COMPOSITION OF ULTRAFILTRATES FROM MILK HEATED AT 80 TO 230° F. IN RELATION TO HEAT STABILITY [1]

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SUMMARY

Analyses of ultrafiltrates collected from milk at temperatures up to 230° F. indicated that temperature changes affect the inorganic composition of milk much more drastically than has previously been demonstrated from the analyses of cooled milks. At 200° F., the amount of calcium passing into the ultrafiltrate was approximately 50%, and the phosphate about 82%, of that found at 80° F. On the other hand, the hydrogen ion concentration of ultrafiltrate collected at 200° F. was at least double that of ultrafiltrate collected at 80° F.

Similar tests made with solutions of calcium and phosphate indicated that these changes in the inorganic composition of milk can be explained entirely on the basis of changing solubility and composition of the insoluble calcium phosphate salts. No change in the dissociation of calcium citrate with temperature could be detected.

No correlation was observed between the changes induced by heat and the heat stability of various samples of milk.

Two studies (5, 15) have led to the belief that the inorganic composition of the milk serum is a major factor which controls the heat stability of the milk colloids. However, because the composition of milk sera separated from the proteins at low temperatures has failed to correlate with the heat stability of the colloids (18), it has been suggested that changes in the inorganic composition of the serum during heating control the heat stability (5). No experimental proof for this suggestion has been obtained.

Modification of our recently published (17) ultrafiltration technique made possible the collection of ultrafiltrates from milk over a wide range of temperatures, and a study of the heat-induced changes in serum composition was, therefore, undertaken. Results of this study are presented herewith.

MATERIALS AND METHODS

Mixed-herd milk, and milk from individual cows, was obtained from morning milkings at the Central Experimental Farm, Ottawa. Bulk milk was separated at 90° F. in a cream separator; milk from individual cows was separated by centrifuging for 15 min. at 1,500 r.p.m., in an International No. 2 centrifuge and withdrawing the skimmilk from below the cream layer by siphon. All tests were made on milk which had not been heated before the beginning of the test.

Ultrafiltrates at 80° F. were obtained by the method previously described (17). At temperatures between 80 and 210° F. the apparatus shown in Figure 1 was used. Approximately 600 ml. of milk, preheated to the desired temperature, was poured into the reservoir and a portion pumped into the filter chamber with the needle valve open. Approximately the first 300 ml. of milk flowing from the return line rinsed residual water or milk (several samples can be filtered without

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changing the membrane) from the unit and were discarded. The return line was then attached to the reservoir as shown, and the needle valve slowly closed until the pressure gauge registered 25 lb. From 0.25 to 1 ml. of ultrafiltrate collected in the receiving flask per minute, the amount increasing with increasing temperature. The stainless steel heating coil assured that the milk was within 1° F. of the desired temperature when it entered the ultrafilter. The need for the rotating brush is discussed below.

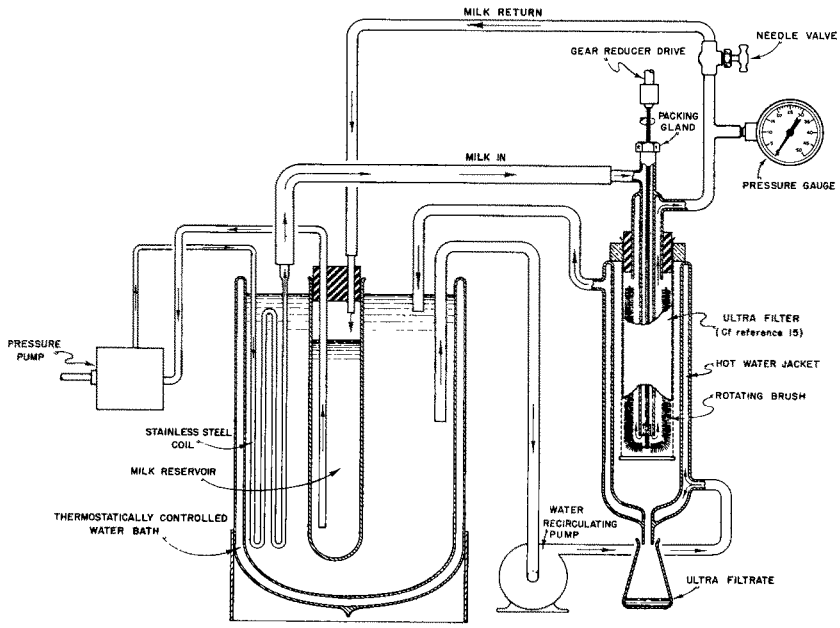

FIG. 1. Apparatus for collecting ultrafiltrate from milk at temperatures up to and including 200° F.

