Note

Effect of milk solids concentration on the pH, soluble calcium and soluble phosphate levels of milk during heating

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Abstract – When milk is processed to dairy products, the concentration of the milk and the temperature/time combinations to which the milk is exposed can be varied. For example, large differences in milk concentrations and temperature/time combinations are used in the manufacture of milk powder or the various concentrated liquid milk products. Although the milk salts, particularly the concentrations and equilibrium states of calcium and inorganic phosphate, are considered to be important in the stability and functionality of dairy products, there have been few studies on the mineral balance in concentrated milk systems and how this is affected by temperature changes. The objective of this study was to examine the effect of milk concentration (9.6–38.4% w/w total solids), temperature (20–80 °C) and time (0–60 min) on the pH and the concentrations of soluble calcium (Ca_{sol}) and soluble inorganic phosphate (P_{sol}). At any given temperature, the level of Ca_{sol} and P_{sol}, as $mmol \cdot kg^{-1}$, increased and the pH decreased as the milk concentration was increased. However, when measured as a percentage of the total, the level of Ca_{sol} and P_{sol} decreased as the milk concentration was increased. At any given milk concentration, the level of Ca_{sol}, P_{sol} and milk pH decreased within the first few minutes of heating, with little further change at longer heating times. For milk samples at each concentration, there was a strong correlation between the final Ca_{sol} and the final P_{sol} at all temperatures. At any particular milk concentration, there was a strong correlation between the final milk pH and the final Ca_{sol} or the final P_{sol} levels. These results indicate that substantial decreases in milk pH, Ca_{sol} and P_{sol} can occur at the temperatures milk will experience in common commercial processes, and the extent of the changes will be dependent on the milk concentration and the processing conditions.

milk / calcium / phosphate / pH / concentration / temperature

摘要 – 牛乳加热过程中乳浓度对 pH、可溶性钙和可溶性磷酸盐含量的影响。在乳品加热过程中，乳浓度和所对应的热处理温度/时间之间的关系是可变的。 例如，生产奶粉或不同浓度的炼乳时，不同乳浓度所对应的热处理温度/时间完全不同。 尽管乳中的盐，特别是钙的浓度和平衡状态以及无机磷酸盐被认为是乳制品稳定和功能特性的重要因素，但是关于炼乳体系中矿物质的平衡以及温度变化对这种平衡影响方面的研究还是非常有限的。 本文目的是研究乳的浓度 （9.6 ~ 38.4%, w/w 总固性物）、温度 （20–80 °C） 和时间 （0 ~ 60 min） 对 pH、可溶性钙浓度 （Ca_{sol}） 和无机磷酸盐浓度 （P_{sol}） 的影响。 在任一给定的

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温度下，Ca$_{sol}$ 和 P$_{sol}$ 的浓度 （以 mmol·kg^{-1} 表示） 是增加的，而 pH 则是随着乳浓度的增加而降低。 Ca$_{sol}$ 和 P$_{sol}$ 随着乳浓度 （以占总固性物的百分含量表示） 的增加而降低。 在任一给定的乳浓度下，Ca$_{s}$、P$_{sol}$ 和 pH 在加热的最初几分钟内是降低，继续加热这三个值的变化幅度较小。 对每一浓度的乳样来讲，在所有的温度下，最终 Ca$_{sol}$ 和最终 P$_{sol}$ 之间具有显著的相关性。 在任一特定的乳浓度下，最终乳制品的 pH 和最终 Ca$_{sol}$ 或最终 P$_{sol}$ 之间具有显著的相关性。 研究结果说明，在乳品工业生产中应该考虑加热所导致乳 pH、Ca$_{sol}$ 和 P$_{sol}$ 发生的变化，这种变化程度将取决于乳的浓度和加工条件。

