# Dairy Chemistry and Physics

**Pieter Walstra**
*Department of Food Science*
*Agricultural University*
*Wageningen, Netherlands*

**Robert Jenness**
*Department of Biochemistry*
*University of Minnesota, St. Paul*

*With a contribution by H. T. Badings*

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The casein micelles also will dissolve when the submicelles dissociate. The molecules in the latter are held together mostly by hydrophobic interaction and by H bonds in the hydrophobic interior of the submicelle. Consequently, addition of large quantities of urea or guanidinium chloride dissolve the micelles, and much smaller quantities of sodium dodecyl sulfate do the same. Electrostatic interactions (i.e., internal salt bridges) also participate in keeping the submicelles together, but they cannot be broken without dissolving the calcium phosphate. Reagents that break –S–S– linkages do not disintegrate the micelles fully, as is to be expected, but it is not known whether less rigorous changes occur.

*Lowering the temperature* (e.g., to 5°C) considerably affects the casein micelles. Hydrophobic interaction becomes much weaker, and part of the casein, particularly of the β-casein, dissociates from the micelles. The voluminosity of the micelles increases, probably in part from increased "hairiness," as β-casein chains may protrude from the micelle surface. A small part of the calcium phosphate dissolves. (See Section 4.5.) These changes may be the cause of the slight disintegration of the micelles; despite the increase in voluminosity, their average size decreases somewhat (e.g., by 15%).

## 13.2. COLLOIDAL STABILITY

The stability of casein micelles is a somewhat confusing subject. Several different treatments may lead to aggregation of the micelles. If the cause if a change in environment, such as pH or salt content, the lack of stability usually can be explained in terms of colloid science; the aggregation can be reversed by restoring the original environment. Aggregation also may be a sequel to a chemical change in the casein micelles; examples are renneting (Section 13.3), age gelation (Section 13.4), and heat coagulation (Section 13.5). In these cases, the aggregation is usually irreversible. Still, the colloidal aspects also need to be considered, as they often interact with other factors in the ultimate stability.

Another problem lies in the definition of stability. Some treatments cause a (partial) dissolution of the casein micelles and may make the milk more stable, but it cannot be stated that the micelles have become so. Other treatments may cause casein micelles to fuse into larger ones; often there is no outward indication of a decreased stability, but a slight change in conditions now may cause the micelles to form a precipitate or a gel.

A third confusing aspect lies in the many factors or treatments that affect the stability and in the fact that most treatments cause more than one change in the micelles. Consequently, a quantitative explanation of casein micelle stability cannot be given.

A caseinate solution is comparatively stable. Boiling, for instance, causes no significant change. Whole casein precipitates at its isoelectric pH (≈4.6),

it loses its net charge and forms internal salt bridges, and its high hydrophobicity then will make the casein insoluble. Casein can be salted out, like most proteins. It is relatively sensitive to $Ca^{2+}$ ions, and a $Ca^{2+}$ activity on the order of 50 m$M$ causes precipitation. Probably, $Ca^{2+}$ makes the casein much more hydrophobic by screening the ester phosphate groups. The stability depends much on casein composition, markedly increasing with increasing proportion of $\kappa$-casein. See further Section 6.3, where the differences between the various caseins also are discussed.

Casein micelles are less stable than caseinate solutions. They are relatively large particles, and Van der Waals attraction becomes important. The decrease in conformational entropy on aggregation is less than in the case of caseinate. Still, the micelles are usually stable under conditions as in fresh milk. When they are sedimented by high-speed centrifuging, they form a gel-like pellet, but this gel can be redispersed again in the supernatant.

The stability of the micelles usually correlates well with their voluminosity $\nu$. This may be because a higher $\nu$ may correspond with a more extensive hairiness, hence to a stronger steric repulsion. (See Section 12.2.4.) But $\nu$ also may be correlated with the compactness of the micelle core, and Van der Waals attraction (Section 12.2.3) then will increase strongly with decreasing $\nu$, because the Hamaker constant is in first approximation proportional to $\nu^{-2}$. Taking the change in radius also into account, the attraction energy would be proportional to $\nu^{-5/3}$. Another factor in micelle stability is presumably electrostatic repulsion. The zeta potential is, for instance $-13$ mV at 20°C and pH 6.7; its absolute value increases with temperature (e.g., 0.3 mV·K$^{-1}$) and with pH (e.g., 0.4 mV per unit).

