Lait 80 (2000) 33–42

Original article

Rennet coagulation of heated milk concentrates

Regina SCHREIBER*, Jörg HINRICHS

Dairy and Food Research Centre Weihenstephan, Institute for Food Process Engineering, Technische Universität München, 85350 Freising, Germany

Abstract —The high temperature heating of cheese milk is a useful tool for preventing late fermentation during cheese ripening if the renneting properties remain unchanged. The aim of the investigations was to find heating conditions which guarantee the inactivation of clostridia spores but still enable rennet coagulation of the heated milk and milk concentrates respectively. The gel strength of the rennet gels increased the more the casein content increased. The native whey proteins did not influence the gel strength. In contrast the denatured whey proteins hinder rennet coagulation and the gel became weaker. The upper limit of denatured whey proteins dependent on the casein concentration in the retentate was able to be determined in order to achieve the gel strength of pasteurized skim milk. Almost "whey protein-free" casein solutions (3.8% casein) were produced by diafiltration. The heating conditions of 100 °C/280 s and 110 °C/24 s were able to destroy spores from *Clostridium tyrobutyricum* by 4 log units while the rennet coagulation was slightly altered.

microfiltration / ultrafiltration / rennet coagulation / high temperature heating / whey protein denaturation

1. INTRODUCTION

A common problem in the manufacturing of semi-hard and hard cheeses is late fermentation caused by spores from *Clostridium tyrobutyricum*. The defective cheeses are puffed-up and show cracks; sensory defects also occur. Therefore in cheese technology microbizids such as nitrate and lysozyme were used in order to prevent late fermentation. A possible alternative to inactivate the spores is through the high temperature heat treatment or ultra high temperature heat treatment of the cheese milk. In this case the temperature-time combinations have to be chosen in such a manner so as to ensure that less than 10 spores of *Clostridium tyrobutyricum* per liter milk survive [21].

On the other hand, it is known that heating cheese milk to temperatures above 70 °C

* Correspondence and reprints. schreiber@charly.lmvt.blm.tu-muenchen.de

leads to whey protein denaturation and therefore impedes rennet coagulation. The main reason for this is the blocking of caseins by disulphide linkages between β-lactoglobulin and κ-casein. If more than 60% of the whey proteins present in milk are denatured and no further measures such as reducing the pH or increasing the concentration of ionic calcium were applied, rennet coagulation is not possible and the milk will stay liquid [19]. Therefore it is impossible to form a rennet gel from UHT-milk. But if an ultrafiltration concentrate with a concentration degree of 2 is UHT-heated, rennet coagulation is still possible [11]. These authors do not state whether the gelling time for forming the rennet gel is lengthened or not. The reason for rennet coagulation in this case, although a part of the whey proteins was denatured by UHT-heating, is the higher casein concentration and consequently the higher collision frequency of the casein micelles [15]. Furthermore, at rising protein concentrations, a lower degree of proteolysis of κ-casein is necessary for the aggregation of the casein micelles [17]. Other authors have also confirmed that rennet coagulation and gel formation of heated milk is possible if the casein fraction was concentrated after the heat treatment. The same was found for retentates which were heated after the concentration process [1, 4, 6, 12–15].

A slight increase of the coagulation time of heated skim milk was ascertained by Dalgleish [5] for low denaturation degrees whereas higher denaturation degrees of more than 50% led to a linear increase of the coagulation time. Other authors [8] determined a linear increase of the coagulation time in the range of 20 to 99% whey protein denaturation. In contrast, some researchers [20] were unable to detect any change in the coagulation time up to a denaturation degree of 60% compared to the unheated sample.

However, there are indications that not only the whey proteins but also the caseins are altered by severe heating conditions [7, 9, 18]. Kinetic investigations for the thermal destabilization of casein solutions are not available at the moment.

In conclusion the problem of the thermal treatment of milk for preventing late fermentation could perhaps be solved by the following:

1. Concentration of the milk by membrane processes leads to a higher casein concentration and therefore to a higher gel strength of the rennet gel. In order to achieve a constant processing time in cheese manufacture attempts should be made to attain the gel strength of pasteurized milk in the heated retentate after a constant coagulation time of 60 min. To reach this value it is possible to denature a part of the whey proteins in the concentrate.

2. The production of casein solutions with a slight amount of or without any whey proteins by microfiltration. In this case, the inhibition of rennet coagulation by the denatured whey proteins would be able to be almost excluded.

