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Sedimentation of Milk Proteins as a Function of Certain Ions

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SOLUBILITY AND DISPERSIBILITY are important factors in spray-dried milk intended for beverage purposes. There is abundant evidence *(17)* that variations in the mineral composition of milk result in both physical and chemical changes, and the decrease in solubility of dry milk as a result of processing has been shown to be related *(9, 13)*, among other factors, to its salt balance. The effect of minerals on coagulation of milk proteins has been emphasized, and calcium especially has been shown to play an important role in relationship to the effects of heat on protein denaturation and flocculation *(2, 5, 18)*.

In the present investigation changes in the nature of milk colloids caused by changes in mineral composition, particularly calcium, and denaturation of proteins due to heat have been followed by examination of the sediment fraction obtained by high speed centrifugation *(3, 16, 19, 20)* of milk with a Sharples Supercentrifuge. Ion exchange processes *(1, 4, 10)*, sequestering agents, and electrolytes have been used to remove or alter the mineral components in the milk under study.

EXPERIMENTAL AND RESULTS

Cations were removed from milk by the use of the mixed bed technique *(1)* employing the resins, Amberlites IR-45 and IRC-50, in the ratio of 3:2. Centrifugation was accomplished using a Sharples Supercentrifuge with steam driven head and a 1.75 inch diameter batch bowl. The speed used, unless otherwise specified, was 40,000 R.P.M. (39,400 x g) for a period of 15 minutes.

When addition of ions was made to milk, concentrated solutions were added to avoid diluting the milk by more than 2%. The treated milk was thoroughly stirred and allowed to stand overnight in a refrigerator before being examined. Analyses for Ca, Na, P, Mg, K and ash were made by published methods *(7, 8, 11, 12, 14, 15)*.

TABLE 1
Optical density and sedimentation as function of calcium level in fresh and dried non-fat milk

Type of milk	Ca++ removed %	O.D.—1:50 DIL.	Sediment—% total milk solids
NFP[1]	0.0	0.60	34.9
NFP[1]	24.0	0.49	27.0
NFP[1]	37.0	0.36	20.0
NFP[1]	44.5	0.28	18.4
NFP[1]	85.3	0.06	2.6
Fluid Fresh	0.0	0.44	26.4
Fluid Fresh	24.0	0.36	23.0
Fluid Fresh	43.5	0.20	7.3
Fluid Fresh	59.0	0.04	6.9
Fluid Fresh	83.6	0.02	0.0

[1] Reconstituted non-fat powder.

Effects of ion exchange. Data in Table 1 and Figures 1 and 2 indicate the effect of removal of calcium on the quantity of sediment obtained by centrifugation. Sediment quantity decreases with increasing demineralization in both reconstituted spray-dried and fresh fluid whole milk. Spray-dried milk[a] yielded more sediment at comparable calcium levels than did fresh whole milk or fresh whole milk heated at 170° F. for 30 minutes. The amounts of sediment obtained from spray-dried samples with approximately 30% calcium removed were comparable to those of non-dried milks of original calcium contents.

[a] The solids content of reconstituted spray-dried milks was adjusted to the levels of the fluid milks used.

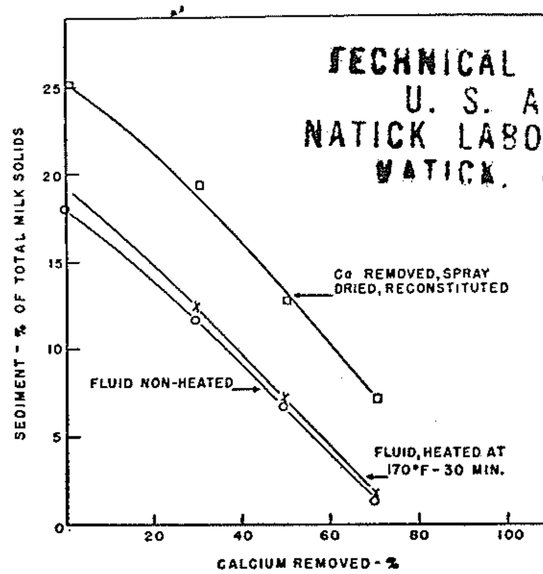

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Figure 1. Relationship of calcium content to quantity of sediment in fluid and reconstituted spray dried whole milk.

