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International Dairy Journal

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https://doi.org/10.1016/j.idairyj.2022.105565

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International Dairy Journal 139 (2023) 105565

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Measuring pH of skim milk and milk permeate at ultra-high temperatures at laboratory and pilot scale

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ARTICLE INFO

Article history:
Received 3 October 2022
Received in revised form
2 December 2022
Accepted 3 December 2022
Available online 10 December 2022

ABSTRACT

Changes in the pH of skim milk and skim milk ultrafiltration permeate on heating from 25 to 140 °C were examined. Results showed that the decrease in pH with increase in temperature up to 140 °C was not linear. Hydrogen ion release due to changes in the milk mineral balance were responsible for the reduction in pH with increase in temperature. The presence of milk proteins offered little buffering against the drop in pH. The precipitation of calcium phosphate resulted in sediment in milk permeate heated above ~70 °C, but this did not occur in skim milk, with the pH remaining lower in milk permeate after heat treatment when measured at 25 °C. This study has shown that in-line pH measurements of milk at ultra-high temperatures is feasible, and could prove useful at laboratory and pilot-scale for studying interactions within, and stability of, more complex formulations with added minerals.

1. Introduction

One of the most significant physicochemical properties of skim milk, affecting its processability and quality of many finished products (e.g., evaporated milk, infant formula) is the heat stability. During dairy processing, milk can be exposed to temperatures anywhere between 4 and 145 °C, designed in most cases to retard or inactivate microbial (pathogenic and spoilage microorganisms, all spore-forming and non-spore-forming bacteria) and enzyme activity (enzyme inactivation for indigenous, exogenous and endogenous enzymes) or to induce desired physical attributes (e.g., improved emulsification, gelation, heat stability) (Deeth, 2021; Lindsay, Robertson, Fraser, Engstrom, & Jordan, 2021; McSweeney, Aydogdu, Hailu, O'Mahony, & McCarthy, 2022). To this effect, the behaviour and stability of milk at high temperatures is critical for maintaining efficient processing and manufacturing products of acceptable and consistent quality.

The most commonly used laboratory technique for determining heat stability of milk is the subjective oil bath method, developed by Davies and White (1966), involving the pH adjustment of milk solutions, prior to submerging the samples in tubes into oil at 120 or 140 °C and recording the elapsed time for coagulation or the appearance of flecks, known as the heat coagulation time (HCT). The heat stability of milk at 140 °C varies widely and is affected by a number of factors, such as microbial quality, protein content and profile, mineral composition, total solids and, in particular, the concentration of hydrogen ions (pH), which has a direct correlation with heat stability (Dumpler, Huppertz, & Kulozik, 2020). Bulk bovine milk and milk samples from most individual cows show as a type A HCT-pH profile, which has a local minimum at ~pH 6.9–7.1 and maximum stability at pH 6.6–6.8. The local minimum observed at ~pH 6.9–7.1 has been reported to be a consequence of dissociation of κ-casein from the micelle with associated whey proteins (Anema & Klostermeyer, 1997; Dumpler et al., 2020; Kudo, 1980; Rose, 1961). Ultimately, there are four main mechanisms that effect heat-induced changes in milk and cause a decrease in the stability of micelles: association of calcium and phosphate with the micelles, association of denatured or aggregated whey proteins to micellar casein, dissociation of κ-casein from the micelles, and/or formation of organic acids through lactose degradation (Anema & Li, 2000; Singh, 1995).

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https://doi.org/10.1016/j.idairyj.2022.105565

There have been numerous studies and review articles describing the heat stability of milk, concentrated milk and factors influencing heat stability, as well as the heat-induced changes in milk prior to coagulation. Previously, Fox (1981) reviewed the heat-induced pH changes in milk, in particular discussing the heat treatment of milk from a study performed by Sweetsur and White (1975). This study used extrapolation of pH data obtained at ≤90 °C to generate pH values at 140 °C, as this was the only feasible method at the time. However, a recent study by Aydogdu, O'Mahony, and McCarthy (2022) studied the pH changes in milk protein systems when heated to 120 °C and found a linear regression between pH and temperature due to the release of hydrogen ions during the formation of calcium phosphate. This study was performed under static conditions using a Peltier heating system, and provided new insights to the actual pH decrease of protein solutions and how the presence of milk salts affect these changes.

Developing this previous work further, the aim of this study was to focus on obtaining pH data in-situ at temperatures up to 140 °C in both a laboratory-designed system for rapid, high throughput analysis and also in-line within a customised pilot-scale tubular heat exchanger.

