selection of the cows

2.1 Materials and methods

About 25 cows were available for the selection of the 8 to 12 which were needed to obtain about 100 litres of milk per milking. Five days prior to each aseptic milking, a sample of milk was taken from each cow about half-

G. KLETER AND TJ. DE VRIES

way through the normal milking procedure, during the phase of maximum milk flow rate. The teats of the cow and the hands of the milker then were disinfected with alcohol and a few squirts of milk were withdrawn. Then from each quarter of the udder a few jets of milk were collected in a sterile flask. A mixed sample of about 40 ml was obtained from each cow. The bacterial count was determined as described in Section 4.1.

2.2 Results and discussion

As a result of the sampling described above, data during a complete lactation period were obtained for 17 cows. All these cows had clinically healthy udders and normal milk production. The figures are given in Table 1.

Although the bacterial count of the samples during this period varied for each cow, it can be seen that the median count differs from cow to cow. Further, the lowest count for some cows is much higher than the highest count ever found for other cows. It is clear that, for our purpose, selection of the cows in the manner described is indeed valuable.

Table 1. Bacterial count per ml of milk, during a complete lactation period. Samples were taken at about half the milking process.

Cow	Number of	Median	Lowest	Highest	
	samples	count	count	count	
Α	19	15	<1	1 300	
В	21	18	1	110	
C	24	21	<1	320	
D	22	24	<1	560	
E	21	42	<1	1 100	
F	26	76	11	1 100	
G	25	130	3	680	
H	21	190	26	1 400	
I	20	210	3	2 200	
J	23	250	85	840	
K	23	290	69	1 600	
L	22	350	41	1 700	
M	22	1 100	230	300 000	
N	20	1 300	190	13 000	
0	20	1 500	550	11 000	
P	20	2 900	450	11 000	
Q	21	13 000	1 900	690 000	

Tabel 1. Kiemgetal per ml melk, gedurende een gehele lactatieperiode. De monsters werden halverwege het melken genomen.

Fig. 1. The milk vat with appendages. 1. milk vat; 2. cover; 3. rubber gasket; 4. clamp; 5. handle; 6. sight glass; 7. connection to pasteurizer; 8. cock; 9. cotton-wool filter; 10. bend (to prevent the driving back of any moisture from the vacuum pipe line); 11. vacuum tube; 12. milk tube; 13. cooling coil; 14. set of teat cups; 15. pulsator tubes.

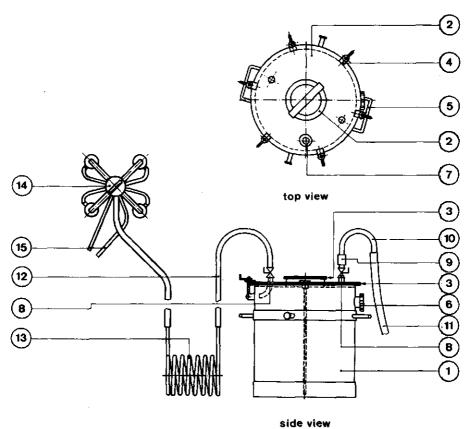


Fig. 1. Het melkvat met toebehoren. 1. melkvat; 2. deksel; 3. rubber ring; 4. knevel; 5. handvat; 6. kijkglas; 7. aansluiting naar pasteur; 8. kraan; 9. watten filter; 10. bocht (om terugslag van vocht uit de vacuümleiding te verhinderen); 11. vacuüm slang; 12. melkslang; 13. koelspiraal; 14. tepelhouders; 15. pulsatorslangetjes.

3 The milk vat

In Fig. 1 the milk vat with appendages is illustrated. It is made from stainless steel and the rubber parts are able to withstand several heat treatments as described below without any damage. Before each milking, the previously thoroughly cleaned apparatus was sterilized as follows.

G. KLETER AND TJ. DE VRIES

The set of rubber teat cups was packed in paper to prevent it from contamination after sterilization. To prevent too much condensation of steam in the apparatus, it was given a preliminary heating by steam on the outside. The sterilization itself was carried out at 120°C for 20 minutes. The amount of condensed water in the apparatus was found to be negligible.

If the milk had to be cooled immediately during milking, the cooling coil was placed in an ice-water bath.

4 Milking of the cows and the treatment of the milk

4.1 Materials and methods

The cows were machine-milked with the apparatus described above. In summer this was done in the meadow in a movable milking parlour and in winter in a clean separate room. To keep the cows quiet during disinfection of the udder and during milking, concentrates were fed.

The first jets of milk from each quarter were withdrawn. The hands of the milker and the udder of the cow were then carefully washed with disinfectant soap, and this was removed by spraying plenty of good quality tap water. After the hands of the milker and the udder of the cow were dried with sterilized towels, some jets of milk from each quarter were again withdrawn. Then the hands of the milker and the teats of the cow were disinfected again, on this occasion with cotton-wool immersed in alcohol (80%). The set of teat cups was then connected with the teats. After the cow was milked, the teat cups were taken off and connected to the teats of the following cow, which in the meantime had been treated by the second milker as described above. In this way 8 to 12 selected cows were milked to obtain 90 to 100 litres of milk.

After the vat had been transported to the laboratory, it was connected by means of a sterilized tube to a sterilized Alfa Laval plate pasteurizer through which the milk could be pumped into the cheese vats. If the milk had to be pasteurized, this was done at 72°C for 15 s. The raw as well as the pasteurized milk was brought to renetting temperature in the pasteurizer.

The bacterial quality of the raw milk was determined as follows:

Bacterial count (in duplicate): plate count agar (Difco) to which 1 g skim milk powder (Oxoid) was added per 1000 ml solution; 1 ml milk or a dilution was mixed with 10 ml of the medium; incubation 3 days at 30°C.

Coliform bacteria (in duplicate): violet red bile agar (Difco); after solidification, 10 ml of the medium inoculated with 1 ml milk or a dilution were covered with 5 ml of the medium; incubation 1 day at 30°C.

Lactobacilli (in duplicate): Rogosa SL agar (Difco); aiter solidification,

10 ml of the medium inoculated with 1 ml milk or a dilution were covered with 5 ml of the medium; incubation 5 days at 30°C.

Gram-negative rods (in quintuplicate): plate count agar (Difco) to which 1 mg crystal violet (Difco) was added per kg after sterilization (38); 0.1 ml milk or a dilution was streaked on 10 ml of dried solidified medium; some drops of glycerine were placed in the cover of the Petri dish; incubation 3 days at 30°C.

The bacteriological quality of the pasteurized milk after being pumped into the cheese vats, was determined by using plate count agar as described for the determination of the total count of raw milk, but it was now done with ten plates. In addition, 5 portions of 100 ml and 5 portions of 10 ml of pasteurized milk were incubated for some weeks at 20°C to determine the most probable number (MPN).

4.2 Results and discussion

As a result of 41 milkings over a period of about two years a total of about 3 500 litres of milk was obtained. At the beginning of the experiments unselected cows were used.

The results are given in Table 2. In the raw milk coliforms and lactobadilli were always absent from 2 ml and Gram-negative rods were always absent from 0.5 ml.

The mean bacterial count of the milkings with unselected cows was 138 per ml, which is somewhat lower than the figures found in literature, but with selected cows it was only 46 per ml. In addition, it was found that the milkings with selected cows (with one exception) had a bacterial count in the raw milk smaller than the mean of the milk from unselected cows. The milkings from unselected cows (with two exceptions) had a bacterial count larger than the mean value in the milk of selected cows.

It is clear that selection of the cows can reduce the bacterial count of the raw milk to an extremely low level, compared to the results obtained with unselected cows.

Table 2.	The	bacterial	count	per ml	of	raw,	aseptical	lly (irawn	milk.
----------	-----	-----------	-------	--------	----	------	-----------	-------	-------	-------

Cows used	Number of milkings	Mean count	Highest count	Lowest count	Median count
Unselected	11	138	170	21	110
Selected	30	46	145	6	32

Tabel 2. Het kiemgetal per ml van rauwe, aseptisch gewonnen melk.

G. KLETER AND TJ. DE VRIES

In the pasteurized milk no bacteria could be detected on plate count agar, although 10 tests with 1 ml of the undiluted milk were made every time. The 100-ml and 10-ml portions of pasteurized milk almost without exception showed no spoilage and no bacterial growth after incubation for weeks at 20°C.

When pasteurized milk was used for cheese making, the requirements as discussed in Section 1 were fulfilled by the extremely low bacterial count of the raw milk and the absence of micro-organisms in the pasteurized milk. When raw milk was used, the low bacterial count and the absence of coliforms and lactobacilli provided a hopeful start. However, in both cases bacteriological control of the cheese made from it was absolutely necessary. In a subsequent publication these aspects will be described.

Acknowledgment

The study of the influence of special bacteria upon the ripening of Gouda cheese was made possible by a grant from the Stichting J. Mesdagfonds, Kaascontrolestation 'Friesland', Leeuwarden, the Netherlands.

Samenvatting

G. Kleter en Tj. de Vries, Aseptisch melken van koeien

Voor het bestuderen van de invloed van bepaalde bacteriën op de rijping van Goudse kaas, onder uitsluiting van de activiteit van alle andere micro-organismen, is het reeds bij de melkwinning nodig om aseptisch te werken. Aangezien het kiemgetal van de melk van individuele koeien met klinisch gezonde uiers onderling in belangrijke mate bleek te verschillen (zie tabel 1), werd selectie van koeien toegepast om melk te verkrijgen welke bruikbaar was voor ons doel.

Met ongeselecteerde koeien was het kiemgetal gemiddeld 138 per ml. Dit is reeds lager dan de getallen welke in de literatuur worden vermeld, waarbij het kiemgetal meestal enkele honderden per ml is. Met geselecteerde koeien waren wij in staat om porties rauwe melk van ongeveer 100 l te winnen met een kiemgetal van bijna altijd < 100, gemiddeld 46 en soms zelfs slechts 6 per ml.

In deze melk konden geen coli-achtigen, andere Gram-negatieve staven of lacto-bacillen worden aangetoond. Na pasteurisatie op 72°C gedurende 15 s werden geen micro-organismen meer gevonden, zelfs niet nadat porties van 100 ml enkele weken bij 20°C werden bewaard.

References

- 1. J. Lister, Pharm. J. Trans. 3 (1878) 555, 572.
- 2. F. W. J. Boekhout & J. J. Ott de Vries, Landbouwk. Tijdschr. 7 (1899) 65.
- 3. L. Schulz, Arch. Hyg. 14 (1892) 260.
- 4. F. W. J. Boekhout & J. J. Ott de Vries, Landbouwk. Tijdschr. 9 (1901) 356.

- 5. A. R. Ward, J. appl. Microsc. 1 (1898) 205.
- 6. A. R. Ward, Bull. Cornell Univ. agric. Exp. Stn 178 (1900) 259.
- 7. Chr. Barthel, Rev. gén. Lait 1 (1902) 505, 529.
- 8. R. H. Burr, CentBl. Bakt. Parasitenk. InfektKrankh. II Abt. 8 (1902) 236.
- 9. E. von Freudenreich, Landw. Ib. Schweiz 17 (1903) 199.
- 10. F. C. Harrison & A. Savage, Rev. gén. Lait 9 (1912) 121.
- 11. W. Steck, Schweiz. Arch. Tierheilk. 62 (1920) 525.
- 12. H. L. Russel, 11th A. Rep. agric. Exp. Stn Univ. Wis. 1894: 150.
- 13. V. Willem & A. Minne, Rev. gén. Lait 4 (1904) 121, 145.
- 14. J. A. Beech & D. Forbes, Vet. Rec. 77 (1965) 1086.
- 15. H. J. Heidrich, D. Grossklaus & M. Mülling, Berl. Münch. tierärtztl. Wochenschr. 77 (1964) 83, 85.
- 16. W. Trautmann, Schweiz. landw. Forsch. 12 (1973) 125.
- 17. A. Tolle & H. Zeidler, Milchwissenschaft 24 (1969) 590.
- H. Grossberger, Zur experimentellen Gewinnung steriler Rohmilch über ein implantiertes Kathetersystem. Thesis, Berlin, 1971.
- 19. B. Reiter, Y. Sorokin, A. Pickering & A. J. Hall, J. Dairy Res. 36 (1969) 65.
- 20. E. von Freudenreich, Landw. Ib. Schweiz 4 (1890) 17.
- 21. E. von Freudenreich, Landw. Jb. Schweiz 11 (1897) 85.
- 22. J. Stadhouders & H. Mulder, Neth. Milk Dairy J. 11 (1957) 164.
- 23. T. Gibson & Y. Abd-El-Malek, Proc. Soc. agric. Bact. (1940) 19.
- 24. T. Gibson & Y. Abd-El-Malek, Proc. Soc. agric. Bact. (1941) 31.
- Y. Abd-El-Malek & A. H. Kirdany, Cairo University, Faculty of Agriculture, Bulletin No 86, 1956.
- 26. J. Stadhouders & H. A. Veringa, Neth. Milk Dairy J. 27 (1973) 77.
- 27. E. von Freudenreich & J. Thöni, Landw. Jb. Schweiz 17 (1903) 232.
- 28. E. von Freudenreich, Landw. Jb. Schweiz 18 (1904) 401.
- E. G. Hastings & C. Hoffmann, CentBl. Bakt. Parasitenk. InfektKrankh. II Abt. 25 (1909) 465.
- 30. R. Burri & J. Hohl, Landw. Jb. Schweiz 31 (1917) 314.
- 31. W. Steck, Schweiz. Arch. Tierheilk. 62 (1920) 525.
- 32. W. Steck, Proc. XIth Int. vet. Congr. (London, 1930).
- 33. W. Dorner, Landw. Ib. Schweiz 44 (1930) 463.
- 34. O. Bang & H. Chr. Bendixen, Z. InfektKrankh. parasit. Krankh. Hyg. Haustiere 42 (1932) 81.
- 35. R. Wayne & H. Macy, J. Dairy Sci. 16 (1933) 79.
- 36. G. A. Cullen & C. N. Hebert, Br. vet. J. 123 (1967) 14.
- 37. Branka Bacic, H. Jackson & L. F. L. Clegg, J. Dairy Sci. 51 (1968) 47.
- 38. F. M. Driessen, pers. comm., 1971.

The bacterial flora in aseptically drawn milk

G. Kleter

Dairying Laboratory, Department of Food Science, University of Agriculture, Wageningen, the Netherlands

Received: 7 November 1974

Summary

The bacterial flora in aseptically drawn mixed milk from selected cows (1) has been subjected to further study.

In 8 out of 30 milkings (1) no strict anaerobic micro-organisms could be detected. In 20 milkings only Gram-positive, catalase-positive cocci were found after examination with plate count agar containing 0.1% (w/v) skim milk powder (PCMA). After the addition of 0.5% (w/v) Tween 80 to this medium, coryneforms were also found.

Of 6 milkings 169 strains were aselectively isolated from plates with PCMA with 0.5% (w/v) Tween 80. Of these strains 45 proved to be coryneforms and 124 cocci. The cocci could grow on PCMA, PCMA + 0.5% (w/v) Tween 80, nutrient agar (NA) and NA + 0.5% (w/v) Tween 80. The coryneforms were able to grow on PCMA and NA, both with 0.5% (w/v) Tween 80, but not on PCMA and NA without Tween 80.

Of the 124 isolated cocci strains, 13 belonged to *Micrococcus* species, 106 were S:aphylococcus epidermidis and 5 Staphylococcus aureus. The 45 coryneforms isolated were all identified as Corynebacterium bovis. During their examination it was found that the C. bovis strains were able to produce acid from glucose in peptone water with Tween 80, but not in peptone water alone, which is normally used for this test.

All isolated strains were destroyed in milk by heating for 30 min at 63°C, starting with an inoculation of millions per ml. Most cocci and all the coryneforms were even killed by a heat treatment of only 5 min at 63°C. Further it was found that a heat treatment of 15 s at 60°C was sufficient to reduce the amount of coryneforms from about 1000 per ml to <1 per ml.

The cocci in the aseptically drawn milk had a lag-phase in this milk, but no bactericidic action could be detected. At 37°C the lag-phase was only a few hours. If the cocci were adapted to the milk, they had no lag-phase at all.

The coryneforms were not able to grow in aseptically drawn, pasteurized (15 s, 82°C) milk.

It is assumed that the bacterial flora present in the udder of clinically healthy cows, and which can therefore be found in aseptically drawn milk, is not in contact with the milk in the udder. After milking, these bacteria have to adapt to the milk, probably without being affected by lactenins.

1 Introduction

As already mentioned in an earlier publication (1), it is now generally ac-

cepted that the milk in the upper parts of clinically healthy udders of cows is usually sterile (2, 3, 4, 5). The theory (e.g. 6, 7) that all parts of the udder of every cow are invaded by micro-organisms and that the bactericidal or bacteriostatic action of the milk prevents the development of real mastitis, seems no longer realistic. This does not exclude the possibility that the milk may have bactericidal or bacteriostatic properties.

The intact meatus of the teat canal is reported to be an effective barrier to the invasion of bacteria. However, exposure of an injured meatus to mastitis organisms readily favours the infection. Numerous reports can be found (e.g. 8, 9, 10, 11, 12) in which this is established. Exposure of the outside of the intact teats to bacteria seldom led to mastitis, although large numbers of mastitis organisms were often used. However, after exposure of the injured meatus to bacteria and especially after injection of bacteria in the cistern, mastitis nearly always occurred. This was also found with bacteria which are not known as mastitis bacteria in the normal sense, and when only very small numbers were used. Newbould (13) has given an excellent review of the factors which affect bacterial invasion of the bovine udder via the teat duct.

The teat canal is a barrier by virtue of its construction (14) and also because of the presence of substances such as lipids (15, 16) and cationic proteins (17, 18, 19, 20), which are thought to be bacteriostatic or bactericidic. At the moment it is not yet clear which substance is really important in this respect (19, 21).

At the same time, the teat canal, particularly the lower part, harbours some special bacteria (e.g. 2, 3, 4, 5, 22, 23), which are obviously less sensitive to these substances. The number of these organisms present in the teat canal of clinically healthy udders may differ considerably. We therefore used selected cows (1) to obtain portions of about 100 litres of aseptically drawn milk with an extremely low bacterial count. In this milk no lactobacilli, coliforms or other Gram-negative rods were found with selective media. After pasteurization (72°C, 15 s) of the milk, no bacteria could be detected at all. In this publication a further study of this bacterial flora is reported.