At temperatures above 210° F., the apparatus previously described (17) was filled with hot milk and hermetically sealed into a metal cylinder in which steam could be generated (Figure 2). The pressure relief valve controlled the steam pressure and, hence, the temperature. Air pressure on the milk sample was kept the same as the steam pressure until the milk attained the desired temperature, as indicated by a thermocouple immersed in the milk. Condensate was then drained off through the outlet valve, the air pressure increased by 25 lb., and ultrafiltrate collected in the stainless steel receiver for periodic removal through the outlet valve into an ice-cooled receiving flask.

Ultrafiltrates were analyzed directly by the following procedures: calcium ion (17), total calcium (11), phosphate (14), citrate (12), magnesium (13), and potassium and sodium by flame photometry.

When required for calculation of the product $(Ca^{++})(HPO_4^{=})$ and $(Ca^{++})^3(PO_4^{\equiv})^2$, concentrations of di- and triphosphate ion were calculated from the dissociation constants derived by Hentola (8) [*cf.* also (17)]. This calculation

ignores the phosphate complexed as undissociated calcium salts, which Bjerrum (2) estimates as between 0.08 and 7%, but the errors thus introduced into the calculated products would not affect the conclusions reached.

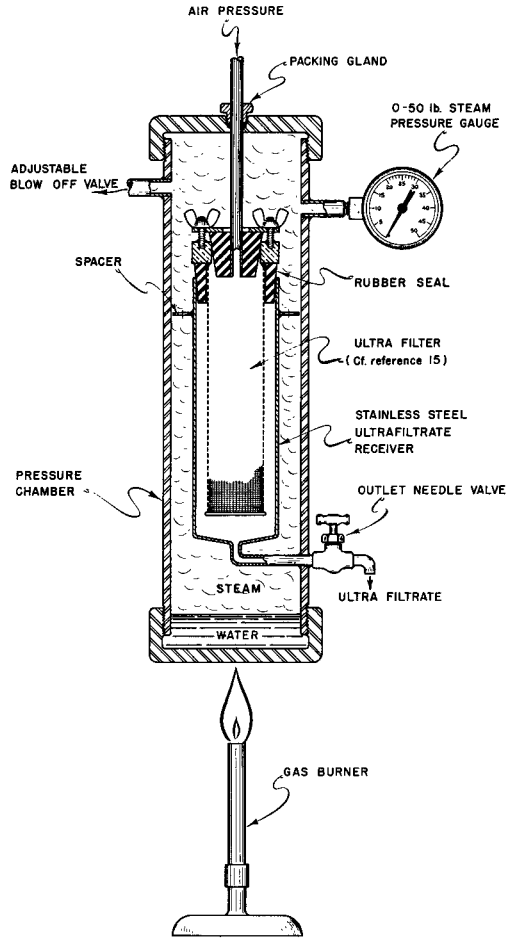

FIG. 2. Apparatus for collecting ultrafiltrate from milk above 210° F.

Heat stability was estimated by sealing 2-ml. aliquots of milk into a 150- by 16-mm. test tube and immersing the tube in a thermostatted oil bath at 284° F. The sample was shaken manually at about 2-sec. intervals, in such a way that milk was splashed on to the sides of the tube. The time required for distinct granulation to show in the milk as it flowed back down the side of the tube was recorded as the "heat stability" of the sample.

RESULTS

Need for the rotating brush. During the early tests with the apparatus, shown in Figure 1, no rotating brush was provided, and a deposit of milk col-

loids formed on the inner surface of the membrane at temperatures above 160° F. Samples of ultrafiltrate collected when this deposit was on the membrane contained abnormally high amounts of several ions, especially potassium, and successive samples collected at one temperature had widely varying compositions. Development of the brushing system shown in Figure 1 made it possible to obtain successive samples of uniform composition, and reduced the potassium content of the serum to that of the original milk.