2. Materials and methods

2.1. Materials

Low-heat skim milk powder was produced in Moorepark Technology Limited, as described by Magan et al. (2019). Total nitrogen (TN) and non-protein-nitrogen (NPN) levels were determined using the Kjeldahl method according to ISO 8968-1 (ISO, 2014). The total protein content of the skim milk powder was 37.2% (w/w), using a nitrogen-to-milk protein conversion factor of 6.38. The NPN level was 0.042% (w/w). All the chemicals used were analytical grade and obtained from Sigma–Aldrich (Arklow, Ireland), unless otherwise stated.

2.2. Rehydration of skim milk, pH adjustment and ultrafiltration

Skim milk powder was reconstituted in water to a final total solids (TS) content of 9% (w/w). The rehydrated skim milk was stored overnight at 4 °C to ensure full hydration. Following overnight agitation, aliquots of skim milk (~1 L; tempered at 25 °C) were pH adjusted from pH 5.6 to 7.2, at 0.2 unit increments using 0.5 M HCl or NaOH under constant stirring. The pH adjusted samples were stirred for 2 h and pH was re-adjusted if necessary. To generate milk permeate from the skim milk samples, ultrafiltration (UF) of the pH-adjusted skim milks was performed at 25 °C using a 10 kDa molecular mass cut-off Vivaflow 200 cross flow cassette membrane (Sartorius AG, Göttigen, Germany) connected to a peristaltic pump. The pH of the milk permeate solutions were not re-adjusted after ultrafiltration to avoid introducing additional ions. The temperature of the skim milk during UF was controlled using a water bath throughout the filtration process to minimise compositional variations in the permeate. Skim milk permeates were collected after UF for further analysis.

2.3. In-situ pH measurement and heat treatment at 140 °C

Aliquots of the pH adjusted skim milks and their corresponding milk permeates were subjected to heat treatment using an oil bath equipped with a custom engineered stainless steel holding cup. The holding cup was fitted with a combination pH probe (Hamilton EasyFerm Bio HB, Station Road, Birmingham, UK) with a VP 120 type connection and built in temperature sensor Pt1000, supplied by Irish Power and Processing (Stoneyford, Co. Kilkenny, Ireland). The processing specifications for the sensor are as follows; temperature 0–140 °C, pH 0–14 and a maximum pressure of 6 bar. The calibration of the pH probe was performed as described previously by Aydogdu et al. (2022). The oil bath (Buchi heating bath B-305, BÜCHI Labortechnik, Flawil, Switzerland) temperature was set to 140 °C prior to analysis, individual samples were placed in a stainless steel holding cup and immersed in the oil and pH and temperature recorded until a sample temperature of 140 °C was reached. After a 5 s holding period, the stainless steel sample holding cell was immediately placed into an ice bath and allowed to cool. Heated samples were subsequently collected and further analysis was carried out within 24 h.

2.4. Heat coagulation time of skim milk

Heat coagulation time (HCT) of skim milk (9% TS) samples were measured at 140 °C as a function of pH (5.6–7.2) as described by Davies and White (1966). pH adjusted samples (Section 2.2) were equilibrated for 1 h at 25 °C prior to measurement and re-adjusted if necessary. Samples were placed in glass tubes and immersed in an oil bath at 140 °C. HCT was monitored and recorded as the time elapsed between immersing the sample in the oil bath and protein aggregation visibly detected.

2.5. Mineral composition

Skim milk permeate samples were collected before and after heat treatment at 140 °C. Milk permeates collected after heat treatment were carefully sampled to collect the serum phase without disturbing any sedimented mineral deposits (centrifugation was not required). Macro elements (calcium, phosphorous, potassium, magnesium and sodium) were determined via inductively coupled plasma-optical emission spectrometry (Agilent ICPMS 7700, Santa Clara, CA, USA) as described by Cruijsen, Poitevin, and Brunelle (2019). Samples were digested in a microwave digestion system (CEM, MARS 6, Dublin, Ireland). Liquid sample (4 mL) was mixed with nitric acid and incubated for 15 min. Samples were then heated to 200 °C and held for 15 min at a pressure of 55 bar.

2.6. Calcium ion concentration

The ionic calcium concentration of skim milk and all milk permeate samples within the pH range 5.6–7.2 were measured before and after heat treatment, and analysed as described by Aydogdu, Ho, Ahrné, O'Mahony, and McCarthy (2021), with a calcium ion selective electrode (sensION+ 9660C, Hach Co., Loveland, CO, USA). The ion selective calcium probe was calibrated with freshly prepared standard calcium solutions at 0.05, 1.00, 2.5 and 5.00 mM at 25 °C. Prior to probe calibration or sample measurements, a 3 M potassium chloride stock solution was prepared, from which 0.1 mL was added to 10 mL of all standard and sample solutions to enhance the ion selectivity of the measurement. All measurements were performed at 25 °C under constant stirring.