The optical densities of both fresh fluid and spray-dried non-fat milk decrease as calcium is removed, Table 1, Figure 2; however, at comparable calcium levels the optical density of the spray-dried product is consistently greater than that of fresh fluid milk. Optical density measurements were made with the Evelyn Spectrophotometer using the 420 mμ filter *(6)*.

Effect of addition of calcium ion. Data shown in Table 2 and Figure 3 indicate that adding calcium to milk altered the colloidal nature of milk protein as shown by the increased quantity of proteins in the sediment, and resulted in the formation of additional calcium-protein complexes *(16)* as indicated by a greater quantity of calcium in the sediment fraction. Removal of calcium by ion exchange *(1)* had the effects as shown in Tables 1, 2, 3a and 3b and Figures 1, 2, and 3.

TABLE 2
Effect of alteration of calcium balance in whole milk on the composition and pH of supercentrifuged sediment fraction

Calcium level (% of normal)	Total solids g./100 ml. milk	A Protein (N x 6.38) g./100 ml. milk	B Calcium mgs./100 ml. milk	A/B Ratio	pH[3]
200[1]	3.22	1.98	91.4	21.7	6.6
155[1]	2.79	1.83	73.1	25.1	6.8
130[1]	2.47	1.73	57.4	30.2	6.8
100 (control)	2.29	1.59	52.4	30.5	7.0
75[2]	1.16	0.79	24.5	32.2	7.0
45[2]	0.75	0.49	12.2	40.1	7.3

[1] Calcium was added as a 15% solution of $CaCl_2$ in water.
[2] Calcium was removed by a mixed bed of Amberlites IR-45 and IRC-50 ion exchangers in a column containing a ratio of 3:1 of these resins.
[3] The pH of the treated milk before centrifugation was maintained constant.

Effect of substitution and addition of various cations. An investigation was conducted to determine the effects of magnesium, sodium, potassium, citrate, and phosphate ions on non-fat fresh milk and reconstituted spray dried milk. Magnesium and calcium ions were added as 1M while salts, sodium and potassium ions were added as 2M solutions of the chloride. Sodium citrate and sodium orthophosphate supplied the citrates and phosphate ions, respectively. Data in Tables 3a and 3b show the effects on the composition and quantity of the sediment following substitution in ion-exchanged milk of equivalent quantities of magnesium, potassium, calcium, and sodium for varying levels of the original native milk calcium in a completely demineralized milk. Figure 5 and Table 6 illustrate the increase in optical densities resulting when these cations were returned to deionized milk. When calcium was added the optical density increased to a value close to that of the original milk. That was not true, however, when magnesium, sodium, or potassium was substituted for calcium. In the latter instances the optical density remained low. Examination of data in Table 3a and 3b shows that while the addition of Mg^{++} ion results in sediment formation equivalent to about 60% of that obtained by addition of Ca^{++} ion, the quantity of mg^{++} ion found in the sediment was about 6% that of Ca^{++} ion, and the optical density was about 20% that obtained by addition of Ca^{++} ion, Table 6, Figure 5.

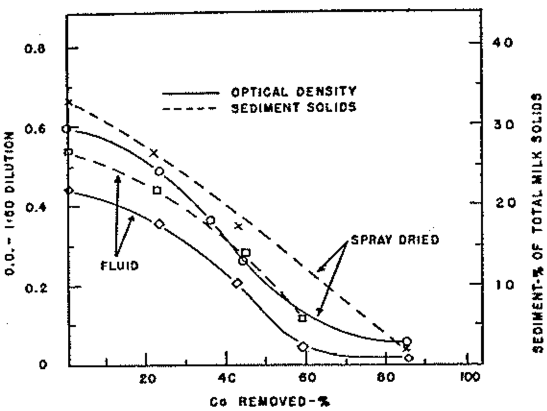


Figure 2. Optical density and sedimentation as functions of calcium level in fluids and spray dried non-fat milk.

