

MATERIALS AND METHODS

L(+)-CaL₂ pentahydrate (Ca(CH₃CHOHCOO)₂·5H₂O) USP grade (purity >99.0%) was purchased from PURAC America, Inc. (Lincolnshire, IL) and Sigma Chemical Co. (St. Louis, MO). Crystals of CaL₂ received from Sigma Chemical Co. were needle-shaped, whereas the specimen of CaL₂ purchased from PURAC America, Inc. was more powderlike. All other chemicals used were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

The solubility of CaL₂ in aqueous model systems was evaluated in terms of temperature, presence of salt, pH, and various calcium and lactate ion concentrations. Saturated solutions of CaL₂ with and without impurities were prepared and analyzed as described below.

Two methods of equilibration at constant temperature were used to obtain the saturation concentration of CaL₂ in solution. The first method involved mixing an excess amount of crystalline CaL₂ with deionized distilled water and allowing the crystals to dissolve until equilibrium was reached (method 1). The second method involved crystallization of CaL₂ from a supersaturated solution by cooling until equilibrium was reached at the desired temperature (method 2). For the pure system (CaL₂ and water), both methods were used to ensure that solutions achieved true equilibrium. Based on the results of equilibration with CaL₂, only method 1 was used for experiments with added impurities since equilibration was approached more rapidly.

Prepared aqueous solutions of CaL₂ were placed in flasks with a stirrer, and the flasks were set in a temperature-controlled water bath. Temperature was controlled by an Isotemp immersion circulator (Fisher Scientific, model 2150) and a refrigerated circulating bath with a digital controller (VWR Scientific, model 1157). Fluctuations of temperature were less than ±0.1°C. After several days of mixing, samples were taken and quickly filtered (to maintain the temperature of the solution) using a syringe with an attached fiberglass filter (Millipore Corp., Bedford, MA). To determine the calcium concentration in the filtrate, the AOAC standard method for calcium determination (AOAC International, method 968.31) based on EDTA complexometric titration was used. A standard curve for calcium was established and used to determine the calcium content in samples. Titration was performed with an automatic buret (Brinkmann/Metrohm, 665 Dosimat) and an excellent reproducibility of results was observed. Samples were allowed to stir until both equilibration methods gave the same results to ensure that the system had come to equilibrium.

Temperatures of 4, 10, and 24°C were used, corresponding to typical storage temperature, typical aging

temperature, and room temperature, respectively. To test the effect of salt on the solubility of CaL₂, we added NaCl at 3, 4, 5, and 6 g/100 g of water prior to the addition of CaL₂. The effect of pH on CaL₂ solubility was tested at 4.8, 5.0, 5.2, 5.4, and 6.5, adjusted to the desired level by dropwise addition of 3.0 N HCl to the solution of CaL₂ once the solution had reached equilibrium. The pH was measured using a pH meter (Accumet, model 815MP) with a relative accuracy of ±0.01. In all cases, solubility was expressed by the amount of anhydrous salt of CaL₂ dissolved in 100 g of water.

The effects of calcium and lactate ion concentrations on the solubility of CaL₂ were investigated using calcium chloride (CaCl₂) to adjust the calcium ion concentration and sodium lactate (NaL) to adjust the lactate ion concentration. Standard solutions of CaCl₂ and NaL were prepared (100 mL each) with 5, 10, 15, or 20% excess concentration of calcium or lactate at saturation. Excess CaL₂ was added to the standard solutions and the mixtures were allowed to equilibrate, as determined by the calcium ion concentration. Changes in the solubility of CaL₂ relative to stoichiometric dissolution were calculated from the dissolved calcium levels. For solutions with excess calcium already added, the solubility of CaL₂ was determined by subtracting the starting calcium level. For solutions with lactate already added, the calcium concentration represented the amount of CaL₂.