2.7. Heat treatment of skim milk using a tubular heat exchanger with in-line pH measurement

Pasteurised liquid skim milk and milk permeate, prepared by ultrafiltration using a 10 kDa molecular mass cut-off polymeric membrane, were obtained from Moorepark Technology Ltd. (Moorepark, Fermoy, Co. Cork). The total solids and protein content of the liquid skim milk was 8.97% and 3.29% (w/w), respectively, with a NPN content of 0.036% (w/w). Milk permeate had a totals solids content of 5.85% (w/w), and a NPN content of 0.030% (w/w).

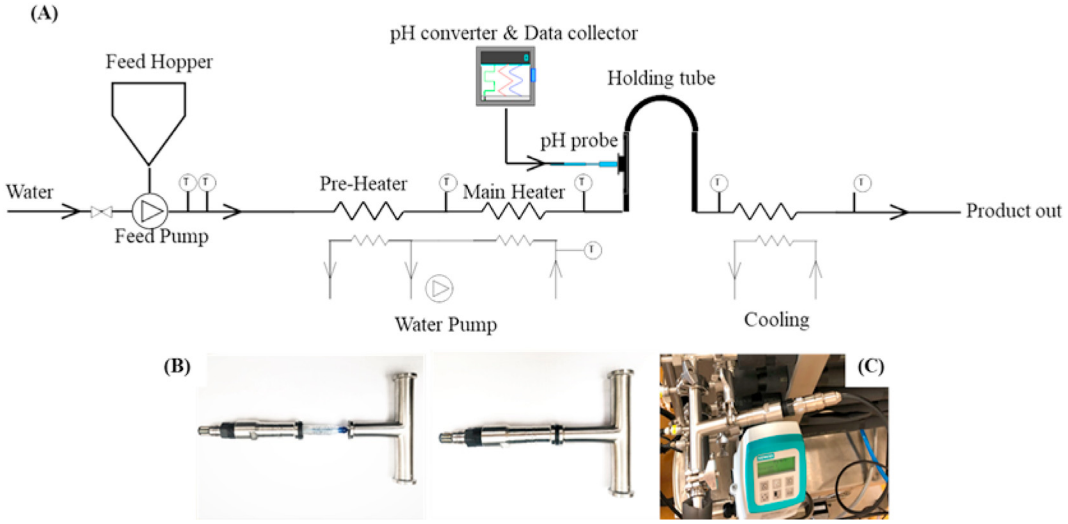

Fig. 1. Line diagram of the pilot-scale heat treatment system with pre-heater, final heater, cooling sections, and modified holding tube with pH probe insert (A) and photographic images of pH probe in a stainless steel Tee-fitting and (B) and photographic image of the pH probe inserted in the holding tube (C).

The macro element composition of the skim milk and milk permeate was as follows: calcium (35.5 and 6.4 mM), magnesium (5.1 and 2.8 mM), phosphorus (37.4 and 12.2 mM), potassium (44.0 and 39.1 mM) and sodium (18.5 and 14.6 mM), respectively.

The skim milk and milk permeate were thermally treated using a pilot-scale tubular heat exchanger (OMVE Netherlands B.V. Gessel 61 3454 MZ De Meern, the Netherlands), equipped with preheater, final heater, holding tubes and two cooling sections. The final heater temperature was incrementally increased from 25 to 65, 75, 85, 90, 100, 120 and finally to 140 °C at a flow rate of 10 L h^{-1} and holding time of 23 s. The holding duration of 23 s was designed to ensure sufficient time and volume of product in the holding tube to obtain a stable pH reading. A custom-built stainless steel T-piece pipe fitting, with pH probe incorporated, was attached to a flexible stainless steel holding tube as shown in Fig. 1. The temperature of the product was concomitantly recorded from the temperature sensor within the pH probe (as described in Section 2.3), which correlated to ±1.5 °C with the reading from the temperature sensor on the holding tube section of the heat exchanger. The back-pressure valve was adjusted from 2.3 bar at temperatures <100 °C to 3.8 bar at temperatures ≥100 °C.