The aim of our investigations was to compare between the rennet coagulation properties of concentrates produced by ultrafiltration (UF) and microfiltration (MF) in order to determine the function of the native whey proteins in rennet coagulation. Afterwards the whey proteins were denatured by heating the concentrate to determine the influence of denatured whey proteins.

Finally, diafiltration (MF) with UF-permeate was used to remove almost all whey proteins. The rennet coagulation properties of what is called “whey protein-free” casein solution are to be described dependent on the heating conditions.

2. MATERIALS AND METHODS

2.1. Manufacture of the milk concentrates

The milk concentrates were produced from pasteurized (72 °C, 15 s) skim milk

purchased from a local dairy by ultrafiltration (UF) or microfiltration (MF).

The UF module (Pall, Dreieich, Germany) consists of a polyethylene sulphone membrane with a total area of 0.35 m^2 and a molecular weight cut-off of 10.000 $g \cdot mol^{-1}$. The MF module (APV, Silkeborg, Denmark) has 7 multichannel elements (SCT, Bazet, France) each consisting of 19 channels. A total area of 1.68 m^2 results from this. The membrane material is α-aluminium oxide and an active layer of zirconium oxide. The pore-size of the membrane was 0.1 µm. The microfiltration plant works according to the UTP (Uniform Transmembrane Pressure) principle, which allows a higher permeation flux compared to traditional cross-flow-plants.

The parameters of the UF and MF plant are shown in Table I. A measure of the concentration of the protein (casein and whey protein) or the casein fraction is the volumic reduction ratio i: i = volume of the milk/volume of the retentate.

Skim milk was concentrated by MF to i = 4 to reduce the whey protein content. Afterwards the resulting retentate was diluted to attain the desired casein content using UF-permeate containing no whey proteins. The lactose and the salt content remained almost unchanged compared to skim milk.

Diafiltration (MF) was used to produce a "whey protein-free" casein solution. Therefore skim milk was continuously washed with UF-permeate at a constant casein concentration [10]. The resulting "pure" casein solution (3.8% casein, 0.02% whey proteins) had almost the same lactose and salt content compared to skim milk.

Table I. Parameters of the UF and the MF plant.

Parameter	UF	MF
Temperature [°C]	50	50
Average shear stress [Pa]	15	110
Trans-membrane pressure [MPa]	0.3	0.04

2.2. Heating

The samples were heated in a pilot heating plant for small tubes [2]. The tubes have a volume of 40 mL each (length 210 mm, diameter 16 mm, material: stainless steal). Due to the small volume the heating up and cooling down time was not longer than 30 s. For heating, saturated steam at a pressure up to 0.6 MPa was used, and ice water was used for cooling down. The heating temperatures ranged between 100 and 120 °C with holding times between 0 and 300 s. Afterwards the samples were stored at 4 °C until the following day.

2.3. Rennet coagulation

The gel strength of the rennet gel was measured the day after heating. The following parameters were chosen for rennet coagulation: pH 6.50 (with lactic acid (9%)); T = 30 °C; 0.02% rennet. The rennet solution consisted of 99% chymosin (P99, Hansen, Lübeck, Germany).

As a measure for the gel strength of the rennet gel the maximum resistance force after 60 min coagulation (F-60-value) was determined using a Texture Analyzer (Stable Micro Systems, Haslemore, England). A soldered crossed wiring (30 mm diameter) penetrates the rennet gel (volume 100 mL) at a velocity of 0.5 $mm \cdot s^{-1}$ to a maximum depth of 15 mm. Each point was determined twice. A variation coefficient of 13% has to be taken into account for the measurement.

The measure used to determine rennet coagulation does not allow a differentiation between the enzymatic hydrolysis and the aggregation of the caseins. Therefore the evaluation of the data based on formal kinetics includes both reaction steps.

2.4. Analysis

The heating load was measured by the degree of whey protein denaturation. The caseins and the denatured whey proteins were precipitated at a pH of 4.6 using HCl. After filtration the concentration of the native whey protein fractions β-lactoglobulin A and B, α-lactalbumin and BSA were determined in the filtrate by RP-HPLC [3]. The degree of denaturation (DD) was calculated by applying the following equation:

$$DD\ [\%] = 1 - \left(\frac{C_{WP\ a.H.}}{C_{WP\ b.H.}} \right) \cdot 100$$

$C_{WP\ a.H.}$: Concentration of native whey proteins after heat treatment.
$C_{WP\ b.H.}$: Concentration of native whey proteins before heat treatment.

