

## Influence of Calcium Salt Supplementation on Calcium Equilibrium in Skim Milk During pH Cycle

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### ABSTRACT

Calcium is a mineral essential for humans, especially for bone constitution. Yet most of the worldwide population does not satisfy their Ca needs. Hence, Ca supplementation is of major importance, even in western countries where some specific populations at risk do not satisfy the recommended daily intake of Ca. More than 70% of dietary Ca comes from dairy products. Calcium supplementation of naturally Ca-rich sources such as skim milk is then of special interest. To our knowledge, few data are available concerning milk Ca (MC) supplementation of milk, particularly when followed by pH cycle. In this paper, MC supplementation is studied and compared with Ca chloride (CC) supplementation as a well-known source of Ca. The effect of Ca salt supplementation followed by pH cycle was studied in reconstituted skim milk. Calcium supplementation was carried out with CC and MC at 25 mmol of Ca/kg of skim milk. Ionized Ca concentration and turbidity variations were followed in situ by Ca ion selective electrode and turbidimetry using light reflection. From normalized data on ionized Ca concentration and turbidity vs. pH, it appeared that hysteresis areas were smaller for CC-supplemented milk, whereas unsupplemented milk and MC-supplemented milk behaved similarly. For these 3 dairy systems, pH cycles to pH 5.0 led to a larger hysteresis area than pH cycles to pH 5.5. The shrinkage of the hysteresis area could be interpreted as a reinforcement of casein micelles with Ca ions over the pH cycle.

**Key words:** calcium supplementation, pH cycle, skim milk

### INTRODUCTION

Calcium is a mineral essential for humans, particularly during certain phases of life such as growth (for

bone constitution), lactation, and during old age (to act against osteoporosis). According to an INCA survey (Volatier et al., 2000), even in Western countries, specific populations at risk (girls from 10 to 19 yr old, women older than 56, and men older than 65 yr old) do not satisfy the recommended daily intake for Ca. This nutritional deficiency in Ca presents a worldwide health risk and could be overcome by Ca supplementation of food. Dairy products, naturally rich in Ca and with a high Ca bioavailability, are appropriate foods for Ca supplementation.

In cow's milk, the mineral fraction represents 9% (wt/wt) of the average composition. It contains, principally, Ca, P, Mg, Na, K, and chloride. Milk comprises 2 phases between which minerals are partitioned. Monovalent ions such as Na, K, and chloride are mostly present in the soluble phase, whereas Ca, P, and inorganic phosphate are much more present in the colloidal phase. In this latter phase, the so-called CN micelles, CN are gathered together with minerals, especially with Ca.

The Ca distribution in milk is 2 to 2.5 times higher in the colloidal phase than in the soluble phase (Rajput et al., 1983; De la Fuente, 1998). Additionally, Ca is present in various forms. For a total concentration of 32 mM, 22 mM (69%) are in the colloidal phase and 10 mM (31%) are in the soluble phase. Only 2 mM of soluble Ca are free ionic Ca (Walstra and Jenness, 1984). The remainder of soluble Ca is essentially associated to citrate, phosphate, and CN monomers. In the colloidal phase, Ca can interact with phosphoesters, carboxyl groups of micellar CN, or colloidal phosphate and citrate associated with CN micelles (Philippe et al., 2003).

When subjected to chemical equilibria, Ca partition in milk varies significantly with physicochemical treatments. Protons and Ca ions are in equilibria with their respective phosphate and citrate salts (Brulé, 1981). Equilibria are shifted toward the colloidal or the soluble phase, notably as a function of pH, temperature, and Ca salt supplementation.

Received October 12, 2006.

Accepted December 27, 2006.

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During acidification, the ionization state of proteic groups is reduced and involves solubilization of micellar minerals (Dalglish and Law, 1989). Calcium, Mg, inorganic phosphate, and citrate ions are transferred from the colloidal phase to the soluble phase of milk (Brulé et al., 1974; Dalglish and Law, 1989; Le Graët and Brulé, 1993; Famelart et al., 1996; Le Graët and Gaucheron, 1999). Calcium phosphate is first solubilized until pH 5.2 (Chaplin, 1984; Le Graët and Brulé, 1993), the pH value at which all mineral phosphate is solubilized. Then, below pH 5.2, Ca bound to phosphoserine residues and the carboxyl group is solubilized. At pH 5.0, almost all the Ca and inorganic phosphate are present in the soluble phase. Calcium is completely solubilized at pH 3.5 (Van Hooydonk et al., 1986; Law, 1996). Consequently, colloidal mineral solubilization induces dissociation of colloidal CN monomers during acidification. Moreover, with temperature decrease, the weakening of hydrophobic interactions increases CN and especially  $\beta$ -CN (Dziuba and Muzinska, 1998) release from micelles. Up to 60% of CN are dissociated at 4°C and pH 5.2 (Dalglish and Law, 1988).

