MILK FOULING IN HEAT EXCHANGERS

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MILK FOULING IN HEAT EXCHANGERS

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Stellingen

1. Bij vervuiling van een roestvaststalen wand tijdens verhitten van melk is eiwit en niet calcium de eerste component die neerslaat.
   Dit proefschrift

2. Lalande et al. suggereren dat het calcium dat samen met eiwit neerslaat op een verhitte wand afkomstig is van het micellair calciumfosfaat in de caseïnemicellen. Dit is slechts in zeer beperkte mate het geval; het overgrote deel is gebonden aan neergeslagen wei-eiwitdeeltjes.
   Lalande et al., *J. Dairy Res.* 51 (1984) 557-568; dit proefschrift

3. Bij de verwijdering van aangebrande melk met natronloog dringen de hydroxyl-ionen het vuil niet louter door middel van diffusie binnen zoals door Gallot-Lavallee et al. wordt aangenomen, maar wordt het binnendringen bevorderd door scheuren die ontstaan in de vuillaag.
   Gallot-Lavallee et al., *Ind. Alim. et Agr.* 105 (1988) 445-456; dit proefschrift

4. De in de praktijk veel voorkomende gedachte dat bij het gebruik van een hogere concentratie alkalisch reinigingsmiddel ook een beter reinigingsresultaat wordt verkregen, is onjuist.
   Dit proefschrift

5. Bij verhitting van melk-, bloed- en eiprodukten is er een grote overeenkomst in vervuilingsgedrag.

6. Gezien de grote variatie in het adsorptiegedrag van de segmenten waaruit melkeiwitten zijn opgebouwd, is het niet verbazend dat er nog geen geschikte coating is gevonden die vervuiling voorkomt.

7. Daar bij de swabmethode slechts een beperkt deel van de op een oppervlak aanwezige bacteriën wordt opgenomen, moeten kiemgetallen die op deze wijze zijn verkregen, met grote omzichtigheid worden geïnterpreteerd.

8. De oplossing voor het wegwerken van het pekeloverschot door ingedampte pekel terug te voeren in het pekelbad zal niet leiden tot een noemenswaardige verhoging van de kaasopbrengst.

9. De spoelproef ter microbiologische controle van de reiniging van een RMO zou niet in aansluiting op de reiniging uitgevoerd moeten worden, maar vlak voordat de RMO weer gebruikt gaat worden voor het inzamelen van melk.

10. Aangezien de fysiologische conditie van topsporters al 25 jaar niet wezenlijk meer is veranderd en daar genetische manipulatie snel terrein wint, dient het Olympisch Comité zich voor te bereiden op controles van al dan niet genetisch gemanipuleerde topsporters.
    Noorderlicht, VPRO, Ned. 3, 4 maart 1996

11. Wie tegen zijn verlies kan, dient van wedstrijdsport te worden uitgesloten.
    Vrij naar Tim Krabbé in "De Renner". Amsterdam: Bert Bakker, 1987, 8e druk, p.127

12. Ook voor de Westerse mens zou het efficiënter zijn om de bagage op het hoofd te dragen in plaats van op de rug.
    Volkskrant, 13 mei 1995

Stellingen bij het proefschrift "Milk fouling of heat exchangers" van Th.J.M. Jeurnink, te verdedigen op 7 mei 1996.

ter nagedachtenis aan mijn ouders

Abstract

Jeurnink, Th.J.M. (1996), Milk fouling in heat exchangers. Ph. D. thesis. Wageningen Agricultural University, The Netherlands (144 pp., English and Dutch summaries).

Keywords:
fouling, cleaning, heat exchangers, evaporators, milk, whey, β-lactoglobulin, denaturation, calcium phosphate, precipitation, air bubbles, heat stability

The mechanisms of fouling of heat exchangers by milk were studied. Two major fouling mechanisms were indentified during the heat treatment of milk: (i) the formation and the subsequent deposition of activated serum protein molecules as a result of the heat denaturation; (ii) the precipitation of calcium phosphate as a result of the decreased solubility of this salt upon heating. Both foulants are formed in the bulk of the solution and are transported to the surface, where they can be deposited.

If the stability of milk is lowered, e.g. by lowering the pH, coagulation of casein micelles can cause extreme fouling. A further cause of fouling is air bubbles, which arise in the milk on heating and stick to the stainless steel wall; they appear to act as nuclei for the formation of protein deposit.

Fouling in heat exchangers can be reduced by controlling the formation of activated serum protein molecules and by preventing the precipitation of calcium phosphate. Various ways to achieve this are given.

Once formed, milk deposits can readily be removed by alkaline cleaning followed by acid cleaning under the right conditions.

Contents

Introduction

1 Introduction to fouling

During heating of milk in a heat exchanger an undesirable deposit is formed on the surface of the stainless steel wall. This process of deposit formation is called fouling. The deposit mainly consists of proteins and minerals. Burton [1] distinguished two different types of deposit (A and B), depending on the intensity of heating. Each type has its own specific character, as summarized in Table 1. Although fouling is an everyday concern to the dairy industry, not much is known about its causes since many product and process parameters play a role. It is known that a direct link exists between fouling and the heat-denaturation of serum proteins when dairy liquids are processed at temperatures above 70 °C. Another major contribution to fouling is the precipitation of minerals as a result of the decreasing solubility of calcium phosphates with increasing temperature. Calcium phosphate may precipitate directly on the stainless steel wall or it may contribute to a deposit via precipitation on depositing protein aggregates.

In the deposits four classes of components can be distinguished. Each has its specific character and requires a different cleaning method, as indicated in Table 2. Alkaline cleaning solutions are commonly used for the removal of proteins and fat, whereas acid cleaning solutions are used, mostly as a second step, to remove calcium salts. The usual procedure for cleaning of heat-treatment equipment in the dairy industry is Cleaning in Place (CIP), so that dismantling of the equipment can be avoided.

2 Fouling problem

Fouling of heat exchangers by dairy liquids is a major problem in the dairy industry. The costs of temporarily halting a process and cleaning are very high. Fouling causes the following undesirable effects:

- a decrease in heat-transfer coefficient;
- an increase in pressure drop, hence a decrease in pumping efficiency;
- loss of product remaining on the heated wall;
- contamination of the processed product by loosened deposits.

Fouling may also enhance the adsorption of bacteria to a surface [2] and by encapsulation in the deposit these microorganisms may become protected against cleaning agents.

Table 1. Types of milk deposits after Burton [1].

Type	Temperature	Characterization
A	70 - 90 °C	spongy, creamy white, wet, 60 % protein, 30 - 40 % minerals
B	110 - 140 °C	compact, crystalline, glassy, 20 % protein, 80 % minerals

Table 2. Deposit components and their specific characteristics, modified and extended after [7].

Component to be cleaned	Solubility of component	Ease of removal when cleaning non-heat-damaged deposit	Effect of heat-induced changes in the deposit component
Sugars	Good in water	Good	Caramelisation: more difficult to clean
Fat	Poor in water, alkaline and acid solutions without surface-active agents	Good with alkaline detergent solutions	Polymerisation: more difficult to clean
Protein	Poor in water, medium in acid solutions, good in alkaline solutions	Poor with water, better with alkaline solutions	Denaturation: more difficult to clean
Mineral salts	Depending on the specific kind, good to poor in water	Reasonably good in acid solutions	Precipitation: reasonably good in acid solutions

To remove a deposit it is common practice to clean the heat-treatment equipment for dairy liquids at least once a day. The extra processing costs as a results of fouling can be divided into [3]:

(1) increased capital costs for oversizing of the heat-transfer equipment, and its early replacement because of a shorter technical life-span;
(2) increased energy (fuel) costs in operation, due to decreasing heat-transfer coefficient and increasing pressure;
(3) costs for product losses, due to product remaining on the wall of the heat exchanger and to plant shut-down for cleaning and start-up procedures;
(4) high maintenance costs, due to the need for a CIP-installation and equipment for effluent treatment;
(5) costs for energy, water and detergents needed for cleaning;
(6) costs of downtime for cleaning, during which no production can take place.

The costs of fouling of heat exchangers for the dairy industry in the Netherlands were estimated in 1986 as follows [4]:

- additional energy costs: 9.3 Mfl/year;
- dairy effluent disposal costs: 22 Mfl/year;
- costs due to product losses: 55.5 Mfl/year.

In summary, fouling is a costly affair. Hence, it would be of great benefit to solve or at least to reduce the problems it causes.

3 Purpose of the study

Fouling has been studied for many years; recent reviews are by Lalande et al. [5] and Fryer et al. [6]. It is clear from these reviews that both the process of serum protein denaturation and that of the precipitation of calcium phosphate play a key role in fouling. Several researchers have studied the link between denaturation of serum proteins and fouling, but there is no agreement on what kinetic data for the denaturation should

be taken. Fouling is not simply a mass-transfer-controlled process, and it is not yet clear whether it is controlled by a surface or by a bulk reaction. The mechanisms by which proteins and minerals are deposited on a stainless steel surface are not precisely understood either. There is also no agreement on which component adsorbs first, protein or mineral.

The aim of this work was to understand the various phases in the process of fouling. By studying the processes taking place in milk during heating both in the bulk and at the heating surface it was hoped to reveal the mechanisms of fouling. Only by knowing these mechanisms can one develop tools to control and to reduce the problem of fouling and devise more effective cleaning procedures.

4 Outline of the thesis

In the first two papers the effect of heating on the behaviour and stability of casein micelles in the bulk of the milk is described. In the third, fourth and fifth papers, experiments are presented in which various conditions were altered in order to establish whether the stability of the casein micelles in milk is related to its fouling behaviour. In this context we examined the effect of proteolysis, change in calcium ion activity and air content in the milk on fouling. In paper six the effect of serum protein concentration on fouling is examined. The seventh paper deals with the processes taking place at the heating surface with emphasis on the influence of the degree of serum protein denaturation on the rate of deposition. The eighth paper analyzes how the removal of a deposit takes place and under what conditions it is possible to realize quick and effective cleaning.

The last paper reviews the reactions related to the fouling process. This paper concludes with recommendations to the dairy industry on measures to prevent or reduce fouling and on how to clean effectively.

5 References

1. H. Burton. Reviews of the progress of dairy science. Section G. Deposits from whole milk in heat treatment plant - a review and discussion. *J. Dairy Res.* 35(1968)317-330.
2. B. Carpentier & O. Cerf. Biofilms and their consequences, with particular reference to hygiene in the food industry. *J. Appl. Bacteriol.* 75(1993)499-511.
3. C. Sandu & R.K. Singh. Energy increase in operation and cleaning due to heat exchanger fouling in milk pasteurization. *Food Techn.* 45(1991)(Dec)84-91.
4. D.W. Brinkman. Energie- en milieuaspecten van het reinigen van verwerkingsapparatuur. In: G.J.M. Wolbers et al., Milieuaspecten van de bereiding van voedingsmiddelen [Environmental aspects of food manufacture]. NIZO-Mededeling M20. Ede (Netherlands): Netherlands Institute for Dairy Research, 1986, pp. 61-76.
5. M. Lalande, F. Rene & J.P. Tissier. Fouling and its control in heat exchangers in the dairy industry. *Biofouling* 1(1989)233-250.
6. P. Fryer, M.T. Belmar-Beiny & P.J.R. Schreier. Fouling and cleaning in milk processing. In: Heat-induced changes in milk (P.F. Fox, ed.). Brussels: International Dairy Federation, 1995, PP. 364-395.
7. Design and use of CIP systems in the dairy industry. *IDF Doc.* 117(1979).

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Changes in milk on mild heating: turbidity measurements

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Keywords: skim milk, denaturation of whey protein, fouling, turbidity

Summary

Changes in casein micelle size due to a heat treatment in conditions in which fouling occurs in heat exchangers were determined by turbidity measurements. No increase in turbidity was found if the heating temperature of skim milk did not exceed 60 °C, nor after heating whey protein-free milk. These results show that the denaturation of β-lactoglobulin (βLg) is responsible for the increase in micelle size after heating. This is achieved by the formation of complexes of βLg with κ-casein at the micelle surfaces. Calcium concentration in the milk influenced the change in micelle size upon heating, probably by its effect on the stability of the complex of βLg and κ-casein.

These results support the view that casein micelles together with the associated βLg deposit on the stainless steel wall.

1 Introduction

When milk undergoes heat teatment, an undesired deposit is formed on the stainless steel wall of the heat exchanger. As a result frequent shut-down of the equipment is required for cleaning. A better understanding of the fouling mechanism may lead to prolonged running times and to improved cleaning processes. Several researchers (1, 2, 3) have found a strong correlation between the amount of deposit and the degree of denaturation of β-lactoglobulin (βLg). Furthermore the ratios of the different caseins found in the deposit layer indicated that they appear in the form of micelles (4). It seems that on heating milk, βLg denatures and deposits on the stainless steel wall (5) as well as on the casein micelles (6). βLg may act as a 'sticking agent', resulting in the deposition of casein micelles and βLg together on the stainless steel wall. On heating milk, calcium phosphate also precipitates on the stainless steel wall or associates with the micelles; it may also act as 'sticking agent' between micelles

(7, 8, 9); consequently it may induce deposition.

On heating milk, the size of the micelles increases as was indeed found by Morr (10), Creamer & Matheson (11) and Singh (12). Heating to temperatures of about 90 °C causes substantial aggregation of whey proteins but produces only minor changes in the dimensions of casein micelles (13). More drastic heat treatments, such as UHT, cause aggregation of casein micelles. In particular, small micelles aggregate with larger micelles or into them (14, 15). Much of the work in the literature has been done on intensive heating of milk, while fouling in heat exchangers occurs under milder conditions. Only Dalgleish (16) reported the formation of βLg/κ-casein aggregates on heating at 75-90 °C for 1-20 min but did not measure the effect on size of casein micelles.

In this study, the extent to which the size of micelles is changed on heating skim milk, under conditions in which fouling occurs (0.5-5 min at 90 °C), was investigated. The size changes were related quantitatively to the denaturation of βLg. Furthermore the influence of the concentration of βLg and of calcium were determined. The changes in particle size were measured by means of turbidity.

2 Theory

2.1 Turbidity measurements

Turbidity τ is the attenuation of a light beam by scattering (or absorption) when it passes through a sample. It is defined as follows:

$$\tau = l^{-1} \ln (I_0/I_t) \qquad (1)$$

where I_0 is the incident light beam intensity, I_t, the transmitted intensity and l the length of the light path in the sample. Increased scattering increases turbidity, i.e. less light is transmitted. A simple conservation equation relates turbidity and scattering.

In a non-absorbing dispersion of uniform small particles, the amount of light scattered from a light beam of intensity I_0 is given in the Rayleigh approximation (17). The so-called Rayleigh ratio $R(K)$ (where K is the scattering wavevector) contains the product of two scattering functions. The form factor $P(K)$ accounts for the interference of light scattered from different parts within one particle. The structure factor $S(K)$ accounts for the interference of light scattered from different particles. This last part again depends on polydispersity and particle interactions. Jansen (18) and Penders (19) have treated the problem of polydispersity and particle interaction quite elaborately and

checked the validity of their approach. Since I am interested in changes rather than in absolute values, suffice it to use a straightforward approach in which polydispersity is neglected, but where any particle interactions are accounted for. Inserting a series expansion for both $P(K)$ and $S(K)$ in the Rayleigh approximation and integrating over the Rayleigh ratio in the conservation equation yields

$$\tau = HcM\left[1 - \frac{8}{3}\left(\frac{\pi n R_g}{\lambda_0}\right)^2\right]\exp\left(-\varphi\left[8 - \frac{2}{\sigma_B} - \frac{8}{5}\left(4 - \frac{5}{3\sigma_B}\right)\left(\frac{\pi n d}{\lambda_0}\right)^2\right]\right) \quad (2)$$

with

$$H = (16/3)\pi K^* \quad (3)$$

$$K^* = 2\pi^2 n^2 (dn/dc)^2 / (N_A \lambda_0^4) \quad (4)$$

where c is mass concentration, M is molar mass of the particles, n is refractive index of the sample, R_g is optical radius of gyration of the particles, λ_0 is wavelength of the light in vacuum, Φ is volume fraction of particles, σ_B is Baxter's stickiness parameter, d is diameter of the particles and N_A is Avogadro number. In the limit of 'zero interaction' or equivalently of high dilution and in the limit of infinite wavelength, this reduces to the well-known result:

$$\tau = HcM \quad (5)$$

In fact, this is the result given by van Boekel et al. (20). From their paper, it is clear that van Boekel et al. (20) realized that they were neglecting polydispersity, finite size of the particles and, more importantly, particle interactions. I will determine Baxter's parameter σ_B in order to account for possible interaction.

2.2 Reaction kinetics

Let us assume that the change in turbidity is related to the denaturation of βLg and approximate this reaction as a first-order reaction. Then the concentration of native βLg is given by

$$C/C_0 = \exp(-kt) \quad (6)$$

where C_0 is initial concentration of native βLg, C_t concentration of native βLg after time t and k rate constant.

Equation 6 can also be written as

$$\ln (C/C_0) = -kt \tag{6a}$$

A plot of ln (C/C_0) against t gives a straight line with slope $-k$.

The temperature dependence of the rate constant was derived from the Arrhenius equation according to

$$k = k_0 \exp\ (-\frac{E_A}{RT}) \tag{7}$$

where k_0 is rate constant in s^{-1} when $1/T = 0$, E_A activation energy in J/mol, R universal gas constant, 8.314 J/mol K, and T absolute temperature in K.

By plotting ln (k) against $1/T$, activation energy can be calculated from the slope of the line.

The kinetics of denaturation of βLg have been studied extensively. Through use of different heating conditions (rate of heating up influences the denaturation), different chemical analysis methods, different βLg concentrations and different batches of milk, the reported kinetic results are not in good agreement. In experiments with skim milk, Luf (21) found a first-order reaction, Dalgleish (16) a pseudo-first-order, Lyster (22) a second-order reaction and Dannenberg & Kessler (23) found the best fit for reaction order $n = 1.5$. De Wit & Klarenbeek (24) found that the two steps of denaturation do not have the same reaction order: the unfolding is a first-order reaction and the subsequent aggregation is of order $n = 1.8$.

Closer consistency is obtained for activation energy (21, 22, 23, 24). The Arrhenius plot shows a break at 90 °C. For temperatures below 90 °C, activation energy was 250-280 kJ/mol. For temperatures above 90 °C, it was 30-55 kJ/mol.

3 Materials and methods

The milk used in this study was skim milk obtained from the institute's experimental dairy. Whey-protein-free skim milk (WPFM; βLg 0.23 g/l and α-lactalbumin 0.11 g/l left) was obtained by reconstituting low-heat WPFM-powder in demineralized water. This powder was prepared by microfiltration of skim milk (S.C.T., Bazet, France, Membralox 7P19-40, 1.4 m^2, pore size 0.5 µm) followed by diafiltration with UF-permeate and spray-drying. Powder of βLg (92% of total protein is βLg) was prepared by the method of Maubois (25)

and then freeze-dried. The powder was dispersed together with WPFM-powder in water to reach a final concentration of βLg in the milk of 3.2 g/l or 6.4 g/l. Simulated milk ultrafiltrate (SMUF) was prepared as described by Jenness & Koops (26).

Calcium concentration was lowered by ion exchange; the milk sample was mixed with the cation-exchange resin Duolite C464 (90:1 w/w), which has a preference for exchanging calcium, though some magnesium was also exchanged. After stirring for 1 h at room temperature, Duolite was separated from the milk by filtration. The treated milk contained 86.2% of the original calcium concentration. Higher concentrations of calcium phosphate (126.6% and 111.9% relative to the original concentration of calcium and phosphate, respectively) were made by adding solutions of calcium and phosphate salts as described by Schmidt et al. (27). Calcium was determined by the method of Raadsveld (28); phosphate was determined by the method of Griswald et al. (29). All treated milks were adjusted to pH 6.7 by addition of NaOH 0.5 mol/l or HCL 0.5 mol/l. The treated milks were then kept in the cold overnight and slowly warmed to room temperature before use. Before heating, pH was measured again and adjusted if necessary. After heating, there was a slight drop in pH (6.65); the decrease in pH as such did not influence the measured turbidity.

The milk samples were kept at room temperature for at least 30 min before heating. Then copper tubes were filled with 20 ml of the milk samples. The copper tubes were 27.0 cm long and 1.4 cm inner diam. Both ends were closed with screw caps. For heating, the tubes were put in a circulating oil bath of the required temperature and kept there for a fixed time. In Figure 1, the temper-

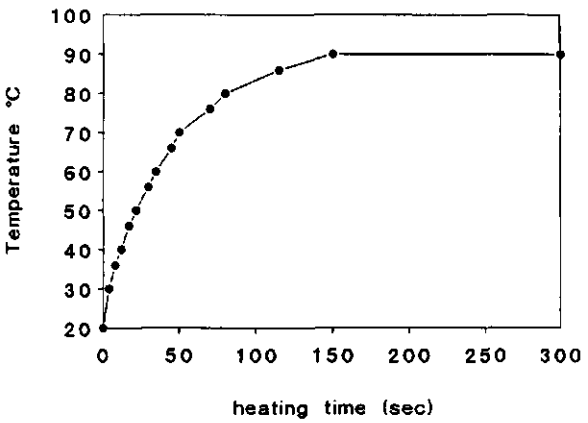

Fig. 1. Temperature in test-tube in an oil bath at 92 °C.

ature inside the tube measured with a thermocouple is shown for an oil bath temperature of 92 °C. Immediately after heating, the tubes were cooled under running tap water. The turbidity of each sample was measured at room temperature after 30 min with a Zeiss spectrometer by the method of van Boekel et al. (8), at a wavelength of 1150 nm in 1-mm cuvettes, accuracy ±1.21%. Although small changes in turbidity were observed, it was possibly, by measuring every milk sample in duplicate and by repeating the turbidity measurements at least three times, to obtain statistical significance in the differences.

4 Results and discussion

4.1 Turbidity of milk heated at different temperatures

Skim milk was heated to different temperatures and, after various heating times (t) (at $t = 0$, the tube was immersed in the oil bath), it was cooled before turbidity measurement. For some samples, an inexplicable initial decrease in turbidity was observed. To avoid this effect, turbidity of the milk after 50 s of heating was taken as τ_0. Turbidity of the milk after time t (seconds) of heat treatment is τ_t. As shown in Figure 2, there was an increase in the ratio τ_t/τ_0 with time of heating. This increase was larger, the longer the heating and the higher the heating temperature. Heating milk to 60 °C hardly increased the turbidity. The increase upon heating to 75 °C or higher probably resulted from a combined effect of denaturation of βLg and subsequent self-aggregation or complexing with the casein micelles (6), and from the precipitation of calcium phosphate on the micelles (9).

4.2 Turbidity of model solutions heated at 90 °C for different holding times

Since a change in turbidity is an overall consequence of changes in milk during heating, model solutions were used to determine the influence of βLg and calcium phosphate separately and together. The turbidities of different model solutions before and after heating at 90 °C for 120 and 300 s are shown in Table 1. SMUF is a clear solution, so there was no scattering. After heating SMUF, the precipitated calcium phosphate caused an increase in turbidity. Also the aggregation of βLg in SMUF caused an increase in τ, especially after 300 s of heating. Since the increase in τ after heating WPFM was smaller than after heating SMUF, part of the calcium phosphate must have associated with the casein micelles, so that it influenced the turbidity only by changing the refractive index of the micelles. Since aggregation of βLg in the presence of casein

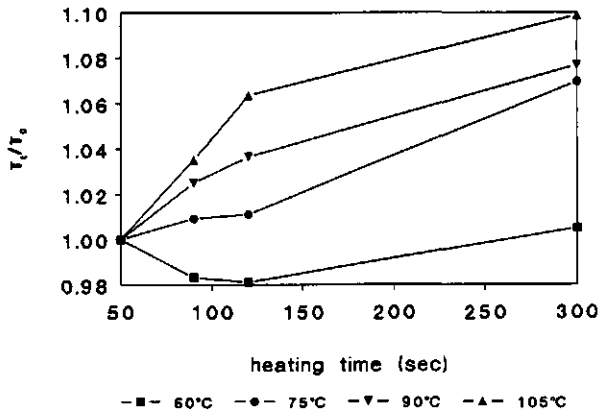

Fig. 2. Influence of heat treatment of skim milk on turbidity.

Table 1. Turbidity (τ) of solutions before and after heating at 90 °C.

Sample	τ, 0 s (m^{-1})	τ, 120 s (m^{-1})	τ, 300 s (m^{-1})
SMUF	0.0	2.3	6.9
SMUF + βLg 3.2 g/l	9.2	16.1	52.9
WPFM	177.3	179.6	181.9
WPFM + βLg 3.2 g/l	172.7	179.9	185.0
Skim-milk	172.7	179.6	186.5

micelles gave a much smaller increase in τ than observed in SMUF, βLg must have associated with the micelles. Another effect may be that the micelles competed with βLg aggregates for βLg molecules, resulting in the formation of much smaller βLg-aggregates whose contribution to the turbidity was relatively small. In summary, I have shown that in the presence of casein micelles the increase in turbidity after heating, due to the denaturation of βLg and the precipitation of calcium phosphate, is much smaller than in solutions without any casein micelles. Therefore βLg and calcium phosphate associate with the micelles.

4.3 Influence of βLg concentrations in heated milk on turbidity

Figure 3 shows the turbidity measurements of WPFM with different amounts of βLg added after heating at 90 °C. (In reconstituted milk, the initial decrease in turbidity was not observed, so τ_0 was taken here as the turbidity of the unheated sample.) The ratio τ_t/τ_0 increased with heating time and with the

amount of βLg by denaturation of βLg, since at 60 °C in the presence of (native) βLg hardly any increase in the ratio τ_t/τ_0 was seen (Figure 2). The results in Table 1 indicate that the aggregates formed by denatured βLg molecules associated with the micelles. This is in agreement with the results of Creamer *et al.* (30) and Mottar *et al.* (31), who found that micelles of heated milk had an irregular surface with appendages or protuberances caused by the heat-induced complexes of βLg and κ-casein at the surface of the casein micelles. Such changes at the surface of the micelles on heating would lead to the change in turbidity measured in the NIZO experiments. Calculation indicated that there was sufficient denatured whey protein in heated milk to associate with the micelles and to cause an increase in turbidity of 8% as found in Figure 2 (Appendix).

Changes in τ can also be caused by interactions between the particles, which

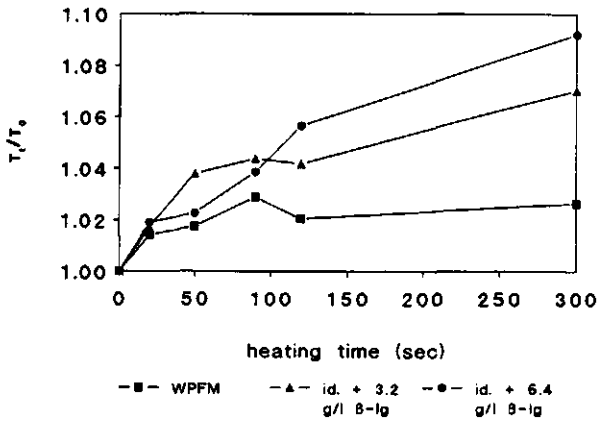

Fig. 3. Influence of β-lactoglobulin concentration on turbidity.

Table 2. Influence of calcium concentration on turbidity (τ/τ_0) after heating WPFM + β-lactoglobulin 3.2 g/l at 90 °C

Concentration relative to skim milk (%)		τ/τ_0 at heating time of		
Ca	PO_4	0 s	120 s	300 s
86	100	1	1.023	1.044
100	100	1	1.042	1.071
125	111	1	1.052	1.089

is accounted for in Equation 2 by Baxter's stickiness parameter, σ_B. In order to check whether the increased turbidity resulted from changed interactions between micelles or from changes in size, one may repeat the measurements with varying volume fraction. However I checked on interactions by viscosity measurements (Th. J. M. Jeurnink, unpublished results), in which changes in milk after heating (5 min at 85 °C) proved to be related to an increase in volume fraction and not to a change in interaction between the micelles.

4.4 Influence of concentration of calcium phosphate in heated milk on turbidity

Before the solutions had reached 70 °C (t = 50 s), the temperature at which βLg denatures, τ_t/τ_0 increased by 2-4% (Figure 3). This can be explained by a change in refractive index difference or by an increase in the number of particles because of the fast precipitation of calcium phosphate on heating; 60% of the calcium was precipitated after 10 s at 90 °C (32).

Table 2 shows the ratio τ_t/τ_0 after heating for WPFM + βLg at 3.2 g/l with different calcium concentrations. The increase in τ_t/τ_0 after heating at 90 °C for 120 s was related to the amount of calcium present. This can be explained as described above. The further change in the turbidity (t = 300 s) cannot be explained by the precipitation of calcium phosphate only: there must be an indirect effect of the precipitated calcium phosphate. Several researchers (6,33) found that the heat-induced association of βLg with casein micelles increased and the complex of βLg with κ-casein was stabilized in the presence of Ca^{2+}. Furthermore most workers assume that the calcium phosphate associated with the casein micelles, rendering them less stable; the calcium phosphate would then act as a 'sticking agent' (7, 8, 9) and cause aggregation of micelles. However van Dijk (9) stated that this happened only at temperatures above 90 °C.

4.5 Reactions kinetics

The experimental design was chosen to simulate the temperature profile in an industrial heat exchanger in order to make a relation with the fouling reaction. I determined the reaction order, though I realized that the design was not ideal for that purpose. A first-order reaction equation best fitted the results obtained at 75, 90 and 105 °C (Figure 2). Equation 6a can be rearranged to

$$\ln (1 - P/P_\infty) = -kt \qquad (8)$$

where $P = \tau_t/\tau_0 - 1$ is the fractional change in turbidity and $P_\infty = \tau_\infty/\tau_0 - 1$ is the

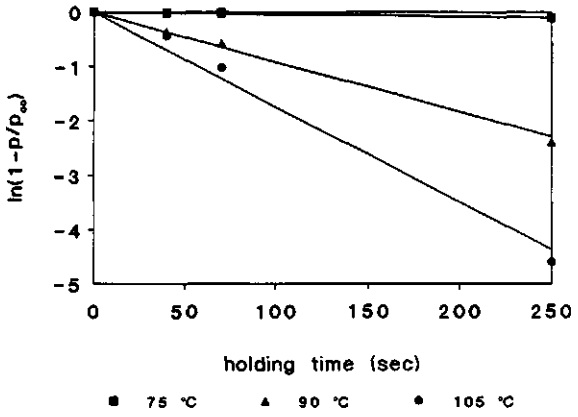

Fig. 4. First-order fit of data from Figure 2.

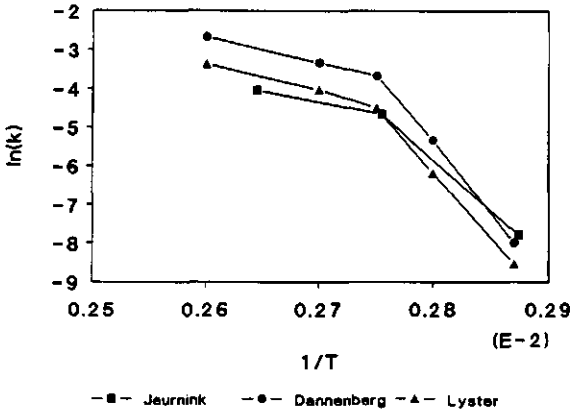

Fig. 5. Arrhenius plot of data from Figue 4.

maximum fractional change in turbidity.

Maximum change in turbidity in the experiments at 75, 90 and 105 °C was reached with complete denaturation of βLg, which was estimated by using the experimental data of Dannenberg (23). In Figure 4, the results were plotted on a semi-logarithmic scale against holding time (heating time minus 50 s, the time to reach 70 °C at which denaturation starts). The straight lines suggest a first-order process. This confirms that the increase in turbidity was controlled by denaturation of βLg, which is reported also to be first-order, although there is no general agreement on the order of denaturation (16). From the slope of the lines in Figure 4, the rate constants (k) were determined and in Figure 5, ln k was plotted against $1/T$ (Equation 7). A line typical for the denaturation

of βLg was found (namely a break at 90 °C) and the activation energy, 217 kJ/mol at 75-90 °C and 49 kJ/mol at 90-105 °C, agrees well with that found by other authors, of which the results of Lyster (22) and Dannenberg (23) are also shown in Figure 5. The results of the kinetic calculations support the conclusion that the denaturation of βLg is responsible for the change in micelle size after heating.

5 Conclusions

The turbidity of heated skim milk increases with temperature and time of heating. No significant increase in turbidity was found in skim milk after heating to 60 °C or after heating to 90 °C in the absence of βLg. So denaturation of βLg is responsible for the change in micelle size with heating, by the association of βLg with casein micelles, probably through the formation of complexes of βLg with κ-casein at the micelle surfaces. Milk contains sufficient denatured whey protein to associate with the micelles to account for the measured increase in turbidity. If the reaction takes place close to the stainless steel wall of the heat exchanger, it is very likely that casein micelles together with the associated βLg would deposit on the wall. This gives a better understanding of the fouling mechanism and supports the view that casein micelles are involved in the fouling reaction (4).

The precipitation of calcium phosphate caused a 2-4% change in turbidity at the beginning of the heating of milk. Also the further change in turbidity was influenced by calcium concentration; the complex of βLg with κ-casein is probably stabilized in the presence of Ca^{2+}.

Acknowledgment

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Appendix

Calculation of the observed increase in turbidity after heating

To show that there are sufficient whey proteins present in milk to cause the increase in turbidity by their association with casein micelles, let us assume that native whey proteins do not contribute to scattering because of their small size and that the refractive index of associated whey proteins does not differ from that of casein micelles.

After heating (300 s at 90 °C) skim milk, there was an increase in τ/τ_0 of 8% (Figure 2). According to Equation 5, τ/τ_0 is proportional to $(d/d_0)^3$, as was also shown by van Boekel et al. (20). This means that the diameter of the micelles increased on average by 2.6%. Heating micelles at 90 °C caused no significant changes in their dimensions (13), so the increase in size must be caused by association of whey protein to the micelles. On average, 1 l of milk contains 10^{17} casein micelles (35) with a volume-average diameter of 120 nm. Increasing this diameter by 2.6% to 123.1 nm means that the whey proteins associated with the casein micelles occupy a volume fraction of 0.007 2.

Snoeren (34) found in ultracentrifuge experiments that native and 100% denatured whey protein had a volume fraction of 0.006 7 and 0.019 5, respectively. Since the product of refractive index difference and specific volume of the whey proteins are almost constant (so that no increase in turbidity would be found after denaturation), the maximum increase in turbidity should correlate with the volume fraction of 0.006 7, which it indeed does.

Milk is not monodisperse. In a polydisperse system, even less associated whey protein would be needed for an increase of 8% in turbidity. The denatured whey proteins not associated with the casein micelles may be present in the serum of the milk as small aggregates, whose contribution to turbidity is negligible.

The above calculation makes oversimplifications but serves to support the view that the increase in turbidity after heating milk must be caused by association of whey proteins with casein micelles.

References

1. M. Lalande, J. P. Tissier & G. Corrieu, Fouling of heat transfer surfaces related to β-lactoglobulin denaturation during heat processing of milk. *Biotechnology Progress* 1 (1985) 131-139.
2. J. Hiddink, M. Lalande, A. J. R. Maas & A. R. Streuper, Heat treatment of whipping cream. I. Fouling of the pasteurization equipment. *Milchwissenschaft* 41 (1986) 542-546.
3. Th. J. M. Jeurnink, D. W. Brinkman & A. D. Stemerdink, Distribution and composition of deposit in heat exchanges. In: H. G. Kessler & D. B. Lund (Eds), Proc. Third Int. Conference on Fouling and Cleaning in Food Processing, Prien (1989) 25-36.
4. Th. J. M. Jeurnink, Effects of proteolysis in milk on fouling in heat exchangers. *Neth. Milk Dairy J.* 45 (1991) 23-32.
5. Th. Arnebrant, K. Barton & K. Nylander, Adsorption of α-lactalbumin and β-lactoglobulin on metal surfaces versus temperatures. *J. Colloid Interface Sci.* 119 (1987) 383-390.
6. P. Smits & J. H. Van Brouwershaven, Heat-induced association of β-lactoglobulin and casein micelles. *J. Dairy Res.* 47 (1980) 313-325.
7. R. J. Carroll, P. Thompson & P. Melnychyn, Gelation of concentrated skim milk: electron microscopic study. *J. Dairy Sci.* (1971) 1245-1252.
8. M. A. J. S. van Boekel, J. A. Nieuwenhuijse & P. Walstra, The heat coagulation of milk. 1. Mechanisms. *Neth. Milk Dairy J.* 43 (1989) 97-127.

9. H. J. M. van Dijk, The properties of casein micelles. 3. Changes in the state of the micellar calcium phosphate and their effects on other changes in the casein micelles. *Neth. Milk Dairy J.* 44 (1990) 125-142.
10. C. V. Morr, Protein aggregation in conventional and ultrahigh-temperature heated skim milk. *J. Dairy Sci.* 52 (1969) 1174-1180.
11. L. K. Creamer & A. R. Matheson, Effect of heat treatment on the proteins of pasteurized skim milk. *N.Z.J. Dairy Sci. Technol.* 15 (1980) 37-49.
12. H. Singh, Effects of high temperatures on casein micelles. *N.Z.J. Dairy Sci. Technol.* 23 (1988) 257-273.
13. H. Singh & P. F. Fox, Heat-induced changes in casein. *Bulletin of the IDF* 238 (1989) 24-30.
14. T. Aoki, C. Hatanaka & T. Imamura, Large casein micelles and soluble casein in heated concentrated whey protein-free milk. *Agr. Biol. Chem.* 41 (1977) 2349-2355.
15. K. S. Mohammad & P. F. Fox, Heat-induced microstructural changes in casein micelles before and after heat coagulation. *N. Z. J. Dairy Sci. Technol.* 22 (1987) 191-203.
16. D. G. Dalgleish, Denaturation and aggregation of serum proteins and caseins in heated milk. *J. Agr. Food Chem.* 38 (1990) 1995-1999.
17. H. C. van de Hulst, Light scattering by small particles. New York, Dover Publications, Inc. (1981).
18. J. W. Jansen, C. G. de Kruif & A. Vrij, Attractions in sterically stabilized silica dispersions. III. Second virial coefficient as a function of temperature, as measured by means of turbidity. *J. Colloid Interface Sci.* 114 (1986) 492-500.
19. M. H. G. M. Penders, A turbidity study on colloidal silica particles in concentrated suspensions using the polydisperse adhesive hard sphere model. *J. Chem. Phys.* 93 (1990) 3704-3711.
20. M. A. J. S. Van Boekel, J. A. Nieuwenhuijse & P. Walstra, The heat coagulation of milk. 2. Kinetic studies. *Neth. Milk Dairy J.* 43 (1989) 129-146.
21. W. Luf, On kinetics of thermal denaturation of α-lactalbumin and β-lactoglobulin A and B in skim milk between 70 °C and 95 °C by means of high-performance liquid chromatography (HPLC). *Österreichische Milchwirtschaft* 43 (1988) 7-12.
22. R. Lyster, The denaturation of α-lactalbumin and β-lactoglobulin in heated milk. *J. Dairy Res.* 37 (1970) 233-243.
23. F. Dannenberg & H. G. Kessler, Application of reaction kinetics to the denaturation of whey proteins in heated milk. *Milchwissenschaft* 43 (1988) 3-7.
24. J. N. de Wit & G. Klarenbeek, Technological and functional aspects of milk proteins. In: C.A. Barth (Ed.), *Milk proteins*, pp. 211-222. Steinkopf Verlag Darmstadt (1989).
25. J. L. Maubois, A. Pierre, J. Fauquant & M. Piot, Industrial fractionation of main whey proteins. *Bulletin of the IDF* 212 (1987) 154-159.
26. R. Jennes & J. Koops, Preparation and properties of a salt solution which simulates milk ultrafiltrate. *Neth. Milk Dairy J.* 16 (1962) 153-164.
27. D. G. Schmidt, J. Koops & D. Westerbeek, Properties of artificial casein micelles. 1. Preparation, size distribution and composition. *Neth. Milk Dairy J.* 31 (1977) 328-341.
28. C. W. Raadsveld, A rapid complexometric method for the estimation of the calcium content of milk. *Neth. Milk Dairy J.* 10 (1956) 114-122.
29. B. L. Griswald, F. L. Humoller, & A. R. McIntyre, Inorganic phosphates and phosphate esters in tissue extracts. *Anal. Chem.* 23I (1951) 192-194.
30. L. K. Creamer, P. Berry & A. R. Matheson, The effect of pH on protein aggregation in heated skim milk. *N. Z. J. Dairy Sci. Technol.* 13 (1978) 9-15.
31. J. Mottar, A. Bassier, M. Joniau & J. Baert, Effect of heat-induced association of whey proteins and casein micelles on yogurt texture. *J. Dairy Sci.* 72 (1989) 2247-2256.
32. Y. Pouliot, M. Boulet & P. Paquin, Observations on the heat-induced salt balance changes in milk. 1. Effect of heating time between 4 and 90 °C. *J. Dairy Res.* 56 (1989) 185-192.

33. Z. Haque & J. E. Kinsella, Interaction between heated κ-casein and β-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation. *J. Dairy Res.* 55 (1988) 67-80.
34. T. H. M. Snoeren, A.J. Damman & H. J. Klok, The viscosity of skim-milk concentrates. *Neth. Milk Dairy J.* 36 (1982) 305-316.
35. P. Walstra & R. Jenness, Dairy Chemistry and Physics. Wiley, New York (1984).

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Changes in milk on heating: viscosity measurements

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SUMMARY. Skim milk was heated at 85 °C for different holding times. As a result of such heating, whey proteins, in particular β-lactoglobulin, denatured and associated with casein micelles. This led to an increase in size of the casein micelles but also to a different interaction between them. Both these changes could be described by using a quantitative model which was developed for the viscosity of so-called adhesive hard spheres. We applied the model successfully to skim milk and were able to describe on a quantitative basis the changes due to the heat treatment of milk. It was shown that after heating the casein micelles became larger and acquired a mutual attraction. The unfolding of the whey proteins and their subsequent association with the casein micelles appeared to be responsible for these changes. How this reaction influences the fouling of heat exchangers is discussed.

When milk is heated, the whey proteins denature and associate with the casein micelles (Smits & van Brouwershaven, 1980). This has two different effects: first the micelle grows in size (Morr, 1969), and second the interaction between micelles changes. Under certain conditions both the change in size and the change in interactions can be determined from viscosity measurements. The viscosity of skim milk as a function of the volume fraction is well described by the Eilers equation (Walstra & Jenness, 1984) or by the equation of Krieger and Dougherty (Griffin *et al.* 1989). Langley & Temple (1985) found a good fit for heated skim milk to an equation of the form $\eta_r = e^{KC}$ where η_r is the relative viscosity, C is the % of total solids (w/v) and K is a constant. However all these equations are semi-empirical relations and do not account for changes in particle interaction. For a quantitative description and understanding we shall use a relation for the viscosity of semi-dilute dispersions of adhesive hard spheres derived by Russel (1984) and refined by Cichocki & Felderhof (1988). With this relation it is possible to distinguish between the contributions of volume fraction and particle interaction to a change in viscosity. The validity of this relation was checked by Woutersen & de Kruif (1991). They used a dispersion of so-called adhesive hard silica spheres. In this study skim milk is considered to be a dispersion of casein micelles in a continuous phase, which includes whey proteins, lactose and salt ions. We apply the theory of adhesive hard spheres to skim milk before and after heating. This allows discrimination between changes in volume fraction and changes in interactions between casein micelles resulting from the heat treatment. Furthermore the influence of β-lactoglobulin (β-lg) on the changes in viscosity after heating skim milk is studied.

