A Comparative Study of pH and Temperature Effects on the Acidic Coagulation of Milks from Cows, Goats, and Sheep

M. M. OULD ELEYA, S. DESOBRY BANON, and J. HARDY
Laboratoire de Physico-Chimie et Génie Alimentaires
Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires
Institut National Polytechnique de Lorraine
2, Avenue de la Forêt de Haye
54500 Vandoeuvre Lès Nancy, France

ABSTRACT

The acidic coagulation of milk from cows, goats, and sheep was studied at various temperatures by a turbidimetric method based on light reflection and capillary viscosimetry. Milk was acidified by hydrolysis of a defined amount of glucono-δ-lactone. Two characteristic behaviors of milk were distinguished by turbidimetry. The first was observed at 15 and 20°C for the three types of milk and at 30°C for goat milk. The typical turbidimetric pattern as a function of pH could be divided into three stages. The first stage, from initial pH of milk to approximately pH 5.9, showed an initial increase in turbidity and a decrease in viscosity. The second stage, from pH 5.9 to approximately pH 5.2, corresponded to a decrease of milk turbidity associated with an increase in viscosity. The third stage was a logarithmic increase in turbidity. The second typical behavior of milk during acidification was obtained at 30 and 40°C for cow and sheep milks and at 40°C for goat milk. The turbidimetric and pH profiles could be divided into two parts: a slight turbidity increase and a rise in turbidity until a plateau was reached.

(**Key words**: acidic coagulation, cow milk, goat milk, sheep milk)

INTRODUCTION

The strong commercial importance of cow milk has caused it to be studied more extensively than goat and sheep milks. In recent years, the growing consumption of dairy products from goat and sheep milks, especially cheese, has required more knowledge of the raw materials. Detailed literature concerning the composition and physicochemical characteristics (1, 17) has been published, but only a few papers dealt with acidification of goat and sheep milks (24, 32).

Sheep milk contains more caseins and minerals (Ca, Mg, and inorganic P) than do cow and goat milks (1, 12, 29); goat milk has the lowest protein and casein contents and the highest proportion of NPN (17, 32). The proportion of calcium and magnesium in goat milk is comparable with that of cow milk, but the quantity of inorganic phosphorus is generally less. The major components of ovine and caprine caseins were identified as α_{s1}-, α_{s2}-, β-, and κ-caseins (16, 19, 20, 22, 23). β-Caseins are, quantitatively, the major protein component of goat milk (17, 20, 22, 23). Compared with bovine caseins, caprine caseins contain much less α_{s1}-casein, a similar amount of α_{s2}-casein, and more β- and κ-caseins (20). Compared with ovine caseins, caprine caseins contain less α_s-casein (α_{s1}- and α_{s2}-) and more β- and κ-caseins (8). According to Richardson et al. (26), the submicelles from milk of cows, goats, and sheep are similar, but the micelles that the submicelles form are different. The caprine casein micelles have a greater diameter (and greater size dispersion) than do bovine and ovine casein micelles (8, 26). Ovine micelles have the narrowest size distribution and smallest particles (about 80 nm in diameter) (8, 26). Furthermore, the mineralization values of casein micelles are, in the order of greatest to least, ovine, caprine, and bovine casein micelles (2, 22, 29, 32). The hydration of casein micelles, which was determined by ultracentrifugation (22, 32) or by viscometry

Received February 22, 1995.
Accepted August 9, 1995.

(29) varied in the opposite direction (2, 22, 29, 32).

The acidic coagulation of casein micelles reflects interactions among proteins that are important in the manufacture of many dairy products. The most important effects of lowering the pH of milk are the solubilization of micellar calcium phosphate (10, 31), the decrease of the net charge of casein molecules (31), and the temperature-dependent dissociation of caseins from micelles (9, 27, 31). Previous studies (27, 30, 31) revealed that some important properties of the casein micelles, such as voluminosity and zeta potential (4), show a particular dependence on pH. Temperature and pH are the two major parameters that affect acidic milk coagulation (18, 33), and their effects on cow milk have been extensively studied (4, 9, 10, 18, 33). However, no studies were carried out on the effects of these parameters on milk with various compositions and different size distributions of casein micelles. The objective of this work was to compare the behavior of cow, goat, and sheep milks during acidification at different temperatures in order to understand more clearly the mechanisms of acidic coagulation of sheep and goat milks.

