UDC 637.1:66.065.2
DOI 10.12737/1512

A PHENOMENOLOGICAL MODEL OF MILK COAGULATION

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(Received March 07, 2013; Accepted in revised form July 23, 2013)

Abstract: A model of additional stabilization for the milk colloid system by means of the micelle electric charge arising owing to dissociation of micellar calcium caseinate is offered. The model allows comprehending the unique role of calcium in milk clotting and describing some features of coagulation temperature dependence, as well as explaining the nature of rennet, acid, heat-acid and heat-calcium coagulation within uniform concepts.

Key words: milk coagulation, sticky hard spheres, calcium caseinate, colloid calcium phosphate, ionized calcium.

INTRODUCTION

Milk clotting is one of important technological processes in the manufacturing of many foodstuffs, in particular, cheeses. This process is based on the coagulation of casein micelles, which may be caused by various factors, such as enzymes, acids, spirits, salts, or high temperature [1].

It is recognized now that the colloid stability of casein micelles in milk is ensured, basically, by the presence of the κ-casein macropeptide hairy layer on the casein micelle surface, sterically restricting the possible clinging together of micelles [2–5]. In essence, this layer represents a quasielastic polyelectric brush formed by negatively charged macropeptide residues [6, 7].

The loss of colloid stability by the casein micellar system may be attributed to different ways of the destruction of the hairy layer. Therefore, under rennet conditions, κ-casein macropeptide hairs are split off by chymosin, which leads to the destruction of the protective layer. During acid coagulation, additional hydrogen ions easily get into the polyelectric brush and shift ion equilibrium to the recombination of dissociated κ-casein macropeptide acid groups, thus reducing the electric charge of macropeptide hairs and finally collapsing the protective layer [8].

Certain distinctions in mechanisms of the destruction of the casein micelle protective layer, as well as a number of factors affecting the micellar casein system's colloid stability, make it very difficult to describe various kinds of milk coagulation with a uniform approach. For example, it is known that the lack of calcium in milk has no significant effect on acid milk coagulation, while it is impossible to coagulate this milk by adding chymosin even after it has completely cut off the protective hairy layer [9, 10].

This research is an attempt to work out a universal model of milk coagulation, which would correctly describe, at least qualitatively, observable features of the milk coagulation phenomenon under various conditions of casein colloid system destabilization.

The background of our model is represented by both well-known experimentally confirmed facts and somehow substantiated but still hypothetical assumptions. In particular, the basic hypothesis rests on the analysis of the outstanding role of calcium ions in the stabilization of the micellar colloid systems in milk.

We hope that this paper will become a stimulus for the direct experimental check of our hypotheses by interested experimentalists.

MATERIALS AND METHODS

Skim milk was reconstituted by mixing up 90 g of low-fat milk powder (Milk Factory, Kemerovo, Russia) with 910 ml of distilled water and 4 cm^3 of 10% solution of calcium chloride. Then, after complete dissolution, the reconstituted milk was left for about 12 hours at 6 ± 2?C.

Milk coagulation was carried out in a thermostatted 200 ml cell.

The chymosin under the trademark of Maxiren® (DSM, Netherlands) was used for rennet coagulation. To prepare the enzyme solution, 0.1 g of dry Maxiren® powder was dissolved in 100 cm^3 of distilled water.

For simulating acid coagulation, a 10% lactic acid solution (Univerkhim, Chelyabinsk, Russia) was slowly brought to milk under careful mixing.

To increase the pH of some milk samples, a 0.5-mM sodium hydroxide solution (NaOH) (Univerkhim, Chelyabinsk, Russia) was used.

Soluble calcium was added to milk in the form of 10% $CaCl_2$ medical solution (Shenlu Pharm, China).

To decrease calcium ion concentration in milk, in a number of experiments Trilon B® (Na_2EDTA) (Khimservis, Ufa, Russia) was used as a chelating agent.

Calcium ion concentration and pH in milk were measured with ELIT (Niko-Analit, Moscow, Russia) ion selective electrodes.

The casein micelles ζ-potential was measured by means of Zetasizer Nano Z - ZEN2600 (Malvern Instruments, Malvern, UK).