Without calcium phosphate, casein submicelles do not form micelles, and the latter are generally less stable if they contain more calcium phosphate. This may be the result of the correlation with micelle size and voluminosity. Generally, a high citrate content of the milk correlates with a low content of calcium phosphate in the micelles. In some regions a cow occasionally may give milk that spontaneously curdles after milking, the so-called Utrecht milk abnormality. It is probably related to an excessive concentration of colloidal calcium phosphate.

There are considerable differences in stability among lots of milk, and there are effects of season (perhaps stage of lactation) and individual cows. Differences in casein composition, content of calcium phosphate, micelle size and voluminosity, and $Ca^{2+}$ activity all may play a part; these variables, of course, are correlated to some extent.

Several factors influence casein micelle stability:

1. Salt composition affects $Ca^{2+}$ activity of the serum and calcium phosphate content of the micelles. These variables are closely related, and it is not known which of them is the more important factor affecting stability. Examples of the effect of various additions are in Table 4.5. Addition of $CaCl_2$, for instance, raises $Ca^{2+}$ activity and

micellar calcium phosphate and decreases pH, all of them being detrimental to stability. Adding NaCl will increase ionic strength somewhat and decrease calcium phosphate content of the micelles; it usually increases stability. Sodium citrate addition enhances stability. Sodium phosphate may work either way: It lowers $Ca^{2+}$ activity, increases phosphate in the micelles, and may affect pH.

2. Lowering the pH leads at first to dissolution of colloidal calcium phosphate (Figure 4.1) and a decrease in micelle voluminosity; some dissolution of micelles may also occur. A still lower pH ($< 5.5$) leads to enlargement of casein micelles, as micelles fuse. This may be caused by loss of surface potential. Figure 12.4 shows that the (negative) zeta potential rises to about zero near pH 5.2. Most but not all of the colloidal phosphate is now lost. At still lower pH more phosphate dissolves, and the casein starts to precipitate, though zeta potential falls again. Presumably, the colloidal calcium phosphate is positively charged in this region, while the casein still has a net negative charge. Near pH 4.8 almost all phosphate is dissolved, and near pH 4.6, the isoelectric point, the solubility of casein is negligible. Now, casein rather than casein micelles precipitates. Acid precipitation is applied commonly to collect casein from skim milk.
3. Temperature has a large effect. Near 0°C, aggregation of casein micelles is very difficult to achieve. Aggregation rate (for conditions where the micelles are unstable) has a $Q_{10}$ of 2–5, according to type of instability and other conditions. Lowering the temperature increases voluminosity, probably increases steric repulsion ($\beta$-casein chains now may protrude from the micelle surface), slightly increases $Ca^{2+}$ activity, somewhat decreases calcium phosphate in the micelles (as solubility increases), and somewhat decreases average micelle size. For increasing temperature these trends are reversed. Temperature also affects the nature of the gel or precipitate formed by acidification. Lower temperatures give a finer, more voluminous precipitate (when the milk is stirred during acidification), and a weaker gel (when the milk is kept still).
4. Heat treatment of the milk to such an extent that the greater part of the whey proteins become associated with the casein micelles (Chapter 10) alters the behavior of the micelles, for instance, on acidification. Fusing of micelles below pH 5.5 is far less, and the gel obtained near pH 4.5 is firmer. Other effects of heat treatment on properties of casein micelles are discussed in Section 13.4 and 13.5; they cannot be explained satisfactorily.
5. Dehydration, for instance, by adding ethanol, leads to aggregation of the micelles. Presumably, the solvent quality for the protruding chains of $\kappa$-casein is lowered, causing a decrease in voluminosity and in steric repulsion. Ethanol to a concentration of 50–70% can be added to normal fresh milk before coagulation is observed. A similar effect

occurs on adding large quantities of salts. The aggregation of casein micelles in frozen milk products also seems to be caused by salting out. (See Section 17.4.)