All solubility measurements were determined in triplicate using a full factorial design. Excellent reproducibility of results was observed with standard deviations in solubility values less than 0.03 g of anhydrous CaL₂/100 g of water. Factors including 3 temperatures (4, 10, and 24°C), 5 salt contents (0, 3, 4, 5, and 6 g/100 g of water), and 5 pH (4.8, 5.0, 5.2, 5.4, and 6.5) were analyzed by Microsoft Excel (using single factor ANOVA, $\alpha = 0.05$) to determine the statistical significance on the solubility of CaL₂.

RESULTS AND DISCUSSION

Temperature and Source Effects

Two different sources of CaL₂ were tested in this research, and significant differences in solubility between the 2 specimens were observed. The solubility values of CaL₂ as supplied by Sigma Chemical Co. were 1.88, 2.38, and 3.89 g of anhydrous CaL₂/100 g of water at 4, 10, and 24°C, respectively. These values were lower than the solubility of CaL₂ supplied by PURAC America, Inc., which were 3.38, 4.04, and 6.41 g of anhydrous CaL₂/100 g of water at 4, 10, and 24°C, respectively. Both specimens contained the same amount of water (5 molecules), were L(+) isomers, and were produced by the sugar beet fermentation process, so there

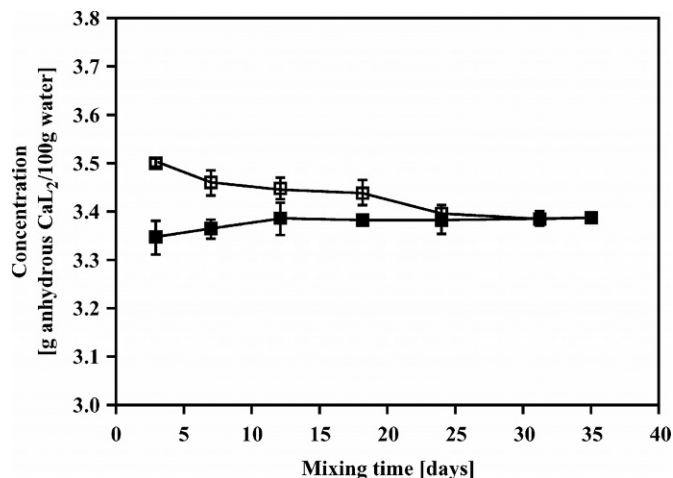


Figure 1. Solubility of calcium lactate (CaL_2) in water at 4°C: ■, method 1 (dissolution of CaL_2 crystals); □, method 2 (crystallization of CaL_2 from supersaturated solution).

should have been no differences in production method. The only possible difference was the amount of impurities in the end product; however, both specimens of CaL_2 met USP specifications. The CaL_2 chosen for these experiments was purchased from PURAC America, Inc., since it was possible to obtain a large quantity of material from the same lot.

Previous research by Glass (1933) and Hill and Cocking (1912) did not find that age, acidity, or different isomeric form of the initial CaL_2 affected its final solubility. Together with Macmorran (1933), Glass (1933) proposed that the differences in solubility are probably due to the method of preparation of the CaL_2 .

The 2 equilibration methods resulted in identical solubility concentrations, as shown in Figure 1, indicating that the system had reached equilibrium with both methods. Method 2 (crystallization) required a longer time to achieve equilibrium than method 1 (dissolution). Method 1 gave stable solubility results within a week, so in all subsequent experiments only method 1 was used. The pH remained unchanged (6.5 to 6.6) throughout the equilibration time.