牛乳 / 钙 / 磷酸盐 / pH / 浓度 / 温度

Résumé – Effet de la concentration du lait en matière sèche sur le pH et les taux de calcium et phosphate solubles au cours du chauffage. Lorsque le lait est transformé en produits laitiers, la concentration du lait et les combinaisons température/temps auxquelles le lait est exposé sont variables. Par exemple, de grandes différences de concentrations du lait et de combinaisons température/temps sont utilisées dans la fabrication de poudre de lait ou de laits concentrés. Bien que les minéraux du lait, particulièrement les concentrations et états d'équilibre du calcium et phosphate inorganique, soient considérés comme importants dans la stabilité et les fonctionnalités des produits laitiers, il y a eu peu d'études sur la balance minérale dans les systèmes laitiers concentrés et sur sa modification par les changements de température. L'objectif de cette étude était d'examiner l'effet de la concentration du lait (9,6 à 38,4 % p/p de matière sèche totale), de la température (20–80 °C) et du temps (0 à 60 min) sur le pH et les concentrations en calcium soluble (Ca$_{sol}$) et phosphate inorganique (P$_{sol}$). À chaque température testée, le taux de Ca$_{sol}$ et de P$_{sol}$ (mmol·kg^{-1}) augmentait et le pH diminuait lorsque la concentration du lait augmentait. Cependant, lorsqu'il était exprimé en pourcentage du total, le taux de Ca$_{sol}$ et P$_{sol}$ diminuait quand la concentration du lait augmentait. À chaque concentration du lait, le taux de Ca$_{sol}$ et P$_{sol}$ et le pH du lait diminuaient au cours des premières minutes de chauffage avec peu de changement ultérieur pour des durées de chauffage plus longues. À chaque concentration du lait, il y avait une corrélation forte entre le Ca$_{sol}$ final et le P$_{sol}$ final à toutes les températures. À chaque concentration du lait il y avait une corrélation forte entre le pH final du lait et les taux finaux de Ca$_{sol}$ et P$_{sol}$. Ces résultats indiquent que des diminutions substantielles du pH du lait, du Ca$_{sol}$ et du P$_{sol}$ peuvent survenir aux températures auxquelles le lait est soumis dans les procédés commerciaux habituels, et l'étendue de ces changements dépendent de la concentration du lait et des conditions de traitement.

lait / calcium / phosphate / pH / concentration / température

1. INTRODUCTION

Bovine milk is a complex biological fluid containing protein, emulsified fat, minerals, carbohydrates and water. The casein proteins, much of the calcium and phosphate, and low levels of other minerals, are found as large macromolecular assemblies called casein micelles [17]. The mineral components that are associated with the casein micelles (predominantly calcium and phosphate) are commonly referred to as colloidal calcium phosphate (CCP) and this CCP is in a dynamic equilibrium with the mineral components in the soluble phase. The soluble calcium (Ca$_{sol}$) and the

soluble inorganic phosphate (P$_{sol}$) are present in the soluble phase as free ions or are associated with other ions, as has been described in detail [6].

The equilibrium state of the calcium and phosphate in milk is markedly dependent on the temperature, concentration and pH of the milk [6]. Increasing the temperature of milk results in the transfer of the Ca$_{sol}$ and P$_{sol}$ to the colloidal phase, with a concomitant decrease in milk pH [14, 16]. Most of this decrease in Ca$_{sol}$ and P$_{sol}$ occurs within the first minutes of heating, with smaller changes on prolonged holding [14, 16]. Under mild heating conditions (below 95 °C/several minutes), the changes

in Ca_{sol}, P_{sol} and milk pH are largely reversible when the temperature of the milk is restored to its initial value [7, 15].

Concentration of milk by evaporation retains all the milk salts, and, as milk is saturated with respect to calcium phosphate, some of the Ca_{sol} and P_{sol} is transferred to the colloidal phase. However, as the milk pH decreases on increasing milk concentration, and, as increasing ionic strengths reduce the activity coefficients of the Ca_{sol} and P_{sol}, the concentrations of calcium and phosphate in the soluble phase do increase but this increase is not proportional to the concentration factor of the milk [6, 12].

Milk concentration can be markedly increased when milk is processed to dairy products, and these concentration changes are usually accompanied by increases or decreases in the temperature of the milk [17]. For example, during the manufacture of skim milk powder, the milk can be concentrated from the natural level (about 10% total solids (TS)) to about 50% TS before spray drying, and the temperature during this processing can range from about 4 °C to temperatures above 100 °C depending on the specification of the powder product [17]. Milk can be concentrated up to 2.5 times to produce evaporated liquid milk products, during which the milk temperature can be varied from about 4 °C to over 140 °C depending on the product being produced. There can be problems while processing milk concentrates (e.g. fouling of surfaces, thickening or coagulation) or storing concentrated milks (e.g. age gelation or sedimentation), and the milk minerals, particularly calcium and inorganic phosphate, are often implicated in these issues [17]. Despite this, there have been few studies on the effect of temperature on the Ca_{sol}, P_{sol} and pH for concentrated skim milk. This study, therefore, examined the effect of milk concentration and temperature on the pH of the milk and the partition of calcium and phosphate between colloidal and soluble phases.