The protein content (crude protein: nitrogen [%]·6.38) was measured according to the method of Dumas. The casein content was calculated from the difference between the protein content and the whey protein content (β-lactoglobulin A and B, α-lactalbumin and BSA) of the unheated sample.

3. RESULTS AND DISCUSSION

3.1. Rennet coagulation of MF- and UF-retentates from pasteurized skim milk

The gel strength of MF- and UF-retentates from pasteurized (72 °C, 15 s) skim milk was examined after 60 min coagulation. As shown in Figure 1, the F-60-value was plotted against the casein concentration of the retentate because the concentration of the native whey proteins had no significant influence on rennet coagulation. This is in accordance with the results of the coagulation time determined by a gelograph. The coagulation time was almost independent of the casein content in a range between 3 and 10% casein (results not shown). Therefore it is not necessary to distinguish between MF- and UF-retentates with the same degree of concentration, i.e. the same casein concentration. The gel strength (F-60-value) increased depending on the casein content of the MF- and UF-retentates (Fig. 1). An increase in the casein content from 3 to 10% led, for example, to a ten times higher F-60-value. The F-60-value correlates directly with the velocity of

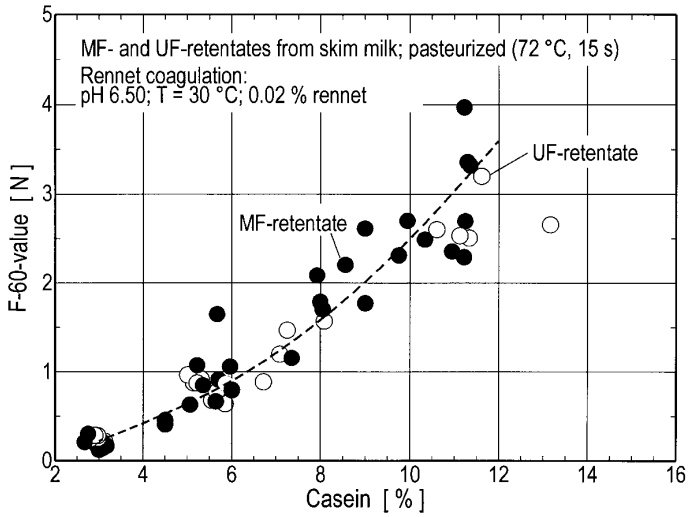

Figure 1. Gel strength of rennet gels dependent on casein concentration of the retentate.

rennet coagulation determined by the increase in gel strength over a time range of 30 to 90 min after the addition of rennet (results not shown). The increase of the F-60-value (Fig. 1) shows a power-law dependence of the casein concentration. An explanation for this is provided by the aggregation theory of Smoluchowski, which states that the aggregation rate, in this case the velocity of rennet coagulation determined as described above, is proportional to the square of concentration of the reactive particles.

3.2. Rennet coagulation in high temperature heated MF-retentates i = 1

Fifty percent of the whey proteins of skim milk were removed by MF up to i = 4 and subsequent dilution of the retentate with UF-permeate was carried out to attain a casein concentration of 3%. The resistance force of the rennet gel after a coagulation time of 60 min decreased the higher the degree of whey protein denaturation (Fig. 2). The decrease in gel strength was less pronounced if the degree of denaturation was lower than 30% (about 1 mg denatured whey protein per 1000 mg MF-retentate (or per 30 mg casein)). However, higher denaturation degrees led to a significant reduction of the F-60-value. Samples with a degree of denaturation of 90% (2.7 mg denatured whey protein per 1000 mg MF-retentate (or per 30 mg casein)) were nearly unable to form a rennet gel during the 60 min coagulation time.

3.3. Rennet coagulation in high temperature heated MF- and UF-retentates i = 2

As previously mentioned, the concentration of native whey proteins had no significant influence on rennet coagulation. Consequently, it is possible to compare the gel strength of MF- and UF-retentates with the same degree of concentration depending on the concentration of denatured whey proteins in a diagram.