These experiments have shown that it takes at least 1 wk at 4°C for a solution to reach equilibrium, even with method 1. At 24°C, equilibration was generally not completed until 4 d of agitation. Several hours of mixing, as reported by Cao et al. (2001) and Pateenko and Smirnov (1974), are not sufficient to ensure equilibrium. The short equilibration time could explain the lower solubility values of Pateenko and Smirnov (1974). In contrast, despite the short mixing times, Cao et al. (2001) obtained the highest (of all reported in the literature) solubility values at a given temperature. We can

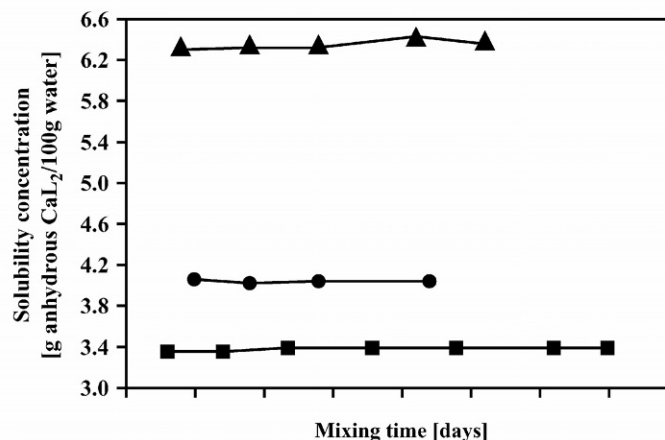


Figure 2. Solubility of calcium lactate (CaL_2) in water as a function of temperature: ■, 4°C; ●, 10°C; ▲, 24°C (standard deviation ≤ 0.01 g of anhydrous CaL_2 /100 g of water).

only speculate that this disagreement may be due to differences in starting materials.

As seen in Figure 2, solubility did not change during 3 wk of agitation at constant temperature, confirming that the true equilibrium was reached. The solubility of CaL_2 was found to be 3.38, 4.04, and 6.41 g of anhydrous CaL_2 /100 g of water at 4, 10, and 24°C, respectively. These data are in general agreement with the results from previous studies (summarized by Kubantseva and Hartel, 2002), which reported solubility values of CaL_2 of about 3, 4, and 6 g of anhydrous CaL_2 /100 g of water at 4, 10, and 24°C, respectively. As expected, solubility increased with an increase in temperature.

Salt Effect

The effect of salt (in the range between 0 and 6 g of NaCl /100 g water) on the solubility of CaL_2 in water is shown in Figure 3. Statistical analysis indicated significant differences ($P < 0.02$) between the solubilities of CaL_2 with various salt concentrations added (see Table 1). However, all differences are quite small and for practical purposes can be considered insignificant. These results are consistent with those reported by Pearce et al. (1973), who also noted only a small effect of salt on the solubility of CaL_2 . Again, no change in pH values (6.5 to 6.6) was observed for any NaCl level.

pH Effect

The effects of pH on CaL_2 solubility are presented in Table 1. A change in pH had only a minor effect on the solubility of CaL_2 within the studied range; however, due to the small standard error, the effect of pH was

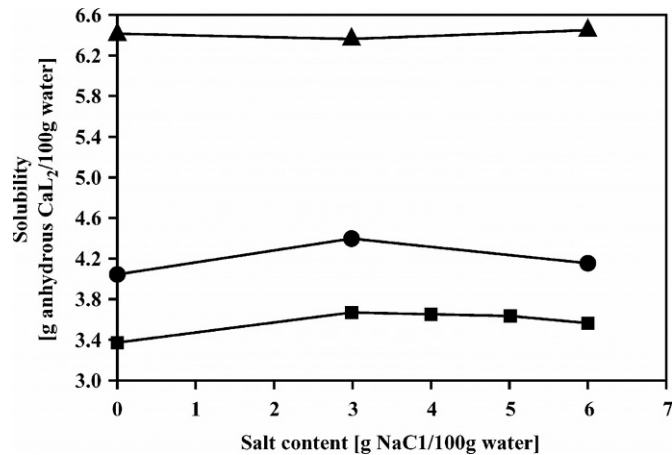


Figure 3. Solubility of calcium lactate (CaL_2) in water as a function of NaCl at various temperatures: ■, 4°C; ●, 10°C; ▲, 24°C (standard deviation ≤ 0.01 g of anhydrous $\text{CaL}_2/100$ g of water).