Milk coagulation was monitored with a computer-driven "thermometric" gauge of our own design [11]. This device measures temperature difference between two thermocouple junctions immersed in milk at a distance of about 3 cm from each other. One of the junctions is attached directly to a small resistor dissipating permanently about 0.5 W of heat. An increase in milk viscosity during coagulation leads to an increase in temperature difference. It is more correct to say that the temperature increase near the warmed-up junction is due not only to the viscosity increase but also to the formation of a gel net structure, which also restricts convection in milk. In a sense, our method is similar to the hot wire method [12]. Hereinafter, the curves obtained by means of the thermometric gauge are called thermograms (by analogy with rheograms).

RESULTS AND DISCUSSION

Figure 1 demonstrates the dependence of rennet coagulation kinetics on ionized calcium concentration. Various doses of the 10% solution of calcium chloride were brought to milk at its reconstitution.

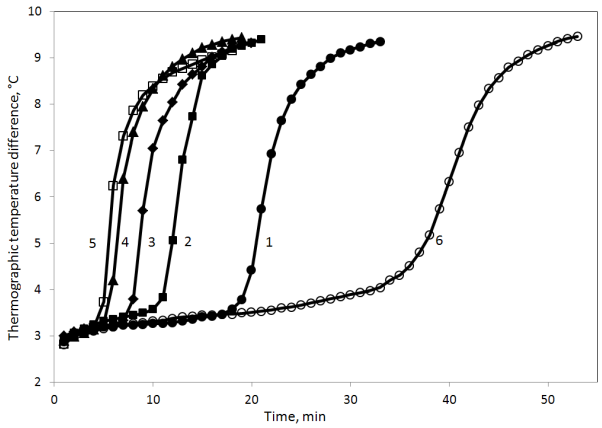

Fig. 1. Thermograms of reconstituted skim milk coagulation by chymosin (25 mg/L) at 30°C.
Curves: 1 – reference sample;
2 – 0.8 g/L of $CaCl_2$ added to reconstituted milk;
3 – 1.2 g/L of $CaCl_2$ added to reconstituted milk;
4 – 1.6 g/L of $CaCl_2$ added to reconstituted milk;
5 – 2.0 g/L of $CaCl_2$ added to reconstituted milk;
6 – no $CaCl_2$ added to milk at its reconstitution.

The reference sample, represented with the curve 1 in Fig. 1, was prepared as specified in the previous section (4 cm^3 of 10% $CaCl_2$ solution was added to 1 *L* of reconstituted milk). The concentration of calcium ions in the reference sample, measured directly before coagulation, was 3.2 ± 0.3 mM.

Enhanced doses of calcium chloride were added to the samples, represented by curves 2–5, as specified in Fig. 1.

The sample represented by curve 6 was reconstituted without adding calcium chloride. Immediately before coagulation, calcium ion concentration in this sample was 1.8 ± 0.3 mM.

All samples were coagulated at 30 ± 1?C by adding 5 ml of the enzyme solution to 200 ml of reconstituted milk.

Predictably, the increase in the concentration of soluble calcium in reconstituted milk considerably reduces the duration of rennet coagulation. However, the increase in the concentration of calcium ions above approximately 10 mM leads to the saturation effect. Thus, for samples 4 and 5, for which the concentration of calcium ions was 9.5 ± 0.4 mM and 11.7 ± 0.4 mM, respectively, the coagulation time was almost the same.

Figure 2 shows thermograms for the coagulation of reconstituted skim milk with a reduced calcium ion concentration by adding Trilon B (Na_2EDTA). The reference sample (curve 1) is the same as in Fig. 1.

The samples represented by curves 2 and 3 in Fig. 2 are prepared as specified in Fig. 2. The calcium ion concentration for these samples was 1.2 ± 0.2 mM. All samples were coagulated at 30 ± 1?C by adding 5 ml of the enzyme solution to 200 ml of reconstituted milk.