The interpretation of this phenomenon is of considerable interest but, because of the variability of the results obtained in the presence of the deposit, firm conclusions can not be drawn, except to the effect that brushing of the membrane is essential for reliable results. It appears probable that the serum in the interstices of the deposit becomes more concentrated because ions are attracted to the deposited proteins, and that this concentrated serum is forced outward through the membrane. As the thickness of the deposit increases, the composition of the ultrafiltrate presumably approaches that of the ionic atmosphere around the protein particles in the deposit; whereas, in the absence of the deposit the ultrafiltrate has the composition of the serum itself.

The brushing system was not used with the apparatus for collecting ultrafiltrate from milk above 210° F. and some error thus may have been introduced. However, little deposit of protein formed on the membrane at 230° F. and it is, therefore, unlikely that this error would be large.

Effect of time and temperature of heating. The composition of ultrafiltrate collected at various times from a sample of skimmilk held at 200° F. is shown in Figure 3. The 0 time samples were collected at room temperature before heating the milk. Collection of hot ultrafiltrate was begun 11 min. after the milk had been heated, and sufficient sample for analysis was collected in a further 15 min. This 11- to 26-min. sample had the same composition, within reasonable limits of error, as the later samples, although the pH was slightly higher. Sufficient sample for a complete analysis could not be collected within a shorter interval, but analyses for calcium and phosphate made on smaller amounts of ultrafiltrate collected at short intervals indicated that equilibrium was established within 5 min. at 180° F.

Increasing temperatures progressively increased the loss of calcium ion, total calcium, and phosphate, and decreased the pH of ultrafiltrates from skimmilk (Figure 4A). There was no significant change in the citrate, magnesium, sodium, or potassium content of the ultrafiltrate at any temperature studied. The loss of phosphate and the decrease in pH appeared to be greater per unit temperature change at the higher temperatures; the loss of calcium ion and total calcium was relatively uniform throughout the temperature range studied.

The composition of ultrafiltrates from 2:1 concentrated skimmilk changed with temperature of filtration in the same manner as the composition of those from skimmilk (Figure 4B). The change in pH and calcium ion concentration was of the same order in concentrated and normal samples, but approximately twice as much total calcium and phosphate became nonultrafilterable.

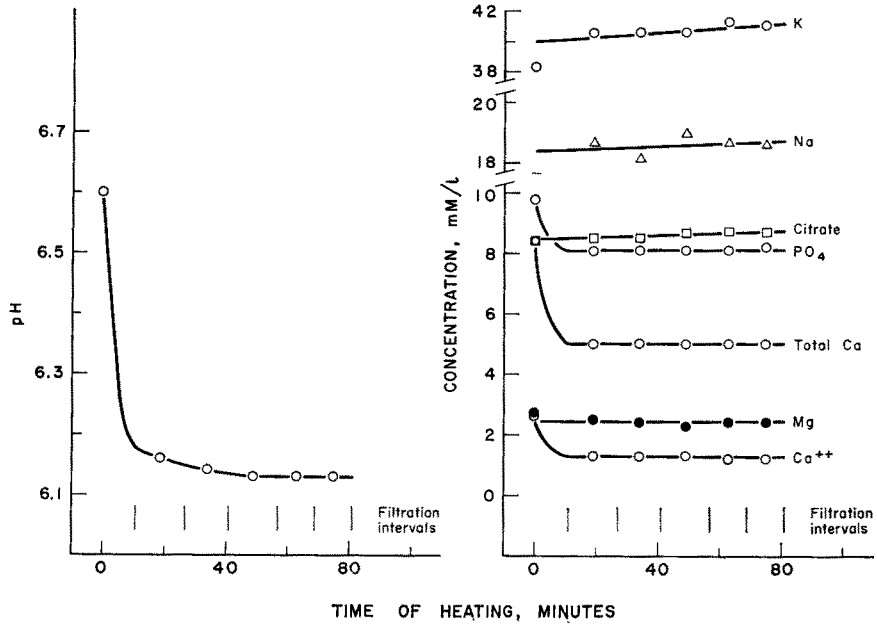

FIG. 3. Composition of ultrafiltrates collected from raw skimmilk and from milk heated to 200° F.