2. MATERIALS AND METHODS

2.1. Milk supply

Skim milk samples of 9.6%, 19.2%, 28.8% and 38.4% TS (w/w) were prepared by dissolving the appropriate quantity of low-heat skim milk powder (Fonterra Co-operative Group Ltd., New Zealand: contains about 34% protein, 4% moisture, 54% carbohydrate, 8% minerals and 0.8% fat) in high purity water. Sodium azide was added to each milk sample at a rate of 0.1 mg·L^{-1}. The milks were allowed to equilibrate at 20 °C for at least 24 h.

2.2. Heat treatment and ultrafiltration permeate collection

A sample of each milk sample was heated from ambient temperature (~ 20 °C) to temperatures ranging from 30 to 80 °C. Heating commenced when the milk sample was pumped from a vessel at room temperature, through the ultrafiltration (UF) unit (10 000 Da (nominal) hollow fibre membrane cartridge and pumping equipment (Amicon, Inc., Beverly, MA, USA)) and a tubular heat-exchange device (at the desired temperature) to a second vessel held in a temperature-controlled water bath set to the desired temperature. Once the required volume of milk had passed through to the second vessel, it was recirculated between the UF unit, the heat-exchange device and the second vessel for the duration of the experiment, thus maintaining the milk at the desired temperature. The heat-up time to the required temperature was < 2 min for all samples. For each sample, a series of UF permeate samples (about 2.5 mL) was collected during a 60-min heating-recirculation period.

Not more than about 10% of the total milk volume was extracted as permeate.

2.3. Measurement of pH

The pH of the milk samples was measured across a range of temperatures and holding times using a combination glass electrode coupled with a temperature probe. At each temperature, standard pH buffers were allowed to equilibrate for about 30 min before being used for calibration of the pH meter. The pH shift of buffers due to temperature was taken into account during calibration. Milk samples were pumped through a heat exchanger to a vessel held at the desired temperature in a thermostatically controlled water bath as described in Section 2.2. The pH of these samples was monitored against time and temperature.

2.4. Calcium, phosphate and TS analysis

Calcium in the milk and permeate samples was determined by an inductively coupled plasma emission spectroscopy method [1]. Phosphate was determined as inorganic phosphate using the molybdo-vanadate method [2]. The TS content of the milk samples was determined by drying samples at 102 °C for 5 h [13].

3. RESULTS

3.1. Time-dependent changes in Ca_{sol} and P_{sol} on heating milk

The results for the time-dependent changes in Ca_{sol} and P_{sol} levels when milk samples were heated from 20 °C to temperatures in the range from 30 to 80 °C are shown in Figures 1A and 1B for the 9.6% and 28.8% TS milk samples, respectively. Similar general results were observed for the milks at the other concentrations. On heating, there was an initial steep decrease in Ca_{sol} and P_{sol}

levels over the first 5 min of heating, followed by a period where only small further changes in Ca_{sol} and P_{sol} were observed. The extent of the decrease in the Ca_{sol} and P_{sol} was greater at higher temperatures. As only small changes in Ca_{sol} and P_{sol} are observed on heating beyond about 10 min, the Ca_{sol} and P_{sol} levels obtained after 60 min heating are referred to as the final Ca_{sol} and final P_{sol} at each particular temperature. Figure 1C shows the effect of temperature on the final Ca_{sol} and final P_{sol} levels. For each milk concentration, there was an inverse linear relationship between the final Ca_{sol} or the final P_{sol} level and temperature. The relative effect of temperature on the change in final Ca_{sol} or final P_{sol} was more pronounced as the milk concentration increased from 9.6% to 28.8% TS, but was similar at milk concentrations of 28.8% and 38.4% TS.