Many of the effects causing instability are additive, for instance, low pH and ethanol. This principle has been applied in the alcohol stability test to detect sour milk; the lower the pH, the lower the ethanol concentration needed to cause coagulation. Another example is the decreased micelle stability in concentrated milk. Removal of water leads to a lower pH (hence a loss of surface potential), a higher $Ca^{2+}$ activity, a higher ionic strength (hence a lower solvent quality and a thinner diffuse double layer), and a higher concentration of colloidal calcium phosphate in the micelles. All of these factors lessen stability. In evaporated milk the micelles have become much larger (up to a new micrometers in diameter), and further concentration eventually leads to gel formation.

Casein micelles may interact with other substances, affecting their stability:

1. Some hydrocolloids, particularly $\kappa$-carrageenan, bind to casein micelles. Electrostatic interaction of the negative carrageenan with positively charged regions of $\kappa$- and $\alpha_{s2}$-casein is involved. (See also Section 16.2.) Carboxymethyl cellulose interacts, possibly in a similar way, at pH 7.5. By adding an excess of such hydrocolloids, the casein can be precipitated and separated from skim milk.
2. Most surfactants bind to proteins, largely through hydrophobic interaction. Small quantities of nonionic surfactants (< 5 mol per mole of casein) have little effect, but cationic ones (e.g., quaternary ammonium compounds) decrease the absolute value of the zeta potential of the micelles, thereby somewhat decreasing their stability. Anionic surfactants (e.g., sodium dodecyl sulfate) have the opposite effect; free fatty acids do the same, but they also may enhance stability by binding $Ca^{2+}$. Many surfactants, when present in high concentration, disintegrate protein aggregates, hence casein micelles.
3. Casein micelles may interact with oil–water and air–water interfaces, for instance, during homogenization and foaming. Some interaction with fat globules is almost unavoidable; consequently, casein preparations always contain some fat (say, 1%), even after washing.

## 13.3. RENNETING

Many proteolytic enzymes are able to clot milk; this means that the milk forms a gel some time after a preparation containing the enzyme has been added. The most used preparation is calf rennet, the active principle of which is chymosin.

Two stages can be distinguished in clotting or renneting:

virtually stops. This is comparable in cause to the much diminished flocculation rate of paracasein micelles at low temperature.

Syneresis (e.g., expressed as the amount of whey expelled) is at first about proportional to $t^{0.7}$ where $t$ is time after cutting the curd. Later on the rate decreases. There is, of course, a lowest possible moisture content. At high temperature (~ 35°C) and low pH (~ 5.2) the lowest obtainable ratio of water to paracasein is about 1.2 (if no fat globules are present), which means that the curd has shrunk to about a fifteenth of its original volume. If the curd is kept at milk pH, shrinkage to about a third of the volume can be achieved. Moisture content after syneresis thus depends primarily on temperature, pH, pressure gradients applied, and fat content. Fat globules, acting as passive spacers, hinder further shrinking. In practice, syneresis usually is stopped at the desired level by lowering the temperature.

Often, we want the shrunken curd grains to fuse into a more or less homogeneous and coherent mass. Close contact between curd grains can be achieved by pressing them together. But for the actual fusing new bonds between paracasein micelles have to be formed. This is possible only if the pH still is decreasing while the grains are pressed together. Pressing after the pH has obtained its final value does not produce a coherent mass. Adding $CaCl_2$ to the milk promotes curd fusion.

### 13.3.5. Effect of Heating

A heat treatment of greater intensity than low pasteurization, thus involving denaturation of whey proteins, causes an increase in rennet clotting time, a weaker curd, and impaired syneresis. If the heat treatment is severe (e.g., 30 min at 90°C) the milk does not clot at all. The clotting time becomes even longer when the heated milk is kept longer and at a lower temperature before adding rennet. Addition of $CaCl_2$ can restore the clotting characteristics of the milk to some extent, if the heating was not too severe. If no whey proteins are present during heating, rennet action is not affected.