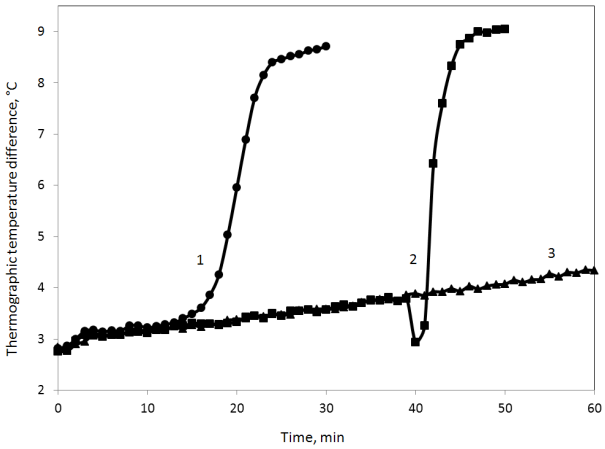

Fig. 2. Thermograms of reconstituted skim milk clotting by chymosin (25 mg/L) at 30°C.
Curves: 1 – reference sample (addition of 0.4 g/L of $CaCl_2$ to milk);
2 – 1.5 g/L of Na_2EDTA and no $CaCl_2$ added to milk at reconstitution, 0.8 g/L of $CaCl_2$ added after 40 minutes of renneting;
3 – 1.5 g/L of Na_2EDTA and no $CaCl_2$ added to milk at reconstitution.

The samples represented by curves 2 and 3 are identical, but 1.6 cm^3 of the 10% calcium chloride solution was brought to sample 2 40 minutes after the addition of the enzyme solution. It is clear from Fig. 2 that coagulation started immediately after the addition of calcium. Note that the sample to which calcium had not been added (curve 3) did not coagulate within 2 hours after the addition of the enzyme solution.

A similar rennet coagulation behavior can be observed if calcium ion concentration in milk decreases one way or another to values less than approximately 1.5 mM: milk can undergo no coagulation for hours after the addition of chymosin (if pH is not decreasing), but, if ionized calcium is brought to milk after κ-casein cleavage by chymosin is over, coagulation begins immediately.

The most common ways to explain the role of calcium in the rennet coagulation of milk are to consider calcium as an agent bridging micelles together or as an

agent affecting the ionic strength of milk and, consequently, the density of the protective electrolytic brush.

However, if bridging is the case, it is difficult to explain why the decrease in calcium ion concentration from 3.2 mM (Fig. 1, curve 1) to 1.8 mM (Fig. 1, curve 6) almost doubles the coagulation time, while the decrease in calcium ion concentration from 3.2 mM (Fig. 2, curve 1) to 1.2 mM (Fig. 2, curve 3) stops coagulation.

Note also that, if, in the case of sample 2 in Fig. 2, one brings to milk solutions of sodium chloride or potassium chloride instead of calcium chloride (with the same ionic strength), it does not lead to coagulation.

We introduce a hypothesis of colloid stabilization for the milk micellar system, based on a simple phenomenological square-potential model. Let micelles be "hairy spheres" with a hard sticky surface and an elastic nonsticky hairy layer attached to the surface and protecting the spheres from sticking together [8]. The potential of interaction between two micelles for this system is shown in Fig. 3.

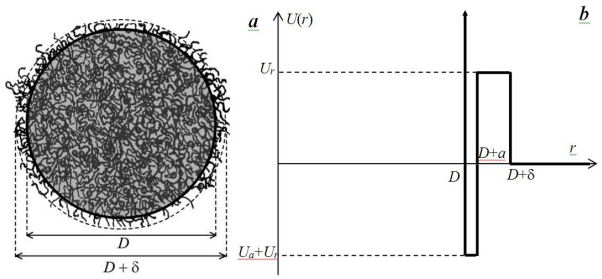

Fig. 3. Schematic micelle image (*a*) and (*b*) the interaction potential depending on distance *r* between the centers of two micelles.

It consists of an infinite hard sphere "wall" at distance *D*, a deep narrow adhesive "well" of width *a*, and a repulsive "step" of width δ:

$$U(r) = U_w(r) + U_a(r) + U_r(r), \quad (1)$$

where

$$U_w(r) = \begin{cases} +\infty, & r \le D \\ 0, & r > D \end{cases},$$

$$U_a(r) = \begin{cases} -U_0 + U_{add}, & r \le D + a \\ 0, & r > D + a \end{cases},$$

$$U_r(r) = \begin{cases} U_r, & r \le D + \delta \\ 0, & r > D + \delta \end{cases} \quad (2)$$

The main idea of our model is an additional micellar potential, U_{add}, resulting from the dissociation of micellar calcium caseinate [13]. In spite of the fact that we are inclined to consider this additional potential as sufficiently screened by Debye layer electrostatic repulsion, it is placed into the "adhesive" part of the whole potential. This is mostly to simplify the coordinate dependence of the whole potential. In this way, the additional potential just varies the adhesive well depth. It is not even forbidden to transform the "well" into "step."