3.2. Relationship between Ca_{sol} and P_{sol}

The relationship between the final Ca_{sol} and the final P_{sol} levels is shown in Figure 2A. At each milk solids concentration, there was a linear relationship between the final Ca_{sol} and the final P_{sol} levels, with the lines displaced to higher Ca_{sol} and P_{sol} as the milk concentration increased. The changes in final Ca_{sol} and final P_{sol} with increasing temperature (relative to the sample at 20 °C) can be plotted against each other to give an indication of the composition of the calcium phosphate material that is transferred from the soluble to the colloidal state (Fig. 2B). With the exception of a couple of points (the 80 °C points for the 28.2% and 38.4% TS milks), all the data fell close to a single line with a slope very close to unity (1.02).

3.3. Time-dependent changes in pH on heating milk

The results for the time-dependent changes in pH when milk samples were
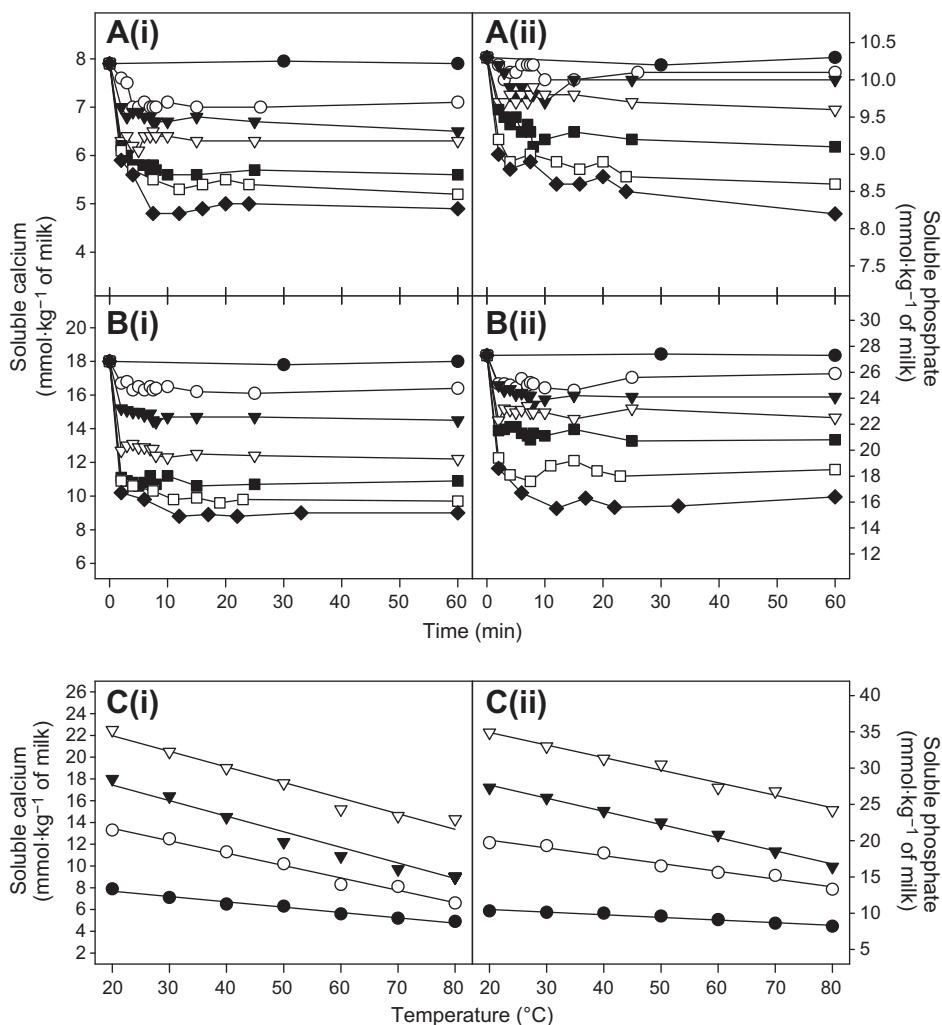
Figure 1. Changes in soluble calcium (i) and soluble phosphate (ii) on heating milk. (A) Changes in soluble calcium or soluble phosphate with heating time for the 9.6% TS milk heated at 20 °C (●), 30 °C (○), 40 °C (▼), 50 °C (▽), 60 °C (■), 70 °C (□) and 80 °C (♦). (B) Changes in soluble calcium or soluble phosphate with heating time for the 28.8% TS milk heated at 20 °C (●), 30 °C (○), 40 °C (▼), 50 °C (▽), 60 °C (■), 70 °C (□) and 80 °C (♦). (C) Changes in final soluble calcium and final soluble phosphate after 60-min heating at a range of temperatures for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples.

