Measurement of ionic calcium, pH, and soluble divalent cations in milk at high temperature

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ABSTRACT

Dialysis and ultrafiltration were investigated as methods for measuring pH and ionic calcium and partitioning of divalent cations of milk at high temperatures. It was found that ionic calcium, pH, and total soluble divalent cations decreased as temperature increased between 20 and 80°C in both dialysates and ultrafiltration permeates. Between 90 and 110°C, ionic calcium and pH in dialysates continued to decrease as temperature increased, and the relationship between ionic calcium and temperature was linear. The permeabilities of hydrogen and calcium ions through the dialysis tubing were not changed after the tubing was sterilized for 1 h at 120°C. There were no significant differences in pH and ionic calcium between dialysates from raw milk and those from a range of heat-treated milks. The effects of calcium chloride addition on pH and ionic calcium were measured in milk at 20°C and in dialysates collected at 110°C. Heat coagulation at 110°C occurred with addition of calcium chloride at 5.4 mM, where pH and ionic calcium of the dialysate were 6.00 and 0.43 mM, respectively. Corresponding values at 20°C were pH 6.66 and 2.10 mM.

Key words: pH, ionic calcium, dialysis, ultrafiltration

INTRODUCTION

When milk temperature is increased, its pH decreases, falling to values below 6.0 when temperature exceeds 100°C. In commercial processes such as HTST pasteurization and UHT treatment, pH returns almost to its original value after cooling. However, following in-container sterilization, the final pH is lower (Augustin and Clarke, 1991). Nieuwenhuise and van Boekel (2003) suggested there was a slight decrease in pH following UHT sterilization and a decrease of pH of 0.2 to 0.3 units during batch sterilization caused by irreversible

degradation of lactose. However, information on pH values of milk at commercial sterilization temperatures is not available, although there are reports showing how pH decreases as temperature increases (Walstra and Jenness, 1984; Chaplin and Lyster, 1988; Fox and McSweeney, 1998; Ma and Barbano, 2003). Mineral partitioning at high temperatures has also received little attention, the most significant reports being those of Pouliot et al. (1989a,b,c). They found that soluble calcium decreased with increasing temperature, measured up to 90°C. This is in accordance with the decrease in calcium phosphate solubility as temperature increases.

For individual milk samples, ionic calcium increases as pH is reduced (Geerts et al., 1983; Tsioulpas et al., 2007). However, at high temperature, this is accompanied and counteracted by a decrease in calcium phosphate solubility and a decrease in soluble calcium. There are no studies on how ionic calcium changes in milk as its temperature increases and how this is influenced by solubility changes of calcium phosphate, induced by changes in both temperature and pH. Another important consideration is that the relationship between milk pH and temperature is not the same for all milk samples, because it is affected by the buffering capacity of the milk. Factors influencing buffering capacity have been reviewed recently by Salaun et al. (2005) and, in this context, include the different casein fractions and their concentrations, and the amounts of phosphate and citrate. Chavez et al. (2004) have reported wide variations in ionic calcium in milk and have shown how this influences ethanol stability and heat stability of milk.

This paper discusses how pH and ionic calcium change, both in permeates from ultrafiltration and in dialysates, over the temperature range from 20 to 120°C. Also measured are total divalent cations and other components that contribute to freezing point depression (FPD). It extends the work of Davies and White (1960), which compared UF and dialysis for analyzing the aqueous phase of milk at 3 and 20°C. Information obtained from these samples should facilitate a better understanding of conditions at or near the surface of the casein micelle at high temperatures.

Received August 10, 2009.

Accepted October 27, 2009.

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MATERIALS AND METHODS

Chemicals and Milk Supply

Sodium azide and calcium chloride (analytical grade) were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, UK). Most of the work in this study was performed with pasteurized bovine semi-skim milk (1.5% fat) from Dairy Crest (Oxford, UK). However, to determine whether heat treatment would change mineral partitioning, raw milk was obtained from the Centre for Dairy Research (CEDAR) at the University of Reading, UK. Raw milk was subjected to a variety of heat-treatment conditions up to UHT sterilization, using an APV junior heat exchanger (APV, Crawley, UK), described by Browning et al. (2001).