It is known that calcium is able to bind chemically with phosphoserine groups of α- and β-caseins [10, 14–16], forming compounds, so to speak, of an ambiguous chemical structure, which are usually called calcium caseinates.

If one assumes the binding of calcium to phosphoserine groups as a chemically reversible process, it is possible to present formally the reaction of calcium caseinate dissociation–recombination as follows:

$$\mathrm{CaCAS} \rightleftarrows \mathrm{Ca}^{2+} + \mathrm{CAS}^{2-}, \quad (3)$$

where symbol "CAS" is chosen to represent a "casein molecule."

The equilibrium constant for reaction (3) is

$$K_{CAS} = \frac{[\mathrm{Ca}^{2+}][\mathrm{CAS}^{2-}]}{[\mathrm{Ca\ CAS}]}, \quad (4)$$

Thus, the decrease in calcium ion concentration $[Ca^{2+}]$ leads to the emergence of an additional micellar negative electric charge, q_{CAS}, proportional to the concentration of dissociated caseinates $[CAS^{2-}]$, which, in turn, is inversely proportional to $[Ca^{2+}]$

$$q_{CAS} \propto -[\mathrm{CAS}^{2-}] = -\frac{K_{CAS}[\mathrm{Ca\ CAS}]}{[\mathrm{Ca}^{2+}]}, \quad (5)$$

Expression (5) may be directly verified by micelle ζ-potential measurement. Figure 4 demonstrates a possible verification. The decrease in calcium ion concentration in our case was attained by adding Trilon B® to milk, and its increase, by adding the calcium chloride solution to reconstituted skim milk. In both cases, the pH of milk was adjusted to 6.7 with a sodium hydroxide solution.

To fit experimental data, the following function for the negative micellar ζ-potential absolute value was used

$$\zeta = \frac{a}{[\mathrm{Ca}^{2+}]} + b, \quad (6)$$

The solid line in Fig. 4 was calculated according to formula (6) with a = 45.7 mV·mM and b = 10.4 mV.

The constant term *b*, in our opinion, is basically attributed to the electric charge arising as a result of the dissociation of κ-casein macropeptide residues and, probably, some other functional groups of casein molecules.

The results shown in Fig. 3 are in quite a reasonable accordance with earlier experimental data [17, 18]. For example, the article [17] states that the negative casein micelle ζ-potential of skimmed bulk milk in absolute value was 24.4 mV, and after the removal of 51% of

calcium from milk through ionic exchange, it became 30.6 mV (in absolute value).

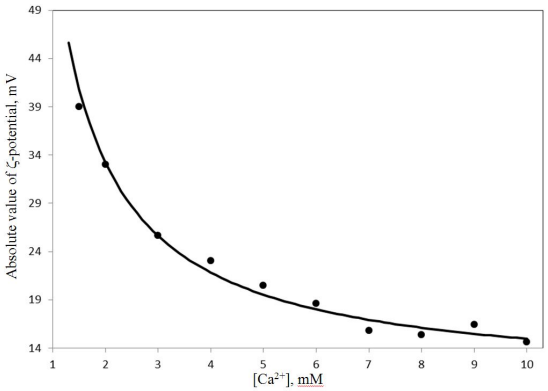

Fig. 4. Dependence of the micellar ζ-potential on calcium ion concentration in reconstituted skim milk at pH = 6.7.

Thus, we can conclude that the lack of calcium ions in milk causes an essential increase in the micelle electric charge as a result of micellar calcium caseinate dissociation. This leads to additional short-ranged (because of Debye screening) electrostatic repulsion. In our square-potential model, it counts as the increase in U_{add} in (2) and, hence, the decrease in the adhesive well depth. Therefore, when calcium ion concentration in milk is decreased by any possible way, micelle "hard spheres" become unstuck and coagulation is impossible even when the "repulsive step" is completely destroyed by chymosin.