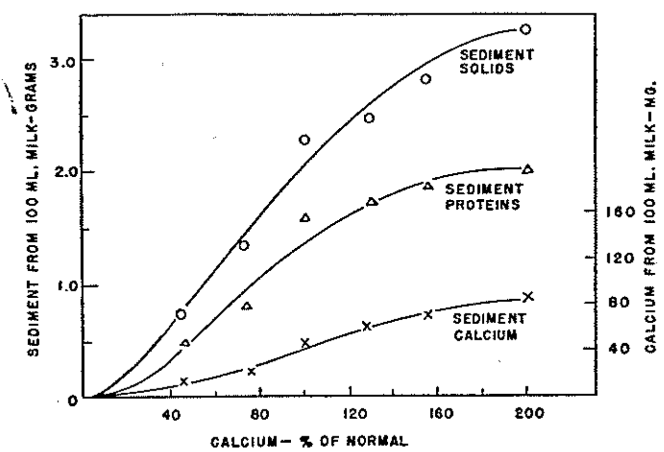


Figure 3. Effect of alteration of calcium in fluid milk on sediment composition.

Effect of addition of phosphate and citrate ions on sedimentation. Addition of phosphate ion, in an aqueous solution, did not affect the quantity of sediment and protein-bound calcium and phosphorus to any significant extent; however, addition of citrate ion, in an aqueous solution, caused a decrease in the quantity of sediment and protein-bound calcium and phosphorus, Table 4, Figure 4.

TABLE 3a

Effect of substitution of cations for calcium on quantity of sediment solids[1]

Percent Ca^{++} removed by ion exchange	Grams sediment from 100 ml. of ion exchanged milk	Grams of sediment solids from 100 ml. milk when Ca^{++}, Mg^{++}, Na^{+} or K^{+2} replaces removed calcium			
		Grams sediment when Ca^{++} is returned	Grams sediment when Mg^{++} replaces Ca^{++}	Grams sediment when Na^{+} replaces Ca^{++}	Grams sediment when K^{+} replaces Ca^{++}
0	2.54	......	......	......	......
15	2.15	2.35	2.22	2.12	2.05
30	1.78	2.17	2.05	1.73	1.58
60	0.58	2.45	1.90	0.68	0.58
100	0	2.50	1.50	......	......

[1] Sediment obtained by centrifuging milk in a Sharples Centrifuge at 40,000 RPM for a period of 15 minutes.
[2] Mg^{++} and Ca^{++} added as 1M solution, Na^{+} and K^{+} added as 2M solution of the chlorides.

TABLE 3b

Effect of substitution of certain cations for calcium on quantity of these cations in sediment[1]

Percent Ca^{++} removed by ion exchange	Mgs. Ca^{++} in sediment from 100 ml. of ion exchanged milk	Mgs. of added[2] cations in sediments from 100 ml. milk in which Ca^{++}, Mg^{++}, Na^{+}, or K^{+} replaces removed calcium			
		Mgs. Ca^{++} in sediment when Ca^{++} is replaced	Mgs. of Mg^{++} in sediment when Mg^{++} replaces Ca^{++}	Mgs. of Na^{+} in sediment when Na^{+} replaces Ca^{++}	Mgs. of K^{+} in sediment when K^{+} replaces Ca^{++}
0	53.5	......	......	......	......
15	40.0	50.0	4.5	3.5	1.3
30	27.2	47.0	6.0	2.0	1.0
60	16.6	51.0	4.0	1.5	0.5
100	0	53.5	3.0	......	......

[1] Sediment obtained by centrifuging milk in a Sharples Centrifuge at 40,000 RPM for a period of 15 minutes.
[2] Mg^{++} and Ca^{++} added as 1M solution, Na^{+} and K^{+} added as 2M solution of the chlorides.

Effect of addition of sequestering agents. Addition of a 10% aqueous solution of a chelating agent[b] sufficient to sequester from 5% to 100% of the total calcium and magnesium in the samples, resulted in a decrease of the optical density of the resultant non-fat milks. The optical density and sedimentation effects were similar to those as a result of ion exchange removal of calcium from milk, Tables 5 and 6.