3. Results

3.1. Laboratory-scale heat treatment (oil bath method)

3.1.1. Mineral and calcium ion concentration of skim milk and milk permeate samples

The mineral profile of the original skim milk and of the corresponding milk permeate solutions obtained at each pH are shown in Table 1. The mineral profile of the original skim milk was typical of that shown before with potassium present at the highest concentration (42.5 mmol) followed by phosphorus (33.4 mmol) and calcium (31.4 mmol). The pH of the original skim milk was 6.7, but its corresponding milk permeate was slightly lower at pH 6.6, with the level of potassium in the milk permeate similar to that in the original skim milk. However, there was a significantly lower level of calcium (7.4 mmol) and phosphorous (11.0 mmol) in the milk permeate at pH 6.6, as the majority of calcium and phosphorus is bound in the casein micelle. Adjusting the pH of skim milk from 6.7 to 7.2 resulted in a reduction in the level of calcium and phosphorus in the resultant milk permeate, alternatively adjusting the pH of skim milk to more acidic values (i.e., < pH 6.6) resulted in an increase in calcium and phosphorous. Similarly, in Fig. 2, the calcium ion concentration increased with decreasing pH for both milk permeate and skim milk, due mainly to the solubilisation of calcium phosphate in the skim milk.

3.1.2. pH and heat coagulation time of skim milk and milk permeate solutions

The pH profile of skim milk and milk permeate solutions measured as a function of temperature is shown in Fig. 3, with the changes in pH inversely related to temperature. The measurement of pH for skim milk was not possible when the pH of the skim milk was pre-adjusted to below pH 6.4, due to significant protein aggregation, with fouling on the probe preventing accurate measurement of pH in this pH range (Fig. 3F–I). Due to the inability to measure pH in skim milk samples at pH <6.4, milk permeate solutions offered insights in to the decrease in pH at 140 °C without the issue of protein aggregation. Plotting the pH of milk permeate solutions as measured at 25 °C versus their corresponding pH values at 140 °C is shown in Fig. 4. For milk permeate at a pH of 7.0 (25 °C) the actual pH at 140 °C was 6.07, while a pH of 5.4 at 25 °C for milk permeate decreased to pH 4.65 at 140 °C. The heat coagulation time (HCT) of skim milk as a function of pH is shown in Fig. 5, with a typical type A profile observed. The HCT of the skim milk was less than 1 min at pH ≤ 6.2 with a maximum HCT at pH 6.8 of 13.3 min. HCT decreased at pH 7.0 to 5.3 min before a subsequent increase at pH 7.2 to 10.3 min. Interestingly, the pH of milk permeate solutions measured at 140 °C (Fig. 4) appear to follow a similar profile with a reduction in pH of milk permeate obtained from skim milk at pH 7.0. The low heat stability at pH values below 6.4 is mainly due to the high concentration of calcium ions in acidified skim milk as shown in Fig. 2.

3.2. Pilot-scale heat treatment in a tubular heat exchanger

3.2.1. pH data of skim milk and milk permeate during heating

The pH profile of the skim milk measured in-line during continuous heat treatment is shown in Fig. 6A. The pH of the skim milk at pH 6.7 (25 °C) gradually decreased to ~pH 6.2 at ~90 °C, where little to no decrease in pH was observed thereafter on

Table 1
Mineral profile (in mmol) of skim milk powder and corresponding ultra-filtration permeates obtained from pH-manipulated skim milk.[a]

Mineral	Skim milk	Milk permeates								
	pH 6.7	pH 7.2	pH 7.0	pH 6.8	pH 6.6	pH 6.4	pH 6.2	pH 6.0	pH 5.8	pH 5.6
Sodium	14.7 ± 1.1	16.0	17.5	16.9	14.5	14.6	14.6	14.4	11.2	11.7
Magnesium	4.60 ± 0.8	2.2	2.5	2.6	2.7	2.8	2.8	3.0	2.5	3.0
Phosphorus	33.4 ± 1.4	9.4	10.1	11.3	11.0	12.1	12.9	14.5	14.7	15.8
Potassium	42.5 ± 1.0	36.6	39.0	41.9	41.5	42.5	39.9	39.8	37.4	34.7
Calcium	31.4 ± 2.7	5.9	6.4	7.8	7.4	8.4	8.5	10.6	11.3	14.3

[a] Data presented are from a single measurement.