The explanation of these observations is probably as follows. $\beta$-lactoglobulin is heat denatured and reacts with $\kappa$-casein. The $\kappa$-casein thus altered is not (or is less) sensitive to cleavage of the Phe–Met bond by chymosin; this pertains in particular to those $\kappa$-casein molecules which are poor in carbohydrate. Consequently, rennet action is incomplete, and the casein micelles remain fairly insensitive to $Ca^{2+}$. On the other hand, the heat-denatured $\beta$-lactoglobulin and most other whey proteins become associated with the casein micelles. (This leads to an increase in cheese yield, if the milk still will clot.) Heat-denatured $\beta$-lactoglobulin is very sensitive to $Ca^{2+}$. Moreover, the calcium phosphate content of the micelles increases appreciably because of the heat treatment. Consequently, the higher stability caused by the diminished cleavage of $\kappa$-casein is compensated for to some

extent by other factors. The dissolution of the excess calcium phosphate during cold storage (see Section 4.5) diminishes the compensating effect.

## 13.4. AGE GELATION

When sterilized concentrated milk products are kept they often gel, for instance, after one to twenty-four months. Usually, the viscosity of the product remains constant for a long period, or even slightly decreases; then it suddenly rises (age thickening), and soon the milk forms a gel that cannot be redispersed. (See Figure 13.6.) The casein micelles have formed a network, but the explanation is still uncertain. It appears as if two different mechanisms may be responsible.

One is proteolytic breakdown of the casein, making the micelles susceptible to aggregation, somewhat as in renneting. This may happen in UHT milk because proteinases, particularly some bacterial proteinases, may be sufficiently resistant to heat treatment. Keeping the raw milk for some days at a low temperature then may aggravate the problem considerably, because of the growth of psychrotrophic bacteria producing heat-resistant proteinases. However, not every proteinase (or combination of proteinases) causes gelation, and sometimes the casein just dissolves, leaving a clear solution (if no fat is present) and a sediment of calcium phosphate. In extreme cases, gelation occurs within two weeks. Milk proteinase (plasmin), if present, may cause dissolution of casein rather than gelation.

The second, more common mechanism is even less understood. Chemical reactions are essential, which points to chemical cross-linking. The principal reaction(s) appears to be different from that causing heat coagulation; several variables have a very different effect on either instability. Age gelation also occurs in the absence of whey proteins. Blocking of thiol groups does not

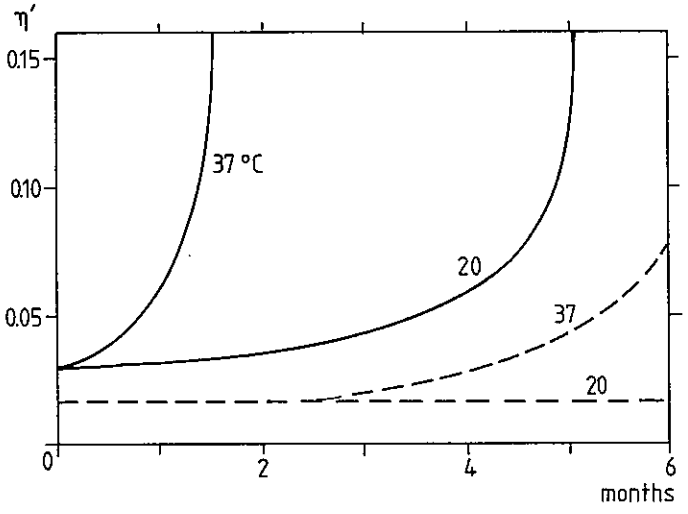


**Figure 13.6.** Age thickening of concentrated milk. Apparent viscosity ($\eta'$, Pa·s) as a function of time of storage at two temperatures (parameter). The broken lines concern milk to which polyphosphate had been added. Approximate results from various sources.

Homogenized UHT cream also is susceptible to age thickening. Now the casein-covered fat globules participate in forming at first aggregates (thereby appreciably increasing viscosity) and eventually a gel. Besides the variables mentioned, those affecting the amount of casein associated with the fat globules influence the rate of gelation. (See Section 14.4.) Addition of Ca-sequestering agents or sweet-cream buttermilk before homogenization thus causes delay of age thickening.

The slow insolubilization of the protein in frozen milk (Section 17.4) may be similar to age gelation, though it is more likely to be a salting-out effect.

