HANDLING, TRANSPORTING, STORAGE AND PROTECTION OF AGRICULTURAL PRODUCTS

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PHOSPHOPROTEINS AS A FACTOR FOR COLLOID STABILITY OF CASEIN MICELLES IN MILK

Research article

Abstract

Understanding the role of calcium in colloidal stability of casein micelles, that is unquestionable, could become the key to control the process of milk coagulation. It is evident that calcium ions can influence milk coagulation, but the molecular mechanism of this influence to micellar casein system is not fully understandable. Methodologically, our research was based on an idea that calcium ions can change the electric charge of casein micelles in the process of dissociation and recombination of some kinds of phosphoproteins, which are components of the casein micelles. A simple quantitative model, which includes kinetic description of the proteolysis process and the thermodynamics of the dissociation process of the functional groups of micellar caseins, was worked out to analyze experimental results. Kinetic and thermodynamic methods of describing the process of stability loss in micellar system were combined in one model, using the concept of solvent quality which is defined by the second osmotic virial coefficient. Our experiments showed that calcium ions are able to connect chemically to caseins in the micelles. Using reasonable assessments for thermodynamic and kinetic parameters, we managed to get quite adequate description of the experimental data. We also demonstrated principal possibility of using our model to describe rennet, acid and mixed acid-rennet clotting of milk as well as heat-calcium and heat-acid coagulation of milk.

Keywords: milk coagulation, casein micelles, colloidal stability, calcium ions, phosphoproteins, calcium caseinates.

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ФОСФОПРОТЕИНЫ КАК ФАКТОР КОЛЛОИДНОЙ СТАБИЛЬНОСТИ КАЗЕИНОВЫХ МИЦЕЛЛ В МОЛОКЕ

Научная статья

Аннотация

Понимание неоспоримой роли кальция в коллоидной стабильности мицелл казеина может стать ключом к контролю процесса коагуляции молока. Очевидно, что ионы кальция влияют на коагуляцию молока, однако молекулярный механизм этого влияния на коллоидную систему казеиновых мицелл в молоке не до конца понятен. Методологически наше исследование было основано на идее о том, что ионы кальция могут изменять электрический заряд мицелл казеина в процессе диссоциации и рекомбинации некоторых видов фосфопротеинов, которые являются компонентами мицелл казеина. Для анализа экспериментальных результатов была разработана простая количественная модель, включающая кинетическое описание процесса протеолиза и термодинамику процесса диссоциации функциональных групп мицеллярных фосфопротеинов. Кинетические и термодинамические методы описания процесса потери устойчивости в мицеллярной системе были объединены в одну модель, используя концепцию качества растворителя, которое определяется вторым осмотическим вириальным коэффициентом. Эксперименты показали, что ионы кальция способны химически связываться с казеинами в мицеллах. Используя разумные оценки для термодинамических и кинетических параметров, удалось получить достаточно адекватное описание экспериментальных данных. Продемонстрирована принципиальная возможность использования модели для описания сычужного, кислотного и смешанного кислотно-сычужного свертывания, а также термокальциевой и термокислотной коагуляции молока.

Ключевые слова: коагуляция молока, мицеллы казеина, коллоидная стабильность, ионы кальция, фосфопротеины, казеинат кальция.

1. Introduction

Milk clotting is one of important technological processes in the manufacturing of many food-stuffs, in particular, cheeses. This process is based on the coagulation of casein micelles, which may be caused by various factors, such as enzymes, acids, alcohol, salts, or high temperature [1, 2].

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 [3–6]. In essence, this layer represents a quasielastic polyelectrolyte brush formed by negatively charged macropeptide residues [7, 8].

The loss of colloid stability by the casein micellar system may be attributed to different ways of the destruction of the hairy layer. Under rennet conditions, κ-casein macropeptide hairs are split off by chymosin, which leads to the destruction of the protective layer [9]. During acid coagulation, additional hydrogen ions easily get into the polyelectrolyte 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 [10, 11]. There are many different cheeses which use joint rennet and acid or heat and acid coagulation in their technology [12, 13]. Heat and calcium coagulation is used mainly to produce protein precipitate from skim milk and this type of milk clotting is quite similar to heat and acid coagulation [14, 15]. Destabilization of milk proteins by alcohol is not widely used in technological processes, thus it is less studied [16].

Certain distinctions in mechanisms of the destruction of the casein micelle protective layer, as well as a number of factors affecting colloid stability of the micellar casein system, make it very difficult to describe various kinds of milk coagulation within a uniform approach. For example, it is known that the lack of calcium in milk has no significant effect on acid coagulation, while it is impossible to coagulate this milk by adding chymosin even after it has completely cut off the protective hairy layer [17, 18].

The investigations described below represent 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 is based on the analysis of the outstanding role of calcium ions in the stabilization of the micellar colloid system in milk.