Milk alkalization involves a reverse effect of acidification; that is, the transfer of minerals from the soluble to the colloidal phase. With pH increase, inorganic phosphate and Ca decrease in the soluble phase (Van Dijk, 1991, 1992).

With Ca salt supplementation in milk, the ionic environment is modified and minerals equilibrate depending on the Ca salt type used and its concentration as well. Indeed, Ca supplementation induces association of Ca ions with soluble phosphate and citrate initially present in milk. Consequently, inorganic phosphate and citrate ion concentrations decrease in the aqueous phase (Philippe et al., 2003). Moreover, the former authors observed that supplemented Ca can absorb into CN micelles. Because of mineral equilibrium modification, CN micelles undergo physicochemical changes such as hydration and charges decrease and increase of whiteness, turbidity, and hydrophobicity (Philippe et al., 2003).

In this paper, the effect of Ca salt supplementation was studied during the pH cycle in reconstituted skim milk. Two Ca salts were compared for their distinct physicochemical properties: Ca chloride (**CC**) for its high solubility and its common use, and milk Ca (**MC**) for its potential use as a natural source of Ca for enrichment of dairy products. Indeed, MC is derived from milk and offers a healthy image in labeling. With a good sensorial profile, this Ca source is used in a wide variety of products at relatively high concentrations without causing an off-flavor.

Calcium addition was analyzed in relation to Ca distribution between the soluble and the colloidal

phases and also to the reversibility of phenomena occurring in skim milk subjected to pH cycle. Two minimal pH values for pH cycle were chosen: 5.5 or 5.0. Indeed, this narrow pH range was reported to be of interest with regard to reversibility in the CN micellar state (Gastaldi et al., 1996). The experimental setup described in Gaiani et al. (2005) permitted data acquisition in situ of various physicochemical parameters, namely, pH, ionized Ca, and turbidity.

## MATERIALS AND METHODS

### *Reconstituted Skim Milk Preparation*

Low-heat skim milk powder (Ingredia, Arras, France) was reconstituted at 12% (wt/wt) in distilled water, stirred for 3 h, and then equilibrated at 4°C overnight before use. Reconstituted skim milk powder was used to prevent mineral variations that could falsify results. Two kilograms of reconstituted skim milk was prepared for each experiment.

### *Ca Supplementation*

Calcium supplementation was performed with CC ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , analytical grade, Carl Roth GmbH, Karlsruhe, Germany) or MC (Fieldgate natural dairy Ca, First District Association, Litchfield, MN). Milk Ca comprised mainly Ca phosphate and contained 28% (wt/wt) Ca but also contained other compounds such as lactose and minerals.

Two kilograms of reconstituted skim milk was supplemented with CC or MC salts at 25 mmol of Ca/kg of skim milk (similar to Guillaume et al., 2002). After a Ca equilibration period (2 h), supplemented skim milk was subjected to pH cycle.

### *pH Cycle Procedure*

Acidification was performed with HCl (1 M) until reaching a minimal pH value of pH 5.5 or 5.0. Then, directly after acidification, NaOH (1 M) was used for a neutralization step. Acid and base were both added to the medium at a rate of 1 mL/min. Both products were purchased at VWR International (Fontenay-sous-Bois, France). The pH cycle was stopped when the initial pH of the milk was reached ( $\text{pH}_i = 6.65$ ). The dairy system was thermostated at 4°C to minimize aggregation and gelation of proteins.

At the end of each step ( $T_1$  = probe stabilization;  $T_2$  = Ca salt equilibrium;  $T_3$  = acidification; and  $T_4$  = neutralization), 20-mL samples were taken for further Ca distribution analyses. Duplicate experimental trials were performed.

### Experimental Setup

Experiments were carried out in a 2-L vessel equipped with a 4-bladed 45° impeller (R 100 impeller: 6 cm in diameter) rotating at 250 rpm (LabMaster mixer, Lightnin, Rochester, NY). The temperature was kept constant at 4°C by using a double-walled jacket vessel.