MATERIALS AND METHODS

Milk Samples

Fresh bulk cow (Holstein-Friesian) and goat milk (French Alpine) samples were collected from the herds of Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (Nancy, France). Bulk sheep milk was obtained from a commercial herd of French Manech sheep. Before use, milk was skimmed by centrifugation at 4°C for 30 min at a centrifugal force of 1000 × g.

Acidification of Milk

To alter the pH of milk, a defined amount of glucono-δ-lactone (Merck, Darmstadt, Germany) was added. The amount of glucono-δ-lactone used for turbidimetric experiments equaled 2.25% (wt/vol) to coagulate milk from various species in 16 h at 15, 20, 30, and 40°C. For casein and mineral analysis and for measurements of viscosity, milk samples were acidified with different amounts of glucono-δ-lactone and left for about 24 h at 20°C for pH equilibration. To prevent bacterial growth, milk was treated with .01% sodium azide.

Turbidimetric Measurements

The turbidimetric method (Analite Novasina, Zurich, Suisse), detailed by Banon and Hardy (3) and Dalgleish and Law (10), has been used in this study. The change in turbidity, $\Delta\tau$, was calculated:

$$\Delta\tau = \tau - \tau_0$$

where τ_0 indicates initial milk turbidity.

Viscosity Measurements

A capillary viscosimeter (Viscosimatic-MS Fica; Le Mesnil Saint-Denis, France) was used to measure dynamic viscosity of milk during acidification at 20°C.

Solubilization of Caseins and Minerals

Ultracentrifugation at 70, 000 × g for 2 h at 20°C was used to separate soluble caseins from micellar caseins in milk samples (ultracentrifuge L5-50B; Beckman Instruments, Palo Alto, CA). After centrifugation, the supernatant liquid was carefully removed. The total N (TN) and noncasein N (NCN) of milk samples and the total N of ultracentrifugation supernatant (TSN) were determined by the Kjeldahl standard method. Assuming that NCN was constant (9), the total casein N (TCN) and the soluble casein N (SCN) were calculated as follows:

$$\text{TCN} = \text{TN} - \text{NCN}.$$
$$\text{SCN} = \text{TSN} - \text{NCN}.$$

The partition between soluble and micellar casein was then calculated.

An ultrafiltration technique was used to prepare serum at 20°C. About 200 ml of milk were ultrafiltered on a membrane (YM10; Amicon, Epernon, France) with a cutoff at molecular mass of 10,000 Da. The first 5 ml were discarded, and the next 5 ml were retained for analysis. Total Ca and Mg contents of the original milk and of permeates from ultrafiltration were determined by atomic ab-

TABLE 1. The mineral and protein composition of cow, goat, and sheep milks.[1]

Variable	Cow		Goat		Sheep	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Protein, g/L	31.01	.45	28.76	.56	49.51	.75
Casein, g/L	23.16	.30	19.31	1.26	37.34	.30
NPN, %	4.75	.31	11.54	.20	4.73	.69
Ca, g/L	1.14	.04	1.34	.06	2.06	.01
Mg, g/L	.11	.01	.10	.01	.21	.02
P, g/L	1.07	.05	.79	.04	1.34	.06

[1]Data are the means of triplicate measurements.

sorption (1100 Atomic Absorption Spectrophotometer; Perkin-Elmer Instruments, Bois d'Arcy, France). The partition of Ca and Mg between micelles and serum was then calculated.

RESULTS

Milk Composition

The mineral and protein compositions of milk samples are summarized in Table 1. These results show that sheep milk contained more casein and minerals (Ca, Mg, and inorganic P) than did cow and goat milks. Goat milk contained less casein, about the same amounts of Ca and Mg, less inorganic P than cow milk, and a particularly high proportion of NPN, 11.5%, in agreement with literature results (1, 12, 32).

Turbidity and pH Recordings

The changes in turbidity of cow, goat, and sheep milks as a function of pH at 15, 20, 30, and 40°C are shown in Figure 1. Depending on the temperature, two types of profiles could be distinguished. At 15 and 20°C for the three types of milk and at 30°C for goat milk, the first typical turbidimetric and pH pattern presented three stages: an initial increase in turbidity, followed by a significant decrease, and then a logarithmic increase in turbidity. The turbidity decrease became more pronounced as temperature decreased. Moreover, the turbidity decrease appeared to be more marked for goat milk than for other milks. At 30 and 40°C for cow and sheep milks and at 40°C for goat milk, the second characteristic profile for turbidity and pH could be divided into two parts: an initial slight increase in turbidity and then a logarithmic increase in turbidity.