DISCUSSION

Data presented in this paper point out the importance of the salt balance and especially of the calcium level in

[b] Disodium Ethylene Diamine Tetracetate dihydrate-sequestrene NA2.

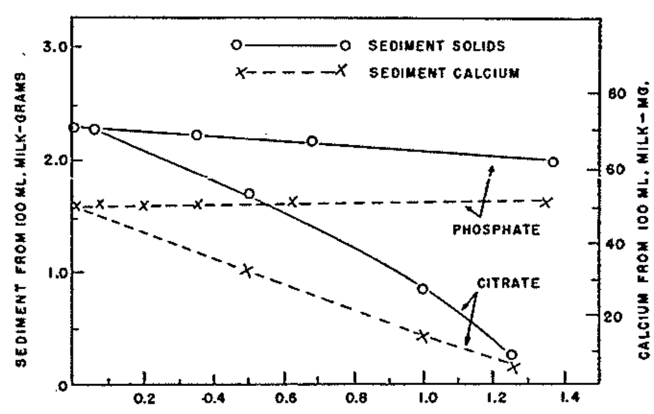


Figure 4. The effect of addition of sodium citrate and disodium phosphate on milk sediment.

TABLE 4

The effect of addition of sodium citrate and sodium phosphate on milk sediment

Sample number	Citrate added gm./100 ml.	Total solids gm./100 ml.	Nitrogen	Ash	Fat	Ca	P	Na
			Mg. per 100 ml. milk					
1	1.00	0.82	76.0	72.0	106.6	14.3	6.8	8.0
2	0.50	1.66	165.4	131.8	300.0	32.7	18.9	5.5
3	0.25	2.16	244.4	169.0	306.7	44.5	27.0	5.0
4	0.15	2.08	215.1	162.3	293.3	42.9	24.8	13.0
5	0.05	2.30	242.1	170.0	333.3	49.6	27.7	5.1
6	0.025	2.34	244.2	170.8	300.0	51.1	28.8	6.7
7	0.0	2.28	242.6	171.0	313.3	50.1	27.7	5.1
	Phosphate added gm./100 ml.							
8	1.35	2.02	195.2	206.2	220.0	51.6	28.1	18.3
9	0.67	2.15	239.3	206.8	280.0	51.7	35.8	8.5
10	0.34	2.18	226.5	169.0	253.3	50.1	31.1	11.3
11	0.20	2.27	235.4	179.3	320.0	50.6	32.7	8.2
12	0.07	2.30	238.2	181.2	326.7	50.6	34.8	7.4
13	0.035	2.29	242.8	167.0	333.3	50.1	29.6	7.0

TABLE 5

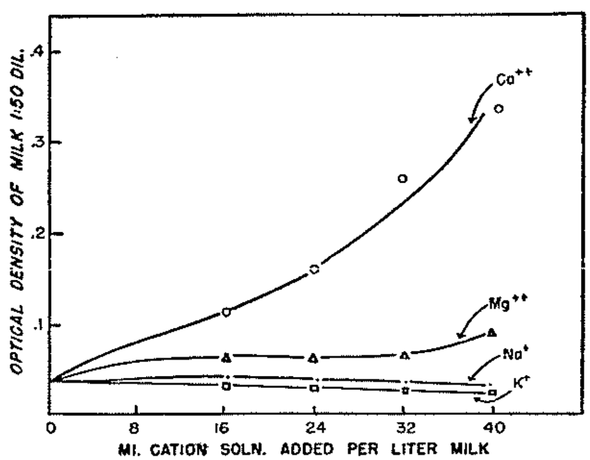
The effect of sequestration of calcium non-fat milk[1] on quantity and composition of milk sediment