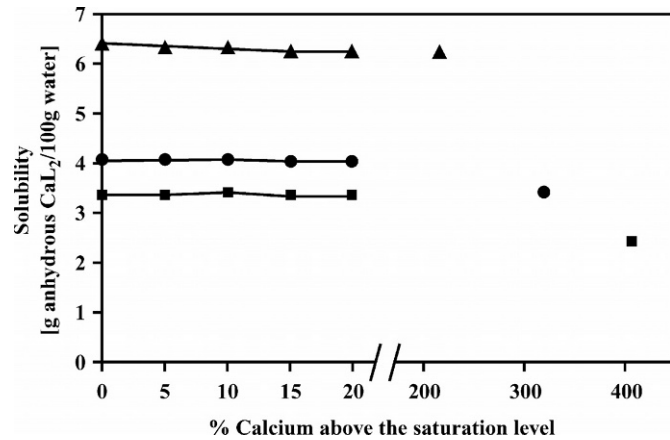


Figure 4. Effect of an addition of calcium ions on the solubility of CaL_2 : ■, 4°C; ●, 10°C; ▲, 24°C (standard deviation = 0.03 g of anhydrous $\text{CaL}_2/100$ g of water).

statistically significant at most pH levels (see Table 1). A slight increase in solubility with a decrease in pH was observed. From the NaCl experiments, it was concluded that chloride ions have almost no effect on the solubility of CaL_2 , therefore it was possible to use HCl to adjust pH and identify the pH effect as a single factor. Roth-Bassell and Clydesdale (1992) compared the solubility of CaL_2 in water at pH values of 2.0 and 7.0 by using HCl to adjust the pH. Their results showed a 20% decrease in the solubility of CaL_2 when the pH was raised from 2.0 to 7.0, a trend generally consistent with our observations.

Calcium and Lactate Ions Effects

The solubility of CaL_2 in water in the presence of additional calcium and lactate ions is shown in Figures 4 and 5, respectively. Additional calcium and lactate ions are defined as the amounts (in %) added above the equilibrium level. At all temperatures, the addition of calcium ions (5 to 20% calcium above the saturation level) to the CaL_2 solution had virtually no effect on the solubility of CaL_2 in water and did not change the pH (between 6.5 and 6.6). Statistically, however, the effect was significant ($P < 0.001$) at some levels of addition due to a small standard error. Dybing et al. (1988) also showed that the addition of calcium ions in the

Table 1. Solubility of calcium lactate (CaL_2) at different pH and temperature in the presence of added NaCl.

Temperature [°C]	pH	g Anhydrous $\text{CaL}_2/100$ g of water ¹				
		NaCl concentration [g NaCl/100 g water]				
		0	3	4	5	6
4	4.8	3.57 ± 0.01 ^{aA}	3.74 ± 0.00 ^{bA}	3.75 ± 0.01 ^{cA}	3.70 ± 0.01 ^{dA}	3.68 ± 0.01 ^{eA}
	5.0	3.44 ± 0.02 ^{aB}	3.67 ± 0.02 ^{bB}	3.68 ± 0.01 ^{bB}	3.65 ± 0.00 ^{cB}	3.63 ± 0.01 ^{dB}
	5.2	3.45 ± 0.00 ^{aB}	3.74 ± 0.00 ^{bA}	3.69 ± 0.01 ^{cB}	3.68 ± 0.01 ^{cC}	3.62 ± 0.00 ^{dC}
	5.4	3.47 ± 0.01 ^{aC}	3.67 ± 0.01 ^{bB}	3.70 ± 0.00 ^{cC}	3.65 ± 0.01 ^{dB}	3.63 ± 0.01 ^{eB}
10	6.6	3.38 ± 0.00 ^{aD}	3.66 ± 0.00 ^{bB}	3.65 ± 0.00 ^{cD}	3.63 ± 0.01 ^{dD}	3.56 ± 0.01 ^{eD}
	5.0	4.18 ± 0.01 ^{aA}	4.39 ± 0.01 ^{bA}	—	—	4.24 ± 0.01 ^{cA}
	6.5	4.03 ± 0.01 ^{aB}	4.24 ± 0.01 ^{bB}	—	—	4.15 ± 0.01 ^{cB}
24	5.0	6.51 ± 0.01 ^{aA}	6.45 ± 0.02 ^{bA}	—	—	6.25 ± 0.01 ^{cA}
	6.5	6.41 ± 0.01 ^{aB}	6.36 ± 0.01 ^{bB}	—	—	6.42 ± 0.01 ^{cB}