2 Micro-organisms present in aseptically drawn milk

2.1 Literature

Research concerning the bacterial flora in cow's milk from clinically healthy udders has mainly been carried out with aseptically drawn samples of milk from one quarter or all the quarters of one cow, and to a smaller extent with larger quantities of aseptically drawn milk from one or more cows. From the

G. KLETER

early beginning (24) micrococci, streptococci and 'bacilli' were reported to be present.

Micrococci were nearly always found and very often they formed the most predominant group of organisms (e.g. 6, 7, 25, 26, 27, 28, 29, 30, 31). Although the milk was obtained from clinically healthy udders, coagulase-positive staphylococci were sometimes found in great numbers (31, 32, 33, 34).

The presence of streptococci has been reported (e.g. 2, 3, 5, 6, 7, 24, 29, 31, 35, 36, 37, 38, 39, 40, 41, 42, 43), although it was claimed that the udders were clinically normal. However, there are other reports (e.g. 25, 26, 30, 44, 45, 46, 47, 48, 49) in which it is stated that streptococci could not be detected in the milk, and that the raw milk did not become sour when kept at room temperature. In this respect the work of Seelemann & Hadenfeldt (50) is of special interest. They state that the presence of streptococci in the milk from cows with clinically normal udders is very infrequent, even after recovering from real mastitis due to streptococci. Thus the presence of streptococci in aseptically drawn milk from clinically healthy udders seems to be questionable.

Concerning what in early times were called 'bacilli', the work of Evans (51, 52, 53, 54) has to be mentioned in particular. She used lactose agar plus blood serum and found organisms which she at first called *Bacillus abortus* according to their morphology, up to 100 000 per ml. These bacteria did not grow on ordinary agar medium plus lactose. As they were able to hydrolyse butterfat she called them *Bacillus abortus* var. *lipolyticus* or *Bacterium lipolyticus*. However, these bacteria differed from *Bacterium abortus* in Gram staining and in some biochemical reactions. That is why she revised her first statement that *Bacterium abortus* is a common organism in aseptically drawn milk. Steck (55), who was also able to culture these organisms from aseptically drawn samples of milk when he used an enriched medium, at first also thought that they were *Bacterium abortus*. Black (56) has shown that *Bacterium lipolyticus* Evans is identical to *Corynebacterium bovis* as described in Bergey's Manual (57).

There are a few publications (4, 58, 59) in which the presence of other types of bacteria, such as *Escherichia coli* and *Aerobacter aerogenes*, are also reported in aseptically drawn milk from clinically healthy udders or in the teat canal itself.

2.2 Materials and methods

The milk. Of the portions of about 100 litres of raw milk obtained from

selected cows as described in an earlier publication (1) a sample was taken and 1 ml of the undiluted and 1 ml of the 1: 10 diluted milk were plated in duplicate.

The media. Plate count agar (Difco) to which 0.1% (w/v) skim milk powder (Oxoid) was added (PCMA), and nutrient agar (Difco) (NA) were used. In some experiments Tween 80 (polyoxy ethylene sorbitan mono-oleate) was added to the media. Plates were incubated for 5 days at 30°C, slopes for 1 to 6 days at 30°C.

Tests on isolated strains. All isolated strains were examined microscopically after 1, 3 and 6 days of incubation, Gram-stained according to the method of van Lochem (60), and the presence of catalase tested by adding a loopful of a 3-day old culture to H_2O_2 10% (w/v). To differentiate the micrococci from the staphylococci, the Hugh-Leifson medium was used (61) and the staphylococci were tested for coagulase (60) by the tube method with rabbit plasma (Difco). For the anaerobic incubation of the Hugh-Leifson medium, anaerobic jars were used, as we found that the use of a layer of liquid paraffin (61) to produce anaerobic conditions very often gave misleading results. The coryneforms were examined for acid production from glucose (62), nitrate reduction (63) and growth in nutrient broth (Oxoid) with 9% NaCl (64).

Reference strains. For comparison a Gram-positive and a Gram-negative strain (Sarcina lutea and Aerobacter aerogenes, respectively, obtained from Dept. of Microbiology, Wageningen), two coagulase-positive staphylococci (S. aureus, Dept. of Food Microbiology, Wageningen), two Microbacterium lacticum strains (NIZO, Ede, Nos 2201 and 2202) and two Corynebacterium bovis strains (A.T.C.C. 7715 and N.T.C.C. 3224) were used.

2.3 Results and discussion

Since the interior of the udder may be anaerobic, in a preleminary experiment with eight of thirty milkings, the total count with PCMA was also determined by incubating the plates under anaerobic conditions. In such conditions the number of colonies developed was always lower than under aerobic conditions, sometimes even considerably, for example 17 and 75, respectively. Of the colonies developed under anaerobic conditions all the colonies of the plates, up to 50 per milking, were picked off and inoculated on slopes with PCMA. All were able to grow on these slopes under aerobic conditions. That is why the anaerobic incubation of the plates was no longer continued.

With twenty milkings, the total count determined with PCMA was compared with the amount of colonies developing in NA. This was done to see whether it was possible to monitor the cheese, which was to be made from

this milk, for the absence of these bacteria in the presence of starter strepto-cocci which will not grow in NA. It was found that the amount of colonies developed in PCMA was always almost the same as in NA. Further, with seven milkings all the colonies of the plates with PCMA, up to 50 per milking, were picked off and inoculated on slopes with PCMA and NA. Although in general the growth on slopes with PCMA was better than on slopes with NA, all colonies were able to grow on NA.

Microscopic examination of the colonies developed in PCMA, showed that only cocci were present. With reference to what is discussed in Section 2.1, this might be due to the fact that PCMA is too poor for the growth of Corynebacterium bovis, an organism which can be expected to be present in this type of milk. The addition of blood serum to the medium, as Evans did (51, 52, 53, 54) to favour the growth of what she called Bacterium lipolyticus, is cumbersome as it has to be added separately just before pouring the plates after being sterilized by filtration. Further we found that the growth of the two C. bovis strains A.T.C.C. 7715 and N.T.C.C. 3224, even on such a medium was poor.

Fleming (65) had discovered that the growth of a *Corynebacterium* species (C. acnes) was greatly favoured by the addition of oleic acid. Cohen et al. (66) found that oleic acid was a growth factor for C. diphtheriae, and Pollock et al. (67), who showed that diphtheroids from the skin required oleic acid, were able to replace this by Tween 80. Since Cobb (68) found that Tween 80 also favours the growth of C. bovis, which was confirmed by others (64, 69, 70, 71, 72), we compared for six milkings the number of colonies developed on PCMA with those on PCMA + 0.5% (w/v) Tween 80, which can be added before sterilization of the medium. The number of colonies developed in PCMA + Tween 80 was always higher than in PCMA.

Further, all colonies developed in PCMA, up to 50 per milking, were picked off and inoculated on slopes with PCMA and PCMA + 0.5% (w/v) Tween 80. All were able to grow on both media and microscopic examination showed that they were cocci.

From the plates with PCMA + Tween 80, all the colonies up to 50 per milking were picked off and inoculated on slopes with PCMA, PCMA + 0.5% (w/v) Tween 80, N.A. and N.A. + 0.5% (w/v) Tween 80. In total, 169 colonies, of which 124 were cocci and 45 were coryneforms, were examined. All the cocci were able to grow on both media with or without Tween 80. However, for the growth of all the coryneforms, the addition of Tween 80 to the medium was absolutely necessary. They were all even able to grow on NA, which is a relatively poor medium, after the addition of Tween 80.

In general, the cocci were found to grow better on PCMA than on NA, mostly without effect due to the addition of 0.5% (w/v) Tween 80. The coryneforms grew better on PCMA + Tween 80 than on NA + Tween 80.

In addition, the growth of the isolated cocci and coryneforms on slopes with PCMA and NA, both with different percentages of Tween 80, was examined. The proportions of Tween 80 used varied from 0 to 1.0% (w/v). It was found that this had almost no influence on the growth of the cocci. Some cocci only were inhibited to a small extent at 0.5 to 1.0% (w/v). The coryneforms were very uniform in this respect. The more Tween 80 was added, the better was the growth.

It is clear that the addition of Tween 80 to PCMA has advantages in the determination of the bacterial count of aseptically drawn milk. Further, to monitor the absence of the bacterial flora of aseptically drawn milk in cheese made from this milk with starter streptococci, the use of NA plus Tween 80 is to be preferred to NA.

The 124 cocci and 45 coryneforms isolated with PCMA + 0.5% (w/v) Tween 80 from six milkings were submitted to the tests described in Sec. 2.2.

The cocci and coryneforms all grew well under aerobic conditions on slopes with PCMA or NA both with 0.5% (w/v) Tween 80, were non-motile, Gram-positive and catalase-positive. The cocci were often present in irregular masses, sometimes singly or in pairs. The coryneforms were granulated rods (about 0.5 by 3 μ m) with an irregular shape. They did not show the formation of cocci, a phenomenon found in *Arthrobacter*.

Of the cocci 13 strains were found to be *Micrococcus* and 111 strains *Staphylococcus*. As there is so much disagreement in the determination of *Micrococcus* species (32, 61, 73, 74, 75, 76), no further identification of the *Micrococcus* strains was carried out. Of the *Staphylococcus* strains 106 were found to be *Staphylococcus* epidermidis (coagulase-negative) and only 5 strains *Staphylococcus* aureus (coagulase-positive).

To test the coryneform strains for the formation of acid from glucose, the use of peptone water as a basic medium can give different results from peptone water plus blood serum (68, 71, 77, 78) or Tween 80 (78, 79). That is why we used the following three media for this test: peptone water + 0.5% glucose, peptone water + 0.1% Tween 80 + 0.5% glucose, and peptone water + 0.1% Tween 80.

In peptone water + 0.5% glucose, there was neither growth nor acid production. In peptone water + 0.1% Tween 80, all strains were able to grow, but there was no acid production. However, in peptone water + 0.1% Tween 80 + 0.5% glucose, all strains were able to grow with the production of acid.

G. KLETER

As there was no growth in peptone water + 0.5% glucose, the use of plain peptone water as a basic medium is of limited value in the determination of these bacteria. With the addition of 0.5% Tween 80, the acid production from glucose was found to be even more pronounced than with 0.1%. However, peptone water + 0.5%. Tween 80 also showed a very slight acid reaction after the growth of the coryneforms. As for the determination of bacteria the test up till now is normally done in plain peptone water as a basic medium, the results obtained with this medium are used by us for the determination of the coryneforms.

All the coryneform strains grew in broth with 9% NaCl and were unable to produce nitrite from nitrate. They were thermolabile as described in Sections 1 and 4.3.

On the basis of these results, the 45 coryneform strains were identified as Corynebacterium bovis.

3 Thermoresistance of the bacteria

3.1 Literature

There are several reports in which it is stated that the bacterial flora from aseptically drawn milk is destroyed by normal pasteurization treatment. The experiments were done with aseptically drawn mixed milk (80, 81), aseptically drawn samples of milk from individual cows or quarters (30, 48, 82, 83) or strains isolated from aseptically drawn milk (32, 53, 64, 84, 85).

In other reports (86, 87, 88, 89, 90, 91) it is concluded that the udder is a source of thermoduric bacteria. This is mainly based on the comparison of the bacterial flora from aseptically drawn milk with that from pasteurized market milk by the usable determination methods. However, the thermoresistance itself was no part of the determination. The reports of Shah et al. (92) and Jacquet et al. (93) in which it is also concluded that the bacterial flora in aseptically drawn milk will not be destroyed by pasteurization, are also doubtful, as their method of examination is vague.

The findings of Bryan et al. (94) give some indication that under special circumstances the udder can be a temporary source of thermoduric bacteria.

3.2 Materials and methods

3.2.1 Determination with the tube method

The method used is a modification of the method Abd-El-Malek & Gibson (95).

Strains. The 126 micrococci and 45 coryneforms isolated from aseptically drawn milk (Section 2.3) and, for comparison, some micrococci isolated from market milk and two *Microbacterium lacticum* strains (Nizo, Ede, Nr. 2201 and 2202) were used.

Pre-incubation. A loopful of a three-day old culture on slopes with plate count agar (Difco) plus 0.1% (w/v) skim milk powder (Oxoid) and 0.5% (w/v) Tween 80, was brought into 20-ml tubes containing 10 ml broth of the same composition without the agar. The tubes were incubated for 3 days at 30° C and then used to inoculate the heating medium. All strains showed good growth in the broth.

Heating medium. Sterile tubes (20 ml) with screw caps were used to contain 10 ml of aseptically drawn (1), pasteurized (72°C, 15 s) milk and completely immersed in a bath with alcohol (96%) at 63°C for 30 min. The tubes were then cooled and kept at 30°C for several days. They proved to be sterile.

Heat treatment. Two tubes with milk were inoculated with each 1 ml of the culture, so that the milk contained some millions of the organism per ml. One of the tubes was completely immersed in the bath with alcohol (96%) at 63°C for a certain time. The time necessary for warming up was found to be 2.5 min and was subtracted from the total time of heating in the bath. After heating, the tubes were immediately placed in ice water for rapid cooling.

Control for thermoresistance. 0.2 ml of the heated and of the unheated tubes were each inoculated in 20-ml tubes with 10 ml of the broth described above and incubated for 3 days at 30°C. They were then checked for growth.

3.2.2 Determination with a plate pasteurizer

As can be seen in Section 3.3, the coryneforms isolated from aseptically drawn milk (Section 2.3) all were killed after heating for only 5 min at 63°C. To obtain more information concerning the thermolability of these bacteria, three of the coryneform strains were further examined with the aid of a plate pasteurizer.

Pre-incubation. This was done as described in Section 3.2.1.

Heating medium. Aseptically drawn (1), pasteurized (72°C, 15 s) milk, which was found to be sterile, was used.

Table 1. Thermoresistance of 1	Table 1. Thermoresistance of bacteria isolated from aseptically drawn milk and of some other strains, determined by heating in tubes.	drawn milk and	of some	other stra	ins, deter	rmined by	y heating	in tubes.
Strain	Source	Number of Number of strains surviving 63°C for	Number	of strain	s survivir	1g 63°C f	JO.	
		strains	5 min	5 min 10 min 15 min 20 min 25 min 30 min	15 min	20 min	25 min	30 min
Micrococcus sp.	aseptically drawn milk	13	0	0	1	1	1	ļ
Staphylococcus aureus	aseptically drawn milk	5	0	0	J	1		1
Staphylococcus epidermidis	aseptically drawn milk	106	20	47	43	17		0
Corynebacterium bovis	aseptically drawn milk	45	0	0	ŀ	1	I	1
Micrococci	market milk	9	4	ec	6	ĸ	m	e
Staphylococcus aureus	Dept. Food Microbiol.,	2	1	0	0	1	1	I
Microbacterium lacticum	Wageningen NIZO, Ede	2	2	2	2	7	7	2

THE BACTERIAL FLORA IN ASEPTICALLY DRAWN MILK

Heat treatment. The milk was inoculated with about 1000 bacteria of a strain per ml and heated with a sterilized plate pasteurizer. The holding time was 15 s. The temperatures used ranged from 56 to 65°C.

Control for thermoresistance. 1 ml milk or a dilution was plated in duplicate before and after heating, with plate count agar (Difco) plus 0.1% (w/v) skim milk powder (Oxoid) and 0.5% (w/v) Tween 80.

3.3 Results and discussion

Tables 1 and 2 give the results of the experiments.

All the strains isolated from aseptically drawn milk, proved to be thermolabile, unlike certain micrococci isolated from market milk and the two *Microbacterium lacticum* strains. Most of the micrococci and all of the coryneforms isolated from aseptically drawn milk were even killed after exposure for only 5 min at 63°C, although the number of bacteria before heating was millions per ml. Further, 15 s at only 60°C was found to be effective in reducing the amount of approximately 1000 coryneforms per ml to less than 1 per ml.

Table 2. Thermoresistance of three Corynebacterium bovis strains isolated from aseptically drawn milk, determined with a plate pasteurizer.

Strain	Bacteria per ml	Bacteria	a per ml a	ifter heat	ing 15 s at	
	before heating	56°C	58°C	59°C	60°C	65°C
B - 3	1 000	_	_	80	< 1	< 1
B - 4	700	145		—	< 1	< 1
B - 36	800	_	75	—	< 1	< 1

4 Growth of the bacteria in milk

4.1 Literature

Fokker (96) had found that lactic acid bacteria grew better in sterilized milk than in fresh, aseptically drawn milk. He concluded that the fresh milk possessed bactericidic properties. This type of experiment, in which mostly streptococci were used, has been carried out by many other workers with the same result (e.g. 7, 97, 98), and the presence of lactenins in milk has had a lot of attention (e.g. 99, 100, 101, 102, 103, 104, 105, 106, 107, 108).

G. KLETER

There are also reports (e.g. 109, 110, 111, 112) in which it is stated that fresh milk is not bacteriostatic or bactericidic at all, not even towards streptococci.

In this respect it must be mentioned that heating, used to destroy lactenins, may also make the medium more favourable to the growth of bacteria, for example by the development of stimulating substances. Further, the circumstances under which the experiments are done, such as the medium used for pre-incubation, growth-phase, are of great importance for the results of the experiments (110, 111, 112, 113).

As aseptically drawn milk can be made sterile by pasteurization at very low temperatures (Section 3.3), which will not destroy lactenins, this type of milk is very suitable for doing experiments in this field. Further we were interested in the growth of the bacterial flora of aseptically drawn milk, as cooling of the milk at the time of milking was found to be necessary when it was to be used for making cheese from raw milk under aseptic conditions.

4.2 Materials and methods

The milk. The milk was drawn aseptically as described in a preceding publication (1). The milk used in these experiments proved to be sterile after heating at 63°C for 15 s.

Strains. The strains used were isolated from aseptically drawn milk.

Agar medium. Plate count agar (Difco) containing 0.1% (w/v) skim milk powder (Oxoid) and 0.5% (w/v) Tween 80.

Pre-incubation of the strains. The strains were pre-incubated in broth with the same composition as described above for the agar medium. The incubation temperature was 30°C. Only cultures which were in the logarithmic growth-phase were used for inoculating the medium.

