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Influence of mineral environment on the buffering capacity of casein micelles

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The buffering capacity of casein micelle suspensions is a physico-chemical characteristic depending on the mineral and protein composition, on the interactions between these compounds and on the physicochemical environment. In this study, buffering capacity was maximum at pH about 5.2 and was mainly due to the acid-base properties of inorganic phosphate solubilised during acidification and organic phosphate from phosphoserine residues of caseins. Addition of calcium chloride to suspensions of casein micelles in water slightly increased buffering capacity, whereas addition of sodium phosphate or sodium citrate increased buffering capacity both qualitatively and quantitatively. These changes were due to intrinsic acid-base properties of the added compounds (citrate and phosphate) and to the physicochemical modifications of micellar caseins induced.

Einfluss der Mineralstoffumgebung auf die Pufferkapazität von Caseinmicellen

Die Pufferkapazität von Caseinmicell-Suspensionen ist eine physikochemische Eigenschaft, die von der Mineral- und Proteinzusammensetzung, den Interaktionen zwischen diesen Bestandteilen und der physikochemischen Umgebung abhängig ist. In dieser Studie war die Pufferkapazität maximal bei einem pH-Wert von ca. 5,2 und beruhte im wesentlichen auf den Säure-Basen-Eigenschaften des anorganischen Phosphats, das während der Säuerung gelöst wurde, und dem organischen Phosphat aus Phosphoserinresten der Caseine. Der Zusatz von Calciumchlorid zu Caseinmicell-Lösungen in Wasser erhöhte leicht die Pufferkapazität, obwohl der Zusatz von Natriumphosphat oder Natriumcitrat die Pufferkapazität sowohl qualitativ als auch quantitativ erhöhte. Diese Veränderungen beruhten auf den intrinsischen Säure-Basen-Eigenschaften der zugesetzten Bestandteile (Citrat und Phosphat) und auf den induzierten physikochemischen Veränderungen der micellaren Caseine.

25 Casein micelles (buffering capacity, minerals)

25 Caseinmicellen (Pufferkapazität, Mineralstoffe)

1. Introduction

Acidification is a fundamental technological step for the manufacture of acid caseins, yoghurts, fermented milks and cheeses. Many physico-chemical modifications of casein micelles have been described during milk acidification e.g. protonation of acid groups inducing demineralisation of casein micelles (1-3) and decreases in solubility, hydration and zeta potential of caseins (4, 5).

These modifications are influenced by the buffering capacity which expresses the ability of a solution to resist variation in pH induced by dilution or by addition of acid or base. For example, it can be evaluated as the molar quantity of acid or base required to change pH by one defined unit. Several authors have defined the elements contributing to the buffering capacity of milk as being soluble phosphate, colloidal calcium phosphate, citrate, bicarbonate, caseins and whey proteins. Compilation of the results published in the literature indicates that the contribution of milk constituents is 35, 5, 40 and 20 % for caseins, whey proteins, soluble minerals and colloidal calcium phosphate, respectively (6-13).

The aim of this study was to evaluate more precisely the contributions of the different milk components to buffering capacity by comparing the buffering capacities of sodium caseinate and casein micelles suspended in water and milk. We then studied the buffering capacity of casein micelles in different mineral environments containing controlled amounts of sodium, calcium, phosphate and citrate. These results

are discussed in relation to the physico-chemical properties of casein micelles and mineral solubilisation induced by acidification.

2. Materials and methods

2.1 Casein micelle, sodium caseinate, milk and mineral solutions

Phosphocaseinate powder was prepared according to SCHUCK *et al.* (14). By dispersing this powder in aqueous solution, the casein micelle suspensions have the same behaviour in terms of acidification as milk casein micelles (15).

Sodium caseinate was prepared from casein micelle suspension by three successive acidifications (to pH 4.6 with 2 M HCl) - alkalinations (to pH 8.0 with 1 M NaOH); the product was then lyophilised.

Different stock mineral solutions were prepared from analytical grade salts (NaCl (500 mM), CaCl₂ (60 mM), trisodium phosphate (250 mM; pH 10.8) and trisodium citrate (90 mM; pH 11.2)).

2.2 Preparation of suspensions containing micellar casein and minerals

Simple mixtures were prepared by adding defined amounts of the stock mineral solutions to casein micelle suspension. The casein concentration of casein micelle suspension and sodium caseinate was 27 g/kg. Raw skimmed milk had a casein concentration of 27.6 g/kg. To prevent bacterial and fungal growth, 200 mg/kg thimerosal (C₉H₉HgO₂SNa, Sigma, St. Louis, USA) was added to the different suspen-

sions stored for 15 h at 4°C before buffering capacity measurements.

