

STUDY OF CALCIUM ROLE IN COLLOIDAL STABILITY OF RECONSTITUTED SKIM MILK UNDER RENNET COAGULATION CONDITIONS

A. M. Osintsev^{a,*}, A. P. Syrtseva^a, R. P. Kolmykov^b, V. I. Braginsky^a,
O. Yu. Lapshakova^a, and M. A. Osintseva^a

^aKemerovo Institute of Food Science and Technology (University),
Stroiteley blvd. 47, Kemerovo, 650056 Russian Federation

^bKemerovo State University,
Krasnaya Str. 6, Kemerovo, 650043 Russian Federation

* e-mail: olex1@mail.ru

Received February 07, 2016; Accepted unrevised form March 20, 2016; Published June 27, 2016

Abstract: The role of calcium in rennet coagulation of milk is unquestionable in production technology of many cheeses. Therefore, understanding the possible mechanism of calcium influence on the colloidal stability of casein micelles may be the key to control the process of milk coagulation. It is evident that calcium ions are involved in maintenance of milk coagulation stability, but the molecular mechanism of how these ions influence micellar caseins system is not fully known. Thus, the role of calcium in maintenance of the colloidal stability of milk is quite an urgent problem. Methodologically, the research was based on analysis of coagulation process of reconstituted skim milk, enriched with ions of calcium, magnesium and sodium. Milk whey separated from the clot after coagulation was investigated for sodium, magnesium, calcium and phosphorus. 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. The experiments showed that calcium and magnesium ions chemically connect to casein micelles. Using reasonable assessments for thermodynamic and kinetic parameters, we managed to get quite adequate description of the experimental data on the coagulation of reconstituted skimmed milk enriched with calcium and magnesium ions. It was stated that the equilibrium constants for the dissociation of magnesium and calcium caseinates should differ by more than two orders of magnitude. The authors demonstrated principal possibility of using the model to describe the rennet, acid and mixed acid-rennet clotting of milk.

Keywords: Reconstituted skim milk coagulation, casein micelles, colloidal stability of milk, the second virial coefficient

DOI 10.21179/2308-4057-2016-1-121-128

Foods and Raw Materials, 2016, vol. 4, no. 1, pp. 121–128.

INTRODUCTION

Coagulation of milk is an important process in the production of many food products. The basis of this process is the coagulation of milk casein, which can be caused by various reasons, for example by acids, proteolytic enzymes, certain salts, alcohols, or high temperature. Coagulative stability of milk and its change in the production process, of course, is due to features of the structure and functional properties of milk as a colloidal system. Understanding these features is the basis for the development of dairy products technology within the modern approach [1].

It is known that during rennet coagulation soluble calcium salt is added into milk with a reduced activity of calcium ions in order to produce clot of necessary density [2]. It is evident that calcium ions are involved in maintenance of milk colloidal stability, the

molecular mechanism of how these ions influence micellar caseins system is not fully known. Most researchers suggest that charge destabilization of micelles is the basis of calcium influence on the coagulation process [3, 4]. Native casein micelles have an electric charge arising in the dissociation or recombination caseins of different functional groups. In general, this charge has a negative sign, which manifests itself in a negative value ζ -potential of micelles in milk. ζ -potential value is associated with the ion composition of milk whey [5, 6]. Changing of ζ -potential may be due to the fact that ions dissolved in milk can either chemically connect to functional groups of caseins, altering the charge of micelles, or they form an electric double layer close to the surface of micelles without contacting them chemically, but shielding their electrostatic interaction.

The aim of this work is to study possible ways how calcium ions influence the stability of the colloidal micelle casein in reconstituted skimmed milk.

OBJECTS AND METHODS OF STUDY

The experimental part

The object of research is reconstituted skim milk. To get it 90 g of skim milk powder was dissolved in 910 ml of distilled water and then it was thoroughly stirred.

Calcium, magnesium, and sodium were added to reconstituted skimmed milk in the form of solutions of their chlorides. $MgCl_2$ and $CaCl_2$ solutions with a concentration of 1 M and $NaCl$ at a concentration of 3 M were used. Thus, 1 ml of solutions of calcium and magnesium chlorides contained 1 mmol of calcium and magnesium. The concentration of sodium chloride was chosen 3 times higher in order to get solutions of equal ionic strength.

After preparation the samples were kept at $6 \pm 2^\circ C$ for 18 hours. Activity of calcium ions was measured potentiometrically immediately after preparation and after storage in all samples by ion-selective electrode ELITE-041Ca (NICO Analyt).

Crystal microbial chymosin CHY-MAX (Chr. Hansen) was used as an enzyme preparation for coagulation of milk. To prepare the solution of the enzyme preparation 0.1 g of dry powder was dissolved in 100 cm^3 of distilled water.

Coagulation of milk samples was at $30^\circ C$ in thermostatted cell volume of which was 130 ml. Duration of clotting time was determined by time between adding of 1 ml of the enzyme preparation and the start of milk coagulation.