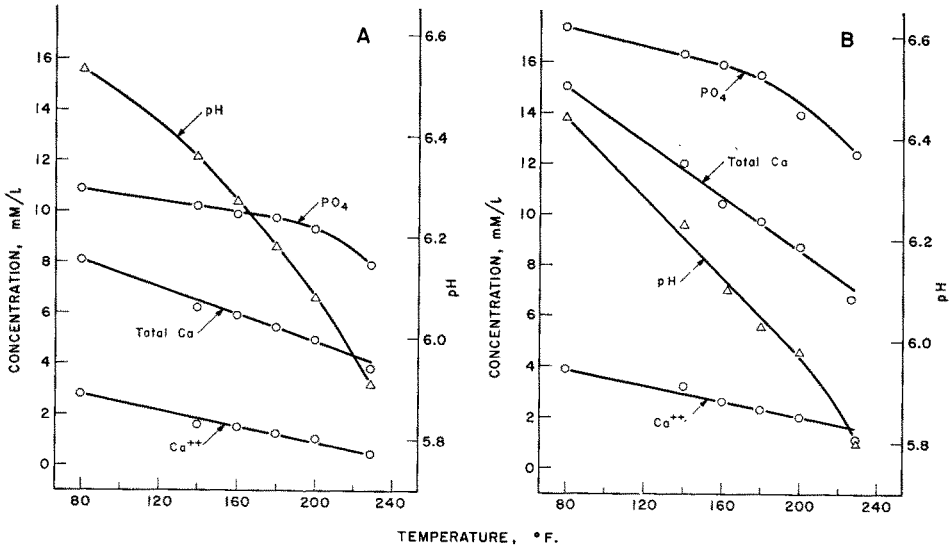

FIG. 4. Composition of ultrafiltrates collected at various temperatures.

A—skimmilk

B—2: 1 concentrated skimmilk (16.3% T.S.)

TABLE 1
Composition of ultrafiltrates from unheated, heated, and cooled skimmilk

Treatment	pH	Ca^{++}	Composition of ultrafiltrate: Total Ca	Total PO_4	$[Ca^{++}] \times [HPO_4^{=}]$	$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$
			(mM/liter)			
Sample I						
Unheated	6.77	2.6	8.7	8.6	11.1×10^{-6}	6.1×10^{-23}
140° F. 130 min.	6.42	1.9	7.4	8.0	4.7×10^{-6}	0.2×10^{-23}
Cooled 1.5 hr.	6.63	2.2	8.2	8.1	7.4×10^{-6}	1.2×10^{-23}
Sample II						
Unheated	6.83	2.9	8.9	10.2	15.7×10^{-6}	18.4×10^{-23}
200° F. 20 min.	6.22	1.1	5.2	7.6	1.8×10^{-6}	0.6×10^{-23}
Cooled 1 hr.	6.64	2.2	8.4	9.0	8.4×10^{-6}	1.6×10^{-23}
Cooled 4 hr.	6.69	2.4	8.7	9.4	10.2×10^{-6}	3.3×10^{-23}
Cooled 22 hr.	6.73	2.7	9.2	9.8	12.6×10^{-6}	6.7×10^{-23}

When heated milk was recooled, the composition of the serum tended to return to that of the original milk, and the composition of ultrafiltrates collected after cooling for 1 hr. or longer (Table 1) agreed with values for heated milk reported by other workers (8, 10). However, the values for (Ca^{++}) $(HPO_4^{=})$ and $(Ca^{++})^3$ $(PO_4^{\equiv})^2$ remained slightly below those of the original milk, even after 22 hr. at 40° F.

Studies with artificial sera. Heating synthetic sera prepared as in (16), which contained suspended calcium phosphate, to 200° F. altered the composition of the ultrafiltrate in much the same way that heating skimmilk altered its ultrafiltrate (Table 2). However, the drop in pH was more marked in the synthetic sera, and the loss of calcium and phosphate appears to have been somewhat greater.

To determine the effect of heating on solutions of calcium phosphate alone, three solutions of varying Ca : P ratio were prepared at ionic strength 0.08

TABLE 2
Composition of ultrafiltrates from synthetic sera at 200° F.