2. Methods

It is known that calcium can chemically bind to phosphoserine groups of caseins [19–21]. Such groups are present in appreciable amounts in α- and β-caseins. The resulting compound is commonly called calcium caseinate. Our hypothesis which is the base for the model is that reversible dissociation of this caseinates releasing calcium ions into serum is able to arise an additional electric charge of micelles thus affecting colloid stability of the micellar system in milk.

2.1. Experimental background

Reconstituted skim milk was chosen as the object for investigation. To prepare 1 kg of milk 90 g of fat-free milk powder were dissolved in 910 mL of distilled water and it was aged for 12 hours at 6±2ºC.

For rennet clotting chymosin in form of Maxiren® or CHYMAX® was used. In both cases 0.1 g of enzyme powder was dissolved in 100 mL of distilled water to prepare the solution.

Calcium, magnesium and sodium were added to the reconstituted skim milk in the form of solutions of their chlorides. Solutions of CaCl2 and magnesium with concentrations of 1 mol/L and NaCl with a concentration of 3 mol/L were used. Thus 1 mL of calcium chloride solutions contained 1 mmol of calcium and magnesium. Concentration of sodium chloride was chosen to be 3 times larger so that the ionic strengths of all solutions were the same.

To decrease calcium ion concentration in milk Trilon B® (Na2EDTA) was used as a chelating agent. Calcium and magnesium ion concentrations and pH in milk were measured with ion selective electrodes.

Milk coagulation was controlled by our thermographic method [22]. It is based on measuring the temperature difference between two thermometers, one of which being heated, immersed into milk at a distance from each other. When coagulation network is being formed in milk convection is hindered and temperature of the heated thermometer is increased. This method is similar to the hot-wire one [23], but, unlike the latter, it is not sensitive to changes in the environmental temperature showing only temperature difference between the heated and unheated thermometers thus making it feasible to use for heat-acid and heat-calcium applications. Hereinafter, the curves, obtained with the help of thermographic method, that characterize the change in milk structure during its coagulation, will be called thermograms (by analogy with rheograms).

Indirect method of analyzing of the amount of calcium and magnesium added to the milk and associated with casein micelles was used. After coagulation, the milk clot was separated from the serum. The serum samples were then dried in a spray dryer and the elemental composition of the resulting powder was examined spectroscopically using an inductively coupled plasma atomic spectrometer.

2.2. Theoretic background

To simulate the experimental results, a model of pair interaction of micelles based on the model of sticky hard spheres was used [10] with two additional rectangular potentials [24]. The total potential energy of the pair interaction of the micelles for the selected model is schematically shown in Figure 1.

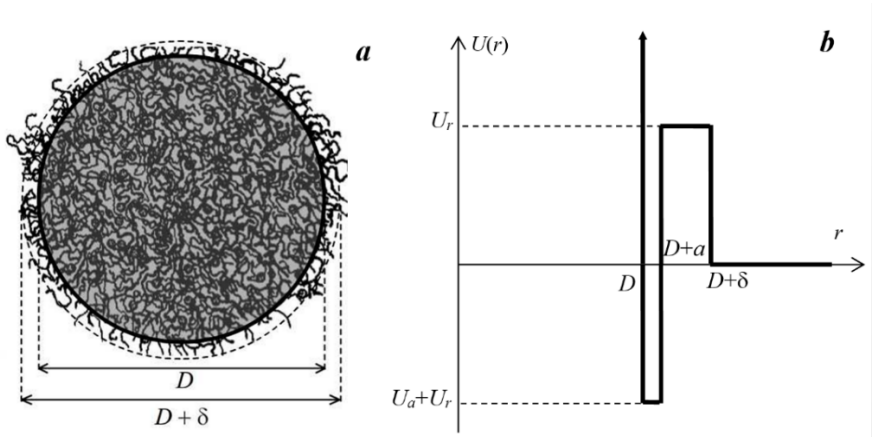

Figure 1. Schematic micelle image (***a***) and interaction potential (***b***) depending on distance r between centers of two micelles.

The potential includes a hard wall at a distance D between the centers of the micelles, a sufficiently deep narrow "attracting" well of width a and a repulsing step with a width of δ: $U(r)=U_w(r)+U_a(r)+U_r(r)$, where

$$U_w(r)=\begin{cases}+\infty, & r\le D\\ 0, & r>D\end{cases},\ U_a(r)=\begin{cases}-U_0+U_{add}, & r\le D+a\\ 0, & r>D+a\end{cases},\ U_r(r)=\begin{cases}U_r, & r\le D+\delta\\ 0, & r>D+\delta\end{cases}. \quad (1)$$

Repulsion energy U_r is due to presence of the elastic hairy layer of κ-casein macro-peptide residues on the surface of micelles. The attraction potential $-U_0$ includes various interactions (van der Waals attraction, hydrophobic interactions, hydrogen bonds, etc.), which ensure the adhesion of micelles when they directly contact their surfaces.