Our own thermographic method, described in detail in [7] was used to determine the moment of milk coagulation. The principle of method is to increase the temperature difference between heated and unheated thermometers immersed into milk during the process of forming a gel-like structure.

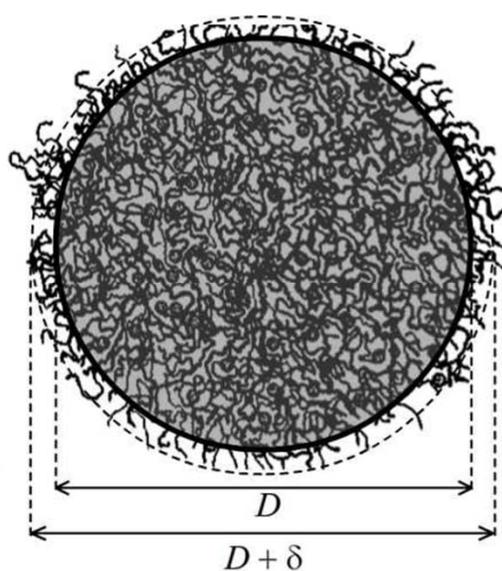
Indirect method for analyzing the concentration of calcium and magnesium in milk whey was used to determine the amount of added into the milk calcium and magnesium which were connected with the casein micelles. After milk coagulation clot was separated from milk whey in tubes of 50 ml with Centrifuge 5430 (Ependorf) for 25 minutes at 7800 rpm. Then 50 ml of the whey samples were dried in Mini Spray Dryer B-290 (BUCHI). The elemental composition of the obtained powder was spectroscopically investigated using an atomic emission spectrometer with inductively coupled plasma iCAP 6500 Duo LA (ThermoFisher Scientific).

Equipment for centrifuging and drying of samples was provided by the Research Institute of Biotechnology of Kemerovo Institute of Food Science and Technology (University).

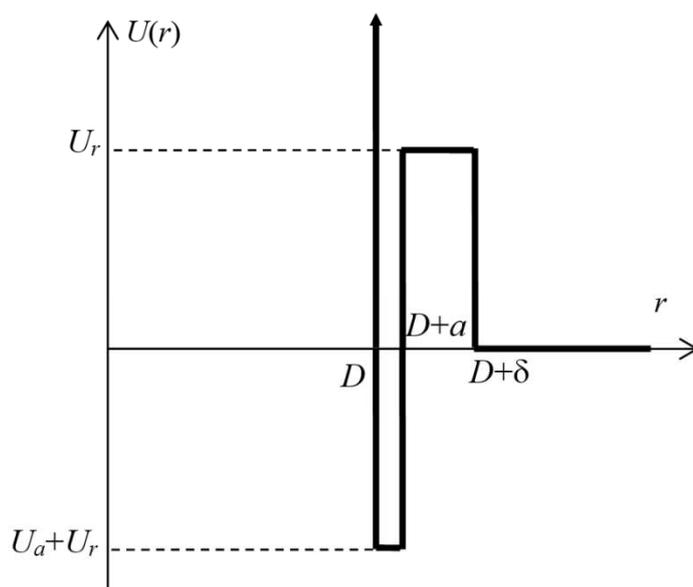
Determination of the elemental composition of samples of dried whey was carried out on the basis of Kemerovo Center for collective use, Kemerovo Research Center, Siberian Branch of the Russian Academy of Sciences.

The theoretical part

To simulate the experimental results we used the model of binary interaction of micelles based on the model of sticky hard spheres [8, 9] with two additional square potentials [10]. The total potential energy of binary interaction of micelles is shown schematically in Fig. 1.



(a)



(b)

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

It includes a completely solid wall at a distance D between the centers of micelles, a rather deep narrow “attractive” well of a -width and repulsive step of δ -width:

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

where

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

$$U_a(r) = \begin{cases} -U_0 + U_{add}, & r \leq D + a \\ 0, & r > D + a \end{cases}, \quad (2)$$

$$U_r(r) = \begin{cases} U_r, & r \leq D + \delta \\ 0, & r > D + \delta \end{cases}.$$

The repulsion, characterized by the potential energy of U_r , is due to the presence of elastic hairy layer of δ depth, consisting of macro-peptide residues of κ -casein, on the surface of micelles.

The attraction, characterized by potential energy $-U_0$, includes various interactions (van der Waals attraction, hydrophobic interactions, hydrogen bonds, etc.), which provide micelles adhesion at direct contact of surfaces.

Additional potential U_{add} describes the repulsion of micelles as a result of similar electrical charge originated from dissociation of micellar calcium caseinate. This repulsion obviously has a short-range character due to strong Debye shielding of protein molecules by ions contained in milk whey. At a sufficiently high degree of dissociation of calcium caseinates the potential of intermolecular attraction U_a can change the sign, becoming repulsive in this case.

Let's take the model of solvent quality, characterized by a second osmotic virial coefficient, to quantify the characteristics of the colloidal stability of the micellar system of skim milk:

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

where k is Boltzmann's constant and T is absolute temperature.