THEORY

The viscosity, η, of a highly dilute dispersion of hard spheres is given by the Einstein equation

$$\eta = \eta_s(1+2{\cdot}5\phi), \tag{1}$$

where η_s is the viscosity of the continuous phase and ϕ is the volume fraction.

In more concentrated dispersions hydrodynamic interactions between particles, which lead to an increase in viscosity, cannot be disregarded. Batchelor (1977) derived an equation for the relative viscosity (η_r) of semi-dilute dispersions of hard spheres (up to a volume fraction of $\phi = 0{\cdot}2$) in the limit of vanishing shear rate

$$\eta_r = \eta/\eta_s = 1+2{\cdot}5\phi + k_H\phi^2, \tag{2}$$

where k_H is the so-called Huggins coefficient which accounts for the hydrodynamic interactions between the hard spheres and has a value of 6·2, but was somewhat refined later (Cichocki & Felderhof, 1988).

Russel (1984) extended this theory to adhesive hard spheres using Batchelor's framework and the Baxter model (Baxter, 1968) for adhesive hard spheres. In this case there are attractions between the hard spheres resulting in the formation of temporary clusters. If the interactions are not too strong there is no permanent clustering. These attractions always lead to an increase in viscosity. The Huggins coefficient takes the form (Cichocki & Felderhof, 1988)

$$k_H = 5{\cdot}913 + 1{\cdot}9/\tau_B, \tag{3}$$

where τ_B is the Baxter interaction parameter; τ_B is infinitely positive for hard spheres and so if it has another value it measures the degree of adhesiveness. In light-scattering and osmotic pressure measurements the second osmotic virial coefficient B_2 is often used, which is related to τ_B as

$$B_2/V_{HS} = 4 - 1/\tau_B \tag{4}$$

where V_{HS} ($= 4/3\pi a^3$) is the volume of the hard sphere of radius a.

Cichocki & Felderhof (1988) extended and refined Russel's calculations. also using Batchelor's framework. The value for the Huggins coefficient in eqn (3) is in fact taken from their work.

The viscosity of dispersions of hard spheres depends on both shear rate and volume fraction. If viscosity decreases with increasing shear rate the system is 'shear thinning'. De Kruif *et al.* (1985) described the shear thinning viscosity of a dispersion of hard spheres as a function of shear rate and volume fraction. Woutersen & de Kruif (1991) found that if hard spheres become adhesive the viscosity of a dispersion at low shear rates increases strongly. This viscosity increase over the hard sphere viscosity could be used to determine the interaction parameter of adhesive hard spheres.

This theory is valid for semi-dilute dispersions of (adhesive) hard spheres. Here skim milk is considered as a semi-dilute dispersion of hard spheres. i.e. casein micelles dispersed in a continuous phase. Eqns (2) and (3) for the viscosity of a dispersion of (adhesive) hard spheres will be applied to skim milk before and after heating. If the viscosity is measured for a dilution series (made by adding permeate) of a certain sample of (heated) skim milk, it is possible to determine both the volume fraction of the micelles and the interaction between the micelles. The continuous phase of skim milk includes whey proteins, lactose and salt ions. Since the whey proteins are so small compared with casein micelles, the Brownian time scale of the former is much

shorter and they can be considered as belonging to the continuous phase. The polydispersity of the micelles is neglected. We found in dynamic light-scattering experiments a standard deviation of the mean size of 0·3–0·4 (second cumulant).

It is known (Walstra & Jenness, 1984) that the viscosity of skim milk is described well by the semi-empirical equation of Eilers (1941)

$$\eta_r = \left(1 + \frac{1{\cdot}25\phi}{1 - \phi/\phi_{max}}\right)^2, \quad (5)$$

where ϕ_{max} is the maximum volume fraction of the dispersed particles. A series development of this semi-empirical equation ($\phi_{max} = 0{\cdot}74$) results in practically the same volume fraction dependence as the theoretical equation of Batchelor (eqn (2))

$$\eta_r = 1 + 2{\cdot}5\phi + 4{\cdot}94\phi^2 + (8{\cdot}78\phi^3) + \ldots \quad (6)$$

This is a first indication that skim milk behaves as a dispersion of hard spheres. Heating such a dispersion may change not only the size of the micelles (and thus the volume fraction, ϕ) but also their interaction.

MATERIALS AND METHODS

Skim milk was obtained from the Institute's experimental dairy. Whey-protein-free skim milk (WPFM; 0·23 g β-lg/l and 0·11 g α-lactalbumin/l remaining) was obtained by reconstituting low-heat WPFM powder in demineralized water. This powder was prepared by microfiltration of skim milk (SCT Membranes, Bazet, France; Mebralox 7P19-40, pore size 0·5 μm, 1·4 m^2) followed by diafiltration with ultrafiltration permeate and spray drying. A β-lg powder (with 92% of total protein β-lg) was prepared by separation according to Maubois *et al.* (1987) followed by freeze drying.

All samples were adjusted to pH 6·7 by addition of 0·5 M-NaOH or 0·5 M-HCl. After heating there was a slight drop in pH (0·05). Decreasing the pH leads to a small decrease in viscosity (Walstra & Jenness, 1984), but this was of no importance here.

Dilution series

Dilutions of the skim milk were made by mixing skim milk with ultrafiltration permeate of the same skim milk. Heated skim milk was diluted with permeate obtained from the same heated skim milk. We used an Amicon (Danvers, MA 01923, USA) hollow fibre cartridge H1MP01-43 ultrafiltration membrane (cut off 0·1 μm). The concentration factor (CF) for diluted skim milk was taken as the ratio of the volume of milk to the total volume of milk and added permeate. Concentrated milk was obtained by using the same ultrafiltration membrane; the CF was calculated from casein determination (Nederlands Normalisatie-Instituut, 1984; N × 6·38) in the skim milk and the concentrated skim milk. Assuming that the size of the micelles did not change on diluting or on concentrating the milk, the following equation was used

$$\phi = \phi_0 \times CF, \quad (7)$$

where ϕ_0 is the calculated volume fraction of the micelles in undiluted milk, which was determined from the Einstein equation (eqn (1)).

Heat treatment

For studying the effects of various heat treatments the samples were treated as follows. The samples were kept at room temperature for at least 30 min before heating. Samples (120 ml) were placed in glass bottles (250 ml) which were closed

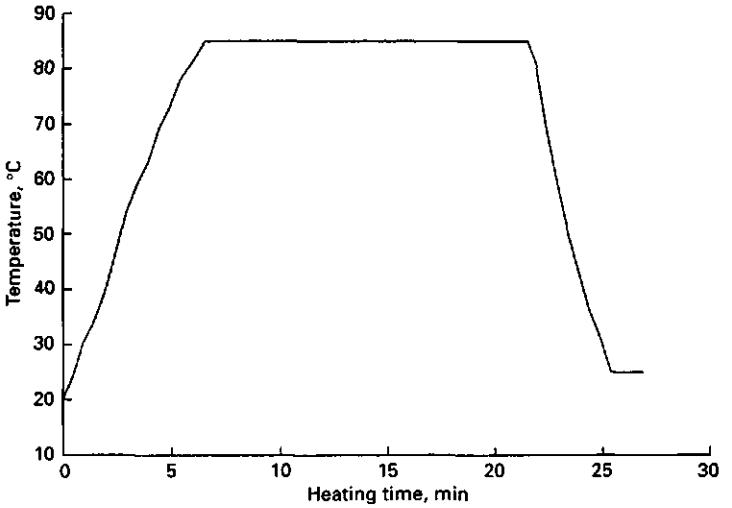

Fig. 1. Temperature profile for heating skim milk at a holding temperature of 85 °C for 15 min, measured with a thermocouple inside the bottle.

with screw caps. The samples were heated in a circulating oil bath (140 °C) until the holding temperature was reached. The bottles were then quickly put into a water bath at the holding temperature and kept there for various holding times. Immediately after heating, the glass bottles were cooled in ice-cold water to room temperature. A typical example of a heating profile at a holding temperature of 85 °C for 15 min, as measured with a thermocouple inside the bottle, is given in Fig. 1. After 30 min viscosity measurements were performed.

Viscosity measurements

An Ubbelohde capillary viscometer was used (Schott Geräte GmbH, Hofheim, Germany; Type 530 10/I, capillary diameter 0·63 mm). Prior to filling the Ubbelohde two drops of octanol (1-octanol reinst; Merck, Darmstadt, Germany) were added to 100 g sample to prevent foaming. The Ubbelohde viscometer was placed in a Tamson (Zoetermeer) water bath (25·00 ± 0·03 °C). Measurements started after 20 min temperature equilibration. The flow time of the sample was automatically determined ten times (variation ⩽ 0·5%) using an AVS 350 viscometer (Schott Geräte GmbH). The average flow time for water was ~ 90 s and for skim milk ~ 140 s. The dynamic viscosity was found by multiplying the kinematic viscosity by the density of the sample measured separately using the Mohrse balance. The Ubbelohde viscometer was cleaned with nitric acid (70%, BDH Analar, Poole, UK) and rinsed with demineralized water and acetone. Overnight the capillary was filled with a 2% Extran MA02 Neutral (Merck) solution to complete the cleaning.

For determining viscosity as a function of shear rate we used a Deer rheometer (Van Bremen BV, Nieuw Leusen) with double cylinder couette geometry. The temperature (25·00 ± 0·03 °C) was controlled through a thermostatted bath.

Degree of denaturation of β-lactoglobulin

For determining the degree of denaturation of β-lg, milk was brought to pH 4·6 and filtered. The filtrate was analysed by high performance gel permeation chromatography (HP-GPC). A Dupont column, Type GF 250 (Rockland Technolo-

gies Inc., Newport, DE 19804, USA), was used at a flow rate of 1·5 ml/min, and the detection was performed at 280 nm. The peak heights of β-lg were compared with those obtained from unheated solutions.

RESULTS

Viscosity of adhesive hard spheres dispersions as applied to skim milk

In order to apply the theory of (adhesive) hard spheres we consider milk to be a dispersion of casein micelles in a continuous phase, i.e. permeate, which included whey proteins, lactose and salts. Several dilution series, made by adding permeate to fresh skim milk, were measured. The permeate contained no caseins but since it contained ~ 50% of the total amount of whey proteins two minor corrections should be made. First it was found that the whey proteins in the continuous phase, i.e. the permeate, constituted a volume fraction of 0·008. Second the whey proteins, which did not pass the ultrafiltration membrane and thus belong to the dispersed phase, contribute only 0·007 to the total volume fraction of the dispersed phase. Because both corrections were of minor influence they were not carried out for the results presented here. Furthermore we think that it is correct to consider the whey proteins as belonging to the continuous phase because not only is their size much smaller but more importantly their Brownian relaxation time differs by a few orders of magnitude from that of the micelles.

Fig. 2 presents the relative viscosity ($\eta_r = \eta/\eta_s$, where η_s is the viscosity of the permeate) of highly diluted samples of skim milk, unheated and after 15 min holding at 85 °C, as a function of the *CF*. From the slope of the line the volume fraction of the casein micelles in undiluted milk (ϕ_0) was calculated using eqns (1) and (7). Because of the increased slope it is clear from Fig. 2 that the volume fraction increased after heating.

In Fig. 3 the relative viscosity of skim milk, unheated and after 15 min holding at 85 °C, is plotted for the whole dilution series, using the value for ϕ_0 calculated from the results in Fig. 2. The curves represent Batchelor's theory (eqns (2) and (3)); by fitting the equations to the experimental results the Baxter interaction parameter (τ_B), or equivalently B_2/V_{HS} could be found. These experiments were repeated with different batches of skim milk. Further batches of milk were heated for 5 min at 85 °C. Representative values of ϕ_0, k_H and B_2/V_{HS} for both heat treatments, together with the protein content of the milk, are summarized in Table 1. From the N content it is clear that Batch II contained more protein, and indeed the volume fraction of casein micelles was found also to be higher in roughly the same proportion. After heating the viscosity increased; from Table 1 it can be seen that this was due to an increase in effective volume fraction as well as in interaction between the particles (B_2/V_{HS} decreased after heating). Each effect contributed ~ 50% to the observed increase in viscosity. The SD of the relative viscosity determination was 0·25% which resulted in an uncertainty for ϕ_0 of ±5%. Owing to the fact that to evaluate k_H the value of ϕ_0 is needed, the errors in ϕ_0 and k_H are correlated. This resulted in an uncertainty for B_2/V_{HS} of ~ 25%. Therefore it is difficult to interpret the absolute values of B_2/V_{HS} and this may explain why we found values higher than the theoretical value of 4. However we are interested in relative changes in interactions before and after heating. Because the SD of η_r was only 0·25% and in all the experiments the curves of η_r against the volume fraction for heated milk were above the curve for unheated milk (i.e. the value for B_2/V_{HS} decreased), we concluded that there was a trend for increased interactions between micelles after heating. This

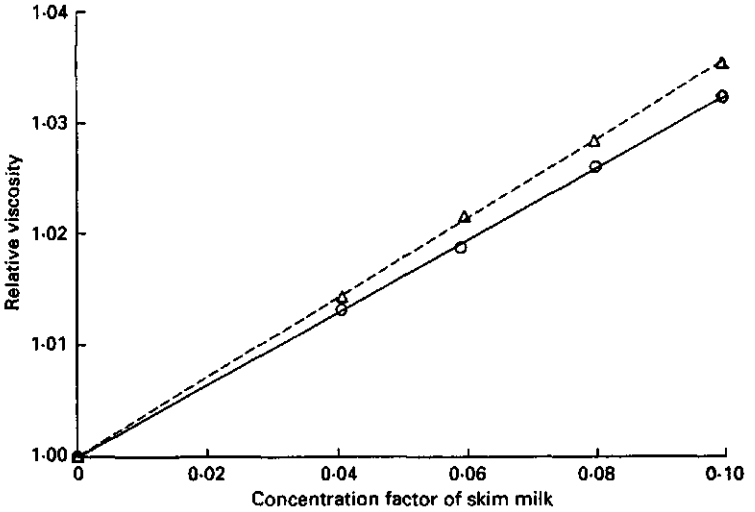

Fig. 2. Relative viscosity of samples of skim milk unheated (○) and after 15 min holding at 85 °C (△), diluted with permeate as a function of the concentration factor.

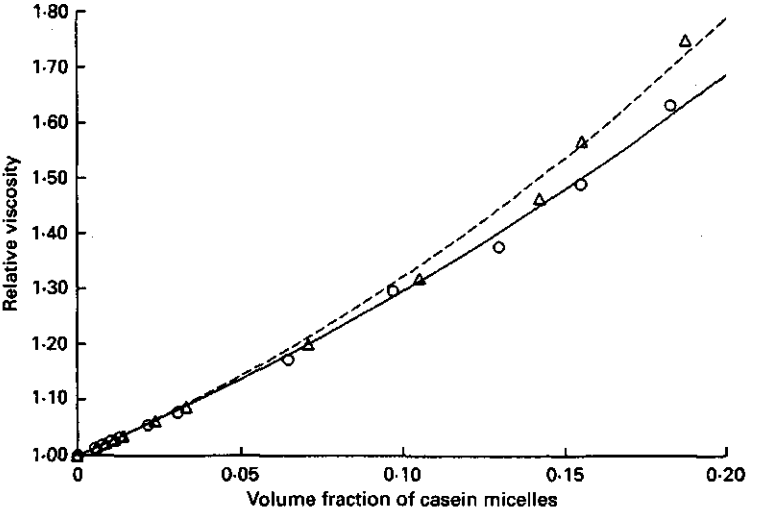

Fig. 3. Relative viscosity of skim milk diluted with permeate and concentrated by ultrafiltration. unheated (○) and after 15 min holding at 85 °C (△), as a function of the volume fraction of casein micelles. The curves are drawn to fit eqn (2); for details, see text.

conclusion, derived from comparing the results for milk before and after heating, is independent of the uncertainty of 25% in the absolute values of B_2/V_{HS}.

Since the theory is valid in the region of vanishing shear rates, outside the range of the Ubbelohde, additional experiments were done with a Deer rheometer (shear rates 5–1000 s^{-1}). In all experiments we found that the relative viscosity of skim milk increased for low shear rates. As expected, skim milk was shear thinning in this region of shear rates (see Table 2; the high shear rate viscosity was fully reached at

Table 1. *Protein content of skim milk and* ϕ_0, k_H *and* B_2/V_{HS} *of skim milk as a function of holding time at 85 °C*

Batch	Heating conditions	Protein concn, %	ϕ_0	k_H	B_2/V_{HS}
I	Unheated	3·03	0·115	3·78	*5·1*
I	5 min, 85 °C	3·03	0·119	4·54	*4·7*
II	Unheated	3·65	0·130	4·60	*4·7*
II	15 min, 85 °C	3·65	0·142	7·13	*3·4*

ϕ_0, Volume fraction of the casein micelles; k_H, Huggins coefficient; B_2, second osmotic virial coefficient; V_{HS}, volume of a hard sphere of radius a. All were calculated from equations and using methods given in the text.

B_2/V_{HS} values > 4 are printed in italics, since strictly the applied theory is not valid for B_2/V_{HS} values > 4.

Table 2. *Relative viscosity of skim milk, before and after heating, for low* ($\dot{\gamma} \longrightarrow 0$) *and higher shear rates* ($\dot{\gamma} \longrightarrow \infty$)

Heating conditions	$\dot{\gamma} \longrightarrow 0$ (s^{-1})	$\dot{\gamma} \longrightarrow \infty$ (s^{-1})
Unheated	1·39	1·33
15 min, 85 °C	1·49	1·40

a shear rate of 800 s^{-1}). As with the measurements with the Ubbelohde viscometer, milk had a higher viscosity after heating. Furthermore, heated milk was more shear thinning than unheated milk, indicating that interactions between micelles had increased (i.e. B_2/V_{HS} decreased) after heating (Woutersen & de Kruif, 1991) and thus confirming, under conditions of vanishing shear rate, the results of the Ubbelohde viscometer.

Influence of heat treatment on viscosity of skim milk

Skim milk was heated at 60 or 90 °C for various holding times (t). Viscosity measurements were then carried out using the Ubbelohde viscometer. If t_0 is the average flow time of unheated skim milk and t_t the average flow time after t s holding time at 60 or 90 °C, we can take t_t/t_0 as a direct measure of the relative change in viscosity $\eta(t)/\eta(t = 0)$. Fig. 4 shows the ratio $\eta(t)/\eta(t = 0)$ as a function of the holding time at 60 and 90 °C. These results were in good agreement with results from similar experiments using turbidity measurements (Jeurnink, 1992). The increase in viscosity of skim milk found after heat treatment is due to denaturation of whey proteins (Walstra & Jenness, 1984). Since no appreciable denaturation of whey proteins occurs up to 70 °C this explains why viscosity did not change much after heating at 60 °C. A pronounced increase in viscosity was observed after heating at 90 °C. After 450 s holding at 90 °C a plateau value was reached which can be explained by the completion of the denaturation of β-lg. According to Dannenberg & Kessler (1988) 99% of the β-lg is denatured after 450 s holding at 90 °C.

The solubility of calcium phosphate decreases rapidly on heating (60% of the Ca is precipitated after 10 s holding at 90 °C (Pouliot *et al.* 1989)) and it is known to precipitate on to the casein micelles (Van Dijk, 1990). This may explain the initial decrease in the ratio $\eta(t)/\eta(t = 0)$ after 30 s holding time (Fig. 4).

Influence of the β-lg concentration on the viscosity

These results showed that denaturation of the whey proteins plays an important role in increasing viscosity. In order to study the effect of the most abundant whey protein, β-lg, on the viscosity, samples of WPFM with various concentrations of

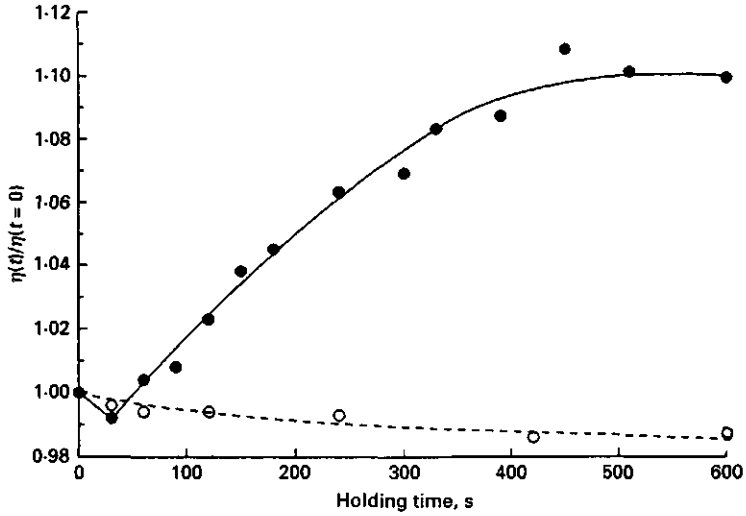

Fig. 4. Relative change in viscosity ($\eta(t)/\eta(t = 0)$) of skim milk as function of the holding time at 60 (○) and 90 °C (●).

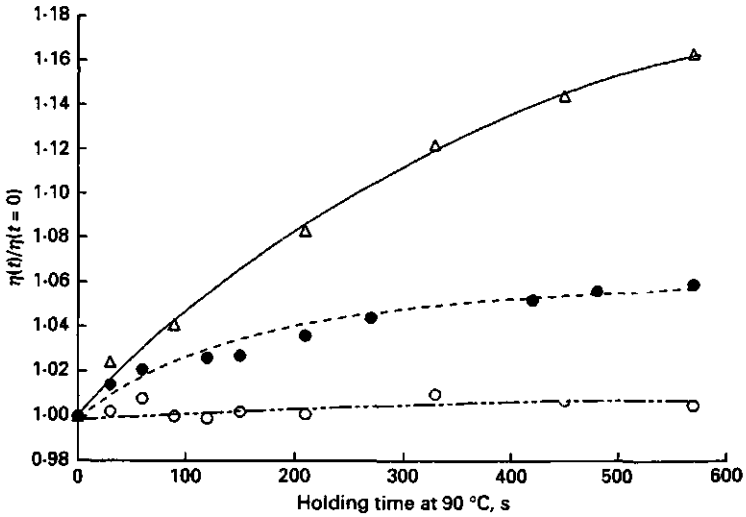

Fig. 5. Relative change in viscosity ($\eta(t)/\eta(t = 0)$) of whey-protein-free milk with various concentrations of β-lactoglobulin (β-lg) as function of the holding time at 90 °C: ○, [β-lg] = 0·2 g/l; ●, [β-lg] = 3·3 g/l; △, [β-lg] = 6·6 g/l.

added β-lg were heated at 90 °C for various holding times. To avoid the disturbing effect of calcium phosphate deposition on the micelles we took as t_0 the average flow time after 30 s holding time rather than that at zero time.

As shown in Fig. 5, no increase in the ratio $\eta(t)/\eta(t = 0)$ was observed in skim milk in which the whey proteins were almost absent. In the presence of β-lg, complexes with the caseins and aggregates were formed, causing an increase in viscosity.

Table 3. *Viscosity of reconstituted skim milk with different degrees of added β-lactoglobulin (β-lg; 3·3 g/l) denaturation*

Sample	Preheat treatment of β-lg	Percentage of β-lg remaining in native form	Viscosity, mPa s
1	—	100	1·523
2	10 s, 70 °C	83·8	1·524
3	240 s, 70 °C	48·6	1·534
4	3600 s, 90 °C	9·8	1·552

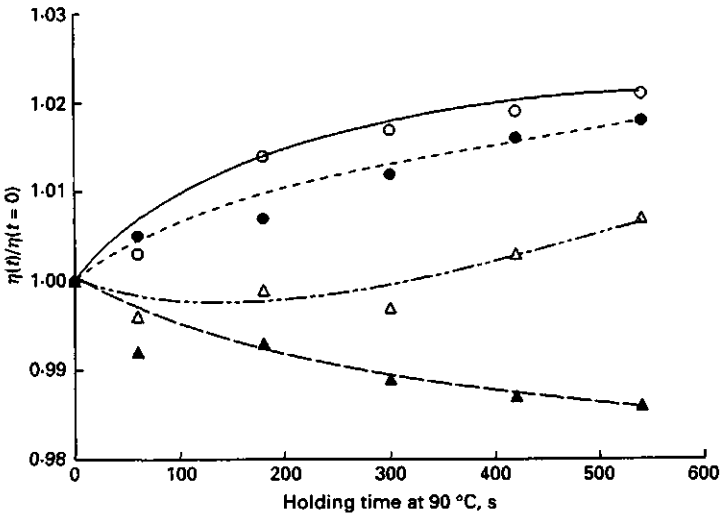

Fig. 6. Relative change in viscosity ($\eta(t)/\eta(t = 0)$) of whey-protein-free milk to which β-lactoglobulin in various degrees of denaturation (3·3 g/l) was added as function of the holding time at 90 °C: ○, 100% native β-lg; ●, 83·8% native β-lg; △, 48·6% native β-lg; ▲, 9·8% native β-lg.

Doubling the β-lg concentration caused a more than proportional increase in viscosity, as expected if it is assumed that by association of the denatured β-lg with the casein micelles the volume fraction of the micelles had increased and was accounted for by the quadratic term in eqn (2). This suggests that β-lg was responsible for the change in viscosity after heating milk.

Influence of the structural form of β-lg on the viscosity

The question arises as to which structural form of β-lg, i.e. native, unfolded or aggregated, was involved in the key process leading to the higher viscosity after heating skim milk. Therefore β-lg solutions in water were given various preheat treatments before being added to WPFM to reach a final concentration of 3·3 g/l of β-lg in the milk. These mixtures were stirred for 1 h at room temperature, adjusted to pH 6·7 and stored at 4 °C overnight. In this way milk samples were obtained containing β-lg with different degrees of denaturation, which was determined by HP-GPC and expressed as a percentage of the native form left (Table 3). The following day the mixtures were heated to 90 °C for various holding times and subsequently

viscosity measurements were carried out in the same way as before. The viscosities of the unheated mixtures are given in Table 3; there was a slight increase in viscosity of the mixtures with the intensity of the preheat treatment. We suggest this was caused by the contribution of aggregated β-lg to the volume fraction of the particles.

Fig. 6 shows the ratio $\eta(t)/\eta(t=0)$ as a function of the holding time at 90 °C. A decrease in final relative viscosity proportional to the degree of denaturation was observed. In sample 4, viscosity even decreased with holding time. Since Fig. 5 showed that in WPFM viscosity did not change after heating, this decrease was probably due to shrinkage of the added β-lg aggregates upon reheating. From Fig. 6 it can be concluded that β-lg that was already aggregated did not cause any increase in viscosity after heating.

DISCUSSION

The relative viscosity of skim milk, before and after heating, as a function of the volume fraction can be described quite well with the Batchelor–Russel theory. We found that the increase in viscosity due to heating skim milk could not be explained by an increase in volume fraction alone. Application of the Batchelor–Russel theory indicates that there is also an increase in attraction between the micelles. One should be aware that the attractions between the micelles are weak: heated milk is still a stable colloidal dispersion. We think that there is not yet permanent clustering between the micelles: the increase in viscosity was caused by clusters of micelles that were only temporary. The results for the samples with the highest volume fraction deviated from the theoretical curve (Fig. 3). Possibly permanent clustering, i.e. aggregation between the micelles, had occurred in these samples and they could no longer be considered as dispersions of hard spheres, thus invalidating the theory. On the other hand, Singh & Fox (1988) stated that heating milk to 90 °C produces only minor changes in the dimensions of the casein micelles. Furthermore if permanent clusters or aggregates were present it would influence the change in ϕ_0 after heating. However, taking into account the change in ϕ_0 after heating we still found an extra increase in viscosity which we interpreted as an attraction between the micelles.

The observed increase in viscosity of skim milk due to heating could theoretically also be caused by an increased repulsion between the micelles. However the turbidity measurements of Van Boekel *et al.* (1989) showed a continuous increase in turbidity upon heating skim milk caused by attraction between the micelles, finally leading to total aggregation of the milk. We therefore conclude that the increased viscosity found here upon heating skim milk was also caused by an attraction.

The polydispersity of casein micelles may cause an error in the B_2/V_{HS} values. We expect that in studying changing interactions, polydispersity will be a second-order effect (Van der Werff & de Kruif, 1989).

The Eilers equation predicted too low a viscosity for heated skim milk (15 min at 85 °C), as was also found by Langley & Temple (1985). Taking into account a smaller ϕ_{max} due to a change in size distribution of casein micelles after heating (Snoeren *et al.* 1982; Mohammad & Fox, 1987) did not result in a better fit for any reasonable ϕ_{max}. This can be explained by the observation that after heating the attractions between the micelles increased and the Eilers equation did not account for this.

The observations that in the absence of whey proteins there was no increase in viscosity, heating skim milk to 60 °C did not result in an increase in viscosity and that after sufficient heating at 90 °C to denature β-lg completely no further increase in viscosity was found indicate that the denaturation of β-lg was responsible for the

change in viscosity after heating milk. As aggregated β-lg did not lead to an increase in viscosity after heating we conclude that the unfolding of β-lg was the key reaction in increasing the viscosity.

The increase in effective volume fraction after heating skim milk was caused by the association of denatured whey proteins with casein micelles. As a result of the heating the composition of the continuous phase (ultrafiltration permeate) changed slightly because of the association of whey proteins with the casein micelles. Indeed, we found that the permeate of heated milk had a lower protein content and a lower dynamic viscosity than the permeate of unheated milk.

The heat treatments applied here also occur in heat exchangers and lead to fouling. The denaturation of β-lg also plays a key role in the fouling reaction (Burton, 1968; Hiddink *et al.* 1986; Jeurnink *et al.* 1989). Arnebrant *et al.* (1987) showed that β-lg, especially if it unfolds, adsorbs on a stainless steel wall. Furthermore it is known that the fouling layer contains caseins, most probably in the form of casein micelles (Jeurnink, 1991). Thus the unfolding state of the whey protein seems to be important in the fouling process. In this transition state the molecule appears to be highly 'reactive', acting as a sticky agent between other β-lg molecules, casein micelles and the stainless steel wall resulting in the formation of a fouling layer. Influencing this transition state reactivity may lead to a diminished fouling.

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REFERENCES

Arnebrant, T., Barton, K. & Nylander, T. 1987 Adsorption of α-lactalbumin and β-lactoglobulin on metal surfaces versus temperature. *Journal of Colloid and Interface Science* **119** 383–390

Batchelor, G. K. 1977 The effect of Brownian motion on the bulk stress in a suspension of spherical particles. *Journal of Fluid Mechanics* **83** 97–117

Baxter, R. J. 1968 Percus-Yevick equation for hard spheres with surface adhesion. *Journal of Chemical Physics* **49** 2770–2774

Burton, H. 1968 Reviews of the Progress of Dairy Science. Section G. Deposits from whole milk in heat treatment plant—a review and discussion. *Journal of Dairy Research* **35** 317–330

Cichocki, B. & Felderhof, B. U. 1988 Long-time self-diffusion coefficient and zero-frequency viscosity of dilute suspensions of spherical Brownian particles. *Journal of Chemical Physics* **89** 3705–3709

Dannenberg, F. & Kessler, H. G. 1988 Application of reaction kinetics to the denaturation of whey proteins in heated milk. *Milchwissenschaft* **43** 3–7

De Kruif, C. G., van Iersel, E. M. F. & Vrij, A. 1985 Hard sphere colloidal dispersions: viscosity as a function of shear rate and volume fraction. *Journal of Chemical Physics* **83** 4717–4725

Eilers, H. 1941 [The viscosity of emulsions of high-viscous materials as function of the concentration.] *Kolloid-Zeitschrift* **97** 313–321

Griffin, M. C. A., Price, J. C. & Griffin, W. G. 1989 Variation of the viscosity of a concentrated, sterically stabilized, colloid: effect of ethanol on casein micelles of bovine milk. *Journal of Colloid and Interface Science* **128** 223–229

Hiddink, J., Lalande, M., Maas, A. J. R. & Streuper, A. R. 1986 Heat treatment of whipping cream. I. Fouling of the pasteurization equipment. *Milchwissenschaft* **41** 542–546

Jeurnink, Th. J. M. 1991 Effect of proteolysis in milk on fouling in heat exchangers. *Netherlands Milk and Dairy Journal* **45** 23–32

Jeurnink, Th. J. M. 1992 Changes in milk on mild heating. Turbidity measurements. *Netherlands Milk and Dairy Journal* **46** 183–196

Jeurnink, Th. J. M., Brinkman, D. W. & Stemerdink, A. D. 1989 Distribution and composition of deposit in heat exchangers. In *Proceedings of the Third International Conference on Fouling and Cleaning in Food Processing*, pp. 25–36 (Eds H. G. Kessler and D. B. Lund). Prien, Augsburg: Walch

Langley, K. R. & Temple, D. M. 1985 Viscosity of heated skim milk. *Journal of Dairy Research* **52** 223–227

Maubois, J. L., Pierre, A., Fauquant, J. & Piot, M. 1987 Industrial fractionation of main whey proteins. *International Dairy Federation Bulletin* no. 212 154–159

Mohammad, K. S. & Fox, P. F. 1987 Heat-induced association-dissociation of casein micelles preceding coagulation. *Journal of Dairy Research* **54** 377–387

MORR, C. V. 1969 Protein aggregation in conventional and ultra high-temperature heated skimmilk. *Journal of Dairy Science* **52** 1174–1180

NEDERLANDS NORMALISATIE-INSTITUUT 1984 Delft: NNI (*NEN* 3198 2nd edn)

POULIOT, Y., BOULET, M. & PAQUIN, P. 1989 Observations on the heat-induced salt balance changes in milk 1. Effect of heating time between 4 and 90 °C. *Journal of Dairy Research* **56** 185–192

RUSSEL, W. B. 1984 The Huggins coefficient as a means for characterizing suspended particles. *Journal of the Chemical Society, Faraday Transactions* 2 **80** 31–41

SINGH, H. & FOX, P. F. 1988 Heat-induced changes in casein. *International Dairy Federation Bulletin* no. 238 24–30

SMITS, P. & VAN BROUWERSHAVEN, J. H. 1980 Heat-induced association of β-lactoglobulin and casein micelles. *Journal of Dairy Research* **47** 313–325

SNOEREN, T. H. M., DAMMAN, A. J. & KLOK, H. J. 1982 The viscosity of skim-milk concentrates. *Netherlands Milk and Dairy Journal* **36** 305–316

VAN BOEKEL, M. A. J. S., NIEUWENHUIJSE, J. A. & WALSTRA, P. 1989 The heat coagulation of milk. 2. Kinetic studies. *Netherlands Milk and Dairy Journal* **43** 129–146

VAN DER WERFF, J. C. & DE KRUIF, C. G. 1989 Hard-sphere colloidal dispersions: the scaling of rheological properties with particles size, volume fraction, and shear rate. *Journal of Rheology* **33** 421–454

VAN DIJK, H. J. M. 1990 The properties of casein micelles. 3. Changes in the state of the micellar calcium phosphate and their effects on other changes in the casein micelles. *Netherlands Milk and Dairy Journal* **44** 125–141

WALSTRA, P. & JENNESS, R. 1984 *Dairy Chemistry and Physics*. New York: Wiley

WOUTERSEN, A. T. J. M. & DE KRUIF, C. G. 1991 The rheology of adhesive hard sphere dispersions. *Journal of Chemical Physics* **94** 5739–5750

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Effect of proteolysis in milk on fouling in heat exchangers

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Summary

Aged milk causes more fouling in a heat exchanger than fresh milk. Experiments with skim milk showed that the action of proteolytic enzymes, produced by psychrotrophic bacteria, is responsible for the increase in deposit. This rise is due to additional protein deposition. A mechanism based on decreased heat stability of casein micelles is proposed.

1 Introduction

It is known (1) that aged milk causes more fouling in a heat exchanger than fresh milk. Burton (1) thought that this rise was due to development of micro-organisms during storage, even under refrigeration; however, further research into this subject has not been reported.

In this study, experiments with skim milk were carried out to determine the mechanism by which micro-organisms cause the increase in deposit.

2 Materials and methods

2.1 Pasteurization of skim milk

Raw milk was skimmed by centrifugation at 45 °C. Skim milk was pasteurized in a laboratory-scale tube heat exchanger or in a pilot-scale plate heat exchanger. The tubular heat exchanger (Fig. 1) consisted of two concentric tubes. The inner tube was of stainless steel, the outer tube of glass. The stainless steel tube was heated on the inside by hot water (T inlet = 95 °C, flow = 180 l/h, velocity (v) = 0.64 m/s). The milk was in counter-current with the hot water and flowed in between the stainless steel tube and the glass tube (flow = 30 l/h, v = 0.095 m/s, Re = 294). The temperature of the milk at the entrance of the annular space was 65 °C and the outlet temperature was ap-

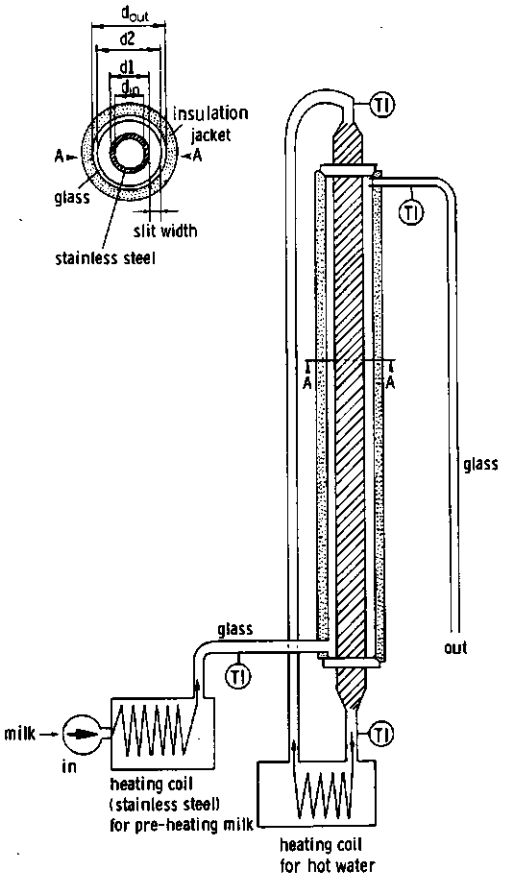

Fig. 1. Experimental set-up of the heat exchanger consisting of two concentric tubes. Inner tube: stainless steel AISI 304, d_{in} = 0.010 m, d_1 = 0.012 m; outer tube: glass, d_2 = 0.016 m, d_{out} = 0.018 m. Length of outer tube 0.75 m. TI = thermocouple.

proximately 80 °C. Usually a batch of 40 l skim milk was pasteurized per run.

The plate heat exchanger was an Alfa-Laval type A3-HRB, with a product flow of 100 l/h. The pasteurization temperature was 85 °C. A run usually took about 5 h.

In both heat exchangers, temperatures were measured to calculate the heat transfer coefficient from the logarithmic mean temperature difference between milk and water. The amount of deposit was determined by weighing the tube or the two plates of the heating section after drying in an oven for 1 h at 95 °C.

2.2 Washed cells

Raw milk (3 l) was stored for 5 days at 10 °C, after which the total bacterial count had reached 1.9×10^9 cfu/ml. The milk was brought to pH 6.5 by adding NaOH (1N); 75 ml of a 40 % sodium citrate solution (4 °C) was also added. The solution was stirred for 5 min and then centrifuged at 4 °C for 15 min at 16 300 *g* (Sorvall RC2-B superspeed centrifuge). The pellet, which contained the cells, was resuspended in 200 ml buffer solution (4 °C, pH = 6.0, 50 mM NaH_2PO_4 and 50 mM NaAc) and again centrifuged. The pellet was resuspended in 6 ml of the same buffer solution.

2.3 Production of extracellular enzymes

Sterilized skim milk was inoculated with *Pseudomonas fluorescens* 22F (isolated from raw milk at NIZO) and incubated for 3 days at 20 °C. Then the milk was heated for 10 min at 65 °C and centrifuged for 10 min at 16 300 *g*. The supernatant, which contained secreted bacterial enzymes was collected and stored at −18 °C. It was found that enzyme solutions made from different batches did not have the same enzyme activity. An indication of the enzyme activity was obtained by measuring the time needed for visual coagulation of the milk. When enzyme solution was mixed with raw skim milk, 400 ppm sodium azide was also added to prevent growth of bacteria.

2.4 Methods of analysis

Protein was determined by the Kjeldahl method according to NEN 3198 (2); protein is N × 6.38.

Ash was determined after 9 h heating in an oven at 550 °C.

Total psychrotrophic count was determined after 1 day incubation at 15 °C and 3 days at 7 °C on a plate count milk-agar.

Heat stability of the skim milk was measured at 140 °C in the Klarograph (3), an apparatus developed at NIZO, based on the Höppler falling ball viscosimeter (4).

Protein composition in the deposit was determined by gel electrophoresis and HPLC.

For HPLC (5) the deposit was treated (15 mg/ml) with 0.02 M 1,3-bis[tris-(hydroxymethyl)-methylamino]propane. HCl containing 4 M urea and 0.1 % 2-mercaptoethanol (pH 7). After 1 h at room temperature, the sample was diluted (1:4, v/v) with HPLC-solvent A (water/acetonitrile/trifluoroacetic acid, 900:100:1, v/v), to which urea had been added to a final concentration of 6 M (pH 2). Then 50 μl of the filtered (Millex GV, Millipore) sample was loaded onto a Hi-Pore RP-318 column (250 × 4.6 mm, Bio-Rad Laboratories) and analysed at 30 °C using a gradient of solvent B, water/acetonitrile/trifluoro-

acetic acid (100:900:0.7, v/v), adapted from reference (5). The flow rate was 0.8 ml/min; detection was at 220 nm.

For gel electrophoresis the deposit was solubilized by a modification of the procedure described by Tissier & Lalande (6); the deposit was treated with nitric acid (1 %), followed by solubilization of the remaining proteins at pH 8.8 in a buffer containing 0.2 M tris (hydroxymethylaminomethane), 0.1 M EDTA, 8 M urea, 0.1 M 2-mercaptoethanol and 0.03 M sodium dodecyl sulphate (SDS). In both treatments ultrasonication was applied for 30 min at 40 °C. Electrophoresis was performed according to Laemmli (7) at pH 8.9 in 15 % polyacrylamide gel containing 4 M urea and 0.003 M SDS. After electrophoresis the gel was stained in Coomassie Blue R250. Staining intensity of bands was quantified using a Shimadzu CS 900 densitometer coupled to a Waters data module integrator.