The additional potential U_{add} describes the repulsion of micelles because of appearance of the same electric charge of the micelles as a result of dissociation of micellar calcium caseinate. This repulsion has evidently a short-range character due to the strong Debye screening of protein molecules by ions contained in the whey. It is clear that at sufficiently high degree of dissociation of calcium caseinates, the whole potential of intermolecular attraction U_a can change its sign becoming repulsive in this case. 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 the "step".

To quantify the colloidal stability of the micellar system we use the formalism of solvent quality characterized by the second osmotic virial coefficient

$$B_2=2\pi\int_0^\infty r^2\left(1-\exp(-U(r)/kT)\right)dr, \quad (2)$$

where k is the Boltzmann constant, and T is the absolute temperature.

Using the value of the specific excluded volume $\beta_2=\frac{B_2}{V_{HS}}$, where $V_{HS}=\pi\frac{D^3}{6}$ is the volume of the hard sphere, and neglecting the terms containing $\frac{a^2}{D^2}$, $\frac{a^3}{D^3}$ and $\frac{\delta^2}{D^2}$, $\frac{\delta^3}{D^3}$, we get from (1) and (2)

$$\beta_2=4+12\frac{a}{D}\left(1-e^{-(U_a+U_r)/kT}\right)+12\frac{\delta-a}{D}\left(1-e^{-U_r/kT}\right). \quad (3)$$

It is believed that the colloidal solution becomes unstable under the condition $\beta_2\approx$-6 [25, 26].

The coagulation kinetics is determined by the fact that the potential energies U_r and U_{add} depend on time. These dependences are determined by the kinetics of variation of the corresponding charges: negative electric charge on the κ-casein hairs q_{CMP} proportional to concentration of dissociated macropeptide residues, and an additional negative electric charge of micelles q_{CAS} proportional to concentration of dissociated molecules of calcium caseinates.

Assuming that the binding of calcium to the phosphoserine groups of the casein molecules is a reversible process, the dissociation-recombination reaction of calcium caseinate may be formally represented as

$$\mathrm{CaCAS} \leftrightarrow \mathrm{Ca^{2+}} + \mathrm{CAS^{2-}}, \quad (4)$$

where symbol CAS is chosen to represent the "molecule" of casein. Thus

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

where K_{CAS} is the equilibrium constant for the reaction (4), e=1.6·10^{-19} C is electron charge and [M] is concentration of casein micelles in milk.

To include acid milk coagulation in our model, we add a couple of highly simplified schemes similar to scheme (4). First, we will consider the negative charge of κ-casein macropeptide hairs q_{CMP} as a result of dehydrogenation

$$\mathrm{CMP} \leftrightarrow \mathrm{CMP^-} + \mathrm{H^+}, \quad (6)$$

$$q_{CMP} = \frac{-e\,[\mathrm{CMP}^-]}{[M]} = -\frac{e}{[M]}\frac{K_{CMP}[\mathrm{CMP}]}{[\mathrm{H}^+]}, \quad (7)$$

where symbol CMP represents the κ-casein macropeptide hydrophilic groups and K_{CMP} is the equilibrium constant for the reaction (6). One may assume that this charge ensures basically micelle steric stability by means of the elastic polyelectrolyte brush.

Another reason for variation of calcium ion concentration as well as milk pH is hydrogenation of micellar colloid calcium phosphate. Because of the complexity of its structure the hydrogenation in our model is considered within the extremely simplified one-step scheme

$$\mathrm{CCP} + 2\mathrm{H}^+ \leftrightarrow \mathrm{CCP}^* + \mathrm{Ca}^{2+}, \quad (8)$$

where 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).

As an approximation, the potential energy associated with the charges is assumed to be proportional to their squares

$$U_r = Aq_{\mathrm{CMP}}^2, \quad U_{add} = Aq_{\mathrm{CAS}}^2. \quad (9)$$

3. Results

3.1. Rennet coagulation

Table 1 and Figure 2 show the results of experiments on rennet coagulation of reconstituted skim milk with different soluble calcium content. The solution of calcium chloride was added so that the concentration of added calcium was 4, 8, 16 and 32 mmol/L.

Table 1. Content of Ca in whey samples

Added $CaCl_2$, mmol/L	4.0±0.1	8.0±0.2	16.0±0.4	32.0±0.8
Detected Ca, mmol/L	2.8±0.2	3.6±0.2	5.4±0.2	8.5±0.2

As it can be seen from the Table 1, the certain amount of calcium added to milk before coagulation does not additively increase its content in the milk serum after coagulation. This fact clearly confirms binding of calcium to casein micelles. In our opinion, it can reflect exactly the exchange of calcium between the serum and caseins according to the scheme described by scheme (4).

