

Mineral partitioning in milk and milk permeates at high temperature

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The soluble phase of milk was separated at 20 and 80 °C using ultrafiltration. The resulting permeates were then subjected to further ultrafiltration and dialysis at close to these two temperatures. It was found that pH, Ca²⁺ and soluble Ca decreased as the separation temperature increased both in original UF permeates and in dialysates obtained from these permeates, but P decreased only slightly. The major reason for these changes was due to the precipitation of calcium phosphate/citrate complexes onto the casein micelle with concomitant release of H⁺. The pH of both permeates and dialysates from milk at 20 °C were slightly higher than for milk. When UF permeates collected at 20 and 80 °C, were each dialysed at both these temperatures, the dialysate collected at 80 °C showed much less temperature dependence for pH and ionic calcium compared with that collected at 20 °C. This is in contrast to milk, which shows considerable temperature dependence for pH and ionic calcium. Further experiments revealed that the pH and Ca²⁺ concentration of permeates showed high temperature dependence above the temperature at which they were separated, but a much lower temperature dependence below that temperature. These findings suggest that dialysis and UF of milk at high temperature provide the best means yet for estimating the pH and ionic calcium of milk at that temperature.

Keywords: Ultrafiltration, dialysis, pH ionic calcium, soluble minerals.

Most studies on mineral partitioning of milk at different temperatures focus on changes taking place as a result of the heat treatment and usually measure properties after the milk has cooled down (Pyne, 1958; Rose & Tessier, 1958, 1959; Kannan & Jenness, 1961; Fox & Morrissey, 1977; Fox & McSweeney, 1998). Those measuring properties whilst the milk is at high temperature are far fewer (Pouliot et al. 1989a–c; Chandrapala et al. 2010; On-Nom et al. 2010).

The partition of mineral constituents of milk between the colloidal phase and soluble phase has been shown to be temperature dependent, i.e. the soluble Ca and P decreased as the temperature employed increased. Pouliot et al. (1989a–c) Jenness & Patton (1959) suggested that temperature dependence of Ca and P exchanges between the colloidal and soluble phases is regulated by the solubilities of Ca phosphates. On heating the calcium phosphates precipitate on to the casein micelles associated with a decrease in the concentration of calcium ions and pH. However, on cooling if the heat treatment was not severe, the concentration of soluble calcium and phosphate increase at the

expense of colloidal calcium phosphate Fox & Morrissey (1977). Rose & Tessier (1959) determined the concentrations of soluble and ionic calcium (Ca²⁺) by UF treatment up to 230 °F and found that heat caused precipitation of calcium phosphate and lowered Ca²⁺ concentration. Kannan & Jenness (1961) studied the effect of heat treatment of skim milk and showed that some of calcium and phosphorous are transferred from the soluble to the colloidal state, however, these constituents returned to their original state when the heated milk was cooled. Hilgeman & Jenness (1951) showed that about 12–18% of the soluble Ca and phosphate were transferred to the colloidal phase on heating at 80 °C for 30 min, and on cooling within 48 h at 5 °C there was a recovery of the loss. Davies & White (1959) reported that, on heating milk for 30 min at 120 °C, soluble Ca, Mg²⁺ and P_i decreased by 30% and Ca²⁺ by 10%. On studying the calcium and phosphate partitions Nieuwenhuijse et al. (1988) showed that more phosphate than calcium became colloidal after preheating for 3 min at 120 °C and the Ca/P ratio in the colloidal calcium phosphate thus decreased. Using ultrafiltration of milk at various temperatures, Rose & Tessier (1959) studied the effects of high temperature treatments and found that, calcium and phosphate precipitate rapidly on heating, essentially complete within 5 min and

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the extent of precipitation depends on temperature but the distribution of Na, K, Mg or citrate was not affected. However, on cooling these changes were partly reversible.

Although it has been reported that the pH of milk will fall as its temperature increases, this has not been properly investigated in terms of understanding the role of pH on heat stability. It has also been reported that this pH change is reversible, even for milk subjected to UHT processing conditions, but not for milk subjected to in-container sterilisation.