2.3 Buffering capacity measurement

Acid titrations were performed on 30 g samples at 25°C from initial pH to 2.5 using 0.5 M HCl added in 0.2 mL increments at 60 s intervals. The evolution of pH was followed on a pH-meter 686 Titroprocessor (Metrohm, Roucaire, Switzerland) for milk and simple mixtures. Buffer indices (dB/dpH) were calculated for each addition of acid according to the formula of VAN SLYKE (16) and indices were plotted against pH values. The resulting buffering curves indicated i) the buffering capacity at a pH value or in a defined pH range, ii) the pH for maximum buffering (determined with a precision of 0.1 pH unit) and iii) the intensity of buffering capacity determined from areas under the buffering curve calculated by integration of peaks in a defined pH range.

3. Results and discussion

3.1 Buffering capacity of sodium caseinate, casein micelles and milk

The buffering capacities of sodium caseinate, casein micelle suspensions and raw skimmed milk were compared in the same experimental conditions (Fig. 1). During acidification of these three suspensions, two buffering peaks were observed in a pH range 4.8–5.2 and at about pH 3.5.

For sodium caseinate, the buffering peak at pH 5.2 corresponded mainly to acid-base properties of organic phosphate from phosphoserine residues and to a lesser extent to histidine residues which have pK values at about 6.6. It should be noted that the pH value of 5.2 is lower than pKa values of phosphoserines in the phosphopeptide (1-25) of β -casein or in β -casein. WALSTRA & JENNESS (10) and BAUMY *et al.* (16) reported pKa of the second ionisation of organic phosphate at about pH 6.8. In other studies, buffering capacities of sodium caseinate and calcium caseinate have been reported to be maximum at pH 5.6 (17, 18). The differences observed may be related to different materials and methods used for their determination.

Interpretation of the buffering capacity curves were more complex for casein micelle suspension and milk than for sodium caseinate because it was necessary to take into account the contributions of constituents present in the aqueous and colloidal phases. These differences in composition induced quantitative and qualitative differences in the buffering capacity. The maximum buffering capacity for these suspensions was observed at pH 5.0–5.2. Similar results were obtained by LUCEY *et al.* (12, 19) and corresponds to the total solubilisation of colloidal phosphate induced by acidification (2, 20, 21). As a consequence, the "free" inorganic and organic phosphates can be associated with H^+ , resulting in a buffering capacity at pH 5.0–5.2. By integrating buffering curves, it was determined that the buffering capacity of milk was higher than that of micellar casein suspension. The buffering capacity of micellar caseins suspended in water was mainly due to the presence of organic and inorganic phosphate. The buffering capacity of milk was higher because there were the supplementary contributions of soluble

minerals (inorganic phosphate and citrate). On the other hand, when the maximum pH value of the buffering capacities of sodium caseinate, micellar caseins and milk were compared, they were slightly different (5.2, 5.2 and 4.8 for sodium caseinate, casein micelle in water and milk, respectively) (Fig. 1). Milk contains more minerals than sodium caseinate or micellar casein suspensions and it is therefore necessary to acidify at lower pH values to solubilise all colloidal minerals (22) and to observe a buffering peak. On the other hand, this shift may be due to differences in the ionic strength of the different suspensions. Indeed, it is accepted that an increase in ionic strength leads to a decrease in the apparent pK of an acid-base group. This is clearly described by BAUMY *et al.* (16) who showed that the increase in ionic strength shifts the pKa value of the second ionisation of phosphate from phosphoserine residues contained in β -casein and its (1-25) phosphopeptide towards lower pH values.

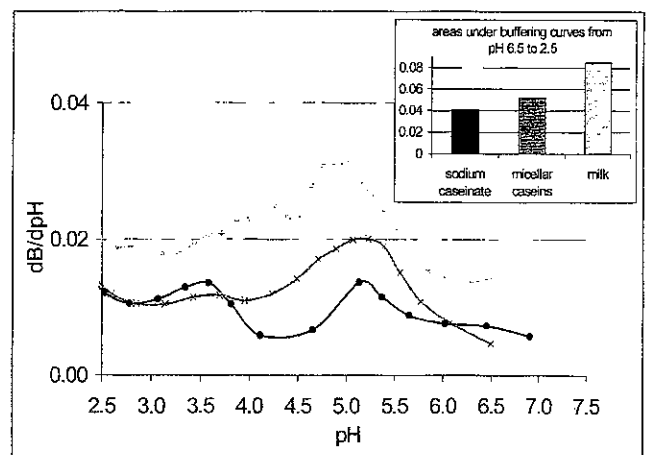


Fig. 1: Buffering capacity of ● sodium caseinate (27 g casein/kg), × casein micelles suspension (27 g casein/kg) and ◆ raw skimmed milk (27.6 g casein/kg).