In the case of potential energy form of (2) integration has following results:

$$B_2 = \frac{2\pi}{3} \left\{ D^3 + (1 - e^{-U_a+U_r/kT}) [(D+a)^3 - D^3] + (1 - e^{-U_r/kT}) [(D+\delta)^3 - (D+a)^3] \right\},$$

As in [8, 9] let's have a look at the value of the specific excluded volume $\beta_2 = \frac{B_2}{V_{HS}}$, where $V_{HS} = \pi \frac{D^3}{6}$ is a solid sphere volume, and furthermore, we will neglect components containing $\frac{a^2}{D^2}$, $\frac{a^3}{D^3}$ and $\frac{\delta^2}{D^2}$, $\frac{\delta^3}{D^3}$:

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

The colloidal solution becomes unstable under condition $\beta_2 \approx -6$ [11, 12].

Kinetics of coagulation is determined by the fact that the potential energies U_r and U_{add} depend on time. These relationships are determined by the kinetics of changes in corresponding charges: a negative electric charge of κ -casein hairs q_{CMP} , proportional to the concentration of dissociated macro-peptide residues and additional negative electric charge of the micelles q_{CAS} , proportional to the concentration of dissociated molecules of calcium caseinates.

In the frame of approach presented in [10], the change in these charges is described by the following expressions:

$$q_{CMP} = -e \frac{[CMP^-]}{[M]} = \frac{-e}{[M]} \frac{K_{CMP}[CMP]_0}{K_{CMP} + [H^+]} \exp(-k_{CMP} \cdot t), \quad (5)$$

$$q_{CAS} = -2e \frac{[CAS^{2-}]}{[M]} = \frac{-2e}{[M]} \frac{K_{CAS}[CAS]_0}{K_{CAS} + [Ca^{2+}]} \exp(-k_{CAS} \cdot t). \quad (6)$$

In the expressions (5) and (6) $e = 1.6 \cdot 10^{-19}$ C is an elementary charge; $[M]$ is casein micelle concentration in milk; K_{CMP} is the equilibrium constant for dissociation reaction of macro-peptide residues of κ -casein ($CMP \leftrightarrow CMP^- + H^+$); K_{CAS} is the equilibrium constant for dissociation reaction of micellar calcium caseinates ($CaCAS \leftrightarrow Ca^{2+} + CAS^{2-}$); k_{CMP} is the rate constant for proteolysis of κ -casein by chymosin; k_{CAS} is the rate constant for additional non-specific proteolysis of α - and β -casein segments, containing phosphoserine groups, by chymosin; $[CMP]_0 = [CMP^-] + [CMP]$ is total concentration of macro-peptide residues of κ -casein; $[CAS]_0 = [CAS^{2-}] + [CaCAS]$ is total concentration of phosphoserine groups of α - and β -casein, which are capable of binding calcium.

When additional magnesium ions are added to milk the micelles charge associated with dissociation of native calcium caseinates, may also be reduced due to the shift of the equilibrium to the left in reaction $MgCAS \leftrightarrow Mg^{2+} + CAS^{2-}$. Denoting the equilibrium constant of this reaction K_{CAS}^* and taking into account the fact that the sum of the concentrations of charged $[CAS^{2-}]^*$ and uncharged $[MgCAS]$ phosphoserine residues of caseins after adding magnesium is equal to concentration of charged residues associated with the dissociation of native calcium caseinates $[CAS^{2-}]$, we have the following expression for the charge of micelles after adding magnesium:

$$q_{CAS}^* = -2e \frac{[CAS^{2-}]^*}{[M]} = \frac{-2e}{[M]} \frac{K_{CAS}^*}{K_{CAS}^* + [Mg^{2+}]} \frac{K_{CAS}[CAS]_0}{K_{CAS} + [Ca^{2+}]} \exp(-k_{CAS} \cdot t). \quad (7)$$

We will consider as a first approximation, as well as in [10] that potential energy associated with charges is proportional to squares of them:

$$U_r = Aq_{CMP}^2, \quad U_{add} = Aq_{CAS}^2. \quad (8)$$

Constant A will be pre-estimated of the following considerations. If one macro-peptide residue of κ -casein has, in average, $40\text{--}50\text{ nm}^2$ of micelle surface area [14], then their total number on the surface of micelle of radius 100 nm will be about 3000 . Let's suggest that at neutral pH each of them has one elementary charge. Coagulation of casein in milk begins when the degree of proteolysis of κ -casein is $80\text{--}90\%$. We assume that when the charge of micelles as a result of proteolysis reduced by 10 times the energy of molecular repulsion U_r , becomes of order of kT . This means that the maximum energy of micelles repulsion, associated with the elastic hairy layer, is about $100kT$. Then it follows from (8) that $kT \approx A(0.1 \cdot 3000e)^2$ or

$$A \approx \frac{kT}{10^5 e^2}.$$

Similarly, we can estimate the equilibrium constant K_{CMP} for the dissociation reaction of macro-peptide residues of κ -casein. If we assume that at $\text{pH} \approx 5$ repulsion energy is becoming of order of kT , then the charge of the hairy layer is reduced by about 10 times.