3 Results and discussion

3.1 Fouling by aged milk and by milk incubated with a psychrotrophic bacterial strain

The first experiments were intended to confirm the results of Burton (1), who imputed the increase in deposit to the growth of micro-organisms. Since milk is stored at low temperature, special attention was given to psychrotrophic bacteria. Table 1 shows the amount of deposit in the plate heat exchanger formed after subjecting skim milk to different pretreatments. Experiment 1 was done with fresh skim milk. In experiment 2 the milk was stored for 10 days at 5 °C. As preservative in experiment 3 sodium azide (400 ppm) was used. In experiment 4 the milk was incubated with psychrotrophic bacteria for 4 days at 5 °C. *Pseudomonas fluorescens* 22F was chosen because it is a good representative of the psychrotrophic flora in milk.

Experiment 2 confirms that aged milk causes more fouling in heat exchangers than fresh milk. From experiment 3 it is concluded that without growth of psychrotrophic bacteria in cooled milk only a small increase of the amount of

Table 1. Psychrotrophic count in milk and amount of deposit formed after heating at 85 °C.

Exp. No.	Test material	Psychr. count (cfu/ml)	Amount of deposit (g)
1	Fresh skim milk	$<10^3$	1.46
2	Skim milk, 10 days at 5 °C	1.4×10^7	15.25
3	Skim milk + preservative, 10 days at 5 °C	$<10^3$	3.85
4	Skim milk + *Ps. fluorescens* 22F, 4 days at 5 °C	1.5×10^6	16.40

deposit takes place. Experiment 4 shows that *Pseudomonas fluorescens* 22F is able to cause an increase in fouling comparable to that obtained in experiment 2. Repeating the experiment gave consistent results.

3.2 Effect of adding washed cells and bacterial enzymes to the milk before pasteurization

To check whether bacterial cells as such contribute to the deposit, so that their increasing number in aged milk is responsible for the increase in fouling, washed cells (see 2.2) were added to milk just prior to pasteurization in the laboratory-scale heat exchanger. Extracellular enzyme solution (see 2.3) and sodium azide (400 ppm) were added to other samples of milk and after 2 h of incubation at 20 °C the milk was pasteurized in the laboratory-scale heat exchanger.

The results of these experiments are shown in Fig. 2, in which the reciprocal of the heat transfer coefficient is plotted against the running time of the experiment; the slope of this line is defined as the fouling rate (8). It can be seen that the fouling rate was not influenced by adding washed cells to the milk. However, the fouling rate of milk incubated with enzymes was significantly increased. It is concluded that not the bacteria as such, but the extracellular enzymes produced by these bacteria, cause the increase in fouling. Psychrotrophic bacteria may produce both proteinases and lipases (9). The experiments here were carried out with skim milk in order to separate the in-

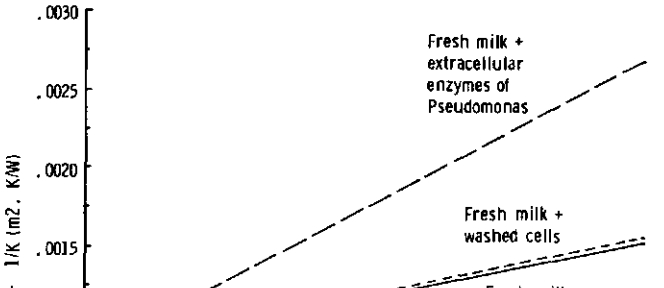

Fig. 2. Plot of the reciprocal of the heat transfer coefficient against time. ——, fresh milk; – – – –, fresh milk + washed cells; — —, fresh milk + extracellular enzymes of *Ps. fluorescens* 22F. The slope is a measure of the rate of fouling.

fluence of proteinases and lipases. In view of the low incidence of bacterially induced rancidity in milk (9), probably due to the inaccessibility of the triglycerides to the lipases (unless the fat globule membrane is damaged), this study focused on the influence of the proteinases only.

3.3 Composition of the deposit

In the experiments described in Table 1, the composition of the deposit was also determined; the results are given in Table 2. Both the fresh skim milk and the milk stored for 10 days with a preservative give a deposit with a composition close to that of type A, as described by Burton (1), though the ash content was somewhat higher. In the other two experiments a strong increase was observed in the percentage of protein in the deposit. From the protein/ash ratio it is concluded that the increase in deposit that occurs upon heating aged milk or milk incubated with *Pseudomonas fluorescens* 22F is due to additional protein deposition.

Summarizing, it is found that the additional deposit formed by heating aged milk is related to the action of proteolytic enzymes produced by psychrotrophic bacteria in the milk. It is known (10) that these enzymes break down κ-casein in particular, thereby forming para-κ-casein-like compounds. Fox and Hearn (11) found that degradation of κ-casein by rennet, forming para-κ-casein, decreased the heat stability of the milk. Combining these results with the fact that the increase in deposit is due to additional protein deposition, the following hypothesis is formulated: in aged milk κ-casein is hydrolysed by proteolytic enzymes, resulting in a decreased heat stability of the degraded casein micelles, which will coagulate on heating and cause an additional protein deposition.

3.4 Effect of proteolysis on fouling and heat stability

In order to check the hypothesis of proteolysis and subsequent fouling the following experiment was done. Skim milk with sodium azide (400 ppm) was di-

Table 2. Composition of the deposit.

Exp. No.	Test material	Dry matter (g)	Protein (%)	Ash %	Protein/ ash ratio
1	Fresh skim milk	1.40	42.3	46.2	0.92
2	Skim milk, 10 days at 5 °C	14.78	69.3	22.0	3.15
3	Skim milk + preservative, 10 days at 5 °C	3.54	45.4	39.0	1.16
4	Skim milk + *Ps. fluorescens* 22F, 4 days at 5 °C	15.66	73.8	12.0	6.15

Table 3. Fouling and heat stability of milk treated with proteolytic enzymes.

Product	Enzyme (%)	Incubation at 20 °C (h)	pH	Heat stability (min)	Deposit (g)
skim milk	0	20.0	6.67	4.4	3.4
skim milk	1	17.5	6.68	0*	5.9

* Coagulated already during heating up to 140 °C.

vided into two batches. To one batch, enzyme solution was added. After keeping both batches overnight at 20 °C the amount of deposit after pasteurization in the laboratory-scale heat exchanger and the heat stability were determined (Table 3).

The results in Table 3 confirm that milk incubated with proteolytic enzymes caused more deposit than untreated milk. Also the heat stability of this milk was lower. This supports the hypothesis that the additional deposit is due to less heat-stable casein micelles.

The deposit in these experiments was analysed for its protein composition by HPLC and gel electrophoresis. The HPLC chromatogram of the deposit of the untreated milk and that of whole casein were similar (Fig. 3), which indicates that the caseins appear in the deposit in the form of micelles. From the

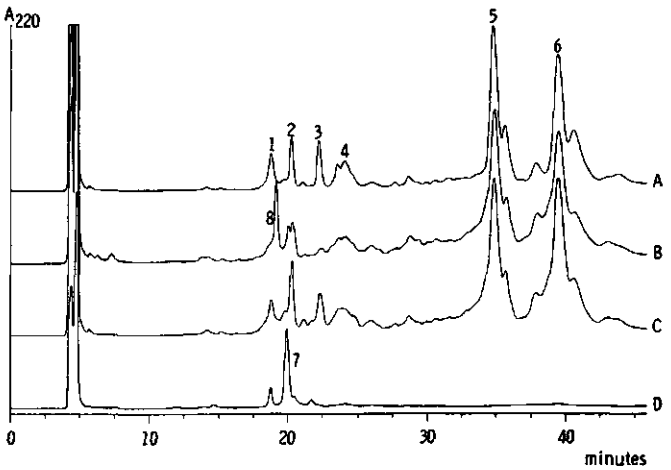

Fig. 3. HPLC chromatogram of whole casein (A), deposit of skim milk after incubation with proteolytic enzymes (B), deposit of untreated skim milk (C), para-κ-casein (D).
1, glucosylated κ_{A+B}-casein; 2, non-glucosylated κ_A-casein; 3, non-glycosylated κ_B-casein; 4, α_{s2}-casein; 5, α_{s1}-casein; 6, β-casein; 7, para-κ-casein; 8, para-κ-casein-like compound.

chromatogram of the deposit of the milk incubated with enzyme it can be seen that the enzyme indeed breaks down κ-casein in particular, forming para-κ-casein and a para-κ-casein-like compound (Fig. 3). Also here the ratio of the caseins is roughly the same as for micelles in milk with the exception of course of κ-casein which is replaced by hydrolysed κ-casein. This indicates that the degraded casein micelles appear in the deposit, supporting the hypothesis that the additional deposition is caused by casein micelles which are less heat stable through the action of the proteolytic enzymes.

Since the deposit sticked to the hot stainless steel wall β-lactoglobulin (β-lg) is heavily denatured; it was not possible to analyse this β-lg properly using HPLC (12).

For casein the results of gel electrophoresis and HPLC confirm each other (Fig. 3 and 4). Fig. 4 shows that in addition to caseins also β-lg is deposited in the fouling layer. However, quantification of the electrophoretic bands revealed that the β-lg content is decreased in the deposit of the milk incubated with enzyme. It is reported (9) that proteolytic enzymes also degrade β-lg. But another explanation for the decrease in the content of β-lg is that in accordance with the hypothesis, degraded casein micelles in the treated milk contribute to the fouling layer, resulting in a relatively lower amount of β-lg; this was confirmed by the decreased ratio of β-lg to casein in the deposit as measured from Fig. 4.

In the deposit of the untreated milk, caseins and β-lg were found. A possible fouling mechanism may be that denatured β-lg deposits on the stainless steel as well as on the casein micelles (13, 14). β-Lg acts as a 'sticking agent' (15), resulting in the deposition of casein micelles and β-lg together on the stainless steel. This means that the appearance of calcium and phosphate in the deposit (1) might not only be due to insoluble calcium phosphate but also to colloidal calcium phosphate from the micelles.

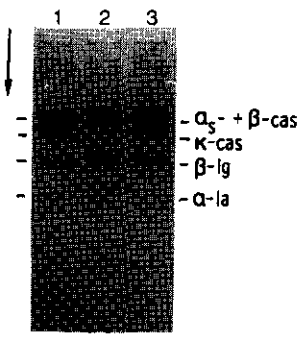

Fig. 4. SDS-polyacrylamide gel electrophoresis of deposit formed by heating milk. 1 total milk protein, 2 deposit of skim milk after incubation with proteolytic enzymes, 3 deposit of untreated skim milk.

4 Conclusion

This study has shown that the increase in deposit which occurs upon heating aged skim milk is caused by the action of proteolytic enzymes produced by psychrotrophic bacteria in the milk. In the deposit of aged skim milk a higher percentage of protein was found. Also through the action of these enzymes the heat stability of the milk is decreased, indicating that there may be a relation between the heat stability and the amount of deposit.

Analysis of the deposit showed that the proteolytic enzymes degrade κ-casein, forming para-κ-casein-like compounds. The ratios of the different caseins in the deposit indicate that they appear in the form of micelles, possibly with denatured β-lg as 'sticking agent' between them and the stainless steel. Further investigations of the behaviour of β-lg are in progress. The appearance of caseins in the deposit in roughly the same ratio as in micelles supports the postulated hypothesis: proteolytic enzymes break down κ-casein, resulting in a decreased heat stability of the degraded casein micelle, which will coagulate when heated, forming an additional protein deposition.

From the practical point of view, to reduce the amount of fouling it is important to prevent psychrotrophic bacteria from producing a significant amount of extracellular enzymes. It is not clear at what bacterial count proteinase production becomes significant; Adams et al. (10) investigated nine strains of *Pseudomonas* isolated from raw milk and showed that with most strains, proteolysis in milk was detectable even before the population had reached 10^4 cfu/ml. Driessen (16) found that *Pseudomonas fluorescens* 22F produced proteinase only towards the end of the exponential growth phase, i.e. at a total count of 10^6 cfu/ml or more. In any case it is recommended that if raw milk has to be stored for a while it is given a thermization treatment in order to kill the psychrotrophic bacteria. If fresh milk is contaminated with aged milk with a high content of proteinase, for example small amounts of milk left behind in tanks over the weekend, it is possible that milk with a relatively low bacterial count may cause additional deposit during pasteurization. Therefore it is important to avoid dead spaces in the processing lines and to drain tanks and pipelines completely.

Acknowledgement

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References

1. H. Burton. Reviews of the progress of dairy science. Section G. Deposits from whole milk in heat treatment plant – a review and discussion. *J. Dairy Res.* 35 (1968) 317-330.
2. Dutch Standard NEN 3198, 2nd edition, October 1984.
3. J. N. de Wit, G. Klarenbeek & C. de Graaf. Klaro-graph: een nieuw apparaat voor het meten van viscositeit, dichtheid en hittestabiliteit in melk en melkconcentraten bij temperaturen tot 140 °C. *Zuivelzicht* 78 (1986) 168-170.
4. W. Weber. Systematische Untersuchung des Fallkugelviskosimeters mit geneigtem Fallrohr. *Kolloid Zeitschrift* 147 (1956) 14-28.
5. S. Visser, K. J. Slangen & H. S. Rollema. High-performance liquid chromatography of bovine caseins with the application of various stationary phases. *Milchwissenschaft* 41 (1986) 559-562.
6. J. P. Tissier & M. Lalande. Experimental device and methods for studying milk deposit formation on heat exchange surfaces. *Biotechnol. Progress* 2 (1986) 218-229.
7. U. K. Laemmli. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680-685.
8. A. C. Ling & D. B. Lund. Fouling of heat transfer surfaces by solutions of egg albumin. *J. Fd Prot.* 41 (1978) 187-194.
9. B. A. Law. Reviews of the progress of dairy science: Enzymes of psychrotrophic bacteria and their effects on milk and milk products. *J. Dairy Res.* 46 (1979) 573-588.
10. D. M. Adams, J. T. Barach & L. Speck. Effect of psychrotrophic bacteria from raw milk on milk proteins and stability of milk proteins to ultrahigh temperature treatment. *J. Dairy Sci.* 59 (1976) 823-827.
11. P. F. Fox & C. M. Hearn. Heat stability of milk: influence of κ-casein hydrolysis. *J. Dairy Res.* 45 (1978) 173-181.
12. S. Visser, NIZO Ede, personal communication.
13. Th. Arnebrant, K. Barton & T. Nylander. Adsorption of α-lactalbumin and β-lactoglobulin on metal surfaces versus temperature. *J. Colloid Interface Sci.* 119 (1987) 383-390.
14. P. Smits & J. H. van Brouwershaven. Heat-induced association of β-lactoglobulin and casein micelles. *J. Dairy Res.* 47 (1980) 313-325.
15. H. Singh. Effects of high temperatures on casein micelles. *N.Z.J. Dairy Sci. Technol.* 23 (1988) 257-273.
16. F. M. Driessen. Lipases and proteinases in milk. Thesis, Agricultural University Wageningen (1983).

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Calcium concentration in milk in relation to heat stability and fouling

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Summary

Some properties of skim milk with varying calcium concentrations were determined before and after heating, to study the effect of calcium on heat stability of the milk and on fouling caused by it. Either increasing or decreasing the calcium concentration in the milk led to a lower heat stability and to more fouling in comparison with normal milk. For normal milk the protein in the deposit consisted mainly of serum proteins. If the calcium concentration was changed there was a shift in the protein composition of the deposit from serum proteins to casein. In low-calcium milk the casein micelles were swollen and a large part of the κ- and β-casein was dissociated from the micelles. These so-called depleted micelles lack (part of) the stabilizing action of κ-casein and are the cause of a lower heat stability and more casein deposition. In high-calcium milk the casein micelles had shrunk and hardly any dissociation was observed. Presumably, the increase in calcium ion activity and in colloidal calcium phosphate reduces electrostatic and steric repulsions between the casein micelles, leading to increased interaction and consequently to a lower heat stability. This instability of the casein micelles, with denatured β-lactoglobulin associated at their surface, would then cause a large increase in protein fouling.

Keywords: fouling; heat stability; calcium ion activity; dissociation of caseins

1 Introduction

Calcium plays a major role in the heat stability of milk [1, 2] and is part of the deposit formed upon heating milk [3, 4]. The latter is not only due to the fact that the solubility of calcium phosphate decreases upon heating, but also to the influence of calcium on the precipitation of the proteins [5]. In order to obtain a better understanding of the effect of the calcium concentration in milk on its heat stability and its fouling behaviour, some properties of milk samples with varying calcium concentrations were determined before and after heating.

2 Materials and methods

2.1 Skim milk

Skim milk was obtained from the institute's experimental dairy. Calcium concentration was lowered by ion-exchange against sodium; to this end the milk sample was mixed with the cation-exchanger Duolite C464 (90:1 w/w; Diamond Chemicals, Vitry-S/Seine, France). After stirring for approximately 30 min at room temperature, Duolite was separated from the milk by filtration. Higher calcium concentrations in the milk were obtained by adding $CaCl_2.2H_2O$ (Merck, Darmstadt, Germany). The treated milks were then kept overnight in the cold and slowly warmed to room temperature before use. In all cases the pH was, if necessary, adjusted to 6.7.

2.2 Heat treatment of the milk

Milk was pasteurized in a plate heat exchanger (Alfa-Laval, type A3-HRB) with a product flow of 100 l/h during 8 h. The milk inlet temperature in the heating section was 69 °C. Pasteurization temperature was 85 °C with a holding time of 15 s. The heating section was composed of two plates with a 'fouling area' of 0.095 m^2. The water flowed counter-current to milk at a rate of 100 l/h and with an inlet temperature of 92 °C.

Heat treatment of portions of milk (120 ml) was carried out as described by Jeurnink and De Kruif [6].

2.3 Centrifugation

Supernatants of the milk were obtained after centrifugation at 90 000 *g* for 1 h at 18 °C (Beckman L8M, rotor TI45). In the case of a heat treatment the milks were centrifuged within 1 h after heating. In this paper casein not ending up in the pellet after centrifugation is called 'dissolved' caseins.

2.4 Viscosity

The viscosity was measured at 25.00 °C with an Ubbelohde capillary viscometer (Schott Geräte GmbH, Hofheim, Germany, Type 530 10/I and Type 530 01/0a) [6]. The dynamic viscosity was found by multiplying the kinematic viscosity by the density of the sample, measured separately using a Mohr's balance. In order to compare the viscosity of different batches, the samples were scaled for their casein content.

2.5 Heat stability

Heat stability of the milk was measured at 140 °C in McCartney bottles according to Australian Standard 1629.3.4 [7]; pH was adjusted using 1N NaOH or 1N

HCl. The dilution caused by this was kept constant for each sample by also adding an appropriate amount of water.

2.6 Analyses

Nitrogen was determined by the Kjeldahl method according to NEN 3198 [8]. Whey proteins have a lower Kjeldahl conversion factor for nitrogen to protein than milk [9]. Because of the high contribution of the whey proteins to the total protein content of the deposits we used 6.3 instead of the commonly used 6.38 as Kjeldahl factor for the deposits. Ash was determined after 9 h heating in an oven at 550°C. Calcium was determined after digestion according to NEN 6465 [10] by atomic absorption spectroscopy (NEN 6446, [11]). Calcium ion activity of the milks was determined with an ion selective electrode (F2110Ca Radiometer Analytical A/S Copenhagen-Denmark) after cold storage overnight and slowly warming to room temperature.

Total phosphorus was determined by photospectroscopy according to NEN 6479 [12]. Fat was determined according to NEN 3235 9.2.1. [13]. Citrate and lactose were determined by HPLC [14]. Magnesium, sodium, potassium, chloride, phosphate (inorganic) and sulphate were determined by capillary ion analysis (Quanta 4000, Waters, Milford, USA). Reversed-phase HPLC (RP-HPLC) was used to determine the casein content in the supernatant, the milk and in the deposit [15]. The degree of denaturation of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) in the supernatant of the milk was determined by High Performance Gel Permeation Chromatography (HP-GPC) [6]. The ratio of casein to serum protein was determined by derivative spectroscopy as described by Luf [16], following the modification of E.A.M. de Jong (personal communication); the amount of serum proteins was calculated using Bipro (a commercial whey protein isolate, Mitchelstown Isolates Ltd., Ireland) as a standard. Electrophoresis was performed according to Laemmli [17] at pH 8.9 in 15% polyacrylamide gel containing 4 M urea and 0.003 M SDS (SDS-PAGE). The dissolution of the deposit for RP-HPLC, derivative spectroscopy and SDS-PAGE is described by Jeurnink [15]. By this method nearly all the protein in the deposit could be passed into solution.

3 Results

3.1 Composition of skim milk with varying calcium concentrations

The average concentrations of protein, fat and lactose in the skim milk were 3.6, 0.04 and 4.9%, respectively. The mineral composition of the milk is given in Table 1. Calcium concentration was lowered by 15% by ion-exchange against sodium and it was increased by 20% by adding $CaCl_2.2H_2O$. Cation-exchanger Duolite C464 specifically binds calcium, although some magnesium was bound as well. Sodium concentration increased by using it as exchange-ion and by using NaOH for adjusting pH. Chloride concentration increased by the addition of

Table 1. Mineral composition of the prepared milks with varying calcium concentrations after pH adjustment to 6.7.

	normal-Ca milk (1230 mg/kg)	low-Ca milk (1050 mg/kg)	high-Ca milk (1470 mg/kg)
magnesium (mg/kg)	124	99	111
sodium (mg/kg)	379	691	451
potassium (mg/kg)	1669	1584	1646
chloride (mg/kg)	875	914	1190
phosphate (inorganic) (mg/kg)	1911	2014	1914
citrate (mg/kg)	1691	1800	1834
sulphate (mg/kg)	123	119	120

$CaCl_2.2H_2O$ or of HCl (for adjusting pH). Potassium, phosphate, citrate and sulphate concentrations (accuracy ± 7%) were not significantly affected by the treatments used for preparing milks with varying calcium concentration.

Although we realize that also the concentrations of sodium and chloride were changed, we think that the differences in properties of the milks tested here can be attributed mainly to the variation in the concentration of the divalent cation calcium.

3.2 Influence of the calcium concentration in milk on its calcium ion activity

The first observation was that when the calcium concentration in milk was changed, the calcium ion activity also changed (accuracy 1-2%, Table 2). The value for normal milk corresponds with data found by Geerts et al. [18]. Because milk is saturated with respect to calcium phosphate and contains a 'buffer' of non-dissolved calcium phosphate, it may be expected that a removal or an addition of calcium ions would lead to a relatively small change in the calcium ion activity, as was observed. The greater part of the changes in calcium level thus occurs in the quantity of micellar calcium phosphate.

3.3 Influence of the calcium concentration in milk on the heat stability

The heat stability of different batches of milk with varying calcium concentrations was determined at 140 °C (accuracy ± 1 min). The results for a representa-

Table 2. Calcium ion activity and heat coagulation time (HCT) at 140 °C of milk with varying calcium concentrations. The dry weight of deposit on two plates of the heater section after pasteurizing 800 l of these milks is also given.

	normal-Ca milk (1230 mg/kg)	low-Ca milk (1050 mg/kg)	high-Ca milk (1470 mg/kg)
Ca^{2+}-activity (mM)	0.84	0.75	1.24
HCT (min)	16.3	12.5	10.5
dry weight of deposit (g)	4.14	4.50	11.24

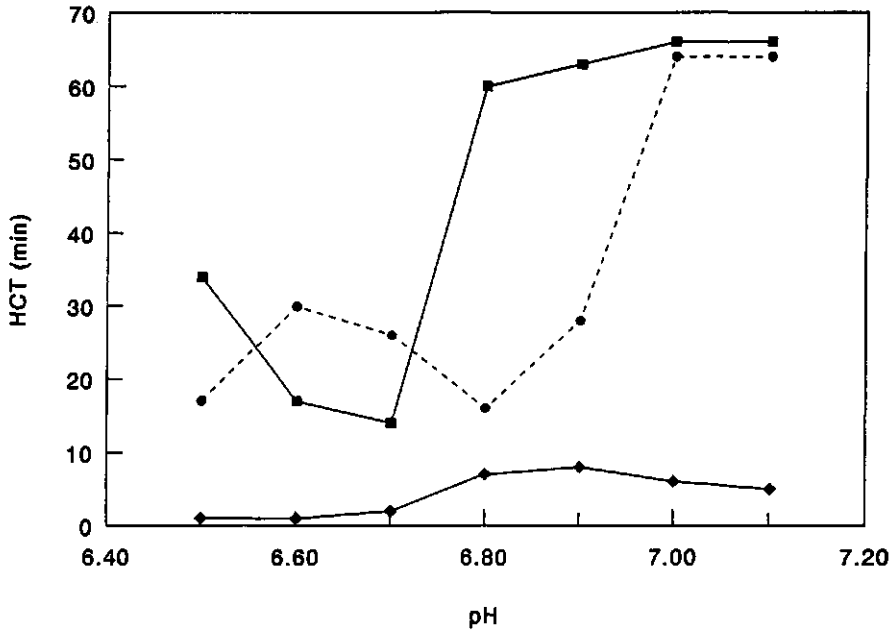

Fig. 1. Heat coagulation time vs. pH (HCT-pH) curve at 140 °C for milk with varying calcium concentrations. ● normal milk; ■ low-calcium milk; ◆ high-calcium milk.

tive batch are shown in Table 2. From the results it can be seen that changing the calcium concentration in milk in either direction decreased the heat stability. The decrease was stronger in high-calcium milk than in low-calcium milk. A decrease in heat stability if calcium and magnesium salts were added to milk was described earlier by Morrissey and O'Mahony [19]. From another batch of milk the heat coagulation time versus pH (HCT-pH) curve was determined for milk with varying calcium concentrations (Fig. 1). Again it is shown that at its own pH (6.7) the normal milk is most heat-stable, followed by low-calcium milk and high-calcium milk, respectively, confirming the results of Table 2. Lowering the calcium concentration moved the minimum in the HCT-pH curve to more acid values; probably the lower calcium ion activity in the milk made it more stable at higher pH. Increasing the calcium concentration destabilized the milk over the whole pH-range; the increased calcium ion activity probably caused a salt-induced coagulation at every pH [1].

3.4 Influence of the calcium concentration in milk on fouling

3.4.1 Amount of deposit. Milk with varying calcium concentrations was pasteurized (85 °C) in a plate heat exchanger. Two plates from the heating section were dismantled and the amount of deposit was determined by scraping it off the surface followed by weighing (accuracy ± 10 mg, Table 2). The experiment was re-

peated with five different batches. The degree of fouling varied from batch to batch, a known phenomenon in fouling studies [20]. With all batches the same trend in the results were obtained; Table 2 shows the results of a representative experiment. For normal milk a mass flux of depositing material of 1.5×10^{-6} $kg.m^{-2}.s^{-1}$ was calculated, which is in the same range as reported by De Jong et al. [21]. Changing the calcium concentration in milk led in both cases to increased fouling; however the effect in the case of high-calcium milk was more pronounced.

3.4.2 Composition of the deposit. Table 3 shows the composition of the deposits found after pasteurization of milk with varying calcium concentrations. The deposit of the normal milk had the appearance of a Type A deposit [22]; however, the ash content was slightly higher. The latter consisted largely (90%) of calcium and phosphate; according to their molar ratio, about 1.6, this would roughly correspond to hydroxyapatite, $Ca_5OH(PO_4)_3$, the most stable form of calcium phosphate salts at the prevailing pH. In addition to calcium and phosphate there are minor contributions of magnesium and citrate. As expected, the contribution of fat (skim milk contained about 0.04% fat) as well as of lactose in the deposits was very small.

It is remarkable that changing the calcium concentration of the milk in either direction, not only affected the minerals in the deposit, but also led to an increase in the deposition of proteins, although this was more pronounced in the high-calcium than in the low-calcium milk. For the high-calcium milk there was relatively more protein in the deposit than for the low-calcium milk.

Figs. 2, 3 and 4 show the protein composition of the deposit and of the milk as determined by RP-HPLC, derivative spectroscopy and SDS-polyacrylamide gel electrophoresis. For fresh milk a RP-HPLC chromatogram as expected is shown (Fig. 2); α-lactalbumin is not mentioned because its peak is hidden under the one for β-casein. An additional peak was found in the RP-HPLC chro-

Table 3. Composition of the dry matter of the deposit on the plates of the heater section after pasteurization of milk with varying calcium concentrations.

	normal-Ca milk (1230 mg/kg)	low-Ca milk (1050 mg/kg)	high-Ca milk (1470 mg/kg)
protein (%)	44.4	45.9	57.3
ash (%)	45.0	47.0	34.5
calcium (%)	15.7	16.5	12.2
phosphate (%)	23.6	24.2	17.8
Ca/PO_4 (mol/mol)	1.58	1.62	1.63
magnesium (%)	0.60	0.35	0.36
citrate (%)	0.49	0.43	0.45
fat (%)	0.4	1.1	0.6
lactose (%)	n.d.	0.01	0.02

n.d. = not determined

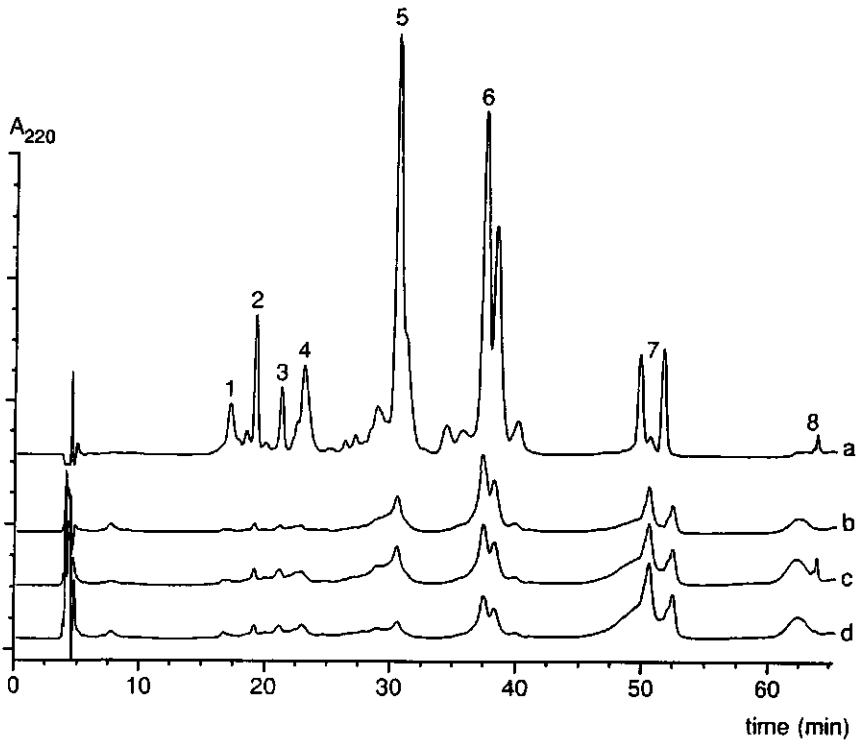

Fig. 2. HPLC chromatograms of fresh skim milk (a) and of deposits of milk with low (b), high (c) and normal (d) calcium concentration, obtained after heating (85 °C) in a pilot-plant-scale plate heat exchanger. 1. glycosylated κ_{A+B}-casein; 2. non-glycosylated κ_A-casein; 3. non-glycosylated κ_B-casein; 4. α_{s2}-casein; 5. α_{s1}-casein; 6. β-casein; 7. β-lactoglobulin; 8. denatured serum proteins.

matogram for the deposits, which probably consisted of denatured β-lg and α-la; this may be explained by the serum proteins in the deposit having been at an elevated temperature for a long time. Due to their denaturation no quantitative results for the serum proteins could be obtained from the RP-HPLC chromatogram. Therefore derivative spectroscopy was also applied; Bipro was used as a standard and because the ratio of β-lg/α-la in the deposit was higher than in Bipro (β-lg/α-la = 3.7) the results (Fig. 3) slightly underestimate the contribution of the serum proteins. The main contribution to the deposit came from the serum proteins, especially from β-lg, while α-la contributed only to a small extent (Fig. 4). All types of caseins were present in the deposits; however, the average casein composition in the deposits differed from that of the casein micelles in the milk (Fig. 3). This suggests that the caseins in the deposit originate from casein micelles and also from the serum phase. For example the presence of α_{s1}– and α_{s2}–casein in the deposit could stem from micelles, but their amount is so low that it also could be the result of a direct deposition on the surface of

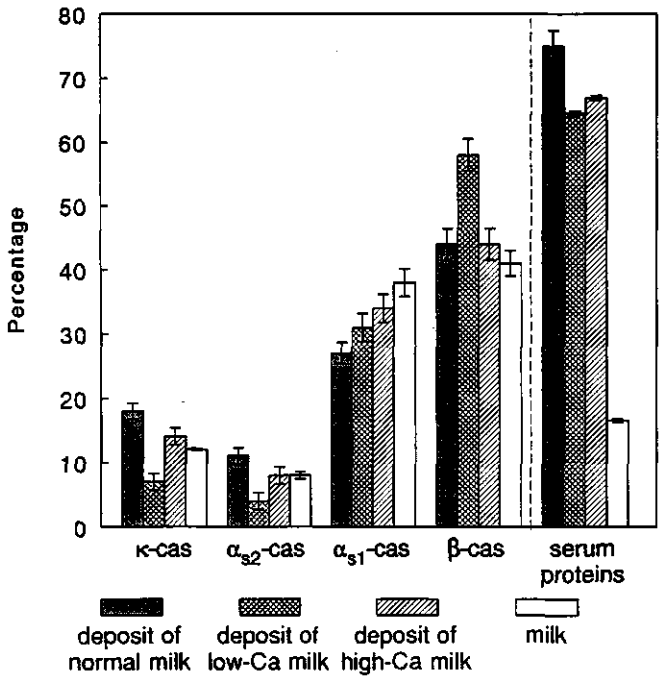

Fig. 3. Composition of the protein in the milk and in the deposit of milk with normal, low and high calcium concentration. The contribution of the different caseins is given as a percentage of the total amount of casein, and the contribution of the serum proteins as a percentage of the total amount of protein in the milk or in the deposits.

'dissolved' caseins. Among the caseins present in milk there was a preference for β-casein to deposit. From the contribution of serum proteins to the deposit it can be seen that if the calcium concentration was changed a shift in the protein composition of the deposit to a higher casein contribution occurred. It is notable that the contribution of the κ-casein to the deposit of the low-calcium milk was significantly lower than for the deposits of the other milks.

3.5 Influence of calcium concentration in milk on the denaturation of serum proteins

The supernatants of milk with varying calcium concentrations were analysed for their native serum protein content before and after heating. The results (Table 4) show that the amount of native β-lg and α-la after 1 and 15 min holding at 85 °C were about the same for the different calcium concentrations. It was concluded that the rate of aggregation of the serum proteins was not significantly influenced by the calcium concentrations in the range as applied here.

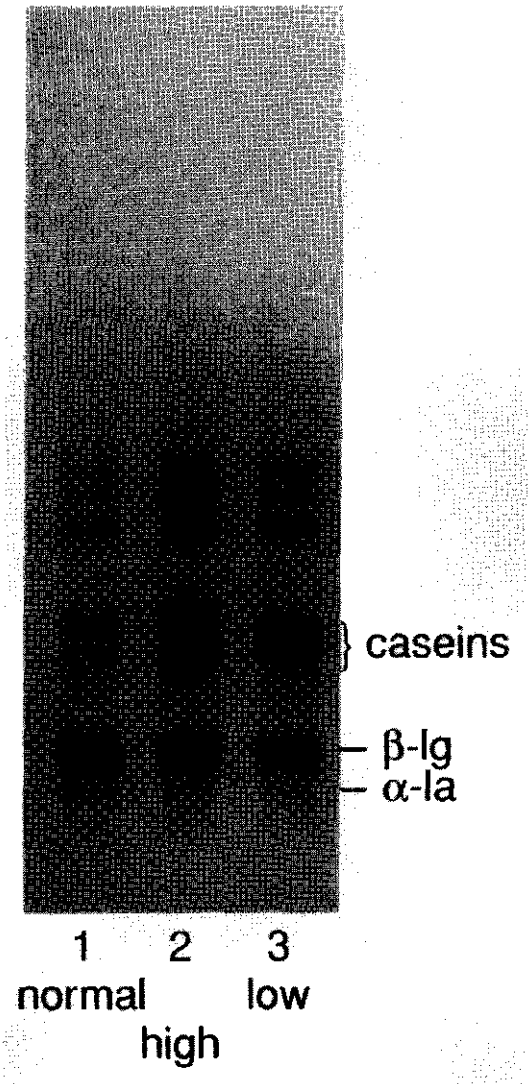

Fig. 4. SDS-polyacrylamide gel electrophoresis of the protein in deposits formed by heating milk with varying calcium concentrations. 1. normal skim milk, 2. high-calcium skim milk, 3. low-calcium skim milk.

Table 4. Percentage of native β-lactoglobulin (β-lg) and α-lactalbumin (α-la) left after 1 and 15 min holding at 85 °C for milk with varying calcium concentrations.

	normal-Ca milk (1230 mg/kg)		low-Ca milk (1050 mg/kg)		high-Ca milk (1470 mg/kg)	
	β-lg	α-la	β-lg	α-la	β-lg	α-la
1 min at 85 °C	32	78	33	70	32	85
15 min at 85 °C	5	26	5	19	5	25

3.6 Milk with varying calcium concentrations as a dispersion of adhesive hard spheres before and after heating

For the viscosity of milk the theory of adhesive hard spheres was applied [6]. By measuring the viscosity of a dilution series of (heated) skim milk it was possible to determine the volume fraction (ϕ, accuracy ± 5%) of the casein micelles and their interaction parameter B_2 (in the case of no interaction except excluded volume, i.e. hard spheres, B_2 has a value of 4; any lower value is a measure of the degree of interaction). Permeate, obtained from the (heated) milk, was used for dilution; it did not contain any casein but since it contained approx. 50% of the total amount of serum proteins two minor corrections were made [6]. By knowing the casein content the voluminosity (in ml/g dry casein) of the casein micelles could be calculated. The results for a representative batch of milk with varying calcium concentrations are shown in Table 5. Decreasing the calcium concentration caused a swelling of the casein micelles, presumably due to increased internal electrostatic repulsion [23] and to dissociation of β-casein some of which may be only partly loosened from the casein micelle, thereby constituting flexible hairs at the casein micelle surface as κ-casein does [24]. Adding calcium to milk caused a decrease in the voluminosity of the micelles. In this case the increase in calcium ion activity and colloidal calcium phosphate probably diminished the electrostatic repulsion within the casein micelles, resulting in a small shrinkage of these particles.

After 1 min holding at 85 °C a lower voluminosity was observed, which must have been due to a change in the dispersed phase because the viscosity of the supernatant had hardly changed. Probably, the cause is linked to a shift in the salt equilibria. In the case of low-calcium milk a lower voluminosity was observed after heating, presumably due to diminished internal repulsion and withdrawal of loosened β-casein molecules leading to shrinkage of the micelles.

After 15 min holding at 85 °C a slightly higher voluminosity was found in normal milk, presumably due to further denaturation of the serum proteins (fully denatured serum proteins have a larger voluminosity than native serum proteins and may associate with the casein micelles). In the case of high-calcium milk had the dispersed particles an even lower voluminosity. Apparently, the addition of calcium salt resulted in more compact casein micelles and/or in the formation of more compact serum protein aggregates.

Table 5. Voluminosity of the casein micelles (ml/g) at 25 °C in milk with varying calcium concentrations before and after 1 or 15 min holding at 85 °C.

	normal-Ca milk (1230 mg/kg)	low-Ca milk (1050 mg/kg)	high-Ca milk (1470 mg/kg)
unheated	4.18	5.01	4.01
1 min at 85 °C	3.96	4.,73	3.71
15 min at 85 °C	4.02	4.41	3.60

Table 6. Interaction parameter (B_2) of micelles in milk at 25 °C with varying calcium concentrations before and after 1 or 15 min holding at 85 °C.

	normal-Ca milk (1230 mg/kg)	low-Ca milk (1050 mg/kg)	high-Ca milk (1470 mg/kg)
unheated	4.7	5.4	4.4
1 min at 85 °C	4.7	5.4	4.4
15 min at 85 °C	3.4	2.9	0.5

Due to the uncertainty in the values obtained for B_2 (uncertainty ~ 25%) it was difficult to interpret absolute values. However, we are interested in relative changes in interactions before and after heating (Table 6). It is assumed, based on former work [6], that after 1 min heating at 85 °C the interactions did not increase, i.e. there was no change in B_2. After 15 min heating at 85 °C certainly a trend for increased interactions was observed (the values for B_2 decreased upon heating). In this case, the casein micelles behave as adhesive hard spheres, caused by the association of the serum proteins with the micelles [6]. The decrease in the B_2-value when high-calcium milk was heated is notable; although the volume fraction decreased, a higher viscosity was found, presumably caused by the strongly increased interactions between the casein micelles.

3.7 Influence of calcium concentration on partition of caseins in milk before and after heating

In milk samples with varying calcium concentrations the percentages of the caseins present in the serum of the milk, i.e. supernatant, were determined before and after heating.

The results are presented in Figs. 5. When the calcium concentration was lowered, a large increase in the percentages of κ-casein and β-casein present in the supernatant was observed; even some α_{s1}– and α_{s2}–caseins were dissociated. If the calcium concentration was increased, less κ- and β-caseins were found in the supernatant; the increase in calcium ion activity and in colloidal calcium phosphate apparently hindered κ- and β-caseins from dissociating at room temperature.

Heating normal skim milk caused a small increase in the dissociation of κ-casein; this is in agreement with findings of Van Hooydonk [25] and was also found in heat stability studies [26]. The percentages of β- and α_{s1}-caseins present in the supernatant hardly changed upon heating skim milk. On heating low-calcium milk there was little change in the percentage of κ-casein, but the percentages of β-, α_{s1}– and α_{s2}–caseins in the supernatant decreased, because they would have returned to the micelles or even because β-casein aggregates would have been formed. In high-calcium milk less κ-casein dissociated upon heating, which may be explained by the formation of a κ-casein/β-lg complex at the surface of the casein micelle being promoted and stabilized by calcium [27, 28, 29].