Sequestrene[2] NA2, grams	Ca^{++} $+Mg^{++}$ Sequestered, %	O. D. 1:50 Dil.	Total Solids	Protein	Ash	Calcium	Phosphorous	Magnesium	Sodium
			g./100 ml. milk		Mgs./100 ml. milk				
1.32	100	.043	0.17	0.11	9.0	0.4	1.3	N.F.	5.2
0.61	50	.082	0.48	0.31	27.0	0.4	3.0	N.F.	1.2
0.31	25	.129	1.53	0.58	99.5	22.6	17.5	1.2	1.5
0.15	10	.141	2.05	0.82	146.5	39.4	26.2	2.3	4.4
0.08	5	.150	2.42	1.76	179.5	50.5	32.4	3.2	4.3
0	0	.161	2.55	1.84	197.0	56.3	36.3	3.5	3.9

[1] Supercentrifuged at 40,000 RPM (39,400 x g) for 15 minutes.
[2] Ethylene diamine tetracetate dehydrate added as 10% solution in water to sequester given percentages of milk calcium plus magnesium.

influencing protein changes which occur during heat processing of milk. Less sediment was found in fresh and in heated milk than in reconstituted spray-dried milk. Calcium removal prior to spray drying decreased the quantity of sediment obtained from the reconstituted product. When calcium was decreased by 30% prior to spray drying, Figure 1, sediments comparable to those from fresh milk were obtained.

Although various cations are removed by the ion exchange process, the evidence obtained by sedimentation studies shows that calcium is of primary importance in determining the particle nature of milk proteins. Calcium continued to combine with the milk proteins when



(1) Mg^{++} AND Ca^{++} AS M SOLN. OF CHLORIDES
Na^{+} AND K^{++} AS 2M SOLN. OF CHLORIDES

Figure 5. The effect of substitution of cations on the optical density of milk.

TABLE 6

The effect of substitution of cations on optical density in demineralized milk[1]

Cation solution added Ml.[2]	Ca^{++}	Mg^{++}	Na^{+}	K^{+}
	Optical density of milk 1:50 dil.			
0	.039	.039	.039	.039
16	.114	.066	.035	.030
24	.158	.066	.035	.028
32	.260	.066	.033	.027
40	.337	.092	.032	.026

[1] Non-fat milk.
[2] Mg^{++} and Ca^{++} as M solution of chlorides per liter of non-fat fluid milk. Na^{+} and K^{+} as 2M solution of chlorides per liter of non-fat fluid milk.

added at higher than normal levels as shown in Figure 3. This may be compared to the effect produced by the addition of sodium and potassium which did not form insoluble complexes. When magnesium was substituted for calcium in equivalent quantities, its effect on sediment formation was less than that of calcium. Since the quality of magnesium in milk is approximately one-tenth that of calcium, the possibility of flocculation due to magnesium would appear to be slight in comparison with that due to calcium. While the addition of calcium resulted in an increase of optical density, the addition of magnesium, sodium or potassium was without effect in this respect, Table 6. The mechanism responsible for the formation of insoluble milk protein-calcium complexes has been stated *(18)* to be due to the combination of calcium with the reactive carbonyl and amino groups of the protein molecule. The resultant intermolecular bridges lead to larger aggregates and consequent coagulation.

SUMMARY

Sedimentation studies, using a Sharples Supercentrifuge, of fluid and reconstituted spray-dried milk indicate that of the chief milk cations only calcium and magnesium tend to form insoluble complexes with proteins. Magnesium has less effect than calcium, whereas sodium and potassium are without effect in this respect.

Addition of calcium over normal levels increased the quantity of sediment and the optical density of milk, but removal of calcium by resinous ion exchange, or the use of a sequestering agent, reversed this effect. Neither the sediment composition nor the optical density of milk changed significantly when disodium phosphate was added up to 1% levels, whereas the addition of sodium citrate to the same levels caused a decrease of more than 50% in both the quantity of sediment and the optical density.

Reconstituted spray-dried whole milk yielded more sediment than fresh whole milk or whole milk heated at 170° F. for 30 minutes; however, by reduction of the calcium content of spray-dried whole milk the quantity of sediment was reduced to the levels obtained with fresh whole milk.

The nature of protein colloid particles in both fresh and spray-dried milk altered with calcium removal so that no separation of sediment was effected under a force of 39,400 x g applied for a period of fifteen minutes in a Sharples Supercentrifuge when the calcium level was one-third of normal.

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