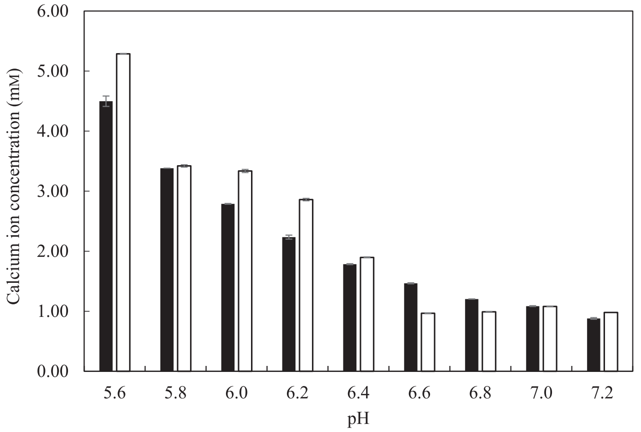

Fig. 2. Ionic calcium concentration of skim milk (■) and corresponding milk permeates (□) as a function of pH from 5.6 to 7.2 at 25 °C.

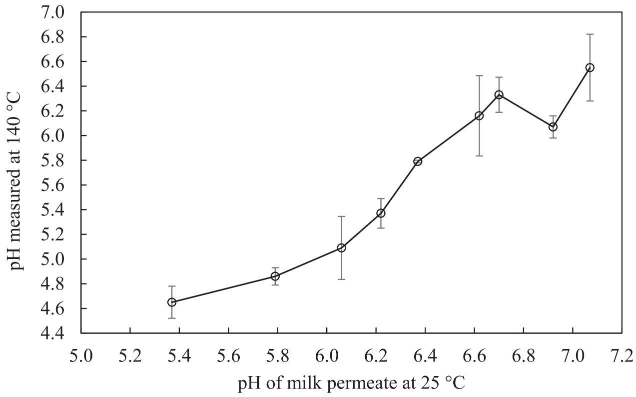

Fig. 4. The pH of milk permeate measured at 140 °C versus its corresponding pH prior to heat treatment.