The observed change in the viscosity of the milk upon heating is not only

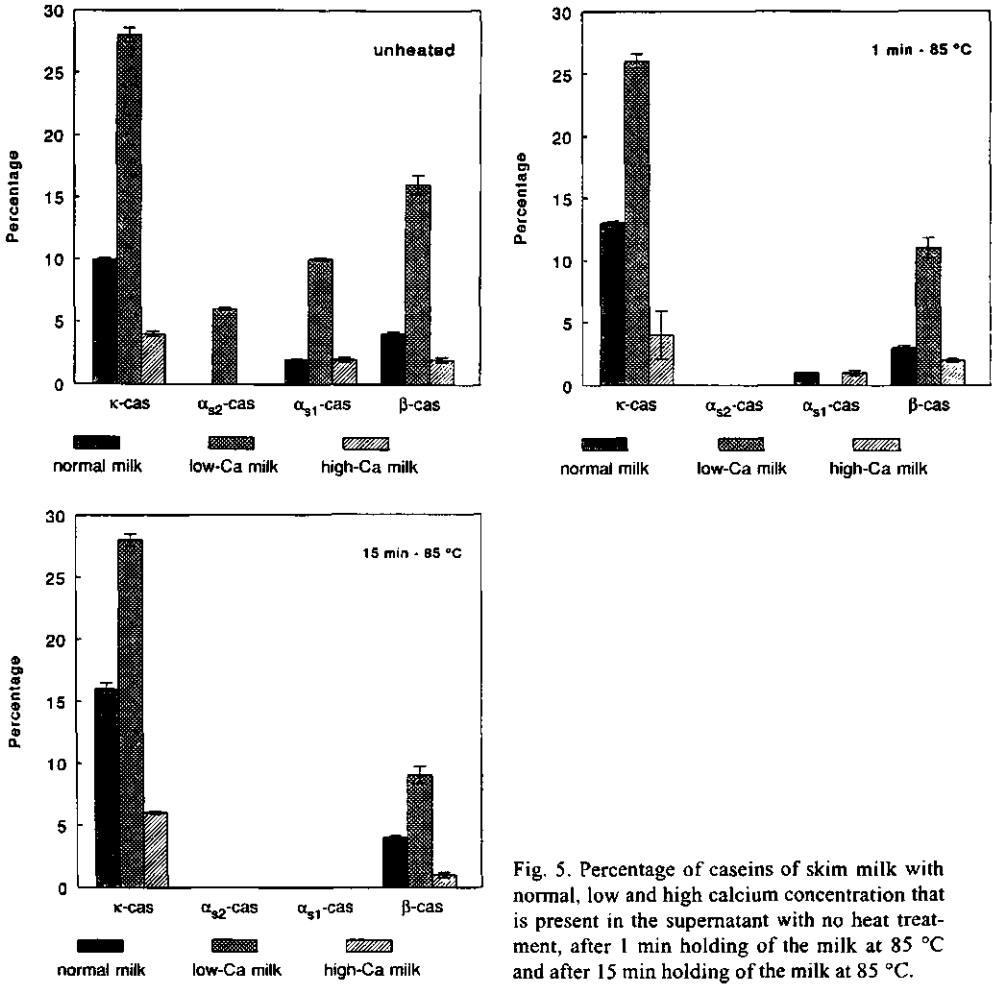

Fig. 5. Percentage of caseins of skim milk with normal, low and high calcium concentration that is present in the supernatant with no heat treatment, after 1 min holding of the milk at 85 °C and after 15 min holding of the milk at 85 °C.

caused by a change in the dispersed phase (swelling or shrinkage of the micelles) but also by a change in the viscosity of the continuous phase, i.e. supernatant, due to the dissociation of casein molecules from the micelles. For low-calcium milk it was found that on average 60% of the increase in viscosity as compared to that of normal milk was caused by a change in the voluminosity of the casein micelles and 40% by the increase in the viscosity of the supernatant.

4 Discussion

Upon heating milk, β-lg is denatured. We presume that the unfolded β-lg can react in three ways: forming aggregates with other β-lg molecules (I), forming a

complex with κ-casein at the surface of the casein micelle (II) and forming a complex with the κ-casein in the serum of the milk (III). Whether the β-lg/κ-casein complex stays at the surface of the micelle or dissociates into the serum will be determined by, among other factors, the calcium ion activity and the pH [28, 30]. This mechanism plays a major role in the explanation of the heat coagulation of milk as a function of the pH [1].

In normal milk, heating caused only a small increase in the dissociation of κ-casein as measured at room temperature. So most probably the micelles were not depleted of κ-casein at pasteurization temperature and the heat coagulation would have been due to a salt-induced coagulation and/or polymerization of the protein molecules [1]. The deposit mainly consisted of serum protein aggregates, strongly suggesting that reaction I is involved in the fouling reaction. Since there was only a small amount of κ-casein in the deposit layer, reactions II and III presumably hardly contributed to the fouling reaction.

Upon heating low-calcium milk, as compared to normal milk, the casein micelles were swollen and a larger part of the κ- and β-caseins was dissociated. Such micelles, the so-called depleted micelles, would no longer have the full stabilizing action of κ-casein and be more sensitive to Ca^{2+} ions. As a result, their mutual interaction would increase (lower B_2-values) and their heat stability decrease as compared to normal milk. Concerning the deposit from the low-calcium milk, there was a shift in protein composition from serum proteins to casein. However, the κ-casein content of the deposit decreased; apparently the depleted micelles contributed to the deposit, whereas the high amount of 'dissolved' κ-casein, being present in β-lg/κ-casein complexes in the serum (reaction III), was not involved in the fouling reaction. Since the contribution of the β-lg to the deposit was also decreased, we presume that κ-casein prevented β-lg from depositing by formation of β-lg/κ-casein complexes in the serum. The depleted micelles contributing to the deposit were probably submicelles, since no increase in the amount of deposited citrate was found (Table 3). The higher contribution of β-casein to the deposit may have been due to a higher amount of β-casein in the serum.

Upon heating high-calcium milk, as compared to normal milk, the casein micelles shrink and hardly any dissociated casein was found in the serum. The increase in calcium ion activity would have reduced electrostatic and steric repulsion and, as a consequence of the lower voluminosity, the Van der Waals attraction would be larger. Hence, the interactions between the micelles increased (lower B_2-values) and as a result the heat stability decreased. The high amount of casein in the deposit was presumably caused by the highly unstable casein micelles; this may be deduced from the observation that the casein in the deposit had about the same composition as in the original casein micelles; also the increase in the absolute amount of deposited citrate is an indication of the presence of micellar calcium phosphate. Visser [28] showed that when the calcium concentration in milk is increased, more serum proteins become associated with the casein micelles on heating. The increased contribution of β-lg to the deposit

may have been the result of this association with the depositing casein micelles (reaction II).

5 Conclusions

Calcium ions changed the structure of the casein micelles in milk in such a way (albeit for low- and high-calcium milk in a different way) that their mutual interaction increased, thereby making them less stable towards heating. The results in this study suggest that if β-lg forms a complex with the κ-casein, either at the surface of (stable) casein micelles or in the serum, it is no longer (or less) available for the fouling reaction.

Since there is a natural variation in the calcium content of milk, the effect of calcium on heat stability and fouling described here may be an explanation for the seasonal variations found in these properties of milk, although we realize that the natural variation in calcium is not achieved by the use of a cation-exchanger or addition of $CaCl_2.2H_2O$.

In former work [15] proteolysis was found to decrease the heat stability of casein micelles and also resulted in more fouling. The results presented here support the view that the degree of protein fouling is correlated with the heat stability of the casein micelles. This result may open the opportunity to predict the fouling behaviour of a given batch of milk by determining its HCT.

6 Acknowledgement

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7 References

1. M.A.J.S. van Boekel, J.A. Nieuwenhuijse & P. Walstra. The heat coagulation of milk. 1. Mechanisms. *Neth. Milk Dairy J.* 43(1989)97–128.
2. D.S. Horne & D.D. Muir. Alcohol and heat stability of milk protein. *J. Dairy Sci.* 73(1990)3613–3626.
3. M. Lalande, J.P. Tissier & G. Corrieu. Fouling of a plate heat exchanger used in ultra-high temperature sterilization of milk. *J. Dairy Res.* 51(1984)557–568.
4. Th.J.M. Jeurnink, D.W. Brinkman & A.D. Stemerdink. Distribution and composition of deposit in heat exchangers. In: Fouling and Cleaning in Food Processing (H.G. Kessler & D.B. Lund, Eds). Augsburg: Druckerei Walch, 1989, pp. 25–36.
5. B.M.A. Delsing & J. Hiddink. Fouling of heat transfer surfaces by dairy liquids. *Neth. Milk Dairy J.* 37(1983)139–148.
6. Th.J.M. Jeurnink & C.G. de Kruif. Changes in milk on heating: viscosity measurements. *J. Dairy Res.* 60(1993)139–150.
7. Australian Standard 1629.3.4 (1978). Standards Association of Australia.
8. Dutch Standards NEN 3198 (1984). Dutch Organization for Standardization. Delft, Netherlands.

9. M.A.J.S. van Boekel & B. Ribadeau-Dumas. Addendum to the evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. *Neth. Milk Dairy J.* 41(1987)281–284.
10. Dutch Standards NEN 6465 (1981). Dutch Organization for Standardization. Delft, Netherlands.
11. Dutch Standards NEN 6446 (1980). Dutch Organization for Standardization. Delft, Netherlands.
12. Dutch Standards NEN 6479 (1981). Dutch Organization for Standardization. Delft, Netherlands.
13. Dutch Standards NEN 3235 9.2.1. (1978). Dutch Organization for Standardization. Delft, Netherlands.
14. Th.J.M. Jeurnink & D.W. Brinkman. The cleaning of heat exchangers and evaporators after processing milk or whey. *Int. Dairy J.* 4(1994)347–368.
15. Th.J.M. Jeurnink. Effect of proteolysis in milk on fouling in heat exchangers. *Neth. Milk Dairy J.* 45(1991)23–32.
16. W. Luf & E. Brandl. On the determination of undenatured whey protein nitrogen in milk and dried milk by means of the UV-derivative spectroscopy. *Milchwissenschaft* 42(1987)275–278.
17. U.K. Laemmli. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(1970)680–685.
18. J. P. Geerts, J.J. Bekhof & W. Scherjon. Determination of calcium ion activities in milk with an ion-selective electrode. A linear relationship between the logarithm of time and the recovery of the calcium ion activity after heat treatment. *Neth. Milk Dairy J.* 37(1983)197–211.
19. P.A. Morrissey & F. O'Mahony. Heat stability of forewarmed milks: influence of κ-casein, serum proteins and divalent cations. *J. Dairy Res.* 43(1976)267–274.
20. A.S. Grandison, UHT processing of milk: seasonal variation in deposit formation in heat exchangers. *J. Soc. Dairy Techn.* 41(1988)43–49.
21. P. De Jong, S. Bouman & H.J.L.J. van der Linden. Fouling of heat treatment equipment in relation to the denaturation of β-lactoglobulin. *J. Soc. Dairy Techn.* 45(1992)3–8.
22. H. Burton. Reviews of the progress of dairy science. Section G. Deposits from whole milk in heat treatment plant- a review and discussion. *J. Dairy Res.* 35(1968)317–330.
23. T.H.M. Snoeren, H.J. Klok, A.C.M. van Hooydonk & A.J. Damman. The voluminosity of casein micelles. *Milchwissenschaft* 39(1984)461–463.
24. P. Walstra. On the stability of casein micelles. *J. Dairy Sci.* 73(1990)1965–1979.
25. A.C.M. van Hooydonk, P.G. de Koster & I.J. Boerrigter. The renneting properties of heated milk. *Neth. Milk Dairy J.* 41(1987)3–18.
26. H. Singh & P.F. Fox. Heat stability of milk: further studies on the pH-dependent dissociation of micellar κ-casein. *J. Dairy Res.* 53(1986)237–248.
27. P. Smits & J.H. van Brouwershaven. Heat-induced association of β-lactoglobulin and casein micelles. *J. Dairy Res.* 47(1980)313–325.
28. J. Visser. A new casein micelle model and its consequences for pH and temperature effects on the properties of milk. In: Protein Interactions (J. Visser, Ed.). New York: Weinheim, 1991, pp. 135–165.
29. H. Singh & P.F. Fox. Heat stability of milk: role of β-lactoglobulin in the pH-dependent dissociation of micellar κ-casein. *J. Dairy Res.* 54(1987)509–521.
30. H. Singh & J.M. Lathum. Heat stability of milk: aggregation and dissociation of protein at ultra-high temperatures. *Int. Dairy J.* 3(1993)225–237.

Fouling of heat exchangers by fresh and reconstituted milk and the influence of air bubbles

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1. Introduction

To exclude seasonal variations (1) as a parameter in fouling, reconstituted milk from the same batch of milk powder is preferably used. However, reconstituted milk differs in properties from fresh milk (*e.g.* in heat stability (2), viscosity and clotting time). In this study it was tested whether evaporation and drying of milk had an effect on its fouling behaviour.

The solubility of air in milk decreases on heating, which may result in the formation of air bubbles. BURTON (3) suggested that air in milk only encourages deposit formation, if it was separated as bubbles on the heating surface. THOM (4) reported that air bubbles were nuclei for the formation of deposit. Here the influence of these air bubbles on the amount of deposit was studied, in both a laboratory-scale and a pilot-plant-scale heat exchanger.

2. Material and methods

2.1 Milk samples

The skim milk used in this study was obtained from the institute's experimental dairy. Reconstituted milk was prepared by dissolving skim milk powder in distilled water at 40°C followed by stirring for 1 h. The milk obtained was stored overnight at 4°C; the pH was adjusted to 6.7 if necessary. The milk was brought to room temperature 1 h before use.

2.2 Heat treatment

To determine the amount of deposit, usually a batch of 6.4 l milk was heated to 85°C in a laboratory-scale tubular heat exchanger (THE) which consisted of 2 concentric tubes (5). The inner tube was of stainless steel, the outer tube of glass. The stainless steel tube was heated from the inside by hot water (inlet temperature 93°C, flow 180 l/h). The milk was pumped (flow 5.5 l/h, Re = 26) counter-current to the hot water between the stainless steel tube and the glass tube. The inlet temperature of the milk was 20°C and the outlet temperature 85°C. This high ΔT was applied in order to obtain a reasonable amount of deposit in a relatively short time (70 min). The fouling area was 0.028 m^2. In- and outlet temperatures were recorded using copper/constantan thermocouples.

In one experiment a pilot-plant-scale plate heat exchanger (PHE) (Alfa-Laval, type A3-HRB) was used. The product flow was 100 l/h for a period of 8 h. The heating temperature was 85°C with a holding time of 15 s. The heating section was composed of 2 plates with a fouling area of 0.095 m^2. The water was counter-current to the milk at a flow rate of 100 l/h and with an inlet temperature of 92°C. The milk inlet temperature in the heating section was 69°C.

After heat treatment of the milk the stainless steel tube of the THE or 1 plate of the heating section of the PHE were cleaned in 2 steps using 1 % NaOH and 0.5% HNO_3, respectively. The cleaning solutions were collected and analysed for nitrogen and calcium content as a measure of the amount of protein and mineral deposit, respectively. In order to analyse the composition of the deposit, it was scraped off the surface.

2.3 Analysis

Nitrogen was determined by the Kjeldahl method according to NEN 3198 (6); protein was calculated via 6.38 * N. Calcium was determined after digestion according to NEN 6465 (7) by atomic absorption spectroscopy (NEN 6446, (8)). Total phosphorus was determined by photospectroscopy (NEN 6479, (9)). Lactose was determined by high-performance liquid chromatography (HPLC); a Polyspher CHPb 18 (90°C) column (E. Merck, Darmstadt, Germany) was used at a flow rate of 0.4 ml/min (eluent H_2O). Refractive index was used for detection. Protein composition in the deposit was determined by SDS-polyacrylamide gel electrophoresis and reversed-phase HPLC (RP-HPLC) as described in (5). For determining the ratio of casein to serum protein, derivative spectroscopy was applied using a modification of the method described by LUF and BRANDL (10); the amount of serum proteins was calculated using Bipro (a commercial serum protein isolate, Mitchelstown Isolates Ltd., Ireland) as a standard.

For scanning electron microscopy (SEM) the deposit was coated with silver and gold (20 nm) in a Baltzer sputter coater. Micrographs were made with a JEOL 1200 scanning electron microscope operating at 60 kV.

3. Results and discussion

3.1 Effect of reconstitution on fouling

After heating (85°C) in the laboratory-scale THE of fresh and reconstituted skim milk, which came from the same batch of milk, the amount of deposit was determined. Table 1 shows that reconstituted milk gave much less fouling than fresh milk. Part of the explanation is found in denaturation of serum proteins during evaporation and drying, since already denatured serum proteins are less active in the fouling reaction. However, since conditions for low-heat milk powder were used, the denaturation

Table 1: Amount of protein and calcium deposition after heating of fresh and reconstituted skim milk (made from the same batch of milk) and of de-aerated fresh skim milk in a laboratory-scale tubular heat exchanger

	Skim milk		
	Fresh	Reco	De-aerated
Protein in deposit (mg)	3480	901	159
Calcium in deposit (mg)	121	34	20

for β -lg was only 25 %; in other words 75 % of the serum protein in the reconstituted milk was still in its native form. The process of powder making and reconstitution presumably caused other changes affecting the fouling behaviour. For example, the calcium concentration and the calcium ion activity in reconstituted milk had decreased by 9 and 11 %, respectively. There is as yet no clear explanation for the decrease in fouling for reconstituted milk compared to fresh milk. It has to be realized that if reconstituted milk is used the absolute figures for the amount of deposit would not apply to situations in which fresh milk is used.

3.2 Effect of air bubbles in milk on fouling

In order to determine the influence of air bubbles on the amount of deposit 1 batch of fresh skim milk was divided into 2 parts, one of which was de-aerated before heating in the laboratory-scale THE. The amount and composition of deposit after heating are given in Tables 1 and 2, respectively. If milk is de-aerated the amount of deposit is far smaller. Visual observations showed that air bubbles were nuclei for the formation of deposit, as was described earlier by THOM (4). From the composition of the deposits (Table 2) there seemed to be little difference between the deposits of normal and de-aerated milk. To obtain a reasonable amount of deposit it was therefore decided not to de-aerate the milk prior to heating. That the calcium to protein ratio in Table 2 is lower than in Table 1 is due to the fact that the former was obtained from deposit that was scraped from the surface and the latter from analysing the cleaning solutions: calcium deposit is more difficult to remove completely by scraping than by the cleaning solutions.

Table 2: Composition (% of dry matter) of deposit after heating of (de-aerated) fresh skim milk in a laboratory-scale tubular heat exchanger

Composition of deposit	Skim milk	
	Normal	De-aerated
Protein (%)	72.8	80.4
Lactose (%)	1.5	0.8
Ash (%)	10.9	17.3
Phosphorus (%)	1.7	2.7
Calcium (%)	3.4	5.5

Since the hydrodynamic conditions in the two heat exchangers are different, air bubbles would behave differently at the surface. Therefore, later on, the protein composition of the deposit of fresh milk from both types of heat exchangers was determined. Figs. 1 and 2 show the RP-HPLC chromatograms for the 2 deposits. The chromatogram of the deposit from the laboratory-scale THE is almost identical to that of heated milk, except that the peaks in the chromatogram of the deposit are slightly moved to the left. This is presumably due to the extensive heat treatment that the proteins, especially the serum proteins, have undergone in the process of fouling. This result suggests that the

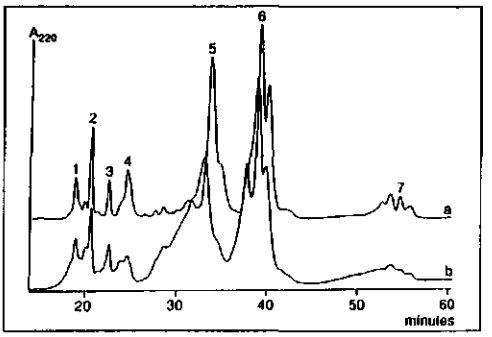

Fig. 1: RP-HPLC chromatogram of heated (85 °C) skim milk in a laboratory-scale tubular heat exchanger (a) and of deposit of that milk (b). 1. glycosylated κ_{A+B}-casein; 2. non-glycosylated κ_A-casein; 3. non-glycosylated κ_B-casein; 4. α_{s2}- casein; 5. α_{s1}-casein; 6. β-casein; 7. β-lactoglobulin.

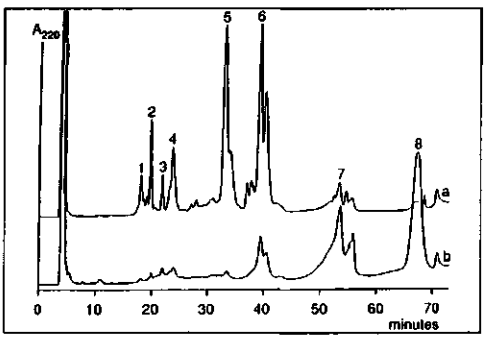

Fig. 2: RP-HPLC chromatogram of fresh skim milk (a) and of deposit of that milk (b) after heating (85°C) in a pilot-plant-scale plate heat exchanger. 1. glycosylated κ_{A+B} -casein; 2. non-glycosylated κ_A -casein; 3. non-glycosylated κ_A -casein; 4. α_{s2}-casein; 5. α_{s1}-casein; 6. β-casein; 7. β-lactoglobulin; 8. denatured serum proteins.

caseins in the deposit are the same as in the milk, *i.e.* they are present in the deposit in the form of casein micelles. Similar results were earlier reported by JEURNINK (5). The chromatogram of the deposit scraped from the pilot-plant-scale PHE differs from that of the milk (Fig. 2) by showing a much higher contribution of serum proteins. Also the ratios of the individual caseins present in the deposit were not the same as in milk; possibly the caseins were not present only in the form of casein

micelles. In Table 3 the protein composition of the deposits is given. A remarkable difference in the ratio of casein to serum protein was found between the 2 deposits, confirming the results of the RP-HPLC chromatogram. Comparing the ratio of β-lg to α-la in milk (ratio 3.3) and in a deposit (ratio 4.4) indicates that there is a preference for β-lg to deposit.

Table 3: Protein composition of fresh skim milk and of its deposit scraped from the laboratory-scale tubular heat exchanger (THE) and from the pilot-plant-scale plate heat exchanger (PHE)

	Serum protein/casein	β-Lactoglobulin/ α-Lactalbumin
Skim milk	0.2	3.3
Deposit (THE)	0.2	4.4
Deposit (PHE)	3	–

– not determined

A possible explanation for the high casein level in the deposit from the laboratory-scale THE surface may be found in the presence of air/vapour bubbles. Air bubbles can contribute to the deposit if the surface to which they may attach becomes dry (Fig. 3, Photo 1). As a consequence, there is an increase in the temperature difference between the hot stainless steel surface and the bulk of the liquid

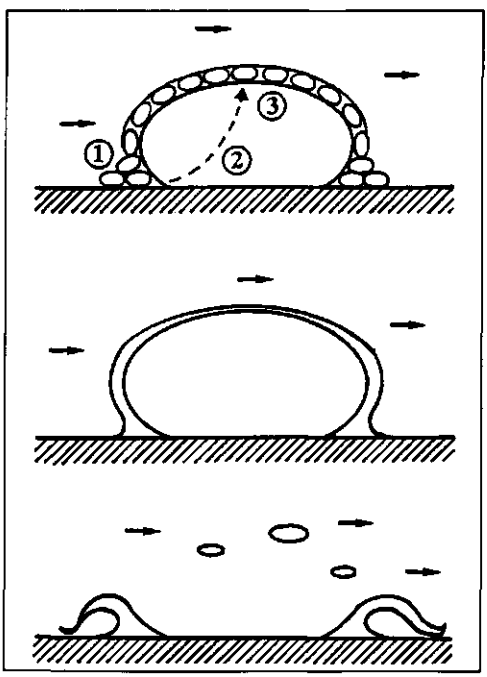

Fig. 3: Schematic representation of the participation of an air bubble in the fouling process of milk at a hot stainless steel surface (see text). 1. adsorption/deposition at the vapour/liquid interface; 2. evaporation; 3. condensation. → = flow direction of the milk.

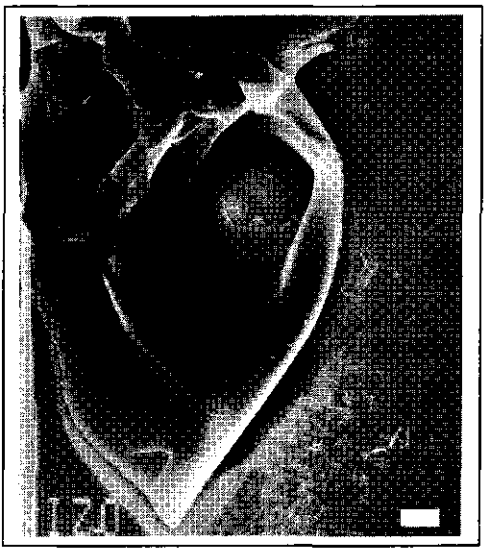

Photo 1: Deposit of fresh skim milk with the remains of air bubble membranes in a laboratory-scale tubular heat exchanger. Bar = 100 µm.

resulting in evaporation of water at the vapour-liquid interface. Due to this evaporation milk is transported from the bulk to the surface where the air/vapour bubble is attached. Here milk protein accumulates and because of the local increase in concentration and the high temperature the protein may coagulate and deposit on the surface. Eventually the air/vapour bubble bursts and part of the membrane is carried away with the liquid. The contribution of air/vapour bubbles to the deposit is determined by the amount of air present in the milk, the temperature difference between the surface and the bulk, the operational pressure in the heat exchanger and the wall shear stress.

Due to the low Re-number in the laboratory-scale THE the air bubbles, which normally arise upon heating milk, were not removed but stuck onto the stainless steel wall. In the pilot-plant-scale PHE, on the other hand, the air bubbles would remain very small due to the high operational pressure. Moreover they are entrained with the flow from the stainless steel surface due to the much higher Re- number. Since in foams caseins tend to adsorb at the air-liquid interface in preference to the serum proteins (11), this may explain the large contribution of caseins to the deposit of the laboratory-scale THE. This view is further supported by scanning electron micrographs of the milk-deposit in the THE and in the PHE, shown in photos 2 and 3, respectively. Photo 2 shows casein micelles with threads (of β-casein?) whereas the globules in photo 3 are much smaller, presumably being serum protein aggregates.

In addition to denatured serum proteins, the proteins present at air/vapour bubble interfaces appear to contribute to the deposit in the laboratory

Photo 2: Deposit of fresh skim milk in a laboratory-scale tubular heat exchanger. N.B.: larger magnification than photo 1: bar = 100 nm.

Photo 3: Deposit of fresh skim milk in a pilot-plant-scale plate heat exchanger. Bar = 100 nm.

scale THE, whereas in the pilot-plant-scale PHE this would be far less so.

4. Conclusions

The process of drying and reconstituting milk was observed to reduce fouling of the milk during heating as compared to fresh milk. Air bubbles at the surface accelerated the fouling process and caused a shift in the protein composition from serum proteins towards caseins. At high shear rates, as in a PHE, the flow conditions may prevent the deposition of casein micelles.

Acknowledgement

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5. References

(1) GRANDISON, A.S.: J. Soc. Dairy Techn. **41** 43-49 (1988)
(2) SINGH, H., CREAMER, L.K.: J. Dairy Res. **58** 269-283 (1991)
(3) BURTON, H.: J. Dairy Res. **35** 317-330 (1968)
(4) THOM, R.: Milchwissenschaft **30** 84-90 (1975)
(5) JEURNINK, T.J.M.: Neth. Milk Dairy J. **45** 23-32 (1991)
(6) Dutch Standards NEN 3198, Dutch Org. for Standardiz. Delft, The Netherlands (1984)
(7) Dutch Standards NEN 6465, Dutch Org. for Standardiz. Delft, The Netherlands (1981)
(8) Dutch Standards NEN 6446, Dutch Org. for Standardiz. Delft, The Netherlands (1980)
(9) Dutch Standards NEN 6479, Dutch Org. for Standardiz. Delft, The Netherlands (1981)
(10) LUF, W., BRANDL, E.: Milchwissenschaft **42** 275-278 (1987)
(11) DICKINSON, E., MAUFFRET, A., ROLFE, S.E., WOSKETT, C.M.: J. Soc. Dairy Techn. **42** 18-22 (1989)

6. Summary

JEURNINK, T.J.M.: **Fouling of heat exchangers by fresh and reconstituted milk and the influence of air bubbles.** Milchwissenschaft **50** (4) 189–193 (1995).

27 Heat exchanger (fouling)

The influence of reconstitution of milk and of air bubbles in milk on fouling was studied. Reconstituted milk gave much less fouling than fresh milk, for reasons that are not yet clear. Air bubbles, which arose in the milk on heating and stuck to the stainless steel wall, appear to act as nuclei for the formation of deposit and influence the composition of the deposit through drying of the membrane of air bubbles containing caseins. High shear rates may prevent air bubbles from sticking to the wall, resulting in less fouling.

JEURNINK, T.J.M.: **Ablagerungen bei Wärmeaustauschern durch frische und rekonstituierte Milch und der Einfluß von Luftbläschen.** Milchwissenschaft **50** (4) 189–193 (1995).

27 Wärmeaustauscher (Ablagerungen)

Es wurde der Einfluß der Rekonstituierung von Milch und von Luftbläschen in der Milch auf Ablagerungen untersucht. Rekonstituierte Milch führte zu weniger „Verschmutzung" als frische Milch aus Gründen, die

noch nicht klar sind. Es hat sich gezeigt, daß Luftbläschen, die in der Milch beim Erhitzen aufstiegen und an der Edelstahlwand hafteten, als „Kerne" für die Bildung der Ablagerungen sorgen. Sie beeinflussen die Zusammensetzung der Ablagerungen durch Trocknen der caseinhaltigen Luftbläschenmembranen. Hohe Scherraten können verhindern, daß Luftbläschen an der Wand haften und damit zu weniger Belagbildung beitragen.

Fouling of heat exchangers in relation to the serum protein concentration in milk

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1. Introduction

If the temperature of the milk in a heat exchanger reaches 70°C and higher a strong increase in the amount of deposit onto the stainless steel wall is found (1). This effect is mainly attributed to the denaturation of the serum proteins in the milk. Several researchers (2, 3, 4, 5, 6) have tried to correlate the fouling rate with the rate of denaturation of the serum proteins. In the work described here, fouling of milk, in the presence and absence of serum proteins, was studied in a laboratory-scale and a pilot-plant-scale heat exchanger to further quantify the role of the serum proteins in the fouling reaction.

2. Material and methods

2.1 Milk samples

Skim milk was obtained from the Institute's experimental dairy. Serum-protein-poor milk (SPPM; 90 mg serum protein/kg milk left) was prepared by microfiltration of skim milk (S.C.T., Bazet, France, Membralox 7P19-40, 1.4 m^2, pore size 0.5 µm) followed by diafiltration with UF-permeate. Freeze-dried powder of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) was prepared by the method of MAUBOIS (7). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO 63178 USA, No A-2153).

Reconstituted milk was prepared by dissolving skim milk powder in distilled water at 40°C followed by stirring for 1 h. The milk obtained was stored overnight at 4°C; the pH was adjusted to 6.7 if necessary. The milk was brought to room temperature 1 h before use.

2.2 Heat treatment

Fouling was studied in a laboratory-scale tubular heat exchanger (THE) and in a pilot-plant-scale plate heat exchanger (PHE). Details of the heat treatment (85°C) and of the determination of the amount and composition of the deposit are described in (8). Experiments were repeated at least twice, except for the one with the PHE.

3. Results and discussion

3.1 Comparison of skim milk and serum-protein-poor milk

Fresh skim milk was divided into 2 batches, from one of which most of the serum protein had been removed (SPPM). Both batches were heated in the pilot-plant-scale PHE or in the laboratory-scale THE, and the amounts of removed deposit were determined (Table 1). It is seen that if serum proteins are almost absent in milk a substantial decrease in fouling results. Apparently, the presence of serum proteins plays an important role in the fouling reaction. In former work (8) the protein composition of the deposit of fresh skim milk was determined (Table 2); unfortunately it was not possible for the deposit of SPPM to collect enough material for analysis. Since in the PHE the deposit of fresh milk mainly consisted of serum proteins, a result of the denaturation of serum proteins, it is to be expected that if serum proteins are almost absent the amount of deposit will be substantially reduced.

Table 1: Amount of protein and calcium deposition after heating of fresh skim milk (SM) and serum-protein-poor milk (SPPM, made from the same batch of milk) in a pilot-plant-scale plate heat exchanger (PHE) and in a laboratory-scale tubular heat exchanger (THE)

	PHE		THE	
	SM	SPPM	SM	SPPM
Protein in deposit (mg)	22880	6020	3480	1103
Calcium in deposit (mg)	1380	400	121	45

Table 2: Protein composition of fresh skim milk and of its deposit scraped from the laboratory-scale tubular heat exchanger (THE) and from the pilot-plant-scale plate heat exchanger (PHE). (Jeurnink (8))

	Serum protein/ casein	β-lactoglobulin/ α-lactalbumin
Skim milk	0.2	3.3
Deposit (THE)	0.2	4.4
Deposit (PHE)	3	–

– = not determined

In the experiment with the laboratory-scale THE another effect also plays a role. The SPPM contained 90 mg serum proteins/kg milk; in total approximately 600 mg serum protein (6.4 l of SPPM) passed the heat exchanger while about 1100 mg of protein deposit was found. This showed that even when all serum proteins are deposited, other proteins than serum protein, *i.e.* caseins, were also involved in the fouling reaction, which is confirmed by the results for the THE in Table 2. This probably took place via drying of the membrane of air/vapour bubbles containing caseins, as was described by JEURNINK (8). That skim milk gave more fouling on the surface of the THE compared to SPPM may possibly be explained by the serum proteins acting as a "sticking agent" between casein micelles, resulting

in a larger amount of casein micelles at the air-liquid interface. Another effect that may have played a role is that the ultrafiltration treatment of the SPPM had reduced the amount of dissolved air before heating, resulting in less fouling (8).

Although the calcium content had not been altered in the milk the parallel decrease in calcium deposition indicates that the deposition of protein is coupled with that of calcium (Table 1). PAPPAS and ROTHWELL (9) showed that serum proteins upon heating bind calcium. Furthermore, casein micelles contain a considerable amount of colloidal calcium phosphate, and heating causes more calcium phosphate to associate with the micelles. In unheated milk there is about 32 mg micellar calcium per g casein. Based upon results of POULIOT *et al.* (10) this value is upon heating increased to 40 mg calcium per g casein. Assuming that the deposit of the SPPM only consisted of casein micelles, it can be calculated that 44 mg of calcium is bound to the casein deposit in the laboratory-scale THE, while 45 mg of calcium deposit was found experimentally. Apparently the direct precipitation of calcium phosphate onto the stainless steel surface hardly contributes to the calcium deposition. Since it was observed that the caseins in the deposit of the pilot-plant-scale PHE were not present in the form of casein micelles (8) it was not possible to do such a calculation. Moreover the volume/surface ratio in the PHE was smaller than in the THE and therefore more calcium phosphate may have precipitated directly at the surface.

3.2 Fouling as a function of the serum protein concentration in milk

Serum protein concentration in milk was varied by adding Bipro (a commercial serum protein isolate) to reconstituted SPPM and to reconstituted skim milk made from the same batch. Reconstituted milk gives less fouling than fresh milk (8); it has to be realized therefore that the absolute figures for the amount of deposit would not apply to situations in which fresh milk is used. After heating of the samples in the laboratory-scale THE the amount of deposit was determined. Fig. 1 shows the amount of protein and calcium in the deposit as a function of the serum protein concentration in the milk. The amount of protein in the deposit increased with increasing serum protein concentration, but was not directly proportional to it. At concentrations of serum proteins above 20 g/l the coagulation of serum proteins was so strong that within a few minutes the channels of the THE were blocked. The curves for protein and calcium deposition are quite similar, implying that the protein/calcium ratio in the deposit is about constant. This, again, is an indication that the depositions of calcium and protein are coupled.

It was not possible to control the product temperature at the outlet of the laboratory-scale THE and this temperature was observed to decrease with fouling. Consequently the heat flux would diminish and so may the amount of deposit. Therefore, the fouling rate according to the method of LING and LUND (11) was also determined. These authors defined the slope of the function of the reciprocal of the heat

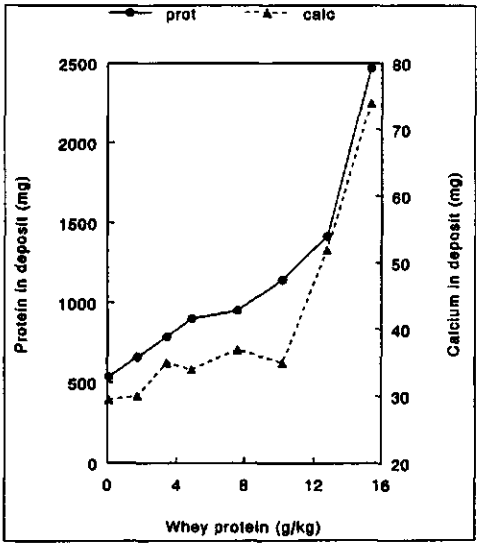

Fig. 1: Protein and calcium deposition after heating in a laboratory-scale tubular heat exchanger of reconstituted skim milk with varying serum protein concentration.

transfer coefficient (K) against the running time of the pasteurization as the fouling rate. Fig. 2 shows the initial fouling rate as function of the serum protein concentration in the milk. The curve is in agreement with the results shown in Fig. 1.

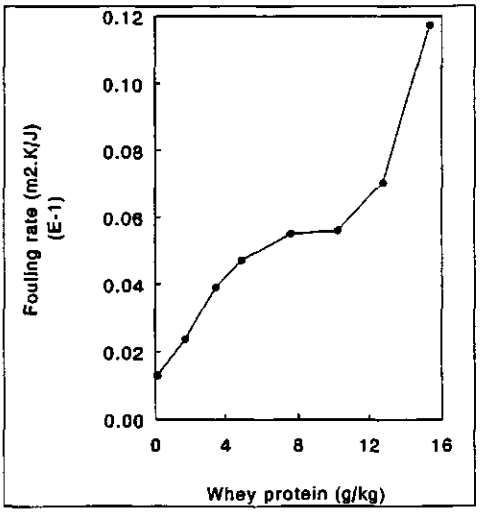

Fig. 2: Initial fouling rate during heating of reconstituted skim milk with varying serum protein concentration.

In other work (8) it was shown that the composition of the deposit in the THE was influenced by the presence of air bubbles; therefore the results ob-

tained in this study can only show a trend of the influence of the serum protein concentration on fouling: fouling increased with serum protein concentration in milk, but the data could not be fitted to a straightforward model since various reactions are involved. First, there is the drying of the membrane of air bubbles onto the surface, which would primarily cause casein deposition (8). Second, serum proteins interact with casein micelles. At a serum protein concentration of about 2.6 g/l there is enough serum protein to cover the surface of the micelles and at higher concentrations, the number of casein micelles may become a limiting factor in the fouling reaction. Third, there is precipitation of aggregated serum proteins directly onto the surface, which becomes more important with increasing serum protein concentration. It seems that at concentrations of serum proteins of 10 g/l or more, the process of coagulation or gelation of the serum proteins is the rate-determining step.

3.3 Influence of the various serum proteins in milk on fouling

In order to study the influence of individual serum proteins in milk on fouling, they were added to reconstituted SPPM separately, in such an amount that the same concentration was reached as in normal milk. After heating in the laboratory-scale THE the amount of deposit was determined. Table 3 shows that all the tested serum proteins, BSA, α-la and β-lg, contributed to the deposit. A concentration of 0.115 % α-la gave more fouling than β-lg at a concentration of 0.318 %. This can probably be explained by α-la denaturing faster than β-lg. If α-la and β-lg were both added, the amount of deposit was less than the sum of their separate contributions. The fact that α-la and β-lg aggregate together may affect the fouling behaviour. Reconstituted SPPM to which α-la, β-lg and BSA had been added gave more fouling than reconstituted skim milk, this may be due to addition of some salts with the serum proteins.

Table 3: Amount of protein and calcium deposition and their ratio after heating in a laboratory-scale tubular heat exchanger of reconstituted skim milk and of reconstituted serum-protein-poor milk (SPPM) to which bovine serum albumin (BSA), α-lactalbumin (α-la) and/or β-lactoglobulin (β-lg) had been added

Serum protein added to SPPM	Protein deposit (mg)	Calcium deposit (mg)	Protein/ calcium
None	536	30	17.9
BSA 0.023 %	644	29	22.2
α-la 0.115 %	893	55	16.2
β-lg 0.318 %	860	50	17.2
α-la 0.115 % + β-lg 0.318 %	1147	–	–
BSA 0.023 % + α-la 0.115 % + β-lg 0.318 %	1371	–	–
Skim milk	901	34	26.5

– = not determined

The protein/calcium ratio was about constant. This showed, again, that the calcium deposition is coupled to that of protein. PAPPAS and ROTHWELL (9) showed that about 3.6 mmol calcium per mmol β-lg was bound. If all the deposited protein of the sample of SPPM + 0.318 % β-lg were β-lg, this would correspond to 6.9 mg calcium. Since 50 mg calcium deposit was found, caseins seem to play an important role in fouling.

4. Conclusions

If serum proteins were almost totally absent in milk, there was a large decrease in fouling compared to normal milk. On the basis of this result it is concluded that the denaturation of serum proteins plays an important role in the fouling reaction. In their denatured state serum proteins can interact with each other, with casein micelles and with the stainless steel surface, resulting in the formation of a deposit layer.

The results further showed that the deposition of calcium was linked with the deposition of proteins. This may be explained by the casein being deposited in the form of micelles, including their colloidal calcium phosphate, and by serum proteins binding calcium on aggregation. The direct deposition of calcium phosphate by precipitation from the bulk onto the surface was relatively small.

Acknowledgement

This study was supported by the Netherlands Agency for Energy and the Environment (NOVEM). Prof. Dr. Ir. P. Walstra (Agricultural University of Wageningen), Dr C.G. de Kruif and Dr J. Visser are thanked for stimulating discussions.

5. References

(1) JEURNINK, T.J.M., BRINKMAN, D.W., STEMERDINK, A.D.: In Proc. Third Int. Conference on Fouling and Cleaning in Food Processing (Ed. H.G. Kessler, D.B. Lund), Prien, Germany 25-36 (1989)

(2) LALANDE, M., TISSIER, J.P., CORRIEU, G.: J. Dairy Res. **51** 557-568 (1984)

(3) HIDDINK, J., LALANDE, M., MAAS, A.J.R., STREUPER, A.: Milchwissenschaft **41** 542-546 (1986)

(4) HEGE, W.U., KESSLER, H.G.: Internat. Zeitschr. Lebensm.technol. Verf.techn. **37** 92-100 (1986)

(5) DANNENBERG, F.: Zur Reaktionkinetik der Molkenproteindenaturierung und deren technologische Bedeutung. Diss. Techn. Univers. Munich, Germany (1986)

(6) RAKES, P.A., SWARTZEL, K.R., JONES, V.A.: Biotechnol. Progr. **2** 210-217 (1986)

(7) MAUBOIS, J.L., PIERRE, A., FAUQUANT, J., PIOT, M.: IDF Bull. 212, 154-159 (1987)

(8) JEURNINK, T.J.M.: Milchwissenschaft **50** (4) 189-193 (1995)

(9) PAPPAS, C.P., ROTHWELL, J.: Food Chem. **42** 183-201 (1991)

(10) POULIOT, Y., BOULET, M., PAQUIN, P.: J. Dairy Res. **56** 185-192 (1989)

(11) LING, A.C., LUND, D.B.: J. Food Prot. **41** 187-194 (1978)

6. Summary

JEURNINK, T.J.M.: **Fouling of heat exchangers in relation to the serum protein concentration in milk.** Milchwissenschaft **50** (5) 257–260 (1995).