Growth medium. If the growth of the total bacterial flora of aseptically drawn milk had to be studied, the raw milk itself was incubated immediately after drawing. About 50 ml of milk was brought into a 100-ml sterile flask and kept at the required temperature in a water bath. If strains were used, pasteurized (63°C or 82°C, 15 s) aseptically drawn milk was used as a growth medium. 50 ml of the milk was brought into a sterile 100-ml flask, inoculated and kept at 30°C in a water bath.

4.3 Results and discussion

The results of two of the experiments in which the growth of the total bacterial flora from aseptically drawn milk was studied by incubating the raw milk at different temperatures, are given in Tables 3 and 4.

At every temperature a lag-phase was found. At 37°C the lag-phase was only a few hours, while at 4°C, for example, no growth could be detected even after two days.

Microscopic examination, Gram staining and catalase test revealed that in fresh aseptically drawn milk micrococci and coryneforms were present. After incubation until the bacterial count was over 1000 per ml, only micrococci could be detected. After inoculation of isolated coryneforms in aseptically

Table 3. Growth of the bacterial flora in aseptically drawn raw milk, kept at different temperatures. The initial bacterial count was 18 per ml.

Incubation	Bacteria	al count per	ml after incu	bation at		
time (h)	45°C	37°C	30°C	25°C	15°C	4°C
1.5	11	18	15	14	10	16
3.5	4	20	15	16	13	18
5.0	3	1400	22	20	22	15
6.0	4	_	38	20	16	15
7.5	6		1500	22	11	18
8.5	8			18	12	15
12.0	_			33	15	15
24.0	_		_	5.105	29	17
48.0				-	6-101	20

Table 4. Growth of the bacterial flora in aseptically drawn raw milk, kept at different temperatures. The initial bacterial count was 12 per ml.

Incubation	Bacteria	l count per	ml after incu	bation at		
time (h)	45°C	37°C	30°C	25°C	15°C	4°C
1.5	10	21	30	20	18	21
3.0	12	24	24	15	_	
4.0	15	110	40	_	-	_
6.0	11	3000	46	—		_
7.5		_	3500	20	17	
9.0			_	20	24	
12.0	_	_		57	28	20
24.0	_	_	_	5.104	80	24
48.0				_	7-101	24

drawn milk pasteurized at 82°C for 15 s, it was found that they were unable to grow in this milk when it was kept at 30°C for several days.

For comparison, the aseptically drawn milk used (Table 4) was also incubated at 37°C and 30°C under anaerobic conditions. After 24 h the bacterial count was respectively 5 · 10⁶ and 2 · 10⁶ per ml. Thus the possible anaerobic condition in the udder of a cow is not able to prevent these bacteria from growing in the milk in the udder.

Since at every temperature a lag phase was found, the presence of lactenins might have been the cause, and might also be responsible for preventing the growth of these bacteria in the milk in the udder of a cow. Another possibility could be that the bacteria are indeed located in the teat canal (see Section 1) and are not in contact with the milk in the udder, so that they have to adapt to the milk. To obtain more information about this, some experiments were done with micrococci isolated from aseptically drawn milk. The results are given in Figs 1 and 2.

It can be seen in Fig. 1 (*Micrococcus* strain 3006 P) that the initial count had no influence upon the length of the lag phase in low-temperature pasteurized milk. The difference in lag-phase between H-milk (82°C, 15 s) and L-milk (63°C, 15 s) was about 5 h. When H-milk was inoculated with bacteria growing in H-milk (A) or L-milk (B), no lag-phase was found (H' and H''). When L-milk was inoculated with bacteria growing in H-milk (A), a

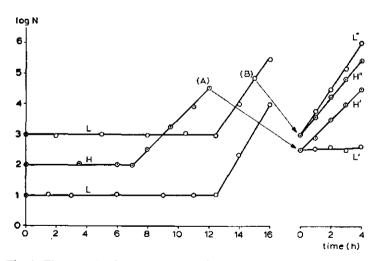


Fig. 1. The growth of *Micrococcus* strain 3006 P in aseptically drawn milk, pasteurized for 15 s at 63° C (L) or 82° C (H). The milk used was always from the same milking. N = number of micro-organisms. Incubation temperature was 30° C.

THE BACTERIAL FLORA IN ASEPTICALLY DRAWN MILK

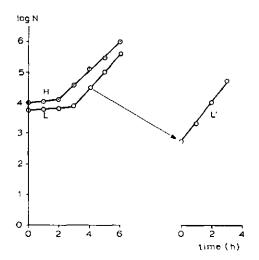


Fig. 2. The growth of Staphylococcus strain 1011 in aseptically drawn milk, pasteurized for 15 s at 63°C (L) or 82°C (H). The milk used was always from the same milking.

N = number of micro-organisms. Incubation temperature was 30°C.

lag-phase was found (L'). However, when L-milk was inoculated with bacteria already growing in L-milk (B), no lag-phase was found (L").

In Fig. 2 the growth of *Staphylococcus* strain 1011 is shown. Again there was a difference in lag-phase between H-milk and L-milk, but now it was only about 1 h. When L-milk was inoculated with bacteria already growing in L-milk, again no lag-phase was found (L').

If in the foregoing experiments the lag-phase of the bacterial flora in aseptically drawn milk and the difference in lag-phase between H-milk and L-milk was caused by lactenins, the bacteria are obviously able to adapt to the lactenins, a phenomenon with which we are unacquainted. Further, the difference in lag-phase between L-milk and H-milk in Fig. 2 of only 1 h does not support the theory of the activity of lactenins. In addition to this, no bactericidic activity was found.

The results found could be explained as follows.

- The bacterial flora in aseptically drawn milk consists of bacteria coming from the teat canal. They have to adapt to the growth substances of the milk after milking. The theory that in clinically healthy udders the milk is sterile and is first infected in the teat canal during milking, might also explain why we were able to obtain raw milk with an extremely low bacterial count (1). If the bacteria were already in contact with the milk in the udder, they could easily grow out to large numbers in the udder, after once becoming adapted to the milk (Figs 1 and 2).

- The difference in lag-phase between milks pasteurized at low and high temperature is due to a difference in growth substances.

In view of the results from Tables 3 and 4 it is clear that cooling of the aseptically drawn milk is necessary when the raw milk has to be maintained for some hours without any growth of its bacterial flora.

Acknowledgments

The aseptic milking was done in co-operation with the Milk Hygiene Research Centre (MOC), Wageningen. The experiments were made possible by the assistance of A. L. H. Daemen and W. L. Lammers.

Samenvatting

G. Kleter, De bacterieflora in aseptisch gewonnen melk.

De bacterieflora in aseptisch gewonnen mengmelk van geselecteerde koeien (1) is verder bestudeerd.

In 8 van de 30 melkmalen (1) konden geen strict anaerobe micro-organismen worden cangetoond. In 20 melkmalen werden na onderzoek met behulp van plate count agar met 0,1% (w/v) ondermelkpoeder (PCMA) alleen Gram-positieve, katalase-positieve coccen gevonden. Na toevoegen van 0,5% (w/v) Tween 80 aan dit medium werden ook coryne-achtigen gevonden.

Uit 6 melkmalen werden op aselecte wijze 169 stammen geïsoleerd vanaf platen met PCMA met 0,5% (w/v) Tween 80. Hiervan bleken er 45 bij de coryne-achtigen en 124 bij de coccen ingedeeld te moeten worden. De coccen waren in staat om te groeien op PCMA, PCMA met 0,5% (w/v) Tween 80, nutrient agar (NA) en NA met 0,5% (w/v) Tween 80. De coryne-achtigen waren in staat om te groeien op PCMA en NA, beide met 0,5% (w/v) Tween 80, maar niet op deze voedingsbodems zonder Tween 80.

Van de 124 geïsoleerde coccenstammen behoorden 13 tot Micrococcus-soorten, 106 waren Staphylococcus epidermidis en 5 waren Staphylococcus aureus. De 45 geïsoleerde coryne-achtigen werden alle geïdentificeerd als Corynebacterium bovis. Gedurende de determinatie bleek dat de Corynebacterium bovis-stammen in staat waren om zuur uit glucose te vormen in pepton-water met Tween 80, maar niet in pepton-water alleen, wat normaliter wordt gebruikt voor deze test.

Alle geïsoleerde stammen werden in melk vernietigd door een verhitting van 30 min bij 63°C, waarbij vóór de verhitting geënt werd met miljoenen bacteriën per ml. De meeste coccen en alle coryne-achtigen werden zelfs gedood door een hittebehandeling van slechts 5 min bij 63°C. Voorts werd gevonden dat een hittebehandeling van 15 s op 60°C reeds voldoende was om het aantal coryne-achtigen van ca. 1000 per ml melk terug te brengen tot <1 per ml.

De in aseptisch gewonnen melk aanwezige coccen vertoonden een lag-phase bij bewaren van deze melk, maar van een bactericide werking was geen sprake. Bij 37°C was de lag-phase slechts enkele uren. Als deze coccen aan de melk aangepast waren, vertoonden ze zelfs helemaal geen lag-phase meer.

De coryne-achtigen bleken niet in staat om te groeien in aseptisch gewonnen, gepasteuriseerde (82°C, 15 s) melk.

Er wordt verondersteld, dat de bacterieflora, die in de uier van gezonde koeien aan-

THE BACTERIAL FLORA IN ASEPTICALLY DRAWN MILK

wezig is en die dientengevolge in aseptisch gewonnen melk aangetroffen wordt, in de uier niet in contact is met de melk. Na de melkwinning moeten deze bacteriën zich aan de melk aanpassen, lacteninen spelen daarbij waarschijnlijk geen rol.

References

- 1. G. Kleter and Tj. de Vries, Neth. Milk Dairy J. 28 (1974) 212.
- H. J. Heidrich, D. Grossklaus & M. Mülling, Berl. Münch. tierärztl. Wschr. 77 (1964) 83.
- H. J. Heidrich, M. Mülling & H. W. Birkner, Berl. Münch. tierärztl. Wschr. 77 (1964) 85.
- 4. J. A. Beech & D. Forbes, Vet. Rec. 77 (1965) 1086.
- 5. W. Trautmann, Schweiz. landw. Forsch. 12 (1973) 125.
- 6. W. Steck, Schweiz. Arch. Tierheilk. 62 (1920) 525.
- 7. W. Steck, Landw. Jb. Schweiz 35 (1921) 511.
- 8. M. Seelemann & K. Siemonsen, Arch. wiss. prakt. Tierheilk. 64 (1932) 457.
- 9. M. Klimmer & H. Haupt, Tierärztl. Rundschau 38 (1932) 828.
- 10. M. Christiansen & F. Nielsen, 12th Int. vet. Congr. 1934, II; 548.
- 11. J. M. Murphy, Corn. Vet. 49 (1959) 411.
- 12. F. H. S. Newbould, Proc. 6th Int. Conf. Cattle Dis. (Philadelphia, 1970): 9.
- 13. F. H. S. Newbould, Dairy Sci. Abstr. 26 (1064) 245.
- W. H. Giesecke, W. H. Gerneke & I. B. J. van Rensburg, J. S. Afr. vet. Ass. 43 (1972) 351.
- 15. E. W. Adams, C. G. Rickard & J. M. Murphy, Corn. Vet. 51 (1961) 124.
- 16. E. W. Adams & C. G. Rickard, Am. J. vet. Res. 24 (1963) 122.
- 17. K. G. Hibbitt & C. B. Cole, Biochem. J. 106 (1968) 39 P.
- 18. K. G. Hibbitt, C. B. Cole & B. Reiter, J. gen. Microbiol. 56 (1969) 365.
- 19. K. G. Hibbitt, Proc. 6th Int. Conf. Cattle Dis. (Philadelphia, 1970): 122.
- 20. K. G. Hibbitt & Margaret Benians, J. gen. Microbiol. 68 (1971) 123.
- 21. J. M. Treece, G. E. Morse & Carol Levy, J. Dairy Sci. 49 (1966) 1240.
- 22. J. Simon, Über Bakterien am und im Kuh-Euter, Thesis, Erlangen, 1898.
- 23. R. T. Black, R. T. Marshall & C. T. Bourland, J. Dairy Sci. 55 (1972) 413.
- 24. H. L. Bolley, CentBl. Bakt. Parasitenk., II Abt. 1 (1895) 795.
- 25. W. Kuntze, CentBl. Bakt. Parasitenk. InfektKrankh., II Abt. 18 (1907) 509 (Ref.).
- 26. W. Kuntze, CentBl. Bakt. Parasitenk. InfektKrankh. II Abt., 20 (1908) 420.
- L. Copeland & T. M. Olson, The bacterial flora of the normal cow's udder, Bull. Dairy Dep. Agric. Exp. Stn S. Dakota St. Coll. 218 (1926).
- Alice F. Breed, Micrococci present in the normal cow's udder, Tech. Bull. N. Y. St. agric. Exp. Stn 132 (1928).
- 29. W. Dorner, Landw. Jb. Schweiz 44 (1930) 463.
- 30. T. Gibson & Y. Abd-El-Malek, Proc. Soc. agric. Bact. (1941) 31 (Abstr.)
- 31. D. Todorov, Dairy Sci. Abstr. 32 (1970) 115, Abstr. 730.
- 32. Y. Abd-El-Malek & T. Gibson, J. Dairy Res. 15 (1948) 249.
- 33. S. Tarkiewicz, Dairy Sci. Abstr. 31 (1969) 206, Abstr. 1389.
- 34. K. Nolting, Dairy Sci. Abstr. 32 (1970) 123, Abstr. 780.
- 35. W. G. Savage, J. Hyg. 6 (1906) 123.
- E. G. Hastings & C. Hoffmann, CentBl. Bakt. Parasitenk. InfektKrankh. II Abs. 25 (1909) 465.
- 37. J. M. Sherman, A. Rep. Pa St. Coll. 1914/15: 303.
- 38. S. H. Ayers & C. S. Mudge, J. infect. Dis. 31 (1922) 40.
- 39. W. Steck, CentBl. Bakt. Parasitenk. InfektKrankh. I Abt. 90 (1923) 61.
- 40. H. Chr. Bendixen, Acta path. microbiol. scand. Suppl. XI (1932) 187.

G. KLETER

- 41. H. Chr. Bendixen, Z. InfektKrankh. parasit. Krankh. Hyg. Haustiere 43 (1933) 106
- 42. R. H. Leitsch, Scott. J. Agric. 17 (1934) 293.
- 43. N. R. Knowles, J. Dairy Res. 7 (1936) 63.
- 44. Chr. Bartel, Rev. gén. Lait 1 (1902) 505, 529.
- 45. E. von Freudenreich, Landw. Jb. Schweiz 16 (1902) 91.
- 46. E. von Freudenreich & J. Thöni, Landw. Jb. Schweiz 17 (1903) 232.
- 47. V. Willem & A. Miele, Rev. gén. Lait 4 (1905) 409.
- 48. T. Gibson & Y. Abd-El-Malek, Proc. Soc. agric. Bact. 1940, 19.
- 49. J. Stadhouders, The hydrolysis of cheese fat in relation to the flavour of cheese. Thesis, Wageningen, 1956.
- 50. H. Seelemann & A. Hadenfeldt, Arch. wiss. prakt. Tierheilk. 64 (1932) 1.
- 51. Alice C. Evans, J. Wash. Ac. Sci. 5 (1915) 122.
- 52. Alice C. Evans, J. infect. Dis. 18 (1916) 437.
- 53. Alice C. Evans, J. Bact. 2 (1917) 185.
- 54. Alice C. Evans, J. infect. Dis. 23 (1918) 354.
- 55. W. Steck, Schweiz. Arch. Tierheilk. 60 (1918) 547.
- 56. L. A. Black, J. Bact. 41 (1941) 99.
- 57. R. S. Breed, E. G. D. Murray & N. R. Smith, Bergey's manual of determinative bacteriology, 1957.
- 58. E. Zschokke, Schweiz. Arch. Tierheilk. 39 (1897) 145.
- 59. C. A. Guerrero & R. Marta Rivas, Dairy Sci. Abstr. 27 (1965) 549, Abstr. 3562.
- 60. E. E. van de Gehuchte, Mikrobiologie praktikum. Vyncke, 1965.
- B. M. Gibbs and F. A. Skinner, Identification methods for microbiologists, Part A. London/New York, 1966; p. 59: A.C. Baird-Parker, Methods for classifying staphylococci and micrococci.
- 62. P. F. Swartling, J. Dairy Res. 18 (1951) 256.
- 63. P. H. Clarke & S. T. Cowan, J. gen. Microbiol. 6 (1952) 187.
- 64. D. J. Jayne-Williams & T. M. Skerman, J. appl. Bact. 29 (1966) 72.
- 65. A. Fleming, Lancet (1909) 1035.
- 66. S. Cohen, J. C. Snijder & J. H. Mueller, J. Bact. 41 (1941) 581.
- 67. M. R. Pollock, S. D. Wainwright & E. E. D. Manson, J. Path. Bact. 61 (1949) 274.
- 68. R. W. Cobb, J. med. Lab. Technol. 20 (1963) 199.
- 69. F. H. S. Newbould, Can. J. Microbiol. 11 (1965) 602.
- 70. T. M. Skerman & D. J. Jayne-Williams, J. appl. Bact. 29 (1966) 167.
- 71. W. F. Harrigan, J. appl. Bact. 29 (1966) 380.
- 72. J. D. Phillips & M. M. Taylor, Br. vet. J. 126 (1970) 210.
- 73. D. A. A. Mossel, J. Bact. 84 (1962) 1140.
- 74. H. Aspergen, Oest. Milchw., wiss. Beil. 20 (1965) 23.
- 75. A. C. Baird-Parker, J. gen. Microbiol. 38 (1965) 363.
- 76. A. C. Baird-Parker, Int. J. syst. Bacteriol. 21 (1971) 61.
- 77. R. W. Cobb & J. K. Walley, Vet. Rec. 74 (1962) 101.
- 78. R. W. Cobb, Vet. Rec. 78 (1966) 33.
- 79. G. Rinzk & P. Janetschke, Monatsh. VetMed. 27 (1972) 470.
- 80. E. von Freudenreich, Landw. Jb. Schweiz 4 (1890) 17.
- 81. E. von Freudenreich, Landw. Jb. Schweiz 5 (1891) 16.
- 82. A. N. Myke & J. C. Olson, J. Dairy Sci. 35 (1952) 484 (Abstr.)
- 83. Y. Abd-El-Malek & A. H. Kirdany, Bull. Cairo Univ. Fac. Agric. 86 (1956).
- 84. V. A. Moore, A. Rep. U.S. Dep. Agric. Ind. 12/13 (1897) 261.
- 85. Y. Abd-El-Malek & T. Gibson, J. Dairy Res. 19 (1952) 153.
- 86. J. L. Hileman, J. Dairy Sci. 23 (1940) 1143.
- 87. J. L. Hileman, H. Leber & M. L. Speck, J. Dairy Sci. 24 (1941) 305.
- 88. F. W. Fabian, J. Milk Technol. 5 (1942) 237.
- 89. W. D. Dotterer, J. Milk Technol. 6 (1943) 269.