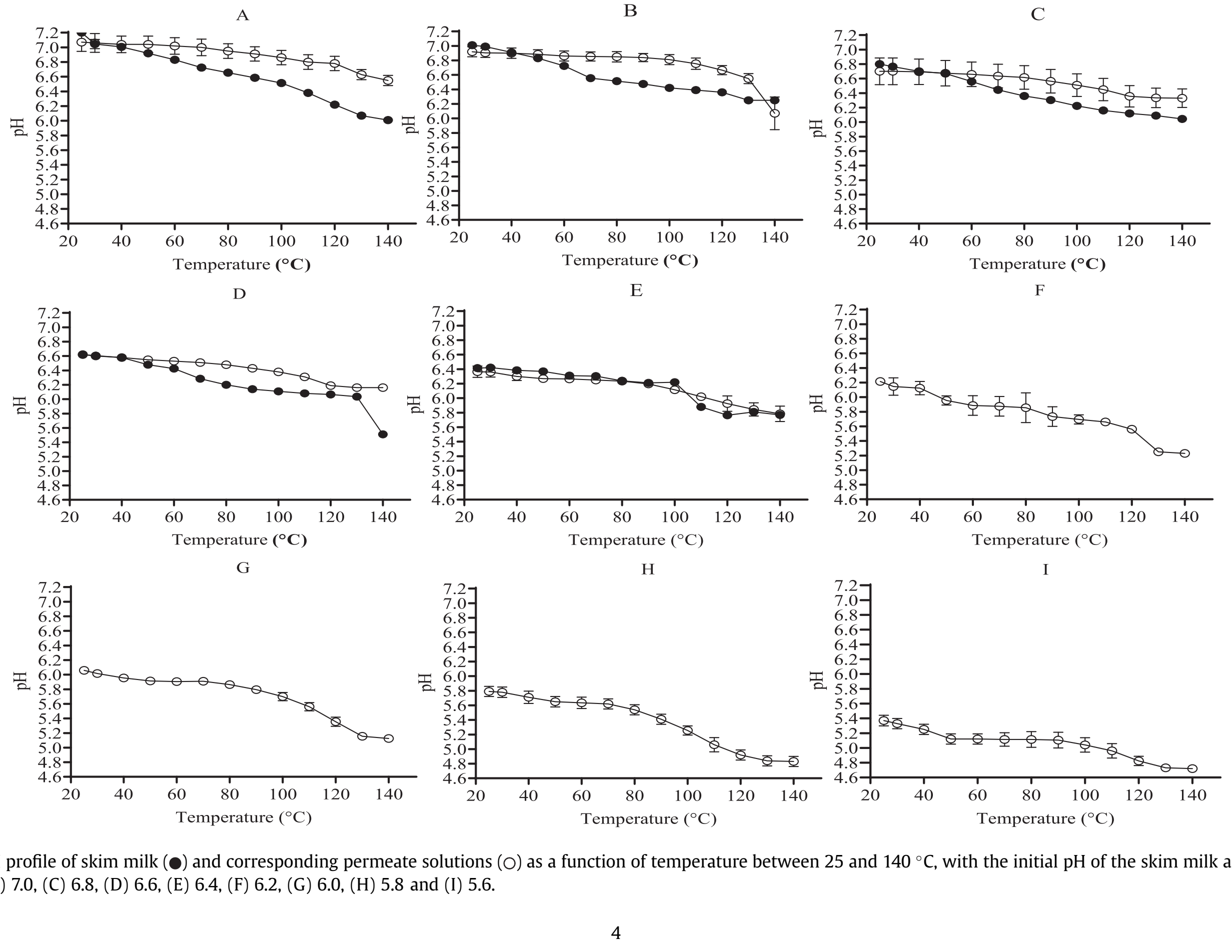

Fig. 3. pH profile of skim milk (●) and corresponding permeate solutions (○) as a function of temperature between 25 and 140 °C, with the initial pH of the skim milk adjusted to (A) 7.2, (B) 7.0, (C) 6.8, (D) 6.6, (E) 6.4, (F) 6.2, (G) 6.0, (H) 5.8 and (I) 5.6.

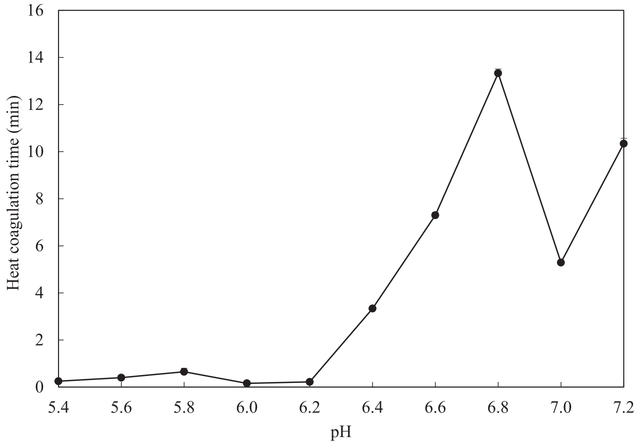

Fig. 5. Heat coagulation time of skim milk measured across a pH range of 5.4–7.2 at 140 °C.

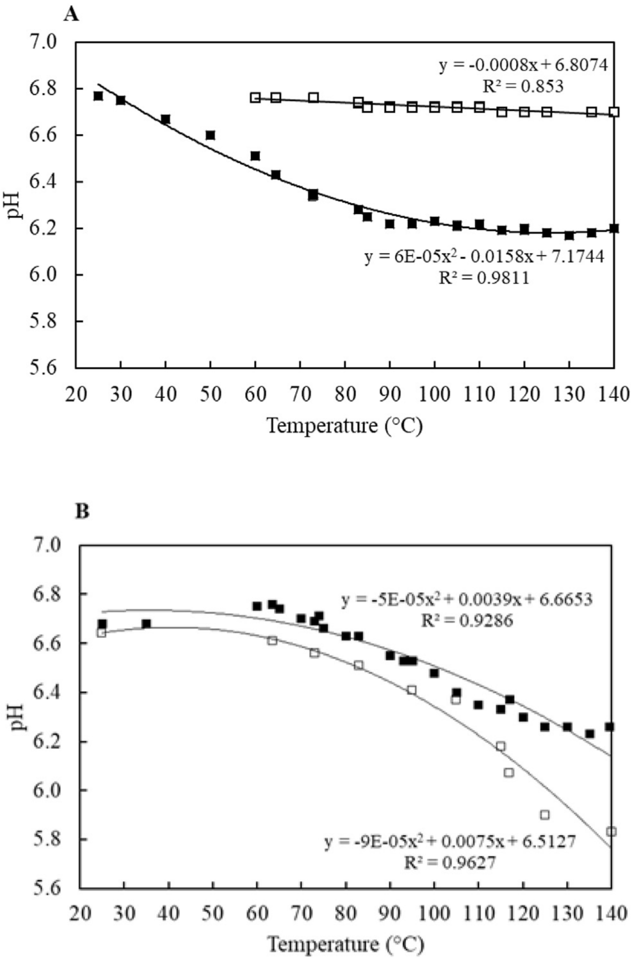

Fig. 6. pH data of skim milk (A) and pH data of milk permeate (B) measured in-line as a function of temperature within a tubular heat exchanger (■) and pH of skim milk and milk permeate measured at 25 °C (□) after each heat treatment temperature.