## 13.5. HEAT STABILITY

When milk is held at temperatures above the boiling point it eventually coagulates, the higher the temperature the sooner. Few subjects in dairy chemistry have been studied more intensively and are understood less. Nevertheless, important practical results have been obtained, and these will be stressed in this section. It is by no means an endeavor to review exhaustively the multitude of observations and hypotheses on heat stability.

### 13.5.1. Phenomena Involved

It is the casein that undergoes heat coagulation. The cause is not heat denaturation as suffered by globular proteins. Casein is not denaturable, and the temperature dependence of the heat coagulation of milk is much weaker than that of the heat denaturation of proteins. (See Section 10.2.) The casein micelles aggregate, and this is not merely a flocculation caused by a lack of colloidal stability of the micelles at the prevailing temperature, because the aggregates do not redisperse after cooling again. The composition of the milk serum is changed considerably because of the heating (Section 10.1), but restoring the original environment does not dissolve the aggregates. Even addition of agents that break H bonds, reduce –S–S– linkages, or dissolve calcium phosphate leaves the aggregates intact. Consequently, chemical change of the casein must have occurred. It is known that casein suffers dephosphorization, partial hydrolysis, and several cross-linking reactions at high temperatures. (See Section 10.3.)

Heat stability usually is defined as the time needed to cause visible coagulation of milk at a given temperature. The unresolved question is what reaction determines the rate of coagulation. Perhaps some chemical reaction renders the micelles unstable, after which they aggregate as soon as they meet, regardless of conditions such as temperature (as with the heat-induced change of $\beta$-lactoglobulin that enables it to react with $\kappa$-casein). In that case the rate of the chemical reaction(s) would be determinant. Perhaps the casein micelles have to come close together before any cross-linking reaction between them can occur. In that case the interaction energy between the micelles, hence colloidal aspects, may be rate determinant. (See Sections

12.2 and 12.3.) It appears that colloidal interactions come into play, though they are not the ultimate cause of the coagulation.

Figure 13.7 shows examples of the dependence of coagulation time on pH, and it suggests that at different pH different reactions are rate determining. It also follows that the pH dependence can differ among individual milks. Most cows give milk of type A; some give type B; mixed milk is generally of type A. But the shape of curve can show more variation than indicated in Figure 13.7. The pH optimum (if present) can vary from 6.6 to 6.9 among lots of milk. Consequently, the pH of the milk itself can be below, at, or above the optimum. To compare the heat stability of lots of milk, we therefore should determine it at a range of pH values, including that giving the maximum stability.

The pH as given in Figure 13.7 is at room temperature before heating. At high temperature the pH will be much lower, but this is mainly a result of the increased dissociation of water, and a lower pH does not imply a more acid environment. (See Section 11.3.) The precipitation of amorphous tricalcium phosphate at high temperature, thereby liberating protons, causes a further decrease in pH. Furthermore, acid is produced during heating, and the pH (as measured at room temperature) at the time of coagulation will be much lowered, the more so as the time needed for coagulation is longer and the temperature higher. The rate of change of pH also depends on the initial pH (Figure 10.1) and on the availability of $O_2$, because this is needed for the formation of acids from lactose. The rate of acid production during heating is certainly the most important factor in the heat coagulation of (unconcentrated) milk. The pH at coagulation is always low (< 6.2, as measured at room temperature). Keeping the pH at its original value during heating by adding alkali prevents heat coagulation, even after several hours