^{a,b,c,d,e}Significant differences among solubilities at a given temperature and pH at different NaCl concentrations (rows).

^{A,B,C,D}Significant differences among solubilities at a given temperature and NaCl content at different pH levels (columns).

¹Mean values (\pm standard deviation) of solubilities at a given temperature, NaCl concentration, and pH level.

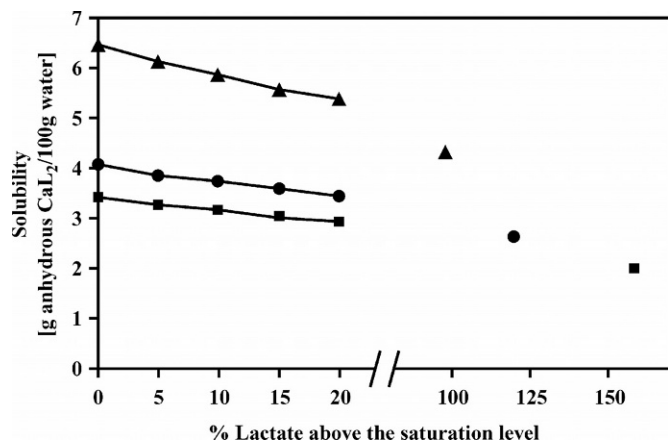


Figure 5. Effect of an addition of lactate ions on the solubility of CaL_2 : ■, 4°C; ●, 10°C; ▲, 24°C (standard deviation ≤ 0.03 g of anhydrous $\text{CaL}_2/100$ g of water).

form of CaCl_2 to cheese serum had no effect on crystal formation.

On the other hand, when calcium ions were added to the CaL_2 solution at much higher concentrations (200% or more calcium above the saturation level), a decrease ($P < 0.001$) in the solubility of CaL_2 was observed at all 3 temperatures. A decrease in pH to 5.7 to 5.8 was also observed.

At all temperatures, the addition of excess lactate ions (5 to 20% lactate above saturation level) caused a decrease ($P < 0.001$) in the solubility of CaL_2 in water, with the effect as large as 14 to 16% depending on the temperature (see Figure 5). The addition of NaL had no significant effect on the pH (between 6.5 and 6.8). When the lactate ion concentration was increased to 100% or more above saturation, the solubility of CaL_2 significantly ($P < 0.001$) decreased (by 33 to 43%), while the pH increased to 6.9 to 7.2. With added lactate ions, the equilibrium shifted towards the formation of solid CaL_2 , according to Le Châtelier's principle, so less CaL_2 was in the soluble form at equilibrium.

CONCLUSIONS

The observed decrease in CaL_2 solubility suggests that an excess level of lactate ions may be a primary reason for CaL_2 crystal appearance on cheese. There-

fore, lactose content should be controlled in the cheese milk to prevent CaL_2 crystals from forming. Temperature fluctuations (from higher to lower) during storage also may be a reason for crystal appearance as the solubility of CaL_2 decreases with a decrease in temperature. Parameters such as pH, NaCl content, and excess calcium ions did not affect CaL_2 solubility and, if they are responsible for CaL_2 crystal formation, it is not due to a decrease in CaL_2 solubility.

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