A significant decrease of the F-60-value depending on the concentration of denatured whey proteins was able to be detected for retentates with a casein concentration of

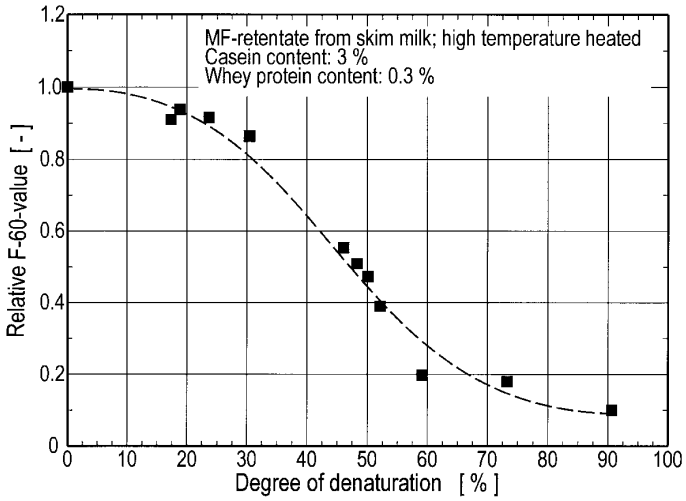

Figure 2. Relative gel strength of rennet gels dependent on their degree of whey protein denaturation (MF-retentate i = 1).

5.3% (Fig. 3). At a concentration of 3 mg/1000 mg retentate (3 mg/53 mg casein), the F-60-value was about half of the initial value. The F-60-value was in the range of 5% compared to the initial value if the whey proteins were totally denatured. MF- and UF-retentates showed the same decrease in gel strength due to the whey protein denaturation.

The limit for retentates with a casein concentration of 5.3% is 6 mg denatured whey protein per 1000 mg retentate to obtain the lowest F-60-value of pasteurized skim milk (0.2 N). Lower F-60-values, i.e. weaker rennet gels, led to higher amounts of curd fines in the whey after cutting the gel or require a longer coagulation time in cheese manufacture.

MF- and UF-retentates had the same disadvantages in rennet coagulation for the same amount of denatured whey proteins. Consequently, a distinction is not made between MF- and UF-retentates in the following passages and only the casein content of the retentates or the degree of concentration of the filtration process is taken into account.

3.4. Rennet coagulation in high temperature heated MF- and UF-retentates i = 1–4

To compare the gel strength of rennet gels with different degrees of concentration and different heating conditions a diagram was drawn depicting the F-60-value plotted against the quotient of denatured whey protein to casein.

The gel strength of MF- and UF-retentates decreased the higher the degree of whey protein denaturation (Fig. 4a) because the caseins were increasingly blocked by denatured whey proteins and formed a weaker and coarser rennet gel. At a rising degree of concentration the retentates with the same ratio of denatured whey protein/casein formed stronger gels. If the concentration of denatured whey proteins was 10% of the casein concentration, retentates (MF-retentates) with a concentration degree of 1 no longer formed a rennet gel. The samples were liquid after 60 min coagulation time. Retentates (MF, UF) with a degree of concentration of 2 and also an amount of 10% denatured whey protein/casein formed

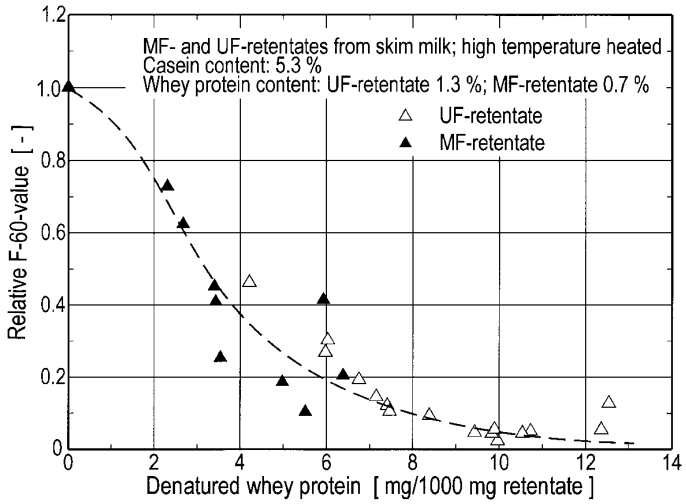

Figure 3. Relative gel strength dependent on the amount of denatured whey protein in the retentate (MF- and UF-retentates i = 2).

rennet gels with a gel strength compared to that of pasteurized skim milk ("optimum" gel strength of a rennet gel for cutting in cheese manufacture). Three-fold concentrated retentates with again a portion of 10% denatured whey protein/casein had twice the F-60-value compared to pasteurized skim milk. A complete denaturation of the whey proteins in an UF-retentate (i = 3) by high temperature heating led to F-60-values which were slightly higher than that of pasteurized skim milk.