Ultrafiltration

Semi-skim milk was heated in a steam-jacketed stainless steel open pan to different temperatures (40 to 80°C). It was then transferred to the UF plant and circulated at constant composition by returning permeate to the feed tank. A tubular UF module (Aquious-PCI Membranes, Basingstoke, UK) was used that contained eighteen 0.9-m long membranes arranged in series (Type ES 625, polyether sulfone, Aquious-PCI) with a diameter of 12.5 mm and molecular weight cutoff of 25 kDa. According to the manufacturer's guidelines, these membranes will withstand temperatures up to 80°C. Ultrafiltration took place at constant pressure and flow conditions, using an inlet pressure of 600 kPa and an outlet pressure of 200 kPa. At each temperature, samples of permeate were taken for analysis after 20 min; in developing the procedure, we established that equilibrium was achieved by that time. The permeation rate and the pH of milk and permeate were measured directly at each experimental temperature. Permeate samples were then cooled to 20°C and analyzed for pH, ionic calcium, FPD, and total divalent cations.

Dialysis

The dialysis tubing used was Visking tubing, size 9, of internal diameter 28.6 mm, having a molecular weight cutoff of 12 kDa (Medicell International Ltd., London, UK). The tubing was secured at one end by a plastic clip (Fisher Scientific, Loughborough, UK), distilled water was added, and then the other end was secured. The prepared tubes were added to milk at temperatures of 20, 40, 60, and 80°C and equilibrated for different times (1, 2, 3, 4, and 5 h) at each temperature, except at 20°C, where an additional time point of 24 h was used. Sodium azide (0.05%) was added to milk samples to prevent microbial growth. Ideally, the volume of milk

should be large compared with the volume of water, so as not to change its composition greatly during the dialysis process. A ratio of 20:1 was used for experiments conducted between 20 and 80°C.

Dialysis was also done at temperatures between 90 and 120°C for 60 min using a milk: water ratio of 12: 1. In this procedure, 120 mL of milk sample was dispensed into a baby food can (52 by 72 mm) and the sealed dialysis tubing, containing 10 mL of water, was also placed into the can, which was then hermetically sealed. Using a mass balance, we calculated that the total loss of divalent cations from the milk to the dialysate would not exceed 2%, taking the volume of milk being dialyzed and the volume of water in the dialysis tubing. This was not considered to radically alter partitioning of these cations.

At 90 and 100°C, cans were heated in temperature-controlled, jacketed, stainless steel open pans. At 110 and 120°C, a steam retort was used at pressures corresponding to those temperatures. Following heating for the desired time period, the retorts were opened as quickly as possible to recover the dialysis bags from the samples before they cooled. This step had to be done carefully to avoid scalding. This was an unavoidable shortfall in the procedure, because the milk temperature decreased slightly before the dialysis bags could be recovered. Dialysis bags were recovered within 5 min of the end of the retorting process. To ensure consistent results, dialysates were left at 20°C overnight before being analyzed.

Analytical Methods

Measurements were taken on permeates and dialysates that had been kept overnight before measurement, except where otherwise stated.

pH Measurement

The pH of milk, dialysates, and permeate samples were measured by using a Hanna Instruments HI 8424 Digital pH meter (Leighton Buzzard, UK). The pH probe was calibrated using standards of pH 4 and 7 (VWR International Ltd., Poole, UK), at 20°C. During UF, pH was also measured directly on milk and on its permeate up to temperatures of 80°C by simply immersing the pH probe into the hot fluid. No temperature compensation was applied. This is further discussed later.

Freezing Point Depression

Freezing point depression was measured for milk, dialysates, and permeate samples by using an Ad-

vanced Milk Cryoscope 4L2 (Advance Instruments Inc., Metuchen, NJ) Results are expressed in terms of thousandths of a degree ($m^{\circ}C$), with a reading of 460 indicating a freezing point of $-0.460^{\circ}C$. Walstra and Jenness (1984) reported that lactose accounts for about 54% of the FPD and Na, K, and Cl for about 34% of FPD.

Ionic Calcium Measurement

Ionic calcium was measured using a Ciba Corning 634 Analyzer (Bayer plc, Newbury, UK), as described by Lin et al. (2006). The instrument was calibrated daily with 5 Ca^{++} standards, selected from the range 0.25 to 3.0 mM. The calibration curve was derived from the log of Ca^{++} ion concentration (mM) and the electrode relative potential difference (mV), which is linear, according to the Nernst equation. The free ionic calcium concentration (mM) was calculated from the regression equation, derived from the calibration curve. All measurements were done at room temperature. The reliability of the electrode was determined by establishing that its potential difference would increase by approximately 9 mV when the calcium ion concentration was doubled.