THE BACTERIAL FLORA IN ASEPTICALLY DRAWN MILK

- 90. F. W. Fabian, J. Milk Technol. 9 (1946) 273.
- 91. C. Agenjo, Proc. 13th int. Dairy Congr. (Den Haag, 1953) II; 153.
- 92. S. Z. A. Shah & L. Laxminarayana, Indian J. Dairy Sci. 2 (1949) 89.
- 93. J. Jacquet, R. Richou, Lottie Steeg, P. Julienne & C. Gerbaux, C.-r. Séanc. Acad. Sci. 234 (1952) 255.
- 94. C. S. Bryan, H. S. Bryan & K. Mason, Milk Plant Mon. 35 (1946) (8): 30.
- 95. Y. Abd-El-Malek & R. Gibson, J. Dairy Res. 15 (1948) 233.
- 96. A. P. Fokker, Z. Hyg. 9 (1890) 41.
- 97. W. Rullmann, Arch. Hyg. 73 (1911) 81.
- 98. F. S. Jones & R. B. Little, J. exp. Med. 45 (1927) 319.
- 99. F. S. Jones & H. S. Simms, J. exp. Med. 51 (1930) 327.
- 100. J. E. Auclair & A. Hirsch, J. Dairy Res. 20 (1953) 45.
- 101. J. E. Auclair & N. J. Berridge, J. Dairy Res. 20 (1953) 370.
- 102. J. E. Auclair, J. Dairy Res. 21 (1954) 323.
- 103. R. C. Wright & J. Tramer, J. Dairy Res. 25 (1958) 104.
- 104. J. E. Auclair & A. Portmann, Proc. 15th int. Dairy Congr. (London, 1959) 2; 580.
- 105. N. Molin, Proc. 4th int. Symp. Fd Microbiol. (Stockholm, 1964).
- 106. J. Stadhouders, Neth. Milk Dairy J. 17 (1963) 96.
- 107. D. Hogg & G. R. Jago, Biochem. J. 117 (1970) 779, 791.
- 108. J. Stadhouders & G. Hup, Neth. Milk Dairy J. 24 (1970) 79.
- 109. F. Basenau, Arch. Hyg. 23 (1895) 44.
- H. Graber, Die Bedeutung der Euterinfektion und der Melkkontamination für die Keimzahl und die Haltbarkeit der Milch. Thesis, Schaffhausen, 1954.
- 111. V. Walser, Untersuchungen über die bacterizide Wirkung roher und keimfreier Frischmilch. Thesis, Schaffhausen, 1964.
- K. Meeder, Versuche über die bacterizide Wirkung von Rohmilch aus gesunden, nicht infizierten Eutern. Thesis, München, 1964.
- 113. F. S. Jones & H. S. Simms, J. exp. Med. 50 (1929) 279.

Apparatus for making cheese under strict aseptic conditions

G. Kleter

Dairying Laboratory, Department of Food Science, University of Agriculture, Wageningen, The Netherlands

Received: 16 May 1975

Summary

Enzymes are of great importance to the chemical and physical changes in cheese during the ripening. As there are so many enzymes involved, such as rennet and enzymes from different types of micro-organisms, it is necessary to have a system in which the effect of these enzymes can be studied separately and in the desired combinations, without the results being complicated by the presence of other enzymes.

To comply with this requirement, a new method of aseptic milking of cows (11) and an apparatus for making cheese under strict aseptic conditions, were developed. A description of the apparatus is given. It consists of two completely closed, identical vats equipped with a knive/stirrer and with a capacity of 40 litres each. The vats can be sterilized at 120 °C for 20 min, and everything necessary during the cheesemaking process is already suspended in the vats before sterilization or can be added aseptically during cheesemaking.

1 Introduction

The ripening of cheese is a very complicated microbiological, chemical and physical occurrence. Enzymes which are present in the milk or are added to it and enzymes which are produced by micro-organisms in the milk and particularly in the cheese, are of great importance in this respect. It is only possible to obtain the correct information concerning the phenomenon of cheese ripening when the different reactions can be studied separately and in the desired combinations.

It is not possible to study the ripening of cheese as a whole, including organoleptic changes, by means of simplified model systems. Besides, model systems have the disadvantage that the circumstances may differ from those in cheese. Also sterilization of the milk or the cheese or the addition of antibiotics and sterilants is unsuitable as well.

To do research under conditions as described above, it is necessary to create

circumstances in which only the desired enzymes and/or added bacterial cultures are able to exercise their influence, exclusive of the development of micro-organisms which have been accidentally introduced into the medium. Therefore, in the first place, it is necessary to milk the cows as aseptically as possible. The bacterial count of the raw milk has to be very low, preferably even nil. Micro-organisms which are possibly present must not have the opportunity to grow and must be destroyed by pasteurization of the milk to give a phosphatase reaction which is just negative. From this milk, cheese has to be made without any contamination. It is especially because we also wanted to carry out experiments with cheese without any bacterial growth, to study the influence of rennet alone and possibly also other enzymes, that the system has to fulfil exacting demands. Even an extremely small contamination with micro-organisms would give them the opportunity to grow in a cheese which contains lactose.

The aseptic method has already been emphasized by von Freudenreich (1). His cows were milked under aseptic conditions and an open, sterilized cheese vat was used for cheesemaking (2). This method was also used by Boekhout & Ott de Vries (3) and later by Stadhouders et al. (4, 5, 6). In spite of possible contamination during cheesemaking in an open vat, it is reported that some of the cheeses were usable (6), although no full description of the bacterial quality of the cheeses is given.

To study the effect of bacteria on the ripening of Cheddar cheese, aseptic cheese vats have been developed (7, 8, 9). Although in this way contamination during cheesemaking could be more readily prevented than in an open vat, the milk used was drawn in the normal way and was therefore not sterile, even after pasteurization. It is reported (10) that fully aseptic conditions could be reached when the milk was drawn by cannula, but this concerned only very small cheeses of 100 g, and a full description of the bacterial quality of the milk and the cheese is not given.

The aseptic milking of the cows as we have done, and the treatment of the milk, are described in an earlier report (11). Here, a description of an apparatus for cheesemaking under strict aseptic conditions is given. The apparatus has been developed and constructed by us, with materials and appendages suitable for this special purpose.

2 The apparatus for aseptic cheesemaking

The apparatus (Figs 1 and 2) consists of two identical vats to enable parallel experiments to be carried out. In one vat, for example, starter only could be added as the bacterial flora and in the other vat the same starter and

lactobacilli. To achieve that the cheeses from both vats will differ only bacteriologically as required, but are identical from a chemical and physical point of view, such as pH and moisture content, the apparatus is equipped with one motor with speed control, which drives the knife/stirrer in both vats in the same way. Further, the required temperature can be maintained at the same value in both vats during cheesemaking by pumping water through the jackets of the vats.

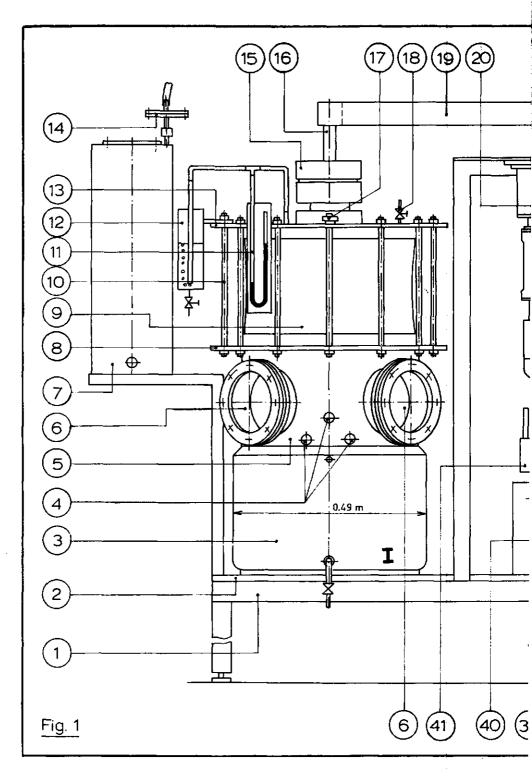
Both vats, each with a capacity of 40 litres, are made of stainless steel, except the upper part which is of glass (Fig. 1, No 9). The heat-resistant

Fig. 1. Apparatus for aseptic cheesemaking. Front view of Vat I. Partial sectional view of Vat II with details of the attachment of the gauntlets and of the sight glasses. The double-walled part of Vat II has been turned through 90° and the single-walled part through 45° with respect to the normal position.

^{1.} Frame; 2. Grating; 3. Double-walled part of the aseptic vat; 4. Quick connection coupling for the supply of milk/water; 5. Single-walled part of the aseptic vat; 6. Sight glass; 7. Water vat; 8. Flange; 9. Glass wall of the aseptic vat; 10. Tie rod; 11. Manometer; 12. Vessel with sterilant solution; 13. Cover; 14. Bacterial filter; 15. Mechanical seal; 16. Driving shaft; 17. Holder with rubber seal for the injection of rennet, bacterial cultures, etc.; 18. Cock for admission of steam to the vat before the sterilization process; 19. Belt drive; 20. Electric motor, speed variator and reduction gear; 21. Sight glass with light; 22. Bacterial filter; 23. Gasket; 24. Nylon gear wheel; 25. Stainless steel gear wheel; 26. Hooks for suspension of moulds, spatula, etc.; 27. Shaft; 28. Hook for placing the perforated metal sheet used for separating curd from whey; 29. A weight for pressing; 30. Holder for a thermometer; 31. Gaskets; 32. Flanges; 33. Outlet circulating water; 34. Knife/stirrer; 35. Cock for draining milk/whey; 36. Rubber gauntlet; 37. Inlet circulating water; 38. Plate to protect gauntlet during sterilization; 39. Tank for circulating water; 40. Cooling coil; 41. Thermostat with circulating pump.

Fig. 1. Apparatuur voor aseptische kaasbereiding. Van vat I is het buitenaanzicht in juiste positie weergegeven. Bij vat II zijn onderdelen van het inwendige van de apparatuur weergegeven, alsmede details van de bevestiging van de rubber handschoenen en de kijkglazen. Het dubbelwandig deel van vat II is 90° en het enkelwandig deel 45° gedraaid t.o.v. de werkelijke positie ervan.

^{1.} Tafel; 2. Rooster; 3. Dubbelwandig deel van het aseptisch vat; 4. Snelkoppelingen voor toevoer melk/water; 5. Enkelwandig deel van het aseptisch vat; 6. Kijkglas; 7. Watervat; 8. Flens; 9. Glazen wand van het aseptisch vat; 10. Trekstang; 11. Manometer; 12. Vaatje met desinfekterende vloeistof; 13. Deksel; 14. Bakteriefilter; 15. Mechanische afdichting; 16. Aandrijfas; 17. Houder met rubber plaatje voor injektie stremsel, bakterie culturen, enz.; 18. Kraan voor doorstomen vóör het steriliseren; 19. Aandrijfriem; 20. Elektromotor met variator en vertragingskast; 21. Kijkglas met verlichting; 22. Bakteriefilter; 23. Pakking; 24. Nylon tandwiel; 25. Roestvrijstalen tandwiel; 26. Haakjes voor ophangen van kaasvaatjes, spatel, enz.; 27. As; 28. Haakje voor aanhaalband; 29. Een gewicht voor het persen; 30. Houder thermometer; 31. O-ringen; 32. Flenzen; 33. Afvoer rondpomp water; 34. Mes/roerder; 35. Kraan voor aftappen melk/wei; 36. Rubber handschoen; 37. Toevoer rondpomp water; 38. Afdekplaat rubber handschoen (bescherming tijdens steriliseren); 39. Voorraadvat rondpomp water; 40. Koelspiraal; 41. Rondpompthermostaat.



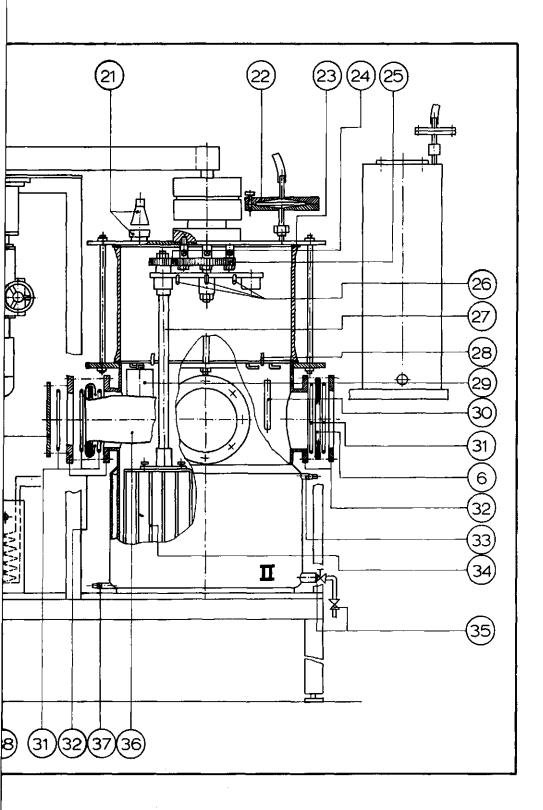


Fig. 2. Apparatus for aseptic cheesemaking.

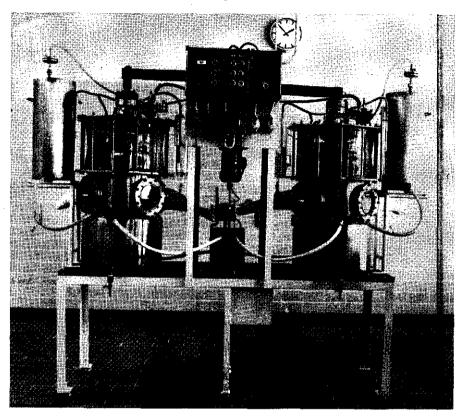


Fig. 2. Apparatuur voor aseptische kaasbereiding.

appendages are of stainless steel, silicone rubber, etc. One of the most delicate constructional details of the apparatus is the passage of the shaft through te cover, as no leakage along the shaft can be tolerated during the whole process of cheesemaking. We have solved this problem by using a mechanical seal consisting of two pairs of annular plates – sliding rings –, one carbon ring being fixed to the shaft and one ceramic ring mounted in the seal housing. The carbon rings rotate while maintaining contact with the ceramic rings. The contact surfaces of the plates have been ground. The seal is surrounded by sterile water.

To carry out such manipulations as are necessary during cheesemaking, each vat has one pair of rubber gauntlets. They are protected against destruction during sterilization by a plate which is connected to the vats after fixing the gauntlets inside (Fig. 1, Nos 36 and 38). Both vats are completely

closed, and before sterilization all the equipment which is required during cheesemaking, including pressing of the cheese, is suspended in the vats (Fig. 1, Nos 26, 28, 29 and 30; Fig. 2), which are then sealed.

The vats are sterilized in an autoclave at 120 °C for 20 min after replacing the air in the apparatus by steam. Before sterilization, the quick connection couplings on the front of the vats (Fig. 1, No 4) are wrapped in paper and a wad of cotton wool is placed in the outlet of the cock at the bottom of the vats (Fig. 1, No 35) and in the pipe for the air outlet. After sterilization, this pipe is placed in a sterilizing solution (Fig. 1, No 12), after removing the cotton wool.

The vats can be filled aseptically with milk which flows from a plate pasteurizer (11) by means of a sterilized tube with quick connection coupling. Bacterial cultures, including starter, and Seitz-filtered rennet, can be added to the milk by means of injection through a rubber seal with sterilizing solution on top of it (Fig. 1, No 17). During cheese making, the wanted quantity of water can be added via a quick connection coupling. After the water has been sterilized at 120 °C for 20 min in stainless steel vessels (Fig. 1, No 7), it is brought to the required temperature before running it into the cheese vats. All manipulations during the filling of the vats with milk, adding the water, etc., are done with all the precautions necessary to ensure complete asepsis.

During the filling of the vats with milk and adding the water, and when the cheese maker puts his gloved hands into the vats, air can escape from the inside of the vats through a pipe which ends in a sterilant solution (Fig. 1, No 12). When the gloved hands are pulled back from the inside of the vats or when milk or whey is drawn off through the cock, sterile air enters the vats through a Seitz filter on top of the vats (Fig. 1, No 22). Care is taken that at all times there is a slight excess pressure in the vats by passing a constant flow of air through this filter. In this way there is a final protection against contamination. The amount of excess pressure in the vats is simply arranged by raising or lowering the pipe in the sterilant solution (Fig. 1, No 12).

Cheese wanted. The cheese can be pressed and kept in the vats at temperatures as required. After that, the cheese has to be taken out under aseptic precautions. If cheese was made which had to be brined, this was done by putting the cheese in fresh brine of special composition (12), which had been sterilized at 120 °C for 15 min. After brining, the surface of the cheese can be covered by a plastic emulsion to protect it against contamination during ripening at the required temperature and relative humidity.

G. KLETER

Bacteriological examination of the milk after being pumped into the vats, which has been described in an earlier publication (11), as well as of the cheeses made, which will be given in subsequent reports together with the other results of our experiments, showed that strict aseptic conditions were indeed attained.

Acknowledgment

The author wishes to express his gratitude to Mr H. Paardekooper and Mr C. Rijpma for technical assistance during the construction of the apparatus.

Samenvatting

G. Kleter, Apparatuur voor het bereiden van kaas onder strikt aseptische omstandigheden.

Enzymen zijn van groot belang voor de chemische en fysische veranderingen in kaas gedurende de rijping. Omdat er zo veel verschillende enzymen bij betrokken zijn, zoals stremsel en enzymen van verschillende bacteriën, is het nodig om een systeem te hebben waarin de invloed van deze enzymen elk apart en in de gewenste combinaties bestudeerd kan worden, zonder door andere enzymen beïnvloed te worden.

