Biochemical Aspects of Syneresis: A Review

M. J. PEARSE and A. G. MACKINLAY
School of Biochemistry
University of New South Wales
Kensington, New South Wales, Australia

ABSTRACT

Syneresis is the process in cheese manufacture whereby the whey component of milk is expelled following curd formation. Factors that interfere with syneresis affect the moisture content and quality of the final product. This article reviews the various reported methods for experimentally measuring syneresis and the effect on syneresis of variables including pH, temperature, salt concentration, milk composition and concentration, and effects of milk pretreatment. A recently developed method for the measurement of syneresis on a small scale has allowed the effect of varying casein composition to be studied. Syneresis is sensitive to the concentration of \( \beta \)-casein and also to low levels of dephosphorylation of \( \beta \)-casein. \( \beta \)-Casein, therefore, seems to play an important role in syneresis and may affect micelle surface properties. Syneresis depends at the protein level on a combination of specific and nonspecific interactions, many of which also occur during curd formation.

INTRODUCTION

Cheese making essentially involves the removal of most of the water in the form of whey from the remaining milk constituents. When milk clots, the coagulum initially encloses the entire aqueous phase of the milk. When the curd is cut and agitated, the coagulum contracts and whey is expelled. This phenomenon is referred to as syneresis. Syneresis represents a key factor in Cheddar cheese manufacture because the moisture content of the curd at whey-off influences the quality of the final product (19). It is surprising that so little is known about the syneresis of milk gels, particularly the mechanism of curd contraction. Most of the research on syneresis has been concerned with determining responses to physical variables. Although this has produced useful information it has done little to elucidate the mechanism of syneresis. An understanding of syneresis at the molecular level would aid in the control and optimization of moisture release during commercial cheese production. It may also suggest reasons why changes in milk composition, such as those encountered seasonally, lead to moisture control problems. This review is concerned primarily with the biochemical aspects of syneresis and is restricted to the syneresis of rennet gels. Other aspects of syneresis, including a comprehensive review of the literature on factors affecting syneresis and the physical aspects of syneresis are covered well in a recent review by Walstra et al. (44).

Among the factors that have hindered an understanding of syneresis is that coagulation and syneresis are not strictly separate; thus, any modification or manipulation of milk components that affects curd structure or formation may also affect syneresis. A further problem resides in the complexity of the casein micelle and the lack of detailed information on its structure and particularly the disposition of components on its surface. There also is a considerable problem associated with the accurate measurement of syneresis, particularly on a small scale.

MEASUREMENT OF SYNERESIS

Existing methods to measure syneresis can be broadly classified into two groups, those measurements involving the physical separation of curd from whey and measurements made while the curd is still in the whey. Syneresis measurements depending on the separation of
curd and whey are performed by measuring either: 1) the percentage of moisture in the curd (6, 2) curd weight (35, 42, 43) and specific gravity (38) or curd volume (5, 16), or 3) weight or volume of whey drained from the curd (18, 24, 26).

The inherent problem with these methods is that curd tends to collapse when handled, which induces rapid expulsion of whey (18). This problem is particularly evident in whey drainage methods, because syneresis is normally measured at a fixed time after rennet addition at which stage gels may not be of equal strength and consequently will not collapse to the same extent.

Because of problems associated with accurately separating the curd and whey phases, numerous attempts have been made to measure syneresis while the curd is still in the whey. Measurement of the progressive dilution of an added compound (tracer) has been tried using glycine (30), noncoagulable formol-treated micelles, used either as such (3) or after labeling with safranine (20) or with blue dextran 2000 (46). The problem, however, has been to find a tracer that does not interfere with the process of syneresis and will not adsorb to or diffuse through the curd. An additional problem for small-scale use is the difficulty of achieving thorough mixing of curd and whey without excessive disturbance of the curd. Because of these disadvantages, few methods have been used by workers other than the original authors. Therefore, when comparing results using different methods for the measurement of syneresis or interpreting data, it is important to consider the methods that have been used.