In Figure 4b the relative F-60-values (that means the F-60-values referred to the not heated sample) were plotted against the ratio of denatured whey protein/casein. In this figure, the procentual decrease in gel

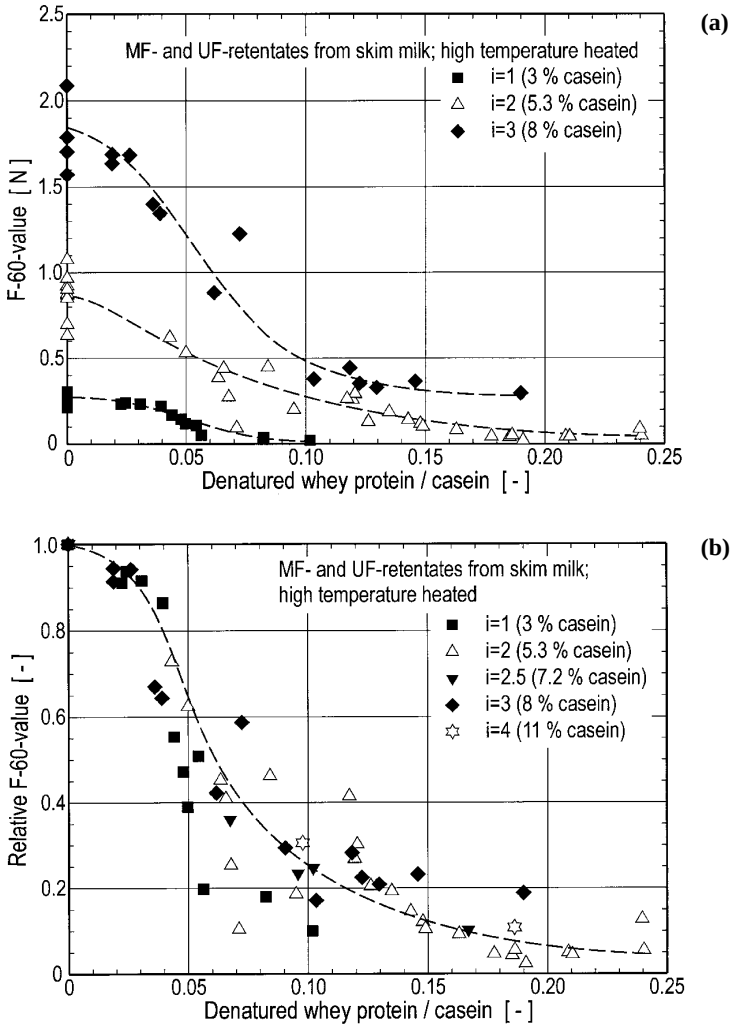

Figure 4. (a) Gel strength of rennet gels dependent on the ratio of denatured whey protein to casein (MF- and UF-retentates i = 1–3). **(b)** Decrease in gel strength for different retentates with varying degree of concentration (MF- and UF-retentates i = 1–4).

strength was independent of the degree of concentration of the retentate and depended only on the ratio of denatured whey protein/casein. If a 3% portion of whey protein/casein was denatured only a slight hindrance in rennet coagulation occurred compared to unheated samples.

The limits for the denaturation of whey proteins or β-lactoglobulin B to achieve the F-60-value of pasteurized skim milk (0.2 N) are shown in Figure 5. In practical experiments the value of pasteurized skim milk after 60 min of coagulation time was found to have the best possible gel strength for cutting in cheese manufacture. In retentates with a casein concentration of 3%, not more than 1 mg whey proteins/1000 mg retentate (3.3 mg/100 mg casein) should be denatured. For retentates with a casein concentration of 5.3% the limit was 6 mg whey proteins/1000 mg retentate (11.3 mg/100 mg casein) and for retentates with a casein content of 7.2% 11 mg whey proteins/1000 mg retentate (15.3 mg/100 mg casein) were allowed to be denatured in order to achieve the gel strength of pasteurized skim milk. A complete denaturation of the whey proteins was possible in UF-retentates with a casein concentration of more than 8%, and the F-60-value was always higher than that of pasteurized skim milk.

The previous results were obtained with retentates with a reduced content of whey proteins. To prevent any influence of whey proteins taking effect in the following investigations, they were almost totally removed by the diafiltration (MF) of skim milk with UF-permeate. The resulting "pure" casein solution with a casein content of 3.8% was subjected to defined heating conditions related to the inactivation of *Clostridium tyrobutyricum* spores by four powers of ten [16]. Figure 6 shows the gel strength of rennet gels dependent on the time after the addition of rennet. No difference could be detected between the unheated and the MF-retentate heated at 110 °C for 24 s. The gel strength of the MF-retentate heated at 100 °C for 280 s was lower.