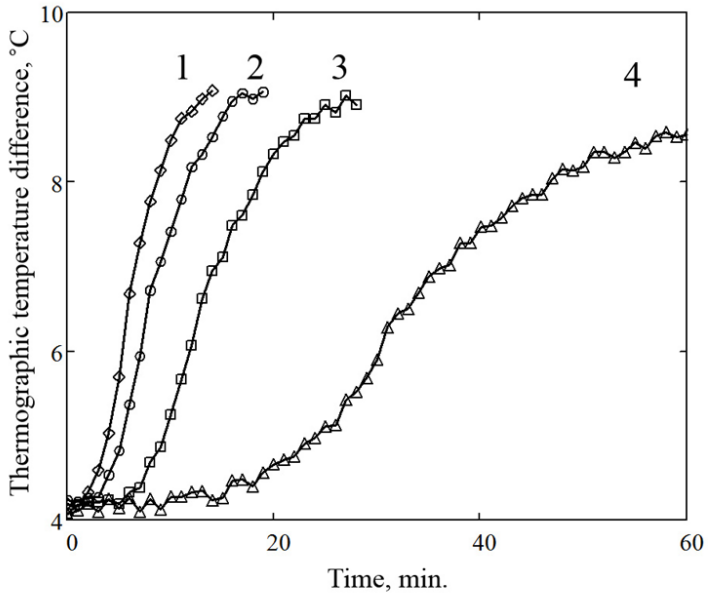

Figure 2. Thermograms of rennet coagulation of reconstituted skim milk with additional Ca^{2+}. Curves:
1 – 32 mmol of $CaCl_2$ added to 1 L of milk sample;
2 – 16 mmol of $CaCl_2$ added to 1 L of milk sample;
3 – 8 mmol of $CaCl_2$ added to 1 L of milk sample;
4 – 4 mmol of $CaCl_2$ added to 1 L of milk sample.

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 8 mmol/L (that corresponds to 32 mmol/L of added $CaCl_2$) leads to the saturation effect.

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 [27, 28]. In this case, the destruction of the protective hairy layer on the micelle

surface for all samples whose thermograms are presented in Fig. 1 should occur simultaneously. One can see that sample 1 and sample 2 coagulate practically in the same time. However, coagulation for sample 4 occurs much later.

Apparently, there should be a process that is able to decrease the additional negative charge of casein micelles, raised in the way described by scheme (4). We assume that this process may be similar to the proteolytic cleavage of κ-casein by chymosin due to the nonspecific proteolytic activity of chymosin directed to charged functional groups of α- and β-caseins [29–31]. Then concentrations of dissociated macropeptide residues [CMP^-] and dissociated molecules of calcium caseinates [CAS^{2-}] should follow simple kinetics:

$$\begin{aligned}[\mathrm{CMP^-}](t) &= [\mathrm{CMP^-}](t=0)\exp(-k_{CMP}\cdot t)\\ [\mathrm{CAS^{2-}}](t) &= [\mathrm{CAS^{2-}}](t=0)\exp(-k_{CAS}\cdot t)\end{aligned}. \tag{10}$$

Here k_{CMP} is the reaction constant for CMP proteolysis by chymosin and k_{CAS} is the reaction constant for the additional nonspecific proteolysis of α-and β-caseins by chymosin.

Taking into account that $[\mathrm{CMP}]_0 = [\mathrm{CMP^-}] + [\mathrm{CMP}]$ is the total concentration of dissociated and undissociated macropeptide residues of k-caseins and $[\mathrm{CAS}]_0 = [\mathrm{CAS^{2-}}] + [\mathrm{CaCAS}]$ is the total concentration of dissociated and undissociated phosphoserine groups we can represent electric charge of the micelles, using (5), (7) and (10) as follows:

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

$$q_{\mathrm{CAS}} = -2e\frac{[\mathrm{CAS^{2-}}]}{[\mathrm{M}]} = \frac{-2e}{[\mathrm{M}]}\frac{K_{\mathrm{CAS}}[\mathrm{CAS}]_0}{K_{\mathrm{CAS}}+[\mathrm{Ca^{2+}}]}\exp(-k_{\mathrm{CAS}}\cdot t). \tag{12}$$

Now it is possible to estimate some quantitive aspects of our model. Figure 3 presents the results of the calculation of the second osmotic virial coefficient in the form (3) characterizing the colloidal stability of the micellar system of caseins as a function of time for different values of the added calcium. Calculations of the potential energy were carried out according to formulas (9), (11) and (12). Parameters used for modeling were: $A = \frac{4.8kT}{10^5 e^2}$; $K_{CMP} = 1.2\cdot10^{-6}$ mol/L; $K_{CAS} = 1.7\cdot10^{-5}$ mol/L; $[\mathrm{CMP}]_0 = 3000[\mathrm{M}]$; $[\mathrm{CAS}]_0 = 1.5\cdot10^5[\mathrm{M}]$; $[\mathrm{H^+}] = 10^{-6.7}$ mol/L; $U_a = 70kT$; $k_{\mathrm{CMP}} = 0.15$ min^{-1}; $k_{\mathrm{CAS}} = 0.014$ min^{-1}.