A microscale syneresis assay, which has a working volume of 10 ml and which avoids the aforementioned problems, has recently been developed by Pearse et al. (27). This assay is based on the light-scattering properties of fat globules in which the “tracer” is whey clarified by ultracentrifugation and layered onto the curd just prior to cutting. Syneresis is determined by measuring the increase in light-scattering properties of the clarified whey as whey containing fat globules is expelled from the curd. The addition of clarified whey is likely to depress syneresis slightly (18). However, this does not detract from the usefulness of this assay in situations where the main emphasis is on determining the effect of defined modifications of milk components on syneresis properties and not on measurements of the absolute rate of syneresis. The optical density of whey decreases slightly as syneresis progresses. This change, however, is not large and, more importantly, it is highly consistent from run to run (Hall and Linklater, unpublished data). In any case the most useful application of this method is the measurement of syneresis on small quantities of fat-free artificial micelle milk (AMM), prepared by the method of Schmidt et al. (32, 33, 34). In this application, this problem is not encountered because whey from curd prepared from AMM is virtually clear throughout the time course of syneresis and it is the dilution of a turbid overlay by essentially clear whey that is measured (27). The reproducibility of this assay, both for raw, reconstituted skim milk and for AMM, is as good or better than that reported for the larger scale laboratory assays currently in use. The availability of a small-scale laboratory assay for syneresis now enables syneresis to be investigated at the protein chemical level.

FACTORS INFLUENCING SYNERESIS

During the manufacture of Cheddar cheese the rate of syneresis is affected by a number of variables such as pH, temperature, ionic strength, degree of agitation, extent of cutting of the curd, and the volume of whey surrounding the curd. The general effects of these parameters are well known; however, the extent of the effect often varies according to the method used to assay syneresis.

pH, Temperature, and Calcium

It has generally been observed (4, 21, 26) that syneresis rate is increased as the pH is lowered, possibly as a result of a reduction in net micelle charge and hence of the electrostatic repulsion between micelles. Syneresis is very temperature-dependent (18, 24, 26, 37). The rate of syneresis accelerates as temperature increases; however, reports vary as to the extent of the effect of temperature.

Similar disagreement exists in the literature regarding the effect on syneresis of CaCl₂ addition. According to Marshall (24), the addition of 2 mM CaCl₂ increased syneresis and 4 mM
CaCl$_2$ gave an additional increase in syneresis only at shorter cutting times (2 to 3× rennet coagulation time). Aiyar and Wallace (1) found that the level of calcium in phosphate-free “artificial milks” greatly influenced syneresis. Raising the calcium form 5.4 to 11.25 mM increased syneresis slightly; further increases (15 mM) caused a sharp drop in syneresis. The validity of translating the effects observed in phosphate-free artificial milks to natural micelle systems, however, is questionable because of the demonstrated differences between the two systems (32, 33). Cheeseman (4) found that syneresis was inhibited at all concentrations of CaCl$_2$ tested (10, 50, and 100 mM). It appears that the addition of very low levels of calcium enhances syneresis, possibly as a result of charge neutralization whereas high levels of CaCl$_2$, as with NaCl and KCl addition (4), inhibit syneresis by ionic effects or possibly as the result of Ca$^{2+}$ occupying sites on the caseins that otherwise would participate in syneresis (41). A similar dependence of rennet coagulation time (RCT) on added calcium is observed—an initial increase up to 50 mM CaCl$_2$, followed by a progressive increase in RCT with further CaCl$_2$ addition (23).

**Milk Composition and Concentration**

Syneresis also depends on the composition of milk. The fat concentration in milk is known to affect syneresis time directly (3, 7, 24, 40). With increased fat content there will be an increased number of interstices within the reticulum occupied by fat globules, thus leading to increased impedance of whey drainage (40). Homogenization of whole milk retards syneresis. Homogenization disperses the fat into an increased number of smaller globules, the surfaces of which are modified by the presence of adhering casein particles. This probably results in a finer coagulum, which gives rise to slower drainage of whey. Homogenization of skim milk, however, has little effect on syneresis (9).

Concentration of milk by either addition of solids (10) or ultrafiltration (24) significantly reduces the rate of syneresis, whereas the dilution of milk causes a massive increase in syneresis rate (24). These effects probably reflect changes in curd structure. Lactose concentration does not significantly affect syneresis time (11).

In the conversion of milk to cheese, casein micelles aggregate to form a network that entraps the aqueous or whey phase. Disruption of this network causes it to shrink, which results in the expulsion of the aqueous phase. Any alteration in the composition of the casein micelles which form this curd network might be expected to affect the coagulation and subsequent syneresis of the milk. The effects of altering the casein composition of AMM on RCT and syneresis, however, are not the same (29). The RCT is dependent on the concentration of β- and κ-caseins, since increasing the concentration of these components reduces RCT, whereas reducing their concentration has the opposite effect. This is consistent with the results of Okigbo et al. (25), who observed that milks coagulating poorly contained more of the hydrolysis products γ and para-κ-casein and less κ- and β-casein. Syneresis, however, is only affected by changes in the concentration of β-casein. Neither RCT nor syneresis are significantly affected by changes in the concentration of α-casein. Increasing the para-κ-casein content of AMM also causes a significant reduction in RCT, which suggests that altering the concentration of κ-casein is influencing the secondary or aggregation phase of coagulation. This dependence of RCT, but not syneresis, on the concentration of κ-casein is supported by the results of experiments involving the incorporation of modified κ-casein into AMM. κ-Casein treated with polyphosphate, diethylpyrocarbonate, which modifies histidine residues, or dansyl chloride, which modifies lysine residues, increased RCT but did not significantly affect syneresis (Pearse and Mackinlay, unpublished results).