Total Divalent Cations (EDTA Titration)

Titration with EDTA is a rapid method for measuring total calcium and magnesium in milk (Davies and White, 1962; Walstra and Jenness, 1984). Calmagite was used as the indicator, because the end-point is clearer than that produced using erichrome black. The reagents used were an ammonia buffer solution [7 g of ammonium chloride and 25 g of ammonia solution (35%, specific gravity of 0.88)], made up to 100 mL with distilled water] and calmagite indicator (0.2 g of calmagite and 5 mL of triethanolamine added to 15 mL of ethanol). The procedure involved adding 1 mL of ammonia buffer and 0.02 mL of calmagite indicator to 5 mL of milk, dialysate, and permeate samples. On mixing, this yields a pink color, indicating the presence of divalent cations. Then, the mixture was titrated with

0.01 M EDTA until the initial color changed from pink to blue.

Lactose

Lactose was measured in permeates and dialysates by polarimetry using a digital polarimeter (model 1, Optical Activity Ltd., Ramsey, UK). The angular rotation was measured and this was used in conjunction with the specific rotation of lactose (52.53°) and the path length of the solution to determine the concentration of lactose.

RESULTS AND DISCUSSION

Ultrafiltration

Ultrafiltration was replicated using 3 milk samples that differed in their natural levels of ionic calcium, but their temperature dependence behavior was consistent. Analyses of permeates showed that pH, ionic calcium, and FPD were no longer changing after 20 min at each experimental temperature. For pH, equilibrium was achieved after only 10 min. This is in line with observations made by Pouliot et al. (1989a). Data taken at 38 and $80^{\circ}C$ are compared in Table 1. Total divalent cations were found to decrease as temperature increased; this is also in agreement with the results of Pouliot et al. (1989a,c) who found that as calcium decreased magnesium also decreased but less so than calcium. Ionic calcium decreased as temperature increased, decreasing by 50 to 60% over the range from 20 to $80^{\circ}C$ (Figure 1). The relationship over this temperature range was linear, and the average gradient was $0.0135 \text{ mM}/^{\circ}C$. Determination of ionic calcium has not been previously reported at high temperatures. Freezing point depression increased with increasing temperature (Table 1); this was not expected, because the contribution from soluble calcium and phosphate should be reduced as temperature increased. It was later confirmed that lactose permeation increased as temperature increased.

During UF, the pH of milk and its corresponding permeate were measured directly at each experimental

Table 1. Changes in ionic calcium, total divalent cations, and freezing point depression (FPD) at 38 and $80^{\circ}C$ in UF permeates¹

Temperature ($^{\circ}C$)	Sample	Ionic calcium (mM)	Total divalent cations (mM)	FPD ($m^{\circ}C$)
38	1	1.41 ± 0.00	8.47 ± 0.01	405 ± 0.03
	2	0.98 ± 0.01	8.80 ± 0.02	435 ± 0.01
	3	1.03 ± 0.01	8.80 ± 0.01	438 ± 0.01
80	1	0.83 ± 0.00	6.00 ± 0.01	431 ± 0.01
	2	0.49 ± 0.01	7.00 ± 0.00	467 ± 0.01
	3	0.54 ± 0.00	7.00 ± 0.00	471 ± 0.00

¹Average of 3 determinations for each milk sample. Note that milk samples 2 and 3 had very similar properties.

Table 2. Changes in pH of milk and its corresponding permeate with temperature measured directly at the temperature and after cooling to 20°C, replicated for 3 milk samples

Temperature (°C)	Sample	Milk pH	Permeate pH	Permeate pH after cooling to 20°C
25	1	6.66 ± 0.01	6.66 ± 0.00	6.70 ± 0.00
	2	6.65 ± 0.00	6.65 ± 0.00	6.68 ± 0.00
	3	6.64 ± 0.00	6.64 ± 0.01	6.69 ± 0.00
70	1	6.28 ± 0.01	6.33 ± 0.00	6.37 ± 0.00
	2	6.13 ± 0.00	6.16 ± 0.00	6.35 ± 0.01
	3	6.12 ± 0.01	6.16 ± 0.01	6.35 ± 0.00
80	1	6.21 ± 0.01	6.26 ± 0.01	6.30 ± 0.01
	2	6.06 ± 0.00	6.10 ± 0.00	6.26 ± 0.00
	3	6.08 ± 0.00	6.10 ± 0.01	6.26 ± 0.00