27 Heat exchangers (fouling)

The influence of the serum proteins in milk on fouling was studied. If serum proteins were (nearly) absent in the milk, fouling was reduced by two-thirds. Fouling increased with increasing serum protein concentration. Not only β-lactoglobulin but also α-lactalbumin, bovine serum albumine and caseins contributed to the deposit. Calcium would deposit jointly with the proteins. The fouling reaction would thus involve denatured serum proteins and calcium through interactions with serum protein aggregates, casein micelles and the stainless steel wall, resulting in a deposit layer.

JEURNINK, T.J.M.: **Ablagerungen bei Wärmeaustauschern unter Berücksichtigung der Serumproteinkonzentration in Milch.** Milchwissenschaft **50** (5) 257–260 (1995).

27 Wärmeaustauscher (Ablagerungen)

Es wurde der Einfluß von Serumproteinen in Milch auf die Bildung von Ablagerungen untersucht. Waren Serumproteine in Milch nahezu nicht vorhanden, sanken die Ablagerungen um 2/3. Die Ablagerungen nahmen mit steigender Serumproteinkonzentration zu. Nicht nur β-Laktoglobulin, sondern auch α-Laktalbumin, bovines Serumalbumin und Caseine trugen zu den Ablagerungen bei. Calcium lagerte sich zusammen mit den Proteinen ab.

Die Belagsbildungsreaktion umfaßt demnach denaturierte Serumproteine und Calcium durch Interaktionen mit Serumproteinaggregaten, Caseinmicellen und der Wand aus rostfreiem Stahl, so daß es zu Depositionen kommt.

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COLLOIDS AND SURFACES B

Deposition of heated whey proteins on a chromium oxide surface

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Abstract

Whey protein solutions were given different heat treatments after which their deposition on a chromium oxide surface (the outer layer of stainless steel) was measured by reflectometry. The deposition was studied under controlled flow conditions by using a stagnation point flow configuration. The rate of deposition is related to a model for heat-induced denaturation. It predicts an activation of β-lactoglobulin (β-lg) and subsequent aggregation. Deposition of more than a monolayer requires the presence of activated β-lg molecules near the surface. It was possible to quantify the rate of deposition by relating it to the concentration of activated molecules. The deposition process is determined by a combination of factors: the reaction by which the activated molecules are formed; their transport to the surface; and the subsequent sticking probability.

Keywords: Adsorption; Deposition; Fouling; Heat; Whey protein

1. Introduction

During heating of milk in a heat exchanger an unwanted deposit is formed on the surface of the stainless steel wall; this is called fouling. This deposit is proteinaceous in character and contains relatively large quantities of β-lactoglobulin (β-lg). Fouling starts by the adsorption of proteins on a hot stainless steel surface, after which deposition of protein (and other substances) on top of the initially adsorbed layer occurs. We make a distinction between adsorption and deposition; the former refers to the process of adhering of proteins to the bare surface with the protein unfolded and attached by strong polar bonds [1]. The latter refers to the adherence of proteins on already attached proteins. We investigated this deposition process on top of that first protein layer with the aim to find ways to prevent or diminish fouling of heat exchangers.

Several researchers have reported a relationship between the denaturation of whey proteins and fouling [2–7]. Lalande et al. [3] predicted the distribution of deposit along the heat transfer surface using the kinetic data for β-lg denaturation given by Lyster [8]. De Jong et al. [7] presented a fouling model based on the denaturation kinetics of β-lg as published by de Wit and Klarenbeek [9] and Dannenberg [5]. In the present study we relate fouling to a model for the denaturation and aggregation of β-lg recently developed by Roefs and de Kruif [10]. This model describes the aggregation of β-lg by analogy with a radical chain reaction. The β-lg contains two disulfide bridges and one free thiol group which is "buried" in the

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interior of the molecule. Upon raising the temperature a conformation change makes this free thiol group accessible for disulfide–thiol exchange reactions, i.e. the free thiol group that is exposed on heating acts as the "radical". This leads to intermolecular bonds between β-lg molecules which thus form aggregates.

We assume that the rate of deposition of the whey proteins ($d\Gamma/dt$) is related to the concentration of "radicals", i.e. activated molecules ([B*]):

$$d\Gamma/dt \propto [B^*]$$

2. Theory

2.1. Denaturation and aggregation model

The denaturation and aggregation of β-lg is considered by Roefs and de Kruif [10] as an ordinary radical addition polymerization reaction, in which the thiol groups play the role of "radical". The reaction scheme contains an initiation, a propagation and a termination step:

initiation: $B \xrightarrow{k_1} B^*$

propagation: $B + B_i^* \xrightarrow{k_2} B_{i+1}^*$

termination: $B_i^* + B_j^* \xrightarrow{k_3} B_{i+j}$

where B are the native whey protein molecules, B_i^* are the activated protein molecules or polymers, B_i are the aggregated polymers, and k_1, k_2 and k_3 are the reaction rate constants of the initiation, propagation and termination, respectively.

In the first step, native whey protein molecules are partially unfolded, whereby the thiol group becomes activated. In the second step these activated molecules can react with native ones to form activated dimers which can react in their turn with other native protein molecules to form larger activated polymers etc. The polymerization process stops when two activated molecules react with each other to form a non-activated polymer.

With the assumptions that the initiation rate is much slower than the propagation rate and that a steady state is reached, this model predicts a reaction order of $\frac{3}{2}$ for the decrease of native β-lg and for the overall reaction rate constant (k'):

$$k' = k_2 \left(\frac{k_1}{2k_3}\right)^{0.5} \quad (1)$$

2.2. Computer calculations

A computer program was used that calculates numerically the concentration of intermediates and reaction products as the aggregation reaction proceeds, in small time steps. At 65°C the experimental overall reaction rate constant k' had a value of $6.9 \times 10^{-4}\,l^{0.5}\,mol^{-0.5}\,s^{-1}$ [10]. The individual reaction rate constants at 65°C were chosen $k_1 = 1 \times 10^{-6}\,s^{-1}$, $k_2 = 975.8\,l\,mol^{-1}\,s^{-1}$ and $k_3 = 1 \times 10^{6}\,l\,mol^{-1}\,s^{-1}$. These values for k_1, k_2 and k_3 are consistent with the theoretical/experimental relation given in Eq. (1) and correspond to values that are given in the literature [11] for normal radical addition polymerization reactions. Moreover, the calculated decrease of native β-lg as a function of heating time at 65°C matches the experimental results, obtained from Ref. [10], quite well (Fig. 1). The temperature dependence of the reaction rate constants is supposed to follow an Arrhenius equation:

$$k_i = k_{0,i}\, e^{E_{a,i}/RT} \quad (2)$$

where $k_{0,i}$ is the pre-exponential factor, E_{a_i} is the activation energy ($J\,mol^{-1}$), R is the universal gas constant ($8.314\,J\,mol^{-1}\,K^{-1}$), and T is the absolute temperature (K).

Hoffmann et al. [12] derived from experimental results that $E_{a_1} = 394\,kJ\,mol^{-1}$ and $2E_{a_2} - E_{a_3} = 386\,kJ\,mol^{-1}$. In our model calculations we used $E_{a_1} = 394\,kJ\,mol^{-1}$, $E_{a_2} = 193\,kJ\,mol^{-1}$ and $E_{a_3} = 0\,kJ\,mol^{-1}$. It is reasonable to assume that the termination step has a very small temperature dependence since it refers to a reaction between two "radicals". The calculated concentrations of native β-lg (B) and of B_i^* as a function of the holding time at various temperatures are shown in Figs. 2 and 3, respectively.

Some model calculations were made to see what the influence of a change in initial β-lg concentration is on the concentration of B_i^* at a heating temper-

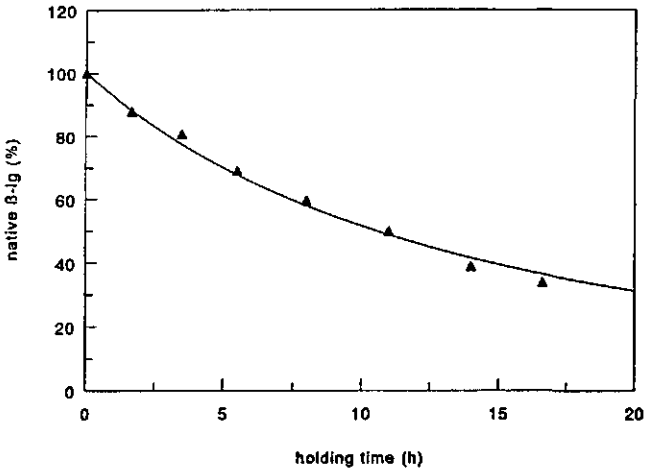

Fig. 1. Calculated and experimentally found (▲) decrease of native β-lg ($c_0 = 16.2\ g\ l^{-1}$) as a function of the holding time at 65°C [10].

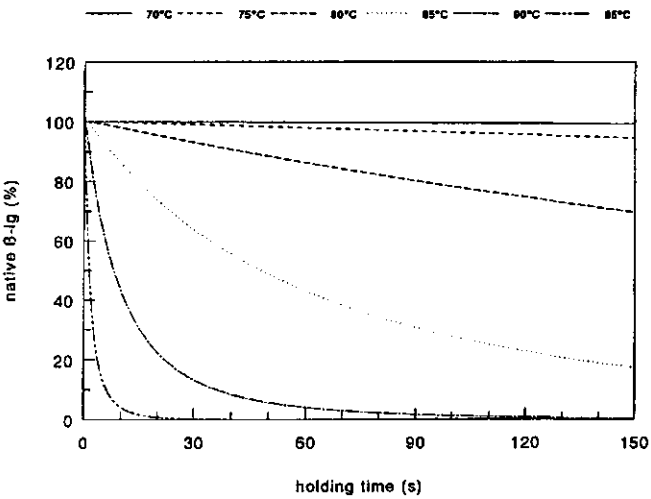

Fig. 2. Calculated concentration of native β-lg as a function of the holding time at various temperatures.

ature of 85°C. These calculations were performed using the same k_i and E_{a_i} values as given before. The initial β-lg concentration was varied from 0.002% to 3%. After 6 s holding at 85°C, model calculations (Fig. 4) indicate that the concentration of B_i^* is proportional to the initial β-lg concentration to the power 0.88 (except for the lower concentrations).

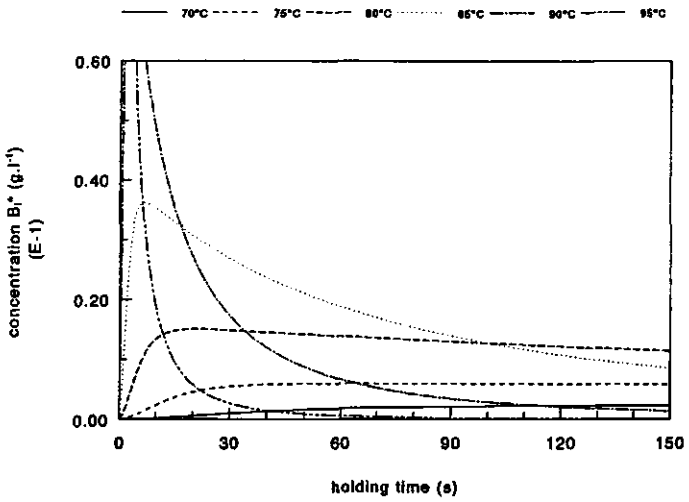

Fig. 3. Calculated concentration of the activated complex (B_f^*) of a 0.15% β-lg solution as a function of holding time at various temperatures.

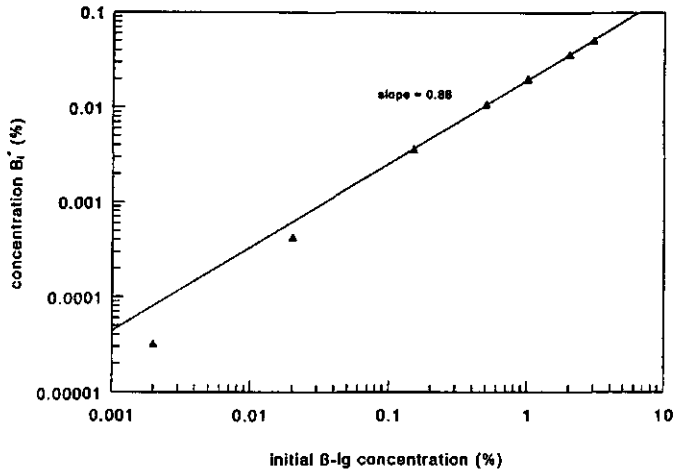

Fig. 4. Calculated concentration of the activated complex (B_f^*) as a function of the initial β-lg concentration after 6 s holding at 85°C on a double logarithmic scale.

2.3. Rate of deposition

The rate of deposition of a component is generally determined by three processes [13,14]: (i) transport by diffusion or convection towards the surface; (ii) attachment to that surface; (iii) changing its conformation to find the condition that minimizes the free energy of binding. The first

process can be controlled by choosing proper flow conditions. The reflectometer cell used to measure protein deposition has a stagnation point flow configuration. In this configuration, the flux of particles towards the surface per unit area is given by [14]

$$J = 0.776\nu^{1/3}R^{-1}D^{2/3}(\alpha\,\mathrm{Re})^{1/3}c \qquad (3)$$

where J is the flux ($\mathrm{kg\,m^{-2}\,s^{-1}}$), ν the kinematic viscosity ($\mathrm{m^2\,s^{-1}}$), R the radius of the inlet tube (m), D the diffusion coefficient ($\mathrm{m^2\,s^{-1}}$), α a parameter reflecting the intensity of the flow near the surface, which depends on Re, Re is the Reynolds number, and c is the concentration ($\mathrm{kg\,m^{-3}}$).

The kinetics for attachment are largely unknown; molecules that touch the surface have a probability (ω) to become attached which depends on interactions between the molecule and the surface, and on the shear rate. For uncharged polymers and low shear rates, one would expect $\omega = 1$, i.e. all particles arriving at the surface will stick. For the adsorption of proteins on various surfaces Weaver and Pitt [15] reported sticking probabilities in the range of 10^{-5}–10^{-8}. For the deposition of proteins on already attached proteins no experimental results for the sticking probability are available.

Conformational changes are of importance for flexible polymers, but we do not expect these to be of major importance for the relatively compact whey proteins.

3. Experimental

3.1. Whey protein

Whey protein solutions were prepared using Bipro, a commercial whey protein isolate (Domo Food Ingredients, Beilen, The Netherlands). A 0.25% Bipro solution in distilled water has a pH of 7.1 and an ionic strength of $I \approx 0.0013$ mol $\mathrm{l^{-1}}$. A β-lg powder was prepared following a modification of the method described by Maubois et al. [16]. The β-lg was further purified in our laboratory by ultrafiltration and diafiltration and finally freeze dried. Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, USA). All whey protein solutions were, if necessary, adjusted to pH 7.1 by the addition of 0.5 M NaOH or 0.5 M HCl.

3.2. Experimental set-up

Whey protein solutions were given heat treatments in the range as applied for pasteurization in the dairy industry. They were heated to the desired temperature while the holding time could be varied. This was achieved by pumping the solution through stainless steel spirals of variable length immersed in a water bath set at the desired temperature. An overview of the experimental set-up is given in Fig. 5. During the experiments the flow rate of the solution through the spiral was measured several times. The results showed that the residence time was not influenced by possible fouling of the spirals. Immediately after leaving the spiral the solution was pumped to the thermostatted reflectometer cell kept at the same temperature as the water bath. Solutions were, unless otherwise stated, not circulated but passed the cell only once. The reflectometer cell is constructed in such a way that a stagnation point flow is obtained. The solution is pumped into the cell through a cylindrical channel of radius R (= 1.1 mm). The distance between the end of the channel and the adsorbing surface is 3.0 mm. The channel is perpendicular to the surface and the intersection of its symmetry axis with the surface is called the stagnation point. This point is positioned such that it coincides with the reflection point of the laser beam [14]. At this point the deposition rate was determined by reflectometry. Due to the finite thickness of the laser beam the deposition was measured not only in the stagnation point itself, but also in a (small) area around it. In the calculation of the flux J we should therefore correct for the radial dependence of α and the intensity profile of the laser beam. We do not correct for this thereby overestimating J somewhat. Also, when the actual alignment of the reflection spot is (somewhat) off-centre J is again overestimated [14].

3.3. Reflectometry

Since the outer layer of stainless steel consists of chromium oxide [17], the deposition of whey

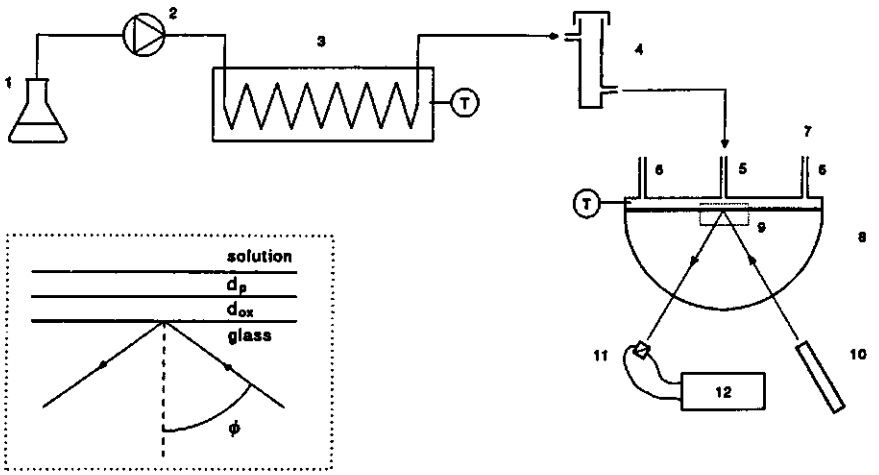

Fig. 5. Schematic diagram of the experimental set-up, including a detailed scheme of the reflectometer cell: (1) vessel with solution; (2) pump; (3) water bath with spiral; (4) de-aeration; (5) inlet; (6) outlet; (7) sampling for HPGPC; (8) thermostatted reflectometer cell; (9) glass prism; (10) laser; (11) beam splitter; (12) data acquisition; T = thermometer.

proteins was studied on a chromium oxide (Cr_2O_3) layer sputtered onto a glass plate, which in turn was adhered to a glass prism using immersion oil. The reflectivity of such a surface depends on the detailed composition of that surface and on the polarization direction of the incident light. In this study, reflectometry is used to measure the deposition as a function of time.

A linearly polarized He/Ne beam ($\lambda = 632.8$ nm) enters the reflectometer cell through a flat semicylindrical glass prism (Fig. 5). The laser beam is reflected from the surface and is split into its parallel and perpendicular components using a polarizing beam splitter. Both components are detected by photodiodes. The output signal, i.e. the ratio of the reflected intensities for parallel and perpendicularly polarized light, I_p/I_s, is continuously measured. A computer calculates the relative increment in the signal S^* defined as

$$S^* = \frac{\left(\frac{I_p}{I_s}\right)_\Gamma - \left(\frac{I_p}{I_s}\right)_0}{\left(\frac{I_p}{I_s}\right)_0} \quad (4)$$

where the subscript Γ indicates the presence of an adsorbed layer and the subscript 0 indicates the initial situation of a bare surface.

Theory shows that I_p/I_s is a function of the following variables [18]:

$$(I_p/I_s) = f(n_g, n_{ox}, n_p, n_s, \phi_i, d_p, d_{ox}, \lambda) \quad (5)$$

where n_g is the refractive index of glass, n_{ox} the refractive index of the Cr_2O_3 layer, n_p the refractive index of the protein layer, n_s the refractive index of the solution, ϕ_i the angle of incidence of the laser beam, d_p the thickness of the protein layer (nm), d_{ox} the thickness of the Cr_2O_3 layer (nm), and λ the wavelength of the laser light (nm).

The variables n_p and d_p are linked by the following relation:

$$\Gamma = \frac{(n_p - n_s)}{(dn/dc)} \cdot d_p \quad (6)$$

where Γ is the deposited amount (mass per unit area) and dn/dc is the refractive index increment (volume per mass).

For the given values of n_g, n_{ox}, n_s, ϕ_i, d_{ox}, λ and

dn/dc a computer program, based on the exact formalism of Abeles [19] and using Eqs. (4), (5) and (6), calculates the relationship between S^* and Γ.

Computer calculations with the optical model showed that at a thickness of 37 nm of the oxide layer (d_{ox}) and at an angle of incidence (ϕ_i) of 51.5° the output signal S^* was most sensitive to adsorption [19]; thus these conditions were applied in our experiments. The sputtering of a chromium oxide layer of 37 ± 0.1 nm on a glass plate was done by Philips Research (Eindhoven, The Netherlands). The values for n_g and n_{ox} were 1.515 and 2.35, respectively. It was assumed that n_g and n_{ox} did not change significantly in the temperature range of the experiments. For n_s the refractive index of water was used at the temperature at which the experiment was made. The refractive increment (dn/dc) was determined experimentally for a Bipro solution in water at 30, 50 and 80°C. The refractive index was determined with an Abbe refractometer ($\lambda = 589$ nm). For all the temperatures studied, dn/dc had a value of 0.193 ml g^{-1} (Fig. 6). For β-lg the same dn/dc was used, in agreement with the value given by Huglin [20]. The fact that dn/dc remained constant up to concentrations as high as 0.18 g ml^{-1} implies that the deposited amount as calculated from reflectometry has a well-defined physical meaning [21]. Using the values given above, the dependence of S^* on Γ is calculated and is shown in Fig. 7.

Experiments always started by flushing with distilled water; the signal S^* was set to zero, i.e. $(I_p/I_s)_0 = 1$, by rotating the laser. When S^* was constant the inlet tube was shifted to the vessel with the protein solution.

At the end of an experiment while rinsing with water, no desorption was observed. The surface was cleaned with 0.5% NaOH at 70°C for 15 min followed by rinsing with water again. If after this procedure the signal of the bare surface was not equal to the signal before the experiment, the plate was dismantled and cleaned for 10 min under a UV lamp with a stream of oxygen over the surface. The contact angle of water on a cleaned chromium oxide surface was 62°.

Deposition experiments were done at least twice, but in many cases more often. The uncertainty in determining Γ in the case of a plateau value was ±10%. The uncertainty in determining the rate of deposition, $d\Gamma/dt$, was ± 0.001 mg m^{-2} s^{-1}.

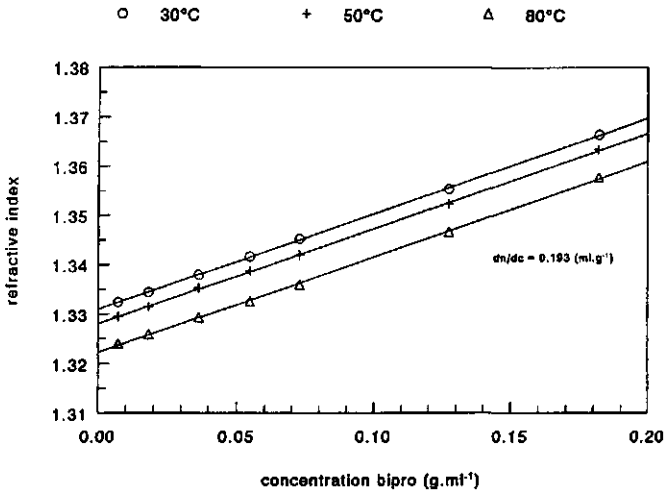

Fig. 6. Refractive index vs. concentration of a Bipro solution in water at various temperatures.

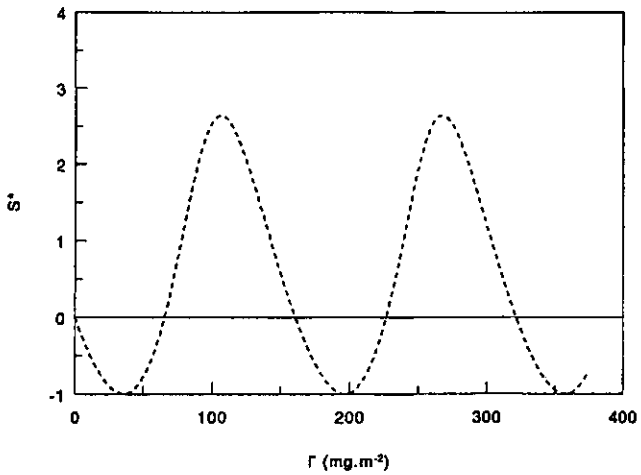

Fig. 7. Computer simulation of the output signal of the reflectometer (S^*) vs. the amount of deposition (Γ; mg m^{-2}), see text.

3.4. Protein analysis

High performance gel permeation chromatography (HPGPC) was used to determine the degree of denaturation of whey proteins [22] in samples taken after the solution had left the reflectometer cell. The samples were cooled immediately in ice water. It was not possible to obtain reliable results at low concentrations of native protein.

4. Results and discussion

4.1. Rate of deposition measured by reflectometry

When running an experiment, S^* was measured as a function of time; a typical example using Bipro (0.25%) heated for 6 s at 80°C, is given in Fig. 8. Due to deposition the thickness of the protein layer increased and a sine-type graph as predicted by theory (Fig. 7) was obtained. With the help of a computer program (see Section 3.3) S^* was converted into the corresponding Γ (mg m^{-2}) as a function of time (Fig. 9). It is seen that deposition increased linearly with time; the slope of the line gives the rate of deposition dΓ/dt (mg m^{-2} s^{-1}).

Because Fig. 8 gives information on the minimum and maximum values of S^* it was possible to estimate the value for n_p. It turned out that on average the best fits of the simulations with the experimental data were obtained if a value of 1.395 for n_p was assumed.

Since the theoretical and experimentally obtained signal S^* can be brought to a close fit we feel that the results obtained are highly reliable. The more so because the height and position of the minima and maxima are very sensitive to the values of the adjustable parameters.

4.2. Adsorption of Bipro and β-lg at room temperature on a cold and hot surface

For a Bipro solution (0.25%) and a β-lg solution (0.15%) the adsorption was measured as a function of time; the solution was kept at an ambient temperature whereas the surface was either at 25 or 85°C (Fig. 10). For both situations the adsorption rate became zero after approximately 1–2 min and a plateau value was reached, probably corresponding to a monolayer. For β-lg a plateau value of approximately 0.6 mg m^{-2} was found. For the situation in which β-lg was used on a hydrophilic

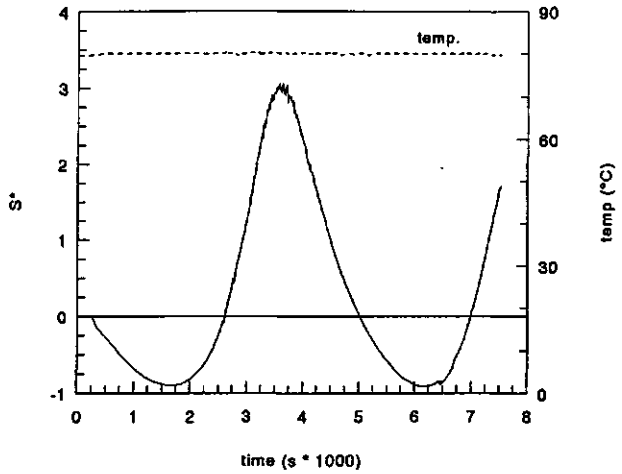

Fig. 8. Output signal of the reflectometer (S^*) as a function of time for a Bipro solution in water (0.25%) heated for 6 s at 80°C. Also the temperature in the reflectometer during the experiment is given.

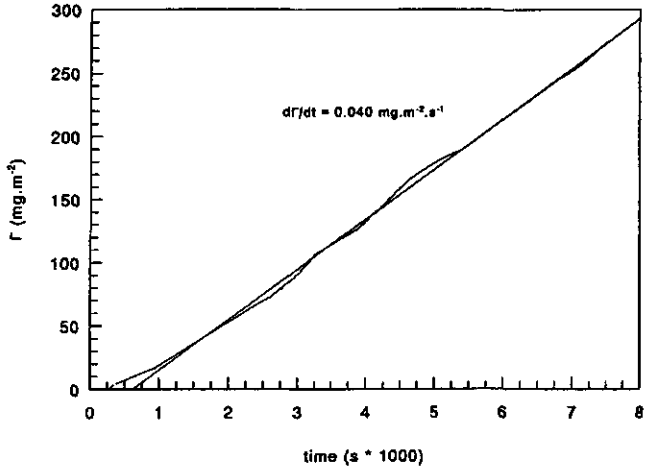

Fig. 9. Amount of deposition (Γ) as a function of time for a Bipro solution in water (0.25%) heated for 6 s at 80°C. The slope of the line gives the rate of deposition ($d\Gamma/dt$).

chromium surface, Arnebrant et al. [1] reported a plateau value of 1.4 mg m^{-2}, which they interpreted as a bilayer. Wahlgren and Arnebrant [23] calculated an adsorbed amount of 2.7 mg m^{-2} for either monomers or dimers of β-lg, adsorbed in a close-packed side-on fashion. Random packing would change this by a factor of 0.6. The experimentally found value of 0.6 mg m^{-2} is below what

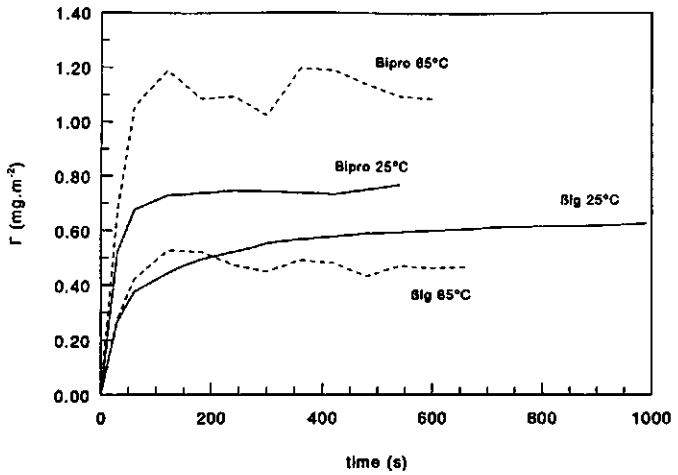

Fig. 10. Amount of adsorption (Γ) as a function of time for a Bipro (0.25%) and a β-lg (0.15%) solution in water at ambient temperature while the surface is at 25 or 85°C.

would be expected for a randomly packed monolayer. This may be explained by electrostatic repulsion between the adsorbed molecules due to the fact that the applied pH of 7.1 is far from their isoelectric point [24,25].

The plateau value for Bipro was higher than for β-lg; possibly a bilayer of different whey proteins was formed in the case of Bipro. It is not clear yet why the plateau value for Bipro increased and that for β-lg decreased somewhat when the wall temperature changed from 25 to 85°C.

If the solutions were at ambient temperature no further deposition on top of the monolayer was observed; this result was independent of the temperature of the surface. This indicates that the deposition process, as observed when the solution was heated (Fig. 9), is controlled by a reaction in the bulk.

In all our experiments it was found that the chromium oxide layer is immediately (within 1 min) covered with protein, probably by a monolayer. In the remaining part of this study we do not focus on the very initial rate of adsorption, but on $d\Gamma/dt$ after a monolayer has been formed, as presented for example in Fig. 9.

4.3. *Influence of preheating on the rate of deposition*

4.3.1. *Bipro*

A Bipro solution (0.25%) was pumped through a spiral (see Fig. 5; holding time 6 s at 85°C) and after leaving the reflectometer cell the solution was fed again to the inlet of the spiral, i.e. the solution was recirculated over the spiral and the reflectometer cell. The effect was that, after heating rapidly, the solution was held at a temperature of approximately 85°C during the whole experiment. Fig. 11 shows the deposition versus time and some percentages of native whey proteins left in the solution. The results show that at the start of the experiment the deposition is very fast. However, after a few minutes the rate of deposition slows to nearly zero, even though more than half of the native whey protein, except for IgG, is still present. This indicates that the rate of deposition is not simply correlated to the concentration of native whey protein.

In a further experiment, Bipro solutions were first given a preheat treatment to cause the whey proteins to be aggravated to a certain extent. A Bipro solution (0.25%) was divided into three

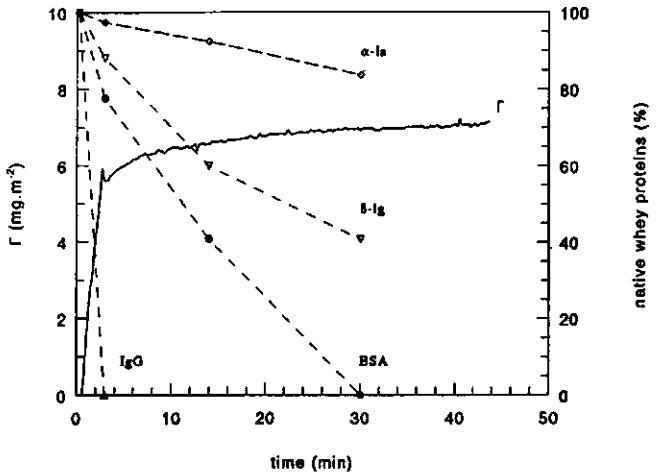

Fig. 11. Amount of deposition (Γ) as a function of time for a Bipro solution in water (0.25%) which has been circulating at 85°C over the surface. At three different times the percentage of native whey protein (IgG, BSA, β-lg and α-la) left in solution is also given.

portions, two of which were heated batchwise at 90°C for 6 s and at 85°C for 2 h, respectively. After cooling and taking a sample to determine the degree of denaturation, the solutions were passed through the spiral in the water bath only once (see Fig. 5; holding time 6 s at 85°C) and the rate of deposition of the whey proteins was measured (Table 1). For the untreated solution it was found that after the formation of a monolayer the deposition increased linearly with time at a rate of 0.043 mg m^{-2} s^{-1}.

If only a mild preheat treatment had been given, i.e. 6 s at 90°C, the deposition rate was markedly reduced. When the Bipro solution had been preheated for 2 h at 85°C, the deposition rate was close to zero, confirming the results shown in Fig. 11 and suggesting that already aggregated molecules are not involved in the process of deposition.

It is noteworthy that the already aggregated molecules behaved like native molecules, i.e. they showed a fast initial adsorption until a "monolayer" was reached, after which the rate of deposition was close to zero (results not shown).

Table 1
The influence of preheating a 0.25% Bipro solution upon the deposition rate ($d\Gamma/dt$, mg m^{-2} s^{-1}) after 6 s holding at 85°C and the corresponding percentage of native individual whey proteins before the 6 s holding at 85°C

Preheating	$d\Gamma/dt$	Degree of nativity (%)			
		α-la	β-lg	BSA	IgG
None	0.043	100	100	100	100
6 s at 90°C	0.029	100	98	83	24
2 h at 85°C	<0.001	<10	<10	0	0

4.3.2. β-lg

A similar experiment as described in the second part of Section 4.3.1 was performed with a pure β-lg solution (0.15%), preheated for 3 h at 95°C (Table 2). Without preheating the deposition continued after adsorption of a monolayer, showing that denatured β-lg molecules are involved in the deposition process. However, if the molecules were aggregated by heating for 3 h at 95°C, they hardly contributed to the deposition process, as was found for Bipro in Section 4.3.1.

The results show that the process of denaturation of the whey proteins near the surface is a prerequisite to obtain more deposition than just a monolayer. However, if all whey proteins are aggre-

Table 2
The influence of preheating a 0.15% β-lg solution upon the deposition rate ($d\Gamma/dt$, mg m^{-2} s^{-1}) after 6 s holding at 85°C and the corresponding percentage of native β-lg before the 6 s holding at 85°C

Preheating	$d\Gamma/dt$	Degree of nativity (%)
None	0.015	100
3 h at 95°C	<0.001	<10

gated the deposition is limited to only "monolayer" adsorption. The fact that preheating the protein solution has such a large effect on the rate and extent of deposition indicates that the deposition depends on a bulk rather than on a surface reaction.

4.4. Rate of deposition versus holding time at various temperatures

4.4.1. Bipro

In order to determine the deposition rate at various degrees of denaturation, Bipro solutions (0.25%) were given different heat treatments. This was achieved by increasing the holding time at various temperatures. The results are shown in Fig. 12. The corresponding degrees of denaturation of IgG, BSA, β-lg and α-la are given in Fig. 13(a)–(d). The concentration decrease predicted by the model calculations (Fig. 2) does not match the experimental results (Fig. 13(c)) exactly, but the trends are similar.

It is seen that a maximum in the deposition rate is obtained if the holding time is varied; the maximum appeared at very short holding times. At 90°C a similar maximum may have been present at even shorter holding times.

4.4.2. β-lg

Fig. 14 shows the rate of deposition for a 0.15% β-lg solution as a function of the holding time at various temperatures. Also in this case a maximum deposition rate was obtained at very short holding times. Under these conditions hardly any β-lg is aggregated: for example after 6 s at 85°C it was found that only 0.5% of the β-lg was aggregated.

4.4.3. BSA

Fig. 15 shows the rate of deposition and the corresponding degree of denaturation for a 0.15% BSA solution as a function of the holding time at 85°C. Again the maximum deposition rate was found at very short holding times.

4.4.4. Discussion

At ambient temperature only a monolayer of protein was found to adsorb at a solid/liquid interface. To obtain more deposition, denaturation of the whey proteins is a prerequisite. However, if all whey proteins were aggregated, again adsorption to a plateau value, corresponding with a monolayer, was found. Also Arnebrant et al. [26] found that both native and preheated β-lg gave a plateau value for the adsorption; he concluded that the surface has to be present during denaturation for deposition to occur. The results for Bipro, β-lg and BSA presented above indicate that some intermediate in the denaturation reaction must be present to let deposition on top of a monolayer occur. As long as the solution had not been heated no intermediates would have been formed yet, whereas if the heat treatment had been too long, the intermediates would have disappeared through the formation of aggregates. The activated molecules (B_f^*) postulated in the denaturation and aggregation model of β-lg can possibly act as such an intermediate. Comparison of Fig. 3 with Figs. 12 and 14 shows that the curves of the calculated B_f^* concentration versus heating time more or less resemble those of $d\Gamma/dt$ versus heating time. So, these results are consistent with the supposition that the rate of deposition is dependent on the concentration of B_f^* in the medium.

Our calculations also provide an explanation for the results shown in Fig. 11: in the beginning of the heat treatment the deposition rate is high, corresponding with a high concentration of B_f^* but soon the concentration of B_f^* becomes very low (Fig. 3) explaining why the deposition rate is close to zero.

The idea that activated molecules, which originate from the denaturation reaction, are involved in the deposition process may also be applicable to whey proteins other than β-lg, since these proteins also contain thiol and/or cysteine groups. There are several possible reasons for the differ-

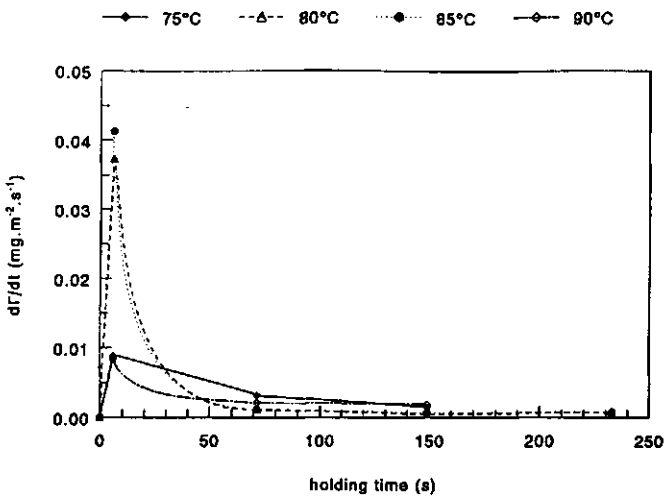

Fig. 12. Rate of deposition ($d\Gamma/dt$) for a Bipro solution in water (0.25%) which had been heated at various temperatures for various times.

ences in deposition rate of whey proteins among Bipro, β-lg and BSA solutions: (1) from Bipro, whey proteins other than β-lg may also be involved in the deposition process; (2) the kinetics of the denaturation of β-lg or BSA in Bipro may be different from that in a pure solution due to differences in, for example, pH, calcium ion activity and ionic strength, resulting in a different concentration of B_f^* for a given heat treatment; (3) in Bipro mutual interactions between the whey proteins may influence the deposition.

4.5. Kinetics of the deposition process

4.5.1. Order of the deposition reaction

To determine the order of the deposition "reaction" with respect to the bulk concentration, the rate of protein deposition was measured for different concentrations of Bipro and β-lg solutions. This was done for the Bipro solutions after 10 s holding at 90°C and for the β-lg solutions after 6 s holding at 85°C. In Fig. 16 the deposition rate $d\Gamma/dt$ is plotted against the concentration c in a double logarithmic plot. From the slope of the lines n, an order of the "reaction", can be obtained: for Bipro and β-lg, n is 0.9 and 0.8, respectively. At very low concentrations deviations from these values were found for unknown reasons.

If deposition is diffusion controlled then the rate of deposition ($d\Gamma/dt$) is related to the flux (J) by $d\Gamma/dt = \omega J$, where ω is the sticking probability. Provided ω is independent of c, the theory predicts that $d\Gamma/dt$ is proportional to c (Eq. (3)). As stated above, our hypothesis is that the rate of deposition is related to the concentration of activated molecules: $d\Gamma/dt \propto [B_f^*]$. In Fig. 16 the concentration of native whey protein (B) is varied. Model calculations showed that $B_f^* \propto B^{0.88}$ (Fig. 4). This implies that one should find an order of $n = 0.88$ if $d\Gamma/dt$ is plotted against the concentration c, which is indeed the case.

Since both the transport of B_f^* to the surface as well as its formation in the bulk have a reaction order (n) (close to) one, it was not possible to conclude, based on n only, whether deposition is a diffusion or a bulk reaction controlled process.

4.5.2. Influence of Reynolds number (Re) on the rate of deposition

In Table 3 the deposition rates from a Bipro solution heated for 90 s at 75°C and a β-lg solution heated for 143 s at 85°C are given at different Re

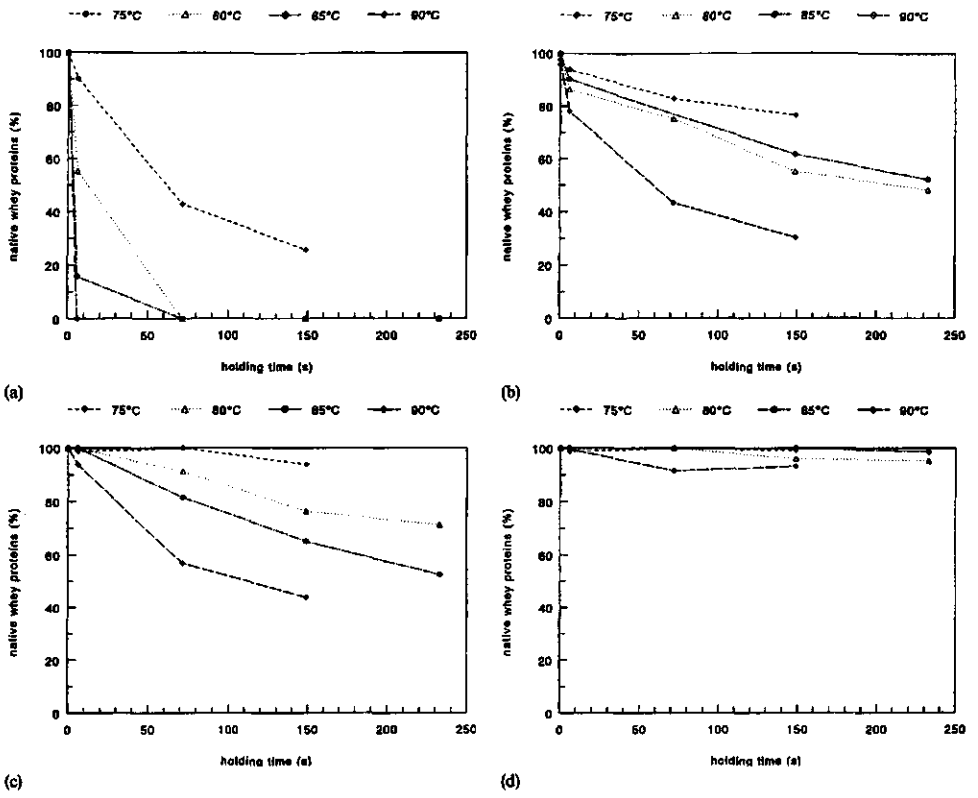

Fig. 13. Percentage of native (a) IgG, (b) BSA, (c) β-lg, and (d) α-la left in solution after heating a Bipro solution in water (0.25%) for various holding times and temperatures.

numbers. The experiments were done in such a way that the holding time was the same at each Re number in order to reach the same degree of denaturation.