A detailed analysis of the effects of the genetic variants of the milk proteins on syneresis has not yet been undertaken. Such an analysis would appear worthwhile and may provide further insight into the process of syneresis.

**Milk Pretreatment**

Ali et al. (2) found that cold storage (4°C) of milk adversely affects its cheese-making properties, including syneresis. In another study (15), cold storage did not affect syneresis. In that investigation, however, syneresis measure-
ments were made using a whey drainage method, so it is possible that any effect on syneresis due to cold storage could have been negated by the greater tendency for the weaker gels to collapse during draining. Cold storage of milk gives rise to an increase in the amount of soluble casein, particularly β-casein (2, 8). Syneresis is highly dependent on the micellar content of β-casein, as indicated, so cold storage would indeed affect syneresis unless reincorporation of β-casein into the micelle on warming is very rapid. According to Ali et al. (2), milk needs to be prewarmed for 30 min at 60°C to reverse the effects of cold dissociation.

Heating milk to temperatures that result in the denaturation of whey proteins (above 65°C) causes a reduction in the rate of syneresis of rennet-treated milk (28, 37). This is caused by the sulfhydryl-mediated complex formation between κ-casein and β-lactoglobulin and to a lesser extent α-lactalbumin (28).

THE MECHANISM OF SYNERESIS

Is Syneresis a Purely Physical Process?

Once the coagulum has been formed it demonstrates very little tendency to contract; only when the coagulum is cut and agitated does syneresis occur. It has been suggested (4, 17) that syneresis proceeds in steps, the first of which is the removal of immobilized water from the micelles as a result of the hydrolysis of κ-casein by rennin. However, evidence from differential scanning calorimetry, gravimetric sorption measurements (31), and nuclear magnetic resonance spectroscopy (22) indicates that changes in the water held in an immobilized form by casein plays a minor role in gelation and that syneresis is not accompanied by an alteration in the nature or extent of protein hydration.

The simplest explanation for syneresis would be that the whey is physically squeezed from the curd as the result of the pressure exerted on the curd particles during agitation. This mechanism, however, does not readily account for the known responses of syneresis to variables such as temperature, pH, ionic strength, and calcium ion concentration. In addition, if whey is physically squeezed from the curd in a similar way as water is squeezed from a sponge, then changing the structure of the curd would be expected to affect syneresis properties. This is not always the case. For example, changing the κ-casein concentration of AMM had a significant effect on curd structure but no effect on syneresis (29). Similarly, curd strength at cut would be expected to affect syneresis with the prediction being that weaker gels should be favorable for syneresis, because they would be more easily compressed during agitation. In a number of investigations however, the rate of syneresis was independent of cut-time and therefore of curd firmness (27, 37, 39). Syneresis therefore would appear to be more than just a physical process. Rather, it is likely to be the result of a range of chemical interactions which are enabled by and dependent upon physical agitation of the curd.

Chemical Interactions During Curd Formation and Syneresis

Curd formation and syneresis are triggered by the specific cleavage of a single peptide bond in the κ-casein molecule during the primary phase of rennin action. It appears that this specific proteolysis makes possible a variety of protein-protein interactions and possibly protein-calcium phosphate interactions that generate a three-dimensional cross-linked network of micelles. The number of other micelles with which any particular micelle can interact may be expected to be limited by geometric considerations. Cutting the curd followed by application of physical agitation would then lead to proliferation of micelle-micelle interactions with the formation of a more highly crosslinked and compacted network. This is accompanied by expulsion or displacement of the aqueous phase through the faces of the curd particle and must also be accompanied by the formation of additional micelle-micelle interactions. One of the properties of casein micelles that will favor this process is their relatively open structure, which is easily deformed when a physical force is applied. The responses of both syneresis and coagulation to variables such as temperature, ionic strength, and pH are similar to those that occur during the aggregation of denatured proteins. It appears likely that most, if not all, of the Interactions known to be important in determining the structure and aggregation of proteins play a role in these processes. The parallel
with aggregation of denatured proteins, which is consistent with the self-associative properties of the caseins themselves, indicates that both curd formation and syneresis may be the result of relatively nonspecific interactions. However, the finding that very limited dephosphorylation of β-casein can have a significant effect on both RCT and syneresis (29) strongly suggests that specific interactions are also involved.