Heating time *(min.)*	pH	Composition: Ca^{++}	Total Ca	PO_4^{---}	Na^{+}	K^{+}	Citrate
			(mM/liter)				
Sample I							
Unheated	6.55	2.1	8.3	9.7	22	37	7.3
5 to 16	5.68	1.1	4.2	6.6	23	38	....
16 to 34	5.64	0.7	3.8	6.8	23	38	7.3
34 to 52	5.60	0.7	3.4	6.5	24	38	7.3
52 to 70	5.57	0.6	3.5	6.5	23	38	7.3
Sample II							
Unheated	6.68	1.8	7.5	7.7	17	28	7.2
5 to 24	5.66	1.0	4.2	5.7	18	32	7.2
50 to 64	5.54	0.8	3.6	5.4	19	33	7.1
90 to 114	5.62	0.6	3.4	4.9	17	31	6.9

(adjusted with potassium chloride), and adjusted to approximately pH 6.7 to induce precipitation. Aliquots of these solutions were then heated for 20 to 30 min. and the precipitate removed in a heated centrifuge. The supernatants were cooled and analyzed. No correction for evaporation of the hot solutions was made, as the increase in concentration (about 10% loss of volume at 200° F.) would merely cause some additional precipitation and not significantly affect either the pH or the composition of the saturated supernatants.

The results (Table 3) indicate that even under these conditions heating the suspensions caused changes to occur in the pH of the supernatants, and in the $(Ca^{++})(HPO_4^{=})$ and $(Ca^{++})^3(PO_4^{\equiv})^2$ products. Precipitation of calcium phos-

TABLE 3

Composition of supernatants from solutions of calcium phosphate centrifuged at various temperatures

(All solutions contained solid calcium phosphate in suspension)

Solution No.	80°	120°	140°	160°	180°	200°
			pH of supernatant			
1[a]	5.98	5.76	5.53	5.19	5.19	4.87
2[a]	6.13	5.83	5.60	5.32	5.22	5.08
3[a]	6.36	6.24	6.19	6.13	6.12	6.07
			Calcium content of supernatant *(mM/liter)*			
1	4.32	4.13	4.26	4.79	4.86	4.93
2	3.22	2.80	2.68	2.66	2.87	2.96
3	0.79	0.40	0.32	0.17	0.16	[b]
			Phosphate content of supernatant *(mM/liter)*			
1	4.50	4.32	4.36	4.70	4.84	5.18
2	6.22	5.94	5.96	6.26	6.40	6.48
3	12.20	12.00	12.31	12.82	12.82	13.56
			Loss of calcium from supernatant *(mM/liter)*			
1	2.58	2.77	2.64	2.11	2.04	1.97
2	2.98	3.40	3.52	3.54	3.33	3.24
3	3.21	3.60	3.68	3.83	3.84	. ...
			Loss of phosphate from supernatant *(mM/liter)*			
1	1.50	1.68	1.64	1.30	1.16	0.82
2	1.78	2.06	2.04	1.74	1.60	1.52
3	2.80	3.00	2.69	2.18	2.18	1.44
			Ration of calcium loss/phosphate loss *(mole basis)*			
1	1.72	1.65	1.61	1.62	1.76	2.40
2	1.67	1.65	1.73	2.03	2.08	2.13
3	1.15	1.20	1.37	1.76	1.76	.....
			$[Ca^{++}]\,[HPO_4{=}] \times 10^6$			
1	2.7	1.6	1.0	0.6	0.6	0.3
2	3.7	1.7	1.0	0.6	0.5	0.4
3	2.7	1.1	0.8	0.4	0.4	....
			$[Ca^{++}]^3\,[PO_4{\equiv}]^2 \times 10^{27}$			
1	156	19	2.9	0.22	0.25	0.015
2	440	20	2.3	0.20	0.11	0.034
3	163	78	2.8	0.28	0.21	.....

[a] Before adjustment to pH 6.70, which initiated precipitation, Solution 1 contained 6.90 m*M*/liter calcium and 6.00 m*M*/liter phosphate, Solution 2, 6.20 m*M* and 8.00 m*M*, and Solution 3, 4.00 m*M* and 15.00 m*M*, respectively.

[b] Too low to determine.

phate in excess of that precipitated at 80° F. was not extensive, and occurred only at 120 and 140° F. The further decrease in pH and in the apparent solubility products at higher temperatures was largely caused by a change in the composition of the precipitate (re-solution of phosphate in excess of calcium).