Under severe heat treatment the pH of milk decreases probably by about 0.1 unit/mm decrease in dissolved calcium phosphate and this is reported to be due to the precipitation of calcium phosphate with concomitant release of H^+ , thermal decomposition of lactose and dephosphorylation of casein with subsequent formation of calcium phosphate and release of H^+ Pyne (1953) and Walstra & Jenness (1984). It was shown that the pH of milk decreased rapidly to about 6 on prolonged heating to 140 °C and this is probably entirely due to changes in Ca phosphate equilibrium (Rose & Tessier, 1959). These changes together with hydrolysis of κ -casein influence reduction of the zeta potential and hydration in the milk protein system which may induce coagulation, Fox & Morrissey (1977). Pyne (1953) showed that, at the point of coagulation, the pH of milk had decreased to 5.5–6.0 and concluded that H^+ and Ca^{2+} were joint factors promoting heat coagulation.

The present study deals with measuring partitioning of calcium and phosphorous at higher temperatures by means of ultrafiltration and dialysis. It aims to establish which separation procedure is most suitable for systematic and routine analysis of a milk-soluble fraction and investigates the changes in pH and Ca^{2+} on heating in the temperature range of 20–80 °C.

Materials and Methods

Milk supply

Pasteurised skimmed milk was obtained from Dairy Crest, Oxford, UK. The skimmed milk was stored at 4 °C until required.

UF treatment and permeate production

For UF at 20 and 80 °C, a tubular UF module (Aquious PCI, Hants, UK) was used. It contained 18 tubular membranes, of length 0.9 m and diameter 1.25 cm, arranged in series (Type ES 625, polyether sulphone), with MWCO 25 kDa. The unit was operated at 6 bar inlet and 2 bar outlet pressure. Forty litres skimmed milk was heated up to the required temperature in a jacketed stainless steel open pan and then transferred to UF unit where ultrafiltration was performed at the same temperature as the heating temperature. Twenty-five litres permeates were collected after 15–20 min and

some of retentates were retained for dialysis and analysis. This is equivalent to a concentration factor (CF) of 2.67. The major part of permeates collected at 20 and 80 °C were UF treated and dialysed. These UF and dialysis operations were performed at the same temperatures as used in the original UF procedures.

Dialysis

Dialysis was also performed at 20 and 80 °C (the same temperatures as the original UF procedures). Dialysis at 20 °C was performed for 24 h with addition of 0.05% sodium azide in samples as preservative while at 80 °C it was performed for 1 h, because at higher temperature equilibrium is established in a shorter time (On-Nom et al. 2010). Dialysis was performed at 20 and 80 °C using skimmed milk, retentates and permeates collected at 20 and 80 °C.

Visking tubing, size 9, of internal diameter 28.6 mm, having a molecular weight cut-off of 12 kDa, (Medicell International Ltd., London, UK) was used for dialysis. The tubing was secured at one end by a plastic clip (Fisher Scientific, Loughborough, UK) and 10 ml distilled water was added and then the other end was secured. At 20 and 80 °C, prepared dialysis tubes were added to 2 litres milk samples contained in buckets. Dialysis tubes were removed immediately at the end of the process and analysed when they had reached 20 °C (room temperature).

Analytical procedures

Milk, UF retentates and samples of the soluble phase produced by UF and dialysis were analysed for pH, Ca^{2+} , freezing point depression, lactose, total calcium and total phosphorous.

Ca^{2+} concentration in milk samples was measured using a selective calcium ion electrode. The procedure involved the use of a Ciba Corning 634 ISE Ca^{2+} /pH analyser (ISE-634) (Bayer Diagnostic Division, Newbury, UK), using the procedure outlined by Lin et al. (2006). The electrode was calibrated on a daily basis using five calcium ion standards.

A glass pH electrode model 420 A (Orion Research UK, East Sussex, UK) was used to measure the pH of samples at ambient temperature. The calibration of the pH probe was done using standard solutions at pH of 4 and 7 which was obtained from VWR International Ltd., Poole, UK. The analysis was done four times.

Total calcium of samples was determined with atomic absorption spectrophotometer at 422.7 nm whereby the AOAC official method of analysis 991.25 (AOAC, 2005) was used and the analysis was done in triplicate. Total phosphorous of samples was determined with spectrometric method at 820 nm whereby International IDF standards 42B method of analysis (IDF, 1990) was used and the analysis was done in triplicate. The concentrations of Ca^{2+} , total calcium, total phosphorous and freezing point depression obtained from standards calibration curves.