The pH meter (Radiometer Analytical, Remiremont, France) and Ca ion selective electrode (Sentek, Braintree, Essex, UK) were immersed into the liquid. A turbidity sensor (Analite NEP 160, McVan Instruments, Mulgrave, Australia) was placed through the vessel wall to avoid disturbances during stirring. This apparatus used light in the near-infrared region (860 nm); the incident beam was reflected back at 180° by any particle in suspension in the fluid to a sensitive electronic receptor. The continuous monitoring (Almemo 8990-8 V5, Ahlborn, Holzkirchen, Germany) of pH, ionized Ca, and turbidity was performed using a data logger coupled with a personal computer equipped with software (AMR WinControl for Almemo). This experimental setup was previously described by Gaiani et al. (2005) in a study on dairy powder rehydration.

### Ionized Ca Determination

Throughout the pH cycle, the Ca ion selective electrode measured ionized Ca concentration through a chemical potential measurement (mV). Calcium activity of calibrating solutions was calculated according to the following equation:

$$a_i = \gamma_i \times C_i,$$

where  $a_i$  = Ca activity ( $M$ );  $\gamma_i$  = activity coefficient (no unit) = 0.40 for an ionic strength value of 0.1  $M$ ; and  $C_i$  = ionized Ca concentration ( $M$ ).

Ionized Ca concentrations were then determined from the previous equation, and the dilution effect due to acid and base addition was corrected.

### Total and Soluble Ca Determination

At the end of each step of the kinetics ( $T_1$  = probe stabilization;  $T_2$  = Ca salt equilibrium;  $T_3$  = acidification; and  $T_4$  = neutralization), total Ca and soluble Ca were quantified by atomic absorption spectrometry (PerkinElmer, Wellesley, MA) according to the procedure described by Brulé et al. (1974) and Dziuba and Muzinska (1998). A Ca detection lamp was set at a wavelength of 422.7 nm with a slit of 0.7 nm.

Colloidal and soluble phases of milk were separated by ultracentrifugation (RC M120 GX, Sorvall, Les Ulis,

France) set at  $110,000 \times g$  during 1 h (Dziuba and Muzinska, 1998) at 4°C. Colloidal Ca was considered as the difference between total Ca and soluble Ca. Calcium was quantified by atomic absorption in triplicate on duplicate experimental trials.

### Statistical Analysis and Normalization Method

Statistical analysis was performed by using Kyplot freeware. Multiple comparison parametric tests were performed. The same superscript letter was used when no differences between 2 Ca concentrations measured were observed ( $P > 0.05$ ). Statistical analysis was performed independently for the colloidal and the soluble phase.

The min-max normalization method corresponded to the ratio of each value to the maximal value measured during the corresponding experiment.