Since temperature could affect the dissociation of any weakly dissociated acids present in ultrafiltrate, a few pH measurements were made at 180° F., with a Beckman Model G instrument and the recommended Beckman procedure. Samples in which a precipitate formed on heating (milk, ultrafiltrate collected at room temperature, synthetic serum) decreased in pH by 0.25 to 0.4 units. Samples in which no precipitate formed, i.e., ultrafiltrate collected at 180° F., supernatant from synthetic serum centrifuged at 180° F., and calcium-free synthetic serum, increased in pH by 0.07 to 0.16 units (average increase, 0.11 unit).

An attempt was also made to utilize the murexide method for the determination of calcium ion (17), to qualitatively estimate whether the dissociation of calcium citrate increased as the temperature increased (Table 4). The small

TABLE 4

Apparent calcium ion concentration of calcium citrate solution

Solutions	O.D. 515 mμ	O.D. 470 mμ	515-470 values[a]	$[Ca^{++}]$	O.D. 515 mμ	O.D. 470 mμ	515-470 values[a]	$[Ca^{++}]$
		80° F.				200° F.[b]		
Standard								
1 m*M*/liter Ca	0.854	0.638	+0.22	1	0.629	0.469	+0.16	1
2 m*M*/liter Ca	0.796	0.745	+0.05	2	0.638	0.561	+0.08	2
3 m*M*/liter Ca	0.782	0.824	−0.04	3	0.629	0.602	+0.03	3
Test								
10 m*M* Ca + 9 m*M* citrate/liter	0.810	0.699	+0.11	1.6	0.653	0.505	+0.15	1.1
1.6 m*M* Ca/liter 0 citrate	0.796	0.699	+0.10	1.6	0.629	0.549	+0.08	2.0

[a] *Cf.* Reference 15.

[b] Murexide solutions fade at 200° F., but reproducible results were obtained by carefully controlling the heating times.

difference in observed optical density on heating indicates an increase in apparent calcium ion in the citrate-free solution (probably because heat-induced fading was slightly greater at 515 than at 470 mμ), but a decrease in the calcium citrate solution.

Relation of ultrafiltrate composition to heat stability. Since the changes induced by heat occur relatively rapidly (Figure 3), and are progressive with increasing temperature (Figure 4), it is reasonable to attempt to relate the effect of heating milk to 200° F. to its stability at higher temperature. Ultrafiltrates were, therefore, prepared from several bulk milks, and from a series of samples from each of four individual cows, at 80 and at 200° F., and the heat stability of the same samples was determined at 284° F. Two of the cows selected

had freshened only a few days before the first sampling, whereas the other two were nearing the end of their lactation period.

None of the attributes tested at either temperature (Table 5) showed any relation to the heat stability, and the differences between the 80 and 200° F. ultrafiltrates also failed to show any relation to heat stability. Grouping the samples in terms of their heat stability failed to indicate any relation between these groups and the composition of the ultrafiltrates. Of particular interest is the observation that even though the samples received four or five days after freshening of the cows had a high calcium ion content (19), they were not unstable in comparison to later samples of normal calcium ion content from the same cows.

DISCUSSION

Most of the results reported in this paper were obtained by the analysis of ultrafiltrates collected from hot milk, but cooled before analysis. Total calcium and phosphate in the ultrafiltrates would not, of course, be affected by the change in temperature after filtration, but interpretation of the pH and calcium ion results is more difficult.

The pH of phosphate buffers passes through a minimum at about 115° F., but would be essentially the same at the two temperatures (80 and 200° F.) used in these studies (1). Similar data were not found for citrate solutions. Data presented in the present paper indicate that the pH of ultrafiltrates may be about 0.1 unit higher at 180° F. than at room temperature. The pH value recorded for cooled samples of ultrafiltrate is, therefore, probably about 0.1 pH unit below that actually developed in the hot milk.