At the end of the experiments no desorption was observed during rinsing, which suggests that increasing the Re number did not disturb the measurement of deposition by shear-induced detachment of already deposited molecules.

The flux equation for stagnation point flow (Eq. (3); assuming $\alpha \propto \mathrm{Re}^{1/2}$) predicts an increase in flux J by a factor 2.9 when the Re number increases from 200 to 1700 and by a factor 1.8 if the Re number increases from 500 to 1700. The experimentally found increase in the rate of adsorption (Table 3) on increasing Re from 200 to 1700 or from 500 to 1700 was 3.0 and 1.1, respectively. In other words, there is a trend at higher Re numbers, and thus at higher J values, that the deposition rate increases. This implies that the transport of particles from the bulk to the surface plays a role in the deposition process.

4.5.3. Sticking probability

The deposition rate was estimated assuming that it is diffusion controlled (Eq. (3)) and that the sticking probability ω equals 1, i.e. $J = \mathrm{d}\Gamma/\mathrm{d}t$. The following values were taken for the variables in

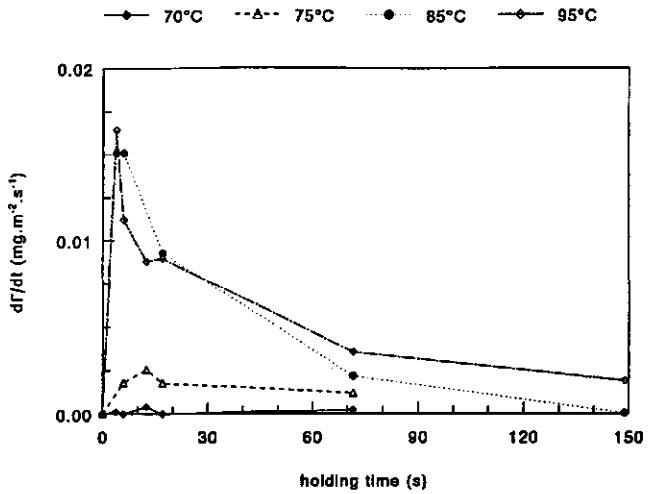

Fig. 14. Rate of deposition ($d\Gamma/dt$) for a β-lg solution in water (0.15%) which had been heated for various holding times and temperatures.

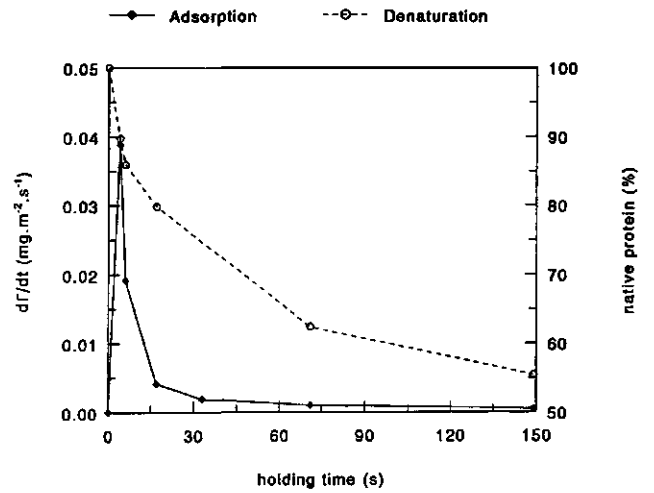

Fig. 15. Rate of deposition ($d\Gamma/dt$) for a BSA solution in water (0.15%) which has been heated for various holding times at 85°C. Also the percentage of native BSA left in solution after the heat treatments is given.

Eq. (3): $\nu = 3.7 \times 10^{-7}$ ($m^2 s^{-1}$), $R = 1.1 \times 10^{-3}$ m, $D = 2.6 \times 10^{-10}$ $m^2 s^{-1}$, $\alpha = 142$ (estimated from a plot of α versus Re number given by Dijt et al. [14] and assuming that $\alpha \propto Re^{1/2}$ for $Re > 10$), $Re = 1035$, $c = 0.06$ kg m^{-3}, i.e. the concentration of active whey proteins (B_l^*), taken from Fig. 3.

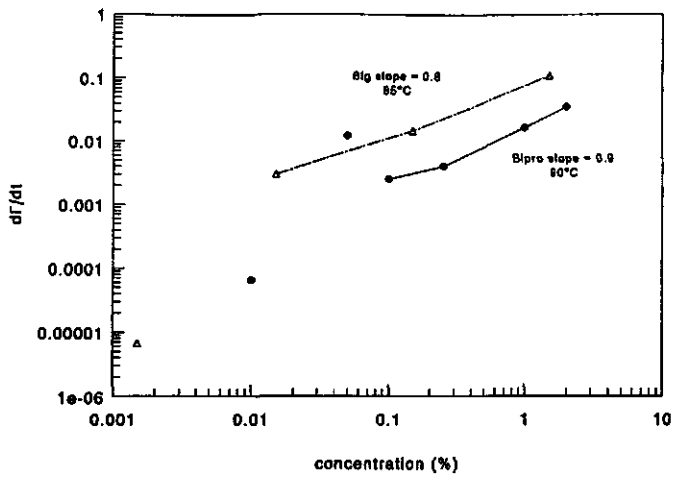

Fig. 16. Rate of deposition ($d\Gamma/dt$) as a function of the concentration of Bipro or β-lg in water. The slope of the lines would give the order of the reaction.

Table 3
Deposition rate ($d\Gamma/dt$, mg m^{-2} s^{-1}) of 0.25% Bipro after 90 s holding at 75°C and of 0.15% β-lg after 143 s holding at 85°C for different Re numbers. Also the percentage of still native whey protein in solution are given

	Re	$d\Gamma/dt$	α-la (%)	β-lg (%)	BSA (%)	IgG (%)
Bipro	200	0.001	97	94	82	35
Bipro	1700	0.003	97	92	82	22
β-lg	500	0.016		54		
β-lg	1700	0.018		57		

This would result in a deposition rate of 6.5 mg m^{-2} s^{-1}, while experimentally values of 0.03 mg m^{-2} s^{-1} were found, i.e. by a factor of 200 smaller. The above-mentioned error in α and, hence, in J is not sufficient to explain this large difference. Therefore we conclude that apparently the sticking probability is less than one.

5. Conclusions

The deposition rate of whey proteins onto a chromium oxide layer is related to the denaturation process; it was shown that the presence of activated molecules near the surface is a prerequisite for continued deposition. It was possible to quantify the rate of deposition by relating it to the concentration of activated molecules in solution, using the model for the denaturation and aggregation of β-lg as presented by Roefs and de Kruif [10].

The fact that preheating the bulk of the solution and changing the flux strongly influenced the rate of deposition led to the conclusion that the deposition process is controlled by reactions in the bulk rather than by a reaction at the surface.

The deposition process on top of an adsorbed monolayer is determined by three steps. (i) Formation of an activated molecule in the bulk of the solution. (ii) Transport of that molecule to the surface. During this transport an activated molecule can be inactivated through a reaction with another molecule in the bulk. (iii) The deposition of an activated molecule by a reaction with an already deposited molecule, most probably through the formation of a disulfide bond. Not every collision with the surface would lead to deposition, resulting in a sticking probability much smaller than one.

The results show that upon heating whey proteins there is a critical period in the residence time, determined by the concentration of activated mole-

cules, where fouling is most severe. The higher the heating temperature the more fouling takes place, albeit over a shorter time period. Fouling in heat exchangers can be reduced by installing a holding section with a high volume-to-surface ratio at the position where the model predicts the largest concentration of B_f^*. This principle was already applied, based on a semiempirical model, by de Jong et al. [7]. Fouling of a heat exchanger could be reduced by more than 50%.

Acknowledgements

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References

[1] T. Arnebrant, B. Ivarsson, K. Larsson and T. Nylander, Prog. Colloid Polym. Sci., 70 (1985) 62–66.
[2] T.J.M. Jeurnink, D.W. Brinkman and A.D. Stemerdink, in H.G. Kessler and D.B. Lund (Eds.), Proc. 3rd Int. Conf. on Fouling and Cleaning in Food Processing, Prien, Germany, 1989, pp. 25–36.
[3] M. Lalande, J.P. Tissier and G. Corrieu, Biotechnol. Prog., 1 (1985) 131–139.
[4] J. Hiddink, M. Lalande, A.J.R. Maas and A. Streuper, Milchwissenschaft, 41 (1986) 542–546.
[5] F. Dannenberg, Dissertation, Technische University Munich, Germany, 1986.
[6] P.A. Rakes, K.R. Swartzel and V.A. Jones, Biotechnol. Prog., 2 (1986) 210–217.
[7] P. de Jong, S. Bouman and H.J.L.J. van der Linden, J. Soc. Dairy Technol., 45 (1992) 3–8.
[8] R.L.J. Lyster, J. Dairy Res., 37 (1970) 233.
[9] J.N. de Wit and G. Klarenbeek, Milk Proteins in Human Nutrition, in Proc. Int. Symp. Kiel, Steinkopff, Darmstadt, 1989, pp. 211–222.
[10] S.P.F.M. Roefs and C.G. de Kruif, Eur. J. Biochem., 226 (1994) 883–889.
[11] P.C. Hiemenz, Polymer Chemistry. The Basic Concepts, Marcel Dekker, New York, 1984.
[12] M.A.M. Hoffmann, S.P.F.M. Roefs, M. Verheul, P.J.J.M. van Mil and C.G. de Kruif, J. Dairy Res., in press.
[13] G.J. Fleer, M.A. Cohen Stuart, J.M.H.M. Scheutjens, T. Cosgrave and B. Vincent, Polymers at Interfaces, Chapman and Hall, London, 1993, Chapter 2.6.
[14] J.C. Dijt, M.A. Cohen Stuart, J.E. Hofman and G.J. Fleer, Colloids Surfaces, 51 (1990) 141–158.
[15] D.N. Weaver and W.G. Pitt, Biomaterials, 13 (1992) 577–584.
[16] J.L. Maubois, A. Pierre, J. Fauquant and M. Piot, IDF Bull., 212 (1987) 154–159.
[17] D. Gorse, J.C. Joud and B. Baroux, Corros. Sci., 33(9) (1992) 1455–1478.
[18] R.M.A. Azzam and N.M. Bashara, Ellipsometry and Polarized Light, Elsevier, Amsterdam, 1992, pp. 269–291.
[19] J.C. Dijt, M.A. Cohen Stuart and G.J. Fleer, Adv. Colloid Interface Sci., 50 (1994) 79–101.
[20] M.B. Huglin, Light Scattering from Polymer Solutions, Academic Press, London, 1972, p. 222.
[21] J.A. de Feijter, J. Benjamins and F.A. Veer, Biopolymers, 17 (1978) 1759–1772.
[22] Th.J.M. Jeurnink and C.G. de Kruif, J. Dairy Res., 60 (1993) 139–150.
[23] M.C. Wahlgren and T. Arnebrant, J. Colloid Interface Sci., 142(2) (1991) 503–511.
[24] M.A. Cohen Stuart, G.J. Fleer, J. Lyklema, W. Norde and J.M.H.M. Scheutjens, Adv. Colloid Interface Sci., 34 (1991) 477–535.
[25] Z. Adamczyk, B. Siwek, M. Zembala and P. Belouschek, Adv. Colloid Interface Sci., 48 (1994) 151–280.
[26] T. Arnebrant, K. Barton and T. Nylander, J. Colloid Interface Sci., 119(2) (1987) 383–390.

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The Cleaning of Heat Exchangers and Evaporators After Processing Milk or Whey

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ABSTRACT

The composition of the deposit and the cleaning rate were studied in heat exchangers and evaporators of a semi-industrial scale, after processing of milk, whey or concentrated whey. Milk deposit is represented as a matrix of protein to which minerals are associated and in which fat is embedded. Under good cleaning conditions, milk deposit in a heat exchanger or evaporator can be removed readily by an alkaline cleaning followed by an acid cleaning. If during the cleaning of an evaporator, the flow rate is too low or the alkali concentration too high, or both, then the cleaning will be slow and incomplete. By optimizing the cleaning procedure, a reduction in energy costs during cleaning of 50% can be achieved.

Whey deposit is represented as a complex of protein together with calcium salts, containing mainly calcium phosphate, but in the case of concentrated whey, calcium citrate is also found. Since whey deposit contains considerably more mineral compounds than milk deposit, it is recommended to start with an acid cleaning followed by an alkaline cleaning after processing (concentrated) whey. As with milk deposit, whey deposit in a heat exchanger or evaporator is removed quickly.

Using a model heat exchanger and evaporator with outer walls of glass, it was possible to follow the cleaning process visually. Based on these observations, together with the analysis of the cleaning solutions, a schematic view of the removal of deposit is presented for both milk and whey.

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INTRODUCTION

Heat processing of milk or whey results in fouling of heat exchangers and evaporators. Cleaning of the equipment requires time, money and energy (Bouman *et al.*, 1988). Understanding and subsequent optimization of the cleaning process leads to reduction of the costs.

Milk deposit in a heat exchanger (70–90°C) contains 50–60% protein, 30–35% minerals and some fat (4–8%); this is classified as type A deposit (Burton, 1968). Tissier and Lalande (1986) found that this fouling layer is built up by two different types of deposit. Near the stainless steel surface, a dense deposit is observed, containing mainly minerals. Over this layer there is a spongy deposit only weakly bound to the mineral-rich layer. The spongy layer consists mainly of protein in which fat globules are enclosed. Indications were found that the protein in the fouling layer consisted not only of denatured whey proteins but also of casein micelles (Jeurnink, 1991).

The composition of milk deposit in an evaporator is not known in detail. Bouman *et al.* (1988) and Palmer and Kelly (1984) reported figures for the chemical oxygen demand (COD) of cleaning solutions used in evaporators, which indicated the presence of organic material.

The deposit formed during heating whey has been the subject of only a few studies. Delsing and Hiddink (1983) found that the contribution of minerals to the deposit was higher than in milk deposit. This was also found after evaporation of whey: Kessler (1989) reported that the mineral content in the deposit increased with the dry matter content of the whey.

Protein and fat are removed by alkaline solutions while minerals are dissolved by acid. Therefore, it is common practice to apply both an alkaline and an acid cleaning for the removal of deposit on heated surfaces. Nowadays, single-stage cleaning is also applied, i.e. sequestering compounds are added to the alkaline solution in order to remove the mineral deposit so that a subsequent acid cleaning is not needed (Timperley & Smeulders, 1987; Stemerdink & Brinkman, 1990).

The mechanism of deposit removal has been studied previously. Grasshoff (1988) observed that a milk deposit showed an initial swelling after contact with the alkaline solution, followed by the removal of the top layer of the swollen deposit. Then, a swelling of the residual deposit layer occurred, the layer broke up and was removed in large pieces. After 10 min, there remained a layer consisting mainly of minerals which was removed by the acid cleaning. Bird and Fryer (1991) observed that a whey deposit swelled upon contact with NaOH, followed by a break-up of the swollen deposit by detachment of protein aggregates. After 20 min, only a layer of minerals remained. These studies were made in heat exchangers

on a laboratory scale. In this study, we present quantitative information on deposit composition and cleaning rate in both heat exchangers and evaporators operated on a semi-industrial scale, using milk, whey or concentrated whey as test solutions. Based on these results and on visual observations, a detailed description of the cleaning process can be given. Furthermore, optimal cleaning conditions with regard to NaOH concentration, flow rate and sequence of cleaning solutions were established.

MATERIALS AND METHODS

Figure 1 shows an overview of the experimental four-effect evaporator (NIRO-250) which is located in the Institute's pilot plant. Throughout a 20-h period, whole milk was pasteurized at 90°C (holding time 315 s) in the tubular heat exchanger (nos. 5 and 6 in Fig. 1) at a flow rate of 2300 litres/h. The logarithmic mean temperature difference in the tubular heat exchanger was 3·0°C. Before the milk reached the heat exchanger it was heated to 45°C in the condenser of the evaporator (no. 10 in Fig. 1). The milk was concentrated to 48% total solids (TS). The vapour and boiling temperature for the four effects were 74–70, 67–63, 60–53 and 52–45°C, respectively.

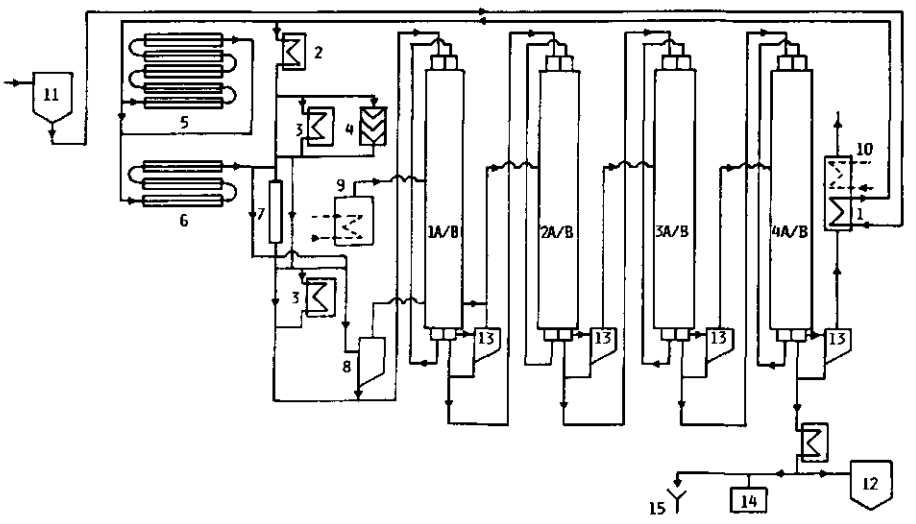


Fig. 1. Overview of the experimental four-effect evaporator (NIRO-250). 1, Preheater; 2, plate heat exchanger low pressure; 3, plate heat exchanger high pressure; 4, direct steam injection; 5, tubular preheater; 6, tubular heat exchanger; 7, holding tube; 8, flash vat; 9, vapour generator; 10, condenser; 11, supply tank; 12, storage tank; 13, vapour separator; 14, sampling device; 15, drain.

Throughout a 20-h period, whey (5·7% TS) was pasteurized at 70°C (holding time 50 s) at a flow rate of 2700 litres/h and subsequently concentrated to 28% TS. The logarithmic mean temperature difference during pasteurization was 3·8°C. The vapour and boiling temperature for the four effects were 70–66, 66–61, 55–48 and 48–40°C, respectively.

Throughout a 10-h period, concentrated whey (28% TS) was pasteurized at 70°C (holding time 90 s) at a flow rate of 1600 litres/h and subsequently concentrated to 55% TS. The logarithmic mean temperature difference during pasteurization was 1·8°C. The vapour and boiling temperature for the four effects were 59–56, 71–68, 65–62 and 60–56°C, respectively.

After processing, the installation was cleaned at a flow rate of 3000 litres/h and a temperature of 70°C. During cleaning, no evaporation of the cleaning solutions occurred. The cleaning solutions were NaOH (1%) and HNO_3 (1%).

For determining the cleaning rate, the cleaning solutions were sampled continuously at the outlet of the evaporator (no. 14 in Fig. 1) using a fraction collector. The samples were subsequently analysed for chemical oxygen demand (COD) and calcium content. Also, the solutions going into the drain were sampled automatically (no. 15 in Fig. 1) in quantities proportional to the flux in order to determine the overall composition of the deposit removed. Before and after cleaning, the heat exchanger and the evaporator were dismantled for visual inspection. Since fouling is unevenly distributed over the surface, total amounts of deposit rather than g/m^2 are reported.

The installation is constructed in such a way that the heat exchanger can be cleaned separately from the evaporator and the effects of the evaporator can also be cleaned separately.

In order to follow visually what happens during cleaning, a model heat exchanger and evaporator were used. The model heat exchanger consisted of two concentric tubes; the inner tube was of stainless steel, the outer tube of glass (Jeurnink, 1991). The model evaporator consisted of one stainless steel pipe, 1 m long. Contrary to normal practice, the steam was led into the inside of the pipe, while the milk was distributed over the outside of the pipe. The stainless steel pipe was surrounded by a glass tube. Milk or whey was concentrated in this evaporator under process conditions simulating the fourth effect of the experimental evaporator. The flow rate at the inlet was 60 litres/h. After processing milk or whey, the model evaporator was cleaned with NaOH and HNO_3 at a flow rate of 100 litres/h. The cleaning process was taped on a video, which is available from the Institute.

Chemical analysis

Chemical oxygen demand (COD) was determined according to Dutch Standards NEN 6633 (1990). Protein was determined according to Dutch Standards NEN 3198 (1984) as nitrogen × 6·38. Calcium was determined after destruction according to Dutch Standards NEN 6465 (1981) by atomic absorption spectroscopy (Dutch Standards NEN 6446, 1980). Total phosphorus was determined by photospectroscopy (Dutch Standards NEN 6479, 1981). Fat was determined according to Dutch Standards NEN 3235 9.2.1. (1978). Citrate and lactose were determined by high performance liquid chromatography (HPLC). For citrate, a HPX-87H column (BioRad, Richmond, USA) was used at a flow rate of 0·6 ml/min (eluent 0·005 M H_2SO_4), and the detection was performed at 215 nm. For lactose, a Polyspher CHPb 18 (90°C) column (E. Merck, Darmstadt, Germany) was used at a flow rate of 0·4 ml/min (eluent H_2O). Refractive index was used for detection.

RESULTS AND DISCUSSION

Removal of milk deposit

Heat exchangers

Cleaning was followed by monitoring the COD and calcium content of the cleaning solution at the outlet of the equipment. COD and calcium are a measure of the organic and mineral compounds in the cleaning solution, respectively. The different stages of the cleaning protocol were: a rinse to remove residual milk, an alkaline cleaning, an intermediate rinse, an acid cleaning and a final rinse. Figure 2 shows the removal of milk deposit as a function of the cleaning time of the heat exchanger after operating for 20 h at 90°C (holding time 315 s). During the first rinse, the cleaning solution did not contain any COD and calcium, i.e. no deposit was removed. However, as soon as the deposit came into contact with the alkaline cleaning solution, a sharp peak in the 'cleaning rate' was observed; the cleaning solution at the outlet of the heat exchanger containing both organic and mineral deposits. When the acid cleaning solution left the heat exchanger (cleaning time = 45 min), a second peak in the cleaning rate was observed but this time only the mineral deposit was removed. Figure 2 shows two remarkable results: the first is that most of the organic deposit was removed in a very short time, and secondly that part of the mineral

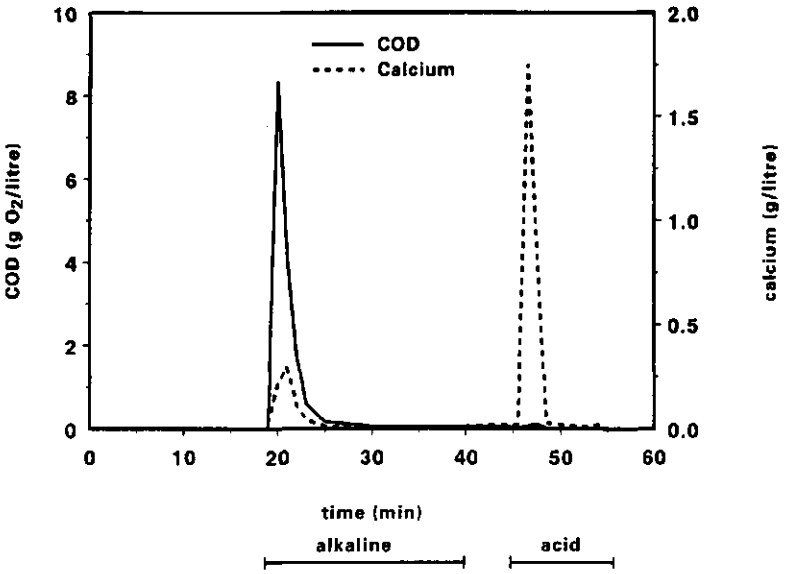


Fig. 2. Milk deposit removal in the heat exchanger after operating for 20 h at 90°C. Cleaning temperature was 70°C and the cleaning solutions were NaOH (1%) and HNO_3 (1%).

deposit was already removed during the alkaline cleaning stage. These results are consistent with findings by Perlat *et al.* (1986).

Evaporator

Figure 3 shows the removal of milk deposit during the cleaning of the evaporator after operating for 20 h with whole milk. The same cleaning procedure as for the heat exchanger was used, except that in the evaporator the cleaning solutions were circulated for a certain period (alkaline solution for 30 min and acid solution for 15 min). Since the evaporator is a much larger apparatus than the heat exchanger, the time needed for cleaning was much longer. As with the heat exchanger, the greater part of the organic deposit and some of the mineral deposit was removed in a short time by the alkaline cleaning of the evaporator. This cleaning solution, containing most of the deposit (the so-called first alkaline flush), was not circulated but discharged directly. Then, the cleaning solution was circulated and finally rinsed out with water. During acid cleaning, some further organic deposit and the remainder of the mineral deposit were removed. In Fig. 3 it is remarkable that during circulation of the alkaline and acid cleaning hardly any deposit was removed.

The cleaning solutions from the heat exchanger and the evaporator were collected and analysed to determine the composition of the deposit

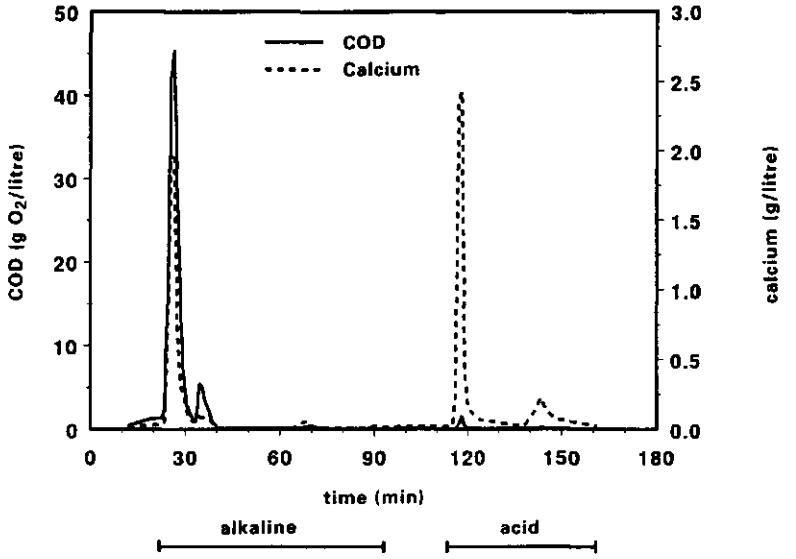


Fig. 3. Milk deposit removal in the evaporator after operating for 20 h. Cleaning temperature was 70°C and the cleaning solutions were NaOH (1%) and HNO_3 (1%).

that was removed (Table 1). These values are averages and do not account for changes in composition with position in the apparatus. This explains why the deposit in the heat exchanger had a relatively high fat content compared to type A deposit (Burton, 1968); visual inspection before the cleaning showed that the fat came from the low-temperature part (< 40°C). The protein and the mineral deposit originated from the heater section, where the temperature reached more than 70°C. The ratio of protein to mineral was somewhat lower than in the typical type A deposit found in previous work using the same heat exchanger (Jeurnink *et al.*, 1989). Presumably, more minerals were deposited due to the relatively long holding time (315 s) at 90°C.

In the evaporator, protein was the main component of the deposit layer. This is somewhat surprising since most of the whey proteins were already denatured during preheating and the process temperature in the evaporator was far below the denaturation temperature. Due to the different circumstances, i.e. the increased concentration of the compounds in the milk, reactions other than the denaturation of the whey proteins are involved in the fouling process in an evaporator.

The value for COD was too high to be caused only by the protein and fat content (1 ppm protein and 1 ppm fat correspond to 1·27 and 2·96 ppm COD, respectively), which indicated that some other organic material was also present in the deposit, e.g. lactose or citric acid.

TABLE 1
Composition of the Deposit from Whole Milk in the Heat Exchanger and in the Evaporator after Operating for 20 h

	COD ($g\ O_2$)	*Protein* (*g*)	*Fat* (*g*)	*Ca* (*g*)	PO_4 (*g*)
Heat Exchanger					
Total	1217	274	297	100	156
Evaporator					
Alkaline flush	8453	2265	1369	229	487
	(96%)	(96%)	(96%)	(52%)	(84%)
Alkaline circulation	167	38	31	26	3
	(2%)	(1·6%)	(2%)	(6%)	(1%)
Acid cleaning	208	57	34	185	86
	(2%)	(2·4%)	(2%)	(42%)	(15%)
Total	8828	2360	1434	440	576

Within parentheses: the percentage of the deposit removed by the different stages of cleaning.

A Ca/P molar ratio in the deposit of 1·5 has been reported (Lyster, 1965; Bouman *et al.*, 1982; Hiddink *et al.*, 1986), suggesting the presence of $Ca_3(PO_4)_2$. For the heat exchanger, this molar ratio was also found, but it is doubtful whether this points to the presence of $Ca_3(PO_4)_2$ since the total phosphorus was determined, i.e. including organic phosphate. In fact, there are two reasons to suggest that the inorganic calcium phosphate in the deposit was present in the form of $CaHPO_4.2H_2O$ (DCPD, brushite): (i) the first precipitation phase of calcium phosphate salts is DCPD (Schmidt & Both, 1987; Van Kemenade & De Bruyn, 1987) and (ii) the calcium phosphate present in the deposit associated with casein micelles, the so-called micellar calcium phosphate, has a brushite-like structure (Holt *et al.*, 1989).

The calcium/phosphate (Ca/PO_4) molar ratio in the cleaning solutions from the evaporator was different from that in $Ca_3(PO_4)_2$, namely 1·8. This may have been caused by the precipitation of calcium salts other than calcium phosphate, e.g. calcium citrate. Also, the presence of casein micelles in the deposit would result in a higher Ca/PO_4 molar ratio than 1·5.

In general, it is very speculative to discuss the form in which calcium salts were present in the deposit because the values for the dissociation constants of the salt equilibria and the solubility products of the calcium salts are not known for milk at high temperatures. In the case of evaporators it becomes even more complex since the concentration factor influences the ionic strength and the ionic activity coefficient. Nieuwenhuijse *et al.* (1988) have tried to calculate the ionic activity coefficient

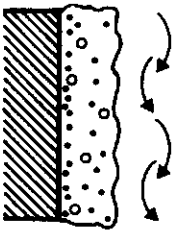
in concentrated milk using a computer model. However, this model holds only for milk at equilibrium, and that is not the case for milk in heat exchangers and evaporators.

For the evaporator, the percentages of the deposit removed by the different stages of cleaning are given in Table 1. As already noted in relation to Figs 2 and 3, large amounts of the deposit (96% of the protein and the fat and 52% of the calcium) were removed with the first alkaline flush. During subsequent circulation of the alkaline solution, only 2% of the organic deposit was removed. The final acid cleaning was necessary to remove the residual mineral deposit (42%). With respect to optimization of the cleaning procedure, it is concluded from Fig. 3 that the length of the alkaline circulation stage can be shortened considerably.

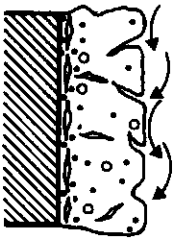
Mechanism of milk deposit removal

Based on the results described above and the visual observations made during the cleaning, the removal of milk deposit is as represented in Fig. 4. The deposit layer is built up of a spongy protein matrix with which minerals are associated and in which fat globules are embedded. Tissier and Lalande (1986) reported a thin dense layer, 15 μm thick after 6 h of pasteurization, adjacent to the stainless steel, consisting mainly of minerals. Measurements made in the present study do not allow us to conclude whether or not such a layer was present.

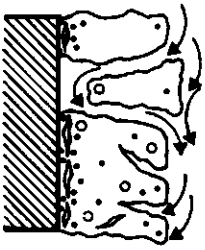
For removal of the deposit layer, two factors are important: (i) a swelling of the deposit observed as soon as it comes into contact with the alkaline cleaning solution, and (ii) mechanical action caused by the shear stress of the flowing cleaning solutions. The fact that during the pre-rinse with water no deposit was removed, but only after contact with the alkaline solution, indicates that swelling is an essential step in the deposit removal process. Diffusion of the alkaline cleaning solution into the deposit layer is slow; reasonable values for the thickness of the fouling layer and for the effective diffusion coefficient are 10 mm and 10^{-8} m^2/s, respectively, which means that it would take at least 1 h before the cleaning solution reached the stainless steel wall by diffusion. The removal process, however, takes only a few minutes. Therefore, it appears that the cleaning solution penetrates into the spongy structure of the deposit layer, causing a swelling from which cracks propagate, which, in turn, accelerates the further penetration of the cleaning solution. Especially near the stainless-steel wall, cracks will arise because the deposit layer swells while the stainless-steel wall does not. Swelling of the deposit layer may be a result of breakage of a number of deposit–deposit attachments or of an increased repulsion in the deposit layer due to the high pH of the alkaline



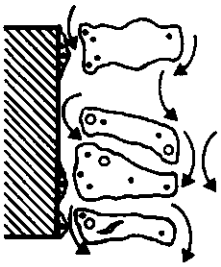
milk deposit layer: a spongy protein matrix with which minerals (•) are associated and in which fat globules (o) are embedded



swelling of the deposit layer and crack formation



loosening of first part of deposit



remaining deposit is removed due to mechanical forces exerted by the cleaning solution. Some calcium phosphate may remain

Fig. 4. Schematic representation of the milk deposit removal during alkaline cleaning.

cleaning solution. Cracks allow the cleaning solution to penetrate into the deposit layer and to disrupt it by the shear stresses and/or the pressure fluctuations (due to turbulence) exerted by the fast-flowing liquid. The deposit is loosened directly from the stainless-steel wall or from the dense mineral layer, which means that the deposit–wall attachment is weaker than the deposit–deposit attachment, and it is carried away in large lumps with the cleaning solution.

The removal in large lumps explains why the mineral deposit is already removed together with the organic deposit by the alkaline cleaning solution. Perlat *et al.* (1986) assumed that this would be the case.

The observations made here are in good agreement with the laboratory-scale observations of Grasshoff (1988) and Bird and Fryer (1991), who also observed an initial swelling of the deposit layer and then removal of parts of the upper deposit layer.

After the alkaline cleaning, the surface was still dull at several spots, indicating that not all the calcium phosphate deposit (possibly from the dense layer) had been removed. This deposit was removed quickly by the subsequent acid cleaning.

Removal of milk deposit in evaporators can be described in a similar way. There may be small differences, since the deposit in an evaporator is more spongy. This would result in a faster penetration of the cleaning solution through the top layer of the deposit and thus in faster cleaning.

It is realized that at heating temperatures higher than studied here, a much harder deposit is formed (type B, Burton, 1968) in the heat exchanger, which may have a different mechanism of removal.

Influence of alkali concentration and flow rate on the cleaning rate in an evaporator

To study the cleaning rate qualitatively, it is desirable to have a high degree of fouling. Since the fourth effect of the evaporator turned out to be fouled most extensively, it was decided to clean this effect separately to study the influence of the alkali concentration and the flow rate on the cleaning rate in an evaporator. Because the evaporator used here was a falling-film evaporator, strictly speaking it is the wetting rate, not the flow rate, that influences the cleaning rate. However, since all the measurements were made on the same effect of the evaporator, the flow rate was set equivalent to the wetting rate. The cleaning took place after evaporating whole milk for 16 h. Four experiments under different cleaning conditions were performed. The removal of organic deposit from the fourth effect during the first 40 min of alkaline cleaning was followed as in Fig. 3. Two parameters were taken as a measure of the rate of cleaning

(Table 2): (i) the width at the base of the peak showing the removal of the organic deposit from the moment the alkaline cleaning solution left the evaporator, and (ii) the percentage of the total amount of removed organic deposit which was removed in the first 40 min of alkaline cleaning (100% is the total removed organic deposit).

Several studies on cleaning rates in heat exchangers (Lalande *et al.*, 1984; Grasshoff, 1988; Timperley & Smeulders, 1988; Bird & Fryer, 1991) have shown that with decreasing flow rate the cleaning rate decreased. Table 2 shows that this was also true for evaporators. If the flow rate was reduced from 3000 to 800 litres/h, which meant that the wetting rate in the fourth effect was reduced from 3100 to 800 litres/h per m (a value often found in industrial evaporators), there was a strong decrease in the cleaning rate. Furthermore, on visual inspection, the evaporator was not clean at the end of the cleaning procedure.

Also, if the alkali concentration was increased to 4·5% but under good flow rate conditions (3000 litres/h), the cleaning rate decreased and the evaporator was not clean afterwards. In the experiments at a flow rate of 800 litres/h, an increase of alkali concentration from 1 to 2% already slowed down the cleaning rate. This shows that the intuitive notion, prevalent in industry, that a higher alkali concentration results in a higher cleaning rate, is not correct. Timperley and Smeulders (1988) and Bird and Fryer (1991) came to a similar conclusion concerning heat exchangers.

It is concluded that under good conditions (for this evaporator, a flow rate of 3000 litres/h and an alkali concentration of 1%), an evaporator can be cleaned completely in a short time. Using too low a flow rate or too high an alkali concentration, or both (as may occur when the cleaning solution is concentrated in the evaporator), gives a much slower cleaning rate and not all of the deposit may be removed. A good flow rate during cleaning can be obtained by using external pumps with a sufficient capacity, as is used for example in the Ralli system (Anon., 1985).

The total amount of organic deposit removed, as measured by total COD, varied from day to day, in spite of constant process conditions. This is a known effect in fouling studies (Timperley & Smeulders, 1987; Grandison, 1988; Stemerdink & Brinkman, 1990) and has not yet been explained.

In addition to COD, the cleaning solutions were also analysed for protein and fat; the ratio of protein to fat in the deposit removed from the fourth effect of the evaporator was approximately 1, which is the same ratio as in the evaporated milk. This suggests that the deposit in this last effect was formed by 'drying on' of the evaporated milk, due, for example, to an insufficient flow rate or an uneven distribution of the milk over the pipes.

TABLE 2

Removal of Milk Deposit in the Fourth Effect of an Evaporator During the First 40 min of the Alkaline Cleaning as a Function of Flow Rate and Alkali Concentration

Flow (litres/h)	*NaOH (%)*	*Width of peak (min)*	*COD in peak (%)*	*COD* (kg O_2/litre)*	*Protein* (kg)*	*Fat* (kg)*	*Visual result*
3000	1	10	98	24·1	5·4	5·3	Clean
800	1	18	88	16·4	3·3	3·0	Fouled
800	2	>25	64	14·3	3·0	2·7	Fouled
3000	4·5	20	95	13·0	2·9	2·7	Fouled

*Total amount of organic deposit removed by the alkaline and acid cleaning.

In the experiments (see Table 2) where the evaporator was still fouled after cleaning, visual inspection showed that the residual deposit had a brown (burned) colour and a rubber-like top layer. Apparently, under the applied cleaning conditions, the deposit layer may have swollen; anyway, some sort of chemical reaction occurred in which the protein had gelled or polymerized into a rubber-like structure (see Fig. 5). Such a 'rubbery' layer presumably prevents or obstructs further penetration of the alkaline solution into the deposit, causing the cleaning rate to decrease markedly. Gel formation due to a reaction between alkali and the deposit layer has been reported before. Perlat *et al.* (1986) found a gel consisting of protein in the heating section of a UHT-sterilizer. Bird and Fryer (1991) also found a smooth, glassy deposit remaining after cleaning with a high alkali concentration (2%). The latter authors used a cleaning temperature of only 50°C, which may explain why they did not find a brown rubber-like gel as we observed at a cleaning temperature of 70°C. It is not known which chemical reaction causes the formation of the rubber-like layer since little research has been done on proteins under the conditions applied during cleaning. It was assumed that good cleaning is achieved if the deposit layer is already loosened from the stainless steel wall before 'polymerization' reaches a degree at which it obstructs the penetration of the cleaning solution and the formation of cracks in the deposit layer.

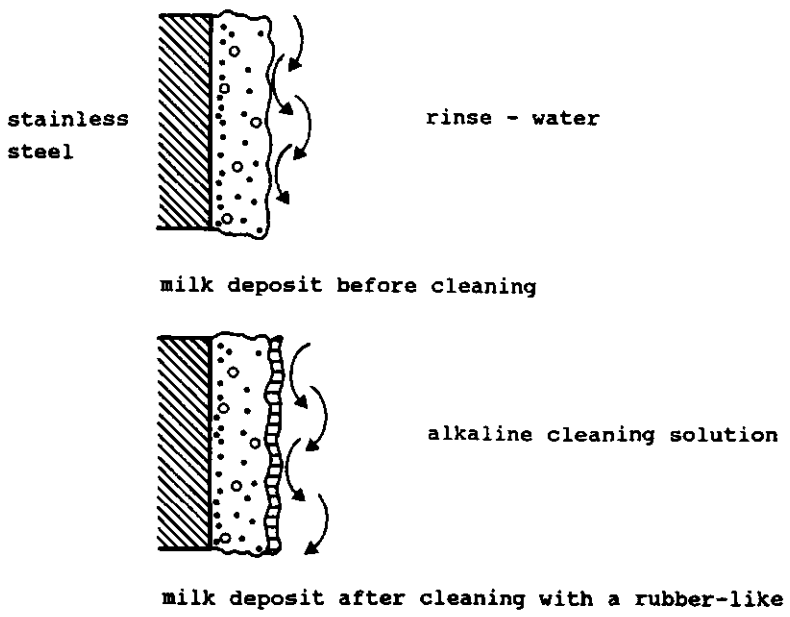


Fig. 5. Schematic representation of the formation of a rubber-like layer at the outside of the milk deposit during alkaline cleaning.