Then, according to (5) $\frac{K_{\text{CMP}}}{K_{\text{CMP}} + 10^{-5}} \approx 0.1$. Then $K_{\text{CMP}} \approx 1.1 \cdot 10^{-6}\text{ M}$. In this case, as it is seen from (5), at $\text{pH} \approx 7$ almost all macro peptide residues are dissociated.

For a preliminary estimation of K_{CAS} we assume that, in accordance with generally accepted notions [15], the micelle has a mass of about $5 \cdot 10^8\text{ Da}$ and, on average, it consists of $2 \cdot 10^4$ casein molecules with mass $2.5 \cdot 10^4\text{ Da}$. If each molecule of casein has an average of 7.5 phosphoserine groups (from 1 for κ -casein and to 15 for α -casein) which can reversibly connect calcium ions, then their concentration is $[\text{CAS}]_0 = 1.5 \cdot 10^5\text{ [M]}$. According to our preliminary estimations, if the concentration of calcium ions in milk whey is approximately $[\text{Ca}^{2+}] = 10\text{ mM}$, then the additional stabilization effect disappears [10]. It means that an additional charge of micelles q_{CMP} decreases, as in the case of estimation of q_{CAS} , lower than, approximately, $300e$. Taking into account that each dissociated group has a charge equal in absolute value to $2e$, we get from

$$(6): \frac{1.5 \cdot 10^5 K_{\text{CAS}}}{K_{\text{CAS}} + 10^{-2}} \approx 150. \text{ Then } K_{\text{CAS}} \approx 1 \cdot 10^{-5}\text{ M. When}$$

the concentration of calcium ions in the whey decreases to $[\text{Ca}^{2+}] \approx 1\text{ mM}$, the additional charge of q_{CAS} micelles increases 10 times, and additional repulsion energy, according to (8), becomes $100kT$. As it is known from [16], with such values of the concentration of calcium ions coagulation of milk does not start even after the proteolysis of κ -casein micelles on the surface is completed. Therefore it is reasonable to estimate the depth of the potential binding $U_0 \approx 100kT$.

Let's estimate the rate constants for proteolysis previously as follows. The time during which the unit dose of chymosin leads milk to the beginning of coagulation process in case of a substantial excess of

calcium ions in whey, i.e. the time of reaching the level of proteolysis of κ -casein on the surface of micelles 90% , is about 5 minutes. Therefore, $\exp(-k_{\text{CMP}} \cdot 5) \approx 0.1$. Then $k_{\text{CMP}} \approx 0.2 \ln(10) \approx 0.5\text{ min}^{-1}$. Coagulation of milk deficient in calcium ions begins only in a few hours. For the initial estimation we assume this time equal to 200 minutes. If we assume this slowdown is due to non-specific proteolysis, reducing the additional charge of the micelles associated with the dissociation of calcium caseinates, then $\exp(-k_{\text{CAS}} \cdot 200) \approx 0.1$. Therefore, $k_{\text{CAS}} \approx 0.005 \ln(10) \approx 0.012\text{ min}^{-1}$.

These preliminary estimates may be corrected while simulating the experimental data.

RESULTS AND DISCUSSION

Fig. 2 shows the results of experiments on milk coagulation with different content of soluble calcium. A solution of calcium chloride was added so that the concentration of calcium added further was $4, 8, 16$ and 32 mM . In all cases, the activity of calcium ions was determined by an ion-selective electrode immediately after preparation of milk samples and after their holding at a temperature of about 6°C for 18 hours. The accuracy of measurement of calcium ion activity was not high due to the complex composition of milk and the presence of interfering ions, especially Mg^{2+} . Perhaps a significant impact on the accuracy was due to the deposition of milk proteins on the measuring diaphragm. In any case, within the sensitivity of the method it was stated that the activity of calcium ion reached an equilibrium value within a few minutes after adding calcium chloride and remained practically unchanged after keeping milk for 18 hours.

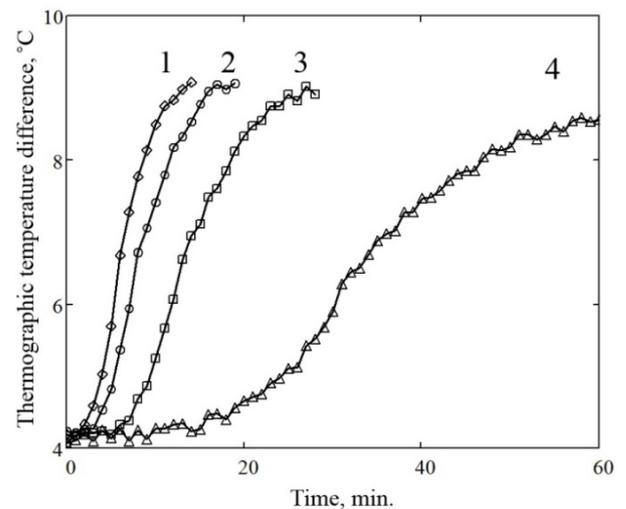


Fig. 2. Thermograms of rennet coagulation of reconstituted skim milk with additional Ca^{2+} . Curves: 32 mmol of CaCl_2 added to 1 l of milk sample (1); 16 mmol of CaCl_2 added to 1 l of milk sample (2); 8 mmol of CaCl_2 added to 1 l of milk sample (3) and 4 mmol of CaCl_2 added to 1 l of milk sample (4).