heated from 20 °C to temperatures in the range from 30 to 80 °C are shown in Figures 3A and 3B for the 9.6% and 28.8% TS milk samples, respectively. Similar

results were observed for the milks at the other concentrations. For each milk concentration, the pH decreased rapidly on initial heating followed by a period where only
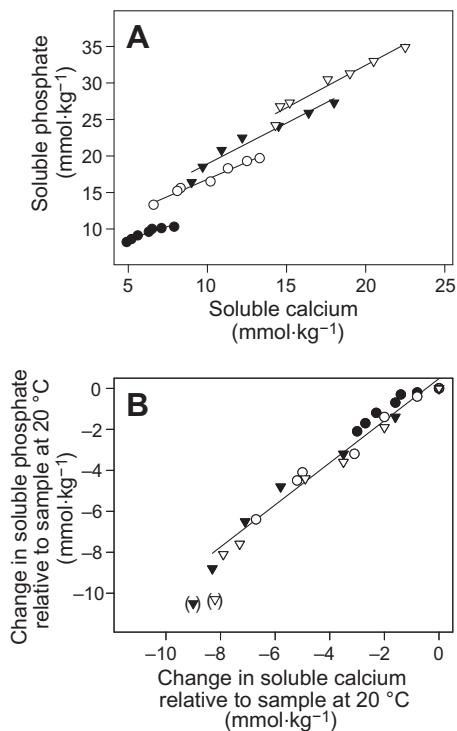
Figure 2. Relationships between soluble calcium and soluble phosphate for milks of different concentrations. (A) Relationship between soluble calcium and soluble phosphate for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples that were heated at temperatures between 20 and 80 °C for 60 min. (B) Relationship between the change in soluble calcium and the change in soluble phosphate (relative to the samples held at 20 °C) for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples that were heated at temperatures between 20 and 80 °C for 60 min.

small further changes in pH were observed. For each milk concentration, a greater decrease in pH was observed at higher temperatures. Only small changes in pH were observed on heating beyond 10 min, therefore, the pH at 60-min heating was referred to as the final pH. At any particular milk concentration, the final pH decreased with increasing temperature, and at any particular temperature, the final pH of the milk decreased with increasing milk solids concentration (Fig. 3).

Figure 3C shows the final pH plotted against temperature. For each milk concentration, the pH decreased essentially linearly with increasing temperature. The observed effect of temperature on the final pH for the 9.6% milk was almost identical to that reported by Chaplin and Lyster [4]. Increasing the milk concentration resulted in a shift in the position of the curve to lower pH; however, the slopes of the curves at all milk concentrations, and therefore the relative effect of temperature on the pH, were very similar.

3.4. Relationship between pH and Ca_{sol}, and pH and P_{sol}

The relationships between the final pH and the final Ca_{sol}, and between the final pH and the final P_{sol} are shown in Figure 4. For each milk concentration, there was a strong linear correlation between the final pH and the final Ca_{sol} or the final P_{sol}, with the curves displaced to higher Ca_{sol} or P_{sol} as the milk concentration is increased. The relative increase in final Ca_{sol} or final P_{sol} with increasing pH was more pronounced (i.e. steeper slope) as the milk concentration increased from 9.6% to 28.8% TS, but was similar at milk concentrations of 28.8% and 38.4% TS.