Removal of whey deposit

In the Dutch dairy industry, whey is concentrated by evaporation in two steps: first at the cheese factory from 5–6% TS to 28% TS to reduce transport costs and then in the whey-processing plant from 28% TS to 55% TS. In either case, fouling occurs which must be removed by cleaning. Two cleaning procedures were tested after heating and evaporation of whey (5·7% TS) or concentrated whey (28% TS): one in which acid cleaning was applied first, followed by an alkaline cleaning, while in the other the sequence of acid and alkaline stages was reversed. The percentages of deposit removal by the different cleaning stages are given in Table 3. As an illustration, Fig. 6 shows the cleaning rate for COD and calcium for both cleaning procedures after evaporating whey with 5·7% TS.

For efficient cleaning of heat exchangers after pasteurizing whey (5·7% TS), the sequence of the acid and the alkaline stage was not important; in both cases most of the deposit was completely removed in a few minutes by the first flush of cleaning solution (Table 3a). If after evaporating whey or after heating and evaporating concentrated whey the cleaning procedure started with an alkaline cleaning, the cleaning rate slowed down considerably (e.g. no peaks were observed in the cleaning rate for whey: Fig. 6(a)); the mineral deposit especially was removed very slowly. If the cleaning procedure started with an acid cleaning, sharp peaks in the cleaning rate were again observed (Fig. 6b) and most of the deposit was removed by the first acid flush (Tables 3a and 3b). The residual deposit after the acid cleaning was quickly removed by the subsequent alkaline cleaning.

In Table 4 the composition of the deposit, expressed in grammes per tonne processed whey, is shown for the heat exchanger and the evaporator. The results for concentrated whey and whole milk (data in Table 1) are also given. From Table 4 it is clear that processing concentrated whey gave much more fouling than did processing whey with 5·7% TS. Furthermore, in contrast to milk, the main component of the whey deposit was minerals; for whey (5·7% TS) this was in the form of calcium phosphate. After evaporating concentrated whey, a considerable amount of calcium citrate (7% of all the citrate in whey) was found and only little calcium phosphate. The change from calcium phosphate to calcium citrate could not readily be understood from the solubility behaviour of the salts. Further research is needed to explain this change.

Comparison of the composition of the whey deposit in the evaporator with that of the processed product, showed that minerals formed most of the deposit while lactose, one of the main components of whey, was found at very low levels in the deposit. The preferential deposition of minerals

TABLE 3a

Percentage of the Compounds of Deposit of Whey (5·7% TS) Removed in a Heat Exchanger for Two Different Cleaning Procedures, One Started with Acid Cleaning (HNO_3) Followed by Alkaline Cleaning (NaOH), The Other Started with NaOH Followed by HNO_3 (Same results for concentrated whey (28% TS))

Compound	*1st procedure*		*2nd procedure*	
	HNO_3	*NaOH*	*NaOH*	*HNO_3*
COD	88 (98)	(100)	12 (2)	99 (95)
Protein	91 (95)	100	9 (5)	99 (80)
Citrate	100	(100)	0 (0)	100
Ca	(100)		3 (0)	(100)
PO_4	97		0 (0)	85 (36)

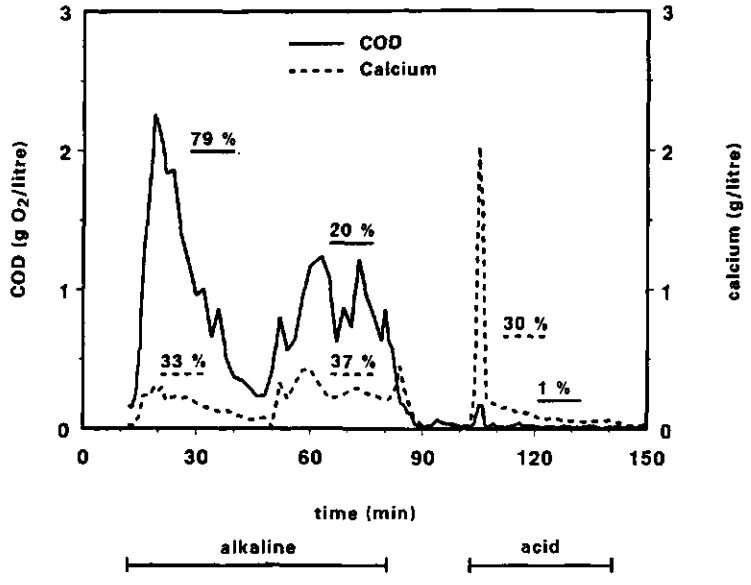
TABLE 3b

Percentage of the Compounds of Deposit of Whey (5·7% TS) (or Concentrated Whey (28% TS)) Removed in an Evaporator for Two Different Cleaning Procedures; One Started with Acid Cleaning (HNO_3) followed by Alkaline Cleaning (NaOH), The Other Started with NaOH Followed by HNO_3 (Same results for concentrated whey (28% TS))

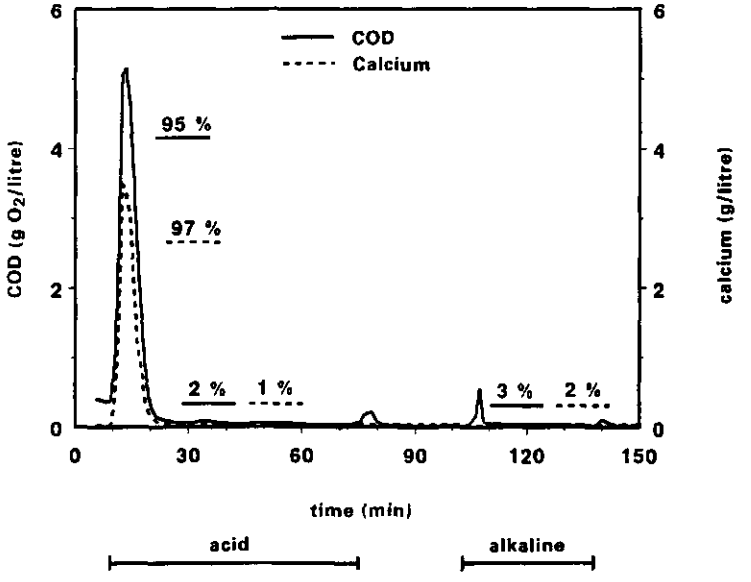
Compound	*1st procedure*			*2nd procedure*		
	HNO_3		*NaOH*	*NaOH*		*HNO_3*
	First flush	*Circulation*		*First flush*	*Circulation*	
COD	95 (99)	2 (0)	3 (1)	79 (96)	20 (3)	1 (1)
Protein	91 (99)	7 (<1)	2 (<1)	73 (97)	23 (1)	4 (2)
Citrate	100 (100)	0 (0)	0 (0)	91 (92)	9 (9)	0 (0)
Ca	97 (100)	1 (0)	2 (0)	33 (84)	37 (13)	30 (4)
PO_4	99 (100)	0 (0)	1 (0)	51 (67)	27 (17)	22 (17)

may be explained by the decrease in solubility product of calcium salts on heating and by the fact that whey contains less compounds than milk that prevent or obstruct the nucleation and crystal growth of these salts. Minerals were the principal components of the (concentrated) whey deposit, which explains why a cleaning procedure starting with acid was more effective.

Table 3 shows that for concentrated whey a large part of the organic deposit was already removed if cleaning was started with the acid cleaning. This was less surprising, because a large part of the deposit consisted of calcium citrate, which contributes to the calcium in, as well as to the COD of, the cleaning solution.



(a)



(b)

Fig. 6. Whey deposit removal in an evaporator after operating for 20 h using (a) an alkaline–acid procedure and (b) an acid–alkaline procedure. Cleaning temperature was 70°C and the cleaning solutions were NaOH (1%) and HNO_3 (1%).

TABLE 4
Composition (g) of the Deposit in the Heat Exchanger and the Evaporator per Tonne Processed Product

	Whey (5·7% TS)	*Whey (28% TS)*	*Milk*
Heat Exchanger			
COD	11	54	27
Protein	6·6	20	6
Fat	0·4	3·0	6·5
Calcium	4·9	25	2·2
Phosphate	7·6	31	3·4
Citrate	1·5	25	nd
Lactose	—	—	nd
Evaporator			
COD	27	806	192
Protein	12	79	52
Fat	0·6	20	34
Calcium	17	178	9·6
Phosphate	23	32	12·6
Citrate	6	631	nd
Lactose	3	77	nd

nd = not determined.

The deposit in the heat exchanger after processing whey with 5·7% TS contained relatively more protein than the deposit in the evaporator, which explains why a cleaning protocol starting with an alkaline stage was still efficient for the heat exchanger but not for the evaporator. In practice, however, the cleaning of the heat exchanger and the evaporator is often done by the same 'cleaning in place' (CIP) programme, so the sequence of cleaning stages is the same. In that case, it is recommended after processing whey to start the CIP-programme with an acid cleaning followed by an alkaline cleaning.

After processing concentrated whey, some COD and calcium were found in the rinse-water, which meant that already during rinsing some deposit was removed. This also happened during the processing of the whey: some deposit was loosened from the wall and disrupted in the pumps into very small particles,which left the evaporator together with the evaporated whey.

Mechanism of whey deposit removal

For the deposit formed during evaporating whey or during heating and evaporating concentrated whey, a different mechanism of removal from

that described for milk deposit is proposed since the components of the deposit are mainly minerals. (Deposit formed during heating whey still contained much protein and is thought to be removed like a milk deposit.) Compared to the milk deposit, the whey deposit was less spongy, had a denser structure and a yellow colour. It is presumed that the deposit layer consists of a complex of protein with calcium salts of phosphate or citrate, or both, in which the calcium has a 'bridge' function between protein molecules and between protein and the salts. At some places, some fat is embedded. Contact of the acid cleaning solution with the protein in the deposit layer did not result in swelling. The bonds within the complex are stronger or more numerous than the attachment of the complex to the stainless steel, which explains why the deposit layer was removed in large lumps. The attachment to the stainless steel is not as strong as between milk deposit and the stainless steel; parts of the deposit were removed simply by mechanical forces (i.e. shear stress) during the processing of whey or during rinsing.

Cost savings

Although it is not the objective of this paper to study cost aspects, it is nevertheless interesting to make a (conservative) estimate of the possible reduction in cleaning costs and energy use that may be realized. If the cleaning procedure for a milk evaporator (including its heat exchanger) is optimized as described in this study, then, during cleaning, a reduction in energy costs of more than 50% can be achieved. Furthermore, the use of cleaning agents can be reduced by 25% and there will be 5% less product losses (FNZ, 1990). For optimization of the cleaning procedure for a whey evaporator, the situation is as follows: energy costs can be reduced by up to 65%, savings on cleaning agents by 25% and a 20% decrease in product losses can be achieved (FNZ, 1991). For the Dutch dairy industry, this would result in an annual saving of more than 5 million Dutch guilders (FNZ, 1990; FNZ, 1991).

CONCLUSIONS

After processing milk, whey or concentrated whey, heat exchangers and evaporators can be cleaned completely in a short time. If the flow rates in evaporators during cleaning are too low or alkali concentrations are too high, or both, then the cleaning will be slow and incomplete. The remaining deposit layer is covered by a brown rubber-like layer of polymerized protein. Therefore, it is recommended to use an optimized cleaning

procedure for heat exchangers and evaporators. By optimizing the cleaning procedure, reductions in energy costs and use of cleaning agents by 50 and 25%, respectively, can be achieved.

Since whey deposit contains considerably more mineral compounds than milk deposit, it is better to reverse the sequence of the cleaning stages after processing whey, i.e. to start with an acid cleaning followed by an alkaline cleaning.

The proposed scheme for the removal of milk deposit, represented as a matrix of protein to which calcium salts are associated and in which fat is embedded, shows that after contact with the alkaline cleaning solution the deposit layer swells and cracks propagate from it. These cracks allow the cleaning solution to penetrate into the deposit and to disrupt it by shear stresses. The deposit is loosened from the stainless steel or a dense layer adjacent to the stainless steel, and removed in large lumps by the cleaning solution.

Whey deposit is thought to consist of a complex of protein, together with calcium salts. After contact with the acid cleaning solution, no swelling is observed but again the deposit is loosened from the stainless steel wall and removed in large lumps with the cleaning solution. Apparently, the weak cohesion of the deposit layer with the stainless steel or the dense layer is an important factor in the removal process.

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REFERENCES

Anon. (1985). The Ralli evaporator cleaning system. *North European Dairy J.*, **51**, 76–8.

Bird, M.R. & Fryer, P. (1991). An experimental study of the cleaning of surfaces fouled by whey proteins. *Trans. I. Chem. Eng.*, **69** (Part C), 13–21.

Bouman, S., Lund D.B., Driessen F.M. & Schmidt, D.G. (1982). Growth of thermoresistant streptococci and deposition of milk constituents on plates of heat exchangers during long operating times. *J. Food Prot.*, **45**, 806–12.

Bouman, S., Brinkman D.W., De Jong, P & Waalewijn, R. (1988). Multistage evaporation in the dairy industry: energy savings, product losses and cleaning. In *Preconcentration and Drying of Food Materials*. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 51–60.

Burton, H. (1968). Reviews of the progress of dairy science. Section G. Deposits from whole milk in heat treatment plant — A review and discussion. *J. Dairy Res.*, **35**, 317–30.

Delsing, B.M.A. & Hiddink, J. (1983). Fouling of heat transfer surfaces by dairy liquids. *Neth. Milk Dairy J.*, **37**, 139–48.

Dutch Standards NEN 3235 9.2.1 (1978). Dutch Organization for Standardization. Delft, The Netherlands.

Dutch Standards NEN 6446 (1980). Dutch Organization for Standardization. Delft, The Netherlands.

Dutch Standards NEN 6465 (1981). Dutch Organization for Standardization. Delft, The Netherlands.

Dutch Standards NEN 6479 (1981). Dutch Organization for Standardization. Delft, The Netherlands.

Dutch Standards NEN 3198 (1984). Dutch Organization for Standardization. Delft, The Netherlands.

Dutch Standards NEN 6633 (1990). Dutch Organization for Standardization. Delft, The Netherlands.

FNZ Royal Dutch Dairy Federation (1990). Reiniging van melkindampers. Rijswijk, The Netherlands.

FNZ Royal Dutch Dairy Federation (1991). Reiniging van wei-indampers. Rijswijk, The Netherlands.

Grandison, A.S. (1988). UHT processing of milk: Seasonal variation in deposit formation in heat exchangers. *J. Soc. Dairy Technol.*, **41**, 43–9.

Grasshoff, A. (1988). The influence of chemical components of caustic cleaners on incrusted milk deposits removal kinetics. *Kieler Milchwirtschaftliche Forschungsberichte*, **40**(3), 139–77.

Hiddink, J., Lalande, M., Maas, A.J.R. & Streuper, A. (1986). Heat treatment of whipping cream. I. Fouling of the pasteurization equipment. *Milchwissenschaft*, **41**, 542–6.

Holt, C., Van Kemenade, M.J.J.M., Nelson, L.S., Jr, Sawyer, L., Harries, J.E., Bailey, R.T. & Hukins, D.W.L. (1989). Composition and structure of micellar calcium phosphate. *J. Dairy Res.*, **56**, 411–16.

Jeurnink, T. J.M. (1991). Effect of proteolysis in milk on fouling in heat exchangers. *Neth. Milk Dairy J.*, **45**, 23–32.

Jeurnink, T. J.M., Brinkman, D.W. & Stemerdink, A.D. (1989). Distribution and composition of deposit in heat exchangers. In *Proc. 3rd Int. Conf. on Fouling and Cleaning*, eds. H. G. Kessler & D. Lund. (Druckerei Walch, Augsberg, Federal Republic of Germany, pp. 25–36.

Kessler, H.G. (1989). Fouling of milk proteins and salts — Reduction of fouling by technological measures. In *Proc. 3rd Int. Conf. on Fouling and Cleaning*, eds. H. G. Kessler & D. Lund. (Druckerei Walch, Augsberg, Federal Republic of Germany, pp. 37–45.

Lalande, M., Gallot-Lavalée, T. & Corrieu, G. (1984). Chemical reaction and mass transfer associated with cleaning of heat exchangers surfaces fouled by milk deposit. In *Engineering and Food*, ed. B. McKenna. Elsevier Applied Science Publishers, London, pp. 59–68.

Lyster, R.L.J. (1965). The composition of milk deposits in an ultra-high-temperature plant. *J. Dairy Res.*, **32**, 203–8.

Nieuwenhuijse, J.A., Timmermans, W. & Walstra, P. (1988). Calcium and phos-

phate partitions during the manufacture of sterilized concentrated milk and their relations to the heat stability. *Neth. Milk Dairy J.*, **42**, 387–421.

Palmer, J. & Kelly, P. (1984). Evaporator waste losses occurring during washdown. *Int. Dairy Fed. Brussels, Bulletin*, **184**, 57–60.

Perlat, M.N., Lalande, M. & Corrieu, G. (1986). Cleaning of milk UHT sterilizer: Kinetics aspects with regard to acid and alkaline detergents. *Le Lait*, **66**, 31–63.

Schmidt, D.G. & Both, P. (1987). Studies on the precipitation of calcium phosphate. I. Experiments in the pH range 5·3 to 6·8 at 25°C and 50°C in the absence of additives. *Neth. Milk Dairy J.*, **41**, 105–20.

Stemerdink, A.D. & Brinkman, D.W. (1990). Single stage cleaning of milk evaporators. *Voedingsmiddelentechnologie*, **11**, 13–16.

Timperley, T.A. & Smeulders, C.N.M. (1987). Cleaning of dairy HTST plate heat exchangers: Comparison of single- and two-stage procedures. *J. Soc. Dairy Technol.*, **40**, 4–7.

Timperley, T.A. & Smeulders, C.N.M. (1988). Cleaning of dairy HTST plate heat exchangers: Optimization of the single-stage procedure. *J. Soc. Dairy Technol.*, **41**, 4–7.

Tissier, J.P. & Lalande, M. (1986). Experimental device and methods for studying milk deposit formation on heat exchange surfaces. *Biotechnol. Progress*, **2**(4), 218–29.

Van Kemenade, M.J.J.M. & De Bruyn, P.L. (1987). A kinetic study of precipitation from supersaturated calcium phosphate solutions. *J. Colloid Interface Sci.*, **118**, 564–85.

Mechanisms of fouling in dairy processing[1]

Th.J.M. Jeurnink; P. Walstra; C.G. de Kruif

Summary

During heating of milk in a heat exchanger an undesirable deposit, mainly consisting of protein and mineral, is formed on the heating surface. Even at room temperature a monolayer of protein is immediately adsorbed. Further deposition of protein on top of this monolayer occurs if serum proteins undergo heat denaturation. Then so-called activated β-lactoglobulin (β-lg) molecules are formed in the bulk. This rate-determining step is followed by the transport of this activated molecule to the surface and its subsequent deposition through a reaction with an already deposited molecule. During its transport to the surface the activated molecule may be inactivated, i.e. no longer be prone to deposition, through a reaction with another activated molecule. On the other hand, a reaction of activated β-lg with another milk component, for example with casein micelles or fat globules, may lead to the incorporation of such components in the deposit layer. Since other serum proteins also undergo heat denaturation they may be involved in the deposition process in a way comparable to β-lg.

A second major cause of fouling is the decreased solubility of calcium phosphate salts upon heating. The precipitation of these minerals is partly driven by the temperature difference between the bulk and the surface. However, part of the minerals will precipitate together with proteins. In its ionic form calcium influences the protein deposition by its effect on the aggregation of whey proteins and on the stability of casein micelles.

Components other than serum proteins and minerals in milk play a minor role in fouling. Only if the colloidal stability of the casein micelles in the milk is decreased, e.g. by lowering the pH, does severe fouling by casein micelles occur. Also the presence of air bubbles at the surface enhances fouling.

From the results obtained we derive recommendations on how to control fouling and on how effective cleaning can be achieved.

Keywords: fouling, heat exchanger, β-lactoglobulin, denaturation, calcium phosphate, precipitation

1 Introduction

In the dairy industry pasteurization is a commonly used method to enhance the keeping quality of milk and to kill pathogenic and psychrotrophic organisms. Unfortunately, a fouling layer, consisting predominantly of (serum) proteins and minerals, accumulates in the heat exchanger. Fryer et al. [1] gave a review of fouling and cleaning in milk processing, from which it is clear that protein denaturation and mineral precipitation play key roles in the process of fouling.

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The rate-determining step in fouling of heat exchangers by milk in the temperature range of 65 - 100 °C appears to be a (chemical) reaction and not mass transfer [2, 3, 4]. Although the final stage in the fouling process consists of a surface reaction at the heated surface it is possible that the rate-determining reaction takes place in the bulk of the solution.

We distinguish three possible mechanisms of fouling.

(1) If fouling is the result of a surface reaction then the process would be controlled by the wall temperature and not by the bulk temperature. The ΔT, that is the temperature difference between the heating surface and the liquid to be heated, results in a faster reaction at the surface than in the bulk, which leads to a concentration gradient and consequently to a continuous transport of molecules to the heating surface. Several researchers have claimed that fouling is a process which takes place in the thermal boundary layer, i.e. the layer adjacent to the wall where the temperature is higher than in the bulk of the liquid [5, 6].

(2) Reactive molecules or particles formed in the bulk of the solution diffuse to the surface where they can deposit. Although the particles diffuse only from a thin layer, continuous supply of "fresh" product may lead to a considerable amount of

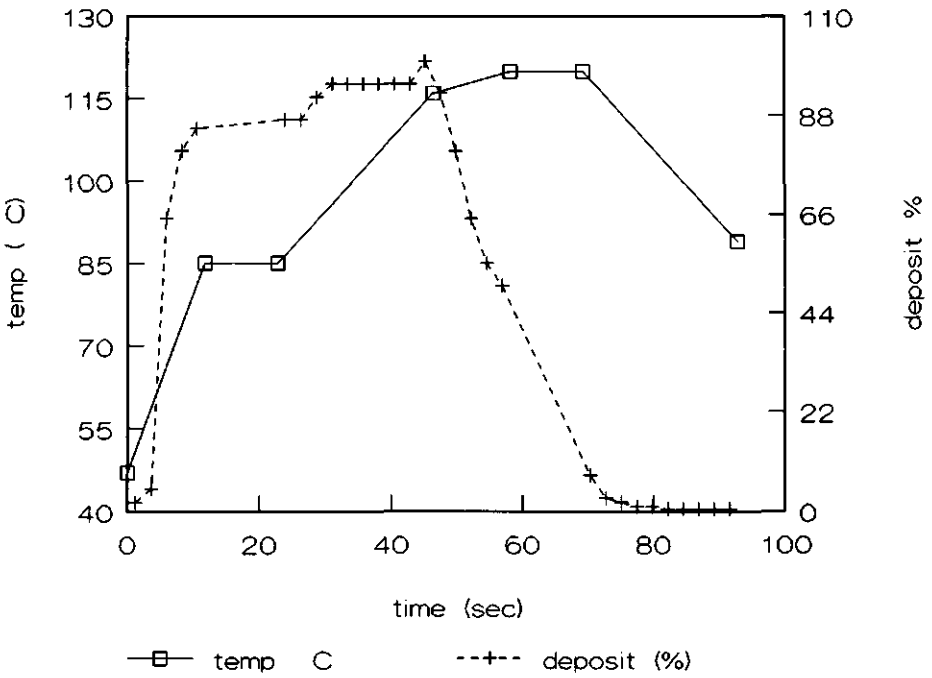

Fig. 1. Temperature profile and deposit distribution in a plate heat exchanger after a running time of 8.5 h at a pasteurization temperature of 120 °C for 11 s. On the horizontal axis the residence time is given. Amount of deposit was determined visually: the most fouled plate was set at 100 %, a visually clean plate at 0 % deposit [22].

deposit after long times. The observation that fouling by dairy liquids also occurs in holding sections (where the wall and the bulk temperature are the same), and even at surfaces with a temperature lower than that in the bulk [7], clearly shows that fouling is not simply driven by a temperature gradient between the wall and the bulk of the solution, but that bulk processes are important in fouling [2].

(3) Fouling can be strongly enhanced through air bubble formation at the heated surface. The bubbles arise in milk on heating as a result of the presence of dissolved air. Proteins, especially casein micelles, at the gas/liquid interface end up in the deposit by drying out of the membrane surrounding the air bubbles.

In this paper we will discuss which of the three mechanisms is the most important in fouling. This will be done by considering the reactions of the various components in milk on heating, which we have studied in our laboratory during the last few years. Further the fouling behaviour of various milk products is discussed. We conclude with recommendations on how to control fouling and how effective cleaning can be achieved.

2 Composition and Structure

2.1 Composition

Fouling of heat exchangers only becomes a serious problem if the milk reaches a temperature of 70 °C or higher (Fig. 1). Burton [8] distinguished two types of deposit: one formed at 70 - 110 °C, the so-called type A deposit, and one formed at 110 °C or higher, the so-called type B deposit. In this study we focus on type A fouling, a soft voluminous curd-like material. A representative composition of such a deposit is given in Table 1.

The total protein content in the dry matter of the deposit is not greatly different from that of milk (Table 1). However, the shift from casein to serum proteins in the composition of the deposit is noticeable. Although all types of caseins are present, the casein composition of the deposit differs from that of the casein micelles in the milk. This suggests that the caseins in the deposit do not only originate from whole casein micelles. In the deposit a large increase in mineral content is observed due to the precipitation of calcium phosphate. It is also seen that fat and lactose hardly contribute to the deposit.

2.2 Structure

A type A deposit can be seen as a spongy protein matrix, to which minerals are bound, and wherein fat globules are embedded [9]. Under this spongy layer, adjacent to the stainless steel, the presence of a thin dense layer (approximately 15 μm), consisting mainly of minerals, is reported [10]. A scanning electron micrograph (Photo 1) of a deposit of skim milk shows the presence of roughly spherical particles which are much smaller in size than casein micelles in milk. Presumably, the deposit is built of serum protein aggregates rather than of casein micelles.

Table 1. Composition (%) of the dry matter of skim milk and its deposit in the heater section (69-85 °C) of a plate heat exchanger after pasteurization, data taken from [15]. The dry matter content of the skim milk was 9.35%.

Component	Skim milk	Deposit
protein	37.2	44.4
serum protein[1,2])	16.5	75
casein[1])	83.5	25
×-casein[3])	12	18
α_{s1}-casein)[3])	38	27
α_{s2}-casein[3])	8	11
β-casein[3])	42	44
mineral	7.4[4])	45.0
calcium	1.32	15.7
PO_4 (inorg.)	2.04	23.0
magnesium	0.13	0.60
citrate	1.81	0.49
fat	<1	0.4
lactose	52.6	0.02[5])
Ca/P inorg.(mol/mol)	1.54	1.62

[1]) The contributions of serum protein and casein are given as a percentage of the total amount of protein present in the milk or in the deposits.
[2]) The main contribution to the deposit of the serum proteins came from β-lg, while α-la contributed only to a small extent (Fig. 4 in [15]).
[3]) The contribution of the different caseins is given as a percentage of the total amount of casein.
[4]) Not determined but taken from Walstra and Jenness [14].
[5]) Estimated from data for deposit of low and high calcium milk [15].

3 Fouling by different milk components

3.1 Serum protein

3.1.1 Introduction. As soon as a stainless steel surface comes into contact with a serum protein solution, even at room temperature, immediately a monolayer of protein adsorbs [11]. On the further deposition of protein on top of this monolayer the following observations were made:

- the total fouling was reduced by two-thirds as compared to normal milk, if serum proteins were (nearly) absent in milk [12];

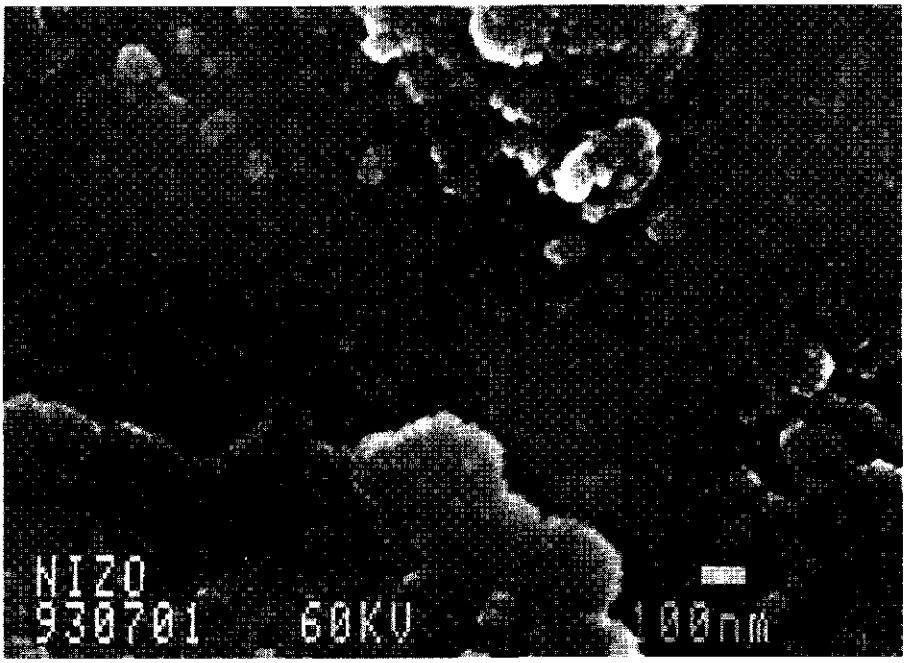

Photo 1. Deposit of fresh skim milk in a pilot-plant-scale heat exchanger [35].

- hardly any deposit was found if the temperature of milk was not higher than 70 °C (Fig. 1);
- not much deposit was found, if the milk had been given a strong preheat treatment in such a way that all the serum proteins had aggregated [11].

Apparently, a prerequisite for continued deposition is the presence of some intermediate of the denaturation reaction of the serum proteins. Protein fouling is controlled by this denaturation reaction taking place in the bulk rather than by a reaction at the surface [11]. The deposition process on top of a monolayer can be described as follows:

(1) formation of some "fouling intermediate" in the bulk of the solution;
(2) transport of this intermediate to the surface;
(3) deposition of the intermediate.

3.1.2 Formation of "fouling intermediate". The partly unfolded and so-called activated β-lg molecules, as described in the model for the denaturation and aggregation of β-lg [13], act as the "fouling intermediate". This model describes the aggregation of β-lg in analogy to a radical chain reaction; upon raising the temperature a thiol group of β-lg is exposed and becomes accessible to disulfide-thiol exchange reactions. This activated molecule acts like a "radical". The rate of deposition is proportional to the concentration of these activated β-lg molecules, as calculated on the basis of the model [11].

3.1.3 Transport to the surface. Transport to the surface is determined by the flow rate and the geometry of the apparatus. During this transport an activated molecule of β-lg can be inactivated through a reaction with another activated β-lg molecule in the bulk or with the κ-casein at the surface of the casein micelles.

Near the stainless steel wall there is a hypothetical layer where an activated β-lg molecule has an equal chance of colliding with the wall or with a casein micelle. The thickness of this layer can be estimated by taking the mean free distance between micelles (z), given by [14]:

$$z = 0.225 d_{vs} \left(\frac{0.74}{\phi} - 1\right)$$

Taking for the volume/surface average diameter (d_{vs}) and for the volume fraction (ϕ) of casein micelles the values of 120 nm and 0.1, respectively, z would be 173 nm. It was calculated that in the plate heat exchanger, as used by Jeurnink [15], diffusion of serum proteins to the surface from a layer with such a thickness would give a contribution of at most 14 % to the serum protein deposition. In other words, there must be a continuous supply of activated molecules to the heating surface from the bulk.

Further calculations, using the same plate heat exchanger [15], showed that at any moment 1 l of milk is in contact with 0.5 m^2 of stainless steel while at the same moment per l of milk 4000 m^2 of casein micelle surface is present [14]. In other words, an activated β-lg molecule has a much larger probability (by a factor 4000/0.5) of encountering a casein micelle than of colliding with the wall of the heat exchanger. On the one hand this may explain, when comparing the amount of denatured β-lg in the milk to the corresponding amount of deposited protein, why only 0.14 % of the denatured β-lg finally ends up in the deposit layer (Table 2). On the other hand it shows that the sticking probability of an activated molecule reacting with a casein micelle would be approximately 10 times smaller than in the case of a reaction with a deposited protein layer at the wall. The steric repulsion by the hairy layer of the casein micelle and the fact that the cysteine residue of the κ-casein, needed for disulfide bonding with β-lg, is located just inside the micelle, may explain this smaller sticking probability.

3.1.4 Deposition. The deposition and binding of an activated protein molecule most probably occurs through the formation of a disulfide bond with a deposited molecule. Not all activated molecules that reach the surface will attach; we presume that for such a reaction the activated β-lg molecule needs a certain orientation. We expect the energy barrier, that it has to overcome for attachment caused by electrostatic or steric repulsion,

Table 2. Amount of denatured β-lg (calculated, [3]) and corresponding total serum protein deposit [15] both in mg per litre pasteurized milk (15 s holding at 85 °C), including their ratio in %.

Denatured β-lg	Deposited serum proteins	Ratio %
1165 mg/l	1.72 mg/l	0.14

to be negligible. The probability of becoming attached would also depend on the shear rate. This shear is of no importance for the relatively small serum protein particles, but may become important if larger aggregates are formed.

3.1.5 Model of protein fouling. Based on the information given above and knowing that there is general agreement that removal of deposited material during the fouling process can be neglected, the deposition process can be represented by:

$$d\Gamma/dt = \omega.k/x.B^*(t)$$

where

$d\Gamma/dt$ is the deposition rate;

ω is the sticking probability of the protein; experimentally we found for a β-lg solution this to be of the order of 1/300 [11];

k is a transport coefficient, which would be constant for a given geometry of the heat exchanger and which comprises both the diffusion coefficient of the protein and the flow rate of the liquid;

x is the fraction of β-lg in the deposit which equals 0.3 (Table 1);

$B^*(t)$ is the bulk concentration of activated β-lg molecules at time t as calculated from the denaturation model [11].

An experimentally found value for the deposition rate in a plate heat exchanger fouled by milk is $1.5*10^{-6}$ $kg.m^{-2}.s^{-1}$ [15].

Not only β-lg but also α-lactalbumin and bovine serum albumin contribute to the fouling process [12]. An immunoglobulin G solution showed during heating a deposition rate comparable to that of a β-lg solution (unpublished results). Since these serum proteins also contain thiol and/or cysteine groups, these may, in a way comparable to β-lg, be involved in the fouling process.

An activated molecule of β-lg may also react with casein micelles or fat globules in the bulk of the solution. If these micelles or globules covered with β-lg molecules reach the wall and the β-lg is still in its active form, they may take part in the fouling reaction.

3.2 Minerals

The solubility products (K_s) of calcium phosphate salts in milk become smaller at higher temperatures. In milk the main calcium salts are several forms of calcium phosphate and calcium citrate. The first precipitating phase of calcium phosphate salts is usually dicalcium phosphate dihydrate (DCPD or brushite; $CaHPO_4.2H_2O$) [16, 17]; this may afterwards be converted into a more stable form of calcium phosphate.

At pH 6.7 Simulated Milk Ultra Filtrate (SMUF) becomes turbid upon heating at 45 °C or higher [18]. Precipitation of calcium phosphate may thus already take place on the stainless steel wall of a heat exchanger from 45 °C upwards. The presence of casein and citrate reduces such a precipitation.

In principle, it is possible to calculate from the change in K_s how much salt would

precipitate upon heating. Holt et al. [19] have developed a computer program which calculates the ion equilibria and solubility products in milk diffusate at room temperature. Unfortunately this model does not hold for the situation in a heat exchanger where high temperatures are applied and where no equilibrium is reached.

Another approach to estimating how much salt precipitates is to use the results of Pouliot et al. [20]. They carried out experiments with a device in which an ultrafiltration unit was coupled to a stainless steel heat exchanger. The advantage of using the method of Pouliot et al. [20] is that the separation between soluble and insoluble salts occurs at the heating temperature while other researchers did this after cooling, with the possibility that part of the insoluble salts has already been redissolved. From the results of Pouliot et al. [20], we calculated, making some assumptions on the type of salts that can be formed, that after heating 1 l of skim milk at 85 °C for 1 min a total of 950 mg salt would have been transferred from the serum to the colloidal phase. This amount was compared to the experimentally found amount of deposited salts of 2.3 mg per l milk, heated in 6.5 s from 71 to 85 °C, Table 3 [15]. Only 0.2 % of the transferred salts then became deposited on the stainless steel wall. As mentioned in section 3.1.3, at any moment 1 l of milk would be in contact with 0.5 m^2 of stainless steel, while at the same moment per l of milk 4000 m^2 of casein micelle surface is present and serum protein aggregates are also formed upon heating. Apparently the transferred salts do have a much higher preference to deposit onto the stainless steel wall than to associate with the proteins. This could mean that mineral deposition is partly driven by the temperature difference between the bulk and the surface (ΔT). This is further confirmed by the observation that a larger amount of minerals is found in the deposit in the final pass of the heating section than in the subsequent holding section, where no difference between bulk and surface temperature exists [6, 21, 22]. These results show that mineral deposition is enhanced if a temperature gradient is present.

Calcium phosphate may precipitate directly on the stainless steel wall or deposit as particles which have been formed in the bulk of the solution, as observed on heating SMUF (unpublished results). In milk, however, we expect that calcium phosphate hardly forms particles but associates with the proteins. Consequently, in milk, calcium phosphate precipitates, in addition to the direct deposition, together with the milk proteins [12, 23]. When serum proteins were removed from milk, while the calcium content was kept constant, the total protein deposit reduced from 23 g to 6 g and the calcium deposit fell from 1.4 g to 0.4 g [12]. Apparently, calcium deposits jointly with the serum proteins. Pappas and Rothwell [24] showed that upon heating milk, about 3.6 mol calcium was bound per mol β-lg. Furthermore, casein micelles, which contain a

Table 3. Amount of transferred salts (calculated from data of Pouliot et al. [20]) and deposited salts (determined by Jeurnink and de Kruif [15]) in mg per litre treated milk after pasteurisation, including their ratio in %.

Transferred salts	Deposited salts	%
950 mg/l	2.3 mg/l	0.2

considerable amount of micellar calcium phosphate (MCP), can take up more calcium phosphate upon heating through association with the MCP already present.
Based on the results of Pouliot et al. [20] in heated milk about 40 mg micellar calcium is present per g casein. However, these amounts of calcium bound to serum proteins as well as to the caseins alone, can not explain the reduction in the level of calcium deposit. Apparently calcium phosphate precipitates at the surface of depositing serum protein aggregates. These findings indicate that a proportion of the minerals present in a deposit is related to the deposition of proteins onto the heating surface.

Apart from the role of calcium (-phosphate) in the process of fouling as a result of its decreasing solubility upon heating, it influences protein deposition also in its ionic form. In fouling experiments with (desalted) whey the presence of calcium ions was essential for the growth of a protein deposit layer [25]. We observed that the rate of protein deposition increased sharply, soon leading to blockage of the heat treatment equipment, if 40 ppm calcium was added to a β-lg solution in water (unpublished results). The presence of positively charged calcium ions leads to a reduction in the electrostatic repulsion and hence to the formation of larger aggregates upon heating serum protein. In the presence of calcium ions the deposition of proteins may also occur through formation of non-covalent bonds. In that case the specific orientation of the approaching molecule for the formation of a disulfide bond with an already deposited serum protein molecule is no longer needed and the sticking probability of serum proteins to the stainless steel surface will increase.

3.3 Caseins

At room temperature almost all the casein molecules in milk are associated into casein micelles. Upon heating to 80 - 90 °C both the size and the mutual interactions of these micelles increase through association with β-lg [26]. Since β-lg is reactive towards casein micelles and to stainless steel, this molecule may act as a sticking agent between the micelles and the stainless steel surface.

Upon heating to 80 - 90 °C also a small increase in the concentration of κ-casein in the serum phase occurs, due to its dissociation from the casein micellar surface [15, 27, 28]. This is of relevance because in section 2.1 we have shown that not all the caseins in a deposit originate from whole casein micelles.

If the colloidal stability of casein micelles in milk is decreased, the deposition process may no longer be controlled by denaturing serum proteins but directly by coagulation of casein micelles starting where the temperature is the highest, i.e. at the stainless steel wall.

Upon decreasing the colloidal stability of the casein micelles in milk through proteolytic action [29] (e.g. renneting), through a change in calcium ion activity [15], or through lowering the pH [30], an increase in fouling has indeed been observed. At the same time under these conditions a decrease in the heat stability of the milk is also observed. This parallel in behaviour suggests a link between these two processes. Such a link is supported by the observed shift in composition of a protein deposit from predominantly serum proteins to predominantly casein under these circumstances. Hence,

the mechanism for deposition of casein micelles upon heating milk may be similar to that of the heat coagulation of milk.

Van Boekel et al. [31] proposed the following mechanism for the heat coagulation of milk. Depending on the pH two types of casein particles emerge upon heating, one type at pH < 6.7, onto which serum proteins are deposited, and another type at pH > 7.0, from which most of the κ-casein has been dissociated, resulting in so-called depleted casein micelles. Between pH 6.7 and 7.0 an intermediate zone exists in which a mixed type of particle can develop. These particles are subject to two reactions that can cause coagulation upon heating:

(1) a salt-induced coagulation, in which especially calcium ions are of importance;
(2) a polymerization of protein molecules in which covalent bonds are involved.

We presume that the first reaction is the cause of the additional casein deposition if the calcium ion activity is raised. When the calcium ion activity was decreased (resulting in depleted micelles) or when κ-casein was broken down by proteolytic enzymes, in both cases the micelles would no longer have the full stabilizing action of κ-casein and would become more sensitive to Ca^{2+} ions, i.e. salt-induced coagulation causes extra deposition. Lowering the pH results in an increase in calcium ion activity and promotes the polymerization reaction, which may (partly) explain the extra deposition found on decreasing the pH.

Summarizing, casein micelles can be entrapped in a deposit through surface bound β-lg or, when the colloidal stability is low, through aggregation near or at the heating surface due to the high temperature.

3.4 Fat

Although fat is present in deposit layers it does not play an important role in the process of fouling. Hiddink et al. [32] have found that in spite of the high fat content of whipping cream (85 % of the total solids) the fouling behaviour of this cream resembles that of whole milk. Transmission electron microscopy analysis of milk deposit showed that some fat globules are entrapped in the spongy deposit individually or in clusters [10]. If milk with predominantly undenatured serum proteins is homogenized and subsequently pasteurized we expect an increase in fat fouling compared to non-homogenized milk due to reactions initiated by activated serum proteins present at the surface of the fat globules.

In the case of recombined milk, fouling is probably related to the nature of the fat globule membrane formed during recombining. Recombined milk made of skim milk powder and anhydrous milkfat and homogenized at 130 bar fouled quite rapidly; the deposit contained up to 60 % fat, present as fat globules. This points to active deposition of the emulsion particles, most likely due to an enhanced activity of the proteinaceous membrane [33].