In this series of experiments, we also prepared samples of milk with different contents of sodium ions. The sodium chloride solution was added so that the concentration of additional sodium was 12, 24, 48 and 96 mM. These samples were also held at a temperature of about 6° C for 18 hours. None of the prepared samples of reconstituted skim milk coagulated under the influence of chymosin during an hour. Possibly reaction $\text{Na}_2\text{CAS} \leftrightarrow 2\text{Na}^+ + \text{CAS}^{2-}$ has a very high value of the equilibrium constant, thus sodium ions are hardly connected with caseins under normal conditions.

Fig. 3 shows the results of experiments on the milk coagulation with different content of soluble magnesium. Magnesium chloride solution was added so that concentration of added calcium was 8 and 16 mM. As it can be seen from the figures, coagulation stability of these samples is comparable to the stability of the milk samples enriched with calcium ions in the same ratio. However, the coagulation duration for samples with magnesium is observably longer than the coagulation duration with the same amount of added calcium. This fact makes it possible to estimate the value of the equilibrium constant for the reaction $\text{MgCAS} \leftrightarrow \text{Mg}^{2+} + \text{CAS}^{2-}$ slightly higher than for the reaction $\text{CaCAS} \leftrightarrow \text{Ca}^{2+} + \text{CAS}^{2-}$.

To determine the accurate proportion of added calcium and magnesium connected with caseins in milk

samples, we measured the elemental composition of centrifuged whey for each sample. The results of whey spectroscopic studies showing the content of calcium, magnesium and phosphorus in whey samples are presented in Table 1.

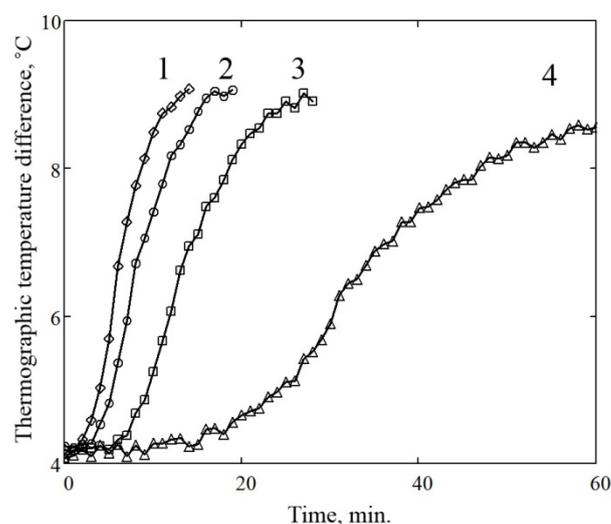


Fig. 3. Thermograms of rennet coagulation of reconstituted skim milk with additional Ca^{2+} . Curves: 32 mmol of CaCl_2 added to 1 l of milk sample (1); 16 mmol of CaCl_2 added to 1 l of milk sample (2); 8 mmol of CaCl_2 added to 1 l of milk sample (3) and 4 mmol of CaCl_2 added to 1 l of milk sample (4).

Table 1. Content of Ca, Mg and P in serum samples

Conditions	Element	Content, mM					
		4.0 ± 0.1	8.0 ± 0.2	16.0 ± 0.4	32.0 ± 0.8	0	0
Added	Ca	4.0 ± 0.1	8.0 ± 0.2	16.0 ± 0.4	32.0 ± 0.8	0	0
	Mg	0	0	0	0	8.0 ± 0.2	16.0 ± 0.4
Detected	Ca	5.6 ± 0.2	5.7 ± 0.2	6.5 ± 0.2	9.1 ± 0.2	4.3 ± 0.2	3.7 ± 0.2
	Mg	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	6.4 ± 0.1	8.9 ± 0.1
	P	7.4 ± 0.2	6.8 ± 0.2	5.9 ± 0.2	5.5 ± 0.2	6.7 ± 0.2	6.0 ± 0.2
Adapted	Ca	2.8	3.6	5.4	8.5	2.3	2.5
	Mg	1.4	1.5	1.4	1.5	6.1	8.7

As it is seen from the table, adding of a certain amount of calcium or magnesium does not increase additively their content in milk whey. This fact clearly confirms the interconnection of some part of added substances with casein micelles. It was noted above that the interchange of calcium between whey and micelles occurs quickly enough. This interchange is difficult to relate to poorly soluble colloidal calcium phosphate. In our opinion, it can reflect exactly the calcium and magnesium interchange between whey and casein according to the scheme described above.