Om aan deze eis te voldoen, werden een nieuwe methode van aseptisch melken van koeien (11) en apparatuur voor de bereiding van kaas onder strikt aseptische omstandigheden ontworpen. Een beschrijving van de apparatuur wordt gegeven. Deze bestaat uit twee volledig gesloten, identieke vaten met een capaciteit van elk 40 liter, voorzien van een mes/roerder. De vaten kunnen worden gesteriliseerd bij 120 °C gedurende 20 min, en alles wat nodig is gedurende het kaasbereidingsproces, is reeds in de vaten gebracht vóór het steriliseren of kan gedurende de kaasbereiding aseptisch worden toegevoegd.

References

- 1. E. von Freudenreich, Landw. Jb. Schweiz 4 (1890) 17.
- 2. E. von Freudenreich, Landw. Jb. Schweiz 11 (1897) 85.
- 3. F. W. J. Boekhout & J. J. Ott de Vries, Landbouwk. Tijdschr. 9 (1901) 356.
- J. Stadhouders, The hydrolysis of cheese fat in relation to the flavour of cheese. Thesis, Wageningen, 1956.
- 5. J. Stadhouders & H. Mulder, Neth. Milk Dairy J. 11 (1957) 164.
- 6. J. Stadhouders & H. A. Veringa, Neth. Milk Dairy J. 27 (1973) 77.
- L. A. Mabbitt, Helen R. Chapman & M. Elisabeth Sharpe, J. Dairy Res. 26 (1959) 105.
- 8. K. D. Perry & W. A. McGillivray, J. Dairy Res. 31 (1964) 155.
- 9. Helen R. Chapman, L. A. Mabbitt & Elisabeth M. Sharpe, Proc. 17th int. Dairy Congr. (München, 1966) D-1: 55.
- 10. B. Reiter, Y. Sorokin, A. Pickering & A. J. Hall, J. Dairy Res. 36 (1969) 65.
- 11. G. Kleter & Tj. de Vries, Neth. Milk Dairy J. 28 (1974) 212.
- 12. Th. J. Geurts, P. Walstra & H. Mulder, Neth. Milk Dairy J. 26 (1972) 168.

The ripening of Gouda cheese made under strict aseptic conditions. 1. Cheese with no other bacterial enzymes than those from a starter streptococcus

G. Kleter

Dairying Laboratory, Department of Food Science, University of Agriculture, Wageningen, the Netherlands

Received: 29 November 1976

Summary

The ripening of Gouda cheeses made under strict aseptic conditions and with no other bacterial enzymes active than those from a starter streptococcus has been studied in comparison with that of cheeses made with the same starter under normal conditions.

On the basis of the amount of water-soluble, non-coagulable nitrogen, amino acid nitrogen, acidity of the fat and organoleptic examination of the cheese, it was found that it is possible to make Gouda cheese with a normal ripening process and normal organoleptic properties, when no other bacterial enzymes than those from a proper starter streptococcus are active in the cheese.

Gouda cheese in which only rennet and heat resistant milk proteases but no bacterial enzymes had been active, differed considerably from cheese with starter streptococci. The amino acid content in particular remained at a very low value in the cheese during ripening, and organoleptic examination revealed no cheese taste and flavour at all. In addition, the cheese became bitter after some months of ripening.

It is concluded that the central role of starter bacteria in the ripening of Gouda cheese has been established.

1 Introduction

It has been argued by us (1) and by several other workers, such as Fryer (2) and Lawrence et al. (3), that only experiments under controlled bacteriological conditions can give conclusive information about the influence of bacterial and other enzymes on the ripening of cheese. Till now experiments under strict aseptic conditions have not been done for Dutch types of cheese, such as Gouda cheese. In earlier publications (1, 4) our technique for making this type of cheese under strict aseptic conditions has been described.

For the production of Gouda cheese, streptococci (Streptococcus cremoris strains), as a rule together with so-called aroma-producing streptococci and/or leuconostocs, are used as starter. In the cheese produced under normal con-

ditions, not only these bacteria are active, but also bacteria which survive pasteurization and may grow out in the cheese. Moreover thermoresistant bacterial enzymes have to be considered. Also contaminants after pasteurization of the milk, especially lactobacilli which normally grow out to very large numbers during cheese ripening, are active.

One of the main purposes of the investigations was to examine if starter bacteria, without the presence of any other bacteria or bacterial enzymes in the cheese, are able to give a normal Gouda cheese ripening process. This is not only important from a scientific point of view but also in relation to cheese making practice. There has been an improvement in the sanitary conditions at the farms, resulting in less bacteria in the raw milk and little opportunity for growth. Moreover there is an increasing interest in the use of closed cheese vats in the plants, which could result in the absence of e.g. lactobacilli in the cheese.

In this publication the ripening of Gouda cheese with only starter bacteria and no other bacteria or bacterial enzymes active in it, is described. In addition the ripening without any bacteria or bacterial enzymes has been studied.

2 Materials and methods

2.1 General

The milk. For each aseptic cheesemaking a portion of about 100 litres of aseptically drawn milk was obtained as described earlier (4). The milk used for reference cheeses was obtained from the same herd on the day following the aseptic milking. The latter milk was drawn in the normal way of good sanitary practice but without special precautions and standardized to the fat content of the aseptically drawn milk.

Pasteurization. Unless otherwise stated, all milk was pasteurized for 15 s at 72 °C as described earlier (4).

Bacteriological examination of the milk. The raw and pasteurized milk were examined as described earlier (4, 5).

Cultures. The starters and lactobacilli were all obtained from the collection of the Netherlands Institute of Dairy Research (NIZO) at Ede, Netherlands.

Cheesemaking. Cheesemaking was done as usual for Gouda cheese. The aseptic cheesemaking was done with the apparatus described earlier (1). The

reference cheeses were made in an open vat under good conditions but without special precautions.

To each portion of 40 litres of milk 10 ml of a commercial, Seitz-filtered rennet (guaranteed milk clotting activity at least 10 000 Soxhlet units) and 240 ml of a starter culture grown in heat-sterilized skim milk prepared from skim milk powder (Oxoid), were added. No other additions such as CaCl₂, nitrate or antibiotics were used.

To study the ripening of Gouda cheese with only rennet and heat-resistant milk proteases, but no proteolytic and lipolytic bacterial enzymes active in it, the starter was omitted in these experiments and the required amount of gluconolactone (Lamers & Indemans) was added to the curd to achieve the required pH in the cheese (6). The use of lactic acid or lactide instead of lactone is impossible due to the instant drop in pH. Gluconolactone and its hydrolysation products are not normal cheese substances, giving an acidification which is somewhat different from acidification by a starter and leaving the lactose in the cheese unchanged. However, we saw no other possibility for acidification without bacteria.

In each aseptic vat and in the open vat two cheeses of on average 2426 g (s = 91 g)* per cheese were made. Cheese brining was done as described earlier (1).

Cheese ripening conditions. The cheeses were stored in a separate cheese cellar at 14 °C and 82 % rel. humidity. Immediately after brining the cheeses were covered by a plastic emulsion (Pliol-Delvocid), and this was repeated several times during ripening. To prevent the loss of too much moisture, the cheeses which were stored for six months were coated with a wax for some months.

Sampling of the cheeses. One cheese from each pair was used for examination during the first months. The second was kept until six months of age. Sampling was done with all precautions necessary to ensure complete asepsis. For bacteriological examinations a borer sample and for chemical and organoleptic examinations a sector sample was taken. After sampling the dissecting surface was immediately covered by a plastic emulsion.

2.2 Bacteriological examination of the cheeses

A 5-g portion of a borer sample was homogenized at 45 °C in a sterile mortar with 5 ml of a 20 % citrate solution and 40 ml of a quarter-strength Ringer

^{*} s = estimated standard deviation.

solution. 1 ml of the homogenized sample or a dilution of it was mixed with 10 ml of each of the given media. After solidification the agar was covered with a layer of 5 ml of the respective medium. The plates were incubated at 30 °C for 5 days, except the plates with violet red bile agar which were incubated for 1 day.

Bacterial count. The bacterial count or total count was determined with tomato dextrose agar according to Naylor & Sharpe (7, 8). In case only streptococci are present or form the major group of the bacterial flora, the number of streptococci in the cheese can be measured with this medium.

Coliform bacteria. Violet red bile agar (Difco) was used to determine coliform bacteria.

Lactobacilli. Lactobacilli and possibly other non-starter lactic acid bacteria were determined with Rogosa SL agar (Difco). Microscopic examinations revealed that under the conditions only rods and no cocci were present.

Non-sugar-requiring bacteria. To monitor the aseptically made cheese for the absence of non-sugar-requiring bacteria, including micrococci and coryne-bacteria from aseptically drawn milk, nutrient agar (Difco) plus 0.5 % Tween 80 was used as described earlier (5).

2.3 Chemical examination of the cheeses

A sector sample was milled twice after removing the rind.

Moisture content. About 3 g of cheese were dried at 105 °C to constant weight according to the IDF method (FIL-IDF 4:1958), without sand.

pH. The pH was measured with a Radiometer pH-meter with combined electrode.

Fat content. 3 g of cheese were used for the butyrometric determination of the fat content according to Gerber-van Gulik (Netherlands Standard NEN 3059).

Salt content. The chloride content was determined according to the IDF method, using about 2 g of cheese. From this the NaCl content was calculated (FIL-IDF 17:1961).

Nitrogen contents. To determine the nitrogen contents for the examination of protein breakdown, the micro-Kjeldahl method was used with about 1:10 of the amounts as described for the macro-Kjeldahl method (NEN 3198) in 50-ml flasks. This method was tested with different solutions containing nitrogen compounds, such as skim milk, and proved to give results equal to the macro-method.

Total nitrogen (TN). This was determined after dissolving the cheese in HCl 25% in a flask in a boiling water bath.

Water-soluble (WN) and water-soluble non-coagulable nitrogen (NCN). The extraction of the cheese to determine the amount of WN according to the method of Sirks (9) has the disadvantage that the circumstances under which the extraction takes place are different from those in cheese. That is why we used a method based on the recommendations of Monib (10). 10 g of cheese dry matter were mixed for 5 min at 15-20 °C and pH 5.1 with an 'Ultra turrax' in 200 g (less the amount of moisture present in the cheese) of an extraction solution containing 4 % NaCl and 0.55 % Ca as $CaCl_2$. The suspension was centrifuged for 10 min at 13 500 g and filtered through a paper filter. However this way of working proved to have the disadvantage that the solution after extraction of the cheese very often stays unclear, even after prolonged centrifugation at higher speed.

NCN was determined by the addition of 0.25 N HCl to the extraction solution and heating for half an hour at 50-55 °C (9). In preliminary experiments it was found that the method used on the basis of the recommendations of Monib has the practical advantage that the amount of coagulable nitrogen (CN) does not have a maximum at a very small pH range, as is the case with the method of Sirks (9, 11). Below a pH of about 3.0, the amount of CN proved to be of constant value. That is why we always used a pH of 2.0.

In the meantime a new method for the determination of protein breakdown has been developed by Noomen (12) on a basis of clear extracts and without the disadvantages of the method of Sirks. As with this method there seems to be scarcely any coagulable nitrogen, WN on basis of the method of Noomen and our value for NCN are about the same (12).

In view of the foregoing we made no use of our figures for WN but of our NCN figures.

Amino acid nitrogen (AN). According to the method of Sirks, to 50 ml of the cheese extract 30 ml of H_2SO_4 25 % were added together with a solution of phosphotungstic acid 50 %. It was found in preliminary experiments that the

addition of 12 ml of the phosphotungstic solution instead of making a whole range (9, 11), gives reliable results.

Acidity of the fat (AF). The degree of fat decomposition was determined according to Stadhouders (13). It is expressed as the number of ml of 0.1 N NaOH required to neutralize 10 g of fat.

2.4 Organoleptic examination of the cheeses

A laboratory panel of about 10 experts on Gouda cheese taste and flavour examined the cheeses, knowing their age. Reference cheeses of the same age and manufactured as described above and sometimes cheeses obtained from cheese-producing factories were used for comparison with aseptically made cheeses. The cheeses were graded for taste and flavour as follows: 3 = very bad; 4 = bad; 5 = insufficient; 6 = normal; 7 = good; 8 = very good. Cheese defects were graded as follows: 0 = none observed; 1 = faint; 2 = distinct; 3 = strong; 4 = very strong.

3 Results and discussion

In the aseptically drawn milk no coliforms, lactobacilli or gram-negative rods were found. Only micrococci and coryneforms were present (4, 5) in low numbers (Table 1: total count). The pasteurized milk proved to be sterile (4). In the cheeses made under aseptic conditions and described in this publication, no coliforms or lactobacilli were found during ripening. Also with nutrient agar plus Tween 80 no bacteria could be detected. Microscopic examinations of the colonies from Tomato Dextrose Agar (TDA) revealed only streptococci. In the experiment in which the starter was replaced by gluconolactone, even on TDA no colonies developed, so that these cheeses were completely sterile. It can be concluded that strict aseptic conditions were indeed reached.

The figures from all the cheeses made under aseptic conditions with only S. cremoris E8 as the bacterial flora in the cheese are given in Table 1. It must be noted that this concerns cheese from one vat of parallel experiments which as such will be reported in a subsequent publication (two aseptic cheese vats: in one vat E8 and in the other vat for example another starter). Fig. 1 and 2 illustrate the summarized results from Table 1.

Although it was intended to make cheese always with the same pH (5.10), moisture content (45%), fat content (49.5%) in d.m.) and NaCl content (4.0%) in moisture at 9 days, this could not be achieved due to unavoidable differences in milk composition and small variations in the circumstances

0												
	Exp. No (total count per ml of raw milk)	Ripening time ¹	Moisture (%)	Hd	Fat in d.m. (%)	NaCl in moisture (%)	NCN in % of TN²	AN in % of TN ²	AF3	Log total count per g of cheese	Taste and flavour (mean)	Taste and flavour defects ¹
	10617 (145)	9d 14d 1m 3m 6m	44.0 42.1 40.1 33.6 31.4	5.07 5.15 5.16 5.17 5.32	46.8	1.1	9.7 11.8 18.7 23.4	2.0 2.7 5.5 8.5	0.9	2 V	6.5 6.4 6.0 6.3	0000
	11026 (54)	3d 9d 14d 21d 2m 6m	44.2 42.9 40.1 31.3	5.17 5.25 5.32 5.32 5.43	47.8	3.7	9.7 16.9 23.5	1.8 3.5 8.3	0.4 0.6 0.7	9.0 5.4 3.0 1.0	6.0 5.8 6.2	000
Neth. Milk	20104	3d 9d 21d 2m 6m	45.1 38.2 32.8	5.07 5.17 5.28	49.9	 	16.9 22.0	4.8 6.5.	0.5	8.7 7.0 6.3	6.1 6.2 6.2	I-2 acid/salt I-2 bitter I n.d.
Dairy J. 30 (1976	20118 (23)	3d 9d 21d 2m 6m	42.4 35.8 31.9	5.00	53.8	4.	15.9 23.4	5.0	0.6	8.9 7.0 6.0 1 >	5.9 6.0 6.1	2 acid 0 0
;)	20201 (41)	3d 9d 21d 2m	42.3 33.8	5.00	52.9	3.9	15.6	4.5	0.4	9.2 6.9 6.0	5.0 5.9	0 1 acid

Table 1. The ripening of aseptically made Gouda cheeses with no other bacterial enzymes than those from Streptococcus cremoris E 8.

RIPENING GOUDA CHEESE MADE UNDER STRICT ASEPTIC CONDITIONS. 1

0	6.0	-	0.7	0.6	28.9			5.20	34.0	ш 9		1
0 1 acid	6.6	6.6 5.2	0.5	4.7	17.2			5.15	36.6	21 d 2 m		26
c	V	27				3.6	53.0	5.06	43.4	p6	(137)	
		9.1								3 d	20725	
0	6.4	$\overline{\lor}$	0.5	7.9	25.7			5.18	35.5	9 m		
l acid	6.5	3.5	0.4	4.3	17.3			5.22	35.8	2 m 2 m		
(·					4.3	50.5	5.15	43.3	p 6	(22)	
		9.0								3 d	20711	
1-3 n.d.	6.4	$\overline{\lor}$	0.7	8.3	25.2			5.25	39.8	e m		
00	6.3	6.7 5.0	0.5		18.3			5.25	37.6	2 m		
						3.8	48.6	5.13	44.3	p 6	(24)	
		9.1								3 d	20627	
2 n.d.	5.9	1.0	0.7	0.6	29.8			5:35	39.7	ш 9		
00	6.1 5.8	6.3 3.6	0.5	8				5.32	37.5	21 d 2 m		
		9.0				3.7	47.1	5.20	43.9	3 d 9 d	20613 (28)	
I −2 n ,d.	7.0	2	9.0 0	4.	79.0			2.20	y./s	E		76)
0	6.2	3.2	0.5	4.7	18.0			5.22	37.0	2 m		(19
C	1.9	6.7				3.9	49.9	5.09	44.2	9 d 21 d	(20)	7. 30
		9.1								3 ф	20509	iry i
I-2 acid	5.5	7	9.0	8.6	26.3			5.09	33.3	w 9		k De
2–3 acid 2 acid	5.8 5.6	7.0 4.6	0.6	4.6	16.2			5.08	33.8	21 d 2 m		Mill
:		;				4.2	53.0	4.91	43.8	96	(34)	eth.
		8.6								3 d	20411	N
1-2 acid	5.6	∇	9.0	7.7	22.1			5,16	33.3	6 m		
1 – 3 acid 1 – 2 acid	5.6 5.6	6.9 5.9	0.5	4.9	14.3			4.99	33.7	2 m 2 m		
F :	0	0				4.2	52.4	4.99	42.7	96	(100)	
)		ŀ

20822 (24) (2005) (102)	Ripening time ¹ 3 d 9 d 21 d 2 m 6 m 1 d 3 d 21 d 2 m 6 m	Moisture (%) 43.2 37.2 34.7 44.5 38.3	5.25 5.25 5.28 5.28 5.19 5.19	Fat in d.m. (%) 50.3	NaCl in moisture (%) 3.7	NCN in % of TN ² 16.4 31.9 17.3 30.8	AN in % of TN² 5.2 9.5 3.7 9.6	AF3 0.4 0.5 0.4 0.7	Log total count per g of cheese cheese 6.3 5.1 <1 <1 <1 <1 <1 <5.9 <5.9 <5.9 <5.9 <5.9 <5.9 <5.9 <5.9	Taste and flavour (mean) 9.2 6.6 6.8 6.8 6.7 6.6 6.8 6.7 7.1 7.1 6.7	
20919 (6)	1d 3d 9d 2ld 2m 6m	44.7 33.8 35.1	5.10 5.16 5.15	48.4	3.9	16.5	4.2 9.2	0.4	9.4 9.3 6.6 5.0	7.0 6.4 6.4	
21003 (8)	3d 9d 21d 2m 6m	42.5 36.2 33.8	5.17 5.28 5.28	49.6	3.6	13.9 25.1	3.3	0.4 0.8	9.4 1.7 1.7 1.7	6.0 6.3 6.3	000
21121 (14)	3d 9d 21d 2m	43.9	5.09	52.3	3.7	16.1	2.7	0.4	9.1 6.7 5.7	6.4	l acid 0

	o		0		0																				
	5.8		5.9		6.4									6.5		6.2	(0.0)	6.4		6.2	(0.4)	0.9		6.2	(0.4)
	6.4		4.1		7		1	9.5	(0.2)	0.6	(0.2)					9.9	(0.4)			4.9	(1.0)	2.0		0.1	(0.4)
			0.4		8.0									0.4						0.5	(0.1)	6.0		0.7	(0.2)
1.1		3.0	3.1	8.9	8.2							1.1		1.9	(0.1)			2.9	(0.2)	4.2	(0.7)	5.5	6.8	8.6	(1.0)
5.2		11.5	13.6	18.8	23.5							5.2		7.6	. (0.0)			11.7	(0.2)	16.3	(1.4)	18.7	18.8	25.8	(3.3)
4.1												3.9	(0.22)												
52.3												9.09	(2.3)							1					
5.10		5.13	5.21	5.24	5.25		n)					5.10	(0.09)	5.20	(0.07)			5.15	(0.05)	5.18	(0.10)	5.17	5.24	5.22	(0.09)
41.1		36.6	32.6	33.4	31.3		aara deviation,					43.5	(0.1)	42.5	(0.5)			38.3	(2.5)	36.3	(2.2)	33.6	33.4	34.3	(2.7)
p 6	21 d	lm	2 m	4 m	6 m		re (and stanz	47 I d		3 d		9 6		14 d		21 d		_ E		2 m		3 ш	4 m	6 m	
(9)							Mean valu	47																	

 2 TN = total nitrogen; NCN = non-coagulable nitrogen; AN = amino acid nitrogen. 3 AF = acidity of the fat. 4 n.d. = not definable. 1 d = days; m = months.