The initial turbidity for cow, goat, and sheep milks at 15, 20, 30, and 40°C is presented in Table 2. At all temperatures, turbidity was apparently least for cow milk and highest for sheep milk. Turbidity of milk gels, at the same temperature, was least for goat milk gels; turbidities for cow and sheep milk gels were comparable (Figure 1). Initial and final turbidity values increased with temperature (Table 2; Figure 1), in agreement with earlier observations (11).

Dynamic Viscosity

Viscosities of cow, goat, and sheep milks at 20°C were measured between pH 6.7 and 5.0. The viscosity and pH profiles shown in Figure 2b had a parabolic shape with a minimum around pH 5.9 for milk from various species. Because of the instrumental limitation, no test was carried out below pH 4.9. The viscosities of sheep milk were generally greater than those of cow and goat milks at similar pH conditions.

Caseins and Minerals Dissociation

Figure 2c shows the dissociation of micellar caseins when pH was lowered for cow, goat, and sheep milks at 20°C. The maximal solubilization occurred around pH 5.5, and caprine casein micelles apparently dissociated more easily than did ovine and bovine casein micelles. Solubilizations of casein of about 30, 15, and 10% were achieved for goat, sheep, and cow milks (Figure 2c). The percentages of solubilized bovine caseins were lower than