It is useful to note that the "chemical" nature of the role of calcium in the stabilization of the milk casein colloid system can be proved by the fact that magnesium acts very similarly when added to milk, while sodium or potassium act differently.

It is known that κ-casein cleavage by chymosin does not depend essentially on the concentration of calcium ions in milk. This may be justified by the conclusions in [19, 20]. In this case, the destruction of the protective hairy layer on the micelle surface for the sample whose thermogram is presented by curve 6 in Fig. 1 occurs practically simultaneously with that for the samples represented by curves 4 or 5. However, coagulation for sample 6 does not occur at this time. It occurs much later.

According to our square-potential model, coagulation is a process of "all-or-nothing" type. Therefore, if the depth of U_a is enough and U_r is not very high, coagulation occurs. However, if the depth of U_a is not enough (whatever U_r is), coagulation does not occur. Then why do both milk samples with calcium ion concentrations of 2 mM and 10 mM coagulate, although in the latter case much faster?

Apparently, there should be a process that is able to decrease the additional negative charge of casein micelles, raised in the way described above. Judging by the form of thermograms recorded at different calcium ion concentrations, this process may be similar to the proteolytic cleavage of κ-casein by chymosin.

Let us now introduce another hypothesis to our model. We assume that the additional negative charge of casein micelles can be decreased due to the nonspecific proteolytic activity of chymosin, directed to the charged functional groups of α- and β-caseins. Researchers are aware of such nonspecific activity [21–23]. In principle, α- and β-caseins located mainly inside micelles can be accessible to chymosin during the primary stage of coagulation. According to modern ideas, the micelle is a porous structure penetrable for small protein molecules [24–27].

To substantiate the hypothesis of chymosin additional proteolytic activity, we performed experiments on renneting calcium-depleted milk with different amounts of chymosin (Fig. 5). One can see that the increase in enzyme concentration leads to an almost proportional reduction of coagulation time. Such dependence is characteristic of enzymatic reactions. Thus, we may suppose that κ-casein proteolysis on the micellar surface and the proteolysis of α- and β-caseins inside micelles are similar processes, although in the latter case the rate of proteolysis is much slower (by about ten times according to our estimates) apparently due to the lower availability of the internal micelle area for chymosin molecules.

Now we can explain the role of calcium ions in milk renneting in the following way. The rennet coagulation time is determined by the rate of κ-casein cleavage by chymosin on the micelle surface only in the case of a sufficient concentration of calcium ions in milk. According to our estimates, this concentration is about 10 mM. As a rule, the concentration of ionized calcium in milk is below this value and chymosin needs some time to cut off the functional groups of α- and β-caseins, negatively charged due to calcium caseinate dissociation, inside casein micelles. Since the additional electric charge of micelles strongly depends on calcium ion concentration, the rennet coagulation time is usually affected dramatically by the quantity of calcium ions added to milk.

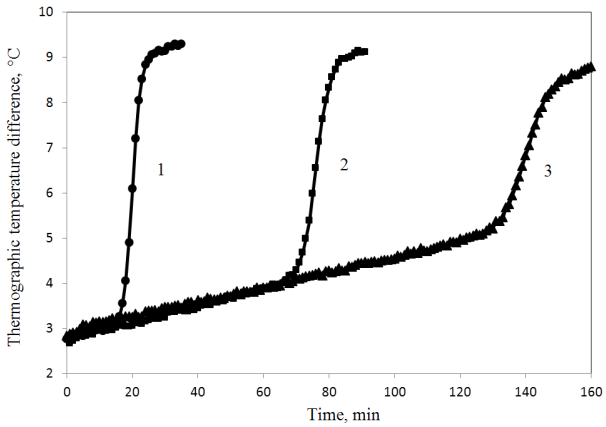

Fig. 5. Thermograms of reconstituted skim milk clotting by different amount of chymosin at 30°C.

Curves: 1 – reference sample (0.4 g/L of $CaCl_2$ added to milk, clotting with 25 mg/L of chymosin);
2 – no $CaCl_2$ added to milk, clotting with 100 mg/L of chymosin;
3 – no $CaCl_2$ added to milk, clotting with 50 mg/L of chymosin.