Table 1. Composition and physical properties of milk-soluble phase after separation at 20 and 80 °C using ultrafiltration (mean values and standard deviation for triplicate analyses of samples for one trial)

Sample	pH \pm SD	[Ca ²⁺] (mM)	Total Ca (mM)	Total P (mM)
Raw milk (control)	6.68 \pm 0.008	1.27 \pm 0.000	31.7 \pm 0.548	24.4 \pm 0.02
Permeate at 20 °C†	6.76 \pm 0.017	1.16 \pm 0.011	8.13 \pm 0.076	11.7 \pm 0.02
Permeate at 80 °C	6.24 \pm 0.006	0.37 \pm 0.000	3.46 \pm 0.079	9.84 \pm 0.02
Retentate at 20 °C‡	6.68 \pm 0.006	1.33 \pm 0.018	62.0 \pm 0.739	
Retentate at 80 °C	6.71 \pm 0.000	1.16 \pm 0.005	82.5 \pm 0.376	
Permeate (20 °C) of P. 20 °C§	6.86 \pm 0.018	1.07 \pm 0.000	5.76 \pm 0.096	9.93 \pm 0.02
Permeate (22.4 °C) of P. 80 °C	6.47 \pm 0.013	0.44 \pm 0.002	3.05 \pm 0.059	8.50 \pm 0.06
Permeate (75 °C) of P. 20 °C	6.42 \pm 0.005	0.34 \pm 0.002	2.70 \pm 0.058	8.55 \pm 0.08
Permeate (73 °C) of P. 80 °C	6.23 \pm 0.006	0.32 \pm 0.004	3.22 \pm 0.079	10.2 \pm 0.01

† Permeate collected on doing ultrafiltration at 20 °C

‡ Milk retained after ultrafiltration at 20 °C

§ Permeate collected on doing re-ultrafiltration at 20 °C from permeate at 20 °C

P. – permeate

Results

UF permeate at 20 °C had a slightly higher pH and slightly lower Ca²⁺ than milk (Table 1). About 26% of total Ca was soluble and about 48% of P. Both the pH and Ca²⁺ values of UF permeate collected at 80 °C were much lower than those collected at 20 °C. Soluble calcium was also considerably reduced, down to 11% of total calcium, whereas soluble P was reduced less, down to 40% of total P. Soluble P seemed much less temperature dependent than soluble calcium. Previous investigations have shown that temperature relationships between Ca²⁺ and pH are linear (Ma & Barbosa 2003; On-Nom et al. 2010). Assuming this to be the case, for permeate collected from UF of milk, the change in pH with temperature is -0.0087 units/°C and the change in Ca²⁺ is -0.0131 mM/°C.

UF retentates (CF=2.67) produced at 20 and 80 °C showed almost similar pH values (Table 1), which were little changed from that of the initial milk, at 6.68. This contrasts with pH of concentrates produced by reverse osmosis or evaporation, which would be substantially lower, due mainly to precipitation of calcium phosphate during the concentration process. Total calcium was higher in the retentate produced at 80 °C, arising from a lower concentration of calcium in permeate at 80 °C. Despite this large increase in total calcium, Ca²⁺ concentrations in the retentates changed relatively little, with the retentate at 80 °C having a slightly lower Ca²⁺ than that produced at 20 °C. It has been shown that heating milk will usually reduce Ca²⁺ (Geerts et al. 1983), but it has been shown to increase following in-container sterilisation (Tsioulpas et al. 2010). This comparatively small increase in Ca²⁺ found for retentates, together with a small change in pH, suggests that UF processing results in little change in the composition of the soluble phase. This was confirmed by dialysis results of retentates and original milk samples, which are discussed later. One benefit of UF at higher temperatures is that calcium retention in the product is improved. Possible drawbacks include higher fouling rates and flavour

defects due to higher Ca²⁺. However, it is interesting that there are no reports on UF permeation rates for milk above 60 °C.

The retentates collected at 20 and 80 °C were also dialysed at these two temperatures. The trends were similar for both these retentates and showed that both pH and Ca²⁺ decreased as dialysis temperature was increased. The pH change of dialysates from retentates was not so temperature dependent as dialysates from milk, most probably due to their higher buffering capacity caused mainly by their increased protein concentration. Ca²⁺ also did not appear to change so much.