It is not difficult to see that the calculation data presented in Figure 3 are in satisfactory agreement with the experimental results on the coagulation of skim milk samples enriched with calcium, shown in Figure 2. If we consider the moment of coagulation t_C as the time in which the rate of increase in the thermographic temperature difference attains it maximum value, then on the basis of the analysis of the curves in Figure 3, the following clotting times can be obtained: $t_{C1} = 6 \pm 1$ min for curve 1; $t_{C2} = 8 \pm 1$ min for curve 2; $t_{C3} = 13 \pm 2$ min for curve 3 and $t_{C4} = 32 \pm 2$ min for curve 4. On the other hand, if, as noted in the previous section, the moment of loss of stability by the colloidal system is the achievement the value $\beta_2 = -6$, then one can see a good coincidence.

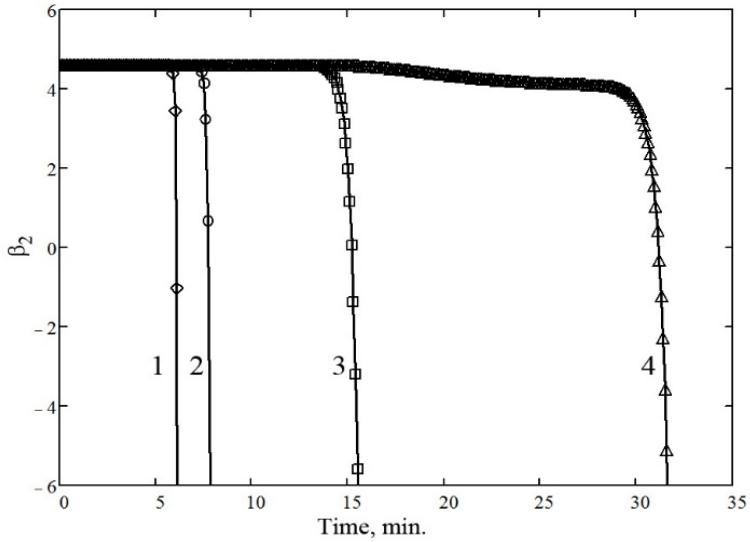

Figure 3. Calculated values of β_2 resulted from fitting to curves 1, 2, 3 and 4 in figure 2.

We also repeated our experimental set using magnesium instead of calcium. Table 2 shows the results of spectroscopic detection of magnesium content in whey after rennet coagulation of reconstituted skim milk with different amount of added magnesium chloride.

Table 2 – Content of Mg in whey samples

Added MgCl2, mmol/L	8.0±0.2	16.0±0.4
Detected Mg, mmol/L	6.1±0.1	8.7±0.1

As one can see comparing Table 1 and Table 2, magnesium seems to demonstrate worse binding to caseins. Note also that the clot quality after adding the same amounts of calcium and magnesium in the samples is higher in the first case.

Figure 4 demonstrates thermogramms obtained during coagulation of skim milk enriched with magnesium.

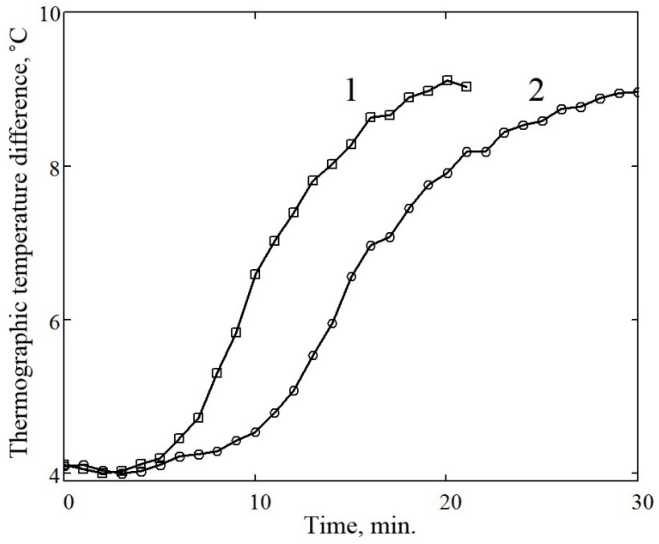

Figure 4. Thermograms of rennet coagulation of reconstituted skim milk with additional Mg^{2+}. Curves:
1 – 16 mmol of $MgCl_2$ added to 1 L of milk;
2 – 8 mmol of $MgCl_2$ added to 1 L of milk.