It is usually believed that acid milk coagulation does not depend on calcium ion concentration and is determined only by the milk pH value. Nevertheless, this is not obvious. It is known that, under acid conditions, casein flocculation in milk begins at a pH value of about 5.2. On the other hand, at such pH values, micellar calcium phosphate is practically entirely ionized. Thus, the mechanism of casein colloid system destabilization during acid milk coagulation is perhaps similar to that described above.

On the one hand, additional hydrogen ions under acid conditions reduce the macropeptide hair charge and make the polyelectric brush collapse (U_r is destroyed in our model). On the other hand, the increase in calcium ion concentration owing to the dissolution of colloid calcium phosphate leads to a decrease in the additional micelle charge (U_a becomes adhesive).

We tried a simple experiment to substantiate this idea. The point is that Na_2EDTA exhibits acid properties under dissolution. Thus, we were able to decrease the milk pH by adding either lactic acid or Trilon B®. In both cases, we decreased it to 4.8.

In both cases, an increase in milk viscosity was observed. However, in case of coagulation with lactic acid, the increase in viscosity was more intensive and a classic acid clot was observed as a result. In case of coagulation with Trilon B®, despite a slow increase in viscosity, clot formation in milk does not occur.

Perhaps, despite the decrease in EDTA's chelating ability at a low pH, the competition between this process and reversible calcium caseinate dissociation leads to the retention of part of the additional charge of casein micelles and, as a consequence, to the reduction of the micelle coagulation ability. Thus, it is quite possible that casein colloid stability in milk is controlled by the same mechanism for both rennet and acid coagulation.

To include acid milk coagulation in our model, we add a couple of highly simplified schemes (similar to scheme (3)) to our phenomenological chemistry. First, we will consider the negative charge of κ-casein macropeptide hairs q_{CMP} as a result of dehydrogenation

$$\mathrm{CMP} \rightleftarrows \mathrm{CMP^-} + \mathrm{H^+}, \qquad (7)$$

with the equilibrium constant

$$K_{CMP} = \frac{[\mathrm{CMP^-}][\mathrm{H^+}]}{[\mathrm{CMP}]}, \qquad (8)$$

Then

$$q_{CMP} \propto -[\mathrm{CMP^-}] = -\frac{K_{CMP}[\mathrm{CMP}]}{[\mathrm{H^+}]}, \qquad (9)$$

The CMP symbol is chosen to represent κ-casein macropeptide hydrophilic groups. We may assume that basically this charge ensures micelle steric stability by means of the elastic polyelectrolyte brush.

Second, because of the complexity of the colloid calcium phosphate (CCP) structure, its hydrogenation is considered within the extremely simplified one-step scheme:

$$\mathrm{CCP} + 2\mathrm{H^+} \rightleftarrows \mathrm{CCP^*} + \mathrm{Ca^{2+}}, \qquad (10)$$

Here CCP^* is the hydrogenated form of CPP. We hope that this "averaged" scheme of CPP hydrogenation is qualitatively correct at least for describing the basic features of the process (for example, dependence on pH).

The mechanism of acid milk coagulation can now be described as follows. A decrease in milk pH or, respectively, an increase in $[H^+]$ shifts, on the one hand, the equilibrium of reaction (7) to the left and, consequently, decreases (in absolute value) the polyelectric brush's negative charge q_{CMP} (according to (9)). On the other hand, the increase in $[H^+]$ leads to the hydrogenation of the micellar colloid calcium phosphate complex according to scheme (10) and, hence, to an increase in calcium ion concentration. As a result of $[Ca^{2+}]$ growth, balance in scheme (3) is shifted to the left, decreasing the absolute value of the additional negative charge of casein micelles q_{CAS} (according to (5)). Eventually, micelles lose both steric stabilization by means of the κ-casein macropeptide hairy layer and stabilization by means of the additional electric charge. Thus, colloid stability is lost, and an acid gel starts to form.