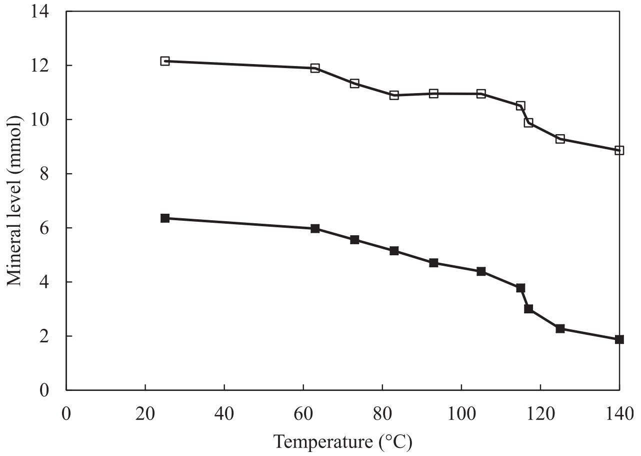

Fig. 7. Level of soluble calcium (■) and phosphorous (□) in milk permeate (initial pH 6.8) after heat treatment at different temperatures in the OMVE tubular heat exchanger. Data presented are from a single measurement.

heating up to 140 °C. The pH-temperature profile allowed for a second-order polynomial regression with a good fit ($R^2 = 0.981$). The pH-temperature profile for milk permeate (Fig. 6B) was different from that of the skim milk, with a slight increase in pH from 6.68 at 25 °C, to ~pH 6.75 at 65 °C before the pH decreased to 6.26 at 125 °C and remained relatively constant thereafter. The pH-temperature profile of milk permeate could be fitted with a polynomial regression (R^2 value ~0.93).

The pH values of skim milk and milk permeate samples collected after heat treatment and measured at 25 °C (Fig. 6A and B) were significantly different from one another. The pH reduction observed in skim milk at 140 °C was reversible upon cooling, compared with milk permeate which resulted in a further reduction in pH upon cooling (Fig. 6B). Although the changes in pH of milk permeate were largely reversible up to ~70 °C, at heat treatment temperatures above this the pH decreased even further upon cooling. The reversibility of pH in skim milk may be due to casein binding the newly formed calcium phosphate, as described by Nieuwenhuijse and Huppertz (2021), whereas casein-free systems such as milk permeate or whey have little ability to stabilise calcium phosphate, as previously shown by Aydogdu et al. (2021) for whey protein isolate dispersed in simulated milk ultrafiltrate. Tercinier, Ye, Anema, Singh, and Singh (2017) showed that α_S- and β-casein had a high affinity for binding hydroxyapatite, through the presence of phosphoserine residues in their primary structure. Pouliot, Boulet, and Paquin (1989a) also showed that formation of calcium phosphate during heat treatment of milk at 85 °C was largely reversible and re-solubilised upon cooling of the milk to 4 °C.

3.2.2. *Mineral composition of milk permeate before and after heat treatment*

The irreversible changes in pH upon heating were reflected in the soluble calcium and phosphorous levels in the milk permeate, as shown in Fig. 7. Above 110 °C, a sharp drop in the concentration of calcium and phosphorus was observed with 1.87 and 8.86 mmol soluble calcium and phosphorous remaining in solution after heat treatment at 140 °C. This equates to a 70% and 27% reduction in calcium and phosphorus, respectively. The sedimentable calcium: phosphorus ratio after heating at 140 °C was 1.76.

4. Discussion

Measurement of heat stability by either the subjective or objective assay are the most commonly used laboratory techniques, as described by O'Connell and Fox (2000), and as heat stability of milk is affected by pH, the most widely used and relevant means of assessing heat stability is over a pH range. However, a poorly understood dimension of milk heat stability is the extent to which pH changes during heat treatment, particularly at temperatures over 100 °C, and how this influences heat stability. The current study has shown that the decrease in pH with temperature is non-linear across the temperature range of 25–140 °C for both skim milk and milk permeate (Fig. 6A and B). In fact, the pH of milk permeate slightly increased when heating from 25 to ~65 °C, before decreasing thereafter as shown in Fig. 6B. This non-linear response of pH to increasing temperature has not been reported to-date, with the vast majority of analyses performed by measuring pH before and subsequently after heat treatment. Of the studies that measured pH at elevated temperatures, Chandrapala, McKinnon, Augustin, and Udabage (2010) found the decrease in pH with temperature to be linear when measured in reconstituted skim milk from 25 to 90 °C. Similarly, Aydogdu et al. (2022) reported that the pH-temperature profile could be fitted as a linear model in milk protein solutions when heated from 25 to 120 °C. Aside from the work of Aydogdu et al. (2022), most studies have focused on measuring pH at temperatures <100 °C or on samples recovered after high temperature processing. On-Nom, Grandison, and Lewis (2010) investigated dialysis and ultrafiltration as methods for monitoring mineral partitioning and pH change at high temperatures. They reported a linear decrease in the pH and ionic calcium level as temperature increased from 20 to 110 °C. However, as shown in Fig. 6 of the present study, the non-linear decrease in pH up to 140 °C is reasonable, given that a linear response does not take in to account the innate buffering capacity of milk.