The Role of β-Casein

Syneresis and curd formation are both dependent on the concentration of β-casein. Furthermore, both these processes depend on the integrity of the β-casein phosphate cluster. Partial dephosphorylation of preformed micelles or the incorporation of dephosphorylated or partially dephosphorylated β-casein into AMM has an adverse effect on both processes. Similar experiments using partially dephosphorylated α-casein and κ-casein have shown no such effect, and this suggests that β-casein and its phosphate cluster play a specific role in both curd formation and syneresis (29). The negatively charged phosphate cluster region may be involved in ionic interactions between negatively and positively charged microenvironments or possibly in calcium phosphate bridging between micelles. Alternatively, the phosphate cluster may be important in determining the orientation of β-casein on the surface of the micelle. In this way, the phosphate cluster may determine the surface properties of the micelle by positioning other determinants of the molecule where they can participate in micelle-micelle interactions. In view of the evidence that both coagulation and syneresis are sensitive to partial dephosphorylation of β-casein, it would be interesting to determine the coagulation and syneresis properties of milk containing the C variant of β-casein, as this species has one less phosphorylated serine residue than the other variants.

The Role of κ-Casein

The detrimental effect on syneresis of heat-induced, sulfhydryl-mediated complex formation between β-lactoglobulin and κ-casein suggests that para-κ casein is directly involved in the micelle-micelle interactions that occur during curd contraction (28). However, and unlike the situation with β-casein, syneresis is not affected by altering the κ-casein concentration nor by incorporating modified κ-casein into AMM prior to renneting. It is possible that the effect of complex formation with β-lactoglobulin on syneresis is an indirect one and that the primary effect is to cause the formation of an altered curd structure, which is then unable to synerese normally. It is well-established that complex formation between κ-casein and β-lactoglobulin interferes with the primary phase of rennin action (45). Therefore, a para-casein gel prepared from milk preheated above 65°C would contain a higher proportion of uncleaved κ-casein and would be more negatively charged, a situation that would be likely to inhibit contraction of the curd. Another possibility is that syneresis depends upon determinants that are masked until rennin releases the macropeptide portion of κ-casein. Hill et al. (12, 13, 14) found evidence for involvement of basic amino acid residues in κ-casein in the coagulation of rennin-treated casein. However, as already mentioned, an effect of the modification of these residues on syneresis has not been demonstrated. The role of κ-casein and of para-κ-casein in syneresis remains an open question and one that should be further investigated, because it is quite possible that either or both proteins may be involved in specific interactions that are an essential part of both curd formation and syneresis.

CONCLUSIONS

With the information currently available, the most economical working hypothesis is that the chemical interactions inducing syneresis of the curd network are in part an extension of the interactions that give rise to curd formation. At present there is no direct evidence to indicate that syneresis involves unique interactions, that is, interactions different from any are also involved in coagulation. Further information may be in disagreement. However, most such interactions will likely be common to both processes. Certainly the unraveling of specific interactions involved in either process will help understanding of both. The finding of an apparently specific effect of the phosphate cluster of β-casein encourages the view that further specific effects will be found. Attempts to identify
which components of renneted micelles interact with the phosphates of β-casein, particularly those molecules on the surface of the micelle, would be valuable as would further information on the role of para-κ-casein.

Curd formation and syneresis are physiological processes. Formation of a firm curd, with rapid syneresis, is necessary for the health and optimal growth of neonatal calves (9). These functions will therefore have been selected for during evolution of the casein complex, and it is likely that they will be a common feature of the milk systems from most species. Mainly as the result of the application of recombinant DNA methods, amino acid sequences are becoming available for caseins from a number of species. Comparisons of these sequences indicate that both β- and κ-casein sequences have been relatively conserved since divergence of species such as the rat, guinea pig and cow but that the α-casein sequences have been subject to a much higher rate of sequence change, including the addition and deletion and rearrangement of whole blocks of sequence (36). These results are consistent with those discussed herein, which indicate that κ-casein is important for curd formation and that β-casein plays a role in curd formation and syneresis, probably as a result of its influence on the surface properties of the micelle.

REFERENCES

SYNERESIS OF MILK CURD