We are of the opinion that our model, including schemes (3), (7), and (10), makes it possible to explain the results discussed in [28], where the authors tried to understand the role of soluble and insoluble calcium in milk coagulation. As is shown above, only ionized (active) calcium is of primary importance for milk colloid stability. In this sense, only calcium hydrogenated from colloid calcium phosphate with lactic acid may be considered as "active." Meanwhile, the authors of [28] consider calcium chelated by EDTA soluble as well (Na_4EDTA could be used in [28], because it seems that it does not change milk pH).

Now we declare that the potential in our model is a function of two charges:

$$U_r = U_r(q_{CMP}) \text{ and } U_{add} = U_{add}(q_{CAS}).$$

The explicit form of these two functions is a problem for a special study. However, for semiqualitative analysis, it would perhaps be enough to put

$$U_r \propto q_{CMP}^2, \quad U_{add} \propto q_{CAS}^2. \qquad (11)$$

Probably, it is also useful to add the rennet way of q_{CMP} reduction in (9). Taking into account the exponential decrease in CMP brush density under rennet conditions (see, for example, [8]) and the fact that $[\mathrm{CMP^-}] + [\mathrm{CMP}] = [\mathrm{CMP}]_0$ is the maximum concentration of CMP hairs, one can easily get from (9):

$$q_{CMP} \propto -\frac{K_{CMP}[\mathrm{CMP}]_0}{K_{CMP}+[\mathrm{H}^+]}\exp(-k_{CMP}\cdot t), \quad (11)$$

Here k_{CMP} is the reaction constant for CMP proteolysis by chymosin.

Walking through similar procedure one can get from (5):

$$q_{CAS} \propto -\frac{K_{CAS}[\mathrm{CaCAS}]_0}{K_{CAS}+[\mathrm{Ca}^{2+}]}\exp(-k_{CAS}\cdot t), \quad (12)$$

Here $[\mathrm{CaCAS}]_0 = [\mathrm{CAS}^{2-}]+[\mathrm{CaCAS}]$ is the full concentration of α- and β-casein phosphoserine groups available for calcium binding and k_{CAS} is the reaction constant for additional nonspecific proteolysis of α- and β-caseins by chymosin.

One more hypothesis in addition to the scheme described above allows explaining some features of temperature dependence for acid and rennet coagulation, as well as the similarity of heat-acid and heat-calcium milk coagulation.

Let us assume that the equilibrium of reactions (3) and (10) is shifted to the left when temperature increases. In other words, at higher temperatures, calcium forms less soluble compounds with both phosphates and caseins. Note that for reaction (10) such dependence is an established fact, while for reaction (3) this assumption is just a working hypothesis. It is based on the possible similarity of the chemical interaction of calcium with phosphate groups and phosphoserine residues of proteins.

Then, if temperature decreases, reaction (3) shifts to dissociation (i.e., K_{CAS} becomes greater) and, as a consequence, to an increase in an additional micellar charge according to expression (5). Indeed, we understand that the temperature dependence of hydrophobic interactions is the main contributor to milk colloid stability at low temperatures. In our model, it counts as the maximal depth of the adhesive well U_0. Apparently, when temperature becomes as low as approximately 6°C, U_0 becomes so low that, even at zero U_{add}, the micelle surface remains unstuck. For this reason, rennet [29] and acid [30] coagulation of milk is impossible at low temperatures.

Temperature increase leads to a decrease in K_{CAS} and, as a consequence, to a decrease in the additional micelle charge, q_{CAS}. Therefore, the rennet coagulation time becomes shorter, and it decreases as temperature grows up to the point of enzyme inactivation. For acid coagulation, temperature growth leads to higher pH values at which coagulation begins.

Figure 6 demonstrates the results of the experiment examining the dependence of temperature at which acid milk coagulation begins on the milk pH value.

The experiment was carried out as follows. The samples of milk were adjusted to a desirable pH value with a lactic acid solution at room temperature and then put into a warmed-up cell equipped with a thermometric viscosity gauge. As one can see in Fig. 6, the coagulation of samples with a lower pH value begins at lower temperatures. These results correspond well to the conclusions made above on the basis of our hypothesis.