As can be seen from the Figure 2 and Figure 4, coagulation stability of the samples is comparable for milk with the same ratio of calcium or magnesium ions. However, samples with magnesium coagulate longer comparing with calcium-added samples with the same amount chlorides. This fact makes it possible to estimate the value of the equilibrium constant for the reaction $MgCAS \leftrightarrow Mg^{2+} + CAS^{2-}$ of dissociation of magnesium caseinate K^*_{CAS} somewhat higher than for the reaction (4). Additional electric charge arising due to dissociation of magnesium caseinate can be represented as follows:

$$q^*_{\text{CAS}} = -2e\frac{[\text{CAS}^{2-}]^*}{[\text{M}]} = \frac{-2e}{[\text{M}]}\frac{K^*_{\text{CAS}}}{K^*_{\text{CAS}}+[\text{Mg}^{2+}]}\frac{K_{\text{CAS}}[\text{CAS}]_0}{K_{\text{CAS}}+[\text{Ca}^{2+}]}\exp(-k_{\text{CAS}}\cdot t). \quad (13)$$

Figure 5 presents the results of the calculation of the second osmotic virial coefficient in the form (3) characterizing the colloidal stability of the micellar system of caseins as a function of time for different values of the added magnesium.

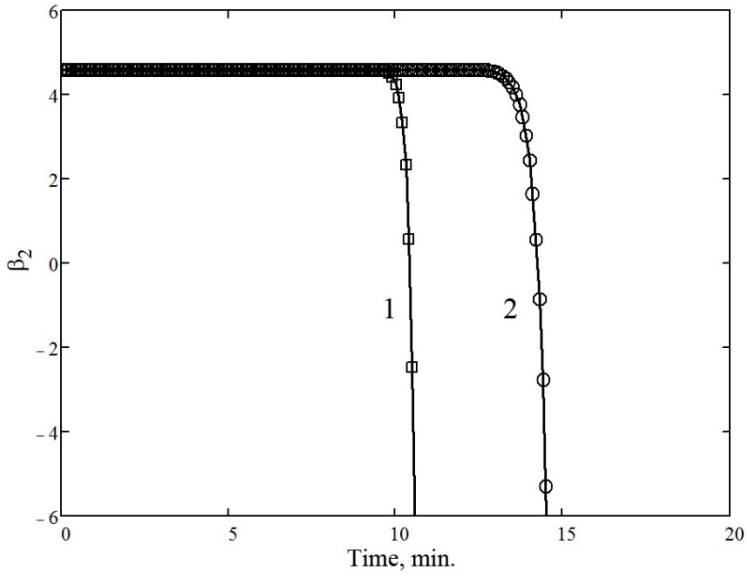

Figure 5. Calculated values of β_2 resulted from fitting to curves 1 and 2 in Figure 4.

All common parameters used for this fit coincide with the parameters used in the previous case. Concentration of calcium ions for both samples was the same and equal to 2.4 mmol/L. The resulting equilibrium constant for the dissociation of magnesium caseinates was found to be $K^*_{CAS}=1.15\cdot10^{-2}$ mol/L. As was expected, this value significantly, almost 700 times, greater than the value of the equilibrium constant for the dissociation of calcium caseinates. One should note again good agreement between the experimental and calculated values of the coagulation time.

Samples of milk with different contents of sodium ions were also prepared in this series of experiments. A solution of sodium chloride was added so that the concentration of added sodium was 12, 24, 48 and 96 mmol/L. None of the samples of reconstituted skim milk prepared in this way were clotted under the influence of chymosin for an hour. In principle, this model easily explains the negative result of milk coagulation with addition of sodium chloride instead of calcium chloride. If the equilibrium constant of dissociation of sodium caseinates is substantially higher than for magnesium caseinates, it will not be possible to coagulate milk with the addition of reasonable amounts of sodium chloride within a reasonable time.

3.2. Acid coagulation

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. On the other hand, at such pH values, micellar calcium phosphate is practically entirely dissociated. 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 charge of the macropeptide hairs, shifting the equilibrium of reaction (6) to the left side. Thus the polyelectrolyte brush collapses and repulsive potential U_r becomes negligible (Figure 6). On the other hand, the increase in calcium ion concentration due to dissociation of colloid calcium phosphate leads to a decrease in the additional micelle charge. Additional positive term U_{add} in potential U_a decreases (Figure 7) and it becomes adhesive.

We tried a simple experiment to substantiate this idea. The point is that Na_2EDTA exhibits acid properties being dissolved in water. Thus, we were able to decrease 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 of a slow increase in viscosity clot formation in milk does not occur.