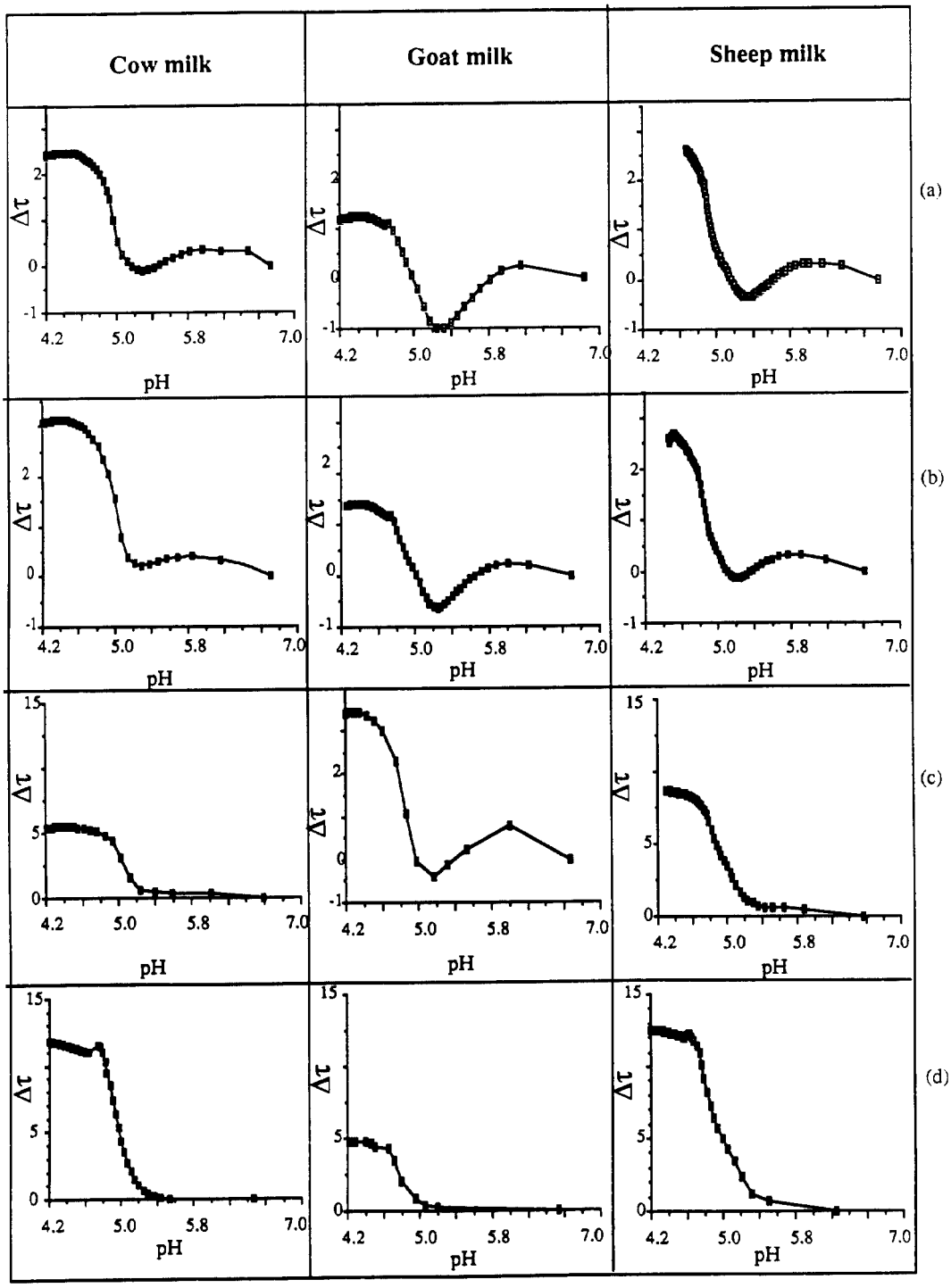

Figure 1. Typical curves of changes in turbidity ($\Delta\tau$) in turbidity units (1000 × NTU) of cow, goat, and sheep skim milks as a function of pH at a) 15°C, b) 20°C, c) 30°C, and d) 40°C.

TABLE 2. Initial turbidity of cow, goat, and sheep skim milks at various temperatures.[1]

Temperature	Cow		Goat		Sheep	
(°C)	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
15	6.64	.21	9.52	.28	10.83	.35
20	6.92	.26	9.93	.29	11.30	.21
30	7.35	.21	10.48	.30	11.71	.44
40	7.63	.17	10.75	.31	12.43	.22

[1]Data are the means of triplicate measurements.

those reported (30%) by Dalgleish and Law (9). The extent of dissolution of colloidal Ca as a function of pH at 20°C for cow, goat, and sheep milks is shown in Figure 2d. Apparently, about 70% of colloidal Ca was solubilized at pH 5.3. Results (not shown) revealed that Ca and Mg dissociated similarly; however, the amount of dissociated Mg was smaller.

DISCUSSION

The differences in turbidity of cow, goat, and sheep milks (Table 2) were related to the differences in casein concentration, in micellar size, and in the optical properties of milks. Indeed, turbidity of milk was a linear function of the product of weight average molecular mass and the mean scattering factor of casein micelles (14, 25). The casein micelles scattering factor was a function of the square of the mean radius. The increase of turbidity with increasing temperature for the three types of milk (Table 2) could be explained then by the reductions in micellar voluminosity and in micellar size. Indeed, Banon and Hardy (4) showed that the size of bovine casein micelles decreased as temperature increased. So, the voluminosity of the casein micelles varied (34). Conversely, the decrease of the mean particle radius led to denser particles with a higher scattering factor (14).

According to the turbidimetric and pH profiles (Figure 1), cow, goat, and sheep milks behaved similarly during acidification. Depending on temperature, two types of profiles were distinguished: the first corresponded to a three-stage turbidimetric and pH profile, and the second corresponded to a two-stage turbidimetric and pH profile. The same differences in turbidity profiles associated with temperature were observed in a recent study (24) on milk acidification from the three species, using 1.5% (wt/vol) of glucono-δ-lactone, an amount lower than that used in the present study.

The acidification of milk from the initial pH to approximately pH 5.9 caused a slight increase in turbidity for the three types of milk at 15, 20, 30, and 40°C. At that pH region, dominated by the increased attraction between positively and negatively charged groups (31), bovine casein micelles retained their integrity, shape, and dimension (33). Vespirini Jaubert (32), in a study of goat milk, showed a dissolution of minerals, a slight solubilization of caseins, a decrease in micellar hydration, and a slight reduction in micellar dimension at this pH region. The increase in turbidity observed in our study was related to a simultaneous decrease in dynamic viscosity at 20°C for cow, goat, and sheep milks (Figure 2b). A similar phenomenon was observed by Banon and Hardy (3, 4) during acidification of reconstituted bovine skim milk at 15, 20, 30, and 42°C. From the initial pH to approximately pH 5.9, the supply of protons was hypothesized to cause partial collapse of the hairy outer layer of micelles (3, 4). Consequently, voluminosity and then dynamic viscosity of micelles decreased according to Eilers's relationship. Moreover, this micellar shrinkage was expected to lead to dense particles that reflect more light. According to our results, the hypothesis of a micellar hairy layer collapse could apply to the first acidification of cow, goat, and sheep milks at 15, 20, 30, and 40°C.