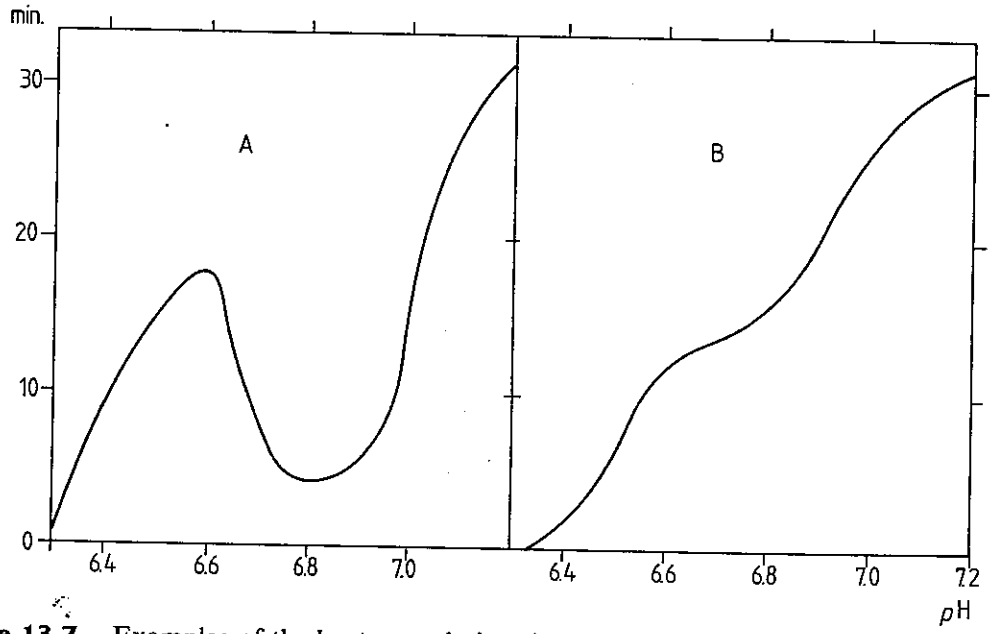


**Figure 13.7.** Examples of the heat coagulation time (minutes at 140°C) of milk as a function of pH.

of heating. The apparent activation energy for heat coagulation is usually equal to that for acid production.

A solution of Na-caseinate is very heat stable (more than 1 h at 140°C and pH 6.7) though it eventually coagulates. A dispersion of casein micelles in a milk–salt solution is far less stable; the heat stability shows a pH dependence like type B. (See Figure 13.7.) Adding lactose to the dispersion decreases heat stability over the full pH range, undoubtedly because more acid is formed during heating. It appears that the colloidal stability of the micelles is important. The lower the pH and the higher the $Ca^{2+}$ activity, the lower the stability. Also, increasing average size, decreasing voluminosity, and increasing content of calcium phosphate of the micelles decrease their heat stability. At high temperatures, the amount of calcium phosphate associated with the micelles increases. (See Section 4.5.) The role of colloidal phosphate is, however, different at higher pH (roughly > 7); it now increases the heat stability, presumably by its buffering action.

If β-lactoglobulin or whey proteins are added to a suspension of casein micelles in protein-depleted milk serum, the heat stability curve usually is converted from type B into type A. At low pH (roughly < 6.7) heat stability is increased, and this may be caused by the β-lactoglobulin diminishing the rate of decrease of the pH. At higher pH coagulation time is remarkably decreased, causing a minimum in the curve. Reactions of thiol groups are involved; addition of a blocking agent (like N-ethyl maleimide) before heating largely eliminates the effect of β-lactoglobulin. Most probably, it concerns the reaction between β-lactoglobulin and κ-casein, because addition of an excess of κ-casein reconverts the type A curve into type B. But other factors also play a part. If the content of colloidal calcium phosphate and/or the $Ca^{2+}$ activity are very low, there is no minimum, despite the presence of sufficient β-lactoglobulin. Heat-denatured β-lactoglobulin is very sensitive to $Ca^{2+}$ ions, and particularly if the latter are present during heating, a $Ca^{2+}$ activity as in milk would suffice to make this protein insoluble. Since the denatured β-lactoglobulin becomes associated with the casein micelles, this may play a part in the heat stability. In the range near the minimum of the (type A) curve coagulation occurs in two steps, and the first rapid step predominantly involves the larger casein micelles, while the coagulum contains a high proportion of calcium phosphate. Other whey proteins that are sensitive to $Ca^{2+}$ ions after heating (e.g., α-lactalbumin) have more or less the same effect as β-lactoglobulin. These proteins also can bind calcium phosphate on heating.

### 13.5.2. Important Variables

Numerous factors affect the heat stability, and some follow from the discussion in the preceding section, notably pH. Unless stated otherwise, we will compare the stability at the optimal pH. The magnitude and even the sign of the effect of some variables may depend on other variables.