When the UF retentate produced at 20 °C was dialysed at 20 °C, its dialysate had very similar Ca²⁺ levels to that of the dialysate from raw milk, although total Ca and total P were slightly higher in those from the retentate. This further suggests that the composition of the soluble phase changes little during the UF process. A similar pattern was observed for dialysates obtained at 80 °C from both retentates, when compared with the dialysate from milk.

Dialysing the two retentates at 80 °C reduced Ca²⁺ concentration by about 50%, compared with dialysis at 20 °C, and soluble calcium by slightly less, showing that at high temperatures, soluble calcium precipitation which lead to the loss of Ca²⁺ and this is in agreement with Fox & McSweeney (1998) and Rose & Tessier (1958).

As shown in Table 1, the pH of permeates and dialysates obtained at 80 °C are consistently lower than pH values of those at 20 °C, even though all measurements were taken at 20 °C. Comparisons of the temperature dependence of pH and Ca²⁺ for permeates removed at these two temperatures were undertaken by subjecting them to further UF treatment at 20 and 80 °C (in reality this was 22 and 75 °C). This is subtly different from UF treatment of milk, because casein micelles, which have an extra reserve of Ca and P, are not involved.

When permeate collected at 20 °C was UF treated at 22 and 75 °C, there was a reduction both in its pH and its Ca²⁺ at the higher UF temperature, although slightly less than

Table 2. Composition and physical properties of milk-soluble phase after separation at 20 and 80 °C using dialysis (mean values and standard deviation for triplicate analyses of samples for one trial)

Sample	pH \pm SD	[Ca ²⁺] (mM)	Total Ca (mM)	Total P (mM)
Raw milk (control)	6.68 \pm 0.008	1.27 \pm 0.000	31.7 \pm 0.548	24.4 \pm 0.02
Dialysate (21 °C) of milk†	6.85 \pm 0.008	1.15 \pm 0.005	7.44 \pm 0.082	11.1 \pm 0.01
Dialysate (80 °C) of milk	6.48 \pm 0.000	0.58 \pm 0.005	5.09 \pm 0.059	10.5 \pm 0.03
Dialysate (21 °C) of P. 20 °C‡	6.89 \pm 0.010	0.97 \pm 0.004	4.93 \pm 0.039	9.18 \pm 0.03
Dialysate (21 °C) of P. 80 °C	6.30 \pm 0.008	0.319 \pm 0.001	2.75 \pm 0.039	8.78 \pm 0.03
Dialysate (80 °C) of P. 20 °C	6.37 \pm 0.013	0.42 \pm 0.002	2.89 \pm 0.039	9.31 \pm 0.04
Dialysate (80 °C) of P. 80 °C	6.13 \pm 0.015	0.33 \pm 0.006	3.23 \pm 0.059	10.6 \pm 0.02
Dialysate (21 °C) of C. 20 °C§	6.78 \pm 0.005	1.14 \pm 0.014	9.21 \pm 0.125	11.9 \pm 0.02
Dialysate (21 °C) of C. 80 °C	6.74 \pm 0.013	1.06 \pm 0.005	10.1 \pm 0.119	12.3 \pm 0.03
Dialysate (80 °C) of C. 20 °C	6.41 \pm 0.000	0.57 \pm 0.003	6.05 \pm 0.039	11.8 \pm 0.02
Dialysate (80 °C) of C. 80 °C	6.40 \pm 0.013	0.54 \pm 0.000	5.86 \pm 0.093	11.3 \pm 0.03

†Dialysate collected after dialysing milk at 21 °C

‡Dialysate collected after dialysis at 21 °C of the permeate collected at 20 °C

§Dialysate collected after dialysis at 21 °C of the retentate collected at 20 °C

C. – retentate; P. – permeate

that experienced by milk. The gradients were $-0.008/^\circ\text{C}$ for pH and $-0.0134 \text{ mM}/^\circ\text{C}$ for Ca²⁺. The corresponding changes for permeate removed at 80 °C were $-0.00471/^\circ\text{C}$ for pH and $-0.0206 \text{ mM}/^\circ\text{C}$ for Ca²⁺. Thus permeates removed at high temperature showed a low temperature dependence, more so for Ca²⁺ than for pH. Maintaining high temperatures was not quite so easy for UF. It was also observed that the pH readings for permeates were always higher than their corresponding retentates, irrespective of whether these were obtained from milk samples or permeate samples.