4. DISCUSSION

The results in Figure 1 indicate that, on heating, the new equilibrium position between soluble and colloidal calcium and phosphate is rapidly approached at all milk concentrations. For the 9.6% TS milks, the changes in Ca_{sol} and P_{sol} with heating time at various temperatures were comparable with those reported by Pouliot et al. [14],
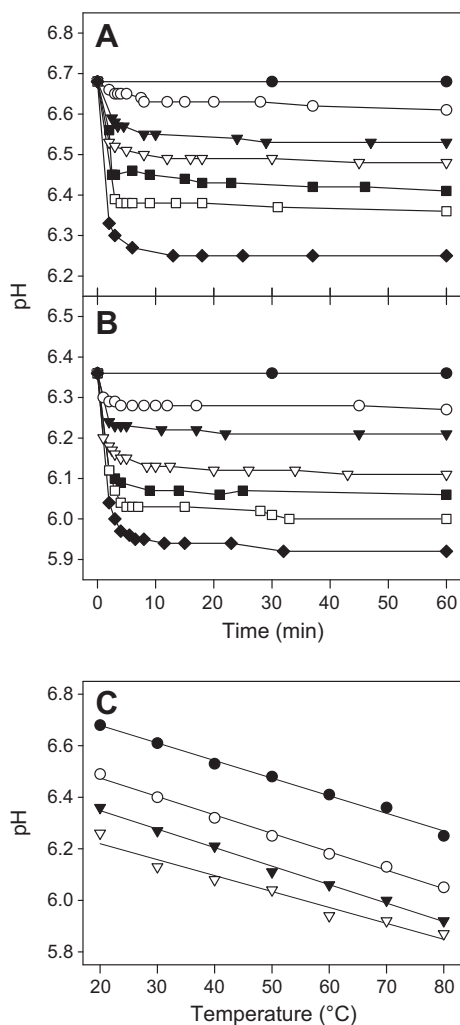
Figure 3. Changes in pH on heating milk. (A) Changes in pH with heating time for the 9.6% TS milk heated at 20 °C (●), 30 °C (○), 40 °C (▼), 50 °C (▽), 60 °C (■), 70 °C (□) and 80 °C (◆). (B) Changes in pH with heating time for the 28.8% TS milk heated at 20 °C (●), 30 °C (○), 40 °C (▼), 50 °C (▽), 60 °C (■), 70 °C (□) and 80 °C (◆). (C) Changes in final pH after 60-min heating at a range of temperatures for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples.

and their final Ca_{sol} and P_{sol} levels were very similar to those obtained in this study (Fig. 1). At any particular temperature, the final Ca_{sol} or final P_{sol} increased, but this increase was markedly less than the concentration factor of the milk (Figs. 1 and 2). Although milk is saturated with respect to Ca_{sol} and P_{sol}, the increased ionic strength on concentration of the milk will reduce the ion activity coefficients, and this, coupled with the reduced pH on increasing the milk concentration, will result in higher Ca_{sol} and P_{sol} concentrations, while maintaining the solubility product relationship between soluble and colloidal calcium and phosphate [6, 12].

Figure 3 shows that the final pH of the milks varied from about pH 6.7 to pH 5.9 depending on the milk concentration and temperature. It may have been expected that this variation in pH would change the nature of the calcium phosphate that is precipitated from solution. However, the results in Figure 2 indicate that the calcium phosphate transferred from the soluble phase during heating milk is similar in composition, regardless of the milk concentration and the temperature to which the milk is heated. This may be explained by the studies of Holt [5, 6] who found that the solubility product of calcium phosphate from milk was independent of pH over the range from pH 6.4 to pH 7.1 and suggested that the solubility product for calcium and phosphate in milk is dictated by the stoichiometry of the equilibrium calcium phosphate phase.

The observation that the heat-precipitated calcium phosphate has a calcium to phosphate ratio close to 1 suggests that this material may have a similar composition to dicalcium phosphate ($CaHPO_4$). These results are consistent with literature reports as Pouliot et al. [14] and Rose and Tessier [16] found a calcium to phosphate ratio close to 1 for the calcium phosphate
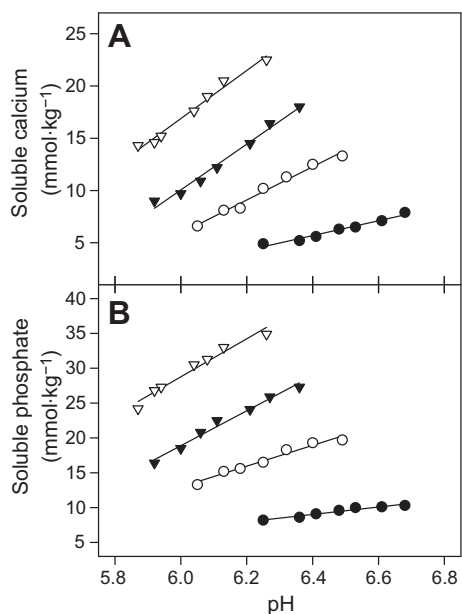
Figure 4. Relationships between pH and soluble calcium or soluble phosphate for milks of different concentrations. (A) Relationship between pH and soluble calcium for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples that were heated at temperatures between 20 and 80 °C for 60 min. (B) Relationship between pH and soluble phosphate for the 9.6% TS (●), 19.2% TS (○), 28.8% TS (▼) and 38.4% TS (▽) milk samples that were heated at temperatures between 20 and 80 °C for 60 min.