temperature. Values for the milk for the 3 replicate trials (Figure 2) showed that pH decreased as temperature increased, falling from about 6.7 at 20°C to 6.21, 6.08, and 6.06, respectively. Data obtained this way (Figure 2) showed a good linear relationship between pH and temperature, with R² values for all 3 sample being greater than 0.985. The gradients for these 3 milk samples were −0.0089, −0.010, and −0.010 respectively. Ma and Barbano (2003) also reported a gradient of −0.0078, whereas Chaplin and Lyster (1988) showed a value of −0.0073. Our results showed a slightly higher temperature dependence than that reported in the other 2 articles. However, when we analyzed data presented by Walstra and Jenness (1984), a gradient of −0.0119 was found. Thus, different authors report different degrees of temperature dependence, ranging from a change of −0.0073 to −0.0119 pH units per °C increase. The results of the current study fall within this range. Note that these values are lower than those reported for water (−0.015 to −0.02). These differences may be because of the different buffering capacities of the 3 milk samples (Salaun et al., 2005) or to the fact that no temperature compensation was applied.

Overall, there were no major differences between values for milk and permeate measured directly (Table 2); in some cases pH was slightly higher, in other cases slightly lower, but it never differed by more than 0.05 pH units. Permeate pH values were similar to those observed by Pouliot et al. (1989a), which were in the range of 6.60 to 6.75 at 40°C and 6.10 to 6.20 at 90°C. Their values were obtained under similar conditions to ours; that is, by ultrafiltering at these temperatures and taking measurements after permeates had cooled to 20°C. Although it has been suggested that protein transmission may increase at high temperature, no whey protein or soluble casein was detected in any of these UF permeates, as measured by SDS-PAGE.

Dialysis

Dialysis experiments were performed in parallel with UF over the temperature range from 20 to 80°C. Initial experiments at 80°C showed that ionic calcium in the dialysate was 0.65 mM after 1 h and remained the same after 2 and 3 h, compared with 1.22 mM measured in

Figure 1. Variations in ionic calcium with temperature, measured in UF permeates.

Figure 2. pH of milk measured during ultrafiltration of milk at different temperatures for 3 different batches of milk. Some values for the corresponding permeates are given in Table 2.

Figure 3. Variations in ionic calcium with temperature, measured in dialysates.

milk at 20°C. It was found that results from dialysis at 20, 40, 60, and 80°C over a period of 1 to 5 h showed considerable temperature dependence. At any temperature, pH equilibrated more quickly than ionic calcium, which in turn was quicker than for total divalent cations. We established that times taken to reach equilibrium for these 3 parameters were (20°C) 12 h; (40°C) 5 h; (60°C) 2 h, and (80°C) 1 h. For 2 milk samples dialyzed at 20°C, ionic calcium in the dialysate (measured after 24 h) was 1.34 and 1.84 mM, compared with 1.38 and 1.88 mM, respectively in the milk, showing that ionic calcium in the dialysate was in close equilibrium with that in the milk.

Ionic calcium in dialysates was found to decrease as temperature increased, in a manner similar to that observed for UF (Figure 3). There was a good linear correlation between ionic calcium and temperature ($R^2 = 0.987$). The average gradient for the 3 milk samples was 0.0119 mM/°C. The pH of dialysates also decreased as temperature increased. The FPD value equilibrated more slowly than other parameters measured, and its value increased with increasing temperature. The

results for dialysis over this temperature range are consistent with results from UF. Again, no whey protein or soluble casein was detected in any of these dialysates, measured by SDS-PAGE.

Thermal Stability of Visking Dialysis Membrane

The thermal stability of the dialysis membrane was evaluated by heating it in a sealed can for 60 min at 120°C. This membrane was then used to dialyze milk at 20 and 80°C and its performance was compared with that of an unheated membrane. There were no significant differences in either pH or ionic calcium values between the heated and untreated membranes (Table 3), thus confirming that this dialysis tubing is suitable for investigating measuring pH and ionic calcium up to 120°C. However, there was a small but significant difference between total divalent cations collected at 20°C but no significant difference for those collected at 80°C.

Dialysis at Temperatures Above 80°C

Dialysis was then investigated in more detail over a temperature range from 90 to 120°C. The experiment was first repeated twice using different milk samples for a heating time of 60 min. It was seen that ionic calcium continued to decrease in dialysates with increasing temperature (Figure 4), decreasing from 0.52 mM at 90°C to 0.26 mM at 120°C. The pH decreased with temperature from 6.37 at 90°C to 6.02 at 120°C, whereas FPD increased from 402 at 90°C to 472 at 120°C. Also, total soluble divalent cations decreased slightly from 6.32 mM at 90°C to 5.12 mM at 120°C. Although soluble calcium and magnesium decreased, the observed increase in FPD suggests that lactose permeation increases at high temperature; this effect was observed consistently during dialysis and during ultrafiltration. This is interesting, because FPD is measuring the totality of low molecular weight dissolved substances. Pouliot et al.