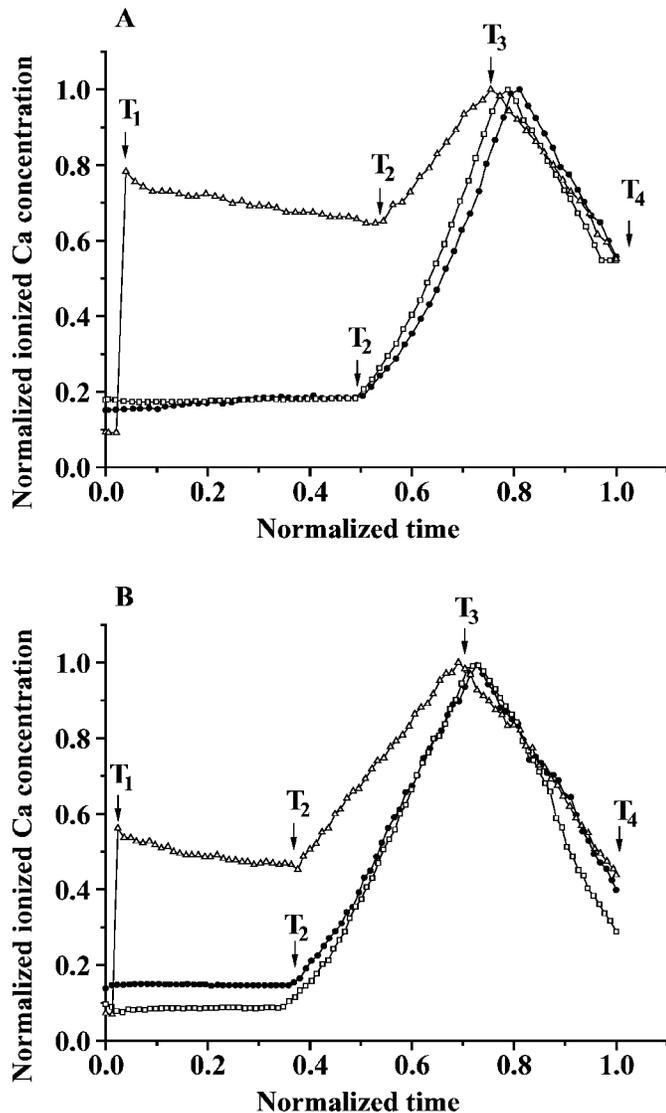
## RESULTS AND DISCUSSION

### Ca Variations as a Function of Time During pH Cycle

Approximately 1.8 mM of ionized Ca was measured in skim milk at pH 6.9 and 4°C, similar to the 2 mM found by Philippe et al. (2003) at pH 6.65 and 25°C and in accordance with the low influence of temperature on mineral solubilization (Dalglish and Law, 1989).

To facilitate comparison between skim milk and Ca salt-supplemented milks (MC and CC), normalized ionized Ca concentration variations are presented as a function of normalized time in Figure 1. The profiles as a whole can be divided into 3 phases: the stabilization of ionized Ca after addition of Ca salts, the increase of ionized Ca concentration with acidification, and the decrease of ionized Ca concentration with neutralization.

From Figure 1, distinct behaviors can be observed for CC- and MC-supplemented milks. Ionized Ca concentration increased just after CC supplementation, whereas no significant variation occurred with MC supplementation. In the meantime, CC addition reduced milk pH from pH 6.9 to 6.4, displacing protons from proteins. During the stabilization period following salt addition ( $T_1$  to  $T_2$ ), a decrease in ionized Ca concentration for CC-supplemented milk was observed from 16 to approximately 13 mM. In this period, the ionized Ca concentration variations in CC milk could be related to Ca ion equilibrium between the soluble and colloidal phases of milk. Then, the decrease should correspond either to the formation of complexes with soluble anions (i.e., citrate or phosphate) or to the absorption of Ca ions into CN micelles. No variations were observed in the case of MC addition. Ionized Ca



**Figure 1.** Normalized ionized Ca concentration ( $M$ ) as a function of normalized time for skim milk ( $\bullet$ ) and for milk Ca-supplemented milk ( $\square$ ) and Ca chloride-supplemented milk ( $\Delta$ ) subjected to pH-cycle (A:  $\text{pH}_{\text{min}}$  5.5, B:  $\text{pH}_{\text{min}}$  5.0).  $T_1$  = end of probe stabilization;  $T_2$  = end of Ca salt equilibrium;  $T_3$  = end of acidification;  $T_4$  = end of neutralization.

variations observed after MC or CC addition fit with the solubility of Ca salts commonly reported in the literature, that is, high for CC and poor for MC. Moreover, the experiment performed with pure Ca phosphate ( $\text{CaHPO}_4$ , Ca initially present in milk and in MC) showed similar behavior as MC supplementation (results not shown).

For the 3 dairy systems in Figure 1, ionized Ca concentration increased and decreased during acidification ( $T_2$  to  $T_3$ ) and neutralization ( $T_3$  to  $T_4$ ), respectively. This reflects the micellar pH-dependent miner-

alization described by many authors (Brulé and Fauquant, 1981; Pierre and Brulé, 1981; Chaplin, 1984; Van Hooydonk et al., 1986; Dalgleish and Law, 1989). Calcium ionized during the acidification step may be endogenous (initially present in the colloidal phase before Ca supplementation), exogenous (supplemented Ca), or both. During pH cycles, normalized profiles of ionized Ca vs. time (Figure 1) varied largely and similarly for control and MC milk compared with the lower variations measured for CC milk. During acidification to pH 5.5 and 5.0, up to 80 and 90% ionized Ca was solubilized for control and MC milk, respectively. In the meantime, 35 to 55% Ca was ionized from CC milk. The Ca released from the colloidal phase to the soluble phase (compared with control and MC milk) was reduced. This can be related to the high concentration of ionized Ca measured in the soluble phase after CC supplementation.

During the neutralization phase, ionized Ca concentration was restored only for CC milk, indicating the reversibility of Ca ionization in this dairy system.