The conclusion to be derived from this is that the heating temperature and time is important for the renneting properties even if whey proteins are not present. To obtain the same inactivation effect on spores of

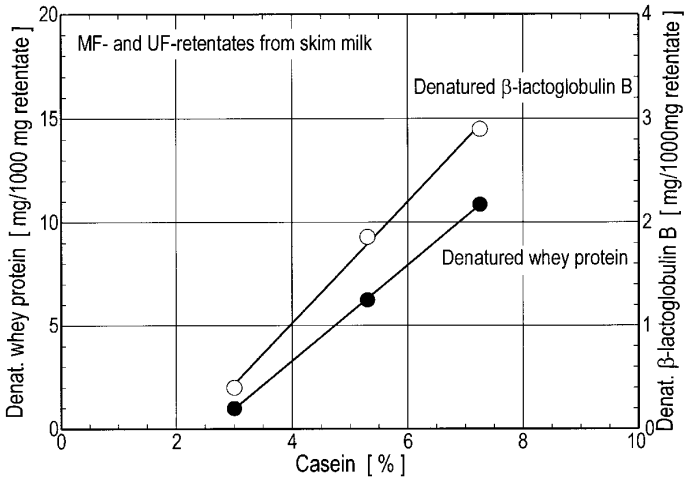

Figure 5. Maximum limits for the denaturation of whey proteins and β-lactoglobulin B dependent on the casein concentration in the retentate for achieving the gel strength of pasteurized skim milk (MF- and UF-retentates i = 1–2.5).

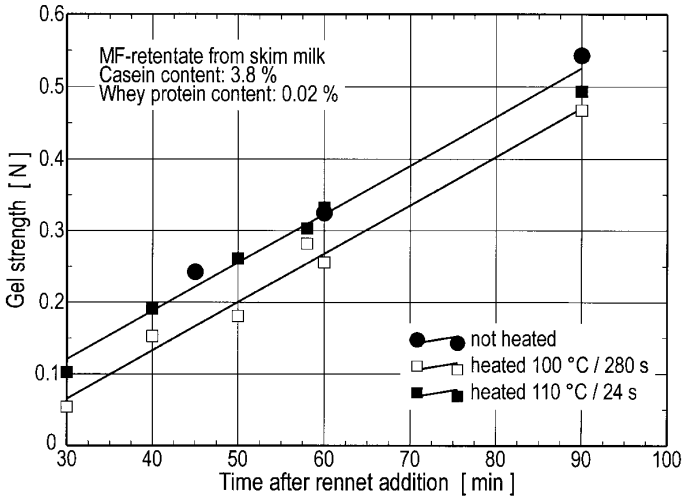

Figure 6. Gel strength of rennet gels dependent on the time after rennet addition for high temperature heated MF-retentates.

Clostridium tyrobutyricum higher temperatures and shorter holding times should be preferred to lower temperatures and longer holding times.

4. CONCLUSION

The investigations have shown that there are two possible ways to thermally inactivate *Clostridium tyrobutyricum* in order to prevent late fermentation and to maintain rennetability:

1. Casein concentration:

With rising degree of concentration, that means with rising casein concentration of the retentate, more whey proteins can be denatured and therefore severe heating conditions may be chosen to attain a certain rennet gel strength. Although the degree of concentration determines the gel strength, the percentage of decrease in gel strength due to the blocking of the caseins by denatured whey proteins does not depend on the casein concentration.

In addition research is necessary to adapt further steps in cheese manufacture if the casein concentration in the retentate rises.

2. Whey protein removal:

If the whey proteins are totally removed by diafiltration (MF) without the concentration of the casein fraction, heating conditions to sufficiently inactivate *Clostridium tyrobutyricum* may be chosen with only slightly or without any altered rennet coagulation properties.

From our results the thermal inactivation of spores from clostridia is possible, on the one hand, by concentrating the casein fraction and, on the other hand, by the removal of almost all whey proteins by diafiltration. Furthermore, a combination of the described methods may be a successful alternative. In each case the consequences of the heat treatment related to the inactivation of ripening enzymes originally present in milk have to be examined.

ACKNOWLEDGEMENT

This paper is dedicated to Prof. Dr. H.G. Kessler. The authors wish to thank P. Huber for his assistance and J. Pfretzschner for his practical work. Further thanks to the colleagues at the institute for discussion and helpful comments.

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