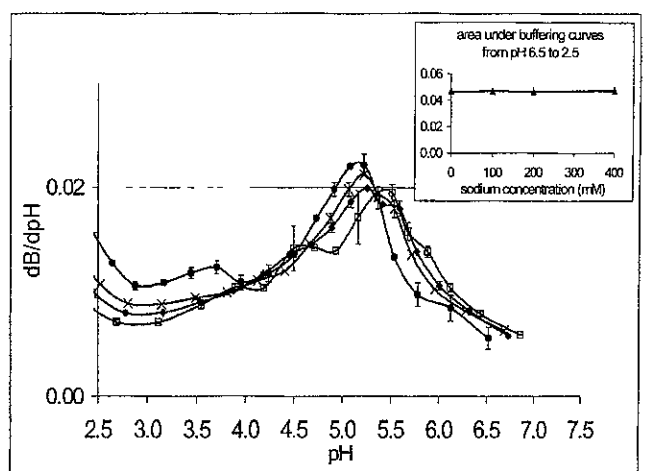


Fig. 2: Effect of the addition of NaCl (×, 100; ◆, 200 and □, 400 mM sodium) on the buffering capacity of casein micelles suspension (●).

Another buffering peak was observed at pH 3.5 especially for sodium caseinate. Although this peak was not specifically visualised for milk and casein micelle suspensions, the buffering capacity at this pH was

high. Buffering capacity at this pH value can be attributed to the acid-base properties of carboxyl groups of the C-terminal amino acid (pKa is 3.7 in milk) and acidic amino acids (pKa of aspartic and glutamic acids are 4.1 and 4.6 in milk, respectively) (10, 13). Moreover, the presence of citrate (pKa in milk 3.0-4.1-4.8) and whey proteins in milk also contribute to a buffering capacity around pH 3.0-3.5.

3.2 Influence of different salts on the buffering capacity of micellar caseins

Addition of NaCl up to a concentration of 400 mM induced changes in the buffering capacity of the casein micelle suspension. A shift in the maximum buffering capacity from pH 5.2 (without NaCl addition) to 5.5 in the presence of 400 mM NaCl was observed. On the other hand, there was a decrease in the buffering capacity at about pH 3.0 (Fig. 2). The solubilisation of micellar phosphate induced by high NaCl concentrations (2, 3, 22-24) could be responsible for a shift in the expression of the buffering capacity at pH higher than 5.2. Similarly, some exchanges between calcium (initially in interaction with carboxyl residues) and sodium could explain the decrease observed at acidic pH. The release of calcium induced by addition of NaCl allowed expression of buffering capacity of carboxyl groups at higher pH values.

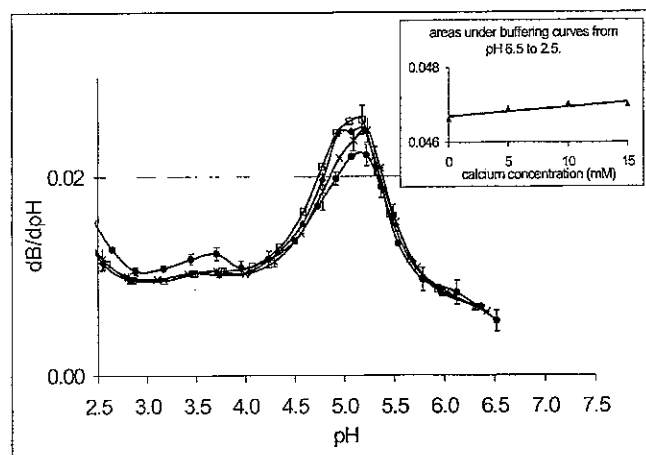


Fig. 3: Effect of the addition of CaCl_2 (x, 5; ◆, 10 and □, 15 mM calcium) on the buffering capacity of casein micelles suspension (●).

Addition of CaCl_2 to the casein micelle suspension induced a slight increase in the intensity of the buffering capacity (Fig. 3). Although casein micelles were suspended in water, the aqueous phase probably contains small quantities of inorganic phosphate (15) which can be associated with added calcium to form insoluble calcium phosphate (1, 24-26). There is then a higher quantity of colloidal calcium phosphate to solubilise by acidification. When these salts are solubilised, especially inorganic phosphate, the buffering capacity is increased. Several authors who studied the behaviour of calcium-supplemented milk during acidification have offered this interpretation. PHILIPPE *et al.* (27), therefore, considered that more acid is required in calcium-supplemented milk to achieve the same micellar mineralisation as in non calcium-enriched

milk. GUILLAUME *et al.* (28) showed that the buffering capacity of milk increases in the pH range 4.5-5.5 after addition from 0 to 17.5 mmol CaCl_2 /kg milk