G. KLETER

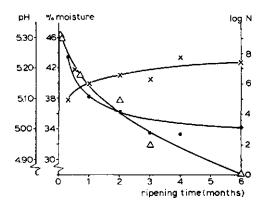


Fig. 1. Moisture content, pH and log number of streptococci in aseptically made Gouda cheeses with no other bacterial enzymes than those from S. cremoris E8.

% moisture; × pH; / log N.

during cheesemaking, which is also the case in cheesemaking under practical conditions. This influenced of course to some extent the cheese ripening process. It must be noted that the differences between the cheeses from one parallel experiment (e.g. to compare two starters or the comparison of starter to starter plus lactobacilli) are much smaller, as will be seen in a subsequent publication.

As the cheeses from the first seven experiments (Table 1) had a relatively low moisture content at six months, in later experiments the cheeses were coated with a wax as described above. The cheeses from the next three experiments were kept for several months in this way which resulted in a somewhat too high moisture content at six months and off-flavours were found. It can

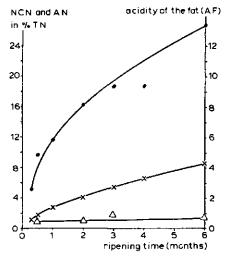


Fig. 2. Protein breakdown as water soluble, non-coagulable nitrogen (NCN) and amino acid nitrogen (AN) in % of total nitrogen (TN), and fat breakdown (AF) as ml 0.1 N NaOH required to neutralize 10 g of fat, in aseptically made Gouda cheeses with no other bacterial enzymes than those from S. cremoris E8.

• NCN; \times AN; \wedge AF.

be remarked that only in these cheeses were streptococci able to survive in small numbers until six months. The rest of the cheeses were coated for about two months (the third and fourth month) with wax, which proved to give enough protection against loss of moisture, and no off-flavours were found.

Due to different methods of examination, it is difficult to compare our figures for NCN with those for WN from literature concerning Gouda cheese (14). The figures for AN are in good agreement with those normally found in Gouda cheese (10, 14).

Fat breakdown in Gouda cheese from pasteurized milk is normally already limited (15). In aseptically made cheeses with only *S. cremoris* it is even practically absent, which will be due to the low bacterial count in the raw milk, the absence of Gram-negative rods and the treatment of the milk which did not allow the natural lipases of the milk to hydrolyse the milk fat to some extent to mono- and diglycerides (15).

Taste and flavour of the cheeses were found to be normal without defects when the pH and moisture content during ripening were correct.

It can be concluded from these results that it is possible to make a normal Gouda cheese, when only a proper S. cremoris strain and no other bacterial enzymes are present in the cheese. This is in agreement with the experiments concerning aseptically made Cheddar cheese with only a S. cremoris strain (16, 17, 18, 19), although with this type of cheese the flavour was of low intensity and developed slowly.

To verify our conclusion and to have comparable figures for NCN in normal Gouda cheese, the results of six experiments with reference cheeses in comparison with aseptically made Gouda cheese with only a *S. cremoris* strain, are given in Table 2.

The aseptically drawn milk had a mean bacterial count of 69 (s = 45) in the raw milk and was sterile after pasteurization. The raw milk which was used for the production of the reference cheeses had a mean bacterial count of 28 400 (s = 13 400) and the mean bacterial count after pasteurization was 1 060 (s = 850). In 1 ml of this pasteurized milk no lactobacilli could be detected. It can be seen from Table 2 that in spite of this very small contamination with lactobacilli, very high numbers of these bacteria were found in the reference cheeses during ripening. The reference cheeses were free from coliforms and showed no defects such as late blowing.

On the basis of the mean pH, moisture content, fat content and salt content at 9 days, the two types of cheese proved indeed to be comparable (Table 2). This is further illustrated by the figures in Table 3. It can be seen from Table 2 that not only the values for AN but also for NCN are nearly the same for both types of cheese. Fat breakdown in the reference cheeses is of a normal value

Table 2. The ripening of aseptically made Gouda cheeses (A) in comparison with Gouda cheeses made under normal conditions (N), both with S. cremoris E8 as starter; mean values (and standard deviations).

Ripening time ¹	Cheese	Number of cheeses exam- ined	Moisture (%)	Hď	Fat in in d.m.	NaCl in in moisture (%)	Log number of strepto- cocci per g of cheese*	Log number of lacto- bacilli per g of cheese	NCN in% of TN ²	AN in% of TN ²	AF3	Taste and flavour** (mean)
3 d	∢	5					8.8 (0.2)	~			•	
	z	5					9.3	1.8				
p 6	∢	9	43.7	5.07	49.5	4.0		ì				
	Z	9	44.2 5.25 5.25	5.07	49.4	3.9						
14 d	4	1 or 2	42.5	5.20	(7. +)	(0.0)			9.7	6:1	0.4	6.5
٠	z	1 or 2	(0.5) 42.8 5	(0.07) 5.25 (0.19)					() () () () () () () () () () () () () ((0.1) 1.8 9.6	6.0	6.5
21 d	∢] :	(61.6)			6.5	<u></u>	(0.5)	(a.v)		5.8
	Z	5					(6.7) - (2×)	5.8				(c.u) 5.9 6.8
							6 / (3×) (04)	(1:1)				(6.9)
l m	∢ 7	 -	49.1	5.16					1.8	2.7		6.4
2 m	z 4	1 4 or 5	36.4	5.12			4.1	$\vec{\lor}$	15.7 16.4	5.5 2.5	0.5	6.4 5.8
	Z	ار.	(2.7)	(0.15)			(1.2)	7.0	(1.6)	(0.6)	(0.1)	(;0.4) 6.1
¢	; -		(3.3)	(0.20)				(0.5)	(0.8)	(0.4)	(0.2)	(0.4)
E T	K Z	- -	33.6 33.9	5.17					18.7	5.2	γ. . . Σ. ∞.	0.0
е ш	¥	5 or 6	34.5	5.25			0.4	<u>~</u>	25.7	œ ;	0.8	6.0
	;		(3.5)	(0.13)			(0.6)	•	(3.2)	(0.8)	(0.3)	(0.3)
, ,	z	9	34.8 8.6	5.30			I	7.0	25.2	%.] (9.4)	- 6	8.5 6.6
				,							,	(

^{* — =} not determined due to high numbers of lactobacilli.

** Taste and flavour defects: sometimes a little acid, or not definable (see also Table 1).

1,2,3 See Table 1.

Neth. Milk Dairy J. 30 (1**97**6)

Experiment No	Moisture (%)	(%)	Hd		Fat in d.m. (%)	:S	NaCl in n	NaCl in moisture (%)
	Z	7	z	4	z	4	z	٥
10617	44.7	+0.7	5.03	-0.04	46.5	-0.3	4.9	+ 0.8
11026	44.3	+0.1	5.25	+0.08	46.0	-1.8	3.2	-0.5
20215	43.8	+1:1	5.01	+0.02	52.1	-0.3	3.8	-0.4
20411	41.1	-2.7	4.97	+0.06	56.4	+3.4	3.8	-0.4
20509	44.9	+0.7	5.07	-0.02	49.0	6'0-	3.6	-0.3
20613	46.0	+2.1	5.09	-0.11	46.2	-0.9	4.1	+0.4

Table 4. Composition at 9 days of aseptically made Gouda cheeses with S. cremoris E8 (E) and gluconolactons (L) instead of starter (Experiment No 21205).

Cheese No	Cultures added	Moisture	Fat in d.m. (%)	Salt in moisture (° ₀)	pН
E	S. cremoris E8 none	41.1	52.3	4.1	5.1 0
L		40.1	49.7	4.5	5.1 0

for Gouda cheese and about 2 times the value for AF in the aseptically made cheeses. Nevertheless the organoleptic properties were about the same. In addition the organoleptic properties of the aseptically made cheeses were compared four times with normal Gouda cheeses of the same age and made in a normal cheese factory. This resulted for the aseptically made cheeses in 6.0 and 6.3 and for the normal practical cheese in 6.2 and 6.1 points (mean) at 2 and 6 months of age respectively.

The experiments with the reference cheeses (Table 2), together with the results obtained in comparison with normal practical cheese, confirm our conclusion that a proper starter streptococcus is able to give a normal Gouda cheese ripening process, without the help of any other bacterial enzymes.

To study the action of rennet and heat-resistant milk proteases alone in comparison with these enzymes plus E8 (both aseptically), the starter was replaced by gluconolactone as described before (Parallel experiment, No 21205). The bacterial count of the raw milk was 6 per ml. The composition of the cheeses at 9 days is given in Table 4. As we were not successful in these experiments in making a lactone-cheese with the wanted moisture content of about 45 %, for comparison we also made cheese with a starter with the same low moisture content. The results of these experiments are given in Fig. 3 and 4.

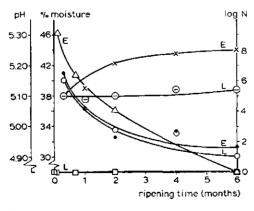


Fig. 3. Moisture content, pH and log number of streptococci in aseptically made Gouda cheese with S. cremoris E8 (E) and gluconolactone instead of starter (L), experiment No 21205, Moisture content: ● (E) and ○ (L). pH: × (E) and ○ (L). Log number of streptococci: △ (E) and □ (L).

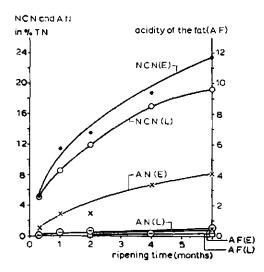


Fig. 4. Protein breakdown as water soluble, non-coagulable nitrogen (NCN) and amino acid nitrogen (AN) in % of total nitrogen (TN) and fat breakdown (AF) as ml 0.1 N NaOH required to neutralize 10 g of fat, in aseptically made Gouda cheese with S. cremoris E8 (E) and gluconolactone instead of starter (L), experiment No 21205.

NCN: \bullet (E) and \bigcirc (L). AN: \times (E) and \bigcirc (L). AF: \triangle (E) and \square (L).

The most remarkable difference between the two types of cheese is the extremely low amino acid content in the lactone-cheese. Also pH, NCN and AF during ripening were lower than in the starter-cheese. Organoleptic examination of the cheeses showed that taste and flavour for the starter-cheese were somewhat lower than normal (5.8) due to the low moisture content, but no defects were observed. The lactone-cheese had no cheese taste and flavour at all and especially at 6 months of age the cheese had become very bitter (3.5).

In the foregoing experiments with S. cremoris E8, the central role of starter bacteria in the ripening of Gouda cheese has been established. In a subsequent publication the comparison of the activity of different starters and the influence of some strains of lactobacilli in cheese will be reported (parallel experiments).

Samenvatting

G. Kleter, De rijping van Goudse kaas bereid onder strikt aseptische omstandigheden. 1. Kaas met geen andere bacteriële enzymen dan die van een zuursel-streptococcus

De rijping van Goudse kaas bereid onder strikt aseptische omstandigheden en met geen andere bacteriële enzymen werkzaam dan die van een zuursel-streptococcus werd bestudeerd in vergelijking met die van kazen met hetzelfde zuursel bereid onder normale omstandigheden.

Op basis van de hoeveelheid water-oplosbaar niet coaguleerbaar stikstof, aminozuur-

G. KLETER

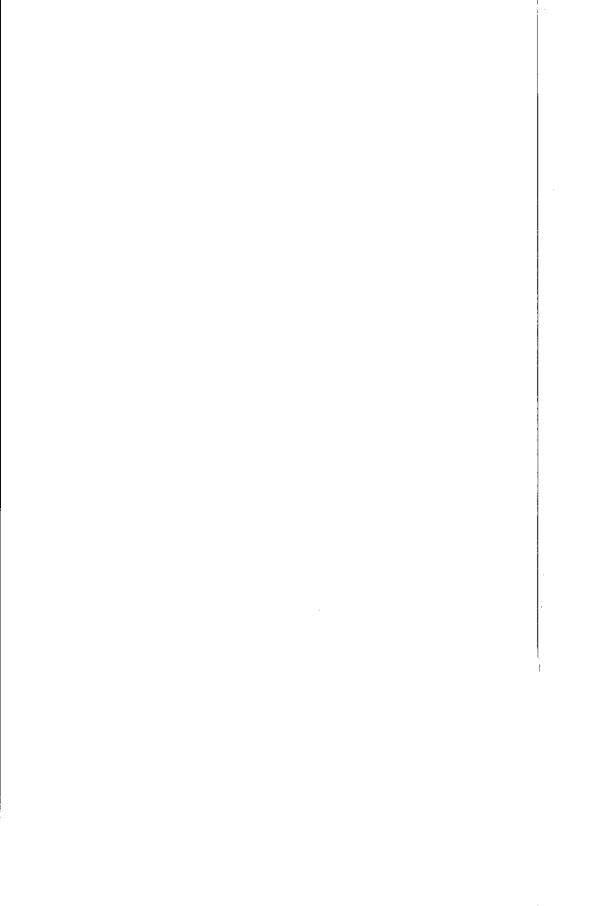
stikstof, zuurgetal van het vet en organoleptische beoordeling van de kaas, bleek dat het mogelijk is om Goudse kaas te maken met een normaal rijpingsproces en normale organoleptische eigenschappen, terwijl geen andere bacteriële enzymen dan die van een passende zuursel-streptococcus in de kaas werkzaam zijn.

Goudse kaas waarin alleen stremsel en hitte-resistente melkproteasen, maar geen bacteriële enzymen werkzaam zijn geweest, verschilde aanzienlijk van kaas met zuurselstreptococcen. Met name het gehalte aan aminozuren bleef erg laag gedurende de rijning van de kaas en uit de organoleptische beoordeling bleek dat er totaal geen kaassmaak aanwezig was.

Er wordt gekonkludeerd dat de centrale rol van de zuurselbacteriën bij de rijping van Goudse kaas is aangetoond.

References

- 1. G. Kleter, Neth. Milk Dairy J. 29 (1975) 295.
- 2. T. F. Fryer, Dairy Sci. Abstr. (rev.) 31 (1969) 471.
- 3. R. C. Lawrence, T. D. Thomas & B. E. Terzaghi, J. Dairy Res. 43 (1976) 141.
- 4. G. Kleter & Tj. de Vries, Neth. Milk Dairy J. 28 (1974) 212.
- 5. G. Kleter, Neth. Milk Dairy J. 28 (1974) 220.
- 6. L. A. Mabbitt, Helen R. Chapman & N. J. Berridge, J. Dairy Res. 22 (1955) 365.
- 7. Jill Naylor & M. Elisabeth Sharpe, J. Dairy Res. 25 (1958) 92.
- 8. P. S. Robertson, J. Dairy Res. 31 (1964) 297.
- 9. H. A. Sirks, Versl. landbouwk. Onderz. 49 (1943) 221.
- 10. A. M. M. F. Monib, The Calcium-paracaseinate-phosphate-complex under conditions similar to those in cheese. Thesis, Wageningen, 1962.
- 11. C. W. Raadsveld & H. Mulder, Neth. Milk Dairy J. 3 (1949) 117.
- 12. A. Noomen, personal communication, 1976.
- 13. J. Stadhouders, The hydrolysis of cheese fat in relation to the flavour of the cheese. Thesis, Wageningen, 1956.
- 14. J. Stadhouders, Neth. Milk Dairy J. 14 (1960) 83.
- 15. J. Stadhouders & H. Veringa, Neth. Milk Dairy J. 27 (1973) 77.
- B. Reiter, T. F. Fryer, A. Pickering, Helen R. Chapman, R. C. Lawrence & M. Elisabeth Sharpe, J. Dairy Res. 34 (1967) 257.
- 17. K. D. Perry & W. A. McGillivray, J. Dairy Res. 31 (1964) 155.
- 18. R. C. Lawrence, N. Z. Jl Dairy Technol. 1 (1966) 122.
- R. J. Lowrie, R. C. Lawrence & M. F. Peberdy, N. Z. Jl Dairy Sci. Technol. 9 (1974) 116.