transferred to the colloidal phase when milk at its natural concentration was heated. Walstra et al. [17] suggested that transformation of Ca_{sol} and P_{sol} to colloidal calcium and phosphate involved the reaction: $Ca^{2+} + H_2PO_4^- \rightarrow CaHPO_4 + H^+$, which also suggests that the heat-precipitated calcium phosphate has a calcium to phosphate ratio close to unity.

It should be noted that milk serum is a complex mixture of mineral components, and some of these other mineral components may also associate with the Ca_{sol}

and P_{sol} (such as citrate and magnesium) on heating to form insoluble salts that are transferred to the colloidal phase [6, 7]. However, Rose and Tessier [16] indicated that there was little change in soluble citrate or soluble magnesium on heating milk at a temperature of ~ 95 °C for up to 80 min, which suggests that little calcium will be precipitated as the citrate salt and little phosphate will be precipitated as the magnesium salt.

The nature of the CCP in milk, the mode of association of CCP with the casein micelles and the relationship between the CCP and the heat-precipitated calcium phosphate have not been unequivocally established [5, 8]. Although CCP has a calcium to inorganic phosphate ratio of about 1.5, when the Ca to (inorganic + organic phosphate) is considered, the ratio is close to unity [8]. This has led to the suggestion that the native CCP in milk may resemble dicalcium phosphate [8, 9]. Holt et al. [10, 11] have prepared calcium phosphate nano-clusters that are similar in size, composition, short-range order and dynamics to the native CCP. Through chemical analyses and infrared spectroscopy, it was suggested that these nano-clusters are composed of amorphous dicalcium phosphate complexed to the organic phosphate groups of the casein proteins. As the heat-precipitated calcium phosphate has a similar calcium to phosphate ratio as the native CCP, it has been suggested that the heating of milk resulted in an increase in the size of the CCP particles within the casein micelles in order to accommodate the precipitated calcium phosphate [7]. It is possible that the native CCP acts as nucleation sites for the heat-precipitated calcium phosphate. This is supported by the observation that the level of casein cross-linked by CCP is not increased by moderate heating [3].

The decrease in pH on increasing milk concentration (Fig. 3) is due to the increase in ionic components in the milk and possibly due to the precipitation of calcium

phosphate. The decrease in milk pH under mild heating conditions (Fig. 3) is reportedly due to the changes in the dissociation of ions within the milk [6, 7, 17] and the conversion of Ca_{sol} and P_{sol} to the colloidal phase (through the reaction $Ca^{2+} + H_2PO_4^- \rightarrow CaHPO_4 + H^+$). On extensive heating, other reactions, such as the degradation of lactose or Maillard reactions, may cause more substantial and irreversible decreases in pH [4, 6]; however, these types of reactions are unlikely to occur to any great extent under the mild heating temperatures used in this study.

This study has demonstrated that the distribution of calcium and phosphate between soluble and colloidal phases is markedly dependent on the temperature and concentration of the milk. Increasing the temperatures resulted in a rapid conversion of Ca_{sol} and P_{sol} to the colloidal phase, and this was accompanied by a concomitant decrease in pH. The Ca_{sol}, P_{sol} and milk pH decreased within the first few minutes of heating and then remained relatively constant at longer heating times. Although the concentrations of Ca_{sol}, P_{sol} and pH were affected by the milk concentration, a similar general effect was observed at all milk concentrations, and as a consequence, interrelationships were observed between Ca_{sol}, P_{sol} and pH. It appears that, at all milk concentrations, the predominant calcium phosphate phase precipitated from milk on increasing the temperature has a calcium to phosphate ratio close to unity. These results indicate that during the processing of milk, rapid decreases in the pH and the Ca_{sol} and P_{sol} occur when the milk is heated. If calcium or phosphate components are implicated in instability problems during the processing or storage of concentrated milks, it will be necessary to consider the concentration of these components as well as the effects of temperature, heating time and milk concentration on the equilibria of calcium and phosphate between the soluble and the colloidal phases.

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