It should be marked that the amount of calcium and magnesium detected in whey need some correction. The table shows that the detected amount of phosphorus in various samples clearly correlates with the amount of added soluble calcium or magnesium: the more ions Ca^{2+} или Mg^{2+} we add to the milk sample, the less of phosphorus we find in whey after its separation from the clot. Apparently, some part of detected phosphorus belongs to micelles left in whey as a result of incomplete coagulation. The obtained data

were processed further to consider calcium and magnesium associated with such micelles. The corrected values are shown in Table 1 in the column "Adapted". Additional calcium remained in whey with non-coagulated micelles was considered as follows. The extrapolated value of the phosphorous concentration in whey at full coagulation of casein was based on experimental data evaluated as $[\text{P}]_0 \approx 5$ mM. The average ratio between calcium and phosphorus in dry milk is about 1.2:1.0. Therefore, some calcium connected with micelles was taken away from calcium concentration directly detected in whey:

$$[\text{Ca}^{2+}]_{\text{adapted}} = [\text{Ca}^{2+}]_{\text{detected}} - 1.2 \cdot ([\text{P}]_{\text{detected}} - [\text{P}]_0).$$

Similarly values of magnesium concentration was processed, but in this case the role of the correction was much smaller because of the significantly lower content of magnesium in micelles.

We should also mark that the clot quality was higher when adding equal amounts of calcium rather than magnesium. Indeed, the phosphorus content in

they samples enriched in magnesium is higher, than in similar samples enriched in calcium. Probably, as it was mentioned above in the analysis of Fig. 3, it is connected with a greater tendency to magnesium caseinate dissociation.

We used processed values of calcium and magnesium concentrations in milk whey samples to simulate the experimental data in order to clarify the basic parameters of the model.

Fig. 4 shows the results of calculation of the second osmotic virial coefficient (4), which characterizes the colloidal stability of the micellar caseins system depending on time for different values of added soluble calcium. Potential energy calculations were carried out according to the formulas (5), (6) and (8). The parameters used for the simulation were: $A = \frac{4.8kT}{10^5 e^2}$,

$$K_{CMP} = 1.2 \cdot 10^{-6} M, \quad [CMP]_0 = 3000[M], \quad [H^+] = 10^{-6.7} M, \quad [CAS]_0 = 1.5 \cdot 10^5 [M], \quad K_{CAS} = 1.7 \cdot 10^{-5} M, \\ U_a = 70kT, \quad k_{CMP} = 0.15 \text{ min}^{-1}, \quad k_{CAS} = 0.014 \text{ min}^{-1}.$$

It is easy to notice that the obtained parameters are not too much different from the estimations given in the previous section. However, calculations of the data presented in Fig. 4 are in satisfactory agreement with the experimental results in Fig. 2 for coagulation of skim milk samples of enriched in calcium. If we assume that the coagulation time t_C is achieved at the maximum value of thermal temperature difference rise rate, then according to the analysis of curves in Fig. 4 we can obtain following clotting time values: $t_C = 6 \pm 1$ min for the curve 1; $t_C = 8 \pm 1$ min for the curve 2; $t_C = 13 \pm 2$ min for the curve 3 and $t_C = 32 \pm 2$ min for the curve 4. On the other hand, as it was noted in the previous section, if we consider achieving value $\beta_2 = -6$ by the second osmotic virial coefficient as the moment of stability loss for colloidal system, then we can see a good match.

Similar calculations were made for data simulation, shown in Fig. 3. The calculation results of the second osmotic virial coefficient (4) using equations (5), (7) and (8) are shown in Fig. 5. All common parameters used for the simulation coincide with the parameters used in the previous case. Moreover, it was believed that the calcium ion concentration was the same for both samples and equal to the average of the data from Table 1: $[Ca^{2+}] = 2.4 \cdot 10^{-3} M$. The obtained equilibrium constant for the dissociation of magnesium caseinate was equal $K_{CAS}^* = 1.15 \cdot 10^{-2} M$. As it was expected, this value was significantly (almost 700 times) bigger than the equilibrium constant for dissociation of calcium caseinates.

We should mark a good coincidence of experimental and calculated values of coagulation time. Analysis of the rate of rise of thermal temperature difference for curves in Fig. 3 allows us to obtain the following values of the coagulation time: $t_C = 10 \pm 2$ minutes for the curve 1 and $t_C = 15 \pm 2$ minutes for the curve 2, which coincides with the results shown in Fig. 5.