The current study also showed using both the oil bath and tubular heat exchanger that because the pH decrease is not linear, the pH of milk remains significantly higher (Figs. 2 and 6) than previously assumed. A review by Fox (1981) of the early work on heat coagulation by Rose and Tessier (1959) stated that the pH value of milk serum was 5.9 at 110 °C and that by extrapolating the data the pH at 140 °C could potentially be as low as pH 5.5. Similarly, Sweetsur and White (1975) were unable to determine that there is a levelling off period in pH in both skim milk and milk permeate at temperatures above 110 °C and therefore, unlike when these authors extrapolated their data to give a potential pH reading of 4.9 for milk at 140 °C, the pH is actually significantly higher as shown in Fig. 6A. Indeed, a number of previous studies have debated how skim milk can remain stable for several minutes at 140 °C if the pH is actually as low as previously thought (Fox, 1981; Rose & Tessier, 1959). However, by combining data from Figs. 2 and 3C in the present study we can assume that the skim milk was stable against heat-induced coagulation for ~13 min at 140 °C at an actual pH of 6.2. Adjusting skim milk to pH values below 6.2 offers little information in terms of heat stability as the pH at 140 °C is potentially pH 5.0 or even lower. A study by Kaombe, Du, and Lewis (2011) proposed a question as to whether UF permeate collected at high temperature and subsequently measured at 20 °C, is representative of the pH of the milk at the higher temperatures during thermal processing. From the data shown in Fig. 6B the pH of milk permeate does not begin to decrease until temperatures above 70 °C, but that once heated and cooled there is a further decrease in pH. Conversely, the increase in pH for skim milk upon cooling, which did not occur for milk permeate, indicates that the calcium phosphate remains insoluble in milk permeate after cooling but either reverses in skim milk or, as previous studies have suggested, the newly formed calcium phosphate is adsorbed within the casein micelle or to casein peptides (Cross, Huq, Palamara, Perich, & Reynolds, 2005; Huppertz & Nieuwenhuijse, 2022; Pouliot, Boulet, & Paquin, 1989b). One aspect arising from the present study that requires future consideration is the low pH value of milk permeate at 140 °C when adjusted to pH 7.0 prior to heat treatment (Fig. 4). The fact that this decrease correlates with the decrease in the HCT of skim milk (Fig. 5) is not clearly understood. The decrease in HCT of skim milk in the range of pH 6.9 to 7.1 is most commonly linked with the dissociation of κ-casein from the micelle, leading to protein aggregation (Anema & Li, 2000). Whether the decrease in pH of the milk permeate is related to the type of calcium phosphate formed when the milk permeate is at pH 7.0 (Fig. 3B) is currently unknown. It should be noted that not only the extent of calcium phosphate precipitation, but also the type of calcium phosphate that precipitates is strongly pH dependent. This will determine the amount of liberated hydrogen ions, the amount of residual buffering salts, and hence the pH of the final solution.

Overall, this study should provide a foundation for similar work across a multitude of applications, such as in high solid concentrates, complex nutritional formulas and ingredient manufacture.

5. Conclusion

This study successfully designed, developed and implemented a real-time, in-line pH measurement system capable of providing data at temperatures up to 140 °C. The measurement of pH during high temperature treatment of skim milk and protein-free milk permeate has shown that the decrease in pH with increase in temperature up to 140 °C is not linear. Therefore, applying a linear pH-temperature coefficient for milk systems is not sufficient to describe the pH changes at ultra-high temperatures. Heat treatment caused a release of hydrogen ions mainly due to the formation of calcium phosphate. The presence of milk proteins offered little in terms of buffering against the drop in pH; however, the presence of casein micelles meant that the heat-induced pH changes were reversible in skim milk upon cooling, compared with milk permeate where the pH was lower after cooling than that measured at 140 °C. The data from this study can act as a benchmark for heat stability measurements, particularly for UHT products, enabling a better understanding on the safety and quality parameters for industrial applications such as UHT processing, sterilisation and cleaning-in-place procedures.

Declaration of competing interest

None.

Acknowledgement

This research was funded by the Teagasc Agriculture and Food Development Authority under the Walsh Scholarship Program (MDDT0184).

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