Table 3. Analysis of dialysates of milk at 20°C and 80°C, using a normal membrane (not sterilized) and a sterilized membrane (sterilized at 120°C for 1 h)¹

Item ²	20°C		80°C	
	Normal	Sterilized	Normal	Sterilized
pH	6.79 ± 0.02 ^a	6.79 ± 0.01 ^a	6.38 ± 0.01 ^a	6.40 ± 0.01 ^b
FPD (m°C)	447 ± 0.01 ^a	456 ± 0.01 ^a	519 ± 0.04 ^a	516 ± 0.01 ^a
Ionic calcium (mM)	1.27 ± 0.02 ^a	1.29 ± 0.02 ^a	0.65 ± 0.03 ^a	0.67 ± 0.02 ^a
TDC (mM)	13.28 ± 0.87 ^a	11.68 ± 0.33 ^b	10.24 ± 1.08 ^a	10.16 ± 0.88 ^a

^{a,b}Means (± SD) within a row and within each temperature followed by the same letter are not significantly different ($P > 0.05$).

¹Results were the average of 3 measurements.

²FPD = freezing point depression; TDC = total divalent cations.

Table 4. Comparison of selected properties of dialysates taken at different temperatures (experiment repeated 3 times)

Item ¹	80°C/1 h	100°C/1 h	110°C/1 h	120°C/1 h
pH	6.47 ± 0.03 ^a	6.29 ± 0.01 ^b	6.09 ± 0.04 ^c	5.92 ± 0.06 ^d
FPD (m°C)	407 ± 12.01 ^a	431 ± 5.03 ^b	451 ± 9.16 ^c	479 ± 12.01 ^d
Ionic calcium (mM)	0.52 ± 0.05 ^a	0.33 ± 0.05 ^b	0.23 ± 0.02 ^c	0.27 ± 0.06 ^{bc}
TDC (mM)	7.16 ± 0.40 ^a	6.53 ± 0.23 ^b	6.22 ± 0.22 ^c	5.87 ± 0.23 ^c

^{a-d}Values with different letters in the same row are significantly different ($P < 0.05$).

¹FPD = freezing point depression; TDC = total divalent cations.

(1989b) reported that sodium and potassium permeation were not affected by temperature, being freely permeating. Because soluble divalent cations (calcium and magnesium) decrease, the FPD increase was considered to be due to lactose. This was confirmed by measuring lactose in permeates collected from milk at 21, 38, 59, and 79°C. Lactose was found to increase steadily as temperature increased, from 4.10% at 21°C to 4.60% at 79°C.

One drawback of the dialysis procedure is that milk needs to be held at high temperatures for a considerable time to achieve equilibrium; during this time its properties may change. Some of these properties were measured in milk that had been heated for 60 min at different temperatures and then allowed to cool to 20°C. The pH decreased from 6.71 to 6.70 for milk heated at 90°C, to 6.66 at 100°C, to 6.52 at 110°C, and to 6.27 at 120°C. The FPD declined slightly for milks heated up to 110°C, probably because of deposition of calcium phosphate on the micelle surface, but increased for milk heated at 120°C, most likely because of lactose breakdown products. An interesting observation was that noticeable browning occurred in milk heated at 110°C and considerable browning at 120°C for 1 h. Because FPD increased between 110 and 120°C, Maillard compounds and lactose breakdown products such as formic

acid may be contributing to this increase in FPD. The dialysate from milk heated at 120°C was also brown, indicating that Maillard products were sufficiently small to diffuse through this membrane. Heat-induced changes in lactose, following 2 possible pathways, are discussed by O'Connell and Fox (2003).

The results for a replicated experiment, in which milk was subjected to dialysis between 80 and 120°C, are summarized in Table 4. These confirm the main trends described earlier, that as temperature increased, ionic calcium decreased, pH decreased, FPD increased, and soluble divalent cations increased. However, in this case, there was no significant difference between ionic calcium measured at 110 and 120°C. Discrepancies between results for ionic calcium at 120°C may have resulted from the reduction in pH caused by the log heating time (1 h). Thus, for more reliable data at 120°C, shorter heating times would be recommended.