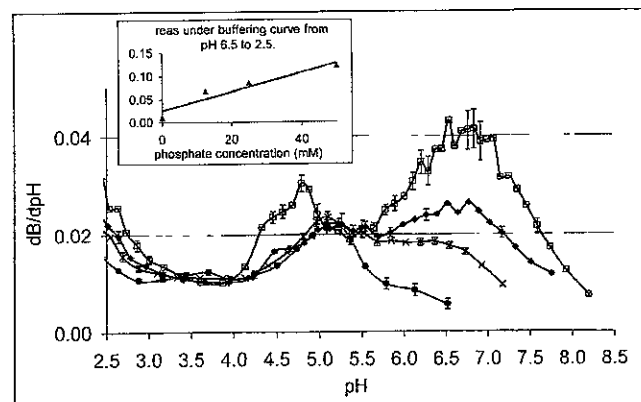


Fig. 4: Effect of the addition of sodium phosphate (x, 12.5; ◆, 25 and □, 50 mM) on the buffering capacity of casein micelles suspension (●).

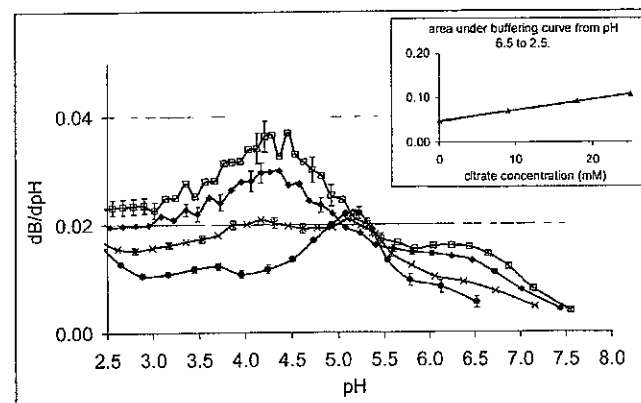


Fig. 5: Effect of the addition of sodium citrate (x, 9; ◆, 18 and □, 25 mM) on the buffering capacity of casein micelles suspension (●).

Addition of sodium phosphate or sodium citrate induces qualitative and quantitative changes in the buffering capacity of micellar caseins during acidification. Addition of 12.5 and 25 mM phosphate to casein micelle suspension increased the intensity of the buffering capacity in the pH range 7.2-5.2 and 7.7-5.2, respectively (Fig. 4). Below pH 5.2, the buffering capacity was unchanged. Addition of 50 mM phosphate produced a more significant increase in buffering capacity in the pH range 8.2 to 5.5 (with a maximum at pH 6.7) and at pH 4.8. The changes in buffering capacity were mainly due to acid-base properties of the added phosphate. For the lowest concentrations (12.5 and 25 mM), this ion slightly interacts with the casein micelles (24) and the acid-base properties expressed at a pH value corresponding to the pKa value (6.8 or 7.2 according to the physico-chemical conditions of determination (10, 13)). When phosphate is at 50 mM, it probably forms new calcium phosphate salts during acid titration and it is necessary to acidify more intensely to solubilise inorganic phosphate. A new buffering peak was therefore observed at pH 4.8 when phosphate was totally solubilised.

When citrate was added to micellar casein, the intensity of the buffering capacity increased below pH 5.2, with a maximum at around pH 4.2 (Fig. 5). Citrate is an anion containing three carboxyl groups which have acid-base properties in a wide pH range. The three pKa values are 3.0, 4.1 and 4.8 in milk (10). As these values are relatively close, buffering peaks were subsequently observed in a pH range between 5.2 to 2.5. On the other hand, the buffering capacity also increased between pH 7.5 and 5.2. This increase was due the chelation properties of citrate towards micellar calcium. As a consequence of this chelation, citrate shifts calcium ions from the micellar phase to the aqueous phase (1). At the same time, as calcium and phosphate are associated in the micellar phase, solubilisation of phosphate ions may occur (20) and consequently the buffering capacity is increased in a pH range corresponding to its pKa value (around pH 6.8-7.2), as previously described when sodium phosphate was added (Fig. 4). These increases in buffering capacity in the presence of phosphate and citrate are very important for the dairy industry. For example, in cheese manufacture the addition of a mixture containing citric acid and disodium phosphate to milk increases the buffering capacity and improves the texture and flavour development during cheese ripening (29). On the other hand, addition of phosphate is a common practice because it improves the stability of milk during heat treatment (30). Addition of this salt is thus used for goat's milk, particularly unstable milk during heat treatment. The buffering capacity of phosphate limits the decrease in pH due to heat-induced reactions such as lactose degradation and calcium phosphate insolubilisation.

4. Conclusion

The buffering capacity of casein micelles depends on several compositional factors. Among these factors, inorganic phosphate, citrate and association of caseins contribute to the buffering characteristic and variations in the composition affect the buffering capacity. Interpretation of these changes must always be taken into account of mineral modifications.

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