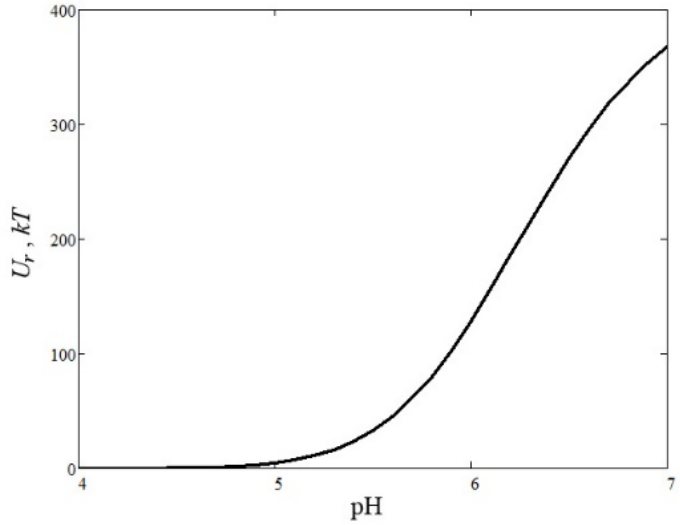

Figure 6. Dependence of U_r on pH

The mechanism of acid milk coagulation can now be described as follows. Decrease in milk pH or, respectively, an increase in $[H^+]$ shifts, on the one hand, the equilibrium of reaction (6) to the left and, consequently, decreases (in absolute value) the polyelectrolyte brush's negative charge q_{CMP} (according to (7)). On the other hand, the increase in $[H^+]$ leads to the hydrogenation of the micellar colloid calcium phosphate complex according to scheme (8) and, hence, to an increase in calcium ion concentration. As a result of $[Ca^{2+}]$ growth, balance in scheme (4) 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.

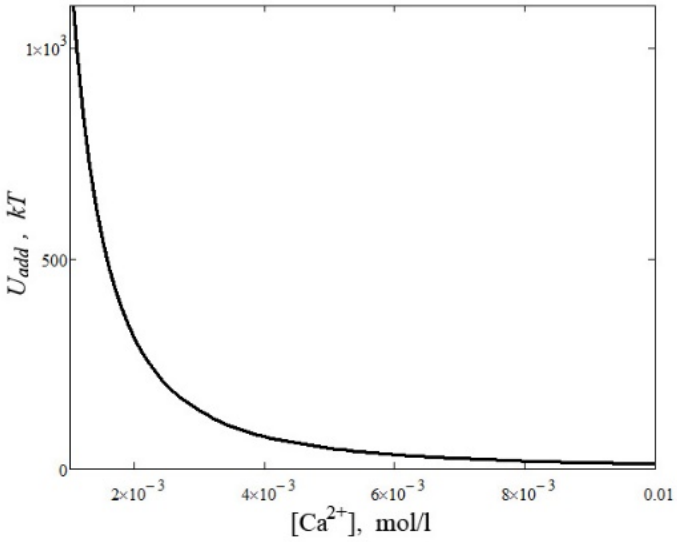

Figure 7. Dependence of Uadd on $[Ca^{2+}]$ (b).

Mechanisms of rennet and acid coagulation can be easily joined in our model to describe acid-rennet type of milk coagulation. One can just add proteolytic decrease of both q_{CMP} and q_{CAS}, as these are described by (11) and (12), to the equation set of (4)-(9).

3.3. Heat-acid and heat-calcium coagulation

One more hypothesis in addition to the scheme described above allows to explain some features of heat-acid and heat-calcium milk coagulation.

Let us assume that the equilibrium of reactions (4) and (8) 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 (8) such dependence is an established fact, while for reaction (4) 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.

Temperature increase leads then to a decrease in K_{CAS} and, as a consequence, to a decrease in the additional micelle charge, q_{CAS}. Therefore, for acid coagulation, temperature growth leads to higher pH values at which coagulation begins.

The direct use of the thermographic method to study the heat-acid or heat calcium coagulation process is complicated by the fact that the application of an acid or calcium chloride solutions to heated milk is accompanied by intensive stirring, which hinders structure formation. As a consequence, no strongly marked change in the temperature difference between the heated and unheated thermometers occurs. In order to solve this problem, we developed the following technique of studying heat-acid and heat calcium milk coagulation. Solutions of lactic acid or calcium chloride are added preliminarily into milk in amounts that admittedly do not cause coagulation at room temperature. The prepared samples are then heated until gel appears under control of thermographic device.

Thermogramms of this type show dependence of thermographic temperature difference on milk temperature. Like for thermogramms on Figure 2 increase in thermographic temperature difference is caused by coagulation structure hindering milk convection.

Figure 8 demonstrates the results of the experiment examining the dependence of temperature at which acid milk coagulation begins on the milk pH value.