Calcium Chloride Addition

From the similarity in the gradient for the linear relationship observed between ionic calcium and temperature for different milk samples, one might speculate that milk with a higher ionic calcium concentration at ambient temperature would produce dialysates with a higher ionic calcium at elevated temperatures. This was investigated by adding calcium chloride to increase ionic calcium and to reduce heat stability. Some properties of these calcium-supplemented milk samples at 20°C and their dialysates collected at 110°C, together with observations on whether milk coagulated on heating at 110°C, are presented in Table 5. Only a relatively small addition of calcium chloride (5.4 mM) was required to cause coagulation of milk samples heated to 110°C. The threshold conditions for coagulation, measured at 20 and 110°C (by dialysis), were pH 6.66 and Ca^{2+} of 2.1 mM and pH 6.00 and Ca^{2+} of 0.43 mM, respectively. Thus, when ionic calcium was increased by addition of calcium chloride, it was also higher when heated to 110°C. There was an inverse linear relationship between ionic calcium and pH, both in milk at 20°C and in dialysates collected at 110°C (Figure 5).

Figure 4. The relationship between ionic calcium and temperature for dialyzed samples over the range from 90 to 120°C; values taken for milk at 20°C are also included.

Table 5. Comparisons of pH and ionic calcium at 20°C and 110°C on addition of calcium chloride, as well as freezing point depression (FPD) in the dialysate at 110°C¹

Added calcium chloride (mM) ²	pH at 20°C	Ionic calcium at 20°C (mM)	pH at 110°C	Ionic calcium at 110°C (mM)	FPD at 110°C (m°C)
0	6.76 ± 0.02	1.31 ± 0.01	6.10 ± 0.01	0.26 ± 0.01	436
1.80	6.72 ± 0.01	1.62 ± 0.03	6.07 ± 0.01	0.35 ± 0.01	447
3.60	6.69 ± 0.01	1.94 ± 0.01	6.01 ± 0.01	0.46 ± 0.00	447
5.40 ³	6.64 ± 0.01	2.27 ± 0.01	6.00 ± 0.01	0.59 ± 0.00	405
7.20 ³	6.58 ± 0.01	2.79 ± 0.01	5.96 ± 0.01	0.84 ± 0.01	394

¹Results are the average of 2 samples; in all cases, variations were very small.²1.80 mM is equivalent to 0.02% addition.³Samples coagulated on sterilization at 110°C.

Effects of Heat Treatment of Milk on the Properties of Dialysates

All UF and dialysis experiments described here were done with pasteurized milk. Although there is no evidence that pasteurization alters the partitioning of minerals, this was further investigated by comparing dialysates taken from raw milk and the same milk subjected to heat treatments of 85 to 140°C for 2 s. These milk samples were stored for 24 h following the heat treatment and then dialyzed at 20°C. The results are presented in Table 6. The pH and ionic calcium of the dialysates were not significantly affected by the heat treatments. However, there were some small but significant differences in FPD and in total divalent cations between the samples, which suggests that some changes in mineral partitioning did occur as a result of the heat treatment, but that these did not influence both pH and ionic calcium. This is worthy of further investigation. Because the pH of dialysates from all heat treatments are similar, this suggests that heat treatments up to 140°C for 2 s do not change the pH of milk, whereas in-container sterilization processes result in a consider-

able reduction in pH (Nieuwenhuise and van Boekel, 2003). Thus, UHT treatment of milk results in minimal pH change to milk, which confirms our own personal experience of UHT treating milk over many years. A final observation is that the pH of dialysates taken from milk samples is higher than that of the original milk and may be a reflection of reduced amounts of calcium and phosphorus in the permeates. This was noticed consistently in dialysis experiments.

General Discussion

The potential for using membrane processes for measuring pH, ionic calcium, and some other properties of the soluble phase of milk at high temperatures has been investigated. Both practical and theoretical information about this is scant (Holt, 2004). Davies and White (1960) investigated dialysis and UF, but only used temperatures of 3 and 20°C. Even so, they observed a close agreement between the methods and found that both pH and ionic calcium were lower at 20°C compared with 3°C.

One important question is whether permeates and dialysates recovered at high temperatures but measured at 20°C, represent the real values in the soluble phase at the high temperature. This would require that there is no change in pH and ionic calcium change upon cooling. In 2 separate trials, it was found that pH and ionic calcium 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 that was dialyzed at 80°C for 1 h. Also, the pH and ionic calcium of this permeate were little changed after heating for 1 h at 80°C. These observations suggest that pH and ionic calcium in permeates and dialysates collected from milk at 80°C showed little temperature dependence. Thus, we propose that pH and ionic calcium measurements taken on permeates and dialysates collected at elevated temperature and measured at 20°C are representative of those values in milk at the elevated temperature. Even if there was slight tempera-

Figure 5. The effects of calcium chloride addition on the relationship between ionic calcium and pH at 20°C and 110°C.