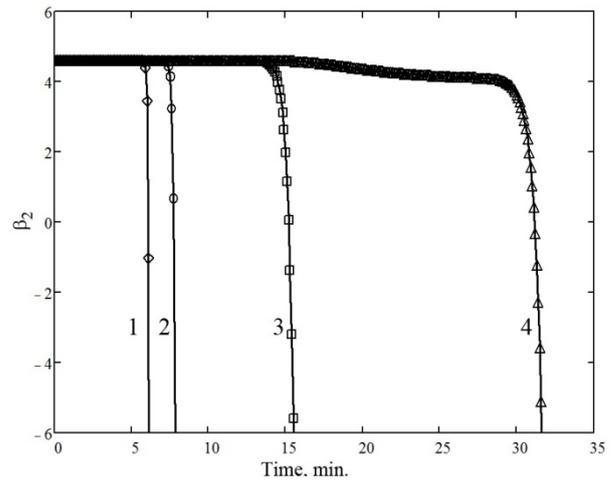


Fig. 4. Calculated values of β_2 resulted from fitting to curves 1, 2, 3 and 4 in Fig 2. See text for details.

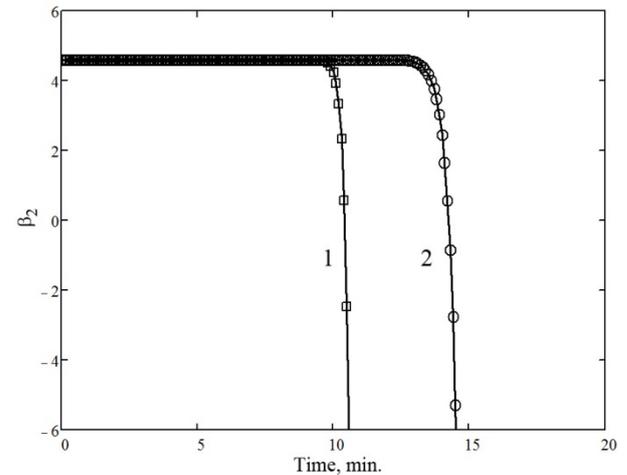
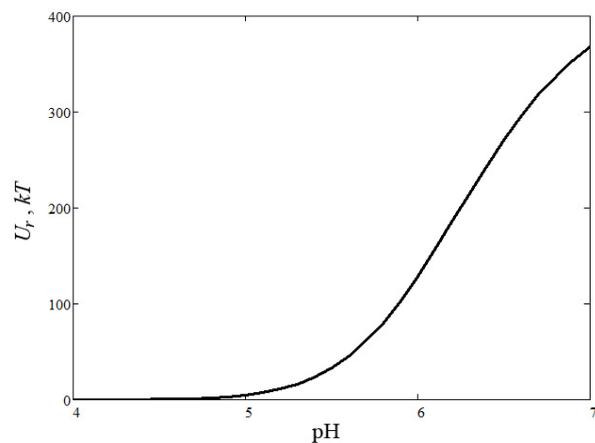


Fig. 5. Calculated values of β_2 resulted from fitting to curves 1 and 2 in Fig 3. See text for details.

We underline one more time that all the calculations for samples enriched in calcium and for samples enriched in magnesium were carried out in the framework of a unified model with the same set of parameters evaluated in the framework of obvious assumptions. Basically, this model easily explains the negative result of coagulation of milk with the addition of sodium chloride. If the equilibrium constant for dissociation of sodium caseinate is substantially higher than of magnesium, it is not possible to coagulate milk adding reasonable amounts of sodium chloride within a reasonable time.

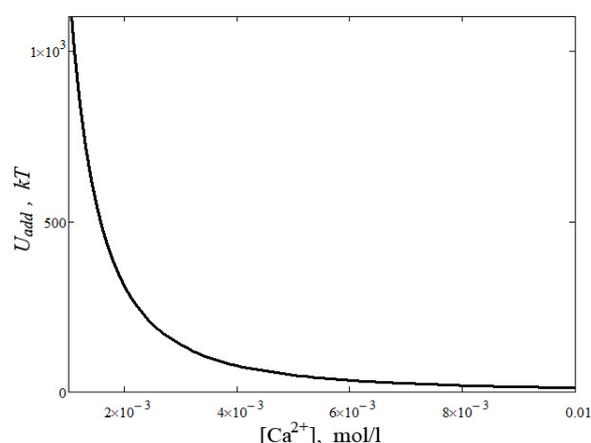
Another advantage of this model is possibility to describe acidic and mixed acid-rennet coagulation of milk. It will be enough to add scheme characterizing the dependence of the solubility of colloidal calcium phosphate on $[H^+]$ concentration to the existing equations. For example, during acid coagulation the repulsive energy of elastic polyelectrolytic brushes from macro peptide residues of κ -casein on the micelles surface decreases with increasing

concentration of $[H^+]$ (Fig. 6a). On the other hand increase in acidity leads to the dissolution of the colloidal calcium phosphate and, consequently, to



(a)

increase of ion $[Ca^{2+}]$ concentration. As a result (Fig. 6b) additional repulsion decreases, and with $pH \approx 5$ the colloidal stability of casein micelles in milk loses.



(b)

Fig. 6. Dependence of U_r on pH (a) and dependence of U_{add} on $[Ca^{2+}]$ (b).