Lowering the pH from 5.9 to 5.2 for cow, goat, and sheep milks at 15 and 20°C caused a decreased turbidity (Figure 1, a and b) and increased viscosity (Figure 2b), casein solubilization (Figure 2c), and mineral dissolution (Figure 2d). The turbidity decrease, which reached a minimum around pH 5.2, has been

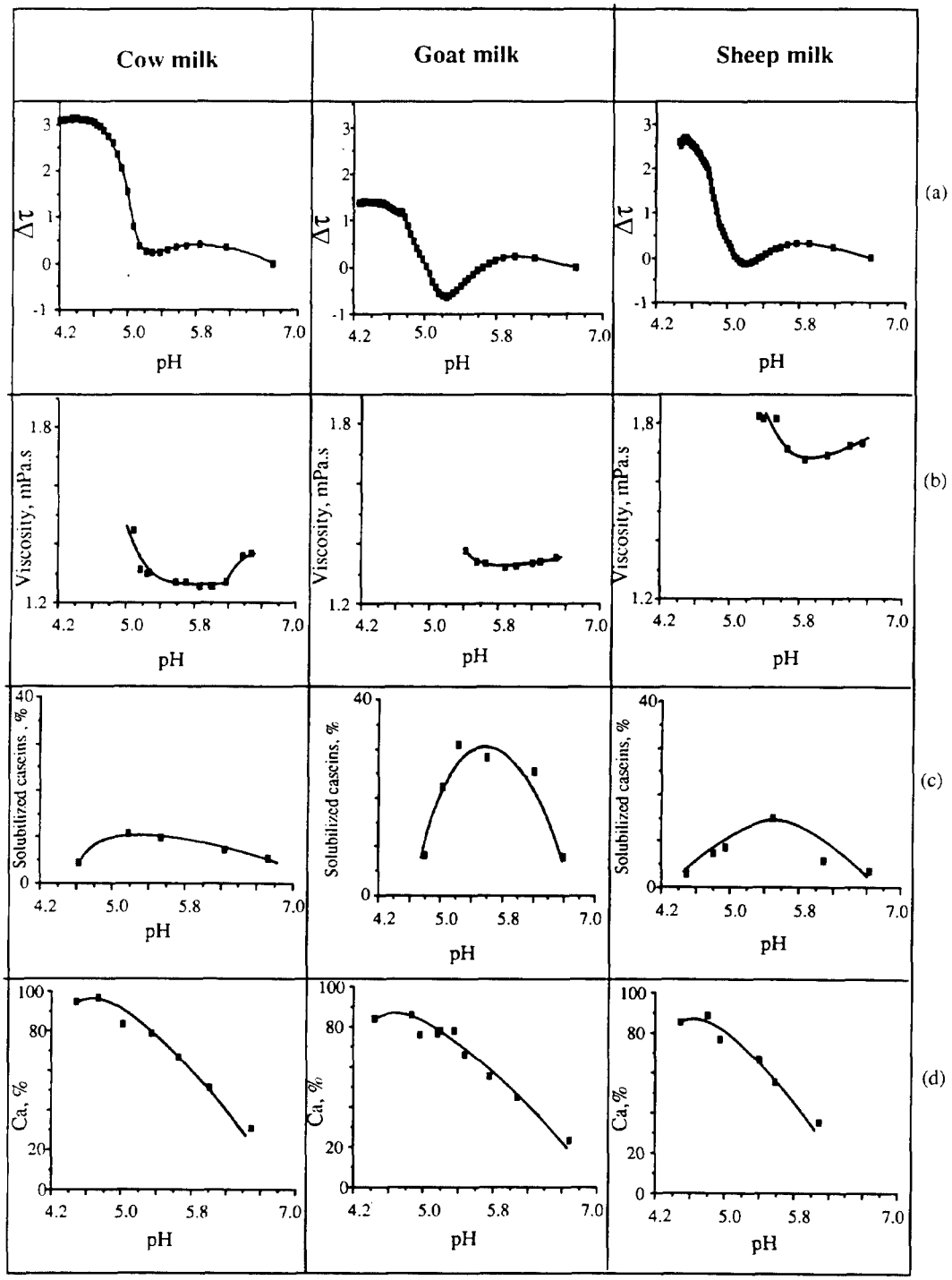

Figure 2. Physicochemical properties of cow, goat, and sheep skim milks at 20°C as a function of pH: a) changes in turbidity ($\Delta\tau$) in turbidity units (1000 × NTU), b) dynamic viscosity (n = 3; SEM = .02), c) percentage of solubilized caseins (n = 3; SEM = .3), and d) percentage of dissociated calcium (n = 3; SEM = 1.8).