Table 6. Dialysis of raw milk and different heat treated milk at 20°C¹

Sample ²	pH	Freezing point depression (m°C)	Ionic calcium (mM)	Total divalent cations (mM)
Raw milk	6.62 ± 0.01	525 ± 0.00	1.38 ± 0.13	33.2 ± 0.28
D Raw milk	6.80 ± 0.04 ^a	508 ± 3.27 ^a	1.18 ± 0.03 ^a	11.36 ± 0.46 ^a
D85°C/15s	6.79 ± 0.01 ^a	487 ± 3.58 ^b	1.17 ± 0.03 ^a	10.72 ± 0.33 ^b
D100°C/2 s	6.81 ± 0.02 ^a	502 ± 1.14 ^c	1.18 ± 0.04 ^a	10.76 ± 0.36 ^b
D110°C/2 s	6.81 ± 0.01 ^a	496 ± 5.26 ^d	1.18 ± 0.02 ^a	10.96 ± 0.22 ^a
D120°C/2 s	6.81 ± 0.02 ^a	512 ± 4.67 ^a	1.20 ± 0.03 ^a	11.20 ± 0.28 ^a
D142°C/2 s	6.84 ± 0.02 ^a	491 ± 4.39 ^b	1.17 ± 0.02 ^a	10.72 ± 0.33 ^b

^{a-d}Means ± SD within a column followed by the same letter are not significantly different ($P > 0.05$).

¹Results are the average of 3 measurements.

²D = dialyzed samples.

ture dependence in these permeates and dialysates on cooling, the real pH and ionic calcium at high temperature would both be even lower, and hence the changes larger, than those values reported in this work.

Measuring properties of dialysates above 100°C poses problems in removing the dialysis bag quickly. Some cooling will inevitably occur as the pressure will be reduced to atmospheric on opening. This is not a problem at 100°C, because it is possible to open the cans immediately. However, in practice, the procedure adopted does appear to give reproducible results because permeation through the dialysis membrane is relatively slow. Again, the real values for pH and ionic calcium would both be slightly lower than the values measured.

From analyses of UF permeates and dialysates, it was found that similar results were found for variations in pH and ionic calcium in milk at different temperatures. Pouliot et al. (1989a,b,c) also measured the pH of UF permeates removed at high temperatures after cooling to room temperatures. Their values are similar to those measured directly at high temperature in our work (Table 2), again suggesting that these values are representative of pH values at the high temperature. The values recorded here for ionic calcium are the first reported at high temperature in milk. Performing UF and dialysis at high temperatures provides samples that can be used to measure salt partitioning at these temperatures. Some researchers have used dialysis to alter milk composition, to evaluate the effects of removing diffusible components on casein micelle stability (Arkora and Bhavadasan, 1984; De la Fuente et al., 1996).

The heat stability evaluation performed for calcium chloride addition showed that coagulation occurred at a pH of 6.00 and ionic calcium of 0.43 mM, as measured in dialysates collected at 110°C. These are probably the first values so reported measured at the coagulation temperature. The corresponding values of the same samples measured at ambient temperature are pH 6.66 and 2.10 mM. We propose that heat coagulation is influenced by both pH and ionic calcium. The

commonality of the relationship between ionic calcium and temperature for different milk samples suggests that milk with a higher ionic calcium concentration at ambient temperature will also have a higher concentration at the sterilization temperature. In this context, the buffering capacity of the milk is also important, because this will influence the extent of pH reduction experienced at the temperature of sterilization.

CONCLUSIONS

When milk is heated, its pH falls, ionic calcium decreases, and soluble divalent cations decrease. Changes in ionic calcium observed from dialysis over the temperature range from 20 to 80°C were in good agreement with those found for ultrafiltration. The mean gradient for all 6 experiments (3 dialysis and 3 UF replicates) was -0.0130 ± 0.001 mM/°C, with a range from -0.0112 to -0.0142 . Dialysis can be used to measure mineral partitioning at high temperature and for measuring ionic calcium and pH up to 120°C. Ionic calcium continued to decrease with increasing temperature. Dialysis was also used to measure pH and ionic calcium in milk supplemented with calcium chloride at its point of coagulation during in-container sterilization. The Visking dialysis tubing was heat stable for up to 1 h at 120°C. There were no noticeable differences in pH and ionic calcium in dialysates obtained at 20°C, from raw milk, HTST pasteurized milk, and from milk heated to UHT processing conditions, so these processes have minimal effect on mineral partitioning in terms of how it influences pH and ionic calcium. Being able to measure pH and ionic calcium at high temperatures will facilitate a better understanding of their role on heat stability of milk.