The parameters for the calculation of the curves in Figure 6 are the same as for the calculation of the curves in Fig. 4 and Fig. 5.

CONCLUSION

We have explained the possible mechanism of how calcium ions influence on the process of milk coagulation within the framework of a simple quantitative model which uses the concept of solvent quality, determined by the second osmotic virial coefficient. Using reasonable estimations for thermodynamic and kinetic parameters of the model we could obtain an adequate description of the experimental data on the coagulation of reconstituted skim milk enriched in calcium and magnesium ions. The difference in the effect of calcium, magnesium and sodium in the coagulation of casein micelles has been explained. The principal possibility of using the model

to describe rennet, acid and mixed acid-rennet coagulation of milk has been shown. Basically, this calculation method may also be used to quantify the magnesium ion content in products produced by coagulation of milk enriched in magnesium ions for special nutrition intended for magnesium deficiency states.

ACKNOWLEDGEMENTS

The authors are grateful to C.G. de Kruif, professor of Applied Physical and Colloid Chemistry at Utrecht University and Research Strategist at NIZO food research, for his fruitful advices on solvent quality formalism.

We also appreciate our cooperation with S.A. Sozinov, Director of Kemerovo Center for Shared use of Scientific Equipment of Siberian Branch of Russian Academy of Sciences.

REFERENCES

- Ostroumov L.A., Osintsev A.M., and Braginsky V.I. Development of fundamentals of cheese making technology. *Proceedings of the Armenian Agricultural Academy*, 2003, vol. 3/4, pp. 150–153 (In Russian).
- Fox P.F., Guinee T.P., Cogan T.M., and McSweeney P.L.H. *Fundamentals of Cheese Science*. Aspen Publishers, Inc., 2000. 639 p.
- Udabage P., McKinnon I.R., and Augustin M.A. Effects of mineral salts and calcium chelating agents on the gelation of renneted skim milk. *Journal of Dairy Science*, 2001, vol. 84, pp.1569–1575.
- Tsioulpas A., Michael J.L., and Grandison A. S. Effect of minerals on casein micelle stability of cows' milk. *Journal of Dairy Research*, 2007, vol. 74, pp. 167–173.
- Rabiller-Baudry M., Gesan-Guizou G., Roldan-Calbo D., Beaulieu S., and Michel F. Limiting flux in skimmed milk ultrafiltration: Impact of electrostatic repulsion due to casein micelles. *Desalination*, 2005, vol. 175, pp. 49–59.
- Zhao Z. and Corredig M. Changes in the physico-chemical properties of casein micelles in the presence of sodium chloride in untreated and concentrated milk protein. *Dairy Science and Technology*, 2015, vol. 95, no. 1, pp. 87–99.
- Osintsev A. Theoretical and practical aspects of the thermographic method for milk coagulation research. *Food and Raw Materials*, 2014, vol. 2, no. 2, pp. 147–155. doi: 10/12737/5473.
- De Kruif C.G. The turbidity of renneted skim milk. *Journal of Colloid and Interface Science*, 1993, vol. 156, pp. 38–42.
- De Kruif C.G. Supra-aggregates of casein micelles as a prelude to coagulation. *Journal of Dairy Science*, 1998, vol. 81, pp. 3019–3028.

10. Osintsev A.M., Gromov E.S., and Braginsky V.I. A phenomenological model of milk coagulation. *Food and Raw Materials*, 2013, vol. 1, no. 1, pp. 11–18. doi: 10/12737/1512.
11. Tuinier R. and de Kruif C.G. Stability of casein micelles in milk. *Journal of Chemical Physics*, 2002, vol. 117, no. 3, pp. 1290–1295.
12. Vliegthart G.A. and Lekkerkerker H.N.W. Predicting the gas-liquid critical point from the second virial coefficient. *Journal of Chemical Physics*, 2000, vol. 112, no. 12, pp. 5364–5369.
13. De Kruif C.G. and Zhulina E.B. κ -Casein as a polyelectrolyte brush on the surface of casein micelles. *Colloids Surfaces A*, 1996, vol. 117, pp. 151–159.
14. Dalgleish D.G. Casein micelles as colloids: Surface structure and stabilities. *Journal of Dairy Science*, 1998, vol. 81, pp. 3013–3017.
15. Walstra P., Jenness R., and Badings H.T. *Dairy chemistry and physics*. Wiley, 1984. 467 p.
16. Tsioulpas A., Michael J.L., and Grandison A.S. Effect of Minerals on Casein Micelle Stability of Cows' Milk. *Journal of Dairy Research*, 2007, vol. 74, pp. 167–173.



Please cite this article in press as: Osintsev A.M., Syrtseva A.P., Kolmykov R.P., Braginsky V.I., Lapshakova O.Yu., and Osintseva M.A. Study of calcium role in colloidal stability of reconstituted skim milk under rennet coagulation conditions. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 121–128. doi: 10.21179/2308-4057-2016-1-121-128.