explained as being a consequence of micelle expansion for reconstituted cow milk (3, 4). Indeed, micellar calcium phosphate and cations (Ca^{2+} and Mg^{2+}), which form salt bridges between the different colloidal casein molecules, were largely dissolved from micelles in this pH range. Moreover, hydrophobic interactions, which contribute to maintain the micellar integrity, became weaker at lower temperatures (34). Weakness of electrostatic and hydrophobic bonds led to the solubilization of all caseins, especially β-casein (9, 32), with a maximum at about pH 5.3 (10, 26, 29, 30). At this pH, all inorganic micellar phosphate has been transferred to serum (10, 21, 31, 32). This progressive micellar disintegration produced porous particles with reduced light reflection properties (3, 4) in accordance with the turbidity decrease. The rise in dynamic viscosity was in agreement with the increase of casein hydration measured in this pH range for cow and goat milks (30, 32).

The turbidity decrease was more pronounced with acidified goat milk than with cow and sheep milks (Figure 1, a and b), which could be explained by the differences in the dissociation of the individual caseins from the various micelles (Figure 2c). Compared with other caseins, β-casein dissociated easily from the caprine micelles (22, 32) and constituted about two-thirds of caprine caseins, half of ovine caseins, and one-third of bovine caseins (1, 32). Therefore, good solubilization of caseins from caprine micelles was expected. Indeed, Vespirini Jaubert (32) showed that about 7.5% of all caseins was dissociated from caprine micelles at 34°C close to the pH range 5.3 to 5.5, but no casein solubilization occurred for bovine micelles (9) or ovine micelles at 30 or 40°C in this pH range (results not shown). This result could explain the decrease in turbidity that was observed only for goat milk at 30°C (Figure 1c).

Little change in the partitioning of bovine casein occurred in the presence of more than 1 m*M* free Ca^{2+}, but, at lower concentrations, soluble casein increased markedly, and the dissociation was in the order, from greatest to least, κ-, β-, α_{s1}-, and α_{s2}-casein (13). Because the Ca^{2+} activity of the serum at the natural pH of milk varied from 1.2 m*M* for sheep milk, to 2 m*M* for cow milk, and to 2.6 m*M* for goat milk (12), further studies are necessary for determination of the influence of mineral balance on casein dissociation.

Below pH 5.2, the turbidity increase for the three types of milk at 15, 20, 30, and 40°C indicated the onset of aggregation. This result was confirmed by the increase in micellar size observed by Banon and Hardy (4) and Vespirini Jaubert (32) for cow and goat milks, respectively. The differences in the turbidity increase and in the final turbidity values (Figure 1) were related not only to the acidification temperature but also to the variations in casein concentration and type and in the salt balance of the three types of milk. Because of the high brownian motion and increasing hydrophobic interactions, coupled with high temperature, casein particle aggregation was favored (6), and gels became firmer (34). The higher casein concentration of sheep milk than that of goat milk, could lead to an increase in the tendency of the casein particles to associate and, subsequently, to form firmer gels (34). Micellar casein composition (34) and mineral balance (7) were very important during the aggregation phenomenon.

According to the occurrence of casein solubilization, aggregation may be between clusters or between clusters and particles. Bringe and Kinsella (6), in their study on dilute milk systems, suggested that aggregation kinetics were typical of coagulation reactions that were limited by diffusion. Horne (15) and Bremer et al. (5) showed that milk aggregation led to flocs of a fractal nature, which formed the network when they occupied the total volume. The formation of a continuous network could be related to the final stabilization of turbidity at a maximum value. According to Roefs et al. (28) and Bremer et al. (5), the acid bovine casein gels have a particulate and heterogeneous structure built of large agglomerates and large cavities filled with serum.

CONCLUSIONS

Despite the differences in casein micelles composition and mineral balance, cow, sheep, and goat milks behaved similarly during acidification in our study. The hypothetical model proposed by Banon and Hardy (4) for acidification of cow milk could then apply to goat and sheep milks. However, the resulting gel structure is expected to be different. The

result of goat milk coagulation at 15 and 20°C appeared to be a dispersion of coarse particles rather than a continuous network; at higher temperatures, goat milk gels were the weakest. Future studies on dynamic rheology will enable the quantification of the viscoelastic behavior of gels from milks of cows, goats, and sheep.

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