REFERENCES

- Arkora, V., and M. K. Bhavadasan. 1984. Influence of dilution and dialysis of buffalo milk on its heat stability-pH relationship. *Indian J. Dairy Sci.* 37:264-266.

- Augustin, M., and P. Clarke. 1991. Effects of added salts on the heat stability of recombined concentrated milk. *J. Dairy Res.* 57:213–226.
- Browning, E., M. J. Lewis, and D. MacDougall. 2001. Predicting safety and quality parameters for UHT-processed milks. *Int. J. Dairy Technol.* 54:111–120.
- Chaplin, L. C., and R. L. J. Lyster. 1988. Effect of temperature on pH of skim milk. *J. Dairy Res.* 55:277–280.
- Chavez, M. S., M. N. Livia, M. A. Taverna, and A. Cuatrin. 2004. Bovine milk composition parameters affecting the ethanol stability. *J. Dairy Res.* 71:201–206.
- Davies, D. T., and J. C. D. White. 1960. The use of ultrafiltration and dialysis in isolating the aqueous phase of milk and in determining the partition of milk constituents between the aqueous and disperse phases. *J. Dairy Res.* 27:171–190.
- Davies, D. T., and J. C. D. White. 1962. The determination of calcium and magnesium in milk and milk difusate. *J. Dairy Res.* 29:285–296.
- De la Fuente, M. A., J. Fontecha, and M. Juarez. 1996. Partition of main and trace minerals in milk: Effect of ultracentrifuging, rennet coagulation and dialysis on soluble phase separation. *J. Agric. Food Chem.* 44:1988–1992.
- Fox, P. F., and P. L. H. McSweeney. 1998. *Dairy Chemistry and Biochemistry*. Blackie Academic Professional, London, UK.
- Geerts, J., J. Bekhof, and J. Scherjon. 1983. Determination of calcium ion activities in milk with an ion selective electrode. *Neth. Milk Dairy J.* 37:197–211.
- Holt, C. 2004. An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. *Eur. Biophys. J.* 33:421–434.
- Lin, M.-J., M. J. Lewis, and A. S. Grandison. 2006. Measurement of ionic calcium in milk. *Int. J. Dairy Technol.* 59:192–199.
- Ma, Y., and D. M. Barbano. 2003. Milk pH as a function of CO₂ concentration, temperature, and pressure in a heat exchanger. *J. Dairy Sci.* 86:3822–3830.
- Nieuwenhuise, J. A., and M. A. J. S. van Boekel. 2003. Protein stability in sterilised milk and milk products. Pages 947–974 in *Advanced Dairy Chemistry*. Volume 1. Proteins. 3rd ed. P. F. Fox and P. L. H. McSweeney, ed. Kluwer Academic/Plenum Publishers, New York, NY.
- O'Connell, J. E., and P. F. Fox. 2003. Heat-induced coagulation of milk. Pages 879–945 in *Advanced Dairy Chemistry*. Volume 1. Proteins. 3rd ed. P. F. Fox and P. L. H. McSweeney, ed. Kluwer Academic/Plenum Publishers, New York, NY.
- Pouliot, Y., M. Boulet, and P. Paquin. 1989a. Observations on the heat induced salt balance changes in milk. 1. Effect of heating time between 4 and 90°C. *J. Dairy Res.* 56:185–192.
- Pouliot, Y., M. Boulet, and P. Paquin. 1989b. An experimental technique for the study of milk salt balance. *J. Dairy Sci.* 72:36–40.
- Pouliot, Y., M. Boulet, and P. Paquin. 1989c. Observations on the heat induced salt balance changes in milk. *J. Dairy Res.* 56:193–199.
- Salaun, F., B. Mietton, and F. Gaucheron. 2005. Buffering capacity of dairy products. *Int. Dairy J.* 15:95–109.
- Tsioulpas, A., M. J. Lewis, and A. S. Grandison. 2007. Effect of minerals on casein micelle stability of cows' milk. *J. Dairy Res.* 74:167–173.
- Walstra, P., and R. Jenness. 1984. *Dairy Chemistry and Physics*. John Wiley and Sons, New York, NY.