The concentrations of soluble and colloidal Ca are indicated in Tables 1 and 2 for pH cycle to pH 5.5 and to pH 5.0, respectively. Colloidal Ca refers either to micellar Ca or to insoluble Ca particles. Soluble Ca represents an ionized form ( $\text{Ca}^{2+}$ ) or complexes with anions such as citrate and phosphate. From Tables 1 and 2, the supplementation of MC in milk appeared to correspond to a higher colloidal Ca concentration compared with CC and skim milk. Because MC solubility was very poor compared with CC, we can expect that a significant amount of MC powder added in skim milk was still insoluble in the colloidal fraction. Additionally, CC addition was accompanied by an increase in soluble Ca, contrary to skim milk and MC milk. Soluble Ca concentrations were similar for skim and MC milk.

For the 3 dairy systems, pH cycles induced an increase in soluble Ca during acidification (from  $T_2$  to  $T_3$ ), related mainly to micellar demineralization and a decrease in soluble Ca during neutralization due to reverse micellar mineralization (from  $T_3$  to  $T_4$ ). The reversibility of Ca solubilization was obtained only for CC milk, as it was observed for the ionized Ca fractions in Figure 1.

#### **Comparison of pH-Dependent Ionized Ca Variations as a Function of Ca Salt Type Added**

To point out the influence of pH on ionized Ca variations, min-max normalized ionized Ca measured during pH cycles was plotted as a function of pH (Figure 2).

During acidification and neutralization, normalized ionized Ca concentration increased and decreased, re-

**Table 1.** Colloidal and soluble Ca concentrations (mmol/kg) during milk pH cycle to pH 5.5<sup>1</sup>

Item <sup>2</sup>	T <sub>1</sub>		T <sub>2</sub>		T <sub>3</sub>		T <sub>4</sub>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Colloidal Ca								
Skim milk	26 <sup>a</sup>	2	25 <sup>ac</sup>	0.7	9	5.5	18 <sup>cd</sup>	2.6
MC milk	24 <sup>ad</sup>	3	48 <sup>b</sup>	1.3	36 <sup>e</sup>	4.6	42 <sup>be</sup>	2.2
CC milk	28 <sup>a</sup>	7	42 <sup>be</sup>	2	26 <sup>acd</sup>	7	39 <sup>e</sup>	4
Soluble Ca								
Skim milk	10 <sup>f</sup>	0.6	11 <sup>f</sup>	1	25 <sup>g</sup>	0.4	19 <sup>h</sup>	0.4
MC milk	10 <sup>f</sup>	1	10 <sup>f</sup>	0.5	23 <sup>g</sup>	1.7	18 <sup>h</sup>	1
CC milk	11 <sup>f</sup>	0.2	26 <sup>g</sup>	4	39	2	24 <sup>g</sup>	2.7

<sup>a-h</sup>The same superscript letter was used when no differences between 2 Ca concentrations measured were observed ( $P > 0.05$ ).

<sup>1</sup>Statistical analysis was performed independently for the colloidal and soluble phases. T<sub>1</sub> = end of probe stabilization; T<sub>2</sub> = end of Ca salt equilibrium; T<sub>3</sub> = end of acidification; T<sub>4</sub> = end of neutralization.

<sup>2</sup>MC = milk Ca-supplemented milk; CC = Ca chloride-supplemented milk.

spectively, and a hysteresis phenomenon was observed. Whatever the minimal pH (i.e., pH 5.5 or 5.0), skim milk (Figure 2, panel A) and MC milk (Figure 2, panel B) showed similar trends, contrary to CC milk (Figure 2, panel C). The hysteresis area was reduced for CC milk (Figure 2, panel C), which means that the reversibility of micellar mineral solubilization was better in these conditions. This could be related to the previous capture of Ca cations into the micelles during the equilibrium phase (T<sub>1</sub> to T<sub>2</sub>) involving a reinforcement of CN micelles and then less variation during pH cycle.

Normalized ionized Ca plotted vs. pH profiles for the 2 pH cycles (i.e., to pH 5.5 or 5.0) showed some differences during the neutralization phase. Indeed, for the pH cycle to pH 5.0, an inflexion point around pH 6.0 to 6.2 was noticeable during neutralization, from which ionized Ca concentration decreased faster to its initial value. When pH was decreased to pH 5.0, Ca bound to phosphoserines and carboxyl groups of CN (Le Graët and Brulé, 1993) was solubilized in addi-

tion to micellar Ca phosphate (as in the case of pH 5.5). These differences in the nature of Ca interactions affected during pH cycles should explain the differences between pH cycles.