The only factor besides pH likely to affect calcium ion concentration during cooling appears to be citrate, and Evenhuis (5) has suggested that calcium dissociates to a greater extent at high temperature. To the best of the authors' knowledge, there is no evidence for this suggestion. The data presented herewith on the optical density of calcium murexide solutions indicate that no increase, but possibly a slight decrease, in calcium ion concentration occurs when calcium citrate solution is heated (Table 4). Thus, it appears probable that the calcium determined after cooling the ultrafiltrates is essentially the same as, or slightly higher than, that of the hot milk.

These considerations lead to the conclusions that heat affects the inorganic composition of milk serum to a much greater extent than has previously been demonstrated by analysis of recooled milks. When milk is heated to 200° F., approximately 50% of the soluble calcium and 18% of the soluble phosphate become nonultrafilterable, calcium ion concentration decreases by approximately 60%, and the acidity (H^+ concentration) increases at least twofold. On cooling, these changes are from 75 to 90% reversible.

Since similar changes were observed when artificial sera and solutions of calcium phosphate were separated from their precipitates at various temperatures (Tables 2 and 3), the primary cause appears to be a heat-induced change in the solubility and composition of the solid calcium phosphate. Association and dissociation of calcium caseinate and calcium citrate probably modify the effect of

TABLE 5

Composition and heat stability of raw skimmilks and composition of their ultrafiltrates collected at 80 and 200° F.

Date	Milk analysis 80°					Ultrafiltrate analysis								
						pH		Ca^{++}		Total Ca		PO_4		Citrate average
	pH	Total Ca	Total PO_4	Inorg. PO_4	Heat stability	80° F.	200° F.	80° F.	200° F.	80° F.	200° F.	80° F.	200° F.	
Bulk milks		*(mM/liter)*			*(min.)*			*(mM/liter)*						
Dec. 9, 1957	6.47	31.6	39.1	20.7	13.3	6.57	6.03	3.8	1.4	8.8	5.0	11.7	10.0	8.8
Dec. 11, 1957	6.57	30.0	30.9	19.1	10.3	6.72	6.13	3.8	1.3	9.0	4.9	10.1	7.8	8.5
Dec. 16, 1957	6.63	30.0	29.1	19.9	8.5	6.72	6.26	2.5	1.1	7.6	3.5	10.2	8.0	7.7
Dec. 18, 1957	6.58	25.8	30.2	19.2	9.0	6.73	6.18	2.8	1.2	8.2	4.5	10.2	8.7	8.7
Jan. 1, 1958	6.59	25.0	27.5	18.5	11.0	6.80	6.19	2.6	1.1	8.5	4.4	10.0	8.2	8.3
Jan. 8, 1958	6.62	24.2	27.8	17.8	9.5	6.77	6.26	2.9	1.2	7.7	3.9	9.9	8.5	8.3
Jan. 13, 1958	6.63	24.8	27.2	18.0	11.6	6.77	6.18	2.7	1.0	7.7	3.8	10.5	8.5	8.1
Jan. 15, 1958	6.60	24.8	27.9	19.4	11.9	6.68	6.10	3.0	1.2	8.7	4.3	10.4	8.1	8.0
Cow No. 1, freshened November 27, 1957														
Dec. 2, 1957	6.54	30.6	38.1	20.3	8.3	6.72	6.06	4.4	1.6	9.7	5.8	9.3	7.1	7.6
Dec. 16, 1957	6.60	28.2	28.8	18.4	9.1	6.82	6.17	2.6	0.8	6.2	4.1	8.9	6.7	5.6
Dec. 23, 1957	6.60	25.4	29.7	...	6.5	6.72	6.23	2.6	1.3	8.2	5.6	9.9	8.8	8.6
Dec. 30, 1957	6.57	28.1	27.5	17.8	5.6	6.76	6.26	3.1	1.5	8.6	5.7	9.3	7.7	8.6
Jan. 6, 1958	6.62	24.5	27.5	18.8	6.3	6.82	6.27	2.8	1.3	7.3	4.7	9.8	8.7	8.4
Jan. 13, 1958	6.62	24.4	27.9	18.7	6.0	6.86	6.21	2.7	0.8	8.3	4.4	10.6	8.3	8.0
Cow No. 2, freshened November 28, 1957														
Dec. 2, 1957	6.40	30.2	35.5	20.0	6.0	6.54	5.94	5.3	1.8	11.1	6.4	10.2	8.1	9.0
Dec. 16, 1957	6.55	28.6	32.0	19.6	5.3	6.70	6.18	2.7	0.9	9.0	4.5	10.5		7.9
Dec. 30, 1957	6.60	27.3	28.5	19.5	6.3	6.76	6.26	2.9	1.4	8.8	4.8	10.4	7.5	9.2
Jan. 6, 1958	6.58	26.3	27.1	18.6	5.8	6.80	6.22	3.0	1.4	8.9	5.7	9.2	7.1	9.9
Jan. 13, 1958	6.59	26.7	27.9	18.7	8.1	6.78	6.14	3.0	1.0	10.3	4.5	10.2	7.8	8.8
Cow No. 3, freshened March 20, 1957														
Dec. 11, 1957	6.69	31.7	28.0	17.3	5.1	7.00	6.40	3.0	1.2	6.5	3.5	7.0	5.4	6.4
Dec. 18, 1957	6.71	24.0	26.0	18.1	3.8	7.12	6.55	2.5	0.8	5.8	3.0	7.2	5.7	6.3
Jan. 8, 1958	6.81	31.0	27.5	17.2	3.3	7.16	6.56	2.7	1.1	5.3	2.7	7.7	4.7	6.0
Jan. 15, 1958	6.59	28.2	27.4	17.9	7.1	7.01	6.44	2.7	1.0	5.3	3.2	6.8	5.5	5.8
Cow. No. 4, freshened March 20, 1957														
Dec. 11, 1957	6.61	26.8	28.4	18.7	5.5	6.79	6.32	2.7	1.0	6.5	3.6	10.6	8.8	7.5
Dec. 18, 1957	6.59	26.0	28.2	20.1	5.0	6.80	6.30	2.4	0.9	7.1	3.9	11.7	10.3	7.8
Jan. 8, 1958	6.60	25.9	30.3	20.2	4.8	6.80	6.30	2.6	1.0	6.6	3.4	11.9	10.4	7.9
Average	6.59[a]	27.3	29.5	18.9	7.4	6.79[a]	6.21[a]	3.0	1.2	7.9	4.4	9.4	7.7	7.9