The ripening of Gouda cheese made under strictly aseptic conditions. 2. The comparison of the activity of different starters and the influence of certain lactobacillus strains

G. Kleter

Dairying Laboratory, Department of Food Science, University of Agriculture, Wageningen, the Netherlands

Received: 10 May 1977

Summary

In this second report about the ripening of Gouda cheese made under strictly aseptic conditions, the activity of various starters and the influence of certain lactobacillus strains is described.

In addition to the results reported earlier (1), a remarkable difference was found between Streptococcus cremoris strains in their ability to produce amino acids and other cheese taste and flavour components, and also in relation to the development of cheese taste and flavour defects.

The comparison of aseptically made Gouda cheese with a proper single strain starter and aseptically made Gouda cheese with mixed strain starters from practice showed that protein and fat breakdown in the two types of cheese were about the same. It is stressed that, from the results of this and the previous publication (1), no definite answer can yet be given to the question whether or not mixed strain starters are preferable to single strain starters for the production of Gouda cheese in relation to the ripening process.

The experiments with types of lactobacilli which are normally present in high numbers in Gouda cheese, showed that these bacteria are not necessary for a good ripening process. Their presence may even be undesirable in relation to the development of taste and flavour defects in Gouda cheese.

1 Introduction

In an earlier publication (1), it was shown that Gouda cheese can have a normal ripening process, including satisfactory taste and flavour characteristics, when no bacterial enzymes are active in the cheese other than those from a proper starter streptococcus. In Gouda cheese ripened without the activity of any bacterial enzymes, much smaller amounts of amino acids were found and even after 6 months storage no cheese taste and flavour could be detected. It was concluded that the central role of starter bacteria in the ripening of Gouda cheese was established.

In this article the results obtained with the Streptococcus cremoris strain

refered to above are compared with those obtained with another S. cremoris strain. For the production of Gouda cheese in the Netherlands, mixed strain starters and no single or multiple strain starters are used. That is why we also carried out some experiments on the influence of mixed strain starters on the ripening of Gouda cheese made under strictly aseptic conditions.

In Gouda cheese made in practice, lactobacilli can be found in high numbers during ripening. These bacteria have often been considered as being important and perhaps even indispensable in the ripening of Gouda cheese. However Stadhouders (2, 3) found that the influence of lactobacilli on the fat and protein breakdown in Gouda cheese was very limited. They originate from contamination after pasteurization of the milk.

High numbers of lactobacilli indeed were also found in our 'reference' cheese (1). Even with less than 1 lactobacillus per ml of pasteurized milk, about 10⁷ per g of cheese were found after 2 and 6 months ripening.

To gain some information about the influence of lactobacilli on the ripening of Gouda cheese, without the results being influenced by any other non-starter bacteria, Gouda cheese was made by us aseptically in parallel experiments with a starter in one aseptic vat and the same starter plus lactobacilli in the other aseptic vat.

2 Materials and methods

The cheeses were made under strictly aseptic conditions as described earlier (4, 5). We confirmed that in the experiments which are reported here we achieved strictly aseptic conditions as described previously (1). The following are additional to the materials and methods as described in a previous publication (1).

The following cultures which were all obtained from the collection of the Netherlands Institute for Dairy Research (NIZO), were used:

E8: single strain starter (S. cremoris strain),

Wg2: single strain starter (S. cremoris strain),

Ur: mixed strain starter (type BD), Fr8: mixed strain starter (type B),

DR: Lactobacillus casei strain,

SKI: Lactobacillus plantarum strain,

AH: Lactobacillus brevis strain.

The mixed strain starters Ur and Fr8 are in use for the production of Gouda cheese in practice. The lactobacillus strains were isolated from Gouda cheese. In the experiments in which the lactobacilli were tested, the pasteurized milk was inoculated with about 5 lactobacilli per ml.

3 Results and discussion

3.1 The activity of different starters

3.1.1 The comparison of Streptococcus cremoris strains. The results of 7 experiments in which only one aseptic cheese vat was used and with no bacterial enzymes active in the cheese other than those from S. cremoris Wg2, are summarized in Table 1 (mean values and standard deviations). If we compare these results with those obtained in several experiments with S. cremoris E8 (already published, 1, Table 1, mean values) remarkable differences can be noted. S. cremoris Wg2 produced in the cheese much less amino acids (AN). Cheeses with Wg2 as starter scored also much lower for taste and flavour.

Parallel experiments with two aseptic vats and the same milk supply were run twice with the strains E8 and Wg2. The results obtained with E8 have been published previously (1, Table 1). The results obtained with Wg2 are given in Table 2. The preliminary conclusions drawn above are confirmed by the results of these parallel experiments. The cheeses from these experiments compared well on the basis of moisture content, pH, fat in dry matter and NaCl in moisture. The results of these parallel trials are also shown in Fig. 1. The panel for judging taste and flavour of the cheese agreed that the difference in scores for E8 and Wg2 was not only due to the fact that the cheeses with Wg2 were bitter, but also due to much less cheese taste and

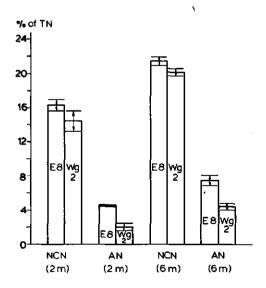


Fig. 1. Protein breakdown in aseptically made Gouda cheeses with Streptococcus cremoris E8 and S. cremoris Wg2, respectively, as starter. Mean values of two parallel experiments: see Table 2 and some figures published earlier (1, Table 1, Exp. 20104 and 20201). TN = total nitrogen; NCN = water-soluble non-coagulable nitrogen; AN = amino acid nitrogen.

Neth. Milk Dairy J. 31 (1977)

Total Riper count per ml time ¹ of raw milk	Ripening time ¹	Moisture (%)	Hd	Fat in dry matter (%)	NaCl in moisture	NCN ii 7% of TN ²	AN in % of TN²	AF3	Log tota count pe	Log total Taste and count per flavour ⁴ g of cheese (mean)
54 (36)	p 6	43.7	5.04 (0.04)	48.6 (4.2)	4.1					
	14 d	42.7	5.07 (0.06)			8.3 (0.4)	1.1 (0.3)			5.5 (0.3)
	н Н	39.3 (-1.3)	5.12 (0.07)			10.8	1.5 (0.3)	0.4	6.3 (0.4)	5.1 (0.4)
	3 m	32.4 (1.4)	5.22 (0.11)			15.8	2.6 (0.3)	0.6 (0.1)	2.1 (0.6)	4.7 (0.4)
	m 9	27.8	5.27 (0.08)			20.8	4.2 (0.7)	0.7	~	4.4 (0.3)

¹ d = days; m = months.

² TN = total nitrogen; NCN = water-soluble non-coagulable nitrogen; AN = amino acid nitrogen.

³ AF = acidity of the fat.

⁴ Very often with bitter defect.

Exp. No (total count per ml of raw milk)	Ripening time ¹	Ripening Moisture time ¹ (%)	ЬН	Fat in dry NaCl in matter moisture (%) (%)	NaCl in moisture (%)	NCN in AN in % of TN ² % of TN ²	AN in % of TN²	AF3	Log total Taste as count per g flavour of cheese (mean)	Taste and Taste and flavour flavour (mean) defects	Taste and flavour defects
20104	9 g	75.0	\$0 \$	- 05	40				9.4		
()	21 d) -	2		P				7.1	5.2	1-2 bitter
	2 m	37.8	5.19			15.6	1.7	0.5	3.4	4.1	2-4 bitter
	e m	32.5	5.28			20.6	4.7	0.7	\ 	4.2	3-4 bitter
20201	3 d								9.4		
41)	9 d 21 d	42.8	5.00	52.8	4.1				8.1	4.9	1 bitter
	2 m	33.6	5.05			13.3	2.5	0.4	2.8	4.2	3-4 bitter
	6 m	32.1	5.13			20.0	4.0	9.0	- - -	3.9	2-4 bitter

^{*} These figures represent half of the results of two parallel experiments. The results obtained with strain E8 have been published earlier (1, Table 1, Exp. 20104 and 20201).

1, 2, 3 See Table 1.

flavour in the cheeses made with Wg2 as starter.

It is clear from the foregoing that the enzymes from the starter bacteria are extremely important in the formation of amino acids and other cheese taste and flavour components. The two S. cremoris strains tested differed considerably in the extent to which these substances were produced. There was also a large difference in the formation of substances which caused bitter defects in the cheeses in which the respective strains were used as starter.

3.1.2 The comparison of mixed strain starters with a single strain starter. The results of two experiments in which one aseptic vat was used and Ur was the starter are given in Table 3 (mean values and absolute variations). The scores for taste and flavour are not given as, due to a temporarily too high relative humidity in the ripening cellar, some of these cheeses developed off-flavours. The comparison of the figures for NCN, AN and AF from Table 3 with those published previously for Gouda cheese made with single strain starter E8 (1, Table 1, mean values) shows that the differences between the two starters, in terms of protein breakdown and fat breakdown in the cheese, are in any case not very large.

To verify the preliminary conclusion drawn above, three parallel experiments were run from the same milk supply, in which we used Ur as starter in one aseptic vat and E8 in the other. The results obtained with E8 have been published earlier (1, Table 1). In Table 4 the results with Ur are given. Comparison of the figures shows that the cheeses compared well on the basis of the composition as determined at 9 days. The figures for NCN, AN and AF in cheeses made with the indicated starters proved to be about the same during ripening (see also Fig. 2). The results of some experiments as described above, but with the use of the starter Fr8, differed only to a small extent. These results give support to our conclusion and that of Visser (6, 7), that a normal Gouda cheese ripening process can be achieved with no bacterial enzymes active in the cheese other than those from a proper S. cremoris strain, although normally mixed strain starters are used in the Netherlands. However it must be stressed that from these results no definite answer can be given to the question whether or not mixed strain starters are indeed preferable to single strain or possibly multiple strain starters for the production of Gouda cheese in relation to the ripening process. In this respect other factors, such as the following, must be taken into account.

Starter E8 gave a cheese ripening process which was qualified as normal for Gouda cheese (1), on the basis of the level of NCN, AN and AF in the cheese, and its taste and flavour. This does not exclude the possibility that other starters could give a higher score for taste and flavour (See scale for

Table 3. The ripening of aseptically made Gouda cheeses with no active bacterial enzymes other than those from mixed strain starter Ur: mean values (and absolute variations) of two experiments.

Total count Ripeni per ml of time ¹ raw milk	45 (60)										
ng	3 d	p 6		14 d		21 d		2 m		ш 9	
Moisture pH (%)		45.7	0.5)	44.5	(0.8)			39.7	(2.0)	32.8	(0.1)
Hd		5.05	(0.01)	5.17	(0.10)			5.31	(0.10)	5.35	(0.10)
Fat in dry NaCl in matter moisture (%)		50.1	(2.5)								
NaCl in moisture (%)		3.8	(0.2)								
NCN in % of TN ²				9.4	(0.3)			19.8	(2.9)	26.3	(0.5)
AN in % of TN²				2.2	(0.0)			5.0	(0.7)	10.2	(0.0)
AF3				9.0	(0.2)			0.7	(0.2)	6.0	(0.3)
Log total count per g of cheese	8.9					7.3	(0.4)	5.4	(0.1)		

1, 2, 3 See Table 1.

Table 4. The ripening of aseptically made Gouda cheese with no active bacterial enzymes other than those from mixed strain starter Ur.* count per g Log total of cheese 7.1 5.6 < 1 _ V 9.3 6.8 5.8 6.6 0.6 AF^3 0.5 0.5 AN in % of TN² 3.5 4.0 NCN in % of TN2 14.8 22.7 14.7 22.6 NaCl in moisture (%) 4.2 3.8 Fat in d.m. (%) 53,9 50.0 52.4 5.02 5.09 4.99 4.97 5.18 5.18 Hd Moisture (%) 36.5 32.2 42.5 43.6 34.1 33.6 42.9 Ripening 21 d 2 m 6 m 3 d 9 d 21 d 2 m 6 m (Total count time1 3 d 9 d 3 d 9 d per ml of raw milk) Exp. No 20215 20118 (23) 21003 <u>@</u>

* These figures represent half of the results of three parallel experiments. The results obtained with strain E8 have been published earlier (1, Table 1, Exp. 20118, 20215 and 21003). 1. 2. 3 See Table 1.

2.3 5.9 1

0.5

3.8

13.0 24.0

5.32

35.2 33.6

2 m 6 m

21 d

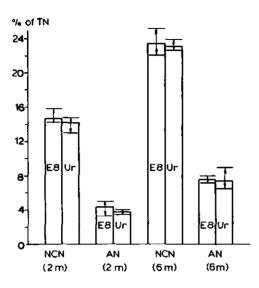


Fig. 2. Protein breakdown in aseptically made Gouda cheeses with Streptococcus cremoris E8 and mixed strain BD starter Ur, respectively, as starter. Mean values of three parallel experiments: see Table 4 and some figures published earlier (1, Table 1, Exp. 20118, 20215 and 21003). TN = total nitrogen; NCN = water-soluble non-coagulable nitrogen; AN = amino acid nitrogen.

scores in Ref. 1). Since the differences between proper single and proper mixed strain starters are expected to be small – as was indicated by our panel –, many experiments with starters will be needed before definite conclusions can be drawn.

In addition it must be remarked that other starter characteristics than protein breakdown, fat breakdown and taste and flavour of the cheese, must also be considered. These are, for example, rate of acid formation, susceptibility to phages, and the formation of eyes in the cheese.

3.2 The influence of certain lactobacillus strains

Four parallel experiments were run with starter (E8) in one aseptic vat and the same starter plus lactobacilli in the other, using the same milk supply. The results obtained with cheeses without lactobacilli have been published earlier (1, Table 1). Table 5 gives the results obtained for cheeses with lactobacilli. Comparison of the figures shows that the influence of the lactobacilli tested on the level of NCN, AN and AF in the cheese during ripening is limited. It can further be seen that the influence of the lactobacilli tested is an undesirable one in relation to cheese taste and flavour, especially at 6 months of age.

Although the number of lactobacillus strains used was limited and they were tested in combination with only one starter, from the results obtained it may be concluded that it is not only possible to achieve a normal Gouda

Table 5. The ripening of aseptically made Gouda cheese with no active bacterial enzymes other than those from S. cremoris E8 (starter) and lactobacilli,*

iaciooaciiii, *												
Exp. No; Ripe Lactobacillus time used; (Number per ml of pas- teurized milk)	Ripening time1	Moisture (%)	Hd	Fat in d.m. (%)	NaCl in moisture (%)	NCN in % of TN2	AN in % of TN2	AF3	Log number of strepto-cocci per g of cheese4	Log number of lacto- bacilli per g of cheese	Taste and flavour (mean)	Taste and flavour defects ⁵
20627 L. casei (9)	3 d 9 d 21 d 2 m 6 m	44.8 37.8 39.5	5.14 5.28 5.25	48.7	3.9	18.6 28.8	4.6 4.6	0.5	9.1	3.6 6.7 8.3 8.2	6.7 6.1 5.4	0 1 n.d. 2-3 n.d.
20509 L. plantarum (6)	3 d 9 d 21 d 2 m	44.5	5.10	49.5	3.7	18.4	5.1	0.4	9.1	3.4 7.5 7.6	6.2 5.9	0 2 bitter
20613 L. brevis (2)	6 m 3 d 9 d 21 d 2 m 6 m	37.8 44.9 38.8 39.3	5.22 5.10 5.21 5.35	46.3	3.9	28.6	8.3 5.0 8.5	0.7	6.6	6.9 6.4 6.6 7.2	5.4 6.5 4.9	2-4 bitter 2-4 bitter 0 1 n.d. 2-3 n.d.
20711 L. casei $+$ L. plant. $+$ L. brevis $(3 \times 4 - 12)$	3 d 9 d 21 d 2 m 6 m	44.6 36.8 35.6	5.12 5.20 5.17	49.8	4.7	16.8	4.0 9.6	0.4	0.6	4.1 7.7 7.9 7.5	6.7 5.8 5.5	2 acid I-2 n.d. 1 acid 2-3 n.d.

⁴ — = not determined due to high number of lactobacilli. 1. 2. 3 See Table 1. L. cas L. pla L. bre 3×1 Neth. Milk Dairy J. 31 (1977)

* These figures represent half of the results of four parallel experiments. The results obtained with cheeses without lactobacilli have been published earlier (1, Table 1, Exp. 20627, 20509, 20613 and 20711).

5 n.d. and definable.

cheese ripening process without the presence of lactobacilli in the cheese, but that certain types of lactobacilli that are normally present in high numbers in Gouda cheese may even be undesirable.

Acknowledgments

The author is greatly indebted to everyone from the Dairying Laboratory who were member of the cheese taste and flavour panel, and the also wishes to express his gratitude to Mr J. L. Uitentuis and to Mr H. J. van der Stege for assistance especially during cheesemaking. The aseptic milking was done in co-operation with the Milk Hygiene Research Centre (MOC), Wageningen.

Samenvatting

G. Kleter, De rijping van Goudse kaas bereid onder strict aseptische omstandigheden. 2. De vergelijking van de activiteit van verschillende zuursels en de invloed van enkele lactobacillus-stammen

In deze tweede publikatie over de rijping van Goudse kaas gemaakt onder strict aseptische omstandigheden wordt de activiteit van verschillende zuursels en de invloed van enkele lactobacillus-stammen beschreven.

In vervolg op resultaten die eerder werden gepubliceerd (1), werd een opmerkelijk verschil gevonden tussen stammen van *Streptococcus cremoris* in hun vermogen om aminozuren en andere kaas geur- en smaakstoffen te produceren, ook met betrekking tot het ontstaan van kaassmaakgebreken.

Uit vergelijking van aseptisch bereide kaas met een goed éénstam-zuursel en aseptisch bereide kaas met meerstam-zuursels uit de praktijk bleek dat eiwit- en vetafbraak in de betreffende kazen vrijwel gelijk was. Er wordt met nadruk op gewezen dat uit de resultaten van deze en de voorgaande publikatie (1) op dit moment nog geen definitief antwoord kan worden gegeven op de vraag of voor de bereiding van Goudse kaas inderdaad beter meerstam-zuursels dan éénstam-zuursels kunnen worden gebruikt met betrekking tot het kaasrijpingsproces.