3.5 Lactose

Lactose is hardly found in milk deposits because it is water soluble. So even if it were incorporated in a deposit it would dissolve again, either in the milk itself or in the water of the first rinsing step of the cleaning. Only at high temperatures (> 100 °C), when caramelization or Maillard reactions can take place, may lactose contribute to a deposit. Schraml [34] found that on heating whey protein concentrates at 25 % dry matter, very spongy deposit layers of protein aggregates were formed, enclosing voids containing liquid. As a result these deposits can contain up to 50 % of lactose.

3.6 Dissolved air

On heating milk, air bubbles arise as a result of the presence of dissolved air. If these bubbles are formed at the heating surface they may act as nuclei for the formation of deposit. The surface becomes dry and as a consequence, there is an increase in the temperature difference between the hot stainless steel surface and the bulk of the liquid (ΔT), resulting in evaporation of water at the vapour/liquid interface. Due to evaporation milk is transported from the bulk to the surface where the air/vapour bubble is attached. Here milk protein accumulates and because of the local increase in concentration and the high temperature the protein may coagulate and deposit on the surface. Eventually the air/vapour bubble may burst and part if the membrane is carried away with the liquid (Fig. 2).

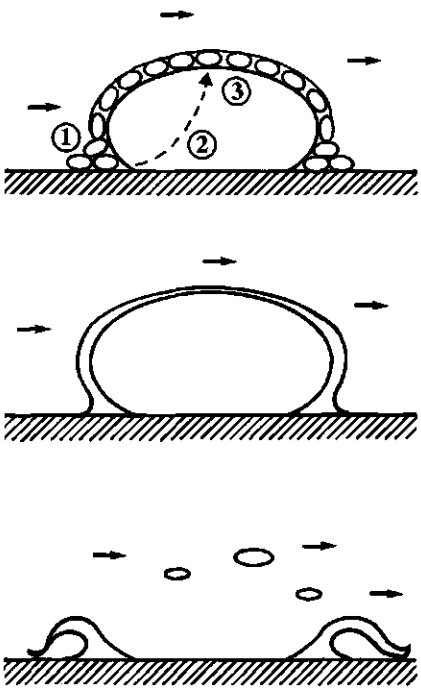

Fig. 2. Schematic representation of the participation of an air bubble in the fouling process of milk on a hot stainless steel surface, see text [35].
1. adsorption/deposition at the vapour/liquid interface; 2. evaporation; 3. condensation.
→ = flow direction of the milk.

The contribution of air/vapour bubbles to the deposit is determined by the amount of air present in the milk, the ΔT, the operational pressure in the heat exchanger and the wall shear stress [35].

In film evaporators for dairy products a similar phenomenon may occur: through an insufficient distribution, parts of the heating surface are not well wetted and become dry. This results in a local increase in the ΔT and the same process as described above will take place.

In these evaporators air bubbles may also arise as a result of nucleate boiling. This occurs at larger ΔT's when vapour bubbles are formed at the metal surface. It appears that the ΔT at which nucleate boiling starts is about 0.5 K for milk [36].

To reduce fouling in film evaporators the liquid should be well distributed over the surface and nucleate boiling should be avoided.

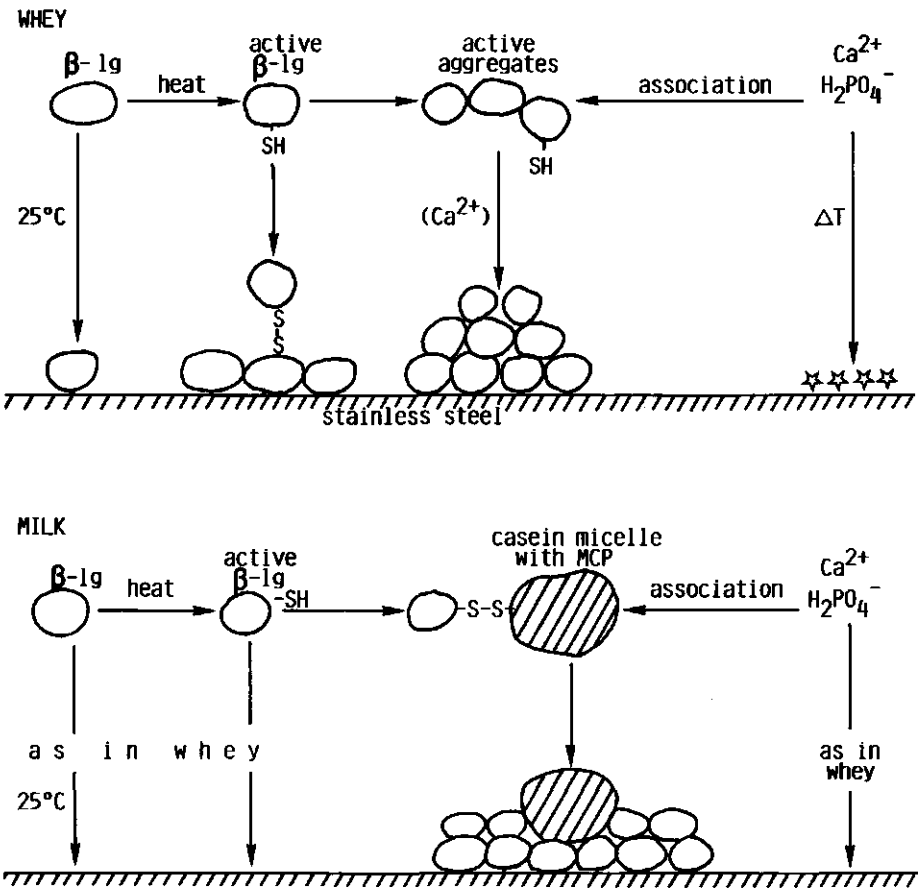

Fig. 3. Schematic representation of the fouling mechanisms during heating of whey and milk, see text.

3.7 Evaluation

The two major mechanisms of fouling during the heat treatment of milk are the formation and the subsequent deposition of activated serum protein molecules and the precipitation of calcium phosphate (directly or together with proteins) as a result of the heat denaturation of the proteins and the decreased solubility of calcium phosphate upon heating. These foulants are formed in the bulk of the solution and are continuously transported to the surface where they can be deposited. A third fouling mechanism, driven by a high wall temperature, i.e. a large ΔT, plays a minor role; it only may enhance the direct precipitation of calcium phosphate particles onto the stainless steel heating surface.

Calcium not only contributes to the deposit by precipitation as calcium salts, but also by its influence on the denaturation and aggregation of the serum proteins and on the stability of the casein micelles.

An overview of the fouling mechanisms during heating of whey and milk is schematically presented in Fig. 3. At room temperature a monolayer of protein is immediately adsorbed. The formation of the active β-lg molecules and aggregates is shown; their deposition is enhanced in the presence of calcium. Calcium phosphate may precipitate directly on the stainless steel wall promoted by a large ΔT or it may associate with β-lg aggregates, or with the micellar calcium phosphate (MCP) if casein micelles are present. In milk the active β-lg molecules may associate with the κ-casein at the surface of the casein micelle and may so entrap the micelles in the deposit.

If conditions in milk are different from normal, other processes than serum protein denaturation and mineral precipitation may take over as the rate-determining step in fouling; for example, the coagulation of casein micelles at low pH; or the formation of protein films when air bubbles, surrounded by proteins, are present at the heating surface and collapse.

3.8 Influence of various parameters on fouling

In Table 4 rough trends are given of the effect of various parameters on fouling, as compared to that of normal milk. Decreasing the pH to 6.4 caused a strong increase in fouling, mainly due to additional deposition of caseins, while an increase in pH to 6.8 resulted in much less deposit [30, 37]. Either increasing or decreasing the calcium concentration in milk led to more fouling, due to the effect of calcium on the stability of the casein micelles [15]. The presence of air bubbles at the surface enhances fouling (see section 3.6). If serum proteins were absent in milk, fouling was reduced by two-

Table 4. Trends of the effect of various parameters on fouling, compared to normal milk, see text [45]. ↑ = increasing the parameter; ↓ = decreasing the parameter; + = more fouling; − = less fouling.

	pH	[Ca]	air	[β-lg]	ΔT	Re	ageing
↑	-	+++	++	+++	+	--	++
↓	+++	++	--	--	-	+	

thirds. Increasing the serum protein concentration led to increased fouling [12]. Increasing the ΔT gave more fouling, especially due to additional calcium salt precipitation. Increasing the Reynolds-number (Re) led to less fouling [2]. Visser (NIZO, unpublished results) recently found on heating SMUF in a rotating disk device, in which the rotational speed was varied, that deposition was prevented if the wall shear stress exceeded 15 $N.m^{-2}$. Aged milk causes more fouling than fresh milk, due to action of proteolytic enzymes produced by psychrotrophic bacteria [29].

4 Fouling by milk products

Based on the trends given in Table 4 and on the results found in the literature the fouling behaviour of some milk products as compared to normal milk can be estimated.

Reconstituted skim milk and recombined milk may give less fouling than fresh milk. This is, among other things, explained by the fact that the serum proteins in the milk powder used have already (partly) been denatured [35]. Cold storage (20-24 h at 5 °C) of such milk and addition of polyphosphates, such as hexametaphosphate (HMP), can reduce fouling markedly. HMP is thought to reduce the available calcium in the system. Heating recombined milk, which contains emulsified milkfat, leads to copious fat fouling [33].

Concentrated milk is usually made of milk that has undergone a heat treatment before the concentration step. Therefore, fouling will not occur via the already denatured serum proteins. Concentrated milk will give fouling as a result of the coagulation of casein micelles. This is promoted through the low pH and the high concentrations of casein and calcium.

Sweet whey will give more fouling than milk. Due to the absence of casein micelles, whey protein aggregates are not hindered in transport to the surface and more calcium ions will associate with serum proteins, resulting in aggregates more prone to fouling.

Acid whey will give more fouling than sweet whey. The pH is closer to the iso-electric pH of the whey proteins, leading to less electrostatic repulsion, which results in a higher probability of sticking onto the deposited protein layer.

Whey protein concentrates will give more fouling simply because of the higher whey protein concentration. Up to 25 % total solids fouling increases; at higher total solids a changed fouling mechanism led to a reduction of the deposited mass [34].

Sour cream/cultured buttermilk will give strong fouling due to the poor stability of the casein micelles at the prevailing pH.

Whipping cream (unhomogenized), in spite of its high fat content, has a fouling behaviour which resembles that of whole milk [32].

Chocolated desserts give rise to deposits containing compounds such as chocolate and starch, which are difficult to remove [38].

5 Prevention of fouling

Upon heating milk there is a region in the heat exchanger where fouling is most severe. This critical region is determined by the concentration of activated serum protein

molecules [11]. Fouling in heat exchangers can be reduced by controlling the formation of these activated molecules. Possible ways of achieving this include:

- installing a holding section with a high volume to surface ratio: De Jong [3] showed that by using a stirred tank as a holder, the amount of deposit was reduced by more than 50 %;
- applying steam injection/infusion to shorten the period during which the concentration of activated molecules is high;
- introduction of a coating on those plates where fouling is most severe. Unfortunately, no successful coating material has yet been reported;
- applying such high shear stresses in the critical region that deposition of foulants will be prevented;
- applying a temperature profile in such a way that fouling is evenly spread over the various passes of a plate heat exchanger to prevent one pass being so rapidly fouled that it blocks the whole equipment [39]. A similar effect is achieved by applying a preheating of milk before pasteurization; in practice, the selection of preheating temperatures is a matter of compromise between reducing downstream fouling without causing overmuch deposition in the preheating section [40].

Other ways to reduce fouling include:

- degassing the milk, applying high operational pressures and high shear rates; these procedures may prevent air bubbles from sticking to the wall, resulting in less fouling;
- preventing psychrotrophic bacteria from producing extracellular proteolytic enzymes in milk by applying thermalization to raw milk, by avoiding dead spaces in the processing lines and by draining tanks and pipelines completely;
- desalting, adding Ca^{2+}-binders and pH adjustment; in certain milk products these may be tools to diminish fouling.

6 Cleaning

6.1 Cleaning mechanism

In the process of cleaning four parameters are of importance: chemical action, temperature, mechanical action and time. A combination of these four, to a certain extent exchangeable, must lead to effective removal of the deposit. The removal of a milk deposit during alkaline cleaning occurs as follows [9, 41, 42, 43]. Diffusion of the alkaline cleaning solution into the deposit layer is slow; reasonable values for the thickness of the fouling layer and for the effective diffusion coefficient are 10 mm and 10^{-8} $m^2.s^{-1}$, respectively, which means that it would take at least 1 h before the cleaning solution reaches the stainless steel wall by diffusion. The removal process, however, takes only a few minutes. Therefore, it appears that the cleaning solution penetrates into the spongy structure of the deposit layer, causing a swelling from which cracks propagate which, in turn, accelerates the further penetration of the cleaning solution. Scanning electron microscopy of whey protein deposits showed that the deposit structure changes dramatically on contact with an alkaline cleaning solution; the dense platelet

structure of the deposit changed in a few minutes to an open hollow matrix [44]. This swelling may be the result of an increased mutual repulsion between protein chains in the deposit layer due to the high pH of the alkaline cleaning solution, thereby breaking a number of deposit-deposit bonds. Moreover, through partial hydrolysis of the proteins the number or strength of bonds within the deposit layer is further reduced. Cracks allow the cleaning solution to penetrate into the deposit layer and to disrupt it by shear stresses and/or the pressure fluctuations (due to turbulence) exerted by the fast-flowing liquid. Finally, the swollen deposit is loosened and carried away in large lumps with the cleaning solution.

A thin transparent sublayer may remain on the stainless steel surface, consisting of calcium salts. This layer is easily removed by a subsequent acid cleaning which simply works via dissolving the deposit layer by layer. If a deposit mainly consists of minerals it is better to start with acid cleaning, followed by alkaline cleaning [9].

6.2 Chemical action

For cleaning milk deposits Bird [44] found an optimum concentration of 0.5 % NaOH in the cleaning solution. At this concentration, the swollen deposit acquires the highest porosity and is thus most susceptible to removal by liquid shear forces. Scanning electron microscopy studies showed that, above the optimum concentration of 0.5 %, NaOH modifies the surface of the deposit in such a way that it obstructs removal. Jeurnink and Brinkman [9] have described this process in terms of a chemical reaction in which the protein is aggregated or polymerized to form a rubber-like structure. Such a 'rubbery' layer presumably prevents or obstructs further penetration of the alkaline solution into the deposit and/or further crack formation, causing the cleaning rate to decrease markedly. This result shows that the intuitive notion, prevalent in industry, that a higher alkali concentration results in a higher cleaning rate is not correct.

6.3 Cleaning temperature

The cleaning temperature should be at least above the melting point of milk fat, so that removal of fat can be promoted by its emulsification. The cleaning rate will further increase with increasing temperature as a result of improved solubility, faster diffusion, faster hydrolysis and a thinner laminar boundary layer (due to a higher Reynolds-number). However, too high a temperature may damage the apparatus (corrosion, gaskets) and lead to reactions of proteins in the deposit (e.g. denaturation, formation of a rubbery layer) that may interfere with effective cleaning. Optimal working temperature therefore lies around 70 °C. This is, in combination with the 0.5 % NaOH-solution, sufficient to kill any pathogenic micro-organisms.

6.4 Mechanical action

A cleaning solution velocity of 1.5 m/s is often quoted as being necessary for cleaning, but this has no theoretical justification [1]. Mechanical energy in a cleaning process is

required for taking away the loosened deposit, splitting large deposit lumps into smaller ones and preventing re-adherence of already loosened deposits [41].

6.5 Cleaning time

Under ideal cleaning conditions, i.e. (i) optimum temperature and detergent concentration, (ii) disposal of the first alkaline flush for heavy fouling and (iii) using flow conditions in which all parts of the equipment are well reached by the cleaning solution, milk deposits in a heat exchanger can be readily removed by alkaline cleaning followed by acid cleaning. In practice, the cleaning solution is often circulated for an unnecessarily long time with the aim (or the hope) of removing deposits that are hardly or not reached by the cleaning solution. In such a case, it is recommended to adjust the flow conditions, to install a sprayball or to achieve a better (hygienic) design of the equipment, rather than to extend the circulating time.

7 Acknowledgment

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8 References

1. P. Fryer, M.T. Belmar-Beiny, & P.J.R. Schreier. Fouling and cleaning in milk processing. In: Heat induced changes in milk (P.F. Fox, ed.). Brussels: Int. Dairy Fed., 1995. pp. 364-395.
2. M.T. Belmar-Beiny, S.M. Gotham, W.R. Paterson, P.J. Fryer & A.M. Pritchard. The effect of Reynolds number and fluid temperature in whey protein fouling. *J. Food Engineering* 19(1993)119-139.
3. P. de Jong, S. Bouman & H.J.L.J. Van der Linden. Fouling of heat treatment equipment in relation to the denaturation of β-lactoglobulin. *J. Soc. Dairy Techn.* 45(1992)(1)3-8.
4. H.G. Kessler. Lebensmittel- und Bioverfahrentechnik - Molkereitechnologie. Freising Weihenstephan (Germany): Verlag A. Kessler, 1988, pp. 478-507.
5. W.R. Paterson & P.J. Fryer. A reaction engineering approach to the analysis of fouling. *Chem. Eng. Sci.* 43(1988)(7)1714-1717.
6. M. Lalande, J.P. Tissier & G. Corrieu. Fouling of heat transfer surfaces related to β-lactoglobulin denaturation during heat processing of milk. *Biotechnology Progress* 1(1985)(2)131-139.
7. P.J.R. Schreier, C. Green, A.M. Pritchard & P.J. Fryer. Heat exchanger fouling by whey protein solutions. In: Proc. Fouling and Cleaning in Food Processing, Cambridge (England), March 1994. In press.
8. H. Burton. Reviews of the progress of Dairy Science. Section G. Deposits from whole milk in heat treatment plant - a review and discussion. *J. Dairy Res.* 35(1969)317-330.
9. Th.J.M. Jeurnink & D.W. Brinkman. The cleaning of heat exchangers and evaporators after processing milk or whey. *Int. Dairy J.* 4(1994)347-368.
10. J.P. Tissier & M. Lalande. Experimental device and methods for studying milk deposit formation on heat exchange surfaces. *Biotechnology Progress* 2(1986)(4)218-229.
11. Th.J.M. Jeurnink, M. Verheul, M.A. Cohen Stuart & C.G. de Kruif. Deposition of heated whey proteins on a chromium oxide surface. *Colloids and Surfaces B: Biointerfaces.* Accepted Dec 1995.
12. Th.J.M. Jeurnink. Fouling of heat exchangers in relation to the serum protein concentration in milk.

Milchwissenschaft 50(1995)(5)257-260.
13. S.P.F.M. Roefs & C.G. de Kruif. A Model for the denaturation and aggregation of β-lactoglobulin. *Eur. J. Bioch.* 226(1994)883-889.
14. P. Walstra & R. Jenness. Dairy Chemistry and Physics. New York: Wiley, 1984.
15. Th.J.M. Jeurnink & C.G. de Kruif. Calcium concentration in milk in relation to heat stability and fouling. *Neth. Milk Dairy J.* 49(1995)151-165.
16. D.G. Schmidt & P. Both. Studies on the precipitation of calcium phosphate. I. Experiments in the pH range 5.3 to 6.8 at 25 °C and 50 °C in the absence of additives. *Neth. Milk Dairy J.* 41(1987)105-120.
17. T. van Kemenade. Influence of casein on precipitation of calcium phosphates. Thesis University of Utrecht, the Netherlands (1988).
18. J. Visser. A new casein micelle model and its consequences for pH and temperature effects on the properties of milk. In: Protein Interactions (J. Visser, ed). New York: Weinheim, 1991, pp. 135-165.
19. C. Holt, D.G. Dalgleish & R. Jenness. Calculation of the ion equilibria in milk diffusate and comparison with experiment. *Analytical Biochemistry* 113(1981)154-163.
20. Y. Pouliot, M. Boulet & P. Paquin. Observations on the heat-induced salt balance changes in milk. I Effect of heating time between 4 and 90 °C. *J. Dairy Res.* 56(1989)185-192.
21. J.P. Tissier, M. Lalande & G. Corrieu. A study of milk deposit on heat exchange surface during UHT treatment. In: Engineering and Food, Vol. 1 (B.M. McKenna, ed.) New York: Elsevier Applied Science Publisher, 1983, pp. 49-58.
22. Th.J.M. Jeurnink, D.W. Brinkman & A.D. Stemerdink. Distribution and composition of deposit in heat exchangers. In: Fouling and cleaning in food processing (H.G. Kessler & D.B. Lund, eds.). Augsburg: Druckerei Walch, 1989, pp. 25-36.
23. M. Lalande, J.P. Tissier & G. Corrieu. Fouling of a plate heat exchanger used in ultra-high-temperature sterilization of milk. *J. Dairy Res.* 51(1984)557-568.
24. C.P. Pappas & J. Rothwell. The effect of heating, alone or in the presence of calcium or lactose, on calcium binding to milk proteins. *Food Chem.* 42(1991)183-201.
25. B.M.A. Delsing & J. Hiddink. Fouling of heat transfer surfaces by dairy liquids. *Neth. Milk Dairy J.* 37(1983)139-148.
26. Th.J.M. Jeurnink & C.G. de Kruif. Changes in milk on heating: viscosity measurements. *J. Dairy Res.* 60(1993)139-150.
27. A.C.M. van Hooydonk, P.G. de Koster & I.J. Boerrigter. The renneting properties of heated milk. *Neth. Milk Dairy J.* 41(1987)3-18.
28. H. Singh & P.F. Fox. Heat stability of milk: further studies on the pH-dependent dissociation of micellar ϰ-casein. *J. Dairy Res.* 53(1986)237-248.
29. Th.J.M. Jeurnink. Effect of proteolysis in milk on fouling in heat exchangers. *Neth. Milk Dairy J.* 45(1991)23-32.
30. P.J. Skudder, B.E. Brooker, A.D. Bonsey & N.R. Alvarez-Guerrero. Effect of pH on the formation of deposit from milk on heated surfaces during ultrahigh-temperature processing. *J. Dairy Res.* 53(1986)75-87.
31. M.A.J.S. van Boekel, J.A. Nieuwenhuijse & P. Walstra. The heat coagulation of milk. 1. Mechanisms. *Neth. Milk Dairy J.* 43(1989)97-128.
32. J. Hiddink, M. Lalande, A.J.R. Maas & A. Streuper. Heat treatment of whipping cream. I. Fouling of the pasteurization equipment. *Milchwissenschaft* 41(1986)(9)542-546.
33. D. Newstead. UHT plant fouling by recombined milk. New Zealand Dairy Research Institute, Report 1994, pp. 19-21.
34. J. Schraml. Zum Verhalten konzentrierter Produkte bei der Belagbildung an heißen Oberflachen. Thesis Munich University (Germany), 1993.
35. Th.J.M. Jeurnink. Fouling of heat exchangers by fresh and reconstituted milk and the influence of air bubbles. *Milchwissenschaft* 50(1995)(4)189-193.
36. S. Bouman, R. Waalewijn, P. de Jong & H.J.L.J. van der Linden. Design of falling-film evaporators

in the dairy industry. *J. Soc. Dairy Techn.* 46(1993)100-106.
37. G.R. Patil & H. Reuter. Deposit formation in UHT plants. III. Effect of pH of milk in directly and indirectly heated plants. *Milchwissenschaft* 43(1988)360-362.
38. F. René, J.C. Leuliet, M. Goldberg & M. Lalande. Encrassement d'un échangeur de chaleur à plaques lors du traitement UHT d'un produit chocolaté. Comparaison avec le lait. *Le Lait* 68(1988)85-102.
39. F. Delplace, J.C. Leuliet & J.P. Tissier. Fouling experiments of a plate heat exchanger by whey proteins solutions. *Trans. I. Chem. Eng.* 72(1994)(Part C)163-169.
40. H. Burton. Fouling of heat exchangers. In: Ultra-high-temperature processing of milk and milk products (H.Burton, ed.) London (England): Elsevier Applied Science, 1988, p. 292.
41. A. Grasshoff. Zum Einfluss der chemischen Komponenten alkalischer Reiniger auf die Kinetik der Ablösung festverkrusteter Beläge aus Milchbestandteilen von Erhitzerplatten. *Kieler Milchwirtschaftliche Forschungsberichte* 40(1988)139-177.
42. M.R. Bird & P.J. Fryer. An experimental study of the cleaning of surfaces fouled by whey proteins. *Trans. I. Chem. Eng.* 69(1991)(Part C)13-21.
43. M.N. Perlat, M. Lalande & G. Corrieu. Cleaning of milk UHT sterilizer: Kinetics aspects with regard to acid and alkaline detergents. *Le Lait* 66(1986)31-63.
44. M.R. Bird. The existence of cleaning agent concentration optima in the removal of food based deposition. In: Proc. Fouling and Cleaning in Food Processing, Cambridge, England, March 1994. In press.
45. T.J.M. Jeurnink & J. Visser. Aanpassing proces en apparatuur vermindert vervuiling warmtewisselaars aanzienlijk. Voedingsmiddelentechnologie 29(1996)(5)11-13.

Summary

Heat treatment of milk and milk products is applied in the dairy industry to obtain microbiologically safe products. During the process in heat exchangers and evaporators an undesirable deposit, mainly consisting of proteins and minerals, is formed on the heating surface. This process of deposit formation is called fouling and is a daily nuisance in the dairy industry. Fouling diminishes the heat transfer and it restricts product flow, causing a rise in operating pressure. Fouling leads to product losses and it may even give rise to microbiological contamination of the product.

In order to remove the deposit a frequent shut-down of the production processes is required for cleaning, which of course requires time, work, materials and energy. In other words, fouling is a costly affair.

The aim of this work was to establish the mechanism of fouling by studying the processes taking place in milk during heating, in the bulk as well as at the heating surface. A better understanding of the fouling and cleaning mechanisms would enable longer processing times and lead to improved cleaning procedures.

Milk consists for the most part of water (approx. 87 %), lactose (4.6 %) fat (4.0 %), protein (3.5 %), and salts (0.7 %). It can be considered as a dispersion of fat globules and proteinaceous particles (casein micelles and serum proteins), in a continuous phase, which includes lactose and dissolved salts. At room temperature milk is a very stable colloidal dispersion. Even at boiling temperature the milk components remain dispersed, although heating causes many changes to occur in milk, for example denaturation of serum proteins, precipitation of calcium phosphate and, at high temperatures, some aggregation of casein micelles. These reactions may cause milk to become less stable and this colloidal instability will lead to fouling. Therefore the fouling process was studied by investigating the colloidal stability of milk in relation to the deposition processes at the heating surface.

In the first two papers the effect of heating on the behaviour and stability of casein micelles in milk as studied by turbidity and viscosity measurements is described. The turbidity and viscosity of skim milk were found to increase with temperature and time of heating. No increase in either parameter was found, however, if the heating temperature did not exceed 60 °C, nor when skim milk was heated to 90 °C in the absence of serum proteins. It thus appeared that denaturation of serum proteins, in particular β-lactoglobulin (β-lg), is responsible for the increase in turbidity and viscosity. Denatured β-lg molecules associate with the casein micelles, leading to an increase in the size of the micelles and in their mutual attraction. The changes in viscosity of skim milk on heating can be described with a quantitative model, which was developed for the behaviour of so-called adhesive hard spheres.

In the third, fourth and fifth papers, experimental results are presented to demonstrate that the heat stability of milk, i.e. the resistance to coagulation during heating, is related to its fouling behaviour:

- Aged milk was found to cause more fouling than fresh milk: due to the action of proteolytic enzymes, produced by psychrotrophic bacteria during cold storage, part of the caseins are broken down. Consequently the heat stability of the milk is decreased so that additional protein deposition takes place.
- Either increasing or decreasing the calcium concentration in normal skimmilk led to a lower heat stability and to more fouling. Changing the calcium concentration produced a shift from serum protein to casein in the composition of the deposit. Apparently calcium ions alter the structure of the casein micelles in milk in such a way (albeit for low- and high-calcium milk in different ways) that their mutual interaction increases and fouling is enhanced.
- Air bubbles, which form in the milk on heating and stick to the stainless steel wall, appear to act as nuclei for the formation of an additional deposit. They influence the composition of the deposit through drying of the membrane that forms around a bubble, which comprises predominantly casein.

In paper six the effect of serum protein concentration on fouling is analysed. Fouling increases with increasing serum protein concentration. If serum proteins are (nearly) absent in the milk, fouling reduces by two-thirds. Although the calcium content remains unaltered, calcium deposition also reduces by two-thirds, indicating that the deposition of calcium is coupled to that of proteins. Not only β-lg but also α-lactalbumin, bovine serum albumin and caseins contribute to the deposit.

The seventh paper deals with the processes taking place at the heating surface. Emphasis is put on the influence of the degree of serum protein denaturation on the rate of deposition. Therefore, serum protein solutions were given various heat treatments before their deposition onto a chromium oxide surface was measured by reflectometry. The deposition was studied under controlled hydrodynamic conditions by applying stagnation-point flow. It was found that for deposition beyond that of a monolayer, denaturation of the serum proteins in the vicinity of the surface is a prerequisite. The rate of deposition can be described by a model developed for the heat-induced denaturation of β-lg, involving the presence of an intermediate activated state, ultimately leading to inactive aggregates. The rate of deposition was found to be quantitatively related to the concentration of activated β-lg molecules.

In the eighth paper the cleaning rate for milk and whey deposits was studied in semi-industrial heat exchangers and evaporators. After processing milk, whey or concentrated whey the equipment can be cleaned completely in a short time. The cleaning mechanism involves penetration of the alkaline cleaning solution (mainly through cracks) into the deposit layer, thereby altering its structural properties, breaking up of the layer, and removal of deposit by carrying it away in large lumps, followed by dissolution of the remaining deposit, mainly mineral, in the subsequent acid cleaning solution. However, if in evaporators the flow rates during cleaning are too small or the alkali concentrations are too high, or both, then cleaning will be slow and incomplete. In that case the remaining deposit layer becomes covered with a brown rubber-like layer of polymerized protein.

Since a whey deposit contains considerably more mineral compounds than a milk deposit it is better to alter the sequence of the cleaning stages after processing whey, i.e. to start with acid cleaning, followed by alkaline cleaning.

The final paper reviews the various reactions that take place during the process of fouling. The two major fouling mechanisms during the heat treatment of milk are: i) the formation and the subsequent deposition of activated serum protein molecules as a result of the heat denaturation, ii) the precipitation of calcium phosphate (directly on the heating surface or together with proteins) as a result of the decreased solubility of this salt upon heating. Both foulants are formed in the bulk of the solution and are continuously transported to the surface, where they can be deposited. A third mechanism, driven by a high wall temperature, i.e. a large ΔT, plays a minor role in fouling, though it appears to enhance the direct precipitation of calcium phosphate onto the stainless steel heating surface.

If the stability of milk is lowered, e.g. by renneting or lowering the pH, the coagulation of casein micelles will cause extreme fouling.

From the various mechanisms of fouling and observations as given above, the following conclusions can be drawn:

- Fouling in heat exchangers can be reduced by controlling the formation of activated serum protein molecules and by preventing the precipitation of calcium phosphate. This can be achieved for example by introducing a holding section with a high volume-to-surface ratio. This holding section should be installed at the site where the heat denaturation model predicts the highest concentration of activated serum protein molecules.
- For some milk products, desalting, addition of calcium binders or pH adjustment may be ways to reduce fouling.
- Degassing of milk and application of high flow rates will prevent air bubbles from sticking to the wall, resulting in less fouling.

Studying the cleaning process revealed that if the following cleaning conditions are applied - (i) optimum temperature and detergent concentration, (ii) disposal of the first alkaline and acid flush in the case of heavy fouling and (iii) flow conditions in which all parts of the equipment are well reached by the cleaning solution - milk deposits in a heat exchanger can be readily removed by alkaline cleaning followed by acid cleaning.

In recent years the expertise obtained in this project has been applied for optimizing cleaning procedures in practice. In many cases a reduction in energy costs of 50 % and a decrease in use of cleaning agents of 25 % was achieved. In this way the task set by the Netherlands Agency for Energy and the Environment (NOVEM), who financially supported much of the research, has been realized.

Samenvatting

Hittebehandelingen van melk(producten) worden in de zuivelindustrie toegepast om houdbare producten te verkrijgen. Tijdens deze behandelingen vormt zich op de verhittingswand van pasteurs en indampers een ongewenste afzetting die voornamelijk uit eiwit en calciumfosfaat bestaat. Deze vervuiling van de apparatuur is een dagelijks terugkerend probleem. Het leidt tot een afname van de warmteoverdracht, tot een toename in de drukval over de procesapparatuur en het kan een bron voor microbiële problemen zijn. Vervuiling gaat gepaard met productverlies en aanzienlijke reinigingskosten, terwijl de tijd benodigd voor reiniging niet als productietijd kan worden gebruikt.

Het doel van dit onderzoek is het ophelderen van het vervuilingsmechanisme, door de reacties te bestuderen die tijdens het verhitten van melk optreden. Deze reacties treden zowel op in de bulk van de oplossing als aan de verhittingswand. Het verworven inzicht in het mechanisme van vervuiling dient te leiden tot langere productietijden en verbeterde reinigingsprocedures.

Melk bestaat hoofdzakelijk uit water (87 %) en bevat lactose (4.6 %), vet (4.0 %), eiwit (3.5 %) en zouten (0.7 %). Melk kan worden beschouwd als een dispersie van vetbolletjes en eiwitdeeltjes (caseïnemicellen en serumeiwitten) in een continue fase waarvan lactose en opgeloste zouten deel uitmaken. Bij kamertemperatuur is melk een zeer stabiele colloïdale dispersie. Zelfs bij kooktemperatuur blijven de melkcomponenten in dispersie, ondanks het optreden van allerlei reacties, zoals het denatureren van de serumeiwitten, het onoplosbaar worden van calciumfosfaat en bij hoge temperaturen enige aggregatie van de caseïnemicellen. Deze reacties maken melk minder stabiel en mede daardoor ontstaat vervuiling. Vandaar ook dat het vervuilingsproces is bestudeerd door het onderzoeken van de colloïdale stabiliteit van melk in relatie tot de afzettingsreacties die plaats vinden aan het verhittende oppervlak.

In de eerste hoofdstukken is het effect van verhitten op het gedrag en de stabiliteit van de caseïnemicellen in melk bestudeerd met behulp van turbiditeits- en viscositeitsmetingen. De turbiditeit en de viscositeit van ondermelk bleken toe te nemen bij stijgende temperatuur en met de duur van de verhitting. Er werd geen toename in deze parameters gevonden als de temperatuur beneden de 60 °C bleef, noch wanneer de ondermelk was verhit tot 90 °C in afwezigheid van serumeiwitten. Blijkbaar is de denaturatie van de serumeiwitten, met name de hoofdcomponent β-lactoglobuline (β-lg), verantwoordelijk voor de toename in turbiditeit en viscositeit. Gedenatureerde β-lg-moleculen associëren met de caseïnemicellen waardoor de micellen groter worden en hun onderlinge attractie toeneemt. De veranderingen in de viscositeit van ondermelk tijdens verhitten konden beschreven worden met een kwantitatief model dat ontwikkeld is voor het gedrag van plakkende harde bollen.

In het derde, vierde en vijfde hoofdstuk worden experimenten beschreven waarin wordt aangetoond dat de hittestabiliteit van melk, d. w. z. het bestand zijn tegen uitvlokken als gevolg van verhitten, en het vervuilingsgedrag van die melk zijn gecorreleerd:

- Lang koel bewaarde melk veroorzaakt bij verhitten meer vervuiling dan verse melk. De verklaring hiervoor is dat als gevolg van de werking van proteolytische enzymen, afgescheiden door psychrotrofe bacteriën tijdens het koud bewaren, een deel van de caseïnes wordt afgebroken. Daardoor neemt de hittestabiliteit van de melk af en vindt er een extra afzetting van eiwitten plaats.
- Zowel verhoging als verlaging van het calciumgehalte in gewone ondermelk leidt tot een verlaagde hittestabiliteit en tot meer vervuiling. In beide gevallen treedt er een verschuiving in de vuilsamenstelling op van serumeiwitten naar caseïnes. Klaarblijkelijk veroorzaken de calcium-ionen een zodanige verandering in de structuur van de caseïnemicellen en in hun onderlinge interacties dat daardoor de vervuiling toeneemt.
- Luchtbellen, die ontstaan bij het verhitten van melk en die hechten aan de wand, werken als kernen voor extra vervuiling. De bellen krimpen en verdwijnen door diffusie van de daarin opgesloten lucht en waterdamp. Het membraan van de luchtbel blijft achter en leidt tot een verhoogde afzetting van eiwit, voornamelijk caseïne.

In het zesde hoofdstuk wordt de invloed van de concentratie aan serumeiwitten in melk op de vervuiling beschreven. Vervuiling neemt toe wanneer de concentratie aan serumeiwitten wordt verhoogd. Als serumeiwitten nagenoeg afwezig zijn, neemt de mate van vervuiling met 2/3 af. Ofschoon de calciumconcentratie ongewijzigd blijft, neemt ook de calciumvervuiling met 2/3 af; dit duidt erop dat de afzetting van calcium en van serumeiwitten sterk aan elkaar gekoppeld zijn. Naast β-lg bleken ook α-lactalbumine, bloedserumalbumine en caseïnes aan de vervuiling bij te dragen.

Het zevende hoofdstuk handelt over de reacties die optreden aan het verhittende oppervlak, waarbij met name de invloed van de denaturatiegraad van de serumeiwitten op hun afzettingsssnelheid is bestudeerd. Daarvoor kregen serumeiwitoplossingen verschillende hittebehandelingen alvorens hun afzetting op een chroomoxidelaag met behulp van reflectometrie te bepalen. De afzetting werd bestudeerd onder gecontroleerde hydrodynamische omstandigheden waarbij gebruik werd gemaakt van "stagnation point flow". Het bleek dat de denaturatie van de serumeiwitten een voorwaarde is voor de afzetting van meer dan een monolaag aan eiwit. De afzettingssnelheid kon worden beschreven met behulp van een model voor hitte-geïnduceerde denaturatie van β-lg. In dit model is sprake van het ontstaan van een actieve tussenvorm van β-lg die verder reageert tot inactieve aggregaten. De afzettingssnelheid van β-lg bij verhitten blijkt kwantitatief gekoppeld te zijn aan de concentratie van actieve β-lg-moleculen.

In het achtste hoofdstuk wordt de reinigingssnelheid van melk- en weivervuiling in semi-industriele pasteurs en indampers beschreven. Na verwerken van melk of wei kan de apparatuur in korte tijd volledig worden gereinigd. Het vervuilingsmechanisme behelst het binnendringen van de alkalische reinigingsoplossing (voornamelijk via scheuren) in de vuillaag waarbij de structuur verandert. Vervolgens breekt de laag op en wordt in grote stukken weggevoerd waarbij de restanten, voornamelijk mineralen, oplossen in de daarop volgende zure reinigingsoplossing. Echter als een indamper gereinigd wordt met een te laag debiet en/of een te hoge loogconcentratie, zal de reiniging langzaam en onvolledig verlopen. Er wordt dan aan de buitenkant van het vuil een rubberachtige laag gevormd als gevolg van polymerisatiereacties van het eiwit.

Omdat weivervuiling aanzienlijk meer mineralen bevat dan melkvervuiling, wordt aangeraden om in dat geval de volgorde van de verschillende reinigingsfasen om te keren, d.w.z. te beginnen met een zuurreiniging gevolgd door een loogreiniging.

Het laatste hoofdstuk geeft een overzicht van de verschillende reacties die plaatsvinden tijdens het vervuilingsproces. De twee belangrijkste vervuilingsmechanismen tijdens het verhitten van melk zijn: (i) de vorming van actieve serumeiwitmoleculen als gevolg van de hittedenaturatie, (ii) het neerslaan van calciumfosfaat (direct op de verhittende wand of op eiwitdeeltjes) als gevolg van een afnemende oplosbaarheid bij verhoogde temperatuur. In beide gevallen worden de vervuilende deeltjes in de bulk van de oplossing gevormd. Vervuiling vindt plaats wanneer deze deeltjes naar het oppervlak worden getransporteerd en zich afzetten. Een derde vervuilingsmechanisme, waarbij de hogere wandtemperatuur de drijvende kracht is, speelt een vrij ondergeschikte rol; het zal mogelijk alleen het rechtstreeks neerslaan van calciumfosfaat op de wand bevorderen.

Als de stabiliteit van de melk wordt verminderd, bijvoorbeeld door proteolyse met stremsel of door pH-verlaging, leidt coagulatie van de instabiel geworden caseïnemicellen tot een sterke toename van de vervuiling.

Uit de boven beschreven vervuilingsmechanismen en waarnemingen kunnen de volgende conclusies worden getrokken:

- Vervuiling in warmtewisselaars kan worden verminderd door de vorming van actieve serumeiwitmoleculen te beheersen en de precipitatie van calciumfosfaat tegen te gaan. Dit kan o.a. worden bereikt door het inpassen in de warmtewisselaar van een houdsectie met een hoge volume/oppervlak-verhouding. Deze houdsectie moet worden geïnstalleerd op die plaats waar het denaturatiemodel de hoogste concentratie aan actieve serumeiwitmoleculen voorspelt.
- Voor sommige melkproducten kunnen ontzouting, toevoeging van calciumbinders en/of wijziging van de pH middelen zijn om de vervuiling te verminderen.
- Ontluchten of het toepassen van een hoge vloeistofsnelheid zal voorkomen dat luchtbellen zich hechten aan de wand, waardoor de vervuiling aanzienlijk afneemt.

Op voorwaarde dat de volgende procescondities worden toegepast, (i) optimale temperatuur en reinigingsmiddelconcentratie, (ii) direct lozen van het eerste deel van de reinigingsvloeistoffen bij sterke vervuiling en (iii) zodanige stromingscondities dat alle delen van de apparatuur goed bereikt worden door de reinigingsvloeistof, zal melkvervuiling snel en volledig kunnen worden verwijderd met een loogreiniging gevolgd door een zuurreiniging.

In de afgelopen jaren is opgebouwde expertise aangewend in de praktijk om reinigingsprocessen te optimaliseren. Daarbij werd in veel gevallen een afname in energiekosten met 50 % en in verbruik van reinigingsmiddelen met 25 % bereikt. Hiermee wordt de opdracht gegeven door de Nederlandse Ontwikkelingsmaatschappij voor Energie en Milieu (NOVEM), die een groot deel van het onderzoek financieel ondersteunde, gerealiseerd.

Curriculum vitae

Theo Jeurnink werd op 26 februari 1962 te Colmschate (gemeente Diepenveen) geboren. In 1980 behaalde hij het diploma Atheneum B aan het Geert Groote College te Deventer. Aansluitend begon hij de studie levensmiddelentechnologie aan de toenmalige Landbouwhogeschool te Wageningen. In maart 1987 slaagde hij voor het doctoraal-examen, met als hoofdvakken melkkunde en levensmiddelenchemie en als bijvak levensmiddelennatuurkunde. Op 13 april 1987 trad hij als wetenschappelijk medewerker in dienst bij het Nederlands Instituut voor Zuivelonderzoek (NIZO) te Ede. Vanaf 1990 werd een groot deel van zijn tijd besteed aan onderzoek waaruit dit proefschrift is voortgevloeid.

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