[a] Average H^+ concentration reconverted to pH.

heat on the composition of the ultrafiltrate, but there is no reason to assume that they play a major role in determining the extent of the changes.

On the basis of analyses made on cooled samples, Pyne and McHenry (15) concluded that only a small portion of the heat-induced decrease in pH resulted from precipitation of calcium phosphate, and that most of the pH change was caused by decomposition of lactose and casein phosphate ester. However, formation of acid from these sources would be expected to occur relatively slowly, particularly at temperatures below the boiling point, and probably accounts for only a small part of the rapid pH change demonstrated by the hot ultrafiltration technique.

Both Evenhuis (5) and Pyne and McHenry (15) have suggested that calcium ion concentration[1] in hot milk should be higher than that of cold milk: Evenhuis assumed an increased dissociation of calcium citrate with increasing temperature, and Pyne and McHenry based their suggestion on the assumption that the dominant effect would be that induced by increasing acidity. However, the data presented herewith show that there is a marked decrease in calcium ion concentration as milk is heated, and it therefore appears that the dominant effect is the decreased solubility and altered composition of calcium phosphate. Explanations of the heat coagulation of milk colloids based on an assumed increase in calcium ion activity (5, 6, 15) are, therefore, unacceptable.

Analyses of ultrafiltrates obtained from cold and hot milk have not provided any indication of the factors controlling heat stability, but make it appear improbable that changes in the inorganic composition of the serum as the milk is heated are a major factor. The extent to which calcium combines with casein may be important, but the assumption that a constant amount of inorganic calcium is attached to casein (3) is not acceptable if the calcium ion activity and the pH are not constant, and Evenhuis' conclusion (7) that there is a constant $\frac{Ca + Mg}{P}$ ratio in the precipitate requires further proof, in view of the known variability of calcium phosphate precipitates (2, 4) and the observed effect of heat on the composition of the precipitate from calcium phosphate solutions (Table 3). Calculation of the caseinate calcium is, therefore, not possible at present.

[1] Evenhuis (5) uses the term activity but, from the context [e.g., "The calcium ion activity of milk was estimated by Smeets by the purpurate method" (5) p. 228], it is apparent that he uses the term as a synonym for concentration.

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