De experimenten met soorten lactobacillen die normaliter in grote aantallen aanwezig zijn in Goudse kaas, toonden aan dat deze bacteriën niet noodzakelijk zijn voor een goed rijpingsproces. Hun aanwezigheid kan zelfs ongewenst zijn in verband met de ontwikkeling van geur- en smaakgebreken in Goudse kaas.

References

- 1. G. Kleter, Neth. Milk Dairy J. 30 (1976) 254.
- 2. J. Stadhouders, The hydrolysis of cheese fat in relation to the flavour of the cheese. Thesis, Wageningen, 1956.
- 3. J. Stadhouders, Neth. Milk Dairy J. 14 (1960) 83.
- 4. G. Kleter & Tj. de Vries, Neth. Milk Dairy J. 28 (1974) 212.
- 5. G. Kleter, Neth. Milk Dairy J. 29 (1975) 295.
- 6. F. M. W. Visser, Neth. Milk Dairy J. 31 (1977) 188.
- 7. F. M. W. Visser, Neth. Milk Dairy J. 31 (1977) 210.

Discussion and conclusions

1 Selection of cows and bacteriological quality of aseptically drawn milk

For aseptic milking, only cows with clinically healthy udders were used. During a complete lactation, samples of milk from 17 cows were drawn aseptically halfway through a normal milking procedure. The bacterial count (number concentration of bacteria) in milk differed widely from cow to cow. For 6 cows, the median ranged from 15 to 76 ml-1, for 6 cows from 130 to 350 and for 5 cows from 1 100 to 13 000 ml-1. The lowest count in milk from some of the 17 cows was much higher than the highest count from other cows. For our purpose, selection of cows is indeed worthwile (Page I-5 and I-6).

The portions, each of about 100 litres raw milk obtained by aseptic milking of the selected cows through a period of about two years, had an extremely low bacterial count. The lowest count in milk was 6, the highest 145, the median 32 and the mean 46 ml⁻¹. These values were all less than for 11 portions of milk obtained in the same way but without selection of the cows, in preliminary tests.

In the aseptically drawn raw milk, no coliforms or other Gram-negative rods and no lactobacilli were found. After pasteurization, the milk proved to be sterile: after keeping portions of 100 ml for weeks at room temperature, no spoilage occurred and no bacteria could be detected.

Our requirements for the bacteriological quality of the milk that was to be used for making cheese in a strictly aseptic way (Page I-5 and I-6), were met by the extremely low bacterial count of the aseptically drawn raw milk and the sterility of the milk after pasteurization.

2 Bacterial flora of aseptically drawn milk from selected cows

In each portion of about 100 litres of aseptically drawn milk from selected cows, no strictly anaerobic bacteria were found. With plate count agar, only micrococci were found. With plate count agar plus 0.5% (w/v) Tween 80, coryneforms were also detected. The micrococci were not inhibited by the Tween 80, so the latter medium is preferable for counts on aseptically drawn milk.

Of the 169 strains isolated from plates with plate count agar plus 0.5% Tween 80, 13 proved to be *Micrococcus* species, 106 were *Staphylococcus epidermidis*, 5 *Staphylococcus aureus* and 45 *Corynebacterium bovis*. All 169 strains grew on nutrient agar plus 0.5% Tween 80, on which starter bacteria did not grow. With this medium, one can therefore check in cheese that bacteria are absent from the aseptically drawn milk, in the presence of starter bacteria. Such a medium is

especially needed when raw milk is used for aseptic cheesemaking to check that these inevitably present bacteria have not multiplied.

All strains were destroyed (= reduced to less than 5 ml-1) in milk that has been inoculated with several million per millilitre, when heat treated at 63 °C for 30 min. Most strains were even destroyed by a heat treatment of 5 min at 63 °C. These results explain why aseptically drawn milk proved sterile after the pasteurization treatment that is usual for cheese milk (Page I-17), and is equal to 63 °C, 30 min.

When the aseptically drawn raw milk was kept at temperatures up to 45 °C, its bacterial flora proved to have a lag-phase. At 37 °C the lag-phase was only some hours. At 4 °C even after two days the bacterial count was still the same as directly after milking (in these cases about 20 ml-1).

The bacteria present in aseptically drawn milk must have to adapt to the milk, but between 30 °C and 40 °C the lag-phase is short. So milk should be cooled at milking if the raw milk has to be kept for some time and the bacterial flora must not develop. Cooling during milking was also necessary, when raw milk was used for aseptic cheesemaking.

Bacteria found in the aseptically drawn milk, were not from the milk in the udder of the cow, but were indeed harboured in the teat canal and passed from there into the milk (1,2,3). Otherwise the bacterial count of aseptically drawn milk would not be so low. In fact, the micrococci grew well in the milk after being adapted to it, especially at 37 °C. The coryneforms did not grow in the milk.

Experiments with isolated micrococci showed that, after the lag-phase, these bacteria did not have any lag-phase at all when they were inoculated into fresh aseptically drawn milk. Therefore the lag-phase of the bacterial flora in aseptically drawn milk must be due only to adaption of the bacteria to the milk. Lactenins seem to play no role.

3 Equipment for aseptic cheesemaking

The equipment that we developed to make cheese under strictly aseptic conditions consists of two completely closed identical vats, each with a capacity of 40 litres, to carry out simultaneous experiments, using the same milk supply in both vats. For example, in the one aseptic vat no other bacteria than the starter bacteria are inoculated and in the other aseptic vat no bacteria at all are added to milk.

The tools needed during cheesemaking can be suspended in the vats before sterilization. Materials such as cultures, and Seitz-filtered rennet can be added aseptically during cheesemaking. All manipulations during cheesemaking, can be done entirely aseptically. The equipment was so constructed, that the cheeses made in a parallel experiment, were exactly the same chemically at the beginning of the ripening period but differed bacteriologically as desired.

When raw, the bacterial count of the milk was extremely low and after pasteurization the milk was sterile (Page I-17). In the cheeses, no other bacteria were present than those added after pasteurization of the milk. The cheeses that were made to study the action of rennet and heat-resistant milk enzymes without the activity of any bacterial enzymes were found to be sterile during ripening of the cheese. The moisture content, pH, salt content and fat content of the cheese that we made under strictly

aseptic conditions, proved to be usual for Gouda cheese. Thus the method for making cheese under strictly aseptic conditions meets requirements (Page I-5 and I-6).

4 Influence of bacterial enzymes on ripening of Gouda cheese made under strictly aseptic conditions

Some properties of the cheese, in which no other bacterial enzymes had been active during ripening than those from a proper Streptococcus cremoris strain (E8), are summarized in table 1 (mean values of 18 aseptic cheesemaking trials). These figures, including those for taste and flavour, are normal for Gouda cheese (4,5). This was confirmed by comparison of the aseptically made cheese with Gouda cheeses that we made under normal conditions and with commercial Gouda cheese. Thus Gouda cheese can ripen normally, if no other bacterial enzymes are active in the cheese than those from a proper starter streptococcus.

To compare the ripening of cheese without any bacterial enzymes with that of cheese with the enzymes from a starter streptococcus, the starter was replaced by gluconolactone (6) in the one aseptic vat. The bacterial-enzyme-free-cheese differed considerably from the cheese with starter bacteria. Hardly any amino acid was found in the bacterial-enzyme-free-cheese up to six months of ripening and no cheese taste and flavour developed. Thus starter bacteria are essential in the ripening of Gouda cheese.

Figure 1 shows the protein breakdown in cheese from simultaneous experiments with no bacterial enzymes other than those from Streptococcus cremoris E8 or S. cremoris Wg2, respectively. The amount of amino acid during ripening of the cheese with S. cremoris Wg2 as starter is only about half that with S. cremoris E8 as starter. Moreover the cheese with S. cremoris Wg2 as starter scored much lower by sensory assessment because of less cheese taste and flavour during ripening and bitterness. Thus S. cremoris strains differ widely in their ability to produce cheese taste and

Table 1. Properties during ripening of Gouda cheese made aseptically, and with no other bacterial enzymes active than those from *Streptococcus cremoris* strain E8 (mean of 18 cheesemaking trials). Initial mean count of bacteria in the raw milk was 47 ml⁻¹. Almost all cheese samples were free from defects and normal to good in taste and flavour.

Ripe+ ning time ¹	Mois- ture (%)	рН	Fat in d.m. (%)	NaCl in mois- ture (%)	NCN in % of TN ²	AN in % of TN ²	AF³	log strep- tococci per g of cheese
3 d								9.0
9 d	43.5	5.10	50.6	3.9.	5.2	1.1		
21 d								6.6
2 m	36.3	5.18			16.3	4.2	0.5	4.9
6 m	34.3	5.22			25.8	8.6	0.7	0.1

^{1.} d = days; m = months.

^{2.} TN = total nitrogen; NCN = water-soluble non-coagulable nitrogen; AN = amino acid nitrogen.

^{3.} AF = acidity of the fat.

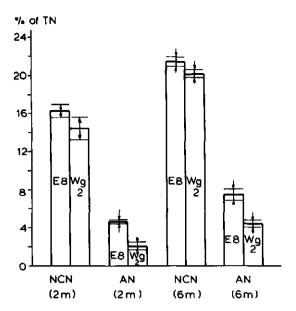


Fig. 1. Protein breakdown in aseptically made Gouda cheese with Streptococcus cremoris E8 or S. cremoris Wg2 as starter (mean values of two simultaneous experiments; the values of two repeated experiments are indicated by the lines at the end of the bars). TN=total nitrogen; NCN=water-soluble non-coagulable nitrogen; AN=amino acid nitrogen; m=months.

flavour by means, for instance, of amino acids, and also in the formation of taste and flavour defects like bitterness.

Cheeses made with mixed strain starters or with the single strain starter S. cremoris E8, differed only to a minor extent in ripening. Protein breakdown and development of cheese taste and flavour were about the same during ripening. Much more research will be needed to confirm whether mixed strain starters are indeed preferable to single strain starters in the production of Gouda cheese (Page I-5), since the differences between a proper single strain starter and mixed strain starters were so small in our experiments. In addition, for the commercial production of cheese other starter characteristics than protein breakdown and taste and flavour development in the cheese, have to be considered like susceptibility to phages, rate of acid production and formation of holes in the cheese.

Little fat was broken down in all the cheeses with no other bacterial enzymes active than those from a starter.

In cheese with bacterial enzymes only from S. cremoris E8, breakdown of fat and protein were about the same as with those from cheese with additionally certain lactobacillus strains. The cheese with lactobacilli scored lower for taste and flavour because of defects, especially at 6 months of age.

Thus Gouda cheese can ripen normally if no other bacterial enzymes are active in the cheese than those from a proper starter. In sterile cheese, hardly any cheese taste and flavour develops during ripening. So the enzymes from the starter bacteria are essential for ripening of Gouda cheese. Lactobacilli, which are normally present in large numbers in Gouda cheese during ripening, are not essential for ripening. They may even be undesirable, since they can give taste and flavour defects.

References

- 1. J. A. Beech & D. Forbes. Pathogens in streak canal. Vet. Rec. 77 (1965) 1086.
- H. J. Heidrich, D. Grossklaus & M. Mülling, Vergleichende bakteriologische Untersuchungen an durch Handmelken und durch Venülenpunktion der Drüsenzisterne gewonnene Milchen. 1. Untersuchungen an Venülenmilchen. 11. Vergleichende Untersuchungen an Handmelk- and Venülenmilchen. Berl. Münch, tierärtzl. Wschr. 77 (1964), 83,85.
- 3. W. Trautmann. Vergleichende bakteriologische Untersuchungen über die primäre und sekundäre Milchflora an durch Handmelken und durch Punktion der Drüsenzisterne gewonnenen Proben. Schweiz.
 landw. Forsch. 12 (1973) 125.
- 4. A. M. M. F. Monib. The Calcium-paracaseinate-phosphate-complex under conditions similar to those in cheese. Thesis, Agricultural University Wageningen. Mededelingen Landbouwhogeschool 62 (1962).
- 5. J. Stadhoudes. De eiwithydrolyse tijdens de kaasrijping. De enzymen die het eiwit in kaas hydrolyseren. Neth. Milk Dairy J. 14 (1960) 83.
- L. A. Mabbitt, Helen R. Chapman & N. J. Berridge. Experiments in cheesemaking without starter. J. Dairy Res. 22 (1955) 365.

Summary

The importance of enzymes in the ripening of cheese is discussed. The enzymes can derive from the milk, the rennet and different types of micro-organisms. To study the influence of the different enzymes separately and in the wanted combinations in the cheese, without the influence of unwanted other enzymes, a method was developed for making cheese in a strictly aseptic way. The influence was studied of the enzymes from certain bacteria on the ripening of Gouda cheese made under strictly aseptic conditions.

Milk drawn aseptically from selected cows had extremely low bacterial counts. It was mostly less than 100 and sometimes only 6 ml-1. After pasteurization at 72 °C for 15 s, as is usual for cheese milk, the milk proved sterile. The types of micro-organisms present, the nutrients needed for growth in media for counting, the heat-resistance and multiplication in milk were studied.

To prevent any contamination with undesirable micro-organisms during cheesemaking process, equipment with two completely closed aseptic cheese vats, capacity 40 litres each, was developed and constructed. With the same milk supply in both vats, simultaneous experiments can be performed. The vats are sterilized in an autoclave before each cheesemaking trial. The tools needed during cheesemaking are suspended in the vats before sterilization. Materials like milk, bacterial cultures and Seitz-filtered rennet can be added aseptically during the cheesemaking. Bacteriological examinations during ripening of the cheeses revealed that conditions were indeed strictly aseptic.

Protein breakdown, and the development of taste and flavour in aseptically made Gouda cheese, in which no other bacterial enzymes had been active than those from a proper Streptococcus cremoris strain as starter, were normal for this type of cheese from pasteurized milk. Bacterial-enzyme-free-cheese differed considerably during ripening from cheese with starter streptococci. Hardly any amino acid were found in the bacterial-enzyme-free-cheese up to 6 months of age and no cheese taste nor flavour developed. Enzymes from the starter bacteria are thus essential for the ripening of Gouda cheese.

S. cremoris strains proved to differ widely in protein breakdown in depth, production of taste and flavour, and defects in the cheese. In protein breakdown, and development of taste and flavour in the cheese, a proper single strain starter and mixed strain starters differed little. For commercial use, however, other properties of starters are also relevant.

Lactobacilli are normally present in large numbers in Gouda cheese during ripening, but proved not to be necessary for ripening. They may even cause taste and flavour defects.

Samenvatting

Methoden voor het strikt aseptisch bereiden van kaas en de invloed van enkele bacteriën op de rijping ervan

Het belang van enzymen bij de rijping van kaas wordt besproken. De enzymen kunnen afkomstig zijn van de melk, het stremsel en verschillende soorten microorganismen. Om de invloed van de verschillende enzymen apart en in de gewenste combinaties in kaas te kunnen bestuderen, zonder de invloed van ongewenste andere enzymen, is een methode ontwikkeld om onder strikt aseptische omstandigheden kaas te bereiden. De invloed van de enzymen van bepaalde bacteriën op de rijping van Goudse kaas die bereid was onder strikt aseptische omstandigheden, is bestudeerd.

Voor aseptisch-melken werden geselecteerde koeien gebruikt. Op deze manier werd bereikt dat het kiemgetal van de rauwe melk extreem laag was. Het was meestal minder dan 100 en soms zelfs slechts 6 ml-1. Na pasteurisatie bij 72 °C gedurende 15 s, zoals te doen gebruikelijk voor kaasmelk, bleek de melk steriel te zijn. De bacterieflora van de aseptisch gewonnen rauwe melk werd verder bestudeerd. Onderzocht werd welke soorten micro-organismen aanwezig waren, de voedingsstoffen die nodig waren voor groei in de media voor het tellen van deze micro-organismen, de thermoresistentie en de groei in melk.

Om iedere besmetting met ongewenste micro-organismen gedurende het kaasbereiden te voorkomen, werd apparatuur met twee volledig gesloten kaasvaten met een capaciteit van elk 40 liter, ontworpen en geconstrueerd. Door in beide vaten dezelfde melk te gebruiken, kunnen synchroon parallel-proeven worden uitgevoerd. Vóór elke kaasbereiding worden de vaten gesteriliseerd in een autoclaaf. Het gereedschap dat nodig is gedurende het kaas-maken, is reeds in de vaten gebracht vóór het steriliseren. De melk, bacterieculturen, gefiltreerd stremsel enz. kunnen aseptisch worden toegevoegd gedurende het kaas-bereiden. De bacteriologische onderzoekingen van de kazen die op deze wijze waren gemaakt, toonden aan, dat inderdaad strikt aseptische omstandigheden waren bereikt.

De eiwitafbraak en de ontwikkeling van geuren smaak in de aseptisch bereide Goudse kaas waarin geen andere bacteriële enzymen werkzaam waren geweest dan die van een goede Streptococcus cremoris-stam als zuursel, bleken normaal te zijn voor dit type kaas uit gepasteuriseerde melk. Kaas vrij van bacteriële enzymen verschilde gedurende de rijping aanzienlijk van kaas met zuursel-streptokokken. In de kaas die geen bacteriële enzymen bevatte, werden vrijwel geen aminozuren gevonden gedurende zes maanden rijpen en er ontwikkelde zich totaal geen kaasgeur en -smaak. Er wordt geconcludeerd, dat de enzymen van de zuurselbacteriën uiterst belangrijk zijn voor de rijping van Goudse kaas.

Verder werd gevonden, dat er een opmerkelijk verschil bestaat tussen de activiteit van verschillende S. cremoris-stammen in hun vermogen tot eiwitafbraak in de diepte, wat betreft de produktie van geur- en smaakstoffen en wat betreft het ontstaan van smaakgebreken in de kaas. Wat betreft de eiwitafbraak en de ontwikkeling van

kaasgeur en -smaak, bleken de verschillen tussen een goed één-stam-zuursel en meer-stam-zuursels gering. Er wordt met nadruk op gewezen, dat bij de vergelijking van de activiteiten van één-stam ten opzichte van meer-stam-zuursels, meer aspecten in acht dienen te worden genomen.

Lactobacillen die normaliter in grote aantallen voorkomen in Goudse kaas gedurende de rijping, bleken niet nodig voor het gewenste rijpingsproces. Verder werd gevonden, dat deze bacteriën zelfs ongewenst kunnen zijn in de kaas, omdat ze smaakgebreken kunnen veroorzaken.