#### **Influence of Salt Nature Supplementation on Turbidity Variation During pH Cycle**

Normalized turbidity variations plotted as a function of pH are presented in Figure 3 for the 2 pH cycles. Turbidity variation measured by light reflection can be related to fluctuations in concentration, size, and optical properties of colloidal particles as CN micelles (Banon and Hardy, 1992). For pH cycles to pH 5.5 and to pH 5.0, CC milk behaved differently from skim milk and MC milk. First, turbidity increased with CC supplementation in accordance with the observations of Green (1982) and Philippe et al. (2003), who reported a turbidity increase with cationic additives. For CC milk, the decrease of ionized Ca in Figure 2, panel C, is concomitant with the increase of turbidity in Figure

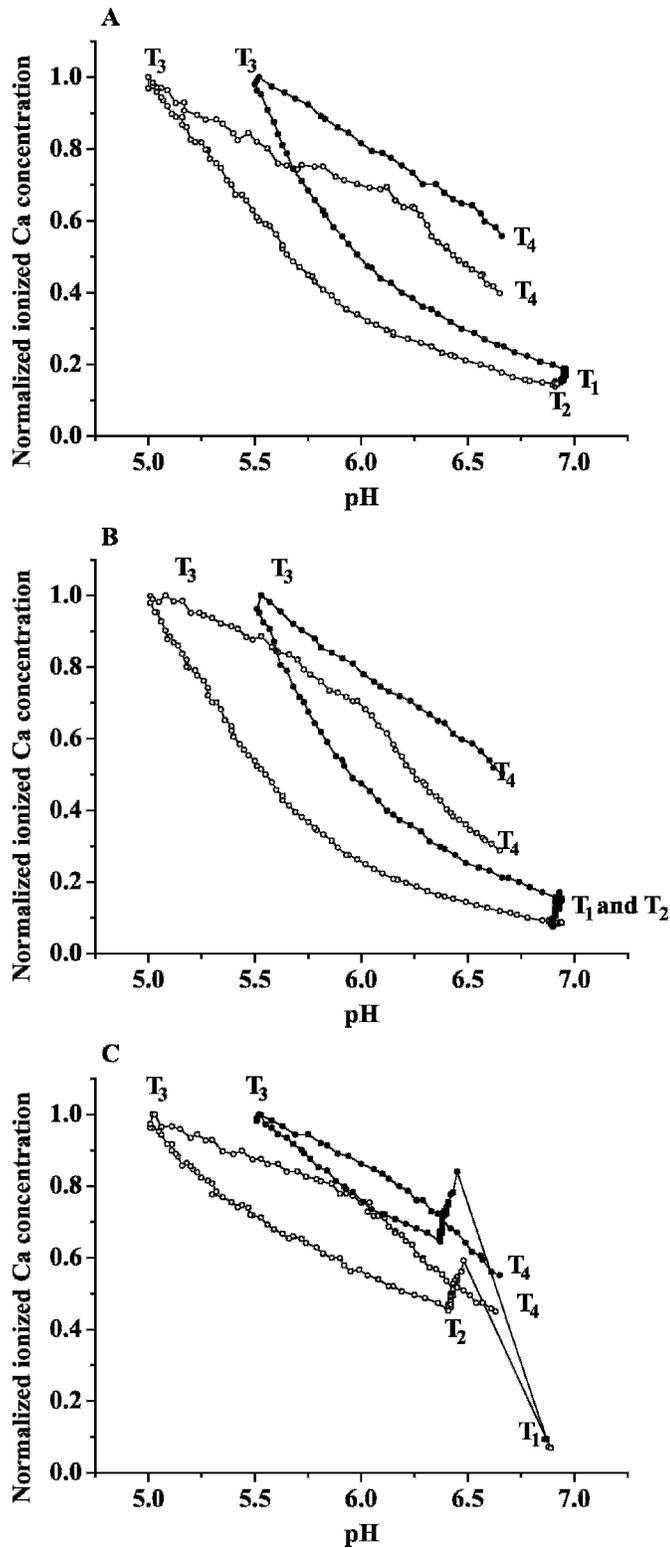
**Table 2.** Colloidal and soluble Ca concentrations (mmol/kg) during milk pH cycle to pH 5.0<sup>1</sup>