When permeate collected at 20 °C was UF treated at 22 °C, its soluble calcium further decreased whereas soluble P decreased only slightly. Again, this might suggest that considerable calcium citrate precipitates when permeate is heated, although this cannot be confirmed as citrate was not analysed. Geerts et al. (1983) showed that precipitation of calcium citrate can also be responsible for the decrease in pH. This is an interesting observation, which fits in with results from our research, since it was observed that reductions in calcium were much higher than reductions in P, suggesting that considerable calcium citrate must have precipitated. Smeets (1955) reported that the average concentration in 40 UF permeates was 2.75 mM; they calculated that 35% was in ionic state, 55% bound to citrate and 10% bound to phosphate.

Results for dialysates obtained from permeates and retentates are shown in Table 2. Dialysate from milk at 20 °C, had a slightly higher pH and slightly lower Ca²⁺ compared with milk. A higher pH was also found for UF permeate from milk but the increase (0.08 pH units) was not as high as that found for dialysis (0.17 pH units). Dialysates from milk at 80 °C had lower pH and Ca²⁺ values compared with dialysates at 20 °C. The temperature dependence for dialysis of milk was $-0.00627/^\circ\text{C}$ for pH and $-9.64 \times 10^{-3} \text{ mM}/^\circ\text{C}$. For Ca²⁺ both values are slightly lower than found for UF treatment of milk.

Dialysates from permeates collected at 20 °C showed a decrease in both pH from 6.89 to 6.37 and for Ca²⁺ from

0.972 to 0.417 mM as dialysis temperature increased from 20 to 80 °C. The temperature dependence for permeate collected at 20 was $-0.00814/^\circ\text{C}$ for pH and $-0.00941 \text{ mM}/^\circ\text{C}$ for Ca²⁺. Soluble calcium decreased from 4.93 to 3.23 mM, whereas soluble P increased slightly.

Dialysates obtained at 20 and 80 °C from permeates collected at 80 °C showed a much smaller decrease in pH (6.30–6.13) and hardly any change in Ca²⁺ (0.309–0.333 mM). Temperature dependence was $-0.00281/^\circ\text{C}$ for pH and $-0.000407 \text{ mM}/^\circ\text{C}$ for Ca²⁺. Both soluble calcium and soluble P increased slightly as dialysis temperature increased. Trends were similar to but not exactly the same as for UF.

To further investigate some of these findings, milk was UF treated at temperatures of 20, 40, 60 and 70 °C. (It would have been ideal to use 80 °C, but it was not possible to maintain temperature constant.) Each of these permeates was then dialysed at 20 and 80 °C. The experiment was repeated for three different batches of milk and results are summarised in Table 3. The pH and Ca²⁺ of permeates decreased as UF temperature increased. When UF permeate collected at 20 °C was dialysed at 20 and 80 °C, there were considerable differences in both pH and Ca²⁺. However, the magnitude of these differences decreased as the UF temperature at which these permeates were collected increased. In contrast dialysates collected at 80 °C from permeates at different temperatures showed only small differences in pH and Ca²⁺.

Discussion

These results confirm that UF permeates obtained from milk at low temperatures, showed considerable temperature dependence for pH and Ca²⁺ when dialysed at a higher temperature. However, permeates collected at the high temperature showed much less temperature dependence for these properties when dialysis was performed at any temperatures below the UF separation temperature. Above that

Table 3. Permeate composition of milk which was UF treated at 20, 40, 60 and 70 °C and the composition of dialysates obtained from these permeates at 20 and 80 °C

UF temp (°C)	Permeate Composition		Dialysis at 20 °C		Dialysis at 80 °C	
	pH	Ca ²⁺ (mM)	pH	Ca ²⁺ (mM)	pH	Ca ²⁺
20	6.77 ± 0.04	1.05 ± 0.16	6.88 ± 0.03	0.88 ± 0.04	6.31 ± 0.04	0.33 ± 0.05
40	6.66 ± 0.04	0.81 ± 0.01	6.77 ± 0.01	0.73 ± 0.03	6.34 ± 0.06	0.35 ± 0.06
60	6.51 ± 0.07	0.61 ± 0.04	6.61 ± 0.02	0.54 ± 0.01	6.34 ± 0.09	0.42 ± 0.05
70	6.45 ± 0.08	0.51 ± 0.03	6.56 ± 0.02	0.47 ± 0.03	6.44 ± 0.06	0.48 ± 0.03