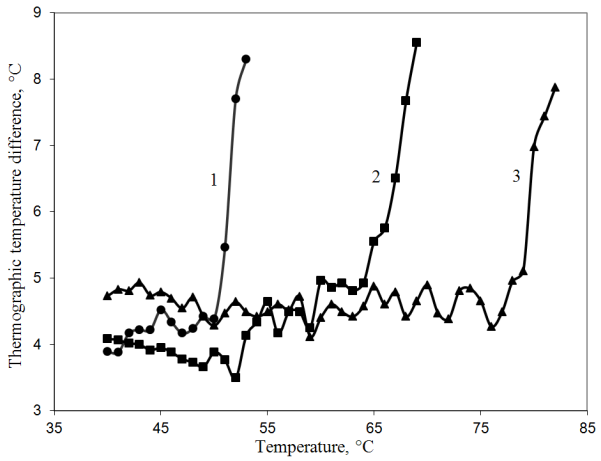

Fig. 6. Thermograms of reconstituted skim milk samples subjected to heating after acidification.

Curves: 1 – sample pH = 5.6;
2 – sample pH = 5.9;
3 – sample pH = 6.2.

Apparently, calcium hysteresis in milk, discussed, for example, in [31], may also be explained within the framework of our scheme, assuming that both the direct and reverse reaction constants in scheme (1) are essentially higher than the similar constants for reaction (10).

In addition, it is noteworthy that the developed approach, based on the analysis of the outstanding role of calcium ions in milk coagulation, can explain the amazing similarity of heat-acid and heat-calcium milk coagulation. It is well known that the addition of acid solutions or calcium chloride to milk heated up to 90–95°C leads to immediate coagulation. Such technologies are used for manufacturing both fresh cheeses and technical casein.

Within the described model, heat-acid and heat-calcium milk coagulation proceeds as follows. As milk is heated, the reverse reaction in (10) leads to an increase in hydrogen ion concentration and, as a consequence, to the shift of reaction (7) to the left. As a result, the charge of the κ-casein macropeptide hairy layer on the micelle surface decreases and the colloid stability of milk is determined only by the additional stabilization due to dissociated calcium caseinate. The reverse reaction in scheme (1) leads to the reduction of the additional negative charge of casein molecules, but the concentration of ionized calcium is usually insufficient for its full neutralization. Adding soluble calcium to heated milk quickly decreases the additional charge. Adding an acid solution to milk leads to the shift of reaction (5) to the right and, hence, to a quick increase in calcium ion concentration, reducing the additional casein charge. Thus, in both cases colloid stability is completely destroyed and coagulation quickly begins.

CONCLUSION

Making use of the hypothesis of the additional electric micelle charge arising owing to the dissociation of micellar calcium caseinate, we managed to describe the outstanding role of calcium in the coagulation of milk and, moreover, to show that various kinds of milk coagulation may be represented as fundamentally very similar processes. In all cases of milk coagulation, two major factors contribute to casein colloid stability:

- steric limitation for the approach of micelles by means of a polyelectrolyte brush consisting of hydrophilic κ-casein macropeptide residues on micelle surfaces; and
- electrostatic (in the sense of the Debye layer) limitation for the approach of micelles owing to the additional electric charge arising as a result of micellar calcium caseinate dissociation.

The destruction of the polyelectrolyte brush can be made either "mechanically," using the proteolytic cleavage of macropeptide hairs with chymosin or "chemically," by neutralizing the negative charge of macropeptide hairs with acid hydrogen ions and thus collapsing the polyelectrolyte brush. In the first case, one has rennet milk coagulation and in the second, acid milk coagulation.

The other stabilizing factor associated with the additional electric micelle charge operates equally under any type of milk coagulation. To decrease the additional charge, it is necessary to bring additional calcium ions to milk (that is why milk coagulation is very sensitive to calcium ion concentration in milk). Hypothetically, the additional charge may also be decreased in the way similar to renneting, i.e., due to nonspecific (or specific in another sense) proteolytic activity of chymosin directed to charged functional groups of α- and β-caseins.

Taking into account the hypothesis of the temperature dependence of calcium caseinate dissociation, we can describe the coagulation of milk and heat-acid coagulation as a high-temperature kind of acid coagulation. In addition, our model shows that heat-acid and heat-calcium milk coagulations are similar because they are based on the same principle: a quick decrease in the additional micelle charge.

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