Item <sup>2</sup>	T <sub>1</sub>		T <sub>2</sub>		T <sub>3</sub>		T <sub>4</sub>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Colloidal Ca								
Skim milk	25 <sup>af</sup>	1.1	27 <sup>ag</sup>	3	2.8	0.1	12 <sup>c</sup>	3
MC milk	27 <sup>a</sup>	1.6	46 <sup>b</sup>	0.6	18 <sup>cf</sup>	5.2	41 <sup>bd</sup>	2.3
CC milk	25 <sup>af</sup>	1.2	40 <sup>be</sup>	3.8	19 <sup>ac</sup>	11.7	35 <sup>deg</sup>	2
Soluble Ca								
Skim milk	11 <sup>h</sup>	0.5	11 <sup>h</sup>	0.7	34 <sup>i</sup>	1.5	24 <sup>k</sup>	2.3
MC milk	11 <sup>h</sup>	0.5	11 <sup>h</sup>	0.2	37 <sup>ij</sup>	1.8	20 <sup>k</sup>	0.5
CC milk	11 <sup>h</sup>	1.1	26 <sup>k</sup>	0.4	40 <sup>j</sup>	10	25 <sup>k</sup>	0.7

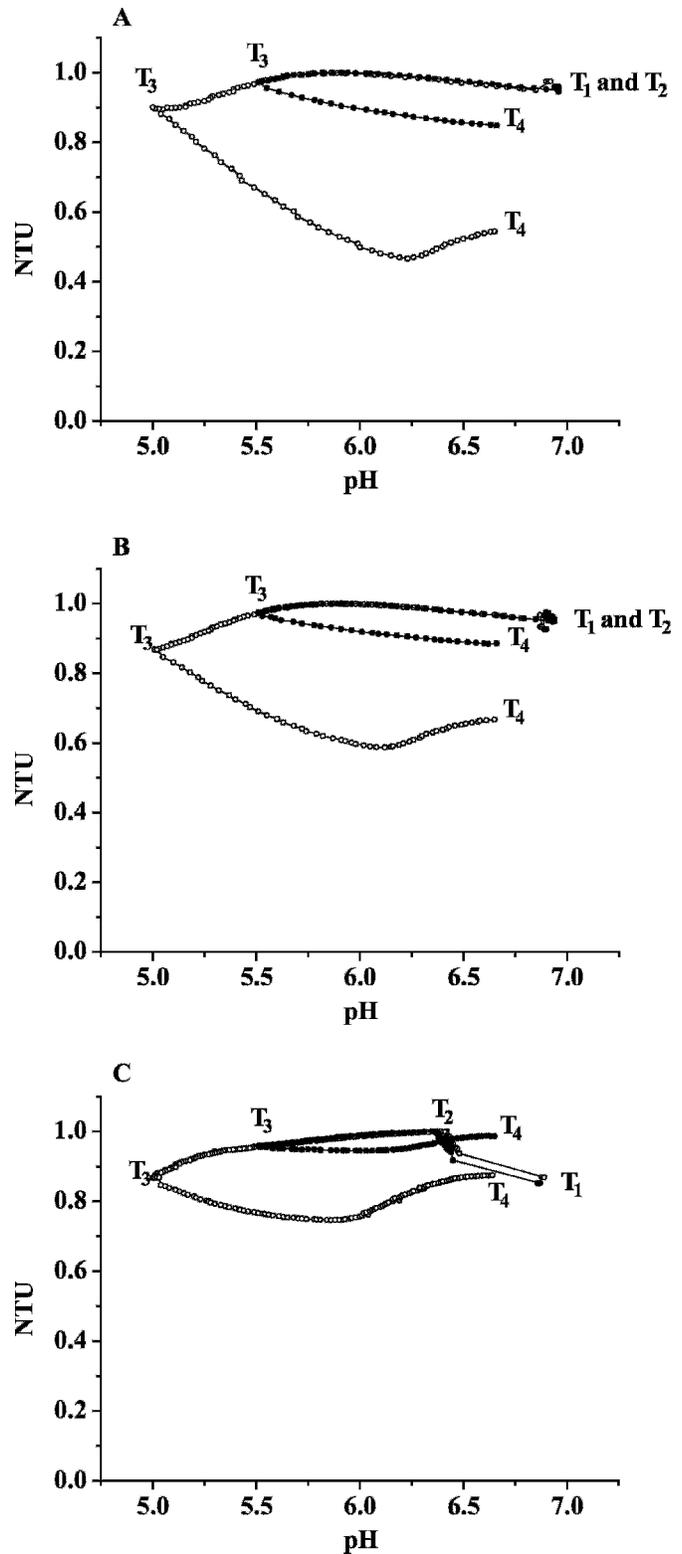
<sup>a-k</sup>The same superscript letter was used when no differences between 2 Ca concentrations measured were observed ( $P > 0.05$ ).