Represents the mean and standard deviation for three replicated trials (using different skim milk samples)

separation temperature, these properties of dialysates were temperature dependent. When UF of milk is performed at any particular temperature, say 60 °C, its permeate will be saturated with calcium phosphate at that temperature. When that permeate cools, there is no solubilisation of calcium phosphate, as would occur in milk, and hence no increase in pH. Thus, it is proposed that calcium phosphate and its precipitation is the main determinant responsible for temperature induced pH changes and when this does not occur then changes are much smaller.

An important question which follows from this is whether UF permeate collected at high temperature, but measured at 20 °C is representative of the pH and temperature of the milk at that high temperature. For dialysates or permeates collected at high temperature, some parameters such as total calcium, phosphate and citrate will not change on cooling, but speciation will and the two simplest parameters to measure are pH and Ca²⁺. These are also important as they will both influence the charge in the vicinity of the micelle. The results show that dialysates of UF permeates obtained at 20 °C show considerable temperature dependence, whereas dialysates obtained from permeates at 70 °C showed much less temperature dependence.

On-Nom et al. (2010) have shown that pH and Ca²⁺ in UF permeates, up to 80 °C and dialysates up to 120 °C decreased as temperature increased. They also showed that pH and Ca²⁺ of UF permeate collected from milk at 80 °C (but measured at room temperature), were similar to values for the dialysate taken from this same permeate which was dialysed at 80 °C for 1 h. Also the pH and Ca²⁺ of this permeate was little changed after heating for 1 h at 80 °C. These observations also suggest that pH and Ca²⁺ in permeates and dialysates collected from milk at 80 °C showed little temperature dependence. In this current work, little temperature dependence was found for Ca²⁺, but slightly more temperature dependence was found for pH. Even if there was slight temperature dependence for these permeates and dialysates when they cooled, the real pH and Ca²⁺ at high temperature would both be even lower, and hence the changes larger, than those values reported in this work.

The decrease in Ca²⁺ as temperature increased resulted from precipitation of tertiary calcium phosphate, which predominates over an increase in Ca²⁺ that might be expected on temperature increase, due to the pH and increased ionisation and increased solubilisation of calcium

phosphate, Augustin & Clarke (1991). Another factor which influences Ca²⁺ concentration is the amount of calcium in casein micelles, which increases with increasing degree of dephosphorylation (Farrell & Kumosinski 1988; Schmidt & Poll 1989), as this will contribute to a decrease in Ca²⁺. The decrease in Ca²⁺ as temperature increases suggests that it may play a lesser role in heat induced sediment or coagulation and that pH reduction may be the key factor.

The differences in mineral composition in milk-soluble phase obtained by separating methods applied reflect the complexity of the mechanisms governing mineral balance in milk. Therefore, it is not easy to recommend any particular procedure to be used for routine analysis. However, due to its speed, ultrafiltration would seem to be more suitable than dialysis. However, dialysis is a simple procedure and it is easy to keep the temperature constant.

Conclusions

Dialysates and permeates produced from milk at temperatures between 75 and 80 °C are different in composition from those produced at 20 °C. Increasing UF temperature will reduce pH, Ca²⁺ and soluble calcium of permeates. Decreases were also observed in soluble P, but results suggest that considerable calcium citrate will precipitate when milk is heated.

Considerable temperature dependence of properties such as pH, Ca²⁺ and soluble calcium were found for milk, UF retentates and UF permeates collected at 20 °C. In contrast, permeates collected at 80 °C showed much less temperature dependence, especially for Ca²⁺ and soluble calcium. It is proposed that these differences are caused mainly by the amount of soluble calcium in the respective permeates.

Thus, when milk is subject to UF or dialysis at high temperature, the pH and Ca²⁺ would provide a reasonable estimate of the values for milk at those high temperature. However, results might suggest that the pH reduction observed when milk temperature is increased may even be underestimated by these methods.

UF of milk at high temperature will result in more calcium being retained in the retentate, thereby increasing the calcium in these products. This is worthy of further investigation.

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