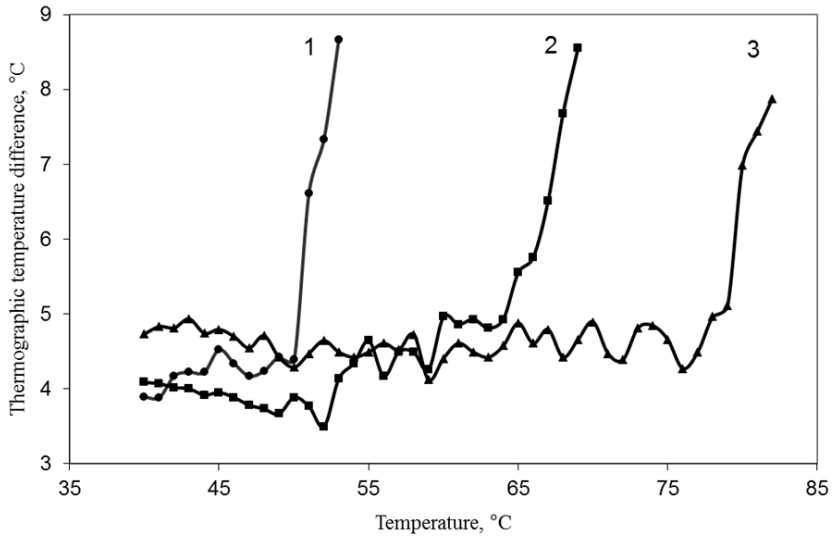

Figure 8. Thermograms of reconstituted skim milk samples subjected to heating after acidification. Curves:
1 – sample pH = 5.6;
2 – sample pH = 5.9;
3 – sample pH = 6.2.

As one can see in Figure 8, 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.

In addition, it is noteworthy that the developed approach, based on the analysis of the out-standing 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.

Within the described model, heat-acid and heat-calcium milk coagulation proceeds as follows. As milk is heated, equilibrium of reaction (8) shifts to the left resulting in increase of hydrogen ion concentration. As a consequence, it shifts of reaction (6) 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 (4) 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 (8) 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.

Figure 9 demonstrates the results of the experiment examining the dependence of temperature at which milk with different concentration of calcium ions begins to coagulate. As one can see, coagulation of sample with higher value of $[Ca^{2+}]$ begins at lower temperatures, as it is predicted by our hypothesis.

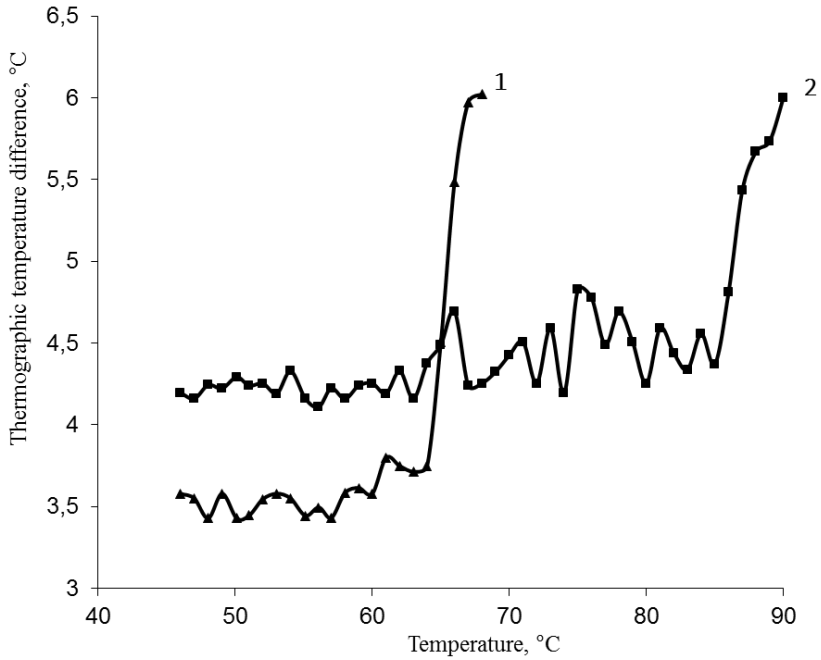

Figure 9. Thermograms of reconstituted skim milk samples subjected to heating after adding of $CaCl_2$. Curves:
1 – $[Ca^{2+}]$ = 4.8 mmol/L;
2 – $[Ca^{2+}]$ = 1.5 mmol/L.

3.4. Conclusion

Within the framework of a simplified model based on the hypothesis of additional micelle charge arising due to dissociation of micellar calcium caseinate we were able to explain the main features of rennet, acid and mixed acid-rennet coagulation of milk.

Using the concept of solvent quality, determined by the second osmotic virial coefficient, and reasonable estimates for the thermodynamic and kinetic parameters of the model we represented some quantative results describing coagulation of reconstituted skim milk enriched with calcium and magnesium ions. It was possible to obtain a quite adequate description of the experimental and explain the difference in the effect of calcium, magnesium and sodium ions on the coagulation of casein micelles.

Hypothesis describing the temperature dependence of equilibrium constant for dissociation of micellar calcium caseinate made it principally possible for us to explain the main features of heat-acid and heat-calcium coagulation of milk.

We believe that our model can be useful not only for scientists, but also for those technologists who wants to enhance .dairy thechnologies using fundamental principles of chemistry and physics.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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