<sup>1</sup>Statistical analysis was performed independently for the colloidal and soluble phases. T<sub>1</sub> = end of probe stabilization; T<sub>2</sub> = end of Ca salt equilibrium; T<sub>3</sub> = end of acidification; T<sub>4</sub> = end of neutralization.

<sup>2</sup>MC = milk Ca-supplemented milk; CC = Ca chloride-supplemented milk.



**Figure 2.** Min-max normalized ionized Ca measured during pH cycles as a function of pH (A: skim milk; B: milk Ca-supplemented milk; C: Ca chloride-supplemented milk). -○-:  $pH_{min} = 5.0$ ; -●-:  $pH_{min} = 5.5$ . T<sub>1</sub> = end of probe stabilization; T<sub>2</sub> = end of Ca salt equilibrium; T<sub>3</sub> = end of acidification; T<sub>4</sub> = end of neutralization.



**Figure 3.** Min-max normalized turbidity (NTU) as a function of pH (A: skim milk; B: milk Ca-supplemented milk; C: Ca chloride-supplemented milk). -○-:  $pH_{min} = 5.0$ ; -●-:  $pH_{min} = 5.5$ . T<sub>1</sub> = end of probe stabilization; T<sub>2</sub> = end of Ca salt equilibrium; T<sub>3</sub> = end of acidification; T<sub>4</sub> = end of neutralization.

3, panel C, and then could reflect the reinforcement of micellar structure. From the variation of other parameters (milk lightness increase, average diameter constant, and hydrodynamic diameter constant), Philippe et al. (2005) related the turbidity increase to a micellar density increase.

During acidification, the 3 dairy systems (Figure 3) showed a similar slight turbidity variation from initial pH 6.9 up to pH 5.0, which could be related to micellar material loss; that is, minerals and CN (Dalglish and Law, 1988, 1989), and consecutive variations in the voluminosity and size of CN micelles (Snoeren et al., 1984; Banon and Hardy, 1991). During neutralization, turbidity variations are larger than during acidification and should reflect an important restructuring of the colloidal phase.

The pH cycle to pH 5.0 amplified the differences between the acidification and neutralization phases, and hysteresis areas were then larger for all dairy systems. These differences should reflect the extent of micellar CN solubilization during acidification (Rose, 1968; Downey and Murphy, 1970; Creamer et al., 1977; Dalglish and Law, 1988) and the micellar components' ability to newly interact during neutralization. Hysteresis areas were systematically smaller for CC milk and almost similar for MC and skim milks. This could be attributed to the initial reinforcement of CN micelles by CC addition related to the diffusion of ionized Ca into the micelle.

Compared with pH 5.5, acidification to pH 5.0 modified the behavior of MC and skim milks during neutralization and the same behavior was observed with CC milk, that is, turbidity decreased drastically to about pH 6.1 to 6.25 then increased to the final pH 6.65 (Figure 3). The minimal value of turbidity at around pH 6.0 to 6.2 was consistent with the inflexion point observed on ionized Ca vs. pH curves in the neutralization phase (Figure 2). This could be related to the reincorporation, via Ca interactions, of CN monomers solubilized during acidification (Dalglish and Law, 1989).

## CONCLUSIONS

In this paper, the influence of Ca salt supplementation on Ca equilibrium was studied in skim milk during the pH cycle at 4°C. Compared with highly soluble CC, MC salt had few effects on Ca equilibrium in milk.

According to ionized Ca and turbidity measurements, CC supplementation would favor the reinforcement of micellar structure and reduce the micellar dissociation during the pH cycle at 4°C.

Further studies on the structure and physicochemical properties of CN micelles after Ca salt supplementa-

tion are necessary to improve the comprehension of Ca-CN micelle interactions and of their stability during technological treatments.

## ACKNOWLEDGMENTS

We thank Unilever Research and Development Center of Vlaardingen for their scientific and financial support. Thanks to Carole Jeandel for helpful technical assistance.

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